2. PŘEDNÁŠKA 7.10. 2013

....ještě doplněk scientometrie

Index h

Index h navrhl Jorge **Hirsch**, University of California, San Diego
(Nature 436 (2005) 900)

je to číslo, ktere udává počet n prací (určitého autora, instituce, apod.) které byly citovány nejméně n-krát

Impact Factor (IF) charakterizuje průměrnou citovanost prací publikovaných v daném časopise

The journal **impact factor** is a measure of the frequency with which the "average article" in a journal has been cited in a particular year. The impact factor will **help you evaluate a journal's relative importance,** especially when you compare it to others **in the same field**.

NOTE: Title changes and coverage changes may result in no impact factor for one or more years.

Impact factor 2011

Cites in 2011 to articles published in: 2010 = 100 2009 = 132 Sum: 232

Number of articles published in: 2010 = 31 2009 = 22 Sum: 53

Calculation:

Cites to recent articles: 232 **IF = 4.377**

Number of recent articles: 53

Problémy časopisů s nízkým IF

1. Nedostatek vysoce kvalifikovaných recenzentů a vyšší pravděpodobnost publikace nekvalitních prací (záleží na šíři problematiky/scope of the journal)
2. Slabé ocenění publikovaných prací při evaluacích a financování výzkumu
3. Menší zájem čtenářů
4. Nestabilita IF atd.

Hrátky s IF

Vybráno z Nature:
The Swiss journal **Folia Phoniatrica et Logopaedica** has a **good reputation among voice researchers** but, with an **impact factor of 0.655 in 2007**, publication in it was unlikely to bring honour or grant money to the authors' institutions.
Now two investigators, one Dutch and one Czech, have taken on the system and fought back.
They published a paper called:

'Reaction of Folia Phoniatrica et Logopaedica on the current trend of impact factor measures'
(H. K. Schutte and J. G. Švec Folia Phoniatr. Logo. 59, 281-285; 2007).

This cited all the papers published in the journal in the previous two years. As 'impact factor' is defined as the number of citations to articles in a journal in the past two years, divided by the total number of papers published in that journal over the same period, their strategy dramatically **increased Folia's impact factor this year to 1.439.**

ALE

San Francisco Declaration on Research Assessment

Putting science into the assessment of research

There is a pressing need to improve the ways in which the output of scientific research is evaluated by funding agencies, academic institutions, and other parties.

To address this issue, the group of editors and publishers of scholarly journals listed below met during the Annual Meeting of The American Society for Cell Biology (ASCB) in **San Francisco, CA, on December 16, 2012**. The group developed a set of recommendations, referred to as the ***San Francisco Declaration on Research Assessment***. We invite interested parties across all scientific disciplines to indicate their support by adding their names to this declaration.

The Journal Impact Factor is frequently used as the primary parameter with which to compare the scientific output of individuals and institutions. The Journal Impact Factor, as calculated by Thomson Reuters, was originally created as a tool to help librarians identify journals to purchase, not as a measure of the scientific quality of research in an article. With that in mind, it is critical to understand that the Journal Impact Factor has a **number of well-documented deficiencies** as a tool for research assessment. These limitations include: **A**) **citation distributions within journals are highly skewed** [1-3]; **B**) the properties of the Journal Impact Factor are **field-specific**: it is a composite of multiple, highly diverse article types, including primary research papers and reviews [1, 4]; C) Impact Factors can be **manipulated** (or “gamed”) **by editorial policy** [5]; and D) **data** used to calculate the Journal Impact Factors are **neither transparent nor openly available** to the public [4, 6, 7].

IF je pouze předběžný údaj o významu určité práce

DŮLEŽITĚJŠÍ je CITOVANOST DANÉ PRÁCE v určitém OBORU

Impaktní faktory (IF) a citační ohlasy

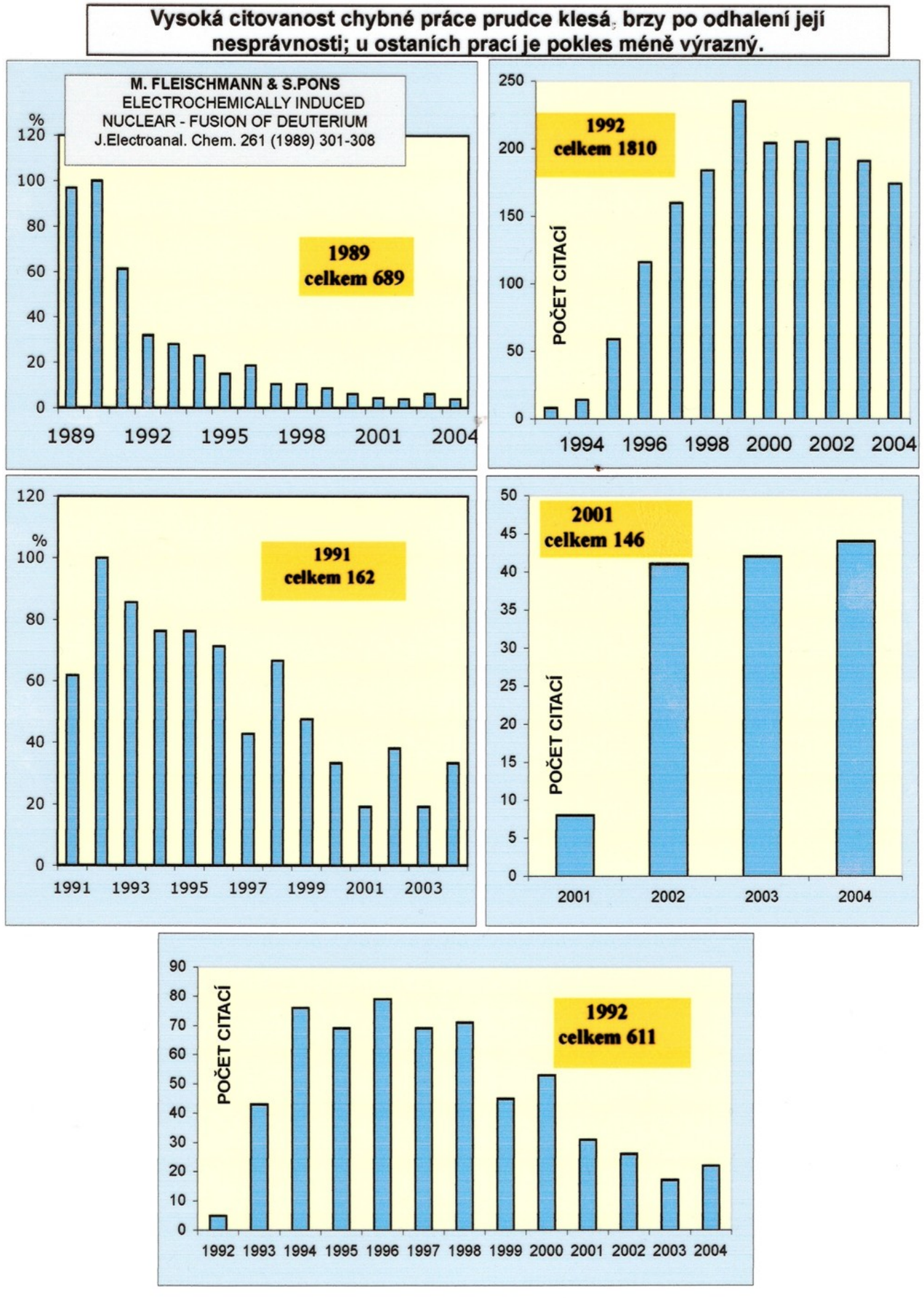
jsou významnými scientometrickými údaji. Tyto údaje jsou zvláště důležité při hodnocení institucí a výkonnosti vědy ve státech a regionech. Při hodnocení jednotlivců a týmů mohou prosté součty citací či IF vést k nesprávným závěrům.

IF reflektují citovanost průměrné práce v daném časopise – vyjadřují tedy naději, že publikovaná práce získá určitý počet ohlasů. Po určitém čase lze posoudit zda skutečné citační ohlasy této naději odpovídají.

Počet citací je dobrým vodítkem pro předběžný odhad (screening) kvality práce vědců v dané vědecké kategorii. Proti jeho využití při hodnocení jednotlivců se ozývají námitky jako např.:

1. Práce je silně citována proto, že byla **kritizována pro závažnou chybu**
2. Spoluator získal spoustu citací, ale jeho zásluhy jsou nepatrné, protože (a) práce byla provedena v době hostováni spoluautora **ve špičkovém zahraničním kolektivu,** kde jeho příspěvek spočíval např. pouze ve schopnosti spolehlivě a rychle pipetovat; (b) kolektiv byl tvořen **příliš velkým počtem autorů**
3. Autor hojně publikuje a **cituje především sám sebe.** Tato námitka je bezpředmětná u prací, které jsou silně citovány.
4. Citace jsou **dokladem minulosti autora**, ale neříkají nic o jeho současných kvalitách
5. Citovaná práce je **přehledný článek** neobsahující (?) žádné nové poznatky
6. Autor **svým objevem předběhl dobu** – jako příklad je často uváděn G J Mendel

Problémy spojené s námitkami pod 1.-5. **lze řešit pomocí citační analýzy**. **V případě 6.** nezbývá než autora politovat nebo mu závidět jeho pozdější slávu. Vhodným řešením může být vstup do kláštera, kde mu/jí budou zajištěny dobré podmínky pro vědeckou práci.



IF, počty citací a další scientometrické údaje by neměly být využívany ke srovnávání úspěšnosti vědců z různých oborů

MATHEMATICS

Search Results -- Summary

\U=(CSORGO S)
)ocType=All document types; Language=All languages; Databases=SCI-EXPANDED, SSCI, A&HCI; Timespan=1980-005

Search within results: Enter a topic SEARCH

73 results found (Set #1) Go to Page: 1 of 8 GO

Records 1 -- 10 [1 | 2 | 3 | 4 | 5 | 6 | 7 | 8]

Use the checkboxes to select individual records for marking, then click Submit to add them to the Marked List.

1. CSORGO M, **CSORGO S**, HORVATH L, et al.
   WEIGHTED EMPIRICAL AND QUANTILE PROCESSES
   ANNALS OF PROBABILITY 14 (1): 31-85 JAN 1986
   Times Cited: 82
2. **CSORGO S**, DEHEUVELS P, MASON D
   KERNEL ESTIMATES OF THE TAIL INDEX OF A DISTRIBUTION
   ANNALS OF STATISTICS 13 (3): 1050-1077 1985
   Times Cited: 79
3. BURKE MD, **CSORGO S**, HORVATH L
   STRONG APPROXIMATIONS OF SOME BIOMETRIC ESTIMATES UNDER RANDOM CENSORSHIP
   ZEITSCHRIFT FUR WAHRSCHEINLICHKEITSTHEORIE UND VERWANDTE GEBIETE 56 (1): 87-112 1981
   Times Cited: 60
4. **CSORGO S**
   LIMIT BEHAVIOR OF THE EMPIRICAL CHARACTERISTIC FUNCTION
   ANNALS OF PROBABILITY 9 (1): 130-144 1981
   Times Cited: 54
5. **CSORGO S**, HORVATH L
   THE RATE OF STRONG UNIFORM CONSISTENCY FOR THE PRODUCT-LIMIT ESTIMATOR
   ZEITSCHRIFT FUR WAHRSCHEINLICHKEITSTHEORIE UND VERWANDTE GEBIETE 62 (3): 411-426 1983
   Times Cited: 52
6. **CSORGO S**, HORVATH L
   ON THE KOZIOL-GREEN MODEL FOR RANDOM CENSORSHIP
   BIOMETRIKA 68 (2): 391-401 1981
   Times Cited: 45
7. **CSORGO S**, MASON DM
   CENTRAL LIMIT-THEOREMS FOR SUMS OF EXTREME VALUES
   MATHEMATICAL PROCEEDINGS OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY 98 (NOV): 547-558 1985
   Times Cited: 39
8. BURKE MD, **CSORGO S**, HORVATH L
   A CORRECTION TO AND IMPROVEMENT OF STRONG APPROXIMATIONS OF SOME BIOMETRIC ESTIMATES UNDER RANDOM CENSORSHIP
   PROBABILITY THEORY AND RELATED FIELDS 79 (1): 51-57 1988

Sort by: Times Cited SORT

Mark: [0 articles marked]
Selected records
All records on this page
Records to
SUBMIT
You can print, save, export, e-mail, and order records after adding them to the Marked List. (The list can hold 500 records.)

Analyze Results:
ANALYZE
View rankings and histograms of the authors, journals, etc. for this set of records. (Up to 2,000 records at a time.)

20. 12x

30x

MOL. BIOL. & GENETICS

Search Results -- Summary

\U=(NASMYTH K)
)ocType=All document types; Language=All languages; Databases=SCI-EXPANDED, SSCI, A&HCI; Timespan=1980-005

Search within results: Enter a topic SEARCH

161 results found (Set #2) Go to Page: 1 of 17 GO

Records 1 -- 10 [1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10]

Use the checkboxes to select individual records for marking, then click Submit to add them to the Marked List.

1. SCHWOB E, BOHM T, MENDENHALL MD, et al.
   THE B-TYPE CYCLIN KINASE INHIBITOR P40(SIC1) CONTROLS THE G1 TO S TRANSITION IN SACCHAROMYCES-CEREVISIAE
   CELL 79 (2): 233-244 OCT 21 1994
   Times Cited: 531
2. SHORE D, **NASMYTH K**
   PURIFICATION AND CLONING OF A DNA-BINDING PROTEIN FROM YEAST THAT BINDS TO BOTH SILENCER AND ACTIVATOR ELEMENTS
   CELL 51 (5): 721-732 DEC 4 1987
   Times Cited: 446
3. Michaelis C, Ciosk R, **Nasmyth K**
   Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids
   CELL 91 (1): 35-45 OCT 3 1997
   Times Cited: 408
4. BREEDEN L, **NASMYTH K**
   REGULATION OF THE YEAST HO GENE
   COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY 50: 643-650 1985
   Times Cited: 403
5. BRAND AH, BREEDEN L, ABRAHAM J, et al.
   CHARACTERIZATION OF A SILENCER IN YEAST - A DNA-SEQUENCE WITH PROPERTIES OPPOSITE TO THOSE OF A TRANSCRIPTIONAL ENHANCER
   CELL 41 (1): 41-48 1985
   Times Cited: 364
6. Zachariae W, **Nasmyth K**
   Whose end is destruction: cell division and the anaphase-promoting complex
   GENES & DEVELOPMENT 13 (16): 2039-2058 AUG 15 1999
   Times Cited: 356
7. Cosma MP, Tanaka TU, **Nasmyth K**
   Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter
   CELL 97 (3): 299-311 APR 30 1999
   Times Cited: 350
8. MOLL T, TEBB G, SURANA U, et al.
   THE ROLE OF PHOSPHORYLATION AND THE CDC28 PROTEIN-

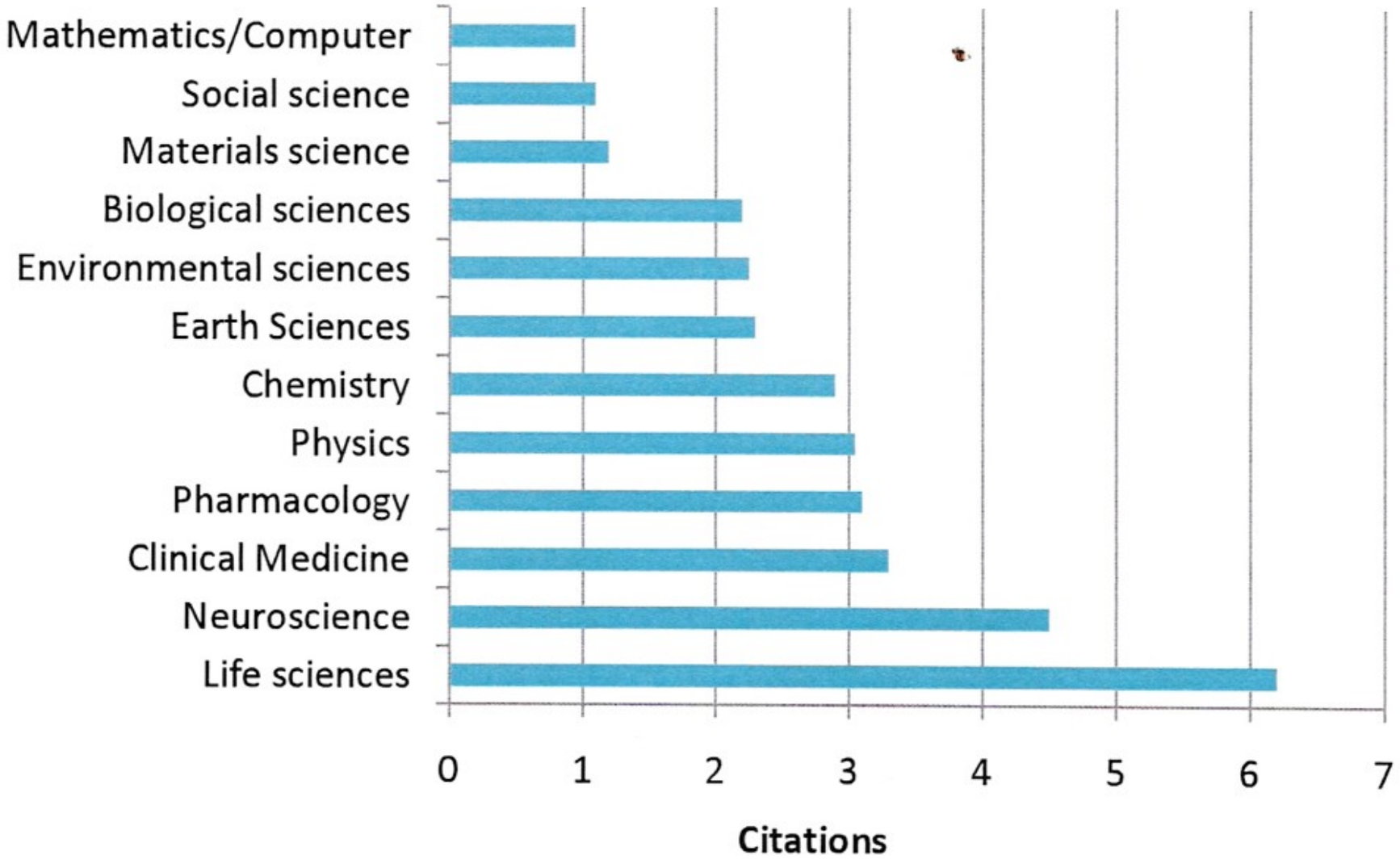
Sort by: Times Cited SORT

Mark: [0 articles marked]
Selected records
All records on this page
Records to
SUBMIT
You can print, save, export, e-mail, and order records after adding them to the Marked List. (The list can hold 500 records.)

Analyze Results:
ANALYZE
View rankings and histograms of the authors, journals, etc. for this set of records. (Up to 2,000 records at a time.)

20. 241x

350x



Average citations per article for different disciplines, showing that citation practices differ markedly. Data from Thompson Scientific [Amin-Mabe 2000].

Příklad analýzy scientometrických dat jednotlivce.

Citace jinými autory x autocitace

1. Do kterého oboru práce spadá

2. Co je hlavním přínosem práce (v době kdy hodnocení probíhá)

3. práce s větším počtem autorů:

a. Kolik členů tým měl a jaké bylo postavení hodnoceného autora v týmu?

b. Byla práce uskutečněna na jeho/jejím pracovišti či jinde – v zahraničí?

c. Čím přispěl(a) hodnocený/á k výsledku?

Analýza zpravidla zahrnuje již určitou míru subjektivity

Vesmír, září 2006

Extra třída bio

Hirschův index a česká věda

neb Domácí realita

irschův index[2] je ze všech indexů hodnotí-ch vědeckou aktivitu nejmladší. Ač zrozen Kalifornii pro hodnocení fyziků, začíná se užívat po celém světě pro hodnocení dal-ch oborů.

V našem předešlém článku (viz Vesmír 81, 08, 2002/9) jsme poukázali na některá úska-užívání celkového počtu citačních ohlasů ací hodnoceného badatele. Výsledky mo-ou být zkresleny například malým počtem lkých „hitů", tedy prací, které se citují řádo-více než ostatní publikace daného autora. lohou to být přehledné nebo metodické prá-, které nemusí věrně vypovídat o skutečné ůrčí aktivitě badatele a jeho dlouhodobém abilním příspěvku k rozvoji oboru.

Právě na tuto skutečnost bere ohled Jorge irsch (fyzik z Kalifornské univerzity v San iegu) ve svém indexu: Vědec má H-index ven *h*, jestliže *h* jeho publikací (z celkové-počtu *N*) bylo citováno nejméně *h*-krát, ostatních *N* – *h* prací je citováno méně než krát. Tedy konkrétně má-li někdo H-index ven 40, znamená to, že každá z jeho 40 nej-tovanějších prací byla dosud citována nej-méně 40krát (nebere se v úvahu, že třeba nej-pší z nich byla citována například 1000krát) zbylé práce (ať jich je dejme tomu 500) jsou továny méně. Jak J. Hirsch uvádí, H-index objektivnější než celkový počet publikací ebo celkový počet citací, protože lépe cha-akterizuje široký dopad práce daného vědce. adatelé s vysokým H-faktorem velmi prav-ěpodobně značně přispívají k rozvoji své isciplíny, protože produkují mnoho hodně tovaných prací.

Důležité je i to, že H-index nelze snadno kreslit samocitacemi. Přitom se dá velmi jed-oduše určit prostým seřazením prací autora odle počtu jejich citací, což lze udělat oprav-u snadno s využitím citační databáze Web f Science (WOS), resp. Web of Knowledge rmy ISI. Samozřejmě se i zde uplatňuje vliv utorova věku (badatelé pracující v oboru dé-by měli mít vyšší index) a vliv badatelova boru (citovanost průměrné práce v molekulární biologii je mnohem vyšší než v matema-ce). Je proto třeba pamatovat, že H-index je hodný spíše pro odrostlejší badatele (asi nad 0 let) a že nelze automaticky srovnávat H-in-exy lidí působících v citačně příliš odlišných borech. Dalším technickým detailem je, že e WOS jsou dobře zpracovány pouze práce yšlé po roce 1980. Starší badatelé, kteří ma-silně citované práce dřívějšího data, budou při jednoduchém zjištění H-indexu z WOS více či méně ochuzeni.

Podívejme se nejprve na původní Hirschův soubor fyziků (tab. I). Hirsch doporučuje používat svůj index pro posouzení kvality badatele, například při obsazování pozic na univerzitách nebo při nominacích nových členů do Národní akademie věd USA (NAS). Hirsch uvádí, že H-index nad 20 (po 20leté vědecké kariéře) je známkou úspěchu; hodnoty 40 a více pak indikují skutečně vynikající badatele, jaké lze nalézt jen ve velmi dobrých laboratořích. H-index roven 12 by měl být dostatečný pro získání pozice na univerzitě, 15–20 pro získání členství v Americké fyzikální společnosti a 45 či vyšší pro členství v NAS (výjimky samozřejmě existují). Fyzici a astronomové nově přijatí do NAS v roce 2005 měli průměrný H-index roven 45. Nositelé Nobelovy ceny za fyziku za posledních 20 let měli medián svých H-indexů roven 35 a nejvíc z nich mělo H-index mezi 35 a 39. Většina laureátů měla vysoký H-index, což ukazuje, že Nobelovy ceny se zpravidla neudělují za jednu vynikající práci, ale za rozsáhlou vědeckou aktivitu. V biovědách (life sciencies) jsou vzhledem k obecně vyšší průměrné citovanosti indexy zhruba 2krát vyšší než u fyziků (tab. II). Medián H-indexů 36 nově přijatých členů NAS (v biologických a medicínských oborech) byl 57, maximální H-index byl 135.

Opusťme nyní svět a obraťme pozornost k české realitě. Upozorňujeme, že uváděné hodnoty H-indexů byly získány jednoduchým hledáním ve WOS,[3] takže u badatelů, kteří začali svoji vědeckou kariéru dříve než v letech 1975–1980, mohou být více nebo méně podhodnoceny. Výběr jmen v této i dalších tabulkách byl subjektivní a přes naši snahu o úplnost se mohlo stát, že jsme na někoho zapomněli. Předem se za to omlouváme. Začněme opět fyzikou (tab. III). Velmi vysoké hodnoty H-indexů (40–55) má ještě řada fyziků elementárních částic, kteří se podílejí na mezinárodních experimentech (např. J. Cvach, J. Žáček, M. Taševský). Publikace, na nichž se tito badatelé podílejí, mají však obvykle několik set spoluautorů, což je činí poněkud atypickými a obtížně srovnatelnými s pracemi, jejichž autory je jen několik málo pracovníků. Na druhé straně je skvělé, že se značný počet našich fyziků podílí na takových náročných experimentech přinášejících opravdu velmi důležité výsledky. Situaci v chemii a v biologii ukazují tab. IV a tab. V. Uvedené přehledy snad umožňují udělat několik následujících poznámek o české vědě:

- Je patrné, že česká chemie je ve světle absolutních hodnot H-indexů srovnatelná s molekulární biologií (výjimkou je J. Bartek z Kodaně). Uvážíme-li však, že průměrná citovanost je v molekulární biologii přinejmenším 1,5–2krát vyšší než v chemických oborech, a tedy je tam snazší dosáhnout vyšších hodnot H-indexu, dospějeme k závěru, že česká chemie zjevně představuje jeden z pilířů české vědy.
- Kurzívou jsou v tab. IV a tab. V uvedena jména badatelů, kteří *nejsou* členy Učené společnosti ČR, tedy elitní české vědecké společnosti. J. Bartek, V. Bondybey, T. Hudlický a J. Bartková pracují v zahraničí, E. Syková je z Ústavu experimentální medicíny AV ČR a Z. Samec a J. Hrušák z Ústavu fyzikální chemie J. Heyrovského AV ČR.
- Mezi 40 vědci uvedenými v tab. III–V jsou jen 3 ženy, všechny pracují v biologických vědách.
- Z českých badatelů uvedených v tabulkách III–V pracuje velká většina v ústavech Akademie věd. Mezi 40 jmény jsou 2 minulí předsedové AV (R. Zahradník a H. Illnerová); současný předseda V. Pačes má také slušný H-index (21). Mezi jmény zcela chybějí akademičtí hodnostáři českých a moravských univerzit.
- Antonín Holý z Ústavu organické chemie a biochemie AV ČR je světově proslulý svými antivirovými léky (mezi jinými proti HIV). Úspěšná patentová a licenční aktivita je u tohoto badatele skloubena i s vysokým H-indexem.
- J. Hirsch navrhuje užívat H-index jako kritérium členství v americké National Academy of Sciences (NAS). Podívejme se, jak to vypadá s členstvím v české obdobě NAS, US ČR. H-index 13 až 20 má 16 členů US z oblasti věd živé přírody, kteří nejsou uvedeni v tab. IV a V. Ze 6 badatelů oceněných Cenou US ČR v posledních 3 letech (I. Hlaváček, V. Petříček, M. Strnad, V. Havlíček, J. Kříž, P. Spurný), tedy potenciálních kandidátů na členství v US, mají H-index v uvedeném rozsahu VP, MS, VH a JK; H-index VP a MS je vyšší (21) než horní hranice limitu.
- Skutečně světová jména v české vědě až na výjimky chybějí; použijeme-li Hirschovo kritérium, pak je mezi českými vědci jen málo badatelů (H-index vyšší nebo roven 40), kteří by byli ozdobou i prestižních světových laboratoří. Vítek, Paldus, Michl, Bondybey, Hudlický, Bartek, Městecký, Lukáš, Hamet, Skamene (a také v tabulce neuvedený J. Klein) pracují dlouhodobě mimo ČR, a tak zůstává jen velmi malý počet skutečně „domácích" jmen. Cesta k zlepšení je nasnadě – systematická nadstandardní podpora vynikajících badatelů. H-index představuje samozřejmě jen jedno z kritérií a nikdy nemůže nahradit řádné recenzní řízení, „peer review". Všichni však víme, jak je toto řízení obtížné a nákladné. Poměrně objektivní, snadno získatelný index tak může vnést důležité srovnání, které by se mělo brát v úvahu třeba při udělování cen za vědu nebo grantových podpor. H-index může poukázat na vynikající badatele, kteří navenek nejsou příliš viditelní, ale také odhalí ty, kteří jsou mediálně velmi zdatní, zatímco jejich skutečný vědecký přínos je poměrně malý.
- Často se poukazuje na potřebu komplexního pohledu při hodnocení vědecké aktivity. Kromě publikací a citací by měla být zohledněna také pozvání k proslovení přednášek na prestižních konferencích, členství v redakčních radách evropských a světových časopisů, zájem našich a zahraničních studentů pracovat v laboratoři daného badatele, recenzní činnost pro významné vědecké časopisy…

Je to samozřejmě správný požadavek. Většina badatelů s vysokým H-indexem tyto požadavky splňuje, a to prostě proto, že všechna uvedená kritéria spolu souvisejí. Nalezení H-indexů je však mnohem rychlejší než složité dotazování na jednotlivé body.

