3. přednáška EP 19.11.13

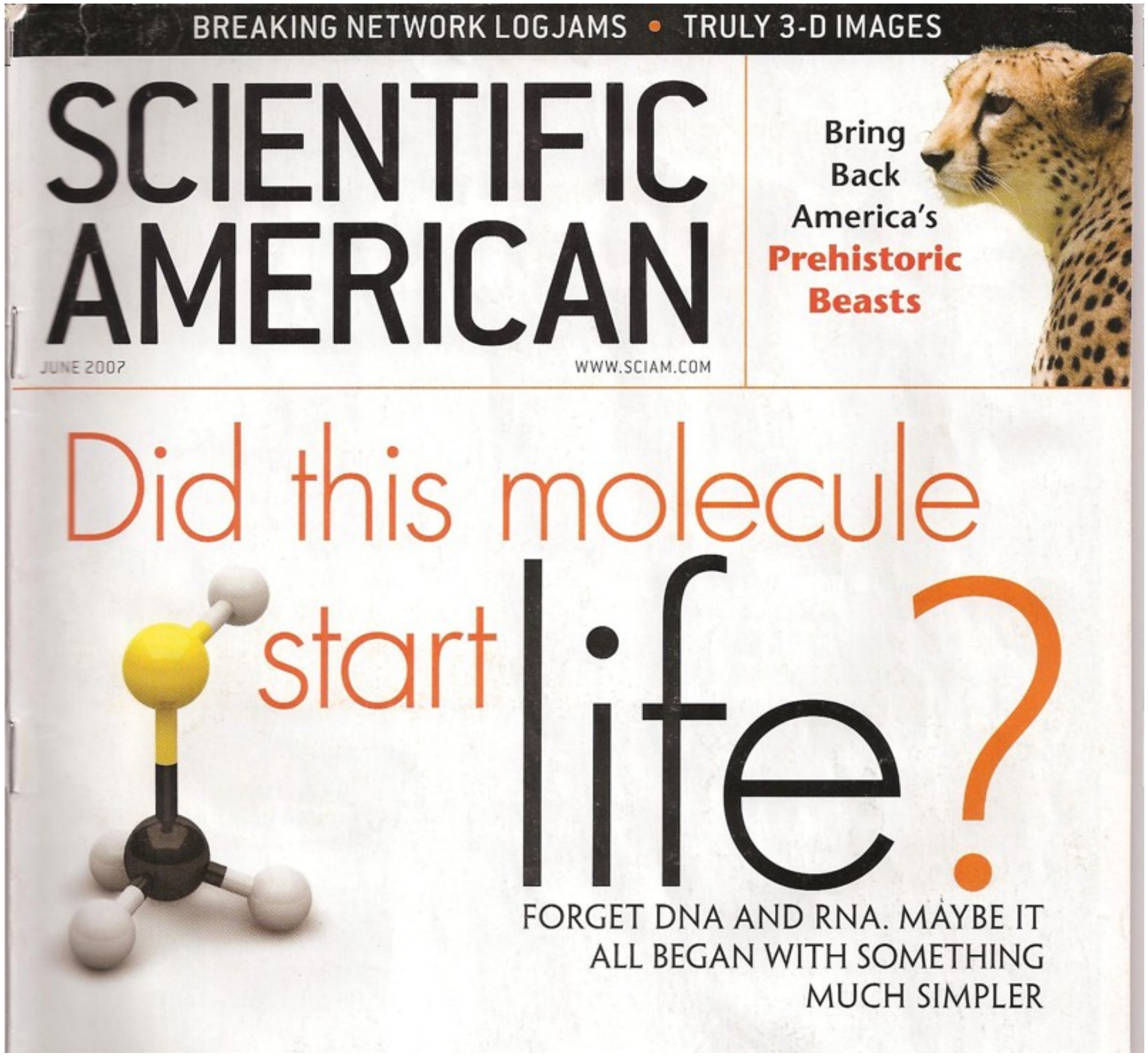
BY ROBERT SHAPIRO

The sudden appearance of a large self-copying molecule such as RNA was exceedingly improbable. Energy-driven networks of small molecules afford better odds as the initiators of life

NOBEL laureate Christian de Duve has called for "a rejection of improbablities so incomensurably high that they only can be called miracles, phenomena that fall outside the scope of scientific inquiry". DNA, RNA and PROTEINS must then be set aside as participants in the origin of life.

Overview/*Origin of Life*

- Theories of how life first originated from nonliving matter fall into two broad classes—replicator first, in which a large molecule capable of replicating (such as RNA) formed by chance, and metabolism first, in which small molecules formed an evolving network of reactions driven by an energy source.
- Replicator-first theorists must explain how such a complicated molecule could have formed before the process of evolution was under way.
- Metabolism-first proponents must show that reaction networks capable of growing and evolving could have formed when the earth was young.



GENETICS FIRST OR METABOLISM FIRST?

Cite this: DOI: 10.1039/c2cs35066a

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CRITICAL REV

Genetics first or metabolism first? The formamide clue†

Raffaele Saladino,*[a] Giorgia Botta,[a] Samanta Pino,[b] Giovanna Costanzo[c] and Ernesto Di Mauro*[d]

Received 6th March 2012
DOI: 10.1039/c2cs35066a

Life is made of the intimate interaction of metabolism and genetics, both built around the chemistry of the most common elements of the Universe (hydrogen, oxygen, nitrogen, and carbon). The transmissible interaction of metabolic and genetic cycles results in the hypercycles of organization and de-organization of chemical information, of living and non-living. The origin-of-life quest has long been split into several attitudes exemplified by the aphorisms "genetics-first" or "metabolism-first". Recently, the opposition between these approaches has been solved by more unitary theoretical and experimental frames taking into account energetic, evolutionary, proto-metabolic and environmental aspects. Nevertheless, a unitary and simple chemical frame is still needed that could afford both the precursors of the synthetic pathways eventually leading to RNA and to the key components of the central metabolic cycles, possibly connected with the synthesis of fatty acids. In order to approach the problem of the origin of life it is therefore reasonable to start from the assumption that both metabolism and genetics had a common origin, shared a common chemical frame, and were embedded under physical–chemical conditions favourable for the onset of both. The singleness of such a prebiotically productive chemical process would partake of Darwinian advantages over more complex fragmentary chemical systems. The prebiotic chemistry of formamide affords in a single and simple physical–chemical frame nucleic bases, acyclonucleosides, nucleotides, biogenic carboxylic acids, sugars, amino sugars, amino acids and condensing agents. Thus, we suggest the possibility that formamide could have jointly provided the main components for the onset of both (pre)genetic and (pre)metabolic processes. As a note of caution, we discuss the fact that these observations only indicate possible solutions at the level of organic substrates, not at the systemic chemical level.

Ernesto Di Mauro

Ernesto Di Mauro was born in Valmontone, Italy, in 1945. In 1967 he obtained his Degree in Biological Sciences from "Sapienza" University of Rome, Italy. In 1969 he joined the Department of Genetics (Seattle), as a post-doctoral fellow. Appointed in 1978 as an associate professor of Enzymology at the University of Rome, he has been a professor of Molecular Biology since 1987. His research interests were centered on gene regulation, DNA and chromatin structure and topology and, at present, on the various aspects of the origin of life.

CHEMICAL SOCIETY REVIEWS 41(2012) 5526-5565

…assumption that both **metabolism and genetics a had a common origin**….

Formamide and catalyzer played a central role …

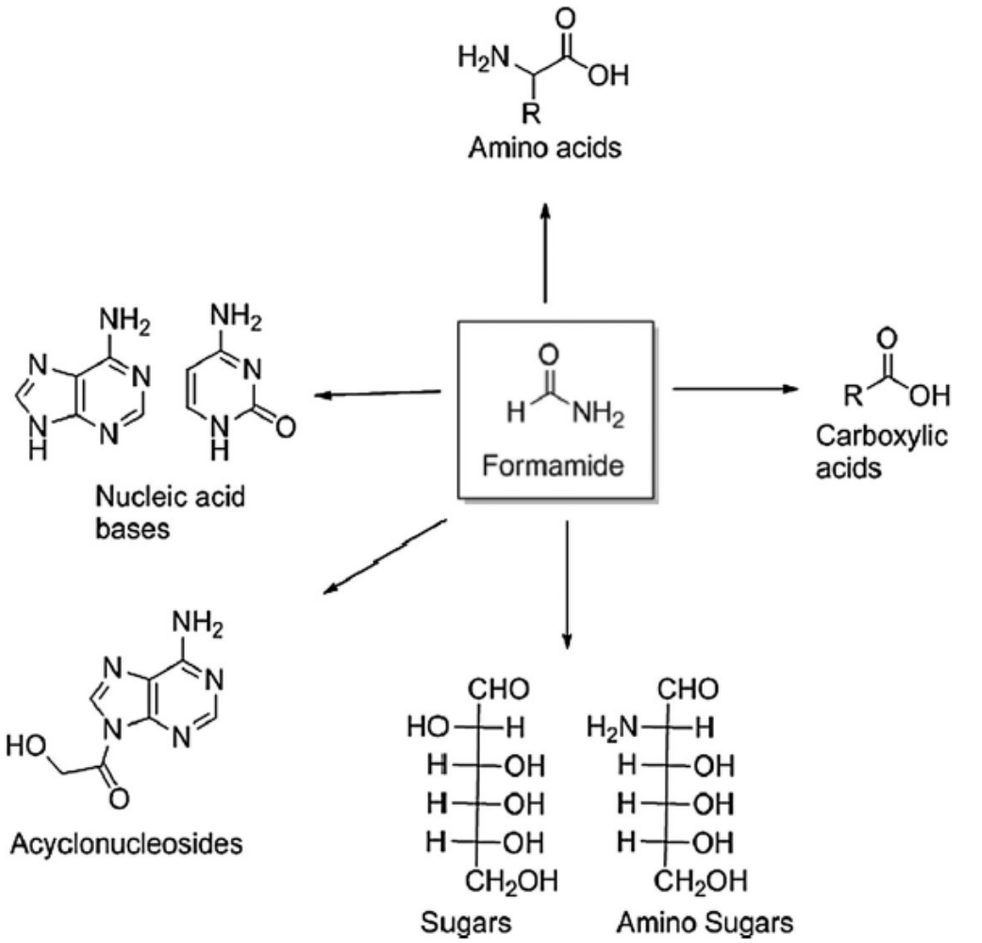
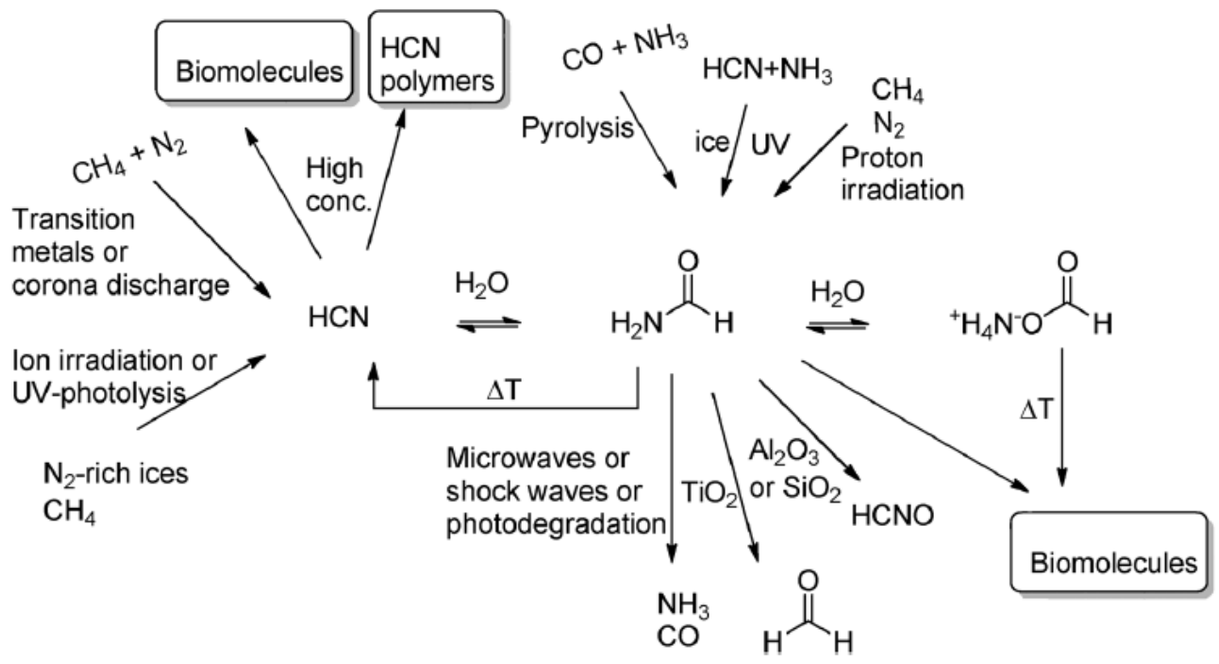


Fig. 1 Syntheses from formamide.



Scheme 1

Zahříváním formamidu za přítomnosti katalyzátorů může vzniknout řada látek, které mohly hrát roli při vzniku života na Zemi

11. Concluding remarks

We have started from the consideration that formamide is the simplest possible amide and that it contains within its diverse chemistry the functional groups and chemical bonds of the central biomolecules. We have considered the sources of formamide (meteorites, comets, interstellar dusts) and we have discussed how formamide can be at the same time solvent and reactant. Having observed the products obtained simply by warming formamide in the presence of one out a large number of different catalysts, we adhere to the conclusion that formamide chemistry is quite versatile and nonfastidiously yields in rich and complex combinations nucleobases, carboxylic acids, amino acids, sugars, amino sugars and condensing agents. These reactions occur in the presence of necessary catalysts, as discussed in the specific sections. If the cradle of life contained formamide, the walls of the cradle were made of one out of many different possible minerals, presumably of combinations thereof. As noted, phosphate minerals were a likely ingredient.

Zdrojem formamidu mohou být meteority, komety i mezihvězdný prach

Pokud kolébka Života obsahovala formamid, její stěny mohly být tvořeny různými minerály, včetně fosfátů

11.1. The limits of the formamide scenario

The contribution that HCN/formamide chemistry provides to the general picture of the origins is limited to the proof-of-principle that a unifying chemistry is at least conceivable. The scenario is far from being fully and satisfactorily sketched. Riddles remain.

The first riddle is the concentration problem. We have mentioned in Section 2 that the steady state concentration of HCN in the primitive ocean was evaluated to be 4×10^{-12} M at 100 °C, that similar values were reported for NH_2CHO and that even at lower temperatures concentrations were too low to foster biomolecular syntheses in solution. Concentration

Thus, the second riddle is the stability problem. The activation free energy value of 31.0 kcal mol^{-1} suggests that the neutral hydrolysis of formamide does not take place at all.[405]

Stejně jako v dřívějších kocepcích vzniku Života na Zemi – i nyní zůstává mnoho hádanek a pochybností. a Dosavadní studie naznačují pouze cesty, kterými Život na Zemi snad mohl vzniknout ???

Third riddle: Optimal temperature problem

Fourth riddle is systemic .....

Electrochemistry of Nucleic Acids is a Booming Field

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

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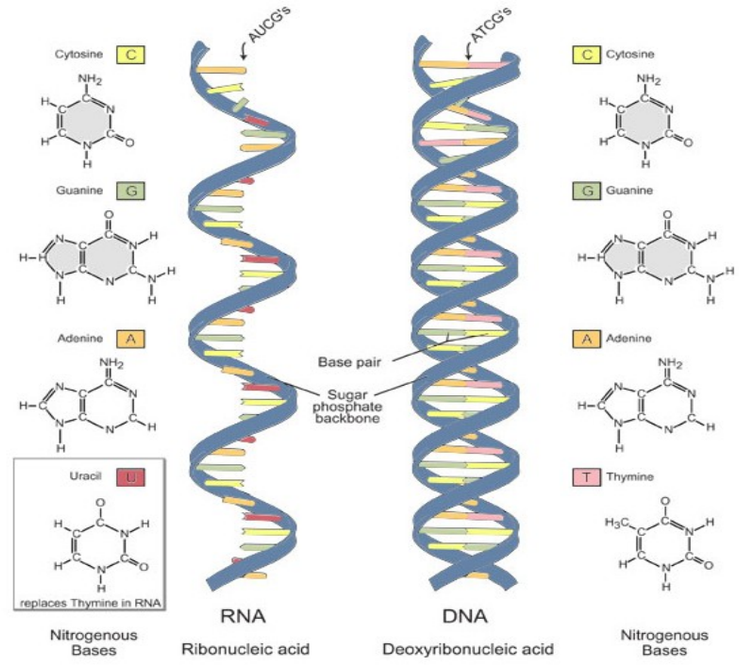


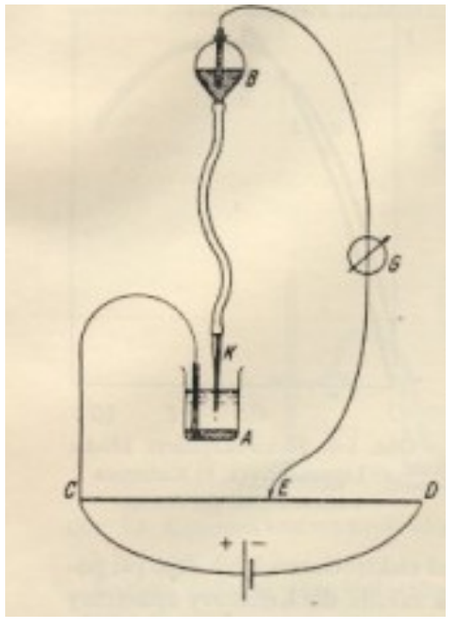
Electroactive Labels can be Introduced in DNA

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

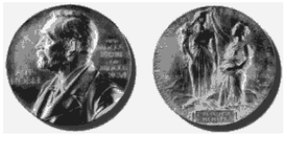
Trefulka, M., et al. (2007): Covalent labeling nucleosides, RNA and DNA with VIII- and VI-valent osmium complexes. Electroanal. 19, 1281-1287

