4. Předn. 3.12. 2013

Chemie, struktura a interakce nukleových kyselin

Fyzikální vlastnosti a izolace DNA

Denaturace, renaturace a hybridizace DNA

Biosyntetické polynukleotidy

Fyzikální vlastnosti DNA

Studium fyz. vlastností DNA *in vitro* vyžaduje její izolaci z buněk či virů do zřeď. vodných roztoků, v nichž nejsou přítomny ostatní celulární komponenty. Takto ztrácíme sice informace o jejich uspořádání *in vivo* (interakce s RNA, bílkovinami, atd.) - získáváme však možnost zodpovědět jiné otázky jako m. v., sekundární struktura ap.

<u>Izolace DNA</u> - pokrok v poznání vlastností DNA postupoval souběžně s pokrokem izolačních technik. Např. zjištění lámavosti dlouhých molekul DNA díky působení střižných sil (shear degradation) - čím větší molekula, tím snadnější degradace (vyfouknutí 1 ml roztoku pipetou o průměru 0,25mm za 2 s zlomí DNA T_2 na poloviny. Při vysoké konc. (500 μg/ml) DNA je možnost zlomení menší. Začátkem 60 let byl vypracovány metody umožňující izolaci nedegradované DNA T_2 a T_4 (130.10^6). Tyto DNA se pak staly standardem pro kalibraci metod stanovení mol. hmotnosti DNA.

Důležitým krokem při izolaci DNA je <u>odstranění bílkovin</u>: vysoká konc. solí, detergent, $CHCl_3$- isoamyl, emulsifikace, proteasy a fenolová extrakce. $CHCl_3$-opakované třepání, degradace; lepší je <u>fenol</u> - DNA o m.v. blízké celému chromosomu *E.coli* ($\sim10^9$) - nebezpečí znečištění fenolu peroxidy (destilace).

<u>Isolace DNA z bakteriofága</u>
a) purifikace fága diferenční centrifugací a/nebo v grad CsCl
b) deproteinace (většinou fenolem)

Dnes nejčastěji je používana <u>plasmidová</u> DNA.

<u>Stupeň čistoty a volba metody izolace</u> jsou velmi <u>závislé na účelu</u>, ke kterému má být DNA použita.

V posledních letech jsou k dispozici komerčně dostupné kolonky využívající imobilizaci DNA na pevném podkladu. K separaci DNA jsou rovněž používány magnetické kuličky (magnetic beads)

Tissue

Cold dilute TCA* or PCA†
Organic solvents

Acid-soluble
+
lipid
fraction

Nucleic acid
+
protein
residue

Alkaline digestion
Acidification

Hot TCA
or PCA

Soluble extract
containing
RNA

Residue
containing
DNA

Soluble extract
containing
RNA + DNA

Residue
(protein)
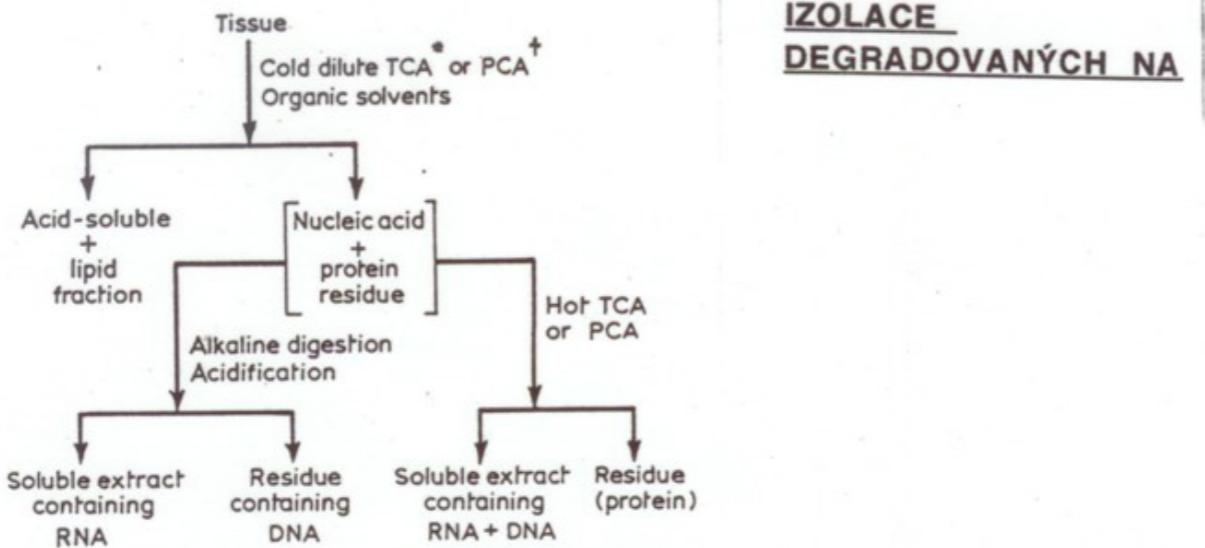
Extraction and fractionation of nucleic
acids from tissues. *TCA − trichloroacetic acid,
†PCA – perchloric acid.

IZOLACE INTAKTNÍ DNA
J. Marmur

a. z virů a bakteriofágů
b. z bakterií
c. z eukaryotních buněk

Plasmidová DNA

Chromosomal
DNA

Plasmid
DNA

RNA
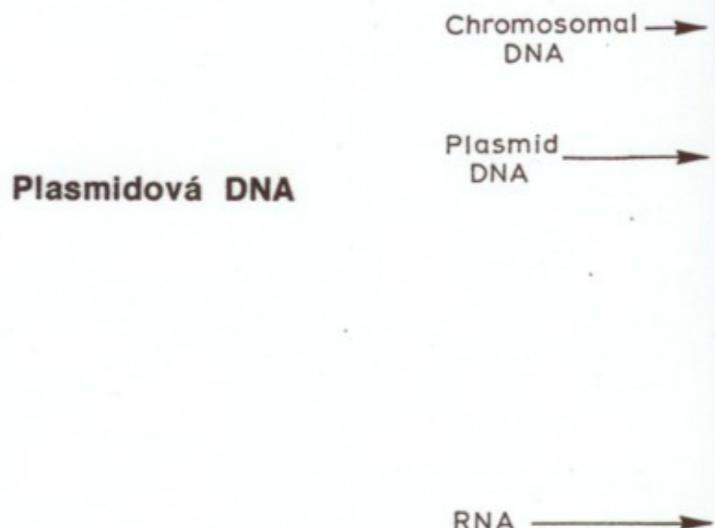
Separation of closed-circular DNA of
plasmid pBR322 from *E. coli* chromosomal DNA by
isopycnic ultracentrifugation in a CsCl density
gradient in the presence of ethidium bromide. The
band marked 'chromosomal DNA' may also contain
nicked plasmid DNA molecules.

J. MARMUR, Harvard Univ./Brandeis Univ., Boston, Mass.

Izolace DNA z bakterií: 1. lysa buněk
          a) mechanicky
          b) enzymaticky (lysozym)
          c) detergenty (SDS)

        2. deproteinace
         a) $CHCl_3$
         b) fenol
         c) enzymaticky
         d) ultracentrifugace v grad CsCl

        3. odstranění RNA
         a) enzymaticky (RNasa)
         b) diferenční srážení
         c) ulracentrifugace v grad CsCl

Jednotlivé kroky při izolaci DNA jsou často kombinovány se srážením etanolem

        4. dialysa

Dnes jsou k dispozici komerčně dostupné přípravky (většinou různé druhy kolonek) pro izolaci DNA z prokaryotních i eukaryotních buněk, které jsou vhodné zejména pro rutinní, seriové izolace DNA

A Procedure for the Isolation of Deoxyribonucleic Acid from Micro-organisms†

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A method has been described for the isolation of DNA from micro-organisms which yields stable, biologically active, highly polymerized preparations relatively free from protein and RNA. Alternative methods of cell disruption and DNA isolation have been described and compared. DNA capable of transforming homologous strains has been used to test various steps in the procedure and preparations have been obtained possessing high specific activities. Representative samples have been characterized for their thermal stability and sedimentation behaviour.

1. Introduction

To facilitate the study of the biological, chemical and physical properties of DNA it is necessary to obtain the material in a native, highly polymerized state. Several procedures have described the isolation of DNA from selected groups of micro-organisms (Hotchkiss, 1957; Zamenhof, Reiner, DeGiovanni & Rich, 1956; Chargaff, 1955). However, no detailed account is available for the isolation of DNA from a diverse group of micro-organisms. The reason for this is that micro-organisms vary greatly

Agarose gel electrophoresis of DNA. (a) Separation of: 1, different forms of DNA of plasmid pBR322; 2, fragments of DNA (lengths indicated in kbp) derived from plasmid pBR322 by double-digestion with restriction endonuclease *Bam* HI and *Bgl* I; (b) Plot of length of DNA fragment (log scale) against distance of migration (linear scale) of data from (a) 2, illustrating linear relationship.
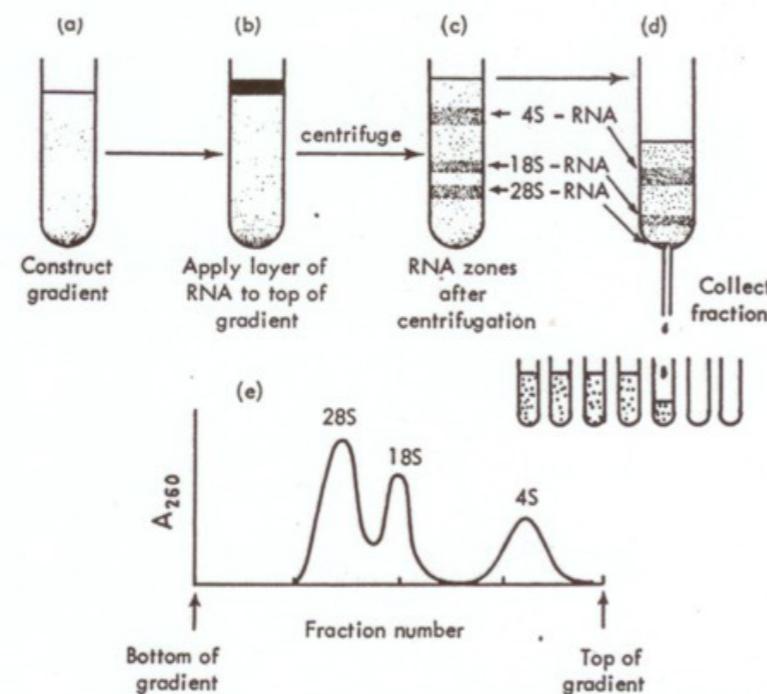
Rate zonal centrifugation of RNA through a sucrose density gradient. A sucrose density gradient is constructed in a centrifuge tube (a) and the RNA solution applied as a layer on top (b). During ultracentrifugation the main components of the RNA separate into zones, primarily on the basis of molecular weight (c). These zones may be recovered by puncturing the bottom of the tube and collecting different fractions in separate tubes (d). The separated RNAs may be visualized and quantitated by measurement of the absorbance at 260 nm (e). Steps (d) and (e) may be conveniently combined by pumping the gradient through the flow-cell of a recording spectrophotometer.

Characterize your DNA sample:

ds x ss, circular x linear
circular: nicked, oc; covalently closed, cc, cd

linear: cohesive or blunt ends
number of base pairs, ssb

Unusual bases, DNA methylation

purity: protein, RNA .... content
analytical methods

Síly ovlivňující konformaci DNA

a) Elektrostatické síly podmíněné ionizací.

V rozmezí pH 5-9, kdy nedochází ve větším stupni k ionizaci bazí je, DNA **aniontovým polyelektrolytem - polyaniontem**, díky záporným nábojům, které nesou fosfátové skupiny). V roztocích solí jsou záporné náboje vystíněny kladnými náboji kationtů (např. Na⁺), které vytvářejí kolem každého záporného náboje iontovou atmosféru. Jestliže je koncentrace kationtů nízká, nabývá na významu odpuzování fosfátových skupin. U **dvoušroubovicové DNA** se toto odpuzování stává faktorem ovlivňujícím významně vlastnosti molekul teprve **při iontových silách nižších než 0,1**. **Při velmi nízkých iontových silách** (kolem 10^{-4} - 10^{-5}) jsou odpudivé síly již tak velké, že mohou zapříčinit **zhroucení dvoušroubovicové struktury** (denaturaci). **Jednořetězcová DNA** (a podobně i RNA) je velmi **citlivá** ke změnám v koncentraci iontů již **při iontových silách nižších jak 1,0**; snižování iontové síly vede ke zvětšování prostoru zaujímaného polynukleotidovým řetězcem.

## b) Síly plynoucí z vertikálního uspořádání bazí

(vrstvení bazí, stacking). Síly působící mezi bazemi pravidelně uspořádanými ve dvojité šroubovici jsou zejména interakce typu dipól - dipól, dipól - indukovaný dipól a Londonovy síly. Existují teoreticky odvozené důkazy, že **tyto síly jsou postačující pro stabilizaci šroubovice**; jejich volná energie odpovídá asi -7 kcal na mól párů bazí. Naproti tomu volná energie vodíkových můstků činí asi -3 kcal pro (G.C) a -2 kcal pro (A.T) pár (na mól párů bazí).

c) Vodíkové vazby

(můstky) - představují **jediný známý způsob zajišťující specificitu párování bazí**. Jsou tedy součástí mechanismu jímž DNA realizuje svoji biologickou funkci. Zpočátku se o nich soudilo, že jsou nejdůležitějším činitelem pro stabilitu dvojité šroubovice; experimentálně i teoreticky bylo však dokázáno, že tomu tak není.

d) Hydrofobní síly

- tento termín se týká **stability dvoušroubovicové DNA plynoucí z její architekrury**: **polární skupiny** jsou **na povrchu**, zatímco **hydrofobní baze** jsou **uvnitř** molekuly a mají větší tendenci interagovat mezi sebou nežli s molekulami vody. Toto uspořádání **stabilizuje** tedy dvoušroubovicovou molekulu DNA **ve vodném prostředí**. Je známo, že molekula DNA je ve vodném roztoku obklopena **hydratační vrstvou**, která hraje významnou úlohu ve stabilizaci dvojité šroubovice. Podrobné znalosti o této hydratační vrstvě jsou nyní získávány zejmena díky výsledkům rtg. strukturní analýzy krystalů DNA.

Denaturation x degradation
aggregation
renaturation/hybridization

DNA DENATURATION and RENATURATION/HYBRIDIZATION

Native DNA

melted DNA

A, B

C

D

melting

melting

+

slow cooling

renaturation

quick cooling

quick cooling

A_{260}

D

C

premelting

A

B

denatured DNA

RENATURED DNA

Temperature

J. Marmur and P. Doty

Native double helix — Strands unwinding — Separated strands — Double helix reformed

Heat — More heat — Cooling

Denaturation — Renaturation

on and renaturation 21

Relative absorbance (260 nm)

Pneumococcus (38%)
E. coli (52%)
S. marescens (58%)
M. phlei (66%)

1.4

1.2

1.0

70   80   90   100
Temperature (°C)

naturation by heat of DNAs isolated from different sources. The figures in brackets indicate the
f the DNA in G + C (%) (from Molecular Genetics by G. S. Stent, W. H. Freeman and Co.
*1 – after [116]).*

STRAND SEPARATION AND SPECIFIC RECOMBINATION IN DEOXYRIBONUCLEIC ACIDS: BIOLOGICAL STUDIES

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Communicated by Paul Doty, February 25, 1960

It is clear that the correlation between the structure of deoxyribonucleic acid (DNA) and its function as a genetic determinant could be greatly increased if a means could be found of separating and reforming the two complementary strands. In this and the succeeding paper[1] some success along these lines is reported. This paper will deal with the evidence provided by employing the transforming activity of DNA from *Diplococcus pneumoniae* while the succeeding paper[1] will summarize physical chemical evidence for strand separation and reunion.
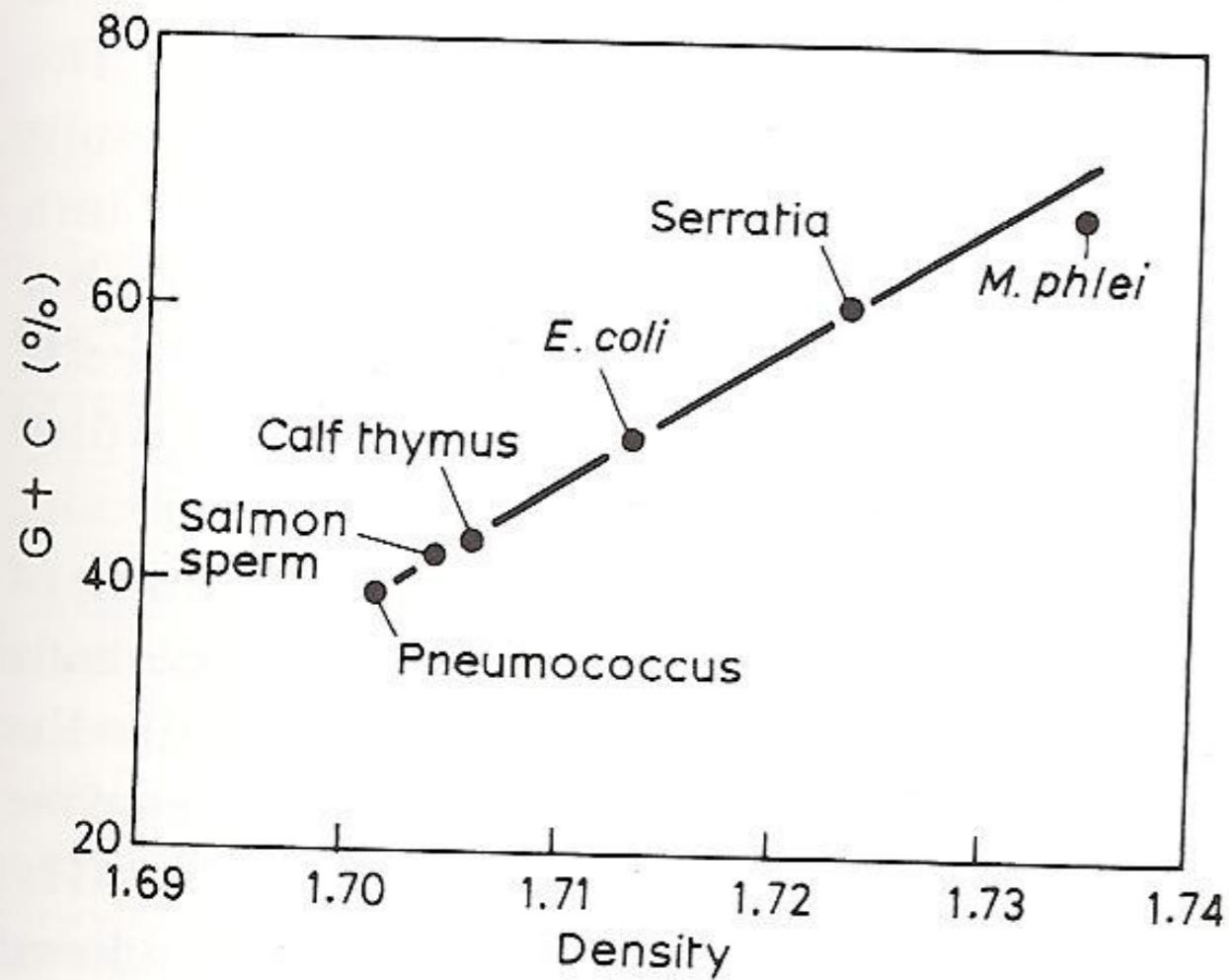
Fig. 2.21 Relationship of density to content of guanine plus cytosine in DNAs from various sources [64].

Source of DNA	Percentage (G + C)
Plasmodium falciparum (malarial parasite)	19
Dictyostelium (slime mould)	22
M. pyogenes	34
Vaccinia virus	36
Bacillus cereus	37
B. megaterium	38
Haemophilus influenzae	39
Saccharomyces cerevisiae	39
Calf thymus	40
Rat liver	40
Bull sperm	41
Diplococcus pneumoniae	42
Wheatgerm	43
Chicken liver	43
Mouse spleen	44
Salmon sperm	44
B. subtilis	44
T1 phage	46
E. coli	51
T7 phage	51
T3 phage	53
Neurospora crassa	54
Pseudomonas aeruginosa	68
Sarcina lutea	72
Micrococcus luteus	72
Herpes simplex virus	72
Mycobacterium phlei	73
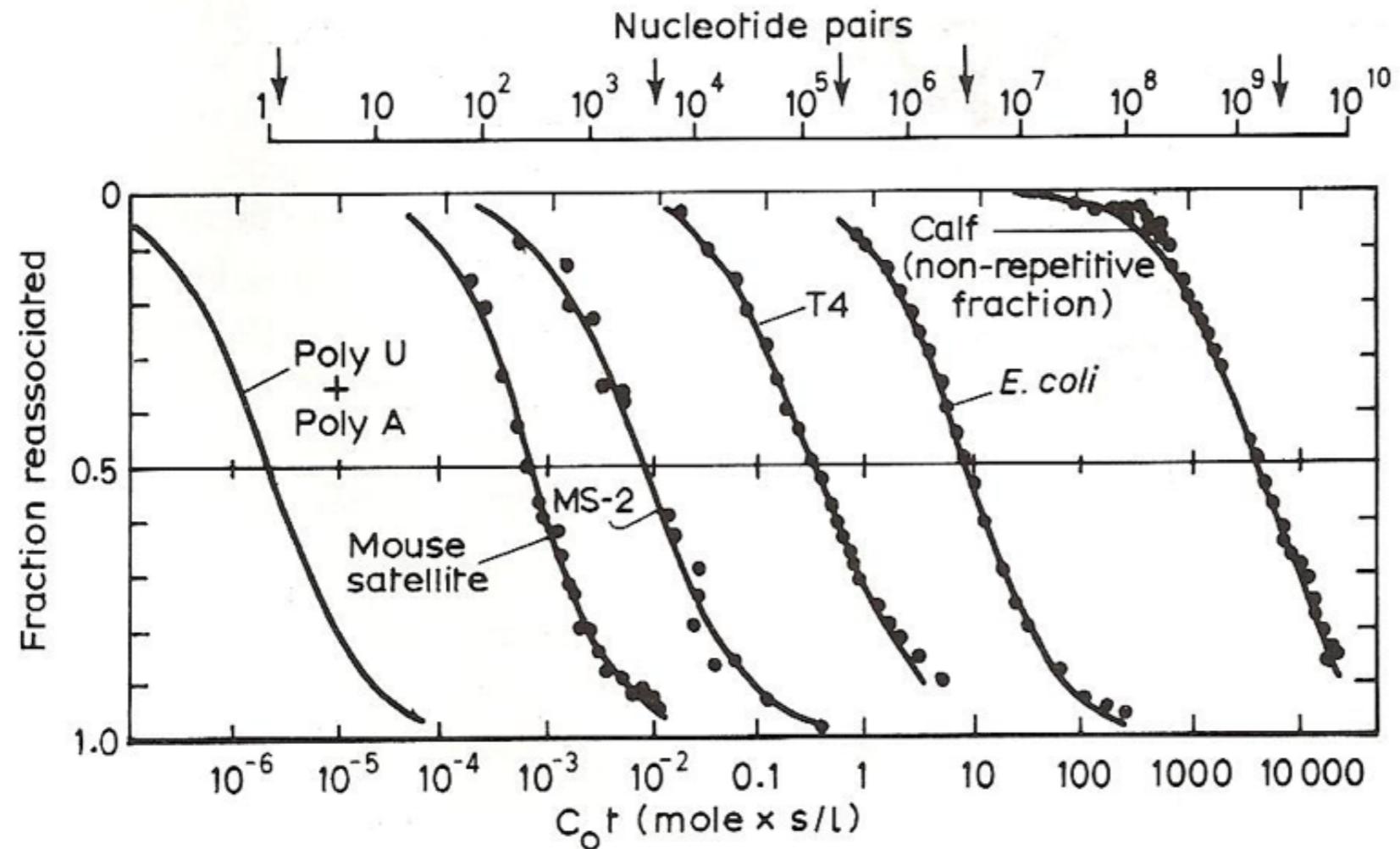
Fig. 2.20 The rate of reassociation of double-stranded polynucleotides from various sources showing how the rate decreases with the complexity of the organism and its genome (from [60]).

