

KEY CONCEPTS

- Scientists long assumed that any DNA mutation that does not change the final protein encoded by a gene is effectively **"silent"**.
- Mysterious exceptions to the rule, in which silent changes seemed to be exerting a powerful effect on proteins, have revealed that **such mutations can affect health through a variety of mechanisms.**
- Understanding the subtler dynamics of how genes work and evolve may reveal further insights into causes and cures for disease.

SILENCE IN THE CODE

[BASICS]

The genetic code, which governs how a cell translates DNA instructions, via RNA, into functional proteins, is unusual in that it is redundant. Genes "written" in RNA nucleotides spell out the sequence of amino acids in an encoded protein using three-letter words called codons that correspond to

one of 20 amino acids (table). With an alphabet of four nucleotide bases, 64 codon triplets are possible—resulting in several codons that specify the same amino acid. A DNA mutation that changes one of these codons to its synonym should therefore be "silent" in protein terms.

▼ **TRANSCRIPTION AND EDITING**
 Inside the cell nucleus, the DNA double helix unwinds to allow an RNA copy of a gene to be made. The resulting transcript is then edited to remove segments that do not encode amino acids, producing a shorter messenger RNA (mRNA) version. Pairing of the bases in the RNA nucleotides causes the mRNA molecule to adopt a folded structure.

▼ **THE CODON-AMINO ACID CODE**
 Because the four RNA bases (A, C, G, U) yield 64 possible triplet combinations, more than one codon can specify a particular amino acid. Often such synonymous codons differ only in their third nucleotide positions.

		Second nucleotide position				
		U	C	A	G	
U	UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine
	UUC	Phenylalanine	UCC	Serine	UAC	Tyrosine
	UUA	Leucine	UCA	Serine	UAA	STOP
	UUG	Leucine	UCG	Serine	UAG	STOP
C	CUU	Leucine	CCU	Proline	CAU	Histidine
	CUC	Leucine	CCC	Proline	CAC	Histidine
	CUA	Leucine	CCA	Proline	CAA	Glutamine
	CUG	Leucine	CCG	Proline	CAG	Glutamine
A	AUU	Isoleucine	ACU	Threonine	AAU	Asparagine
	AUC	Isoleucine	ACC	Threonine	AAC	Asparagine
	AUA	Isoleucine	ACA	Threonine	AAA	Lysine
	AUG	Methionine	ACG	Threonine	AAG	Lysine
G	GUU	Valine	GCU	Alanine	GAU	Aspartate
	GUC	Valine	GCC	Alanine	GAC	Aspartate
	GUA	Valine	GCA	Alanine	GAA	Glutamate
	GUG	Valine	GCG	Alanine	GAG	Glutamate
					UGU	Cysteine
					UGC	Cysteine
					UGA	STOP
					UGG	Tryptophan
					CGU	Arginine
					CGC	Arginine
					CGA	Arginine
					CGG	Arginine
					AGU	Serine
					AGC	Serine
					AGA	Arginine
					AGG	Arginine
					GGU	Glycine
					GGC	Glycine
					GGA	Glycine
					GGG	Glycine

▼ **TRANSLATION TO PROTEIN**
 In the cellular cytoplasm, ribosomes unfold and read the mRNA and produce the encoded amino acid chain with the help of transfer RNA (tRNA) molecules. Each tRNA delivers a single amino acid to the ribosome, binding to the corresponding mRNA codon to confirm that the correct amino acid is being added. The growing amino acid chain begins folding into its three-dimensional protein shape even as it is still forming.

Silence in the code

The genetic code, which governs how a cell translates DNA instructions via RNA into functional proteins, is unusual in that it is redundant. Genes "written" in RNA nucleotides spell out the sequence of amino acid in an encoded protein using three-letter words called codons that correspond to one of **20 amino acids**. With an alphabet of four nucleotide bases, **64 codon triplets** are possible - resulting in **several codons that specify the same amino acid**. A DNA mutation that **changes one of these codons to its synonym** should therefore be "**silent**" in protein terms.

Translation to protein

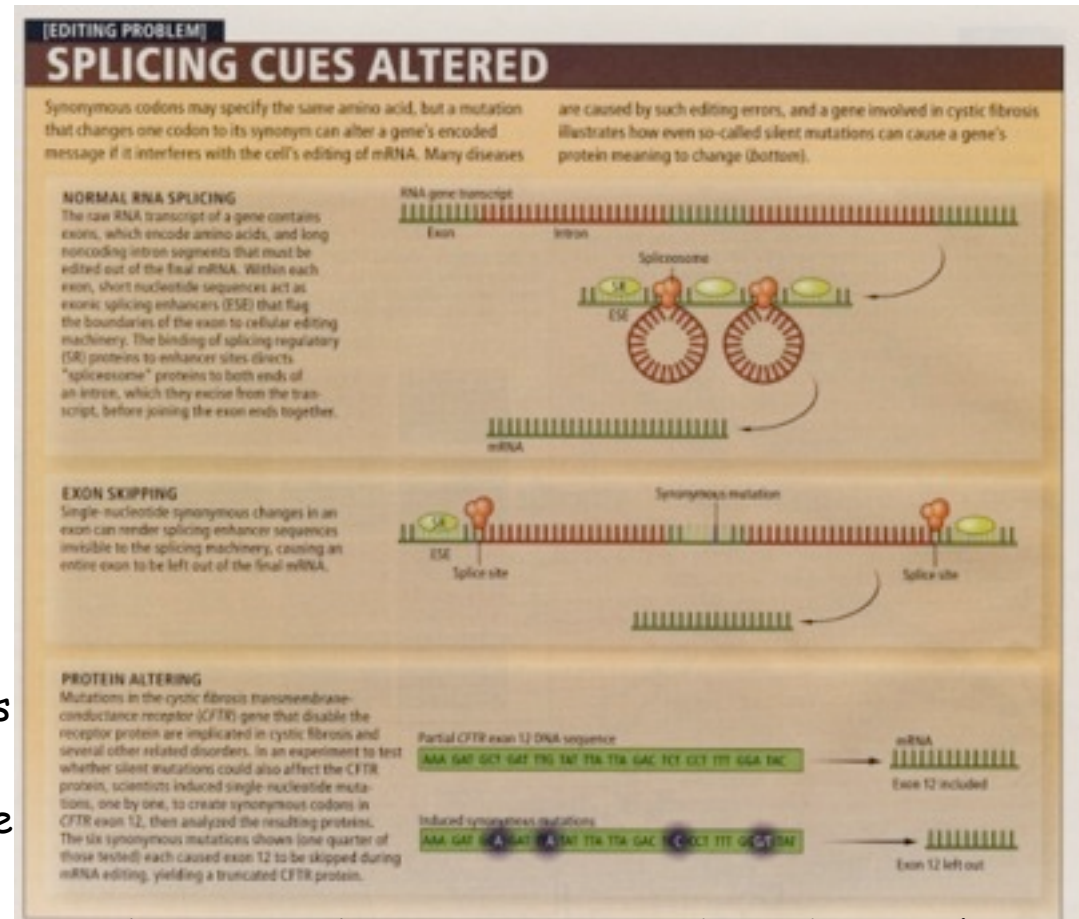
In the cellular cytoplasm, **ribosomes unfold and read the mRNA** and produce the encoded amino acid chain with the help of transfer RNA (tRNA) molecules. Each tRNA **delivers a single amino acid to the ribosome, binding to the corresponding mRNA codon** to confirm that the correct amino acid is being added. The **growing amino acid chain begins folding into its three-dimensional protein shape even as it is still forming**.

Synonymous codons may specify the same amino acid, but a **mutation that changes one codon to its synonym can alter a gene's encoded message if it interferes with the cell's editing of mRNA**. Many diseases are caused by such editing errors, and a gene involved in cystic fibrosis illustrates how even so-called silent mutations can cause a gene's protein meaning to change.

Normal RNA splicing. The raw RNA transcript of a gene contains **exons**, which encode amino acids, and long **noncoding intron** segments that must be edited out of the final mRNA. Within each exon, short nucleotide sequences act as **exonic splicing enhancers (ESE)** that **flag the boundaries of the exon** to cellular editing machinery. The binding of **splicing regulatory (SR) proteins** to enhancer sites directs "**spliceosome**" proteins to both ends of an intron, which they excise from the transcript, before **joining the exon ends together**.

Exon skipping. Single-nucleotide synonymous changes in an exon can render **splicing enhancer sequences invisible to the splicing machinery**, causing an entire exon to be **left out of the final mRNA**.

Protein altering. Mutations in the **cystic fibrosis transmembrane-conductance receptor (CFTR) gene** that disable the receptor protein are implicated in cystic fibrosis and several other related disorders. In an experiment to **test whether silent mutations could also affect the CFTR protein**, scientists induced single-nucleotide mutations, one by one, to create synonymous codons in CFTR exon 12, then analyzed the resulting proteins. The **six synonymous mutations shown (one quarter of those tested)** each caused exon 12 to be skipped during mRNA editing, yielding a **truncated CFTR protein**.



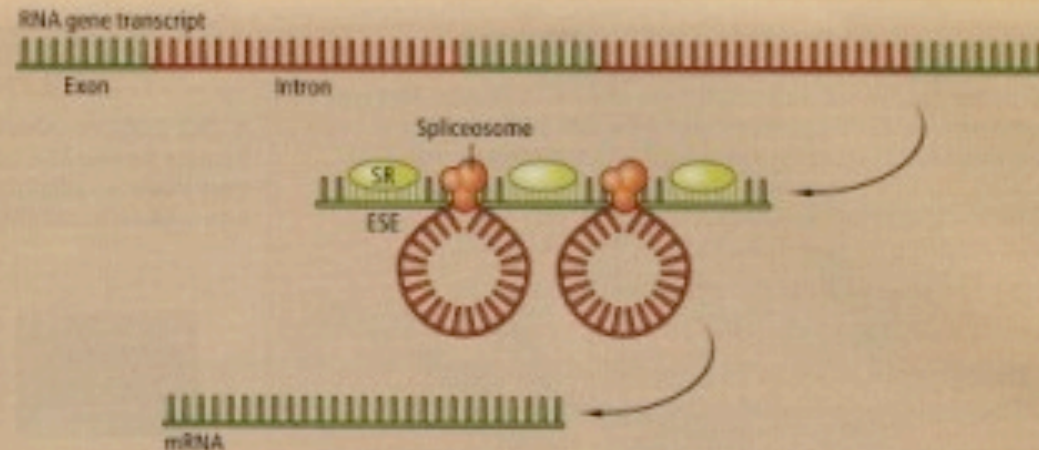
SPLICING CUES ALTERED

Synonymous codons may specify the same amino acid, but a mutation that changes one codon to its synonym can alter a gene's encoded message if it interferes with the cell's editing of mRNA. Many diseases

are caused by such editing errors, and a gene involved in cystic fibrosis illustrates how even so-called silent mutations can cause a gene's protein meaning to change (bottom).

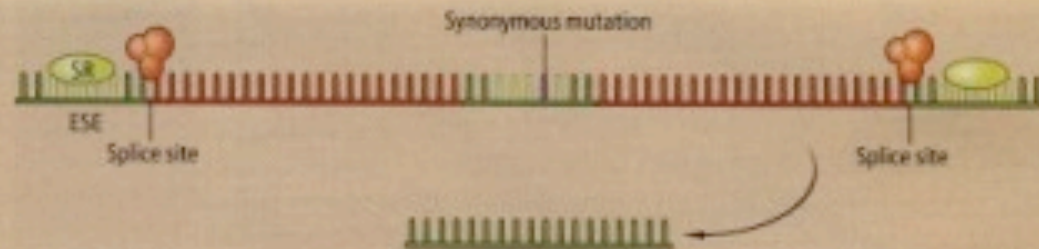
NORMAL RNA SPLICING

The raw RNA transcript of a gene contains exons, which encode amino acids, and long noncoding intron segments that must be edited out of the final mRNA. Within each exon, short nucleotide sequences act as exonic splicing enhancers (ESE) that flag the boundaries of the exon to cellular editing machinery. The binding of splicing regulatory (SR) proteins to enhancer sites directs "spliceosome" proteins to both ends of an intron, which they excise from the transcript, before joining the exon ends together.



EXON SKIPPING

Single-nucleotide synonymous changes in an exon can render splicing enhancer sequences invisible to the splicing machinery, causing an entire exon to be left out of the final mRNA.



PROTEIN ALTERING

Mutations in the cystic fibrosis transmembrane-conductance receptor (CFTR) gene that disable the receptor protein are implicated in cystic fibrosis and several other related disorders. In an experiment to test whether silent mutations could also affect the CFTR protein, scientists induced single-nucleotide mutations, one by one, to create synonymous codons in CFTR exon 12, then analyzed the resulting proteins. The six synonymous mutations shown (one quarter of those tested) each caused exon 12 to be skipped during mRNA editing, yielding a truncated CFTR protein.

Partial CFTR exon 12 DNA sequence

AAA GAT GCT GAT TTG TAT TTA TTA GAC TCT CCT TTT GGA TAC

mRNA

Exon 12 included

Induced synonymous mutations

AAA GAT GCA GAT TAA TAT TTA TTA GAC TCC CCT TTT GGGT TAT

mRNA

Exon 12 left out

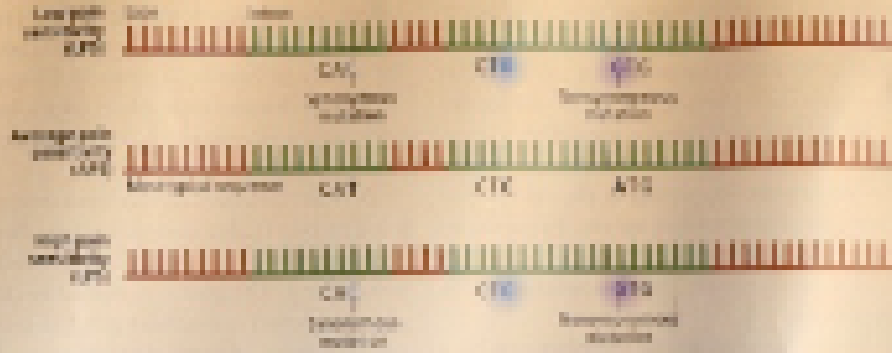
MUFFLED MESSAGE

A synonymous mutation was found to affect pain sensitivity by changing the amount of an important enzyme that cells produced. The difference results from alterations in the shape of mRNA, that can influence how

easy ribosomes are able to unpackage and read the strand. The folded shape is caused by base-pairing of the mRNA's nucleotides; therefore, a synonymous mutation can alter the way nucleotides match up.

COMPLEX MESSAGES

There is a vast array of the genes for control of mRNA production (CMT) are associated with low GPC, average GPC or high pain sensitivity (HPS). Differences between the most typical sequence (GPC) and alternative variants occur within the gene's main, however, only some of the changes (single genes) affect the encoded amino acid. That mutation may occur through a change in the amino acid sequence, but it is not clear how the amino acid sequence from the same 5' initial position, or it is not clear how the amino acid sequence from the same 5' initial position, or it is not clear how the amino acid sequence from the same 5' initial position, or it is not clear how the amino acid sequence from the same 5' initial position.



MORE COMPLEX STRUCTURE PRODUCE DIFFERENT

Experiments showed that the synonymous mutation change and the second generation of large protein-coded RNA changes dramatically different from the typical response. The resulting amino acid sequence also have a nearly 20-fold difference in levels of the CMT response in the cells at low and high sensitivity levels.

CMT with 2 amino acids



MUFFLED MESSAGE

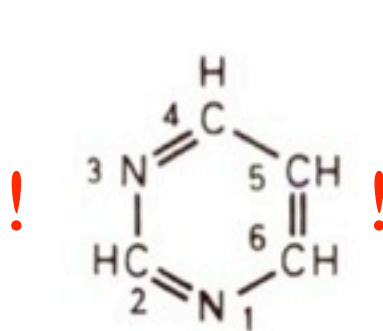
A synonymous mutation was found to affect pain sensitivity by changing the amount of an important enzyme that cells produced. The difference results from alteration in the shape of mRNA that can influence how easily ribosomes are able to unpackage and read the strand. The folded shape is caused by base-pairing of the mRNA's nucleotides; therefore, a synonymous mutation can alter the way nucleotides match up.

Chemie, struktura a interakce nukleových kyselin

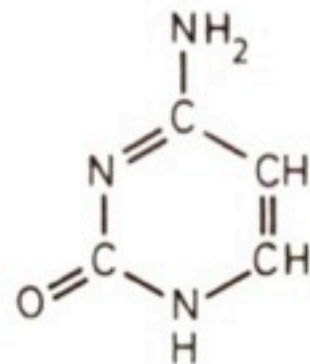
2009-10 2.ep PŘEDNÁŠKA 30.9.09

Složky nukleových kyselin

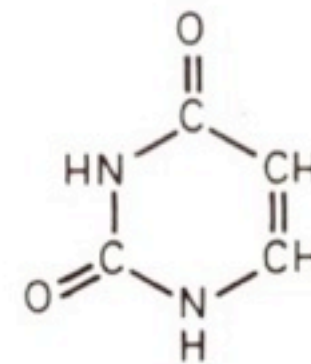
Pyrimidinové báze



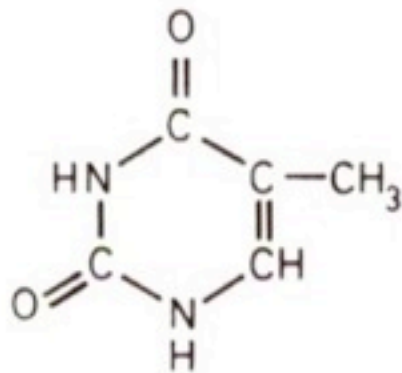
Pyrimidine



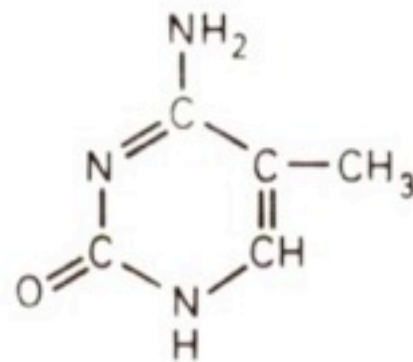
Cytosine



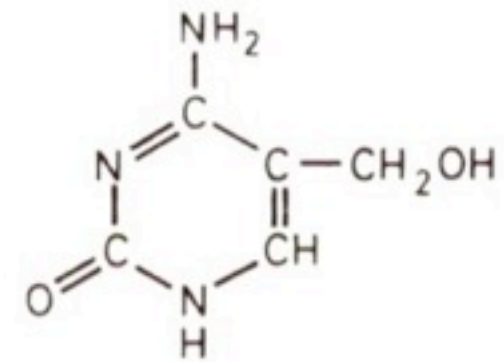
Uracil



Thymine
(5-methyl-uracil)



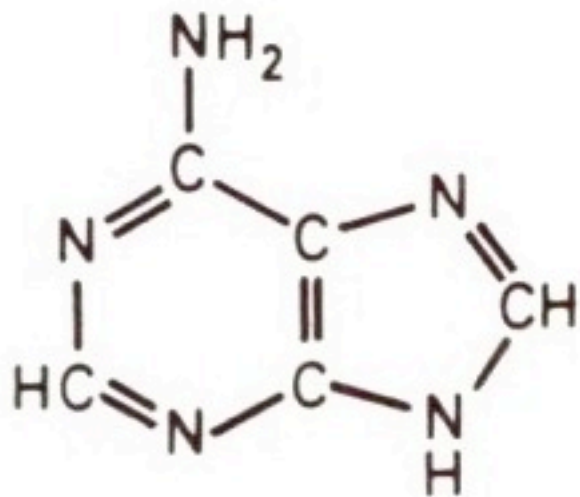
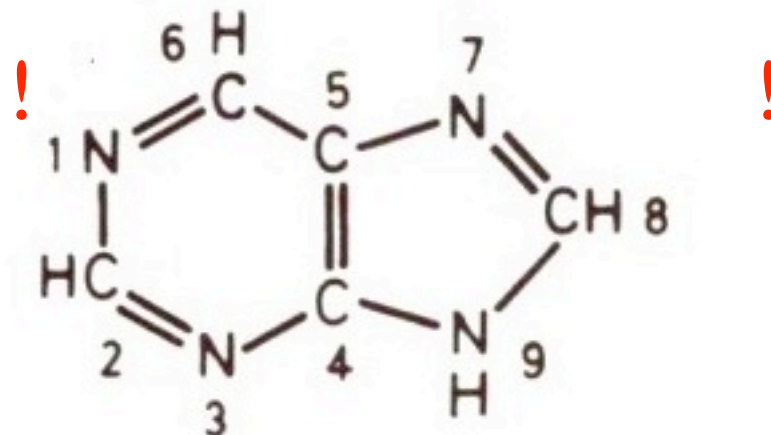
5-methyl
cytosine



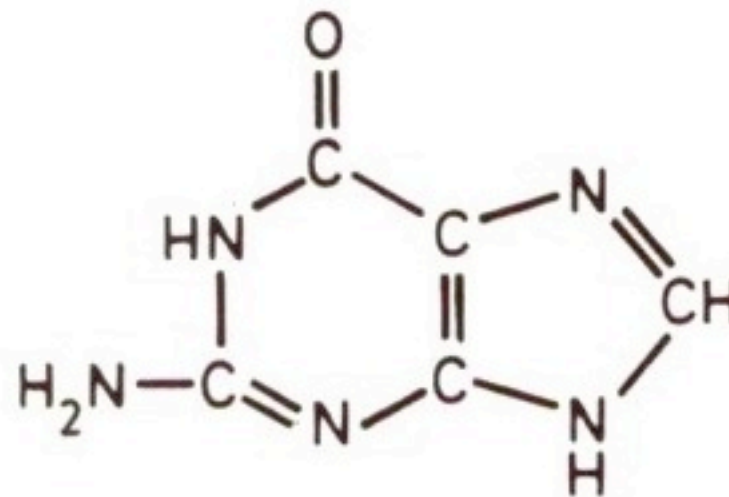
5-hydroxymethyl
cytosine

Fig. 2.1

Purinové báze



Adenine
(6-aminopurine)



Guanine
(2-amino 6-hydroxypurine)

Fig. 2.3

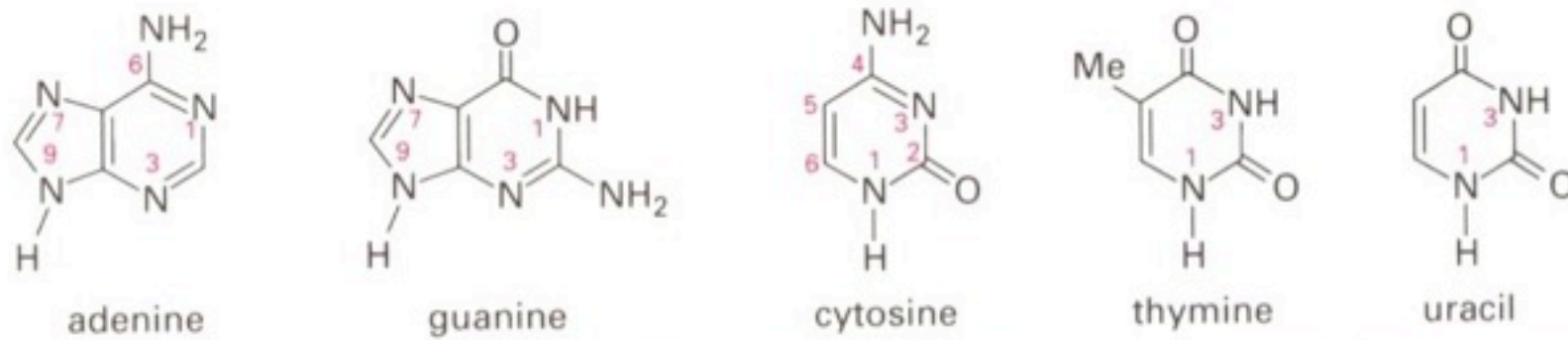


Fig. 2.1 Structures of the five major purine and pyrimidine bases of nucleic acids in their dominant tautomeric forms and with the IUPAC numbering systems for purines and pyrimidines.