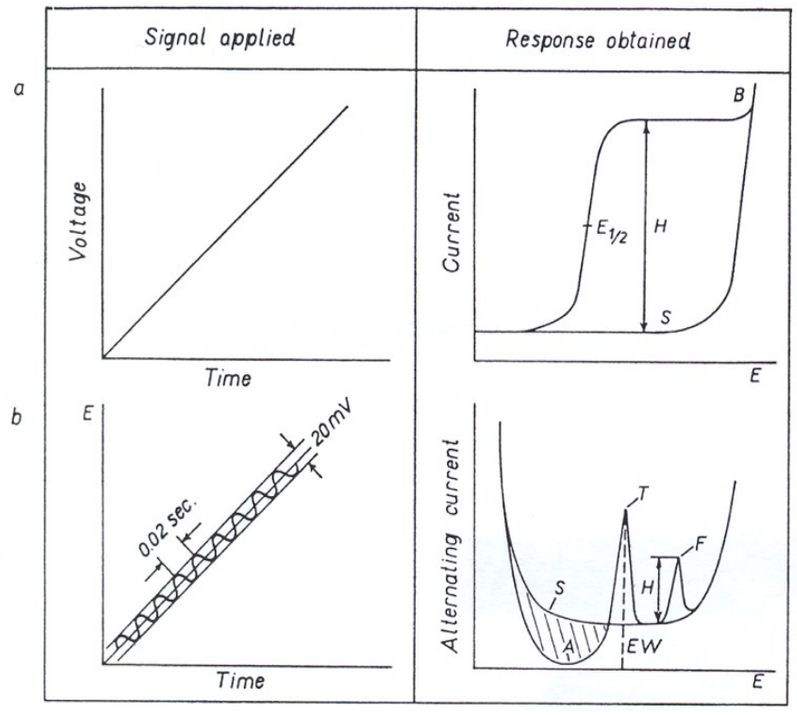
Jaroslav Heyrovský 1890-1967 invented POLAROGRAPHY in 1922

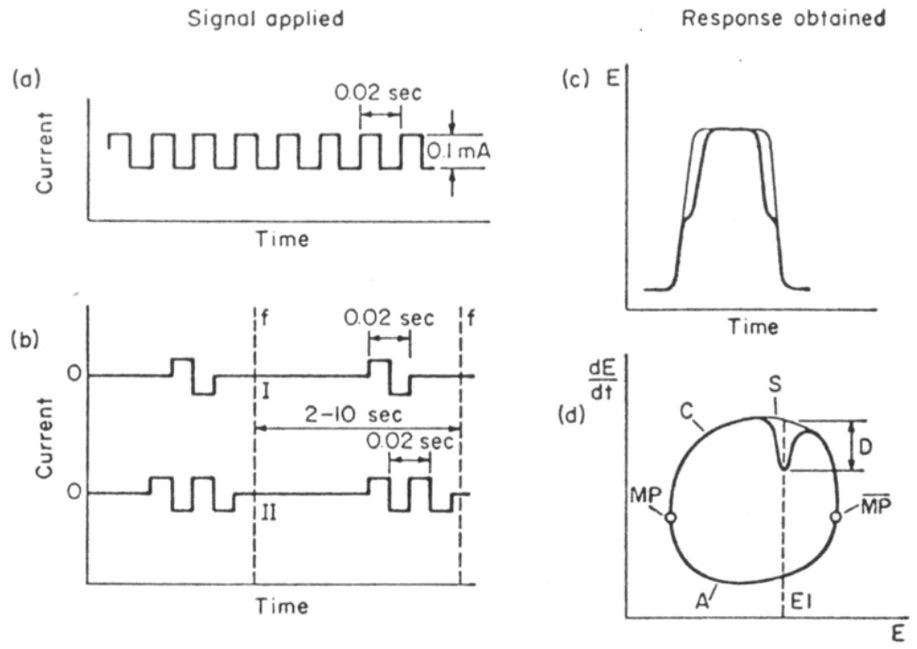
Present electrochemical analysis stems from Heyrovský's polarography



Nobel Prize 1959



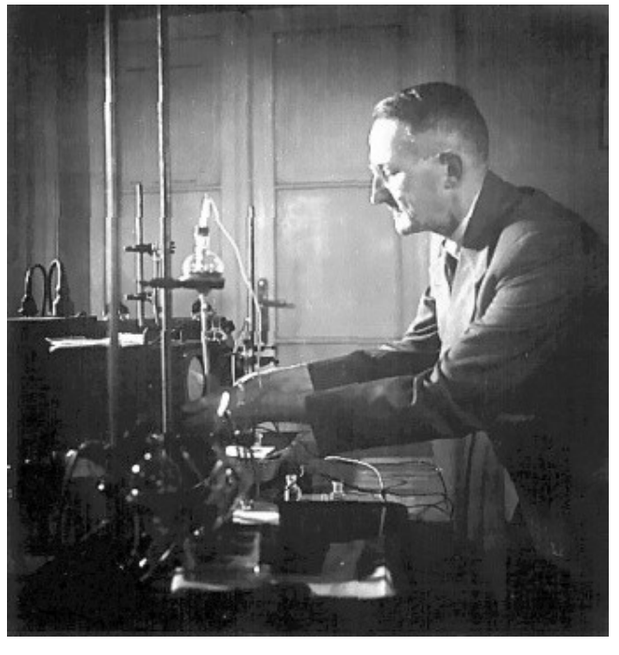




Oscillographic polarography at controlled a.c
(cyclic a.c. chronopotentiometry)

complete analyses on a single mercury drop 1941

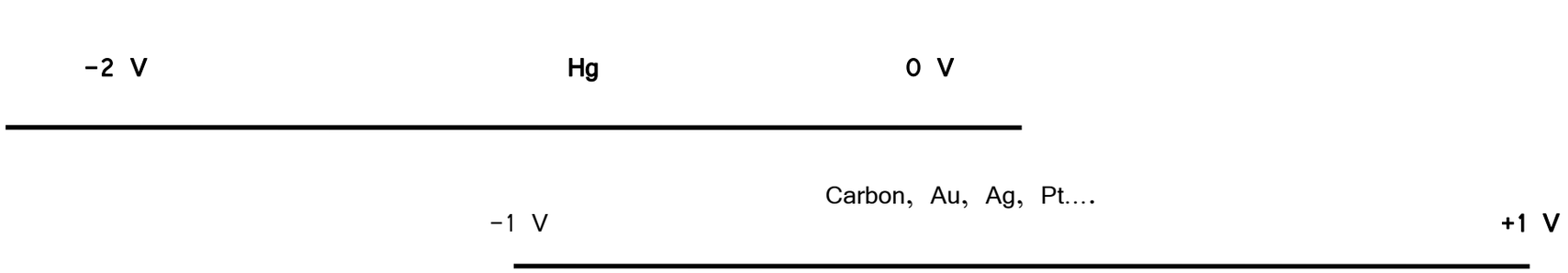
J Heyrovsky S Ochoa A Kornberg



J. Heyrovský

Electrodes

Heyrovsky's polarography was based on mercury electrodes. At present a number of different electrodes is used in electrochemical analysis, incl. bimacromolecule 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 NA electroactivity has been observed. Mercury electrodes and most of the solid electrodes greatly differ in their potential windows. Are Hg electrodes toxic?



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 functional groups may greatly affect adsorption of DNA and proteins

90 years of polarography and ~55 years of nucleic acid electrochemistry

This year we commemorate the 90th Anniversary of the invention of polarography by J. Heyrovsky. In 1941 he invented oscillographic polarography with controlled a.c. (cyclic a.c. chronopotentiometry). By the end of the 1950's oscillographic polarography was the method of choice f the DNA electrochemical analysis:

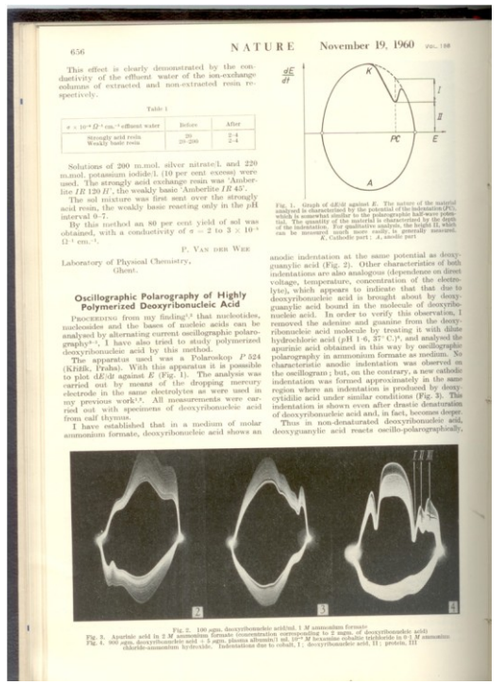
1958: Nucleic acid bases, DNA and RNA are electroactive

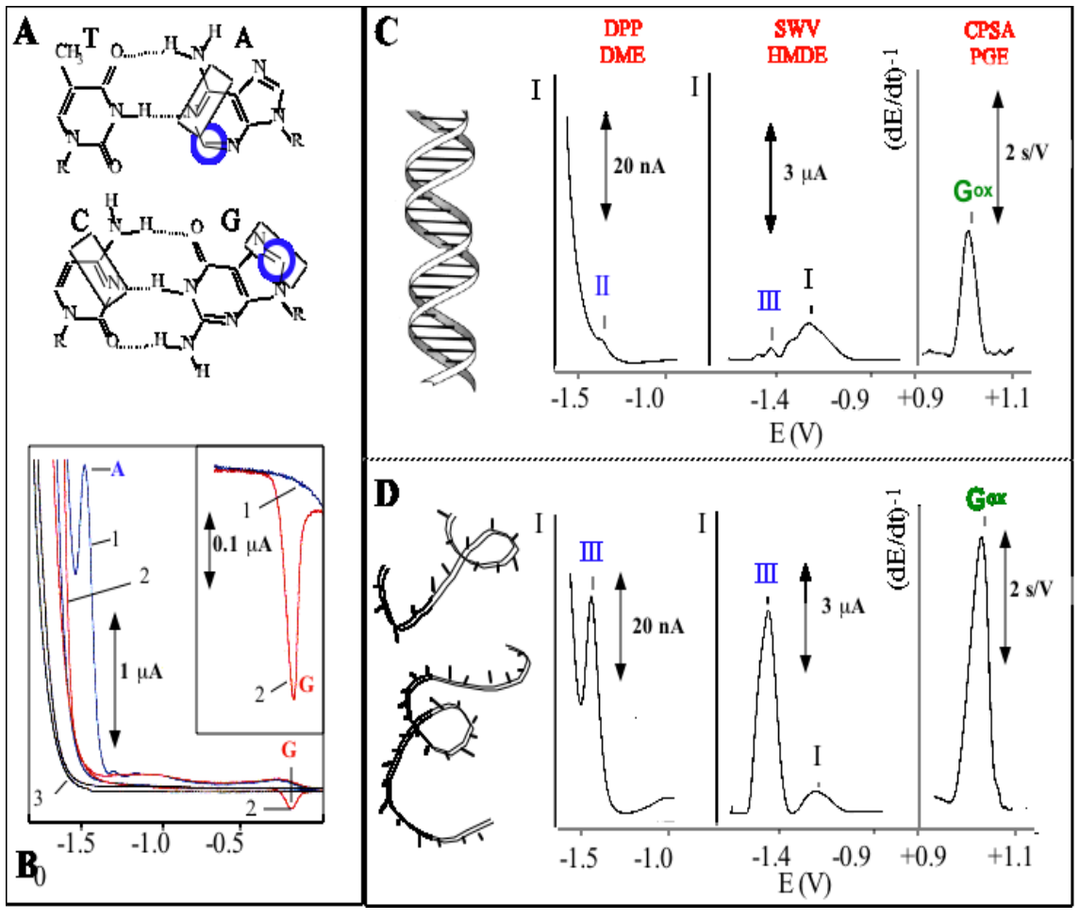
1960: Relations between the DNA structure and electrochemical responses

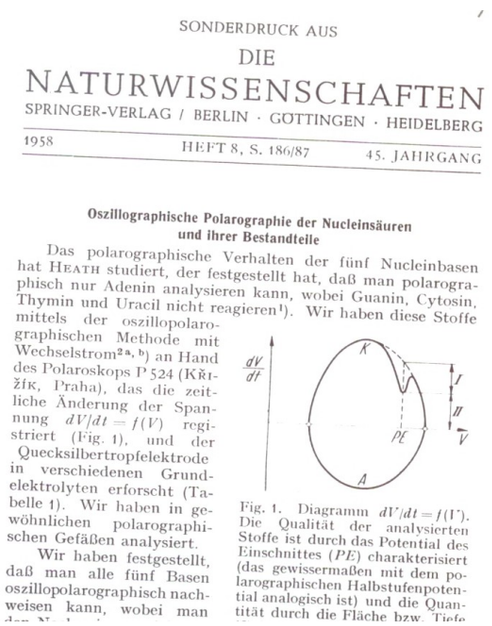
1955 :Adenine is polarographically reducible at strongly acid pH while other NA bases are inactive. J.N.Davidson and E.Chargraff: The Nucleic Acids, Vol.1, Academic Press, New York 1955

1957: NO response of RNA and DNA on oscillopolarograms

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







SONDERDRUCK AUS

DIE

NATURWISSENSCHAFTEN

SPRINGER-VERLAG / BERLIN · GÖTTINGEN · HEIDELBERG

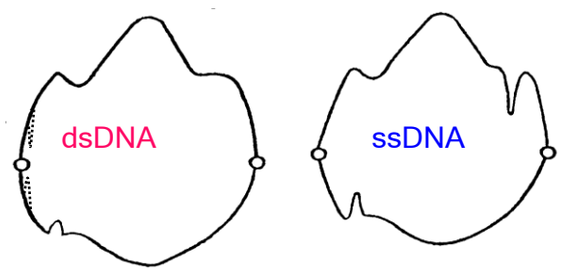
1958 HEFT 8, S. 186/87 45. JAHRGANG

Oszillographische Polarographie der Nucleinsäuren und ihrer Bestandteile

Das polarographische Verhalten der fünf Nucleinbasen hat HEATH studiert, der festgestellt hat, daß man polarographisch nur Adenin analysieren kann, wobei Guanin, Cytosin, Thymin und Uracil nicht reagieren[1]). Wir haben diese Stoffe mittels der oszillopolarographischen Methode mit Wechselstrom[2a, b]) an Hand des Polaroskops P 524 (KŘIŽÍK, Praha), das die zeitliche Änderung der Spannung $dV/dt = f(V)$ registriert (Fig. 1), und der Quecksilbertropfelektrode in verschiedenen Grundelektrolyten erforscht (Tabelle 1). Wir haben in gewöhnlichen polarographischen Gefäßen analysiert.

Wir haben festgestellt, daß man alle fünf Basen oszillopolarographisch nachweisen kann, wobei man

Fig. 1. Diagramm $dV/dt = f(V)$. Die Qualität der analysierten Stoffe ist durch das Potential des Einschnittes (PE) charakterisiert (das gewissermaßen mit dem polarographischen Halbstufenpotential analogisch ist) und die Quantität durch die Fläche bzw. Tiefe



Using these techniques in the 1960's and and 1970's DNA denaturation and renaturation was followed and early evidence of DNA premelting and POLYMORPHY OF THE DNA DOUBLE HELIX was obtained

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

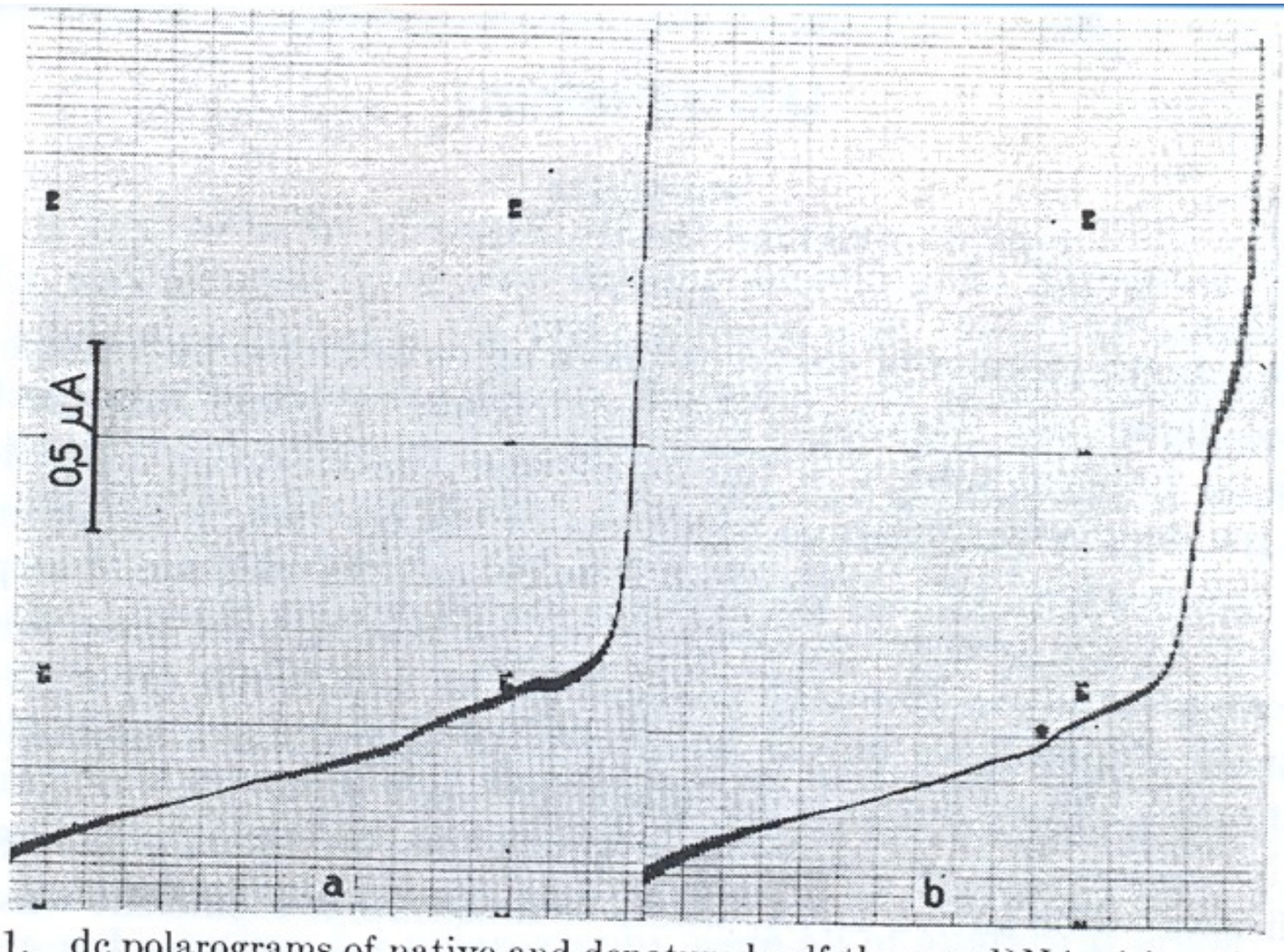


Fig. 1. dc polarograms of native and denatured calf thymus DNA: (*a*) native DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0); (*b*) denatured DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0). DNA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