DNA renaturation/reassociation depends on the concentration of the DNA molecules and the time allowed for reassociation. Often imperfect matches may be formed which must again dissociate to allow the strands to align correctly. $C_o t$ value of DNA is defined as the initial concentration C_0 in moles nucleotides per Litre multiplied by time t in seconds. $C_o t$ reflects complexity of DNA. Methods: S1, hydroxyapatite - dsDNA binds more strongly

Biosyntetické polynukleotidy -

modely pro výzkum fyzikálních a **chemických vlastností** a struktury nukleových kyselin

POLYRIBONUKLEOTIDY

byly syntetizovány většinou pomocí **polynukleotid fosforylázy**, která polymerizuje nukleotid-5'-difosfáty (při čemž se uvolňuje anorganický fosfát)

Po počáteční syntetické fázi, dochází k rovnováze mezi syntézou a degradací (fosforolyzou) a vytvářejí se polymery s poměrně malým rozptylem délek

Polynukleotid fosforyláza polymerizuje mnohá analoga nukleosid difosfátů jako 2'-O-metyl, 2'-chloro-, 2'-fluoro- a dokonce i arabinonukleosid-5'-difosfáty a nukleosid difosfáty s různě modifikovanými bazemi.

Nukleozidy mající konformaci syn- (např. 8-bromoguanosin) polymerizovány nejsou. Enzym vyžaduje konformaci cukru 3'-endo.

Tento enzym nevyžaduje pro svoji funkci matrici (někdy očko/primer). Vhodný zejména pro syntézu **homopolynukleotidů.** Heteropolymery mají náhodnou sekvenci nukleotidů.

Příprava polynukleotidů s **definovanou** sekvencí nukleotidů vyžaduje **RNA-polymerázu** (závislou na DNA) nebo **DNA-polymerázu** (pro syntézu polydeoxyribonukleotidů)

Důležité modely vlivu sekvence nukleotidů na vlastností DNA

nukleosid-difosfáty
nevyžaduje primer ani matrici

nukleosid-trifosfáty

Homopolynukleotidy

Poly(U) a poly(dT) při pokojové teplotě mají málo výraznou sekundární strukturu, při vyšší teplotě tuto strukturu ztrácejí

Poly(C) v kyselém prostředí tvoří dvojřetězovou protonizovanou strukturu s paralelními řetězci. V neutrálním prostředí tvoří jednořetězovou strukturu stabilizovanou vertikálním vrstvením bazí (stacking)

Poly(A) tvoří v kyselém prostředí dvojřetězovou strukturu s paralelními řetězci (podobně jako poly (C). Párování bazí je ve struktuře poly(A) zajištěno jinak než v poly(C). V neutrálním prostředí má poly(A) strukturu jednořetězovou.

Poly(G) a poly(I) tvoří čtyřvláknové struktury

poly(A)
poly(rC)
poly(dG)
poly(U)
poly(rT)
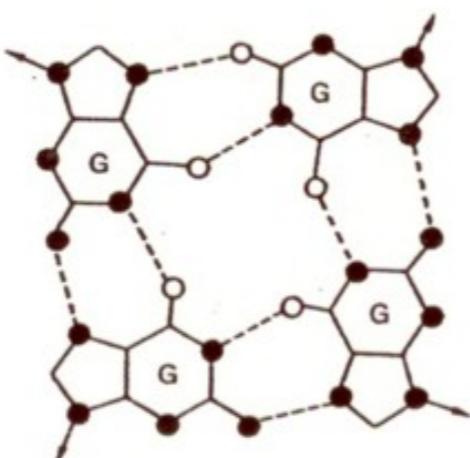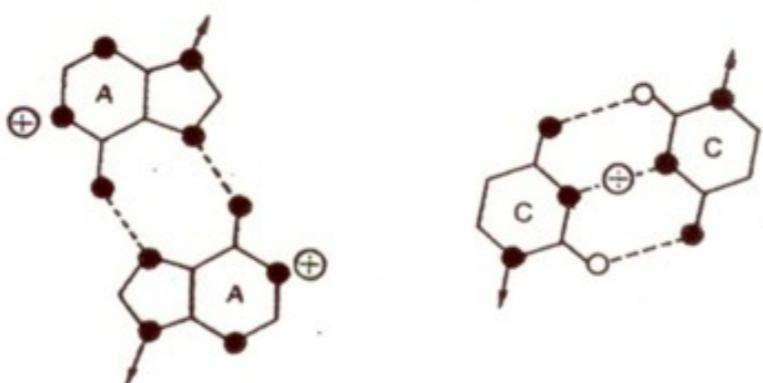
Polynukleotidové komplexy

Smícháním polynukleotidů (za vhodných iontových podmínek) vznikají dvou- a víceřetězové komplexy

Poly(A)·poly(U) tato dvojitá šroubovice vzniká při fyziologické iontové síle za nepřítomnost Me^{2+}. Při vyšších iontových silách může vzniknout trojřetězová struktura poly(A)·poly(U)·poly(U) [poly(A)·2 poly(U)]
(Hoogsteen)

Poly(G)·poly(C), poly(I)·poly(C) tyto dvojité šroubovice vznikají při neutrálním pH. V kyselém prostředí se tvoří trojřetězové struktury
poly(G)·poly(C)·poly(C^+) v nichž je jeden řetězec
poly(C) protonizovaný. Podobně interaguje i poly(C) s poly(I)

Tyto interakce jsou silně závislé na iontové síle

Studium vlastností biosyntetických polynukleotidů přineslo v minulosti důležité informace o vztazích mezi sekvencí nukleotidů a strukturou DNA a RNA, např.:

t_m: (rI)·(rC) > (rI)·(dC) > (dI)·(dC) > (dI)·(rC)

poly(dI-dC) a poly (dG-dC) jsou stabilnější nežli odpovídající komplexy homopolynukleotidů

Směsné křivky:
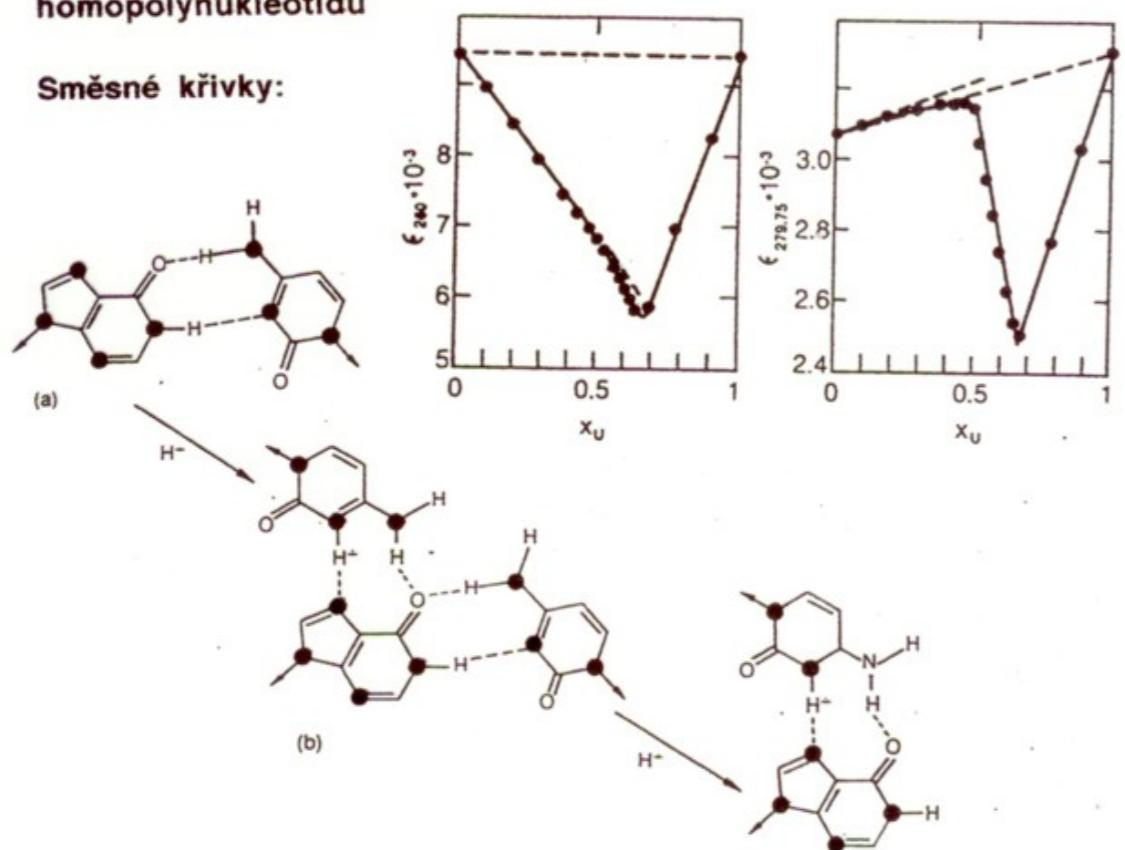
Mirror Molecules

NA bases x L-amino acids (aa) x D-carbohydrates

D-aa already known in the late 1800's

In chemistry of life L-aa were the rule (only gly achiral)

Ribosomes compatible only with L-aa but not with D-aa

For a long time the only exceptions to this pattern were found in bacteria.

Recently biologically active D-aa's have been found to perform important roles in human physiology.

1990's   S. Snyder D-compounds serve as neurotransmitters

„Like most of science, whenever there is something really new or different, some people say some people say: That's ridiculous"

Later:
D-aspartate shown to be a neurotransmitter involved in normal brain development

D-serine teams up with L-glutamate to activate neuronal molecules essential for synaptic plasticity – key to learning and forming memories

2002   P.Kuchel: Platypus poison contains D-aa
2009   D-aa's play unexpected function in bacterial cell walls
2010   complex assemblies of bacteria use D-aa when the biofilms should disperse

D-serine important factor in schizofrenia (lower amount of this aa) x higher amounts: increase brain damage in stroke

Review 2012

Since d-amino acids were identified in mammals, **d-serine has been one of the most extensively studied** "unnatural amino acids". This brain-enriched transmitter-like molecule plays a pivotal role in the human central nervous system by modulating the activity of NMDA receptors. **Physiological levels of d-serine are required for normal brain development and function**; thus, any alterations in neuromodulator concentrations might result in NMDA receptor dysfunction, which is known to be involved in several pathological conditions, including neurodegeneration(s), epilepsy, schizophrenia, and bipolar disorder. In the brain, the **concentration of d-serine stored in cells is defined by the activity of two enzymes: serine racemase (responsible for both the synthesis and degradation) and d-amino acid oxidase (which catalyzes d-serine degradation)**. Both enzymes emerged recently as new potential therapeutic targets for NMDA receptor-related diseases. In this review we have focused on **human d-amino acid oxidase and provide an extensive overview of the biochemical and structural properties of this flavoprotein and their functional significance.** Furthermore, we discuss the mechanisms involved in modulating enzyme activity and stability with the aim to substantiate the **pivotal role of d-amino acid oxidase in brain d-serine metabolism in physiological and pathological conditions and to highlight its great significance for novel drug design/development.**

Only L-aa are produced in cells

Brain cells produce
an enzyme that flips the handedness of L-serine to its D-form

Platypus venom is made in a similar way: ribosome builds up the peptide from regular L-aa. Then an enzyme flips an aa into its D-form

2005 Kreil (Austrian Academy of Sciences, Wien) making D-aa's in tree frog venom. Without a single D-aa, the peptide has no hallucinogenic effect

D-aa's found in poisons of a wide range of organisms but these aa's have also more peaceful purposes, e.g. in lobsters they keep salt levels in order

Biggest users of D-aa's are microbes

For example, peptidoglycan in cell walls may contain D-ala, D-met or D-leu

Important research task:
Understanding how bacteria exploit D-aa's for communication
Attempts to develop drugs that break-up the biofilms in our teeth, in the lungs of cystic fibrosis or in medical equipment
Addition of D-aa's to therapeutic peptides or proteins may prevent their enzymatic degradation

Speculations:
D-aa's in bacterial cells living on our skin or elsewhere in the body might be important for our well-being and behavior

Preliminary reports:
Yoko Nagata, Tokyo: D-aa in human saliva
D-ala in the insulin-secreting beta cells in rats

Enzymes converting L-aa into D-aa found in human and rat hearts. Precise role of such enzyme in human physiology is still "a total mystery".

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The role of D-amino acids in amyotrophic lateral sclerosis pathogenesis: a review
Author(s): Paul, Praveen; de Belleroche, Jacqueline
Source: AMINO ACIDS Volume: 43   Issue: 5   Pages: 1823-1831   DOI: 10.1007/s00726-012-1385-9   Published: NOV 2012

Structure-function relationships in human d-amino acid oxidase
Author(s): Sacchi, Silvia; Caldinelli, Laura; Cappelletti, Pamela; et al.
Source: AMINO ACIDS Volume: 43   Issue: 5   Pages: 1833-1850   DOI: 10.1007/s00726-012-1345-4   Published: NOV 2012
Times Cited: 2 (from Web of

Nutritional and medicinal aspects of D-amino acids
Author(s): Friedman, Mendel; Levin, Carol E.
Source: AMINO ACIDS Volume: 42   Issue: 5   Pages: 1553-1582   DOI: 10.1007/s00726-011-0915-1   Published: MAY 2012

Lokální struktury DNA a metody jejich analýzy

Local Supercoil-Stabilized DNA Structures

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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 sequence.[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 sam-

ples 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-

POLYMORFIE DVOJITÉ ŠROUBOVICE DNA

Polymorphy of the DNA double helix

Až do konce 70. let bylo všeobecně předpokládáno, že DVOJITÁ ŠROUBOVICE DNA (DNA DOUBLE HELIX) je velmi pravidelná a nezávislá na sekvenci nukleotidů. Tento názor byl založen především na výsledcích rtg.-strukturní analýzy VLÁKEN - metody, která byla používána po více jak 2 desetiletí k analýze struktury DNA.

V průběhu 60. a 70. let se však začaly hromadit výsledky empirických metod, nasvědčující tomu, že koncepce jedinečné (unique) struktury DNA je neudržitelná a že existuje vztah mezi sekvencí nukleotidů DNA a jejím prostorovým uspořádáním.

Začátkem 70. let **S. Bram** - rtg.-strukturní analýza VLÁKEN DNA s velmi rozdílným obsahem bazí

F. Pohl a T. Jovin - CD poly(dG)·(dC)

EP - elektrochemická analýza DNA

Koncem 70. let rtg.-strukturní analýza KRYSTALU

VISWAMITRA et al. d(pATAT)

A. Rich   d(CGCGCG)   d(CGCG) levotočivá Z-DNA

R. Dickerson   d(CGCGAATTCGCG) pravotočivá B-DNA

Prokázána
závislost struktury na sekvenci nukleotidů,
která je velmi výrazná u B-DNA

Kromě sekvenční informace je možno uvažovat
i informaci KONFORMAČNÍ

B. sublilis and B. brevis DNAs have the same
G+C content and different nucleotide sequence
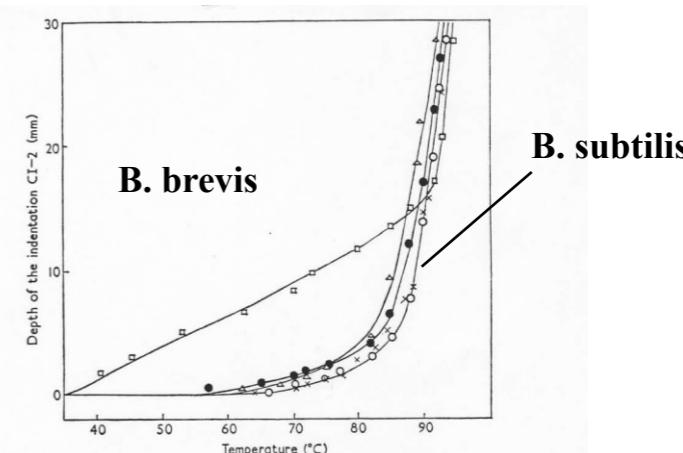
FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 μg/ml. in 0·25 M-ammonium formate plus 0·025 M-sodium phosphate (pH 7·0).
—●——●—, *B. subtilis* 168; —×——×—, *B. natto*; —○——○—, *B. subtilis* var. *niger*; —△——△—, *B. subtilis* var. *aterrimus*; —□——□—, *B. brevis* (ATCC 9999).
P 524 polarascope, dropping mercury electron polarized with repeated cycles of a.c. The measurements were carried out in the laboratory of Prof. J. Marmur, Department of Biochemistry, Brandeis University, Waltham, Mass., U.S.A.

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Premelting Changes in DNA
Conformation

E. PALEČEK

### 6. POLYMORPHY OF DNA SECONDARY STRUCTURE

On the basis of the preceding discussion, a schematic picture of the structure of natural linear DNA in solution under physiological conditions (e.g., at 36°C, moderate ionic strength, and pH 7) can be drawn. We can assume that the double-helical structure of the very long (A + T)-rich regions differs from the structure of the major part of the molecule and that some of the (A + T)-rich segments are open (Fig. 20). An open ds-structure can be assumed in the region of chain termini and/or in the vicinity of ss-breaks and other anomalies in the DNA primary structure. The exact changes in the open ds-regions will depend on the nucleotide

sequence as well as on the chemical nature of the anomaly. Most of the molecule will exhibit an average Watson–Crick B-structure with local deviations given by the nucleotide sequence. Elevating the temperature in the premelting region (Fig. 20) is likely to lead to the opening of other regions and, eventually, to expansion of the existing distorted ds-regions and to further structural changes. Thus the course of the conformational changes as a function of temperature (premelting) will be determined by the distribution of the nucleotide sequences and anomalies in the primary structure, and may have an almost continuous character.

Consequently, even if we do not consider "breathing," not only the architecture of a DNA double-helical molecule, but also its mechanics or dynamics can be taken into account.

To determine whether, e.g., only the (A + T)-rich molecule ends will be open at a certain temperature or also long A + T regions in the center of the molecule, further experimental research with better-defined samples of viral and synthetic nucleic acids will be necessary. Further work will undoubtedly provide new information on the details of the local arrangement of nucleotide residues in the double helix, as well as on DNA conformational motility. Thus a more accurate picture of DNA structure will emerge, whose characteristic feature will be polymorphy of the double helix, in contrast to the classical, highly regular DNA structure models.