I tato metoda má – jako všechny metody hodnocení – jasné limitace: je vhodná hlavně pro starší badatele, neodlišuje vždy dobře pracovníky, kteří jsou opravdu vůdčími duchy týmů, od těch, kdo pracují spíše na dílčích úkolech ve velkých týmech, a samozřejmě se v ní musí velice brát v úvahu značné oborové odlišnosti. Tato metoda také znevýhodňuje vědce, kteří mají menší počet vysoce citovaných prací (a třeba i velmi vysoký průměr citovanosti na publikaci). I tuto metodu – tak jako všechny ostatní – musíme aplikovat opatrně, s rozmyslem, nikoli mechanicky. Ve velké většině případů však evidentně něco velmi důležitého říká.

I. FAKTOR[1]

ČEŠI a. Fyzici

Nobel

E. Witten	110
M. L. Cohen	94
P. W. Anderson	91
S. Weinberg	88
M. Cardova	86
P.-G. de Gennes	79
F. Wilczek	68
C. Vafa	66
M. B. Maple	66
D. Gross	66
M. S. Dresselhaus	62

Tab I. Jména a hodnoty H-indexu vybraných badatelů, kteří představují světovou extratřídu (řada z nich získala Nobelovu cenu).

S. H. Snyder	191
D. Baltimore	160
R. C. Gallo	154
B. Vogelstein	151
C. A. Dinarello	138
R. Evans	127
A. Ullrich	120

Tab. II. H-indexy předních badatelů v biovědách.

V. Vítek (Z)	42
J. Tauc (Z)	33
V. Červený	25
J. Peřina	21
P. Harmanec	20
P. Hořava (Z)	18
J. Bičák	18

Tab. III. Vybraní čeští fyzikové pracující v ČR nebo převážně v zahraničí (Z) a jejich H-indexy. H-indexy prvních dvou klasiků (pevná fáze) převyšují 30, V. Vítek překročil hranici 40, což je ve fyzice velmi vysoké číslo; P. Hořava je ještě dosti mladý (43 let). Je třeba poznamenat, že velmi vysoké hodnoty H-indexů (40–55) má řada fyziků elementárních částic z FZÚ AV ČR a MFF UK, kteří se dlouhodobě podílejí na velkých mezinárodních experimentech v CERN, DESY a FERMILAB (např. J. Cvach, J. Žáček, M. Taševský).

b. Chemici

J. Paldus (Z)	53
P. Hobza	52
J. Michl (Z)	48
V. Bondybey (Z)	48
E. Paleček	44
A. Holý	40
T. Hudlický (Z)	40
J. Šponer	38
Z. Samec	34
F. Tureček (Z)	34
R. Zahradník	31
K. Ulbrich	31
V. Sklenář	31
V. Mareček	30
V. Špirko	27
J. Hrušák	27

Tab. IV. Čeští chemikové pracující v ČR nebo převážně v zahraničí (Z) a jejich H-indexy (kurzívou jsou jména badatelů, kteří nejsou členy Učené společnosti ČR). U chemiků (a podobně u biologů v tab. V) jsou uvedeni pouze vědci s indexem vyšším nebo rovným 27; seznam určitě není kompletní mj. proto, že u některých běžných jmen (Růžička, Svoboda, Klein, Novák…) se ve WOS špatně hledá. Ke zkreslení H-indexu také může dojít, pokud má více autorů stejné příjmení a iniciálu křestního jména.

J. Bartek (Z)	71
J. Městecký (Z)	54
J. Lukáš (Z)	50
P. Hamet (Z)	47
E. Skamene (Z)	44
V. Hořejší	41
J. Bartková (Z)	38
J. Bureš	35
P. Martásek	33
J. Lom	32
M. Malkovský (Z)	32
P. Démant (Z)	32
J. Závada	30
E. Syková	29
H. Illnerová	29
I. Vořechovský (Z)	28
B. Vojtěšek	27

Tab. V. Čeští biologové pracující v ČR nebo převážně v zahraničí (Z) (kurzívou badatelé, kteří nejsou členy Učené společnosti ČR). O problémech s hledáním nositelů některých jmen viz popisku tab. IV. Extrémně vysokou hodnotu H-indexu (větší než 70) má určitě např. světový imunogenetik Jan Klein.

c. Bio

1) Pozn. red: Vzhledem k tomu, že se na přípravě tohoto textu podílelo různou měrou více autorů, vyjímečně jsme připustili „kolektivní" jméno I. Faktor. Poděkování patří všem, kteří se textem kriticky zabývali. Odpovědnost za korektnost údajů v tomto případě nese ovšem redakce. **Ivan Boháček**

2) H-index; http://xxx.arxiv.org/abs/physics/0508025, viz též Nature 436, 900, 2005.

3) WOS – Science Citation Index Expanded – Cited Ref Search; zahrnujeme „černé" i „modré" záznamy.

Vědecké týmy, vědecká spolupráce

Společné a **vlastní** a publikace
ÚLOHA JEDNOTLIVCE

Když si budete vybírat **téma své diplomky/dizertace** snažte se zjistit jak je váš budoucí **školitel** ve vědě **úspěšný** a jak **aktuální a zajímavé** jsou problémy, které řeší

Již během svého studia můžete dělat vědecké objevy!

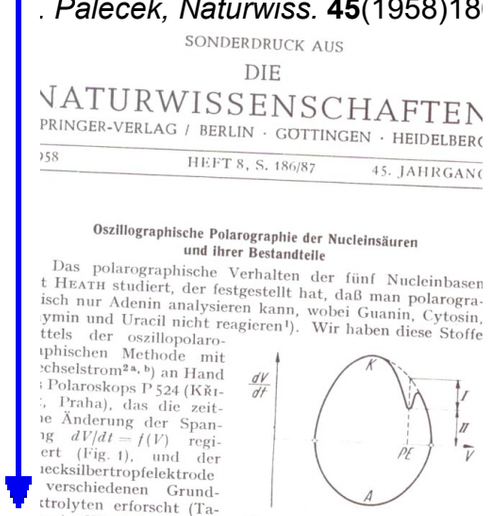
90 years of polarography and ~55 years of nucleic acid electrochemistry

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 polarograph was the **method of choice for** the **DNA electrochemical analysis**:

1958: Nucleic acid bases, DNA and RNA are electroactive

1960: Relations between the DNA structure and electrochemical responses

. *Palecek, Naturwiss.* **45**(1958)18



SONDERDRUCK AUS

DIE

NATURWISSENSCHAFTEN

PRINGER-VERLAG / BERLIN · GÖTTINGEN · HEIDELBERG

)58 HEFT 8, S. 186/87 45. JAHRGANG

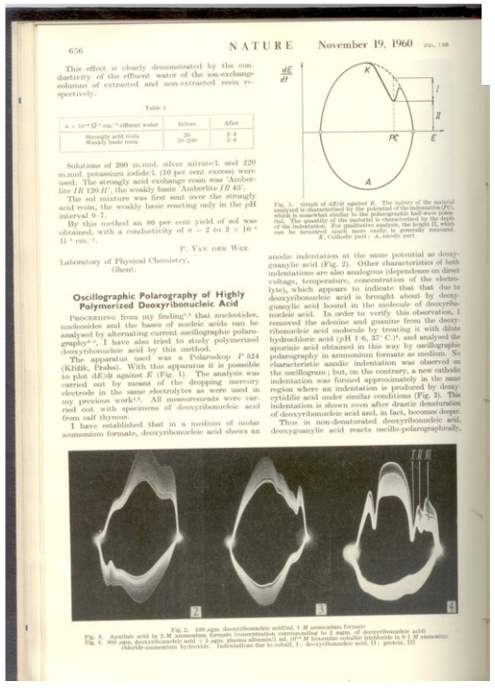
Oszillographische Polarographie der Nucleinsäuren und ihrer Bestandteile

Das polarographische Verhalten der fünf Nucleinbasen t HEATH studiert, der festgestellt hat, daß man polarographisch nur Adenin analysieren kann, wobei Guanin, Cytosin, ymin und Uracil nicht reagieren[1]). Wir haben diese Stoffe ttels der oszillopolarographischen Methode mit chselstrom[2a, b]) an Hand Polaroskops P 524 (Kři- Praha), das die zeitänderung der Spang $dV/dt = f(V)$ regiert (Fig. 1), und der iecksilbertropfelektrode verschiedenen Grundtrolyten erforscht (Tae 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

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



656 NATURE November 19, 1960 Vol. 188

This effect is clearly demonstrated by the conductivity of the effluent water of the ion-exchange columns of extracted and non-extracted resin respectively.

Table 1

$\sigma \times 10^{-6}\ \Omega^{-1}\,cm^{-1}$ effluent water	Before	After
Strongly acid resin / Weakly basic resin	20 / 20–200	2–4 / 2–4

Solutions of 200 m.mol. silver nitrate/l. and 220 m.mol. potassium iodide/l. (10 per cent excess) were used. The strongly acid exchange resin was 'Amberlite *IR* 120 *H*', the weakly basic 'Amberlite *IR* 45'.

The sol mixture was first sent over the strongly acid resin, then weakly basic reacting only in the *p*H interval 0–7.

By this method an 80 per cent yield of sol was obtained, with a conductivity of $\sigma = 2$ to 3×10^{-6} $\Omega^{-1}\,cm^{-1}$.

P. VAN DER WEE

Laboratory of Physical Chemistry, Ghent.

Oscillographic Polarography of Highly Polymerized Deoxyribonucleic Acid

PROCEEDING from my finding[1,2] that nucleotides, nucleosides and the bases of nucleic acids can be analysed by alternating current oscillographic polarography[3–5], I have also tried to study polymerized deoxyribonucleic acid by this method.

The apparatus used was a Polaroskop *P* 524 (Křižík, Praha). With this apparatus it is possible to plot dE/dt against E (Fig. 1). The analysis was carried out by means of the dropping mercury electrode in the same electrolytes as were used in my previous work[1,5]. All measurements were carried out with specimens of deoxyribonucleic acid from calf thymus.

I have established that in a medium of molar ammonium formate, deoxyribonucleic acid shows an

Fig. 1. Graph of dE/dt against E. The nature of the material analysed is characterized by the potential of the indentation (PC), which is somewhat similar to the polarographic half-wave potential. The quantity of the material is proportional to the area of the indentation. For qualitative analysis, the height II, which can be measured much more easily, is generally measured. K, Cathodic part; A, anodic part

anodic indentation at the same potential as deoxyguanylic acid (Fig. 2). Other characteristics of both indentations are also analogous (dependence on direct voltage, temperature, concentration of the electrolyte), which appears to indicate that that due to deoxyribonucleic acid is brought about by deoxyguanylic acid bound in the molecule of deoxyribonucleic acid. In order to verify this observation, I removed the adenine and guanine from the deoxyribonucleic acid molecule by treating it with dilute hydrochloric acid (pH 1·6, 37° C.)[6], and analysed the apurinic acid obtained in this way by oscillographic polarography in ammonium formate as medium. No characteristic anodic indentation was observed on the oscillogram; but, on the contrary, a new cathodic indentation was formed approximately in the same region where an indentation is produced by deoxycytidilic acid under similar conditions (Fig. 3). This indentation is shown even after drastic denaturation of deoxyribonucleic acid and, in fact, becomes deeper.

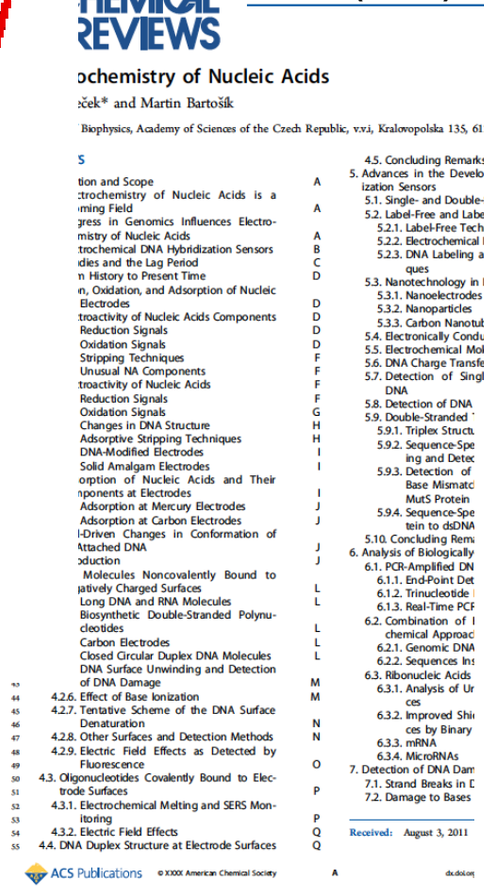
Thus in non-denaturated deoxyribonucleic acid, deoxyguanylic acid reacts oscillo-polarographically,

Fig. 2. 100 µgm. deoxyribonucleic acid/ml. 1 *M* ammonium formate

Fig. 3. Apurinic acid in 2 *M* ammonium formate (concentration corresponding to 2 mgm. of deoxyribonucleic acid)

Fig. 4. 900 µgm. deoxyribonucleic acid + 5 µgm. plasma albumin/ml. 1 *M* hexamine cobaltic trichloride in 0·1 *M* ammonium chloride–ammonium hydroxide. Indentation due to cobalt. I, deoxyribonucleic acid. II, protein. III

lectrochemistry of nucleic acids is now a booming field



HEMICAL REVIEWS

112 (2012) 3427-3481

Review

pubs.acs.org/CR

ochemistry of Nucleic Acids

ček* and Martin Bartošík

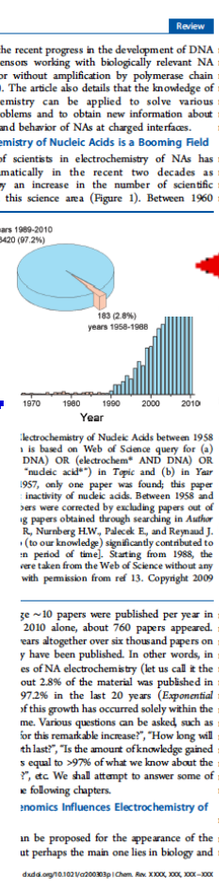
Biophysics, Academy of Sciences of the Czech Republic, v.v.i, Kralovopolska 135, 612 65 Brno, Czech Republic

S

tion and Scope A
trochemistry of Nucleic Acids is a ming Field A
gress in Genomics Influences Electro- mistry of Nucleic Acids A
trochemical DNA Hybridization Sensors B
idies and the Lag Period C
n History to Present Time D
n, Oxidation, and Adsorption of Nucleic Electrodes D
troactivity of Nucleic Acids Components D
Reduction Signals D
Oxidation Signals D
Stripping Techniques F
Unusual NA Components F
troactivity of Nucleic Acids F
Reduction Signals F
Oxidation Signals G
Changes in DNA Structure H
Adsorptive Stripping Techniques H
DNA-Modified Electrodes I
Solid Amalgam Electrodes I
orption of Nucleic Acids and Their nponents at Electrodes I
Adsorption at Mercury Electrodes J
Adsorption at Carbon Electrodes J
l-Driven Changes in Conformation of ttached DNA J
oduction J
Molecules Noncovalently Bound to atively Charged Surfaces L
Long DNA and RNA Molecules L
Biosynthetic Double-Stranded Polynucleotides L
Carbon Electrodes L
Closed Circular Duplex DNA Molecules L
DNA Surface Unwinding and Detection of DNA Damage M
4.2.6. Effect of Base Ionization M
4.2.7. Tentative Scheme of the DNA Surface Denaturation N
4.2.8. Other Surfaces and Detection Methods N
4.2.9. Electric Field Effects as Detected by Fluorescence O
4.3. Oligonucleotides Covalently Bound to Electrode Surfaces P
4.3.1. Electrochemical Melting and SERS Monitoring P
4.3.2. Electric Field Effects Q
4.4. DNA Duplex Structure at Electrode Surfaces Q
4.5. Concluding Remarks R
5. Advances in the Development of DNA Hybridization Sensors R
5.1. Single- and Double-Surface Techniques R
5.2. Label-Free and Label-Based DNA Sensing S
5.2.1. Label-Free Techniques T
5.2.2. Electrochemical Impedance Spectroscopy T
5.2.3. DNA Labeling and Label-Based Techniques U
5.3. Nanotechnology in DNA Sensors W
5.3.1. Nanoelectrodes W
5.3.2. Nanoparticles W
5.3.3. Carbon Nanotubes X
5.4. Electronically Conducting Polymers X
5.5. Electrochemical Molecular Beacons Y
5.6. DNA Charge Transfer Z
5.7. Detection of Single-Base Mismatches in DNA
5.8. Detection of DNA
5.9. Double-Stranded
5.9.1. Triplex Structu
5.9.2. Sequence-Spe ing and Detec
5.9.3. Detection of Base Mismatc MutS Protein
5.9.4. Sequence-Spe tein to dsDNA
5.10. Concluding Rem
6. Analysis of Biologically
6.1. PCR-Amplified DN
6.1.1. End-Point Det
6.1.2. Trinucleotide
6.1.3. Real-Time PCF
6.2. Combination of chemical Approac
6.2.1. Genomic DNA
6.2.2. Sequences Ins
6.3. Ribonucleic Acids
6.3.1. Analysis of Ur ces
6.3.2. Improved Shi ces by Binary
6.3.3. mRNA
6.3.4. MicroRNAs
7. Detection of DNA Dam
7.1. Strand Breaks in D
7.2. Damage to Bases

Received: August 3, 2011

ACS Publications © XXXX American Chemical Society A dx.doi.org



Chemical Reviews Review

8. Biodevices: DNA Microarrays and Lab-on-a-Chip AL
9. Summary and Conclusion AL
Author Information AO
Corresponding Author AO
Biographies AO
Acknowledgments AP
List of Abbreviations AP
References AP
Note Added in Proof BC

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

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 g 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

ge ~10 papers were published per year in 2010 alone, about 760 papers appeared. ears altogether over six thousand papers on y have been published. In other words, in es 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* of this growth has occurred solely within the me. Various questions can be asked, such as for this remarkable increase?", "How long will th last?", "Is the amount of knowledge gained s equal to >97% of what we know about the ?", etc. We shall attempt to answer some of e following chapters.

nomics Influences Electrochemistry of

an be proposed for the appearance of the ut perhaps the main one lies in biology and

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

IF ~40

WHY?

progress in GENOMICS increasing importance of parallel **nucleotide sequencing**

electrochemistry can complement optical detection in arrays and particularly in chips **for decentralized analysis**

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

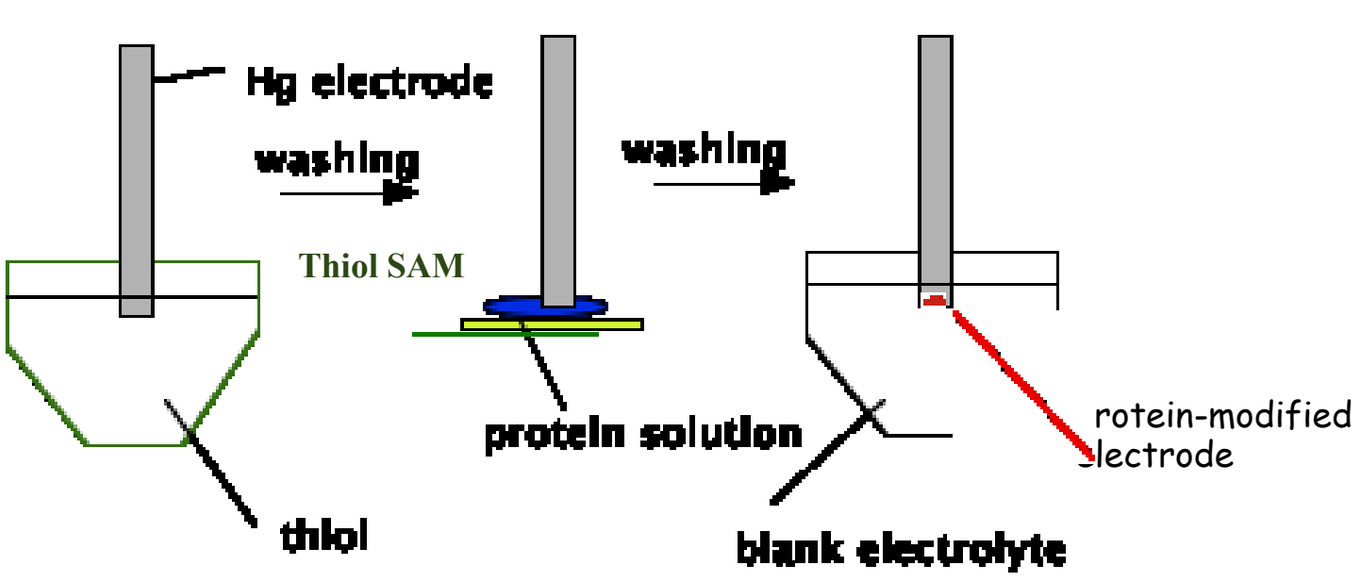
1986-88 DNA-modified electrodes

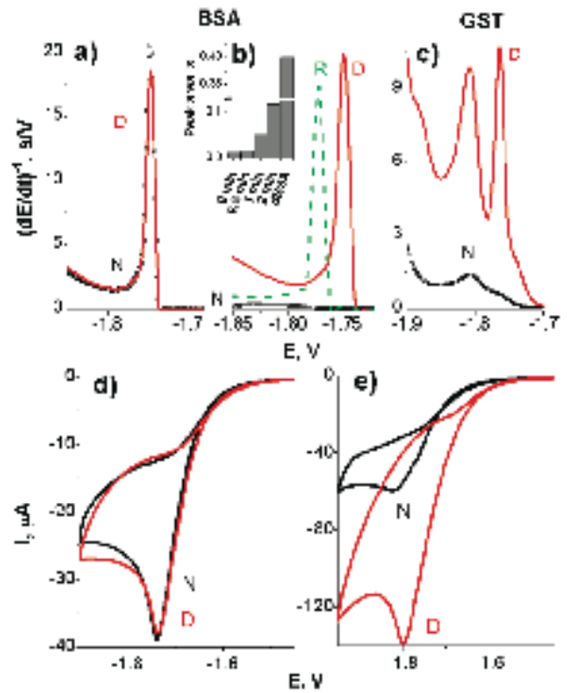
V posledních letech se zabýváme interakcemi **bílkovin** a jejich elektrochemii

Electrochemical analysis of proteins and peptides at Hg electrodes in the presence of large excess of thiols was difficult or impossible.

Recently we have found that peak H is produced by proteins adsorbed at mercury and solid amalgam electrodes modified by different kinds of thiol self-assembled monolayers (SAMs). For practical reasons we were primarily interested in DTT SAMs.

V. Ostatná, H. Cernocká, E. Palecek (2010) J.Am. Chem. Soc., 132, 9408-9413





Temperature, at which the electrode process is taking place, greatly influences the electrochemical behavior of the surface-immobilized proteins.

Tumor suppressor protein p53 declared „**The Molecule of the Year" by Science magazine in 1993** perhaps the **most important protein in the development of cancer**. This protein p53 plays a critical role in the cellular response to DNA damage by regulating the expression of genes involved in controlling cell proliferation, DNA repair, and apoptosis. P53 protein is **inactivated by mutation** in about 50 % of human malignancies. Most **mutations** are located in the DNA-binding **core domain** of the protein. p53 protein is biologically **active in its reduced state** and is usually **stored with** mM concentrations of **reducing agent - dithiothreitol** (DTT).

EU 6th FP: **Mutant p53 as target for improved cancer therapy**

Journal of the American Chemical Society | 132 | ver.9 | 12/4/2011 | 22:53 | Mac Js-2011-00000s | TEID: doc00 | BATID: 00000 | Pages: 6-4

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

ARTICLE

pubs.acs.org/JACS

Electrocatalytic Monitoring of Metal Binding and Mutation-Induced Conformational Changes in p53 at Picomole Level

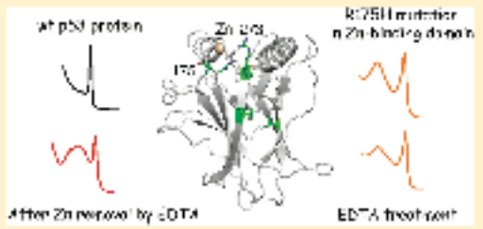
Emil Paleček,*[†] Veronika Ostatná,[†] Hana Černocká,[†] Andreas C. Joerger,[‡] and Alan R. Fersht[‡]

[†]Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Královopolská 135, 612 65 Brno, Czech Republic

[‡]MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, U.K.

Supporting Information

ABSTRACT: We developed an innovative electrochemical method for monitoring conformational transitions in proteins using constant current chronopotentiometric stripping (CPS) with dithiothreitol-modified mercury electrodes. The method was applied to study the effect of oncogenic mutations on the DNA-binding domain of the tumor suppressor p53. The CPS responses of wild-type and mutant p53 showed excellent correlation with structural and stability data and provided additional insights into the differential dynamic behavior of the proteins. Further, we were able to monitor the loss of an essential zinc ion resulting from mutation (R175H) or metal chelation. We envisage that our CPS method can be applied to the analysis of virtually any protein as a sensor for conformational transitions or ligand binding to complement conventional techniques, but with the added benefit that only relatively small amounts of protein are needed and instant results are obtained. This work may lay the foundation for the wide application of electrochemistry in protein science, including proteomics and biomedicine.



INTRODUCTION

The tumor suppressor protein p53 plays a critical role in the cellular response to DNA damage by regulating the expression of genes involved in controlling the cell cycle, DNA repair, and apoptosis.[1,2] It is directly inactivated by mutation in about 50% of human cancers, with most oncogenic mutations being located in the DNA-binding core domain of the protein.[3,4] It is essential to understand the molecular basis of p53 inactivation in cancer in order to develop novel anticancer strategies.[5] The structural effects of many oncogenic p53 mutants have been intensively studied by X-ray crystallography and complementary techniques (reviewed in ref 6). Yet, the most frequent cancer-associated mutant, R175H, which is highly destabilized, has eluded a detailed structural characterization so far, highlighting the need for complementary techniques to study conformationally unstable mutants.

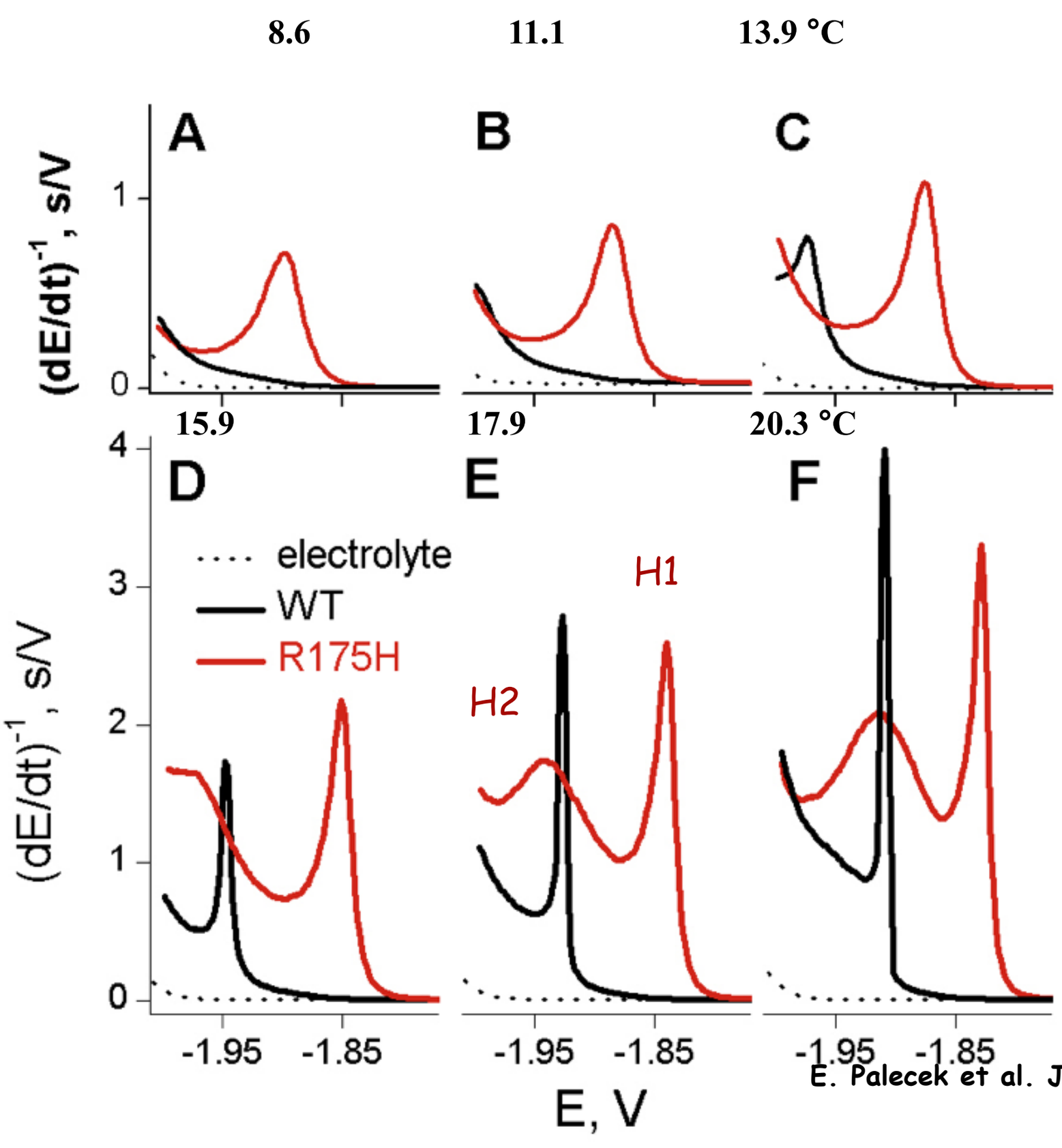
In recent decades, electrochemistry of proteins was limited to relatively small conjugated proteins containing nonprotein redox centers yielding reversible electrochemistry,[7−10] and a majority of proteins were neglected. We have proposed a new electrochemical method for analysis of practically all proteins, which is sensitive to changes in protein structure.[11−20] This method is based on the ability of proteins to catalyze hydrogen evolution at mercury electrodes[9,21−23] and relies on constant current chronopotentiometric stripping (CPS, involving very fast potential changes) and mercury-containing electrodes.[19,24] With this method, a number of proteins in their native and denatured and/or reduced and oxidized forms were analyzed displaying protein structure-sensitive responses (denominated as peaks H).[11,15] We used CPS to study aggregation of α-synuclein (important in Parkinson's disease), and we detected changes in the interfacial behavior of this protein preceding fibril formation.[15]

To our knowledge, the only paper using electrochemical analysis to study the p53 protein was limited to determination of traces of glutathione-S-transferase in the C-terminal domain of p53.[25] Studies of the full-length p53 protein or its core domain were difficult because of DTT (dithiothreitol, usually present in these p53 samples), which interfered with the electroanalysis at mercury electrodes.[19] Replacement of DTT by other reducing agents, such as tris(2-carboxyl-ethyl)phosphine hydrochloride, was laborious, risking damaging the labile proteins. Recently, we have proposed thiol-modified mercury electrodes.[19] Thiol self-assembled monolayers (SAM) at the Hg surface do not interfere with the electrocatalytic reaction responsible for peak H and make analysis of reduced proteins (usually stored with mM concentrations of DTT) easier.