RENATURATION OF RNA AS DETECTED BY DPP
Time dependence

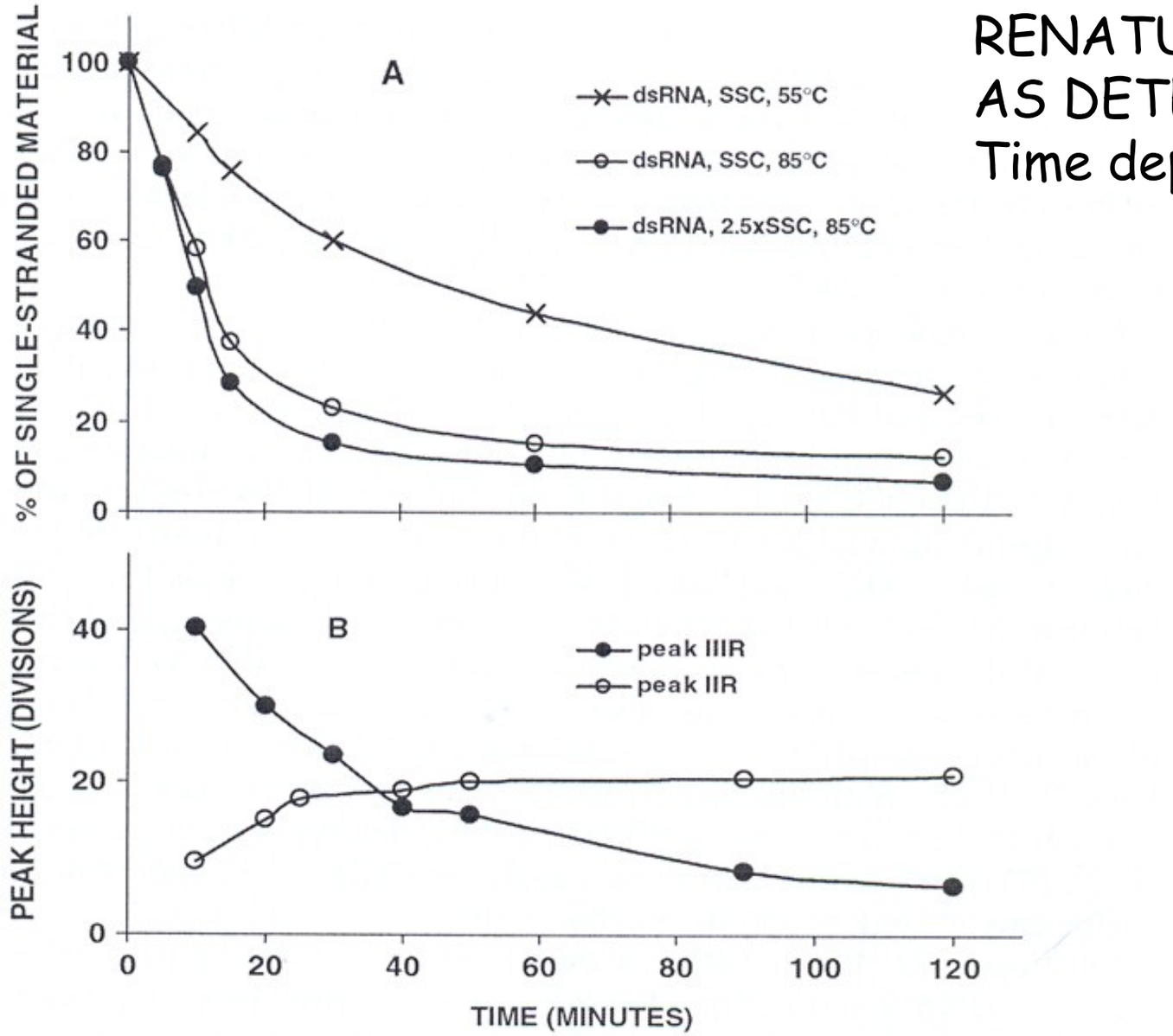


Fig. 10. Time-course of renaturation of phage f2 dsRNA. (A) Thermally denatured ssRNA was incubated (●—●) at 85°C in 2.5 × sodium saline citrate (SSC) or (o—o) at 85°C in SSC, and (x—x) at 55°C. Samples were withdrawn in time intervals given in the graph and quickly cooled. DPP measurements were performed 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 IIIR. 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 Doskocil (1974). Copyright 1974, with permission from Academic Press.

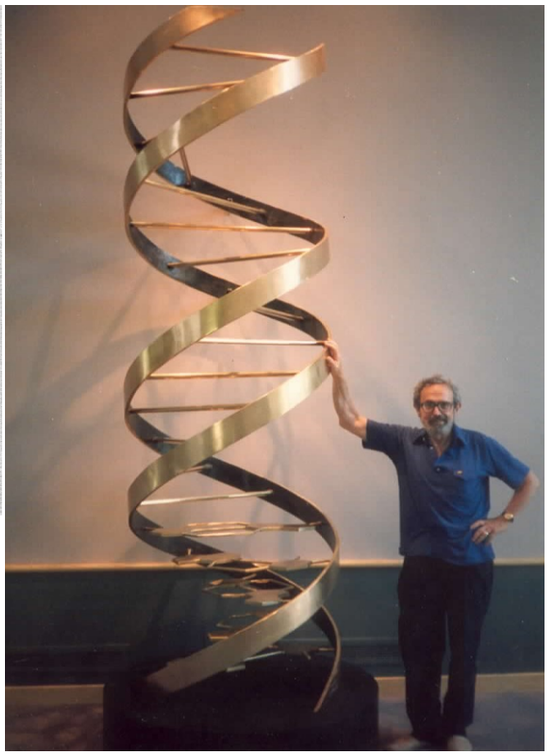
In 1960 when I published my NATURE paper on electrochemistry of DNA I obtained invitations from 3 emminent US scientists:
J. Marmur - Harvard Univ.
L. Grossman - Brandeis Univ.
J. Fresco - Princeton Univ.
To work in their laboratories as a postdoc

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 Reccommendation 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 (1926–1996)

J. 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

1. Understanding how heat denaturation of native DNA results in separation of the DNA strands with concomitant loss of biological (transforming) activity.
2. Showing that the native DNA structure could be restored by annealing the separated strands, with simultaneous regaining of transforming activity.
3. Showing that density-labeled DNA strands of one DNA could be annealed with strands of another but homologous DNA, forming a biologically active hybrid double helix.

W. SZYBALSKI

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. PALECEK, F. M. KAHAN†, J. LEVINE, and M. MANDEL‡
Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts

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.

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PROCEDURE FOR ISOLATION OF DEOXYRIBONUCLEIC ACID FROM MICRO-ORGANISMS
JOURNAL OF MOLECULAR BIOLOGY 3 (2): 208& 1961
Times Cited: 9234

2. **MARMUR J**, DOTY P
DETERMINATION OF BASE COMPOSITION OF DEOXYRIBONUCLEIC ACID FROM ITS THERMAL DENATURATION TEMPERATURE
JOURNAL OF MOLECULAR BIOLOGY 5 (1): 109& 1962
Times Cited: 3210

3. SCHILDKRAUT CL, DOTY P, **MARMUR J**
DETERMINATION OF BASE COMPOSITION OF DEOXYRIBONUCLEIC ACID FROM ITS BUOYANT DENSITY IN CSCL
JOURNAL OF MOLECULAR BIOLOGY 4 (5): 430& 1962
Times Cited: 1619

4. **MARMUR J**, DOTY P
HETEROGENEITY IN DEOXYRIBONUCLEIC ACIDS .1. DEPENDENCE ON COMPOSITION OF THE CONFIGURATIONAL STABILITY OF DEOXYRIBONUCLEIC ACIDS
NATURE 183 (4673): 1427-1429 1959
Times Cited: 427
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9. **MARMUR J**, LANE D
STRAND SEPARATION AND SPECIFIC RECOMBINATION IN DEOXYRIBONUCLEIC ACIDS - BIOLOGICAL STUDIES
PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 46 (4): 453-461 1960
Times Cited: 246

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Early evidence of DNA Premelting and Polymorphy 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

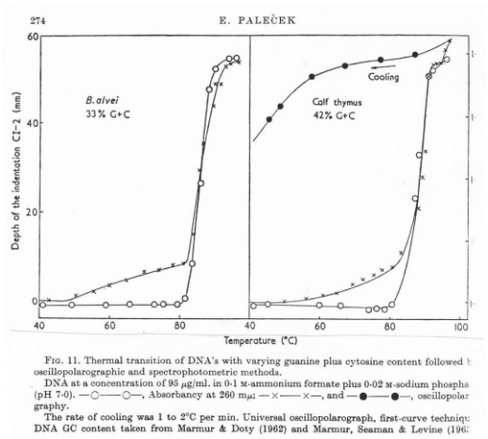


FIG. 11. Thermal transition of DNA's with varying guanine plus cytosine content followed b oscillopolarographic and spectrophotometric methods.
DNA at a concentration of 95 μg/ml. in 0·1 M-ammonium formate plus 0·02 M-sodium phospha (pH 7·0). —○——○—, Absorbancy at 260 mμ; —×——×—, and —●——●—, oscillopolar graphy.
The rate of cooling was 1 to 2°C per min. Universal oscillopolarograph, first-curve techniq DNA GC content taken from Marmur & Doty (1962) and Marmur, Seaman & Levine (196

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

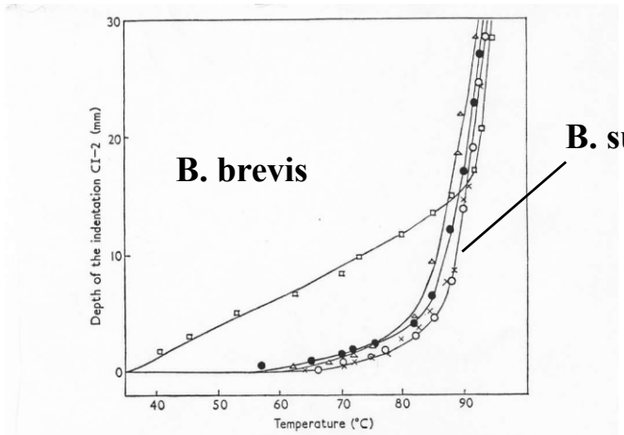
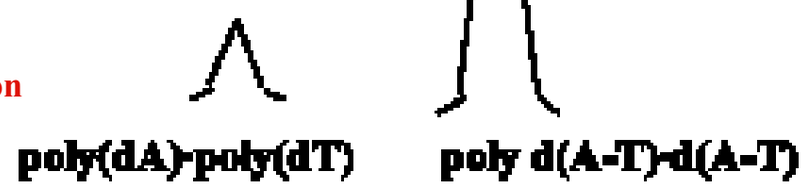


FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 μg/ml. in 0·25 M-ammonium formate plus 0·025 M-sodium phosphate (pH 7·0).
—●——●—, *B. subtilis* 168; —×——×—, *B. natto*; —○——○—, *B. subtilis* var. *niger*; —△——△—, *B. subtilis* var. *aterrimus*; —□——□—, *B. brevis* (ATCC 9999).
P 524 polaroscope, 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.

POLAROGRAPHIC BEHAVIOR OF dsDNA
At roomand premeltig temperaturse 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

After 1980
Is not it strange that such an obscure technique can recognize POLYMORPHY OF THE DNA DOUBLE HELIX?

1976

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PROGRESS IN NUCLEIC ACID RESEARCH AND MOLECULAR BIOLOGY, VOL. 18
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ACADEMIC PRESS, INC
New York San Francisco London

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.

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

December 3, 1976

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

Dear Professor Palecek,

I do apologise for taking so long to reply to your letter of September 29 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
Perkauf Foundation Visiting Professor

FHCC:lt

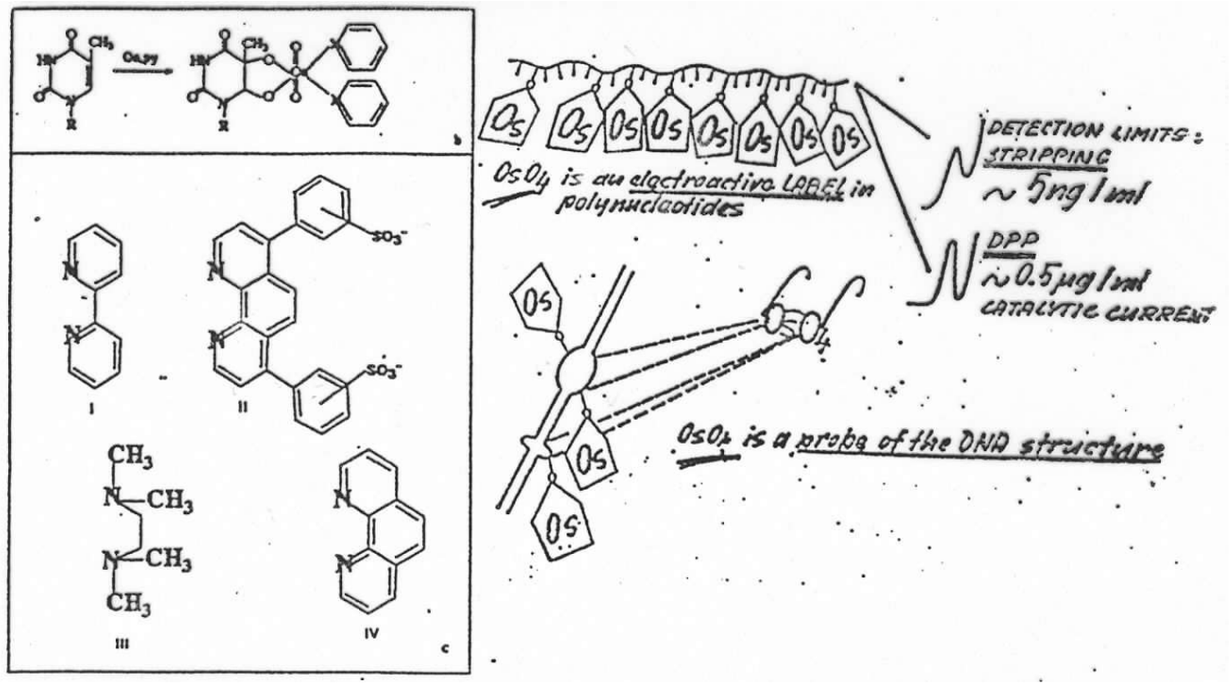
Prof. F. CRICK se omlouvá, že se nebude moci zúčastnit symposia v Brně a doporučuje prof. H. Sobell(a)

Post Office Box 1809, San Diego, California 92112 • Telephone (714) 453-4100

Electroactive labels can be introduced in nucleic acids

Os(VIII)L complexes are sensitive to the DNA structure (CHEMICAL PROBES OF THE DNA STRUCTURE)

they react with single-stranded and distorted but NOT with intact double-stranded DNA in vitro and in cells



In the beginning of the 1980's Os,L 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

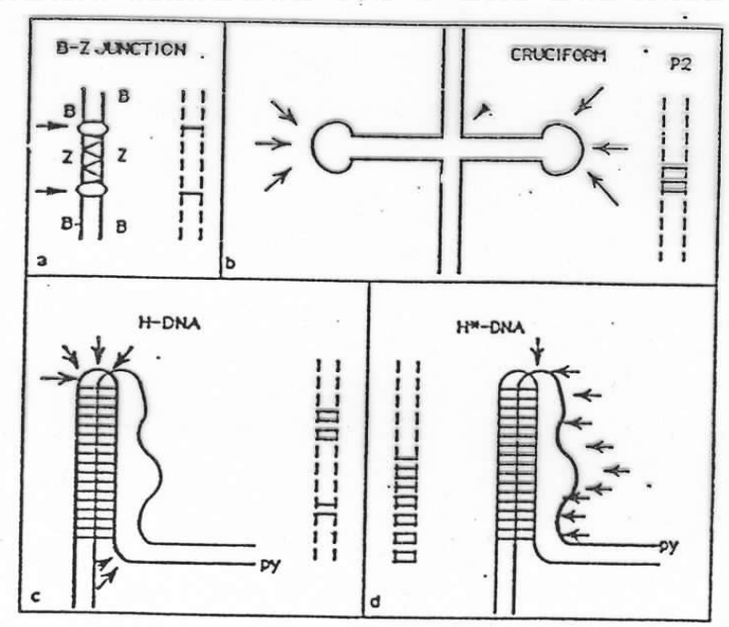
Critical Reviews in Biochemistry and Molecular Biology, 26(2):151—226 (1991)

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

We developed methods of chemical probing of the DNA structure based on osmium tetroxide complexes (Os,L). Some of the Os,L complexes react with single-stranded DNA but not with