Dvojitá šroubovice DNA je POLYMORFNÍ
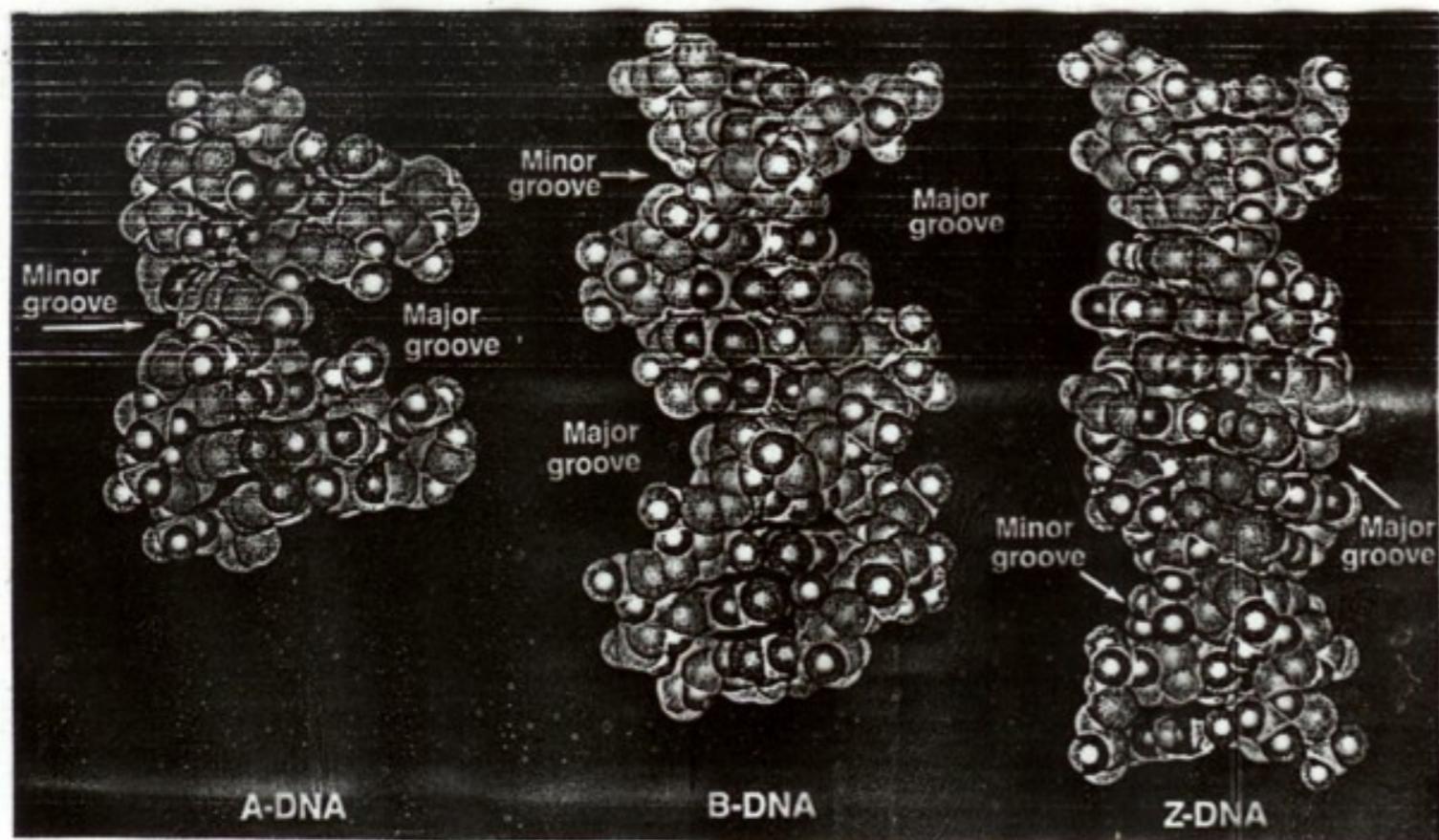
A-DNA   B-DNA   Z-DNA

DNA structures from X-ray crystal analysis

DNA double helix is polymorphic
depending on the nucleotide sequence

DNA se v BUŇKÁCH vyskytuje převážně
v NEGATIVNĚ SUPERHELIKÁLNÍ
(nadšroubovicové) formě

ÚROVEŇ SUPERHELICITY je
homeostaticky KONTROLOVÁNA
TOPOISOMERASAMI

Topo I   Topo II (Gyrase)
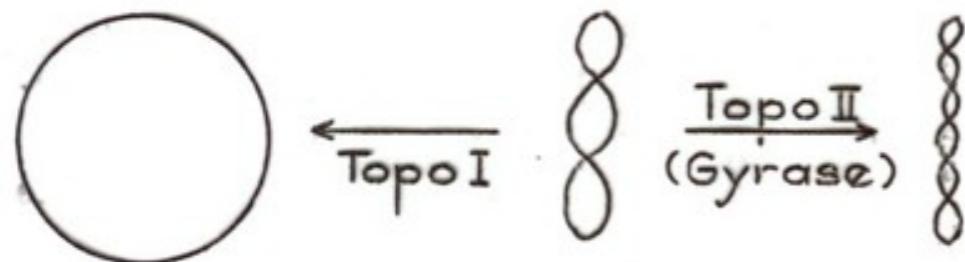
SUPERHELICITA DNA OVLIVŇUJE základní
biochemické děje jako TRANSKRIPCI.

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 70s, when organic synthesis had been developed to the point where oligodeoxynucleotides (ODN) could be produced in the purity and quantity necessary for the preparation of single crystals for X-ray diffraction (and NMR) studies. Three main families of DNA forms were identified by crystallographic analysis of ODN: right-handed A and B-forms and the left-handed Z-form.

B-, A- and Z-helices

The A-, B- and Z-helices have distinctly different shapes which 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 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.

A   B   Z

Double helical conformations of DNA: (left) *A-DNA,* (center) *B-DNA,* (right) *Z-DNA.*
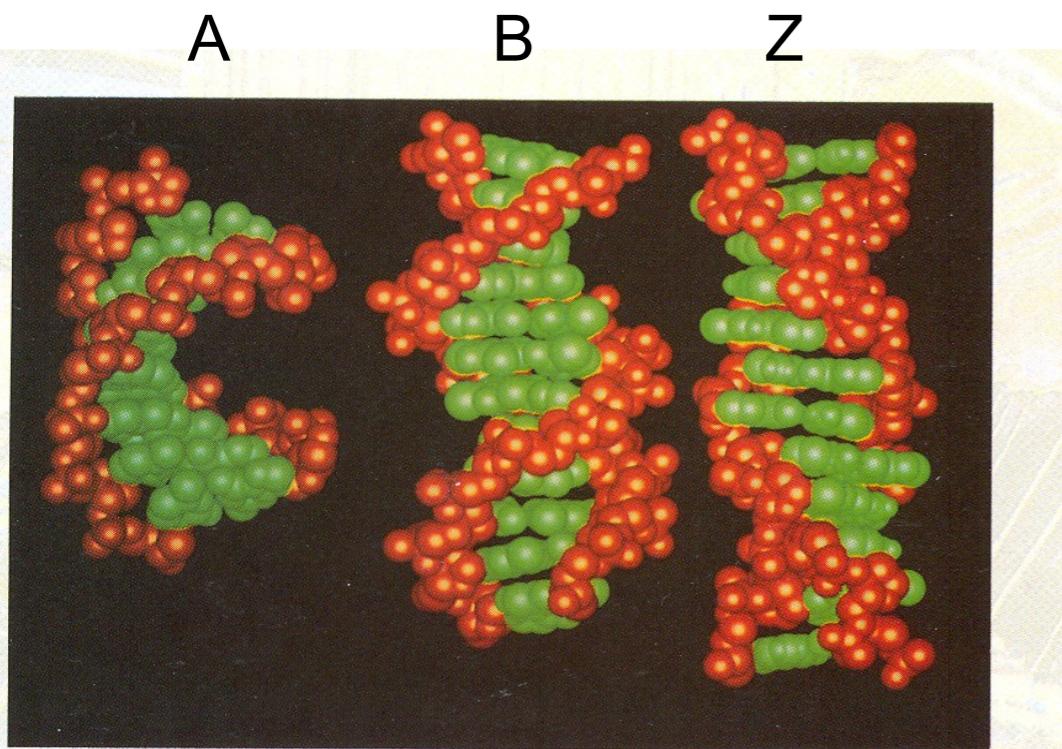
In A-duplexes base pairs are heavily tilted in contrast to base pairs in B-duplexes which are almost perpendicular to the helical axis. (Table 1). Many of the structural differences between the helices arise from the puckering of the sugar ring; C3'-endo is typical for A-DNA, while in Z-DNA C3'-endo alternates with C2'-endo. In B-DNA sugar pucker tends to favor the C2'-endo or C1'-exo, 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 Z-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 zig-zag 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 Z-DNA. Methylation or bromination of cytosines at position 5 (studied mainly in ODNs with alternating C-G sequence) stabilizes Z-DNA. Under certain conditions even non-alternating sequences of purines and pyrimidines can assume the conformation of Z-DNA with thymines in a syn orientation. The outer surface features of such a Z-helix are different at the non-alternating sites but the backbone is similar to that observed with alternating sequences.

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

Helix sense	A-DNA[a] 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 (Å)	2.9	3.4	3.5, 3.3	3.7
Helix pitch (Å)	32	34	34	45
Base pair tilt (°)	13	0	0	−7
P distance from helix axis (Å)	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.

Single-strand selective chemical probes of the DNA structure

```
CHEMICAL  PROBES  of  the  DNA  STRUCTURE
reacting  preferentially  with
single-stranded  and  non-B  DNA
regions


Base  specificity              Probe
-----------------------------------------------

T>>C,G                         Os,py,  Os,bipy
.                              KMnO4

T>>C                           OsO4  (alone)

A>G                            DEPC*


A,C                            BAA**,  CAA**


G                              glyoxal*
.                 N-hydroxylaminofluorene

C                              NaHSO3
.                              hydroxylamine
.                              methoxylamine
-----------------------------------------------

*  reacts  also  with  Z  DNA  (purines  in
syn  conformation)

**  disturbance  of  Watson-Crick
pairing  required
```

TABLE 2
Average Helical Parameters for Selected Right-Handed Structures

	Helix twist (°)	Rise per base pair (Å)	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
B-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-bromcytosine
Adapted from Kennard, O. and Hunter, W. N., *Q. Rev. Biophys.*, 22, 327, 1989. With permission.

Dvojitá šroubovice DNA je POLYMORFNÍ
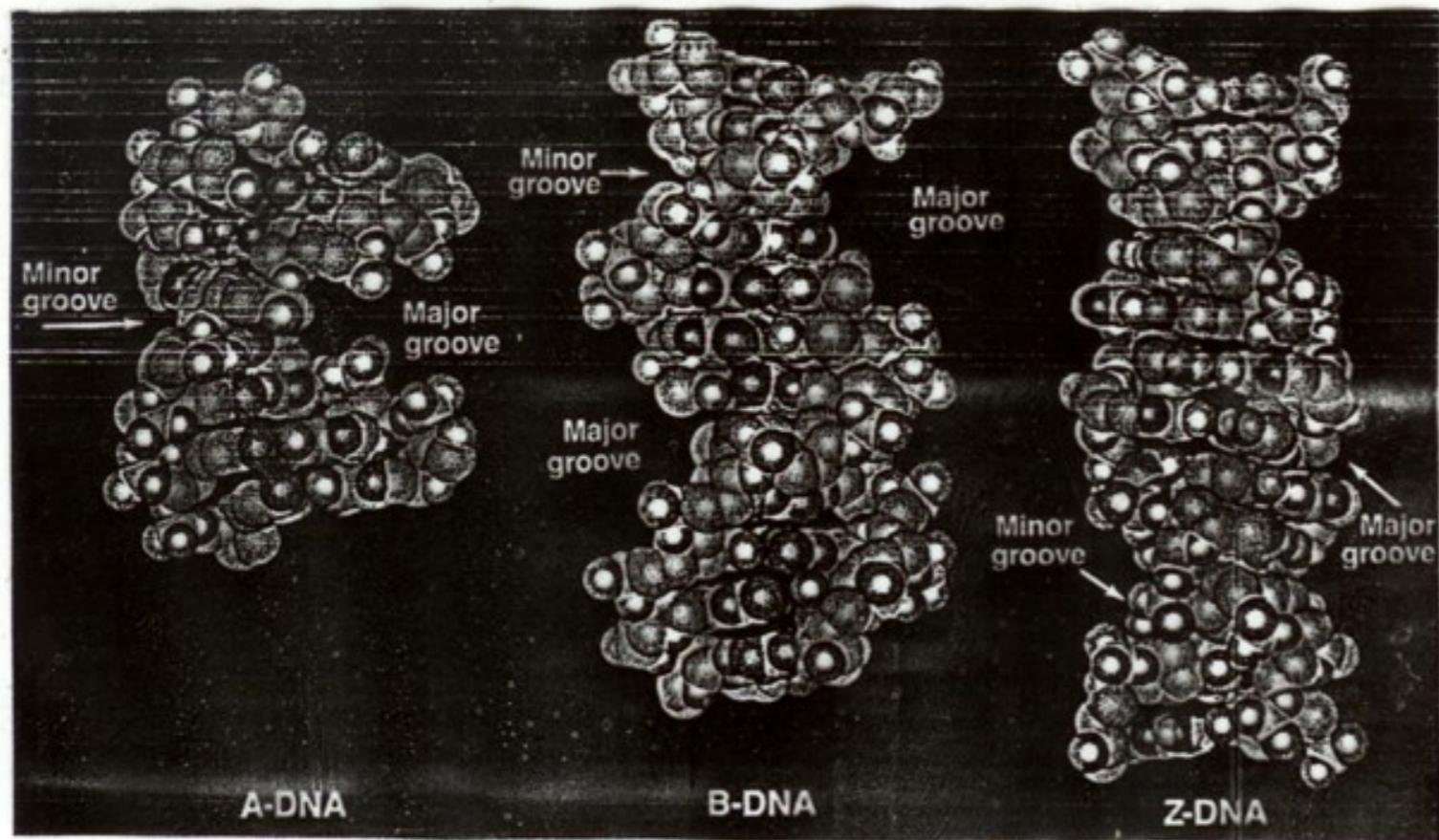
A-DNA   B-DNA   Z-DNA

DNA structures from X-ray crystal analysis

DNA double helix is polymorphic depending on the nucleotide sequence

DNA se v BUŇKÁCH vyskytuje převážně v NEGATIVNĚ SUPERHELIKÁLNÍ (nadšroubovicové) formě

ÚROVEŇ SUPERHELICITY je homeostaticky KONTROLOVÁNA TOPOISOMERASAMI

Topo I   Topo II (Gyrase)
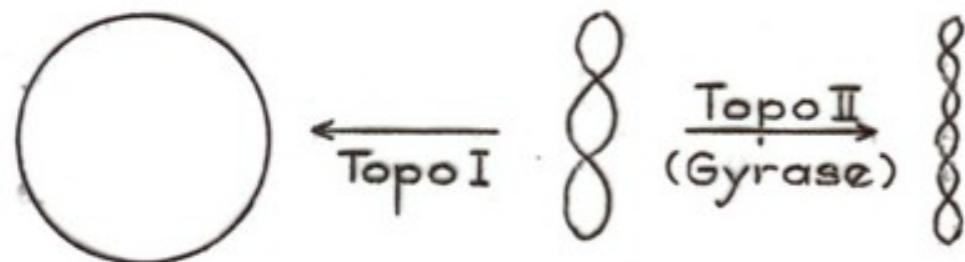
SUPERHELICITA DNA OVLIVŇUJE základní biochemické děje jako TRANSKRIPCI.

DNA footprinting

mapping of DNA interactions
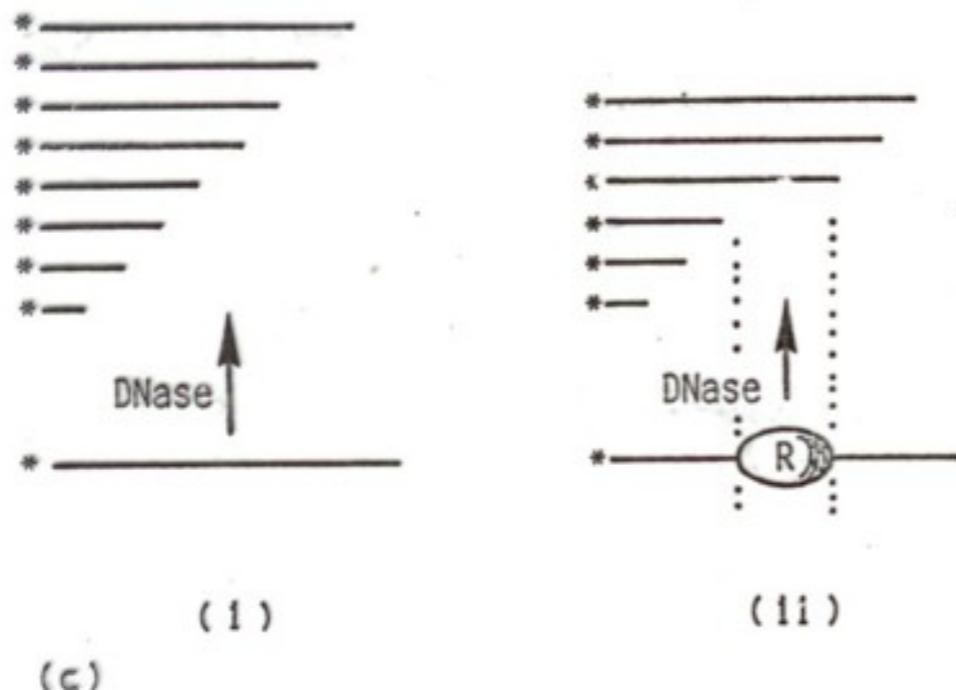
Enzymatic probe (DNAase I)

(c)

(i)   (ii)

(b)
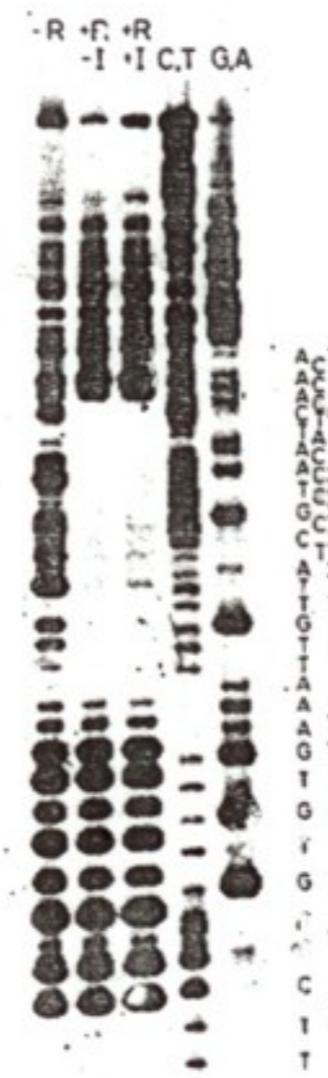
Fig. A.24 Example of DNase I footprinting. (a) Partial digestion of a piece of end-labelled DNA to which a protein (R) is bound (ii) results in the absence of the end-labelled fragments cleaved in this region but found in the digest (i) of the unprotected DNA; (b) the results of separation of the products of such an experiment on a polyacrylamide sequencing gel. The example is with *lac* operator DNA and the *lac* repressor (R). I is IPTG (0.3 M), which does not prevent the binding of a mutant repressor used in this study. C,T and G,A represent the results of Maxam-Gilbert C + T and A + G reactions on the undigested end-labelled fragment. Adapted from [105], with permission.

Chemical probe - DEPC
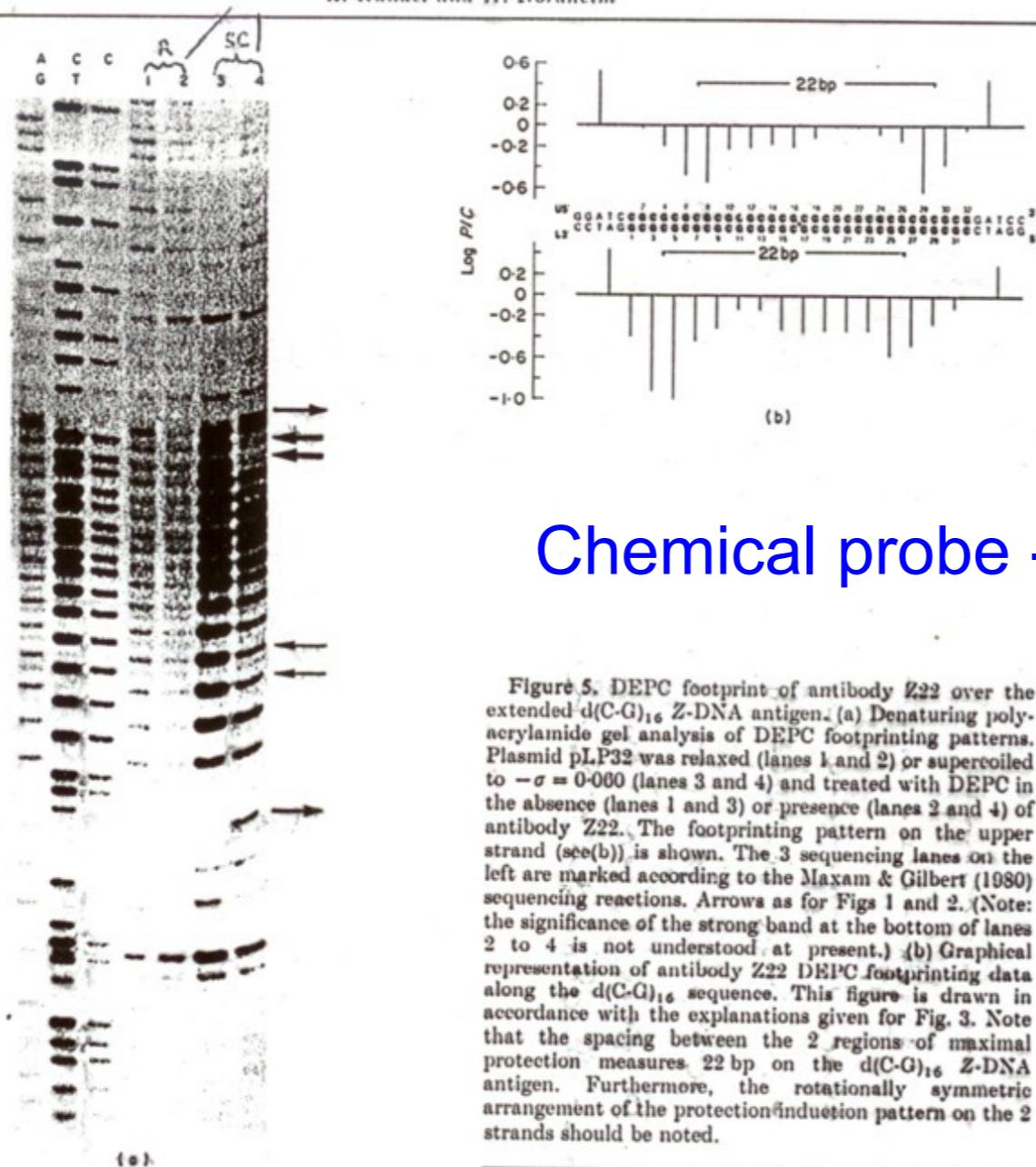
Figure 5. DEPC footprint of antibody Z22 over the extended d(C-G)$_{16}$ Z-DNA antigen. (a) Denaturing polyacrylamide gel analysis of DEPC footprinting patterns. Plasmid pLP32 was relaxed (lanes 1 and 2) or supercoiled to $-\sigma = 0.060$ (lanes 3 and 4) and treated with DEPC in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of antibody Z22. The footprinting pattern on the upper strand (see(b)) is shown. The 3 sequencing lanes on the left are marked according to the Maxam & Gilbert (1980) sequencing reactions. Arrows as for Figs 1 and 2. (Note: the significance of the strong band at the bottom of lanes 2 to 4 is not understood at present.) (b) Graphical representation of antibody Z22 DEPC footprinting data along the d(C-G)$_{16}$ sequence. This figure is drawn in accordance with the explanations given for Fig. 3. Note that the spacing between the 2 regions of maximal protection measures 22 bp on the d(C-G)$_{16}$ Z-DNA antigen. Furthermore, the rotationally symmetric arrangement of the protection/induction pattern on the 2 strands should be noted.