Fig. 2.6 Keto–enol tautomers for 2-pyridone : 2-hydroxypyridine (left) and amine–imine tautomerism for 2-aminopyridine (right)

Neobvyklé báze a nukleosidy

vyskytují se např v tRNA;
jaké další v chromosomálních DNA

(i) prokaryotních a

(ii) eukaryotních buněk?

(iii) v DNA virů?

Může se v DNA vyskytovat uracil?

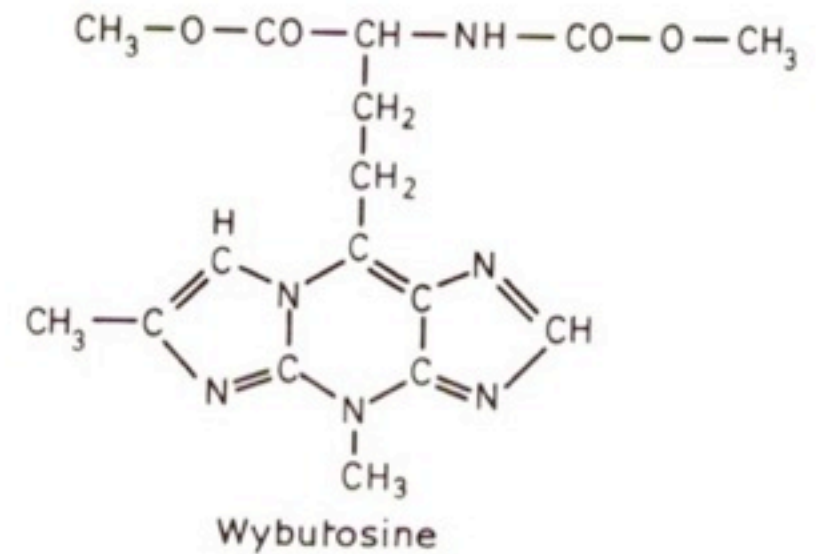


Fig. 2.5

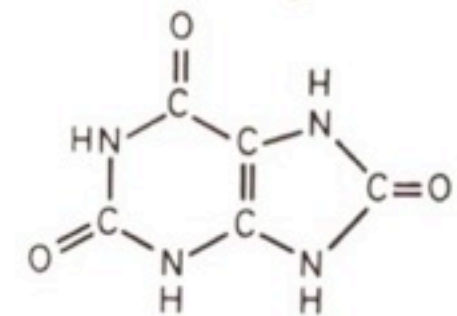
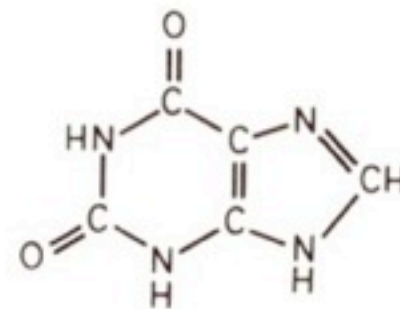
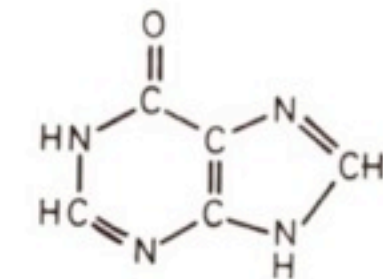
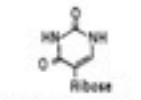
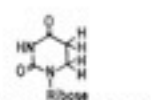


Fig. 2.4

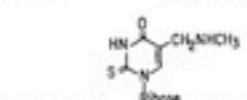
Neobvyklé báze a nukleosidy v tRNA



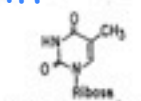
1. Pseudouridine (Ψ)



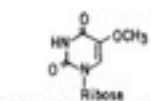
2. Dihydrouridine (D or U)



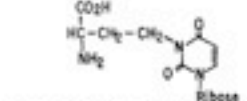
10. 5-Methylaminomethyl-2-thiouridine (mnm²Ψ)



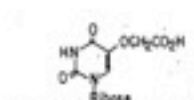
3. Ribothymidine (T)



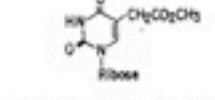
4. 5-Methoxyuridine (moU)



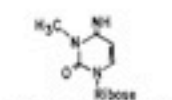
11. 3-(3-Amino-3-carboxypropyl)uridine (acp³U)



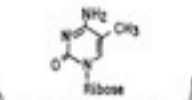
5. Uridin-5-oxycetic acid (U)



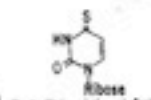
6. 5-(Methoxycarbonylmethyl)uridine (mcm²U)



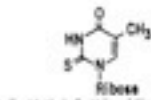
12. 3-Methylcytidine (m³C)



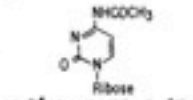
13. 5-Methylcytidine (m⁵C)



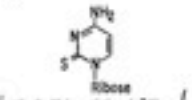
7. 4-Thiouridine (s⁴U)



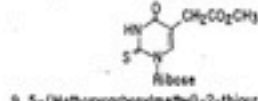
8. 5-Methyl-2-thiouridine (m⁵s²U)



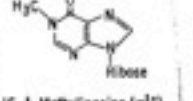
14. N⁶-Acetylcytidine (ac⁴C)



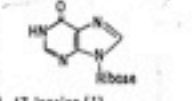
15. 2-Thiocytidine (s²C)



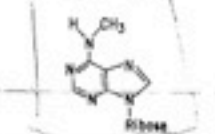
9. 5-(Methoxycarbonylmethyl)-2-thiouridine (mcm⁵s²U)



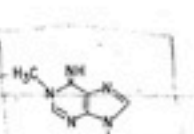
16. 1-Methylinosine (m¹I)



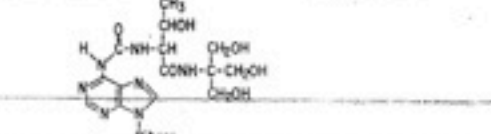
17. Inosine (I)



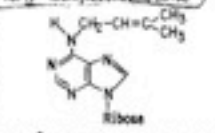
18. N⁶-Methyladenosine (m⁶A)



19. 1-Methyladenosine (m¹A)



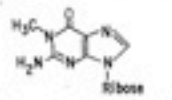
24. 5-[γ-(3-β-D-Ribofuranosylpurin-6-yl) carbonyl]threonyl 2-amido-2-hydroxymethylpropane-1,3-diol (K)



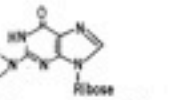
20. N⁶-Isopentenyladenosine (i⁶A)



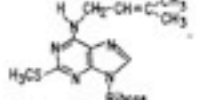
19. 1-Methylidenosine (m¹I)



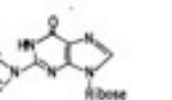
25. 1-Methylguanosine (m¹G)



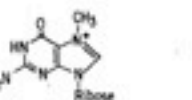
26. N⁷-Methylguanosine (m⁷G)



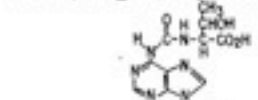
21. 2-Methylthio-N⁶-isopentenyladenosine (m²i⁶A)



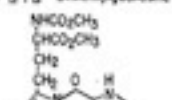
27. N⁷, N²-Dimethylguanosine (m⁷m²G)



28. 7-Methylguanosine (m⁷G)



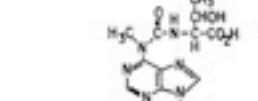
22. 5-[γ-(3-β-D-Ribofuranosylpurin-6-yl) carbonyl]threosine (K)



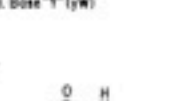
29. Base Y¹ (Y¹)



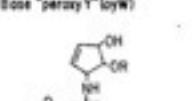
30. Base Y² (Y²)



23. 5-[γ-(3-β-D-Ribofuranosylpurin-6-yl) 5-methylcarbamoyl]threosine (m⁵K)



31. Base Y³ (Y³)



32. R-H 2-Isoinosine or Quai¹⁶

R1-Q 2-mannosyl man²Q35
R2-D 2-galactosyl gal²Q35

Neobvyklé báze v DNA bakteriofágů

Table 1 Properties of phage DNAs containing modified bases

Phage	Host	DNA molecular weight ($\times 10^{-6}$)	Mol % G+C		Base change	Extent of change ^a (%)	T_m^b		Buoyant density ^c		References
			Phage	Host			Observed	Expected ^d	Observed	Expected ^d	
T4	<i>Escherichia coli</i>	110	34	50	5-hmCyt for Cyt	100	84	84	1.700	1.694	61, 87, 122, 154
ϕ e	<i>Bacillus subtilis</i>	100	39	43	5-hmUra for Thy	100	77.5	85.3	1.742	1.703	54, 119, 138
PBS2	<i>B. subtilis</i>	150	28	43	Ura for Thy	100	76.5	81.5	1.722	1.690	51, 74, 128, 129
χ P12	<i>Xanthomonas oryzae</i>	30	67	64	5-mCyt for Cyt	100	101.5	95.4	1.710	1.726	29, 68
S2L	<i>Synechococcus elongatus</i>	28	69	70	2-nAde for Ade	100	101.9	98.3	1.731	1.728	60
SP15	<i>B. subtilis</i>	250	42	43	5-dhpUra for Thy	41	61.7	86.2	1.761	1.702	10, 86, 139
SP10	<i>B. subtilis</i>	59 ^e	43	43	α -gluThy for Thy	15–20	81.5	86.9	1.723	1.703	66, 77
ϕ W14	<i>Pseudomonas acidovorans</i>	92	51 ^f	67	α -putThy for Thy	50	99.3	90.3	1.666	1.716	9, 102

^a The extent to which the modified base replaces the normal base.

^b Thermal transition temperatures are extrapolated to the value in 0.15 M NaCl.

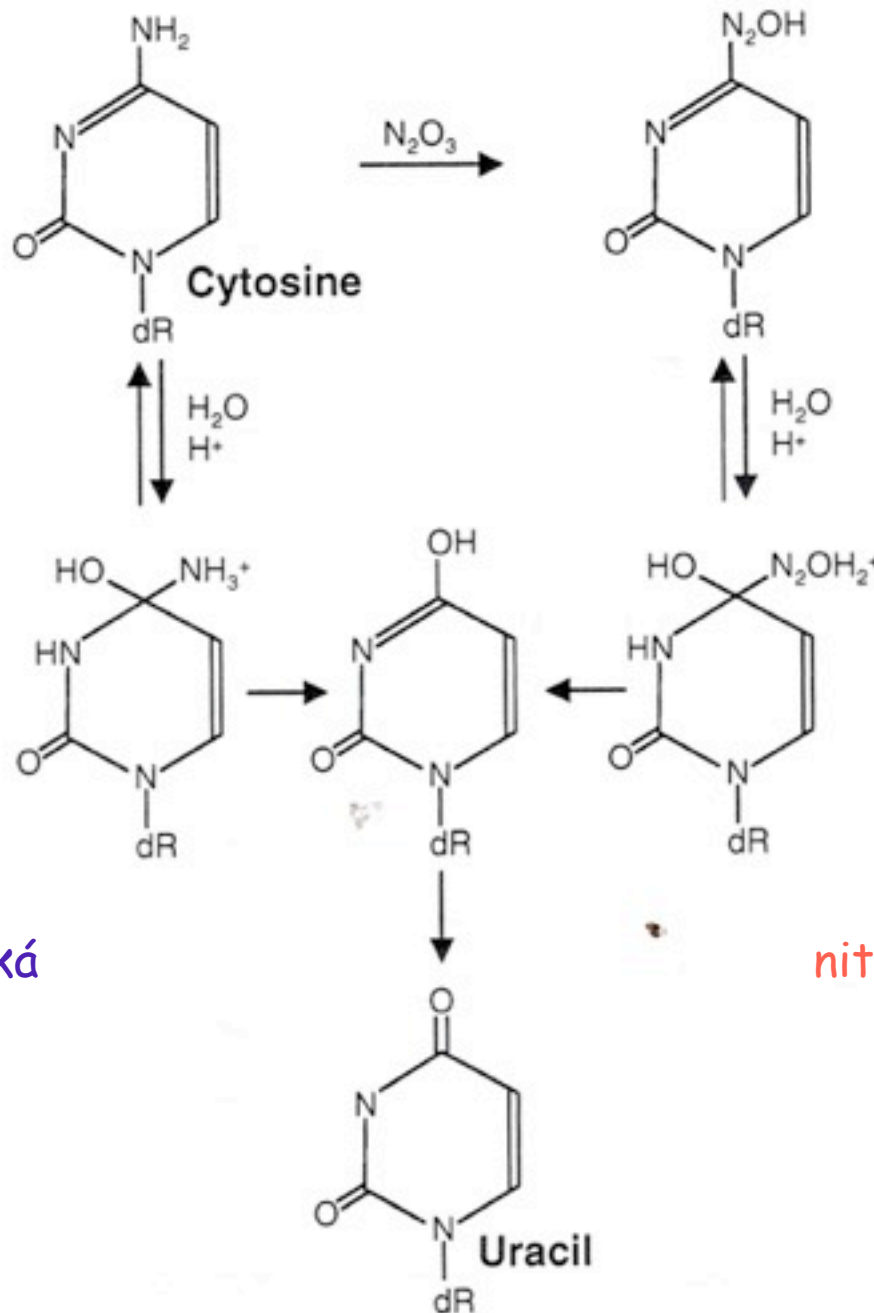
^c Buoyant densities in neutral CsCl, assuming a value of 1,710 g ml⁻¹ for *E. coli* DNA.

^d These are the values expected for a DNA of the same mol % G+C and of normal composition.

^e Not reported, but may be the value given [K. Bott, personal communication in (6)].

^f M. Mandel, personal communication.

Deaminace cytosinu (C - U)



Zbytky uracilu v DNA, vznikající v důsledku nesprávné inkorporace nebo díky deaminaci cytosinu, jsou v lidském organismu odstraňovány pomocí **uracil-DNA glykosylázy**, která je jedním z nejúčinnějších enzymů v systému reparujícím DNA (base-excision DNA repair)

Hydrolytická

nitrosativní

3. Reaction pathways for hydrolytic and nitrosative deamination of cytosine (Caulfield et al., 1998).

Review

DNA-uracil and human pathology

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Received 20 March 2007; accepted 26 April 2007

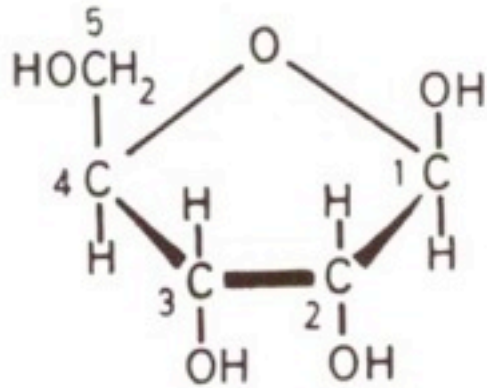
Abstract

Uracil is usually an inappropriate base in DNA, but it is also a normal intermediate during somatic hypermutation (SHM) and class switch recombination (CSR) in adaptive immunity. In addition, uracil is introduced into retroviral DNA by the host as part of a defence mechanism. The sources of uracil in DNA are spontaneous or enzymatic deamination of cytosine (U:G mispairs) and incorporation of dUTP (U:A pairs). Uracil in DNA is removed by a uracil-DNA glycosylase. The major ones are nuclear UNG2 and mitochondrial UNG1 encoded by the *UNG*-gene, and SMUG1 that also removes oxidized pyrimidines, e.g. 5-hydroxymethyluracil. The other ones are TDG that removes U and T from mismatches, and MBD4 that removes U from CpG contexts. UNG2 is found in replication foci during the S-phase and has a distinct role in repair of U:A pairs, but it is also important in U:G repair, a function shared with SMUG1. SHM is initiated by activation-induced cytosine deaminase (AID), followed by removal of U by UNG2. Humans lacking UNG2 suffer from recurrent infections and lymphoid hyperplasia, and have skewed SHM and defective CSR, resulting in elevated IgM and strongly reduced IgG, IgA and IgE. UNG-defective mice also develop B-cell lymphoma late in life. In the defence against retrovirus, e.g. HIV-1, high concentrations of dUTP in the target cells promotes misincorporation of dUMP-, and host cell APOBEC proteins may promote deamination of cytosine in the viral DNA. This facilitates degradation of viral DNA by UNG2 and AP-endonuclease. However, viral proteins Vif and Vpr counteract this defense by mechanisms that are now being revealed. In conclusion, uracil in DNA is both a mutagenic burden and a tool to modify DNA for diversity or degradation.

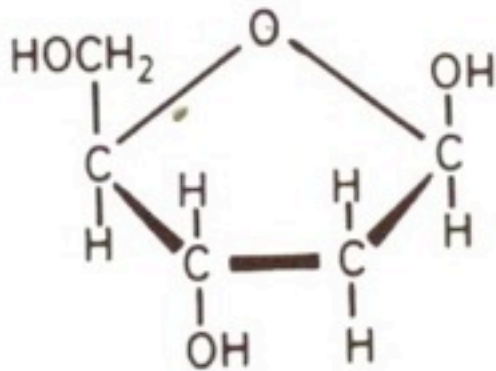
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Uracil v lidské DNA může představovat **mutační zátěž** nebo **nástroj pro odlišení nebo degradaci DNA**

Číslování **uhlíků** ve zbytcích **cukru**:
1', 2' ...5' na rozdíl od bází 1, 2, ... 5, 6 ..



β -D-ribofuranose

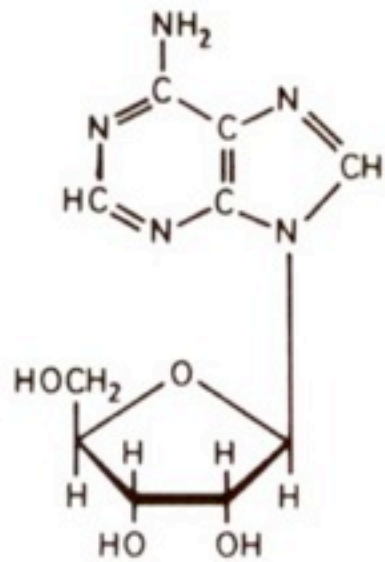


β -D-2-deoxyribofuranose

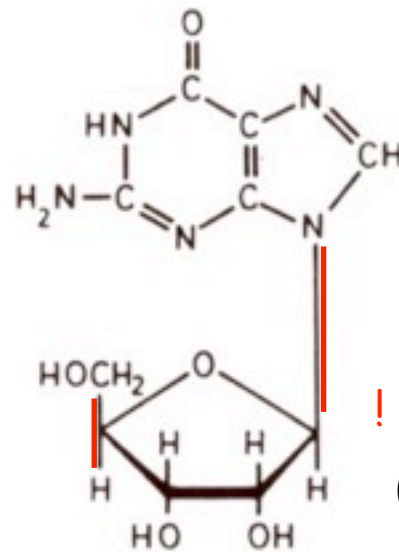
Fig. 2.6

Sugar pucker

Nucleosides/nukleosidy



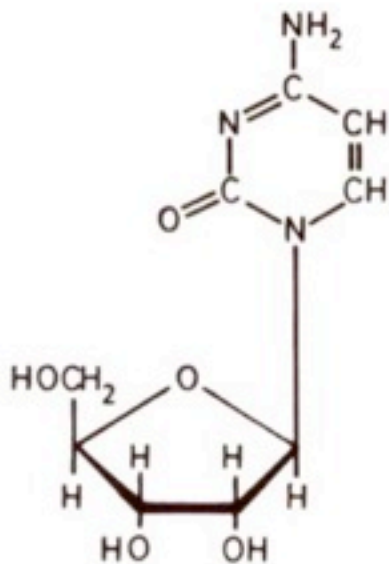
Adenosine
(9-β-D-ribofuranosyl adenine)



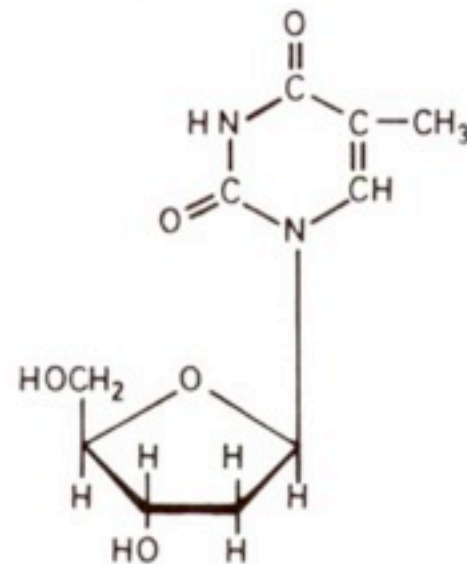
Guanosine
(9-β-D-ribofuranosyl guanine)

! beta-glykosidická vazba
(obě vazby na stejné straně kruhu cukru)

Guanine riboside Guanosine



Cytidine
(1-β-D-ribofuranosyl cytosine)

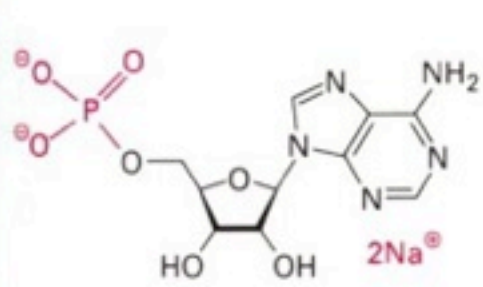


Thymidine
(1-β-D-2-deoxyribofuranosylthymine)

Thymine deoxyriboside

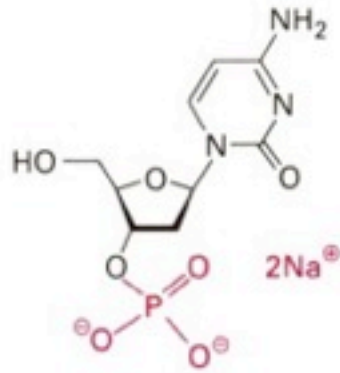
Fig. 2.7

Nucleotides/nukleotidy



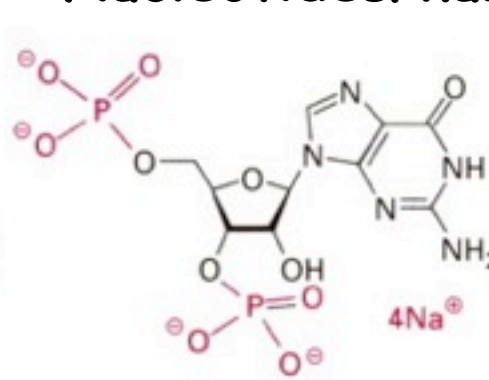
adenosine 5'-phosphate

AMP

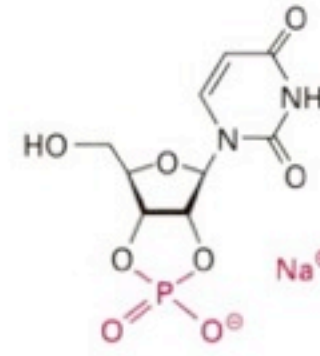


deoxycytidine 3'-phosphate

CMP



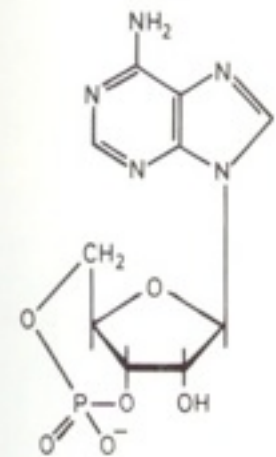
guanosine 3', 5'-bisphosphate



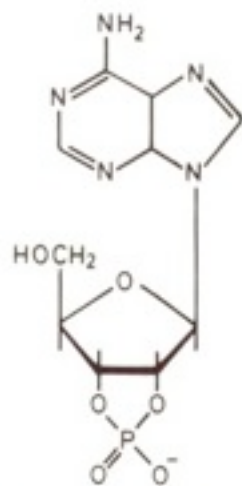
uridine 2', 3'-cyclic phosphate
cyclic p
cUMP

Fig. 2.4 Structures of some common nucleotides. All are presented as their sodium salts in the state of ionization observed at neutral pH.

