Local Supercoil-Stabilized DNA Structures

E. Paleček

Max-Planck Institut für Biophysikalische Chemie, Gbttingen, BRD and Institute of Biophysics, Czechoslovak Academy of Sciences, 61265 Brno, CSFR

ABSTRACT: The DNA double helix exhibits local sequence-dependent polymorphism at the level of the single base pair and dinucleotide step. Curvature of the DNA molecule occurs in DNA regions with a specific type of nucleotide sequence periodicities. Negative supercoiling induces *in vitro* local nucleotide sequence-dependent DNA structures such as cruciforms, left-handed DNA, multistranded structures, etc. Techniques based on chemical probes have been proposed that make it possible to study DNA local structures in cells. Recent results suggest that the local DNA structures observed *in vitro* exist in the cell, but their occurrence and structural details are dependent on the DNA superhelical density in the cell and can be related to some cellular processes.

KEY WORDS: supercoil-stabilized DNA structures, DNA double helix polymorphy, probing of DNA structure, DNA structure in cells.

I. INTRODUCTION

Until the end of the 1970s, it was generally accepted that the DNA double helix is very regular and independent of the nucleotide se-.' quence.[1-3] This conclusion was based mainly on data obtained by means of the X-ray fiber diffraction technique that had been used to study DNA structure for more than 2 decades. During the 1960s and 1970s, evidence based chiefly on the results of empirical techniques gradually mounted,[4-10] e.g., suggesting that the structure of the DNA double helix is sequence dependent and influenced by environmental conditions.[10] In the early 1970s Bram[11,12] reached a similar conclusion based on his studies using X-ray fiber diffraction. Due to its limited resolution,'.this technique yields only an averaged DNA conformation; it cannot detect local variations in the double helix induced by the particular nucleotide sequence.[13] Using this technique and DNA samples with extremes of base composition, however, Bram[12] was able to predict an almost infinite polymorphy of DNA in the B state. At about the same time, Pohl and Jovin[14,15] obtained circular dichroism (CD) spectra of poly(dG-dC)·poly(dG-dC), which suggested that this polynucleotide at high salt concentrations assumes a structure differing from B-DNA and possibly left-handed.

The untenability of the single DNA structure conception became obvious in the mid-1970s. Based on results obtained with various techniques, it was suggested that the DNA double helix is polymorphic,[10,12] depending on the duplex nucleotide sequence and its anomalies as well as on environmental conditions.[10] This conclusion, however, received little attention at the time of its publication.

The situation changed dramatically by the end of the 1970s, when the first results from single-crystal X-ray analysis of short deoxyoli-

gonucleotides were reported. Unlike fibers, crystals are ordered in three dimensions and can diffract X-rays at or near atomic resolution, providing substantially more data. Due to these factors, minute details of the DNA double helix can be observed. The crystal structure of d(pATAT) with different sugar phosphate conformation at adenine and thymine residues reported by Viswamitra et al.[16] in 1978 led to the proposal of an alternating B-DNA structure for poly(dA-dT)·poly(dA-dT) with a dinucleotide repeat unit.[16-18] The left-handed Z-DNA structure of d(CGCGCG)[19] and d(CGCG) crystals[20,21] solved at high resolution came as a surprise and immediately transformed DNA structure studies into a flourishing field. Shortly afterward it was shown that the structure of a DNA dodecamer d(CGCGAATTCGCG) was right-handed;[22-24] its structure differed, however, from that deduced by studying DNA fibers. Particularly interesting was the dependence of local structure on the nucleotide sequence. At the present time close to 80 deoxyoligonucleotides containing four or more base pairs have been studied by single-crystal X-ray analysis, conclusively demonstrating the nucleotide sequence-dependent polymorphy of the !, DNA double helix.''

Further aspects of DNA structure polymorphy were uncovered soon after the discovery of Z-DNA. These included sequence-directed DNA curvature[26,27] and local DNA structures stabilized by supercoiling.[28-35] In 1980, correlation analysis of chromatin DNA nucleotide sequence revealed that certain dinucleotides,[26] e.g., AA or TT, occur at regular intervals correlated with the pitch of the DNA double helix. The regular in-phase occurrence of these nucleotides was supposed to be responsible for the unidirectional curvature of DNA in chromatin. A few years later the anomalously slow electrophoretic mobility of a DNA fragment from *Leishmania tarantolae* kinetoplast was explained by DNA curvature.[27] The determination of the nucleotide sequence of 'this' fragment revealed runs of adenines and thymines in phase with turns of the DNA helix.

Cruciforms[28-31] and left-handed DNA segments[32,33] were among the earliest discovered supercoil-stabilized local DNA structures. A necessary condition for the formation of this type of structure is a suitable nucleotide sequence and a superhelix density sufficiently negative to stabilize the given structure. In the past decade great progress has been made in the elucidation of the relations between the DNA supercoiling, local structures, and their dynamics, interactions, extrusion kinetics, and other properties (reviewed in References 31 and 34 to 36). On the other hand, understanding the biological role of local structures has lagged considerably. Greater progress can be expected in this area within the next few years due to the recent development of research techniques for the study of DNA structure in the cell.[37-43] This article deals mainly with local DNA structures stabilized by supercoiling *in vitro* and in the cell; other aspects of DNA structure polymorphy are briefly summarized.

II. MICROHETEROGENEITY OF THE DNA DOUBLE HELIX FORMS

Studies of the detailed relationships between nucleotide sequence and DNA structure became feasible by the end of the 1970s, when organic synthesis had been developed to the point that deoxyoligonucleotides could be produced in the purity and quantity necessary for the preparation of single crystals for X-ray diffraction (and nuclear magnetic resonance, [NMR]) studies.'' Three main families of DNA forms were identified by crystallographic analysis of deoxyoligonucleotides (for review see References 13, 25, and 45 to 47): right-handed A- and B-forms and the left-handed Z-form.

A. A-, B-, and Z-Helices

The A-, B-, and Z-helices have distinctly different shapes that are due to the specific positioning and orientation of the bases with respect to the helix axis. In A-DNA the base pairs are displaced (0.4 nm) from the helix axis, the major groove is very deep, and the minor groove is very shallow. In B-DNA the major and minor grooves are of similar depths and the helix axis is close to the base pair center. In Z-DNA the minor groove is deep and the major groove is convex. In A- and B-DNA a single nucleotide can be considered as the repeat unit, while in Z-

DNA the repeat unit is a dinucleotide. In A-duplexes base pairs are heavily tilted in contrast to base pairs in B-duplexes, which are almost perpendicular to the helical axis. Definitions and nomenclature of nucleic acid structure parameters were published recently.[48]

The distinguishing averaged helical parameters of the DNA forms are given in Table 1. Many of the structural differences between the helices arise from the puckering of the sugar ring; C3'-endo is typical for A-DNA, while in 2-DNA C3'-endo alternates with C2'-endo. In B-DNA sugar pucker tends to favor the C2'-endo or C1'-ex ~ : but the distribution of conformations is much broader than in A- and Z-DNA. The right-handed A- and B-forms have the anti glycosidic bond, whereas in the left-handed 2-helix the orientation alternates between syn (for purines) and anti (for pyrimidines). In the latter structure the orientation around the C4'-C5' bond with respect to the C3' atom alternates between gauche ± and trans conformations for cytidine and guanosine, respectively. The alternating features of Z-DNA result in the zigzag shape of its sugar-phosphate backbone, from which the name was derived. The changes in the backbone and glycosidic-bond conformations are accompanied by substantial variations in the stacking interactions between

successive base pairs in 2-DNA. Methylation or bromination of cytosines at position 5 (studied mainly in oligonucleotides with alternating C-G sequence) stabilizes 2-DNA. Under certain conditions even nonalternating sequences of purines and pyrimidines can assume the conformation of Z- with thymines in a syn orientation.[49,50] The outer surface features of such a 2-helix are different at the nonalternating sites, but the backbone is similar to that observed with alternating sequences.

B. Local DNA Structure and Nucleotide Sequence

Average helix parameters for some right-handed structures are given in Table 2. The significant variations in some of these global parameters dependent on nucleotide sequence result in local changes along the DNA double helix. Such relations have been analyzed in detail by several authors and reviewed by Shakked and Rabinovich.[13] In A- and B-DNA these variations seem to be determined mainly by the specific interactions between the stacked base pairs and also to some extent by neighboring bases. In particular, homopolymer dinucleotide steps show a

TABLE 1
Comparison of A-, B-, and Z-DNA

Helix sense	A-DNA. right-handed	B-DNA[a] right-handed	B'-DNA[b] right-handed	Z-DNA[c] left-handed
Base pairs per turn	11	10	10	12 (6 dimers)
Helix twist (°)	32.7	36.0	34.1, 36.8	−10, −50
Rise per base pair (A)	2.9	3.4	3.5, 3.3	3.7
Helix pitch (A)	32	34	34	45
Base pair tilt (°)	13	0	0	−7
P distance from helix axis (A)	9.5	9.3	9.1	6.9, 8.0
Glycosidic orientation	anti	anti	anti	anti, syn
Sugar conformation	C3'-endo	Wide range	C2'-endo	C2'-endo, C3'-endo[d]

[a] Numerical values for each form were obtained by averaging the global parameters of the corresponding double-helix fragments.

[b] B'-DNA values are for a double helix backbone conformation alternating between conformational states I and II.

[c] The two values given correspond to CpG and GpC steps for the twist and P distance values, to cytosine and guanosine for the others.

[d] Two values correspond to the two conformational states. From Kennard, O. and Hunter, W. H., Q. Rev. Biophys., 22, 3427, 1989. With permission.

TABLE 2
Average Helical Parameters for Selected Right-Handed Structures

	Helix twist (°)	Rise per base pair (A)	Base pair tilt (°)	Propeller twist (°)	Groove width (Å)		Displacement Da (Å)
					Minor	Major	
A-form							
d(GGTATACC)	32	2.9	13	10	10.2	6.3	4.0
d(GGGCGCCC)	32	3.3	7	12	9.5	10.1	3.7
d(CTCTAGAG)	32	3.1	10	11	8.7	8.0	3.6
r(GCG)d(TATACGC)	33	2.5	19	12	10.2	3.2	4.5
r(UUAUAUAUAUAUAA)	33	2.8	17	19	10.2	3.7	3.6
Fiber A-DNA	33	2.6	22	6	11.0	2.4	4.4
9-form							
d(CGCGAATTCGCG)	36	3.3	2	13	5.3	11.7	−0.2
d(CGCGAATTBrCGCG)	36	3.4	−2	18	4.6	12.2	−0.2
Fiber B-DNA	36	3.4	2	13	6.0	11.4	−0.6

BrC = 5-bronecytosimo.
Adapted from Kennard, O. and Hunter, W. N., *Q. Rev. Biophys.*, 22, 327, 1989. With permission.

wide spectrum of stacking characteristics that are markedly neighbor dependent. On the other hand, pyrimidine-purine steps in A-DNA (especially the C-G steps) often display a low twist and high slide that are only slightly dependent on neighboring steps. In Z-DNA the shape of the helix surface changes significantly due to deviations in the regular alteration of the purine-pyrimidine sequence, while the sugar-phosphate backbone does not change. The effect of the nucleotide sequence on the fine geometrical features of each DNA form has been clearly demonstrated but not fully elucidated. The emerging rules, however should be considered as tentative since they were based on a relatively small number of examples. The well-known ''Calladine's rules''[51] are now perceived to be incomplete and to neglect important factors other than the steric clash of purine ring~.~

C. DNA Hydration

Information about the organization of water molecules in DNA forms has recently been gained (reviewed in References 25, 52, and 53) from X-ray diffraction analysis of crystals. Distinct hydration patterns were observed in the major and minor grooves and around the sugar-phosphate backbone. It was proposed that in DNA with a

mixed nucleotide sequence hydration of the backbone is related to global conformation.[52] In A- and Z-DNA a chain of water molecules can bridge the phosphate oxygens along the backbone. There are more water molecules around each phosphate group in the B-DNA, but almost no water bridges between the phosphate oxygens, as the distance between phosphate oxygens in this DNA form are too great to be linked with a single water molecule. It appears that specific nucleotide sequences that create local changes in the DNA double helix may also affect the backbone hydration pattern.[54,55] Even greater dependence of the hydration patterns on nucleotide sequence has been found in the DNA grooves. In A-DNA specific hydration patterns occur in the major grooves.[25] A string of well-ordered water molecules hydrogen bonded to oxygen and nitrogen atoms in the minor groove has been found in the central AATT sequence of the B-DNA dodecamer d(CGCGAATTCGCG).[56,57] This specific hydration of B-DNA, the ''spine of hydration'', significantly contributes to DNA stability. Studies of further B-DNA helices (Table 3) revealed two ribbons of water molecules along the walls of wide regions of the minor groove, while narrow regions of the minor groove contained an ordered zigzag spine of hydration.[46] It appears that the interdependence between nucleic acid structure and the solvent represents one of the

TABLE 3
Summary Comparison of Properties of B-DNA Helices

Nucleotide sequence	I.D. code[a]	Resolution (Å)	Minor groove width[b]			Mean propeller twist[c]			Minor groove hydration[d]			Helix bend at/ (if present)
CGCGAATTCGCG	HD	1.9	W	N	W	L	H	L	b	S	b	Bent, CGCG/AATTCGCG
CGCAAATTTGCG	MC	2.2	W	N	W	L	H	L	b	u	b	Bent, CGC/AAATTTGCG
CGCAAAAAAGCG	HN	2.5	W	N	W	L	H	L	b	u	b	Bent, CGC/AAAAAAGCG
CGCAAAAATGCG	AD	2.6	W	N	W	L	H	L	b	u	b	Bent, CGC/AAAAATGCG
CGCGAATTBrCGCG	MK	2.3	W	N	W	L	H	L	b	S	b	Straight
CGCATCTCTGCG	CY	2.2	W	N	W		I		b	u	b	Bent, CGC/ATATATGCG
CGCGATATCGCG	ZS	2.2	W	N	W	L	I	L	b	S	b	Bent, CGCG/ATATCGCG
GpsCGpsCGpsC	WC	2.2		W			L			R		Straight
CCAAGATTGG	GA	1.3		IWWWI		L	H	L		R		Straight
CCAACGTTGG	CG	1.4		WWNWW		L	I	L	R	S	R	Straight
CGATCGATCG	KK	1.8		WNWNW		L	I	L	S	R	S	Straight
CCAGGCCTGG	UH	1.6		IWIWI		L	(I)	L	R	S	R	Straight

[a] Identification code usually from initials of first author: HD = Horace Drew; MC = Miquel Coll; HN = Hillary Nelson; AD = Anna DiGabrieli; MK = Mary Kopka; CY = Chun Yoon; ZS = Zippora Shakked (personal communication); WC = William Cruse; GA,CG = Gilbert Prive; KK = Kazunori Yanagi and Kasimietz Gtzeskowiak; UH = Udo Heinemann.

[b] W = wide minor groove (>12 Å), I = intermediate minor groove, N = narrow minor groove (<10 Å).

[c] H = high propeller twist (>15°), I = intermediate propeller twist, L = low propeller twist (<10°).

[d] S = central spine of hydration; R = two ribbons along groove walls; d = hydration irregular or disordered; b = groove blocked, no information; u = hydration state uncertain because of lack of resolution. From Dickerson, R. E. in *Structure and Methods, Vol. 3, DNA* and RNA, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, N.Y., 1990, 1. With permission.

bases for DNA double helix polymorphy (reviewed in References 25, 52, and 53).

D. Continuum of Right-Handed DNA Conformation

The original concept of unique global conformations of right-handed DNA based on X-ray fiber diffraction analysis is undergoing significant change. Instead of discontinuous states of A-, B-, and C-forms that are stable only in conditions that differ significantly from one another, a continuum of right-handed DNA conformation is being considered.[25,58] A recent comparison of the published A- and B-crystal structures revealed[58] that certain structural features such as major groove width, rise per base pair, and base pair inclination change in an almost continuous fashion upon going from A- to B-DNA. A- and B-DNA can be distinguished, however, by lateral displacement of the base pairs from the helix axis (Table 2) that changes discontinuously between the two helical families. It has been shown that in the crystal structure of d(GGBrUABrUACC) A and B conformations can coexist.[59] Coexistence of these two forms was detected also in d(GGGGGTTTTT)·d(AAAAACCCCC) in 6 M NaCl by Raman spectroscopy showing an A-B junction occurring over a small span of bases.[60] This may imply that adjacent segments of DNA might adopt different global conformations in vivo, for example, as a result of a specific DNA protein interaction in the absence of drastic environmental changes.

E. DNA Structure, Crystal Packing, and Environmental Conditions .

In spite of the great progress made in the crystallographic analysis of DNA in the last decade, some basic problems still remain to be clarified, including questions of the influence of molecular packing in the crystal and the effect of temperature, hydration, etc. Quite recently it was revealed that the DNA molecular conformation and molecular packing are closely related.[61] The global DNA structure of the same oligonucleotide may vary substantially between different crystalline states; however, it also appears that the great sensitivity of DNA structure to crystal packing might be limited to only short A-DNA fragments (up to about *8 bp).*[62] Furthermore, the structure of a B-DNA decamer can be nearly the same in two very different crystal lattices.[63]

Local DNA structure in the given crystal environment is determined not only by nucleotide sequence but also by hydration and temperature effects.[46,61] An interesting question is the relation between the DNA structure in crystals and in solution. GC-rich oligonucleotides often crystallize as A-DNA,[58] but their solution structure as determined by Raman or NMR spectroscopy[64,65] at moderate ionic strengths is predominantly B-DNA. A possible explanation may be seen in the presence of both forms in solution (a smaller amount of A-form being undetectable due to the limited sensitivity of spectroscopic techniques) combined with preferred oligonucleotide crystallization as A-DNA (which would represent a more favorable way of crystal packing than the B-form).[58] Dependence of the DNA crystal structure on temperature[46,61,66,67] and other environmental conditions as well as partial opening of the base pairs[u] in B-DNA under conditions far below melting might be related to premelting changes in DNA conformation observed in solution.[10]

III. DNA CURVATURE AND BENDING

The helical axis of a DNA segment can be unidirectionally bent if tracts of adenine residues occur at regular intervals (around 10.5 bp) of the helical repeat (reviewed in References *68* to 72).

Bending of DNA can also be induced by an external force; protein-induced bending has been reviewed recently.[73,74] Compared with normal DNA, intrinsically curved DNA has a decreased electrophoretic mobility;[69,71,72,75,76] this property has proven to be a useful experimental criterion, but it should not be taken as an absolute indication of DNA curvature. Other factors can result in altered DNA mobility and under some conditions curved DNA molecules may display normal electrophoretic mobility.[72] DNA curvature can also be visualized in the electron microscope.[70,77,78]

A. Nucleotide Sequence Requirements and Phasing of the Sequence Motif

From a detailed analysis of the sequence requirements for DNA bending a general picture may be drawn:

1. An intact tract of four to six adenine residues is important for the phenomenon, with six A's supplying the maximum effect.[71]
2. Interrupting the sequence with G, C, or T strongly decreases the effect (T is the least effective);
3. Flanking of the A-tract by T on the 3'-side and by C on the 5'-side results in the greatest degree of curvature.
4. Adenine can be replaced by inosine in the A-tract[79,80] (curvature remained detectable for a small number of I in sequences such as AIAIA and AAIAA; it disappeared, however, in oligomers composed only of I·C pairs).
5. Deletion of the 2-amino group from the minor groove of the B-DNA helix increases DNA curvature, while displacement of an amino group from the major groove to the minor one may eliminate curvature.[81]
6. Methylation of adenine can increase DNA bending.[82] Pyrimidine 5-methyl groups influence the magnitude of the DNA curvature;[83,84] such effects are position dependent.[83]

Proper phasing of the sequence motif is of crucial importance. If the motif was repeated every

9 or 12 bp, DNA bending was significantly reduced, whereas repetition every 1.5 helical turns resulted in the disappearance of electrophoretically detectable bending.[85,86] The possibility that the A-tract might provide a flexible universal hinge for bending was excluded by the latter experiment. DNA curvature is influenced by environmental conditions. Increasing temperature[69] or NaCl concentration[69,87] decreases DNA bending, while addition of divalent ions increases bending in some cases.[69,88]

B. Structural Models

Intrinsic curvature represents an interesting aspect of the sequence-dependent polymorphy of the DNA double helix. Attempts have been made to explain this phenomenon and structural models proposed. "Wedge models",[89] based on Trifonov and Sussman's[26] suggestion that the angle between base planes (the wedge angle) is sequence dependent, emphasize the smooth deformation of DNA by a series of small tilt and roll components between adjacent base pair planes. Bolshoy et al.[90] recently showed that in addition to AA·TT, other base pair stacks, namely, AG·CT, CG·CG, and GA·TC, have large wedge angles that induce appreciable curvature into DNA molecules as detected by their anomalous gel mobilities. They considered the possibility that formation of transient kinks (as a result of forces applied to DNA during gel electrophoresis), especially in CA·TG and AC·GT stacks, may influence the anomalous mobilities of curved DNAs. In the "junction model"[71,91] a deflection of the helix axis is expected at the junction between B- and non-B- (A-tract) DNA. The wedge model is more general,[71] as it can include some aspects of the junction model.[89]

C. Structure of A-Tracts

Despite numerous studies (reviewed in References 71 and 72), uncertainty remains regarding the structure of A-tracts. The original suggestion that poly(dA)·poly(dT) adopted a heteronomous structure[g2] was abandoned after further X-ray fiber diffraction studies[g3] and reexamination of the original data.[92] It was shown that the results of the X-ray fiber diffraction studies are most consistent with the B-type helix with a distinctly narrow minor groove, large positive propeller twist, and negative tilt of the base pairs (B'-DNA). According to energy calculation,[94] stacking energies of AT pairs in poly(dA)·poly(dT) are not optimal, but the lost stacking energy seems to be compensated for by a formation of a water spine in a minor groove; such a hydration network cannot form in GC-rich sequences where the amino group of guanine intrudes into the minor groove.

Single-crystal X-ray analysis of several A-tract-containing DNA duplexes confirmed the presence of the narrow minor groove and a markedly pronounced propeller twist of the AT pairs.[95-99] In addition, a changed Watson-Crick base pairing was observed in which the C6 amino group of adenine formed an additional hydrogen bond with the O_4 of the 3'-neighboring thymidine. It has been suggested[72] that the presence of bifurcated hydrogen bonds is not required for DNA curvature since AIAIA sequences display a significant curvature but are not able to form bifurcated hydrogen bonds.[79] The single-crystal X-ray measurements produced important information regarding short-range conformation of the A-tract but do not provide unambiguous evidence for the structural basis of curvature. The observed junctional distortions may be a result of crystal lattice constraint. It appears that the A-tract curvature in crystals is due to the intramolecular packing constraints of the crystal lattice and that the structure of this tract is dependent on its length. Lengthening of an A-tract resulted in a cooperative switch from B-DNA structure to one more characteristic of poly(dA)·poly(dT);[71] in longer A-tracts the minor groove narrowed over the first three base steps and then remained almost constant. Measurements of imino proton exchange rates showed anomalously long lifetimes in A4- and A5-tracts.[100] Thymidine protons in shorter tracts tended to have shifts similar to those of B-DNA.

Using CD and UV absorption spectroscopy, Herrera and Chaires[101] provided evidence of temperature or dimethylsulfoxide-induced premelting conformational transition of poly-(dA)·poly(dT). Their results suggested the existence of at least two species at equilibrium

under conditions far below the melting transition. A distinct premelting transition in the duplex GA_4T_4C was observed by Park and Breslauer[72] by means of differential scanning 'calorimetry. These results are consistent with the hydration model proposed by Chuprina.[94] Disruption of the spine of water in the minor groove induced by the temperature or dimethylsulfoxide would result in loss of curvature and normalization of the electrophoretic mobility actually observed at elevated temperatures.[69]

D. Biological Role

Intrinsic DNA curvature has attracted great attention in recent years, but the question of its biological role has not been solved. Numerous papers have been published (reviewed in Reference 72) reporting loci of curvature in a wide variety of organisms, in many cases in regions of functional importance. Poly(dA)·poly(dT) and DNA containing A_n-tracts from *Leishmania tarantolae* were shown to be immunogenic[102] and specifically detectable by antibodies. Yet, is a curvature per se of biological importance, or is it the A-tract sequence only that has an important function? Recent results obtained by Linial and Shlomai[103-107] suggest that the endonuclease from C. *fasciculata* binds in a specific manner to curved DNA, recognizing specific local variations in helix trajectory. It has been shown that a number of curvature loci appear near prokaryotic (and Simian virus 40, SV40) origins of replication and that the degree of curvature of such loci is increased due to binding of specific replication proteins, suggesting a possible relation between curvature and replication.[72] Transcription activity can be significantly influenced by the presence of a curved DNA segment in the vicinity of a promoter.[108,109] Binding of the catabolite activator protein to upstream sites in many E. *coli* promoters induces DNA bending and transcription activation. Introduction of intrinsically curved DNA upstream from the promoter resulted in transcription activation in the absence 'of catabolite activator protein binding,[109] suggesting that DNA curvature plays an important role in the transcription activation. Involvement of DNA

curvature in DNA recombination was also demonstrated recently.[110,111]

Considering the biological relevance of curved DNA, the properties of negatively supercoiled DNA-containing curvature-inducing sequences are of particular interest; however, little is known about the relations between supercoiling and DNA curvature. It has been shown that the presence of curved segments can influence writhing of the supercoiled DNA.[87] Minicircle kinetoplast DNA-containing curvature-inducing sequences were linearized by single-strand selective mung bean nuclease in the presence of 50% formamide.[112] Supercoiled plasmid pK5/6T217 was site-specifically modified under physiological conditions with osmium tetroxide, pyridine[113] within the curvature-inducing sequence dATATATTTTTTAGAGATTTTT. Such site-specific modification was detected neither in linearized pK5/6T217 nor in supercoiled and linearized plasmids containing a slightly less curved dGACAAAACTC sequence. The site-specific modification pK5/6T217 with the single-strand selective chemical probe suggests structural distortion in the curvature-inducing sequence resulting from the superhelical stress. It is, however, not clear whether this distortion is related to DNA curvature or only to the specific AT-rich sequence (see Section VII.D.2).

IV. DNA SUPERCOILING

Vinograd and co-workers[114] reported the discovery of the twisted circular form of polyoma viral DNA 25 years ago. Their results were closely connected to the earlier work of Weil[115] and Lebowitz,[116] who had demonstrated some unusual properties of polyoma DNA, as well as to the findings of Vogt and Dulbecco,[117] who had shown that conformation of this DNA can be changed by a single DNaseI cleavage event. Weil and Vinograd,[118] in collaboration with Stoeckenius, visualized three forms (linear, open circular, and twisted) of polyoma DNA in the electron microscope. By means of a series of experiments that 'included measurements of pH-induced changes in DNA sedimentation velocity, Vinograd and co-workers[114] explained the twisted

appearance of **polyoma** DNA by the formation of superhelical turns in the circular molecule. It soon became apparent that circular DNAs from many sources were supercoiled and that even linear DNA could supercoil inside the cell if constrained into topologically distinct domains[119,120] (reviewed in References 31, 45, and 121 to 125).

A. Decomposition of Linking Number to Twisting and Writhing

In linear DNA, the ends are free and the molecule can easily accommodate changes in helical pitch. If, however, the two strands become linked to form a loop or a covalently closed circular molecule, the number of times the strands wind about each other (the linking number, Lk) cannot by changed. Lk is an integer that may be decomposed[127-133] as

$$Lk = Tw + Wr \qquad (1)$$

where Tw (twisting) is the number of times one strand rotates around the DNA duplex axis, which corresponds to about 10.5 pb per unit twist for B-DNA in solution, and Wr (writhing), a measure of the global tertiary structure of the molecule, describes the deformation of the duplex axis. If the DNA duplex were able to under- or overwind easily, Lk would always be equal to Tw (as is the case with a relaxed DNA) and no supercoiling would arise. DNA tends, however, to retain its B-form, thus limiting Tw to a narrow range, and changes in Lk can be compensated for by changes in superhelicity and by local changes in DNA secondary structure and formation or absorption of some specific local structures, as explicated in the following section. Closed circular DNA molecules can be characterized by the linking difference ΔLk, the difference between Lk and Lk_o

$$\Delta Lk = Lk - Lk_o \qquad (2)$$

Lk_o is the linking number of a relaxed reference DNA (lacking superhelical turns) under the given conditions; ΔLk is often referred to as the number of superhelical turns. Equation 2 can be transformed into

$$\sigma = \Delta Lk/Lk_o \qquad (3)$$

where α is the specific linking difference (independent of the number of base pairs per DNA molecule), frequently termed superhelix density.

Isomeric DNA forms that result from topological differences are called topoisomers. Individual topoisomers of **plasmid** and viral DNAs can be resolved by one- or two 2-D gel electrophoreses[75,134] (due to the different shapes of each topoisomer molecule)-(see Section V.A). Each topoisomer can also be characterized by a difference in free energy arising from the deformations required to accommodate the linking difference. The free energy (ΔG_s) is related quadratically to ΔLk

$$\Delta G_s = \frac{K \cdot RT}{N} \Delta Lk^2 \qquad (4)$$

where K is the proportionality constant equal to 1050 for molecules larger than about 2 kb, R is the gas constant, T is the absolute temperature, and N is the number of base pairs per molecule. In naturally supercoiled DNAs the free energy is appreciable. At $\sigma = -0.05$, plasmid pBR322 has a free energy of about 100 kcal/mol.[31] In recent years the mathematical analysis of DNA supercoiling has expanded significantly.[133,135-140] It has been shown that the division of the linking number into three rather than two components is more convenient, as this helps to clarify relationships between experimentally measurable quantities and topological and geometric DNA properties. This new subdivision has been recently broadly developed;[128] its detailed discussion is beyond the scope of this article.

B. Negatively and Positively Supercoiled DNA

Equation 2 was applied to numerous purposes that included, in addition to the determination of the extent of supercoiling, determination of the free energy associated with DNA supercoiling,[121] determination of the average duplex rotation angle in DNA and its dependence on temperature and salt concentration,[121,141] analysis of the ex-

tent of DNA winding in nucleosomes,[141,142] screening of DNA binding modes in various drugs,[143] etc. In most of the naturally supercoiled DNAs $Lk < Lk_o$, such DNAs are called negatively supercoiled. They are underwound and the form of their superhelix is right-handed. A typical superhelix density of isolated **plasmid** or viral circular DNAs is around -0.06. Positively supercoiled (overwound, left-handed superhelical turns) DNA with $Lk > Lk_o$ was recently found in archaebacteria *Sulfolobus acidocaldarius*.[144] In E. *coli* and other prokaryotes, positively supercoiled DNA can be generated during transcription in front of the transcription ensemble.[145]

C. Physical Properties of Supercoiled DNA Molecules

Properties of **supercoiled** DNA and DNA interactions with single-strand binding proteins **as** well **as** with other proteins were studied by means of physical and physicochemical techniques.[147-165] The recent results of **Raman** spectroscopy measurements of supercoiled and nicked ColEl DNA suggest that accommodation of supercoiling occurs **mainly** in AT base pairs and backbone moieties.[161] Premelting effects might account for these changes, including a slight change in the band known to be responsible for base pair breakage. The major part of the alteration of the backbone geometry takes place in the **C-O** linkage between the **C5'** and adjacent phosphate group. **Raman** spectra obtained with supercoiled **pBR322**[159] and its derivative pFb100[160] did not completely agree with those of ColEl DNA; the reasons for this disagreement are not known. It is tempting to suggest that the presence of AT-rich C-inducing sequences[3'] in ColEl (See Section **VI.A**) **might** be connected with the observed **Raman** spectra of this DNA. Positively supercoiled pBR322 induced a negative contribution to CD of the main bands at 270 and 187 nm and showed a higher electrophoretic mobility when compared with the negatively supercoiled **topo**isomeric sample ($\sigma = 0.07$ and -0.07, respectively).[165] As expected, cruciform structure did not extrude in **the** positively supercoiled DNA.

D. Shape of Supercoiled DNA Molecules

Negatively supercoiled DNA can exist in two basic forms:[128,143] solenoidal (toroidal) and plectonemic (interwound). The latter form has been assumed to be the most probable form of negatively supercoiled DNA free in solution. Solenoidal supercoiling can be exemplified by the wrapping of DNA along **histones** in nucleosomes. There are some important differences between these two forms. The solenoidal form is left-handed, while the plectonemic (interwound) is right-handed. Plectonemic supercoiled DNA can be naturally branched, bringing together nucleotides that are distant from one another in the primary structure; this is not the case with solenoidal supercoils. It is probable that these two forms are in a dynamic equilibrium in **eukaryotic** cells,. Not all protein-bound supercoils must be necessarily solenoidal, as the supercoil form is dictated by the winding surface; well-characterized plectonemic **DNA/protein** complex can be represented by the synaptic intermediate of the Tn3 resolvase.[128,146]

Recent computer statistical-mechanical simulations of moderately and highly supercoiled DNA molecules (treating supercoiled DNA within the **wormlike** model with excluded volume) showed irregularly shaped molecules with characteristics of branched interwound helices at higher superhelix densities.[140] The calculations showed that the quadratic dependence of the superhelical free energy on Lk (Equation 4) is valid for a variety of conditions but is not universal. Significant deviations can be expected at high superhelix density under ionic conditions where effective diameter of DNA is small.

Experimental data concerning the shape of supercoiled DNA in solution are not free of ambiguity. The results of small-angle X-ray scattering are consistent with the toroidal shape but not with an interwound shape,[149,150] while dynamic light-scattering[152-154] and neutron diffraction in liquid crystalline solution[158] favor the interwound superhelical structure. Most electron microscopy work[162,163] displayed images consistent with the interwound model, but changes in the DNA shape due to the DNA absorption

and drying on the supporting film were not excluded. Quite recent observations by Adrian et al.[164] made by cryoelectron microscopy of vitrified specimens demonstrated the interwound form of the protein-free negatively supercoiled DNA. These authors also showed that the shape of the supercoiled DNA molecules was strongly affected by Mg^{2+} ions; in 10 mM Tris the diameter of the pUC18 DNA molecules was about 12 nm, while it was reduced to 4 nm in 10 mM $MgCl_2$. Approximate values of the partition of the linking difference (ΔLk) between the changes of writhe (ΔWr) and twist (ΔTw) were calculated from a single projection of pUC18 topoisomer with a $\Delta Lk = -12$ (in Tris solution with no added $MgCl_2$). The partition between ΔTw and ΔWr was estimated to be between 1:3 and 1:4. These are the frrst data of ΔTw and ΔWr partitions based on direct measurements.

E. Topoisomerases

Supercoiling is controlled by enzymes called topoisomerases (reviewed in References 166 to 168) that may be divided into two classes. The type I enzymes do not require an external source of energy; the transient interruption of a DNA phosphodiesteric bond is accompanied by the formation of an enzyme-DNA covalent intermediate that conserves the free energy of the bond. This energy is then used for resealing the broken strand after its rotation around the unbroken strand. Type II enzymes transiently break both strands, forming two covalent bonds between the DNA duplex and the enzyme molecule. The latter type of enzymes usually require ATP energy. Besides their basic ability to modify DNA linking number, topoisomerases can perform a number of topological reactions involving both double- and single-stranded DNAs, folding them into various kinds of topological knots and catenates.

V. METHODS OF ANALYSIS OF LOCAL DNA STRUCTURES

In natural supercoiled DNA molecules such as plasmids, local structures represent only a very small part of the whole molecule, usually around 1%. Application of conventional physical techniques to the study of these molecules is thus very limited. In the last decade new methods were developed that have made it possible not only to detect a local structure in the DNA molecule, but also to provide its exact location and information about its chemical reactivity at single-nucleotide resolution. In the early studies[29,30,32,33] analysis of DNA electrophoretic mobility, antibodies, and enzymatic probes represented the main tools in local DNA structure research. In the past 5 to 7 years chemical probes have been increasingly applied, partially replacing the enzymatic probes.

A. Analysis of DNA Electrophoretic Mobility

Individual topoisomers can be resolved on agarose gels (see Section IV). It was shown[169] that the electrophoretic mobility of circular DNA molecules containing long, inverted repeats increased regularly with supercoiling to a certain threshold level and then collapsed back. This collapse corresponded to the extrusion of the cruciform structure, presumably reflecting the decrease in writhing that accompanies the extrusion. In this way gel electrophoresis can yield information about cruciform extrusion and other transitions in supercoiled DNA molecules. In 2-D gel electrophoresis[75,170,171] a family of topoisomers in which ΔLk ranges from about zero to relatively large negative values is prepared first. Then electrophoresis is performed in the frrst dimension, followed by electrophoresis in the direction perpendicular to that of the first dimension. The electrophoresis in the second dimension is carried out in the presence of an intercalator, chloroquine, which reduces the twisting number Tw in all topoisomers. This decreases the supercoil-induced stress, so that any DNA segment that might have turned into a cruciform or another unusual structure reverts to its original B-form DNA. Thus, in the second dimension the mobility is governed only by the Lk of the topoisomers. As a result of this procedure an arc of topoisomers is formed in which a discontinuity is indicative of the local structural transition. From the results of 2-D gel electrophoresis, the superhelix density necessary for the structural transition as well as the change in Tw induced by this

transition can **be** calculated. In an experiment performed by Wang et al.,[171] for example, due to the flipping of a $(dG\text{-}dC)_{32}$ segment from a 10.5-fold helix into a left-handed 12-fold **Z-helix,** the observed ΔTw was equal to 6, corresponding well to the expected decrease by ~~(32/10.5)~~ **+** ~~(32112) or 5.7.~~ In addition, coupling between the free energy changes and local structural transitions in supercoiled DNA molecules can be studied[31,171] by 2-D gel electrophoresis, which" has been applied to studies of cruciforms,[172,173] Z-DNA,[171,174] and triplexes[175] in supercoiled plasmids. 2-D gel electrophoresis performed at various first dimension temperatures provided a sensitive assay for the detection of local structural transitions in closed duplex DNAs.[134]

The possibility of applying electric fields in two orientations was utilized in a different way in pulsed field eletrophoresis, developed over the past several years for the resolution of very large DNA molecules (ranging from 20 to 2000 kb).[176-178] This technique makes possible the separation of intact chromosomal DNA molecules from lower eukaryotes as well as large human chromosomal DNA fragments (References 179 to 181 and references therein) and can also be applied to studies of closed circular DNA molecules and their topology.[180,182,183]

Another method introduced recently is denaturing gradient gel electrophoresis,[184-186] using solvents[184] (urea, formamide), temperature,[185,187] or their combination[188] as denaturing agents. The latter technique was applied to the study of early melting of supercoiled DNAs.[188] Increased application of denaturing gradient gel electrophoresis can be expected in the near future, as this technique has many assets, including the possibility of studying DNA structural transitions, even in partially purified samples and in cell extracts containing small amounts of DNA.

The procedure based on the difference in electrophoretic mobility between a specific DNA-protein complex and free DNA is widely known as gel retardation analysis.[189,190] In the last decade this technique has been increasingly applied in DNA-protein interaction studies, including interactions with antibodies,[191-194] RNA polymerase,[195] etc. Practical protocols for gel retardation experiments[196] and reviews of recent application~ and factors affecting the lifetime and

mobilities of protein-DNA complexes[197] were published within the last 2 years. The gel retardation technique has been widely used in studies of DNA curvature (see Section III). The combination of curved stretches in phase with non-curved segments makes it possible to detect small structural changes involving changes in the DNA helix axis.[198]

B. Antibodies Recognizing Local DNA Structures

Nucleic acid immunochemistry provides valuable reagents for studies of complex biological materials and of purified nucleic acids (reviewed in References 199 to 201). Most abundant nucleic acids are only weakly immunogenic, whereas chemically damaged nucleic acids and less usual DNA structures show greater effectivity in inducing antibody formation. The low immunogenicity of B-DNA may be due to its rapid degradation by serum nucleases.[202] On the other hand, antibodies reacting with B-DNA were found in sera of patients with systemic lupus erythematosus.[200] Monoclonal antibodies have been isolated from hybridomas derived from mice with a disease similar to human lupus erythematosus. Some of them showed an ability to recognize B-DNAs with different nucleotide sequences.

Local DNA denaturation can be recognized by antibodies induced by insoluble complexes of denatured DNA with methylated bovine serum albumin or by conjugates of base nucleotides or nucleotides with proteins.[203,204] The most prominent subject of DNA structure immunochemical studies has been left-handed 2-DNA (reviewed in References 34, 199 to 201, and 205). Antibodies have been induced mainly by brominated or methylated poly(dG-dC)·poly(dG-dC) in Z-form. Monoclonal antibodies recognizing various nucleotide sequences or a single sequence have been generated.

Antibodies to triplex structures have been induced by triple-stranded polyribonucleotides and mixed polyribo- and polydeoxyribonucleotides.[205] Lee et al.[206] recently generated a monoclonal antibody against poly(dT-d^{5m}C)·poly(dG-dA)·poly(dT-d^{5m}C) capable of forming a triplex at neutral pH.[207] By use of this antibody, the presence of triplex structures in supercoiled

plasmids[206,208] at pH 5 as well as in cells and chromosomes[206] was demonstrated. Triplex structures were also sensitively detected by immunoblotting.[209] Monoclonal antibodies against cruciform structures have also' become available.[210]

Antibodies represent a very useful tool in local DNA structure research due to their high specificity and sensitivity and applicability in complex biological media. On the other hand, at higher concentrations of antibodies, the equilibrium between a B-form and a specific local DNA structure recognized by the antibody (e.g., Z-DNA) can be strongly shifted in favor of the latter structure. In cytological studies antibodies are applied after fixation, which may significantly influence the presence of the DNA structure detected by the antibody. These facts should be kept in mind when using DNA structure-specific antibodies to report the presence of local DNA structures in various biological materials (see Section VII).

C. Enzymatic Probes

The application of enzymes for probing local DNA structures is based on the ability of some nucleases to recognize and cleave DNA at sites with a more or less single-stranded character without cleaving the intact B-DNA. Properties of some single-strand selective nucleases were previously summarized.[10] Among these enzymes, nuclease S1 has been most frequently used for local DNA structure studies.[211,212] This enzyme cleaves DNA (at acid pH in the presence of Zn^{2+} ions) in the cruciform loops,[29,31] at the B-Z junctions,[213] at triplex sites,[175] etc. It appears that the enzyme recognizes distortions in the sugar-phosphate backbone,[214,215] but the exact structure of the sites is not known. It has been shown that nuclease S1 does not cleave at a single base mismatch, but two mismatches are sufficient for cleavage.[216,217] Mung bean nuclease displayed properties similar to those of S1 nuclease;[218,219] however, it has been used in local DNA structure studies to a lesser extent.[220-223] In contrast to these two enzymes, endonuclease from Neurospora crassa[224,225] and P1 nuclease[226] can be applied at neutral pH. Interest has increased in the latter

enzyme, which is commercially available.[226-228]

Enzymes do not seem to be ideal DNA probes for many reasons, including the limitation of their application to the conditions of optimum enzyme activity, their mostly unknown mechanism of action, the possibility of induction of secondary structural changes in DNA, etc. For these reasons, attempts have been made to find other DNA probes.