Lokální struktury DNA a metody jejich analýzy

Local Supercoil-Stabilized DNA Structures

E. Paleček

Max-Planck Institut für Biophysikalische Chemie, Göttingen, 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.

Parametry různých typů ds DNA

Metody analýzy lokálních struktur DNA

Ohyby v DNA

Typy lokálních struktur stabilizovaných nadšroubovicovým vinutím

Strukturní rozhraní

Výskyt lokálních struktur DNA in vivo

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 sequence.[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 sam-

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

METHODS OF ANALYSIS OF LOCAL DNA STRUCTURES

Metody analýzy lokálních stuktur DNA

1. ANALYSIS OF DNA ELECTROPHORETIC MOBILITY
two-dimensional gel electrophoresis

2. ANTIBODIES recognizing local DNA STRUCTURES
anti-Z-DNA, anti-crucifom, anti-triplex

3. ENZYMATIC PROBES
Nucleases and especially single-strand selective nucleases
nuclease S1, nuclease P1, mung bean nuclease, etc.

4. Ultraviolet radiation

5. CHEMICAL PROBES

a. Probes reacting with double-stranded DNA / FOOTPRINTING

i. Dimethyl sulfate (DMS) / G - N7, A - N3

ii. N-Ethyl-N-nitrosourea (ENU) / phosphates

iii. Probes with nuclease activities: 1,10-phenanthroline copper ion, methidiumpropyl-EDTA, Fe(II).EDTA - hydroxyl radical induced chain cleavage

iv. Photochemical probes: Psoralens, acridines, EtdBr, uranyl salts, tris-phenanthroline transition metal complexes

v. Complementary addressed modification and cleavage of DNA

b. Single-strand selective probes

Single-strand selective chemical probes of the DNA structure

CHEMICAL PROBES of the DNA STRUCTURE
reacting preferentially with
single-stranded and non-B DNA
regions

Base specificity	Probe
T>>C,G	Os,py, Os,bipy KMnO$_4$
T>>C	OsO$_4$ (alone)
A>G	DEPC*
A,C	BAA**, CAA**
G	glyoxal* N-hydroxylaminofluorene
C	NaHSO$_3$ hydroxylamine methoxylamine

* reacts also with Z DNA (purines in syn conformation)

** disturbance of Watson-Crick pairing required

Discovery of the cruciform
in sc DNA

D M J LILLEY, 1981

a

$Os O_4$ / pyridin

b

c

d

S1 NUCLEASE

1

CHEM CLEAVAGE

2

3

TERMINATION

R II

4   CLEAVAGE
     INHIBITION

RI

A

OsO_4 / PYRIDINE

B

5   IMMUNOASSAY

EM   S1

A

I

OsO₄ bipy → EcoRI (RA) → S1, BamHI (RR)

II

OsO₄ bipy → EcoRI (RA) → S1, BamHI (RR)

B

1 2 3

I

1 2 3

II
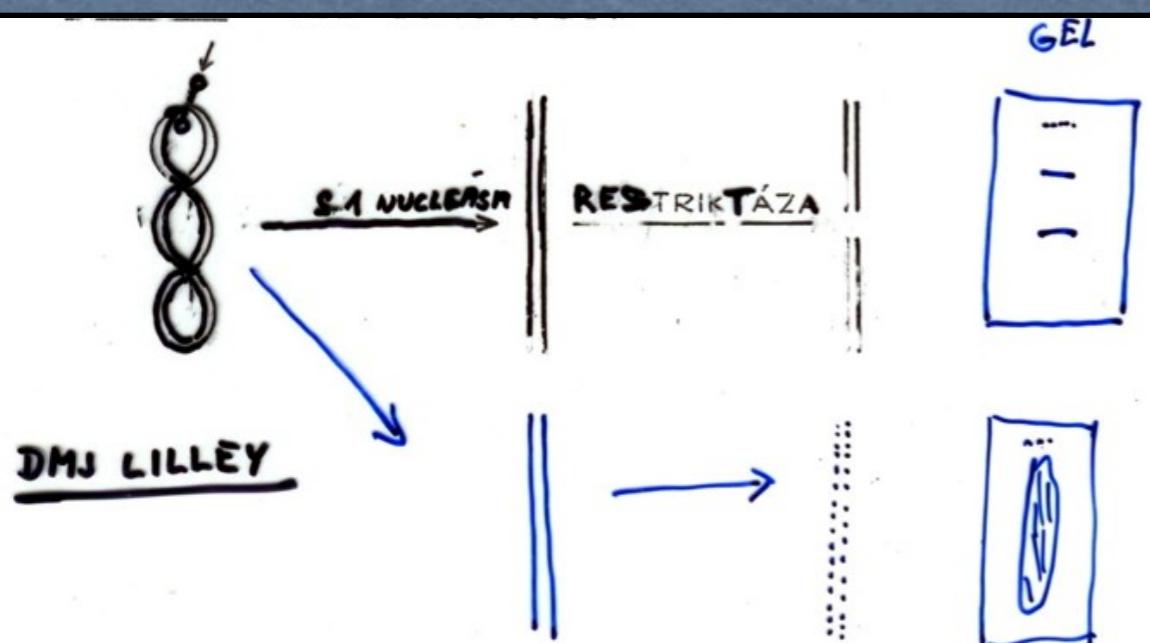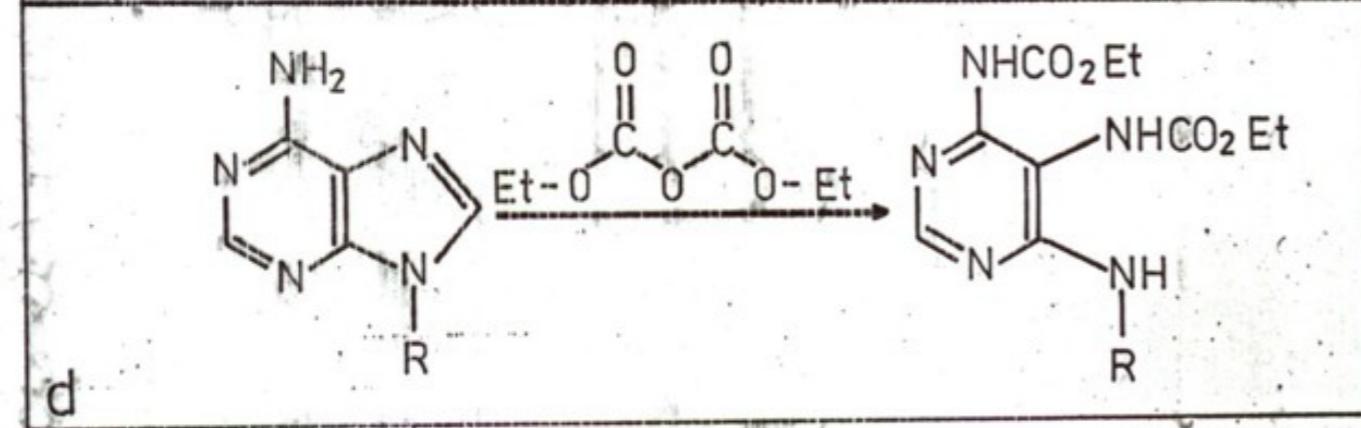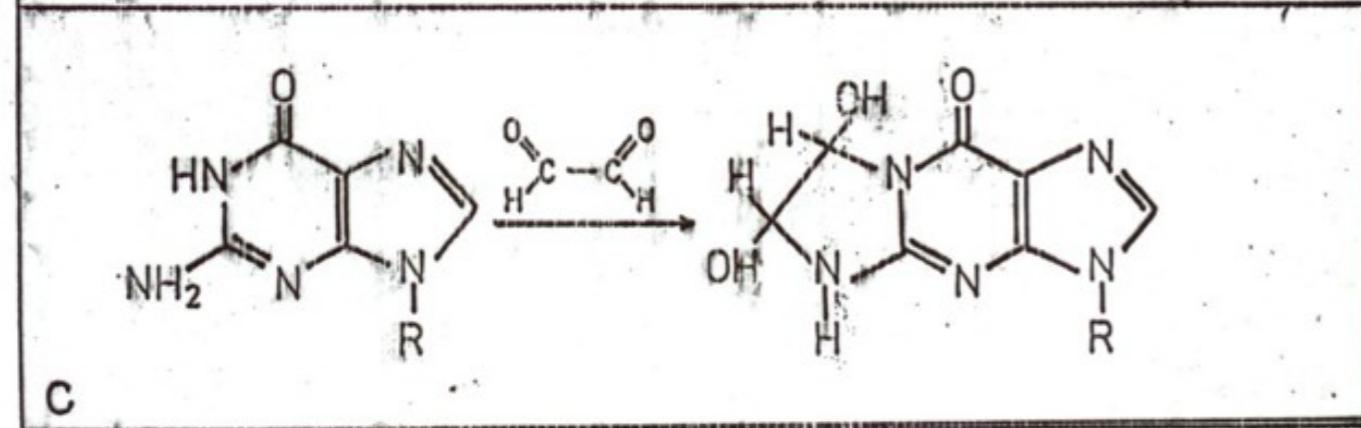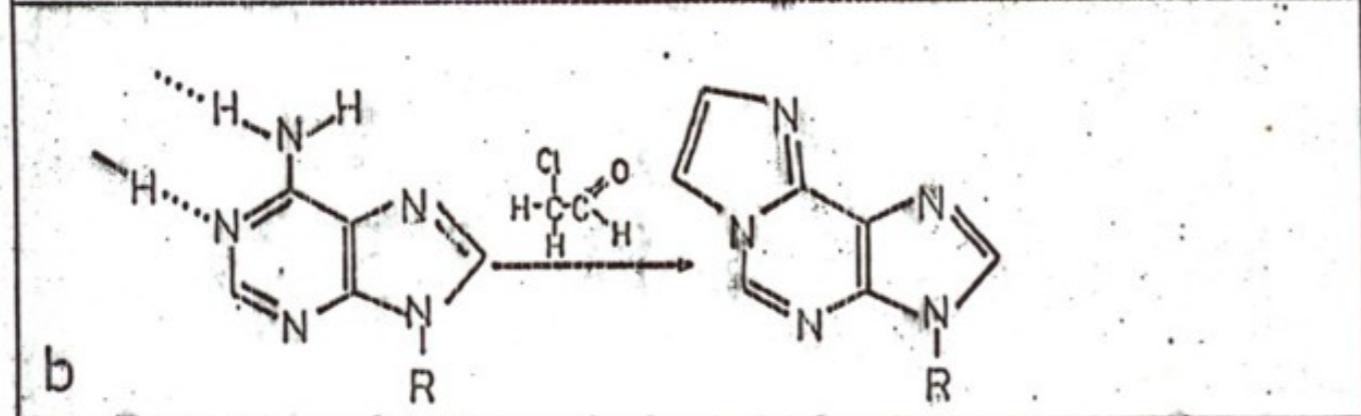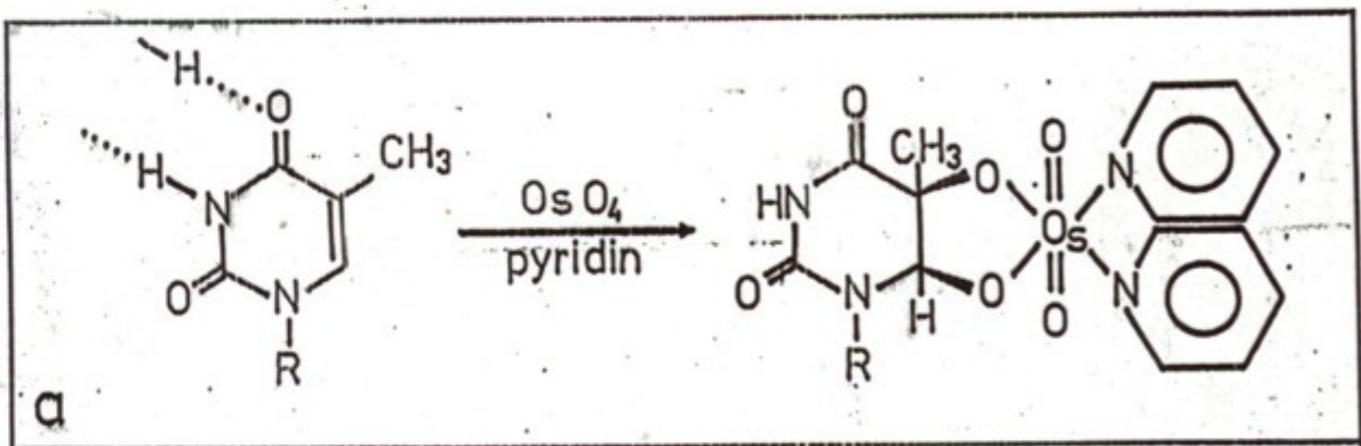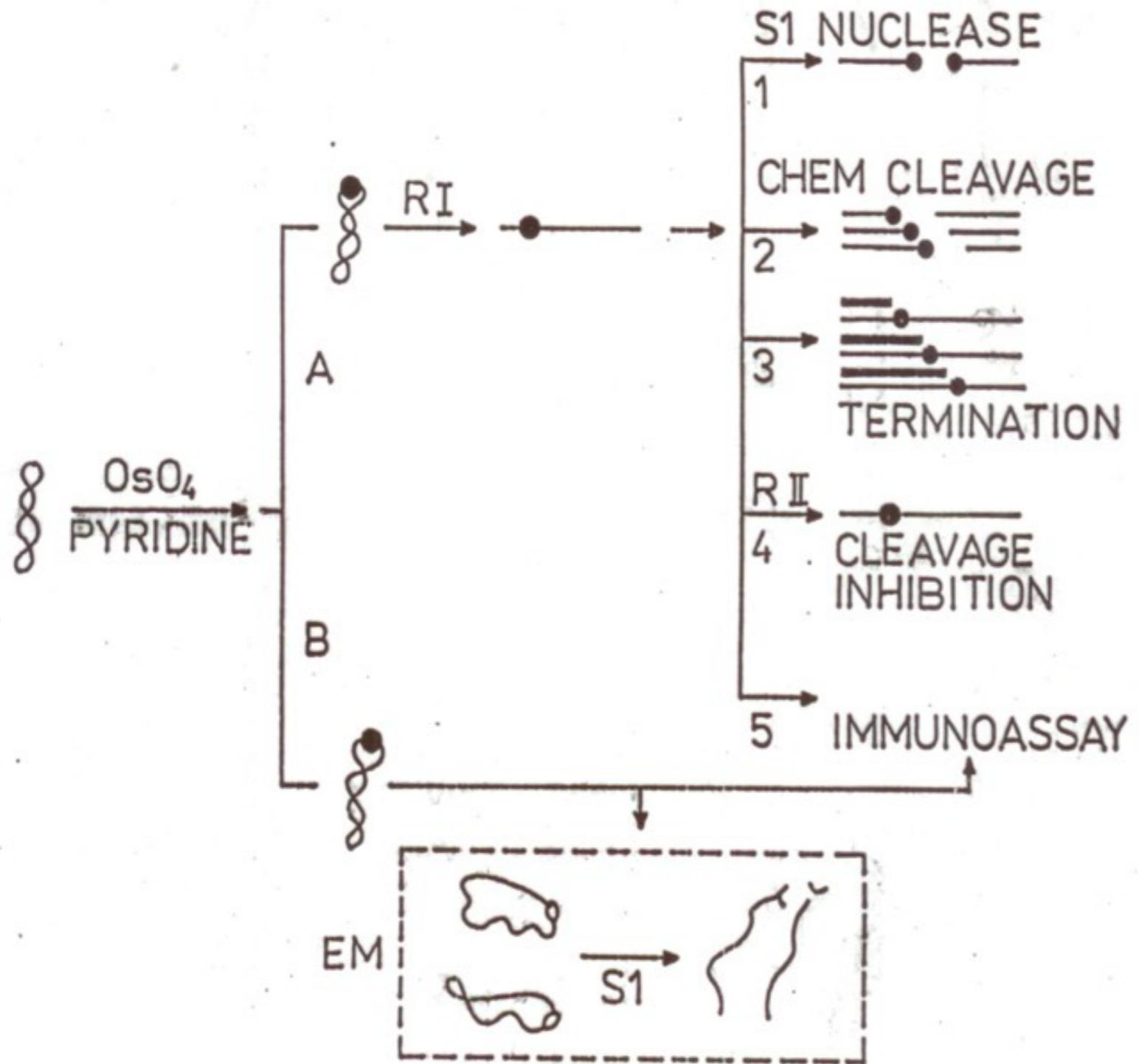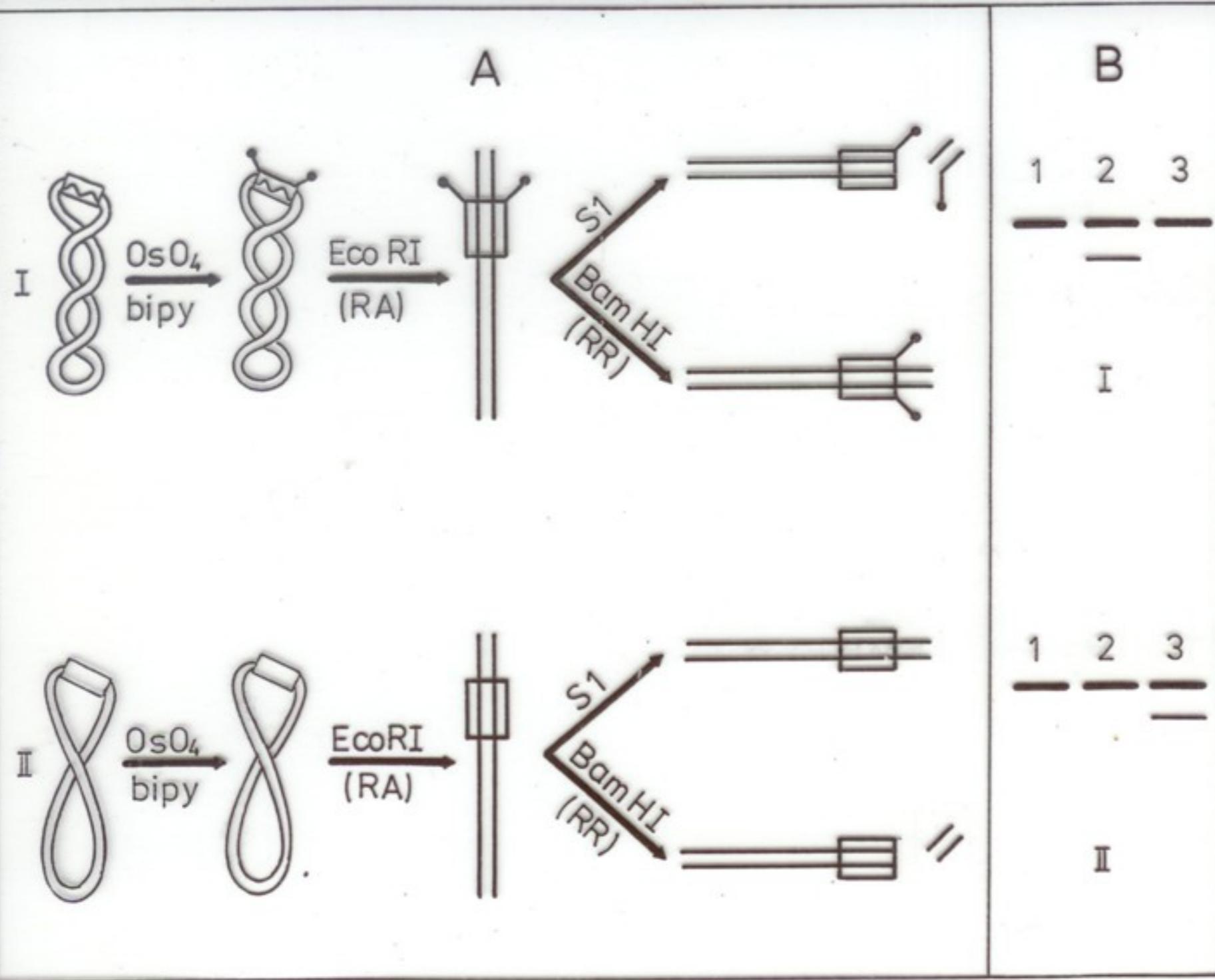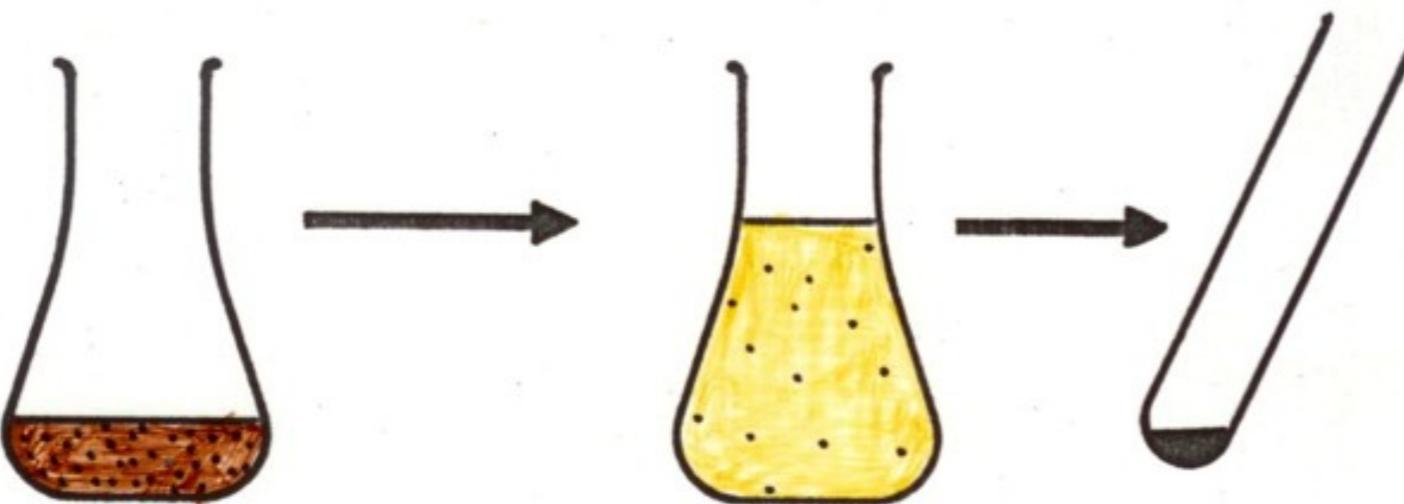
CELLS+Os,bipy
(1-2mM)
(e.g.in 0.5M
phosphate pH 7.4)
37° or 26 °C
10-60 min
2 mg cells/ml