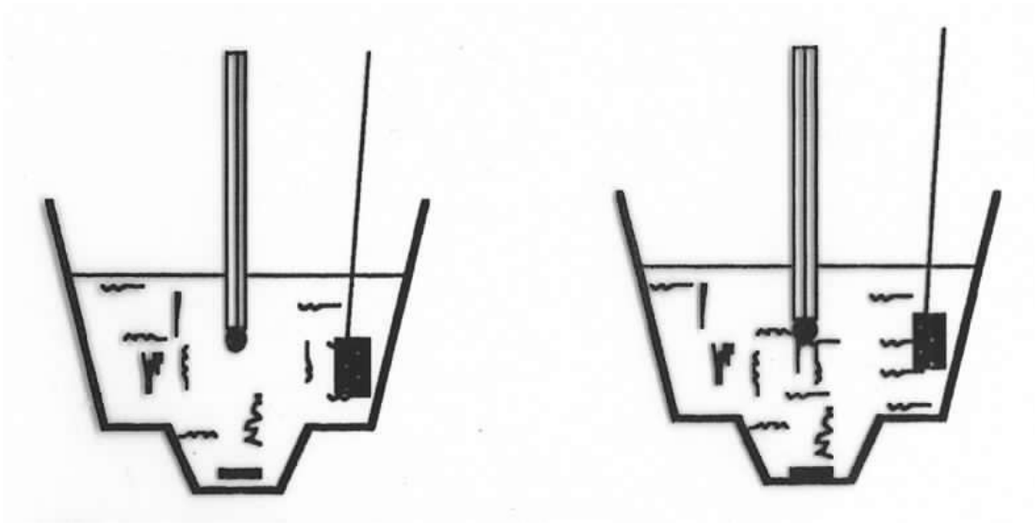
These methods yielded information about the distorted and single-stranded regions in the DNA double helix at single-nucleotide resolution. DNA probed both in vitro and

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

By EMIL PALEČEK

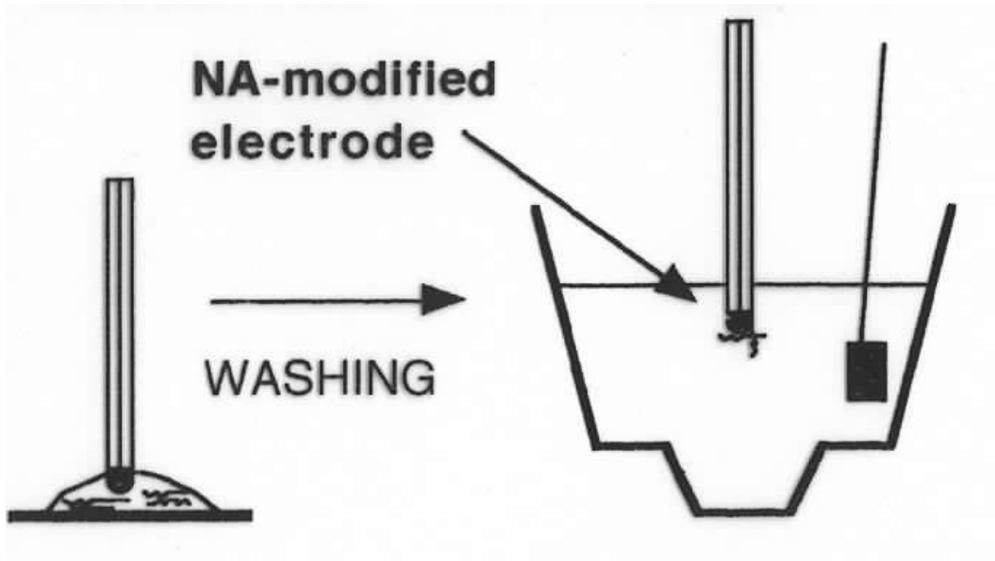
METHODS IN ENZYMOLOGY, VOL. 212

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 ⌈l)

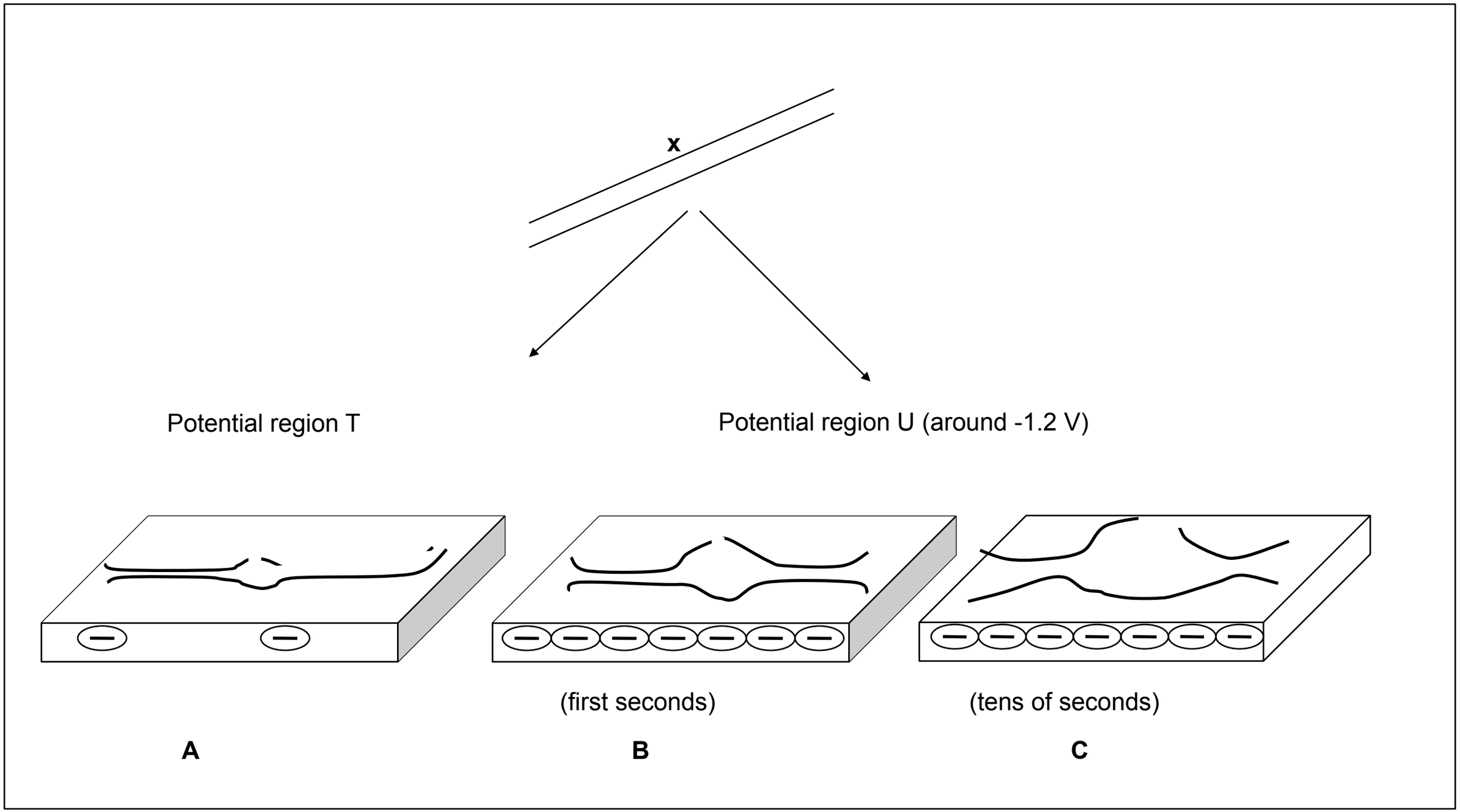
NA is at the electrode but the electrolytic cell contains only blank electrolyte

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

AdTSV has many advatages 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.

DNA can be unwound at negatively charged surfaces



DNA unwinding was found at Hg electrodes in 1974 and later at other surfaces

peak G

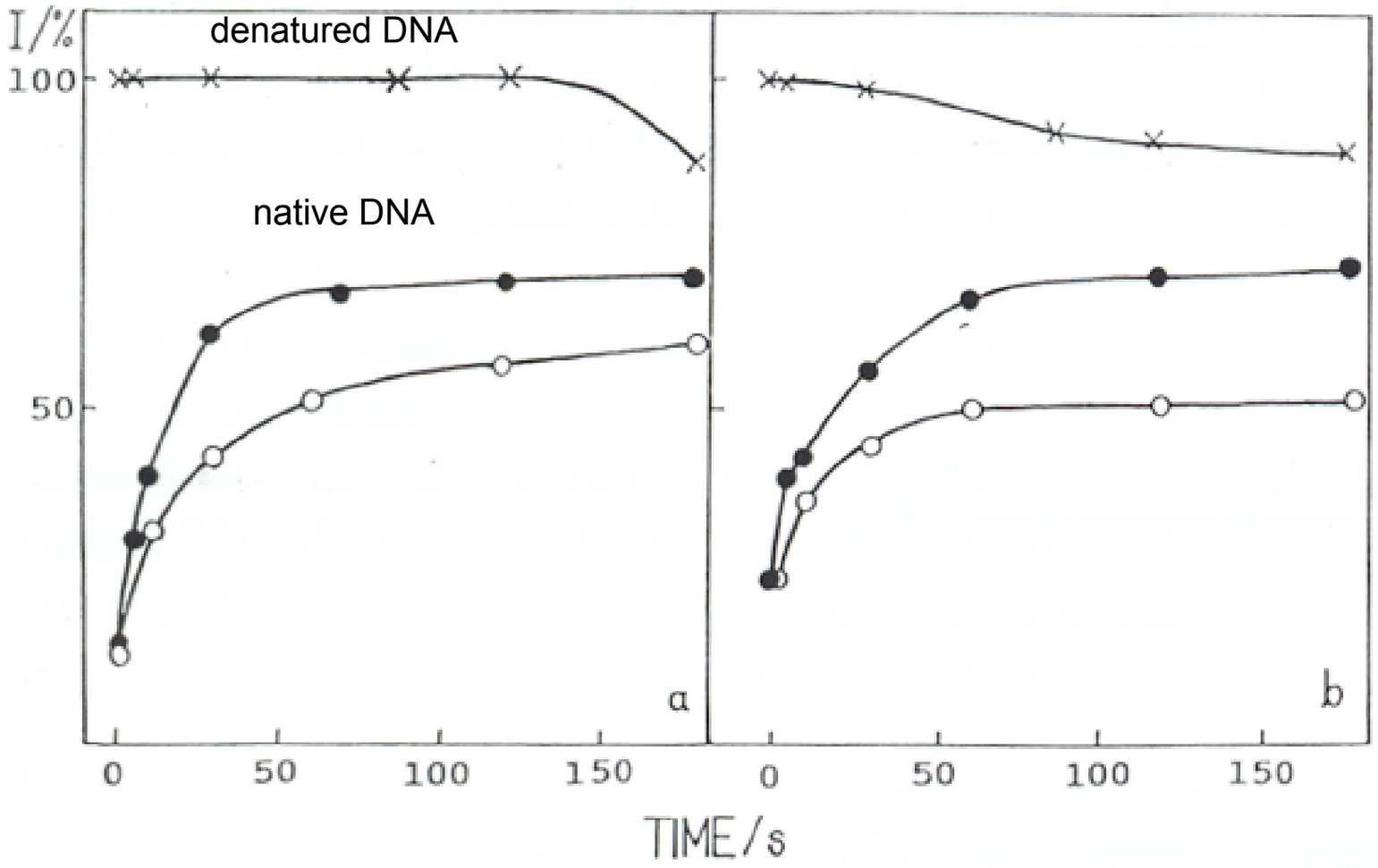


Fig. 6. The dependence of the relative heights of (a) the AdTSCV anodic peak G and (b) the cathodic peak AC on time t_b at potentials $E_b = -1.2$ V (○———○), and $E_b = -1.3$ V (●———●) for native DNA and for denatured DNA (×———×). The HMDE charged to a potential $E_a = -0.25$ V was immersed into the solution of native DNA (at a concentration of 292 μg ml^{-1}) or into the solution of denatured DNA (140 μg 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%.

Effect of nucleotide sequence on DNA unwinding

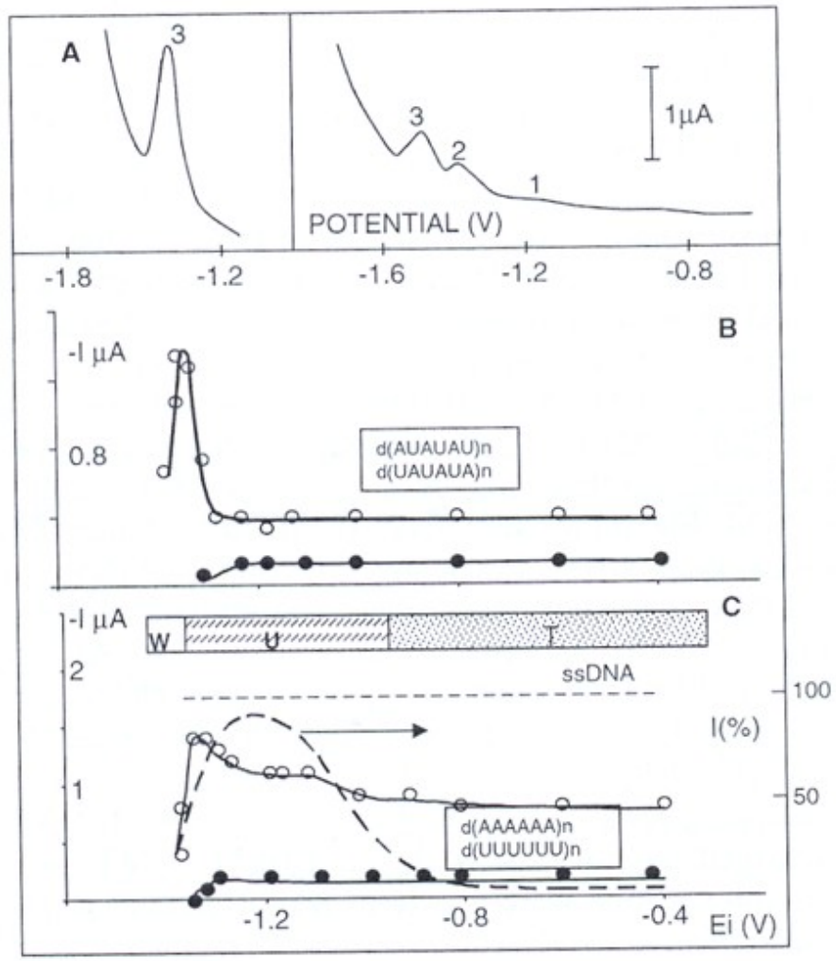
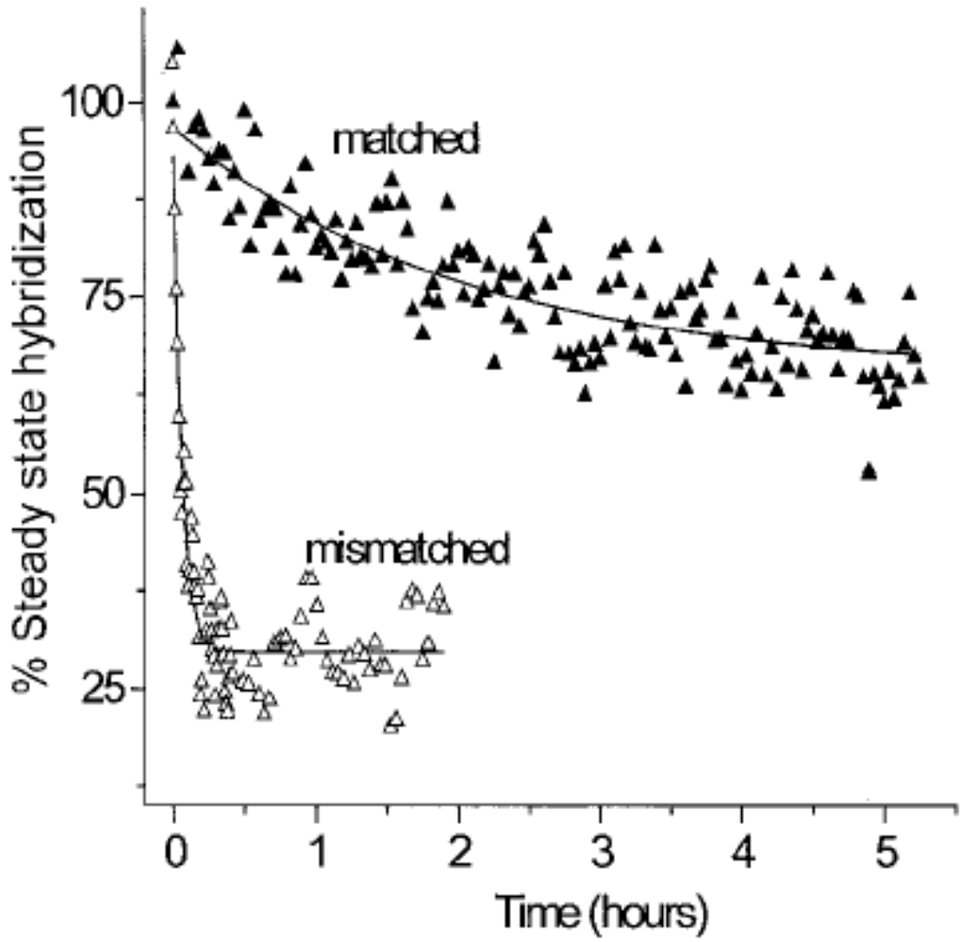


Fig. 16. Dependence of the voltammetric behavior of biosynthetic polynucleotides with different nucleotide sequences on the initial potential (E_i). (A): voltammetric peaks of poly (dA–dU) · poly (dA–dU). $E_i = -0.6$ V (left), $E_i = -1.35$ V (right); (B): ●—● , peak 2; ○—○, peak 3; (C): poly (rA) · 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.

DNA unwinding was observed also at negatively charged Au and applied in DNA sensors



In DNA containing mismatched bases
DNA unwinding is faster

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

Foundations of nucleic acid electrochemistry

were laid down in 1960-1980's using mercury and carbon electrodes

After the discovery of the DNA electroactivity i t was shown that:
Signals of ds and ss DNA and RNA greatly differ . This made it possible
to follow the course of : DNA denaturation/melting, renaturation/hybridization
to detect: traces of ssDNA in dsDNA samples, DNA damage, single-strand breaks, chem. modification, depurination...