D. Chemical Probes of the DNA Structure

A common feature of the local DNA structures studied to date is the presence of bases that, compared with the bases in the B-form, have better accessibility for interaction with the environment, e.g., bases in the cruciform loop, at the B-Z junction, etc. One may thus expect that these bases will display enhanced reactivity toward some chemicals. In the beginning of the 1980s[229,230] we looked for a chemical probe of the DNA structure that could (1) bind specifically to single- and distorted double-stranded DNA regions under nearly physiological conditions, (2) form a covalent bond sufficiently stable even after the removal of the unreacted agent, (3) form a DNA adduct that is easily detectable even if only a very small friction of bases in the DNA molecule undergo the reaction, and (4) be potentially applicable for studies of DNA structure in situ. We have shown that osmium tetroxide, pyridine reagent (Os,py) fulfills the above requirement~.~~~~~.~~~

In addition to single-strand selective probes, chemicals that react with double-stranded B-. DNA, such as dimethylsulfate (see Section V.D.2) and agents with nuclease activity, can be applied to DNA structure studies using mainly the foot-printing approach (comparing the reactivities of DNA itself and its complex with a protein molecule or some other ligand). Photochemical reagents represent another class of DNA structure probes. We confine ourselves here mainly to the single-strand selective probes that have been demonstrated to have specific advantages in the study of local supercoil-stabilized DNA structures. Other probes are mentioned only briefly.

1. Single-Strand Selective Probes

Table 4 shows the most important probes that react preferentially with single-stranded and some non-B DNA regions. These probes can be subdivided into two groups: probes reacting with sites involved in Watson-Crick hydrogen bonding and probes reacting with bases at other sites. The former group includes chloroacetaldehyde (CAA) (Figure 1c), bromoacetaldehyde (BAA), glyoxal (Figure 1d), and N-cyclohexyl-N′-β(4-methylmofpholinium)ethylcarbodiimide-p-toluene (CMC) (Figure 1e); these probes cannot react within an intact B-DNA as they require hydrogen bond breakage (Figures 1c to e). The latter group includes osmium tetroxide complexes, KMnO₄, diethylpyrocarbonate (DEPC),

TABLE 4
Chemical Probes of the DNA Structure Reacting Preferentially with Single-Stranded and Non-B-DNA Regions

Probe	Base specifity	Ref.
A. Probes reacting with sites involved in Watson-Crick hydrogen bonding		
BAA, CAA	C, A	268, 286, 287, 288, 290, 291, 308, 309
Glyoxal	G	**264**, 293,310
CMC	T, G	294
B. Probes reacting in other sites		
Os,py, Os,bipy	T ≫ C, G	27, 37, 38, 230— 234, 245, 263, 268—270, 295, 296
KMnO₄	T ≫ C, G	298—300, **302**, 303, 304
OsO₄ (alone)	T ≫ C	305—307
DEPC	A > G	245, 278—282, 303
Hydroxylamine	C	245
Methoxylamine	C	313
NaHSO₃	C	310, 314, **315**
Ozone	T, G	316

■ Bold numbers refer to papers containing important methodological aspects; other represent examples of the probe application. Further references are given in text.

hydroxylarnine, and methoxylarnine. With the exception of DEPC, their reactions involve mainly C5 and C6 of the pyrimidine ring. Nonreactivity of bases in B-DNA is probably due to steric reasons.

All of the above-mentioned probes are highly specific, showing almost no reaction with B-DNA, provided the reaction conditions are properly chosen. The reaction sites in the DNA chains can be detected in various ways. Polyclonal antibodies were elicited in rabbits for the reaction products of the following probes: bisulfite, O-methylhydroxylarnine mixture,[235] CMC,[236] osmium tetroxide, pyridine (Os,py),[194] and osmium tetroxide,2,2′-bipyridine.[194,237] Monoclonals against the latter DNA adduct were generated in 1990.[237]

The availability of these antibodies opens up new possibilities in DNA structure studies, especially with respect to the detection of open local structures in situ, and makes possible the determination of specific DNA adducts at high sensitivity.

Thus far, osmium tetroxide complexes, DEPC, and BAA(CAA) have been applied to the largest extent to research local DNA structures. Properties of these probes are discussed in greater detail in the following paragraphs.

a. Osmium Tetroxide and Its Complexes

Osmium (VIII) tetroxide is a versatile electron acceptor and an effective reagent for the cis hydroxylation of alkenes under stoichiometric conditions (reviewed in Reference 238). In DNA osmium (VI) esters are formed (Figure 2) via addition to the 5-6 double bond of the pyrimidine ring.[37,239] In the 1970s this reaction was utilized for the introduction of a heavy metal stain with the intention of developing an electron microscopic method of DNA sequencing.[240] Ligands profoundly alter the nature of the osmium tetroxide reaction, changing the structures, kinetics of formation, and hydrolytic stability of the products. The reaction of Os,py with monomeric nucleic acid components have been studied in detail, chiefly by Behrman et al.[239,241] Os,py reacts most readily with thymine moieties in single-stranded DNA.[38] The structure
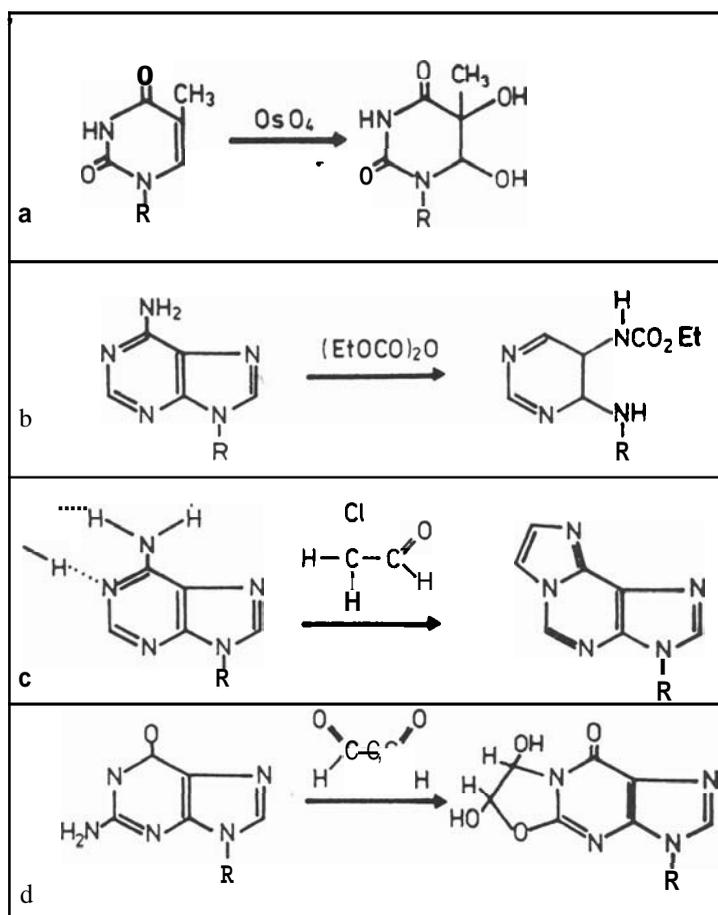
FIGURE 1. Formation of the adducts between DNA bases and single-strand selective probes: (a) osmium tetroxide (alone), (b) diethyl pyrocarbonate, (c) chloroacetaldehyde, (d) glyoxal, (e) N-cyclohexyl-N'-β-(4-methylmorpholinium) ethylcarbodiimide-p-toluene (CMC), ..., hydrogen bonding in the Watson-Crick base pair.

of the thymine-Os,py *cis* ester (Figure 2) was determined by single-crystal X-ray diffraction analysis;[242,243] osmium in this ester is approximately octahedral. Osmium binding sites in DNA chains can be detected in various ways (Figure 3), including detection at single-nucleotide resolution based either on the labilization of the sugar-phosphate backbone (at the site of the thymine osmate ester formation) to piperidine cleavage,[38,244-246] the ability of the adduct to terminate the transcription,[247] or the DNA primer extension in *vitro*.[248] Adducts of DNA and RNA with various osmium tetroxide complexes are reducible at the mercury electrode,[229-252] producing catalytic currents at negative potentials; these adducts can be determined at low concentrations by means of polarographic (voltammetric) techniques.

In the B-DNA double helix the target C5-C6 double bond of thymine is located in the major groove where it is not accessible to the bulky electrophilic osmium probe.[231] Local changes in helix geometry may render the C5-C6 double bond accessible to the out-of-plane attack of the Os,py molecule on the base π-orbitals. Such changes include base unstacking in the four-way junction at low ionic strengths,[253,254] single-base mismatches and bulges,[217,255-259] local changes in twist in (A-T)$_n$ sequences,[260] helix distortions in the vicinity of single-strand interruptions,[230,231] premelting of AT-rich sequences in supercoiled DNA,[261] etc. Os,py has shown an ability to recognize minute local changes in the DNA molecule that are not detectable by chemical probes such as BAA, CAA (both requiring rupture of

165

FIGURE 1E
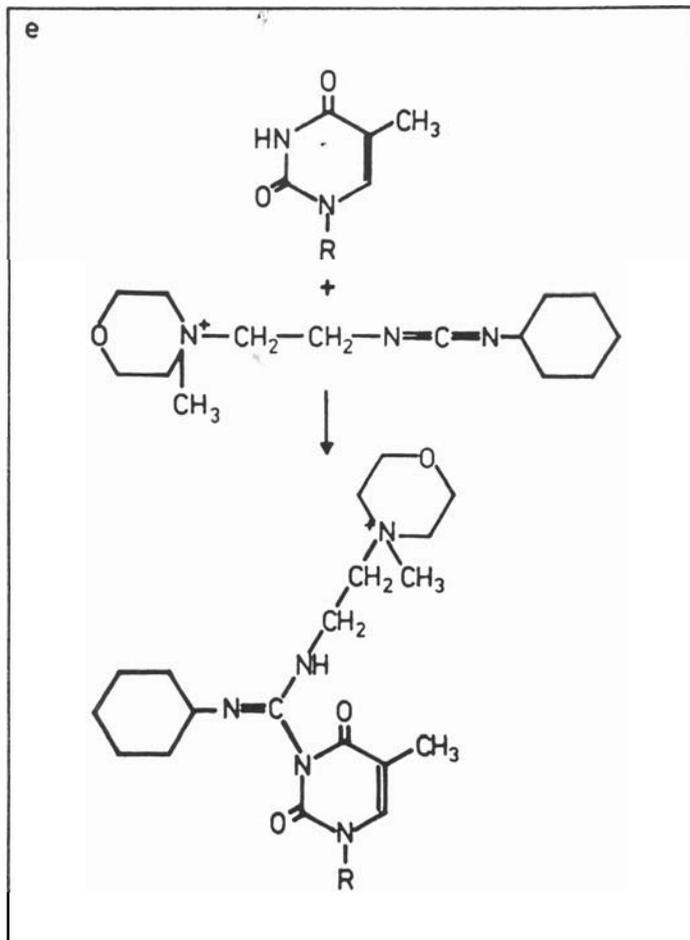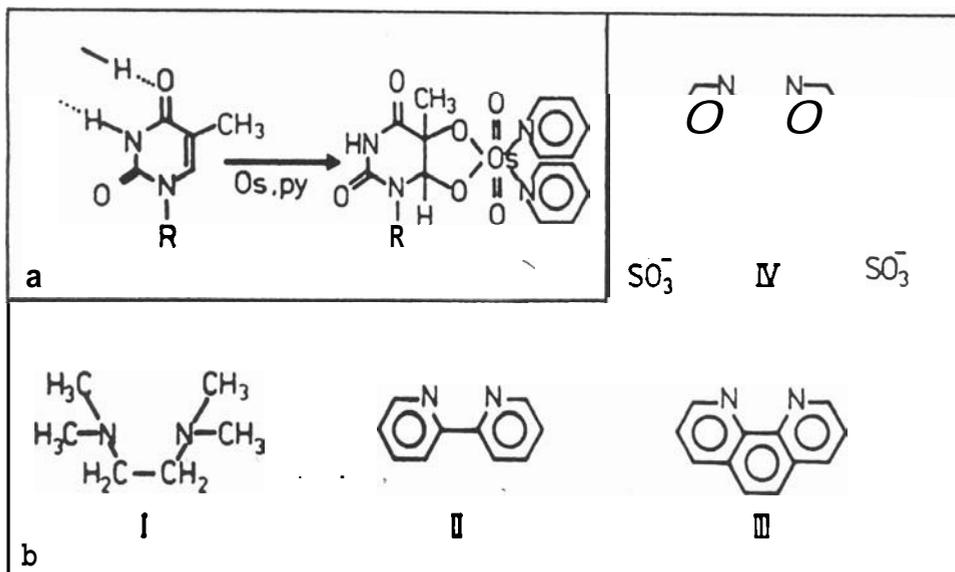

FIGURE 2. (a) Formation of the adduct between thymine and osmium tetroxide, pyridine; . . . , hydrogen bonding in the Watson-Crick base pair; (b) some ligands which can replace pyridine in the osmium complex: I, tetramethylethylenediamine (TEMED); II, 2,2'-bipyridine (bipy); III, 1,10-phenanthroline (phe); IV, bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) disulfonic acid (bpds).
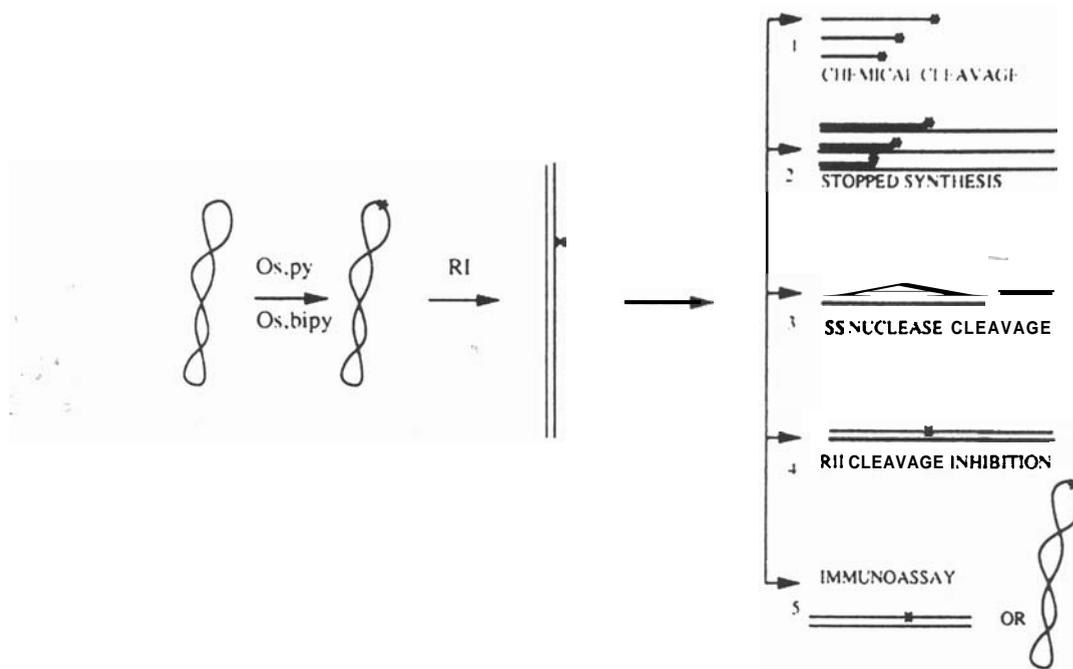
FIGURE 3. Scheme of treatment of supercoiled DNA with osmium tetroxide, pyridine (Os,py), or 2,2'-bipyridine (Os,bipy) and mapping of the osmium binding sites. To map the osmium binding sites DNA is cleaved by restrictase (RI) followed (1) by cleavage with hot piperidine and sequencing using the principles of the Maxam-Gilbert method; (2) by primer extension or transcription *in* vitro (terminated at modified bases in the template) and nucleotide sequencing; (3) by digestion with single-strand selective nuclease and (nbndenaturing) gel electrophoresis of the resulting DNA fragments; or (4) if a structural distortion (e.g., in the B-Z junction) is expected to occur within the recognition site of a particular restrictase (RII), the site-specific modification of the distortion can be manifested by inhibition of the restriction cleavage; (5) osmium binding in plasmids and DNA fragments can be determined by immunoassay (e.g., by DNA gel retardation, immunoblotting, or ELISA). Modification of large DNA regions may result in partial or full relaxation of the molecule (accompanied by changes in the electrophoretic mobility) and formation of a denaturation "bubble" visible by electron microscopy; the amount of bound osmium can be determined electrochemically.

Watson-Crick hydrogen bonds)[261] (Figure 1c), or DEPC.[217,253] Within a short time Os,py has proven to be a useful probe of DNA structure in *vitro*.[37,38]

At high pyridine concentrations in the Os,py reagent, low ionic strengths, and long reaction times, the initial attack of the probe may be followed by the formation and propagation of the "denaturation bubble" manifested by changes in the DNA electrophoretic mobility.[231,233,234] Large "bubbles" can be visualized in the electron microscope.[233,262] Under properly chosen reaction conditions (e.g, 1 mM osmium tetroxide, 2% pyridine, in 0.2 M NaCl, 25 mM Tris, 5 mM EDTA, pH 7.8 15 min at 26°C), secondarily induced changes are negligible; under these conditions, site-specific modification at the B-Z junctions[245,246,263] cruciform loops,[234,261] protonated triplexes,[264,265] etc. were detected without any sign of changes induced by the probe. CD measurements showed no changes in the CD band in the initial stage of the Os,py reaction with calf thymus DNA.[231] Marked changes in CD and a steep increase in the amount of modified bases occurred under the: given conditions only after more than 34 h of the reaction.[231] It may thus be concluded that Os,py is suitable as a probe of DNA structure, but the reaction conditions must be carefully chosen if reliable data are to be obtained. On the other hand, the ability of pyridine to destabilize the DNA double

helix at higher concentrations may help to detect some very small changes in DNA structure.[38,230,231,266,267]

In Os,py reagent osmium tetroxide is usually applied at 1 to 2 mM concentrations, while the concentration of pyridine is higher by two orders of magnitude. Replacing monodentate pyridine in Os,py reagent by bidentate 2.2'-bipyridine (bipy) results in more stable adducts with DNA and makes it possible to work with osmium tetroxide and bipy at equimolar concentrations;[268] even subrnillimolarconcentrations of Os,bipy are sufficient for the detection of local open structures such as the B-Z junctions. In contrast to Os,py with Os,bipy, no secondary effects were observed. Os,bipy can be used to probe the DNA structure in E. *coli* cells (see Section VII).

To improve the versatility of the osmium probes we recently tested different ligands for their ability to site-specifically modify the B-Z junction and the cruciform loop in supercoiled plasmids.[269,270] In addition to Os,bipy (Figure 2) bathophenathroline disulfonic acid (bpds) and tetramethylethylenediamine (TEMED) can site specifically modify the above-mentioned structures at millimolar and submillimolar concentrations. Under the same conditions, a complex of osmium tetroxide with 1,10-phenanthroline (phe) displayed a lower specificity and also reacted at other sites of the supercoiled and linear DNA molecules. Os,phe may thus become useful in DNA footprinting *in vitro* and *in situ*. Differences in size and other properties of Os,bipy, Os,bpds, Os,TEMED, and Os,phe molecules may come into play in the selectivity of these probes for specific DNA structures *in vitro* and in the probe penetration into the cell and transport to its target DNA structure, The great diversity in the properties of osmium (VI) esters offers a number of possibilities (so far largely unexploited) that are not available in other chemical probes.

b. Diethylpyrocarbonate (DEPC)

DEPC, an enzyme inhibitor and bactericidal agent, has been applied in nucleic acid research (reviewed in Reference 271) and protein research,[272,273] due mainly to its ability to react chemically with some amino acids and nucleic acid bases. DEPC carbethoxylates N7 of purines (Figure 1b) in DNA, but under certain conditions reactions at other sites and with other bases may occur.[271,274] In RNA, purines in single-stranded regions are accessible to this reagent, while those contained in double-stranded regions do not react.[275,276] It has been shown that purines in *syn* conformation in left-handed Z-DNA react with DEPC much faster than those in B-DNA.[277,278] Enhanced reactivity of Z-DNA toward DEPC provided a new approach to the studies of the formation and distribution of Z-DNA segments within a Z-DNA molecule.[245,278] DEPC also reacts with single-stranded DNA, showing a specific reaction with bases in the cruciform loop.[278,280] As a result of carbethylation and ring opening (Figure 1b) the N-9 glycosidic bond is labilized, and due to alkali treatment chain scission occurs at the modified base site. This makes it possible to detect modified bases (similarly as with Os,py and Os,bipy) at single nucleotide resolution using DNA sequencing techniques. Other ways of detecting the reaction sites in DNA strands have been little exploited. DEPC has been used for footprinting DNA-protein complexes;[281] however, this type of experiment should be interpreted with caution, as DEPC reacts much faster with proteins than with' single-stranded nucleic acids.[271,282] DNA footprinting is referred to again in Section V.D.2.

c. Bromo- (BAA) and Chloroaceetaldehyde (CAA)

BAA and CAA react with adenine and cytosine,[283-285] forming fluorescent cyclic ethenoderivatives (Figure 1c). BAA was applied to study local changes in the DNA structure[286-288] using nuclease S1 to cleave DNA at the BAA-modified sites. Both BAA and CAA were used to detect unpaired bases in cruciform loops,[287] B-Z junctions,[268,289] and triplex structures.[290] For several years it was difficult to obtain a pattern at single-nucleotide resolution by means of the chemical cleavage at the modified sites. Only recently has a chemical cleavage procedure been developed that gives a resolution comparable to that obtained with DEPC and Os,py.[290] It has been generally accepted that CAA and BAA react only

with bases not included in Watson-Crick base pairing. Recent results showing CAA reactivity within certain stretches of Z-DNA[291] might be explained by a CAA reaction with a specific "open" state of the (dC-dA)$_n$ segments related to the dynamic equilibrium between B and Z DNA (see Section VI.B.2).

d. Advantages and Disadvantages

Chemical probes (Table 4) have some advantages over enzymatic probes:

1. They usually can be applied under a wide range of conditions, including pH, temperature and ionic strength values, presence of nonaqueous solvents, etc.
2. Their molecules are smaller and can diffuse to various parts of the DNA structure.
3. Their structures and reaction mechanisms are known and induction of secondary changes in DNA structure is less probable that with large protein molecules. Chemical probes differ in their specificity toward bases (Table 4, Figures 1 and 2) and their functional groups; proper combination of the chemical probes can thus provide detailed information about spatial organization of the local DNA structure.
4. They do not induce DNA chain scissions, making possible the occurrence of simultaneous reactions at several sites of the supercoiled DNA molecule.
5. The reaction sites in DNA strands can be detected in various ways (Figure 3).
6. They are potentially applicable to probe DNA structure *in vivo.*

Chemical probes also have some disadvantages: all chemical probes reacting with DNA bases are potentially mutagenic and working with these reagents may represent a health hazard. CAA is carcinogenic;[284,285] DEPC reacts with ammonia, producing the carcinogenic urethane;[274] OsO$_4$ vapors irritates eyes and mucous membranes; etc. The reagents should be thus handled with care.

2. Probes.Reacting with Double-Stranded DNA

There are a number of chemicals capable of reacting with double-stranded DNA (reviewed in References 318 to 324) but only those that have found significant application in the study of local DNA structure research are mentioned here.

a. Dimethylsulfate (DMS)

In B-DNA, DMS methylates N-7 of guanine and N-3 of adenine, i.e., the sites not involved in Watson-Crick hydrogen bonding.[318,325] In single-stranded DNA, N-1 of adenine and to a lesser extent N-3 of cytosine are also methylated. In Hoogsteen pairing N-7 is involved in hydrogen bonding; it does not react with DMS. This makes DMS suitable for probing structures involving Hoogsteen pairing, such as protonated triplex structures.[326-328] DMS also yields information about ligandlguanine contacts in the major groove of B-DNA, where N-7 of guanine is located.[329-331]

b. Nitrosourea

N-Ethyl-N-nitrosourea (ENU) reacts with DNA backbone phosphates, forming triesters. The reaction sites can be recognized by the triester alkaline hydrolysis.[332] Ethylation of phosphates has been used for interference studies of ligand interactions with DNA phosphates.[332,333] The technique does not detect direct contacts in the DNA complex, but rather that phosphates that are located too close to the ligand to accommodate the ethyl group. The conditions of the reaction are far from physiological, thus significantly limiting the use of ENU in DNA studies.[332]

Modification of DNA with N-methyl-N-nitrosourea (MNU) under physiological conditions showed that the initial attack of MNU is strongly dependent on DNA conformation, suggesting that in addition to phosphates, bases might be involved in the reaction.[334]

c. Probes with "Nuclease" Activities

Some chemicals are capable of inducing DNA chain scission, thus simulating to a certain extent the behavior of natural nucleases. Nucleases such as DNaseI, DNaseII, and micrococcal nuclease were applied in DNA footprinting,[320,321] i.e., the technique used in the analysis of specific protein and drug binding to DNA. In footprinting, DNA fragments are cleaved with a nuclease and the products are analyzed using the Maxam and Gilbert technique,[335] in which the sites protected with specifically bound protein or drug molecules are observed as gaps in the DNA cleavage pattern. In addition to natural nucleases, chemicals have been applied recently to cleave DNA, including 1,10-phenanthroline-copper ion,[322] methidium-propyl-EDTA,[323] and Iron (II)·EDTA.[320] With the two latter chemicals DNA reacts to generate the hydroxyl radical (·OH), which induces the chain cleavage. In contrast to methidiumpropyl-EDTA, Iron (II)·EDTA does not bind to DNA, which is cut by freely diffusible ·OH. With Iron(II)·EDTA footprint at very high resolution can be obtained. On the other hand, specific binding of phenanthroline-copper to B-DNA can be utilized in DNA structure studies.[322] This chemical cleaves A-DNA slower than B-DNA, while Z-DNA and single-stranded DNA are not cleaved under the same conditions. More details can be obtained in References 332 and 336 to 341.

d. Photochemical Probes

Psoralens intercalate within the double helical DNA and on irradiation they are covalently added to the thymine 5,6 double bond.[342] One psoralen molecule can photoreact with two thymines at nucleotide sequences containing adjacent thymines in opposite strands, creating a DNA interstrand crosslink. The crosslinking sites can be analyzed by electron microscopy. To improve the limited resolution of this technique, enzyme-based procedures were recently developed that make it possible to analyze sites of psoralen adducts at single-nucleotide resolution.[343-346]

Barton et al.[324,347-350] developed a series of transition metal complexes that bind specifically to local DNA structures; their photoactivation results in a site-specific DNA strand cleavage. For example, the tris (phenanthroline) metal complexes are favored for intercalation into right-handed helices, while the bulkier isomer of tris (4,7-diphenylphenanthroline) ruthenium binds to left-handed Z-DNA or to any other conformation that is unwound sufficiently to accommodate the bulky complex. Rhodium(III) complex binds to the cruciform and upon photoactivation it cleaves DNA at and near the cruciform.[348] A probe for A-DNA was developed by matching the shape of the ruthenium(II) complex to that of the shallow minor groove of the A-DNA form.[349,350]

e. Complementary Addressed Modification and Cleavage of DNA

The ability of DNA strands to renature and hybridize was recognized more than 30 years ago.[351-353] Utilization of this principle has become one of the main driving forces in the development of specific molecular biological techniques, of which one is the technique of complementary addressed modification and cleavage of DNA. Here, a relatively short oligonucleotide equipped with a chemically reactive group, intercalating agent, and/or nucleic acid cleaving agent binds to its complementary target sequence in single-stranded DNA or RNA (reviewed in References 354 to 359). In double-stranded DNA, sequence-specific modification with alkylating oligonucleotides was observed in a negatively supercoiled plasmid but not in its relaxed form.[360] The double helix in relaxed DNA molecules can, however, be a target for pyrimidine (or purine) oligonucleotides that recognize homopurine~homopyrimidine(homopu·py) sequences via the major groove and form triplex structures.[355-359,361-363] The oligonucleotide is bound in a parallel orientation to the purine strand of the DNA double helix.

An oligonucleotide of about 15 to 19 nucleotides should be sufficiently long to recognize a single specific sequence in the human genome.[356] This technique may be of great use for mapping genomes over large distances to study local DNA structures, namely, the DNA triplexes both in *vitro* and *in vivo,* etc.

While in experiments in *vitro* the usual deoxyoligonucleotides can be applied, for ex-

periments *in vivo* several modifications have been introduced into oligonucleotides to prevent their degradation by nucleases. The phosphate group was replaced by methylphosphonate and phosphothionate,[364] and the α-nucleotide anomers were substituted for the natural β-anomers.[365] The covalently linked intercalating agents include acridine, phenanthroline, proflavin, and furocoumarin derivatives.[357,366-368] Proflavin and furocoumarins are photoactive and can be attached to the oligonucleotides by irradiation.[356] As a nucleic acid-cleaving agent, metal complexes such as Iron-EDTA, Iron-porphyrin, and 1,10-phenanthroline-copper have been used.[356]

Oligonucleotide binding can be irreversible when the covalent bond is formed. An irreversible and well-detectable change is a site-specific DNA chain scission induced by a DNA-cleaving agent such as Iron(II)·EDTA attached to the oligonucleotide.[356,357,359] Recently, an irradiation-induced cleavage was reported with an I1-residue homopyrimidine oligonucleotide covalently linked to ellipticine derivative that introduced cleavage of the two strands of the target homopu·py sequence.[369] Development of the sequence-specific artificial photonucleases of this type represents a new approach in which the cleavage reaction can be very well controlled by light.

Oligonucleotide conjugates that bind specifically to mRNA inhibit its translation in cell-free extracts.[356,358] The use of antisense oligonucleotide to selectively control gene expression is a very promising approach of potentially great significance that may provide the basis for the development of highly specific therapeutic agents. At present, however, some basic questions remain unanswered.[370]

Site-specific DNA chain scissions have also been accomplished by incorporation of DNA cleaving agents into sequence-specific DNA binding peptides and proteins, e.g., the E. *coli* catabolite gene activator, a helix-turn-helix motif sequence-specific binding protein.[371]

f. Ultraviolet Irradiation

The formation of UV light-induced cyclobutane pyrimidine dimers in DNA requires a proper alignment of the 5.6 double bond in the two reacting pyrimidines. This requirement is not fulfilled in B-DNA; thus, the dimer formation can serve as a DNA structural probe.[320,372,373] Formation of the interstrand crosslinking dimers was used more than 20 years ago to study DNA premelting,[10,375,376] but in the years hence, this approach was little exploited. Only recently was a renewed interest in this technique shown. It has been demonstrated that peaks of pyrimidine dimer formation occur in nucleosome core particles with an average periodicity of 10.3 bases,[377,378] and that the rate of thymine dimer formation is affected by the direction and degree of DNA bending.[379] Homopyrimidine inserts [with (dT-dC)$_x$ or (dC)$_m$ sequences] in plasmid DNA are good targets for UV-induced [6-41-pyrimidine dimer formation,[380] as demonstrated by photoprints of fragments produced by DNA piperidine cleavage. The dimerization in these sequences was almost completely abolished when homopyrimidine oligonucleotides with (T-C), or (C), sequences were added to form the triplex structure. This technique may become very useful in the research of DNA triplex structures, both *in vitro* and *in vivo.*

UV irradiation has also been applied as a photocrosslinking and footprinting agent in studies of protein-DNA interactions.[319,374,381,382] Pulsed laser (nanosecond pulses) and flash irradiation (microsecond pulses) have been increasingly applied for time resolution studies.[383-385] It appears that thymines photoreact with primary amines, including lysine residues, in proteins, but the photochemistry of protein-DNA crosslinking is not yet fully understood.

3. Conclusions

In contrast to the early 1980s, when the arsenal of methods suitable for the analysis of local supercoil stabilized structures was rather limited, a large number of methods are currently available. Among them chemical probes and gel electrophoresis are perhaps the most important. In studies of oligonucleotides modeling a specific DNA structure, physical techniques and particularly NMR[386] can yield detailed structural information. It can be expected that scanning tun-

neling microscopy, whose usefulness in the DNA structure studies was recently demonstrated,[387-389] will soon become a very useful tool in the research of local DNA structures and their interactions. Attempts to improve our knowledge. of DNA structure inside the cell will require the development of new techniques. The first results in this respect have been obtained with chemical probes and specific molecular genetic approaches (see Section VII). It is anticipated that further, rapid development of these techniques will continue in the near future.

VI. LOCAL CHANGES IN DNA SECONDARY STRUCTURE STABILIZED BY SUPERCOILING

According to Equation 1, supercoiled DNA with $\Delta Lk \neq 0$ must either twist or writhe, or both, away from its relaxed form. Changes in Tw may involve torsional deformations of existing secondary structures and/or transitions of B-DNA segments to supercoil-stabilized specific local structures depending on nucleotide sequence,. These transitions include B-Z transitions at alternating purine-pyrimidine sequences, triplex formation at homopwpy tracts, cruciform extrusions at inverted repeat sequences, etc.; changes in Wr involve bending and the formation of superhelical turns. It should be noted that local changes resulting from conformational transitions must be compensated for elsewhere in the molecule (supercoiled domain) to preserve the linking number.

Thus far the best understood structural change in a negatively supercoiled DNA molecule may be the cruciform extrusion,[31,390,391] discussed in detail in Section VI.A; here, it is mentioned as an example of formation of a local structure assisted by free energy of the negative supercoiling. Inverted repeat sequences can, in principle, undergo a transition to a structure in which the regions with intrastrand complementarity can pair, forming the cruciform structure. Such a structure is energetically unfavorable compared with perfectly base-paired continuous DNA duplex. The energetic disadvantage of cruciform formation, however, can be overcome if the inverted repeat is placed in the negatively supercoiled molecule,

in which the free energy of superhelix formation can provide the driving energy required to facilitate the cruciform extrusion. An inverted repeat of, for example, 21 bp, will in its unperturbed state contribute to the total Tw by 21110.5 or 2. The inverted repeat contribution will decrease to zero after the cruciform extrusion provided the backbone conformation adopted within the hairpin itself is relevant to the topological properties of the whole molecule.[122] A similar decrease in Tw can be expected if the same sequence forms a melted region, while formation of Z-DNA would result in a DNA unwinding almost twofold greater.

In natural supercoiled DNAs usually more than one nucleotide sequence in the molecule is able to undergo transition to a specific local supercoil-stabilized structure. Once the first transition occurs, the probability of further transition is changed due to the partial relaxation of the molecule induced by the first transition. Moreover, a single nucleotide sequence may itself be able to form several alternative structures, depending on various factors, including environmental conditions,[253,392] superhelix density,[253] etc. Thus, complex multistate equilibria may arise in supercoiled DNA molecules; such equilibria may take part in DNA-controlled regulatory processes *in vivo*.

Presence of the local structures in supercoiled DNAs complicates their geometric and topological analysis. Equation 1 was formulated in terms of winding of either DNA strand about the duplex axis that is often difficult to define. To overcome this difficulty, new models were proposed (see Section IV) capable of evaluating quantitatively complex phenomena connected with the presence of local structures such as cruciform-in supercoiled DNA molecules. In this section conformations and properties of cruciforms, left-handed segments, triplexes, and other local structures *in vitro* are discussed. The question of the existence of these structures *in vivo* and their biological relevance is a subject of Section VII.

A. Cruciforms

Cruciform structure was predicted 35 years

ago,[393,394] but the importance of the negative supercoiling for its formation was suggested and theoretically derived much later.[395-398] The first experimental evidence of the cruciform structure was obtained about 10 years ago with the observation of very large artificial cruciforms in the electron microscope[28] and by nuclease S1 probing of supercoiled natural plasmids and phage DNAs.[29,30,32] The possibility that the structure might be artificially induced by acid pH and $Zn^{2}+$ ions of the enzyme probe buffer and/or by interaction of the enzyme molecule with DNA was soon excluded by chemical probing[234] and 2-D gel electrophoresis[172,399] at neutral pH in the absence of Zn^2+ ions. Cruciform is now well established and various aspects of its structure were reviewed in a number of papers.[31,35,122,123,138,390,391,400] The formation of a cruciform requires a nucleotide sequence with a local twofold symmetry represented usually by a sufficiently long inverted repeat. Two structural features are characteristic for the cruciform, i.e., the formally unpaired loop and the four-way junction.

1. Loop

Optimal size of the formally single-stranded loop lies between four and six bases.[79,401,402] The loop adopts some structure involving probably base stacking[280,403] and even non-Watson-Crick base pairing.[402] Hairpin loops may affect the stem conformation close to the loop with the stem perturbation stronger for smaller loops.[404]

2. Four-Way Junction

The four-way junction[3'] is normally fully base paired,[79,279,280,405] asymmetric,[406] and X-shaped.[254] It introduces a bend into the molecule whose nature differs from the intrinsic sequence-directed curvature.[407,408] In the absence of ions thymines at the junction are reactive to Os,py, probably due to base unstacking,[254] and the helical arms of the junction are fully extended in a square, configuration. The four-way junction is formally, equivalent to the Holliday junction — a fundamental structure in the genetic recombination. A

series of papers was published in 1989 and 1990 by Lilley et al.[409-414] demonstrating for the first time the structural details of the Holliday junction. Their experimental approach was based on hybridization of four oligonucleotides (each of 80 nucleotides) to assemble a four-way helical junction. This complex and its variants (containing small changes in nucleotide sequence or substitution of electrically neutral methylphosphonate at the central phosphodiester linkages) were investigated using various techniques, including gel electrophoresis, chemical probes, nuclease cleavage,[411-413] and the resulting model was confirmed and refined on the grounds of fluorescence energy transfer[414] and model building studies.[411] The model is called the stacked X-structure in which the four arms are associated in pairs, stacked in a coaxial manner to generate quasi-continuous helices. The nucleotide sequence at the junction center determines the choice of the stacking partners. Binding of metal ions is important for the stability of the X-structure. Resolvase (from T_4 phage) binds at one side of the X-structure cleaving the exchanging strands two or three nucleotides from the junction. More details can be found in Lilley and co-workers' recent review.[414]

Information about the details of the cruciform loop, stem, and four-way junction was obtained not only by investigation of supercoiled DNAs by techniques specific for local supercoil-stabilized structures (this section), but also by studying oligonucleotide constructs modeling cruciform structure or its specific parts[401,402,404,405,407-416] by physical techniques,[401,402,404,408,415] namely, NMR. Using this approach a crystal structure of a hexadecanucleotide CGCGCGTTTCGCGCG was solved at 2.1 Å resolution showing a hairpin configuration with Z-DNA hexamer stem.[416] In contrast to this type of research studies of the cruciform extrusion had to be done with supercoiled DNA molecules and the applied techniques were thus limited mainly to those described in Section V.

3. Cruciform Extrusion

Extrusion of the cruciform requires significant rearrangement of the DNA structure, including complete reorganization of the base pair-

ing. Compared with the regular DNA duplex, cruciform is thermodynamically unstable due to the energetic cost of base unpairing in the loop and formation of the four-way junction. Free energy of the cruciform formation was determined to be 15 to 20 kcal/mol.[138,172,399,417]

The first studies demonstrated a significant kinetic bamer and showed that the cruciform extrusion could be a rather slow process.[418,419] Further work with other DNAs revealed,[404] however, that extrusion could proceed under some conditions more easily. Detailed studies showed two types of cruciform extrusion kinetics. Cruciforms requiring the presence of salt for their extrusion (showing maximum extrusion rates at about 50 mM NaCl) are termed S-type (related to salt dependency). S-type cruciforms are more common than C-type cruciforms, so far represented only by ColE1 as the only natural member of the Ctype (C for ColEl) class. C-type cruciforms extrude maximally in the absence of salt, showing a marked dependence on temperature, which is in contrast to the rather low temperature dependence of the extrusion of the S-type cruciforms. Striking differences in the kinetic properties of S- and C-type cruciforms suggested the possibility of two different mechanistic pathways for the extrusion process (Figure 4). The S-type extrusion is initiated by a helix opening at the center of the inserted repeat, followed by the formation of a small protocruciform and branch migration resulting in elongation of the cruciform stems up to the complete extrusion. Such extrusion was explained theoretically by Vologodskii and Frank-Kamenetskii[398] and documented by a wealth of experimental evidence (Reference 391 and references therein). Recent results[422] suggest that the initial opening may correspond to about 10 bp and that the nucleotide sequence at the center of the inverted repeat significantly influences the rate of cruciform extrusion.[423-425] High A + T content of this region or destabilization of the helix by methylation of adenine at N6 enhanced the extrusion rate, while an increase in the G + C content or stabilization of the helix by cytosine methylation resulted in a decrease of the extrusion rate. These results, as well as cation binding analysis, support the concept of the S-type cruciform extrusion.[426]

The C-type extrusion (Figure 4) is supposed to start with coordinate unpairing of a much larger duplex segment followed by intrastrand reassociation to form the fully extruded cruciform. This pathway would be expected to be facilitated by low ionic strength and characterized by a large activation energy, i.e., the characteristics experimentally observed, but what are the reasons for the two different extrusion mechanisms? Comparison of the nucleotide sequence of ColEl cruciform with that of a typical S-type cruciform does not show differences that may help answer this question. The explanation of the C-type extrusion offered recently by Lilley et al.[31,261,424,426-429] is based on the presence of very (A + T)-rich sequences flanking the ColEl inverted repeat. Replacing ColE1 cruciform with another inverted repeat (originally S-type) resulted in a typical C-type extrusion[429] Similarly replacing an S-type cruciform in its natural environment with a ColEl inverted repeat exhibited S-type extrusions. This clearly demonstrated that for the C-type extrusion, the sequences flanking the ColEl repeat are responsible.