10x DILUTION
0 °C

RINSED

DNA ISOLATION

(BOILING METHOD)

LINEARIZED

RESTRICTASE

BamHI

S1

INHIBITION

sequencing

SITE - SPECIFIC
CLEAVAGE
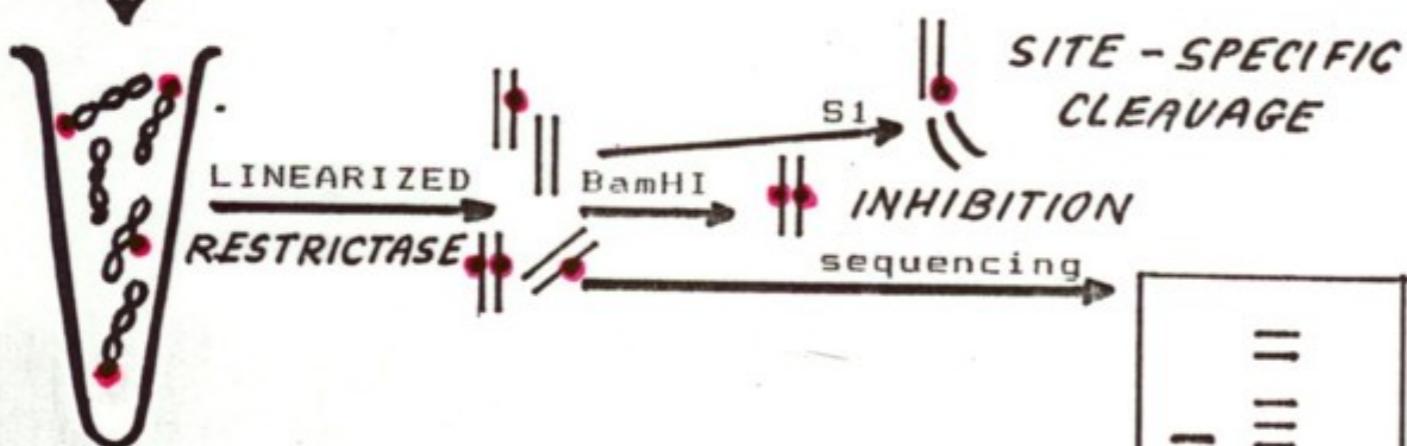
A. No structural transition:
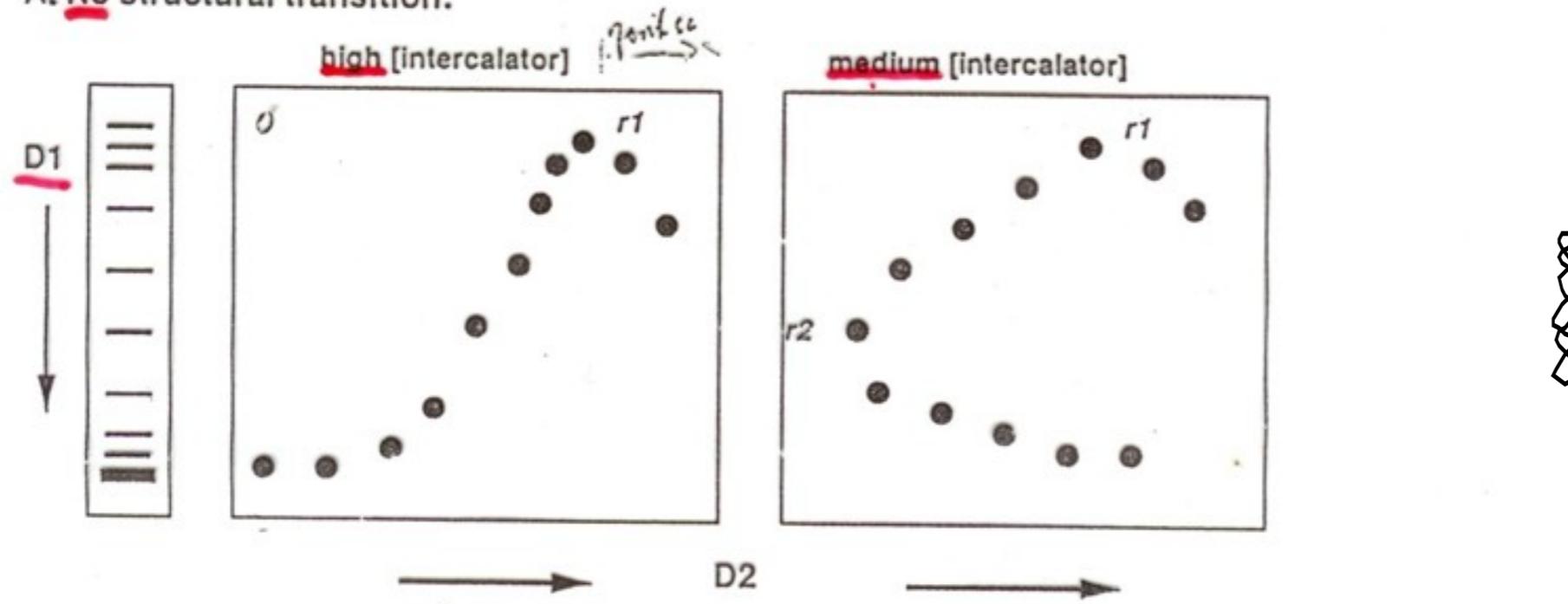
B. With structural transition:
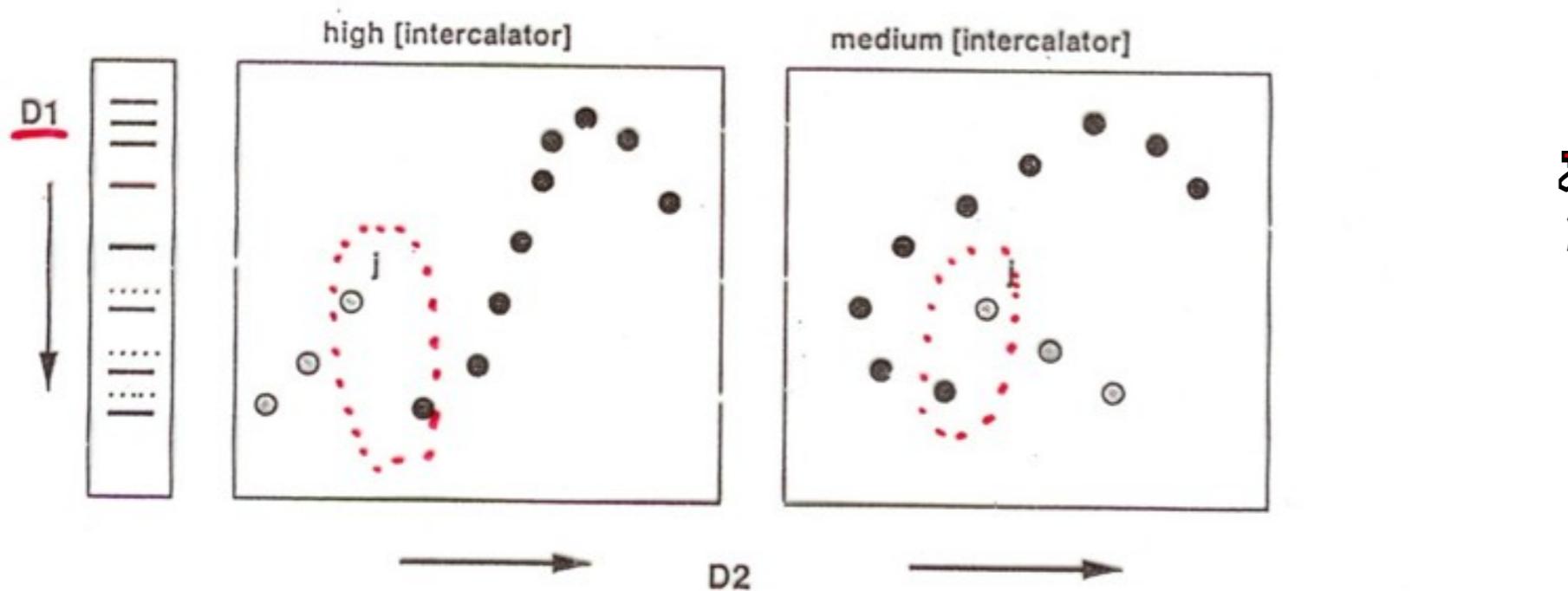
FIG. 2. Scheme to demonstrate the principle of two-dimensional gel electrophoresis. (A) Generation of two-dimensional gels from a circular DNA molecule that undergoes no structural transition. *Left*, One-dimensional gel electrophoresis (comparable to that in Fig. 1); *middle*, two-dimensional gel using a high chloroquine concentration in the second dimension; *right*, two-dimensional gel using a medium chloroquine concentration in the second dimension. (B) Equivalent to (A), except that we have now introduced a structural transition, leading to "jumps" of the topoisomer spots. See text for further details.

DNA CURVATURE AND BENDING
(OHYBY V DNA)

SEKVENCE

EXT. SÍLY

KORELAČNÍ ANALÝZA NUKLEOTIDOVÝCH SEKVENCÍ DNA
CHROMATINU   (TRIFONOV)

AA a TT se vyskytují v pravidelných intervalech korelujících se
zdvihem šroubovice DNA

Snížená pohyblivost fragmentů DNA z kinotoplastů *Leishmania
tarantolae* vysvětlena ohybem DNA

Osa helixu segmentu DNA může být jednosměrně ohnutá, jestliže se v
něm vyskytují úseky adeninových zbytků v pravidelných intervalech,
odpovídajích zdvihu šroubovice (10,5 bp).   Max. efekt 4-6 A

Jiné sekvence

KINK

Wedge model (klín)

Junction   model (rozhraní)

Sekvence tvořící ohyby DNA se vyskytují v různých organizmech
ve funkčně důležitých oblastech

např. v blízkosti počátku replikace (origin of replication)

přítomnost ohybu v blízkosti promotoru může ovlivnit transkripční aktivitu

některé bílkoviny se vážou specificky na místa ohybu a příp. ohyb
zvětšují, jiné svou vazbou ohyby vytvářejí

Negative SUPERCOILING stabilizes local DNA structures

CRUCIFORM
inverted repeat

LEFT-HANDED Z-DNA
alternating pu-py

SUPERCOIL

TRIPLEX structure
homopu·homopy

Text

CURVATURE
4-6 A's in phase with
the helix turns

HAIRPIN

SINGLE-STRANDED region
AT-rich
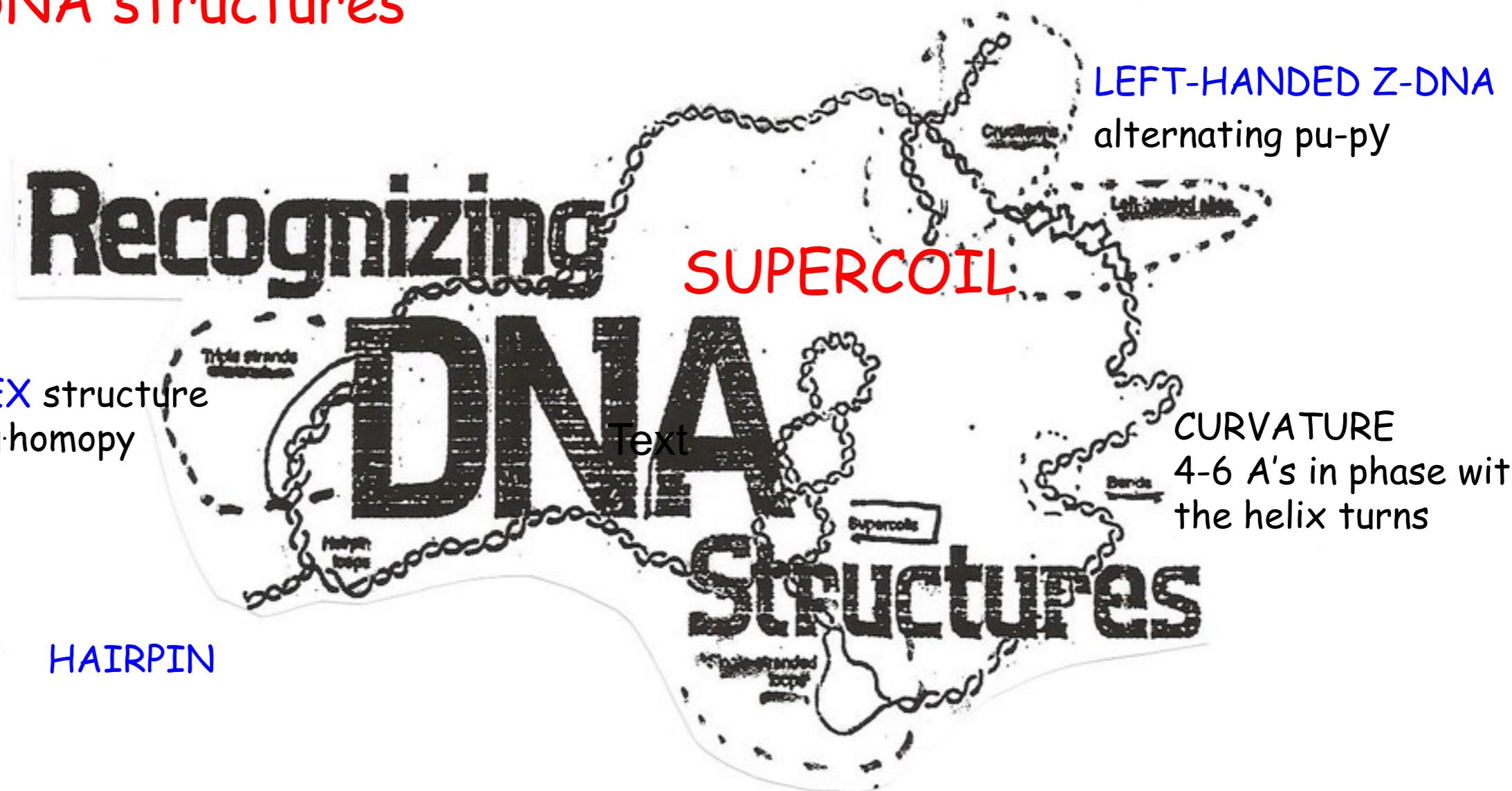
Physical methods such as NMR and X-ray analysis indispensable in the research of linear DNA structures are of limited use in studies of local structures stabilized by supercoiling

5'-AAATGCGCATAATCATGCGCATTT- 3'

3'-TTTACGCGTATTAGTACGCGTAAA- 5'

INVERTED REPEAT

```
        A — T
      A       C
      T - A
      A - T
      C - G
      G - C
      C - G
      G - C
      T - A
5'-AAA - TTT- 3'

3'-TTT - AAA-5'
      A - T
      C - G
      G - C
      C - G
      G - C
      T - A
      A - T
     T       G
        T — A
```
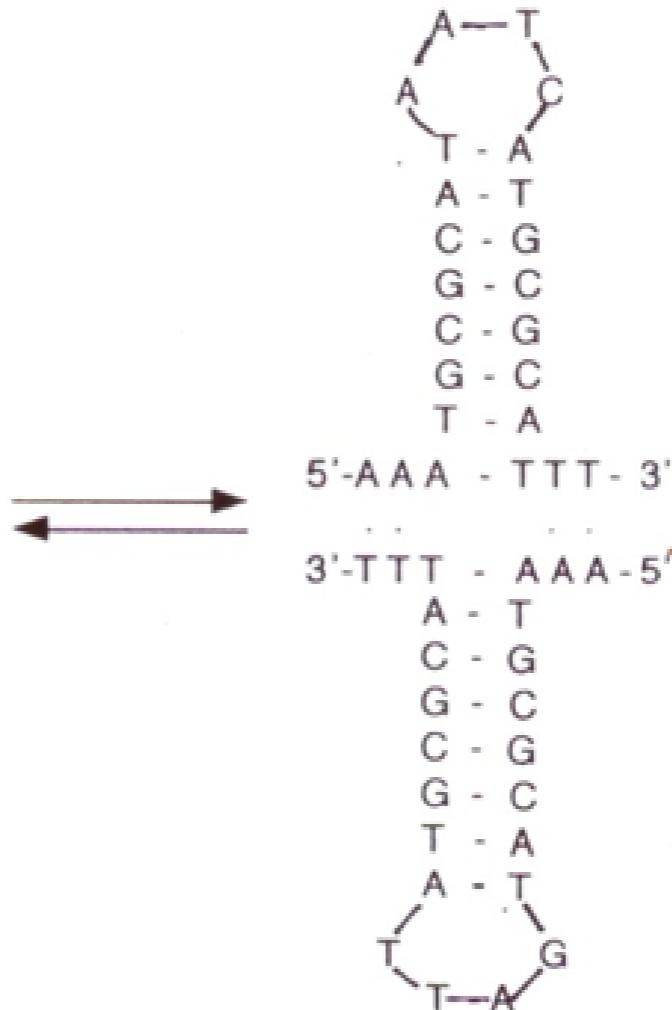
Palindromes and cruciforms. The arrows indicate the palindromic sequence which can fold back on self to form the cruciform structure.
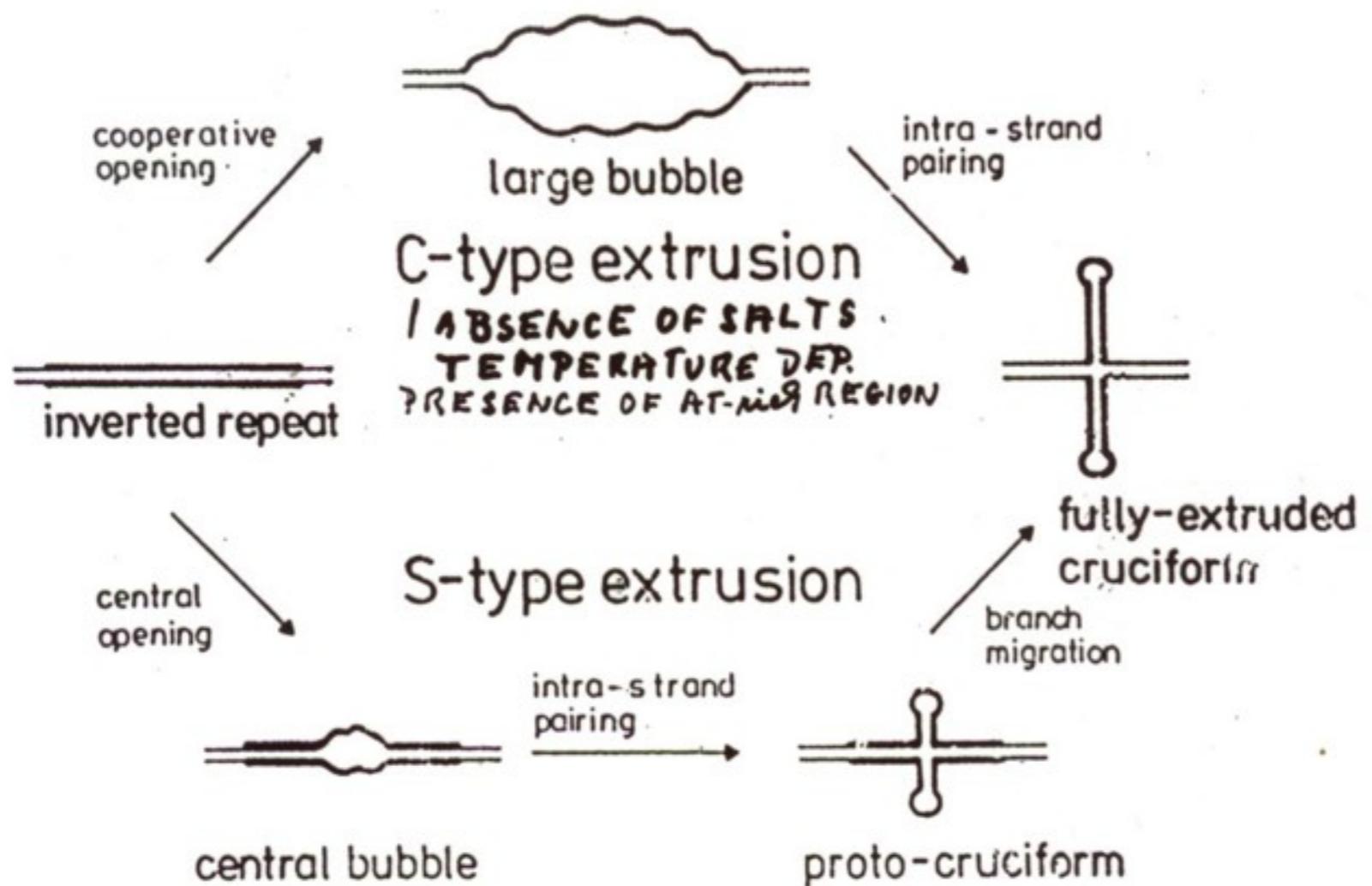
FIGURE 4. Mechanism of cruciform extrusion. The inverted repeat, repre-
sented by the thicker line, is shown in the unextruded form on the left. C-type
cruciforms (top) initiate the extrusion process with a coordinate opening of many
base pairs to form a large bubble. An intrastrand reassociation then 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 trans-
ferred 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.)
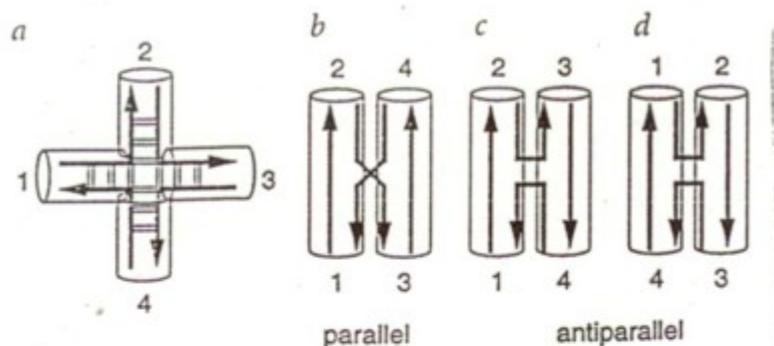
Fig. 1. Schematic of a four-way helical junction. *a*, The junction comprises four strands, indicated by different colors, forming four helical arms numbered 1–4. The polarity of the strand is indicated by the arrows drawn at the 3' termini. In this form the junction is unstacked with a square geometry. The junction can be folded by pairwise helical stacking of arms, as depicted in (*b*) through (*d*). *b*, A parallel-stranded form of the junction. In stacked forms there are two types of strand, continuous and exchanging, and in this structure the continuous strands are parallel to each other. *c*, An antiparallel structure, which can be obtained from (*b*) by a 180° rotation of the axis of the 3/4 helices relative to that of the 1/2 helices, thereby opening the center of the junction where the strands exchange between the helices. *d*, There are two conformers of these stacked forms, and the alternative form of the antiparallel structure is illustrated. In this form the stacking partners have exchanged, and thus helix 1 is now coaxially stacked on helix 4. Note that this changes the character of all the strands, so that the strands that were continuous are now exchanging, and *vice versa*.
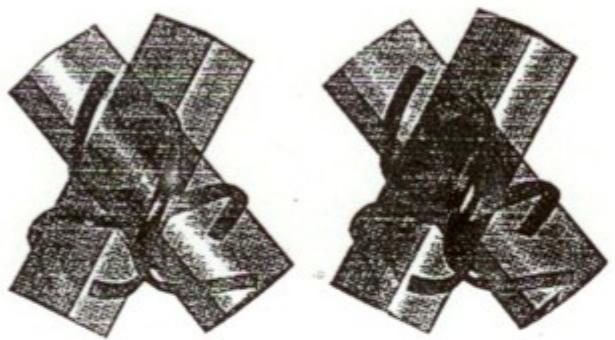
Fig. 3 The junction adopts a right-handed cross of helices, illustrated for the DNAzyme junction[2]. The view is that of the face of the junction, at 90° to that shown in Fig. 2*b*, for example. Cylinders have been fitted to the axes of the two pairs of stacked helices, and the path of the ribose-phosphate backbones shown by the ribbon as before. The angle between the axes is 55°, which compares well with previous biophysical estimates of ~60°. This angle should give optimal alignment between strands and grooves. However, the smaller angle found in the all-DNA junction[3] suggests that this angle may be easily altered, and perhaps crystal packing can distort this feature of the junction.