Shorthand notation



Adenosine 3' 5'-cyclic phosphate (cAMP)



Adenosine 2' 3'-cyclic phosphate

Fig. 2.11

5'-TAGGTCGA-3'
3'-ATCCAGCT-5'

Cp (C-3') x pC (C-5')
UpUp U-Up UpU

bis- x di-phosphates (e.g. ADP)

2 fosfáty na jedné pentose

Ionization of AMP

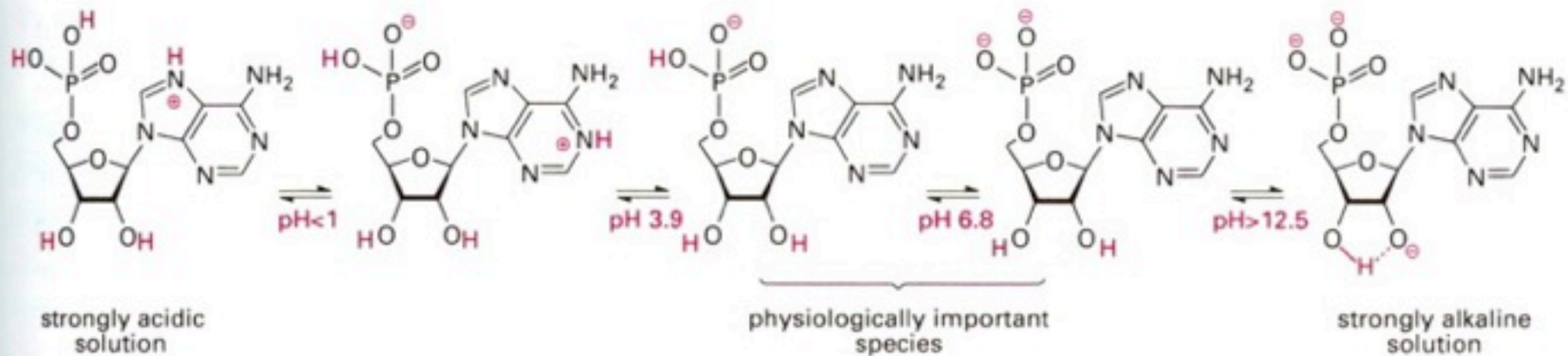


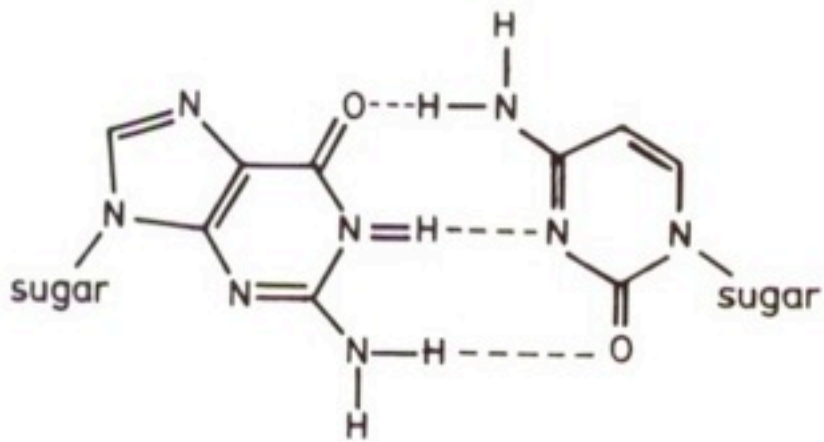
Fig. 2.5 States of protonation of adenosine 5'-phosphate (AMP) from strongly acidic solution (left) to strongly alkaline solution (right).

BUT, pK of base residues
in DNA may significantly differ!

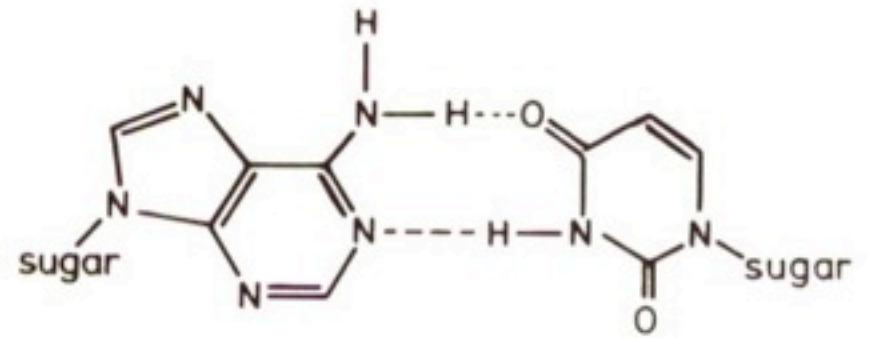
Table 2.1. pK_a values for bases in nucleosides and nucleotides

Base (site of protonation)		Nucleoside	3'-Nucleotide	5'-Nucleotide
Adenine	(N-1)	3.52	3.70	3.88
Cytosine	(N-3)	4.17	4.43	4.56
Guanine	(N-7)	3.3	(3.5)	(3.6)
Guanine	(N-1)	9.42	9.84	10.00
Thymine	(N-3)	9.93	—	10.47
Uracil	(N-3)	9.38	9.96	10.06

These data relate to 20°C and zero salt concentration. They correspond to *loss* of a proton for $pK_a > 9$ and *capture* of a proton for $pK_a > 5$.



G : C base pair



A : T base pair

Fig. 2.14 The normal base-pairing arrangement found in DNA.
(The dashed lines indicate hydrogen bonds).

DNA base pairing

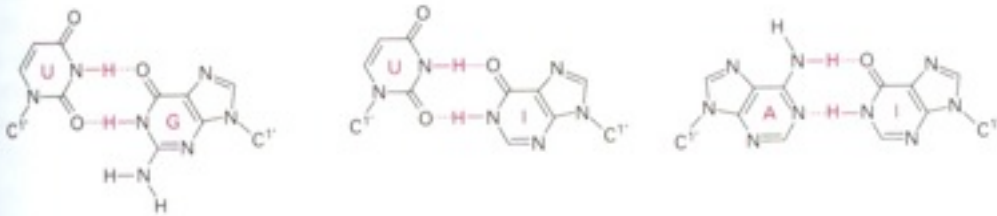


Fig. 2.9 'Wobble' pairings for G-U (left), I-U (centre), and I-A (right).

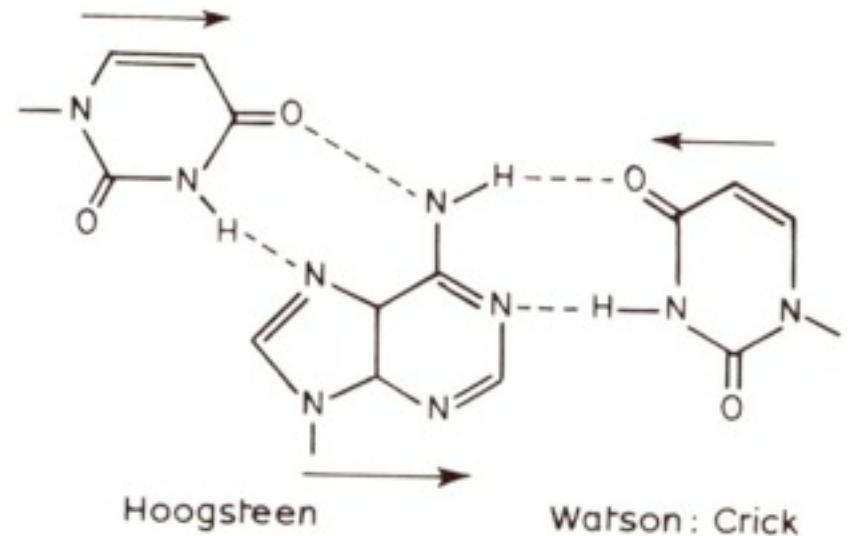


Fig. 2.27 The triple-stranded structure formed by two poly(U) and one poly(A) strand involves a Watson:Crick base paired poly(A)·poly(U) with the second poly(U) strand running in the same direction as

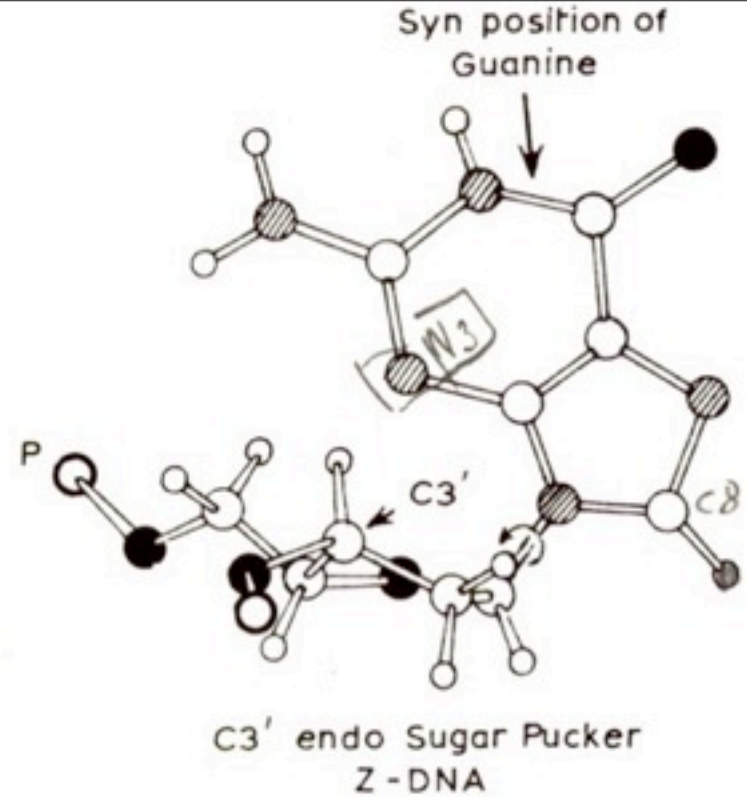
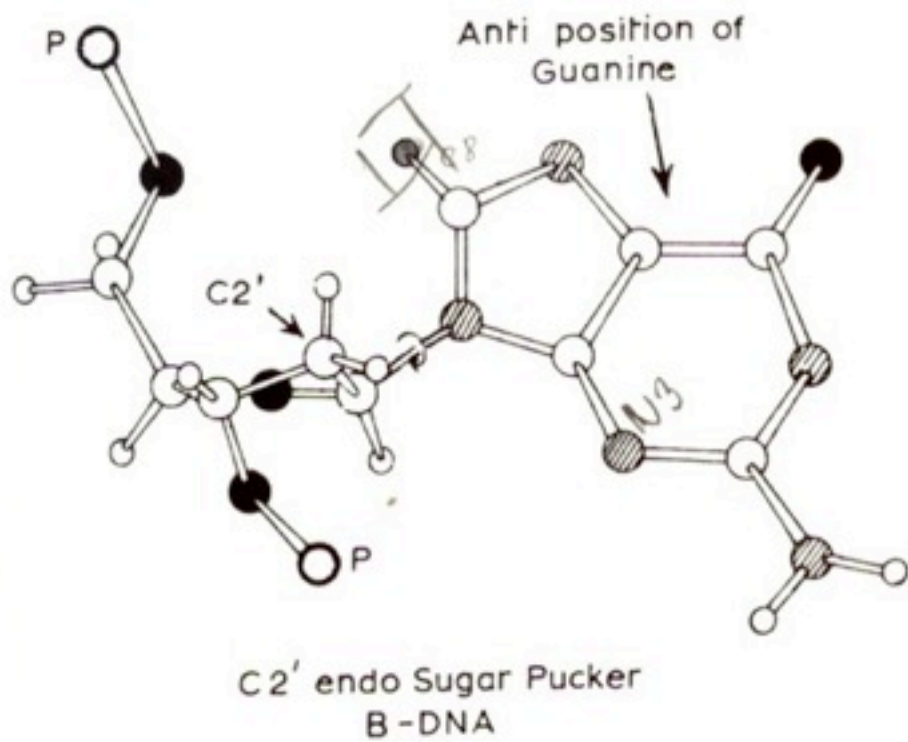
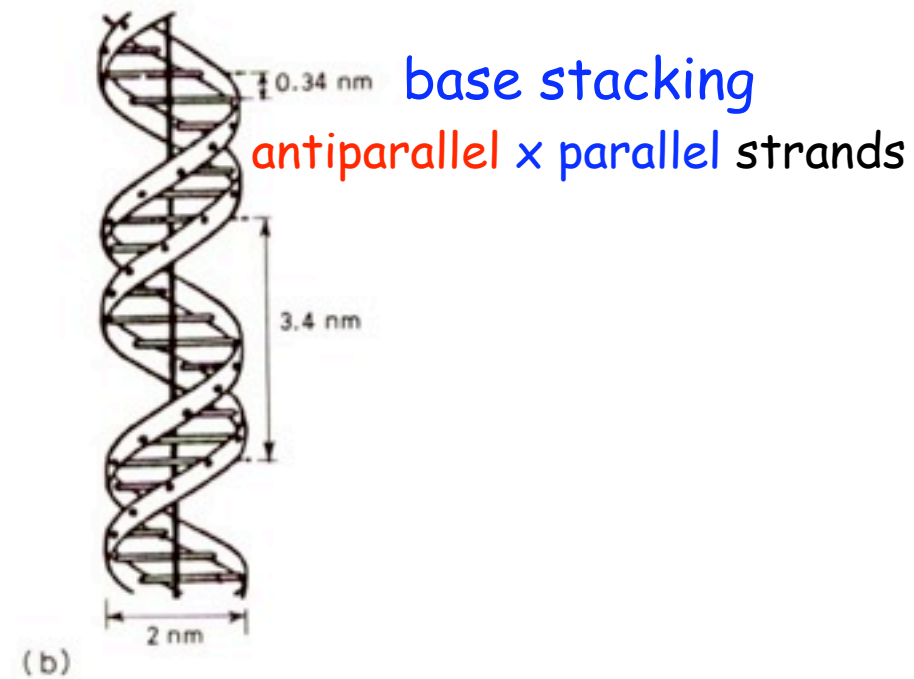
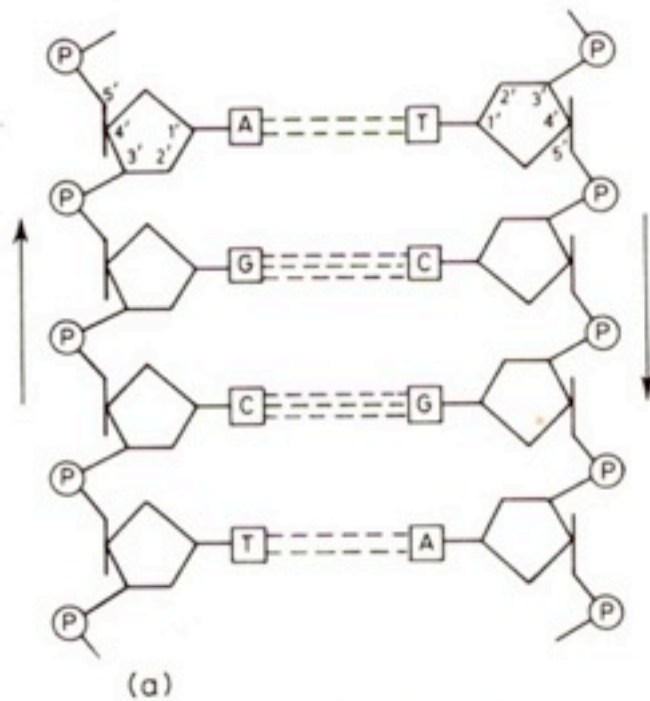


Fig. 2.16 Conformation of the base-sugar linkage. Guanine is shown in the *anti* conformation linked to C2' *endo* deoxyribose as in B-DNA and in the *syn* conformation linked to C3' *endo* deoxyribose as in Z-DNA reproduced with permission, from Rich *et al.* [115] copyright Annual Reviews Inc.



Between pH 5 and 9
DNA is a polyanion
 with a single negative
 charge per nucleotide

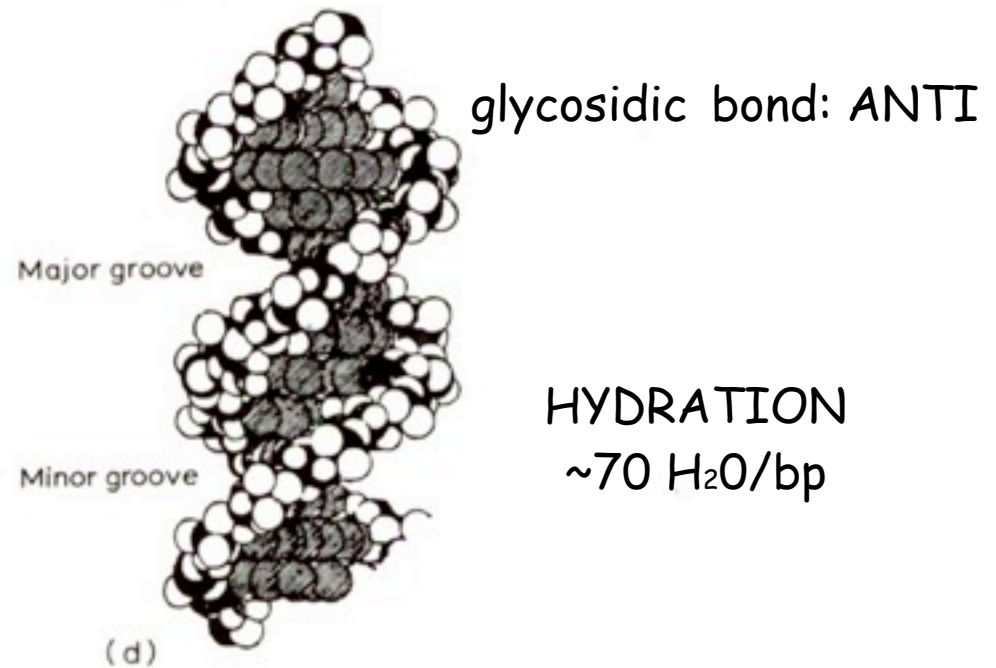
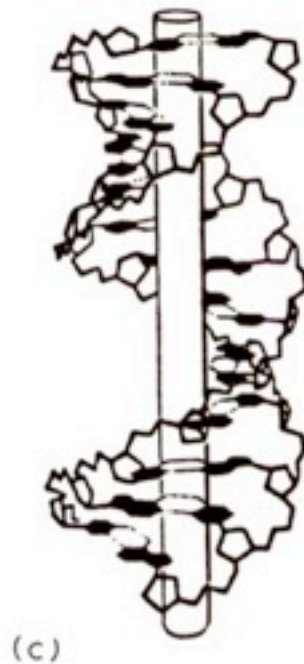
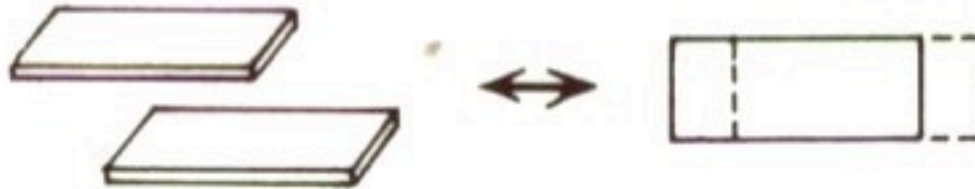


Fig. 2.15 Various diagrammatic ways of representing DNA: (a) showing polarity and base pairing but no helical twist; (b) showing helical twist and helix parameters but not base pairs; (c) showing helix and base pairs; (d) space-filling representation showing major and minor grooves.

Roll



Slide



Twist



Fig. 2.17 Ways in which adjacent bases may move relative to one another (seen from two different positions).

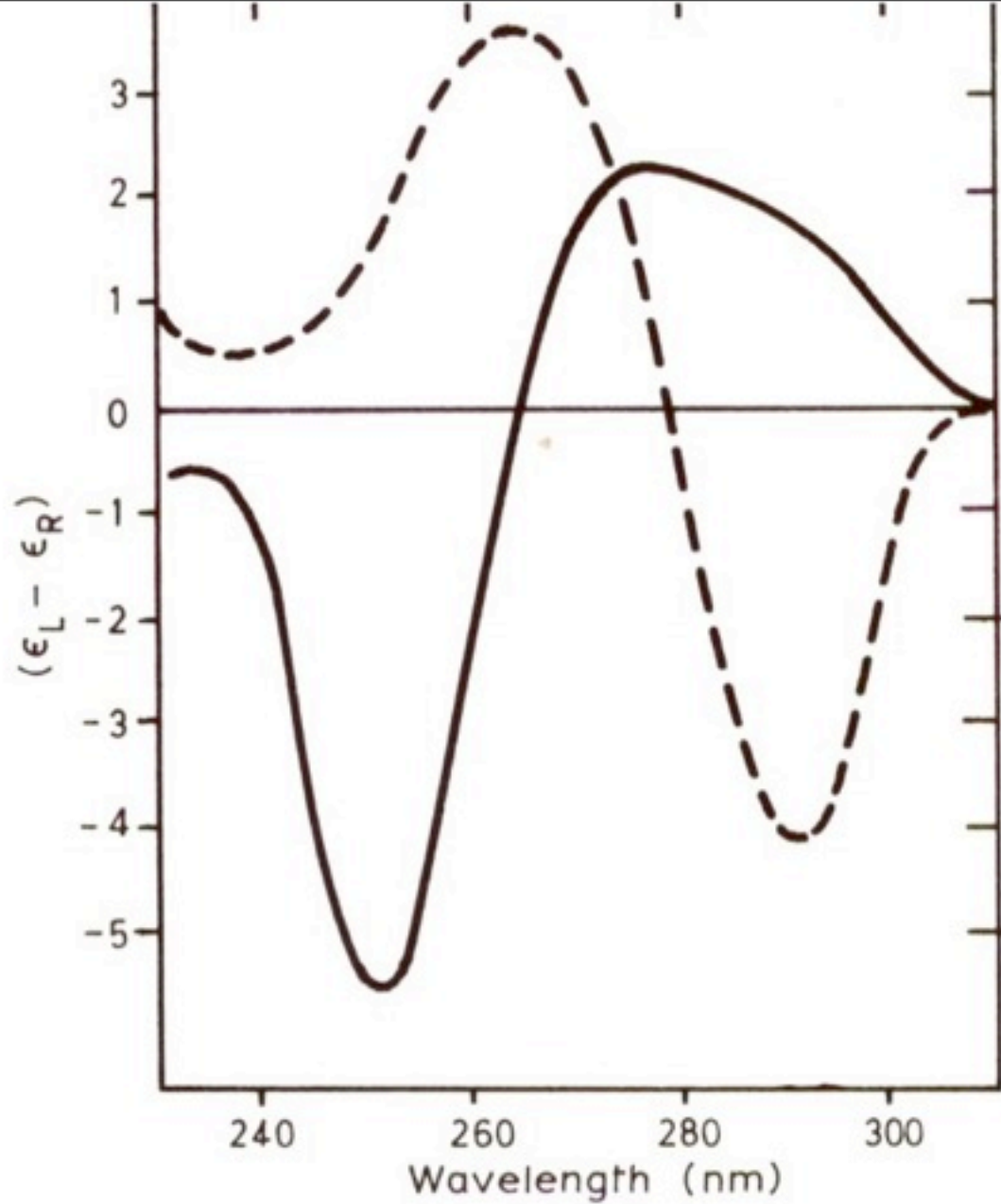


Fig. 2.18 Circular dichroism spectra of poly(dG-dC) in low-salt (0.2 M NaCl)—and high-salt (3.5 M NaCl)—conditions (reproduced, with permission, from Pohl and Jovin [55] copyright Academic Press, Inc.).

Summary

The building blocks of nucleic acids are nucleotides, which are the phosphate esters of nucleosides. These are formed by condensation of a base and a pentose. In RNA, the pentose is D-ribose and is linked in its furanose form from C-1' to N-9 of a purine, adenine, or guanine, or N-1 of a pyrimidine, cytosine, or uracil. In DNA, 2-deoxy-D-ribose is joined in the same way to the four bases, among which thymine takes the place of uracil. The phosphate esters are strong acids and exist as anions at neutral pH. The 'bases' are, in reality, only very weakly basic and A, C, and G become protonated only below pH 4. The amide NHs in G, T, and U are deprotonated at pHs above 9.