It was further shown that

1. The transition energy of the cruciform extrusions decreases with the length of the C-type inducing sequence[431]
2. The polarity of the sequence can be unimportant and its length can be reduced to 12[427,430]
3. If the inverted repeat is flanked both by S- and C-type sequences, the salt concentration dominates the type of kinetics (i.e., C-type in absence of NaCl)
4. The effect may be manifested at a significant distance (around 100 bp)
5. Insertion of a (G + C) segment between the C-type inducing sequence and the inverted repeat may block the C-type extrusion

How can the (A + T)-rich C-type inducing sequence, perform a remote control of the cruciform extrusion? It was proposed that these sequences are responsible for a coordinate destabilization of a large domain in the supercoiled DNA, thus increasing the probability of large-scale base opening in the inverted repeat.[31,261,390,427] In agreement with this proposal, duplex stabilization in (A-T)-rich sequences with
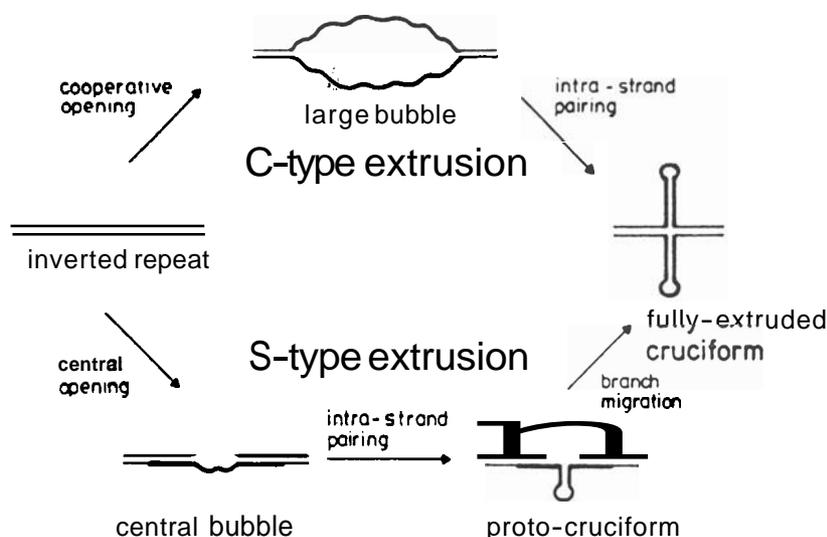
FIGURE 4 Mechanism of cruciform extrusion. The inverted repeat, represented by the thicker line, is shown in the unextruded form on the left. C-type cruciforms (top) initiate the extrusion process with a coordinate opening of many base pairs to form a large bubble. An intrastrand reassociation then forms the mature cruciform structure. The extrusion of S-type cruciforms (bottom), is initiated by a smaller opening event. Intrastrand pairing generates a smaller protocruciform, which may undergo branch migration. Base pairing is transferred from unextruded sequence to the growing cruciform stem in a multistep process to form the fully extruded structure. The principal differences between the two mechanisms lie in the initial opening and the degree of tertiary folding in the transition state. (From Lilley, D. M. J., Chem. *Soc. Rev.,* 18, 53, 1989. With permission.)

distamycin and NaCl resulted in S-type extrusion. Conversely, helix destabilizing agents induced in DNA with S-type extrusion a quasi-C-type extrusion kinetics. Thus, changing the helical stability results in interconversion between S- and C-type mechanisms.

Bases in the C-type inducing sequence in supercoiled DNA are hyperreactive toward Os,py, BAA, glyoxal, NaHSO$_3$,[261] and DEPC.[431] This hyperreactivity requires a threshold level of negative supercoiling and changes in the chemical reactivity induced by various agents correlate with those in cruciform extrusion.[261] BAA hyperreactivity was detected at temperatures above 26°C (with a maximum around 40°C), while that of Os,py was observed above 0°C reaching a maximum of 20°C. The BAA hyperreactivity was explained by a cooperative but transient helix opening. Os,py hyperreactivity at much lower temperatures might be due at least in part to helix destabilization by 3% pyridine at low ionic strength.[267]

Hypersensitivity toward BAA, DEPC, and KMnO$_4$ was observed in a "random sequence" region centered 10 to 30 bases away from the junction of the (A-T),, cruciform;[432] this hypersensitivity was pH-dependent. (A-T), sequences adopt the cruciform structure at low energies of formation without a detectable kinetic barrier.[173,288,433] The unusual extrusion kinetics of the (A-T), cruciform may be due to specific properties of (A-T), duplexes that are very easily deformable and denaturable and whose structure differs from the regular B-DNA.[260,434] It has been suggested that these (A-T), sequences may represent simultaneously the inverted repeat and an effective inducing sequence, being thus a special subclass of the C-type extrusion.[31] The hypersensitivity of the region in the vicinity of the (A-T),, cruciform was related to the cruciform extrusion, but its relation to the C-type extrusion remained unclear.

The C-type cruciform extrusion is a very interesting phenomenon and some of its aspects

may have more general consequences. It was shown quite recently that the AT-rich flanking sequences may influence the structure of left-handed DNA (see Section VI.B.2).[435] At the present time it would be desirable to have more detailed information on the way in which the inducing sequence materializes the remote control of the cruciform extrusion. The suggested[3'] telestability effect[4326] would at least require some considerations concerning DNA supercoiling and the explanation based on the soliton-like states in DNA seems to be even less acceptable.[437,438] There is no doubt that the ability of some DNA segments to induce local changes in DNA conformation far away from the segment may be of great biological importance. Their better understanding, however, requires further work.

4. Multiple Structures in Inverted Repeats

Some inverted repeats may be composed of alternating purine-pyrimidine sequences and can (in principle) form either a cruciform or a 2-DNA structure. It was shown that the $(CATG)_{10} \cdot (CATG)_{10}$ insert selectively forms a cruciform structure when integrated in a negatively supercoiled plasmid.[439] Similarly, $(TCGA)_5 \cdot (TCGA)_5$ and $(TG)_6 \cdot (CA)_6$ inserts preferentially adopted cruciform at low ionic strength rather than Z-DNA.[440] However, the latter two sequences were induced to form left-handed DNA (after removal of local structures by ethidium bromide) when the negative supercoiling necessary for the transition was generated at higher ionic strength (e.g., 0.2 M NaCl).

Although 2-DNA formation in (C-G) inserts requires more free energy than cruciform formation these sequences preferred the left-handed form.[226] (GTAC), flanked with two (G-C), sequences adopted left-handed DNA.[441] (A-T) sequences showed a strong preference for cruciform formation,[260,434] but under specific conditions (in the presence of Ni^{2+} ions) left-handed structure was observed in a supercoiled plasmid (see Section VI.B.1).[392] These results suggest that equilibria between B, Z, and cruciform structures exist in alternating pu·pyr sequences depending on the superhelicity and environmental conditions.

The possibility that a given sequence may undergo more than one structural transition may be of great biological significance as these transitions provide specific recognition sites (loops and four-way junctions of the cruciform and B-Z junctions and altered helical sense of 2-DNA) for DNA binding proteins and cause different levels of DNA relaxation, which may transmit long-range structural effects influencing regulatory processes.[260,392,440]

B. Left-Handed Z-DNA

About 8 years after publication of the CD spectra of the left-handed DNA in poly(dG-dC)·poly(dG-dC) by Pohl and Jovin,[14,15] Z-DNA structure was solved by single-crystal X-ray diffraction studies (see Section II).[19-21] This discovery stimulated enormous scientific effort, resulting in hundreds of papers that have been surveyed in numerous reviews.[34-36,138,191,442-447] It would be useless to try to present a more or less comprehensive review of the broad field of 2-DNA in this article; instead I shall touch on some problems studied in recent years that have not been 'fully covered in the preceding reviews. These include the question of formation of left-handed DNA within $(dA-dT)_n$ sequences and the B-Z junctions.

1. Left-Handed DNA in $(dA-dT)_n$ Sequences

Regular alternations involving anti and syn nucleoside conformations are one of the main characteristics of Z-DNA (see Section II). Such alternations are formed most easily in alternating pu·pyr nucleotide sequences (with purines in syn conformation). $(dC-dG)_n$ sequences easily adopt the Z-DNA structure, as has been shown both by solution and crystal studies[w2] The surprising reluctance of the corresponding $(dG-dC)_n$ oligonucleotides to adopt Z-DNA structure has not yet been fully explained. The ability of $(dA-dC)_n \cdot (dT-dG)_n$ sequences to form left-handed helices in solution both in linear[448-451] and supercoiled DNAs[33,192,213,452] is well known, but no crystal structure has been reported up to now.

Consecutive AT pairs can be incorporated into the Z helix (reviewed in References 442 and 445) and a maximum of six AT pairs can be tolerated, including the (T-A), sequence. In longer (dA-dT) sequences no left-handed structure was observed under conditions where (dC-dG)$_n$ and' (dT-dG)$_n$·(dA-dC)$_n$ sequences adopted this struc-ture. Inversion of the CD spectra of poly(dA-dT)·poly(dA-dT) in concentrated CsF solutions was not due to the B-Z transition but to the so-called B-X transition.[453,454]

The first evidence of the B-Z transition was obtained in 1986 in Tallandier's laboratory by investigating poly(dA-dT)·poly(dA-dT) in films by IR spectroscopy in the presence of different counter ions and a wide variety of water content ~IR-spectra observed in the presence of Ni^{2+} at high polynucleotide concentration and low water activity were assigned to a Z-type structure of poly(dA-dT)·poly(dA-dT). CD of poly(dA-dT)·poly(dA-dT) solution at high NaCl concentration (5 M) in the presence of NiCl$_2$ (90 mM) displayed almost total spectrum inversion with no negative band around 250 nm, suggesting Z conformation of the polynucleotide.[456] The nickel-induced Z-form of poly(dA-dT)·poly(dA-dT) obtained further support from UV absorption[456] and especially from Raman[457] and resonance Raman measurements.[458] It was proposed that poly(dA-dT)·poly(dA-dT) forms Z-DNA due to interaction of nickel ion with adenine N7.[457,458] This interaction is possible thanks to the screening of negatively charged phosphates by high sodium concentration that results in stabilization of adenosine in *syn* conformation and reorganization of the water distribution along the molecule that stimulates the B-Z transition. At NiCl$_2$ concentrations close to 0.1 M pH cannot be neutral, but it must be weakly acidic. The role of acid pH in the formation of Z-DNA in (dA-dT) sequences, however, was not considered.

Recently it was shown[392] that (dA-dT)$_{16}$ insert can adopt left-handed structure in a super-coiled plasmid under conditions (2 M NaCl, 0.2 M NiCl$_2$, or 1 M NiCl$_2$ alone) not sufficient to induce Z-DNA in the linear plasmid or poly(dA-dT)·poly(dA-dT). (dA-dT)$_5$ block placed in the center of the 32 bp self-complementary alternating purine-pyrimidine insert adopted a left-handed structure[459] at substantially lower NiCl$_2$ concen-trations (10 mM NiCl$_2$, 0.2 M NaCl) as detected by DEPC probing. These results suggest in agreement with the recent predictions that all simple alternating purine-pyrimidine sequences such as G-C, A-C, and A-T may adopt left-handed conformation under some environmental conditions in linear DNAs;[138,442] in supercoiled DNAs these conditions must be combined with a proper superhelix density.

2. B-Z Junctions

Segments of left-handed Z-DNA can be contiguous with B-DNA both in *vitro* and in vivo (see Section VII). The boundary between these two structures has been specified as B-Z junction (reviewed in References 34, 36, 442, 444 to 446, and 460). Structural models of the B-Z junction suggest that at least one base pair at the junction, must be different from the B or Z conformation~. ~Experimental data obtained mainly with supercoiled plasmids show that the B-Z junction may be a rather polymorphic structure,

Cleavage of the B-Z junctions in supercoiled plasmids with single-strand selective nucleases (such as nuclease S1) as well as site-specific modification with single-strand selective chemical probes (see Sections V.C and V.D.1), including Os,py[245,268,293,466-468,470] and hydroxyl-amine,[245,468] indicated open distorted structures, but the data could not be unambiguously interpreted in terms of a presence of unpaired bases (not required in the structural models), as the above nucleases and chemicals can interact even with distorted base-paired regions. To detect unpaired bases in the B-Z junction chemicals reacting specifically with sites involved in Watson-Crick hydrogen bonding were applied.[289,291,308,466,471]

In 1987 it was shown[268,291,293,308,470,471] that such chemicals, including BAA, CAA, and glyoxal, site-specifically modify the boundary between B- and Z-DNA formed in supercoiled plasmids either by (G-C)$_n$[268,293,308,471] or (T-G)$_n$[471] sequences. This implies that the B-Z junctions in supercoiled DNA contain unpaired bases. On the other hand, further conclusions regarding, for example, the exact number of unpaired bases, must be considered with caution because the nuclease

S1 applied for the detection of BAA or CAA-modified sites may not recognize solitary nucleotides modified by the probe.[268,293] To obtain more accurate data, the recently developed method of chemical cleavage at the sites of BAA modification should be applied.[290]

No crystallographic data have thus far been published describing structure of the B-Z junction. Recently, however, other physical techniques[472-475] were applied to study the B-Z junction in a linear DNA fragment[472] and oligonucleotide~. It was shown that the hydrodynamic dimensions of the 153 bp fragment are not affected by the transition of two (C-G), segments to Z-DNA, induced by 15 mM [Co(NH$_3$)$_6$]$^{3+}$.[472] This may imply that the B-Z junctions were, under the given conditions, neither strongly bent nor particularly flexible. On the other hand, the results of IR spectroscopy of nucleotide films suggest that flexibility in the junction region (resulting from the presence of one no-base residue) stimulates the B-Z transition in six nucleotide residues of a double-stranded tridecamer.[475]

Recently, a 16 bp oligonucleotide

^{m}C	G	^{m}C	G	^{m}C	G	^{m}C	G	A	C	T	G	A	C	T	G
G	^{m}C	G	^{m}C	G	^{m}C	G	^{m}C	T	G	A	C	T	G	A	C
1	2	3	4	5	6	7	8	9	10	11	12'	13	14	15	16
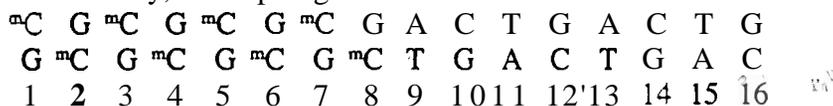
was synthesized with 5-methylcytosine (^{m}C) incorporated in the (C-G), segment, which adopted a Z-conformation at high (>3 M) NaCl concentration~. Studies of this oligonucleotide by UV absorption and CD and NMR spectroscopy revealed three base pairs involved in the B-Z junction with only one of them being dramatically distorted.[473,474] The proton resonances for base pairs 7, 8, and possibly 9 became observable only at temperatures higher than approximately 30°C, suggesting that at lower temperatures the base pairs are accessible to exchange with solvent. At 30 to 50°C, all internal hydrogen bonds in base pairs 2 to 14 were intact. It has been shown that methylation of a plasmid with HhaI methylase in vitro decreases unwinding in the B-Z junction by half as compared to the unmethylated plasmid.[476] The question thus arises to what extent are the structural features of the B-Z junction observed by NMR in the tridecamer influenced by cytosine methylation.[474]

The results of chemical probing of the B-Z junctions in supercoiled plasmids[246,263,268,289,293,308,466-471] suggest that the B-Z junctions adjacent to (C-G), segments are narrow in good agreement with the results of the oligonucleotide proton NMR.[474] B-Z junctions adjacent to (G-T), segments in supercoiled plasmids may contain more bases hypersensitive to the chemical probe and might be structurally different.[268,467-470] It was shown that in (dC-dA)$_{31}$ sequence around a −0.06 only about half of the 62 bp tract exists in Z-form anywhere along the CA/TG segment and is probably constantly in motion.[452,468-470] Under these conditions the hypersensitivity toward chemical probes associated with B-Z junctions can be distributed over many bases. At more negative a the junction hypersensitivity begins to appear at one end of the Z-forming sequence, suggesting that the structure of a B-Z junction can be assumed at less energy costs at one boundary than at the other.

Do these data suggest that the left-handed structure of (T-G)$_n$·(A-C)$_n$ segments is different from Z-DNA observed in (C-G), sequences? The available experimental data do not exclude such a possibility; some of them [e.g., the free energy of formation of Z-helices in (T-G)$_n$·(C-A)$_n$ sequences is higher than for (C-G), stretches of the same length] might even support it.[445] Quite recent results of Rajagopalan et al.[435] makes the possibility of the existence of various left-handed structures even more probable. These authors showed that flanking of Z-forming (C-G) sequences with AT-rich ColE1 sequences (see Section VI.A.2) resulted in site-specific Os,bipy and BAA modifications within the left-handed (C-G) tracts of 36 and 40 bp in length. To explain this unexpected result it was suggested that the (C-G) tract adopted two conformations that created a new junction.

Regarding the B-Z junctions, at the present time we may only conclude that their structure' is polymorphic depending on nucleotide sequence, superhelical density, and environmental conditions. Bases in the junction show not only

hypersensitivity to single-strand selective chemicals (Table 4), but also represent sites of enhanced intercalation of the psoralen probe.[447] Structure and properties of the B-Z junctions may profoundly influence the B-Z transition, and with their open structures differing markedly from those of the adjacent helices, these junctions themselves may play a significant biological role (representing, for instance, a binding site for single-strand binding proteins).[478,479] Further work will be needed, including X-ray crystallography, NMR, and other physical techniques, as well as chemical probing to understand better the structural features of the B-Z junctions.

C. Triplexes

In only a few years triplexes have become one of the most studied aspects of the DNA polymorphic structure. Intramolecular triplexes at weakly acidic and neutral pH values require supercoiling, while intermolecular triplexes from duplex DNAs and oligonucleotides can be formed under the same conditions in relaxed DNA molecules. As this article deals with supercoil-stabilized structures, I shall focus on intramolecular triplexes in an attempt to present a more detailed review. Intermolecular complexes are only briefly summarized.

1. Early Studies of Multistranded Structures in Synthetic Polynucleotides

More than 30 years ago poly(A) and poly(U) were shown to form a triple-stranded structure (at neutral pH, in the presence of $MgCl_2$) with the stoichiometry 1A:2U where the second poly(U) strand was bound via Hoogsteen pairing.[480–482] Studies using poly(C) and poly(G) or poly(I) showed triplex formation at weakly acidic pH values (with one homopurine and two homopyrimidine strands).[483-385] Later, the possibility of formation of poly(dG)·poly(dG)·poly(dC) at neutral pH was demonstrated.[486] It was shown that base triplets T-A-T and C-G-C$^+$ can be formed in both polyribo- and polydeoxyribonucleotide series.[487–492] Triplex structures were observed not only in homopolymers, but also in copolymers with alternating sequence containing all purines or all pyrimidines in each strand; for example, poly(dT-dC)·poly(dA-dG)·poly(dT-dC) was formed below pH 6 in the presence of $MgCl_2$. Recently it was shown that these polynucleotides can adopt triple-stranded structure even at neutral pH if the cytosine residues are methylated.[207] It was proposed that in the triplex the third strand was associated with a duplex through Hoogsteen pairing in the major groove with parallel orientation to the homopurine strand.[485,492,493] Polynucleotides with mixed purine-pyrimidine sequences such as, for example, poly(dA-dT)·poly(dA-dT), did not form triplex structures. Guanosine and its derivatives are known to form tetrameric aggregates at high concentration ~Poly(G) and poly(I) form quadruple helices.[501-503] The ability to form four-stranded structures was observed also in guanine-rich polypurine copolymers.[504]

DNA and RNA multistranded structures have long been considered mainly an interesting property of some synthetic polymers with no biological relevance. Recent discoveries of supercoil-stabilized triplex structures in plasmid DNAs and the occurrence of quadruplex structures in G-rich telomeric sequences (see Section VI.D.1) turned the multistranded structures into the latest "hits" of DNA structure research.[505,505a]

2. Intramolecular Triplexes

The discovery of Z-DNA in synthetic poly- and oligonucleotides by means of physical techniques (see Sections I, II, and VI.B) induced a search for Z-forming nucleotide sequences in various genomes and studies of relations between their location, structure, and biological function. The history of the discovery of the supercoil-stabilized triplex DNA followed a different path. First, a great number of papers were published in the first half of the 1980s describing the location and properties of the polypurine-·polypyrimidine (polypu·py) sequences (reviewed in Reference 506; see Section VII.C). Such sequences were found in a variety of eu-. karyotic organisms and tissues located mainly within the regulatory regions of active genes, Many of these sequences displayed a marked hy-

persensitivity to the single-strand selective nuclease S1,,suggesting the presence of non-B DNA structure. To explain this hypersensitivity several models were postulated (reviewed in Reference 328), including slipped structure[507,508] left-handed non-Z DNA,[509] a structure in which A·T Watson-Crick pairs alternate with Hoogsteen G·C pairs (with G in *syn* conformation),[215] and a heteronomous structure with a dinucleotide repeat unit.[327]

a. H-DNA Model

In 1985 Lyarnichev et al.,[175] using 2-D gel electrophoresis, observed a sharp structural transition within the insert (from the histone gene unit of sea urchin) of the recombinant plasmid pEJ4 containing (A-G),, in the polypurine strand. This transition depended on pH and on the degree of negative supercoiling, while the mobility drop was pH-independent (within the given pH range), consistent with the presence of noninterwound complementary strand throughout the (dA-dG)$_n$·(dT-dC)$_n$ stretch but not with cruciform or Z DNA. On the basis of their results they suggested a new structure called H form, stabilized by hydrogen ions and including a hairpin.[175] Considering the results of Lee et al.[510] obtained with poly (dT-dC)·poly(dG-dA) at weakly acid pH they proposed a protonated triplex H-DNA. Stemming from different methodological approaches Christophe et al.[511] suggested a protonated triplex structure. In the H-DNA proposed by Lyamichev[512] the Watson-Crick duplex extends to the center of (dT-dC)$_n$·(dG-dA)$_n$ tract (Figure 5) and the second half of the homopyrimidine strand folds back upon itself, winding down in the major groove of the helix. This returning strand forms Hoogsteen pairs with the purines of the Watson-Crick duplex cytosines being protonated. The second half of the homopurine strand also folds back, but is probably unstructured. Neither the experimental data of Christophe · et al.[511] based on mapping of the nuclease S1 hypersensitive sites nor those of Lyarnichev et al.[175,512] represented sufficient proof of the H-DNA triplex. Such proof was obtained **2** years later by means of chemical probes.[247,264,265,290,313,326,513]

b. Chemical Probing: Strong Evidence for H-DNA Model

In 1987 we probed supercoiled plasmid pEJ4 (constructed in the laboratory of M. Frank-Kamenetskii) using Os,py as a probe for the homopyrimidine strand and glyoxal for the homopurine strand in combination with nuclease S1 to cleave DNA at the site of the modified bases.[264] We demonstrated the dependence of the site-specific modification on pH, NaCl concentration (this dependence was observed at pH 6, but not at pH 4), and supercoiling. At pH 5.6 a major site-specific modification was observed in the middle of the homopyrimidine strand and a minor site close to the end of the tract. On the basis of these results we concluded that under the given conditions, protonated triplex H-DNA is present in the supercoiled pEJ4 DNA. At pH 4.0 similar site-specific modification was observed even in linear DNA molecules.[247,264] Employing basically the same approach several laboratories, including ours, published in 1988 modification patterns at single-nucleotide resolution (using chemical cleavage of DNA or termination of transcription at modified bases instead of nuclease S1) of several recombinant plasmids containing (dA-dG)$_n$·(dT-dC)$_n$ tracts. These patterns displayed strong Os,py modification in the middle of the homopyrimidine strand (Figure 5) (corresponding to the hairpin loop in H-DNA) and a minor modification of the 3'-end of the (dT-dC)$_n$ tract (consistent with the reaction at the B-H junction), plus a strong DEPC modification of the 5'-half of the homopurine strand corresponding to the 5'-half of the unpaired sequence not included in the triplex: This hypersensitivity of the 5'-half was observed regardless of the insert orientation within the vector.[513] Other single-strand selective chemical probes such as hydroxylamine and methoxylarnine yielded more or less the same modification patterns, showing that the results are independent of the nature of the chemical probe.[247,313] Modification with DMS showed that guanines of the 3'-half of the purine strand are protected against alkylation in agreement with their assumed involvement in the Hoogsteen pairing.[215,313,326,513] The interconversion between duplex and triplex occurred within a few minutes, as detected by nuclease P1 site-specific cleav-
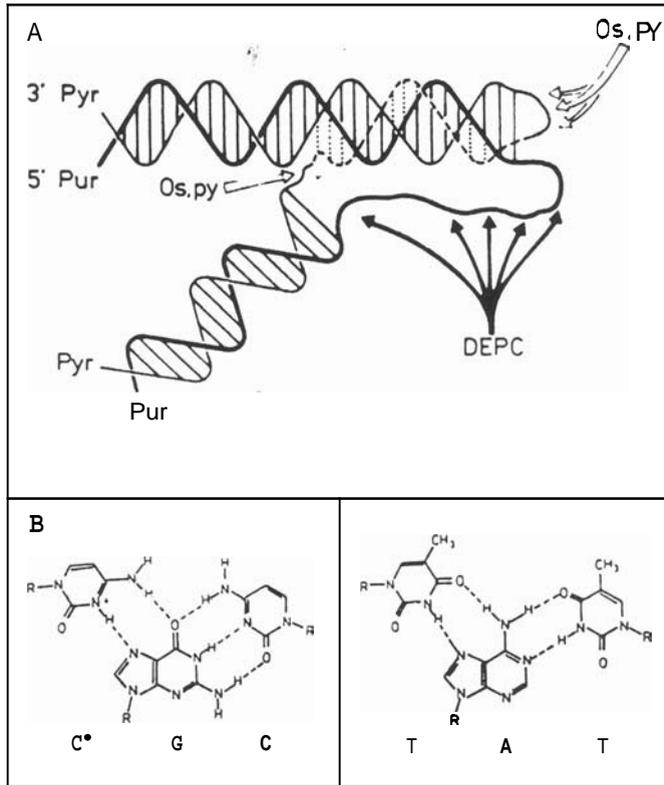
FIGURE 5. Structure of H-DNA. (A) Schematic representation of H-DNA in (TC·AG)$_{18}$, with 3'-half of the pyrimidine (dT-dC) repeat donated to the triplex, forming the H-y3 conformer. The 5'-half of this repeat, plus the complementary 3'-half of the (dA-dG)$_n$, polyurine repeat, act as the acceptor helix in this conformation. The two halves of the polypyrimidine strand in the triplex (——— and – – – –) are antiparallel. Watson-Crick base pairs are shown as lines, Hoogsteen base pairs as dots. (B) Hydrogen-bonding schemes for base triplets in H-DNA. (C) Alternative use of 3'- or 5'-half of the (dT-dC) repeat as the donated strand, to form H-y3 or H-y5 conformers of H-DNA. Watson-Crick base pairs are shown as lines and Hoogsteen base pairs between the acceptor purines and uncharged T or protonated C$^+$ pyrimidines are shown by · and +, respectively. Boxed letters and letters in circles indicate nucleotides that are reactive to single-strand selective probes (e.g., Os,bipy or DEPC) when the DNA is in the H conformation.

age.[514] The energy parameters of the B-H transition in supercoiled DNA were obtained by Lyamichev et al.[515] for (dA-dG)$_n$ and (G)$_n$ sequences. The energy of nucleation of the H form in (A-G), sequences $F_n = 18$ kcal/mol was close to the corresponding value for the cruciform[399] Because of these results, Lyamichev et al.[515] considered the possibility of H-form extrusion in homopu·py tracts shorter than 15 bp as very improbable. This is in agreement with the recent results of Kohwi,[309] who demonstrated triplex formation in (G$_{16}$)·(C$_{16}$) but not in (G$_{14}$)·(C$_{14}$). Triplex formation in both (C-T), and (C)$_n$ sequences effectively protected the DNA duplex from UV-induced pyrimidine dimerization.[380] The degree of protection depended on the degree of supercoiling and acidity.[516]

Displacement of the single-stranded region, a characteristic feature of H-DNA, was questioned by some authors.[328] Htun and Dahlberg[517]

181

FIGURE 5C

demonstrated an ability of the $(dT-dC)_{18}$ sequence cloned into circular M13 phage DNA to form a complex with H-DNA in supercoiled plasmid containing $(dT-dC)_{18}\cdot(dA-dG)_{19}$ sequence. The complex was studied by electron microscopy, 2-D gel electrophoresis, and chemical probing. The latter technique showed Os,py reactivity in the pyrimidine strand similar to that of uncomplexed supercoiled DNA; the DEPC reactivity of the purine strand was significantly reduced, however, suggesting the single-strandedness of the 5'-half of the (A-G),, stretch in H-DNA, a result not consistent with other hypothetical models.[328] A similar approach has been applied to reveal the presence of intramolecular triplexes in (dA-dG) stretches in the initiation region of a dehydrofolate reductase replicon of Chinese hamster cells.[518] Formation of the complex with the (T-C), stretch in M13 DNA requires the presence

of triplex structure, in contrast to complexes between short (T-C), oligonucleotides and (A-G)·(T-G) sequences in duplex DNA.[363,517]

The results summarized above (1) provided strong evidence in favor of the intramolecular triplex H-DNA and (2) suggested that from the two possible isomers of H-DNA (Figure 5), only that in which the donated strand comes from the 3'-half of the pyrimidine strand prevailed under the given conditions.

c. Nucleotide Sequence Requirements

The homopu·py nature of the sequence suitable for the formation of H-DNA is determined by the necessity of forming base triads TAT and CGC[+] or alternatively ATA and GCG (see below). The sequence must contain a mirror repeat (i.e., to be the same in the 3'-5' as well as in the 5'-3' directions along a single strand), which is also termed H-palindrome to distinguish it from normal palindromes (inverted repeats) extruding the cruciform structure.[506,514,519-521] H-palindrome may contain a nonpalindromic segment (which may even disturb the homopu·py character of the sequence) in its center.[519] In triplexes with identical 12-base triads in the stem, 4 to 10 base interruptions in the center were tolerated.[521] Longer triplex loops, however, required higher supercoil energy for triplex formation and were less thermostable than triplexes with shorter loops. For example, the thermal stability of the triplex with 10-base loop was lower by 3 to 4°C and the free energy was about 5 kcal/mol higher than in the most stable triplex with a 4-base loop. A 12-base nonhomopurine spacer between two $(dA-dG)_7$ segments did not prevent the formation of H-DNA (stable over a broad pH range), while the presence of a 46-base spacer with a random sequence completely abolished the H-DNA formation under the same conditions.[522,523] If, however, the spacer was composed of a regular $(dA-dT)_{46}$ tract, a complex H-DNA with a cruciform structure formed by the spacer.

The presence of bases disturbing the H-palindrome in its noncentral region was not so well tolerated.[514,520] Mirkin et al.[520] constructed sequences

5'-AAGGGAGAAXGGGGTATAGGGGYAAGAGGGAA-3'

in which X and Y were either A or G. When X = Y this sequence (inserted in pUC19 DNA) exhibited facile transition to H-DNA. For X ≠ Y this transition was much more difficult or im-' possible, as detected by 2-D gel electrophoresis. A more detailed picture was obtained with Os,py and DEPC modifications. For example, a 42-bp pwpy sequence with three consecutive interruptions formed a mixture of two smaller triplexes instead of a large triplex containing unpaired bases in the stem. A shorter sequence with a single interruption formed a triplex with one unpaired base in the stem. The presence of this interruption resulted in a decrease of thermostability by 7°C and the requirement of a higher energy of supercoiling for the triplex formation. Lower requirements for supercoiling and increased thermostability were observed with increasing G+C content of a homopwpy sequence, suggesting that the presence of CGC triads is very important for triplex formation. No triplex formation was observed in $(dA)_{20} \cdot (dT)_{20}$ even at superhelix densities more negative than -0.09.[513] It was shown in 1990 that much longer $(T_{69}) \cdot (A_{69})$ segments adopted the triplex form.[524]

d. Hinged H-DNA

Htun and Dahlberg[247] proposed a three-dimensional model for H-DNA, from which they predicted that H-DNA would introduce a severe kink in DNA molecules. Studying the electrophoretic mobility of DNA fragments containing H-DNA in (A-G), sequence (at pH 4) at different sites of the molecule, they showed that such a kink does exist and that it possesses a limited flexibility; thus, it can also be termed a hinge. The presence of a kink or a bend in supercoiled pEJ4 plasmid containing H-DNA was demonstrated at pH 5.6 by electron microscopy.[525]

e. Conformers of H-DNA

Htun and Dahlberg[517,526] proposed a standard nomenclature for describing H-DNA structures. According to this nomenclature, H-DNA refers

to conformations with triple-stranded and displaced single-stranded regions (not considering the nature of the base pairing). To distinguish between two possible conformers, H-DNAs in which the donated polypyrirnidine strands come from the 5'-half were termed H-y5 and those from the 3'-half H-y3. The number of base triplets within H-DNA can be indicated in brackets, e.g., H-y5(16). In H-DNAs where the polypurine strand is donated, y (for pyrimidines) is replaced by r (for purines), e.g., H-r5.

Once formed, H-DNA may be trapped in a local energy minimum; the initial nucleation event can thus determine which conformer can be formed. To form the nucleation site, rotation of the helices flanking the donated pyrimidines must occur. Formation of H-y3 conformer from (dT-dC)$_{18}$·(dA-dG)$_{18}$ requires the relaxation of about one more negative supercoil than does formation of H-y5 due to the necessity of additional rotation prior to nucleation. To form H-y5 conformer, the donor duplex only folds back on the acceptor, while H-y3 nucleation requires one complete turn of the two strands around each other. Thus, H-y3 nucleation would be promoted at higher levels of negative supercoiling when compared with nucleation of H-y5. DEPC modification of topoisomers containing H-DNA confirmed this assumption,[526] which can be used to explain the reported bias toward utilizing of the 3'-half of the polypyrimidine strand as a donor.[247,265,313,513]

Relative energetics costs of H-DNA formation decrease with increasing length of the (dT-dC)·(dA-dG) repeats.[526] Longer repeats support generation of multiple conformers and relax a larger amount of negative supercoiling upon H-DNA formation.[517,526] Multiple forms may result from the long repeats in which the mutation did not occur at the repeat center.[526] The pPP1 plasmid containing (dA-dG)$_7$ATCGATATATATCG(dA-dG)$_7$ sequence, which is not very long by itself but contains a long spacer region, displayed at pH 4.5 DEPC modification patterns consistent with the presence of both conformers at a wide range of $-\sigma$ values (0.02 to 0.1).[522] With shorter purpyr sequences of about 25 to 30 bp, only the H-y3 conformer was observed at pH 5 in a wide range of superhelix densities.[514,521] Similarly, the (CAA)$_9$TCC(GAA)$_8$ sequence yielded only H-

y3 conformer at σ from -0.001 to -0.09 at pH 6 and 7.[526a] On the other hand, increasing the length of the regular (A-G), sequence resulted in the formation of multiple conformers.[526b] Their presence was manifested by complex modification patterns of (G-A)$_n$, (G-A)$_{nm}$. and (AG-GAG)$_{12}$ segments at pH 5.5 and below at moderate and high superhelix densities. It thus appears that the mechanism suggested by Htun and Dahlberg[526] might be limited to certain sequences, while in other pur·pyr sequences different mechanisms may come into play and give rise to different triplex structures.[526a]

f. Effect of pH, Ions, Supercoiling, Nucleotide Sequence, and Length of the Homopu·py Tracts

Low pH, negative supercoiling, and increasing length of the homopu-py sequences act interdependently to stabilize H-DNA.[247,264,433,526b,527] With increasing lengths of the homopu·py tract (at pH 5.2), less negative superhelix densities were required to induce the triplex formation.[526,526b] As the pH was increased (at a constant insert length), more supercoiling was necessary to drive the B-H transition.[526,526b]

The homopu-py sequences capable of adopting H-form triplexes can be composed either of mixed sequences forming H-palindromes or of homopolynucleotide stretches such as (G)$_n$·(C)$_n$.

Mixed Homopwpy Sequences: With highly supercoiled DNA modification patterns characteristic for H-DNA were observed even at neutral pH.[247] On the other hand, in plasmids (containing segments of regions flanking the C2b and C2a immunoglobulin constant region genes) where (dC-dT)$_n$ tracts were adjacent to alternating pu·pyr segments, increasing the superhelix density did not result in triplex formation at neutral pH.[528] This was explained by the ability of the Z-DNA flanking sequence to prevent triplex formation. The absence of triplex in pyrimidine sequences at pH 8 at high superhelix density, combined with the formation of left-handed DNA in an alternating purine pyrimidine region located 76 bp to the 5'-side of the CT segment, was observed by Johnston.[313]
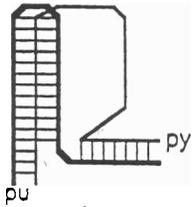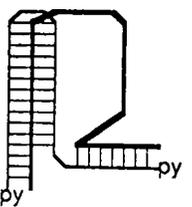
Using nuclease P1, site-specific cleavage was observed starting from the mean superhelix density of about -0.04 at pH 4.6 and about -0.06 at pH 7.5 with both synthetic (dT-dC)$_{12}$·(dA-dG)$_{12}$ and (dG)$_{19}$·(dC)$_{19}$.[513] Similar results were obtained using Os,py modification.[521] At pH >7.8 the reactivity of the homopyrimidine strand was lost,[247,526b] but the homopurine strand remained reactive to DEPC even above pH 9. This result was explained by the presence of a new conformation (J-DNA)[247] and alternatively by different conditions under which DEPC and Os,py reactions were conducted.[526b] It should not be difficult to solve this question experimentally using different chemical probes.

Evans and Efstratiadis[327] showed that nuclease S1 cleavage of (dG-A)$_n$·(dC-dT)$_n$ sequences is length dependent; they observed hypersensitivity of (dG-A)$_{38}$·(dC-dT)$_{38}$ sequences to venom phosphodiesterase from *Crotalus adamanteus* at pH 9 (this enzyme possesses an intrinsic endonuclease single-strand selective activity). Recently it was shown[526b] by Os,py and DEPC probing that increasing the length of the insert decreases the dependence on acid pH for triplex formation. The (dG-dA)$_{37}$ sequence (constructed by Evans and Efstratiadis[327]) adopted a triplex structure at neutral pH and a moderate level of supercoiling ($\sigma = -0.049$). The pK of the triad C$^+$GC is between 7 and 8; thus, at more alkaline pH values unprotonated CGC triads may contribute to triplex stability.[510,529]

An interesting observation was recently made by Bernues et al.,[530,530a] who showed that the (G-A·C-T)$_{22}$ sequence cloned in SV40 can adopt an altered structure at neutral pH in the presence of Zn^{z+} ions (at millimolar concentrations). On the basis of Os,py and DEPC modification patterns the authors proposed a triplex structure denoted as *H-DNA stabilized by GGC and AAT triads (Table 5). GGC and AAU triads were shown to be stable at neutral pH.[531,532] At pH 4.5 the d(GA·CT)$_{22}$ stretch assumed the usual H-form.[530] It thus appears that this sequence can assume two different structures, depending on pH and ions present in solution. *H-DNA was observed also in the presence of Mn^{2+} and Co^{z+} at both neutral and acid pH.[526b] The switch region of IgA immunoglobulin in mice cloned into a recombinant plasmid showed hypersensitivity in the (AG-GAG)$_{28}$ direct repeat to nuclease S1 (at acid pH)

TABLE 5
Effect of pH and Ions on Formation of Protonated and Unprotonated H-DNA[a]

Form:	py·py·pu	pu·pu·py

Sequence:
(A-G)$_n$·(C-T)$_n$

pH	Acid	Neutral
Me^{2+}	No	Zn^2+ Mn^2+ Co^2+

(G)$_n$·(C)$_n$

pH	Acid	Neutral
Me^{2+}	No	Mg^2+Mn^2+Ca^2+

(A)$_n$·(T)$_n$ In n = 69, but not in n = 33

pH	Neutral	Not observed
Me^{2+}	Mg^2+	

[a] See text for details.

and P1 (at neutral pH) at a more negative than −0.002.[527] The nuclease S1 hypersensitivity was retained for the shorter repeat (AGGAG)$_6$, which displayed at acid pH Os,py and DEPC modification patterns characteristic for the H-y3 conformer of H-DNA.

Long mirror repeats (GAA)$_9$TCC(GAA)$_8$ and (GGA)$_9$TCC(GGA)$_8$ form H triplexes at pH 6.0 and 7.0 in plasmids at −σ as isolated from E. coli.[526a] With an increase of −a and/or lowering of the pH, Os,py and DEPC modification patterns were observed that were inconsistent with the formation of a usual H triplex. It has been suggested that a structure is formed that simultaneously contains two triplexes in the given sequence. One possibility is that Hy-5 forms in the (GAA), and Hy-3 in the (GAA), segment, but other triplex models are possible. Similarly, (GGA)$_9$TCC(GAA)$_8$, which is not a mirror repeat, formed non-B conformation at acid pHs. The energy required for the transition was about the same as with the H triplex formed by the H-palindrome sequence, but the Os,py and DEPC modification patterns of the former structure were not consistent with H triplex DNA. A non-B structure hypersensitive to single-strand selective

nucleases was observed in a long AT-rich polypu·py tract (found downstream of the chicken myosin heavy chain gene) at pH 4.5 and 7.5.[533] Recent CD studies of poly(dA-dG)·(poly(dT-dC) demonstrated six different conformational states of this polymer at pH values between 8.0 and 2.5.[534] These results suggest that local structures other than H triplexes may be formed in pur·pyr sequences at various pH values.

(dG)$_n$·(dC)$_n$ Sequences: After Lyamichev et al.[519] demonstrated the existence of protonated H triplex in (G)$_n$·(C)$_n$ sequences by 2-D gel electrophoresis, Kohwi and Kohwi-Shigematsu[290] showed that the (dG)$_{30}$·(dC)$_{30}$ sequence can adopt another triplex structure at neutral pH in the presence of Mg^{2+} ions (at millimolar concentrations) formed by unprotonated base triads GGC (Table 5). (dG)$_{16}$·(dC)$_{16}$ tract within the 5'-flanking region of the adult chicken β^A globin gene displayed similar properties. In addition to Mg^2+ ions, Ca^2+ and Mn^2+ also induced the (G)$_{16}$·(C)$_{16}$ sequence into a GGC triplex. Recent results suggest that in forming the dG·dG·dC triplex structure the potential of poly(dG)·poly(dC) depends on the length of the (dG)·(dC) tract.[535] Zn^{2+}, Cu^2+, and Co^2+ ions did not induce triplex structure in the (G)$_{16}$·(C)$_{16}$ sequence, but did influence the structure of the direct repeat sequence adjacent to the (G)$_{16}$·(C)$_{16}$ sequence. d[(G)$_{24}$C(G)$_{21}$]·d[(C)$_{24}$G(C)$_{21}$] in pG46C plasmid displayed structural transition at neutral pH in the presence of Mg^Z+ at ΔLk − 15 accompanied by a release of five turns as detected by 2-D gel electrophoresis.[537]

A 64-bp GC-rich polypu·py tract from the rat long interspersed DNA element displayed two classes of supercoil-dependent reactivity toward chemical probes.[538] One class consisted of highly sensitive bases (whose sensitivity was strongly affected by pH and Mg^2+ ions) probably contained in H-DNA triplexes observed earlier in (dG)$_n$·(dC)$_n$ sequences.[290,519,536] The other class comprised moderately sensitive bases independent of reaction conditions. This reactivity suggests the presence of non-B-DNA, but the structural basis for this reactivity is not known.