Here, we applied CPS in combination with DTT-modified HMDE (DTT-HMDE) to study the DNA-binding domain of human p53 and cancer-associated mutants. We observed striking differences between the CPS responses of the wild-type like protein T-p53C and its R175H mutant, which has a perturbed zinc-binding region. Removal of the zinc ion from T-p53C resulted in a CPS response resembling that of the R175H mutant. Studies of other T-p53C mutants showed some

Received: February 9, 2011

p53 core domain



Mutation in **R175H** induces **structural perturbation at the zinc-binding site,** destabilizes the core domain by 3 kcal/mol and eliminates p53 sequence specific DNA binding. The **same effect** can be observed in the **wt** core domain **upon removal of the zinc ion**.

We tested **other mutants** such as V145A, F270L, R273H and Y220C and we always observed CPS **responses different from** the **wt protein**

E. Palecek et al. JACS 2011, 133, 7190–7196

The Protein Group

Hana Cernocka
Mojmir Trefulka
Petra Mittnerova
Emil Palecek
Lida Rimankova
Martin Bartosik
Veronika Ostatna
Veronika Vargová

Problems of life origin

What was first - DNA, RNA or protein?

Well-known Oxford zoologist Professor Richard Dawkins (who declares himself to be passionate fighter for the truth) writes in his book River out of Eden:

"At the beginning of Life Explosion there was no mind, no creativity, no intent, there was only chemistry"

Let us try to summarize what chemistry it was

New York Times
June 13, 2000, Tuesday
SCIENCE DESK

Life's Origins Get Murkier and Messier; Genetic Analysis Yields Intimations of a Primordial Commune

By By NICHOLAS WADE (NYT) 2179 words

The surface of the earth is molten rock. The oceans are steam or superheated water. Every so often a wandering asteroid slams in with such energy that any incipient crust of hardened rock is melted again and the oceans are reboiled to an incandescent mist. Welcome to Hades, or at least to what geologists call the Hadean interval of earth's history. It is reckoned to have lasted from the planet's formation 4.6 billion years ago until **3.8 billion years ago,** when the rain of ocean-boiling asteroids **ended.**

The Isua greenstone belt of western Greenland, one of the oldest known rocks, was formed as the Hadean interval ended. And amazingly, to judge by chemical traces in the Isuan rocks, life on earth was already old.

Everything about the origin of life on earth is a mystery, and it seems the more that is known, the more acute the puzzles get.

The dates have become increasingly awkward. **Instead of there being a billion or so years for the first cells to emerge from a warm broth of chemicals, life seems to pop up almost instantly after the last of the titanic asteroid impacts that routinely sterilized the infant planet.** Last week, researchers reported discovering microbes that lived near volcanic vents formed 3.2 billion years ago, confirming that heat-loving organisms were among earth's earliest inhabitants.

The chemistry of the first life is a nightmare to explain. No one has yet devised a plausible explanation to show how the earliest chemicals of life -- thought to be RNA, or ribonucleic acid, a close relative of DNA -- might have constructed themselves from the inorganic chemicals likely to have been around on the early earth. The **spontaneous assembly of small RNA molecules on the primitive earth "would have been a near miracle,"** two experts in the subject helpfully declared last year.

A third line of inquiry into the beginnings of life has now also hit an unexpected roadblock. This is phylogeny, or the drawing of family trees of the various genes found in present-day forms of life. The idea is to run each gene tree backward to the ancestral gene at the root of the tree. The collection of all these ancestral genes should define the nature of the assumed universal ancestor, the living cell from which all the planet's life is descended. The universal ancestor would lie some distance away from life's origin from chemicals, but might at least give clues to how that process started.

"It is not so preposterous anymore **to think of the common ancestor as a sort of Noah's ark, where pretty much every protein domain has been represented,**" Dr. Koonin said. The proteins of living organisms are composed of mix-and-match functional units known as domains.

Still, this idea is a disturbing concept. **Evolutionists are accustomed to portraying the evolutionary process in terms of neatly branching trees, not Noah's arks.** .....

BIOLOGICKÉ LISTY 68 (1): 3-14, 2003

Problémy vzniku života na Zemi

EMIL PALEČEK

Biofyzikální ústav Akademie věd České republiky, Královopolská 135, 61265 Brno

Přijato do tisku

1. Úvod

V úterý 13. června 2000 vyšel v *New York Times* článek „Life's Origins Get Murkier and Messier; Genetic Analysis Yields Intimations of a Primordial Commune" („Původ života se stává mlhavější a zmatenější; genetická analýza naznačuje prvotní (buněčnou) komunu" , překlad EP) (Wade 2000). Vzhledem k tomu, že nemám vždy úplnou důvěru k novinovým článkům zabývajícím se vědeckými problémy, rozhodl jsem se trochu podívat, co se o otázce vzniku života na Zemi píše ve vědecké literatuře. Nakonec jsem článku v *New York Times* musel dát za pravdu.

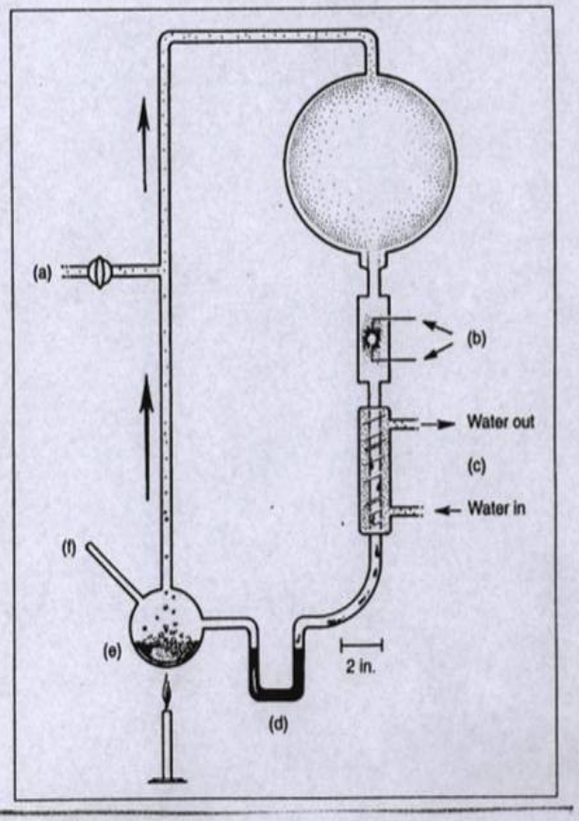
Mám v živé paměti přednášku, kterou přednesl před mnoha lety v Liblicích Harold Urey o vzniku aminokyselin v laboratorních podmínkách, napodobujících podmínky předpokládané na Zemi v době, kdy pravděpodobně vznikl život. Přednáška byla jednoduchá a elegantní a dávala tušit, že během několika málo desetiletí budou problémy vzniku života vědecky zcela objasněny. Experimenty Ureyho studenta Stanley Millera vycházely z předpokladu, že v době vzniku života existovala na Zemi silně redukční atmosféra (Miller 1953, Ring *et al.* 1972, Wolman *et al.* 1972). Literatura z pozdější doby však nasvědčuje tomu, že prebiotická atmosféra nebyla silně redukční, jak vyžadují experimenty zaměřené na prebiotickou syntézu stavebních kamenů bílkovin a nukleových kyselin, a že obsahovala kyslík (Florkin 1975, Lumsden a Hall 1975, Towe 1978, 1996, Carver 1981,

Woese, C.R. 2002. - Proc. Natl. Acad. Sci. USA **99**: 8742.

Wolman, Y., Haverland, W.J., Miller, S.L. 1972. - Proc. Nat. Acad. Sci. USA **69**: 809.

E. Paleček (Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic) **Problems of life origin on the Earth**

There are three popular hypotheses attempting to explain the origin of prebiotic nucleic acid building blocks, *i.e.* (a) synthesis in a reducing atmosphere, (b) input in meteorites and (c) synthesis on surfaces of metal sulfides in deep sea vents. At present it is hard to say whether any of these hypotheses is correct. It is particularly difficult to imagine the prebiotic synthesis of cytosine based on the known chemistry; similarly the prebiotic synthesis of pyrimidine nucleosides and nucleotides represent unsolved problems. The progress in RNA chemistry and elucidation of their catalytic functions offer an interesting system that might play an important role in the origin of life but it appears highly impro-bable that such a complicated molecule as RNA could have appeared *de novo* on the primitive Earth. Unfortunately, it is unclear whether the RNA world was preceded by some simpler world. Darwin´s idea that all living species have a single cell common ancestor is questionable. Recently Woese has suggested that the universal ancestor was probably not a single-celled organism but a commune – a loosely built conglomerate of diverse cells in which the horizontal transfer of genes played a critical role. New important discoveries are necessary for better understanding of the origin of life on Earth.



Abiotic synthesis of small organic molecules.

Miller, a graduate student who was working with Harold Urey, began the modern era in the study of the origin of life at a time when most people believed that the atmosphere of the early earth was strongly reducing. Miller[6] subjected a mixture of methane, ammonia and hydrogen to an electric discharge and led the products into liquid water. He showed that a substantial percentage of the carbon in the gas mixture was incorporated into a relatively small group of simple organic molecules and that several of the naturally occurring amino acids were prominent among these products. This was a surprising result; organic chemists would have expected a much-less-tractable product mixture. The Urey–Miller experiments were widely accepted as a model of prebiotic synthesis of amino acids by the action of lightning.

PROBLEMS OF LIFE ORIGINS

S. Miller and H. Urey subjected mixture of methane, ammonia and hydrogen to an electric discharge and led the product into water ...

The Miller-Urey experiment attempted to recreate the chemical conditions of the primitive Earth in the laboratory, and synthesized some of the building blocks of life

but geologists showed that prebiotic atmosphere was not strongly reducing and not oxygen-free, differring from that expected by Miller and Urey

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 4396–4401, April 1999
Biochemistry

Prebiotic cytosine synthesis: A critical analysis and implications for the origin of life

ROBERT SHAPIRO*

Department of Chemistry, New York University, 100 Washington Square East, New York, NY 10003

Communicated by Leslie Orgel, The Salk Institute for Biological Studies, San Diego, CA, January 25, 1999 (received for review November 19, 1998)

ABSTRACT **A number of theories propose that RNA, or an RNA-like substance, played a role in the origin of life. Usually, such hypotheses presume that the Watson–Crick bases were readily available on prebiotic Earth, for spontaneous incorporation into a replicator. Cytosine, however, has not been reported in analyses of meteorites nor is it among the products of electric spark discharge experiments. The reported prebiotic syntheses of cytosine involve the reaction of cyanoacetylene (or its hydrolysis product, cyanoacetaldehyde), with cyanate, cyanogen, or urea. These substances undergo side reactions with common nucleophiles that appear to proceed more rapidly than cytosine formation. To favor cytosine formation, reactant concentrations are required that are implausible in a natural setting. Furthermore, cytosine is consumed by deamination (the half-life for deamination at 25°C is ~340 yr) and other reactions. No reactions have been described thus far that would produce cytosine, even in a specialized local setting, at a rate sufficient to compensate for its decomposition. On the basis of this evidence, it appears quite unlikely that cytosine played a role in the origin of life. Theories that involve replicators that function without the Watson–Crick pairs, or no replicator at all, remain as viable alternatives.**

Cytosine synthesis would not be possible even strongly in reducing prebiotic atmosphere.

Similar problems arise with the abiotic synthesis of nucleotides

Abiotic synthesis of a complicated molecule such as RNA is highly improbable

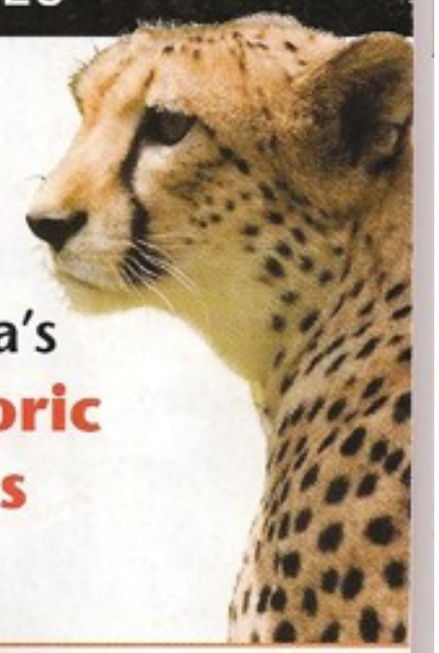
BREAKING NETWORK LOGJAMS • TRULY 3-D IMAGES

SCIENTIFIC AMERICAN

JUNE 2007

WWW.SCIAM.COM

Bring Back America's **Prehistoric Beasts**



Did this molecule start life?

FORGET DNA AND RNA. MAYBE IT ALL BEGAN WITH SOMETHING MUCH SIMPLER

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.

DNA drugs come of age - new DNA vaccines

Viral **gene(s)** are brought **to** the **cells via** an appropriate **plasmid**. Once plasmids are inside, the **cells manufacture the protein encoded by the gene**. In the case of an **antiviral DNA vaccine**, the resulting viral proteins elicit an **immune response** that **prevents** future **infection by that virus**.

MAKING THE VACCINE PROTEINS: A DNA vaccine delivered into the skin enters (tranfects) local skin cells and some immune cells. The transfected cells make the plasmid-encoded viral protein; immune cells engulf the antigen proteins as they are exiting cells.

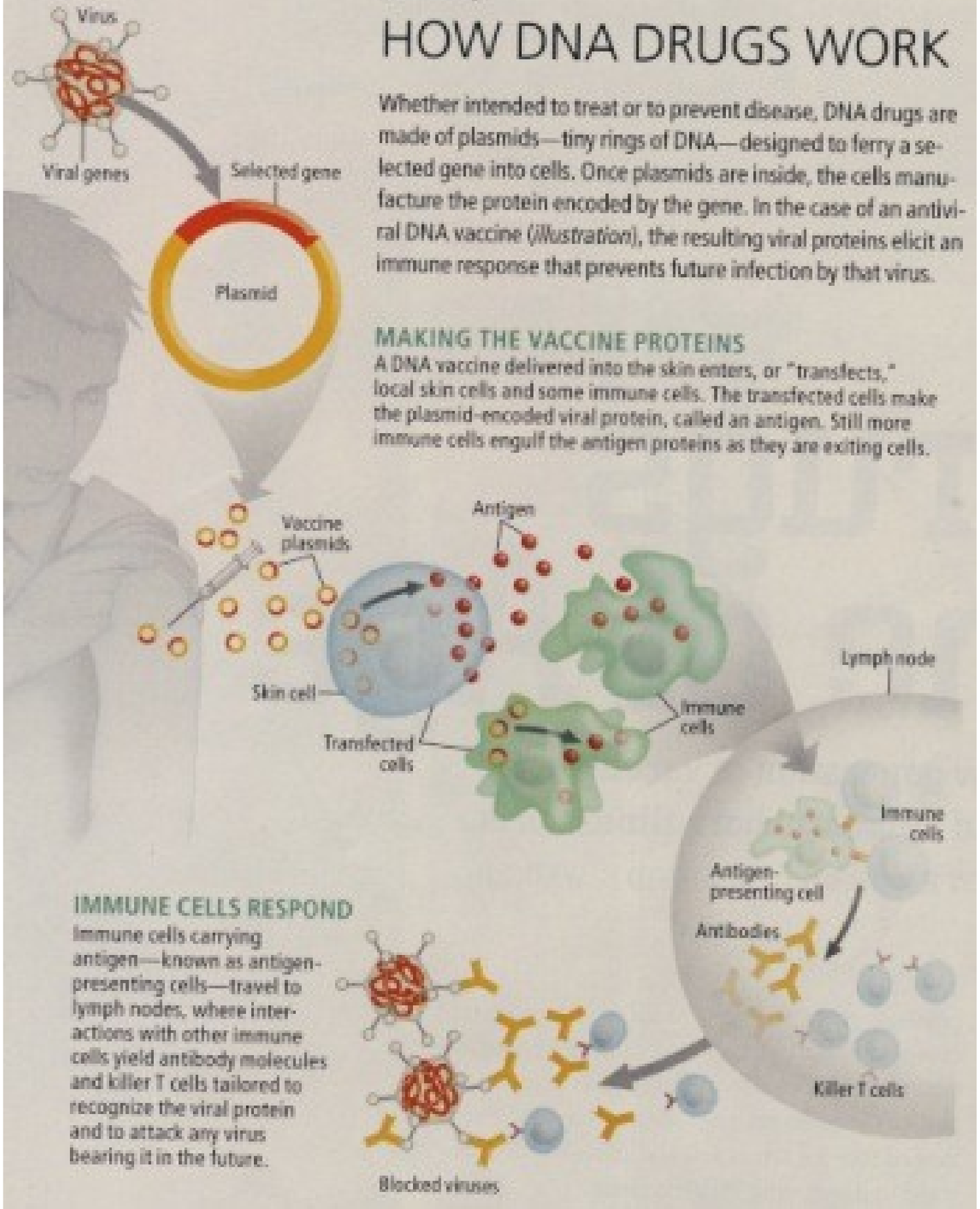
A GOOD IDEA
The **immune system does not perceive the plasmids as foreign material**.
The **protein encoded by the plasmid gene** elicits proper **immune reaction against this protein.**
However, the **early DNA vaccines** evoke a **weak immune response** only. The reasons were
(i) vaccine plasmids were **not getting into enough cells,**
(ii) the cells were **not producing enough of** the encoded **proteins**.
Simply, the **immune system** was **not** being sufficiently **stimulated**.

2007 FAILURE (STEP TRIAL)
Merck tried to use as vector an **adenovirus** called AdHu5 to deliver HIV viral genes. Unfortunately, people who got the vaccine were no better protected than those who received the placebo, and eventually they appeared to be **more vulnerable to being infected by HIV**. The discouraging result was **49 out of 914 men** in the **vaccine group** became HIV positive, whereas **33 out of 922 men in the placebo** group did not.

[BASICS]

HOW DNA DRUGS WORK

Whether intended to treat or to prevent disease, DNA drugs are made of plasmids—tiny rings of DNA—designed to ferry a selected gene into cells. Once plasmids are inside, the cells manufacture the protein encoded by the gene. In the case of an antiviral DNA vaccine (*illustration*), the resulting viral proteins elicit an immune response that prevents future infection by that virus.



MAKING THE VACCINE PROTEINS

A DNA vaccine delivered into the skin enters, or "transfects," local skin cells and some immune cells. The transfected cells make the plasmid-encoded viral protein, called an antigen. Still more immune cells engulf the antigen proteins as they are exiting cells.

IMMUNE CELLS RESPOND

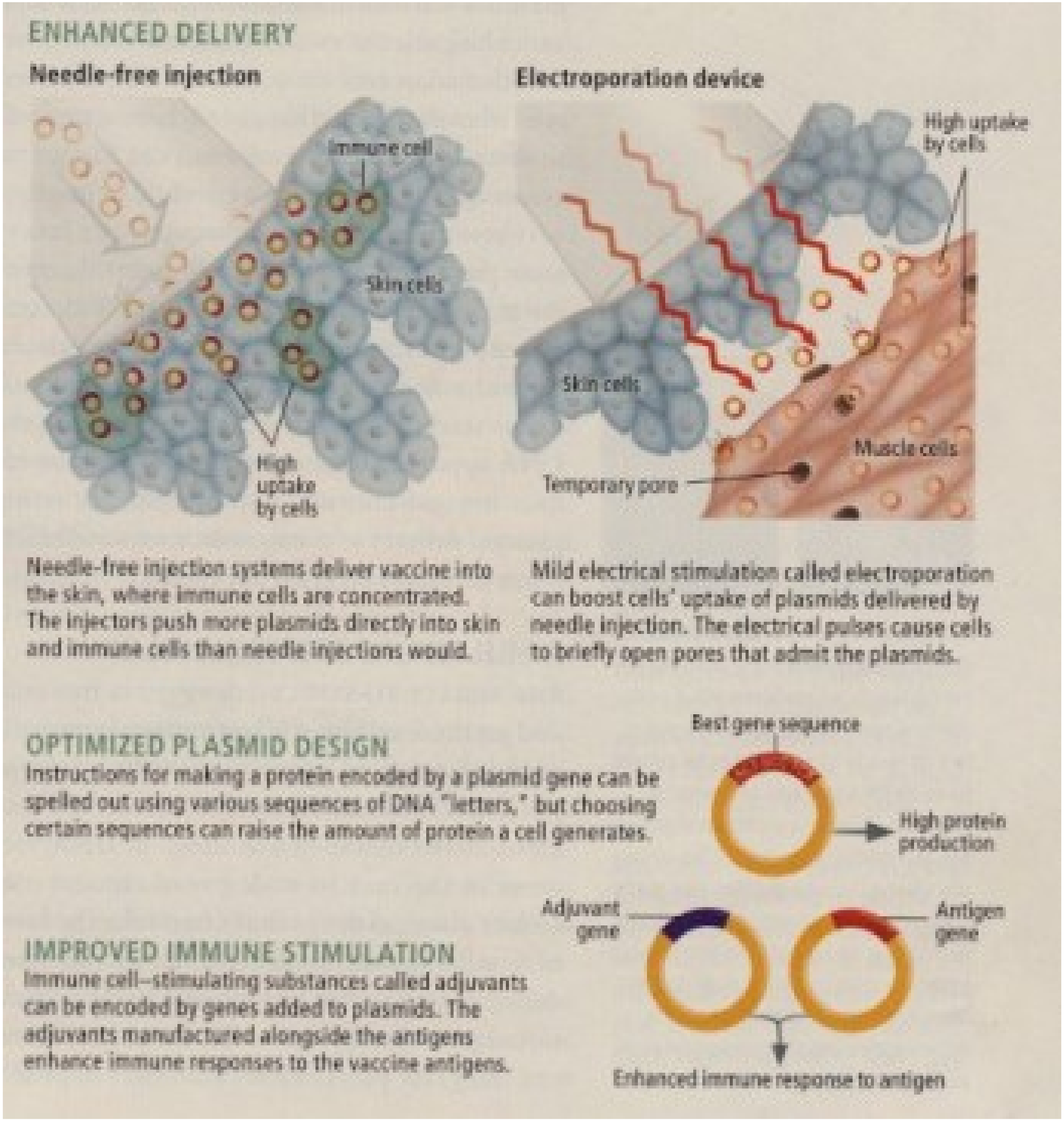
Immune cells carrying antigen—known as antigen-presenting cells—travel to lymph nodes, where interactions with other immune cells yield antibody molecules and killer T cells tailored to recognize the viral protein and to attack any virus bearing it in the future.

THE REBIRTH OF DNA

The search for improvement was directed into several directions how to boost all aspects of the plasmids´ activity namely **new methods of getting them into cells or new ways of increasing protein production**.

New vaccine delivery methods get considerably more cells – including immune cells themselves – to take up plasmids. For instance, **needle-free systems**, such as **GeneGun and Bioject** that use **pressurized air to inject vaccine,** deliver plasmids into the skin where immune entries called antigen-presenting cells are highly concentrated.
To achieve a similar result the injection can be followed by **electroporation**. That is application of a series of electrical pulses that cause cell membranes to temporarily open pores that allow plasmids to enter more easily. Electroporation can i**ncrease uptake of plasmids by as much as 1,000 fold**.

The plasmid-gene constructs themselves have also been improved through **refinements to the DNA sequences of the genes** they carry. **Codon optimization** results in the gene´s instructions in a way the cell will execute most readily. Certain amino acids are designated by more than one codon, but **cells typically favor one of** these **synonymous codons** and **translate it more efficiently** than the others. Thus optimal codons increase production of the desired protein. Also the **stability and accuracy the messenger RNA gene transcripts** plays a role. A leader sequence near the start of each gene is the first to be translated by the cell into the beginnings of a protein molecule, and optimizing a gene´s leader sequence can improve the stability of the final protein molecules. It is even possible to mark a protein as one that the cell should secrete.



The search for improvement was directed into several directions how to boost all aspects of the plasmids´ activity namely **new methods of getting them into cells or new ways of increasing protein production**.

New vaccine delivery methods get considerably more cells – including immune cells themselves – to take up plasmids. For instance, **needle-free systems**, such as **GeneGun and Bioject** that use **pressurized air to inject vaccine,** deliver plasmids into the skin where immune entries called antigen-presenting cells are highly concentrated. To achieve a similar result the injection can be followed by **electroporation**. That is application of a series of electrical pulses that cause cell membranes to temporarily open pores that allow plasmids to enter more easily. Electroporation can i**ncrease uptake of plasmids by as much as 1,000 fold.**

A final important improvement involves **additions of adjuvants**, which are typically added to traditional vaccines **to boost immune system responses**. An adjuvant **can even steer the immune system toward one response over another**. For instance, **greater production of T cells** can kill pathogen-infected cells. On the other hand **antibody** proteins **block pathogens from entering cells**.
Vaxfectin has been shown to increase antibody responses to a DNA vaccine against influenza 200-fold. **Resiquimod** provokes strong reaction including T cells and antibodies. It is also possible to **incorporate** the **gene for an adjuvant** molecule directly **into a vaccine plasmid**.

A MULTIPURPOSE TECHNOLOGY
Unlike classical drugs that often take the form of small chemical molecule, **DNA therapies deliver a gene** to treat an ailment. **The plasmid does not integrate permanently into** the recipient´s cellular **genome**.
Successes in plasmid-based therapies have been **in animals**. In **pigs** the application of growth hormone-releasing hormone supported the gestating fetuses´ survival. A single injection was sufficient.

Large **clinical trials for human DNA therapies** are now **under way**. One of them is **growth factors that mobilize stem cells to treat** congestive **heart failure**.

- critical limb ischemia
- new blood vessels
- hepatitis C virus
- veterinary applications are more advanced than human – e.g. melanoma in dogs (achieved six-fold increase of the medial survival time
- flu vaccine: in animals protects against common flu strains inclusive the highly lethal **H5N1 avian fl**u
- main reason: plasmids contain so-called consensus sequences of **flu virus** genes
- experimental DNA version of an H1N1 vaccine is now in early human trials with encouraging results
- HIV: Pennvax-B: 3 viral genes + adjuvant molecules, applied by electroporation
- HIV in NIH: DNA-based HIV vaccine with one of two adenovirus-based HIV vaccines as boosts

With optimized plasmids and improved delivery methods we can see a comeback by the start of the STEP trial. The DNA approach has begun to show promise for uses beyond classical vaccination, including plasmid delivery of some medications an of immune therapies targeted at cancers.