Hydrogen bonds can be formed between the major *amino-keto* tautomers of the bases to link A with T and C with G in Watson-Crick base-pairing. Such hydrogen bonds are largely electrostatic in character. 'Wobble' and Hoogsteen base-pairs offer minor variations to Watson-Crick pairing, and are seen in tRNA structures.

Nucleotides have defined shapes with a general preference for the *anti*-conformers of the glycosylic bond χ , for the C^{4'}–C^{5'} bonds γ , and for the two C–O(P) bonds β and ϵ . The furanose ring is puckered to relieve strain and can adopt either the C^{2'}-*endo* or the C^{3'}-*endo* conformation, which are in rapid equilibrium at room temperature. XXXXXXXXXX

Electrochemistry of DNA

If you want to do research into the DNA electrochemistry it is not sufficient to know well methods of electrochemical analysis, you have to know something about DNA. Since the beginning of the 1950's DNA has become one of the most studied objects. It has been studied not only by chemists, physicists and biologists but also by MD's, anthropologists, biotechnologists, etc. because of its utmost biological importance and also for its interesting properties such as self-complementarity of its strands, strand separation and restoration of its double-helical structure, etc.

I therefore decided to start my lecture series with DNA

DNA electrochemistry

Co je polarografie?

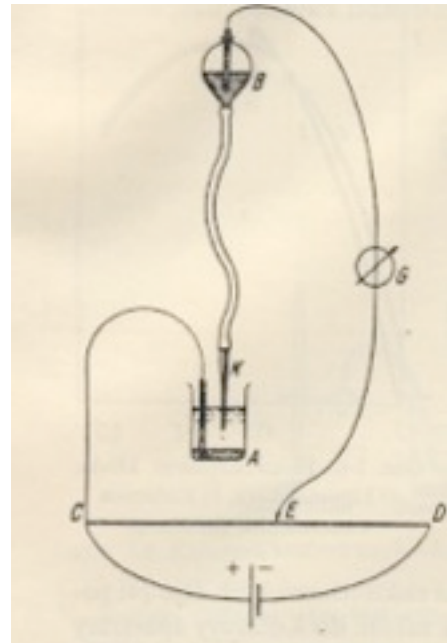
Kdo ji objevil?

Moderní elektrochemické metody

Může být elektrochemie užitečná při výzkumu nukleových kyselin a bílkovin?

Jaroslav Heyrovský

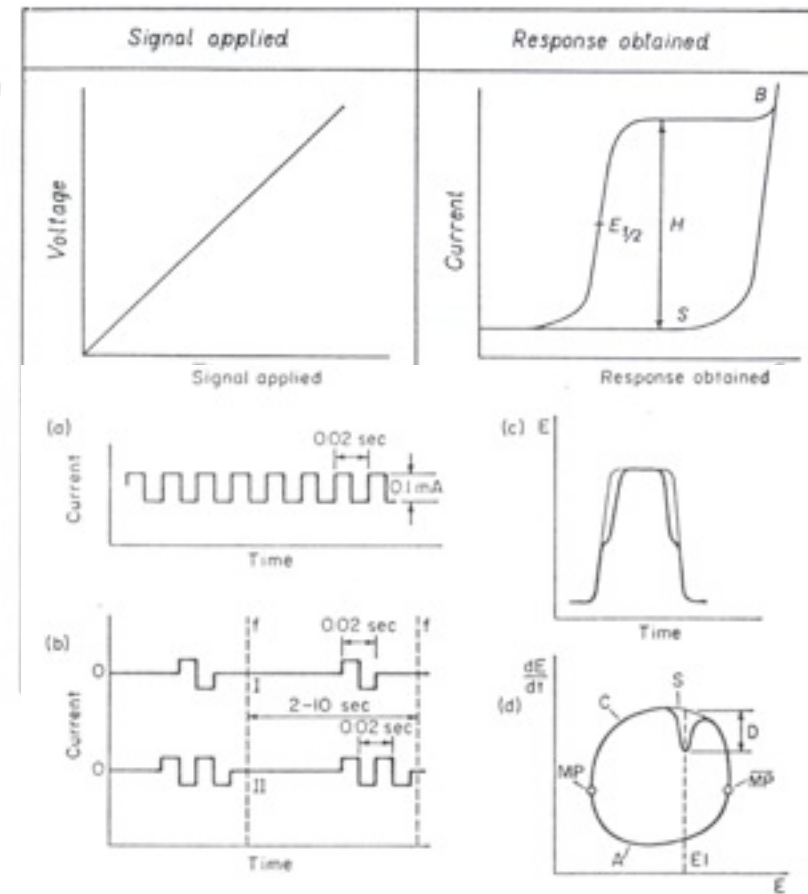
1890-1967



d.c. polarography
1922

J. Heyrovský

Nobel Prize 1959

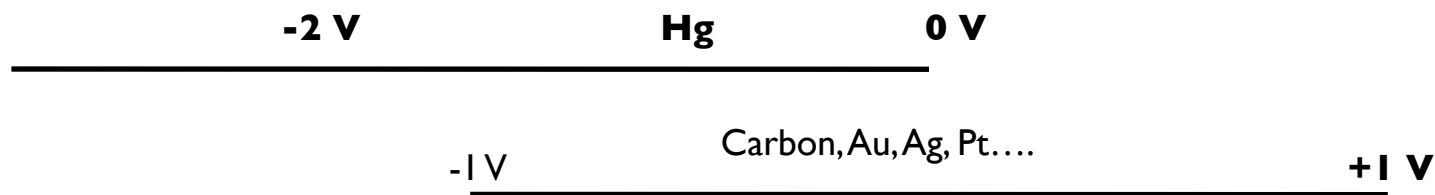


Oscillographic polarography at controlled a.c.
(cyclic a.c. chronopotentiometry)

complete analyses on a single drop **1941**

Electrodes

A number of electrodes have been used in electrochemical **NA and protein studies**, such as liquid mercury and solid mercury-containing electrodes (such as film and solid amalgam, incl. dental amalgam electrodes), carbon, gold, indium-tin oxide, silver, etc. Only with **mercury**-containing and **carbon** electrodes well-behaved **electroactivity** was observed. Mercury electrodes and most of the solid electrodes greatly differ in their **potential windows**



Hg electrodes thus suits better for **reductions** while **solid** electrodes (e.g. carbon, Au,...) are better for **oxidation** processes. **Material of the electrode** is also very important. Hydrophobicity/hydrophilicity as well reactive atoms and functional groups may greatly affect **adsorption** of DNA and proteins

DNA and RNA are Electroactive Species

producing faradaic and other signals on interaction with electrodes

Cytosine (C)

Adenine (A) A, C, G are reduced at **MERCURY** electrodes

Guanine (G) reduction product of guanine is oxidized back to G

All bases (A, C, G, T, U) yield sparingly soluble compounds with mercury and can be determined at concentration down to 10^{-11} M.

Solid amalgam electrodes can be used instead of the mercury drop electrodes.

A and G as well as C and T are oxidized at **CARBON** electrodes

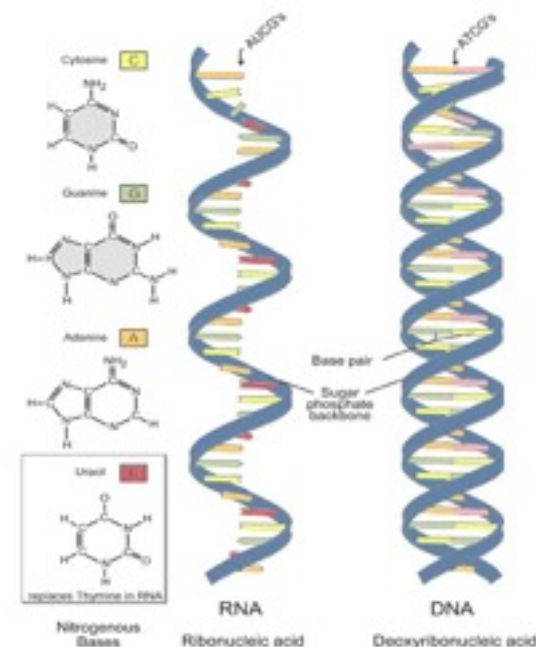
PEPTIDE NUCLEIC ACID (PNA) BEHAVES SIMILARLY TO DNA AND RNA

Microliter volumes of the analyte are sufficient for analysis

Electroactive Labels can be Introduced in DNA

Fojta, M., et al.. (2007): „Multicolor“ electrochemical labeling of DNA hybridization probes with osmium tetroxide complexes. *Anal. Chem.* 79, 1022-1029

Trefulka, M., et al. (2007): Covalent labeling nucleosides, RNA and DNA with VIII- and VI-valent osmium complexes. *Electroanal.* 19, 1281-1287



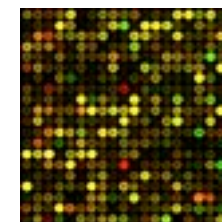
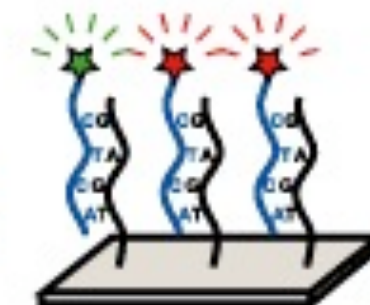
Progress in genomics affects electroanalysis

Many areas of science are influenced by the fast development of the genomics and by the success of the Human Genome Project.

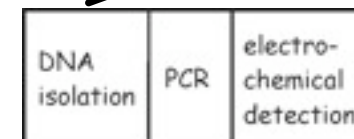
Classical sequencing of individual human genomes with 3×10^9 base pairs is too difficult.

Sequencing by DNA hybridization is gaining importance

Relatively expensive DNA hybridization **ARRAYS** with optical detection are currently applied in research labs
It is believed that **electrochemistry** can complement the optical detection providing new **LESS EXPENSIVE** hybridization detection for **decentralized DNA analysis** in many areas of practical life



LOW DENSITY CHIPS

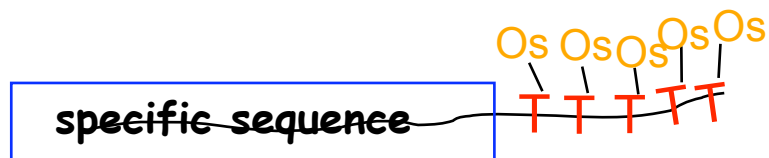


LAB-ON-A-CHIP

END-LABELING of DNA and RNA

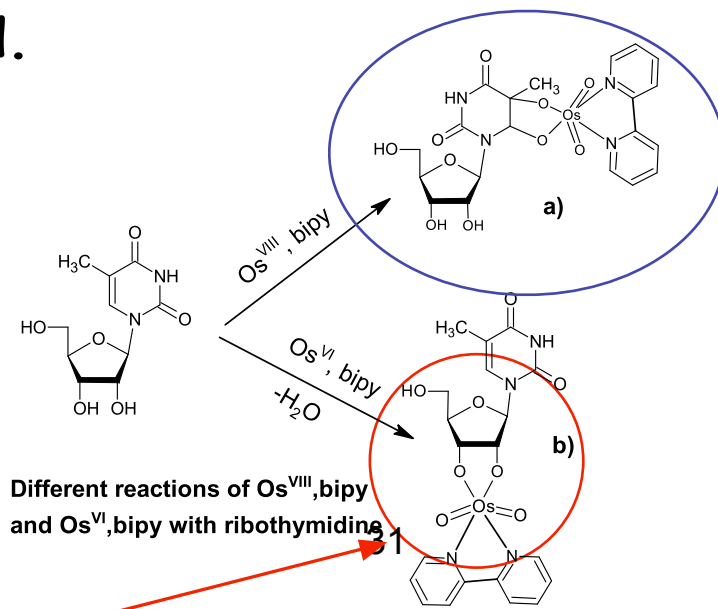
Electroactive labels such as ferrocene, daunomycin, viologen, thionine, etc. were covalently bound to DNA to obtain electrochemical signals closer to zero charge and/or to increase the sensitivity of the analysis. These labels are expensive and can hardly be used for labeling of longer NAs, such as plasmid DNAs.

Osmium tetroxide complexes with nitrogen ligands ($\text{Os}^{\text{VIII}}, \text{L}$) can be used for DNA labeling regardless of the DNA length, in an average biochemical or biological laboratory without any special equipment. DNA- $\text{Os}^{\text{VIII}}, \text{L}$ adducts produce redox signals at mercury, amalgam, carbon and gold electrodes; in addition, electrocatalytic signals can be obtained at mercury and amalgam electrodes. Multiple labels can be easily introduced.



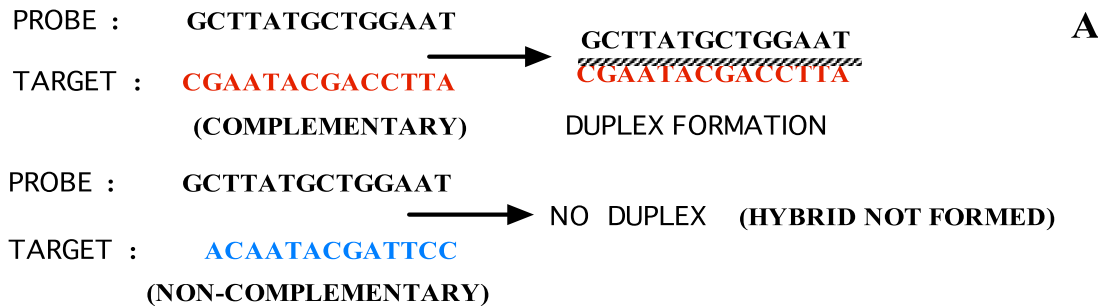
Trefulka, M., et al. (2007): Covalent labeling of nucleosides, RNA and DNA with VIII- and VI-valent osmium complexes. *Electroanalysis* 19 (No.12) 1281-1287.

With six-valent $\text{Os}(\text{VI})\text{L}$ ribose residue can be modified



Electrochemical sensors/detectors for DNA hybridization

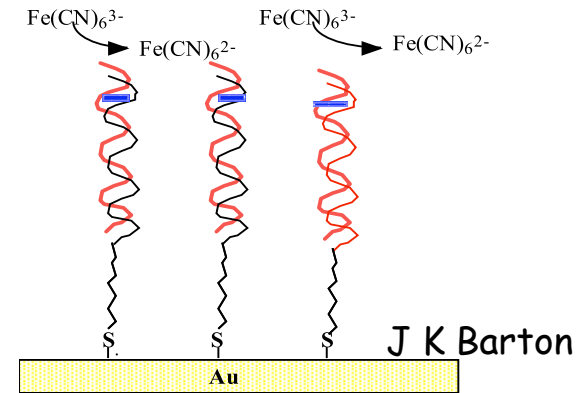
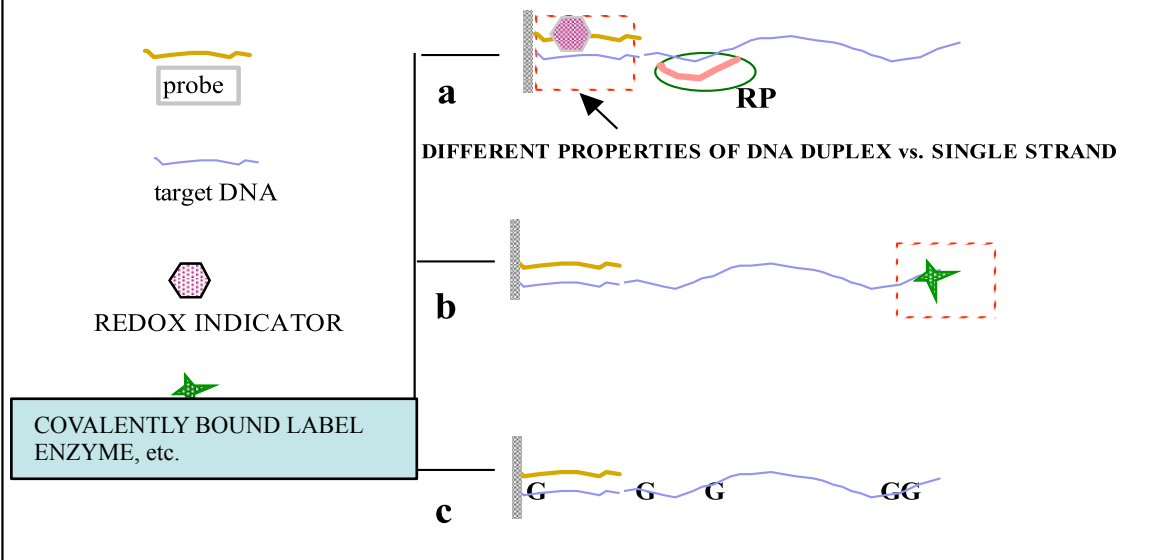
Single-Surface Technologies:



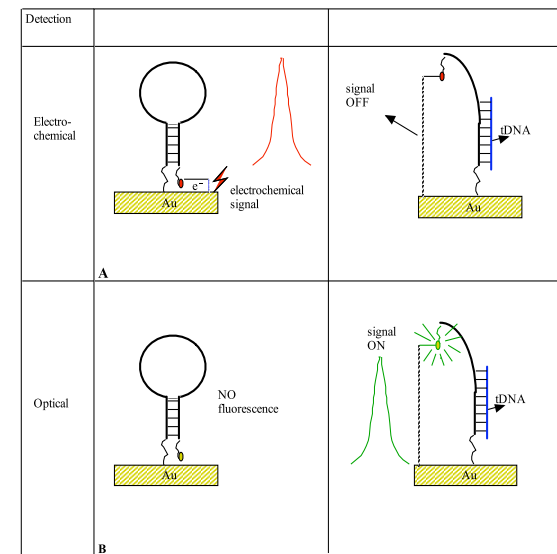
A

DNA HYBRIDIZATION AND ELECTROCHEMICAL DETECTION AT THE SAME SURFACE

B



Surface-attached molecular beacons



A Heeger

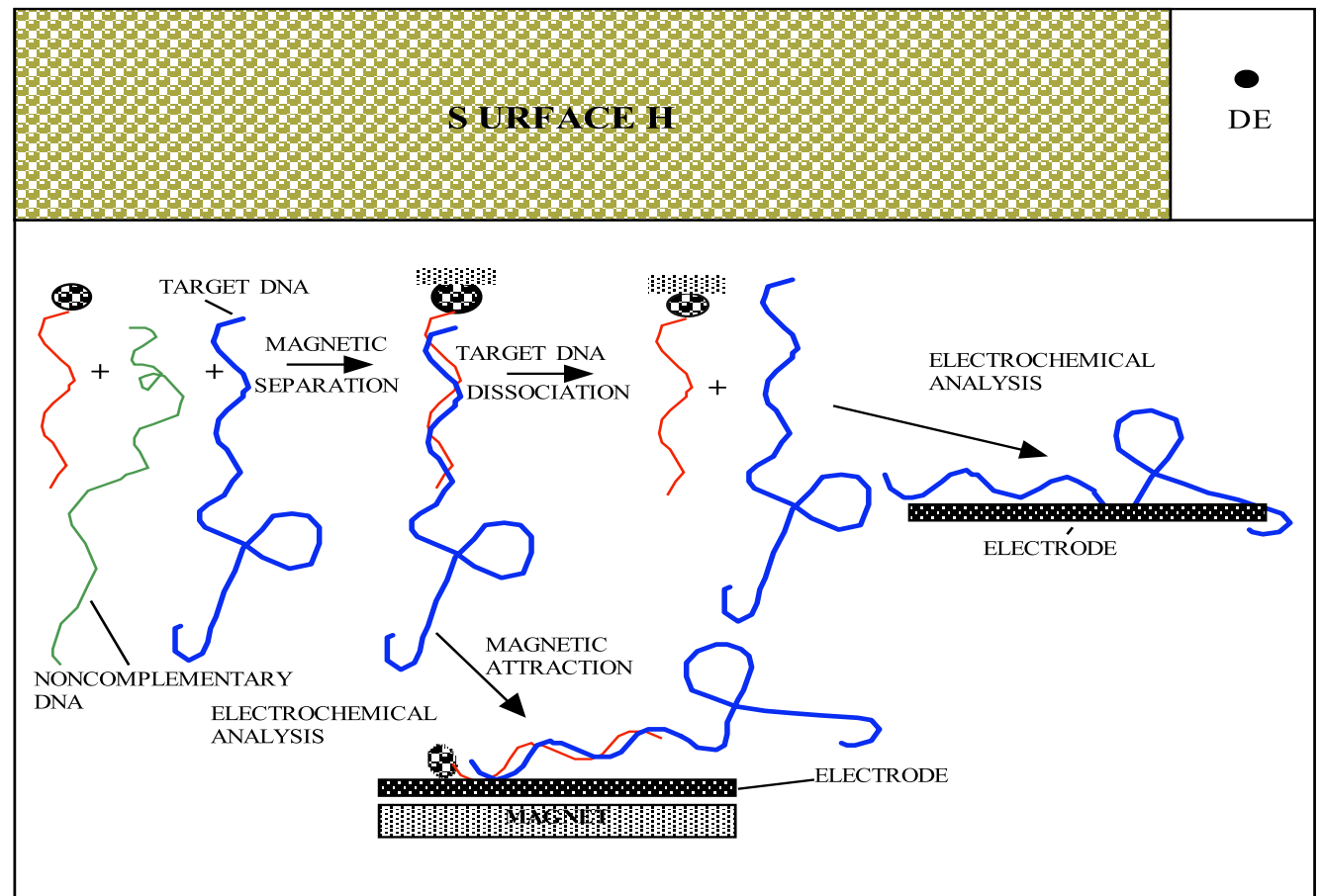
In the last decade nucleic acid electrochemistry was oriented predominantly to DNA sensors for (a) DNA hybridization and (b) DNA damage.

This trend has been accompanied not only by interesting discoveries but also by a number of poor papers lacking the necessary control experiments, claiming sequence detection without PCR amplification but using synthetic oligos as target DNA, etc.

Double-surface technique

Few years ago we proposed a new technique in which (in difference to previous techniques) DNA hybridization is separated from electrochemical detection. Optimum properties of the hybridization surface (H) and the detection electrode (DE) are not identical. We used magnetic beads optimized for hybridization as surface H and chose optimum DE for the given electrode process.

With single-surface techniques analysis of long DNA target molecules and in large excess of noncomplementary DNA may be difficult



With spherical magnetic beads non-specific binding of NAs is minimized. 20 microl of the bead suspension gives 3 to 7 cm² area. Beads can be incorporated into microfluidic systems and chips

Electrochemical sensors for DNA hybridization

At present both single- and double-surface techniques can be used for DNA sequencing of longer oligonucleotides and PCR products.

Electrochemical detection of point mutations is also possible.

Optimization of the procedures are now necessary to develop commercially successful devices.

Challenges:

1) Sequencing **eukaryotic** DNA without amplification (by PCR).
Great **sensitivity** and **specificity** of the analysis is required

2) Development of electrochemical sensors for **DNA-protein**
protein-protein interactions for proteomics and biomedicine

The results of the DNA electrochemistry studies and development of the **electrochemical DNA hybridization sensors** in the last decade suggest that these sensors **can complement DNA sensors with optical detection**

How and when the DNA electrochemistry begun?

How did it begin?

1955: Adenine is polarographically reducible at strongly acid pH while other NA bases are inactive. J.N.Davidson and E.Chargraff: The Nucleic Acids, Vol.1, Academic Press, New York 1955

1957: **NO response** of RNA and DNA on oscillograms

Brdicka's Co-solution (background electrolyte)

0.5 mg of RNA per ml of Brdicka's Co-solution

0.5 mg of RNA plus 0.5 mg of BSA per ml of Brdicka's Co-solution

278 H.Berg: Polarographische Untersuchungen



Abb. 1. Die drei Polarogramme P (die vertikale Achse) zeigen die Stromstärke in Mikroampere bei einer Stromstärke von 10⁻⁴ A. Die Kurven sind in der Reihenfolge: 1. RNA in Brdicka's Co-solution, 2. RNA + BSA in Brdicka's Co-solution, 3. RNA + BSA in Brdicka's Co-solution. Die Kurven sind in der Reihenfolge: 1. RNA in Brdicka's Co-solution, 2. RNA + BSA in Brdicka's Co-solution, 3. RNA + BSA in Brdicka's Co-solution.