(A)$_n$·(T)$_n$ Sequences: Fox[574] showed that (dT)$_{69}$·(dA)$_{69}$ sequences adopt an intramolecular triplex structure in supercoiled plasmid (at native a) in the presence of Mg^2+ at pH 8. The structure displayed a characteristic Os,py modification in

the center of the (T_r) segment and a DEPC modification of the purine strand characteristic for the H-y3 conformer. No chemical modification resembling the triplex structure was observed in $(A_{33})\cdot(T_{33})$ and $(A_{23})\cdot(T_{23})$ segments. The nuclease S1 cleavage and DEPC modification patterns of $(A_{69})\cdot(T_{69})$ suggested that the displaced half of the purine strand might weakly interact with the triplex. 2-D gel electrophoresis failed to detect any structural change; this was said to be due to the relatively small fraction of molecules containing the triplex. Compared with $(G_n)\cdot(C_n)$ and $(A\text{-}G)_n\cdot(T\text{-}C)_n$ sequences, formation of triplex in $(A_n)\cdot(T_n)$ segments requires much longer homopu·py stretches. Fox[524] suggested that the requirements for long $(A)\cdot(T)$ tracts may be due to the high stability and rigidity of propeller-twisted $(A_n)\cdot(T_n)$ helix containing bifurcated hydrogen bonds (see Section III). If this explanation is correct, why did $(A_{15}T_{15})$ segments show facile C-type cruciform extrusion[253] and why did thermally more stable $(G_n)\cdot(C_n)$ sequences with $n \leqslant 69$ form triplex?[290,513,519] Further work is necessary to better understand recently discovered triplex structure in long $(A)\cdot(T)$ segments.

3. Infermolecular Triplexes

a. Complexes of Oligonucleotides with DNA

The possibility of complexing oligonucleotides with DNA has been long anticipated.[492,539] Formation of intermolecular triplexes resulting from the interaction of oligonucleotides with the complementary homopurine sequence in duplex DNA was demonstrated only recently.[356-359,363,540] Interest in intramolecular triplexes has recently greatly intensified (see Section V.D.2.e), mainly due to their potential application as inhibitors of gene expression and as recognition and cleaving elements in chromosome mapping.

It has been shown that a 27-base-long oligonucleotide probe binds to duplex DNA at a single site within the 5'-end of the human *c-myc* gene, forming a colinear triplex with the duplex binding site.[540] The triplex formation correlates with repression of *c-myc* transcription in *vitro.* A shorter 11-mer oligopyrimidine d(TTTCCTCCTCT) formed a complex with a fragment of double-stranded DNA containing a complementary sequence.[357,366,369] The complex was stabilized by additional binding energy resulting from intercalation of the aromatic ring system (acridine, phenanthroline) attached to the oligonucleotide 5'-end. The oligomer was bound to the major groove of DNA in a parallel orientation with respect to the purine strand. This kind of highly sequence-specific interaction can be used to control DNA expression.

A similar approach has been adopted to develop a new strategy of genomic DNA mapping.[359] Oligonucleotides with attached cleaving agent EDTA·Iron(II) at the 5'-end can induce sequence-specific double-strand breaks in DNA. An oligonucleotide of about 15 to 19 nucleotides should be sufficiently long to recognize a specific sequence in the human genome,[356,359] providing in a formal sense 10^6 times better resolution than natural restriction nucleases. More details can be found in recent reviews and also in Section V.D.2.e.[356-359,541]

b. Physical Studies of Triplexes

In addition to the studies mentioned above, structural information on the triple helices has been derived from poly- and oligonucleotide studies by means of physical techniques. Early X-ray fiber diffraction studies of homopolynucleotides resulted in a triplex model in which the third strand lies in the major groove, interacting with the duplex via Hoogsteen pairing.[493,542] The duplex portion of the structure adopts an A-like conformation with a C3'-*endo* sugar pucker. the third strand can be homopurine or homopyrimidine, depending on experimental conditions. If the third strand is homopyrimidine it is parallel to the homopurine strand in the triplex.

Recent studies of oligonucleotides with various nucleotide sequences by means of NMR and other techniques confirmed in the principle triplex model, resulting from the X-ray fiber diffraction measurements.[493,542-548] It has been shown by NMR, CD, and other measurements that at neutral pH in the presence of $MgCl_2$, triple-helical $(dA)_{10}\cdot 2(dT)_{10}$ is formed.[546] In this triplex thymine N3-H iminoprotons are involved in both Watson-Crick and Hoogsteen base pairing.

d(GA)$_4$ and d(TC)$_4$ octarners are able to form B-DNA duplex as well as triplexes dependent on

experimental conditions.[543,545] pyr·pu·pyr triplex was observed at low pH and in an excess of pyrimidine strand. The results unambiguously confirmed that the second pyrimidine strand binds via a Hoogsteen pairing (with cytosines protonated at N3) in the major groove of a Watson-Crick duplex. The conformation of the pyrimidine sugars was A-DNA-like (see Section 11), while purines had a B-DNA-type sugar pucker. Under the given conditions the TA Hoogsteen base pairs appeared more stable than GC+ Hoogsteen base pairs. Results of independent 2-D NMR studies of 11-mers were in good agreement with those of octamer studies showing A-helical base stacking conformation in the oligopurine strand of the 11-mer triplex.[547] Quite recently, Sklenar and Feigon[548] constructed a 28-base DNA oligomer with a sequence that could potentially form a triplex containing C^+GC and TAT triads. Their 2-D NMR experiment showed that this oligonucleotide forms an intramolecular triplex at pH 5.5 and that a significant amount of triplex remains at neutral pH.

The 26-mer d(GAAGGAGGAGATTTTTCTCCTCCTTC) formed a hairpin in solution.[549] If this oligonucleotide was mixed with d(TCTCCTCCTTC) at pH 5, a triplex is formed as detected by gel electrophoresis and CD measurements. The triple-stranded structure melted in a biphasic profile. The duplex-to-triplex transition was accompanied by an average change in enthalpy of -73 (± 5) kcal/mol. The equimolar mixture of d(CTCTTCTTTCTTTTCTTTCTTCTCT) and d(GAGAAGAAAGA) formed a triplex at pH 5 as detected by gel electrophoresis, ethidium bromide interactions, DNaseI digestion, and CD spectra. Cooperative thermal transition was observed that was attributed to the disruption of H-DNA-like structure into single strands.[550]

X-ray fiber diffraction studies of poly(dG)·poly(dC) complexes with N-a-acetyl-L-arginine ethylamide displayed a B-form pattern, although these polynucleotides favor (in the absence of arginine) the A-DNA form.[551] Upon dehydration a triplex was observed, most likely formed by poly(dC+)·poly(dG)·poly(C).

4. Conclusions

The triplex H-DNA structure suggested by the Frank-Kamenetskii group is now well established.[175,512] The structure can be formed in any homopu·py sequence containing a mirror repeat. Strong evidence for the protonated triplex structure (Figure 5) has been supplied by chemical probing.[264,265,290,313,326,513] Important structural information has been extracted from recent NMR studies on synthetic oligonucleotides.[543-548] In spite of a large amount of data, it is still difficult to ascertain the structure of the half of the strand not involved in the triplex base pairing. Is it truly single-stranded and free of any interactions? This does not seem very probable. At least some results of chemical probing suggest that bases in this part of H-DNA in supercoiled plasmids are involved in interactions that are most probably substantially weaker than those within the triple~.~ Do these interactions involve the triplex or are they limited to, for example, stacking interactions within the displaced single strand itself? In the former case we would have to consider the possibility of the formation of a loosely bound tetraplex.[522]

D. Other Structural Changes

, The structures of cruciform, left-handed DNA, and triplexes, as well as their relations to DNA supercoiling and other factors, were well established in the 1980s. These structures are undoubtedly not the only ones stabilized by negative supercoiling. Recent experimental data suggest that a number of other structural changes may occur.[552-555] Presently, some data cannot be interpreted in terms of a well-defined structure, while others do not reveal the relation of a structural change to DNA supercoiling. It is to be expected that these points will be clarified and new local DNA structures discovered soon.

Among the DNA local structural changes that have recently attracted the greatest attention are base-unpaired regions, structures of telomeric sequences, and parallel-stranded DNA. These are briefly discussed in the following paragraphs.

1. Structure of Telomeric Sequences

Telomeres (reviewed in References 505 and 556 to 558), the ends of eukaryotic chromo-

somes, all have a similar type of nucleotide sequence, i.e., tandemly repeated GC-rich sequences with a pronounced strand-specific base composition asymmetry such as $d(C_4A_2) \cdot d(T_2G_4)$ (repeated at least 50 times) in ciliated protozoan Tetrahymena or $d(C_4A_4) \cdot d(T_4G_4)$ of *Oxytricha*. Telomeres are involved in the replication and maintenance of the chromosomal ends, and a higher order structural theme shared by all telomeric sequences has been suggested to be critical for their function.[559] In the last 2 to 3 years evidence has accumulated showing specific local structures in telomeric sequences.

a. C,A Hairpin

Budarf and Blackburn[560] demonstrated that the telomeric sequence poly $d(C_4A_2)_n \cdot d(T_2G_4)_n$ of Tetrahymena inserted in a plasmid is hypersensitive to nuclease S1 under conditions of negative supercoiling. No such hypersensitivity was observed in linear DNA. As in the case of homopu·py sequences, mapping of nuclease S1 hypersensitive sites did not yield a basis sufficient to deduce the higher structure of the given sequence. Using 2-D gel electrophoresis and chemical probing, Lyamichev et al.[561,561a] proposed a novel protonated DNA conformation, the C,A hairpin. In this structure two independent hairpins are formed that are stabilized by $C \cdot C^+$ and $A \cdot A$ base pairs.

The model of (C,A) hairpin structure is based primarily on the results of chemical probing. DMS, Os,py, and $KMnO_4$ showed no protection of G's and T's from modification, suggesting that the G_4T_2 strand is virtually unstructured. In the C_4A_2 strand the strongest reaction was observed in the central A's, while other adenine residues were less reactive. The nonequivalence of the two strands in the (C,A) hairpin is supported by the binding of the oligonucleotide $(C_3A_2C_4C)$ complementary to the G-rich strand, which occurred only at acid pH. $(G_4T_2)_2$ complementary to the C-rich strand showed no binding.

The data presented by Lyamichev may also be consistent with triplex H-DNA carrying CGA and ATC triads (in addition to TAT and CGC^+), if two conformers are present in comparable amounts.[561a] Further studies, including modifi-

cation of C in various topoisomers, may help to solve this problem.

b. Quadruplexes

In the last few years studies of synthetic oligonucleotides containing telomeric sequence motifs have resulted in new models of DNA structures based on non-Watson-Crick base-pairing. In 1987 Henderson et al.[559] investigated the structure of G-rich strands of several telomeric sequences and demonstrated the formation of novel intramolecular structures containing $G \cdot G$ pairs with guanosine residues in *syn* conformation as determined by NMR spectroscopy. Sen and Gilbert[562] probed the structure of self-associated single-stranded DNA oligonucleotides (which displayed a decreased electrophoretic mobility when compared with single strands) by DMS and concluded that four-stranded structures were formed in which the strands run in parallel fashion and guanines are bonded to each other by Hoogsteen pairing.

Quite recently compelling evidence was gathered independently from three laboratories showing that G-rich oligonucleotides may form antiparallel quadruplexes containing cyclic guanine base tetrads.[563-565] Sundquist and Klug[563] prepared a series of oligonucleotides containing duplex sections with a TTGGGG repeating sequence (found in Tetrahymena telomeres) followed by a single-stranded 3'-terminal overhang of two repeats and showed that these oligonucleotides dimerize to form stable complexes in solution. The complexes were uniquely stabilized by potassium ion. The dimerization was mediated by the 3'-terminal overhang. In these complexes the N7 of every guanine in the 3'-terminal overhang was inaccessible to DMS and DEPC, while both pairs of thymines were accessible to osmium tetroxide. The authors proposed that telomeric DNA dimerizes by hydrogen bonding between two intramolecular hairpin loops, forming antiparallel quadruplexes.

In contrast to Sundquist and Klug,[563] Williamson et d.$@used single-stranded oligonucleotides composed of the telomeric sequence repeats from *Oxytricha* and *Tetrahymena,* while Panyutin et al.[565] used fragments of DNA con-

taining $(dG)_{23}$, $(dG)_{27}$, and $(dG)_{37}$, respectively. The results obtained by different methods (chemical probing,[562-564] UV crosslinking,[564] electrophoretic mobility[559,562-564]) induced proposals of remarkably similar structures with guanosine bonds of adjacent chains in opposite conformations: *syn* vs. *anti.* Thus, in any tetrad two guanosine residues have anti and two have *syn* conformations. The suggested[559,562-565] Hoogsteen base pairing (Figure 5) received further support from experiments with replacement of dG with dI at various positions of the telomeric sequence.[566] The oligonucleotide complexes prepared by Sundquist and Klug[563] opposed those of Williamson et al.[564] by the different effect of monovalent ion on their stabilization, suggesting differences in sizes of cavities created within the structures.

Do these results mean that the original model of a parallel-stranded quadruplex (G4-DNA; Figure *6*) proposed by Sen and Gilbert[562] should be abandoned? The recent work of these authors suggests that this should not be the case.[567] Sen and Gilbert[567] showed that both parallel-stranded G4-DNA (with all of its guanosine residues in the *anti* conformation) and antiparallel quadruplexes can be formed. They expected sequences with four or more separated runs of G's to yield four-stranded intramolecular fold-back structures, sequences with two runs to produce dimer fold-back structures, and sequences with single runs to yield G4-DNA. Complex sequences formed parallel-stranded G4-DNA in the pres-, ence of sodium and rubidium but not not the presence of potassium ions. This anomaly (which was not found in oligonucleotides containing short, single runs of three or more Gs) arose because potassium ions stabilized quadruplex structures so strongly that transient intermediates were trapped, preventing formation of the G4 structure. Formation of G4-DNA was very strongly dependent on the ratio of Na^+/K^+. Two phases of G4 formation were shown: at low molar ratios of potassium, a progressive increase in the G4 formation rate was observed, while at higher potassium ratios G4 DNA formation was inhibited.

Telomere DNA sequences were observed in phylogenetically diverse organisms including protozoa, fungi, and even higher eukaryotes.[558] It now seems that telomeres of all eukaryotic nuclear chromosomes may have basically similar structures that can undergo transitions in dependence on their ionic environment. We can, however, only speculate about the relations between their spatial organization and biological function.

2. Base Unpaired Regions

Early melting was perhaps the first recognized structural change assisted by the free energy of negative supercoiling.[143,567a] Local base pair disruption was observed[124,220] under various conditions far from melting but differing from physiological conditions. Hypersensitivity to single-strand selective nucleases (such as mung bean and P1 nucleases) was reported at physiological conditions in AT-rich regions of supercoiled plasmids.[223,568,569] The hypersensitive sites did not correlate strictly with AT content and appeared in replication origins and transcriptional regulatory regions in both pro- and eukaryotic DNAs. Until recently it was not known whether this local DNA hypersensitivity was due to permanent or transient unwinding of the given DNA region. Recent experiments with 2-D gel electrophoresis showed that the unwound structure is thermodynamically stable (prevailing at equilibrium over the B-form).[570,571]

Transition to stable unwinding occurred[571] at about $-\sigma = 0.05$ and the average extent of unwinding was sufficient to completely unwind the region recognized by the nuclease at $-\sigma$ 0.067.[570] The formation of completely unwound DNA segments in replication origins and transcriptional regulatory regions is a very attractive suggestion, since DNA must unwind to initiate replication and transcription. The evidence based on nuclease hypersensitivity and 2-D gel electrophoresis, however, is not sufficient to rule out other alternatives.[569-571]

Kohwi-Shigematsu[535] showed that the yeast replication origin sequence is reactive to CAA;[569,572,573] this result suggests that the reactive region is truly unpaired. It also has been shown that stable unpaired regions hypersensitive to CAA modification surround the immunoglobulin heavy chain (IgH) enhancer. These regions are AT-rich and contain negative regulatory elements. Unpaired CAA-hypersensitive, AT-rich
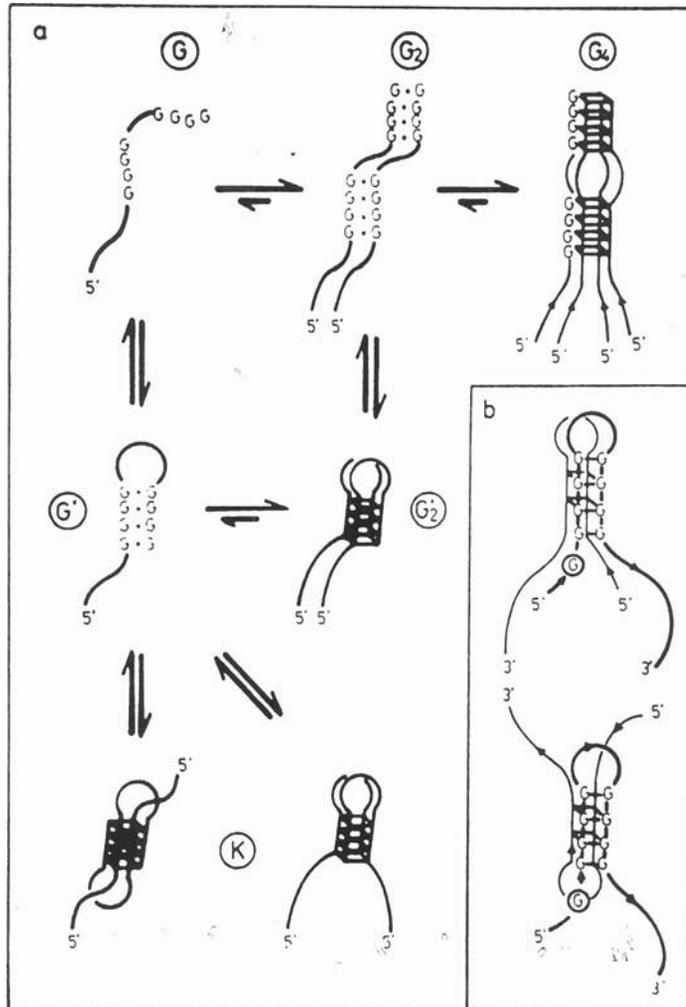
FIGURE 6. (a) Scheme for the formation of G_4-DNA. The formation of the dimer structure G_2 must be rate-limiting, and it must **be** rapidly converted into G. Only three possible structures (including K and G_2') of fold-back intermediates are shown, but other structures may also be formed. (b) Structures of product K most compatible with its methylation-protection pattern. The methylation-enhanced guanine is circled. The arrows indicate the 5'-3' direction of the sugar-phosphate backbone. (Reprinted by permission from Sen, D. and Gilbert, W., *Nature*, **244**, 410, 1990.)

regions are also included within matrix association regions. Similar to mung bean nuclease hypersensitive sites of the E. *coli* replication origin,[571] the structural transition in the IgH enhancer region occurs at σ about −0.05, and increasing the negative superhelix density results in extension of the unpaired region.[573] A local high **AT** content is not sufficient to cause the **DNA** segment to adopt the unpaired structure. Mutation of three adenines (to either G or C) in the sequence ATATAT in the CAA-hypersensitive region resulted in a marked reduction of its sensitivity to CAA. It was proposed that the ATATAT motif may be kinked, serving as a nucleation site for base unpairing.

Various sequences required different environmental conditions to form unpaired region~;'' @ for example, at 22°C the 3'-sequence of the IgH enhancer was very sensitive to CAA, while the autonomously replication sequence of

the yeast origin was unreactive,[572] showing some CAA hypersensitivity at 37°C.[573] Base unpairing is involved in the C-type cruciform extrusion (see Section VI.A.2). Hypersensitivity to single-strand chemical probes under superhelical stress was observed also in other AT-rich sequences[3'] and the curvature-inducing ATATATTTTTAGA-GATTTTT sequence.[113]

The coincidence of the base unpaired regions with various functional elements such as negative regulatory elements[573] (repressing IgH enhancer activity in fibroblasts but not in β cells), nuclear matrix association regions, replication origins,[570-572] and transcriptional regulatory regions suggests that DNA structure may play an active role in replication and transcription and other biological functions rather than serving only as a passive source of nucleotide sequence information.

3. Parallel-Stranded DNA

Multistranded structures containing parallel strands were discussed in preceding paragraphs. Recently it has been shown that parallel-stranded duplexes can be formed by oligonucleotides consisting of AT pairs under physiological,-conditions.[574-576] Parallel-stranded DNA was formed in hairpin molecules (1) with stems stabilized in a parallel orientation by 5'-5' or 3'-3' phosphodiester linkages in the hairpin loop[574,577-579] and (2) with crosslinked ends.[580-582]

From the biological point of view the most interesting parallel-stranded DNA appears to be that formed by hybridization of two complementary oligonucleotides with appropriate sequences (partially homooligomeric A·T sequences). Such parallel-stranded molecules were recently designed, synthetized, and characterized.[575-577,583-587] The conformational constraints in these oligonucleotides was obtained by using overhangs or by appropriate combination of block and mixed sequences.[575] Runs of alternating (A-T), segments[579] as well as interspersed GC pairs are compatible with the parallel-stranded helix but induce its destabilization.[586]

a. Structural Features of Parallel-Stranded DNA

The structure of parallel-stranded DNA has been studied in solution using various physical and chemical techniques. (Crystallographic data are not yet available). The results of gel electrophoresis, W absorption, and CD spectra measurements, as well as thermal melting and chemical probing, suggested that parallel-stranded DNA contains a distinct secondary structure.[574-587] Differences between antiparallel- and parallel-stranded DNA behavior were observed upon binding intercalating drugs chemical probing and nuclease cleavage.[574-578,583-585,587]

According to the theoretical force field calculation,[588] reversed (trans) Watson-Crick AT base pairs are formed in parallel-stranded DNA in which the adenine 6-amino group is hydrogen bonded to thymine via the O, instead of the O, of the keto group [in the normal (cis) Watson-Crick pair]. The reverse (Watson-Crick base pairing) has been supported by ^{1}H-NMR[578] DMS probing,[574] and Raman spectra.[579] The Raman spectra,[589] NOESY measurements,[578] and molecular modeling[574,586] suggested that the furanose rings are mainly in C2'-endo conformation and bases are in anti orientation. ^{31}P-NMR of the intramolecular parallel-stranded hairpin (containing (A)·(T) stem and four cytosines in the loop) and a 25 bp parallel-stranded duplex composed of two complementary strands [with a $(A)_{10}·(T)_{10}$ block and sequences containing TA and AT steps] produced different results.[578,587] In the former parallel-stranded hairpin no drastic differences between parallel- and antiparallel-stranded DNAs were observed,[578] whereas the backbone structure of the latter parallel-stranded duplex differed from that of the antiparallel-stranded duplex.[587] No clear explanation of these results has been yet offered.

Measurements of fluorescence resonance energy transfer of 5'-fluorescence labeled oligonucleotides confirmed that the strands in parallel-stranded DNA indeed have the same polarity.[590] Parallel-stranded DNA inserted in a supercoiled plasmid underwent a transition to a $(pu)_2·(pyr)$

triplex as detected by 2-D gel electrophoresis and chemical probing.[591] The data confirmed that parallel-stranded DNA forms a right-handed duplex. Parallel-stranded duplexes formed between unnatural a-anomers and natural β-oligonucleotides[365,592] as well as duplexes containing T·T pairs and phosphate-methylated backbone have been reported.[593]

b. Biological Role

Does parallel-stranded DNA occur in vivo, and, if so, what is its biological role? This question cannot yet be answered, but the stability of parallel-stranded DNA at physiological conditions suggests that such a structure might be formed in vivo. It has been suggested that parallel-stranded DNA would arise most readily in the course of exchange reactions involving interactions between separated segments of DNA, including recombination and genomic rearrangements.[576,587] In principle, parallel-stranded DNA can be formed in several ways, the simplest of which involves intrastrand loop formation.[576]

Parallel complementary sequences were found in the Drosophila genome[593a] and the ability of very similar sequences to form parallel-stranded DNA in *vitro* was demonstrated.[581] Thus, the possibility of the existence of local parallel-stranded DNA regions in vivo does not seem to be unrealistic. More details concerning parallel-stranded DNA structure and properties can be found in recent reviews.[587,594]

E. Conclusions

It has been shown that negative supercoiling stabilizes left-handed DNA segments, cruciform, triplex and C,A-hairpin structures, and base unpaired regions. These structures are frequently called "unusual structures". Are these structures unusual only to us, because we recognized them much later than B- and A-DNA, or are they really unusual in nature? Does, for example, Z-DNA or the triplex structure occur in vivo substantially less frequently than A-DNA? Until now we have not been able to answer such questions.

A common feature of cruciforms, triplexes,

C,A hairpins, and base unpaired regions is the accessibility of some bases for interactions with the environment (in contrast to bases hidden inside the B- or A-DNA double helices). The accessibility of bases in Z-DNA is less marked than in other unusual structures, but the results of chemical modification experiments suggest that at least in (A-C)·(G-T) sequences left-handed DNA segments (in supercoiled plasmids) may contain a number of exposed bases.[467-470] In addition, accessible bases were found at B-Z junctions regardless of the nucleotide sequence of Z-DNA. Local accessibility of a small fraction of bases was observed earlier even in nonsupercoiled DNAs far below melting conditions.[10] Regions containing accessible bases have been called "open" DNA regions. Similarly, cruciforms, triplexes, hairpins, and B-Z junctions may be called open DNA structures. It is not clear whether the base unpaired regions are completely structureless (this does not seem to be probable) or whether they adopt some structure. In the latter case they can be included among open DNA structures.

If the open DNA structures play some biological role, it would be surprising if the accessibility of their bases would not take part in biological processes. Exposed bases in open DNA structures may represent targets for specific interactions with other DNA and RNA strands; they can facilitate or prevent recognition of a given sequence by specific proteins and other substances interacting with DNA. Many mutagenic chemicals would react preferentially with exposed bases in a way similar to that of chemical probes. In addition to open structures discussed above other open structures and complexes can be transiently formed during various biological processes, including DNA replication, transcription, and recombination. An example of such structures is an open complex of RNA polymerase with promoter sequences. This complex contains a so-called melted region 12 to 16 bases in length. Recently DEPC, DMS, and Os,py have been applied to characterize this region in an open complex of the lac UV 5 promoter.[259,282] It has been shown that bases in the template strand from -10 to $+3$ react with DEPC (A residues) and DMS (C residues).[259] Thymines at positions -8, -9, and -11 reacted with Os,py in contrast to those at $+1$ and $+2$ (assumed to be in a single-

stranded region) that did not react. On the other hand, thymine at -11 showed hypersensitivity toward Os,py even though this base is expected to be outside the locally "melted" region. These results suggest that the locally melted region is not completely structureless. Its detailed characterization by means of chemical probes may provide important information, including those about the strength of the given promoter. Work with this goal in mind is underway.[259] Similar approaches can be applied to study other open structures and complexes both *in vitro* and *in situ*.

VII. DNA STRUCTURE IN THE CELL

Much information has been gained concerning DNA structure in solution, fibers, and crystals, although information about DNA structure in its natural environment, i.e., in the cells, is scarce. Such information is, however, vital for a better understanding of the biological role of DNA. The preceding sections showed that DNA supercoiling stabilizes various local structures such as cruciform, triplexes, left-handed DNA, etc., and that extrusion of these structures in turn changes the DNA superhelix density. Extrusion and absorption of the local structure may represent an efficient way of regulation of the biological processes involving DNA, but do these structures really exist *in vivo?*

Early experiments suggested that a certain portion of DNA isolated from various organisms had a single-stranded character (reviewed in Reference 10). It was shown that the amount of DNA cleaved with single-strand selective nuclease S1 increased during the period of DNA synthesis in human diploid fibroblasts.[595] In experiments carried out with isolated DNA it was difficult to exclude the possibility that regions with a single-stranded character were formed secondarily (e.g., due to nuclease cleavage after the cell disruption). Techniques have become available that make possible the study of DNA structure inside the cell.

A. Methods of Analysis of DNA Structure in the Cell

Both indirect and direct ways of demonstrat-

ing the formation of open local structures in the cells have been employed. The former include: (1) methods of studying certain genetic consequences of the formation of such a structure, including the modulation of transcription by Z-DNA[Sg%] and cruciform,[597] and the susceptibility of potential Z-forming[Sg8] and inverted repeat sequences[599-601] to deletions: and (2) methods based on changes in DNA topology induced by the formation of a local structure[288,602] and on compensation of the simultaneous partial DNA relaxation by intracellular topoisomerases (linking number assay). The indirect methods do not significantly disturb cell life, but interpretation of the results may not be free of ambiguity; for instance, the linking number assay of the cruciform extrusion *in vivo* relies upon the accurate regulation of superhelix density in the cell. It is expected that the change in the superhelix density induced by the structural transition will be compensated for by the regulatory system, which reduces the DNA linking number so that the level of supercoiling is reestablished[288] Unfortunately, other changes in DNA superhelix density (e.g., due to a specific protein binding to the inverted repeat) may be compensated for in a similar way.

Perhaps the most efficient way to demonstrate 'the formation of a local DNA structure in the cell is by probing DNA *in situ*. This has been achieved by molecular genetic[42,603] and chemical approaches.[39] The molecular genetic method induces an enzyme in the cell and the consequences of its specific interaction with the local DNA structure in the cell are determined. For example, cleavage of the cruciform loop by 'I7 endonuclease was used to demonstrate cruciform structure in *E. coli* cells.[603] Single-strand selective chemical probes have been applied successfully in DNA structure studies *in vitro* (Section *V*). We have shown[39] that Os,bipy enters E. *coli* cells without disturbing their integrity and site-specifically reacts with bases in open regions of the cellular DNA such as B-Z junctions[39,40] or triplex structures.[604] Testing of other osmium tetroxide complexes revealed that in addition to Os,bipy, Os,TEMED (Figure 2) can be applied to probe DNA structure *in situ*.[605] $KMnO_4$ was recently introduced as another single-strand selective probe of the DNA structure in E. *coli* cells.[41] Chemicals reacting with double-stranded DNA such as

DMS[330,331,606] and copper-1,10-phenanthroline[607] were used to obtain footprints of intracellular DNAs. DNA footprinting can also be carried out by irradiating cells with short exposures of UV light.[372,608,609] With the use of UV irradiation it may also be possible to obtain information about local DNA structures *in vivo.*

After the treatment of cells with a chemical probe (or UV light), DNA is usually isolated and the probe reaction sites determined as in experiments *in vitro* (Figure 3). It is also possible to detect the probe binding in the cell by means of immunofluorescence techniques without isolating DNA.[610] By using Os,bipy it has been shown that Z-DNA,[39,40,248,447] cruciform,[39,611] and triplex structures[38,604] can exist in E. *coli* cells.

B. Cruciform Structures

Inverted repeats, the potential cruciform sequences, are frequently found within genetic regulatory regions.[260,612,613] Can all of these sequences extrude cruciforms *in vivo?* Reports published *in* 1983 suggested[399,613] that in bacterial cells cruciform may be rare because of its slow formation. On the other hand, the tendency of some inverted repeats to undergo deletion was observed,[418,419,599-601] suggesting the possible presence of cruciforms in the cell.

1. Evidence of Cruciform Structure in Bacterial Cells

Using the linking number assay, Hanniford and Pulleyblank[288] concluded that the (A-T),, sequence extruded cruciform in E. *coli* cells under conditions of blocked protein synthesis. In 1987, Panayotatos and Fontaine[603] showed cleavage of the ColE1 inverted repeat in the intracellular pLAT75 plasmid by T7 endonuclease induced in E. *coli* cells. The digestion site coincided with the cruciform loop cleavage by T7 and S1 nuclease *in vitro.* The ColE1 inverted repeat in the pLAT75 was' placed in its native environment within the coding sequence of the colicin resistance gene and was actively transcribed from its natural promoter. The sequence surrounding the palindrome was highly AT-rich. Thus, the com-

bined effects of active transcription[145] and presence of adjacent AT-rich sequences[3'] (see Section VI.A) could significantly contribute to cruciform extrusion in the cell. By means of the Os,bipy probe, the presence of cruciform in E. *coli* cells was demonstrated recently in (A-T), inserts of different lengths.[40,611] In the system not undergoing active transcription, biased in favor of cruciform formation (using salt shock or topoisomerase mutation to increase the superhelix density) cruciform was detected in (A-T)$_{34}$, (A-T),,, and (A-T)$_{15}$ but not in (A-T),,. These experiments made it possible to calculate the effective DNA superhelix density inside the cell that responded directly to genetic and environmental influences.

The above data seem to be sufficient to warrant the conclusion that at least some inverted repeats can form cruciform in E. *coli* cells.[603,611] The presence of AT-rich C-type-inducing sequence and superhelix density increased above the average intracellular level appears to be important for cruciform extrusion in the cell.

2. Biological Role

If we admit that cruciforms can exist in the cell, a question concerning their biological function may arise. It has been suggested that one of the signals involved in the initiation of DNA synthesis is a specific local DNA structure that is recognized by the enzyme necessary for replication.[210,614] Features common to many replication origin sequences include inverted repeats and AT-rich regions.[615,616] Thus, cruciform structures may be one of the candidates that might be involved in signaling initiation of DNA synthesis. Quite recently Ward et al.[616] observed some correlation between the distribution of activated origins of replication and the distribution of cruciform using immunofluorescence of eukaryotic nuclei labeled with monoclonal anticruciform antibody. By means of fluorescence flow cytometry they determined the number of cruciforms to be around 10^5 per nuclei. It was shown that human and rat cells contain cruciform-binding protein that is structure-specific and sequence-independent.[617,618] This protein was identified as nuclear HMG1.[619] HMGl is an abundant component of

the nucleus involved in transcriptions and DNA replication; its interaction with the cruciform structure points to an important biological role of this structure.

It has been shown that a synthetic E. *coli* promoter containing a cruciform in a − 10 region may regulate transcription in a supercoil-dependent manner.[597] Transcription from this promoter *in vitro* was repressed as the cruciform [50 bp highly (88%) AT-rich inverted repeat] was extruded. Transcription *in vivo* was induced as supercoiling was relaxed due to DNA gyrase inhibition [which was less (64%) AT-rich]. A cruciform in the − 35 promoter region behaved similarly *in vitro*;[620] however, the same inverted repeat had little effect on the transcription *in vivo*. A 48-bp inverted repeat placed in the J-F intercistronic region of $ x 174 replicative form DNA gave identical transcripts *in vitro* with extruded and unextruded cruciform.[621] Unusual local structure, most probably cruciform, prevented both transcriptional initiation and elongation in form V of pBR322 DNA.[622]

It has been shown that transcription induces the generation of positive supercoils in front of the transcription complex and formation of negative supercoils behind it.[145,623-625] Thus, cruciform structures can be absorbed or extruded during transcription. S-type extrusions *in vivo* are rather improbable, while the occurence of C-type extrusion *in vivo*, especially in highly AT-rich inverted repeats, appears probable. Therefore, the difference observed in the effect of inverted repeats contained in the − 10 and − 35 regions on transcription *in vivo* may be due to different extrusion pathways of the two cruciforms.[597] *In vitro* transcription proceeds in a less complex milieu (e.g., no membrane attachment sites are present), which may result in the formation of supercoil domains differing from those created *in vivo* by their size, degree of supercoiling, etc. It is thus not surprising that the effect of an inverted repeat on the transcription *in vitro* and *in vivo* is not always the same.

The recent data suggest that cruciform structures can exist in the cell and might be involved in the regulation of DNA replication and transcription.[597,603,611,616-620] The possibility of involvement in other biological processes cannot be excluded.[626,627]

C. Left-Handed DNA

The presence of Z-DNA in fixed eukaryotic chromosomes was reported in the early 1980s.[628-630] By means of anti-Z-DNA antibodies patterns of different intensity bands in polytene chromosomes of *Drosophila* and *Chironomus* were observed by indirect immunofluorescence.[628-633] Shortly after this occurred it was shown that Z-DNA could not be detected in unfixed chromosomes isolated by micromanipulation,[632,634] and that solvents used in fixation procedures induced different stable DNA tracts, resulting in the reproducible patterns. Therefore, antibody binding results can only be taken as evidence for the presence of potential Z-DNA sequences in the eukaryotic genomes. Attempts to demonstrate the existence of Z-DNA in (G-C)$_n$·(G-C)$_n$ segments of plasmid DNA in E. *coli* by the linking number assay[635] did not solve the problem of the existence of Z-DNA *in vivo* since the results of this assay cannot be interpreted unambiguously (see above).

1. Existence of Left-Handed DNA in Prokaryotic Cells

In 1987 we used Os,bipy to search for left-handed DNA in (C-G)$_n$·(C-G)$_n$ segments of an intracellular plasmid.[39,40] We showed that Os,bipy reacts at the boundaries of these segments, recognizing site-specifically the B-Z junction inside the E. *coli* cell. At the same time Jaworski et al.,[42] using a special molecular genetic technique, showed independently that left-handed DNA exists in the plasmid (C-G)$_n$·(C-G)$_n$ stretches and elicits a biological response in E. *coli* cells. Their *in vivo* assay was based on the *in vitro* observation that a EcoRI recognition site was not methylated when it was near or in the Z-helix. In the *in vivo* assay a plasmid encoding the gene for a temperature-sensitive EcoRI methylase (MEcoRI) was cotransformed with any one of several plasmids containing (C-G), or (T-G), blocks of different lengths with target EcoRI sites in the center or at the end of the blocks. Inhibition of methylation by the MEcoRI was observed for the inserts, with the longest (G-C), segments long enough to form 56 bp left-handed helices. These

results provided evidence that left-handed DNA can exist in the living cell; however, the possibility that the observed inhibition of methylation was due to a specific protein binding cannot completely be excluded. On the other hand, the site-specific Os,bipy modification of the B-Z junction in the cell could not be due to protein binding, although this assay did not allow the study of the biological response of left-handed DNA.[40,139] Considering the results of the genetic[42] and chemical[35] studies performed in 1987, it may be concluded that strong evidence has been obtained showing that left-handed DNA can exist in the cell. This conclusion is supported by further studies of the inhibition of MEcoRI *in vivo* and a thorough linking number assay[602,636] using systems similar to those used by Jaworski et al.[42] as well as by the insert deletion analysis.[598,637] Using the linking number assay and MEcoRI inhibition assay, Zacharias et al.[636] showed that cytosine methylation stabilized left-handed DNA in E. *coli* cells just as occurred in the *in vitro* experiments. Insert deletion analysis showed that sequences capable of adopting Z-DNA were generally stable when cloned into an untranslated site of pBR322 (EcoRI), but suffered deletions when cloned into a site (BamHI) located in the tetracycline resistance structural gene.[598]

Using Os,bipy, Rahmouni and Wells[248] recently showed that (C-G) segments as short as 12 bp adopted left-handed DNA when cloned upstream from the *tet* gene, whereas no left-handed DNA was found when the (C-G)blocks (up to 74 bp long) were cloned downstream. These studies strongly support the notion of domains with varying degrees of supercoiling in E. *coli* cells, perhaps related to transcriptional activity. The important contribution of R. D. Wells' laboratory to studies of Z-DNA in bacterial cells was reviewed recently,[43,447] The progress made in the last few years allows us to conclude that left-handed DNA can exist in bacterial cells [at least in (G-C), segments], and that its existence is dependent on the level of intracellular supercoiling.

2. Left-Handed DNA in Eukaryotic Cells

Studies of Z-DNA in eukaryotic cells by

means of antibodies are more difficult, because of the possibility of the secondary induction of Z-DNA by (1) direct effect of fixatives, (2) removal of proteins during fixation procedures, which results in changes in DNA superhelix density, and (3) perturbance of the B-Z equilibrium by the anti-Z-DNA antibody. To overcome these difficulties, Wittig et al.[638,639] encapsulated permeabilized myeloma nuclei (that were active in transcription and replication) in agarose microbeads and probed the extent of Z-DNA formation in dependence on supercoiling. Upon binding Z-DNA specific antibody, they observed a broad plateau of constant binding that was taken as a measure of preexisting Z-DNA in the nuclei. They calculated that about 0.04% of the base pairs were in the Z conformation.[639] Inhibition of topoisomerase I with camptothecin resulted in a higher Z-DNA content, while cleavage with DNaseI induced the complete loss of preexisting Z-DNA in the nuclei.

Soyer-Gobillard et al.[639a] localized Z-DNA in limited areas inside the chromosomes of the dinoflagellate *Prorocentrum micans* by immunocytochemistry on squashed fixed, unfixed, and frozen cells. This organismus is a primitive eukaryote whose chromosomes show a permanently well-organized DNA structure with no histones and a nucleosomal system that would modulate DNA supercoiling. This makes the dinoflagellate chromosome a highly suitable model for studying Z-DNA and other local structures *in vivo*. Z-DNA was localized often at the periphery or near the segregation fork of dividing chromosomes. In the nucleolus, Z-DNA was observed only in the nucleolus organizer region and never in the fibrillogranular area. Positive results obtained with unfixed and frozen cells provide strong evidence for the existence of Z-DNA in eukaryotic cells. These results cannot be simply correlated with the previous data obtained from eukaryotic chromosomes because of substantial differences in their chromosome organization.

$(C-G)_n$ and (C-A), sequences were cloned into SV40.[640,641] $(C-G)_n$ was highly unstable compared with that of $(C-A)_n$.[640] This instability, however, was not related to the formation of Z-DNA in eukaryotic cells, as no signs of this structure in (C-G), and $(C-A)_{30} \cdot (T-G)_{30}$ inserts *in vivo* were detected by the linking number assay.[641] It

has been pointed out in this article that the results of this assay cannot be unambiguously interpreted; the possibility that changes induced by Z-DNA formation were compensated for by, for example, dissociation of some protein molecule, cannot be excluded.

Studies based on the dinoflagellate model introduced by Soyer-Gobbilard[639a] as well as by the technique developed by Wittig et al.[638,639] represent a substantial advance in the study of Z-DNA in eukaryotic cells. Further techniques are, however, needed to elucidate the question of the presence, distribution, and biological function of left-handed DNA and other local structures in eukaryotic cells. Application of chemical probes and antibodies specific to DNA-probe adducts may represent a new and useful approach to these studies. An even more interesting approach can be seen in a novel confocal Raman microspectrometry;[642] by means of this technique the Raman spectra of a single intact cell, a chromosome, or a polytene chromosome band and interband can be obtained, providing information about DNA structure and DNA/protein ratio. This technique brings new perspectives for future DNA structure research in vivo.