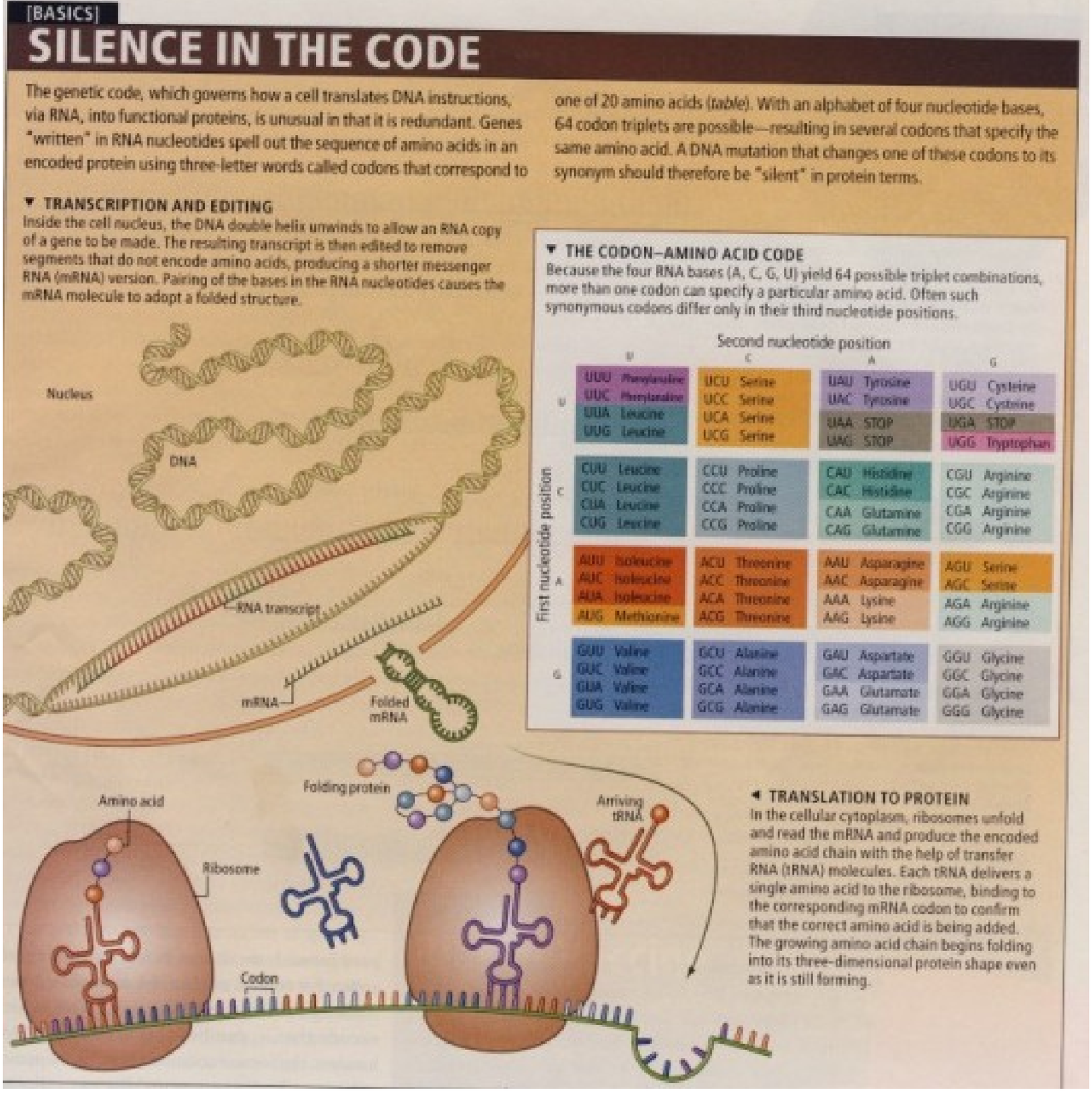
DNA vaccine projects continues but original ideas may not be so simple .....

Cells typically favor one of the **synonymous codons**
and **translate it more efficiently** than the others

WHY?

KEY CONCEPTS

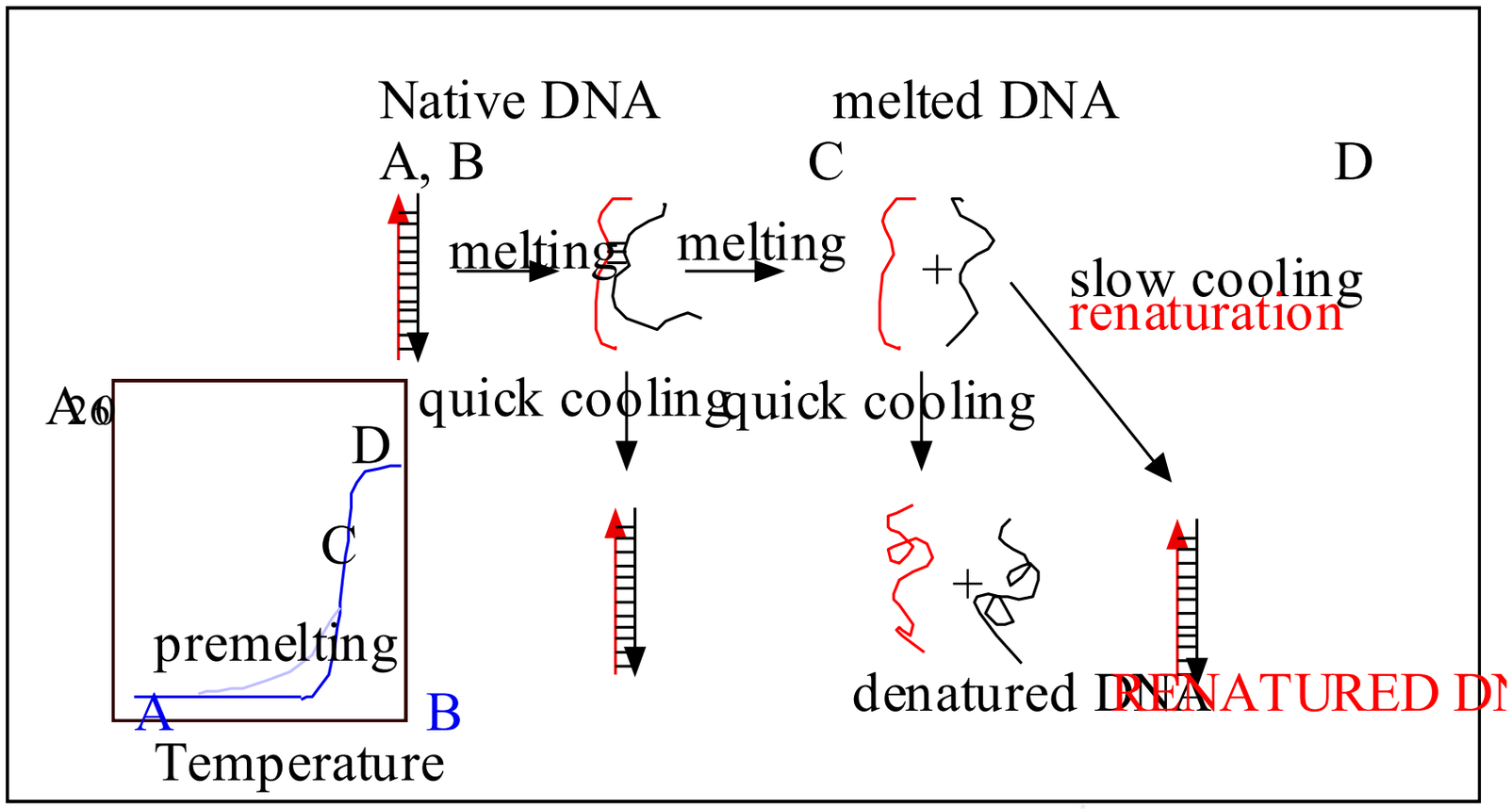
- Scientists **long assumed** that any **DNA mutation that does not change the final protein** encoded by a gene **is effectively "silent"**.
- Mysterious **exceptions to the rule**, in which silent changes seemed to be exerting a powerful effect on proteins, have revealed that **such mutations can affect 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.

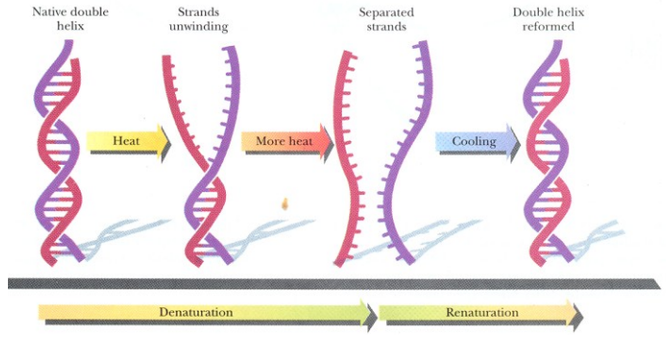


MUFFLED MESSAGE

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.

DNA DENATURATION and RENATURATION/HYBRIDIZATION





J. Marmur and P. Doty, around 1960
Harvard Univ, Cambridge, Mass

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.

158 results found (Set #1) Go to Page: 1 of 16 GO

Records 1 -- 10 Show 10 per page [1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10]

Use the checkboxes to select records for output. See the sidebar for options.

1. **MARMUR J**
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
VIEW FULL TEXT

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

Sort by:
Times Cited SORT

Analyze Results:
ANALYZE
View rankings of the authors, journals, etc. for these records.

Citation Report:
CITATION REPORT
View detailed citation counts and the h-index value for the results.

Output Records:
- Selected records on page
- All records on page
- Records to

Bibliographic Fields

PRINT E-MAIL SAVE

EXPORT TO REFERENCE SOFTWARE

SAVE TO MY EndNote Web

[Sign in to access EndNote Web]

Or add them to the Marked List for later output and more options.

ADD TO MARKED LIST

[0 articles marked]

Microbiologist, biochemist and molecular biologist

Julius Marmur – dicovered renaturation of DNA

*22 March, 1926 Bialystok (Poland) – +20 May, 1996 New York, NY

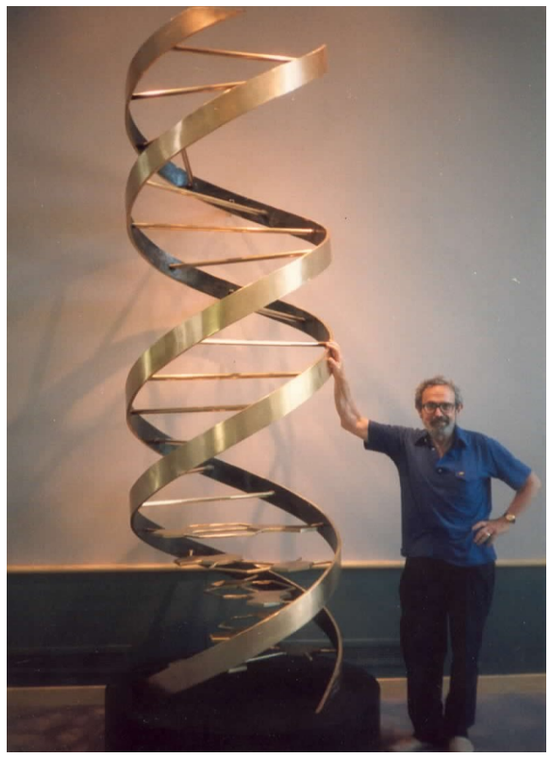
Oswald Avery — 1944 - DNA is a genetic material (Rockefeller Institute, New York, NY)

↓

Rollin D. Hotchkiss

↓

Julius Marmur



1993

The double helix: a personal view

Francis Crick

Medical Research Council Laboratory for Molecular Biology, Hills Road, Cambridge, UK

Francis Crick reviews the papers published 21 years

crystalline A structure, but only briefly, except for the claim

The double helix: a personal view

Francis Crick

Medical Research Council Laboratory for Molecular Biology, Hills Road, Cambridge, UK

Francis Crick reviews the papers published 21 years ago on the structure of DNA and the reaction to them.

For this anniversary I thought it might be appropriate to look back, in a rather informal way, at the original papers on the structure of DNA to see how they appear today in the light of 21 years of research.

During the spring and summer of 1953 Jim Watson and I wrote four papers on the structure and function of DNA. The first appeared in *Nature* on April 25 accompanied by two papers from King's College London, the first by Wilkins, Stokes and Wilson, the other by Franklin and Gosling. Five weeks later we published a second paper in *Nature*, this time on the genetic implications of the structure. A general discussion was included in the volume that came from that year's Cold Spring Harbor Symposium, the subject of which was viruses. We also published a detailed technical account of the structure, with rough coordinates, in an obscure journal[1] in the middle of 1954.

The first *Nature* paper was both brief and restrained. Apart from the structure itself the only feature of the paper which has excited comment was the short sentence: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material". This has been described as 'coy', a word that few would normally associate with either of the authors, at least in their scientific work. In fact it was a compromise, reflecting a difference of opinion. I was keen that the paper should discuss the genetic implications. Watson was against it. He suffered from periodic fears that the structure might be wrong and that he had made an ass of himself. I yielded to his point of view but insisted that something be put in the paper, otherwise someone else would certainly write to make the suggestion, assuming we had been too blind to see it. In short, it was a claim to priority.

Why, then, did we change our minds and, within only a few weeks, write the more speculative paper of May 30? The main reason was that when we sent the first draft of our initial paper to King's College we had not yet seen their own papers. Consequently we had little idea of how strongly their X-ray evidence supported our structure. The famous 'helical' X-ray picture of the B form, reproduced by Franklin and Gosling in their paper, had been shown to Watson, but he certainly had not remembered enough details to construct the arguments about Bessel functions and distances which the experimentalist gave. I myself, at that time, had not seen the picture at all. Consequently we were mildly surprised to discover that they had got so far and delighted to see how well their evidence supported our idea. Thus emboldened, Watson was easily persuaded that we should write a second paper.

The papers in *Nature*

The two experimental papers of April 25 overlap to a considerable extent. Rosalind Franklin's paper mentions the crystalline A structure, but only briefly, except for the claim that the Patterson superposition function (which was in the press at the time) supported two chains rather than three. Both papers stress that there must be more than one chain in the structure. Indeed Maurice Wilkins had personally told Chargaff that a year or so earlier. Both present the argument that the positions of the intensity maxima ruled out two (parallel) chains related by a dyad parallel to the fibre axis. Neither gave the neat argument, due to Watson, that their own density measurement, together with the observed change in length between the two forms, supported two chains rather than three. Franklin noted that if there were several chains they could not be equally spaced and that 'equivalence' favoured two rather than three. It was not explicitly stated, however, that equivalence implies dyad axes perpendicular to the fibre axis and that therefore the two chains must run in opposite directions. Nor did she realise that the monoclinic unit cell of the A form also suggested this, although we had deduced this from her own experimental data.

Both papers correctly concluded from the intensity positions that the phosphate–sugar backbone was on the outside of the structure and that the bases were stacked on the inside. Franklin repeated the argument, which she had made to us verbally a year earlier, that the phosphates would be hydrated (in which she was perfectly right) and therefore that they would probably be on the outside of the molecule. In short, both the groups at King's College had obtained a fairly general idea of the structure but they had done no proper model building. Mainly because of this they had missed the pairing of the bases and they had completely overlooked the significance of Chargaff's rule.

The omissions in the paper by Watson and myself are also striking. The structure is produced like a rabbit out of a hat, with no indication as to how we arrived at it. No dimensions are given (let alone coordinates) except that the base pairs were 3.4 Å apart and that the structure had 10 base pairs in its repeat. The exact nature of the base pairing was not immediately obvious; nor even unambiguous since at that time there were two systems for numbering pyrimidine rings. Most of this information was provided in the subsequent papers. However the general nature of the structure was clear enough, though the tone of the paper ("it must be regarded as unproved until it has been checked against more exact results") was, apart from the short first paragraph, rather muted.

Although a casual reader could easily have overlooked the significance of the first set of papers, especially as they were full of obscure crystallographic jargon, he could hardly miss the impact of our second one. The biologically important features of the proposed structure were explicitly described. The base pairs were listed with the minimum of hedging about tautomerism and were illustrated in scale diagrams. The proposed duplication mechanism was spelt out in simple terms, unmarred by any trace of algebra. In spite of the discussion of the difficulties of unwinding, the list of unsolved problems and the reservations about the unproved nature of the structure, the final paragraph leaves little doubt that the authors thought they had a good idea.

How do they stand today?

How have these early papers stood the test of time? It can now be taken as firmly established that DNA usually consists of two chains, wound together and running in opposite directions. The evidence for this statement is so extensive that it would take too long to quote it all here. The fact that normally A pairs with T, and G with C, is also well established but the details were less certain until recently. The G:C pair was never in serious doubt. Watson and I drew this with only two hydrogen bonds but mentioned in our technical paper[1] that three was also a possibility. This was made almost certain by the theoretical arguments of Pauling and Corey[2] and was confirmed by X-ray structure determinations of single crystals of base pairs. The same technique showed that the A:T (or A:U) pair in single crystals usually did not have the configuration Watson and I suggested. The matter was only finally resolved about a year ago when Rich and his colleagues published two crystal structures; that of GpC paired with itself[3] and ApU paired with itself[4] (the backbone in each case was ribose), both to about 0.9 Å. They show not only the expected configurations for the base pairs but also make it highly likely that, as we claimed, nucleic acid helices are right handed.

In 1953 it was uncertain whether RNA could form a double helix. Watson and I stated that we thought we could not build our model for the B form of DNA with an RNA backbone. The discovery of double-stranded RNA viruses proved, however, that biological RNA too could form a double helix, though with slightly different parameters. The detailed coordinates we had (tentatively) suggested for DNA were soon shown to be incorrect (we had put the backbone at too big a radius) and much more accurate coordinates were provided by Wilkins and his colleagues, using fairly sophisticated methods of handling their much improved X-ray data. The general correctness of this work has been strongly supported recently by the single-crystal studies, mentioned above, of Rich and his coworkers.

Recently, Bram[5] has put forward evidence that the parameters of a DNA double helix may vary somewhat with base composition, though whether this is a trivial variation or has deep biological implications is at present uncertain. Watson and I were so impressed with the apparent uniformity of the double helix from different biological sources and the regularity of the backbone of our model that we had no hesitation in saying that it "seems likely that the precise sequence of the bases is the code that carries the genetic information", an idea which gave me plenty to think about in the next 10 or 12 years.

Nothing was said about the possibility that the two chains might be melted apart and then annealed together again, correctly lined up. The discovery of this by Marmur and Doty has provided one of the essential tools of molecular biology. I can still remember the excitement I felt when Paul Doty told me about it at breakfast one day in New York in a hotel overlooking Central Park. But in other respects we were almost too far sighted, as witness our remark that recombination would probably depend upon base pairing. We struggled for several years to produce neat models for this, all to no avail, partly because we accepted copy choice too easily but also because we were trying to invent a mechanism which did not need additional enzymes. This showed a gap in our overall grasp of molecular biology, which can also be glimpsed in our tentative suggestion that DNA synthesis might not need an enzyme, a remark I should certainly not make today except perhaps in the context of the origin of life.

As to DNA replication, our earliest description was mainly schematic. We realised that plain nucleotides were not likely to be the immediate precursor but missed the rather obvious idea that they were nucleoside triphosphates, again a lack of insight into biochemistry. We did suggest the so-called Y mechanism (in the Cold Spring Harbor paper) but did not mention the difficulties due to the direction of synthesis of antiparallel chains, though I frequently emphasised it a few years later. Looking back, I think we deserve some credit for not being inhibited by the difficulty of unwinding which we clearly recognised and for our forthright stand against paranemic (as opposed to plectonemic) coiling. In this instance our grasp of X-ray diffraction was invaluable.

The functions of DNA

It is, of course, somewhat a matter for surprise that DNA synthesis is not fully understood even today. It would take too much space to discuss the complex and rapidly moving field here. Semiconservative replication in many instances is firmly established. The process certainly occurs as if base pairing were taking place, but I have often asked myself what evidence would make it certain that base pairing really occurs rather than some elaborate allosteric mechanism, even though the latter seems unlikely. Perhaps only an X-ray determination of the structure of the polymerase will finally answer the question. Meanwhile the topics of Okazaki fragments, rolling circle models, RNA primers and the exact roles of the various polymerases will keep many people busy. Even at that early period we did at least ask whether the DNA of a chromosome was in one long molecule, though the idea of circular DNA never occurred to us. Nor did we suggest that a virus might have single-stranded DNA. There is however one remark which may turn out to be perspicacious ". . . we suspect that the most reasonable way to avoid tangling is to have the DNA fold up into a compact bundle as it is formed". As we struggle with the structure of the *E. coli* chromosome and the even more formidable problem of the structure of the chromosomes of higher organisms—probably the major unsolved problem of molecular biology today—it might be worth remembering this tentative suggestion from the distant past.

The other topic we touched on was mutation. This was of the base-substitution type—there is no hint of frameshift mutants. We totally missed the possible role of enzymes in repair although, due to Claud Rupert's early very elegant work on photoreactivation, I later came to realise that DNA is so precious that probably many distinct repair mechanisms would exist. Nowadays one could hardly discuss mutation without considering repair at the same time.

There is no hint in these early papers that nucleic acid might form a complex three-dimensional structure such as we now find in transfer RNA nor even the idea of the hypothetical Gierer loops. Our message was that DNA was simple and alone carried the genetic information. We saw no reason to complicate it till we had to. For the same reason although we must have drawn a G:U pair we attached no importance to it. "Wobble" was still far in the future, but these, it seems to me, are forgivable oversights.

Reactions to the structure

It is really for the historian of science to decide how our structure was received. This is not an easy question to

Francis Crick 21 years after invention of the DNA double helix structure about the discovery of DNA renaturation

Nothing was said about the possibility that the two chains might be melted apart and then annealed together again, correctly lined up. The discovery of this by Marmur and Doty has provided one of the essential tools of molecular biology. I can still remember the excitement I felt when Paul Doty told me about it at breakfast one day in New York in a hotel overlooking Central Park.

Nature 248(1974) 766

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

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-

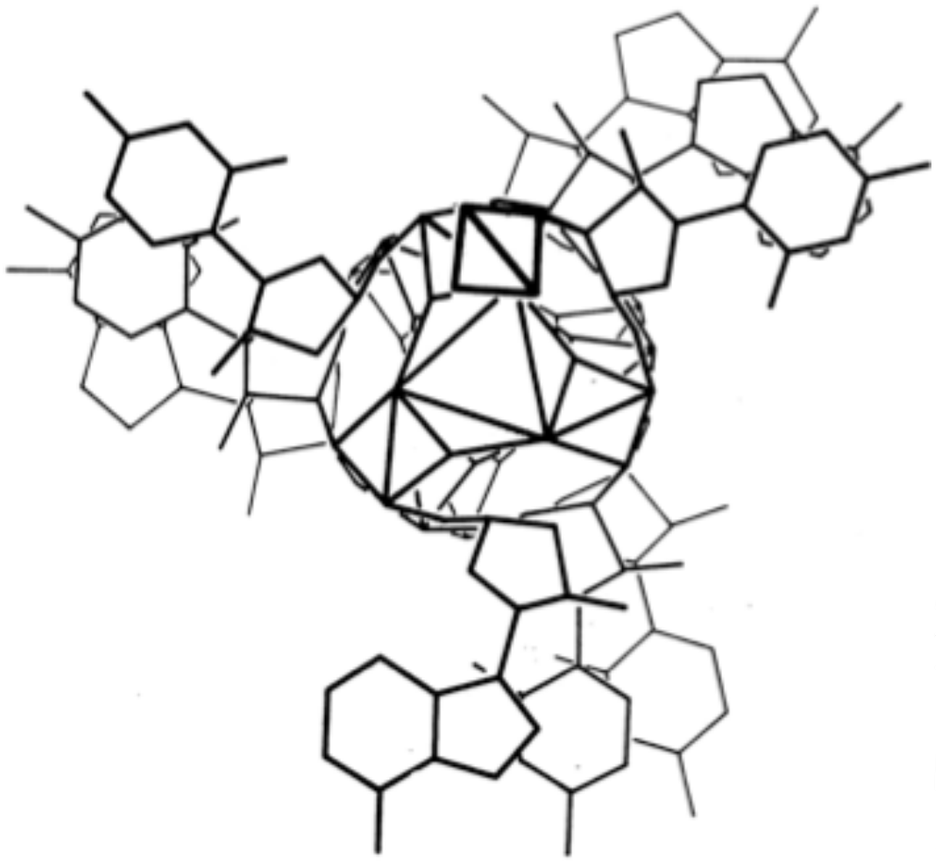


FIGURE 6

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

REALITY

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 propose this structure.
Polarography clearly showed that bases must be hidden in the interior of native DNA molecule and become accessible when DNA is denatured/single-stranded

Negative SUPERCOILING stabilizes local DNA structures

CRUCIFORM
inverted repeat

LEFT-HANDED Z-DNA
alternating pu-py

TRIPLEX structure
homopu·homopy

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

HAIRPIN

SINGLE-STRANDED region
AT-rich

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

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**

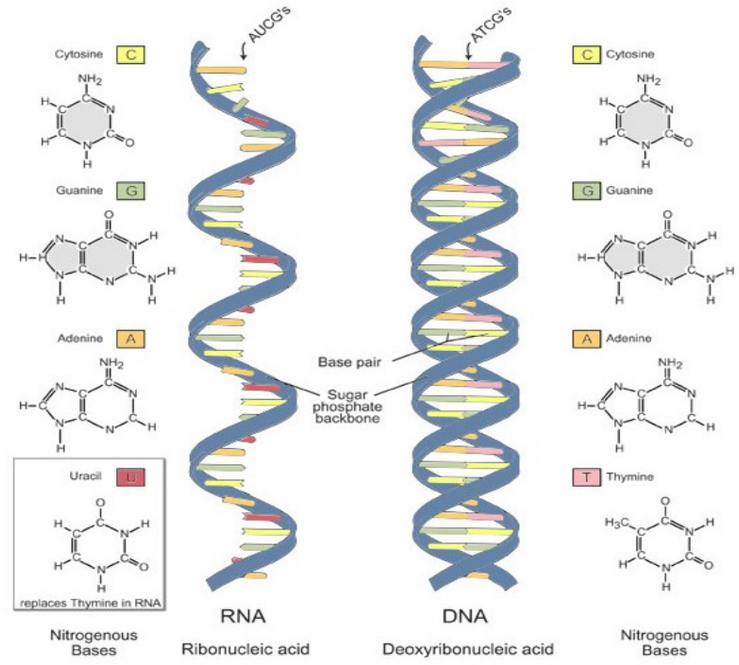
All bases (A, C, G, T, U) yield sparingly soluble compounds with mercury and can be determined at concentration down to 10^{-11}M.
Solid amalgam electrodes can be used instead of the mercury drop electrodes.