H. BERG, Biochem. Z. 329 (1957) 274

1958: all bases, DNA and RNA are electroactive

Tab. 1

Stoff	Grundelektrolyt*)				
	H ₂ SO ₄	HCOOH	HCOONH ₄	KCl	NaOH
Adenin	+	+ (0,4 µg)	+	+	+
Guanin	+	+	+ (5 µg)	+	+
Cytosin	+	+	+	+	+
Thymin	—	—	—	—	+ (5 µg)
Uracil	—	—	—	—	+ (5 µg)
Adenylnsäure	+	+	+	+	+
Guanylsäure	+	+	+	+	+
Desoxythymidylsäure	+	+	+	+	+
DNS	—	—	+ (2 µg)	+ (15 µg)	+
RNS	—	—	+	+	+

*) + reagiert durch einen Einschnitt; — reagiert nicht; {} Stoffmenge auf 1 ml, bei der der Einschnitt noch bemerkbar ist.

Analyse der Nucleotidfraktionen angewandt werden, die aus der Ionenaustauschersäule durch Gradientenelution mit HCOOH und HCOONH₄ gewonnen wurden. Das Elutionsmittel dient hier als Grundelektrolyt.

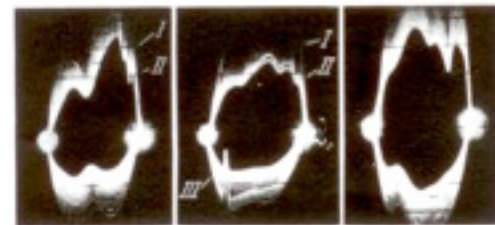
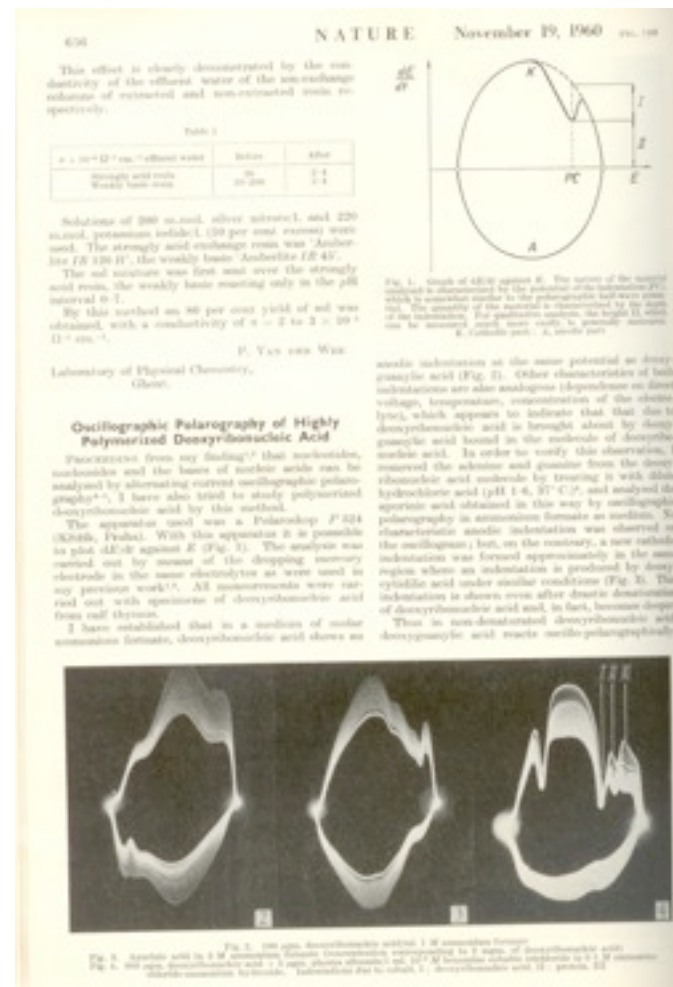


Fig. 2—4. Oscillogramme dI/dt = dI/F. Fig. 2. 8 µg Adenylnsäure (I) + 20 µg Cytosin (II) in 2 n H₂SO₄, Wechselstrom 0,3 mA, Empfindlichkeit 4. Fig. 3. 18 µg Adenylnsäure (I) + 60 µg Guanylsäure (I, III) in 2 n HCOONH₄, 0,15 n HCOOH, Wechselstrom 0,3 mA, Empfindlichkeit 4. Fig. 4. 200 µg DNS in 2 n KCl, Wechselstrom 0,4 mA, Empfindlichkeit 4.

E. PALECEK, Naturwiss.45 (1958) 186-187

1960: Native (ds) and denatured (ss) DNA yield different responses



E. PALECEK, Nature 188 (1960) 656-657

Science in Czechoslovakia after the IIInd World War

After **February 1948** life in Czechoslovakia was increasingly affected by the **stalinist ideology** and heavily controlled by the **Party and Government**.

Many **scientists and scholars** were **fired from Universities** but some of them got employment in the Institutes of the **Czechoslovak Academy of Sciences** established in **1952**. This was possible particularly at the Institutes whose Directors were influential Party members but serious scientists.

PRAHA/PRAGUE

Institute of Organic Chemistry and Biochemistry/

Director: F. Šorm

Chemistry and Biochemistry of **Proteins** and **Nucleic Acids**

B. Keil, B. Meloun, O. Mikes, J. Doskocil, D. Grunberger, A. Holy, I. Rychlík, J. Ríman, J. Sponar, V. Paces, Z. Sormová, S. Zadrazil



For many years **Czech scientists** were efficiently **isolated from the West**
In this respect the situation in Brno was much worse than in Prague

Institute of Biophysics, Brno

Director: F. Hercík

Founded in 1955 for radiobiological research it gradually turned into an institute devoted mainly to DNA

For a long time we received **50 - 100 US \$** for materials/chemicals **per year** and Department. The **orders** of materials from the West had to be **planned 1-2 years ahead**

Taking part in **meetings in western countries** was **difficult** not only because of currency problems



50 years of nucleic acid electrochemistry

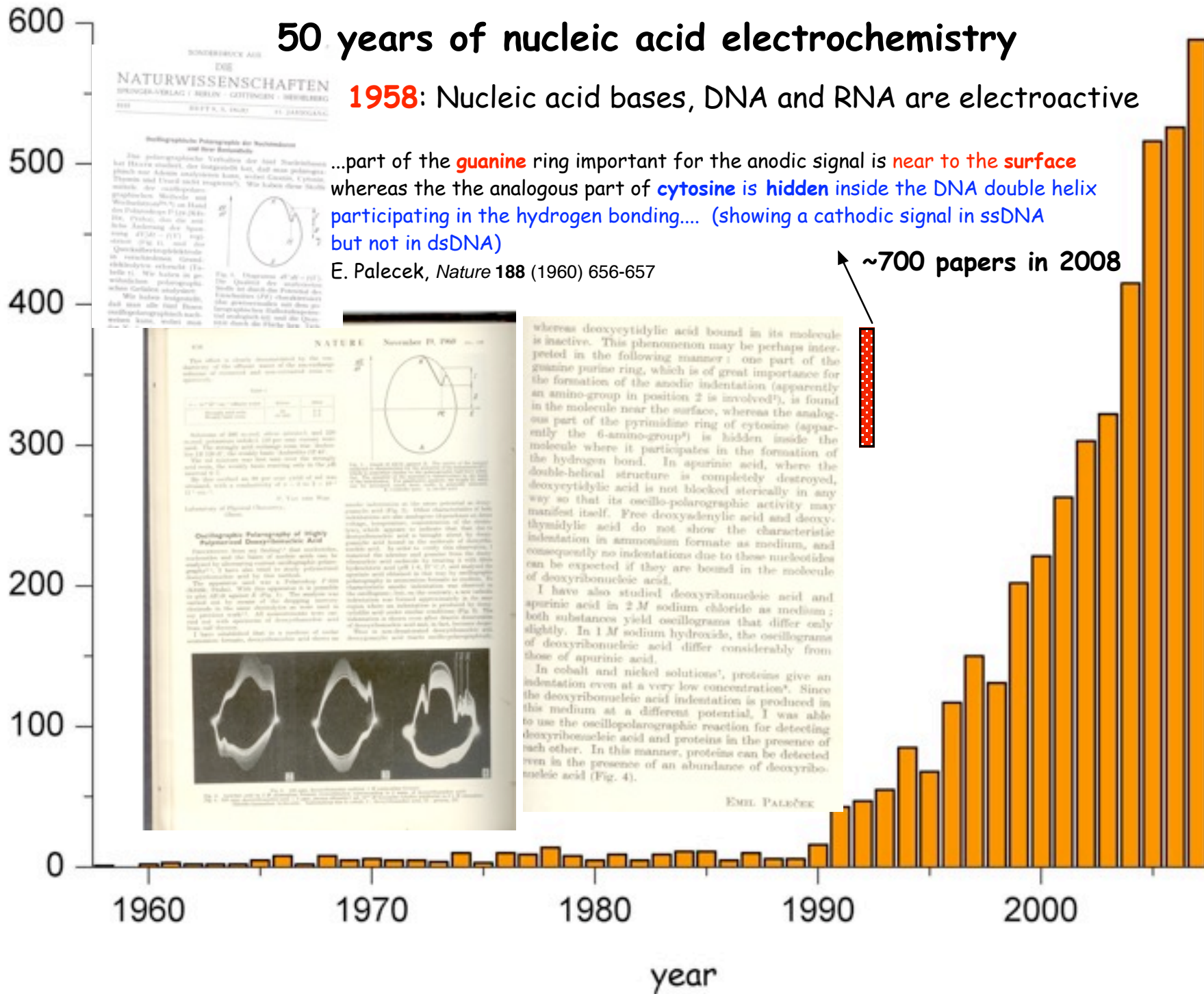
1958: Nucleic acid bases, DNA and RNA are electroactive

...part of the **guanine** ring important for the anodic signal is **near to the surface** whereas the the analogous part of **cytosine** is **hidden inside** the DNA double helix participating in the hydrogen bonding.... (showing a cathodic signal in ssDNA but not in dsDNA)

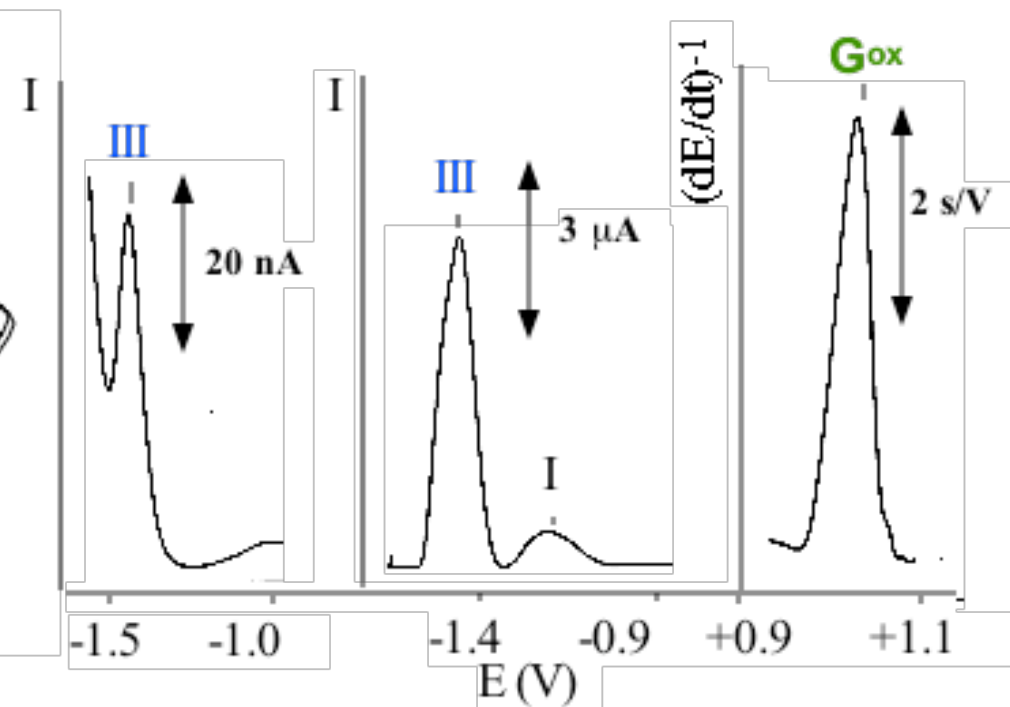
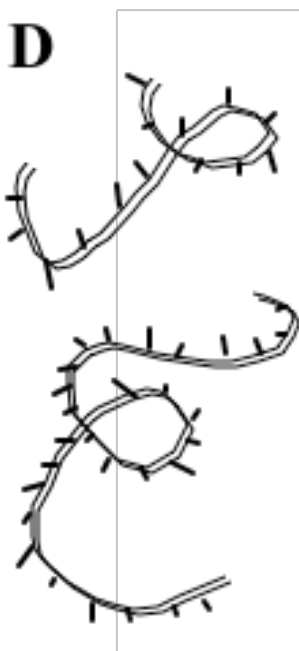
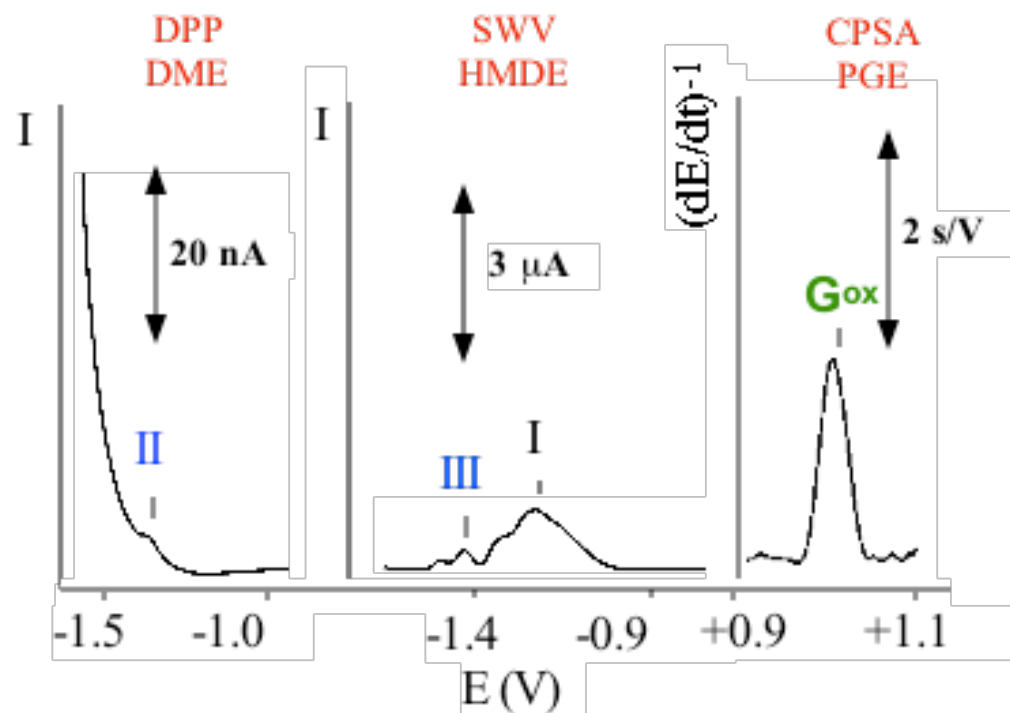
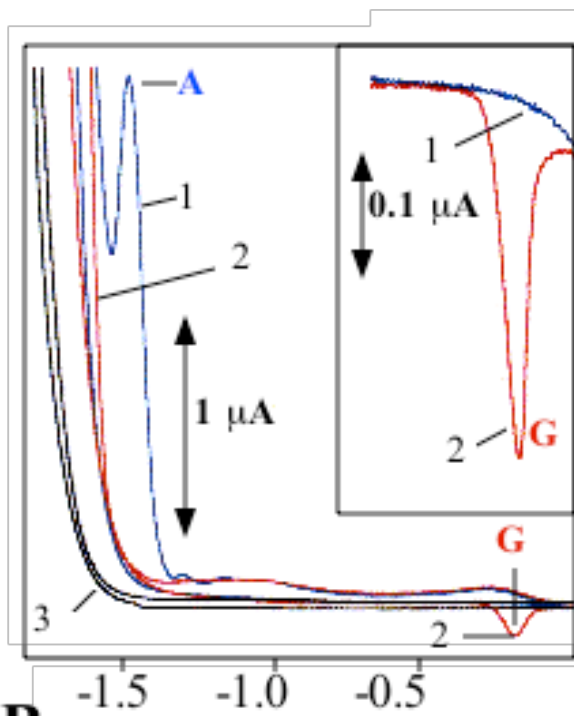
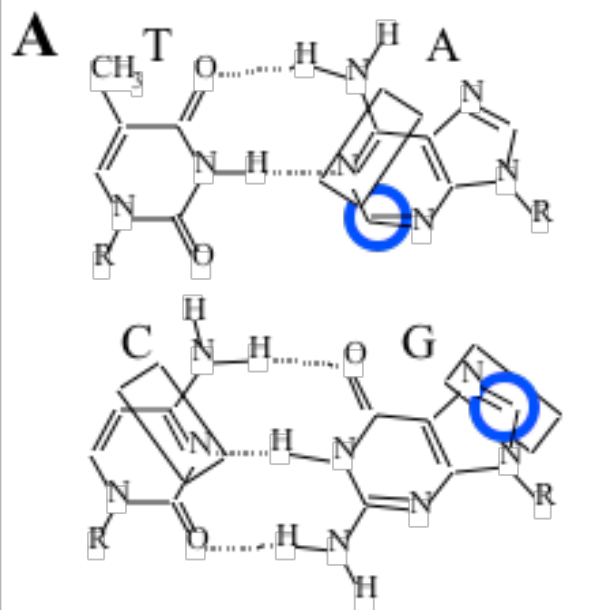
E. Palecek, *Nature* 188 (1960) 656-657

~700 papers in 2008

Number of publications

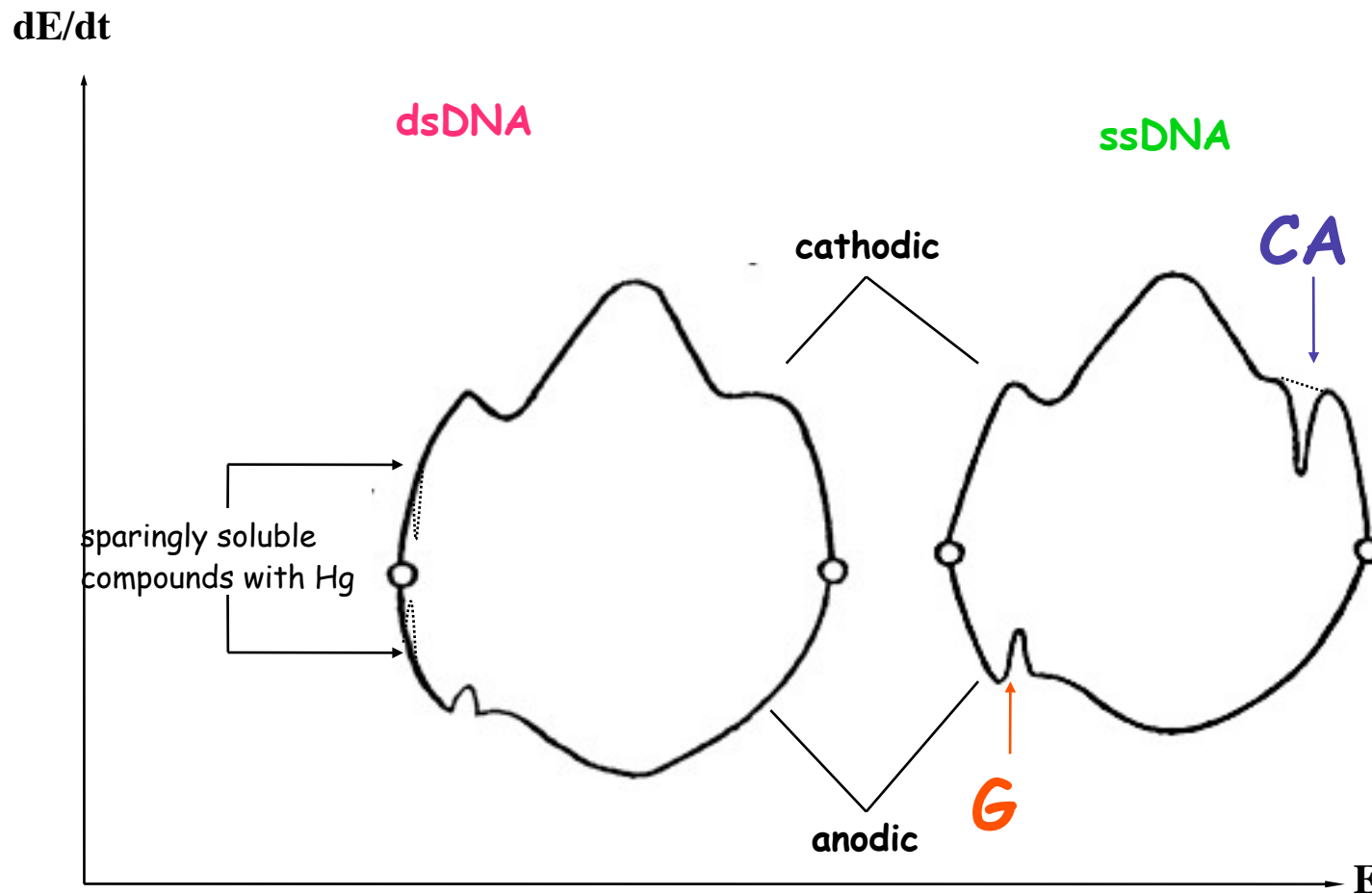


E. Palecek, Fifty years of nucleic acid electrochemistry, *Electroanalysis* 2009, 21, 239-251.



OSCILLOGRAPHIC POLAROGRAPHY

At controlled alternating current (constant current chronopotentiometry)



LITERATURE in 1958: **Adenine** is polarographically **reducible** at strongly acid pH while **other NA bases** as well as **DNA** are **inactive**

J.N.Davidson and E.Chargraff: *The Nucleic Acids*, Vol. 1, Academic Press, New York 1955

Palecek E.: *Oszillographische Polarographie der Nucleinsauren und ihrer Bestandteile*; *Naturwiss.* 45 (1958), 186

Palecek E.: *Oscillographic polarography of highly polymerized deoxyribonucleic acid*; *Nature* 188 (1960), 656

J. Heyrovsky invented **POLAROGRAPHY** in 1922.
After 37 years he was awarded a Nobel Prize

In difference to most of the electrochemists I met in the 1960's and 1970's, **J Heyrovsky was interested** in nucleic acids and he greatly stimulated my polarographic studies of DNA

J Heyrovsky S Ochoa A Kornberg

Nobel Prizes 1959



J. Heyrovsky



D.c. polarography vs. oscillopolarography (OP)

Why d.c. polarography was rather poor in DNA analysis?