news and views
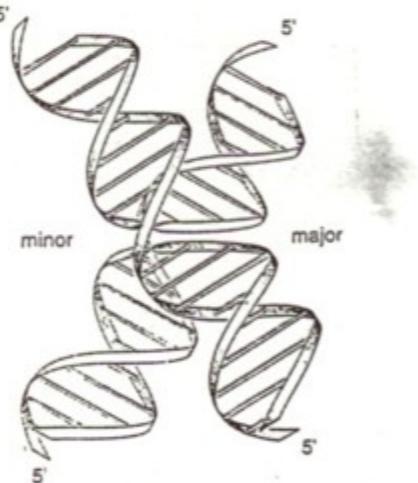
Fig. 4 The original proposal for the four-way junction structure was a stacked X-structure, as depicted here. The cartoon of the junction shows the face view, corresponding to that shown in Fig. 3. The view from the left would look into the minor groove, while that from the right looks into the major groove. The original cartoon was hand-drawn by H. Sebesse[3].

Levotočivá Z-DNA

Asi 8 let po uveřejnění CD spekter F. Pohla a T. Jovina (1971-72) byla vyřešena struktura levotočivé Z-DNA tvořené sekvencí (dC-dG)n pomocí rtg.-strukturní analýzy krystalu.

Z-DNA se stala středem zájmu vědců - v krátké době stovky publikací Zprvu Z-DNA pozorována pouze za nefyziologických podmínek, brzy však zjištěno, <u>v negativně superhelikální DNA se může Z-DNA vyskytovat za podmínek blízkým fyziologickým in vitro a také in vivo.</u>

Přes toto velké úsilí její funkce není dosud zcela objasněna

Zde se dotkneme pouze několika problémů, studovaných v posledních 10-15 letech

1. Sekvence nukleotidů - levotočivá DNA v (C-G) x (A-T)
Max. 6 AT párů d(A-T)3 v Z-DNA tvořené (dC-dG)n
Specifický vliv iontů Ni^{2+}

2. Strukturní rozhraní mezi B a Z-DNA (B-Z junction)

Strukturní rozhraní
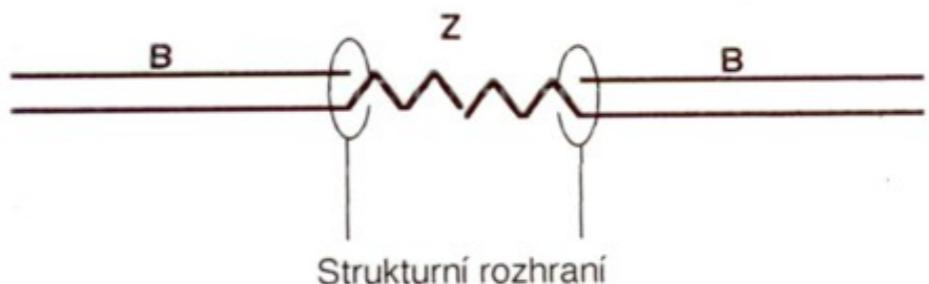
(baze více dostupné pro interakce s okolím)

3. Existence Z-DNA in vivo

PROBING OF DNA STRUCTURE IN CELLS BY IMMUNOFLUORESCENCE

A. USUAL WAY: DNA PROBING **AFTER** FIXATION (M. ROBERT-NICOUD / T. JOVIN, GOTTINGEN)

	FIXATION	PROBING FIRST Ab	SECOND Ab–FITC	MICROSCOPY
CELLS				
SALIVARY GLAND	SQUASH FIXATION			
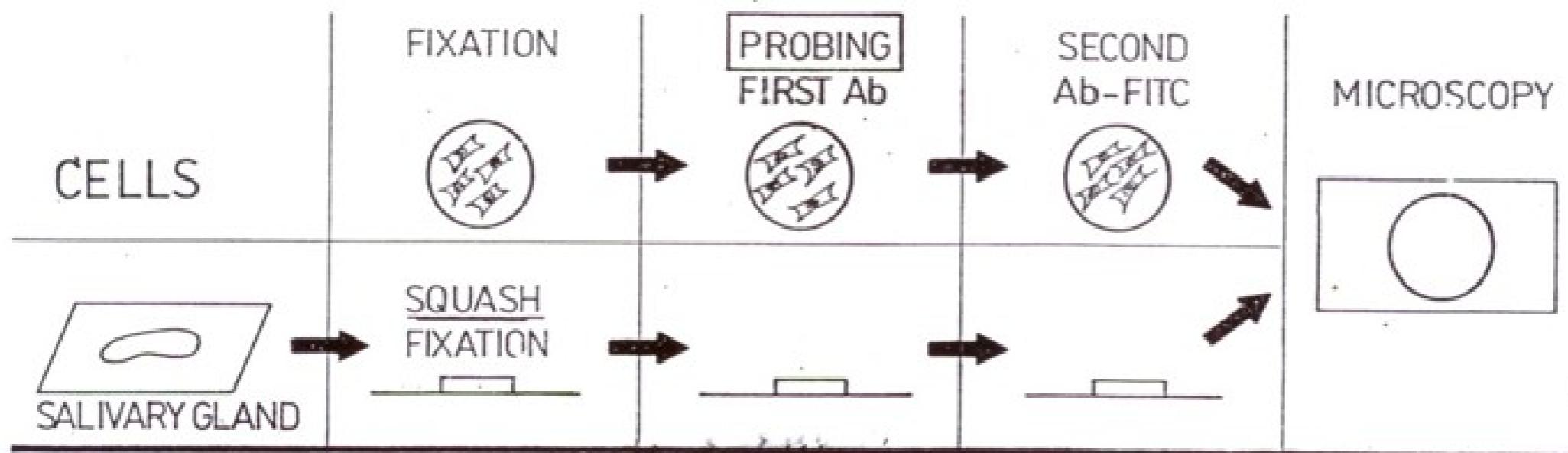
B. NEW APPROACH: DNA PROBING **PRIOR** TO FIXATION

CHEMICAL PROBING (Os, bipy)

Ab RECOGNIZING CHEMICALLY MODIFIED DNA

CELLS				
SALIVARY GLAND				

1 2 3 4　5　6 7 8 9　10

8

13

1 2 3

1709—
1537—
1396—

1118—

13

8

GAGTCC　GAGTCC
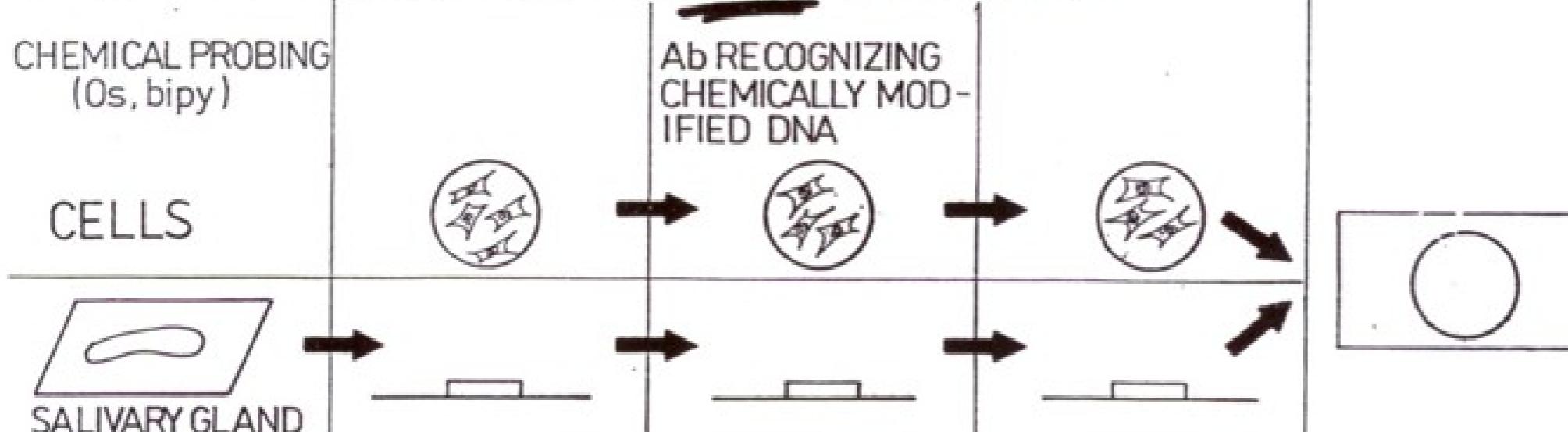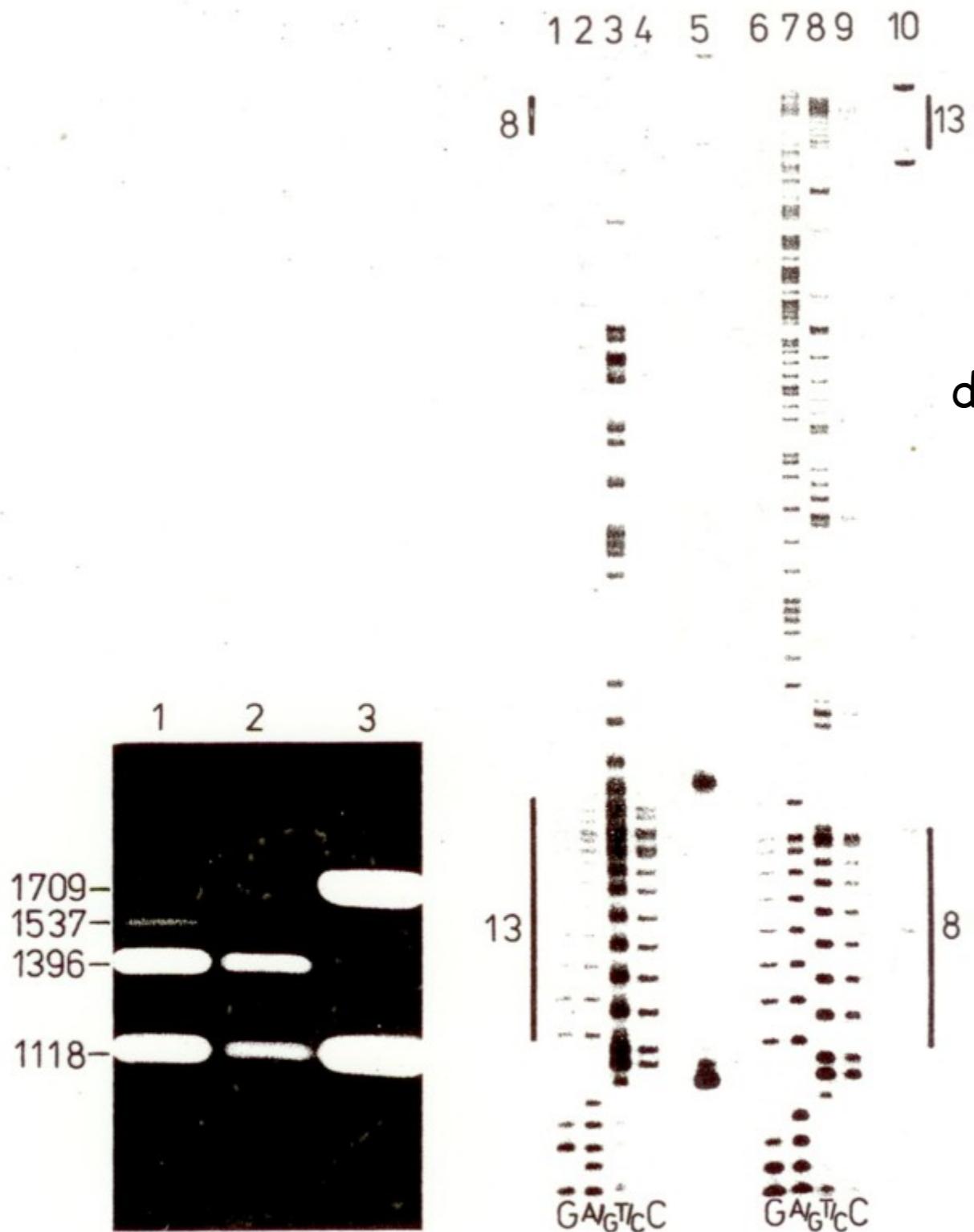
DNA B-Z junction

Strukturní rozhraní mezi B- a Z-DNA
detekce pomocí chemické strukturni sondy

Figure 4: Sequence-mapping of osmium modified (15 min., 26°C) native pPK2 plasmid multiple cloning site (containing the insert, Figure 1). (A) DNA was digested with *Bgl*I and cleaved with either *Bam*HI (lane 1) or nuclease S1 (lane 3). Lane 2 contains the standard fragments derived by cleavage of unmodified pPK2 DNA with *Bgl*I plus *Bam*HI. (B) DNA was cleaved by *Hind*III and *Eco*RI restrictases, the *Eco*RI-*Hind*III fragment was handled according to Materials and Methods. The left part of the autoradiograph (lanes 1-5) contains the sequencing of the bottom strand (OsO$_4$ modified sample in lane 5), the right half (lanes 6-10) deals with the upper strand (OsO$_4$ modified sample in lane 10). The vertical lines span the (dC-dG)$_n$ segments, the adjoining numbers stand for n values in respective segments.

INTRAmolecular

py
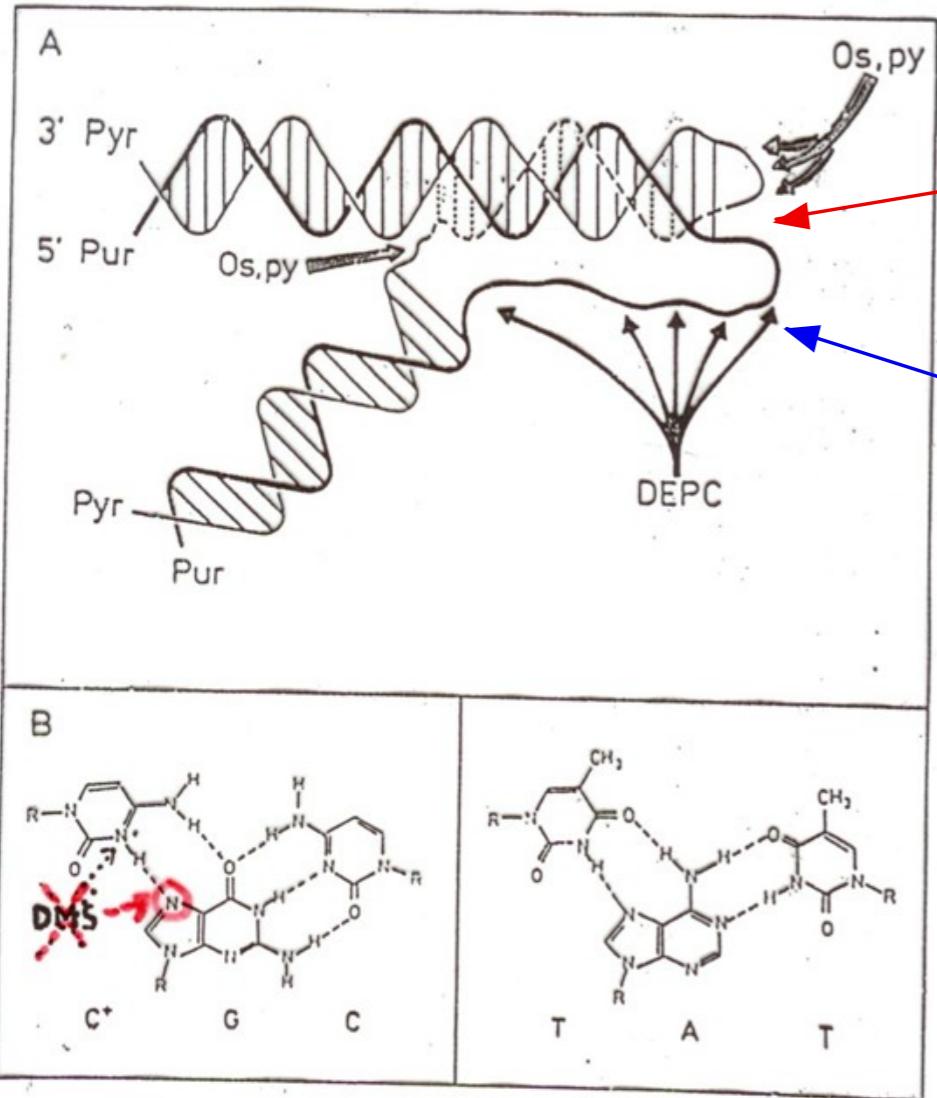
DNA triplexes

their identification by chemical probes

pu

INTERmolecular
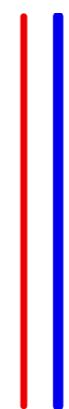
FIGURE 5. Structure of H-DNA. (A) Schematic representation of H-DNA in (TC·AG)$_{10}$, with 3'-half of the pyrimidine (dT-dC) repeat donated to the triplex, forming the H-y3 conformer. The 5'-half of this repeat, plus the complementary 3'-half of the (dA-dG)$_n$, polyurine repeat, act as the acceptor 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.
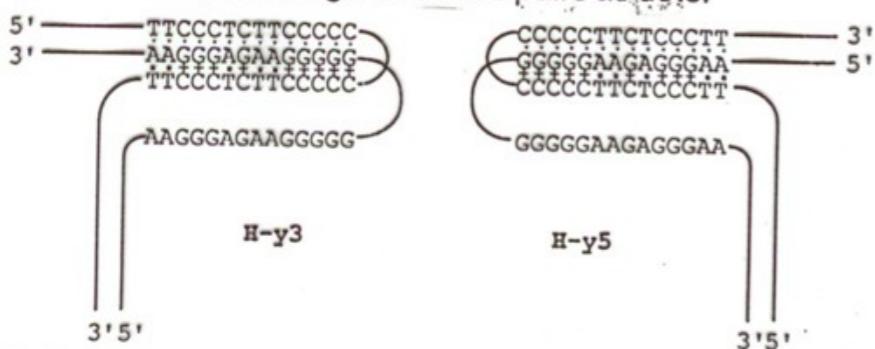
Figure 5 Two isoforms of H-DNA (91). Watson-Crick hydrogen bonds are labeled by points, nonprotonated Hoogsteen hydrogen bonds are shown by squares, and protonated Hoogsteen hydrogen bonds are shown by plus symbols.