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

---

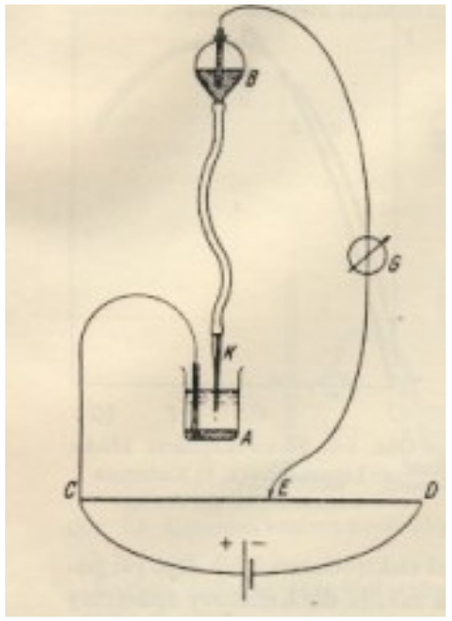


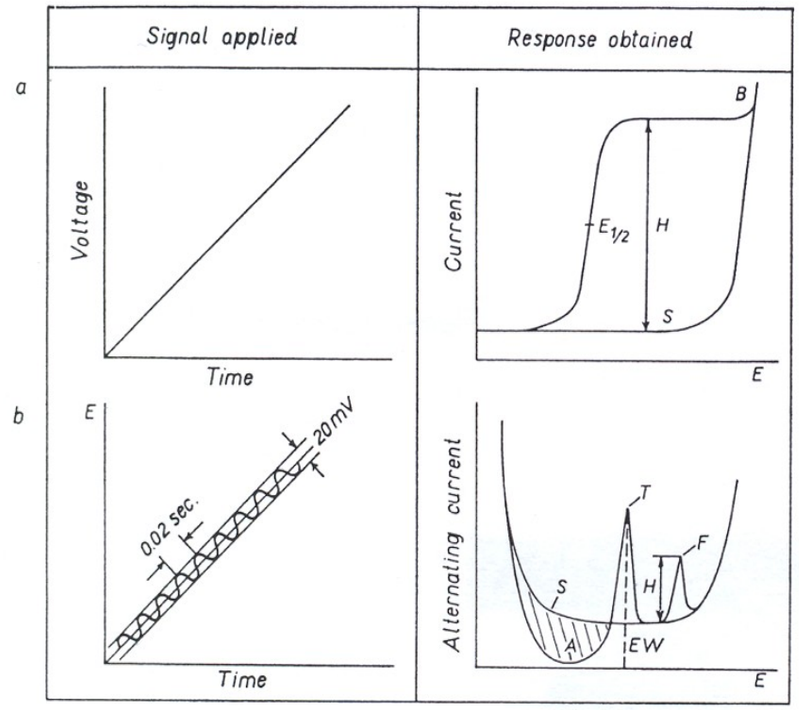
Electroactive Labels can be Introduced in DNA

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

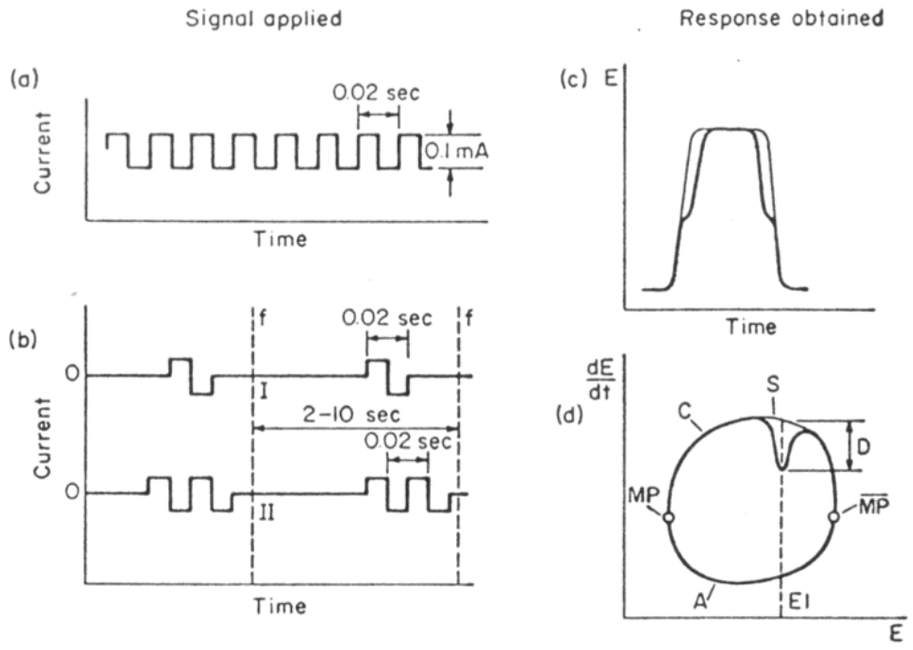




J Heyrovsky S Ochoa A Kornberg

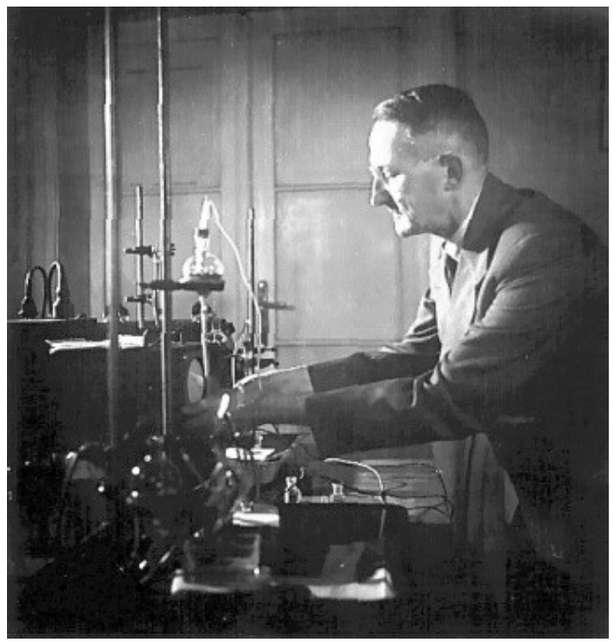
Nobel Prize 1959

T. Heyrovský



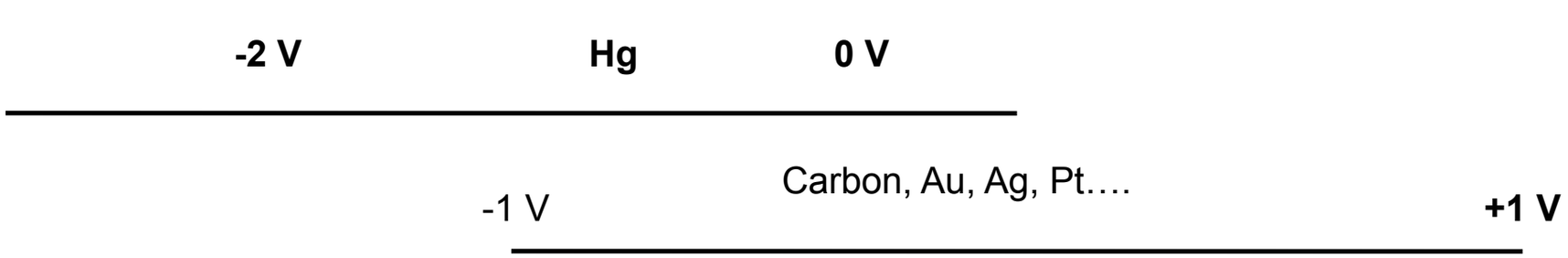
Oscillographic polarography at controlled a.c
(cyclic a.c. chronopotentiometry)

complete analyses on a **single mercury drop** 1941



Electrodes

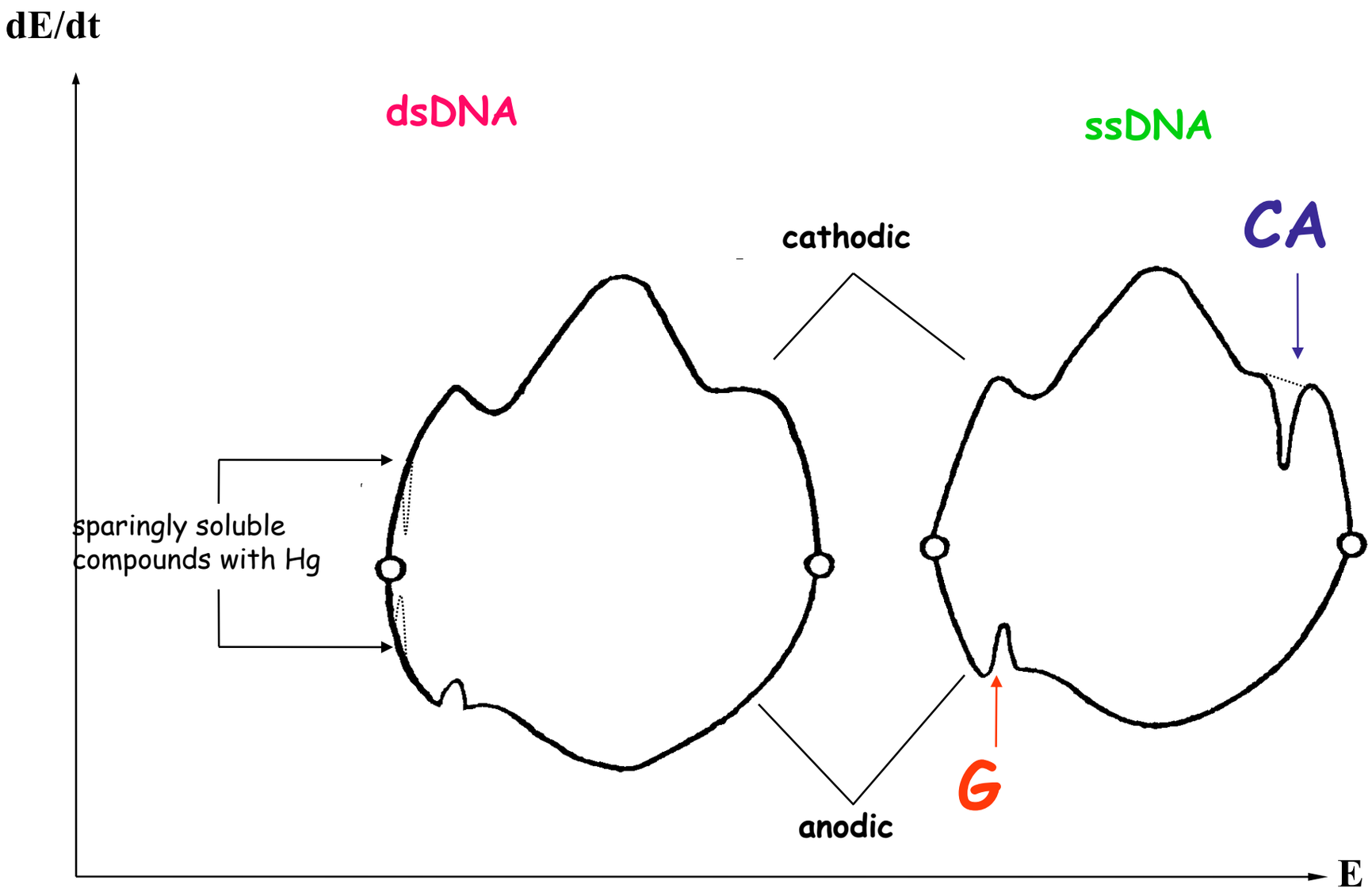
Heyrovsky's polarography was based **on mercury electrodes**. **At present** a number of different electrodes is used in electrochemical analysis, incl. bimacromolecule studies, such as liquid mercury and solid mercury-containing electrodes (such as film and solid amalgam, incl. dental amalgam electrodes), **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



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

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.: Oszillographiche *Polarographie der Nucleinsauren und ihrer Bestandteile*; **Naturwiss**. 45 (**1958**), 186
Palecek E.: Oscillographic *polarography of highly polymerized deoxyribonucleic acid*; **Nature** 188 (**1960**), 656

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

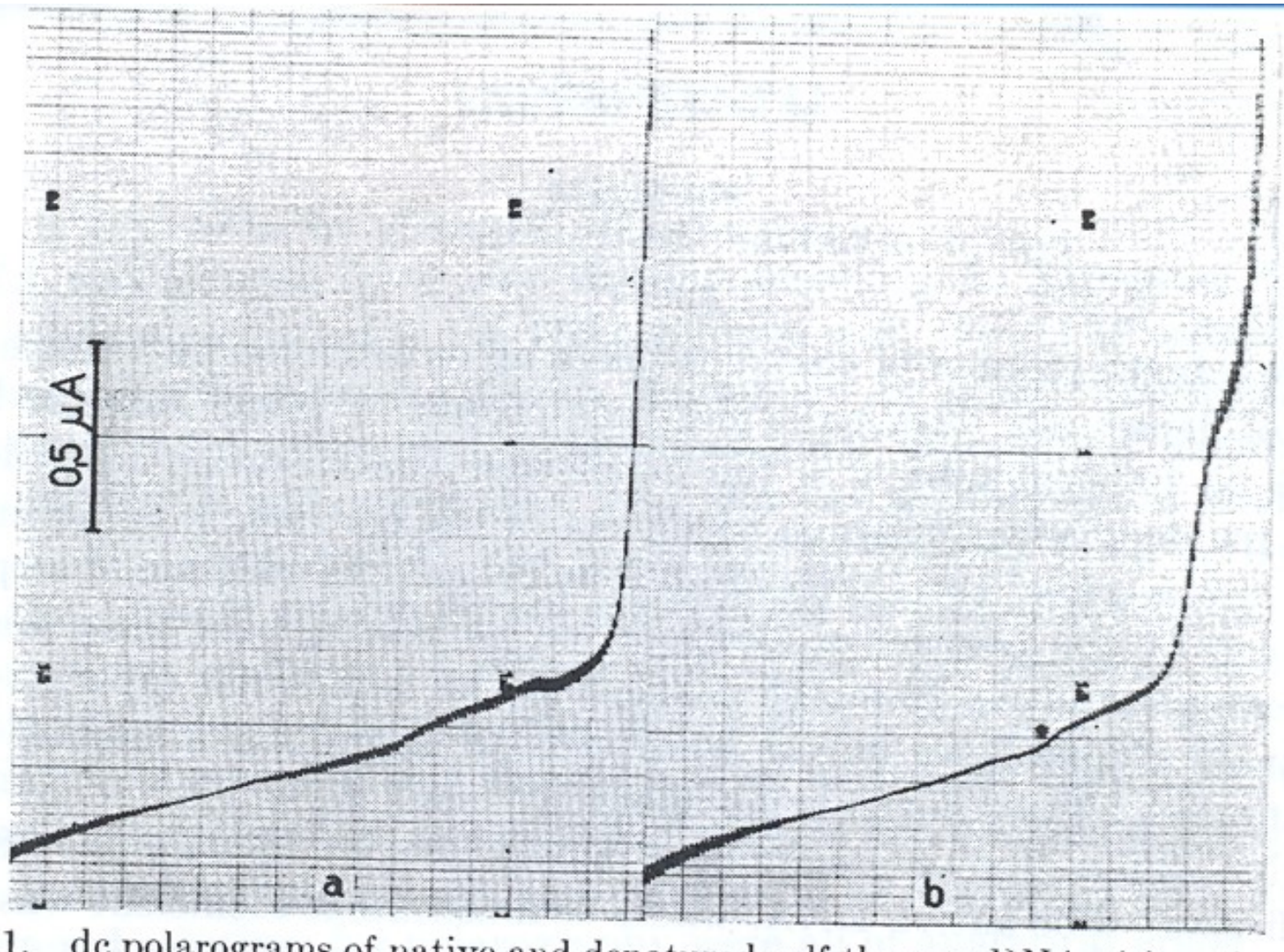
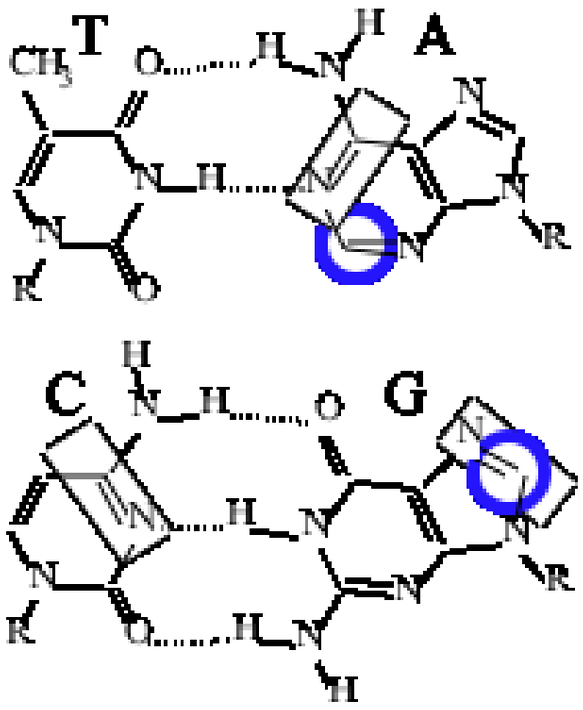
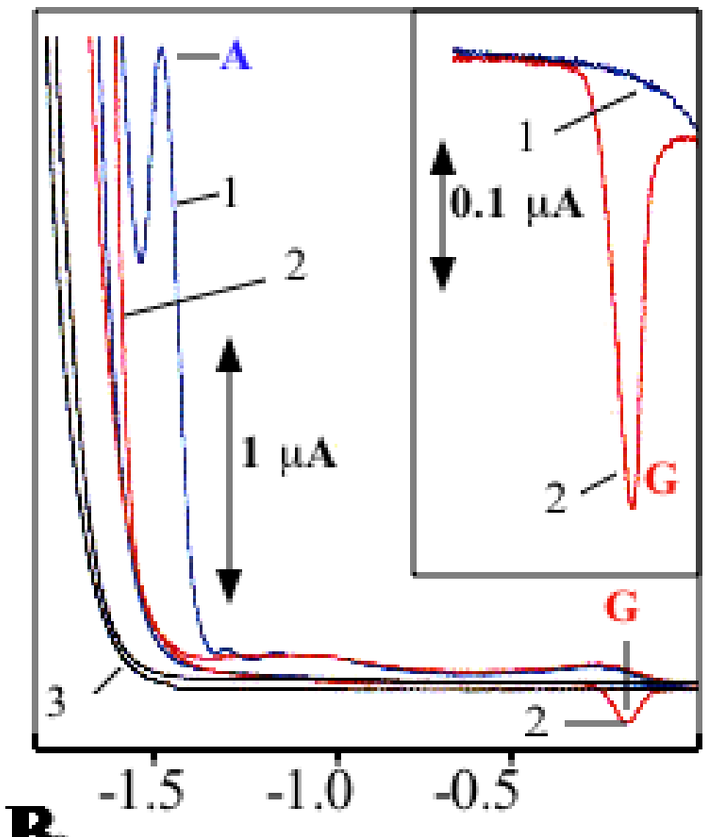


Fig. 1. dc polarograms of native and denatured calf thymus DNA: (*a*) native DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0); (*b*) denatured DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0). DNA 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.

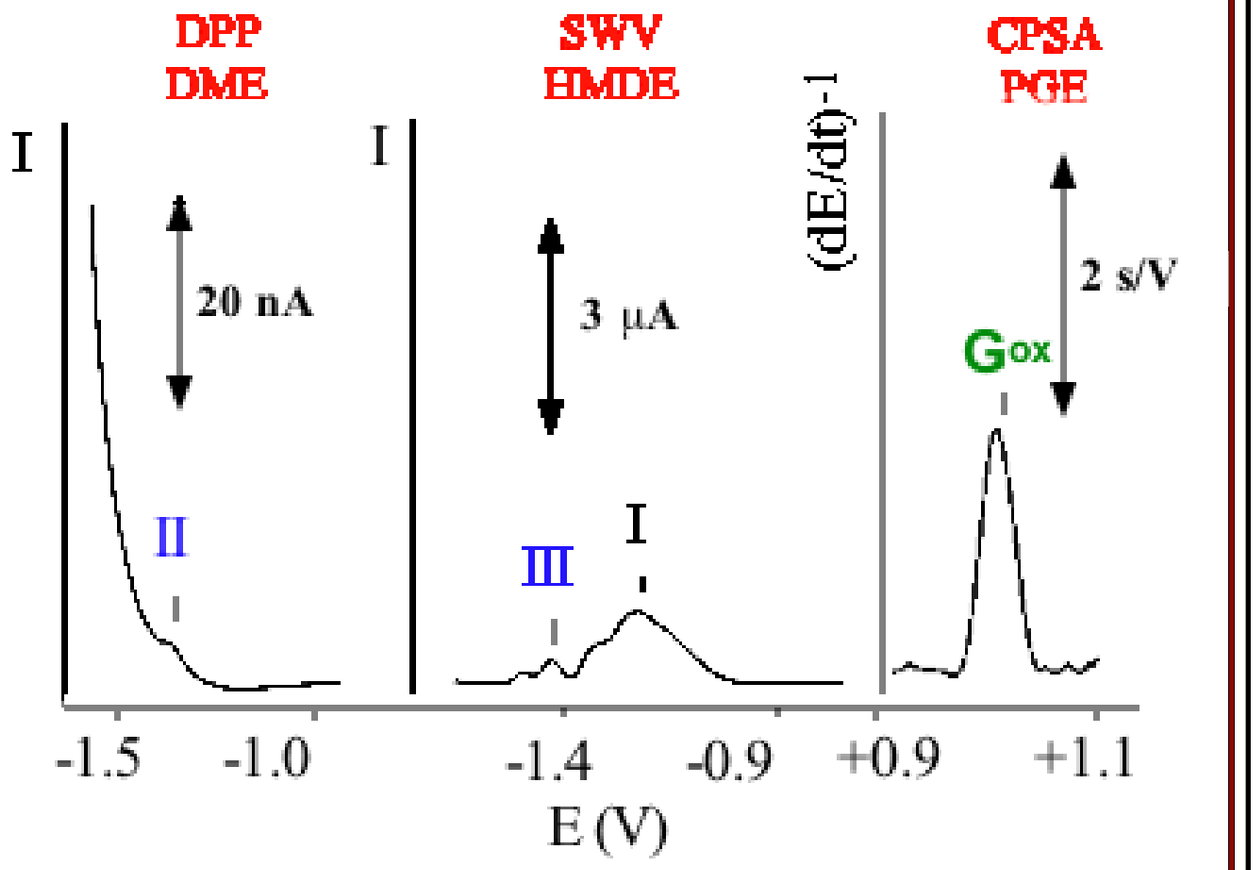
A


T
A
C
G

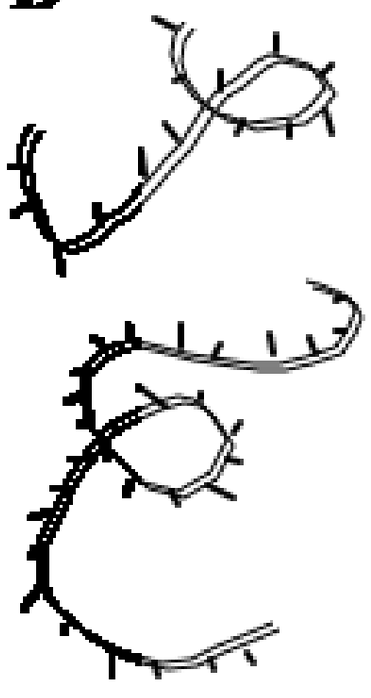
B

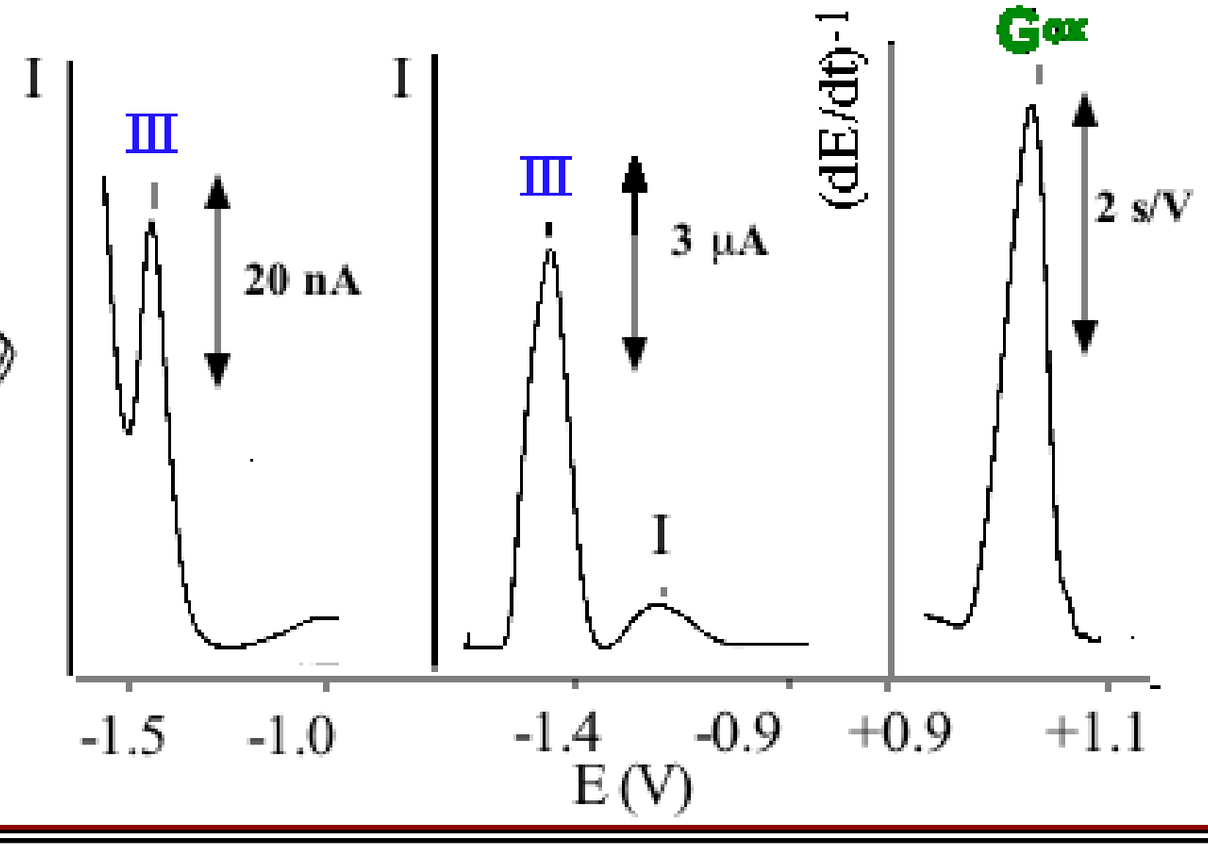

A
1
2
1 µA
0.1 µA
G
3
-1.5
-1.0
-0.5

C


DPP
DME
SWV
HMDE
CPSA
PGE
I
I
20 nA
3 µA
(dE/dt)-1
2 s/V
Gox
II
III
I
-1.5
-1.0
-1.4
-0.9
+0.9
+1.1
E (V)

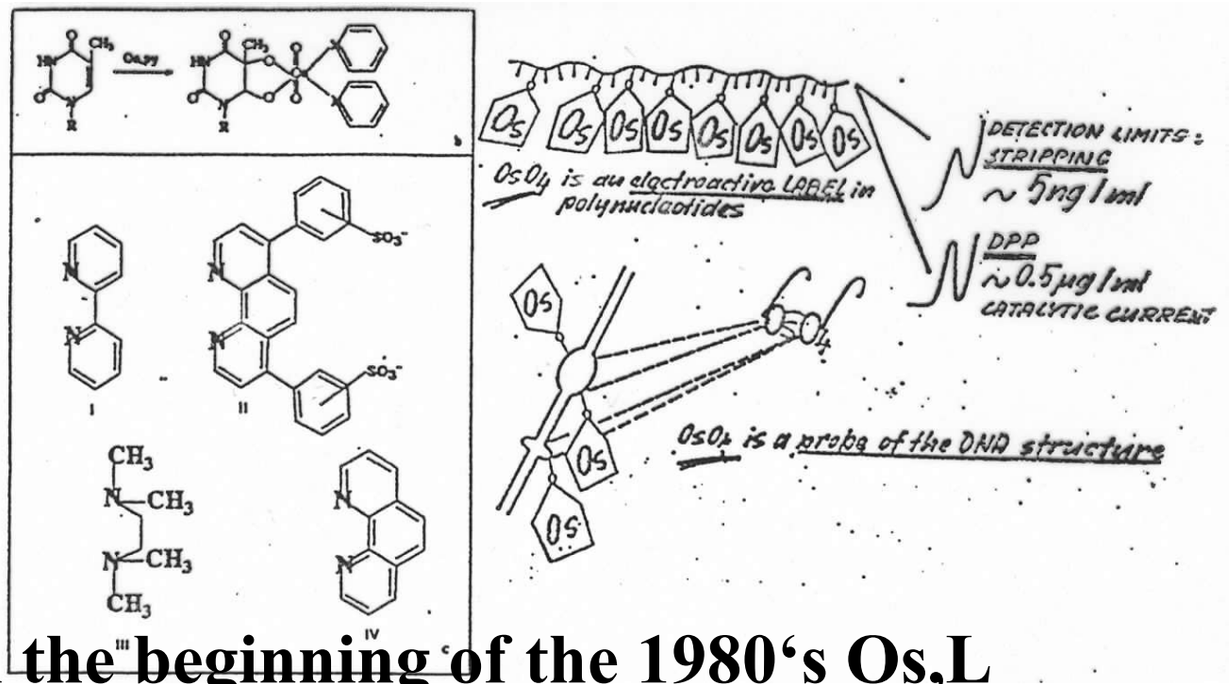
D



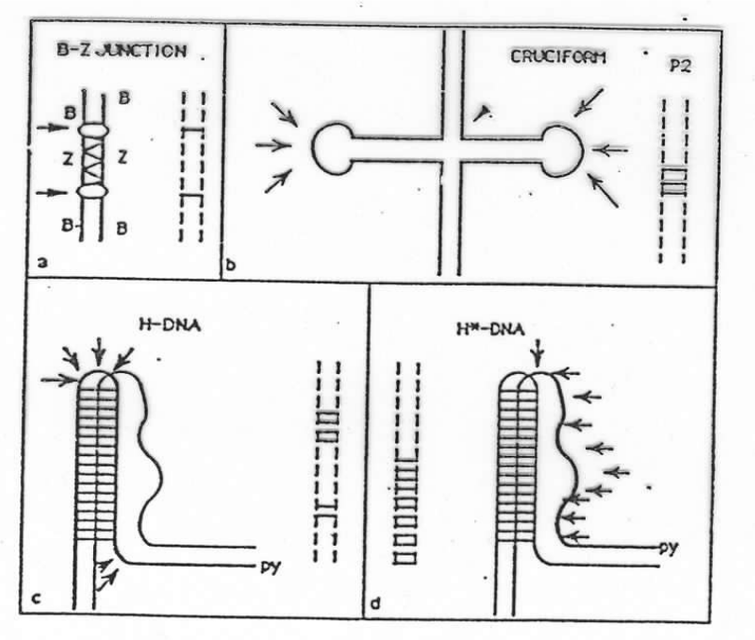

Gox
I
I
III
III
20 nA
3 µA
(dE/dt)-1
2 s/V
I
-1.5
-1.0
-1.4
-0.9
+0.9
+1.1
E (V)

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



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 the double-stranded B-DNA.