Important findings :

DNA premelting : beginning of the 1960's

DNA unwinding at the electrode surface : middle of 1970's

Polymorphy of the DNA double helix : middle of 1970's

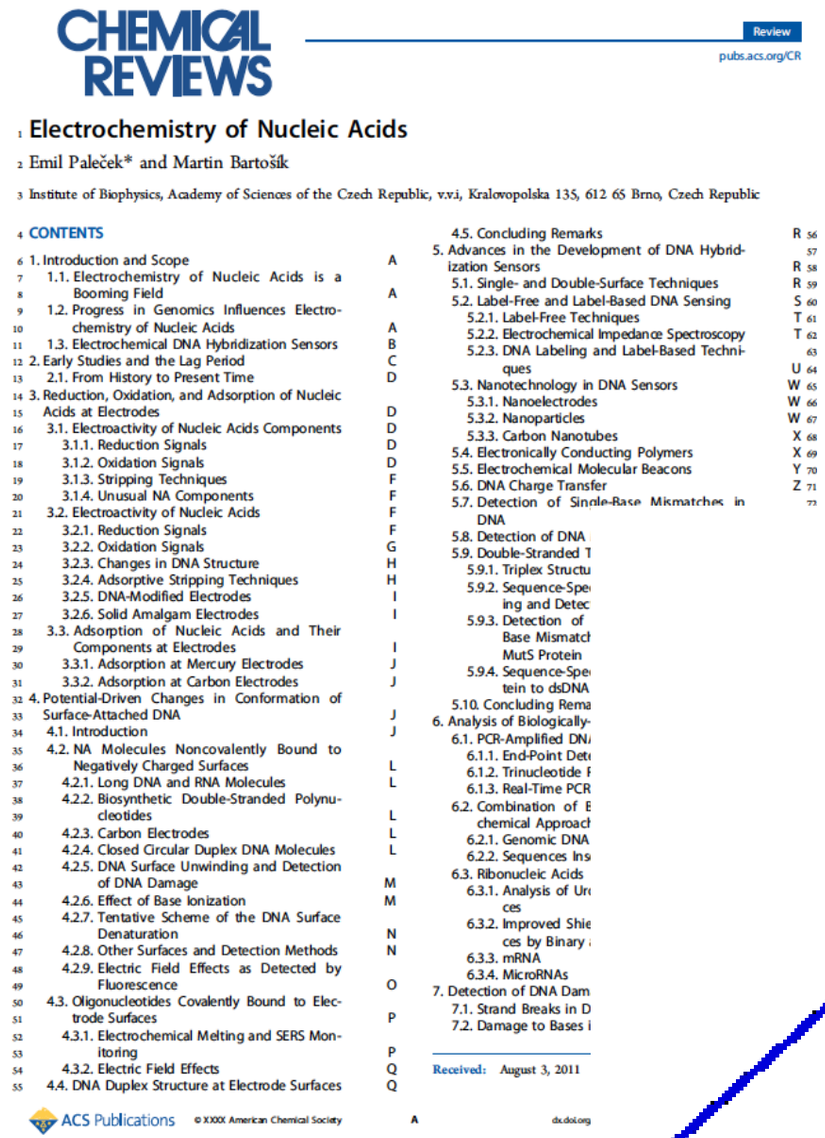
New approaches later utilized in DNA sensors :

First covalently bound electroactive DNA labels : beginning of the 1980's

First DNA-modified electrodes : middle of the 1980's

Electrochemistry of nucleic acids is now a booming field

112 (2012) 3427-3481



CHEMICAL REVIEWS

Review

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Electrochemistry of Nucleic Acids

Emil Paleček* and Martin Bartošík

Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i, Kralovopolska 135, 612 65 Brno, Czech Republic

CONTENTS

Received: August 3, 2011

dx.doi.org

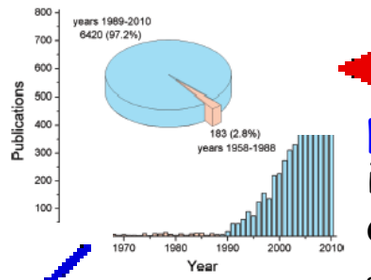
1. INTRODUCTION AND SCOPE

The present boom in electrochemical studies of nucleic acids (NAs) is closely related to DNA sequencing techniques, which are indispensable in genomics. In the first half of the 1960s, it was believed that in difference to the RNA nucleotide sequencing,[1] sequencing of large genomic DNA would not be possible. Methods of DNA renaturation/hybridization were, however, available since 1960.[2,3] The capacity of DNA to form molecular hybrids was used to test the genetic relatedness of some organisms, to study the specificity of hybridization of DNA with mRNA[2−4] and for other purposes.[5] The discovery of sequence-specific restriction endonucleases by the end of the 1960s[6] opened the door to specific cleavage and manipulation of DNA.[7] In about a decade, Maxam and Gilbert[8] and Sanger et al.[9] invented the technology underlying DNA sequencing

DNA and (b) the recent progress in the development of DNA hybridization sensors working with biologically relevant NA samples with or without amplification by polymerase chain reaction (PCR). The article also details that the knowledge of NA electrochemistry can be applied to solve various biochemical problems and to obtain new information about the properties and behavior of NAs at charged interfaces.

1.1. Electrochemistry of Nucleic Acids is a Booming Field

The interest of scientists in electrochemistry of NAs has increased dramatically in the recent two decades as documented by an increase in the number of scientific publications in this science area (Figure 1). Between 1960



lectrochemistry of Nucleic Acids between 1958 is based on Web of Science query for (a) DNA) OR (electrochem* AND DNA) OR "nucleic acid*") in *Topic* and (b) in *Year* 957, only one paper was found; this paper inactivity of nucleic acids. Between 1958 and ers were corrected by excluding papers out of papers obtained through searching in *Author* R, Nurnberg H.W., Palecek E., and Reynaud J. (to our knowledge) significantly contributed to n period of time]. Starting from 1988, the ere taken from the Web of Science without any with permission from ref 13. Copyright 2009

e ~10 papers were published per year in 2010 alone, about 760 papers appeared. ears altogether over six thousand papers on have been published. In other words, in s of NA electrochemistry (let us call it the out 2.8% of the material was published in 97.2% in the last 20 years (*Exponential* f this growth has occurred solely within the ne. Various questions can be asked, such as or this remarkable increase?", "How long will th last?", "Is the amount of knowledge gained equal to >97% of what we know about the ", etc. We shall attempt to answer some of e following chapters.

nomics Influences Electrochemistry of

n be proposed for the appearance of the it perhaps the main one lies in biology and

dx.doi.org/10.1021/cr200303p | Chem. Rev. XXXX, XXX, XXX–XXX

WHY?

progress in GENOMICS
increasing importance
of parallel nucleotide
sequencing
electrochemistry can
complement optical
detection in arrays
and particularly in chips
for decentralized
analysis

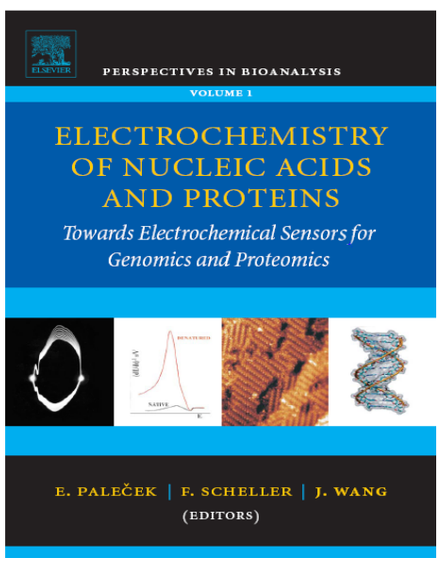
Findings important for present development of electrochemical DNA sensing

1960−66 Relation between the DNA structure and electrochemical responses

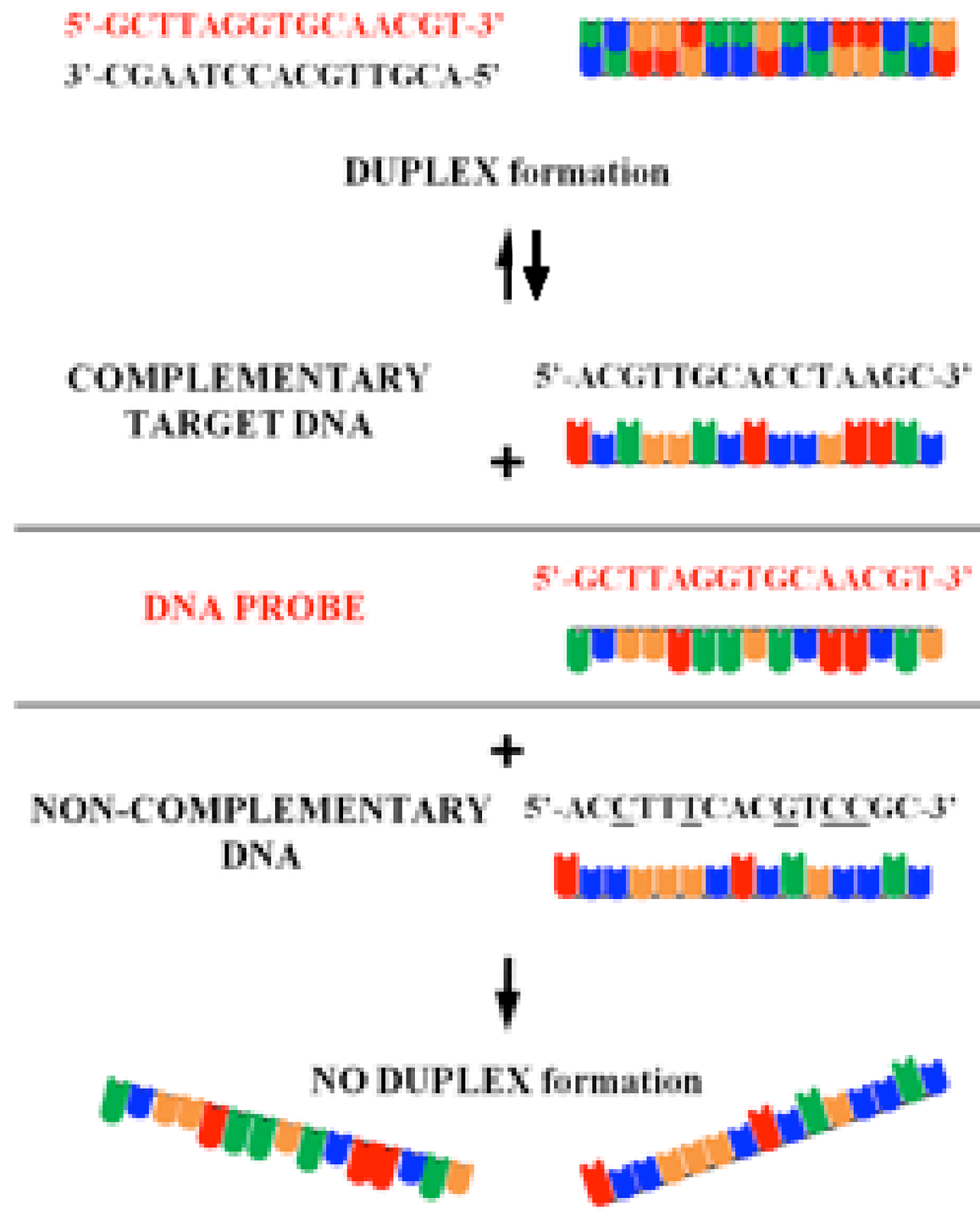
1974 DNA unwinding at negatively charged surfaces

1981−83 Electroactive markers covalently bound to DNA

1986−88 DNA−modified electrodes

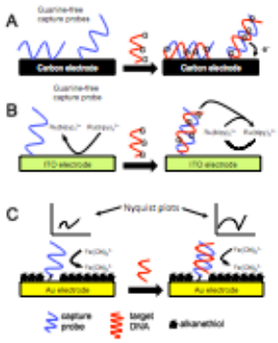


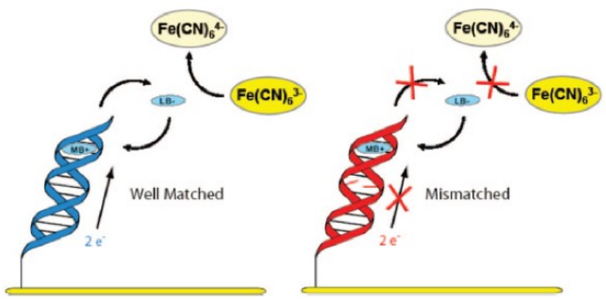
The ability of DNA to reform its double-helical structure was discovered in the early 1960's by J. Marmur and P. Doty at Harvard University. Its principles are applied in many biotechnologies

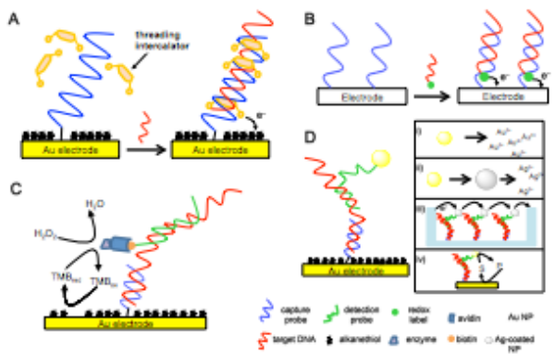


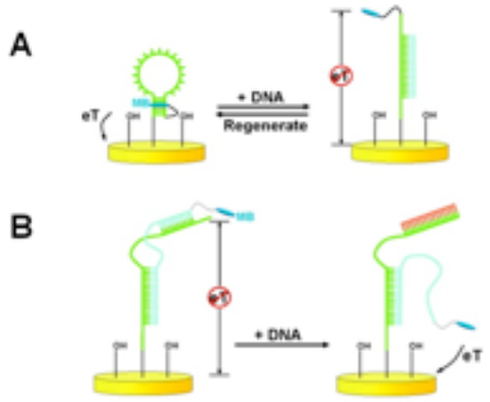
Electrochemical sensors for DNA hybridization are coming of age

At present electrochemical detection of any nucleotide sequence, including detection of point mutations is possible in PCR-amplified DNAs. Detections of DNA methylation and microRNA's are gradually getting ground.









Challenges:

1) Detection of a specific nucleotide sequences in biological materials without PCR amplification.

Exploitation of natural amplification of DNA and RNA sequences for electrochemical analysis of DNA and RNA.

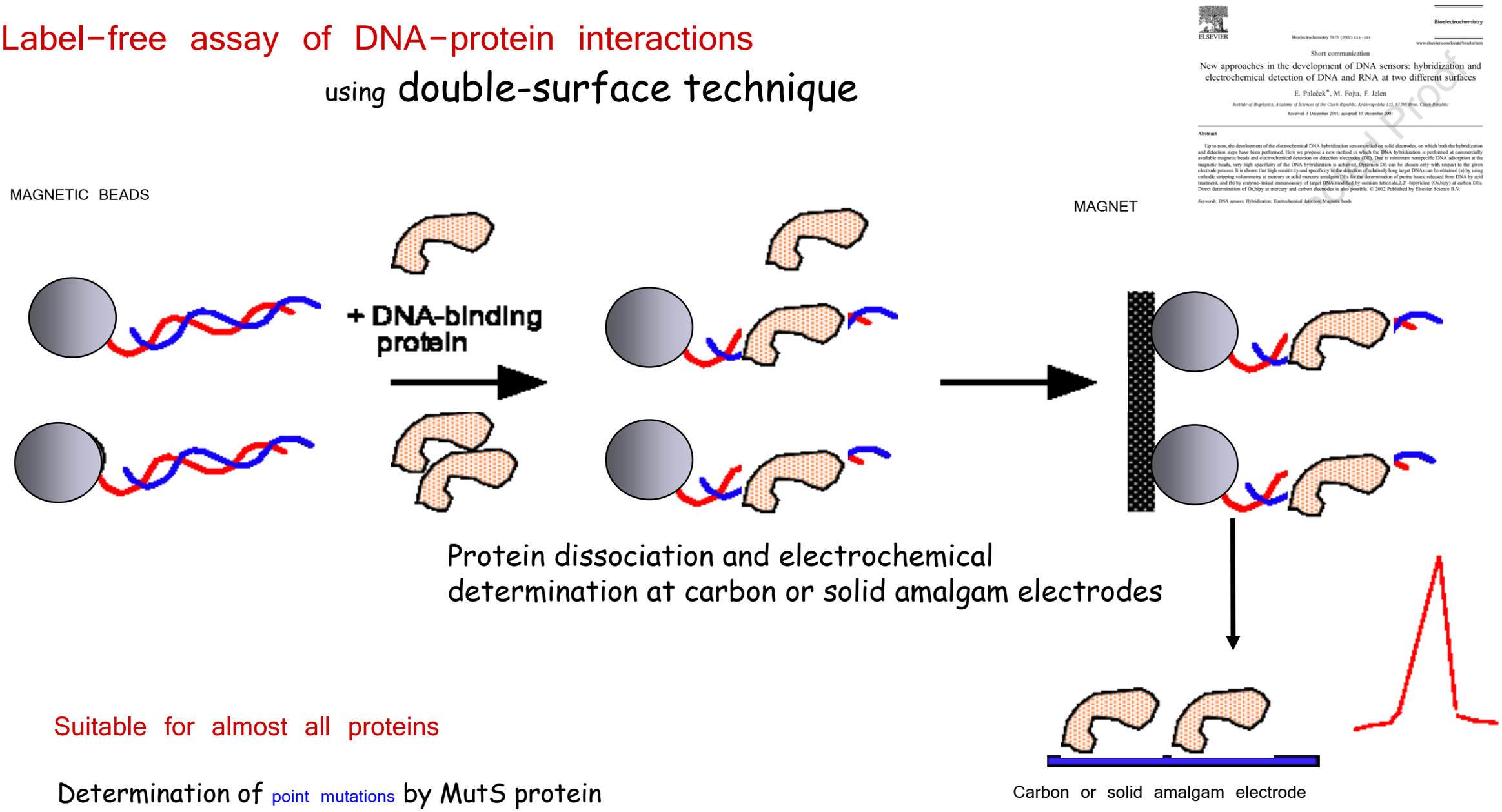
High sensitivity (signal amplification) and specificity (elimination of non-specific interactions) of the analysis is required.