3. Biological Role of Z-DNA

While the possibility of the existence of left-handed DNA in vivo has been reliably established,[39,42] the biological role of the structure remains unclear. Long $(dC-dG)_n$ tracts are not widely found in biological systems, but $(dT-dG)_n \cdot (dA-dC)_n$ sequences were found in both pro- and eukaryotic genomes (reviewed in References 34 and 643 to 647) associated with many genes (reviewed in References 34, 36, and 648 to 653). Proteins that bind to Z-DNA in vitro were isolated (reviewed in References 34, 36, and 648 to 653). Up to the present time, however, no Z-DNA-dependent in vivo functions of these proteins have been identified.

Among biological roles suggested for ZDNA are its participation in transcription, recombination, chromatin structure, etc.[36,134,654,655] It was shown that E. coli RNA polymerase could transcribe through (C-G),, sequence in the B-form.[596] When this sequence was flipped to the left-handed form, the RNA polymerase together with its nascent transcript was blocked at the boundary of the $(C-G)_n$ tract. In vivo, however, the entire sequence was transcribed, suggesting some mechanism that removes or prevents the blocking of transcriptional elongation in the cell. It was recently shown that HMG1 protein removes in. vitro the transcriptional block caused by the (C-G),, sequence in the left-handed form.[656]

Insertion of (C-G), in the lac Z gene of E. coli inhibited expression of β-galactosidase in vivo.[657] The (C-G),, showed a 34-fold decrease of β-galactosidase synthesis when inserted instead of a lac operator and a 24-fold decrease when inserted between codons 5 and 6 of the lac Z gene. With shorter (C-G), sequences the decrease was substantially smaller. It might be interesting to try to compare the observed inhibition with the actual presence of left-handed DNA in the cell.

In contrast to (C-G),,, which caused a transcriptional block in negatively supercoiled plasmid in vitro, the $(C-A)_{21} \cdot (T-G)_{21}$ sequence induced no strong hindrance to transcription.[596] Longer $(C-A)_n \cdot (T-G)_n$ sequences (with n = 60 and 179 bp, located upstream of the rat prolactin gene) formed left-handed DNA and inhibited gene transcription in vitro.[658] Nucleosome assembly at the (G-C),, insert was prevented when the insert adopted Z-DNA in a supercoiled plasmid.[659]

These results suggest that left-handed DNA may be involved in important biological processes. Further work is, however, necessary to elucidate the role of this DNA form in transcription, recombination, and other biological processes.[660]

D. Triplexes

1. Occurrence of Polypu·py Tracts

The occurrence of polypu·py sequences in genomes of various organisms has been studied intensively in the past few years. $(G-A)_n \cdot (T-C)_n$ tracts constitute 0.4, 0.3, and 0.4%, respectively, of the rat, hamster, and mouse genomes, but only 0.7 and 0.5% of the human and monkey genome.[661] These tracts were also found in other organisms.[662] $(T)_n \cdot (A)_n$ and $(G)_n \cdot (C)_n$ sequences

are present in the human genome with 0.3 and 0.0002% frequencies, respectively.[663] In mice and rats the transcription units are flanked by $(A)_n \cdot (T)_n$ and $(G)_n \cdot (C)_n$ sequences.[664] Tracts of (C), and (G), blocks have been found in the vicinity of mouse immunoglobin light chain genes[M5] and within the 5'-flanking region of the adult chicken β^A globin gene.[309] $(T)_n$ sequences are found 5' upstream of the human $\epsilon\Delta$-globin gene intermingled with $(T-G)_n$.[666] $(T-C)$, and $(T-G)$, blocks are adjacent in the third intron of the apolipoprotein CII gene.[667] In *Drosophila* chromosomes $(T-C)$, and $(T-G)$, blocks show a nonrandom distribution, with the highest occurrence on the X chromosome.[668-670] Blocks containing (GAA), sequence units are present on all human chromosomes except the Y chromosome.[671] The $(GGA)_n$ sequence family may be ubiquitous in the genomes of higher eukaryotes. Pentamers T,, $(T)_4C$, TTCTT, TTTCT, and TCTTT are represented with over 125 copies in the ovalbumin gene locus.[672] (CCT), sequences occur at recombination sites of the complex satellite DNA of the Bermuda land crab.[673] homopu·py sequences are also found in numerous virus genomes.[672,674]

Homopu·py regions 1 1 0 bp in length were found in human β-globin region and six other human genes with an average of about one string per 170 to 250 bp.[675] A high bias in favor of homopu·py strings in a human genome may affect nucleosome stability and placement.[663,675-677] Nucleosomes were reconstituted with all homopwhomopyr sequences tested,[675,678] with the exception of poly(dA)·poly(dT), which was not able to form nucleosomes.[679,680]

Further details on the occurrence of homopu·homopyr sequences can be found in recent reviews.[506,670]

2. Existence of H-DNA In Vivo

In contrast to cruciform and left-handed Z-DNA, whose supercoil-stabilized structures were uncovered in the early 1980s, H-DNA structure was proposed only a few years ago, therefore, attempts to identify this structure *in vivo* have only a short history. In 1987 Lee et al.[206] generated monoclonal antibody specific to triplex DNA. They reported binding of this antibody to mouse metaphase chromosomes and interphase nuclei. In fixed mouse and human chromosomes a positive correlation between immunofluorescent staining patterns, G- and/or C-banding patterns, and Hoechst 33258 banding was observed.[681] Unfixed isolated mouse chromosomes were only weakly fluorescent. These results are interesting, but they do not represent unequivocal evidence of triplex structure in the cell, as their interpretation suffers from drawbacks similar to those of Z-DNA immunofluorescent staining.[628,634]

Using Os,bipy we did not observe any sign of triplex formation in pEJ4 intracellular plasmid at neutral pH.[38] If E. *coli* cells were preincubated in pH 4.5 or 5.0, a modification pattern characteristic for H-DNA was obtained. The shift of the intracellular superhelix density to more negative values (by cultivating the cells in media supplemented with 0.35 M NaCl) resulted in a stronger site-specific modification.

A more detailed analysis of the pL153 plasmid (containing the homopu·py sequence from pEJ4 not undergoing active transcription) showed differences between the Os,bipy modification in *vitro* and *in situ*.[604] In *situ*, more bases were modified in the triplex loop and modification of two thymines at the 3'-end of the $(C-T)_{16}$ sequence (forming the B-H junction of the H-3y conformer) was weaker (Figures 5 and 7). On the other hand, modification of three cytosines at the 5'-end of the $(C-T)$,, sequence was observed; these cytosines were unmodified *in vitro*. The modification of these cytosines can be expected in H-y5 conformer, which is formed at lower superhelix density than H-y3 observed mainly in isolated plasmids. It was therefore tentatively suggested that H-y5 conformer prevails in the cell. Differences in triplex loop modification, which might be due to some intracellular interactions, suggest that the triplex structure in the cell may differ in some details from that observed in *vitro*.

Basically the same modification patterns (Figure 7) were found in the pH range 4.5 to 5.2; at pH 5.4 no characteristic triplex modification was observed. When compared with extracellular pH, the intracellular pHs were higher by about 0.5 as determined by the fluorescein method. These results were obtained at pH values that are

A

(in vitro)

3' ATCTCCTTCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCCCCCCC 5'

130   140   150   160   170   180

5' – C-C-C-C-C ———————————————— T-C-T 157
3' – G-G-G-G-G ———————————————— A-G-A C   H-y3
                                       T-C-T 155

T138
T137

A-A

B

3' C-T-C-T-C-T-C ———————————————— T-C-T-C-T 155
5' G-A-G-A-G-A-A ———————————————— A-G-A-G-A C   H-y5
                                             T-C-T-C-T 157

C 174
C 176

(in situ)

3' ATCTCCTTCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCCCCCCC 5'

130   140   150   160   170   180

FIGURE 7.   Modification of the polypyrimidine strand of the insert in pL153 plasmid with Os,bipy (A) *in vitro* and (B) *in situ*.[604] The length of the vertical lines in the nucleotide sequence represents the relative intensities of the bands on the sequencing gel obtained by densitometric tracing after (A) treatment of supercoiled pL153 DNA *in vitro* at pH 5.0 and (B) after Os,bipy treatment of E. *coli* cells harboring the pL153 plasmid (at external pH 5.0). Two conformers of the H-DNA triplex are shown in which is indicated the direction of the donated strand by full triangles. The arrows show the strongest modified base in the triplex loop and different modification of bases at the potential B-H junctions consistent with presence H-y5 conformer in the cell and H-y3 conformer *in vitro*.

not fully physiological for E. *coli,* but *E. çoli* can grow at pH 5.0 and 5.2, and while a transfer of cells from pH **6.9** to **4.3** results in an induction of acid shock proteins, no such induction occurs due to the transfer to pH 5.[682] Intracellular pH depends on the life cycle of the cells (low pH is common to resting cells), and in eukaryotic cells pH values in different cell compartments can significantly vary.[683] In the cell, requirements for protonation might be decreased by specific protein binding and/or more negative superhelix density in the given DNA domain. It may thus be expected that triplex structures will be detected at higher pH values, especially with longer po-

lypu·py tracts, which have demonstrated the ability to form triplexes at neutral pH.

The possibility of the existence of H-DNA in E. coli cells under physiological conditions is supported by the results of the recent insert deletion analysis of mixed polypu·py sequences capable of forming H-DNA in vitro.[598] Similar to Z-forming sequences, the former sequences were stable when cloned into a nontranslated region, while inserts located in the tetracycline resistance structural gene were deleted. Parniewski et al.[523] showed that polypu·py sequences capable of forming H-DNA in vitro are undermethylated in vivo within the potential triplex loop when grown in the JM dam' strain. dam undermethylation was suppressed by the administration of chloramphenicol to the cells. The results were explained by H-DNA formation in vivo and protection of the triplex loop from methylation by interaction with a specific protein. This explanation is very attractive, but formation of H-DNA with the given plasmids at neutral pH requires a σ of −0.06; so far, only less negative values in vivo have been reported.[248,611] Other interpretations are possible: presence of a partially extruded H-DNA trapped specifically in vivo or formation of a different structure which is reabsorbed due to chloramphenicol treatment.

Application of chemical probes in situ might help to clarify this problem. It has been shown that H-DNA can exist in a bacterial cell at acid intracellular pH values.[38,604] Further work is necessary to establish whether this structure does exist under physiological conditions and in eukaryotic cells, where the location and high occurrence of polypu·py sequences suggest their biological importance.

3. Structural-Functional Relations

Polypyrimidine sequences in vitro were observed in a number of naturally occurring sequences,[506,684,685] some of which are involved in known biological functions. Carcinogen-induced amplification of the integrated polyomavirus DNA was arrested within a specific cell DNA segment containing $(G-A)_{27}·(C-T)_{27}$ tract.[684] Single-stranded (T-C)n and (G-A)n tracts of various length were cloned into M13 phage and replicated

by extension of the M13 primer to determine whether these tracts act as stop signals for DNA replication in vitro.[686] Specific arrest of replication was detected around the middle of (T-C)n and (G-A)n with n >16. In (T-C)n tracts the arrests were more prominent at pH 6.5 to 7.5 than at pH 8.0. It was concluded that the arrests are due to triplex formation between partially replicated (T-C)n or (G-A)n tracts and the unreplicated portion of these sequences. Cooney et al.[540] showed that a 27-base-long purine-rich oligonucleotide binds to duplex DNA at a single site within the 5'-end of the human c-myc gene 115 bp upstream from the transcription origin P1. Correlation between the triplex formation at −115 bp and repression of c-myc transcription in vitro was shown. If such a triplex formation can also occur in vivo, it may represent an alternative method of gene control in the cell.

Using Os,py, DEPC, and DMS probes Kinniburgh[685] identified H-DNA in vitro in a mixed polypu·py sequence from a positive cis-acting transcription element of the human c-myc gene. This sequence binds several transcription factors. Kinniburgh[685] speculated that hybridization of the RNA component of one of these factors may represent the first step in H-DNA formation in vivo and that H-DNA would increase the transcriptional activity of the c-myc gene. An unusual structure, probably H-DNA, was formed by the $(G)_6·ATT(G)_6$ sequence in a supercoiled plasmid containing the genome of the human immunodeficiency virus type 1.[687] This sequence is located at the integration site of a human immunodeficiency virus (HIV) provirus.

Kohwi-Shigematsu[535] showed quite recently that $(G)_n·(C)_n$ sequences enhance CAT gene expression in eukaryotic cells. The level of enhancement was highest for n = 28 to 30, comparable to the polyoma enhancer. Any shorter or longer tracts were less active. In vivo competition assay suggested the existence of a transacting factor that interacts with the $(G)_n·(C)_n$ sequence.

In Drosophila nuclei a protein binding to multiple GAGA DNA sequence motifs was found. This protein activates the transcription of Ubx promoter in a binding-site-dependent manner.[688] Further proteins from the same source bind to regions of $(C-T)_n$ in the promoters of heat shock and histone genes.[689] Interaction of the purified

protein fraction with the intergenic region located between promoters occurred on linear DNA fragments, indicating that supercoiling was not required. Interaction of these proteins with supercoiled DNA *in vitro* was not studied.

In principle, specific protein interaction with the polypu·py region might stabilize the duplex, preventing triplex formation and *vice versa*. On the other hand, formation of a triplex can prevent specific protein-DNA interactions normally occurring in the duplex. Oligonucleotide-directed triplex formation can inhibit recognition of the DNA double helix by prokaryotic restriction/ modification enzymes and by eukaryotic transcription factor at homopurine target sites.[690,691]

E. Conclusions

Among the local DNA structures discussed in this section, triplexes appear to be the best candidates to play a role in gene expression. Intermolecular triplexes seem to have a better chance, to form *in vivo*, as their requirements for specific environmental conditions and negative supercoiling are less stringent. Moreover, they can be formed not only between DNA molecules, but also between DNA and RNA, including various nucleoproteins. Involvement of intermolecular triplexes in biological processes *in vitro* such as DNA replication,[684,686] transcription,[540] and restriction/modification[690,691] has been demonstrated.

The formation of intramolecular triplexes, Z-DNA, and cruciforms *in vivo* is strongly dependent on the intracellular DNA superhelix density. Until recently the probability of the formation of these structures *in vivo* was considered to be rather low, as the (average) effective level of (unconstrained) supercoiling is about one half that of purified DNA.[173,596,599,602,692]

The recent discovery of transcriptional waves of supercoiling makes the extrusion of local DNA structures much more probable.[145,623,625,693] In fact, if a suitable sequence is present in a sufficiently negatively supercoiled DNA domain, a corresponding local structure should be formed *in vivo* unless its extrusion is prevented by some other factor (e.g., protein binding, kinetic barrier). Detection of a local structure *in vivo* can be exploited

to study the level of effective supercoiling in a given DNA domain.[248,611]

Therefore, in addition to a question frequently asked in recent years, "Do these (local, unusual) structures exist *in vivo?",* we may now also ask, "Why is this structure (e.g., Z-DNA) not formed in the given nucleotide sequence *in vivo?"* Asking questions is usually easier than answering them. To answer the above questions, further development of techniques suitable for DNA structure studies in the cell is necessary. We recently applied osmium tetroxide complexes to study the presence of open local DNA structures in eukaryotic cells by means of immunofluorescence.[610] As the chemical probe can be applied to cells and even glands prior to fixation, the adverse effects of fixation are eliminated. Using this technique we observed selective binding of the osmium probe to DNA in the cells, suggesting a wide occurrence of open local structures in eukaryotic cells. These structures are probably not limited to cruciforms, B-Z junctions, and triplexes. They may include further known and unknown structures and their junctions as well as open transcription complexes and other structures connected with DNA replication, recombination; and other biological processes.[302,555,694-700] The permanent or transient availability of bases contained in these structures for interaction with the environment (which is important for their detection) may also play a significant biological role.

VIII. PERSPECTIVES

The question of recognition of DNA nucleotide sequences by specific proteins is one of the most important problems of contemporary molecular biology. There is no doubt that the specific proteins do not read the nucleotide sequence as such; rather, they recognize the three-dimensional structure of DNA. Nussimov[701] believes that DNA regions may be distinguished by the thermodynamics or flexibility of the DNA double helix and not by different local structures, because they might not be trapped as such. Evidence has been presented suggesting that different protein motifs might interact with the DNA grooves and/or backbone, recognizing their spe-

cific sequence-dependent spatial arrangement~.'~ In fact, the amount of data about the relations between nucleotide sequences and their locations in pro- and eukaryotic genomes is much larger than the amount of data concerning the presence and location of DNA local structures. The reason for this difference has been due principally to the difficulties with obtaining the latter data. The results summarized in this review indicate that local DNA structures can be trapped both in vitro and in vivo, and that sequences from which these structures may be extruded are frequently located in biologically significant sites of the genomes. It would thus be rather surprising if the local DNA structures did not represent any signal in the DNA recognition. I believe that the recent progress in the development of techniques of probing the DNA structure in the cells will soon result in a great advance in our knowledge of the relation between local DNA structures and their biological function. The present situation seems to resemble that close to the end of the 1970s, when the suggested polymorphy of the DNA double helix was rather reluctantly accepted.[10] This was at a time when the crystals of the Z-DNA were probably already growing.

List of Abbreviations

Chemical Probes

Os,py: osmium tetroxide,pyridine reagent
Os,bipy: osmium tetroxide complexed with 2,2'-bipyridine
phe: 1,10-phenanthroline
bpds: bathophenanthroline disulfonic acid
TEMED: tetramethylethylenediamine
DEPC: diethylpyrocarbonate
BAA(CAA): bromo(chloro)acetaldehyde
DMS: dimethylsulfate
ENU: ethylnitrosourea
MNU: methylnitrosourea
CMC: N-cyclohexyl-N'-β(4-methylmorpholinium) ethylcarbodiimide-p-toluene

Other Abbreviations

homopu·py: homopurine·homopyridine
polypu·py: polypurine·polypyrimidine
2-D: two dimensional

DEDICATION

This article is dedicated to Professor Julius Marmur on the occasion of his 65th birthday.

ACKNOWLEDGMENTS

REFERENCES

1. Wilkins, M. H. F., Physical studies of the molecular structure of deoxyribose nucleic acid and nucleoprotein, *Cold Spring Harbor Symp. Quant. Biol.*, 21, 75, 1956.
2. Crick, F. H. C., The double helix: a personal view, *Nature*, 248, 766, 1974.
5. Zimmerman, S. B., The three-dimensional structure of DNA, *Annu. Rev. Biochem.*, 51, 395, 1982.
4. Jovin, T. M., Recognition mechanisms of DNA-specific enzymes, *Annu. Rev. Biochem.*, 45, 889, 1976.
5. Palecek, E., Changes in oscillopolarographic behaviour of deoxyribonucleic acids at temperatures below denaturation temperature. *J. Mol. Biol.*, 11, 839. 1965.
6. Palecek, E., Polarographic behaviour of native and denatured deoxyribonucleic acid, *J. Mol. Biol..* 20, 263. 1966.
7. Palecek, E., Deoxyribonucleic acid conformational changes at temperatures below melting temperature, *Arch. Biochem. Biophys.*, 125, 142, 1968.
8. Johnson, P. H. and Laskowski, M., Sugar-unspe-

cific mung bean nuclease I, *J. Biol. Chem.*, 243, 3421, 1968.

9. **Palecek, E. and Fric, I.,** Correlation between polarographic reducibility and circular dichroism of DNA at submelting temperatures, *Biochem. Biophys. Res. Commun.*, 47, 1262, 1972.

10. **Palecek, E.,** Premelting changes in DNA conformation, *Progr. Nucl. Acid Res. Mol. Biol.*, 18, 151, 1976.

11. **Bram, S. and Tougard, P.,** Polymorphism of natural DNA, *Nature*, 239, 128, 1972.

12. **Bram, S.,** The polymorphism of DNA, *Progr. Mol. Subcell. Biol.*, 4, 1, 1976.

13. **Shakked, Z. and Rabinovich, D.,** The effect of the base sequence on the fine structure of the DNA double helix, *Progr. Biophys. Mol. Biol.*, 47, 159, 1986.

14. **Pohl, F. M.,** Isomerization of a double-stranded DNA, in *1st Eur. Biophys. Cong.*, Broda, E. and Locker, H., Eds., Springer-Lederer, Vienna, 1971, 343.

15. **Pohl, F. M. and Jovin, T. M.,** Salt-induced cooperative conformational change of a synthetic DNA: equilibrium and kinetic studies with poly(dG-dC), *J. Mol. Biol.*, 67, 375, 1972.

16. **Viswamitra, M. A., Kennard, O., Shakked, Z., Jones, P. G., Sheldrick, G. M., Salisbury, S., and Falvello, L.,** Crystal structure of d(A-T) and sequence specific DNA-protein recognition, *Curr. Sci.*, 47, 298, 1978.

17. **Viswamitra, M. A., Kennard, O., Jones, P. G., Sheldrick, G. M., Salisbury, S. A., Favello, L., and Shakked, Z.,** DNA double helical fragment at atomic resolution, *Nature*, 273, 687, 1982.

18. **Viswamitra, M. A., Shakked, Z., Jones, P. G., Sheldrick, G. M., Salisbury, S. A., and Kennard, O.,** Structure of the deoxytetranucleotide d-pATAT and a sequence-dependent model for poly(dA-dT), *Biopolymers*, 21, 513, 1982.

19. **Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., and Rich, A.,** Molecular structure of a left-handed double helical DNA fragment at atomic resolution, *Nature*, 282, 680, 1979.

20. **Drew, H. R., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R. E.,** High salt d(CGCG): a left-handed Z-DNA double helix, *Nature*, 286, 567, 1980.

21. **Crawford, J. L., Kolpak, F. J., Wang, A. H.-J., Quigley, G. J., van Boom, J. H., van der Marel, G., and Rich, A.,** The tetramer d(CpGpCpG) crystallizes as a left-handed double helix, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 4016, 1980.

22. **Wing, R., Drew, H. R., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E.,** Crystal structure analysis of a complete turn of B-DNA, *Nature*, 287, 755, 1980.

23. **Dickerson, R. E. and Drew, H. R.,** Structure of a B-DNA dodecamer: influence of base sequence on helix structure, *J. Mol. Biol.*, 149, 761, 1981.

24. **Lomonossoff, G. P., Butler, P. J. G., and Klug, A.,** Sequence-dependent variation in the conformation of DNA, *J. Mol. Biol.*, 149, 745, 1981.

25. **Kennard, O. and Hunter, W. N.,** Oligonucleotide structure: a decade of results from single crystal X-ray diffraction studies, *Q. Rev. Biophys.*, 22, 327, 1989.

26. **Trifonov, E. N. and Sussman, J. L.,** The pitch of chromatin DNA is reflected in its nucleotide sequence, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 3816, 1980.

27. **Marini, J. C., Levene, S. D., and Crothers, D. M.,** Bent helical structure in kinetoplast DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 7664, 1982.

28. **Gellert, M., Mizuuchi, K., O'Dea, M. H., Ohmori, H., and Tomizawa, J.,** DNA gyrase and DNA supercoiling, *Cold Spring Harb. Symp. Quant. Biol.*, 43, 35, 1978.

29. **Lilley, D. M. J.,** The inverted repeat as a recognizable structural feature in supercoiled DNA molecules, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 6468, 1980.

30. **Panayotatos, N. and Wells, R. D.,** Cruciform structures in supercoiled DNA, *Nature*, 289, 466, 1981.

31. **Lilley, D. M. J.,** Structural isomerization in DNA: the formation of cruciform structures in supercoiled DNA molecules, *Chem. Soc. Rev.*, 18, 53, 1989.

32. **Klysik, J., Stirdivant, S. M., Larson, J. E., Hart, P. A., and Wells, R. D.,** Left-handed DNA in restriction fragments and a recombinant plasmid, *Nature*, 290, 672, 1981.

33. **Peck, L. J., Nordheim, A., Rich, A., and Wang, J. C.,** Flipping of cloned d(pCpG)n.d(pCpG)n DNA sequences from right- to left-handed helical structure by salt, Co(III), or negative supercoiling, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 4560, 1982.

34. **Rich, A., Nordheim, A., and Wang, A. H. J.,** The chemistry and biology of left-handed Z-DNA, *Annu. Rev. Biochem.*, 53, 791, 1984.

35. **Wells, R. D.,** Unusual DNA Structures, *J. Biol. Chem.*, 263, 1095, 1988.

36. **Nordheim, A., Herrera, R. E., Meese, K., Runkel, L., Scholten, P. M., Schroter, H., and Shaw, P. E.,** Structural and topological polymorphism of DNA, in *Architecture of Eucaryotic Genes*, Kahl, G., Ed., 1988, 3.

37. **Palecek, E.,** Local open DNA structures *in vitro* and in the cell as detected by chemical probes, in *Highlights of Modern Biochemistry*, Kotyk, A., Skoda, J., Paces, V., and Kostka, V., Eds., VSP International Science, Zeist, 1989, 53.

38. **Palecek, E., Boublikova, P., Jelen, F., Krejcova, A., Makaturova, E., Nejedly, K., Pecinka, P., and Vojtiskova, M.,** Chemical probing of the DNA polymorphic structure *in vitro* and *in situ*, in *Structure and Methods, Vol. 3, DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 237.

39. **Palecek, E., Boublikova, P., and Karlovsky, P.,** Osmium tetroxide recognizes structural distorsions at junctions between right- and left-handed DNA in a

bacterial cell, *Gen. Physiol Biophys.*, 6, 593, 1987.

40. **Palecek, E., Rasovska, E., and Boublikova, P.,** Probing of DNA polymorphic structure in the cell with osmium tetroxide, *Biochem. Biophys. Res. Commun.*, 150, 731, 1988.

41. **Sasse-Dwight, S. and Gralla, J. D.,** $KMnO_4$ as a probe for *lac* promoter DNA melting and mechanism *in vivo*, *J. Biol. Chem.*, 264, 8074, 1989.

42. **Jaworski, A., Hsien, W.-T., Blaho, J. A., Larson, J. E., and Wells, R. D.,** Left-handed DNA *in vivo*, *Science*, 238, 773, 1987.

43. **Wells, R. D., Amirhaeri, S., Blaho, J. A., Collier, D. A., Hanvey, J. C., Jaworski, A., Larson, J. E., Rahmouni, A., Rajagopalan, M., Shimizu, M., Wohlrab, F., and Zacharias, W.,** Left-handed Z-DNA *in vivo*, in *Structure and Methods, Vol. 2, DNA Protein Complexes and Proteins*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 25.

44. **Itakura, A., Rossi, J. J., and Wallace, R. B.,** Synthesis and use of synthetic oligonucleotides, *Annu. Rev. Biochem.*, 53, 323, 1984.

45. **Saenger, W.,** *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, 1983.

46. **Dickerson, R. E.,** What do we really know about B-DNA?, in *Structure and Methods, Vol. 3, DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 1.

47. **Drew, H. R. and McCall, M. J.,** New approaches to DNA in the crystal and in solution, in *DNA Topology and Its Biological Effects*, Cozzarelli, N. R. and Wang, J. C., Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1990, 1.

48. **Dickerson, R. E. et al.,** Definitions and nomenclature of nucleic acid structure parameters, *EMBO J.*, 8, 1 1989.

49. **Feigon, J., Wang, A. H.-J., van der Marel, G. A., van Boom, J. H., and Rich, A.,** Z-DNA forms without an alternating purine-pyrimidine sequence in solution, *Science*, 230, 82, 1985.

50. **Wang, A. H.-J., Gessner, R. V., van der Marel, G. A., van Boom, J. H., and Rich, A.,** Crystal structure of a Z-DNA without an alternating purine-pyrimidine sequence, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 3611, 1985.

51. **Calladine, C. R.,** Mechanics of sequence-dependent stacking of bases in B-DNA, *J. Mol. Biol.*, 161, 343, 1982.

52. **Saenger, W.,** Structure and dynamics of water surrounding biomolecules, *Annu. Rev. Biophys. Chem.*, 16, 93, 1987.

53. **Westhof, E.,** Water: an integral part of nucleic acid structure, *Annu. Rev. Biophys. Chem.*, 17, 125, 1988.

54. **Heinemann, U., Lauble, H., Frank, R., and Bloker, H.,** Crystal structure analysis of an A-DNA fragment at 1.8 A resolution: d(GCCCGGGC), *Nucleic Acids Res.*, 15, 9531, 1987.

55. **Hunter, W. N., D'Estaintot, B. L., and Kennard, O.,** Structural variation in d(CTCTAGAG):

56. **Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E.,** Structure of a B-DNA dodecamer; conformation and dynamics, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 2179, 1981.

57. **Kopka, M. L., Fratini, A. V., Drew, H. R., and Dickerson, R. E.,** Ordered water structure around a B-DNA dodecamer. A quantitative study, *J. Mol. Biol.*, 163, 129, 1983.

58. **Heinemann, U., Alings, C., and Lauble, H.,** Structural features of G/C-rich DNA going A or B, in *Structure and Methods, Vol. 3, DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 039.

59. **Doucet, J., Benoit, J.-P., Cruse, W. B. T., Prange, T., and Kennard, O.,** Coexistence of A- and B-form DNA in a single crystal lattice, *Nature*, 337, 190, 1989.

60. **Wang, Y., Thomas, G. A., and Peticolas, W. L.,** A duplex of the oligonucleotides d(GGGGGTTTTT) and d(AAAAACCCCC) forms an A to B conformational junction in concentrated salt solutions, *J. Biomol. Struct. Dyn.*, 6, 1177, 1989.

61. **Shakked, Z., Guerstein-Guzikevich, G., Zaytzev, A., Eisenstein, M., Frolow, F., and Rabinovich, D.,** Polymorphism and isomorphism in DNA crystals: the effect of crystal packing, hydration and temperature on the DNA double helix, in *Structure and Methods, Vol. 3, DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 055.

62. **Heinemann, U.,** A note on crystal packing and global helix structure in short A-DNA duplexes, *J. Biomol. Struct. Dyn.*, 8, 801, 1991.

63. **Heinemann, U. and Alings, C.,** The conformation of a B-DNA decamer is mainly determined by its sequence and not by crystal environment, *EMBO J.*, 10, 35, 1991.

64. **Benevides, J. M., Wang, A. H.-J., Rich, A., Kyogoku, Y., van der Marcel, G. A., van Boom, J. H., and Thomas, G. J.,** Raman spectra of single crystals of r(GCG)d(CGC) and d(CCCCGGGG) as models for A DNA, their structure transitions in aqueous solutions, and comparison with double helical poly(dG)·poly(dC), *Biochemistry*, 24, 41, 1986.

65. **Wolk, S., Thurmes, W. N., Ross, W. S., Hardin, C. C., and Tinoco, I.,** Conformational analysis of d(C_3G_3), a B- family duplex in solution, *Biochemistry*, 28, 2452, 1989.

66. **Timsit, Y., Westhof, E., Fuchs, R. P. P., and Moras, D.,** Unusual helical packing in crystals of DNA bearing a mutation hot spot, *Nature*, 341, 459, 1989.

67. **Eisenstein, M., Frolow, F., Shakked, Z., and Rabinovich, D.,** The structure and hydration of the A-DNA fragment d(GGGTACCC) at room temper-

implications for protein-DNA interactions, *Biochemistry*, 28, 2444, 1989.

ature and low temperature, *Nucleic Acids Res.*, 18, 3185, 1990.

68. **Trifonov, E. N.,** Curved DNA, *Crit. Rev. Biochem.*, 19, 89, 1985.

69. **Diekmann, S.,** DNA curvature, in *Nucleic Acid and Molecular Biology*, Vol. 1, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1987, 138.

70. **Sundaralingam, M. and Sekharudu, Y. C.,** Sequence directed DNA bending and curvature — an overview, in *Structure and Expression, Vol. 3, DNA Bending and Curvature*, Olson, W. K., Sarma, M. H., Sarma, R. H., and Sundaralingam, M., Eds., Adenine Press, Schenectady, NY, 1988, 9.

71. **Crothers, D. M., Haran, T. E., and Nadeu, J. G.,** Intrinsically bent DNA, *J. Biol. Chem.*, 265, 7093, 1990.

72. **Hagerman, P. J.,** Sequence-directed curvature of DNA, *Annu. Rev. Biochem.*, 59, 755, 1990.

73. **Travers, A. A. and Klug, A.,** Bending of DNA in nucleoprotein complexes, in *DNA Topology and its Biological Effects*, Cozzarelli, N. R. and Wang, J. C., Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1990, 57.

74. **Travers, A. A.,** DNA conformation and protein binding, *Annu. Rev. Biochem.*, 58, 427, 1989.

75. **Stellwagen, N. C.,** Electrophoresis of DNA in agarose and polyacrylamide gels, in *Advances in Electrophoresis*, Vol. 1, Chrambach, A., Dunn, M. J., and Radola, B. J., Eds., VCH Publishers, Weinheim, 1987, 172.

76. **Abagyan, R. A., Mironov, V. N., Chernov, B. K., Chuprina, V. P., and Ulyanov, A. V.,** Electrophoretic behavior of d(GGAAAAAAGG)n, d(CCAAAAAACC)n, and (CCAAAAAAGG)n and implications for a DNA bending model, *Nucleic Acids Res.*, 18, 989, 1989.

77. **Griffith, J., Bleyman, M., Rauch, C. A., Kitchin, P. A., and Englung, P. T.,** Visualization of the bent helix in kinetoplast DNA by electron microscopy, *Cell*, 46, 717, 1986.

78. **Griffith, J. D., Laundon, C. H., Rauch, C. A., Englund, P. T., Hsieh, W.-T., and Wells, R. D.,** Use of electron microscopy to examine sequence-directed DNA bending, in *Structure and Expression, Vol. 3, DNA Bending and Curvature*, Olson, W. K., Sarma, M. H., Sarma, R. H., and Sundaralingam, M., Eds., Adenine Press, Schenectady, NY, 1988, 025.

79. **Diekmann, S., Von Kitzing, E., McLauglin, L., Ott, J., and Eckstein, F.,** The influence of exocyclic substituents of purine bases on DNA curvature, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 8257, 1987.

80. **Koo, H. S. and Crothers, D. M.,** Chemical determinants of DNA bending at adenine-thymidine tracts, *Biochemistry*, 26, 3745, 1987.

81. **Diekmann, S.,** DNA curvature in native and modified EcoRI recognition sites and possible influence upon the endonuclease cleavage reaction, *J. Mol. Biol.*, 202, 823, 1988.

82. *Diekmann, S.,* DNA methylation can enhance or induce DNA curvature, *EMBO J.*, 6, 4213, 1987.

83. **Hagerman, P. J.,** Pyrimidine 5-methyl groups influence the magnitude of DNA curvature, *Biochemistry*, 29, 1980, 1990.

84. **Unemoto, K., Sarma, M. H., Gupta, G., and Sarma, R. H.,** Effect of the methyl group on DNA bending and curvature: structure of $d(GA_4U_4C)_2$ in solution, *Biochemistry*, 29, 4714, 1990.

85. **Hagerman, P. J.,** Sequence dependence of the curvature of DNA: a test of the phasing hypothesis, *Biochemistry*, 24, 7033, 1985.

86. **Koo, H. W., Wu, H. M., and Crothers, D. M.,** DNA bending at adenine·thymidine tracts, *Nature*, 320, 501, 1986.

87. **Diekmann, S. and Wang, J. C.,** On the sequence determinants and flexibility of the kinetoplast DNA fragment with abnormal gel electrophoretic mobilities, *J. Mol. Biol.*, 186, 1, 1985.

88. **Zhurkin, V. B., Ulyanov, N. B., and Ivanov, V. I.,** Mechanism of DNA bending in the free state and in the nucleosome, in *Structure and Expression, Vol. 3, DNA Bending and Curvature*, Olson, W. K., Sarma, M. H., Sarma, R. H., and Sundaralingam, M., Eds., Adenine Press, Schenactady, NY, 1988, 169.

89. **Diekmann, S. and von Kitzing, E.,** On the structural origin of DNA curvature, in *Structure and Expression, Vol. 3, DNA Bending and Curvature*, Olson, W. K., Sarma, M. H., Sarma, R. H., and Sundaralingam, M., Eds., Adenine Press, Schenactady, NY, 1988, 57.

90. **McNamara, P., Bolshoy, A., Trifonov, E. N., and Harrington, R. E.,** Curved DNA without AA: sequence-dependent kinks induced in curved DNA experimental estimation of all 16 DNA wedge angles, *a. Biomol. Struct. Dyn.*, 3, 529, 1990.

91. **Levene, S. D. and Crothers, D. M.,** A computer graphics study of sequence-directed bending in DNA, *J. Biomol. Struct. Dyn.*, 1, 429, 1983.

92. **Arnott, S., Chandrasekaran, R., Hall, I. H., and Puigjaner, L. C.,** Heteronomous DNA, *Nucleic Acids Res.*, 11, 4141, 1983.

93. **Alexeev, D. G., Lipanov, A. A., and Skuratovskii, I. Y.,** Poly(dA)·poly(dT) is a B-type double helix with a distinctively narrow minor groove, *Nature*, 325, 831, 1987.

94. **Chuprina, V. P.,** Anomalous structure and properties of poly(dA)·poly(dT). Computer simulation of the polynucleotide structure with the spine of hydration in the minor groove, *Nucleic Acids Res.*, 15, 293, 1987.

95. **Kopka, M., Fratini, A. V., Drew, H. R., and Dickerson, R. E.,** Ordered water structure around a B-DNA dodecamer: a quantitative study, *J. Mol. Biol.*, 163, 129, 1983.

96. **Dickerson, R. E., Kopka, M. L., and Pjura, P.,** A stochastic model for helix bending in B-DNA, *J. Biomol. Struct. Dyn.*, 1, 755, 1983.

97. **Nelson, H. C. M., Finch, J. T., Bonaventura,**

F. L., and Klug, A., The structure of an oligo(dA)·oligo(dT) tract and its biological implications, *Nature,* 330, 221, 1987.

98. **Coll, M., Frederick, C. A., Wang, A. H.-J., and Rich, A.,** A bifurcated hydrogen-bonded conformation in the d(A·T) base pairs of the DNA dodecamer (d(CGCAAATTTGCG) and its complex with distamycin, *Proc. Natl. Acad. Sci. U.S.A.,* 84, 8385, 1987.

99. **Di Gabriele, A. D., Sanderson, M. R., and Steitz, T. A.,** Crystal lattice packing is important in determining the bend of a DNA dodecamer containing an adenine tract, *Proc. Natl. Acad. Sci. U.S.A.,* 86, 1816, 1989.

100. **Leroy, J.-L., Charratier, E., Kochoyan, M., and Gueron, M.,** Evidence from base-pair kinetics for two types of adenine tract structures in solution: their relation to DNA curvature, *Biochemistry,* 27, 8894, 1988.

101. **Herrera, J. E. and Chaires, J. B.,** A premelting conformational transition in poly(dA)·poly(dT) coupled to daunomycin binding, *Biochemistry,* 1983, 28, 1989.

102. **Zarling, D. A., Diekmann, S., and Calhoun, J. S.,** Protein recognition of B'-(dA)n·(dT)n sequences and kinetoplast, in *Structure and Expression, Vol. 3, DNA Bending and Curvature,* Olson, W. K., Sarma, M. H., Sarma, R. H., and Sundaralingham, M., Eds., Adenine Press, Schenectady, NY, 1988, 087.

103. **Shlomai, J. and Linial, M.,** A nicking enzyme from trypanosomatids which specifically affects the topological linking of duplex DNA circles, *J. Biol. Chem.,* 261, 16219, 1986.

104. **Linial, M. and Shlomai, J.,** The sequence-directed bent structure in kinetoplast DNA is recognized by an enzyme from *Crithidia fasciculata, J. Biol. Chem.,* 262, 31, 15194, 1987.

105. **Linial, M. and Shlomai, J.,** Sequence-directed bent DNA helix is the specific binding site for *Crithidia fasciculata* nicking enzyme, *Proc. Natl. Acad. Sci. U.S.A.,* 84, 8205, 1987.

106. **Linial, M. and Shlomai, J.,** A unique endonuclease from *Crithidia fasciculata* which recognizes a bend in the DNA helix, *J. Biol. Chem.,* 263, 290, 1988.

107. **Linial, M. and Shlomai, J.,** Bent DNA structures associated with several origins of replication are recognized by a unique enzyme from trypanosomatids, *Nucleic Acids Res.,* 16, 6477, 1988.

108. **Collis, C. M., Molloy, P. L., Both, G. W., and Drew, H. R.,** Influence of the sequence-dependent flexure of DNA on transcription in *E. coli, Nucleic Acids Res.,* 17, 9447, 1989.

109. **Bracco, L., Kotlarz, D., Kolb, A., Diekmann, S., and Buc, H.,** Synthetic curved DNA sequences can act as transcriptional activators in *Escherichia coli, EMBO J.,* 8, 4289, 1989.

110. **Goodman, S. D. and Nash, H. A.,** Functional replacement of a protein-induced bend in a DNA recombination site, *Nature,* 341, 251, 1989.

111. **Snyder, U. K., Thompson, J. F., and Landy, A.,** Phasing of protein-induced DNA bends in a recombination complex, *Nature,* 341, 1989, 255.

112. **Muhich, M. L. and Simpson, L.,** Specific cleavage of kinetoplast minicircle DNA from *Leishmania tarentolae* by mung bean nuclease and identification of several additional minicircle sequence classes, *Nucleic Acids Res.,* 13, 5531, 1986.

113. **Palecek, E., Makaturova-Rasovska, E., and Diekmann, S.,** Osmium tetroxide probing of local DNA structure in linear and supercoiled plasmids containing curvature-inducing sequences, *Gen. Physiol. Biophys.,* 7, 379, 1988.

114. **Vinograd, J., Lebowitz, J., Radloff, R., Watson, R., and Laipis, P.,** The twisted circular form of polyoma viral DNA, *Proc. Natl. Acad. Sci. U.S.A.,* 53, 1104, 1965.

115. **Weil, R.,** The denaturation and the renaturation of the DNA of polyoma virus, *Proc. Natl. Acad. Sci. U.S.A.,* 49, 480, 1963.

116. **Lebowitz, J.,** Through the looking glass: the discovery of supercoiled DNA, *Trends Biochem. Sci.,* 15, 202, 1990.

117. **Vogt, M. and Dulbecco, R.,** Virus-cell interaction with a tumor-producing virus, *Proc. Natl. Acad. Sci. U.S.A.,* 46, 365, 1963.

118. **Weil, R. and Vinograd, J.,** The cyclic helix and cyclic coil forms of polyoma viral DNA, *Proc. Natl. Acad. Sci. U.S.A.,* 50, 730, 1963.

119. **Stonington, O. G. and Pettijohn, D. E.,** The folded genome of *Escherichia coli* isolated in a protein-DNA-RNA complex, *Proc. Natl. Acad. Sci. U.S.A.,* 68, 6, 1971.

120. **Worcel, A. and Burgi, E.,** On the structure of the folded chromosome of *Escherichia coli, J. Mol. Biol.,* 71, 127, 1972.

121. **Bauer, W. R.,** Structure and reactions of closed duplex DNA, *Annu. Rev. Biophys. Bioeng.,* 7, 287, 1978.

122. **Lilley, D. M. J.,** Hairpin-loops in supercoiled DNA, *Topics Nucleic Acid Struct.,* 2, 173, 1982.

123. **Lilley, D. M. J.,** DNA: sequence, structure and supercoiling, *Biochem. Soc. Trans.,* 12, 127, 1984.

124. **Wang, J. C.,** Superhelical DNA, *Trends Biochem. Sci.,* 5, 219, 1980.

125. **Wang, J. C.,** DNA supercoiling and its effects on the structure of DNA, *J. Cell Sci. Suppl.,* 1, 21, 1984.

126. **Wang, J. C.,** DNA topoisomerases: nature's solution to the topological ramifications of the double-helix structure of DNA, *Harv. Lect. Ser.,* 81, 93, 1987.

127. **Benham, C. J.,** Theoretical analysis of conformational equilibria in superhelical DNA, *Annu. Rev. Biophys. Biophys. Chem.,* 14, 23, 1985.

128. **Cozzarelli, N. R., Boles, T. C., and White, J. H.,** Primer on the topology and geometry of DNA supercoiling, in *DNA Topology and Its Biological Effects,* Cozzarelli, N. R. and Wang, J. C., Eds., Cold

Spring Harbor Laboratory, Cold Spring Harbor, NY, 1990, 139.

129. **Fuller, F. B.,** The writhing number of a space curve, *Proc. Natl. Acad. Sci. U.S.A.,* 68, 815, 1971.

130. **Vinograd, J. and Lebowitz, J.,** Physical and topological properties of circular DNA, *Gen. Physiol.,* 49, 103, 1966.

131. **Vinograd, J., Lebowitz, J., and Watson, R.,** Early and late helix-coil transitions in closed circular DNA: the number of superhelical turns in polyoma DNA, *J. Mol. Biol.,* 33, 173, 1968.

132. **White, J. H.,** Self-linking and the Gauss integral in higher dimensions, *Am. J. Math.,* 91, 693, 1969.

133. **White, J. H.,** An introduction to the geometry and topology of DNA structure, in *Mathematical Methods for DNA Sequences,* Waterman, M. S., Ed., CRC Press, Boca Raton, FL, 1989, 225.

134. **Lee, F. S. and Bauer, W. R.,** Temperature dependence of the gel electrophoretic mobility of superhelical DNA, *Nucleic Acids Res.,* 13, 1665, 1985.

135. **White, J. H. and Bauer, W. R.,** Calculation of the twist and writhe for representative models of DNA, *J. Mol. Biol.,* 189, 329, 1986.

136. **White, J. H.,** Superhelical DNA with local substructures: a generalization of the topological constraint in terms of the intersection number and the ladderlike correspondence surface, *J. Mol. Biol.,* 195, 205, 1987.

137. **White, J. H.,** The helical repeat of nucleosome-wrapped DNA, *Cell,* 56, 9, 1989.

138. **Frank-Kamenetskii, M. D.,** DNA supercoiling and unusual structures, in *DNA Topology and Its Biological Effects,* Wang, J. C. and Cozzarelli, N., Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1990, 185.

139. **Tan, R. K. Z. and Harvey, S. C.,** Molecular mechanics model of supercoiled DNA, *J. Mol. Biol.,* 205, 573, 1989.

140. **Klenin, K. V., Vologodskii, A. V., Anshelevich, V. V., Dykhne, M., and Frank-Kamenetskii, M. D.,** Computer simulation of DNA supercoiling, *J. Mol. Biol.,* submitted.

141. **Wang, J. C.,** Variation of the average rotation angle of the DNA helix and the superhelical turns of covalently closed cyclic λ DNA, *J. Mol. Biol.,* 43, 25, 1969.

142. **Germond, J. E., Hirt, B., Oudet, P., Gross-Belard, M., and Chambon, P.,** Folding of the DNA double helix in chromatin-like structures from simian virus 40, *Proc. Natl. Acad. Sci. U.S.A.,* 72, 1843, 1975.

143. **Bauer, W. and Vinograd, J.,** The interaction of closed circular DNA with intercalative dyes. I. The superhelix density of SV40 DNA in the presence and absence of dye, *J. Mol. Biol.,* 33, 141, 1968.

144. **Nadal, M., Mirambeau, G., Forterre, P., Reiter, W. D., and Duquet, M.,** Positively supercoiled DNA in a virus-like particle of an archaebactericum, *Nature,* 321, 256, 1986.

145. **Wu, H.-Y., Shyy, S., Wang, J. C., and Liu, L. F.,** Transcription generates positively and negatively supercoiled domains in the template, *Cell,* 53, 433, 1988.

146. **Benjamin, H. W. and Cozzarelli, N. R.,** Isolation and characterization of the Tn3 resolvase synaptic intermediate, *EMBO J.,* 7, 1897, 1988.

147. **Benight, A. S., Langowski, J., Wu, P.-G., Wilcoxon, J., Shibata, J. H., Fujimoto, B. S., Ribiero, N. S., and Schurr, J. M.,** Structures and dynamics of supercoiled DNAs, in *Laser Scattering Spectroscopy of Biological Objects, Studies in Physical and Theoretical Chemistry,* Vol. 45, Stepanek, J., Anzenbacher, P., and Sedlacek, B., Eds., Elsevier, Amsterdam, 1987.

148. **Krueger, S., Zaccai, G., Wlodawer, A., Langowski, J., O'Dea, M., Maxwell, A., and Gellert, M.,** Neutron and light-scattering studies of DNA gyrase and its complex with DNA, *J. Mol. Biol.,* 211, 211, 1990.

149. **Brady, G. W., Fein, D. B., and Brumberger, H.,** X-ray diffraction studies of circular superhelical DNA at 300-10,000-A resolution, *Nature,* 264, 231, 1976.

150. **Brady, G. W., Satkowski, M., Foos, D., and Benham, C. J.,** Environmental influences on DNA superhelicity: the effect of ionic strength on super helix conformation in solution, *J. Mol. Biol.,* 195, 185, 1987.

151. **Vojtiskova, M., Lukasova, E., Jelen, F., and Palecek, E.,** Polarography of circular DNAs, *Bioelectrochem. Bioenerg.,* 8, 487, 1981.

152. **Langowski, J., Benight, A. S., Fujimoto, B. S., Schurr, M., and Schomburg, U.,** Change of conformation and internal dynamics of supercoiled DNA upon binding of *Escherichia coli* single-strand binding protein, *Biochemistry,* 24, 4022, 1985.

153. **Langowski, J., Fujimoto, B. S., Wemmer, D. E., Benight, A. S., Drobny, G., Shibata, J. H., and Schurr, M.,** Deformational dynamics and NMR relaxation of supercoiled DNAs, *Biopolymers,* 24, 1023, 1985.

154. **Langowski, J., Giesen, U., and Lehmann, C.,** Dynamics of superhelical DNA studied by photon correlation spectroscopy, *Biophys. Chem.,* 25, 191, 1986.

155. **Langowski, J.,** Salt effects on internal motions of superhelical and linear pUC8 DNA. Dynamic light scattering studies, *Biophys. Chem.,* 27, 263, 1987.

156. **Langowski, J. and Giesen, U.,** Configurational and dynamic properties of different length superhelical DNAs measured by dynamic light scattering, *Biophys. Chem.,* 34, 9, 1989.

157. **Langowski, J., Giesen, U., and Farago, B.,** Studies of the structure and internal motions of supercoiled DNAs using dynamic scattering techniques, in *Proc. 2nd Int. Conf. Laser Scattering Spectroscopy of Biological Objects,* Néwat, B., Ed., Pecs, Hungary, 1989, 191.

158. **Torbet, J. and DiCapua, E.,** Supercoiled DNA is interwound in liquid crystalline solutions, *EMBO J.,* 8, 4351, 1989.

207

159. **Vasmel, H.,** Influence of supercoiling on DNA structure: laser raman spectroscopy of the plasmid pBR322, *Biopolymers,* 24, 1001, 1985.

160. **Hayashi, H., Nishimura, Y., Tsuboi, M., Sekimizu, K., Nakanishi, Y., and Ntori, S.,** Raman spectrum of a closed-circular DNA, *Biopolymers,* 24, 1107, 1985.

161. **Christens-Barry, W. A., Martin, J. C., and Lebowitz, J.,** Raman spectroscopy of supercoiled and nicked ColE1 plasmid, *Biopolymers,* 28, 1515, 1989.

162. **Rhoades, M. and Thomas, C. A., Jr.,** The P22 bacteriophage DNA molecule. II. Circular intracellular forms, *J. Mol. Biol.,* 37, 41, 1968.

163. **Boles, T. C., White, J. H., and Cozzarelli, N. R.,** Structure of plectonemically supercoiled DNA, *J. Mol. Biol.,* 213, 931, 1990.

164. **Adrian, M., Heggeler-Bordier, B. T., Whali, W., Stasiak, A. Z., Stasiak, A., and Dubochet, J.,** Direct visualization of supercoiled DNA molecules in solution, *EMBO J.,* in press.

165. **Brahms, J., Nakasu, S., Kikuchi, A., and Brahms, J. G.,** Structural changes in positively and negatively supercoiled DNA, *Eur. J. Biochem.,* 184, 297, 1989.

166. **Gellert, M. and Menzel, R.,** DNA supercoiling as a regulator of bacterial gene expression, in *New Frontiers in the Study of Gene Functions,* Poste, G. and Croce, S. T., Eds., Plenum Press, New York, 1987, 51.

167. **Wang, J. C.,** DNA topoisomerases, *Annu. Rev. Biochem.,* 54, 665, 1985.

168. **Lilley, D. M. J.,** The genetic control of DNA supercoiling, and *Vice versa,* in *Symposium of the Society for General Microbiology, Regulation of Gene Expression,* Both, I. and Higgins, D., Eds., Cambridge University Press, London, 1986, 105.

169. **Panyutin, I. G., Lyamichev, V. I., and Lyubchenko, Y. L.,** A sharp structural transition in pA03 plasmid DNA caused by increased superhelix density, *FEBS Lett.,* 148, 297, 1982.

170. **Lee, C.-H., Mizusawa, H., and Kakefuda, T.,** Unwinding of double-stranded DNA helix by dehydration, *Proc. Natl. Acad. Sci. U.S.A.,* 78, 2838, 1981.

171. **Wang, J. C., Peck, L. J., and Becherer, K.,** DNA supercoiling and its effects on DNA structure and function, *Cold Spring Harbor Symp. Quant. Biol.,* 47, 85, 1983.

172. **Lyamichev, V. I., Panyutin, I. G., and Frank-Kamenetskii, M. D.,** Evidence of cruciform structures in superhelical DNA provided by two-dimensional gel electrophoresis, *FEBS Lett.,* 153, 298, 1983.

173. **Greaves, D. R., Patient, R. K., and Lilley, D. M. J.,** Facile cruciform formation by an (A-T)$_{34}$ sequence from a *Xenopus* globin gene, *J. Mol. Biol.,* 185, 461, 1985.

174. **O'Connor, T. R., Kang, D. S., and Wells, R. D.,** Thermodynamic parameters are sequence-dependent for the supercoil-induced B to Z transition in recombinant plasmids, *J. Biol. Chem.,* 261, 13302, 1986.

175. **Lyamichev, V. I., Mirkin, S. M., and Frank-Kamenetskii, M. D.,** A pH-dependent structural transition in the homopurine-homopyrimidine tract in superhelical DNA, *J. Biomol. Struc. Dyn.,* 3, 327, 1985.

176. **Schwartz, D. C. and Cantor, C. R.,** Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis, *Cell,* 37, 67, 1984.

177. **Anand, R.,** Pulsed field gel electrophoresis: a technique for fractionating large DNA molecules, *Trends Genet.,* 278, 1986.

178. **Cantor, C. R.,** Pulsed-field gel electrophoresis of very large DNA molecules, *Annu. Rev. Biophys. Biophys. Chem.,* 17, 287, 1988.

179. **Sutherland, J. C., Emrick, A. B., and Trunk, J.,** Separation of chromosomal length DNA molecules: pneumatic apparatus for rotating gels during electrophoresis, *Electrophoresis,* 10, 315, 1989.

180. **Hightower, R. C. and Santi, D. V.,** Migration of circular DNAs using orthogonal-field-alternation gel electrophoresis, *Electrophoresis,* 10, 283, 1989.

181. **Gardiner, K. and Patterson, D.,** Transverse alternating field electrophoresis and applications to mammalian genome mapping, *Electrophoresis,* 10, 296, 1989.

182. **Chu, G.,** Pulsed field electrophoresis in contour-clamped homogeneous electric fields for the resolution of DNA by size or topology, *Electrophoresis,* 10, 290, 1989.

183. **Serwer, P. and Hayes, S. J.,** Atypical sieving of open circular DNA during pulsed field agarose gel electrophoresis, *Biochemistry,* 28, 5827, 1989.

184. **Lerman, L. S., Fischer, S. G., Hurley, I., Silverstein, K., and Lumelsky, N.,** Sequence-determined DNA separations, *Annu. Rev. Biophys. Bioenerg.,* 13, 399, 1984.

185. **Rosenbaum, V. and Riesner, D.,** Temperature-gradient gel electrophoresis. Thermodynamic analysis of nucleic acids and proteins in purified form and in cellular extracts, *Biophys. Chem.,* 26, 235, 1987.

186. **Riesner, D., Steger, G., Zimmat, R., Owens, R. A., Wagenhöfer, M., Hillen, W., Vollbach, S., and Henco, K.,** Temperature-gradient gel electrophoresis of nucleic acids: analysis of conformational transitions, sequence variations, and protein-nucleic acid interactions, *Electrophoresis,* 10, 377, 1989.

187. **Hecker, R., Wang, Z., Steger, G., and Riesner, D.,** Analysis of RNA structures by temperature-gradient gel electrophoresis: viroid replication and processing, *Gene,* 72, 59, 1988.

188. **Lyubchenko, Y. L. and Shlyakhtenko, L. S.,** Early melting of supercoiled DNA, *Nucleic Acids Res.,* 16, 3269, 1988.

189. **Fried, M. and Crothers, D. M.,** Equilibria and kinetics of *lac* electrophoresis, *Nucleic Acids Res.,* 9, 6505, 1981.

190. **Garner, M. M. and Revzin, A.,** A gel electrophoresis method for quantifying the binding of proteins

to specific DNA regions: application to components of the *Escherichia coli* lactose operon regulatory system, *Nucleic Acids Res.,* 9, 3047, 1981.

191. **Jovin, J. C., McIntosh, L. P., Arndt-Jovin, D. J., Zarling, D. A., Robert-Nicoud, M., van de Sande, J. H., Jorgenson, K. F., and Eckstein, F.,** Left-handed DNA: from synthetic polymers to chromosomes, *J. Biomol. Struct. Dyn.,* 1, 21, 1983.

192. **Nordheim, A., Lafer, E. M., Peck, L. J., Wang, J. S., Stollar, B. D., and Rich, A.,** Negatively supercoiled plasmids contain left-handed Z-DNA segments as detected by specific antibody binding, *Cell,* 31 309, 1982.

193. **Pohl, F. M.,** Hysteretic behaviour of a Z-DNA-antibody complex, *Biophys. Chem.,* 26, 385, 1987.

194. **Palecek, E., Krejcova, A., Vojtiskova, M., Podgorodnichenko, V., Ilyina, T., and Poverennyi, A.,** Antibodies to DNAs chemically modified with osmium structural probes, *Gen. Physiol. Biophys.,* 8, 491, 1989.

195. **Ceglarek, J. A. and Revzin, A.,** Studies of DNA-protein interactions by gel electrophoresis, *Electrophoresis,* 10, 360, 1989.

196. **Gardner, M. M. and Revzin, A.,** Gel retardation analysis of nucleic acid-protein interactions, in *The Practical Approach Series,* Rickwood, D. and Hames, B. D., Eds., IRL Oxford University Press, Oxford, 1990, 201.

197. **Fried, M. G.,** Measurement of protein-DNA interaction parameters by electrophoresis mobility shift assay, *Electrophoresis,* 10, 366, 1989.

198. **Diekmann, S.,** The migration anomaly of DNA fragments in polyacrylamide gels allows the detection of small sequence-specific DNA structure variations, *Electrophoresis,* 10, 354, 1989.

199. **Leng, M.,** Immunological detection of lesions in DNA, *Biochimia,* 67, 309, 1985.

200. **Stollar, D.,** Antibodies to DNA, *Crit. Rev. Biochem.,* 20, 1, 1986.

201. **Eilat, D.,** Anti-DNA antibodies: problems in their study and interpretation, *Clin. Exp. Immunol.,* 65, 215, 1986.

202. **Braun, P. B. and Lee, J. S.,** Immunogenic duplex nucleic acids are nuclease resistant, *J. Immunol.,* 141, 2084, 1988.

203. **Munns, T. W. and Liszewski, M. K.,** Antibodies specific for modified nucleosides: an immunochemical approach for the isolation and characterization of nucleic acids, *Progr. Nucleic Acid Res. Mol. Biol.,* 24, 109, 1980.

204. **Strickland, P. T. and Boyle, J. M.,** Immunoassay of carcinogen-modified DNA, *Progr. Nucleic Acid Res. Mol. Biol.,* 31, 1, 1984.

205. **Stollar, D.,** Immunochemical approaches to the definition of unusual nucleic acid structures, in *Unusual DNA Structures,* Wells, R. D. and Harvey, S. C., Eds., Birmingham, 1988, 256.

206. **Lee, J. S., Burkholder, G. D., Latimer, L. J. P., Haug, B. L., and Braun, R. P.,** A monoclonal antibody to triplex DNA binds to eukaryotic chromosomes, *Nucleic Acids Res.,* 15, 1047, 1987.

207. **Lee, J. S., Woodsworth, M. L., Latimer, L. J. P., and Morgan, A. R.,** Poly(pyrimidine)·poly(purine) synthetic DNAs containing 5-methylcytosine form stable triplexes at neutral pH, *Nucleic Acids Res.,* 12, 6603, 1984.

208. **Lee, J. S., Latimer, J. L. P., Haug, B. L., Pulleyblank, D. E., Skinner, D. M., and Burkholder, G. D.,** Triplex DNA in plasmids and chromosomes, *Gene,* 82, 191, 1989.

209. **Lee, J. S., Latimer, L. J. P., and Sibley, J. T.,** Detection of unusual nucleic acids with monoclonal antibodies specific for triplexes, poly(ADP-ribose), DNA-RNA hybrids, and Z-DNA, *Anal. Biochem.,* 178, 373, 1989.

210. **Frappier, L., Price, G. B., Martin, R. G., and Zannis-Hadjopoulos, M.,** Monoclonal antibodies to cruciform DNA structures, *J. Mol. Biol.,* 193, 751, 1987.

211. **Ando, T.,** A nuclease specific for heat-denatured DNA isolated from a product of *Aspergillus oryzae, Biochem. Biophys. Acta,* 114, 158, 1966.

212. **Vogt, V. M.,** Purification and further properties of single-strand-specific nuclease from *Aspergillus oryzae, Eur. J. Biochem.,* 33, 192, 1973.

213. **Singleton, C. K., Klysik, J., Stirdivant, S. M., and Wells, R. D.,** Left-handed Z-DNA is induced by supercoiling in physiological ionic conditions, *Nature,* 299, 312, 1982.

214. **Drew, H. R.,** Structural specifities of five commonly used DNA nucleases, *J. Mol. Biol.,* 176, 535, 1984.

215. **Pulleyblank, D. E., Haniford, D. B., and Morgan, A. R.,** A structural basis for S1 hypersensitivity in double stranded DNA, *Cell,* 42, 271, 1985.

216. **Dodgson, J. B. and Wells, R. D.,** Action of single-strand specific nucleases on model DNA heteroduplexes of defined size and sequence, *Biochemistry,* 16, 2374, 1966.

217. **Bhattacharya, A. and Lilley, D. M. J.,** Single base mismatches in DNA. Long- and short-range structure probe by analysis of axis trajectory and local chemical reactivity, *J. Mol. Biol.,* 209, 583, 1989.

218. **Johnson, P. H. and Laskowski, M.,** Sugar-unspecific mung bean nuclease I, *J. Biol. Chem.,* 243, 3421, 1968.

219. **Johnson, P. H. and Laskowski, M.,** I. Mung bean nuclease. II. Resistance of double stranded deoxyribonucleic acid and susceptibility of regions rich in adenosine and thymidine to enzymatic hydrolysis, *J. Biol. Chem.,* 245, 891, 1970.

220. **Kowalski, D. and Stanford, J. P.,** Action of mung bean nuclease on supercoiled PM2 DNA, *J. Biol. Chem.,* 257, 7820, 1982.

221. **Sheflin, I. G. and Kowalski, D.,** Mung bean nuclease cleavage of a dA + dT-rich sequence or an inverted repeat sequence in supercoiled PM2 DNA on ionic environment, *Nucleic Acids Res.,* 12, 7087, 1984.

222. **Kowalski, D.,** Changes in site specificity of single-strand-specific endonucleases on supercoiled PM2 DNA with temperature and ionic environments, *Nucleic Acids Res.,* 12, 7071, 1984.

223. **Sheflin, L. G. and Kowalski, D.,** Altered DNA conformations detected by mung bean nuclease occur in promoter and terminator regions of supercoiled pBR322 DNA, *Nucleic Acids Res.,* 13, 6137, 1985.

224. **Linn, S. and Lehmann, I. R.,** An endonuclease from *Neurospora crassa* specific for polynucleotides lacking an ordered structure. I. Purification and properties of the enzyme, *J. Biol. Chem.,* 240, 1287, 1965.

225. **Linn, S.,** An endonuclease from *Neurospora crassa* specific for polynucleotides lacking an ordered structure, *Methods Enzymol.,* 12, 247, 1967.

226. **Blaho, J. A., Larson, J. E., McLean, M. J., and Wells, R. D.,** Multiple DNA secondary structures in perfect inverted repeat inserts in plasmids, *J. Biol. Chem.,* 263, 14446, 1988.

227. **Camilloni, G., Seta, F. D., Negri, R., Ficca, A. G., and di Mauro, E.,** Structure of RNA polymerase II promoters. Conformational alterations and template properties of circularized *Saccharomyces cerevisiae* GAL1-GAL10 divergent promoters, *EMBO J.,* 5, 763, 1986.

228. **Amirhaeri, S., Wohlrab, F., Major, E. O., and Wells, R. D.,** Unusual DNA structure in the regulatory region of the human papovavirus JC virus, *J. Virol.,* 62, 922, 1988.

229. **Palecek, E.,** Electrochemical analysis of polynucleotides, *Bioelectrochem. Bioenerg.,* 8, 497, 1981.

230. **Lukasova, E., Jelen, F., and Palecek, E.,** Electrochemistry of osmium-nucleic acids complexes: a probe for single-stranded and distorted regions in DNA, *Gen. Physiol. Biophys.,* 1, 53, 1982.

231. **Lukasova, E., Vojtiskova, M., Jelen, F., Sticzay, T., and Palecek, E.,** Osmium-induced alternation in DNA structure, *Gen. Physiol. Biophys.,* 3, 175, 1984.

232. **Palecek, E. and Hung, M. A.,** Determination of nanogram quantities of osmium-labeled nucleic acids by stripping (inverse) voltammetry, *Anal. Biochem.,* 132, 236, 1983.

233. **Glikin, G. C., Vojtiskova, M., Rena-Deszalzi, L. N., and Palecek, E.,** Osmium tetroxide: a new probe for site-specific distorsions in supercoiled DNAs, *Nucleic Acids Res.,* 12, 1725, 1984.

234. **Lilley, D. M. and Palecek, E.,** The supercoil-stabilised cruciform of ColE1 is hyper-reactive to osmium tetroxide, *EMBO J.,* 3, 1187, 1984.

235. **Poverenny, A. M., Podgorodnichenko, V. K., Bryksina, L. E., Monastyrskaya, G. S., and Sverdlow, E. D.,** Immunochemical approaches to DNA structure investigation — I, *Mol. Immunol.,* 6, 313, 1979.

236. **Wani, A. A., Sullivan, J. K., and Lebowitz, J.,** Immunoassay for carbodiimide modified DNA-detection of unpairing transitions in supercoiled ColE1 DNA, *Nucleic Acids Res.,* 17, 9957, 1989.

237. **Krejcova, A., Poverennyi, A., and Palecek, E.,** unpublished data, 1990.

238. **Schröder, M.,** Osmium tetroxide *cis* hydroxylation of unsaturated substrates, *Chem. Rev.,* 80, 187, 1980.

239. **Subbaraman, L. R., Subbaranam, J., and Behrman, E. J.,** The reaction of osmium tetroxide-pyridine complexes with nucleic acid components, *Bioinorg. Chem.,* 1, 35, 1971.

240. **Cole, M. D., Wiggins, J. W., and Beer, M.,** Molecular microscopy of labeled polynucleotides: stability of osmium atoms, *J. Mol. Biol.,* 117, 387, 1977.

241. **Chang, C.-H., Ford, H., and Behrman, E. J.,** Reaction of cytosine and 5-methylcytosine with osmium(VII) reagents: syntheses and deamination to uracil and thymine derivatives, *Inorg. Chim. Acta,* 55, 77, 1981.

242. **Neidle, S. and Stuart, D. I.,** The crystal and molecular structure of an osmium bispyridine adduct of thymine, *Biochim. Biophys. Acta,* 418, 226, 1976.

243. **Kistenmacher, T. J., Marzilli, L. G., and Rossi, M.,** Conformational properties of the osmium tetroxide bispyridine ester of 1-methylthymine and a comment on the linearity of the trans $O = Os = O$ group, *Bioinorg. Chem.,* 6, 347, 1976.

244. **Friedman, T. and Brown, D. M.,** Base-specific reactions useful for DNA sequencing: methylene blue-sensitized photooxidation of guanine and osmium tetroxide modification of thymine, *Nucleic Acids Res.,* 5, 615, 1978.

245. **Johnston, B. H. and Rich, A.,** Chemical probes of DNA conformation: detection of Z-DNA at nucleotide resolution, *Cell,* 42, 713, 1985.

246. **Galazka, G., Palecek, E., Wells, R. D., and Klysik, J.,** Site-specific OsO_4 modification of the B-Z junctions formed at the $(dA-dC)_{32}$ region in supercoiled DNA, *J. Biol. Chem.,* 261, 7093, 1986.

247. **Htun, H. and Dahlberg, J. E.,** Single strands, triple strands, and kinks in H-DNA, *Science,* 241, 1791, 1988.

248. **Rahmouni, A. R. and Wells, R. D.,** Stabilization of Z DNA *in vivo* by localized supercoiling, *Science,* 246, 358, 1989.

249. **Palecek, E., Vojtiskova, M., Jelen, F., and Kozinova, M.,** Introduction of electroactive marker into polynucleotide chains: a sensitive probe for DNA structure, in *Charge and Field Effects in Biosystems,* Allen, M. J. and Usherwood, P. N. R., Eds., Abacus Press, 1984, 397.

250. **Palecek, E.,** Chemical and electrochemical probes of the DNA polymorphic structure, *Stud. Biophys.,* 114, 39, 1986.

251. **Palecek, E.,** Electrochemical behaviour of biological macromolecules, *Bioelectrochem. Bioenerg.,* 15, 275, 1986.

252. **Boublikova, P.,** Chemical modification of single-stranded polynucleotides by osmium tetroxide complexes, *Stud. Biophys.,* 131, 185, 1989.

253. **McClellan, J. A. and Lilley, D. M. J.,** A two-state

conformational equlibirium for alternating (A-T)n sequences in negatively supercoiled DNA, *J. Mol. Biol.*, 197, 707, 1987.

254. **Duckett, D. R., Murchie, A. I. H., Diekmann, S., von Kitzing, E., Kemper, B., and Lilley, D. M. J.,** The structure of the Holliday junction, and its resolution, *Cell,* 55, 79, 1988.

255. **Bhattacharyya, A. and Lilley, D. M. J.,** The contrasting structures of mismatched DNA sequences containing looped-out bases (bulges) and multiple mismatches (bubbles), *Nucleic Acids Res.,* 17, 6821, 1989.

256. **Cotton, R. G. H. and Campbell, R. D.,** Chemical reactivity of matched cytosine and thymine bases near mismatched and unmismatched bases in a heteroduplex between DNA strands with multiple differences, *Nucleic Acids Res.,* 17, 4223, 1989.

257. **Cotton, R. G., Rodrigues, N. R., and Campbell, R. D.,** Reactivity of cytosine and thymine in single base-pair mismatches with hydroxylamine and osmium tetroxide and its application to the study of mutations, *Proc. Natl. Acad. Sci. U.S.A.,* 85, 4397, 1988.

258. **Schwartz, A., Marrot, L., and Leng, M.,** Conformation of DNA modified at a d(GG) or a d(AG) site by the antitumor drug *cis*-diamminedichloroplatinum(II), *Biochemistry,* 28, 7975, 1989.

259. **Buc, H., Lilley, D. M. J., and Buckle, M.,** personal communication, 1990.

260. **McClellan, J. A., Palecek, E., and Lilley, D. M. J.,** (A-T)n tracts embedded in random sequence DNA — formation of a structure which is chemically reactive and torsionally deformable, *Nucleic Acids Res.,* 14, 9291, 1986.

261. **Furlong, J. C., Sullivan, K. M., Murchie, A. I. H., Gough, G. W., and Lilley, D. M. J.,** Localized chemical hyperreactivity in supercoiled DNA: evidence for base unpairing in sequences that induce low-salt cruciform extrusion, *Biochemistry,* 28, 2009, 1989.

262. **Vojtiskova, M., Stokrova, J., and Palecek, E.,** Modification of ColE1 DNA with osmium tetroxide generates positively supercoiled molecules, *J. Biomol. Struct. Dyn.,* 2, 1013, 1985.

263. **Nejedly, K., Kwinkowski, M., Galazka, G., Klysik, J., and Palecek, E.,** Recognition of the structural distortions at the junction between B and Z segments in negatively supercoiled DNA by osmium tetroxide, *J. Biomol. Struct. Dyn.,* 3, 467, 1985.

264. **Vojtiskova, M. and Palecek, E.,** Unusual protonated structure in the homopurine·homopyrimidine tract of supercoiled and linearized plasmids recognized by chemical probes, *J. Biomol. Struct. Dyn.,* 5, 283, 1987.

265. **Vojtiskova, M., Mirkin, S., Lyamichev, V., Voloshin, O., Frank-Kamenetskii, M., and Palecek, E.,** Chemical probing of the homopurine·homopyrimidine tracts in supercoiled DNA at

single-nucleotide resolution, *FEBS Lett.,* 234, 295, 1988.

266. **Palecek, E.,** unpublished data, 1990.

267. **Bowater, R. and Lilley, D. M. J.,** personal communication, 1990.

268. **Palecek, E., Boublikova, P., Galazka, G., and Klysik, J.,** Inhibition of restriction endonuclease cleavage due to site-specific chemical modification of the B-Z junction in supercoiled DNA, *Gen. Physiol. Biophys.,* 6, 327, 1987.

269. **Palecek, E., Boublikova, P., and Nejedly, K.,** Probing of DNA structure with osmium tetroxide. Effect of ligands, *Biophys. Chem.,* 34, 63, 1989.

270. **Boublikova, P. and Palecek, E.,** Probing of B-Z junctions in recominant plasmids *in vitro* and in the cell with different osmium tetroxide complexes, *Gen. Physiol. Biophys.,* 8, 475, 1989.

271. **Ehrenberg, L., Fedorcsak, I., and Solymosy, F.,** Diethyl pyrocarbonate in nucleic acid research, *Progr. Nucleic Acids Res. Mol. Biol.,* 16, 198, 1976.

272. **Abdulwajid, A. W. and Wu, Y.-H. F.,** Chemical modifiction of *Escherichia coli* RNA polymerase by diethyl pyrocarbonate: evidence of histidine requirement for enzyme activity and intrinsic zinc binding, *Biochemistry,* 25, 8167, 1986.

273. **Pasta, P., Mazzola, G., and Carrea, G.,** Chemical modification of $3\alpha,20\beta$-hydroxysteroid dehydrogenase with diethyl pyrocarbonate. Evidence for an essential, highly reactive, lysyl residue, *Biochemistry,* 26, 1247, 1987.

274. **Vincze, A., Henderson, R. E. L., McDonald, J. J., and Leonard, N. J.,** Reaction of diethyl pyrocarbonate with nucleic acid components. Bases and nucleosides derived from guanine, cytosine, and uracil, *J. Am. Chem. Soc.,* 95, 2677, 1973.

275. **Peattie, D. A. and Gilbert, W.,** Chemical probes for higher-order structure in RNA, *Proc. Natl. Acad. Sci. U.S.A.,* 77, 4679, 1980.

276. **VanStolk, B. J. and Noller, H. F.,** Chemical probing of conformation in large RNA molecules, *J. Mol. Biol.,* 180, 151, 1984.

277. **Herr, W., Corbin, V., and Gilbert, W.,** Nucleotide sequence of the 3' half of AKV, *Nucleic Acids Res.,* 10, 6931, 1982.

278. **Herr, W.,** Diethyl pyrocarbonate: a chemical probe for secondary structure in negatively supercoiled DNA, *Proc. Natl. Acad. Sci. U.S.A.,* 82, 8009, 1985.

279. **Scholten, P. M. and Nordheim, A.,** Diethyl pyrocarbonate: a chemical probe for DNA cruciforms, *Nucleic Acids Res.,* 14, 3981, 1986.

280. **Furlong, J. C. and Lilley, D. M.,** Highly selective chemical modification of cruciform loops by diethyl pyrocarbonate, *Nucleic Acids Res.,* 14, 3995, 1986.

281. **Runkel, L. and Nordheim, A.,** Chemical footprinting of the interaction between left-handed Z-DNA and anti-Z-DNA antibodies by diethylpyrocarbonate carbethoxylation, *J. Mol. Biol.,* 189, 487, 1986.

282. **Buckle, M. and Buc, H.,** Fine mapping of DNA single-stranded regions using base specific chemical

probes: study of an open complex formed between RNA polymerase and the *lac* UV5 promoter, *Biochemistry*, 28, 4388, 1989.

283. **Krzyzosiak, W. J., Biernat, J., Ciesiolka, J., Gulewicz, K., and Wiewiorowski, M.,** The reactions of adenine and cytosine residues in tRNA with chloroacetaldehyde, *Nucleic Acids Res.*, 9, 2841, 1981.

284. **Kusmierek, J. T. and Singer, B.,** Chloroacetaldehyde-treated ribo- and deoxyribopolynucleotides. I. Reaction products, *Biochemistry*, 21, 5717, 1982.

285. **Kusmierek, J. T. and Singer, B.,** Chloroacetaldehyde-treated ribo- and deoxyribopolynucleotides. II. Errors in transcription by different polymerases, *Biochemistry*, 21, 5723, 1982.

286. **Kohwi-Shigematsu, T., Gelinas, R., and Weintraub, M.,** Detection of an altered DNA conformation at specific sites in chromatin and supercoiled DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 4389, 1983.

287. **Lilley, D. M. J.,** Structural pertubation in supercoiled DNA: hypersensitivity to modification by a single-strand-selective chemical reagent, *Nucleic Acids Res.*, 11, 3097, 1983.

288. **Haniford, D. B. and Pulleyblank, D. E.,** Transition of a cloned d(AT)n-d(AT)n tract to a cruciform, *in vivo*, *Nucleic Acids Res.*, 13, 4343, 1985.

289. **Kang, D. S. and Wells, R. D.,** B-Z DNA junctions contain few, if any, nonpaired bases at physiological superhelical densities, *J. Biol. Chem.*, 260, 7783, 1985.

290. **Kohwi, Y. and Kohwi-Shigematsu, T.,** Magnesium ion-dependent triple-helix structure formed by homopurine·homopyrimidine sequences in supercoiled plasmid DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 3781, 1988.

291. **Vogt, N., Marrot, L., Rousseau, N., Malfoy, B., and Leng, M.,** Chloracetaldehyde reacts with Z-DNA, *J. Mol. Biol.*, 201, 773, 1988.

292. **Nejedly, K., Pivec, L., Formanek, P., Rozkot, F., and Palecek, E.,** Specific changes in high resolution melting curves of ColE1 DNA due to modification by osmium tetroxide, *Stud. Biophys.*, 114, 49, 1986.

293. **Palecek, E., Boublikova, P., Nejedly, K., Galazka, G., and Klysik, J.,** B-Z junctions in supercoiled pRW751 DNA contain unpaired bases or non-Watson-Crick base pairs, *J. Biomol. Struct. Dyn.*, 5, 297, 1987.

294. **Anin, M.-F. and Leng, M.,** Distortions induced in double-stranded oligonucleotides by the binding of *cis*- or *trans*-diamine-dichloroplatinum(II) to the d(GTG) sequence, *Nucleic Acids Res.*, 18, 4395, 1990.

295. **Leng, M.,** DNA bending induced by covalently bound drugs. Gel electrophoresis and chemical probe studies, *Biophys. Chem.*, 35, 155, 1990.

296. **McLean, M., Seela, F., and Waring, M. J.,** Echinomycin-induced hypersensitivity to osmium tetroxide of DNA fragments incapable of forming Hoogs-

teen base pairs, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 9687, 1989.

297. **Hayatsu, H. and Ukita, T.,** The selective degradation of pyrimidines in nucleic acids by permanganate oxidation, *Biochem. Biophys. Res. Commun.*, 29, 556, 1967.

298. **Palecek, E.,** Oscillographic polarography of deoxyribonucleic acid degradation products, *Biochim. Biophys. Acta*, 51, 1, 1961.

299. **Bayley, C. R. and Jones, A. S.,** Nucleotide sequence: the oxidation of DNA with potassium permanganate, *Trans. Faraday Soc.*, 55, 492, 1959.

300. **Sagelsdorff, P. and Lutz, W. K.,** Sensitivity of DNA and nucleotides to oxidation by permanganate and hydrogen peroxide, *Arch. Toxicol. Suppl.*, 11, 84, 1987.

301. **O'Halloran, T. V., Frantz, B., Shin, M. K., Ralston, D. M., and Wright, J. G.,** The MerR heavy metal receptor mediates positive activation in a topologically novel transcription complex, *Cell*, 56, 119, 1989.

302. **Borowiec, J. A., Zhang, L., Sasse-Dwight, S., and Gralla, J. D.,** DNA supercoiling promotes formation of a bent repression loop in *lac* DNA, *J. Mol. Biol.*, 196, 101, 1987.

303. **Jeppesen, C. and Nielsen, P. E.,** Detection of intercalation-induced changes in DNA structure by reaction with diethyl pyrocarbonate or potassium permanganate. Evidence against the induction of Hoogsteen base pairing by echinomycin, *FEBS Lett.*, 231, 172, 1988.

304. **Fox, K. R. and Grigg, G. W.,** Diethylpyrocarbonate and permanganate provide evidence for an unusual DNA conformation induced by binding of the antitumour antibiotics bleomycin and phleomycin, *Nucleic Acids Res.*, 16, 2063, 1988.

305. **Beer, M., Stern, S., Carmalt, D., and Mohlhenrich, K. H.,** Determination of base sequence in nucleic acids with the electron microscope. V. The thymine-specific reactions of osmium tetroxide with deoxyribonucleic acid and its components, *Biochemistry*, 5, 2283, 1966.

306. **Burton, K. and Riley, W. T.,** Selective degradation of thymidine and thymine deoxynucleotides, *Biochem. J.*, 98, 70, 1966.

307. **McLean, M. J., Dar, A., and Waring, M. J.,** Differences between sites of binding to DNA and strand cleavage for complexes of bleomycin with iron and cobalt, *J. Mol. Recog.*, 1, 184, 1989.

308. **Kohwi-Shigematsu, T., Manes, T., and Kohwi, Y.,** Unusual conformational effect exerted by Z-DNA upon its neighbouring sequences, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 2223, 1987.

309. **Kohwi, Y.,** Cationic metal-specific structures by the poly(dG) region and the direct repeats in the chicken adult bA-globin gene promoter, *Nucleic Acids Res.*, 17, 4493, 1989.

310. **Lilley, D. M. J.,** Cyclic adduct formation at structural perturbations in supercoiled DNA molecules, in

The Role of Cyclic Nucleic Acid Adducts in Carcinogenesis and Mutagenesis, Singer, B. and Bartsch, H., Eds., International Agency for Research on Cancer, Lyon, 1986, 83.

311. **McLean, M. J. and Wells, R. D.,** The role of DNA sequence in the formation of Z-DNA versus cruciforms in plasmids, *J. Biol. Chem.,* 263, 7370, 1988.

312. **McLean, M. J., Lee, J. W., and Wells, R. D.,** Characteristics of Z-DNA helices formed by imperfect (purine-pyrimidine) sequences in plasmids, *J. Biol. Chem.,* 263, 7378, 1988.

313. **Johnston, B. H.,** The S1-sensitive form of d(C-T)n·d(A-G)n. Chemical evidence for a three-stranded structure in plasmids, *Science,* 241, 1800, 1988.

314. **Lukasova, E. and Palecek, E.,** Reaction of double-helical DNA with sodium bisulfite, *Stud. Biophys.,* 60, 61, 1976.

315. **Gough, G. W., Sullivan, K. M., and Lilley, D. M. J.,** The structure of cruciforms in supercoiled DNA: probing the single-stranded character of nucleotide bases with bisulphite, *EMBO J.,* 5, 191, 1986.

316. **Sawadaishi, K., Miura, K., Ohtsuka, E., Euda, G., Shinriki, N., and Ishizaki, K.,** Structure- and sequence-specificity of ozone degradation of supercoiled plasmid DNA, *Nucleic Acids Res.,* 14, 1159, 1986.

317. **Hale, P., Woodward, R. S., and Lebowitz, J.,** Carbodiimide inactivation of *Escherichia coli* RNA polymerase promoters on supercoiled simian virus 40 and ColE1 DNAs occurs by a one-hit process at salt concentrations in the physiological range, *J. Biol. Chem.,* 258, 7828, 1983.

318. **Brown, D. M.,** Chemical reactions of polynucleotides and nucleic acids, in *Basic Principles in Nucleic Acid Chemistry,* Ts'O, P., Ed., Academic Press, New York, 1974.

319. **Nielsen, P. E.,** Chemical and photochemical probing of DNA complexes, *J. Mol. Recog.,* 3, 1, 1990.

320. **Tullius, T. D.,** Physical studies of protein-DNA complexes by footprinting, *Annu. Rev. Biophys. Biophys. Chem.,* 18, 213, 1989.

321. **Portugal, J.,** Footprinting analysis of sequence-specific DNA-drug interactions, *Chem.-Biol. Interact.,* 71, 311, 1989.

322. **Sigman, D. S. and Chen, C. B.,** Chemical nucleases: new reagents in molecular biology, *Annu. Rev. Biochem.,* 59, 207, 1990.

323. **Dervan, P. B.,** Sequence specific recognition of double helical DNA. A synthetic approach, in *Nucleic Acids and Molecular Biology,* Vol. 2, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1988, 49.

324. **Fleisher, M. B., Mei, H.-Y., and Barton, J. K.,** Metal complexes which target DNA sites: coupling recognition to reactivity, in *Nucleic Acids and Molecular Biology,* Vol. 2, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1988, 65.

325. **Lawley, P. D.,** Effects of some chemical mutagens and carcinogens on nucleic acids, *Progr. Nucleic Acid Res. Mol. Biol.,* 5, 89, 1966.

326. **Voloshin, O. N., Mirkin, S. M., Lyamichev, V. I., Belotserkovskii, B. P., and Frank-Kamenetskii, M. D.,** Chemical probing of homopurine-homopyrimidine mirror repeats in supercoiled DNA, *Nature,* 333, 475, 1988.

327. **Evans, T. and Efstratiadis, A.,** Sequence-dependent S1 nuclease hypersensitivity of a heteronomous DNA duplex, *J. Biol. Chem.,* 261, 14771, 1986.

328. **Pulleyblank, D. E.,** Non-commensurate polypurine/polypyrimidine DNA structures, in *Nucleic Acids and Molecular Biology,* Vol. 2, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1988, 31.

329. **Gilbert, W., Maxam, A., and Mirzabekov, A.,** Contacts between the lac-repressor and DNA revealed by methylation: control of ribosome synthesis, in *Alfred Benzo Symposium,* Kjeldgaard, N. O. and Maaloe, O., Eds., Copenhagen, 1976, 139.

330. **Church, G. M. and Gilbert, W.,** Genomic sequencing, *Proc. Natl. Acad. Sci. U.S.A.,* 81, 1991, 1984.

331. **Saluz, H. P. and Jost, J. P.,** Genomic sequencing and *in vivo* footprinting, *Anal. Biochem.,* 176, 201, 1989.

332. **Siebenlist, U. and Gilbert, W.,** Contacts between *Escherichia coli* RNA polymerase and an early promoter of phage T7, *Proc. Natl. Acad. Sci. U.S.A.,* 77, 122, 1980.

333. **Heuer, C. and Hillen, W.,** Tet repressor-tet operator contacts probed by operator DNA-modification interference studies, *J. Mol. Biol.,* 202, 407, 1988.

334. **Lukasova, E., Palecek, E., Kruglyakova, K. E., Zhizhina, G. P., and Smotryaeva, M. A.,** Properties of DNA modified with N-methyl-N-nitrosourea, *Rad. Environ. Biophys.,* 14, 231, 1977.

335. **Maxam, A. M. and Gilbert, W.,** Sequencing end-labeled DNA with base-specific chemical cleavages, *Methods Enzymol.,* 65, 499, 1980.

336. **Tullius, T. D. and Dombroski, B. A.,** Iron(II) EDTA used to measure the helical twist along any DNA molecule, *Science,* 230, 679, 1985.

337. **Chen, J.-H., Churchill, M. E. A., Tullius, T. D., Kallenbach, N. R., and Seeman, N. C.,** Construction and analysis of monobile DNA junctions, *Biochemistry,* 27, 6032, 1988.

338. **Vrana, K. E., Churchill, M. E. A., Tullius, T. D., and Brown, D. D.,** Mapping functional regions of transcription factor TFIIIA, *Mol. Cell. Biol.,* 8, 1684, 1988.

339. **Hayes, J., Tullius, T. D., and Wolffe, A. P.,** A protein-protein interaction is essential for stable complex formation on a 5 S RNA gene, *J. Biol. Chem.,* 264, 6009, 1989.

340. **Kimball, A. S., Milman, G., and Tullius, T. D.,** High-resolution footprints of the DNA-binding domain of Epstein-Barr virus nuclear antigen 1, *Mol. Cell. Biol.,* 9, 2738, 1989.

341. **Shafer, G. E., Price, M. A., and Tullius, T. D.,**

Use of the hydroxyl radical and gel electrophoresis to study DNA structure, *Electrophoresis*, 10, 397, 1989.

342. Cimino, G. D., Gamper, H. B., Isaacs, S. T., and Hearst, J. E., Psoralens as photoactive probes of nucleic acid structure and function: organic chemistry, photochemistry, and biochemistry, *Annu. Rev. Biochem.*, 54, 1151, 1985.

343. Zhen, W.-P., Buchardt, O., Nielsen, H., and Nielsen, P. E., Site specificity of psoralen-DNA interstrand crosslinking determined by nuclease Bal31 digestion, *Biochemistry*, 25, 6598, 1986.

344. Sage, E. and Moustacchi, E., Sequence content effects on 8-methoxypsoralen photobinding to defined DNA fragments, *Biochemistry*, 27, 3018, 1987.

345. Ostrander, E. A., Karty, R. A., and Hallick, L. M., High resolution psoralen mapping reveals an altered DNA helical structure in the SV40 regulatory region, *Nucleic Acids Res.*, 16, 213, 1988.

346. Ostrander, E. A., Kurkinen, N. A., and Hallick, L. M., DNA structural alternations in the SV40 enhancer region are retained *in vivo*, *Virology*, 165, 274, 1988.

347. Barton, J., Metals and DNA: molecular left-handed complements, *Science*, 233, 727, 1986.

348. Barton, J. K., Tribolet, R., and Kirshenbaum, M. R., Rh(DIP)$_3^{3+}$: a shape-selective metal complex which targets cruciforms, *Nucleic Acids Res.*, 16, 7943, 1988.

349. Mei, H.-Y. and Barton, J. K., Chiral probe for A-form helices of DNA and RNA: tris(tetramethylphenanthroline)-ruthenium(II), *J. Am. Chem. Soc.*, 108, 7414, 1986.

350. Mei, H.-Y. and Barton, J. K., Tris(tetramethylphenanthroline) ruthenium(II): a chiral probe that cleaves A-DNA conformations, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 1339, 1988.

351. Marmur, J. and Lane, D., Strand separation and sepecific recombination in deoxyribonucleic acids: biological studies, *Proc. Natl. Acad. Sci. U.S.A.*, 46, 453, 1960.

352. Doty, P., Marmur, J., Eigner, J., and Schildkraut, C., Strand separation and specific recombination in deoxyribonucleic acids: physical chemical studies, *Proc. Natl. Acad. Sci. U.S.A.*, 46, 461, 1960.

353. Marmur, J., Schildkraut, C. L., and Rownd, B., Denaturation and renaturation of deoxyribonucleic acid, *Progr. Nucleic Acids Res.*, 1, 231, 1963.

354. Knorre, D. G. and Vlassov, V. V., Complementary-addressed (sequence-specific) modification of nucleic acids, *Progr. Nucl. Acids Res. Mol. Biol.*, 32, 291, 1985.

355. Helene, C. and Thuong, N. T., Oligo-α-deoxyribonucleotides covalently linked to intercalating agents. A new family of sequence-specific nucleic acid reagents, in *Nucleic Acids and Molecular Biology*, Vol. 2, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1988, 105.

356. Helene, C., Artificial control of gene expression by oligodeoxynucleotides covalently linked to intercalating agents, *Br. J. Cancer*, 60, 157, 1989.

357. Helene, C., Sun, J. S., Francois, J. C., Saison-Behmoaras, T., Montena Garestier, T., Asseline, U., and Thuong, N. T., Sequence-specific recognition and cleavage of the DNA double-helix by triple helix formation and intercalation, in *Structure and Methods, Vol. I: Human Genome Initiative and DNA Recombination*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 51.

358. Helene, C. and Toulme, J.-J., Specific regulation of gene expression by antisense, sense and antigene nucleic acids, *Biochim. Biophys. Acta*, 1049, 99, 1990.

359. Dervan, P. B., Chemical methods for the site-specific cleavage of genome DNA, in *Structure and Methods, Vol. I: Human Genome Initiative and DNA Recombination*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 37.

360. Vlassov, V. V., Gaidamakov, S. A., Zarytova, V. F., Knorre, D. G., Levina, A. S., Nikonova, A. A., Podust, L. M., and Fedorova, O. S., Sequence-specific chemical modification of double-stranded DNA with alkylating oligodeoxyribonucleotide derivatives, *Gene*, 72, 313, 1988.

361. Le Doan, T., Perrouault, L., Chassignol, M., Thuong, N. T., and Helene, C., Sequence-targetted chemical modification of nucleic acids by complentary oligonucleotides covalently linked to porphyrins, *Nucleic Acids Res.*, 15, 8643, 1987.

362. Moser, H. and Dervan, P. B., Sequence specific cleavage of double strand DNA by triple strand formation, *Science*, 238, 645, 1987.

363. Lyamichev, V. I., Mirkin, S. M., Frank-Kamenetskii, M. D., and Cantor, C. R., A stable complex between homopyrimidine oligomers and the homologous regions of duplex DNAs, *Nucleic Acids Res.*, 16, 2165, 1988.

364. Marcus-Sekura, C. J., Woerner, A. M., Shinozuka, K., Zon, G., and Quinnan, G. V., Comparative inhibition of chloramphenicol acetyltransferase gene expression by antisense oligonucleotide analogues having alkyl phosphotriester, methylphosphonate and phosphorothioate linkages, *Nucleic Acids Res.*, 15, 5749, 1987.

365. Thuong, N. T., Asseline, U., Roig, V., Takasugi, M., and Helene, C., Oligo(α-deoxynucleotide)s covalently linked to intercalating agents: differential binding to ribo- and deoxyribopolynucleotides and stability towards nuclease digestion, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 5129, 1987.

366. Francois, J.-C., Saison-Behmoaras, T., Chassignol, M., Thuong, N. T., and Helene, C., Sequence-targeted cleavage of single- and double-stranded DNA by oligothymidylates covalently linked to 1,10-phenanthroline, *J. Biol. Chem.*, 264, 5891, 1989.

367. Francois, J.-C., Saison-Behmoraras, T., Thuong, N. T., and Helene, C., Inhibition of restriction en-

donuclease cleavage via triple helix formation by homopyrimidine oligonucleotides, *Biochemistry*, 28, 9617, 1989.

368. **Slama-Schwok, A., Rougee, M., Ibanez, V., Geacintov, N. E., Montenay-Garestier, T., Lehn, J.-M., and Helene, C.,** Interactions of the dimethyldiazaperopyrenium dication with nucleic acids. I. Binding to double-stranded polynucleotides, *Biochemistry*, 28, 3234, 1989.

369. **Perrouault, L., Asseline, U., Rivalle, C., Thuong, N. T., Bisagni, E., Giovannangeli, C., Le Doan, T., and Helene, C.,** Sequence-specific artificial photoinduced endonucleases based on triple-forming oligonucleotides, *Nature*, 344, 358, 1990.

370. **Cazenave, C., Stein, C. A., Loreau, N., Thuong, N. T., Neckers, L. M., Subasinghe, C., Helene, C., Cohen, J. S., and Toulme, J.-J.,** Comparative inhibitionn of rabbit globin mRNA translation by modified antisense oligodeoxynucleotides, *Nucleic Acids Res.*, 17, 4255, 1989.

371. **Ebright, R. H., Ebright, Y. W., Pendergast, P. S., and Gunasekera, A.,** Conversion of a helix-turn-helix motif sequence-specific DNA binding protein into a site-specific DNA cleavage agent, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 2882, 1990.

372. **Becker, M., Wang, Z., Grossmann, G., and Becherer, K. A.,** Genomic footprinting in mammalian cells with ultraviolet light, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 5315, 1989.

373. **Becker, M. M. and Wang, Z.,** B-A transitions within a 5S ribosomal RNA gene are highly sequence-specific, *J. Biol. Chem.*, 264, 4163, 1989.

374. **Cadet, J., Voituriez, L., Grand, A., Hruska, F. E., Vigny, P., and Kan, L.-S.,** Recent aspects of the photochemistry of nucleic acids and related model compounds, *Biochimie*, 67, 277, 1985.

375. **Glisin, V. R. and Doty, P.,** The effect of temperature on the cross-linking of deoxyribonucleic acid strands by ultraviolet radiation, *Biochim. Biophys. Acta*, 61, 458, 1962.

376. **Palecek, E., Lukasova, E., Vorlickova, M., and Ambrova, H.,** Conformational changes preceding DNA denaturation, *Stud., Biophys.*, 24/25, 123, 1970.

377. **Gale, J. M., Nissen, K. A., and Smerdon, M. J.,** UV-induced formation of pyrimident dimers in nucleosome core DNA is strongly modulated with a period of 10.3 bases, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 6644, 1987.

378. **Gale, J. M. and Smerdon, M. J.,** Photofootprinting for nucleosome core DNA in intact chromatin having different structural states, *J. Mol. Biol.*, 204, 949, 1988.

379. **Pehrson, J. R.,** Thymine dimer formation as a probe of the path of DNA in and between nucleosomes in intact chromatin, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 9149, 1989.

380. **Lyamichev, V. I., Frank-Kamenetskii, M. D., and Soyfer, V. N.,** Protection against UV-induced py-rimidine dimerization in DNA by triplex formation, *Nature*, 344, 568, 1990.

381. **Shetlar, M. D.,** Cross-linking of proteins to nucleic acids by ultraviolet light, *Photochem. Photobiol. Rev.*, 5, 105, 1980.

382. **Budowsky, E. I. and Abdurashidova, G. G.,** Polynucleotide protein cross-links induced by ultraviolet light and their use for structural investigation of nucleoproteins, *Progr. Nucleic Acids Res. Mol. Biol.*, 37, 1, 1989.

383. **Singer, P. and Wu, C.-W.,** Promoter search by *Escherichia coli* RNA polymerase on a circular DNA template, *J. Biol. Biochem.*, 262, 14178, 1987.

384. **Hockensmith, J. W., Kubase, W. L., Voracheck, W. R., and von Hippel, P. H.,** Laser cross-linking of nucleic acids to proteins. Methodology and first applications to the phage T4 DNA replication system, *J. Biol. Chem.*, 261, 3512, 1986.

385. **Panyutin, I. G., Kovalsky, O. I., and Budowsky, E. I.,** Irradiation of the template with high-intensity (pulse-laser) ultraviolet light results in DNA-polymerase termination events at deoxyguanosine residues, *FEBS Lett.*, 258, 274, 1989.

386. **Clore, G. M. and Groneborn, A. M.,** Determination of three-dimensional structures of protein and nucleic acids in solution by nuclear magnetic resonance spectroscopy, *Crit. Rev. Biochem.*, 24, 479, 1989.

387. **Amrein, M., Stasiak, A., Gross, H., Stoll, E., and Travaglini, G.,** Scanning tunneling microscopy of recA-DNA complexes coated with a conducting film, *Science*, 240, 514, 1988.

388. **Beebe, T. P., Jr., Wilson, T. E., Ogletree, D. F., Katz, J. E., Balhorn, R., Salmero, M. B., and Siekhaus, W. J.,** Direct observation of native DNA structures with the scanning tunneling microscope, *Science*, 243, 370, 1989.

389. **Cricenti, A., Selci, S., Felici, A. C., Generosi, R., Gori, E., Djaczenko, W., and Chicarotti, G.,** Molecular structure of DNA by scanning tunneling microscopy, *Science*, 245, 1226, 1989.

390. **Lilley, D. M. J., Sullivan, K. M., and Murchie, A. I. H.,** the extrusion of cruciform structures in supercoiled DNA — kinetics and mechanism, in *Nucleic Acids and Molecular Biology*, Vol. 1, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1987, 126.

391. **Lilley, D. M. and Murchie, A. I. H.,** The mechanism of salt-dependent cruciform extrusion in supercoiled DNA molecules, in *Highlights of Modern Biochemistry*, Vol. 1, Kotyk, A., Skoda, J., Paces, V., and Kostka, V., Eds., VSP International Science Publishers, Zeist, 1989, 73.

392. **Nejedly, K., Klysik, J., and Palecek, E.,** Super-coil-stabilized left-handed DNA in the plasmid (dA-dT)$_{16}$ insert formed in the presence of Ni^{2+}, *FEBS Lett.*, 243, 313, 1989.

393. **Platt, J. R.,** Possible separation of intertwined nu-

cleic acid chains by transfer twist, *Proc. Natl. Acad. Sci. U.S.A.*, 41, 181, 1955.

394. **Gierer, A.,** A model for DNA-protein interactions and the function of the operator, *Nature,* 212, 1480, 1966.

395. **Beerman, T. A. and Lebowitz, J.,** Further analysis of the altered secondary structure of superhelical DNA. Sensitivity to methylmercuric hydroxide a chemical probe for unpaired base, *J. Mol. Biol.,* 79, 451, 1973.

396. **Hsieh, T.-S. and Wang, J. C.,** Thermodynamic properties of superhelical DNAs, *Biochemistry,* 14, 527, 1975.

397. **Vologodskii, A. V., Anshelevich, V. V., Lukashin, A. V., and Frank-Kamenetskii, M. D.,** Statistical mechanics of supercoils and the torsional stiffness of the DNA double helix, *Nature,* 220, 294, 1979.

398. **Vologodskii, A. V. and Frank-Kamenetskii, M. D.,** Theoretical study of cruciform states in superhelical DNA, *FEBS Lett.,* 143, 257, 1982.

399. **Courey, A. J. and Wang, J. C.,** Cruciform formation in a negatively supercoiled DNA may be kinetically forbidden under physiological conditions, *Cell,* 33, 817, 1983.

400. **Lilley, D. M. J.,** DNA supercoiling and DNA structure, *Biochem. Soc. Trans.,* 14, 211, 1984.

401. **Hilbers, C. W., Haasnoot, C. A. G., de Bruin, S. H., Joordens, J. J. M., van der Marel, G. A., and van Boom, J. H.,** Hairpin formation in synthetic oligonucleotides, *Biochimie,* 67, 685, 1985.

402. **Blommers, M. J. J., Walters, J. A. L. I., Haasnoot, C. A. G., Aelen, J. M. A., van der Marel, G. A., van Boom, J. B., and Hilberts, C. W.,** Effects of base sequence on the loop folding in DNA hairpins, *Biochemistry,* 28, 7491, 1989.

403. **Haasnoot, C. A. G., Hilberts, C. W., van der Marel, G. A., van Boom, J. H., Singh, U. C., Pattabiraman, N., and Kollman, P. A.,** On loop-folding in nucleic acid hairpin-type structures, *J. Biomol. Struct. Dyn.,* 3, 843, 1986.

404. **Germann, M. W., Kalisch, B. W., Lundberg, P., Vogel, H. J., and van de Sande, J.,** Perturbation of DNA hairpins containing the EcoRI recognition site by hairpin loops of varying size and composition: physical (NMR and UV) and enzymatic (EcoRI) studies, *Nucleic Acids Res.,* 18, 1498, 1990.

405. **Wemmer, D. E., Wand, A. J., Seeman, N. C., and Kallenbach, N. R.,** NMR analysis of DNA junctions: imino proton NMR studies of individual arms and intact junction, *Biochemistry,* 24, 5745, 1985.

406. **Cooper, J. P. and Hagerman, P. J.,** Gel electrophoretic analysis of the geometry of a DNA four-way junction, *J. Mol. Biol.,* 198, 711, 1987.

407. **Gough, G. W. and Lilley, D. M. J.,** DNA bending induced by cruciform formation, *Nature,* 313, 1480, 1985.

408. **Diekmann, S. and Lilley, D. M. J.,** The anomalous gel migration of a stable cruciform: temperature and salt dependence, and some comparisons with curved DNA, *Nucleic Acids Res.,* 15, 5765, 1987.

409. **Parsons, C. A., Murchie, A. I. H., Lilley, D. M. J., and West, S. C.,** Resolution of model Holliday junctions by yeast endonuclease: effect of DNA structure and sequence, *EMBO J.,* 8, 239, 1989.

410. **Duckett, D. R. and Lilley, D. M. J.,** The three-way DNA junction is a Y-shaped molecule in which there is no helix-helix stacking, *EMBO J.,* 9, 1659, 1990.

411. **von Kitzing, E., Lilley, D. M. J., and Diekmann, S.,** The stereochemistry of a four-way DNA junction: a theoretical study, *Nucleic Acids Res.,* 18, 2671, 1990.

412. **Murchie, A. I. H., Carter, W. A., Portugal, J., and Lilley, D. M. J.,** The tertiary structure of the four-way DNA junction affords protection against DNase I cleavage, *Nucleic Acids Res.,* 18, 2599, 1990.

413. **Murchie, A. I. H., Clegg, R. M., von Kitzing, E., Duckett, D. R., Diekmann, S., and Lilley, D. M. J.,** Fluorescence energy transfer shows that the four-way DNA junction is a right-handed cross of antiparallel molecules, *Nature,* 341, 763, 1990.

414. **Duckett, D. R., Murchie, A. I. H., Clegg, R. M., Zechel, A., von Kitzing, E., Diekmann, S., and Lilley, D. M. J.,** The structure of the Holliday junctions, in *Structure and Methods, Vol. 1: Human Genome Initiative and DNA Recombination,* Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 157.

415. **Wemmer, D. E., Chou, S. H., Hare, D. R., and Reid, B. R.,** Duplex-hairpin transition in DNA: NMR studies on CGCGTATACGCG, *Nucleic Acids Res.,* 13, 3755, 1985.

416. **Chattopathyaya, R., Ikuta, S., Grzeskowiak, K., and Dickerson, R. E.,** X-ray structure of a DNA hairpin molecule, *Nature,* 334, 175, 1988.

417. **Lilley, D. M. J. and Hallam, L. R.,** The thermodynamics of the ColE1 cruciform: comparisons between probing and topological experiments using single topoisomers, *J. Mol. Biol.,* 180, 179, 1984.

418. **Mizuuchi, K., Mizuuchi, M., and Gellert, M.,** Cruciform structures in palindromic DNA are favored by DNA supercoiling, *J. Mol. Biol.,* 156, 229, 1982.

419. **Gellert, M., O'Dea, M. H., and Mizuuchi, K.,** Slow cruciform transitions in palindromic DNA, *Proc. Natl. Acad. Sci. U.S.A.,* 80, 5545, 1983.

420. **Sinden, R. R. and Pettijohn, D. E.,** Cruciform transition in DNA, *J. Biol. Chem.,* 259, 6593, 1984.

421. **Lilley, D. M. J.,** The kinetic properties of cruciform extrusion are determined by DNA base-sequence, *Nucleic Acids Res.,* 13, 1443, 1985.

422. **Courey, A.,** Influence of DNA sequence and supercoiling on the process of cruciform formation, *J. Mol. Biol.,* 202, 35, 1988.

423. **Zheng, G. and Sinden, R. R.,** Effect of base composition at the center of inverted repeated DNA sequences on cruciform transition in DNA, *J. Biol. Chem.,* 263, 5356, 1988.

424. **Murchie, A. I. and Lilley, D. M. J.,** Base meth-

ylation and local DNA helix stability effect on the kinetics of cruciform extrusion, *J. Mol. Biol.*, 205, 593, 1989.

425. **Merchlinsky, M., Garon, C. F., and Moss, B.,** Molecular cloning and sequence of the concatemer junction from vaccinia virus replicative DNA, *J. Mol. Biol.*, 199, 399, 1988.

426. **Sullivan, K. M. and Lilley, D. M. J.,** Influence of cation size and charge on the extrusion of a salt-dependent cruciform, *J. Mol. Biol.*, 193, 397, 1987.

427. **Sullivan, K. M., Murchie, A. I. H., and Lilley, D. M. J.,** Long range structural communication between sequences in supercoiled DNA, *J. Biol. Chem.*, 263, 13074, 1988.

428. **Sullivan, K. M. and Lilley, D. M.,** Helix stability and the mechanism of cruciform extrusion in supercoiled DNA molecules, *Nucleic Acids Res.*, 16, 1079, 1988.

429. **Sullivan, K. M. and Lilley, D. M. J.,** A dominant influence of flanking sequences on a local structural transition in DNA, *Cell,* 47, 817, 1986.

430. **Wang, Y. and Sauerbier, W.,** Flanking AT-rich sequences may lower the activation energy of cruciform in supercoiled DNA, *Biochem. Biophys. Res. Commun.*, 158, 423, 1989.

431. **Voloshin, O. N., Shlyakhtenko, L. S., and Lyubchenko, Y. L.,** Localization of melted regions in supercoiled DNA, *FEBS Lett.*, 243, 377, 1989.

432. **Glover, J. N. M., Haniford, D. B., and Pulleyblank, D. E.,** Intermediate range effects in DNA I: low pH/stress induced conformational changes in the vicinity of an extrudes d(AT)n·d(AT)n cruciform, *Nucleic Acids Res.*, 16, 5473, 1988.

433. **Panyutin, I., Lyamichev, V., and Mirkin, S.,** A structural transition in d(AT)n·d(AT)n inserts within superhelical DNA, *J. Biomol. Struct. Dyn.*, 2, 1221, 1985.

434. **Lilley, D. M. J. and McClellan, J. A.,** Alternating adenine-thymine tracts in supercoiled and linear DNA molecules, in *Structure and Expression, Vol. 2: DNA and Its Drug Complexes,* Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1987, 73.

435. **Rajagopalan, M., Rahmouni, A. R., and Wells, R. D.,** Flanking AT-rich tracts cause a structural distortion in Z-DNA in plasmids, *J. Biol. Chem.*, 265, in press.

436. **Burd, J. F., Larson, J. E., and Wells, R. D.,** Further studies on telestability in DNA, *J. Biol. Chem.*, 250, 6002, 1975.

437. **Englander, S. W., Kallenbach, N. R., Heeger, A. J., Krumhansl, J. A., and Litwin, S.,** Nature of the open state in long polynucleotide double helices: possibility of soliton excitations, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 7222, 1980.

438. **Sobell, M. H., Sakore, T. D., Jain, S. C., Banerjee, A., Bhandary, K. K., Reddy, B. S., and Lozansky, E. D.,** β-Kinked DNA — a structure that gives rise to drug intercalation and DNA bending — and its wider significance in determining the premelting and melting behavior of DNA, *Cold Spring Harbor Symp. Quant. Biol.*, 47, 293, 1982.

439. **Naylor, L. H., Lilley, D. M. J., and van de Sande, J. H.,** Stress-induced cruciform formation in a cloned d(CATG)$_{10}$ sequence, *EMBO J.*, 5, 2407, 1986.

440. **McLean, M. J. and Wells, R. D.,** The role of DNA sequence in the formation of Z-DNA versus cruciforms in plasmids, *J. Biol. Chem.*, 263, 7370, 1988.

441. **Vlach, J. and Sponar, J.,** Flexibility of A·T pairs in left-handed DNA helices, *J. Biomol. Struct. Dyn.*, 7, 1083, 1990.

442. **Jovin, T. M., Soumpasis, D. M., and McIntosh, L. P.,** The transition between B-DNA and Z-DNA, *Annu. Rev. Phys. Chem.*, 38, 521, 1987.

443. **Soumpasis, D. M. and Jovin, T. M.,** Energetics of the B-Z DNA transition, in *Nucleic Acids and Molecular Biology,* Vol. 1, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1987, 86.

444. **Leng, M.,** Left-handed Z-DNA, *Biochim. Biophys. Acta,* 825, 339, 1985.

445. **McLean, M. J. and Wells, R. D.,** The role of sequence in the stabilization of left-handed DNA helices *in vitro* and *in vivo, Biochim. Biophys. Acta,* 950, 243, 1988.

446. **Blaho, J. A. and Wells, R. D.,** Left-handed Z-DNA and genetic recombination, *Progr. Nucleic Acids Res. Mol. Biol.*, 37, 107, 1989.

447. **Wells, R. D., Amirhaeri, S., Blaho, J. A., Collier, D. A., Dohrman, A., Griffin, J. A., Hanvey, J. C., Hsieh, W.-T., Joworski, A., Larson, J. E., McLean, M. J., Rahmouni, A., Rajagopalan, M., Shimizu, M., Wohlrab, F., and Zacharias, W.,** Biology and chemistry of Z-DNA and triplexes, in *The Bacterial Chromosome,* Drlica, K. and Riley, M., Eds., American Society for Microbiology, Washington, D.C., 1990, 1987.

448. **Vorlickova, M., Kypr, Z. J., Stokrova, S., and Sponar, J.,** A Z-like form of poly (dA-dC)·poly(dG-dT) in solution?, *Nucleic Acids Res.*, 10, 1071, 1982.

449. **Zimmer, C., Marck, C., and Guschlbauer, W.,** Z-DNA and other non-B-DNA structures are reversed to B-DNA by interaction with netropsin, *FEBS Lett.*, 154, 156, 1983.

450. **Ridoux, J.-P., Liquier, J., and Taillandier, E.,** Z form of poly d(A-C)·poly d(G-T) in solution studied by Raman spectroscopy, *Nucleic Acids Res.*, 15, 5813, 1987.

451. **Thomas, T. J. and Messner, R. P.,** A left-handed (Z) conformation of poly(dA-dC)·poly(dG-dT) induced by polyamines, *Nucleic Acids Res.*, 14, 6721, 1986.

452. **Haniford, D. B. and Pulleybank, D. E.,** Facile transition of poly [d(TG)°d(CA)] into a left-handed helix in physiological conditions, *Nature,* 302, 632, 1983.

453. **Vorlickova, M., Kypr, J., Kleinwachter, V., and Palecek, E.,** Salt-induced conformational changes of poly(dA-dT), *Nucleic Acids Res.*, 8, 3965, 1980.

454. **Kypr, J. and Vorlickova, M.,** Conformations of DNA duplexes containing (dA-dT) sequences of bases and their possible biological significance, in *Structure and Expression,* Vol. 2, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1988, 105.

455. **Adam, S., Liquier, J., Taboury, J. A., and Taillandier, E.,** Right- and left-handed helixes of poly[d(A-T)]·poly[d(A-T)] investigated by infrared spectroscopy, *Biochemistry,* 25, 3220, 1986.

456. **Bourtayre, P., Liquier, J., Pizzorni, L., and Taillandier, E.,** Z form of poly d(A-T)·poly d(A-T) in solution studied by CD and UV spectroscopies, *J. Biomol. Struct. Dyn.,* 5, 97, 1987.

457. **Ridoux, J. P., Liquier, J., and Taillandier, E.,** Raman spectroscopy of Z-form poly[d(A-T)]·poly[d(A-T)], *Biochemistry,* 27, 3874, 1988.

458. **Miskovsky, P., Chinsky, L., Laigle, A., and Turpin, P. Y.,** The Z-conformation of poly(dA-dT)·poly(dA-dT) in solution as studied by ultraviolet resonance Raman spectroscopy, *J. Biomol. Struct. Dyn.,* 7, 623, 1989.

459. **Klysik, J., Zacharias, W., Galazka, G., Kwinkowski, M., Uznanski, B., and Okruszek, A.,** Structural interconversion of alternating purine-pyrimide inverted repeats cloned in supercoiled plasmids, *Nucleic Acids Res.,* 16, 6915, 1988.

460. **Wells, R. D., Amirhaeri, S., Blaho, J. A., Collier, D. A., Hanvey, J. C., Hsieh, W.-T., Jaworski, A., Klysik, J., Larson, J. E., McLean, M. J., Wohlrab, F., and Zacharias, W.,** Unusual DNA structures and the probes used for their detection, in *Unusual DNA Structures,* Wells, R. D. and Harvey, S. C., Eds., Birmingham, 1988, 1.

461. **Arnott, S. and Chandrasekaran, R.,** Fibrous polynucleotide duplexes have very polymorphic secondary structures, in *Biomolecular Sterodynamics,* Vol. 11, Sarma, R. H., Ed., Adenine Press, Albany, NY, 1981, 99.

462. **Arnott, S., Chandrasekaran, R., Hall, I. H., Puigjaner, L. C., Walker, J. K., and Wang, M.,** DNA secondary structures: helices, wrinkles, and junctions, *Cold Spring Harbor Symp. Quant. Biol.,* 47, 53, 1982.

463. **Sasisekharan, V., Bansal, M., Bramachari, S. K., and Gupta, G.,** Conformational flexibility of DNA: a theoretical formalism, in *Proc. 2nd SUNYA Conversation in the Discipline Biomolecular Stereodynamics,* Vol. 1, Sarma, R. H., Ed., Adenine Press, Albany, NY, 1981, 123.

464. **Hingerty, B. and Broyde, S.,** AAF linked to the guanine amino group: a B-Z junction, *Nucleic Acids Res.,* 11, 3241, 1983.

465. **Harvey, S. C.,** DNA structural dynamics: longitudinal breathing as a possible mechanism for the B-Z transition, *Nucleic Acids Res.,* 11, 4867, 1983.

466. **Nejedly, K., Matyasek, R., and Palecek, E.,** Site-specific chemical modification of B-Z junctions in supercoiled DNA as detected by nuclease S1 diges-tion, inhibition of restriction cleavage and nucleotide sequencing, *J. Biomol. Struct. Dyn.,* 6, 161, 1988.

467. **Galazka, G., Klysik, J., Kwinkowski, M., Szala, S., and Palecek, E.,** B-Z transition within the mouse repetitive sequence, in *Biophosphates and Their Analogues — Synthesis, Structure, Metabolism and Activity,* Bruzik, K. S. and Stec, W. J., Eds., Elsevier, Amsterdam, 1987, 515.

468. **Johnston, B. J.,** Chemical probing of the B-Z transition in negatively supercoiled DNA, *J. Biomol. Struct. Dyn.,* 6, 153, 1988.

469. **Johnston, B. H., Ohara, W., and Rich, A.,** Stochastic distribution of a short region of Z-DNA with a long repeated sequence in negatively supercoiled plasmids, *J. Biol. Chem.,* 263, 4512, 1988.

470. **Vogt, N., Rousseau, N., Leng, M., and Malfoy, B.,** A study of the B-Z transition of the AC-rich region of the repeat unit of a satellite DNA from Cebus by means of chemical probes, *J. Biol. Chem.,* 263, 11826, 1988.

471. **McLean, M. J., Larson, J. E., Wohlrab, F., and Wells, R. D.,** Reaction conditions affect the specificity of bromoacetaldehyde as a probe for DNA cruciforms and B-Z junctions, *Nucleic Acids Res.,* 15, 6917, 1987.

472. **Porschke, D.,** B-Z DNA junctions are neither highly flexible nor strongly bent, *Biopolymers,* 26, 1971, 1987.

473. **Sheardy, R. D.,** Preliminary spectroscopic characterization of a synthetic DNA oligomer containing a B-Z junction at high salt, *Nucleic Acids Res.,* 16, 1153, 1988.

474. **Sheardy, R. D. and Winkle, S. A.,** Temperature-dependent CD and NMR studies on a synthetic oligonucleotide containing a B-Z junction at high salt, *Biochemistry,* 28, 720, 1989.

475. **Adam, S., Ridoux, J. P., Bourtayre, P., Taillandier, E., Pochet, S., Huynh-Dinh, T., and Igolen, J.,** IR and UV studies on stability and conformations of short DNA duplexes containing a no-base residue: coexistence of B and Z conformations, *J. Biomol. Struct. Dyn.,* 6, 167, 1988.

476. **Zacharias, W., O'Connor, T. R., and Larson, J.,** Methylation of cytosine in the 5-position alters the structural and energetic properties of the supercoil-induced Z-helix and of the B-Z junction, *Biochemistry,* 27, 2970, 1988.

477. **Kochel, T. J. and Sinden, R. R.,** Hyperreactivity of B-Z junctions to 4,5',8-trimethylpsoralen photobinding assayed by an exonuclease III/photoreversal mapping procedures, *J. Mol. Biol.,* 205, 91, 1989.

478. **Hamada, H. and Bustin, M.,** Hierarchy of binding sites for chromosomal proteins HMG 1 and 2 in supercoiled deoxyribonucleic acid, *Biochemistry,* 24, 1428, 1985.

479. **Lohman, T. M., Bujalowski, W., and Overman, L. B.,** *E. coli* single strand binding protein: a new look at helix-destabilizing proteins, *Trends Biochem. Sci.,* 13, 250, 1988.

480. Felsenfeld, G., Davies, D. R., and Rich, A., Formation of a three-stranded polynucleotide molecule, *J. Am. Chem. Soc.*, 79, 2023, 1957.

481. Michelson, A. M., Massoulie, J., and Guschlbauer, W., Synthetic polynucleotides, *Progr. Nucleic Acids Res. Mol. Biol.*, 6, 83, 1967.

482. Guschlbauer, W., Polynucleotides, in *Encyclopedia of Polymer Science and Engineering*, Vol. 12, John Wiley & Sons, New York, 1988, 699.

483. Lipsett, M. N., Complex formation between polycytidylic acid and guanine oligonucleotides, *J. Biol. Chem.*, 239, 1256, 1964.

484. Courtois, Y., Fromageot, P., and Guschlbauer, W., Protonated polynucleotide structures. A. An optical rotatory dispersion study of the protonation of DNA, *Eur. J. Biochem.*, 6, 493, 1968.

485. Thiele, D. and Guschlbauer, W., Polynucleotides protones. VII. Transitions thermiques entre different complexes de l'acide polyinosinique et de l'polycytidylique en milieu acide, *Biopolymers*, 8, 361, 1969.

486. Marck, C. and Thiele, D., Poly(dG)·poly(dC) at neutral and alkaline pH: the formation of triple stranded poly(dG)·poly(dG)·poly(dC), *Nucleic Acids Res.*, 5, 1017, 1978.

487. Miller, J. H. and Sobell, H. M., A molecular model for gene repression, *Proc. Natl. Acad. Sci. U.S.A.*, 55, 1201, 1966.

488. Inman, R. B. and Baldwin, R. L., Helix-random coil transition in DNA homopolymer pairs, *J. Mol. Biol.*, 8, 452, 1964.

489. Thiele, D., Marck, C., Schneider, C., and Guschlbauer, G., Protonated polynucleotide structures. The acid-base hysteresis of poly(dG)·poly(dC), *Nucleic Acids Res.*, 5, 1997, 1978.

490. Chamberlin, M. J., Comparative properties of DNA, RNA, and hybrid homopolymer pairs, *Fed. Proc.*, 24, 1446, 1965.

491. Riley, M., Maling, B., and Chamberlin, M. J., Physical and chemical characterization of two- and three-stranded adenine-thymine and adenine-uracil homopolymer complexes, *J. Mol. Biol.*, 20, 359, 1966.

492. Morgan, A. R. and Wells, R. D., Specificity of the three-stranded complex formation between double-stranded DNA and single-stranded RNA containing repeating nucleotide sequences, *J. Mol. Biol.*, 37, 63, 1968.

493. Arnott, S. and Selsing, E., Structures for the polynucleotide complexes poly(dA)·poly(dT) and poly(dT)·poly(dA)·poly(dT), *J. Mol. Biol.*, 88, 509, 1974.

494. Gellert, M., Lipsett, M. N., and Davies, D. R., Helix formation by guanylic acid, *Proc. Natl. Acad. Sci. U.S.A.*, 48, 2013, 1962.

495. Sarkar, P. D. and Yang, J. T., Optical activity of 5'-guanylic acid gel, *Biochem. Biophys. Res. Commun.*, 20, 346, 1965.

496. Chantot, J.-F. and Guschlbauer, W., Physico-chemical properties of nucleosides. Gel formation by 8-bromoguanosine, *FEBS Lett.*, 4, 173, 1969.

497. Chantot, J.-F., Sarocchi, M.-T., and Guschlbauer, W., Physico-chemical properties of nucleosides. Gel formation by guanosine and its analogues, *Biochimie*, 53, 347, 1971.

498. Chantot, J.-F. and Guschlbauer, W., Mechanism of gel formation by guanine nucleosides, in *The Purines: Theory and Experiment, Vol. 4*, The Jerusalem Symposia on Quantitative Chemistry and Biochemistry, Jerusalem, 1972, 205.

499. Chantot, J.-F., Nucleoside conformations. IX. A calorimetric study of gel formation by guanylic acids, *Arch. Biochem. Biophys.*, 153, 347, 1972.

500. Pinnavaia, T. J., Miles, H. T., and Becker, E. D., Self-assembled 5'-guanosine monophosphate: nuclear magnetic resonance evidence for a regular, ordered structure and slow chemical exchange, *J. Am. Chem. Soc.*, 97, 7198, 1975.

501. Thiele, D. and Guschlbauer, W., The structures of polyinosinic acid, *Biophysik*, 9, 261, 1973.

502. Zimmerman, S. B., Cohen, G. H., and Davies, D. R., X-ray fiber diffraction and model-building study of polyguanylic acid and polyinosinic acid, *J. Mol. Biol.*, 92, 181.

503. Miles, H. T. and Frazier, J., Poly(I) helix formation. Dependence on size-specific complexing to alkali metal ions, *J. Am. Chem. Soc.*, 100, 8037, 1978.

504. Lee, J. S., Evans, D. H., and Morgan, A. R., Polypurine DNAs and RNAs form secondary structures which may be tetra-stranded, *Nucleic Acids Res.*, 8, 4306, 1980.

505. Frank-Kamenetskii, M., DNA structure — the turn of the quadruplex, *Nature*, 342, 737, 1989.

505a. Guschlbauer, W., Chantot, J.-F., and Thiele, D., Four-stranded structures 25 years later: from guanosine gels to telomer DNA, *J. Biomol. Struct. Dyn.*, 8, 491, 1990.

506. Wells, R. D., Collier, D. A., Hanvey, J. C., Shimizu, M., and Wohlrab, F., The chemistry and biology of unusual DNA structures adopted by oligopurine·oligopyrimidine sequences, *FASEB J.*, 2, 2939, 1988.

507. McKeon, C., Schmidt, A., and Cromobrugghe, B., A sequence conserved in both the chicken and mouse a 2(I)collagen promoter contains sites sensitive to S1 nuclease, *J. Mol. Biol.*, 259, 6636, 1984.

508. Mace, H. A. F., Pelham, H. R. B., and Travers, A. A., Association of an S1 nuclease-sensitive structure with short direct repeats 5' of *Drosophila* heat shock genes, *Nature*, 304, 555, 1983.

509. Cantor, C. R. and Efstratiadis, A., Possible structures of homopurine-homopyrimidine S1-hypersensitive sites, *Nucleic Acids Res.*, 12, 8059, 1984.

510. Lee, J. S., Johnson, D. A., and Morgan, R. A., Complexes formed by (pyrimidine)n·(purine)n DNAs on lowering the pH are three-stranded, *Nucleic Acids Res.*, 6, 3073, 1979.

511. **Christophe, D., Carber, B., Bacolla, A., Targovnik, H., Pohl, V., and Vassat, G.**, An unusually long poly(purine)-poly(pyrimidine) sequence is located upstream from the human thyreoglobulin gene, *Nucleic Acids Res.*, 13, 5127, 1985.