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 concentratio

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

Local Supercoil-Stabilized DNA Structures

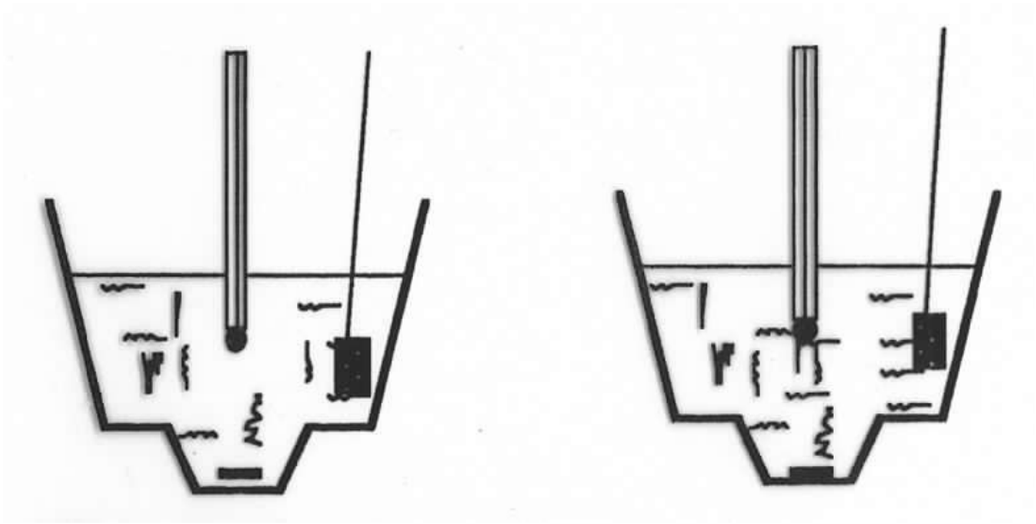
E. Paleček
Max-Planck Institut für Biophysikalische Chemie, Göttingen, BRD and Institute of Biophysics, Czechoslovak Academy of Sciences, 61265 Brno, CSFR

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

By EMIL PALEČEK

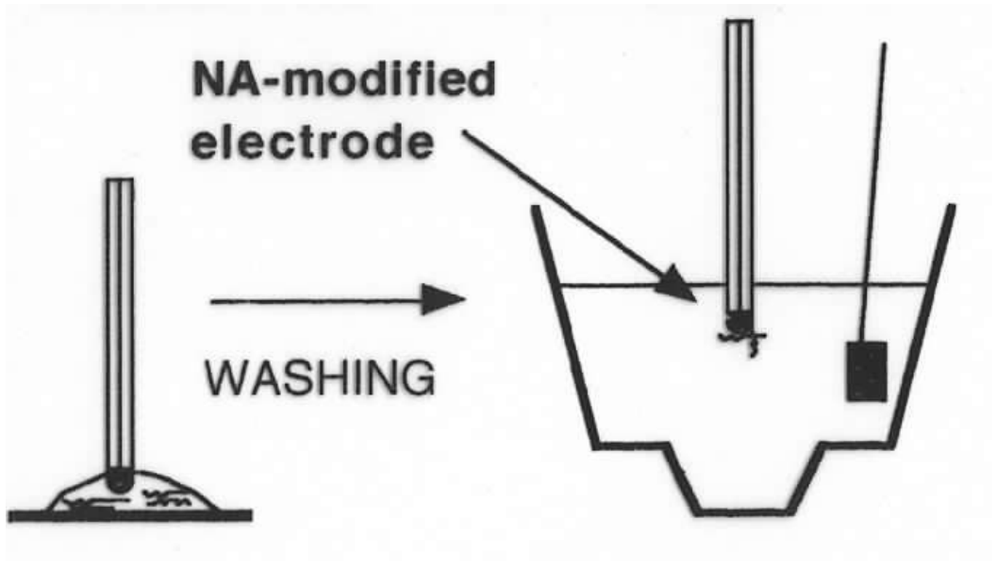
These methods yielded information about the distorted and single-stranded regions in the DNA double helix at single-nucleotide resolution. DNA probed both in vitro and directly in cells.

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.

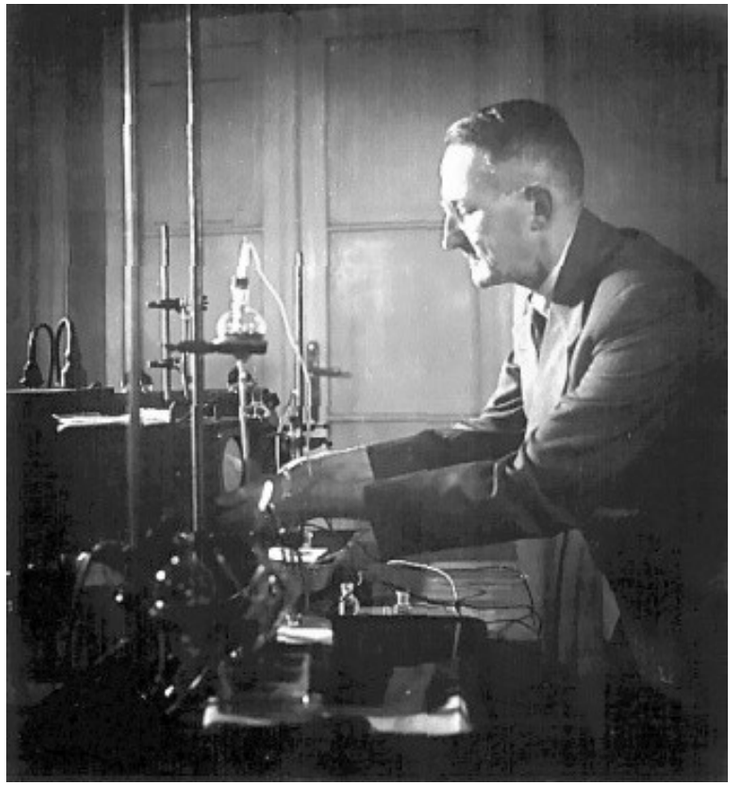
J. Heyrovsky **invented POLAROGRAPHY in 1922.**
After 37 years he was awarded a Nobel Prize

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

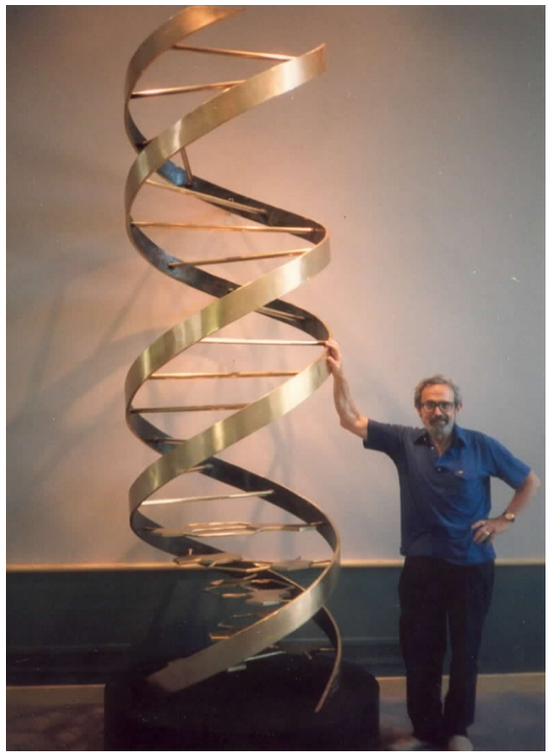


In 1960 when I published my NATURE paper on electrochemistry of DNA I obtained invitations from 3 emminent US scientists:
J. Marmur - Harvard Univ.
L. Grossman - Brandeis Univ.
J. Fresco - Princeton Univ.
To work in their laboratories as a postdoc

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

RENATURATION OF RNA AS DETECTED BY DPP
Time dependence

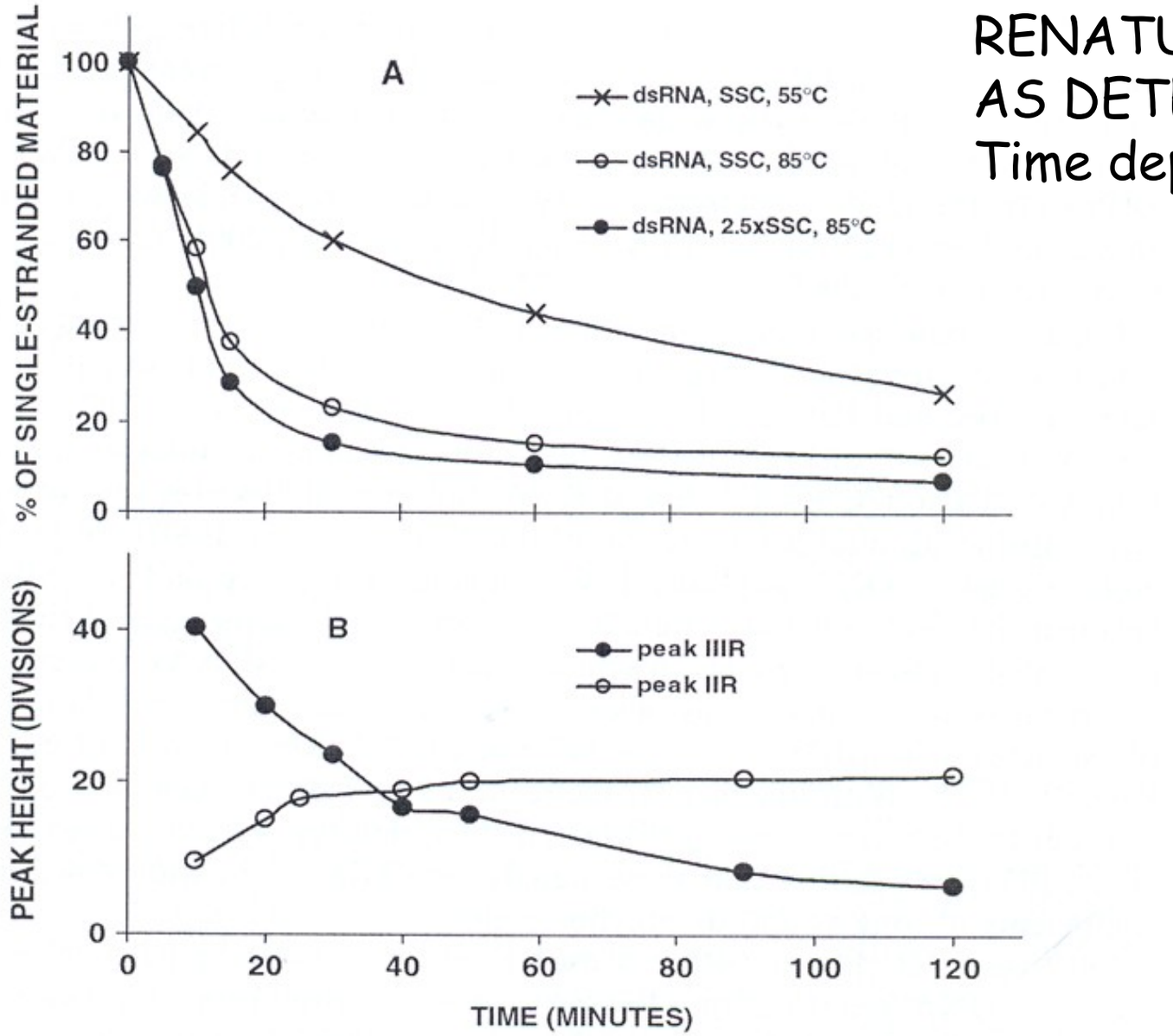


Fig. 10. Time-course of renaturation of phage f2 dsRNA. (A) Thermally denatured ssRNA was incubated (●—●) at 85°C in 2.5 × sodium saline citrate (SSC) or (o—o) at 85°C in SSC, and (x—x) at 55°C. Samples were withdrawn in time intervals given in the graph and quickly cooled. DPP measurements were performed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

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

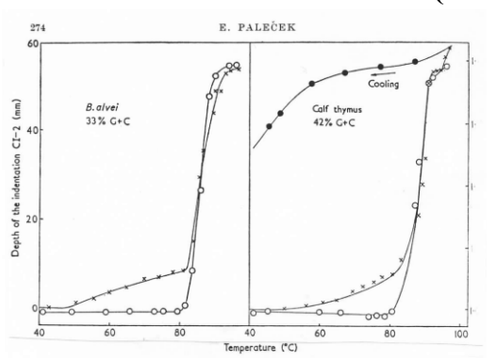


FIG. 11. Thermal transition of DNA's with varying guanine plus cytosine content followed b oscillopolarographic and spectrophotometric methods.
DNA at a concentration of 95 μg/ml. in 0·1 M-ammonium formate plus 0·02 M-sodium phospha (pH 7·0). —○——○—, Absorbancy at 260 mμ; —×——×—, and —●——●—, oscillopolar 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

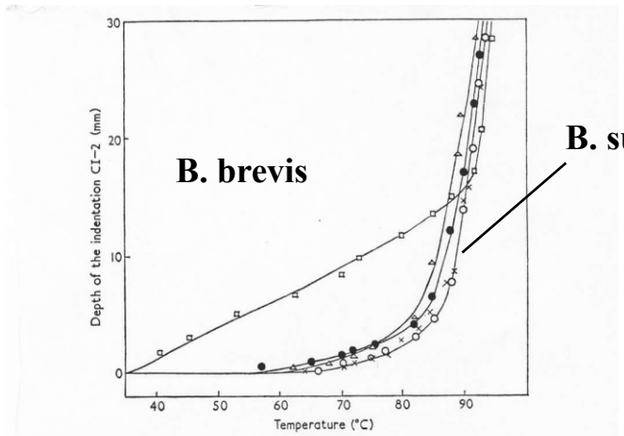


FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 μg/ml. in 0·25 M-ammonium formate plus 0·025 M-sodium phosphate (pH 7·0).
—●——●—, *B. subtilis* 168; —×——×—, *B. natto*; —○——○—, *B. subtilis* var. *niger*; —△——△—, *B. subtilis* var. *aterrimus*;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

1976

Reprinted from:
PROGRESS IN NUCLEIC ACID RESEARCH AND MOLECULAR BIOLOGY, VOL. 18
© 1976
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.

POLAROGRAPHIC BEHAVIOR OF dsDNA
At roomand premeltig temperaturse depended on DNA nucleotide SEQUENCE

poly(dA)•poly(dT)

poly d(A-T)•d(A-T)

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
Ferkauf Foundation Visiting Professor

FHCC:lt

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

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?

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

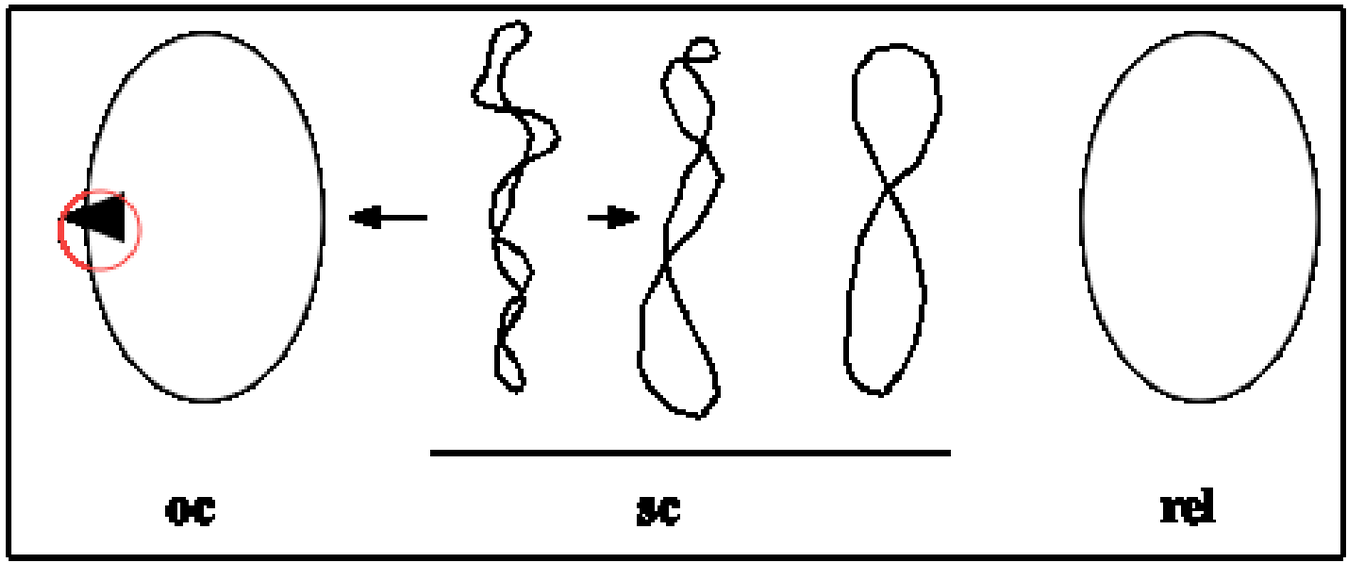
For two decades only mercury electrodes were used in NA electrochemistry

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

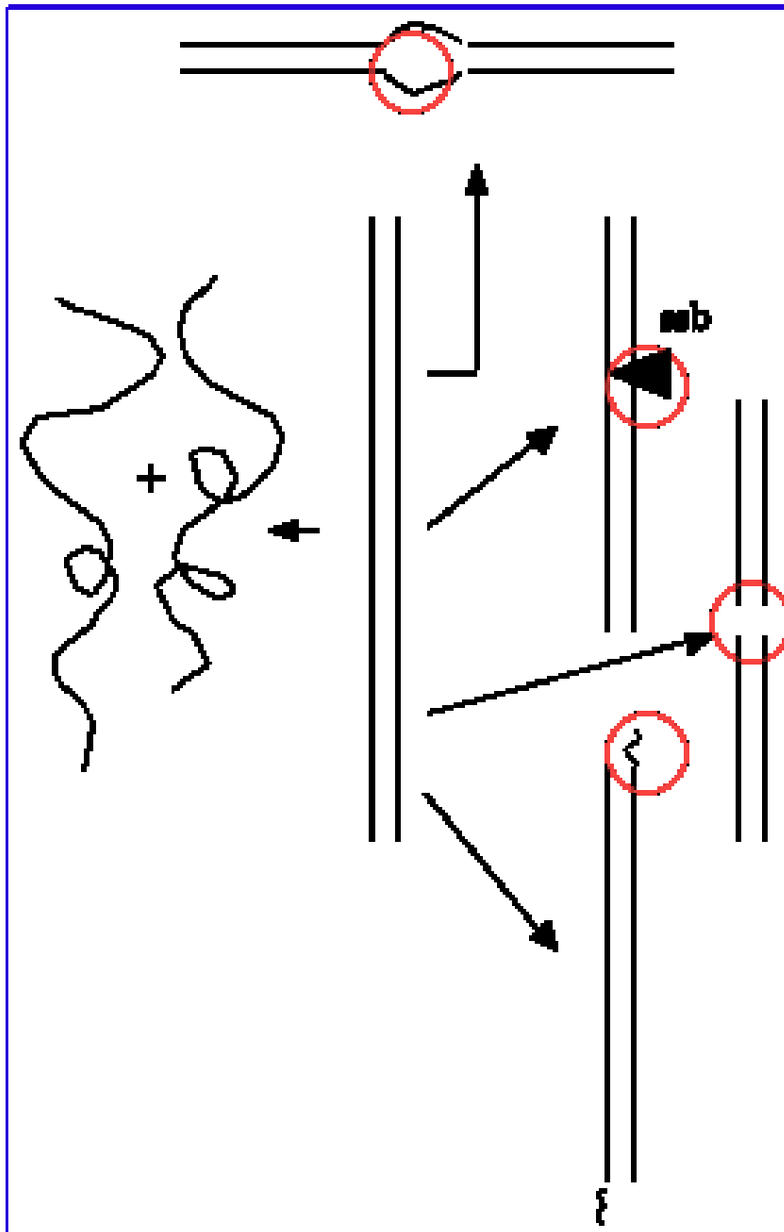
Results obtained at: IBP, Brno or elsewhere (author's name is given); the results which have been utilized in the DNA sensor development are in **blue**

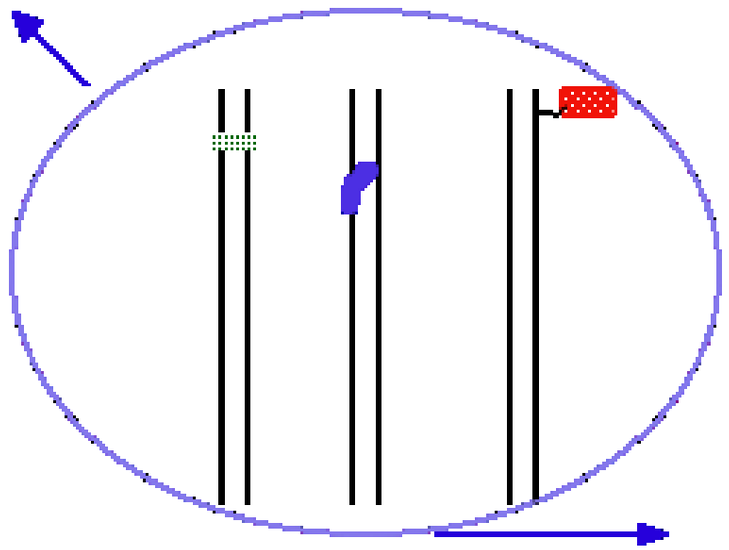
ELECTROCHEMICAL METHODS RECOGNIZE SMALL CHANGES IN DNA STRUCTURE AND DETERMINE TRACES OF IMPURITIES IN DNA SAMPLES

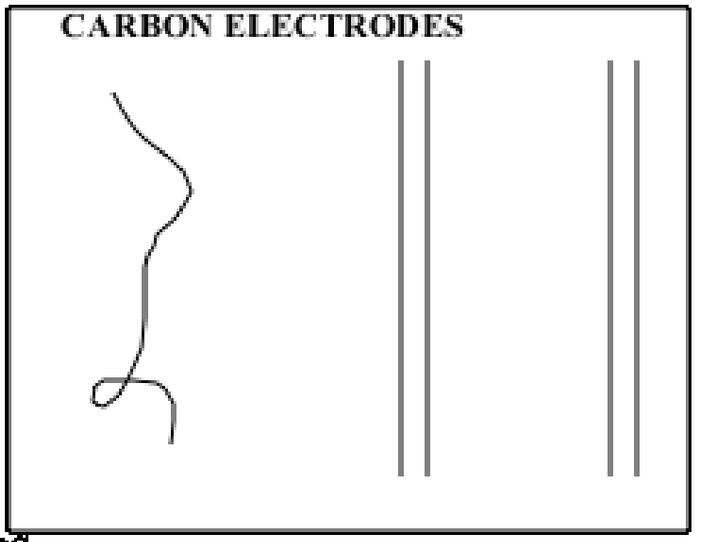
MERCURY ELECTRODES ARE PARTICULARLY SENSITIVE



DETERMINATION OF TRACES (< 1%) OF







INTERCALATORS COVALENT MODIFIERS

GROOVE BINDERS

DNA unwinding at negatively charged surfaces

native denatured

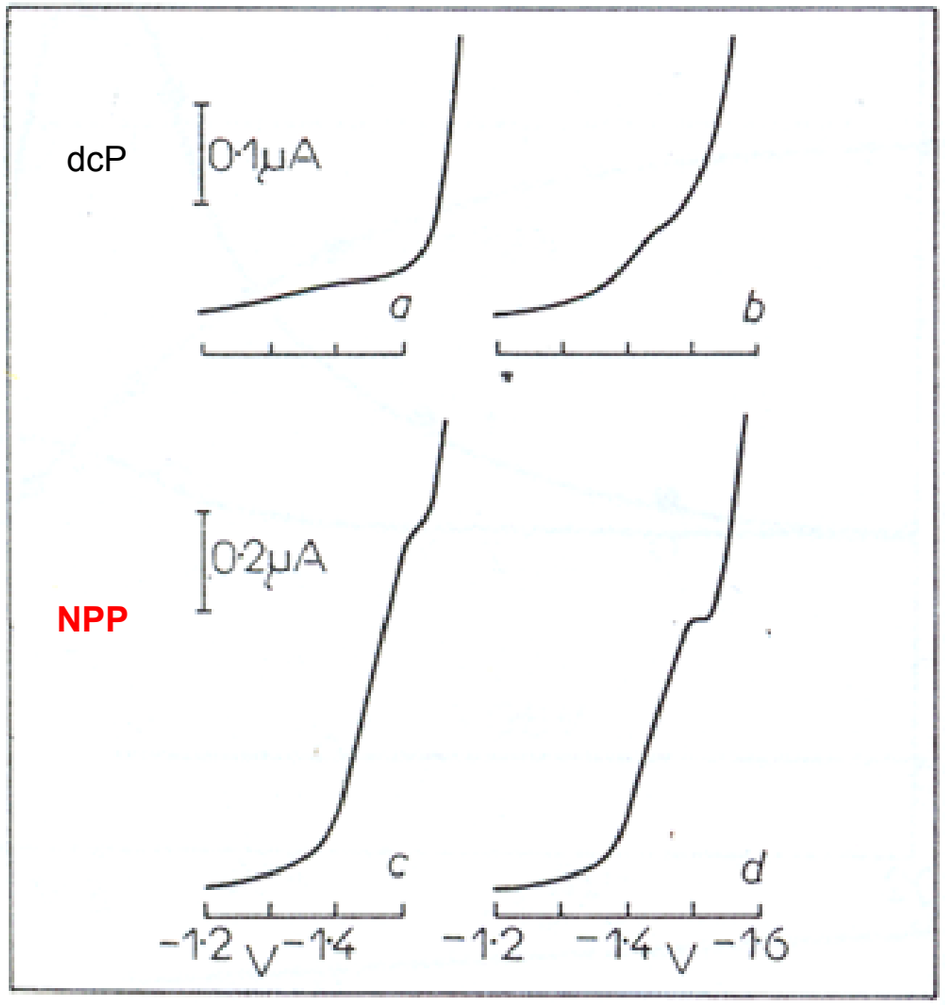


FIG. 1

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.