- (a) no DNA accumulation at the electrode
- (b) DNA adsorption at negatively charged DME (~-1.4V) compared to open current potential in OP

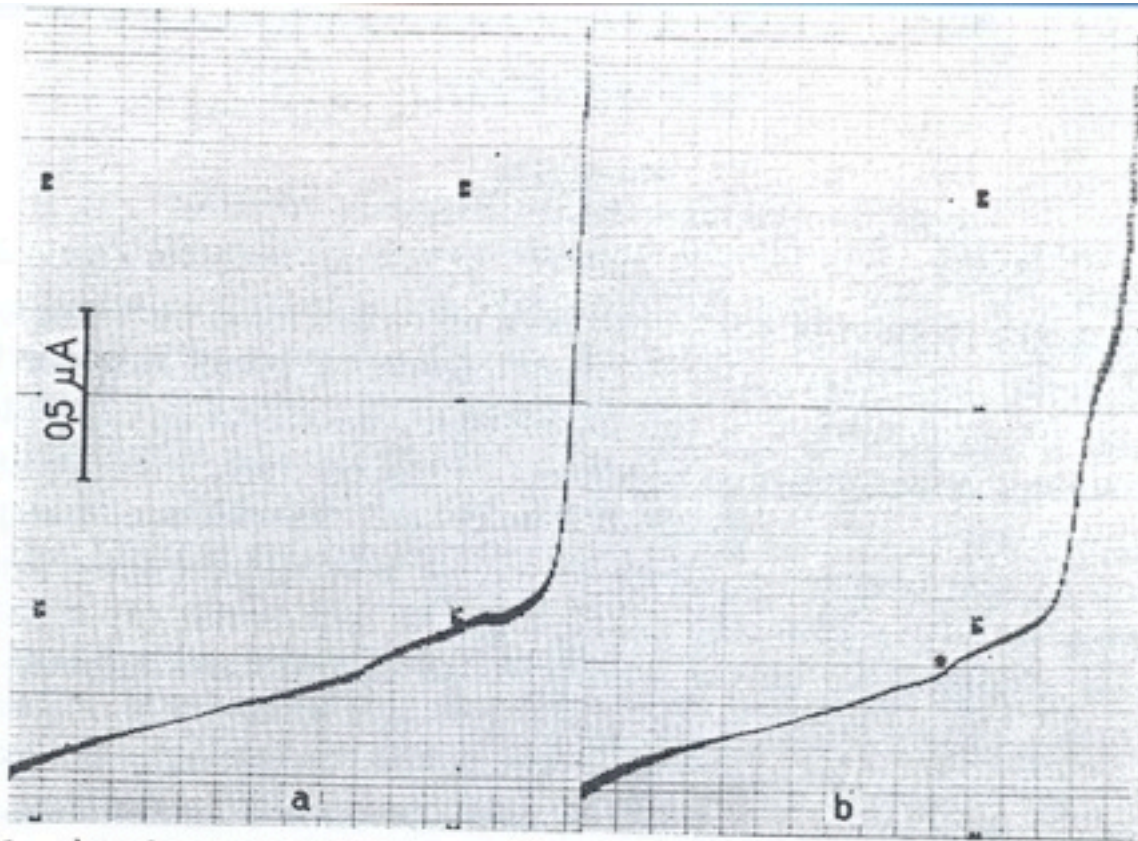


Fig. 1. dc polarograms of native and denatured calf thymus DNA: (a) native DNA at a concentration of 500 $\mu\text{g}/\text{ml}$ in 0.5M ammonium formate with 0.1M sodium phosphate (pH 7.0); (b) denatured DNA at a concentration of 500 $\mu\text{g}/\text{ml}$ in 0.5M ammonium formate with 0.1M sodium phosphate (pH 7.0). DNA was denatured by heat at the concentration of 666 $\mu\text{g}/\text{ml}$ in 0.007M NaCl with 0.7 mM citrate. Both curves start at 0.0 V, 100 mV/scale unit, capillary I, saturated calomel electrode.

In 1960 when I published my NATURE paper on electrochemistry of DNA I obtained invitations from 3 eminent US scientists:

J. Marmur - Harvard Univ.

L. Grossman - Brandeis Univ.

J. Fresco - Princeton Univ.

To work in their laboratories as a postdoc

In 1960 new techniques were sought to study DNA Denaturation and Renaturation. To those working with DNA Oscillographic Polarography (OP) appeared as a very attractive tool. Invented by J. Heyrovsky, it was fast and simple, showing large differences between the signals of native and denatured DNA. The instrument for OP was produced only in Czechoslovakia.

I accepted the invitation by Julius Marmur but for more than two years I was not allowed to leave Czechoslovakia.

In the meantime JM moved from Harvard to Brandeis Univ.

By the end of November 1962 I finally got my exit visa and with Heyrovsky Letter of Recommendation in my pocket I went to the plane just 24 hours before expiration of my US visa. Before my departure I sent my OP instrument by air to Boston. It arrived after 9 months completely broken. Instead of OP I had to use ultracentrifuges and microbiological methods.

Julius Marmur discovered DNA Renaturation/Hybridization and proposed (in JMB) a new method of DNA isolation which was widely applied. His paper was quoted > 9000x.



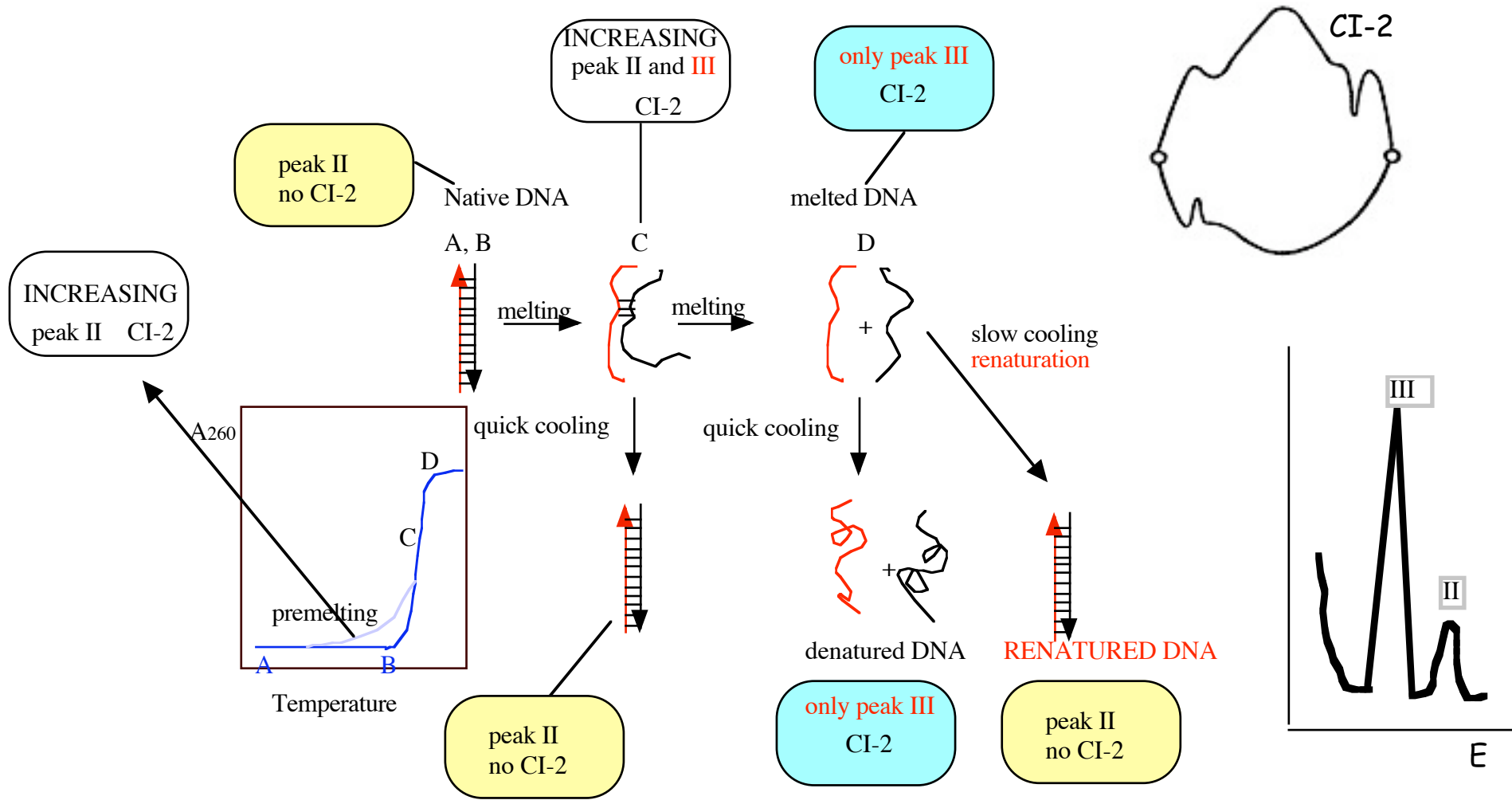
J M at the 40th Anniversary of the Discovery of the DNA Double Helix

At the end of my stay at Brandeis I did some OP experiments which I finished in Brno and published in J. Mol. Biol. in 1965 and 1966.

Reprinted from COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY
Volume XXVIII, 1963
Printed in U.S.A.

Specificity of the Complementary RNA Formed by
Bacillus subtilis Infected with Bacteriophage SP8

J. MARMUR*, C. M. GREENSPAN, E. PALECKE, F. M. KAHAN†, J. LEVINE, and M. MANDEL‡
Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts



DNA Premelting and Polymorphism of the DNA Double Helix

Before my departure to the US I observed **Changes in the polarographic behavior of DNA far below the denaturation temperature. These changes were later called DNA Premelting**

J. Mol. Biol.
20 (1966) 263-281

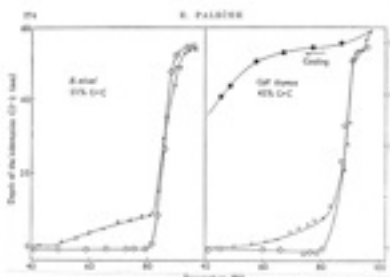


Fig. 11. Thermal transition of DNA's with varying guanine plus cytosine content followed by ultraviolet and optical rotatory methods. DNA was a concentration of 0.4 mg/ml in 0.05 M sodium borate plus 0.01 M sodium phosphate pH 7.0. The rate of cooling was 1 to 2°C per min. (Reprinted with permission from J. Mol. Biol. 20, 263-281, 1966, Academic Press, Inc., New York, N.Y.)

POLAROGRAPHIC BEHAVIOR OF dsDNA
At room and premelting temperature **depended on DNA nucleotide SEQUENCE**

What the people said

Before 1980

No doubt that this **electrochemistry must produce artifacts** because we know well that the **DNA double helix has a unique structure INDEPENDENT** of the nucleotide **SEQUENCE**

B. subtilis and B. brevis DNAs have the same G+C content and different nucleotide sequence

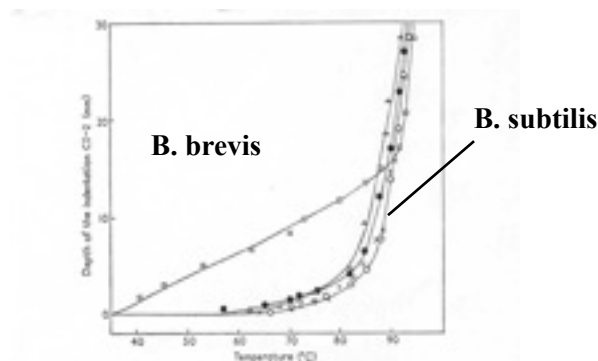
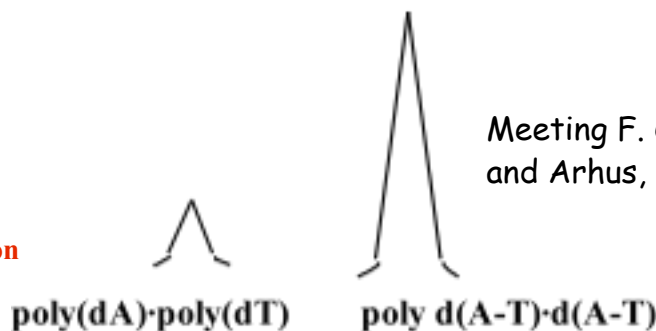


Fig. 13. Thermal transition of DNAs isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 250 μ g/ml in 0.05 M sodium borate plus 0.01 M sodium phosphate (pH 7.4). —●—●—, *B. subtilis* 168; —○—○—, *B. subtilis*; —□—□—, *B. subtilis* var. *spiz*; —△—△—, *B. subtilis* var. *sterilis*; —○—○—, *B. brevis* (ATCC 25695). F. H. J. Polakowski, dropping mercury electrode, polarized with repeated cycles of a.c. The measurements were carried out in the laboratory of Prof. J. Moras, Department of Biochemistry, Brunel University, Uxbridge, Middx., U.S.A.



Meeting F. Crick in Copenhagen and Arhus, 1977 (B. Clark)

1976

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AND MOLECULAR BIOLOGY, VOL. 73
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ACADEMIC PRESS, INC.
New York San Francisco London

Premelting Changes in DNA Conformation

E. PALECEK

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. **That 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.**

December 3, 1974

Professor Emil Palecek
Institute of Biophysics
Czechoslovak Academy of Sciences
Brno 12, Kralovopolska 135
Czechoslovakia

Dear Professor Palecek,

I do apologize for taking so long to reply to your letter of September 19 and the very interesting review you sent with it. Unfortunately I myself will not be able to attend the Symposium you plan for September, 1977 and my Cambridge colleague Aaron Klug tells me that he too is unable to be present. Had you considered the possibility of asking Dr. Hank Sobell? He has just published in PNAS an account of the other (base-paired) kink and has ideas about premelting conformations. I have no idea whether he would be able to come but should you wish to invite him his address is: Department of Chemistry, The University of Rochester, River Station, Rochester, New York 14627.

Yours sincerely,

Francis Crick

F. H. C. Crick
Felschl Foundation Visiting Professor

PHOC:16

RENATURATION OF RNA AS DETECTED BY DPP Time dependence

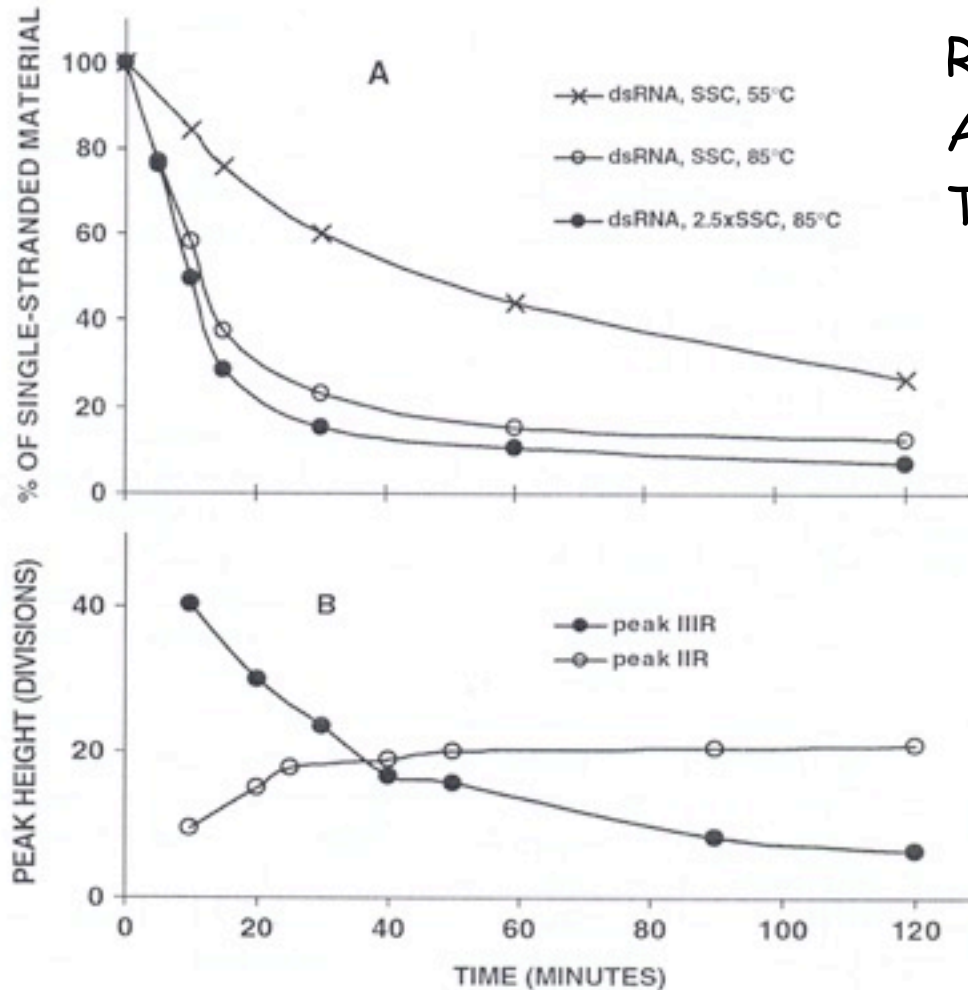


Fig. 10. Time-course of renaturation of phage f2 dsRNA. (A) Thermally denatured ssRNA was incubated (●—●) at 85°C in 2.5 × sodium saline citrate (SSC) or (o—o) at 85°C in SSC, and (x—x) at 55°C. Samples were withdrawn in time intervals given in the graph and quickly cooled. DPP measurements were performed at room temperature at a RNA concentration of 3.2 μg/mL in 0.3 M ammonium formate with 0.2 M sodium acetate, pH 5.6; PAR 174. (B) (o—o) peak IIR. (●—●) peak IIIIR. ssRNA (108 μg/mL) in 0.01 × SSC was heated for 6 min at 100°C. Then it was placed into a thermostated polarographic vessel with the same volume of 0.6 M ammonium formate with 0.2 M sodium phosphate, pH 7, preheated to 58°C. The pulse polarograms were measured at 58°C in times given in the graph. Southern-Harwell A 3100, amplifier sensitivity 1/8. Adapted from Palecek and Duskocil (1974). Copyright 1974, with permission from Academic Press.

Firsts in Electrochemistry of Nucleic Acids during the initial three decades

1958 DNA and RNA and all free bases are electrotractive

1960-61 assignment of DNA electrochemical signals to bases, relation between the DNA structure and electrochemical responses

1961 adsorption (ac impedance) studies of DNA (IR Miller, Rehovot)

1962-66 DNA premelting, denaturation, renaturation/hybridization detected electrochemically, traces of single stranded DNA determined in native dsDNA. Nucleotide sequence affects dsDNA responses

1965 Association of bases at the electrode surface (V. Vetterl)

1966 application of pulse polarography to DNA studies

1967 detection of DNA damage

1967-68 Weak interactions of low m.w. compounds with DNA (P.J. Hilsson, M.J. Simons, Harrow, UK and H. Berg, Jena)

1974 DNA is unwound at the electrode surface under certain conditions (EP and H.W. Nürnberg, Jülich, independently)

1976 Evidence for polymorphy of the DNA double-helical structure

For two decades only mercury electrodes were used in NA electrochemistry

1978 Solid (carbon) electrodes introduced in nucleic acid research (V. Brabec and G. Dryhurst, Norman)

1980 Determination of bases at nanomolar concentrations by cathodic stripping

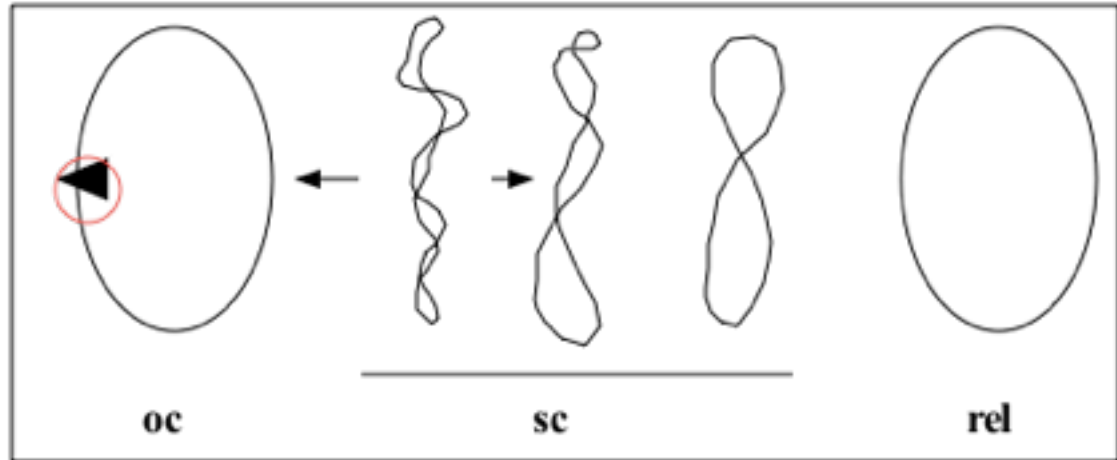
1981-83 Electroactive markers covalently bound to DNA

1986-88 DNA-modified electrodes

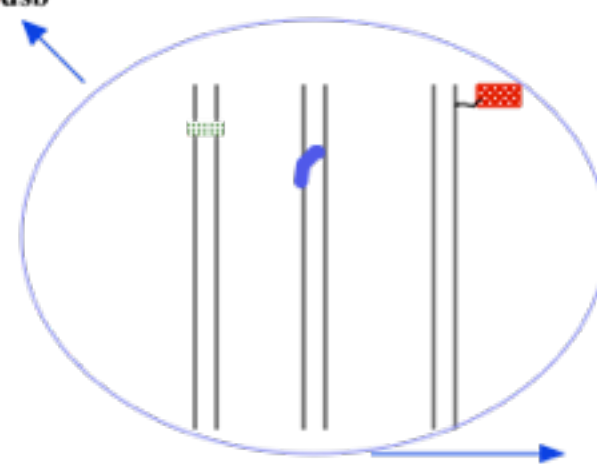
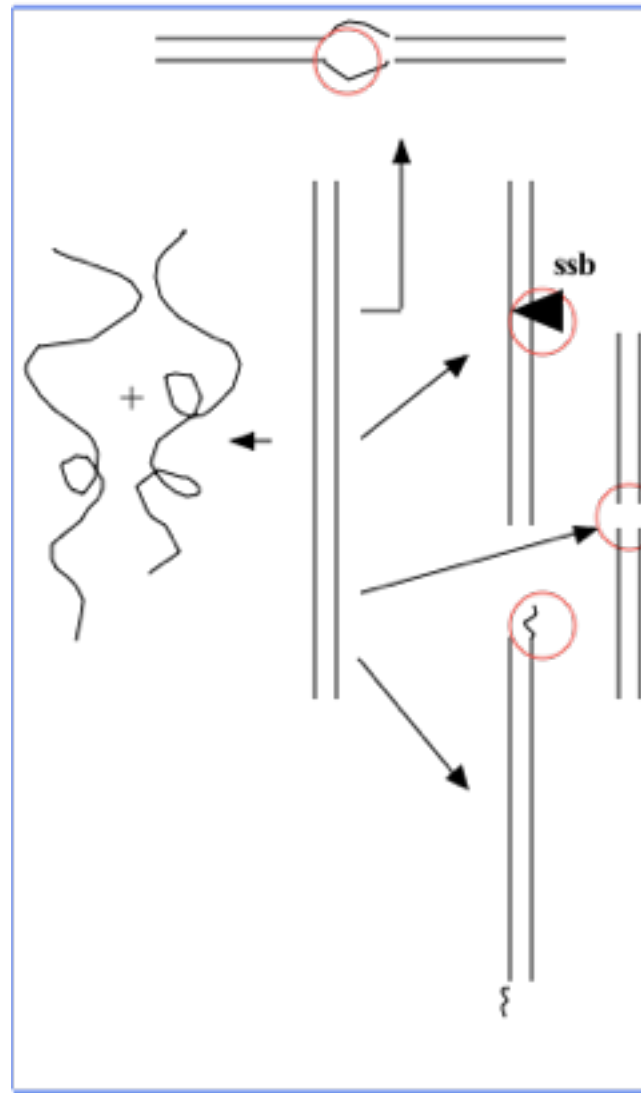
Results obtained at: IBP, Brno or elsewhere (author's name is given); the results which have been utilized in the DNA sensor development are in blue

ELECTROCHEMICAL METHODS RECOGNIZE SMALL CHANGES IN DNA STRUCTURE AND DETERMINE TRACES OF IMPURITIES IN DNA SAMPLES

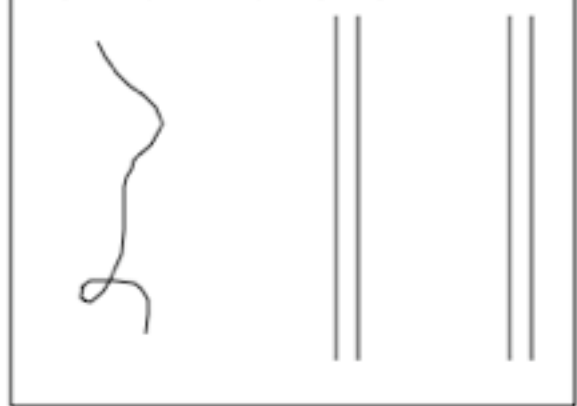
MERCURY ELECTRODES ARE PARTICULARLY SENSITIVE



DETERMINATION OF TRACES (< 1%) OF



CARBON ELECTRODES



DNA unwinding at negatively charged surfaces

native denatured

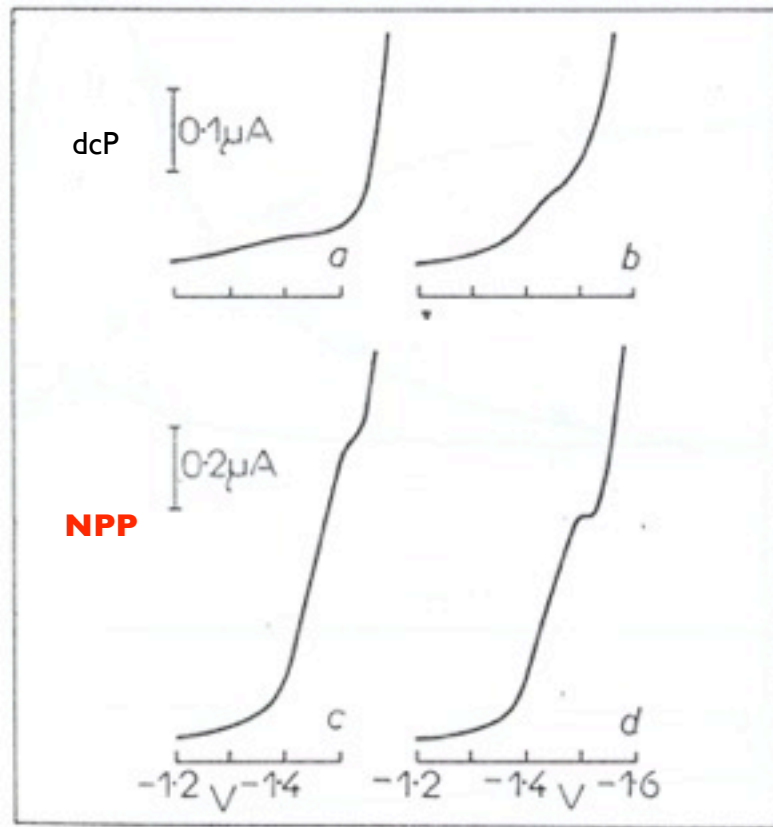


FIG. 1

Polarograms of Native and Denatured DNA

Upper curves: current-sampled d.c. polarography; lower curves: normal pulse polarography. *a*, *c* native DNA 500 μg/ml; *b*, *d* denatured DNA 50 μg/ml. 0.6M ammonium formate with 0.1M sodium phosphate pH 6.8. PAR 174.