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

DNA analysis in complex biological media

About 10 years ago no electrochemical methods were available for this purpose. In 2001 we proposed the double-surface method, in which the hybridization is performed at one surface (optimized for hybridization and including a separation step) and electrochem. determination at another surface (detection electrode). Later we applied this method for DNA-protein interaction studies.

Label-free assay of DNA-protein interactions using double-surface technique



Bioelectrochemistry

Bioelectrochemistry 56(73 (2002) xxx–xxx

www.elsevier.com/locate/bioelechem

Short communication

New approaches in the development of DNA sensors: hybridization and electrochemical detection of DNA and RNA at two different surfaces

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Received 3 December 2001; accepted 10 December 2001

Abstract

Up to now, the development of the electrochemical DNA hybridization sensors relied on solid electrodes, on which both the hybridization and detection steps have been performed. Here we propose a new method in which the DNA hybridization is performed at commercially available magnetic beads and electrochemical detection on detection electrodes (DE). Due to minimum nonspecific DNA adsorption at the magnetic beads, very high specificity of the DNA hybridization is achieved. Optimum DE can be chosen only with respect to the given electrode process. It is shown that high sensitivity and specificity in the detection of relatively long target DNAs can be obtained (a) by using cathodic stripping voltammetry at mercury or solid mercury amalgam DEs for the determination of purine bases, released from DNA by acid treatment, and (b) by enzyme-linked immunoassay of target DNA modified by osmium tetroxide,2,2′-bipyridine (Os_2bipy) at carbon DEs. Direct determination of Os_2bipy at mercury and carbon electrodes is also possible. © 2002 Published by Elsevier Science B.V.

Keywords: DNA sensors; Hybridization; Electrochemical detection; Magnetic beads

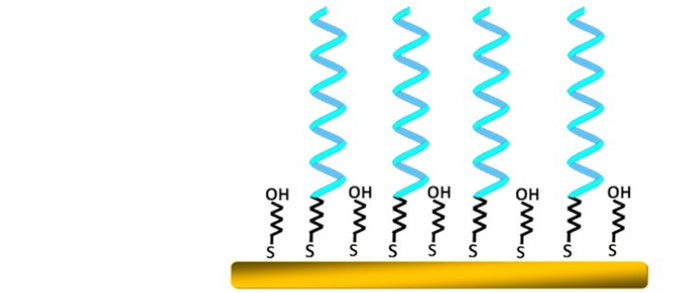
Suitable for almost all proteins

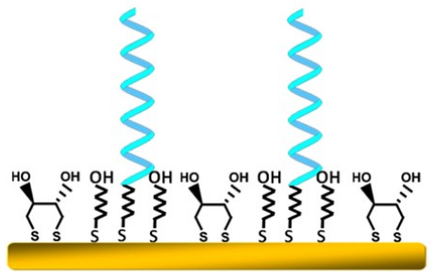
Determination of point mutations by MutS protein

Paleček, E. et al. (2004). "Sensitive electrochemical determination of unlabeled MutS protein and detection of point mutation in DNA." ***Anal. Chem***. **76**(19): 5930-5936.

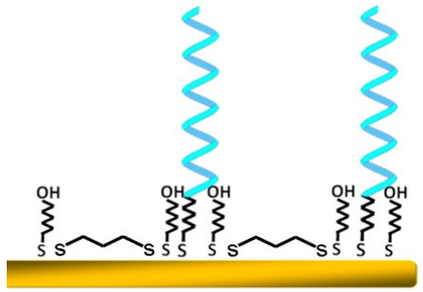
Back to single-surface technologies ....

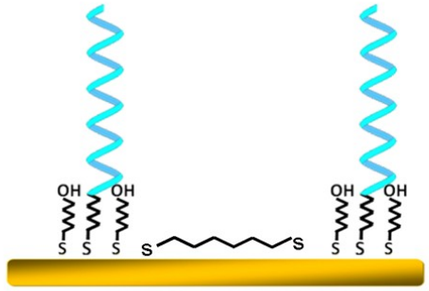
Better shielding of Au electrode surfaces is necessary for DNA analysis in biological materials

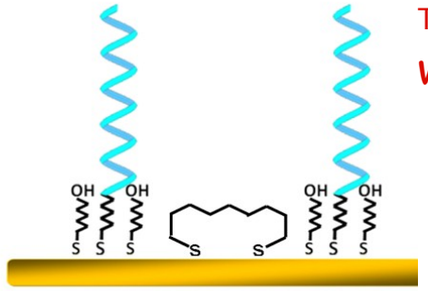




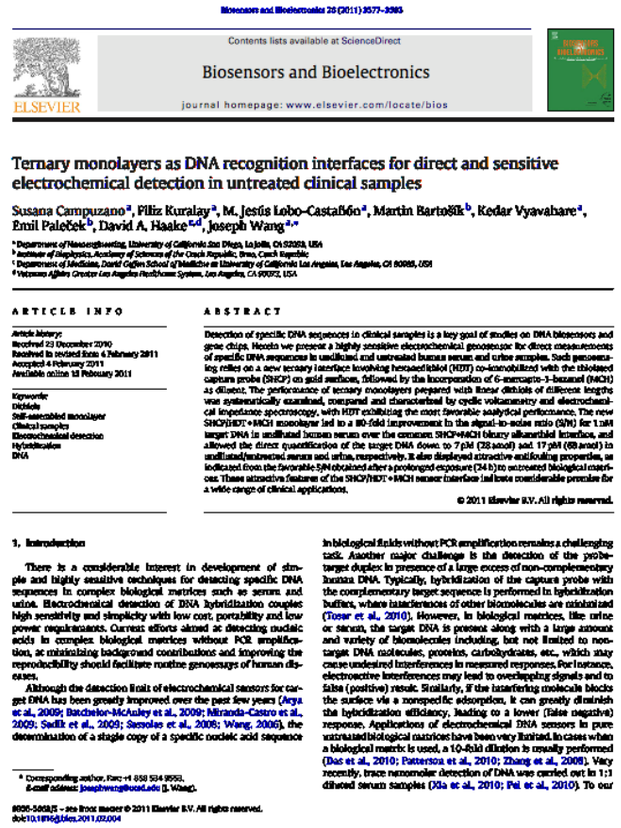
Binary layers







Ternary layers with dithiols



Biosensors and Bioelectronics 26 (2011) 3577–3583

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Ternary monolayers as DNA recognition interfaces for direct and sensitive electrochemical detection in untreated clinical samples

Susana Campuzano[a], Pilliz Kuralay[a], M. Jesús Lobo-Castañón[a], Martin Bartošík[b], Kedar Vyavahare[a], Emil Paleček[b], David A. Haake[c,d], Joseph Wang[a,*]

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[b] Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
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Hybridization
DNA

ABSTRACT

Detection of specific DNA sequences in clinical samples is a key goal of studies on DNA biosensors and gene chips. Herein we present a highly sensitive electrochemical genosensor for direct measurements of specific DNA sequences in undiluted and untreated human serum and urine samples. Such genosensing relies on a new ternary interface involving hexanedithiol (HDT) co-immobilized with the thiolated capture probe (SHCP) on gold surfaces, followed by the incorporation of 6-mercapto-1-hexanol (MCH) as diluent. The performance of ternary monolayers prepared with linear dithiols of different lengths was systematically examined, compared and characterized by cyclic voltammetry and electrochemical impedance spectroscopy, with HDT exhibiting the most favorable analytical performance. The new SHCP/HDT+MCH monolayer led to a 80-fold improvement in the signal-to-noise ratio (S/N) for 1 nM target DNA in undiluted human serum over the common SHCP+MCH binary alkanethiol interface, and allowed the direct quantification of the target DNA down to 7 pM (28 amol) and 17 pM (68 amol) in undiluted/untreated serum and urine, respectively. It also displayed attractive antifouling properties, as indicated from the favorable S/N obtained after a prolonged exposure (24 h) to untreated biological matrices. These attractive features of the SHCP/HDT+MCH sensor interface indicate considerable promise for a wide range of clinical applications.

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1. Introduction

There is a considerable interest in development of simple and highly sensitive techniques for detecting specific DNA sequences in complex biological matrices such as serum and urine. Electrochemical detection of DNA hybridization couples high sensitivity and simplicity with low cost, portability and low power requirements. Current efforts aimed at detecting nucleic acids in complex biological matrices without PCR amplification, at minimizing background contributions and improving the reproducibility should facilitate routine genoassays of human diseases.

Although the detection limit of electrochemical sensors for target DNA has been greatly improved over the past few years (Arya et al., 2009; Bandyopadhyay-McAuley et al., 2009; Miranda-Castro et al., 2009; Sadik et al., 2009; Sassolas et al., 2008; Wang, 2006), the determination of a single copy of a specific nucleic acid sequence in biological fluids without PCR amplification remains a challenging task. Another major challenge is the detection of the probe-target duplex in presence of a large excess of non-complementary human DNA. Typically, hybridization of the capture probe with the complementary target sequence is performed in hybridization buffers, where interferences of other biomolecules are minimized (Tosar et al., 2010). However, in biological matrices, like urine or serum, the target DNA is present along with a large amount and variety of biomolecules including, but not limited to non-target DNA molecules, proteins, carbohydrates, etc., which may cause undesired interferences in measured responses. For instance, electroactive interferences may lead to overlapping signals and to false (positive) result. Similarly, if the interfering molecule blocks the surface via a nonspecific adsorption, it can greatly diminish the hybridization efficiency, leading to a lower (false negative) response. Applications of electrochemical DNA sensors in pure untreated biological matrices have been very limited. In cases when a biological matrix is used, a 1Q-fold dilution is usually performed (Das et al., 2010; Patterson et al., 2010; Zhang et al., 2008). Very recently, trace nanomolar detection of DNA was carried out in 1:1 diluted serum samples (Xia et al., 2010; Pei et al., 2010). To our

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E-mail address: josephwang@ucsd.edu (J. Wang).

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doi:10.1016/j.bios.2011.02.004

… new ternary interface involving hexanedithiol (HDT) co-immobilized with the thiolated capture probe (SHCP) on gold surfaces, followed by the incorporation of 6-mercapto-1-hexanol (MCH) as diluent. The new SHCP/HDT+MCH monolayer led to a 80-fold improvement in the signal-to-noise ratio (S/N) for 1 nM target DNA in undiluted human serum over the common SHCP+MCH binary alkanethiol interface, and allowed the direct quantification of the target DNA down to 7 pM (28 amol) and 17pM (68 amol) in undiluted/untreated serum and urine, respectively.

University of California, San Diego in La Jolla

Department of Nanotechnology, Prof. Joseph Wang

Atkinson Hall, UCSD

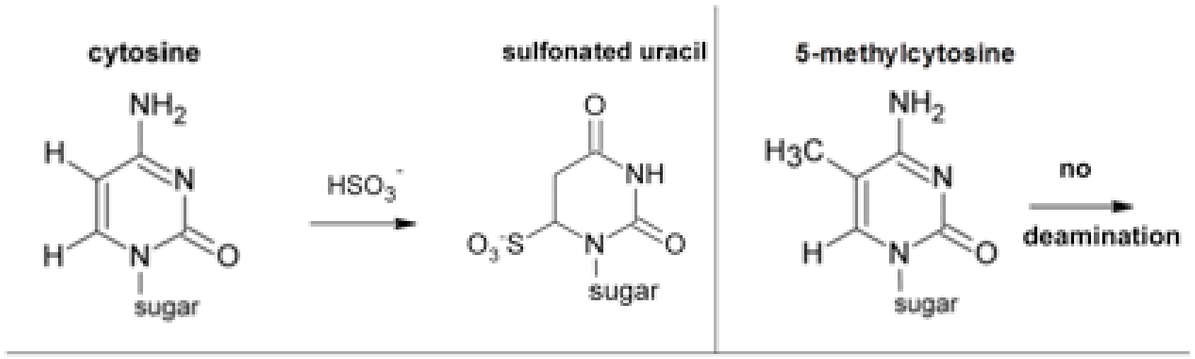
Bioengineering Hall, UCSD

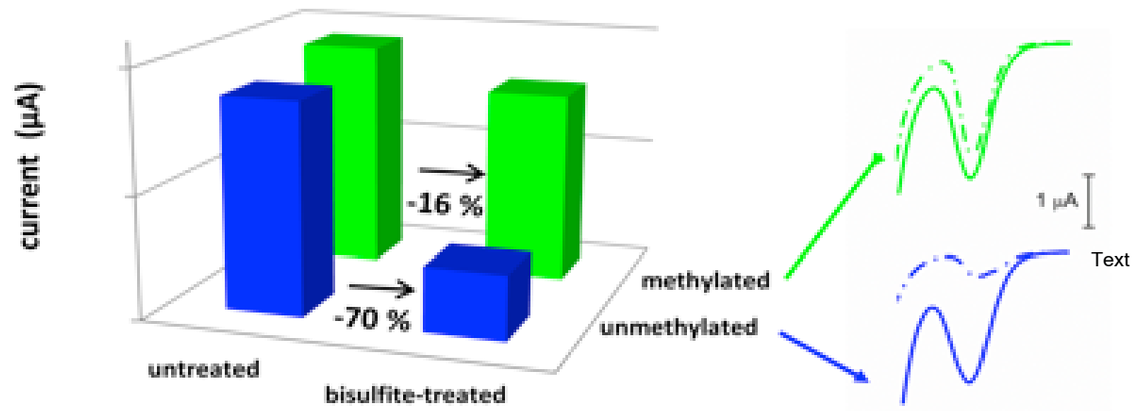
UCSD Campus

View from hotel

San Diego Sea World

Detection of 5-methylcytosine in bisulfite-treated DNA at Hg electrodes





DNA methylation is an important epigenetic event playing crucial roles in physiologic and pathologic processes. Methylation of cytosine (C) residues in DNA can be easily detected using Hg or solid amalgam electrodes.

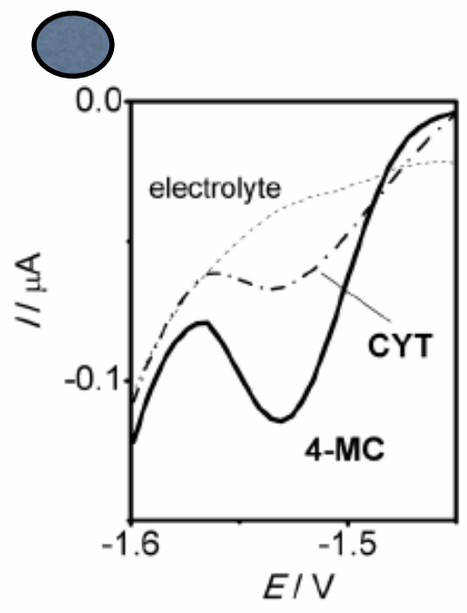
Reduction peaks of untreated single-stranded methylated and unmethylated oligodeoxynucleotides do not significantly differ. Using DNA bisulfite treatment, reducible C's are transformed into nonreducible uracil residues, strongly decreasing C reduction peaks. On the other hand, 5-methylcytosine (mC) residues resist the bisulfite treatment and display almost unchanged reduction peak.

Adenine, cytosine and guanine residues in NAs are reducible at Hg electrodes while thymine and URACIL are INACTIVE

By combining DNA bisulfite treatment with square wave voltammetry, DNA methylation can be determined quantitatively at nanomolar and subnamolar DNA concentrations.

The analysis can be done either at HMDE or

at solid amalgam electrodes



500 pM, tA 300 s

M Bartošík, M Fojta, E Paleček

Electrochim. Acta 78 (2012) 75– 81

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 of its analysis but miniaturization of electrodes decreases these volumes to nL.
Sensitivity of the analysis has greatly increased in recent years.

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.

Izolace DNA - 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 konce. (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 odstranění bílkovin: vysoká konc. solí, detergent, $CHCl_3$- isoamyl, emulsifikace, proteasy a fenolová extrakce. $CHCl_3$-opakované třepání, degradace; lepší je fenol - DNA o m.v. blízké celému chromosomu *E.coli* ($\sim 10^9$) - nebezpečí znečištění fenolu peroxidy (destilace).

Isolace DNA z bakteriofága

a) purifikace fága diferenční centrifugací a/nebo v grad CsCl

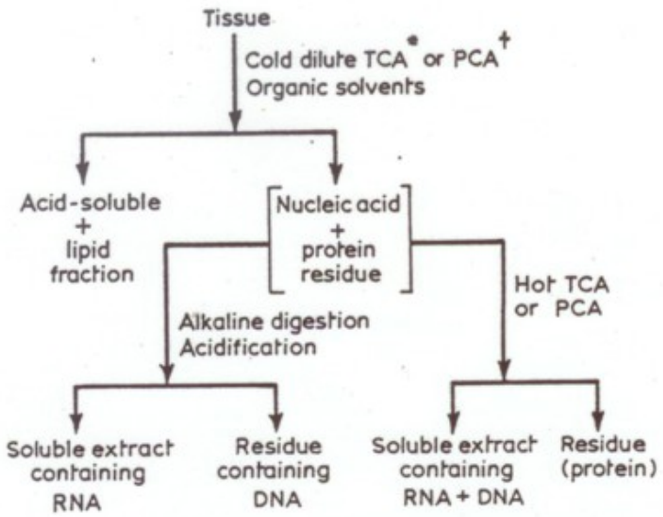
b) deproteinace (většinou fenolem)

Dnes nejčastěji je používana plasmidová DNA.