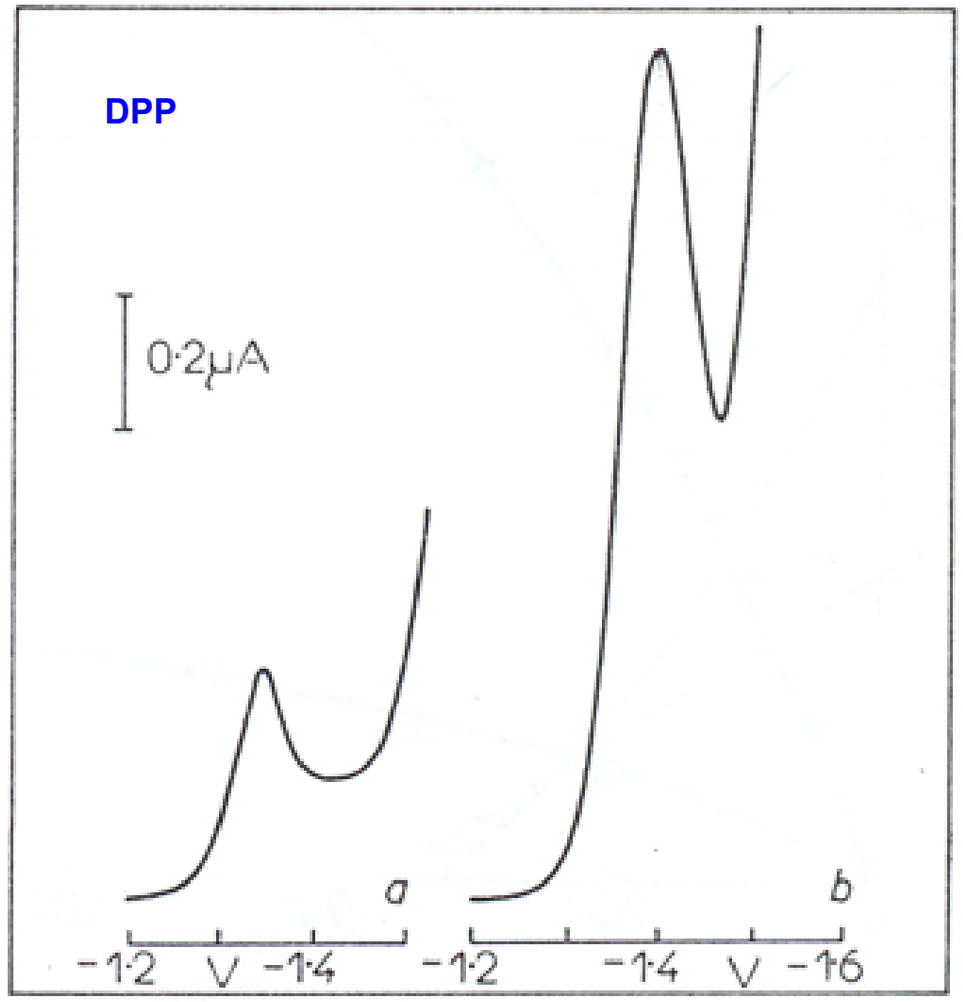


FIG. 2

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

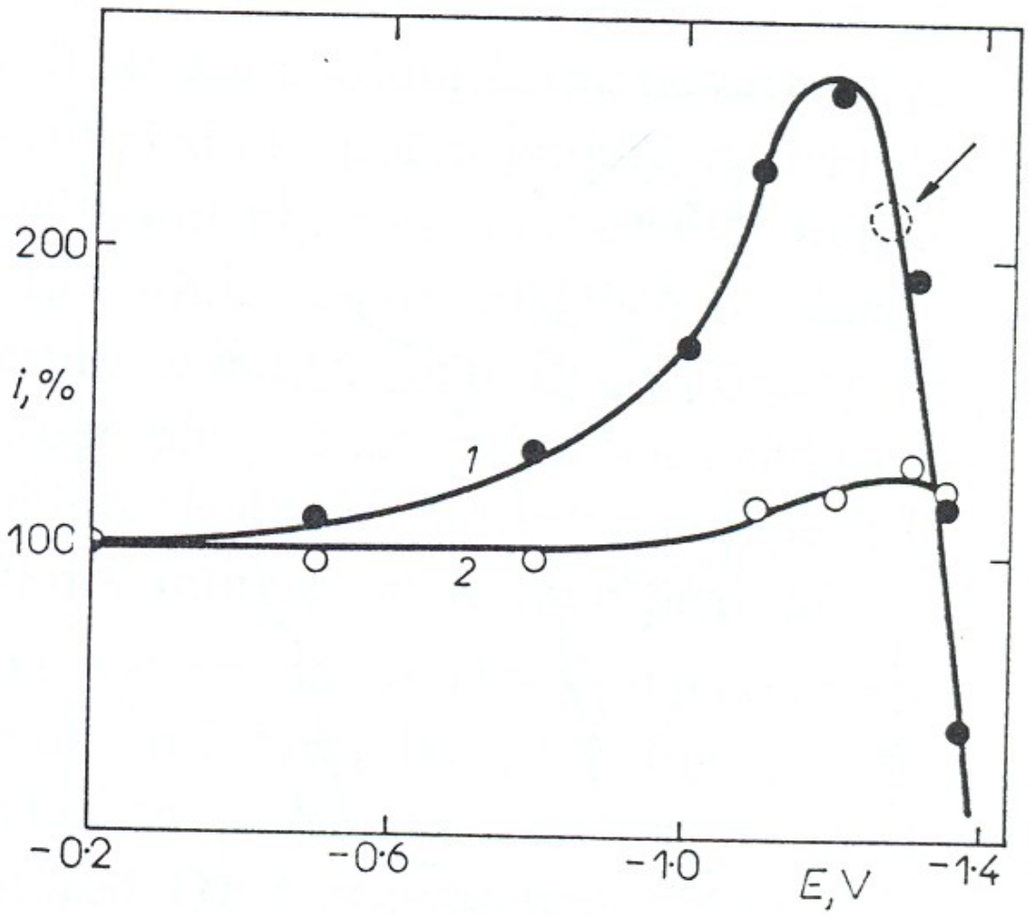
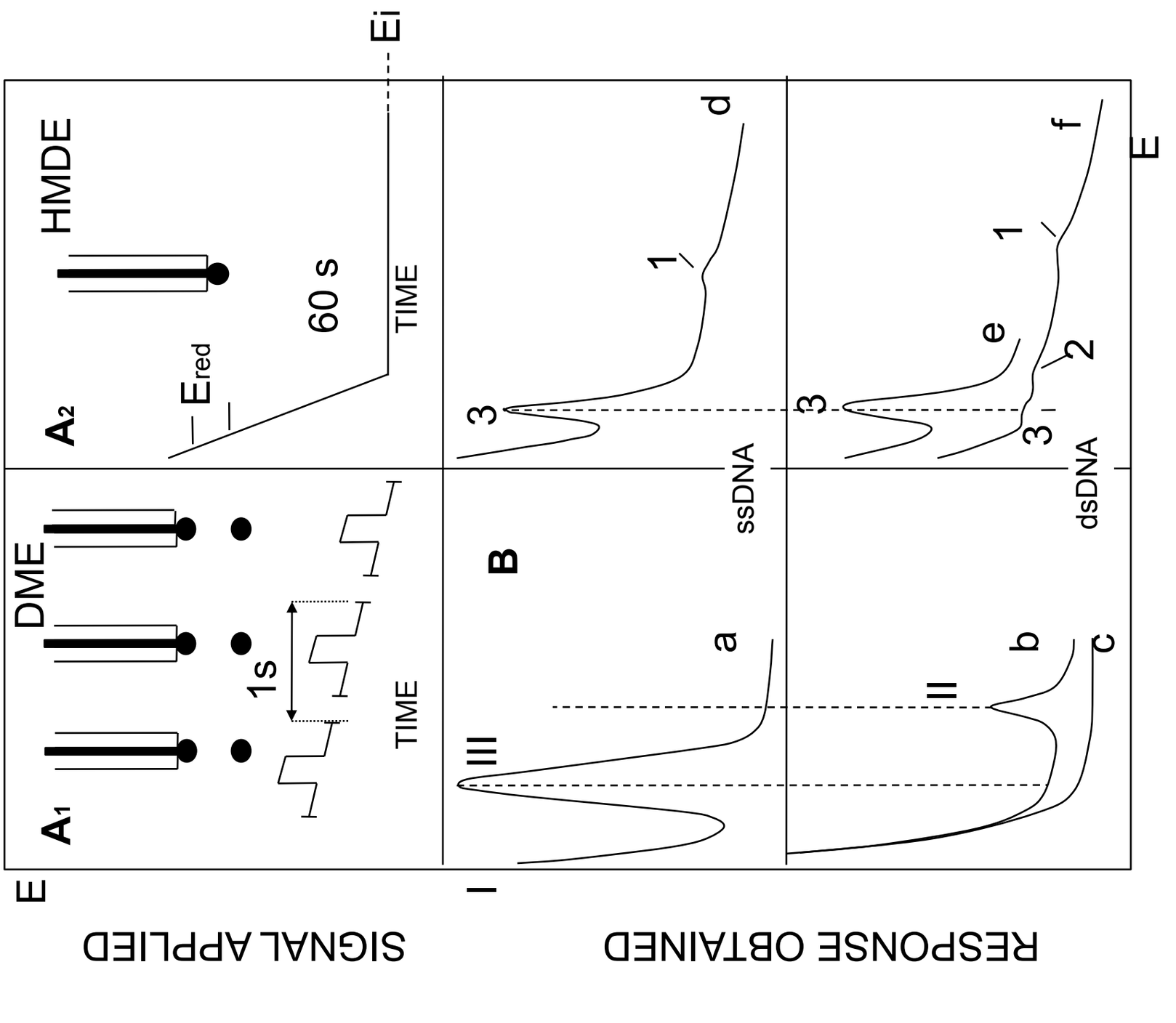


FIG. 4

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

1 Native DNA 500 µg/ml; *2* denatured DNA 50 µg/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.

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


SIGNAL APPLIED
RESPONSE OBTAINED
E
I
A1
DME
1s
TIME
A2
HMDE
Ered
60 s
TIME
B
ssDNA
dsDNA
III
II
I
a
b
c
d
e
f
1
2
3
E

Effect of pH on DNA unwinding

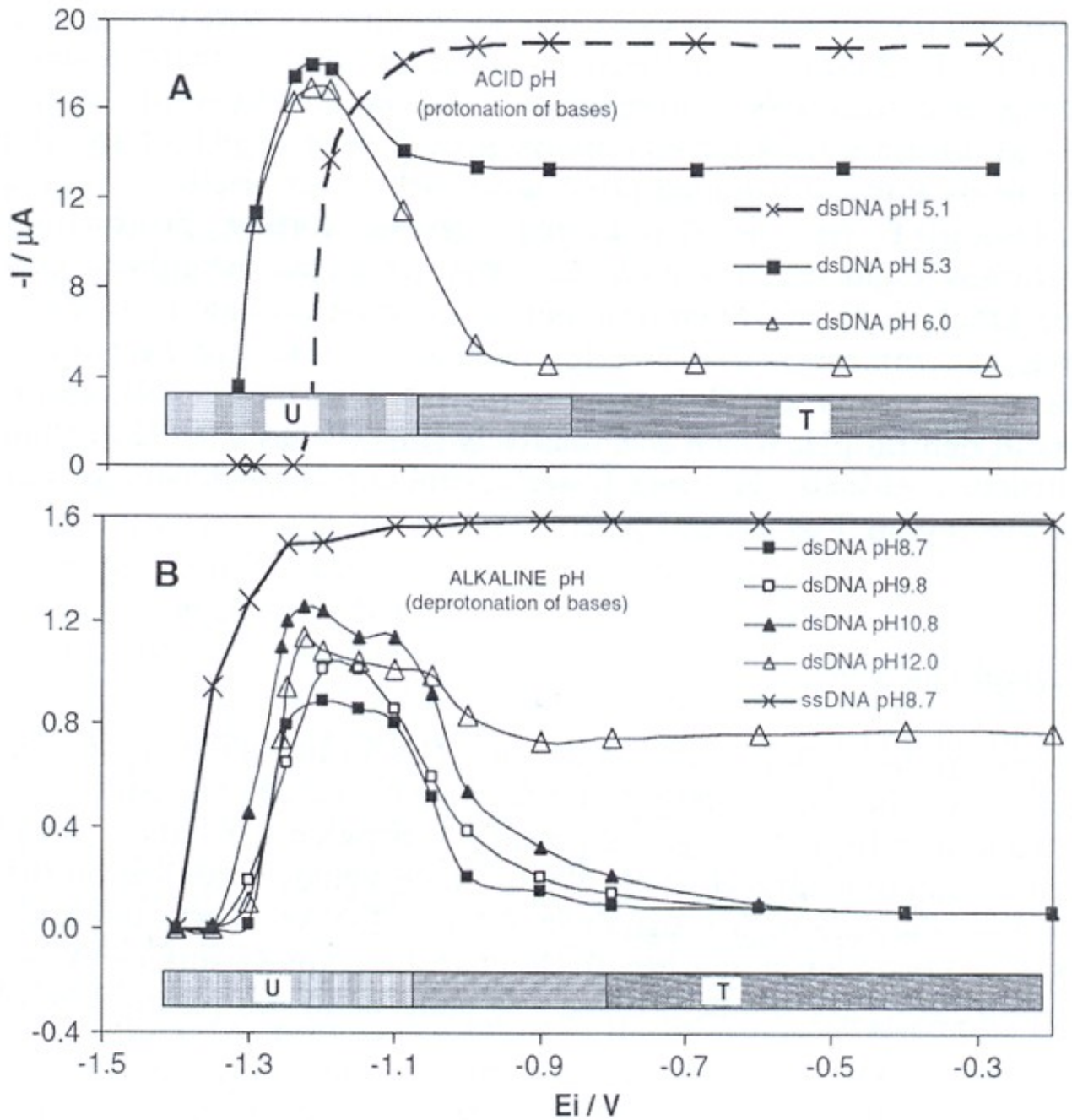


Fig. 17. Dependence of the height of the DNA voltammetric peak 3 on initial potential E_i (A) at acid pHs. dsDNA at concentration of 420 µg/mL: △—△, pH 6.0; ■—■, pH 5.3; x—x, pH 5.1. The graphical indication of the region T and U is valid only for the curve of dsDNA at pH 6.0. (B) at alkaline pH's. dsDNA: ■—■, pH 8.7; □—□, pH 9.8; ▲—▲, pH 10.8; △—△, 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

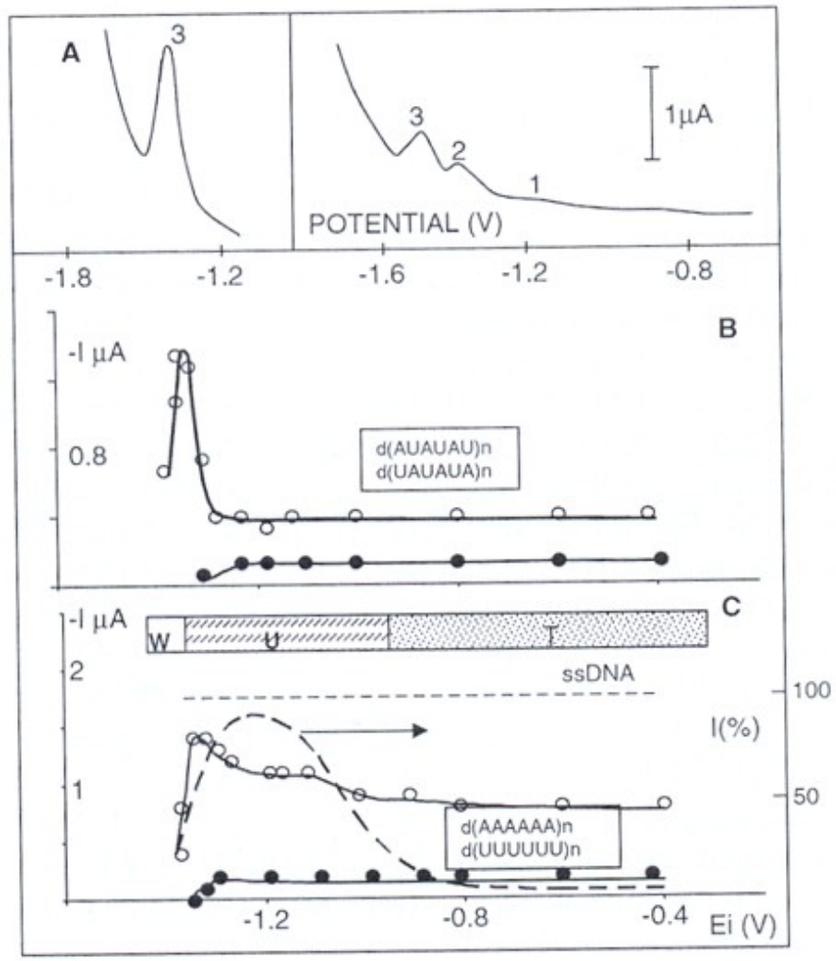


Fig. 16. Dependence of the voltammetric behavior of biosynthetic polynucleotides with different nucleotide sequences on the initial potential (E_i). (A): voltammetric peaks of poly (dA–dU) · poly (dA–dU). $E_i = -0.6$ V (left), $E_i = -1.35$ V (right); (B): ●—● , peak 2; ○—○, peak 3; (C): poly (rA) ·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

peak G

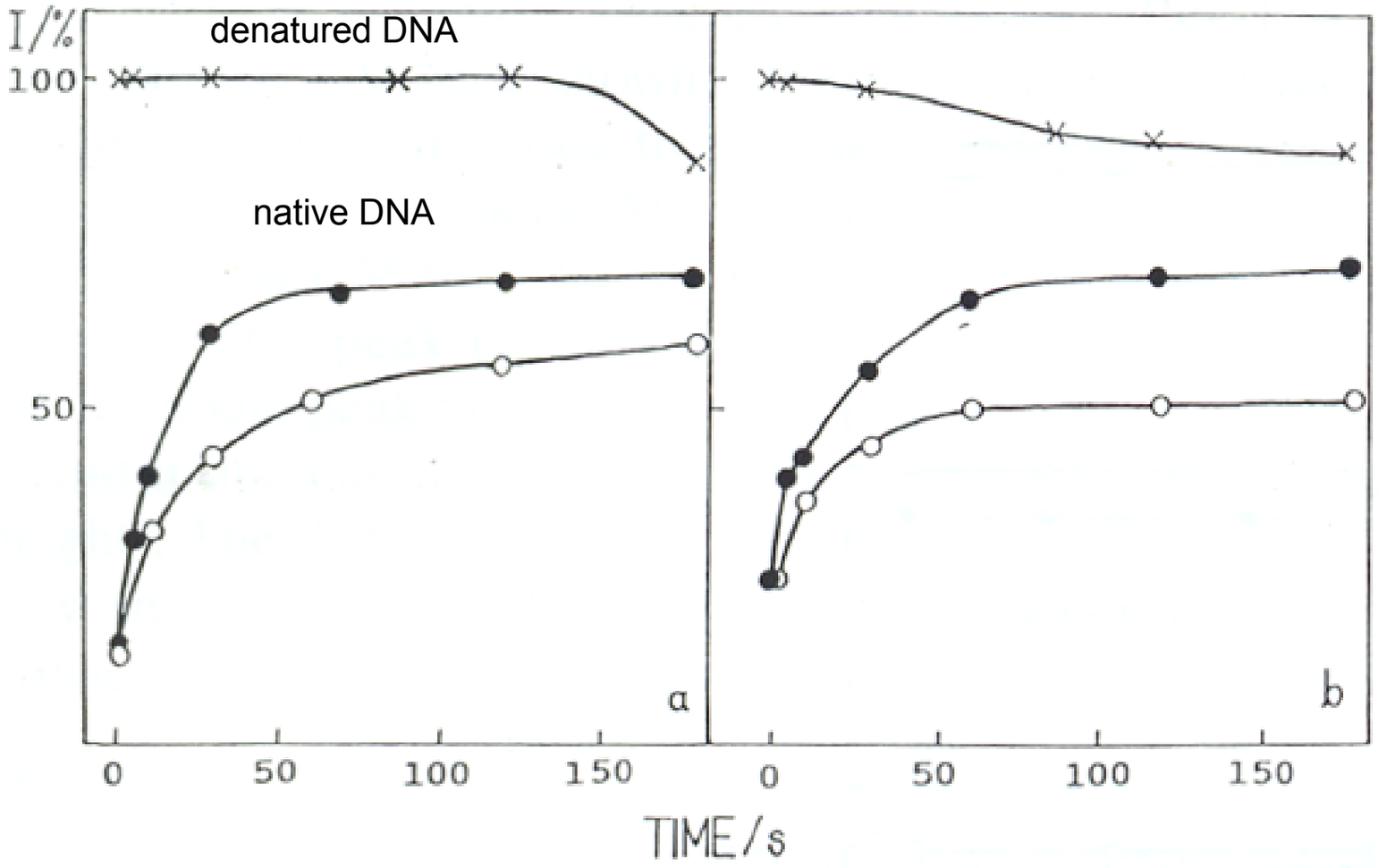


Fig. 6. The dependence of the relative heights of (a) the AdTSCV anodic peak G and (b) the cathodic peak AC on time t_b at potentials $E_b = -1.2$ V (○———○), and $E_b = -1.3$ V (●———●) for native DNA and for denatured DNA (×———×). The HMDE charged to a potential $E_a = -0.25$ V was immersed into the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

Scheme 1

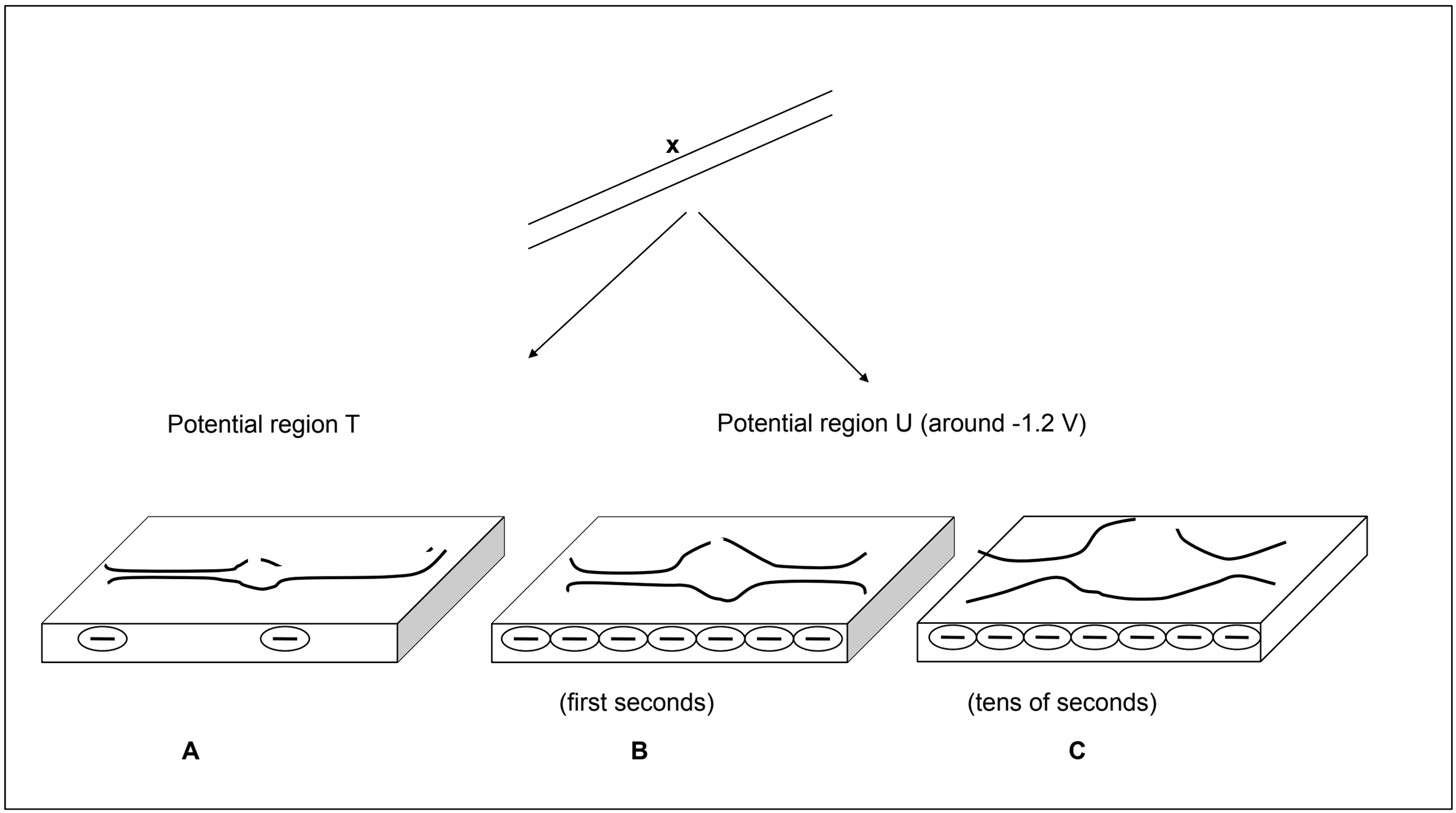
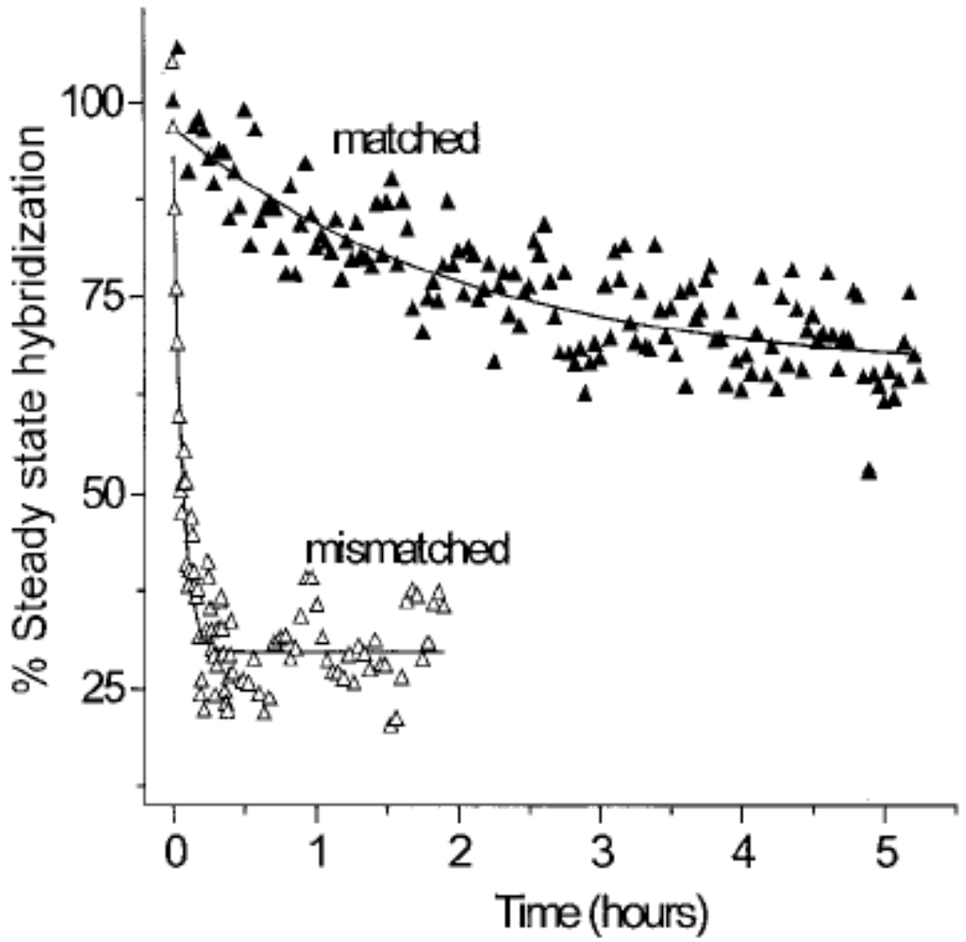

x
Potential region T
Potential region U (around -1.2 V)
(first seconds)
(tens of seconds)
A
B
C

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

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

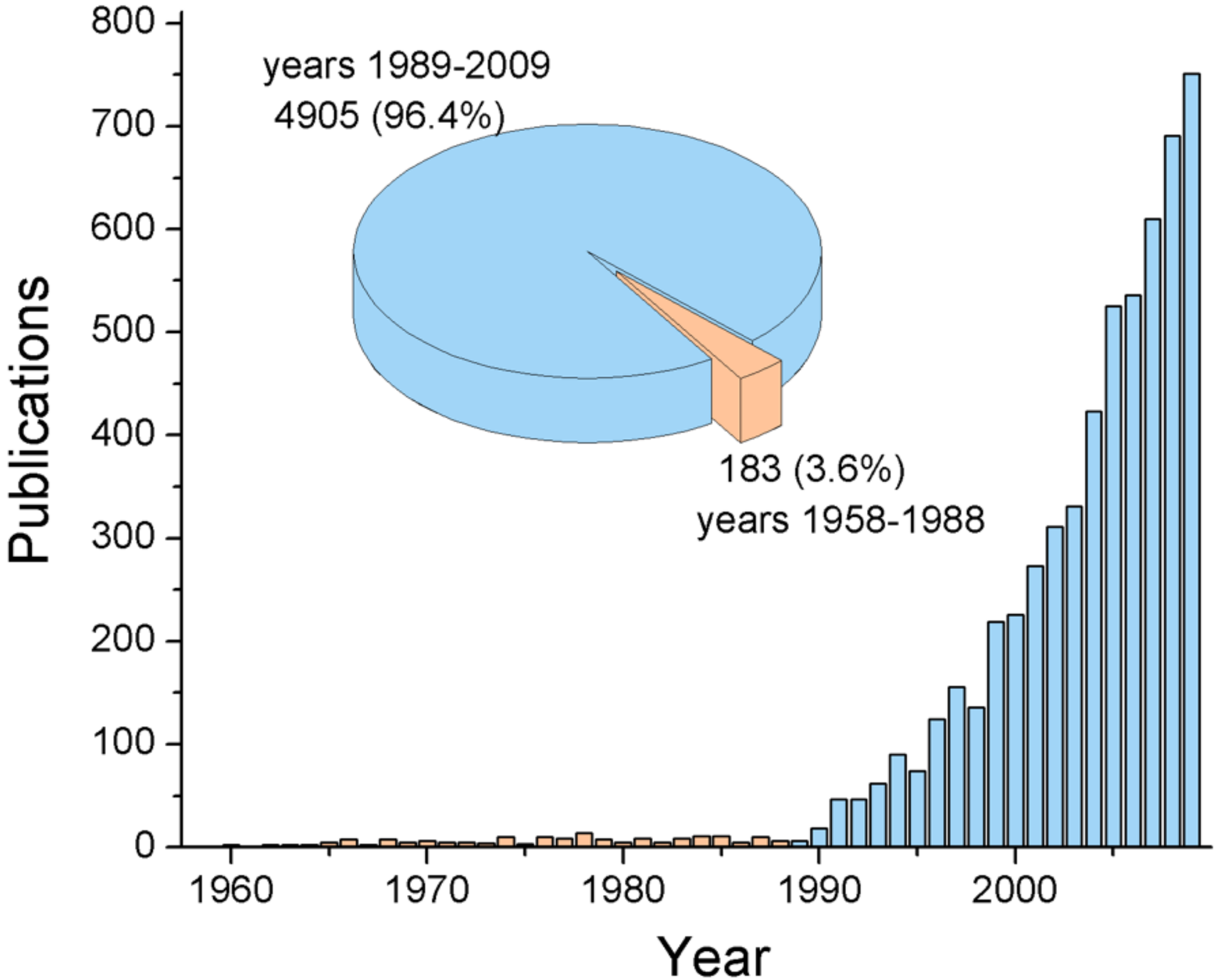
Polymorphy of the DNA double helix : middle of 1970's

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

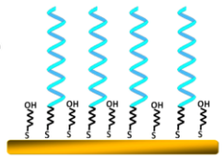


Electrochemical sensors for DNA hybridization are coming of age

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.