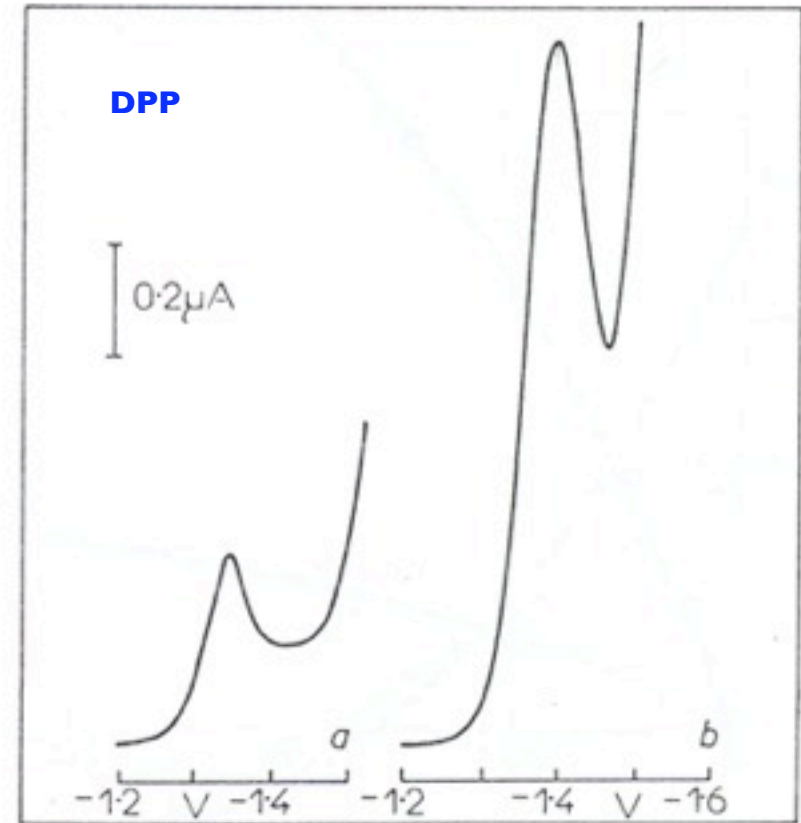
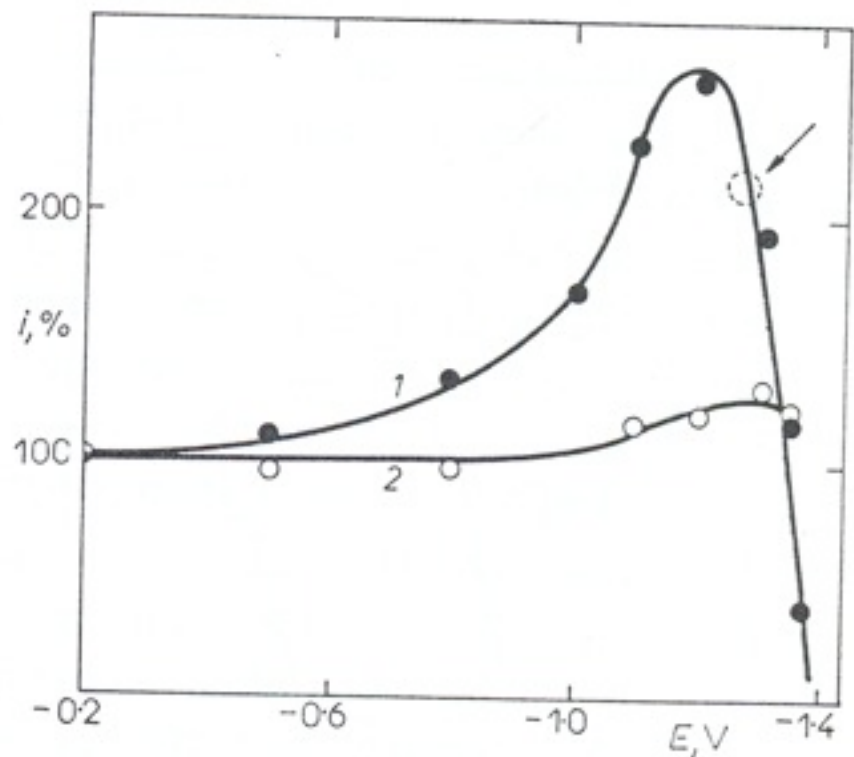


FIG. 2

Derivative Pulse Polarograms of Native and Denatured DNA

a Native DNA 500 μg/ml; *b* denatured DNA 50 μg/ml. Other conditions as in Fig. 1.

1974

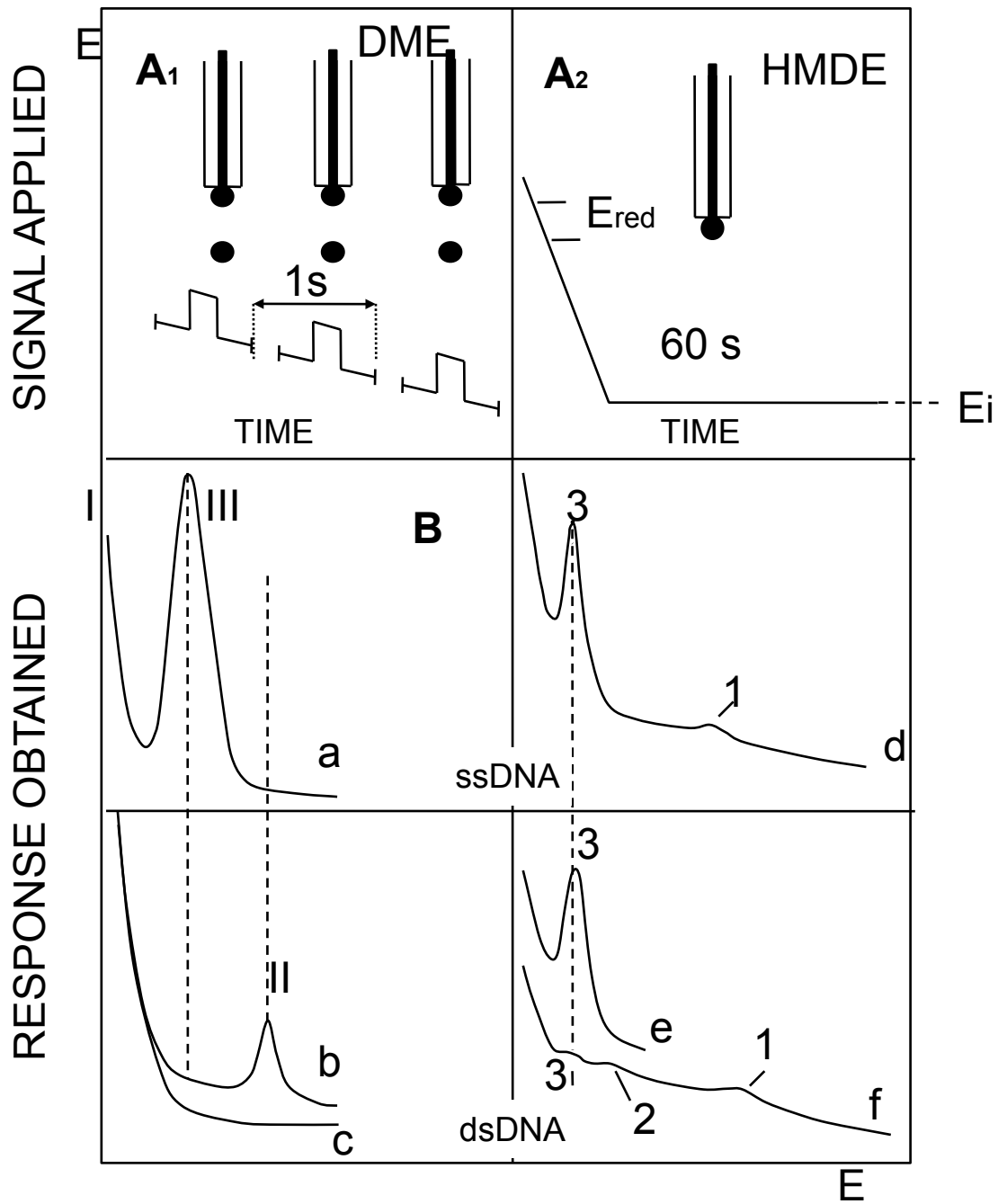


In native DNA its NPP responses depended on the initial potential, E_i

FIG. 4

Dependence of the Normal Pulse-Polarographic Wave Height of DNA on Starting Potential

1 Native DNA 500 $\mu\text{g}/\text{ml}$; 2 denatured DNA 50 $\mu\text{g}/\text{ml}$. The wave heights of native and denatured DNA at a starting potential of -0.2 V were taken as 100%. Scan range 1.5 V, other conditions as in Fig. 1.



Effect of pH on DNA unwinding

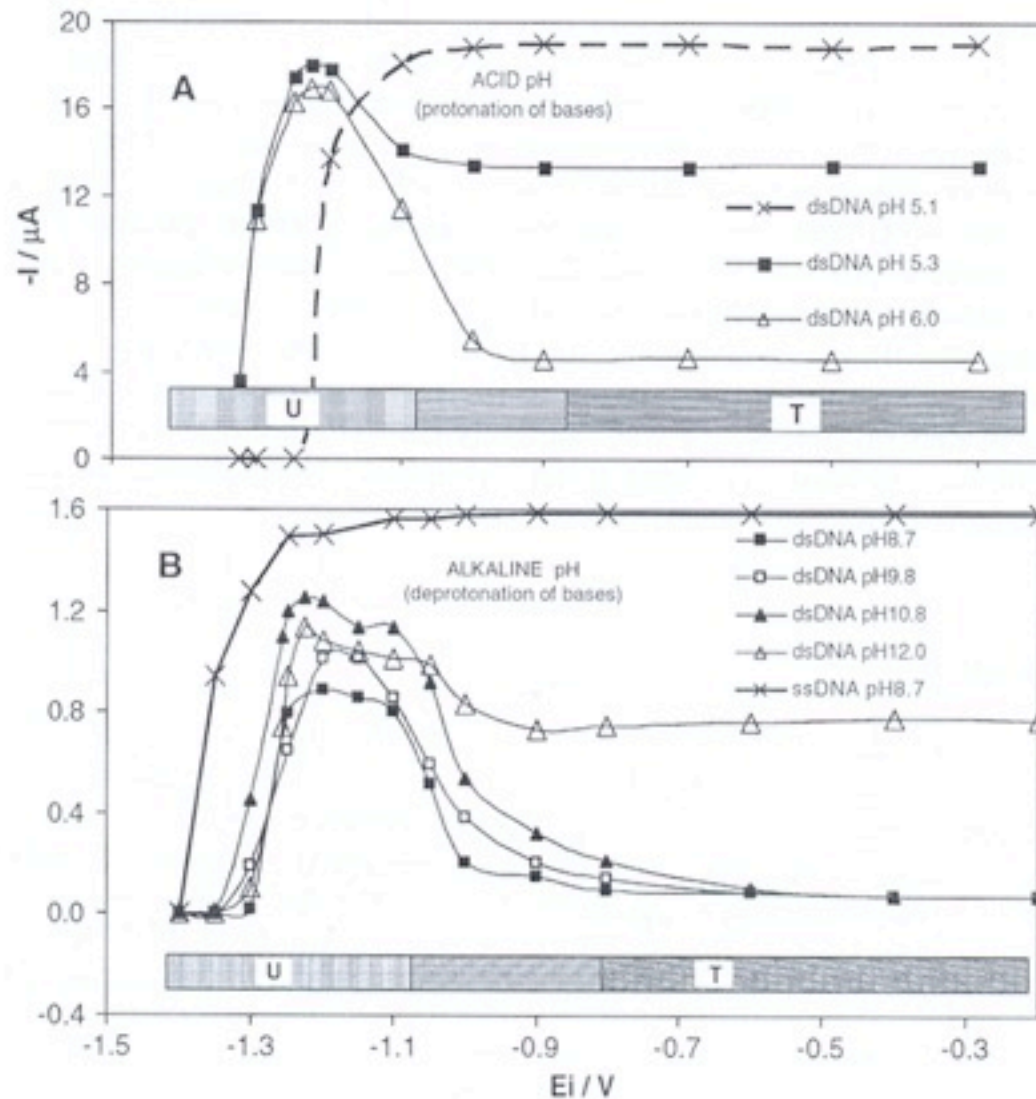


Fig. 17. Dependence of the height of the DNA voltammetric peak 3 on initial potential E_i ; (A) at acid pH's. dsDNA at concentration of $420 \mu\text{g/mL}$: $\Delta-\Delta$, pH 6.0; $\blacksquare-\blacksquare$, pH 5.3; $x-x$, pH 5.1. The graphical indication of the region T and U is valid only for the curve of dsDNA at pH 6.0. (B) at alkaline pH's. dsDNA: $\blacksquare-\blacksquare$, pH 8.7; $\square-\square$, pH 9.8; $\blacktriangle-\blacktriangle$, pH 10.8; $\triangle-\triangle$, pH 12.0. ssDNA: $x-x$, pH 8.7. PAR 174, DME, LSV, scan rate 5 V/s, waiting time 60 s. Potentials were measured against SCE. Adapted from Brabec and Palecek (1976b) and Palecek (1983). Copyright 1976, with permission from John Wiley and Sons Ltd.

Effect of nucleotide sequence on DNA unwinding

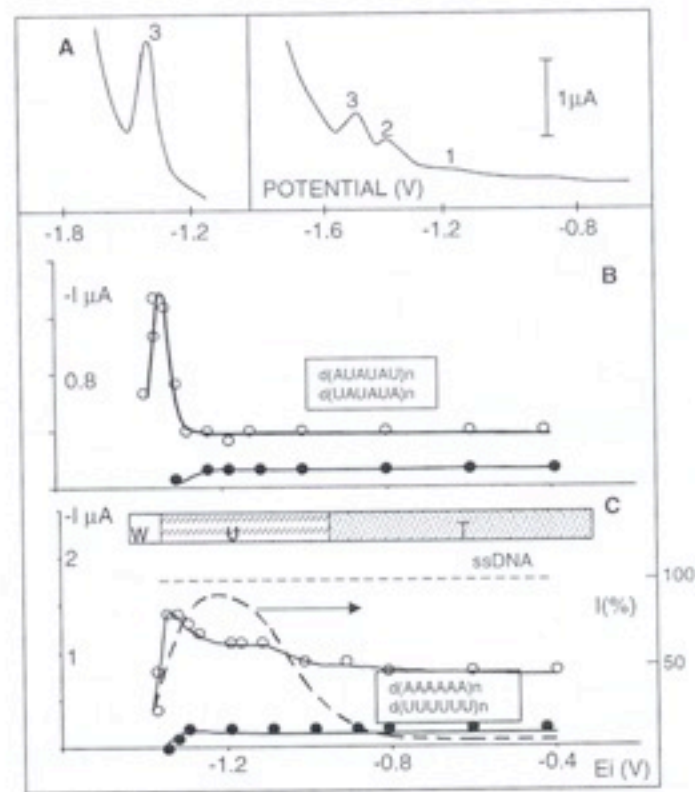
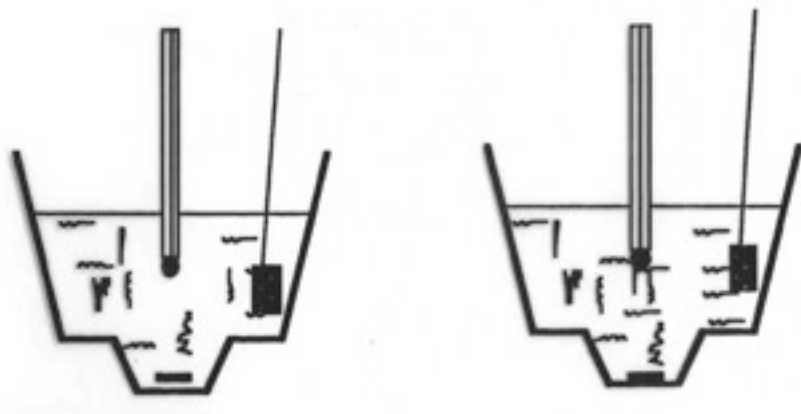


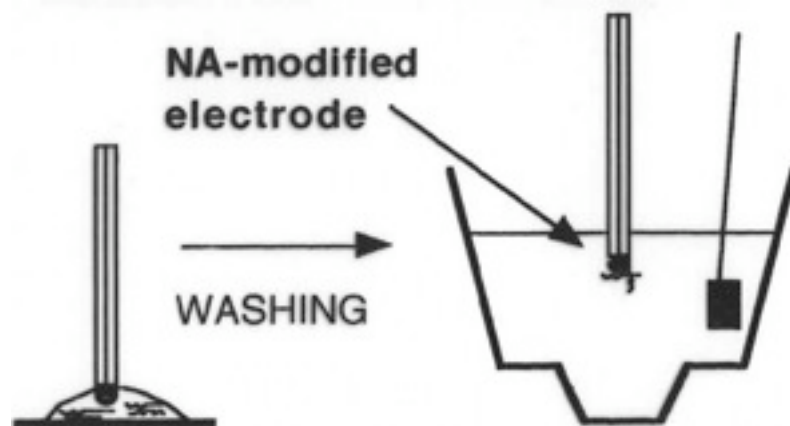
Fig. 16. Dependence of the voltammetric behavior of biosynthetic polynucleotides with different nucleotide sequences on the initial potential (E_i). (A): voltammetric peaks of poly (dA-dU) · poly (dA-dU). $E_i = -0.6$ V (left), $E_i = -1.35$ V (right); (B): ●—●, peak 2; ○—○, peak 3; (C): poly (rA) · poly (rU), ●—●, peak 2; ○—○, peak 3; ---, calf thymus DNA (data extracted from Palecek and Kwee (1979), peak height expressed in percents of the height of peak of thermally denatured DNA. DNA at a concentration of 100 μg/mL, concentration of other polynucleotides was 5×10^{-5} M (related to phosphorus content). Background electrolyte: 0.3 M ammonium formate with 0.05 M sodium phosphate (pH 6.9). HMDE, scan rate 0.5 V/s, waiting time 60 s. U is the potential region in which relatively slow opening of the DNA double helix occurs, involving an appreciable part of the molecule (provided the time of DNA interaction with the electrode is sufficiently long). T is the potential region where fast opening of the DNA double helix takes place; it is limited to several percents of the molecule in the vicinity of certain anomalies in the DNA primary structure (e.g. single-strand breaks). W is the potential region where no changes in the DNA conformation were detected. Potentials were measured against SCE. Reproduced from Jelen and Palecek (1985). Copyright 1985, with permission from the Slovak Academy of Sciences.

ADSORPTIVE STRIPPING



NA is in the electrolytic cell and accumulates at the electrode surface during waiting

ADSORPTIVE TRANSFER STRIPPING



NA is attached to the electrode from a small drop of solution (3-10 μl)

NA is at the electrode but the electrolytic cell contains only blank electrolyte

In 1986 we proposed **Adsorptive Transfer Stripping Voltammetry (AdTSV)** based on easy preparation of DNA-modified electrodes

AdTSV has many advantages over conventional voltammetry of NAs:

- 1) Volumes of the analyte can be reduced to few microliters
- 2) NAs can be immobilized at the electrode surface from media not suitable for the voltammetric analysis
- 3) Low m.w. compounds (interfering with conventional electrochemical analysis of NAs) can be washed away
- 4) Interactions of NAs immobilized at the surface with proteins and other substances in solution and influence of the surface charge on NA properties and interactions can be studied, etc.

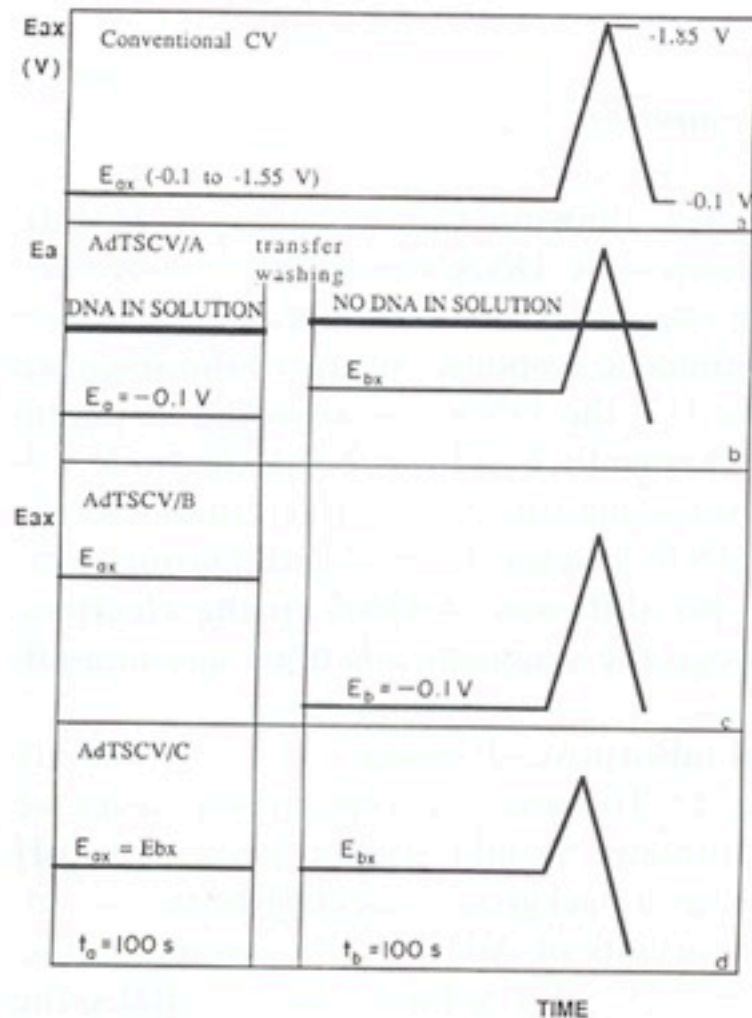


Fig. 1. Schematic diagram of HMDE polarization in (a) conventional (adsorptive stripping) CV and (b–d) variants A, B and C of AdTSCV. (b) AdTSCV variant A: the HMDE charged to a potential $E = -0.1$ V was immersed in a DNA solution for a time $t = 100$ s, the electrode was then washed and transferred to the background electrolyte (0.3 M ammonium formate with 50 mM sodium phosphate, pH 6.9 not containing DNA, medium 0). A potential E (varying in the range between -0.1 V and -1.55 V) was then applied to the HMDE for $t = 100$ s followed by a triangular voltage sweep in the cathodic direction from E to -1.85 V and back in the anodic direction to -0.1 V. (c) AdTSCV variant B: this variant differs from variant A in that DNA is adsorbed at potentials E (varying between -0.1 V and -1.55 V) and kept in medium 0 at $E = -0.1$ V. (d) AdTSCV variant C: in contrast to variant B both potentials E_{ax} and E_{bx} were variable but they were always the same in a given experiment. This variant thus resembles conventional CV (a) where the HMDE was kept for $t_a = 200$ s at the potential E_{ax} followed by CV measurements during which the electrode was immersed in the DNA solution.