512. **Lyamichev, V. I., Mirkin, S. M., and Frank-Kamenetskii, M. D.**, Structures of homopurine-homopyrimidine tract in superhelical DNA, *J. Biomol. Struct. Dyn.*, 3, 667, 1986.

513. **Hanvey, J. C., Klysik, J., and Wells, R. D.**, Influence of DNA sequence on the formation of non-B right-handed helices in oligopurine·oligopyrimidine inserts in plasmids, *J. Biol. Chem.*, 263, 7386, 1988.

514. **Hanvey, J. C., Shimizu, M., and Wells, R. D.**, Intramolecular DNA triplexes in supercoiled plasmids. II. Effect of base composition and noncentral interruptions on formation and stability, *J. Biol. Chem.*, 264, 5950, 1989.

515. **Lyamichev, V. I., Mirkin, S. M., Kumarev, V. P., Baranova, L. V., Vologodskii, A. V., and Frank-Kamenetskii, M. D.**, Energetics of the B-H transition in supercoiled DNA carrying d(CT)$_x$·d(AG)$_x$ and d(C)$_n$·d(G)$_n$ inserts, *Nucleic Acids Res.*, 17, 9417, 1989.

516. **Dahlberg, J. E.**, personal communication, 1990.

517. **Htun, H. and Dahlberg, J. E.**, The structure and formation of triple-stranded H-DNA, in *Structure and Methods, Vol. 3: DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 185.

518. **Caddle, M. S., Lussier, R. H., and Heintz, N. H.**, Intramolecular DNA triplexes, bent DNA and DNA unwinding elements in the initiation region of an amplified dihydrofolate reductase replicon, *J. Mol. Biol.*, 211, 19, 1990.

519. **Lyamichev, V. I., Mirkin, S. M., and Frank-Kamenetskii, M. D.**, Structure of d(G)$_n$·(dC)$_n$ under superhelical stress and acid pH, *J. Biomol. Struct. Dyn.*, 5, 275, 1987.

520. **Mirkin, S. M., Lyamichev, V. I., Drushlyak, K. N., Dobrynin, V. N., Filippov, S. A., and Frank-Kamenetskii, M. D.**, DNA H form requires a homopurine-homopyrimidine mirror repeat, *Nature*, 333, 495, 1987.

521. **Shimizu, M., Hanvey, J. C., and Wells, R. D.**, Intramolecular DNA triplexes in supercoiled plasmids. I. Effect of loop size on formation and stability, *J. Biol. Chem.*, 264, 5944, 1989.

522. **Parniewski, P., Galazka, G., Wilk, A., and Klysik, J.**, Complex structural behavior of oligopurine-oligopyrimidine sequence cloned within the supercoiled plasmid, *Nucleic Acids Res.*, 17, 617, 1989.

523. **Parniewski, P., Kwinkowski, M., Wilk, A., and Klysik, J.**, Dam methyltransferase sites located within the loop region of the oligopurine-oligopyrimidine sequences capable of forming H-DNA are undermethylated *in vivo*, *Nucleic Acids Res.*, 18, 605, 1990.

524. **Fox, K. R.**, Long (dA)n·(dT)n tracts can form intramolecular triplexes under superhelical stress, *Nucleic Acids Res.*, 18, 5387, 1990.

525. **Stokrova, J., Vojtiskova, M., and Palecek, E.**, Electron microscopy of supercoiled pEJ4 DNA containing homopurine·homopyrimidine sequences, *J. Biomol. Struct. Dyn.*, 6, 891, 1989.

526. **Htun, H. and Dahlberg, J. E.**, Topology and formation of triple-stranded H-DNA, *Science*, 243, 1571, 1989.

526a. **Shimizu, M., Hanvey, J. C., and Wells, R. D.**, Multiple non-B-DNA conformations of polypurine polypyrimidine sequences in plasmids, *Biochemistry*, 29, 4704, 1990.

526b. **Collier, D. A. and Wells, R. D.**, Effect of length, supercoiling, and pH on intramolecular triplex formation, *J. Biol. Chem.*, 265, 10652, 1990.

527. **Collier, D. A., Griffin, J. A., and Wells, R. D.**, Non-B right-handed DNA conformations of homopurine·homopyrimidine sequences in the murine immunoglobulin C$_\alpha$ switch region, *J. Biol. Chem.*, 263, 7397, 1988.

528. **Weinreb, A., Collier, D. A., Birshtein, B. K., and Wells, R. D.**, Left-handed Z-DNA and intramolecular triplex formation at the site of an unequal sister chromatid exchange, *J. Biol. Chem.*, 265, 1352, 1990.

529. **Howard, F. B., Frazier, F., Lipsett, M. N., and Miles, H. T.**, Infrared demonstration of two- and three-strand helix formation between poly C and guanosine mononucleotides and oligonucleotides, *Biochem. Biophys. Res. Commun.*, 17, 93, 1964.

530. **Bernues, J., Beltran, R., Casasnovas, J. M., and Azorin, F.**, Structural polymorphism of homopurine-homopyrimidine sequences: the secondary DNA structure adopted by a d(GA·CT)$_{22}$ sequence in the presence of zinc ions, *EMBO J.*, 8, 2087, 1989.

530a. **Bernues, J., Beltran, R., Casasnovas, J. M., and Azorin, F.**, DNA-sequence and metal-ion specificity of the formation of *H-DNA, *Nucleic Acids Res.*, 18, 4067, 1990.

531. **Broitman, S. L., Im, D. D., and Fresco, J. R.**, Formation of the triple-stranded polynucleotide helix, poly(A·A·U), *Proc. Natl. Acad. Sci. U.S.A.*, 84, 5120, 1987.

532. **Marck, J., Thiele, D., Schneider, C., and Guschlbauer, G.**, Protonated polynucleotides structures. CD study of the acid-base titration of poly(dG)·poly(dC), *Nucleic Acids Res.*, 5, 1979, 1978.

533. **McCarthy, J. C. and Heywood, S. M.**, A long polypyrimidine/polypurine tract induces an altered DNA conformation on the 3′ coding region of the adjacent myosin heavy chain gene, *Nucleic Acids Res.*, 15, 8069, 1987.

534. **Antao, V. P., Gray, D. M., and Ratliff, R. L.**, CD of six different conformational rearrangements of poly[d(A-G)·d(C-T)] induced by low pH, *Nucleic Acids Res.*, 16, 719, 1988.

535. **Kohwi-Shigematsu, T.**, personal communication, 1990.

536. **Kohwi-Shigematsu, T. and Kohwi, Y.**, The intramolecular poly(dG)-poly(dG)-poly(dC) triple helix structure forms at a neutral pH in the presence of

Mg^{++} ions, in *Structure and Methods, Vol. 3: DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 225.

537. **Panyutin, I. G., Kowalsky, O. I., and Budowsky, E. I.,** Magnesium-dependent supercoiling-induced transition in (dG)n (dC)n stretches and formation of a new G-structure by (dG)n strand, *Nucl. Acid. Res.*, 17, 8257, 1989.

538. **Usdin, K. and Furano, A. V.,** The structure of the guanine-rich polypurine:polypyrimidine sequence at the right end of the Rat L1 (LINE) element, *J. Biol. Chem.*, 264, 15681, 1989.

539. **Sarocchi-Landousy, M.-T., Haas, B. L., and Guschlbauer, W.,** Interaction of oligoribocytidylates with T7 DNA in neutral and acid media, *Biochemistry*, 16, 5414, 1977.

540. **Cooney, M., Czernuszewicz, G., Postel, E. H., Flint, S. J., and Hogan, M. E.,** Site-specific oligonucleotide binding represses transcription of the human c-*myc* gene *in vitro*, *Science*, 241, 456, 1988.

541. **Strobel, S. A. and Dervan, P. B.,** Site-specific cleavage of a yeast chromosome by oligonucleotide-directed triple-helix formation, *Science*, 249, 73, 1990.

542. **Arnott, S., Bond, P. J., Selsing, E., and Smith, P. J. C.,** Models of triple-stranded polynucleotides with optimised stereochemistry, *Nucleic Acids Res.*, 3, 2459, 1976.

543. **Rajagopal, P. and Feigon, J.,** NMR studies of triple-strand formation from the homopurine-homopyrimidine deoxyribonucleotides d(GA)$_4$ and d(TC)$_4$, *Biochemistry*, 28, 7859, 1989.

544. **Rajagopal, P. and Feigon, J.,** Triple-strand formation in the homopurine:homopyrimidine DNA oligonucleotides d(G-A)$_4$ and d(T-C)$_4$, *Nature*, 339, 637, 1989.

545. **Feigon, J., Gilbert, D., Rajogopal, P., Wang, E., van der Marel, G. A., and van Boom, J. H.,** Triplexes, duplexes, and homopurine complexes formed from homopurine and homopyrimidine DNA oligonucleotides, in *Structure and Methods, Vol. 3: DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 207.

546. **Pilch, D. S., Levenson, C., and Shafer, R. H.,** Structural analysis of the (dA)$_{10}$·2(dT)$_{10}$ triplex helix, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 1942, 1990.

547. **de los Santos, C., Rosen, M., and Patel, D.,** NMR studies of DNA (R$^+$)$_n$·(Y$^-$)$_n$·(Y$^+$)$_n$ triple helices in solution: imino and amino proton markers of T·A·T and C·G·C$^+$ base-triple formation, *Biochemistry*, 28, 7282, 1989.

548. **Sklenar, V. and Feigon, J.,** Formation of a stable triplex from a single DNA strand, *Nature*, 345, 836, 1990.

549. **Manzini, G., Xodo, L. E., Gasparotto, D., Quadrifoglio, F., van der Marel, G. A., and van Boom, J. H.,** Triple helix formation by oligopurine-oligopyrimidine DNA fragments, *J. Mol. Biol.*, 213, 833, 1990.

550. **Xodo, L. E., Manzini, G., and Quadrifoglio, F.,** Spectroscopic and calorimetric investigation on the DNA triplex formed by d(CTCTTCTTTCTTTCTTTCTTCTC) and d(GAGAAGAAAGA) at acidic pH, *Nucleic Acids Res.*, 18, 3557, 1990.

551. **Campos, J. L. and Subirana, J. A.,** The complex of poly(dG)·poly(dC) with arginine: stabilization of the B form and transition to multistranded structures, *J. Biomol. Struct. Dyn.*, 5, 15, 1987.

552. **Kilpatrick, M. W., Torri, A., Kang, D. S., Engler, J. A., and Wells, R. D.,** Unusual DNA structures in the adenovirus genome, *J. Biol. Chem.*, 261, 11350, 1986.

553. **Wohlrab, F., McLean, M. J., and Wells, R. D.,** The segment inversion site of herpes simplex virus type 1 adopts a novel DNA structure, *J. Biol. Chem.*, 262, 6407, 1987.

554. **Wohlrab, F. and Wells, R. D.,** Slight changes in conditions influence the family of non-B-DNA conformations of the herpes simplex virus type 1 DR2 repeats, *J. Biol. Chem.*, 264, 8207, 1989.

555. **Higgins, N. P., Collier, D. A., Kilpatrick, M. W., and Krause, H. M.,** Supercoiling and integration host factor change the DNA conformation and alter the flow of convergent transcription in phage mu, *J. Biol. Chem.*, 264, 3035, 1989.

556. **Blackburn, E. H. and Szostak, J. W.,** The molecular structure of centromeres and telomeres, *Annu. Biochem.*, 53, 163, 1984.

557. **Blackburn, E. H.,** Telomeres: do the ends justify the means?, *Cell*, 37, 7, 1984.

558. **Cech, T. R.,** G-strings at chromosome ends, *Nature*, 332, 777, 1988.

559. **Henderson, E., Hardin, C. C., Walk, S. K., Tinoco, I., Jr., and Blackburn, E. H.,** Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine·guanine base pairs, *Cell*, 51, 899, 1987.

560. **Budarf, M. and Blackburn, E.,** S1 nuclease sensitivity of a double-stranded telomeric DNA sequence, *Nucleic Acids Res.*, 15, 6273, 1987.

561. **Lyamichev, V. I., Mirkin, S. M., Danilevskaya, O. N., Voloshin, O. N., Balatskaya, S. V., Dobrynin, V. N., Filippov, S. A., and Frank-Kamenetskii, M. D.,** An unusual DNA structure detected in a telomeric sequence under superhelical stress and at low pH, *Nature*, 339, 634, 1989.

561a. **Lyamichev, V. I., Mirkin, S. M., Danilevskaya, O. N., Voloshin, L. N., Balatskaya, S. V., Dobrynin, V. N., Filippov, S. A., and Frank-Kamenetskii, M. D.,** Telomeric sequence under superhelical stress and low pH: a novel DNA structure with non-Watson-Crick pairing, in *Structure and Methods, Vol. 3: DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Albany, NY, 1990, 175.

562. **Sen, D. and Gilbert, W.,** Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis, *Nature*, 334, 364, 1988.

563. **Sundquist, W. I. and Klug, A.,** Telomeric DNA

dimerizes by formation of guanine tetrads between hairpin loops, *Nature*, 342, 825, 1989.

564. **Williamson, J. R., Raghuraman, M. K., and Cech, T. R.,** Monovalent cation-induced structure of telomeric DNA: the G-quartet model, *Cell*, 59, 871, 1989.

565. **Panyutin, I. G., Kovalsky, O. I., Budowsky, E. I., Dickerson, R. E., Rikhirev, M. E., and Lipanov, A. A.,** G-DNA: a twice-folded DNA structure adopted by single-stranded oligo(dG) and its implications for telomeres, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 867, 1990.

566. **Henderson, E. R., Moore, M., and Malcolm, B. A.,** Telomere G-strand structure and function analyzed by chemical protection, base analogue substitution, and utilization by telomerase *in vitro*, *Biochemistry*, 29, 732, 1990.

567. **Sen, D. and Gilbert, W.,** A sodium-potassium switch in the formation of four-stranded G4-DNA, *Nature*, 344, 410, 1990.

568. **Iacono-Connors, L. and Kowalski, D.,** Altered DNA conformations in the gene regulatory region of torsionally-stressed SV40 DNA, *Nucleic Acids Res.*, 14, 8949, 1986.

569. **Umek, R. M. and Kowalski, D.,** Yeast regulatory sequences preferentially adopt a non-B conformation in supercoiled DNA, *Nucleic Acids Res.*, 15, 4467, 1987.

570. **Kowalski, D., Natale, D. A., and Eddy, M. J.,** Stable DNA unwinding, not "breathing," accounts for single-strand-specific nuclease hypersensitivity of specific A + T-rich sequences, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 9464, 1988.

571. **Kowalski, D. and Eddy, M. J.,** The DNA unwinding element: a novel, *cis*-acting component that facilitates opening of the *Escherichia coli* replication origin, *EMBO J.*, 8, 4335, 1989.

572. **Umek, R. M. and Kowalski, D.,** The ease of DNA unwinding as a determinant of initiation at yeast replication origins, *Cell*, 52, 559, 1988.

573. **Kohwi-Shigematsu, T. and Kohwi, Y.,** Torsional stress stabilizes extended base unpairing in suppressor sites flanking immunoglobulin heavy chain enhancer, *Biochemistry*, 29, 9551, 1990.

574. **van de Sande, J. H., Ramsing, N. B., Germann, M. W., Elhorst, W., Kalisch, B. W., von Kitzing, E., Pon, R. T., Clegg, R. M., and Jovin, T. M.,** Parallel stranded DNA, *Science*, 241, 551, 1988.

575. **Germann, M. W., Kalish, B. W., and van de Sande, J. H.,** Relative stability of parallel- and antiparallel-stranded duplex DNA, *Biochemistry*, 27, 8302, 1988.

576. **Ramsing, N. B. and Jovin, T. M.,** Parallel stranded duplex DNA, *Nucleic Acids Res.*, 16, 6659, 1988.

577. **Ramsing, N. B., Rippe, K., and Jovin, T. M.,** Helix-coil transition of parallel-stranded DNA. Thermodynamics of hairpin and linear duplex oligonucleotides, *Biochemistry*, 28, 9528, 1989.

578. **Germann, M. W., Kalish, B. W., Pon, R. T., and**

van de Sande, J. H., Characterisation of a parallel-stranded DNA hairpin, *Biochemistry*, 28, 6220, 1989.

579. **Germann, M. W., Kalish, B. W., Pon, R. T., and van de Sande, J. H.,** Length dependent formation of parallel-stranded DNA in alternating AT segments, *Biochemistry*, 29, 9426, 1990.

580. **Shchyolkina, A. K., Lysov, Y. P., Il'ichova, I. A., Chernyi, A. A., Golova, Y. B., Chernov, B. K., Gottikh, B. P., and Florentiev, V. L.,** Parallel stranded DNA with AT base pairing, *FEBS Lett.*, 244, 39, 1989.

581. **Tchurikov, N. A., Chernov, B. K., Golova, Y. B., and Nechipurenko, Y. D.,** Parallel DNA: generation of a duplex between two *Drosophila* sequences *in vitro*, *FEBS Lett.*, 257, 415, 1989.

582. **Jovin, T. M. and Vojtiskova, M.,** unpublished data, 1990.

583. **Rippe, K., Ramsing, N. B., and Jovin, T. M.,** Spectroscopic properties and helical stabilities of 25-nt parallel-stranded linear DNA duplexes, *Biochemistry*, 28, 536, 1989.

584. **Rippe, K. and Jovin, T. M.,** substrate properties of 25-nt parallel-stranded linear DNA duplexes, *Biochemistry*, 28, 9542, 1989.

585. **Klysik, J., Rippe, K., and Jovin, T. M.,** Reactivity of parallel-stranded DNA to chemical modification reagents, *Biochemistry*, 29, 9831, 1990.

586. **Rippe, K., Ramsing, N. B., Klement, R., and Jovin, T. M.,** A parallel stranded linear DNA duplex incorporating dG·dC base pairs, *J. Biomol. Struct. Dyn.*, 7, 1199, 1990.

587. **Jovin, T. M., Rippe, K., Ramsing, N. B., Klement, R., Elhorst, W., and Vojtiskova, M.,** Parallel stranded DNA, in *Structure and Methods, Vol. 3: DNA and RNA*, Sarma, R. H. and Sarma, M. H., Eds., Adenine Press, Schenectady, NY, 1990, 155.

588. **Pattabiraman, N.,** Can the double helix be parallel?, *Biopolymers*, 25, 1603, 1986.

589. **Otto, C., Thomas, G. A., Rippe, K., Jovin, T. M., and Peticolas, W. L.,** The hydrogen bonding structure in parallel-stranded duplex DNA is reverse Watson-Crick, *Biochemistry*, 30, 3062, 1991.

590. **Dotsch, V. and Jovin, T. M.,** unpublished data, 1990.

591. **Klysik, J., Rippe, K., and Jovin, T. M.,** unpublished data, 1990.

592. **Morvan, F., Rayner, B., Imbach, J. L., Lee, M., Hartley, J. A., Chang, D.-K., and Lown, J. W.,** A-DNA-V. Parallel annealing, handedness and conformation of the duplex of the unnatural α-hexadeoxyribonucleotide α-[d(CpApTpGpCpG)] with its β-complement β-[d(GpTpApCpGpC)] deduced from high field 1H-NMR, *Nucleic Acids Res.*, 15, 7027, 1987.

593. **Koole, L. H., van Genderen, M. H. P., and Buck, H. M.,** A parallel right-handed duplex of the hexamer d(TpTpTpTpTpT) with phosphate triester linkages, *J. Am. Chem. Soc.*, 109, 3916, 1987.

593a. **Tchurikov, N. A., Ebralidze, A. K., and Georgiev,**

G. P., The suffix sequence is involved in processing the 3' ends of different mRNAs in *Drosophila melanogaster*, *EMBO J.*, 5, 2341, 1986.

594. Jovin, T. M., Parallel-stranded DNA with trans-Crick-Watson base pairs, in *Nucleic Acids and Molecular Biology*, Vol. 5, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, in press.

595. Collins, J. M., Deoxyribonucleic acids structure in human diploid fibroblast stimulated to proliferate, *J. Biol. Chem.*, 252, 141, 1977.

596. Peck, L. J. and Wang, J. C., Transcriptional block caused by a negative supercoiling induced structural change in an alternating CG sequence, *Cell*, 40, 129, 1985.

597. Horwitz, M. S. Z. and Loeb, L. A., An *E. coli* promoter that regulates transcription by DNA super-helix-induced cruciform extrusion, *Science*, 241, 703, 1988.

598. Jaworski, A., Blaho, J. A., Larson, J. E., Shimizu, M., and Wells, R. D., Tetracycline promoter mutations decrease non-B DNA structural transitions, negative linking differences and deletions in recombinant plasmids in *Escherichia coli*, *J. Mol. Biol.*, 207, 513, 1989.

599. Lilley, D., *In vivo* consequences of plasmid topology, *Nature*, 289, 380, 1981.

600. Lilley, D., A new twist to an old story, *Nature*, 230, 14, 1986.

601. Greaves, D. R. and Patient, R. K., RecBC, sbsB independent, (AT)n-mediated deletion of sequences flanking a *Xenopus laevis* gamma-globin gene on propagation in *E. coli*, *Nucleic Acids Res.*, 14, 4147, 1986.

602. Zacharias, W., Jaworski, A., Larson, J. E., and Wells, R. D., The B-Z DNA equilibrium *in vivo* is perturbed by biological processes, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 7069, 1988.

603. Panayotatos, N. and Fontaine, A., A native cruciform DNA structure probed in bacteria by recombinant T7 endonuclease, *J. Biol. Chem.*, 262, 11364, 1987.

604. Karlovsky, P., Pecinka, P., Vojtiskova, M., Makaturova, E., and Palecek, E., Protonated triplex DNA in *E. coli* cells as detected by chemical probing, *FEBS Lett.*, 274, 39, 1990.

605. Boublikova, P. and Palecek, E., Osmium tetroxide, N,N,N',N'-tetramethylethylendiamine. A new probe of DNA structure in the cell, *FEBS Lett.*, 263, 281, 1990.

606. Nick, H. and Gilbert, W., Detection *in vivo* of protein-DNA interaction within the lac operon of *Escherichia coli*, *Nature*, 313, 795, 1985.

607. Lickl, E., Chao, S.-C., and Chang, W.-C., Intracellular oxidative cleavage of DNA in *Escherichia coli* by the copper-1,10-phenanthroline complex, *Free Rad. Res. Commun.*, 8, 37, 1989.

608. Becker, M. M. and Wang, J. C., Use of light to footprinting DNA *in vivo*, *Nature*, 309, 682, 1984.

609. Axelrod, J. D. and Majors, J., An improved method for photofootprinting yeast genes *in vivo* using taq polymerase, *Nucleic Acids Res.*, 17, 171, 1989.

610. Robert-Nicoud, M., Jovin, T. M., Poverennyi, A., and Palecek, E., unpublished data, 1990.

611. McClellan, J. A., Boublikova, P., Palecek, E., and Lilley, D. M. J., Superhelical torsion in cellular DNA response directly to environmental and genetic factors, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 8373, 1990.

612. Muller, U. R. and Fitch, W. M., Evolutionary selection for perfect hairpin structures in viral DNAs, *Nature*, 298, 582, 1982.

613. Sinden, R. R., Broyles, S. S., and Pettijohn, D. E., Perfect palindromic *lac* operator DNA sequence exists as a stable cruciform structure in supercoiled DNA *in vitro* but not *in vivo*, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 1797, 1983.

614. Hand, R., Eucaryotic DNA: organization of the genome for replication, *Cell*, 15, 317, 1978.

615. Campbell, J. L., Eukaryotic DNA replication, *Annu. Rev. Biochem.*, 55, 733, 1986.

616. Ward, G. K., McKenzie, R., Zannis-Hadjopoulos, M., and Price, G. B., The dynamic distribution and quantification of DNA cruciforms in eukaryotic nuclei, *Exp. Cell Res.*, 188, 235, 1990.

617. Elborough, K. M. and West, S. C., Specific binding of cruciform DNA structures by a protein from human extracts, *Nucleic Acids Res.*, 16, 3603, 1988.

618. Bianchi, M. E., Interaction of a protein from rat liver nuclei with cruciform DNA, *EMBO J.*, 7, 843, 1988.

619. Bianchi, M. E., Beltrame, M., and Paonessa, G., Specific recognition of cruciform DNA by nuclear protein HMG1, *Science*, 243, 1056, 1989.

620. Horwitz, M. S. Z., Transcription regulation *in vitro* by an *E. coli* promoter containing a DNA cruciform in the −35' region, *Nucleic Acids Res.*, 17, 5537, 1989.

621. Morales, N. M., Cobourn, S. D., and Muller, U. R., Effect of *in vitro* transcription on cruciform stability, *Nucleic Acids Res.*, 18, 2777, 1990.

622. Bagga, R., Ramesh, N., and Brahmachari, S. K., Supercoil-induced unusual DNA structures as transcriptional block, *Nucleic Acids Res.*, 18, 3363, 1990.

623. Pruss, G. J. and Drlica, K., DNA supercoiling and prokaryotic transcription, *Cell*, 56, 521, 1989.

624. Tsao, Y.-P., Wu, H.-Y., and Liu, L. F., Transcription-driven supercoiling of DNA: direct biochemical evidence from *in vitro* studies, *Cell*, 56, 11, 1989.

625. Frank-Kamenetskii, M., Waves of DNA supercoiling, *Nature*, 337, 206, 1989.

626. Kimura, T. and Takeya, T., Nuclease S1-sensitive sites on superhelical DNA molecules carrying the LTR region of Moloney murine leukemia virus, *Biochim. Biophys. Acta*, 908, 285, 1987.

627. Battistoni, A., Leoni, L., Sampalolese, B., and Savino, M., Kinetic persistence of cruciform struc-

tures in reconstituted minichromosomes, *Biochim. Biophys. Acta*, 950, 161, 1988.

628. **Nordheim, A., Pardue, M. L., Lafer, E. M., Muller, A., Stollar, B. D., and Rich, A.,** Antibodies to left-handed Z-DNA bind to interband regions of *Drosophila* polytene chromosomes, *Nature*, 294, 417, 1981.

629. **Pardue, M. L., Nordheim, A., Lafer, E. M., Stollar, B. D., and Rich, A.,** Z-DNA and the polytene chromosome, *Cold Spring Harbor Symp. Quant. Biol.*, 47, 171, 1982.

630. **Jovin, T. M., van de Sande, J. H., Zarling, D. A., Arndt-Jovin, D. J., Eckstein, F., Fuldner, H. H., Greider, C., Grieger, I., Hamori, E., Kalisch, B., McIntosh, L. P., and Robert-Nicoud, M.,** Generation of left-handed Z-DNA in solution and visualization in polytene chromosomes by immunofluorescence, *Cold Spring Harbor Symp. Quant.Biol.*, 47, 143, 1982.

631. **Arndt-Jovin, D. J., Robert-Nicoud, M., Zarling, D. A., Greider, C., Weimer, E., and Jovin, T. M.,** Left-handed Z-DNA in bands of acid-fixed polytene chromosomes, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 4344, 1983.

632. **Robert-Nicoud, M., Arndt-Jovin, D. J., Zarling, D. A., and Jovin, T. M.,** Immunological detection of left-handed Z-DNA in isolated polytene chromosomes. Effects of ionic strength, pH, temperature and topological stress, *EMBO J.*, 3, 721, 1984.

633. **Lancillotti, F., Lopez, M. C., Arias, P., and Alonso, C.,** Z-DNA in transcriptionally active chromosomes, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 1560, 1987.

634. **Hill, R. J. and Stollar, B. D.,** Dependence of Z-DNA antibody binding to polytene chromosomes on acid fixation and DNA torsional strain, *Nature*, 305, 338, 1983.

635. **Haniford, D. B. and Pulleyblank, D. E.,** The *in vivo* occurrence of Z-DNA, *J. Biomol. Struct. Dyn.*, 1, 593, 1983.

636. **Zacharias, W., Jaworski, A., and Wells, R. D.,** Cytosine methylation enhances Z-DNA formation *in vivo*, *J. Bacteriol.*, 172, 3278, 1990.

637. **Freund, A. M., Bichara, M., and Fuchs, R. P. P.,** Z-DNA-forming sequences are spontaneous deletion hot spot, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 7465, 1989.

638. **Wittig, B., Dorbic, T., and Rich, A.,** The level of Z-DNA in metabolically active, permeabilized mammalian cell nuclei is regulated by torsional strain, *J. Cell. Biol.*, 108, 755, 1989.

639. **Wittig, B., Dorbic, T., and Rich, A.,** Measurement of left-handed Z-DNA in permeabilized, metabolically active mammalian nuclei, in *Structure and Methods, Vol. 2: DNA Protein Complexes and Proteins*, Sarma, R. H. and Sarma, M. H., Ed., Adenine Press, Schenectady, NY, 1990, 001.

639a. **Soyer-Odile, M.-O., Geraud, M.-L., Coulaud, D., Barray, M., Theveny, B., Revet, B., and Delain, E.,** Location of B- and Z-DNA in the chromosomes of a primitive eukaryote dinoflagellate, *J. Cell. Biol.*, 111, 293, 1990.

640. **Casasnovas, J. M., Ellison, M. J., Rodriguez-Campos, A., and Azorin, F.,** The obtention of simian virus 40 recombinants carrying d(CG·GC)n, d(CA·GT)n and d(CT·GA)n sequences. Stability of the inserted simple repeating sequences, *Eur. J. Biochem.*, 167, 489, 1987.

641. **Casasnovas, J. M., Ellison, M. J., Rodriguez-Campos, A., Martinez-Balbas, A., and Azorin, F.,** *In vivo* assessment of the Z-DNA-forming potential of d(CA·GT)n and d(CG·GC)n sequences cloned into SV40 minichromosomes, *J. Mol. Biol.*, 208, 537, 1989.

642. **Puppels, G. J., de Mul, F. F. M., Otto, C., Greve, J., Robert-Nicoud, M., Arndt-Jovin, D. J., and Jovin, T. M.,** Studying single living cells and chromosomes by confocal Raman microspectroscopy, *Nature*, 347, 301, 1990.

643. **Hoheisel, J. D. and Pohl, F. M.,** Searching for potential Z-DNA in genomic *Escherichia coli* DNA, *J. Mol. Biol.*, 193, 447, 1987.

644. **Argentin, S., Nemer, M., Drouin, J., Scott, G. K., Kennedy, B. P., and Davies, P. L.,** The gene for rat atrial natriuretic factor, *J. Biol. Chem.*, 260, 4568, 1985.

645. **Hayes, T. E. and Dixon, J. E.,** Z-DNA in the rat somatostatin gene, *J. Biol. Chem.*, 260, 8145, 1985.

646. **Hamada, H. P., Petrino, M. G., Kakunaga, T., Seidman, M., and Stollar, B. D.,** Characterization of genomic poly(dT-dG)·poly(dC-dA) sequences, structure, organization and conformation, *Mol. Cell. Biol.*, 4, 2610, 1984.

647. **Weinreb, A., Katzenberg, D. R., Gilmore, G. L., and Birshtein, B. K.,** Site of unequal sister chromatid exchange contains a potential Z-DNA-forming tract, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 529, 1988.

648. **Azorin, F. and Rich, A.,** Isolation of Z-DNA binding proteins from SV40 minichromosomes: evidence for binding to the viral control region, *Cell*, 41, 365, 1985.

649. **Krishna, P., Kennedy, B. P., van de Sande, J. H., and McGhee, J. D.,** Yolk proteins from nematodes, chickens, and frogs bind strongly and preferentially to left-handed Z-DNA, *J. Biol. Chem.*, 263, 19066, 1988.

650. **Fishel, R. A., Detmer, K., and Rich, A.,** Identification of homologous pairing and strand-exchange activity from a human tumor cell line based on Z-DNA affinity chromatography, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 36, 1988.

651. **Kitayama, S., Matsumura, O., and Masuda, S.,** Isolation of a DNA-binding protein from *Deinococcus radiodurans* having an affinity for a Z-form polynucleotide, *J. Biochem.*, 104, 127, 1988.

652. **Lafer, E. M., Sousa, R. J., and Rich, A.,** Z-DNA-binding proteins in *Escherichia coli* purification, gen-

eration of monoclonal antibodies and gene isolation, *J. Mol. Biol.*, 203, 511, 1988.

653. **Leith, I. R., Hay, R. T., and Russell, W. C.,** Detection of Z DNA binding proteins in tissue culture cells, *Nucleic Acids Res.*, 16, 8277, 1988.

654. **Nordheim, A.,** Z-DNA: conformational flexibility of the DNA double helix, in *The Impact of Gene Transfer Techniques in Eukaryotic Cell Biology*, Springer-Verlag, Berlin, 1985, 35.

655. **Nordhein, A.,** Z-DNA: exploring the biological significance, in *Nucleic Acids and Molecular Biology*, Vol. 1, Eckstein, F. and Lilley, D. M. J., Eds., Springer-Verlag, Berlin, 1987, 112.

656. **Waga, S., Mizuno, S., and Yoshida, M.,** Nonhistone protein HMG1 removes the transcriptional block caused by left-handed Z-form segment in a supercoiled DNA, *Biochem. Biophys. Res. Commun.*, 153, 334, 1988.

657. **Horbach, E. and Muller-Hill, B.,** Insertion of d(pCpG)n·d(pCpG)n into the *lac* Z gene of *Escherichia coli* inhibits expression of gamma-galactosidase *in vivo*, *J. Mol. Biol.*, 202, 157, 1988.

658. **Naylor, L. H. and Clark, E. M.,** d(TG)n·d(CA)n sequences upstream of the rat prolactin gene form Z-DNA and inhibit gene transcription, *Nucleic Acids Res.*, 18, 1595, 1990.

659. **Casasnovas, J. M. and Azorin, F.,** Supercoiled induced transition to the Z-DNA conformation affects the ability of a d(CG/GC)$_{12}$ sequence to be organized into nucleosome-cores, *Nucleic Acids Res.*, 15, 8899, 1987.

660. **Kmiec, E. B. and Holloman, K.,** Homologous pairing of DNA molecules by ustilago rec1 protein is promoted by sequences of Z-DNA, *Cell*, 44, 545, 1986.

661. **Manor, H., Rao, B. S., and Martin, R. G.,** Abundance and degree of disperion of genomic d(GA)n·d(TC)n sequences, *J. Mol. Evol.*, 27, 96, 1988.

662. **Birnboim, H. C., Sederoff, R. R., and Paterson, M. C.,** Distribution of polypyrimidine·polypurine segments in DNA from diverse organisms, *Eur. J. Biochem.*, 98, 301, 1979.

663. **Tautz, D. and Renz, M.,** Simple sequences are ubiquitous repetitive components of eukaryotic genomes, *Nucleic Acids Res.*, 12, 4127, 1984.

664. **Braaten, D. C., Thomas, J. R., Little, R. D., Dickson, K. R., Goldberg, I., Schlessinger, D., Ciccodicola, A., and D'Urso, M.,** Locations and contexts of sequences that hybridize to poly(dG-dC)·(dC-dA) in mammalian ribosomal DNAs and two X-linked genes, *Nucleic Acids Res.*, 16, 865, 1988.

665. **Gebhard, W. and Zachau, H. G.,** Simple DNA sequences and dispersed repetitive elements in the vicinity of mouse immunoglobulin k light chain genes, *J. Mol. Biol.*, 170, 567, 1983.

666. **Shen, S. and Smithies, O.,** Human globin ΨB2 is not a globin-related sequence, *Nucleic Acids Res.*, 10, 7809, 1982.

667. **Das, H. K., Jackson, C. L., Miller, D. A., Leff, T., and Breslow, J. L.,** The human apolipoprotein CII gene sequence contains a novel chromosome 19-specific minisatellite in its third intron, *J. Biol. Chem.*, 262, 4787, 1987.

668. **Huijser, P., Hennig, W., and Dijkhof, R.,** Poly(dC-dA/dG-dT) repeats in the *Drosophila* genome: a key function for dosage compensation and position effects?, *Chromosoma*, 95, 209, 1987.

669. **Pardue, M. L., Lowenhaupt, K., Rich, A., and Nordheim, A.,** (dC-dA)n·(dG-dT)n sequences have evolutionary conserved chromosomal locations in Drosophila with implications for roles in chromosome structure and function, *EMBO J.*, 6, 1781, 1987.

670. **Lowenhaupt, K., Rich, A., and Pardue, M. L.,** Nonrandom distribution of long mono- and dinucleotide repeats in Drosophila chromosomes: correlations with dosage compensation, heterochromatin, and recombination, *Mol. Cell. Biol.*, 9, 1173, 1989.

671. **Vogt, P.,** Potential genetic functions of tandem repeated DNA sequences blocks in the human genome are based on a highly conserved ''chromatin folding code'', *Hum. Genet.*, 84, 301, 1990.

672. **Bodnar, J. W. and Ward, D. C.,** Highly recurring sequence elements identified in eukaryotic DNAs by computer analysis are often homologous to regulatory sequences or protein binding sites, *Nucleic Acids Res.*, 15, 1835, 1987.

673. **Fowler, R. F. and Skinner, D. M.,** Eukaryotic DNA diverges at a long and complex pyrimidine·purine tract that can adopt altered conformations, *J. Biol. Chem.*, 271, 8994, 1986.

674. **Beasty, A. M. and Behe, M. J.,** An oligopurine sequence bias occurs in eukaryotic viruses, *Nucleic Acids Res.*, 16, 1517, 1988.

675. **Behe, M. J.,** The DNA sequence of the human B-globin region is strongly biased in favor of long strings of contiguous purine or pyrimidine residues, *Biochemistry*, 26, 7870, 1987.

676. **Hoffman-Liebermann, B., Liebermann, D., Troutt, A., Kedes, L. H., and Cohen, S. N.,** Human homologs of TU transposon sequences: polypurine/polypyrimidine sequences elements that can alter DNA conformation *in vitro* and *in vivo*, *Mol. Cell. Biol.*, 6, 3632, 1986.

677. **Liu, Q.-R. and Chan, P. K.,** Identification of a long stretch of homopurine·homopyrimidine sequence in a cluster of retroposons in the human genome, *J. Mol. Biol.*, 212, 453, 1990.

678. **Jayasena, S. D. and Behe, M. J.,** Competitive nucleosome reconstitution of polydeoxynucleotides containing oligoguanosine, *J. Mol. Biol.*, 208, 297, 1989.

679. **Rhodes, D.,** Nucleosome cores reconstituted from poly (dA-dT) and the octamer of histones, *Nucleic Acids Res.*, 6, 1805, 1979.

680. **Simpson, R. T. and Künzler, P.,** Chromatin and core particles formed from the inner histones and

synthetic polydeoxyribonucleotides of defined sequences, *Nucleic Acids Res.*, 6, 1387, 1979.

681. **Burkholder, G. D., Latimer, L. J. P., and Lee, J. S.,** Immunofluorescent staining of mammalian nuclei and chromosomes with a monoclonal antibody to triplex DNA, *Chromosoma,* 97, 185, 1988.

682. **Heyde, M. and Portalier, R.,** Acid shock proteins of *Escherichia coli, FEMS Microbiol. Lett.,* 69, 19, 1990.

683. **Madshus, I. H.,** Regulation of intracellular pH in eukaryotic cells, *Biochem. J.,* 250, 1, 1988.

684. **Baran, N., Lapidot, A., and Manor, H.,** Unusual sequence element found at the end of an amplicon, *Mol. Cell. Biol.,* 7, 2636, 1987.

685. **Kinniburgh, J.,** A *cis*-acting transcription element of the c-*myc* gene can assume an H-DNA conformation, *Nucleic Acids Res.,* 17, 7771, 1989.

686. **Lapidot, A., Baran, N., and Manor, H.,** (dT-dC)n and (dG-dA)n tracts arrest single stranded DNA replication *in vitro, Nucleic Acids Res.,* 17, 883, 1989.

687. **Gama Sosa, M. A., Hall, J. C., Schneider, K. E., Lukaszewicz, G. C., and Ruprecht, R. M.,** Unusual DNA structures at the interaction site of an HIV provirus, *Biochem. Biophys. Res. Commun.,* 161, 134, 1989.

688. **Biggin, M. D. and Tjian, R.,** Transcription factors that activate the Ultrabithorax promoter in developmentally staged extracts, *Cell,* 53, 699, 1988.

689. **Gilmour, D. S., Thomas, G. H., and Elgin, S. C. R.,** *Drosophila* nuclear proteins bind to regions of alternating T residues in gene promoters, *Science,* 245, 1487, 1989.

690. **Maher, III, L. J., Wold, B., and Dervan, P. B.,** Inhibition of DNA binding proteins by oligonucleotide-directed triple helix formation, *Science,* 245, 725, 1989.

691. **Hanvey, J. C., Shimizu, M., and Wells, R. D.,** Site-specific inhibition of EcoRI restriction/modification enzymes by a DNA triple helix, *Nucleic Acids Res.,* 18, 157, 1989.

692. **Bliska, J. B. and Cozzarelli, N. R.,** Use of site-specific recombination as a probe of DNA structure and metabolism *in vivo, J. Mol. Biol.,* 194, 205, 1987.

693. **Giaever, G. N., Snyder, L., and Wang, J. C.,** DNA supercoiling *in vivo, Biophys. Chem.,* 29, 7, 1988.

694. **Chan, P. T., Sullivan, J. K., and Lebowitz, J.,** Site-directed chemical modification for probing DNA-protein interactions, *J. Biol. Chem.,* 264, 21277, 1989.

695. **Camilloni, G., Della Seta, F., Negri, R., and Di Mauro, E.,** Structure of RNA pollymerase II promoters, *J. Biol. Chem.,* 261, 6145, 1986.

696. **Borowiec, J. A. and Gralla, J. D.,** High-resolution analysis of *lac* transcription complexes inside cells, *Biochemistry,* 25, 5051, 1986.

697. **Spassky, A., Rimsky, S., Buc, H., and Busby, S.,** Correlation between the conformation of *Escherichia coli* −10 hexamer sequences and promoter strength: use of orthophenanthroline cuprous complex as a structural index, *EMBO J.,* 7, 1871, 1988.

698. **Krämer, H., Amouyal, M., Nordheim, A., and Müller-Hill, B.,** DNA supercoiling changes the spacing requirement of two *lac* operators for DNA loop formation with *lac* repressor, *EMBO J.,* 7, 547, 1988.

699. **Gama Sosa, M. A., Hall, J. C., and Ruprecht, R. M.,** Slipped DNA structures within the enhancer region of the Moloney murine leukemia virus, *Biochem. Biophys. Res. Commun.,* 156, 417, 1988.

700. **Yagil, G. and Sussman, J.,** Structural models for non-helical DNA, *EMBO J.,* 5, 1719, 1986.

701. **Nussinov, R.,** Sequence signals in eukaryotic upstream regions, *Crit. Rev. Biochem. Mol. Biol.,* 25, 185, 1990.