THE TRIPLEX STORY

Since 1982 (Larsen & Weintraub; Hentschel) hypersensitivity to nuclease S1(associated with homopurine homopyrimidine sequences) has been reported in active chromatin (mainly within the regulatory regions) and in supercoiled plasmids. Various structures were proposed to explain the S1 sensitivity

1986: M. Frank-Kamenetskii et al. showed by 2-D gel electrophoresis of pEJ4 plasmid containing $d(GA)_{16} \cdot d(CT)_{16}$ sequence that the structure is stabilized by hydrogen ions and proposed a model of H-DNA triplex

1987: Vojtíšková and Paleček applied for the first time single-strand selective chemical probes to study at low resolution the H-DNA in pEJ4 and confirmed the Frank-Kamentskii model

1988: Several laboratories (Dahlberg, Frank-Kamenetskii, Johnston, Paleček, Wells) applied this approach at single-nucleotide resolution bringing a convincing evidence of the H-DNA structure
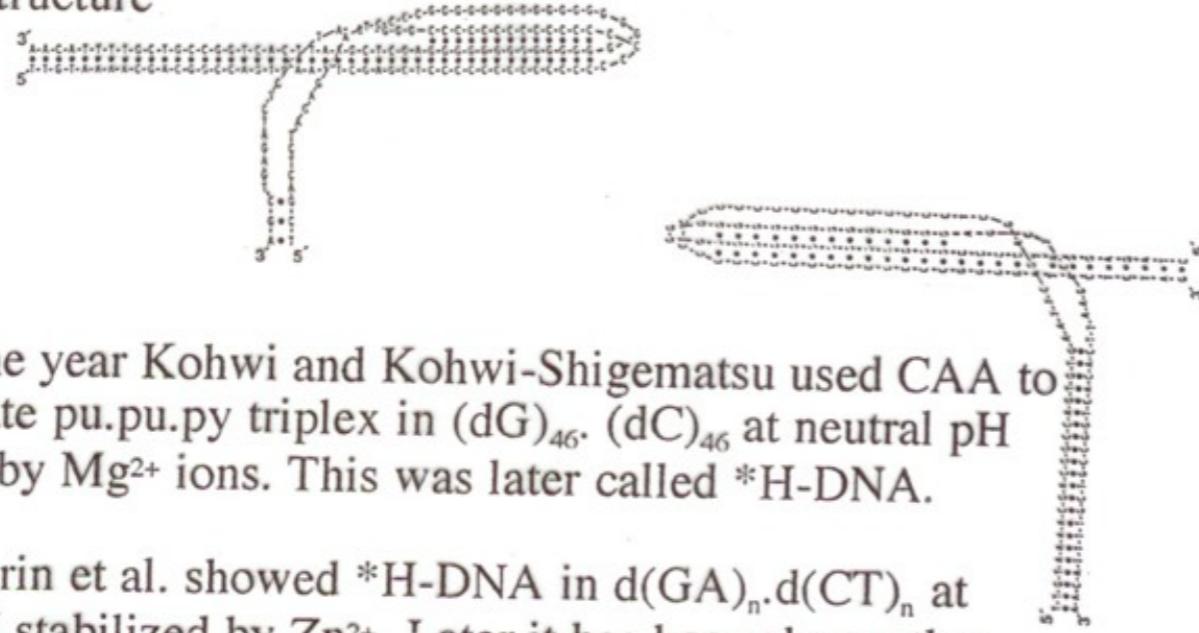
In the same year Kohwi and Kohwi-Shigematsu used CAA to demonstrate pu.pu.py triplex in $(dG)_{46} \cdot (dC)_{46}$ at neutral pH stabilized by Mg^{2+} ions. This was later called *H-DNA.

1989: Azorin et al. showed *H-DNA in $d(GA)_n \cdot d(CT)_n$ at neutral pH stabilized by Zn^{2+}. Later it has been shown that *H-DNA can be formed in various sequences and can be stabilized by various bivalent ions

1990 existence of triplex H-DNA in cells was demostrated in Paleček's laboratory

Homopurine.homopyrimidine mirror repeat sequence (H palindrome) is necessary for the formation of H-DNA.

Mirror repeat (H palindrom)

```
AAGGGAGAAGGGGG TATA GGGGG AAGAGGGAA
TTCCC TCTTCCCCC ATAT CCCCCTTCT CCCTT
* *              *       *        * *
```

H-DNA: CG*C$^+$ and TA*T

---

*H-DNA is more versatile as compared to H-DNA with respect sequence requirements.

*H-DNA: CG*G and TA*A but also TA*T, CGA$^+$ and less stable TA*C and GC*T
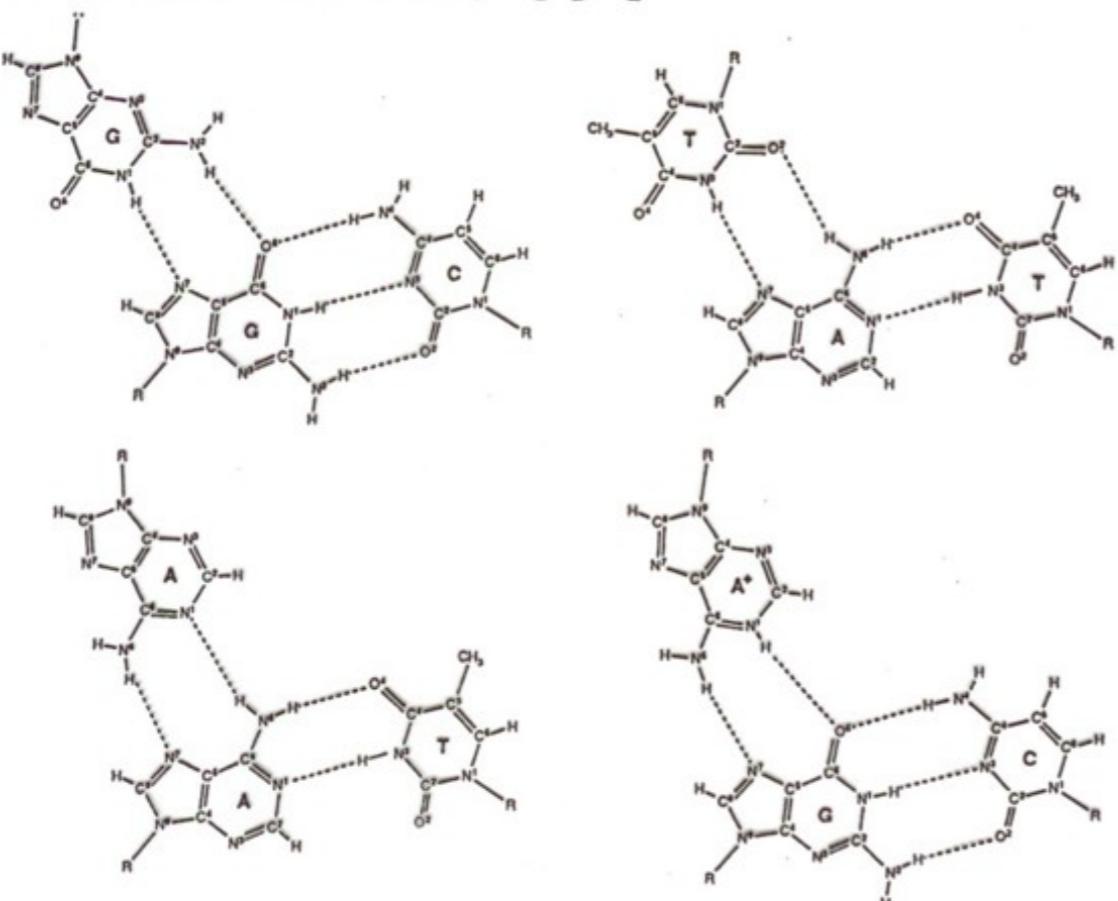
therefore *H-DNA can be formed by sequences that are neither homopurine.homopyrimidine nor mirror repeat

---

Homopy.homopy sekvence se zrcadlovou symetrii je potřebná pro vznik triplexu H-DNA

*H-DNA může vzniknout i v jiných sekvencích

E. Trifonov, Weizmann Inst, Rehovot
M. Frank-Kamenetskii, Boston Univ
S. Mirkin, Univ. Illinois, Chicago
D. Lyamichev ....

všichni původně v Moskvě

G-quartet

G-quartet

This structure (**Figure 11a**) can be formed by direct repeats containing tandemly arranged runs of guanines. The building elements are stacked G4 runs that are stabilized by certain monovalent cations (**Figure 11b**). This structure is definitely formed by single-stranded G-rich direct tandem repeats (such as telomeric repeats in eukaryotes) and is extensively characterized at atomic resolution. However, there are only fragmentary indications that it exists in superhelical DNA.

DOPLNĚK k předn. prof. M. Vorlíčkové

$(C_4A_2)_n \cdot (T_2G_4)_n$ TETRAHYMENA $n = 50$ and > 50
$(C_4A_4)_n \cdot (T_4G_4)_m$ OXYTRICHA E. BLACKBURN

ACID q H

SC -STABILIZED

Rb and N but NOT K

PS

G4

W. GILBERT

APS

K.

G·G PAIRS

NO SC
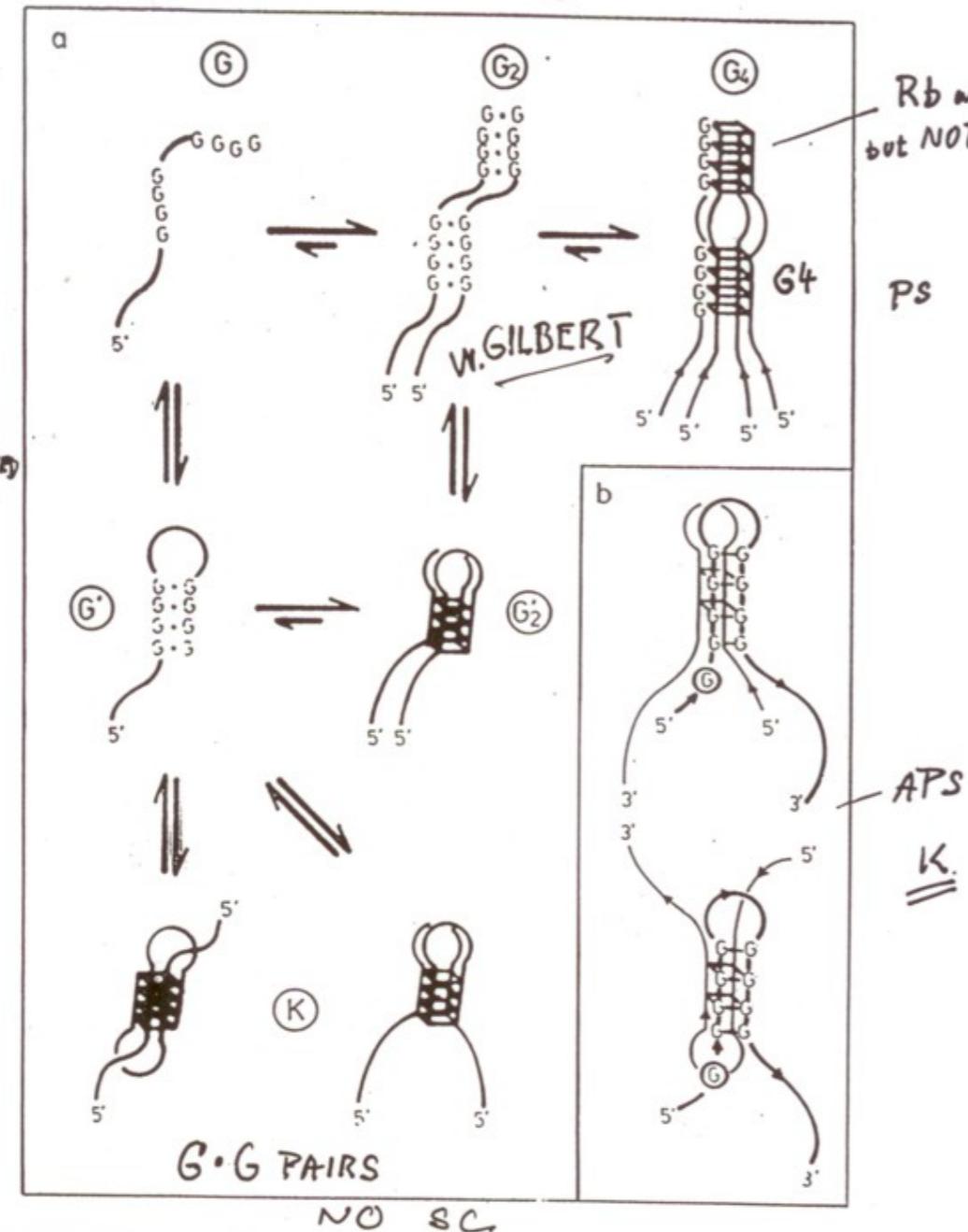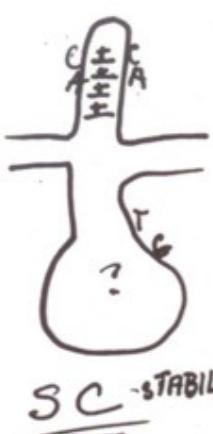
FIGURE 6. (a) Scheme for the formation of G$_4$-DNA. The formation of the dimer structure G$_2$ must be rate-limiting, and it must be rapidly converted into G$_4$. Only three possible structures (including K and G$_2'$) of fold-back intermediates are shown, but other structures may also be formed. (b) 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.)

TELOMERS - REPLICATION AND MAINTENANCE OF CHROMOSOMAL ENDS

Figure 11 G-quartet. (a) General overview. The black line is the DNA strand and the purple rectangles are the stacked G-quartets. (b) Structure of a G-quartet.

Oblasti s nespárovanými bazemi
[Base unpaired (unwound) regions]

v sekvencích bohatých páry AT, stabilizovány superhelicitou
(supercoil-stabilized)

Paralelní řetězce (parallel-stranded, ps) DNA
připraveny synteticky jako např. vlásenkové struktury (hairpin)
v nichž smyčky (loops) smyčky s fosfodiesterickými vazbami 5'-
5' nebo 3'-3' stabilizovali paralelní orientaci řetězců

triplexy, kvadruplexy

Unusual structures - jsou opravdu neobvyklé?
Společným rysem lokální struktur stabilizovaných

superhelicitou je jejich spojení s otevřenými oblastmi - baze
dostupné pro interakci s okolím "open DNA structures".

Výskyt lokálních struktur in vivo

Některé sekvence potřebné pro vznik těchto struktur jsou
abundantní

Metodické prostředky pro analýzu struktur DNA in vivo jsou
omezené (ve srovnání s analýzou in vitro)

Prokázána existence křížových forem, Z-DNA i triplexů in vivo.

ZÁVĚRY

PROSTOROVÉ USPOŘÁDÁNÍ DNA JE ZÁVISLÉ NA SEKVENCI NUKLEOTIDŮ, PODMÍNKÁCH PROSTŘEDÍ A HUSTOTĚ NADŠROUBOVICOVÉHO VINUTÍ

CHEMICKÉ SONDY JSOU V POSLEDNÍCH LETECH STÁLE VÍCE POUŽÍVÁNY PŘI STUDIU LOKÁLNÍCH STRUKTUR DNA STABILIZOVANÝCH NADŠROUBOVICOVÝM VINUTÍM (na př. KŘÍŽOVÉ FORMY, TRIPLEXY A SEGMENTY LEVOTOČIVÉ DNA)

KOMPLEXY OXIDU OSMIČELÉHO PATŘÍ K NEJČASTĚJI POUŽÍVANÝM CHEMICKÝM SONDÁM STRUKTURY DNA

K JEJICH VÝHODÁM PATŘÍ:

1. SNADNÁ DETEKCE MÍSTA VAZBY SONDY V POLYNUKLEOTIDOVÉM ŘETĚZCI NA ÚROVNI ROZLIŠENÍ JEDNOTLIVÝCH NUKLEOTIDŮ

2. JEDNODUCHÁ PŘÍPRAVA SOND S RŮZNÝMI VLASTNOSTMI, ZÁMĚNOU DUSIKATÝCH LIGANDŮ

3. DOSTUPNOST POLYKLONÁLNÍCH A MONOKLONÁLNÍCH PROTILÁTEK S VYSOKOU SPECIFICITOU VŮČI ADUKTŮM SOND S DNA

4. POUŽITELNOST KE STUDIU STRUKTURY DNA *in vivo* (PŘÍMO V PROKARYOTNÍCH A EUKARYOTNÍCH BUŇKÁCH)

VÝZKUM STRUKTURY DNA PŘÍMO V BUŇKÁCH (POMOCÍ CHEMICKÝCH SOND) OTEVÍRÁ NOVÉ MOŽNOSTI STUDIU VZTAHŮ MEZI SEKUNDÁRNÍ A TERCIÁRNÍ STRUKTUROU DNA na straně jedné a JEJÍ FUNKCÍ na straně druhé

Výskyt lokálních struktur DNA
v prokaryotních a eukaryotních buňkách

Superhelical torsion in cellular DNA responds directly to environmental and genetic factors

(DNA supercoiling/cellular DNA topology/cruciform/topoisomerase/osmium tetroxide)

James A. McClellan*, Pavla Boublíková†, Emil Paleček†, and David M. J. Lilley*‡

*Department of Biochemistry, The University, Dundee DD1 4HN, United Kingdom; and †Institute of Biophysics, Czechoslovak Academy of Sciences, Kralovopolska 135, 612 65 Brno, Czechoslovakia

Chemická modifikace DNA v buňkách pomocí komplexu OsO_4 (Os,bipy) a její využití pro testování - $\int \Delta NA$

Strukturní přechod DNA duplex – křížová forma v buňce může informovat o superhelikální hustotě DNA a o jejich změnách
působených změnami prostředí nebo genetickými faktory

ABSTRACT   Superhelical tension of DNA in living bacteria is believed to be partially constrained by interaction with proteins. Yet DNA topology is a significant factor in a number of genetic functions and is apparently affected by both genetic and environmental influences. We have employed a technique that allows us to estimate the level of unconstrained superhelical tension inside the cell. We study the formation of cruciform structures by alternating adenine-thymine sequences in plasmid DNA by *in situ* chemical probing. This structural transition is driven by superhelical torsion in the DNA and thus reports directly on the level of such tension in the cellular DNA. We observe that the effect of osmotic shock is an elevation of superhelical tension; quantitative comparison with changes in plasmid linking number indicates that the alteration in DNA topology is all unconstrained. We also show that the synthesis of defective topoisomerase leads to increased superhelical tension in plasmid DNA. These experiments demonstrate that the effect of environmental and genetic influences is felt directly at the level of torsional stress in the cellular DNA.

FIG. 1. Chemical modification of a cruciform structure adopted by (A-T)$_{34}$ sequence present in *E. coli*. (A) Map of pXG540 showing the (A-T)$_{34}$ sequence (stippled). In the *in situ* modification experiments, the plasmid was cleaved with *Eco*RI and radioactively labeled at the resulting 3' termini. For *in vitro* modification of pXG540 as a marker, the DNA was cleaved with *Eco*RI and *Bam*HI and labeled at the resulting 3' termini. (B) *In situ* modification of pXG540 with and without salt shock. The DNA in the left-most lane was obtained by *in vitro* modification of pXG540 with osmium tetroxide under conditions where there is uniform reactivity of all thymine bases in the (A-T)$_{34}$ tract (43) and can be used to identify the extent of the alternating sequence, indicated by the arrowheads on the left side. Note the strong modification at the center of the (A-T)$_{34}$ tract by osmium tetroxide, provided that the cells were subjected to the salt-shock procedure. The middle and right lanes show *in situ* modification with (+) and without (−) salt shock. (C) Effect of salt shock on the linking number of plasmid extracted from *E. coli*. Cells were grown as above, with and without salt shock, and pXG540 DNA was prepared. This was electrophoresed in a 1% agarose gel in 90 mM Tris·borate (pH 8.3), 10 mM EDTA, and chloroquine (4 μg/ml). Note that the salt-shocked DNA is more supercoiled than that of the untreated cells, by approximately six turns.

We conclude that these differences reflect different extents

Salt shock

Cruciform structures in E. coli cells
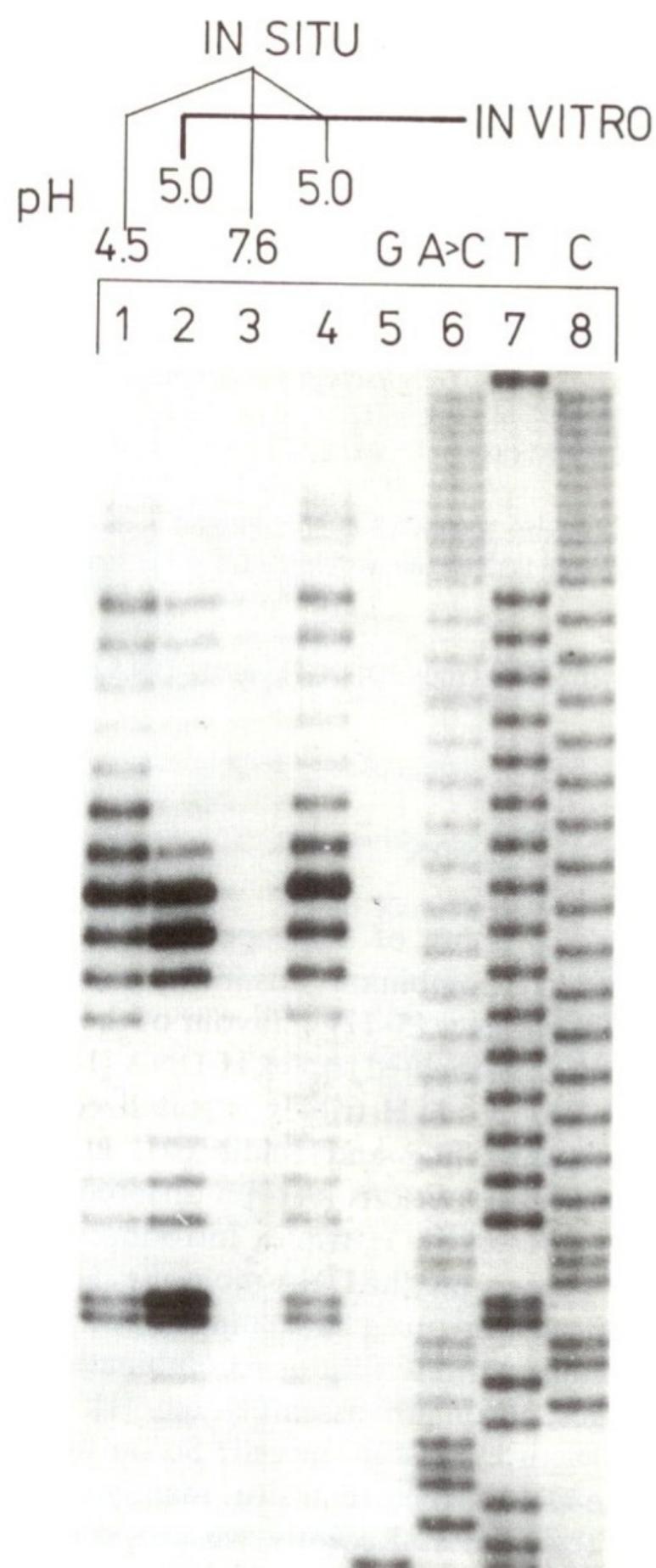
TRIPLEX DNA V BUŇKÁCH PROKÁZANÝ POMOCÍ Os,bipy

P. Karlovský, P. Pecinka, M. Vojtísková, E. Makaturová, E. Palecek,
FEBS Letters 274 (1990)39-42
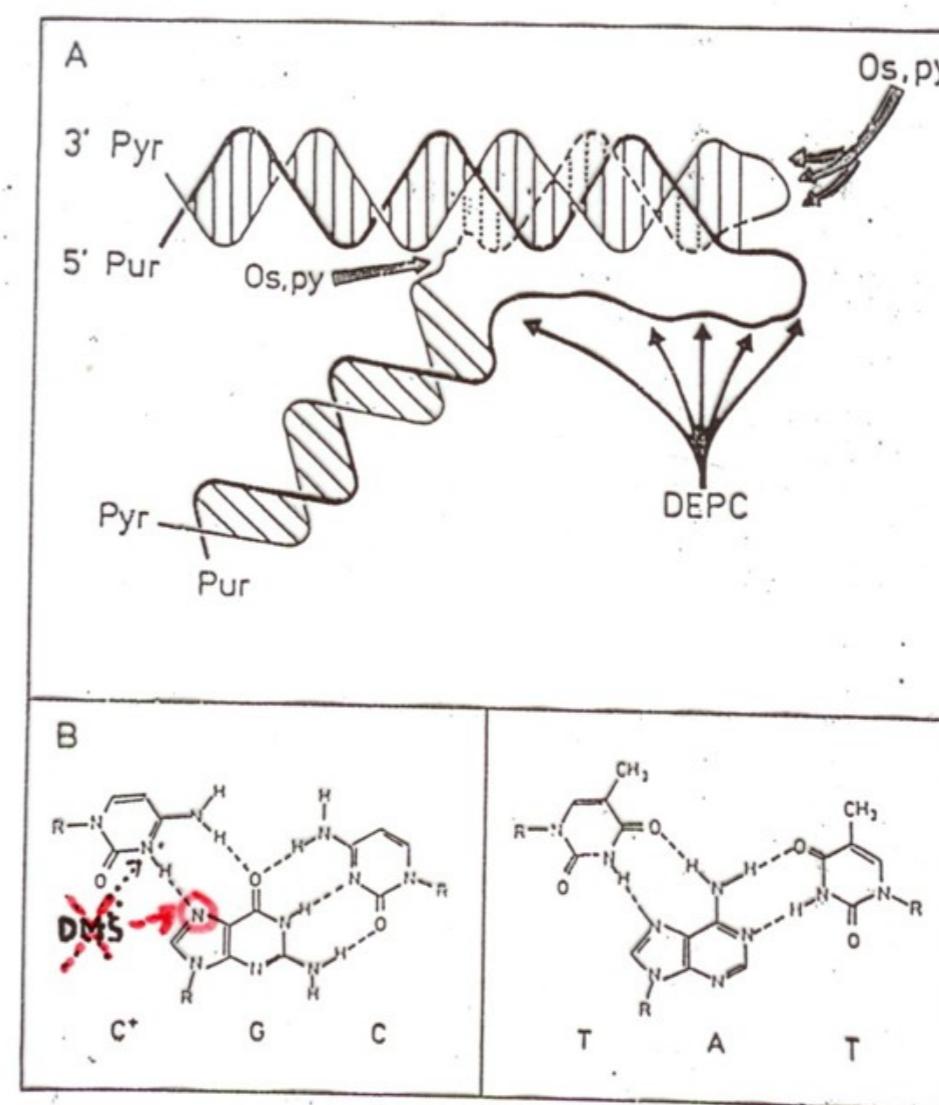
Fig. 2. Osmium binding sites in the homopurine–homopyrimidine region of pL153. *E. coli* JM109 (pL153) treated with Os,bipy at 4.5 (lane 1), pH 5.0 (lane 4) and pH 7.6 (lane 3). pL153 DNA modified in vitro at pH 5.0 (lane 2).


FIGURE 5. Structure of H-DNA. (A) Schematic representation of H-DNA in (TC·AG)₁₀, 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, polypurine 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

Fig. 3. Osmium binding sites in the homopurine–homopyrimidine sequence of pL153 present in *E. coli* cells. (A) pH dependence: cells were treated with Os,bipy at pH 5.0 (lane 2), pH 5.2 (lane 3) and pH 5.4 (lane 4). Lane 1, unmodified control. (B) Time dependence: cells were treated with Os,bipy at pH 5.0 for 0 (lane 9), 5 (lane 10), 10 (lane 11), 20 (lane 12), 30 (lane 13) and 60 min (lane 14). R, purine; Y, pyrimidine.

Konformer H-y5
pravděpodobně
v buňkách převažuje
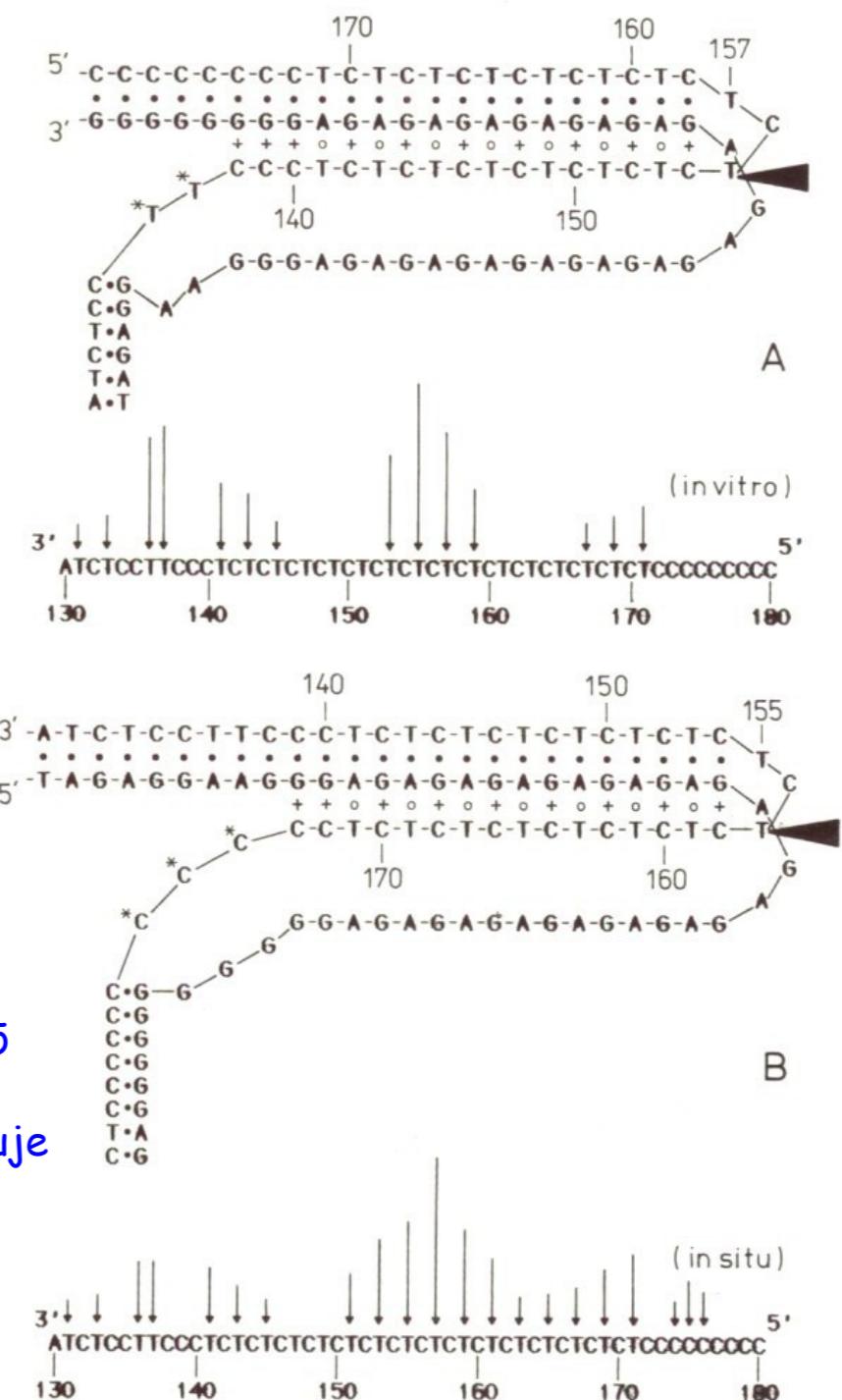
Fig. 4. (A) H-y3 and (B) H-y5 conformers [23] of the H-DNA triplex [4] and the Os,bipy modification of bases in the polymyrimidine tract of the pL153 insert (A), in vitro and (B), in situ. The lengths of the vertical arrows in the nucleotide sequence represent the relative intensities of the bands on the sequencing gel (Fig. 2) obtained by densitometric tracing, (A) after Os,bipy treatment of the supercoiled plasmid pL153 DNA in vitro (Fig. 2, lane 2) and (B) after treatment of cells with Os,bipy at external pH 5.0 (Fig. 2, lane 4). The main element of both H conformers is a triple helix [4] which includes the Watson-Crick duplex (.) associated with the homopyrimidine strand by Hoogsteen base pairing (o, +) where cytosines are protonated. The triangle shows the strongest modified base in the triplex and the asterisks denote the modification at the B–H junctions.

Unconstrained supercoiling in eukaryotic cells
In difference to the prokaryotic genome the eukaryotic genome was for years believed not to be under the superhelical stress due to the accommodation of the DNA writhing around histone octamers in nucleosomes (Pearson, 1996, Van Holde, 1994). The actively transcribing portion of the eukaryotic genome was, however, shown to contain unconstrained supercoiling, part of which can be attributed to the process of transcription per se.


Figure 28.10
Levels of chromatin structure. The beaded string structure is a 10-nm fiber, which folds into a "solenoidal" 30-nm fiber with about six nucleosomes per turn. This can further fold to form thick 200-nm fibers that can be observed in electron micrographs of chromosomes or nuclei.

POTENTIAL SITES OF SC-STABILIZED NON-B STRUCTURES

Using prokaryotic cells it has been recently shown that the effects of transcriptionally driven supercoiling are remarkably large scale in vivo(in a kbp range). Similarly to the transcription effects, in DNA replication intermediates supercoils are formed both behind and in front of the replication fork and superhelical stress is distributed throughout the entire partially replicated DNA molecule. and gyrase activity of topoisomerases.

Figure 28.9
The elements of chromatin structure. At the top, the extended beaded string structure shown in Figure 28.10 is diagrammed. Light digestion with nuclease first releases mono- and oligo-nucleosomes; then, as linker DNA is further digested, nonhistone proteins and H1 are released, to yield the core particle whose structure is shown in Figure 28.8.
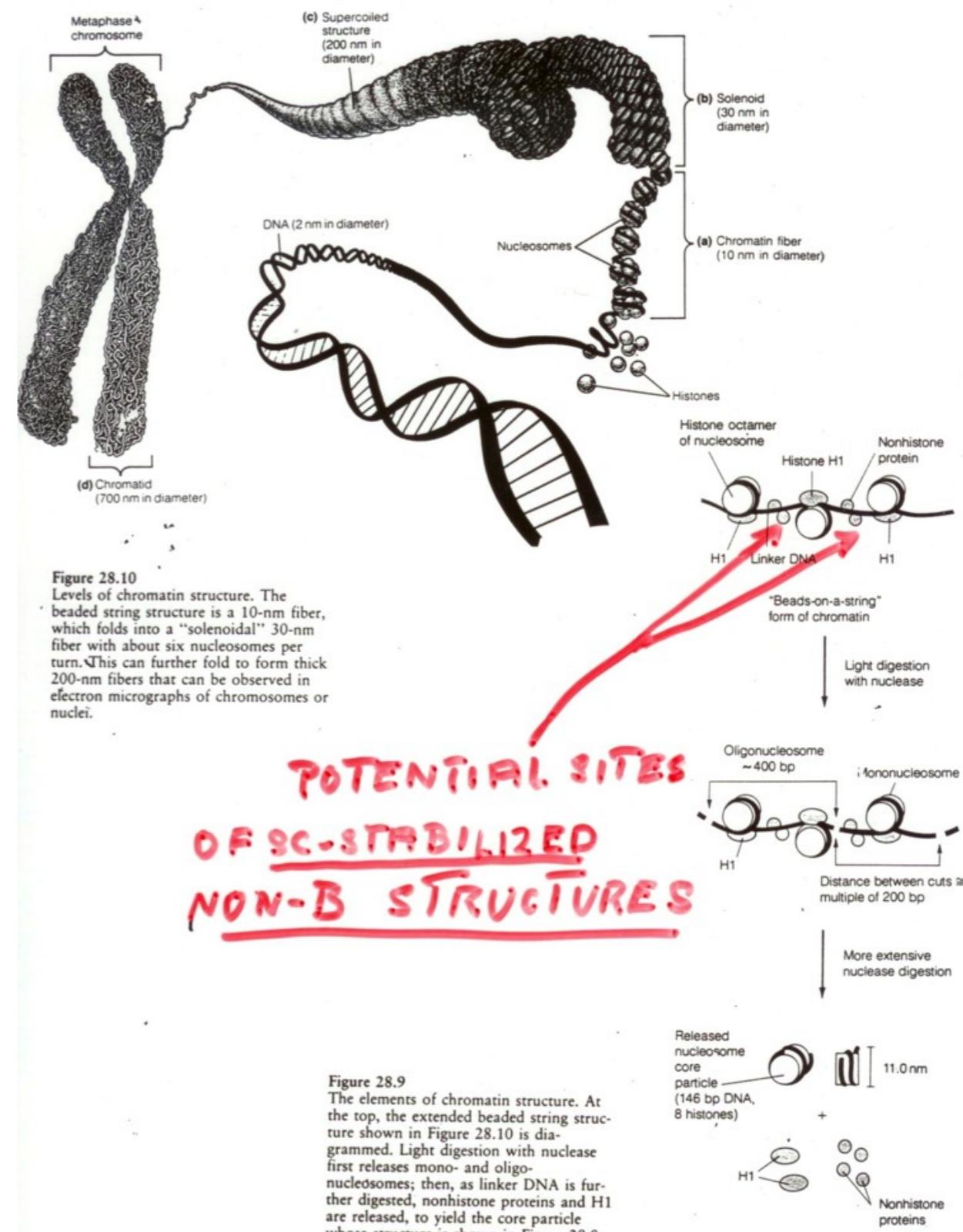
Unconstraint negative supercoiling stabilizes local DNA structures such as cruciforms, Z-DNA segments and intramolecular triplexes. Mounting evidence of the existence of these structures in vivo both in prokaryotic and eukaryotic cells has been reviewed. It appears that alternative DNA structures are located in extranucleosomal regions such as linkers and DNase hypersensitive site but probably not within the DNA wrapped around DNA octamer.

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Figure 28.10
Levels of chromatin structure. The beaded string structure is a 10-nm fiber, which folds into a "solenoidal" 30-nm fiber with about six nucleosomes per turn. This can further fold to form thick 200-nm fibers that can be observed in efectron micrographs of chromosomes or nuclei.

POTENTIAL SITES OF SC-STABILIZED NON-B STRUCTURES

Figure 28.9
The elements of chromatin structure. At the top, the extended beaded string structure shown in Figure 28.10 is diagrammed. Light digestion with nuclease first releases mono- and oligonucleosomes; then, as linker DNA is further digested, nonhistone proteins and H1 are released, to yield the core particle whose structure is shown in Figure 28.8.

Recently **mechanisms by which the effect of supercoil-stabilized non-B DNA structures on transcription can be exerted in eukaryotic cells have been proposed** [van Holde, 1994 #72] .

If the promoter region of the gene is blocked by a nucleosome and sites for transcription factors are inaccessible, nucleosome dissociation can result in transcriptional activation provided transcription factors are available; such dissociation is accompanied by formation of negative supercoils capable to induce alternative structure in a linker. Similar transcriptional activation may be produced by binding of a specific protein, shifting the equilibrium in favor of an alternative DNA structure, followed by formation of positive supercoils and loss of a nucleosome. For this mechanism, denominated as *conformational compensation* , precise location or orientation of the potential non-B sequence is not critical.

Another mechanism based on binding of proteins to potential non-B sequences has been proposed by Hatfield at al. [Sheridan, 1999 #70; Sheridan, 1998 #69; Parekh, 1996 #71].

Supercoiling can locally destabilize B-DNA structure and drive transitions to other structures at susceptible sequences. In principle the supercoil driven local structural transitions can be either inhibited or facilitated by proteins that bind at or near potential transition sites [Sheridan, 1999 #70; Sheridan, 1998 #69; Parekh, 1996 #71]. If a DNA segment, susceptible to forming a supercoil-induced alternative structure, is stabilized in the B-form by a DNA-binding protein, the propensity of this segment for structural transition will be transferred to another site within the same DNA domain. Positioning of this site in the promoter region may facilitate open complex formation and activation of gene expression.

Transkripce může být aktivována vazbou specif. proteinu (např. Z-binding) posunující rovnováhu ve prospěch lokální struktury, následované vznikem pozitivní nadšroubovice a ztrátou nukleosomu
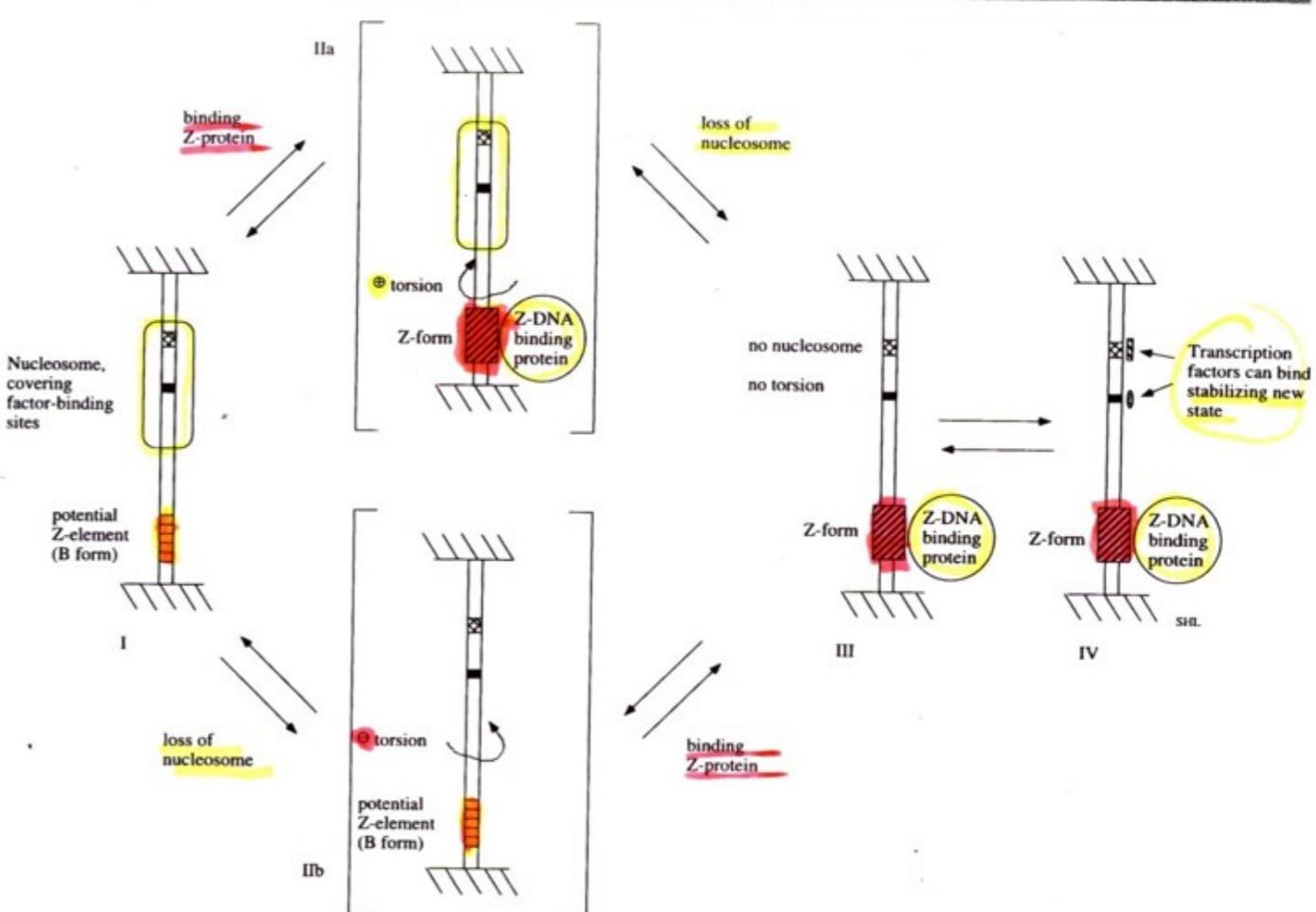

Fig. 2. Conformational compensation as a potential model for gene activation. A region of chromatin is constrained by surrounding structures in the nucleus. Here for specificity we imagine a segment containing one nucleosome in the promoter regions of a gene with a potential Z-element in an adjacent linker (state I). Either binding of a Z-protein or transient dissociation of the nucleosome lead to energetically unfavorable transition states (IIa or IIb) with DNA under torsion. This can be relaxed as shown in III, a state which could then be stabilized by the binding of transcription factors to uncovered sites (state IV).

Disociace nukleosomu vede k aktivaci transkripce a tvorbě negativní nadšroubovice (supercoil) schopné indukovat lokální struktury.

Fig. 2. Conformational compensation as a potential model for gene activation. A region of chromatin is constrained by surrounding structures in the nucleus. Here for specificity we imagine a segment containing one nucleosome in the promoter regions of a gene with a potential Z-element in an adjacent linker (state I). Either binding of a Z-protein or transient dissociation of the nucleosome lead to energetically unfavorable transition states (IIa or IIb) with DNA under torsion. This can be relaxed as shown in III, a state which could then be stabilized by the binding of transcription factors to uncovered sites (state IV).