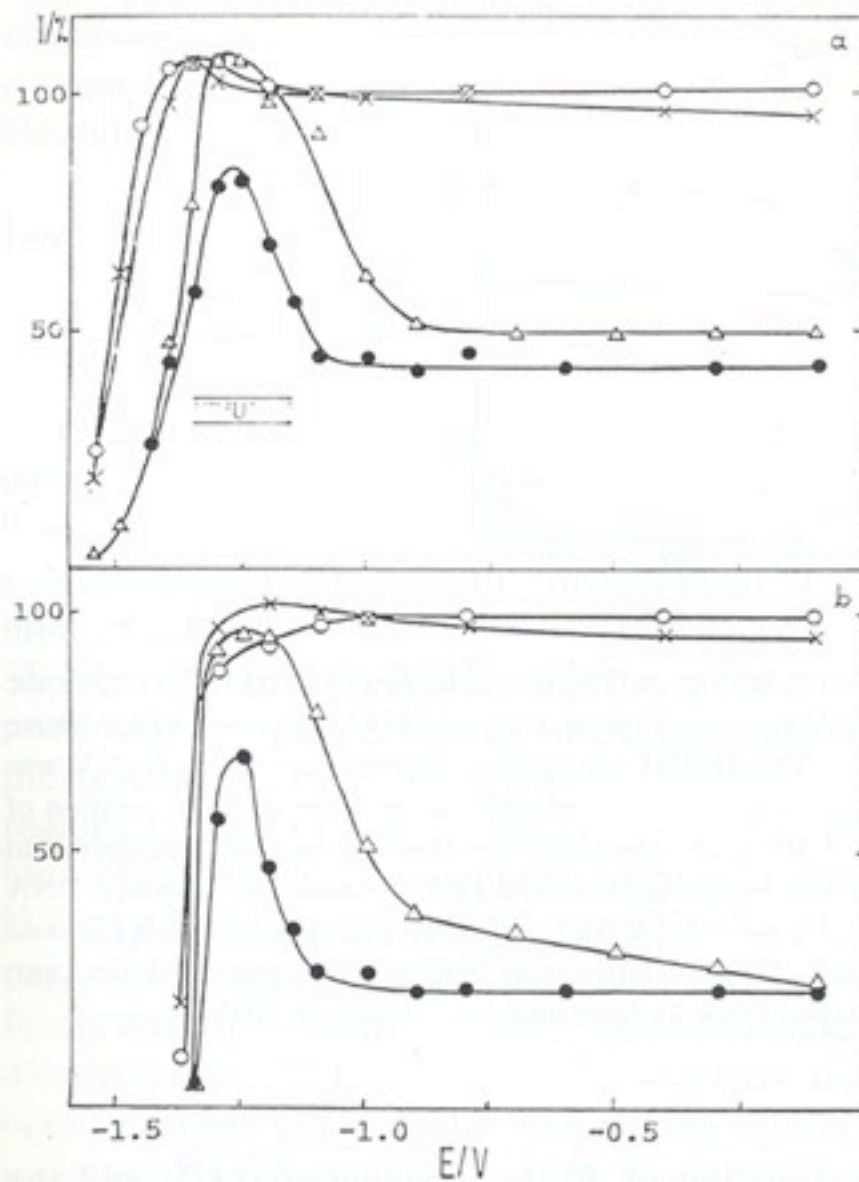
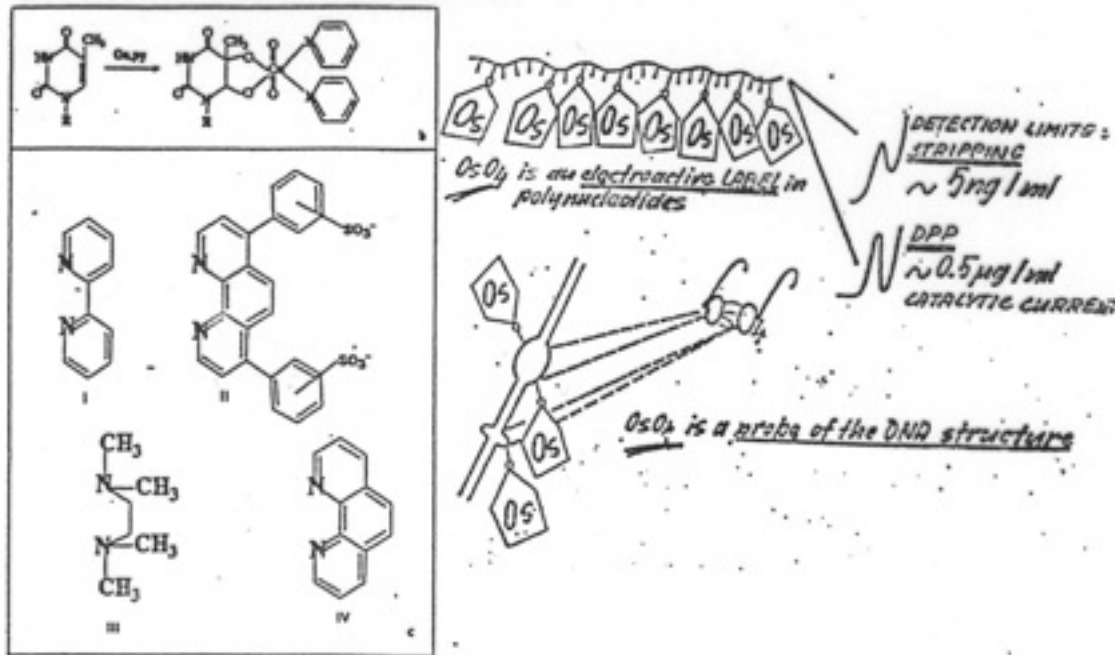
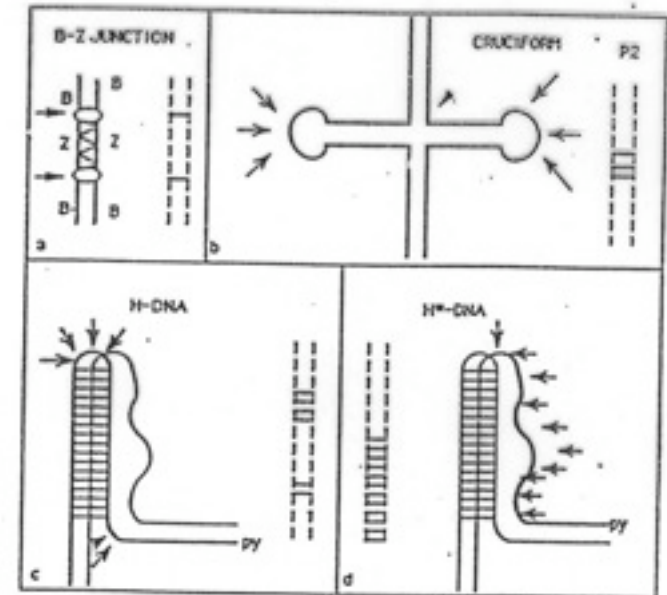


Fig. 5. The dependence of the relative peak heights of (a) the anodic peak G and (b) the cathodic peak AC of native (Δ — Δ , \bullet — \bullet) and denatured (\times — \times , \circ — \circ) DNA on the HMDE potential obtained by conventional CV (Δ — Δ , \circ — \circ) and by AdTSCV variant A (\bullet — \bullet , \times — \times) (for details see Figs. 1 and 2). The relative peak heights are expressed in per cent; the height of the peak of thermally denatured DNA obtained by conventional CV at $E_a = -0.1$ V was taken as 100%. Region U is shown for AdTSCV variant A.

Probing of DNA structure with osmium tetroxide complexes



We developed methods of **chemical probing of the DNA structure** based on osmium tetroxide complexes (Os_4L). Some of the Os_4L complexes react with single-stranded DNA but not with the double-stranded B-DNA.



In the beginning of the 1980's Os_4L complexes were the **first electroactive labels** covalently bound to DNA. These complexes produced catalytic signals at Hg electrodes allowing **determination of DNA at subnanomolar concentrations**

Critical Reviews in Biochemistry and Molecular Biology, 36(2):131-126 (1991)

Local Supercoil-Stabilized DNA Structures

E. Paleček

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[17] Probing of DNA Structure in Cells with Osmium Tetroxide-2,2'-Bipyridine

By EMIL PALEČEK

METHODS IN ENZYMOLOGY, VOL. 212

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These methods yielded information about the **distorted and single-stranded regions** in the DNA double helix **at single-nucleotide resolution**. DNA probed both **in vitro** and **directly in cells**.

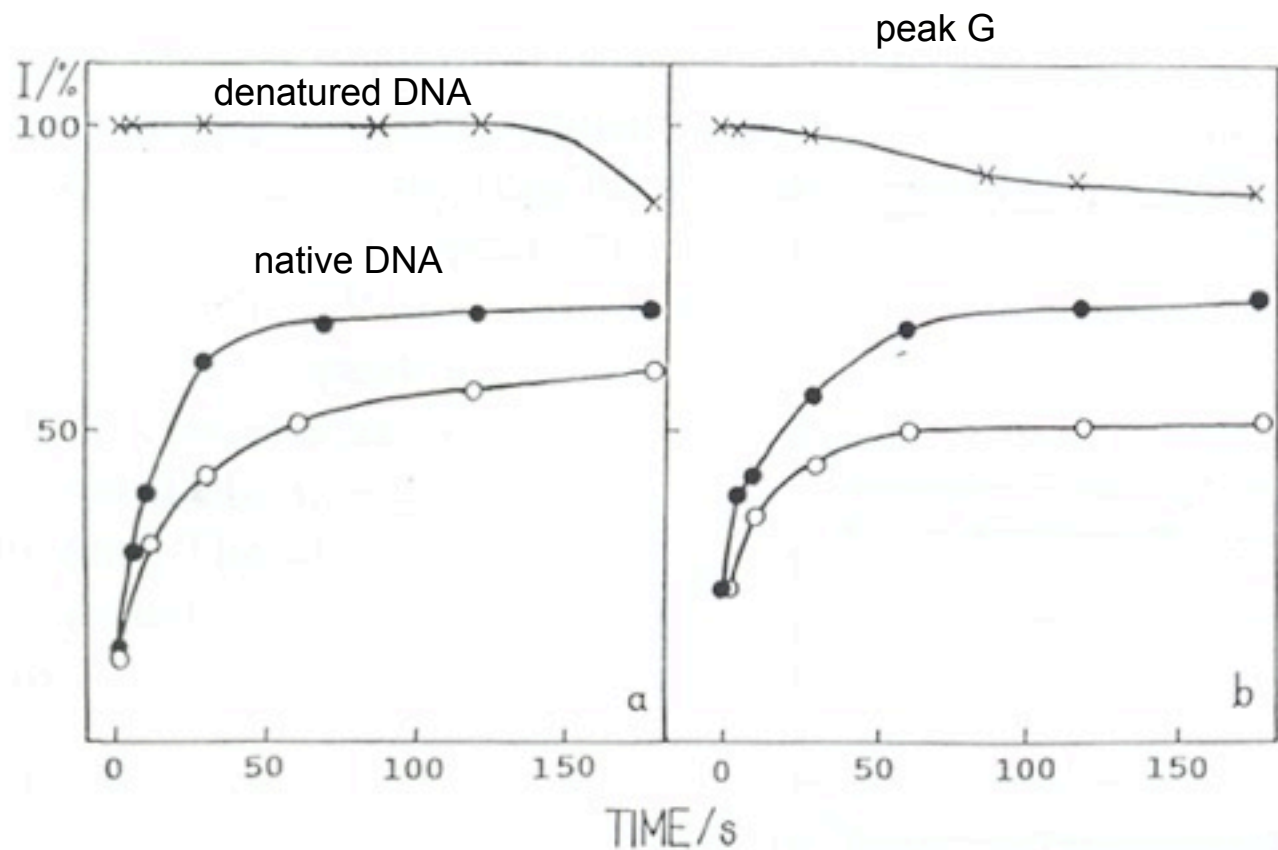


Fig. 6. The dependence of the relative heights of (a) the AdTSCV anodic peak G and (b) the cathodic peak AC on time t_b at potentials $E_b = -1.2$ V (\circ — \circ), and $E_b = -1.3$ V (\bullet — \bullet) for native DNA and for denatured DNA (\times — \times). The HMDE charged to a potential $E_a = -0.25$ V was immersed into the solution of native DNA (at a concentration of $292 \mu\text{g ml}^{-1}$) or into the solution of denatured DNA ($140 \mu\text{g ml}^{-1}$) for a time $t_b = 100$ s; the electrode was then washed and transferred to the background electrolyte not containing DNA. In this medium the HMDE (with the adsorbed DNA layer) was exposed to the potentials $E_b = -1.2$ V or -1.3 V for the time t_b given in the graph followed by CV measurement (for details see Figs. 1 and 2). The relative peak heights are expressed in per cent; the heights of peaks AC and G of the denatured DNA at zero time were taken as 100%.

Scheme 1

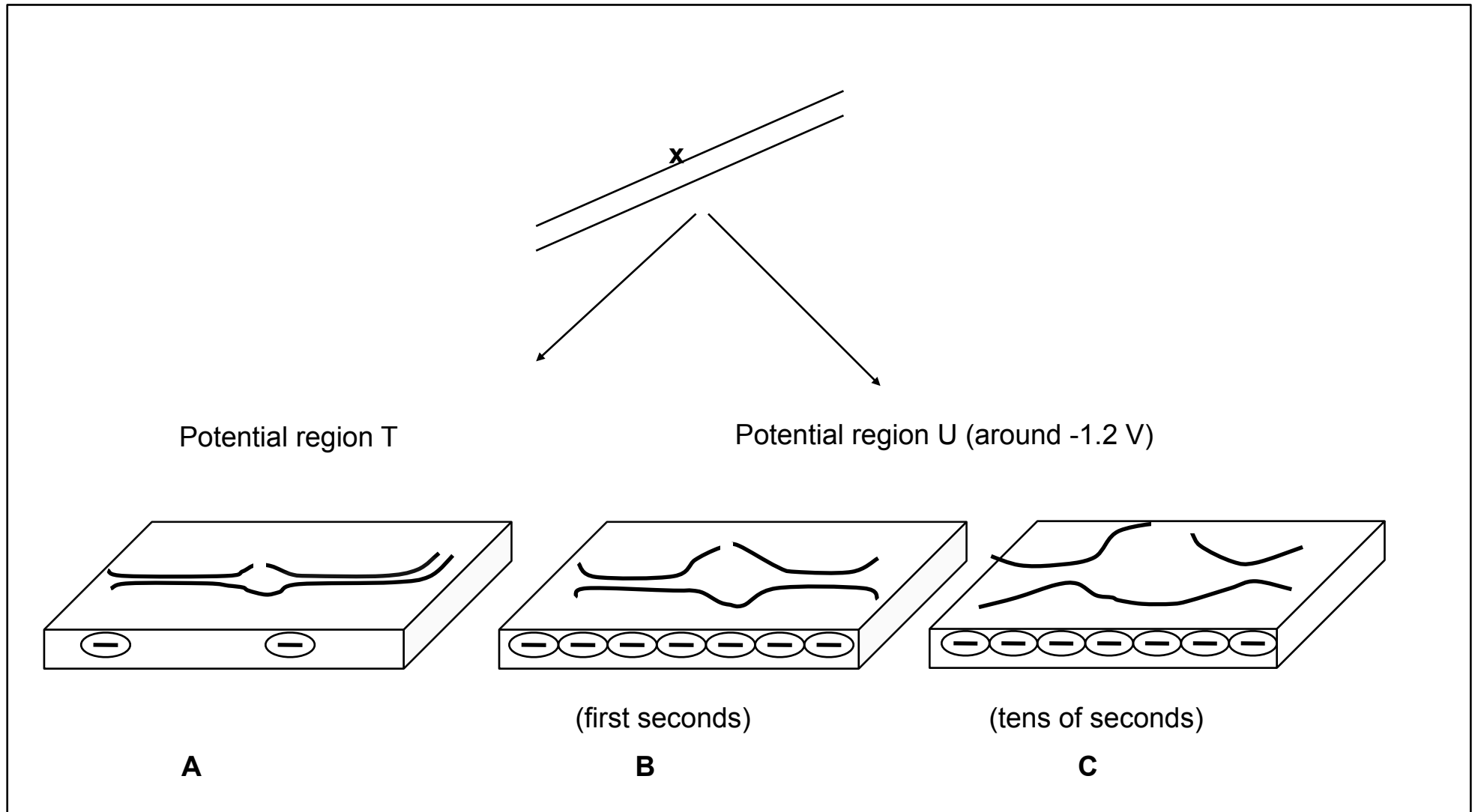
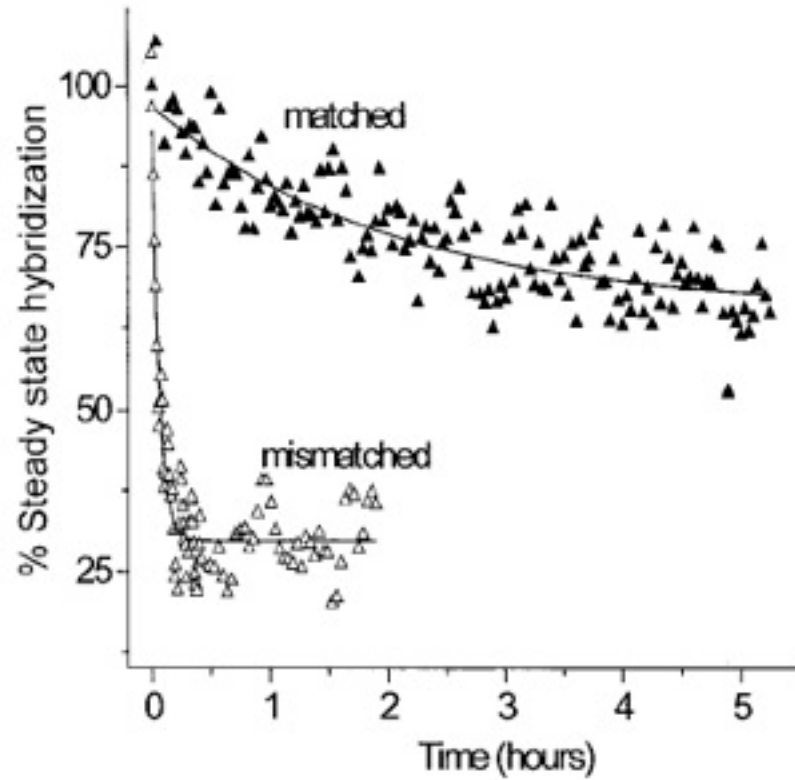


Figure 19

DNA unwinding at negatively charged Au surfaces was recently observed by R. Georgiadis et al. and applied in DNA sensors



Heaton RJ, Peterson AW, Georgiadis RM, PNAS 98 (2001) 3701

IFFY stories

On this day 50 years ago, Watson and Crick published their double-helix theory. **But, what if...**
By Steve Mirsky (2003)

"I am now astonished that I began work on the triple helix structure, rather than on the double helix," wrote [Linus Pauling](#) in the April 26, 1974 issue of Nature.

In February 1953, [Pauling proposed a triple helix structure](#) for DNA in the Proceedings of the National Academy of Sciences (PNAS). He had been working with [only a few blurry X-ray crystallographic images from the 1930s and one from 1947](#).

If history's helix had turned slightly differently, however, perhaps the following timeline might be more than mere musing...

August 15, 1952: [Linus Pauling](#) (finally allowed to travel to England by a US State Department that thinks the words "chemist" and "communist" are too close for comfort) [visits King's College London and sees Rosalind Franklin's X-ray crystallographs](#). He immediately [rules out a triple helical structure](#) for DNA and [concentrates on](#) determining the nature of what is undoubtedly a [double helix](#).

February 1953: [Pauling and Corey describes the DNA double helix structure in PNAS](#)

A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

BY LINUS PAULING AND ROBERT B. COREY

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,* CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated December 31, 1952

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CHEMISTRY: PAULING AND COREY

PROC. N. A. S.

which are involved in ester linkages. This distortion of the phosphate group from the regular tetrahedral configuration is not supported by direct experimental evidence; unfortunately no precise structure determinations have been made of any phosphate di-esters. The distortion, which corresponds to a larger amount of double bond character for the inner oxygen atoms than for the oxygen atoms involved in the ester linkages, is a reason-

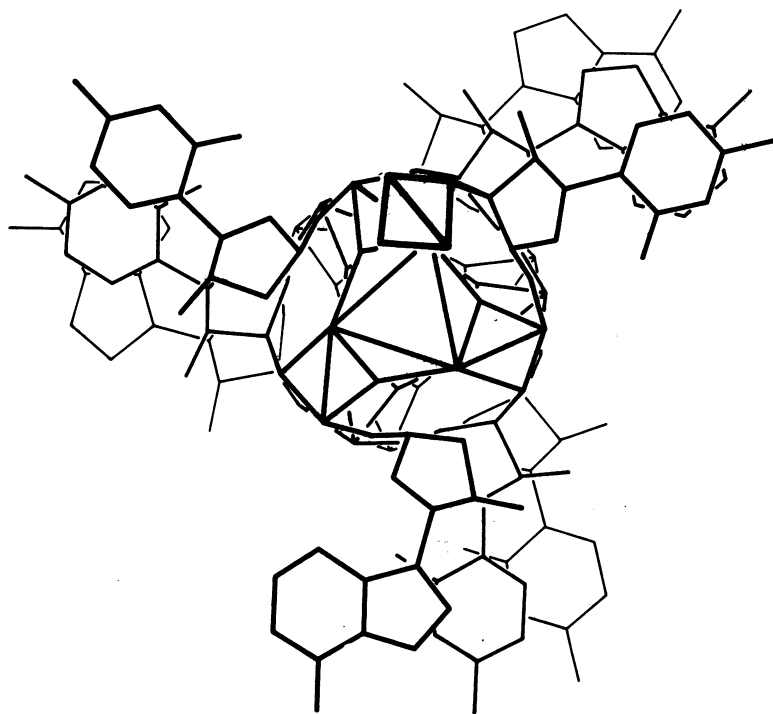


FIGURE 6

Plan of the nucleic acid structure, showing several nucleotide residues.

Triple helix

with bases on the outside and sugar-phosphate backbone in the interior of the molecule

My IFFY story:

If L. PAULING had in his lab an oscillopolarograph in 1952 he would never proposed this structure. Polarography clearly showed that bases must be hidden in the interior of native DNA molecule and become accessible when DNA is denatured

SUMMARY

Electroactivity of nucleic acids was discovered about 50 years ago. Reduction of bases at Hg electrodes is particularly sensitive to changes in DNA structure. The course of DNA and RNA denaturation and renaturation can be easily traced by electrochemical methods.

At present electrochemistry of nucleic acids is a booming field, particularly because it is expected that **sensors for DNA hybridization** and for **DNA damage** will become important tools in biomedicine and other regions of practical life in the 21st century.

DNA-modified electrodes can be easily prepared; microL volumes of DNA are sufficient for its analysis but miniaturization of electrodes decreases these volumes to nL. Sensitivity of the analysis has greatly increased in recent years.