Stupeň čistoty a volba metody izolace jsou velmi závislé na účelu, 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)

IZOLACE DEGRADOVANÝCH NA



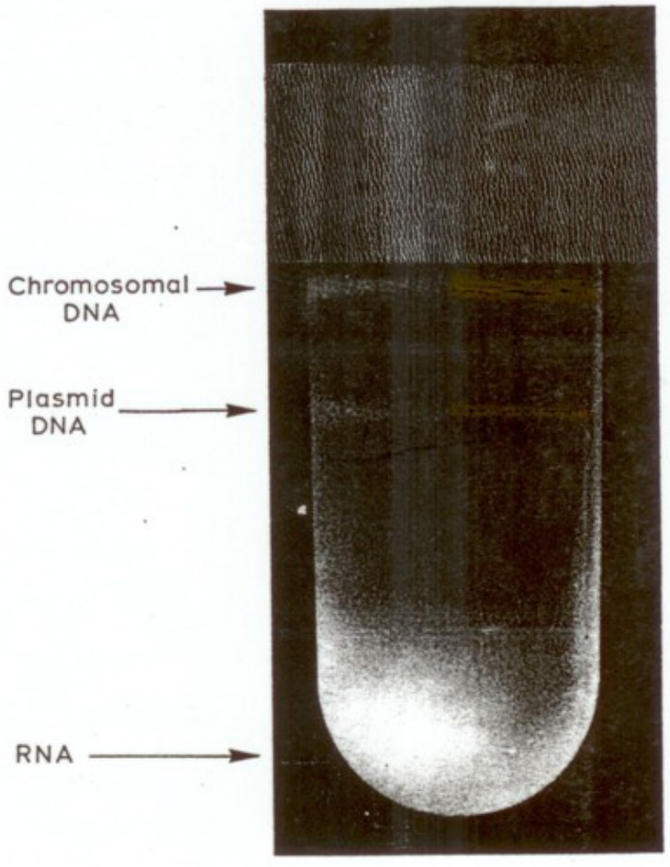
Extraction and fractionation of nucleic acids from tissues. *TCA – trichloroacetic acid, †PCA – perchloric acid.

IZOLACE INTAKTNÍ DNA

J. Marmur

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

Plasmidová DNA



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†

J. MARMUR‡

Department of Chemistry, Harvard University, Cambridge, Massachusetts, U.S.A.

(*Received 6 December 1960*)

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

Characterize your DNA sample:

ds x ss, circular x linear

circular: nicked, oc; covalently closed, cc, cd

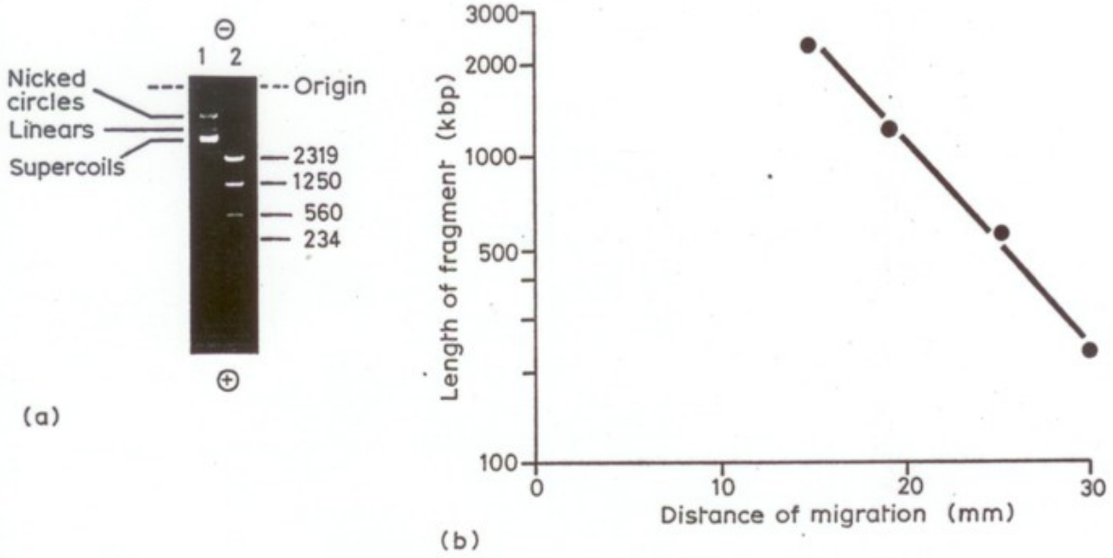
linear: cohesive or blunt ends

number of base pairs, ssb

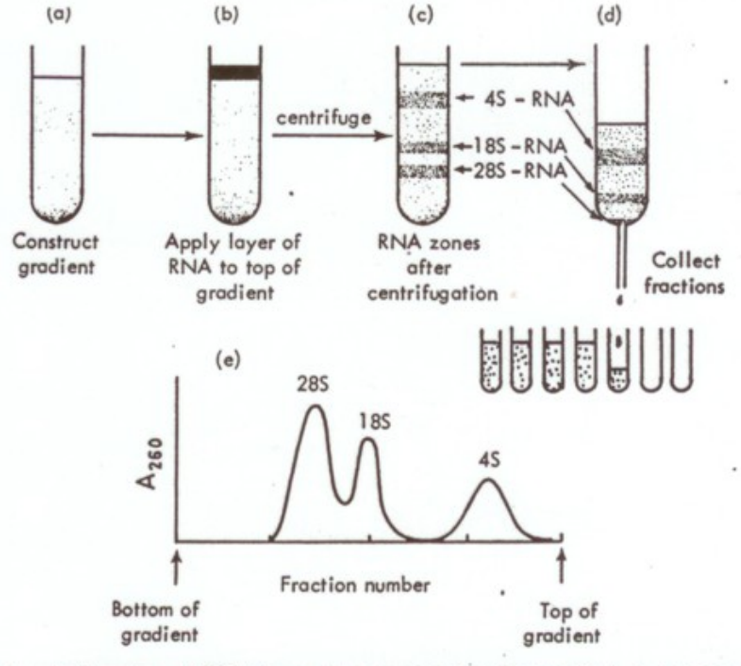
Unusual bases, DNA methylation

purity: protein, RNA .... content

analytical methods



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.

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)

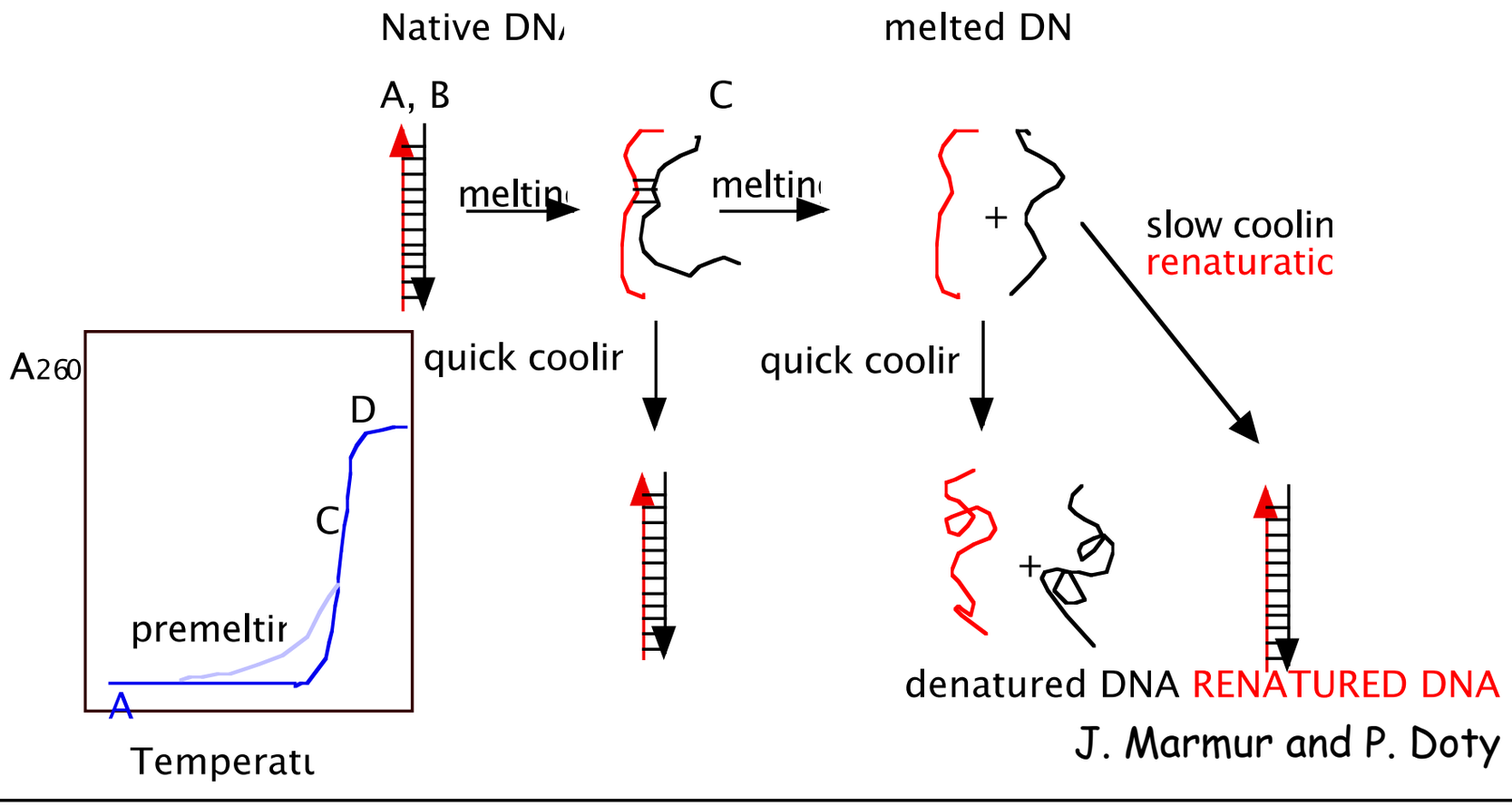
- představují **jediný známý způsob zajišťující specificitu párování bazí**. Jsou tedy součástí mechanismu jímž DNA realizuje svoji biologickou funkci. Zpočátku se o nich soudilo, že jsou nejdůležitějším činitelem pro stabilitu dvojité šroubovice; experimentálně i teoreticky bylo však dokázáno, že tomu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

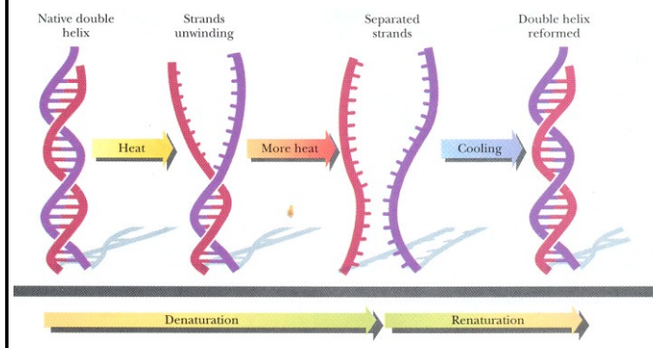
d) Hydrofobní síly

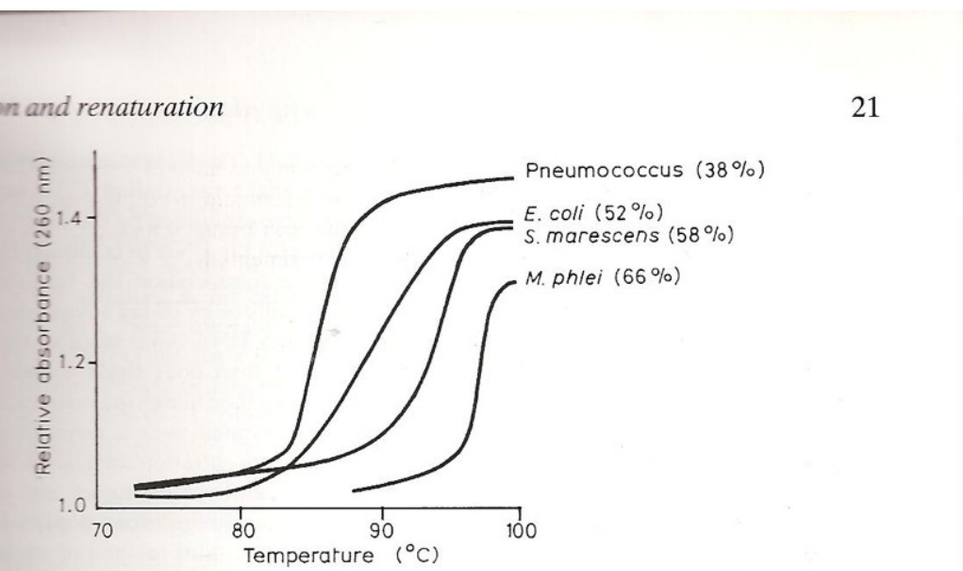
- tento termín se týká **stability dvoušroubovicové DNA plynoucí z její architekrury**: **polární skupiny** jsou **na povrch**u, zatímco **hydrofobní baze** jsou **uvnitř** molekuly a mají větší tendenci interagovat mezi sebou nežli s molekulami vody. Toto uspořádání **stabilizuje** tedy dvoušroubovicovou 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







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

By J. Marmur and D. Lane

CONANT LABORATORY, DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY

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.

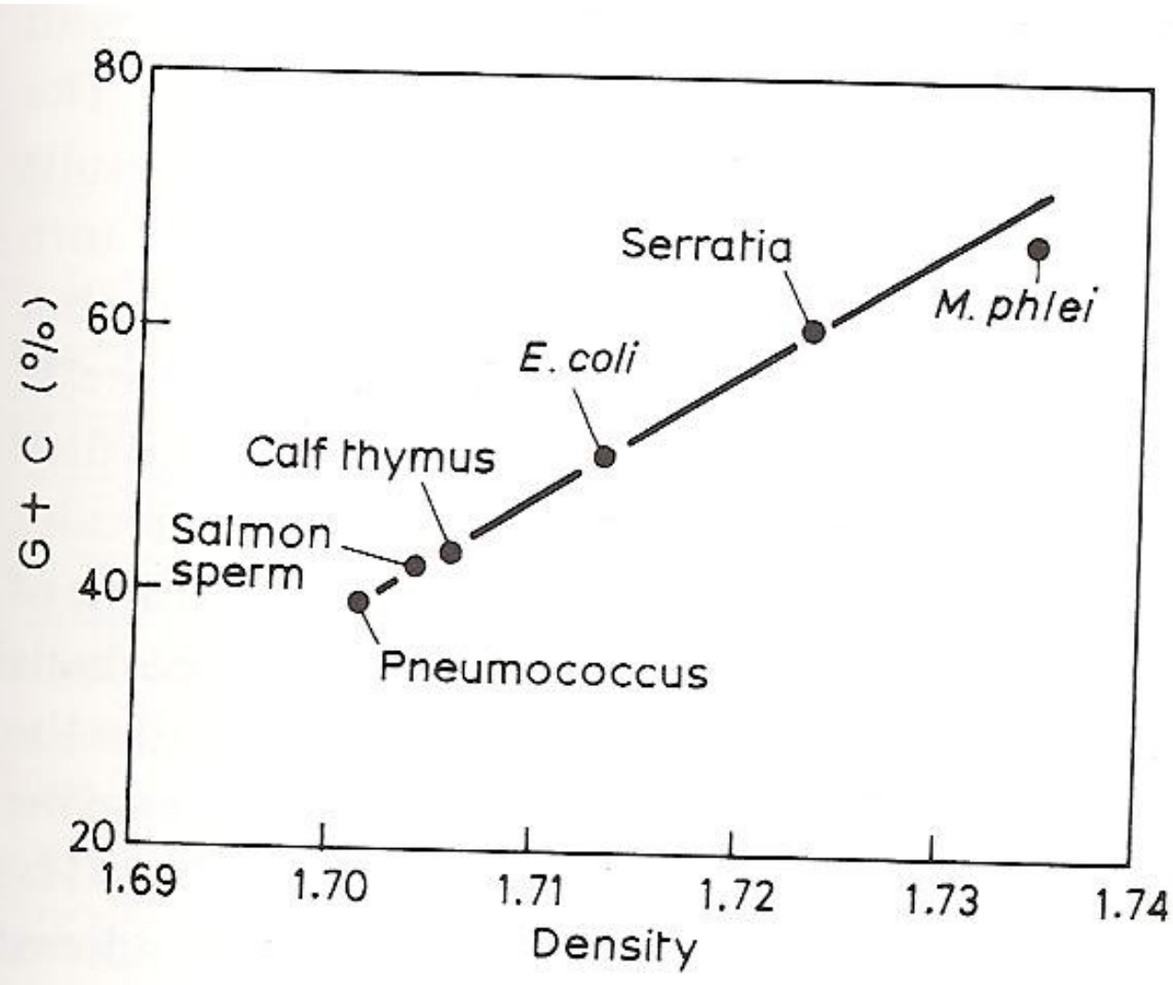


Fig. 2.21 Relationship of density to content of guanine plus cytosine in DNAs from various sources [64].

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

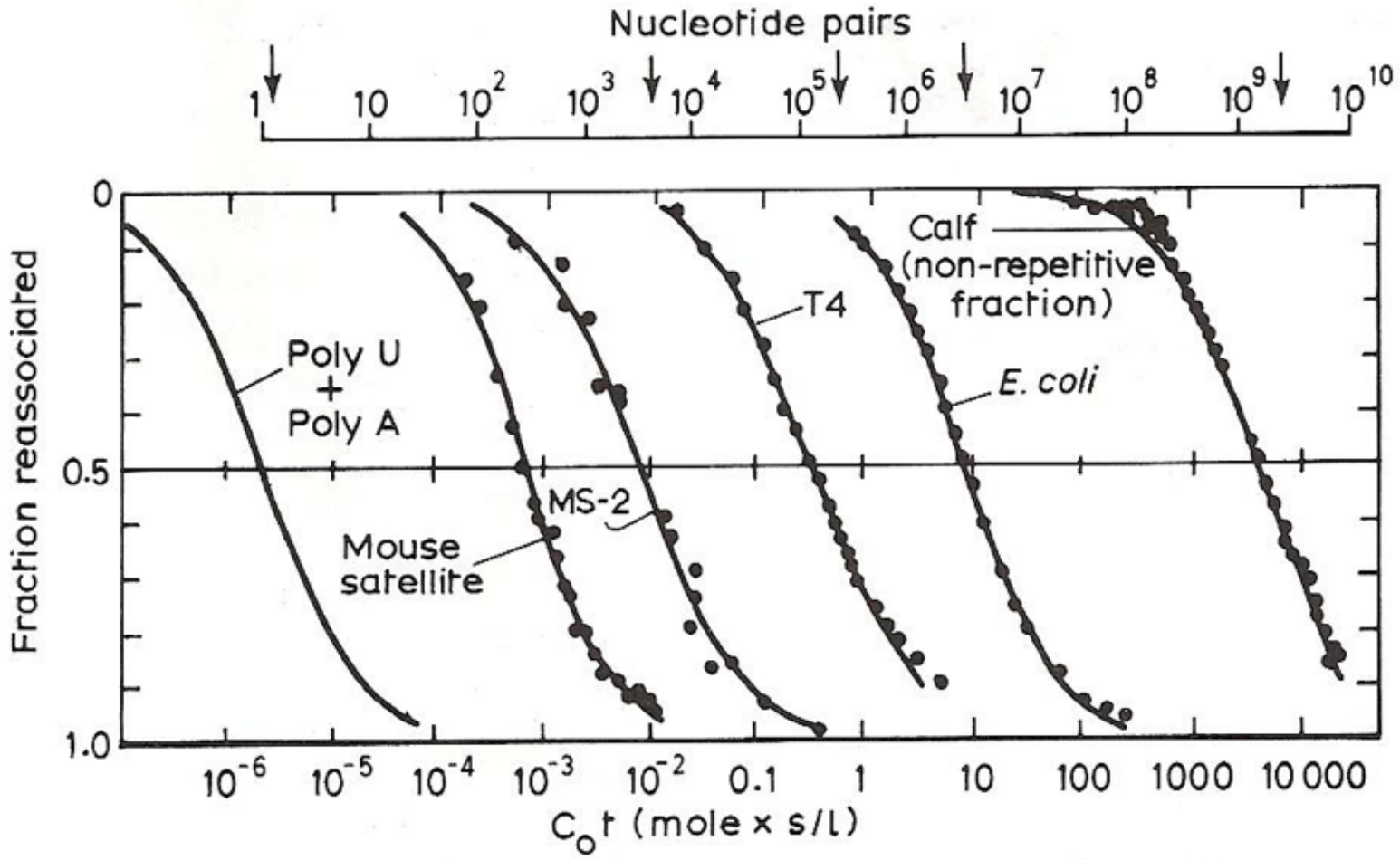


Fig. 2.20 The rate of reassociation of double-stranded polynucleotides from various sources showing how the rate decreases with the complexity of the organism and its genome (from [60]).

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_0t value of DNA is defined as the initial concentration C_0 in moles nucleotides per Litre multiplied by time t in seconds. C_0t reflects complexity of DNA. Methods: S1, hydroxyapatite - dsDNA binds more strongly

Biosyntetické polynukleotidy -

modely pro výzkum fyzikálních a chemických vlastností a struktury nukleových kyselin

Důležité modely vlivu sekvence nukleotidů na vlastností DNA

POLYRIBONUKLEOTIDY

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

nukleosid-difosfáty

nevyžaduje primer ani matrici

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.

nukleosid-trifosfáty

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

Homopolynukleotidy

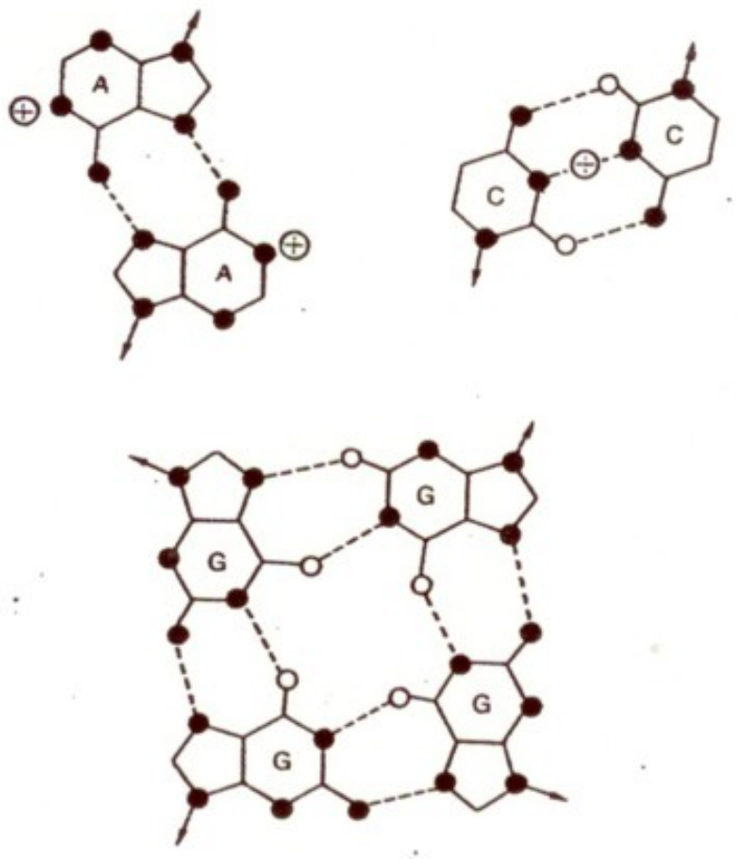
Poly(U) a poly(dT) při pokojové teplotě mají málo výraznou sekundární strukturu, při vyšší teplotě tuto strukturu ztrácejí

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 podminek) 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)

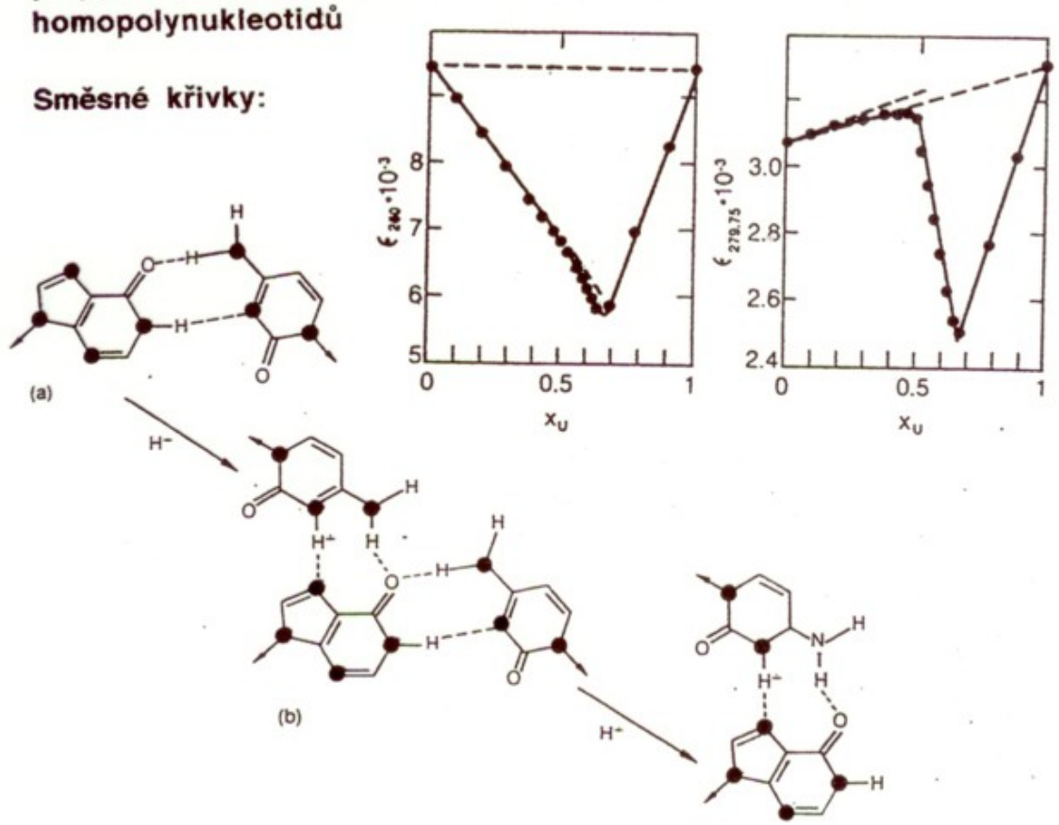
Tyto interakce jsou silně závislé na iontové síle

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:



Lokální struktury DNA a metody jejich analýzy

Critical Reviews in Biochemistry and Molecular Biology, 26(2):151–226 (1991)

Local Supercoil-Stabilized DNA Structures

E. Paleček

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Referee: James E. Dahlberg, Dept. of Physiological Chemistry, 587 Med. Sci. Bldg., University of Wisconsin, 1300 University Ave., Madison, WI 53706

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 nadšroubovi 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 samples with extremes of base composition, however, Bram[12] was able to predict an almost infinite polymorphy of DNA in the B state. At about the same time, Pohl and Jovin[14,15] obtained circular dichroism (CD) spectra of poly(dG-dC)·poly(dG-dC), which suggested that this polynucleotide at high salt concentrations assumes a structure differing from B-DNA and possibly left-handed.

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

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

POLYMORFIE DVOJITÉ ŠROUBOVICE DNA

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

Polymorphy of the DNA double helix

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

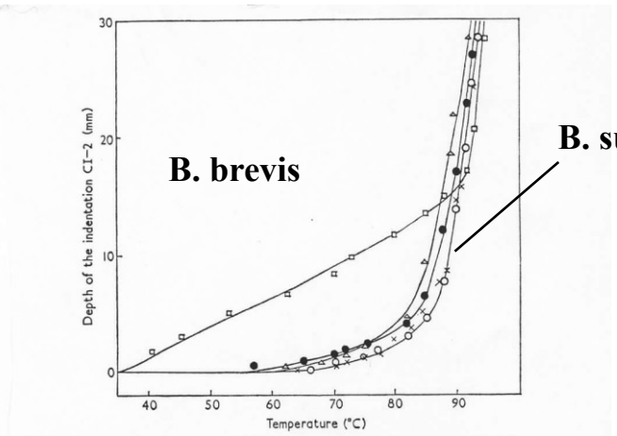

FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 μg/ml. in 0·25 M-ammonium formate plus 0·025 M-sodium phosphate (pH 7·0).
—●—●—, *B. subtilis* 168; —×—×—, *B. natto*; —○—○—, *B. subtilis* var. *niger*; —△—△—, *B. subtilis* var. *aterrimus*; —□—□—, *B. brevis* (ATCC 9999).
P 524 polaroscope, 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

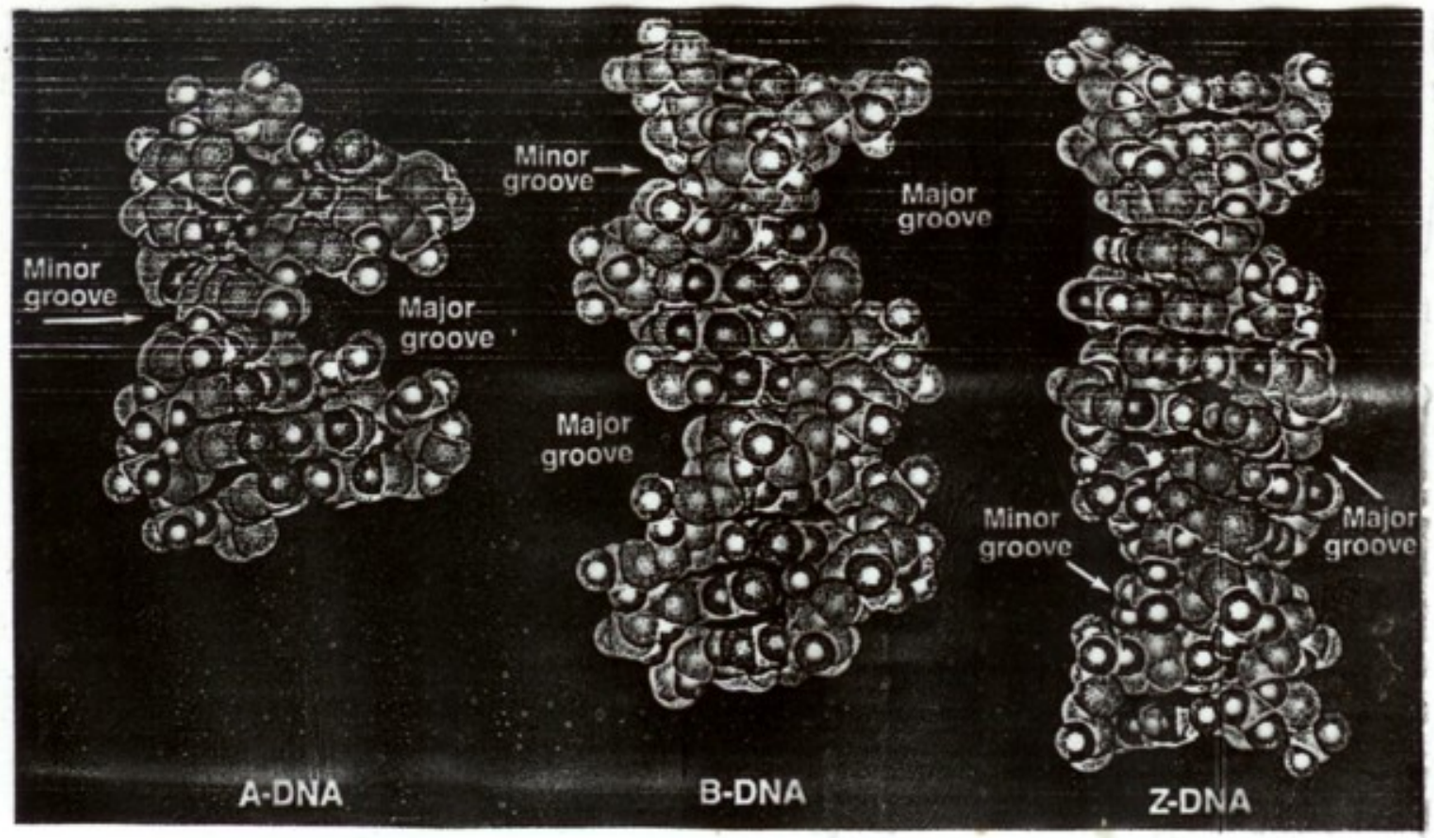
6. POLYMORPHY OF DNA SECONDARY STRUCTURE

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

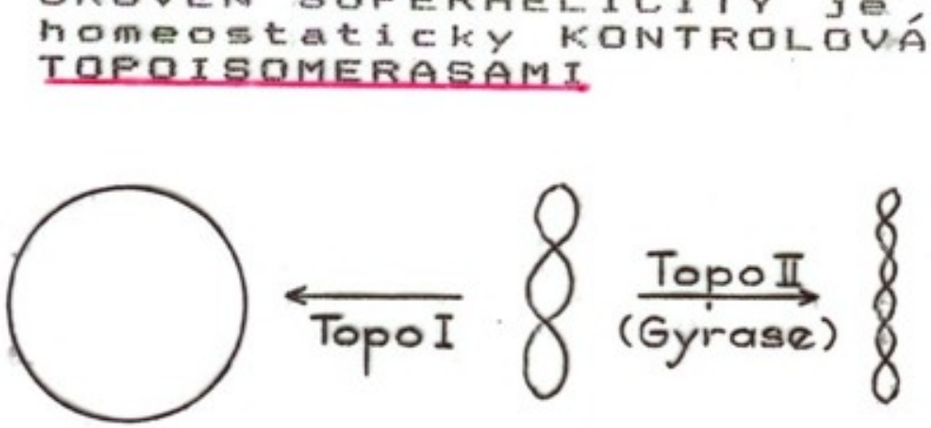


DNA structures from X-ray crystal analysis

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



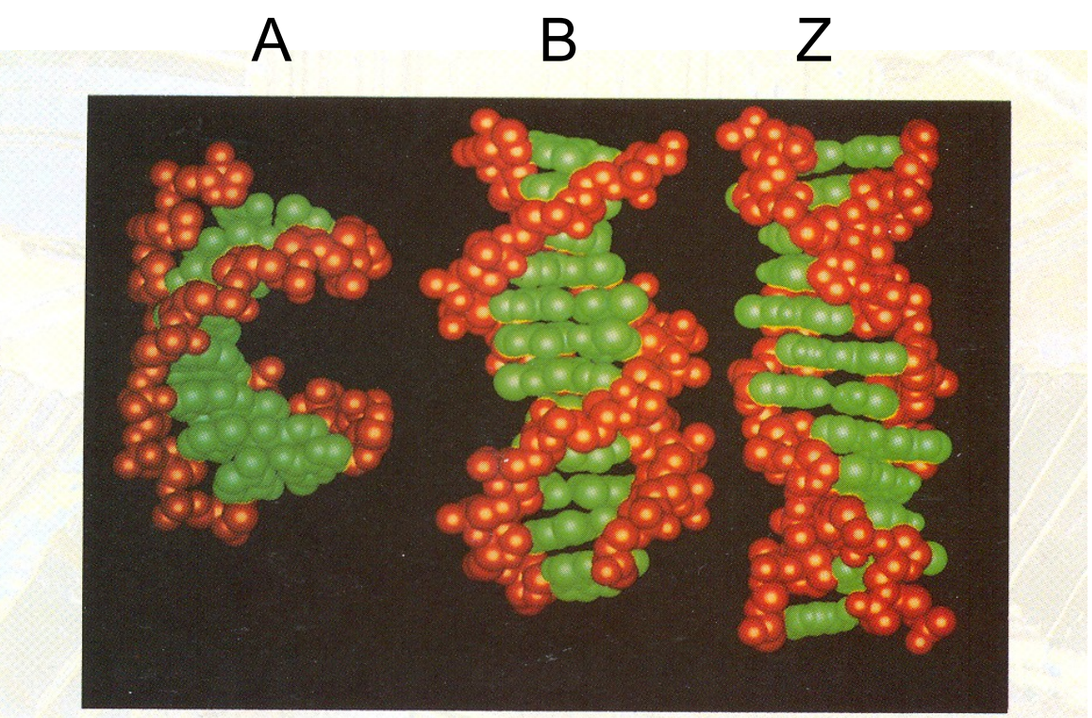
SUPERHELICITA DNA OVLIVŇUJE základní biochemické děje jako TRANSKRIPCI,

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.



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 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.