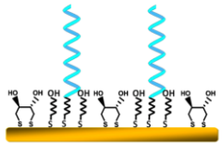
… new ternary interface involving **hexanedithiol (HDT)** co-immobilized with the thiolated capture probe (SHCP) on gold surfaces, followed by the incorporation of 6-mercapto-1-hexanol (MCH) as diluent. The **new SHCP/HDT+MCH monolayer** led to a **80-fold improvement in the signal-to-noise ratio** (S/N) 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.

Binary layer

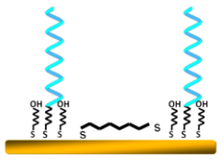


a) SHCP + MCH

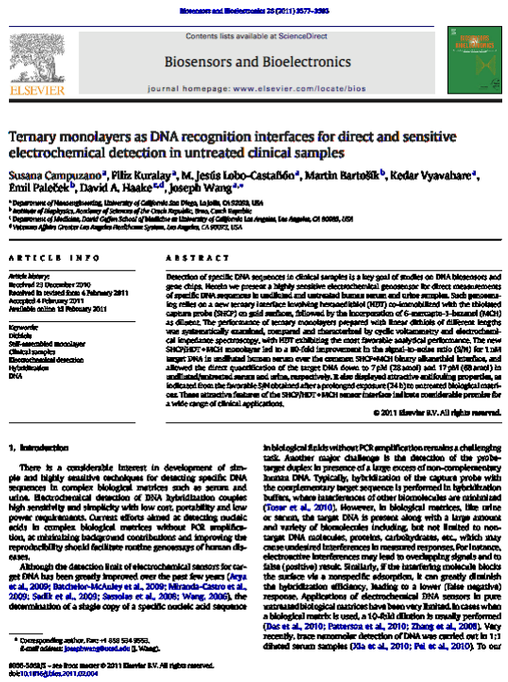
Ternary layers with **dithiols**



b) SHCP/DTT + MCH



c) SHCP/HDT + MCH



Biosensors and Bioelectronics 26 (2011) 3577–3585

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

[a] Department of Nanoengineering, University of California San Diego, La Jolla, CA 92093, USA
[b] Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[c] Department of Medicine, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA 90095, USA
[d] Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA 90073, USA

ARTICLE INFO

Article history:
Received 23 December 2010
Received in revised form 4 February 2011
Accepted 4 February 2011
Available online 18 February 2011

Keywords:
DNAzyme
Self-assembled monolayer
Clinical samples
Electrochemical detection
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 unaltered and untreated human serum and urine samples. Such genosensing relies on a new ternary interface involving hexanethiol (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 three 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.

© 2011 Elsevier B.V. All rights reserved.

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 genosensys 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; Bacchler-McAuley et al., 2009; Miranda-Castro et al., 2009; Sadik et al., 2009; Semaan 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 sequences 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 10-fold dilution is usually performed (Dai et al., 2010; Fanreza 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; Fei et al., 2010). To our

* Corresponding author. Fax: +1 858 534 9553.
E-mail address: josephwang@ucsd.edu (J. Wang).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.02.004

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**

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

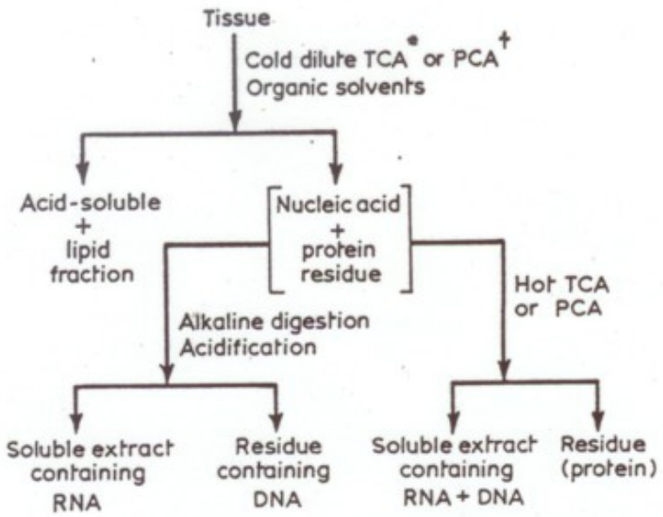
b) deproteinace (většinou fenolem)

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



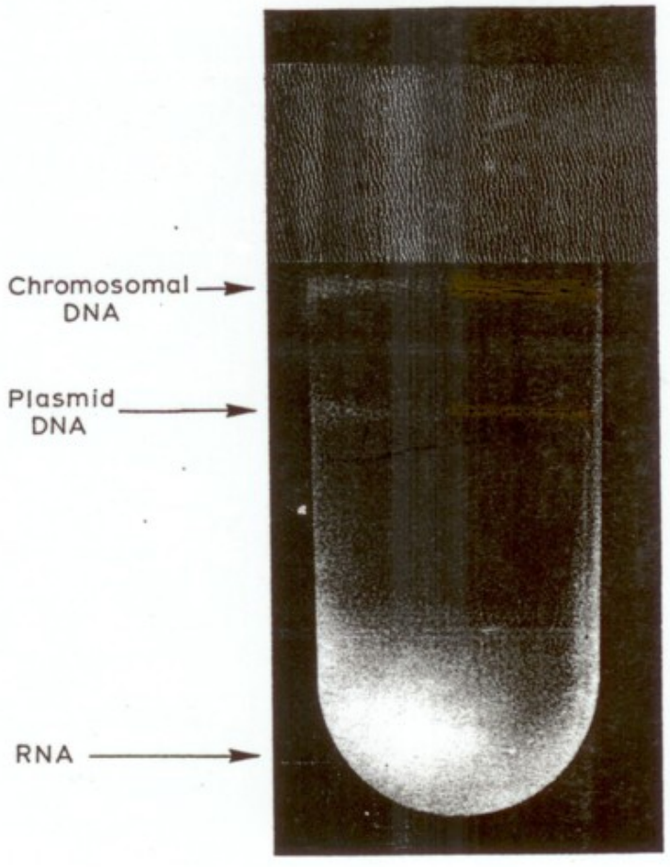
Extraction and fractionation of nucleic acids from tissues. *TCA – trichloroacetic acid, †PCA – perchloric acid.

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‡

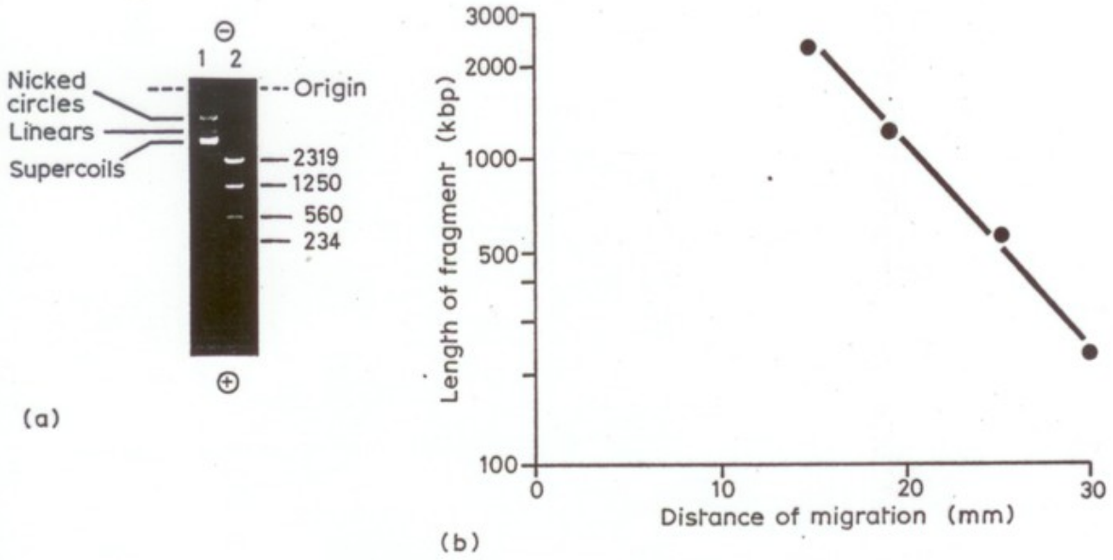
Department of Chemistry, Harvard University, Cambridge, Massachusetts, U.S.A.

(*Received 6 December 1960*)

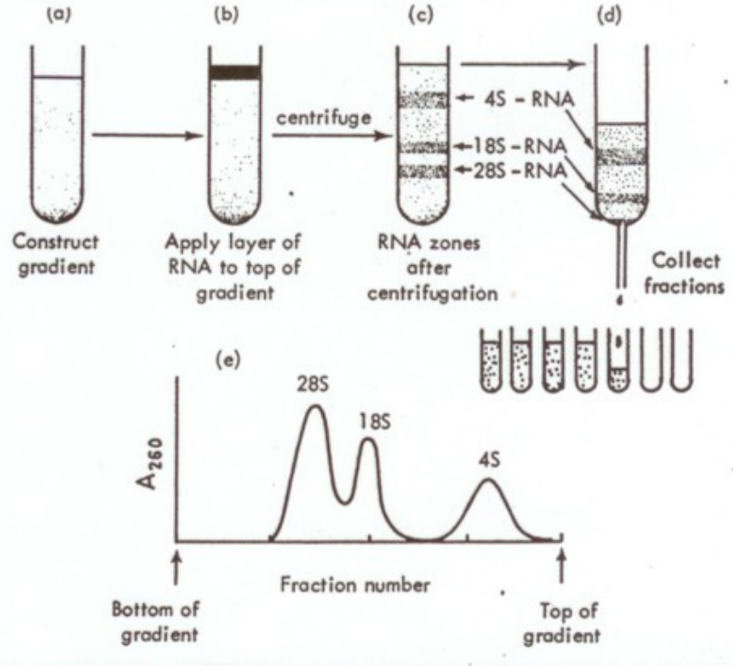
A method has been described for the isolation of DNA from micro-organisms which yields stable, biologically active, highly polymerized preparations relatively free from protein and RNA. Alternative methods of cell disruption and DNA isolation have been described and compared. DNA capable of transforming 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



Agarose gel electrophoresis of DNA. (a) Separation of: 1, different forms of DNA of plasmid pBR322; 2, fragments of DNA (lengths indicated in kbp) derived from plasmid pBR322 by double-digestion with restriction endonuclease *Bam* HI and *Bgl* I; (b) Plot of length of DNA fragment (log scale) against distance of migration (linear scale) of data from (a) 2, illustrating linear relationship.



Rate zonal centrifugation of RNA through a sucrose density gradient. A sucrose density gradient is constructed in a centrifuge tube (a) and the RNA solution applied as a layer on top (b). During ultracentrifugation the main components of the RNA separate into zones, primarily on the basis of molecular weight (c). These zones may be recovered by puncturing the bottom of the tube and collecting different fractions in separate tubes (d). The separated RNAs may be visualized and quantitated by measurement of the absorbance at 260 nm (e). Steps (d) and (e) may be conveniently combined by pumping the gradient through the flow-cell of a recording spectrophotometer.

Characterize your DNA sample:

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

linear: cohesive or blunt ends
number of base pairs,

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

Síly ovlivňující konformaci DNA

a) Elektrostatické síly podmíněné ionizací.

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

b) Síly plynoucí z vertikálního uspořádání bazí

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

c) Vodíkové vazby (můstky)

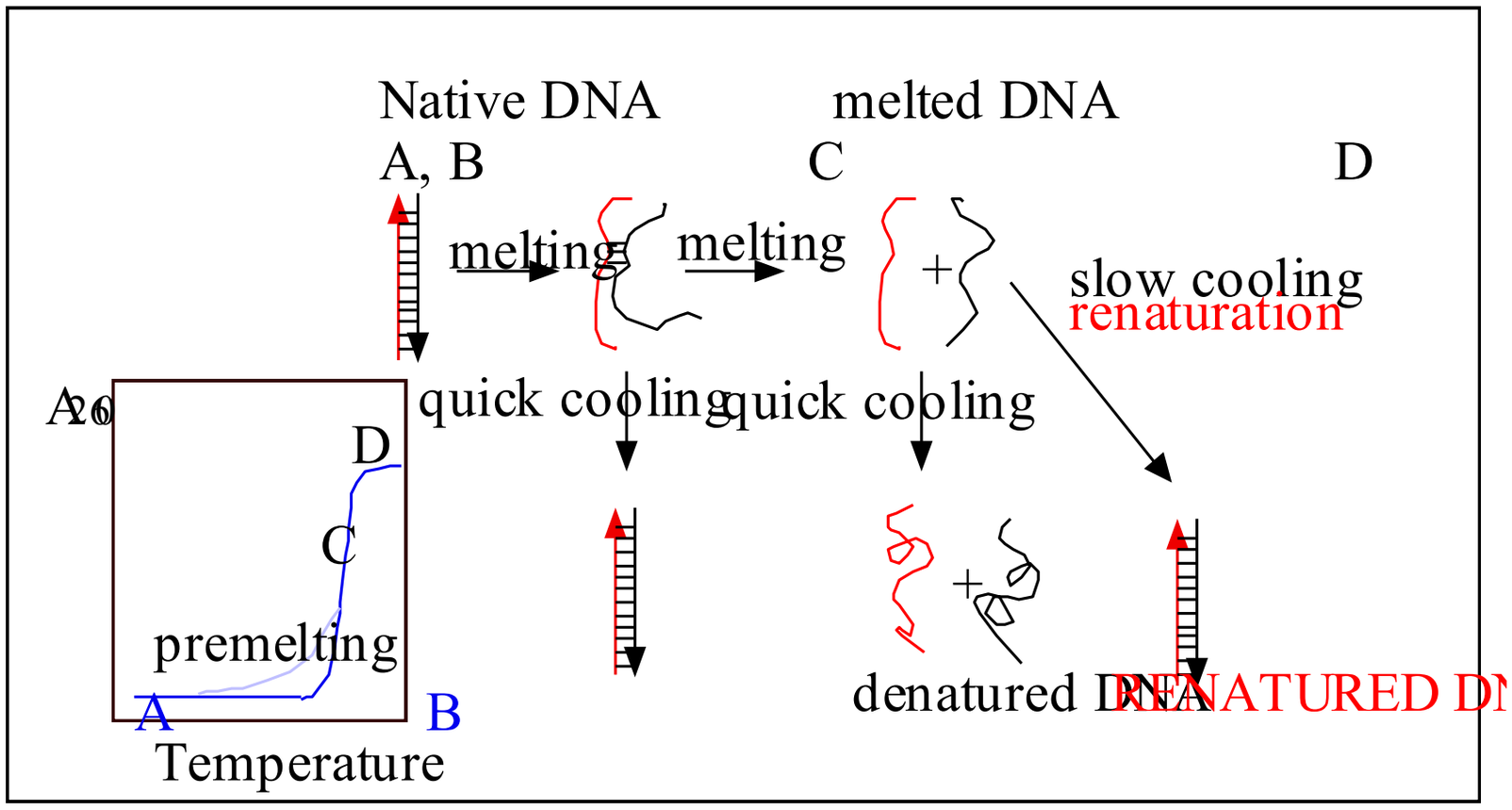
- představují **jediný známý způsob zajišťující specificitu párování bazí**. Jsou tedy součástí mechanismu jímž DNA realizuje svoji biologickou funkci. Zpočátku se o nich soudilo, že jsou nejdůležitějším činitelem pro stabilitu dvojité šroubovice; experimentálně i teoreticky bylo však dokázáno, že tomu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

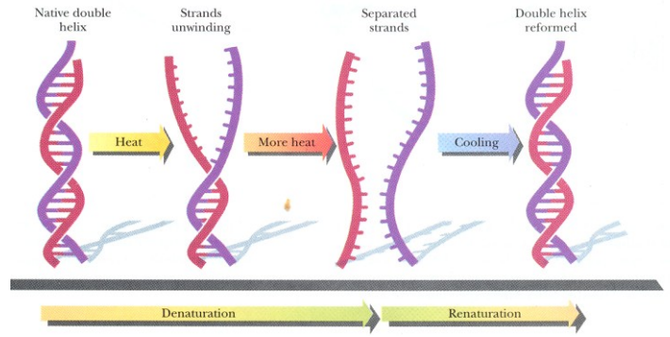
d) Hydrofobní síly

- tento termín se týká **stability dvoušroubovicové DNA plynoucí z její architekrury**: **polární skupiny** jsou **na 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.

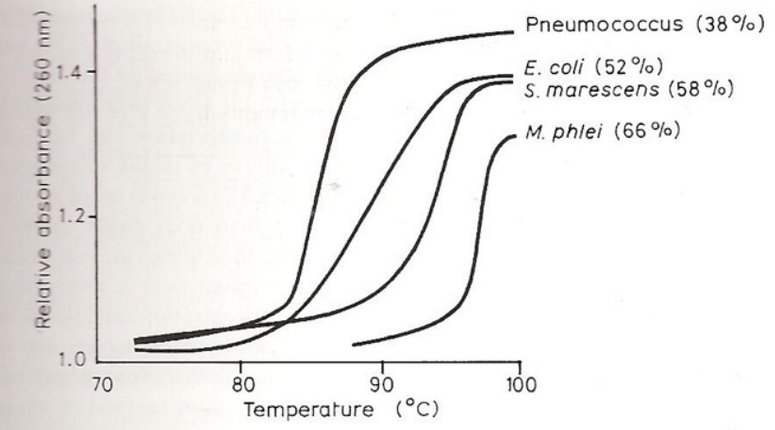
Denaturation x degradation
aggregation
renaturation/hybridization

DNA DENATURATION and RENATURATION/HYBRIDIZATION





J. Marmur and P. Doty



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. l – after [116]).

n and renaturation 21

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.

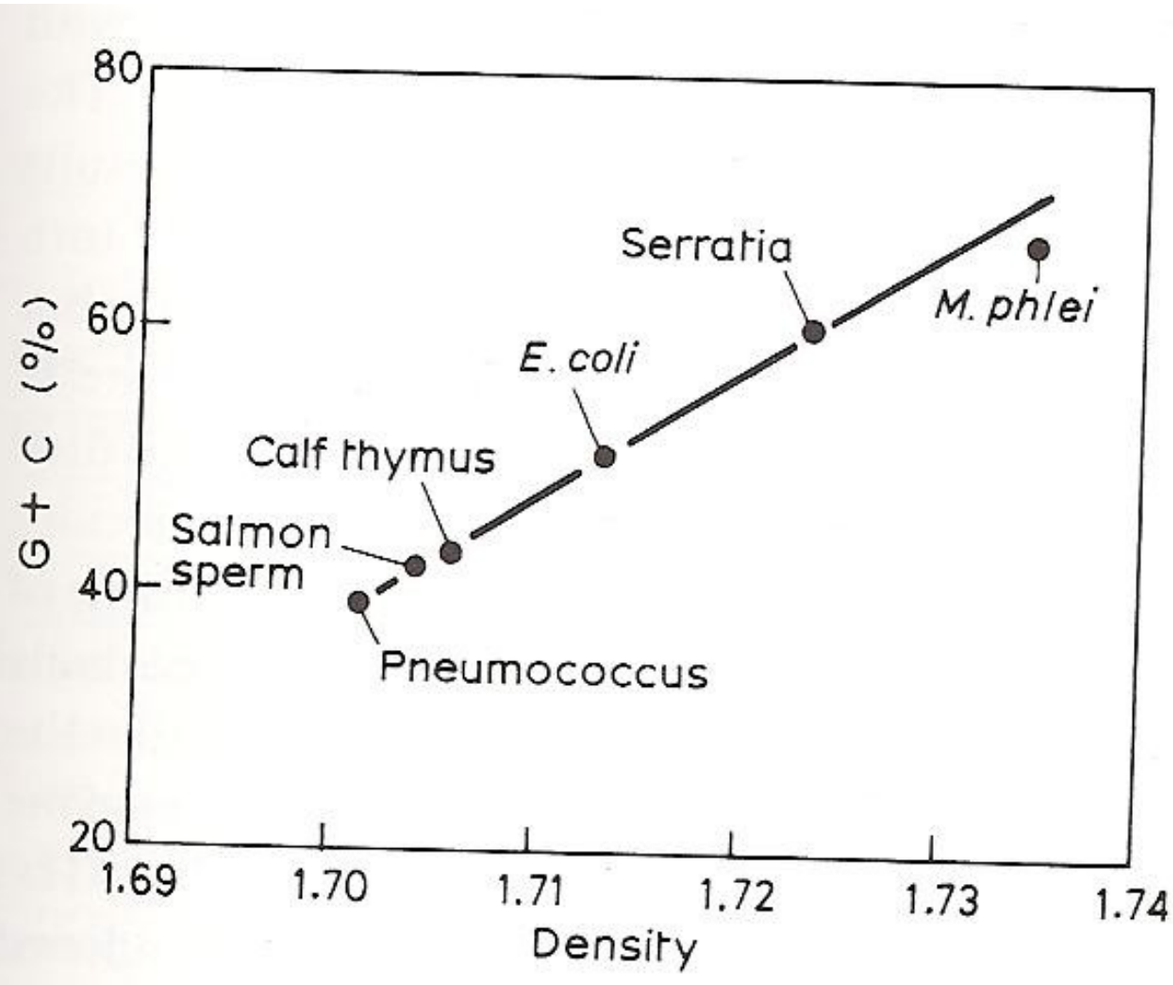


Fig. 2.21 Relationship of density to content of guanine plus cytosine in DNAs from various sources [64].

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

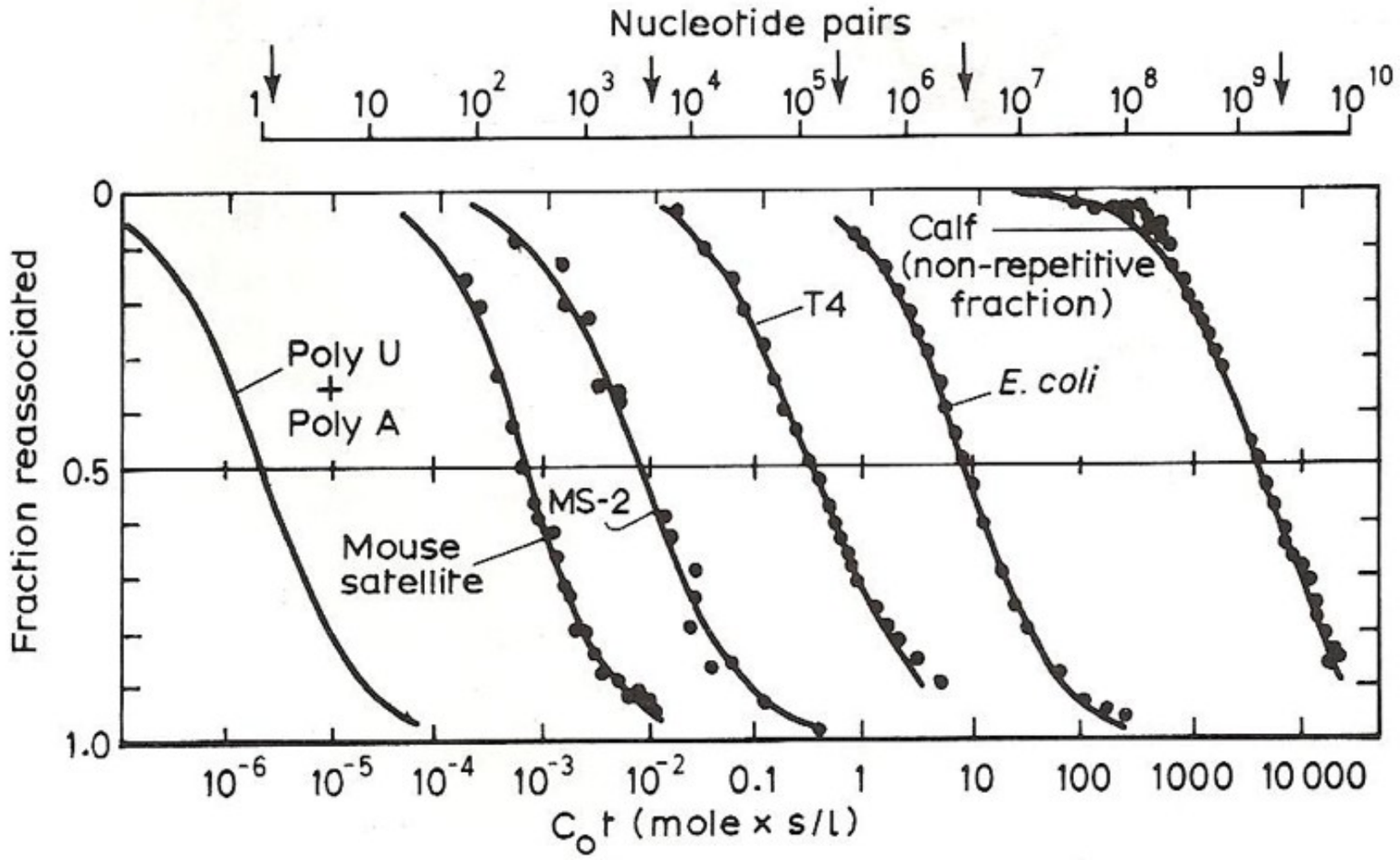


Fig. 2.20 The rate of reassociation of double-stranded polynucleotides from various sources showing how the rate decreases with the complexity of the organism and its genome (from [60]).

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)

158 results found (Set #1) Go to Page: 1 of 16 GO

Records 1 -- 10 Show 10 per page [1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10]

Use the checkboxes to select records for output. See the sidebar for options.

1. **MARMUR J**
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
VIEW FULL TEXT

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

Sort by:
Times Cited SORT

Analyze Results:
ANALYZE
View rankings of the authors, journals, etc. for these records.

Citation Report:
CITATION REPORT
View detailed citation counts and the h-index value for the results.

Output Records:
- Selected records on page
- All records on page
- Records to

Bibliographic Fields

PRINT E-MAIL SAVE

EXPORT TO REFERENCE SOFTWARE

SAVE TO MY EndNote Web

[Sign in to access EndNote Web]

Or add them to the Marked List for later output and more options.

ADD TO MARKED LIST

[0 articles marked]

Homopolynukleotidy

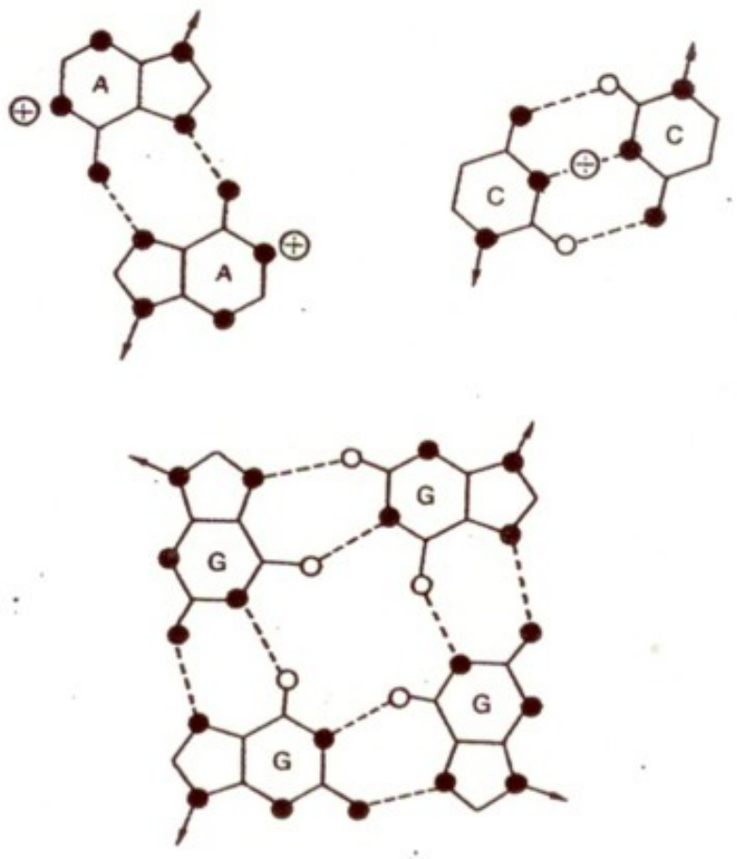
Poly(U) a poly(dT) při pokojové teplotě mají málo výraznou sekundární strukturu, při vyšší teplotě tuto strukturu ztrácejí

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: