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

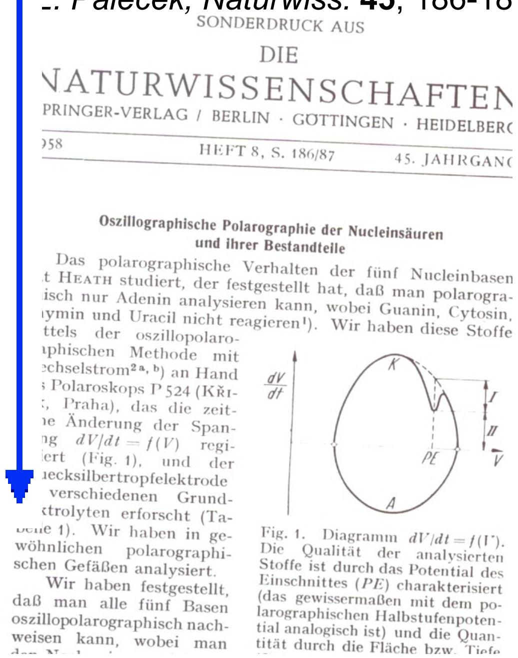
This year we commemorate the 90th Anniversary of the invention of polarography by J. Heyrovsky. In **1941 he invented oscillographic polarography with controlled a.c.** (cyclic a.c. chronopotentiometry). By the end of the 1950's oscillographic polarograp was the **method of choice** for the DNA electrochemical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

1958: Nucleic acid bases, DNA and RNA are electroactive

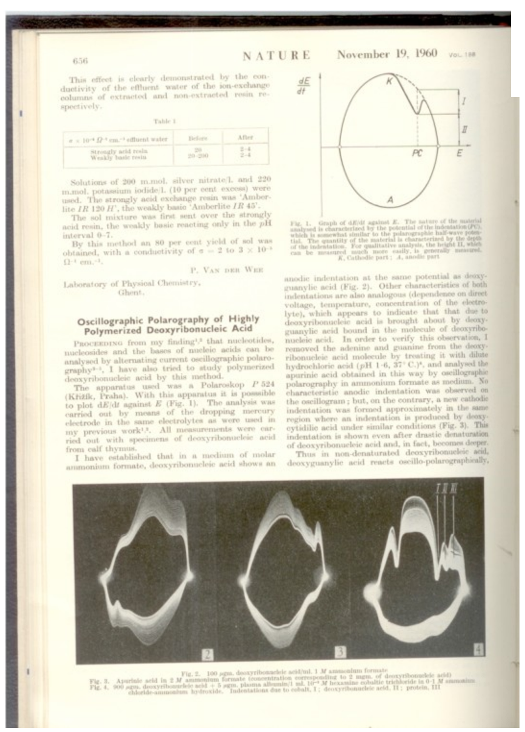
2. PŘEDNÁŠKA 25.9. 2014

1960: Relations between the DNA structure and electrochemical responses

E. Palecek, *Naturwiss.* **45**, 186-18



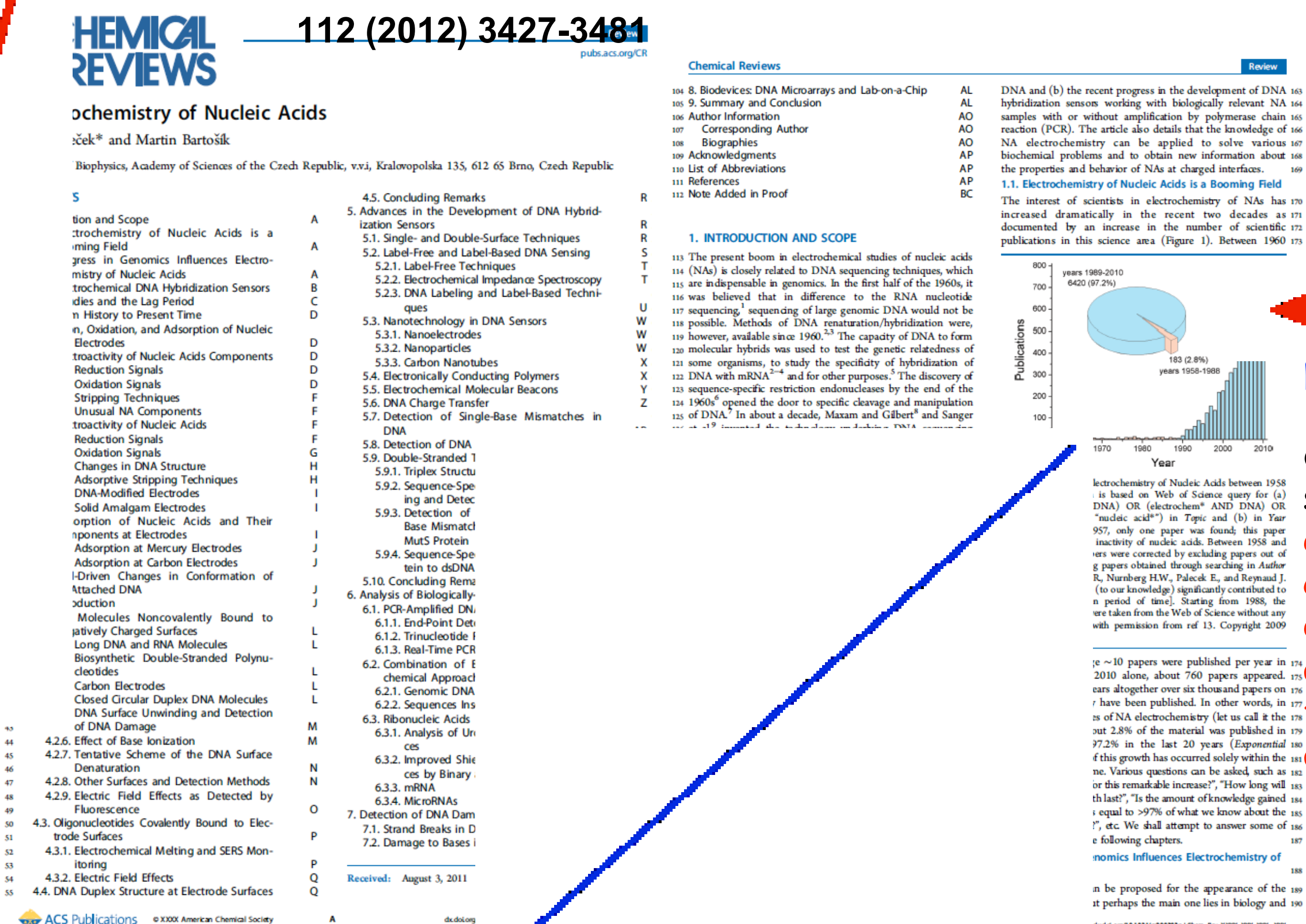
E. Palecek, *Nature* **188** (1960) 65



Electrochemistry of nucleic acids is now a booming field

112 (2012) 3427-3481

IF 33



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

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**

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[‡]

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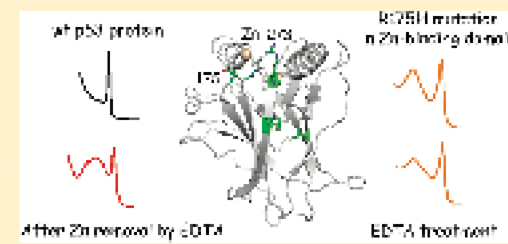
Supporting Information

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.

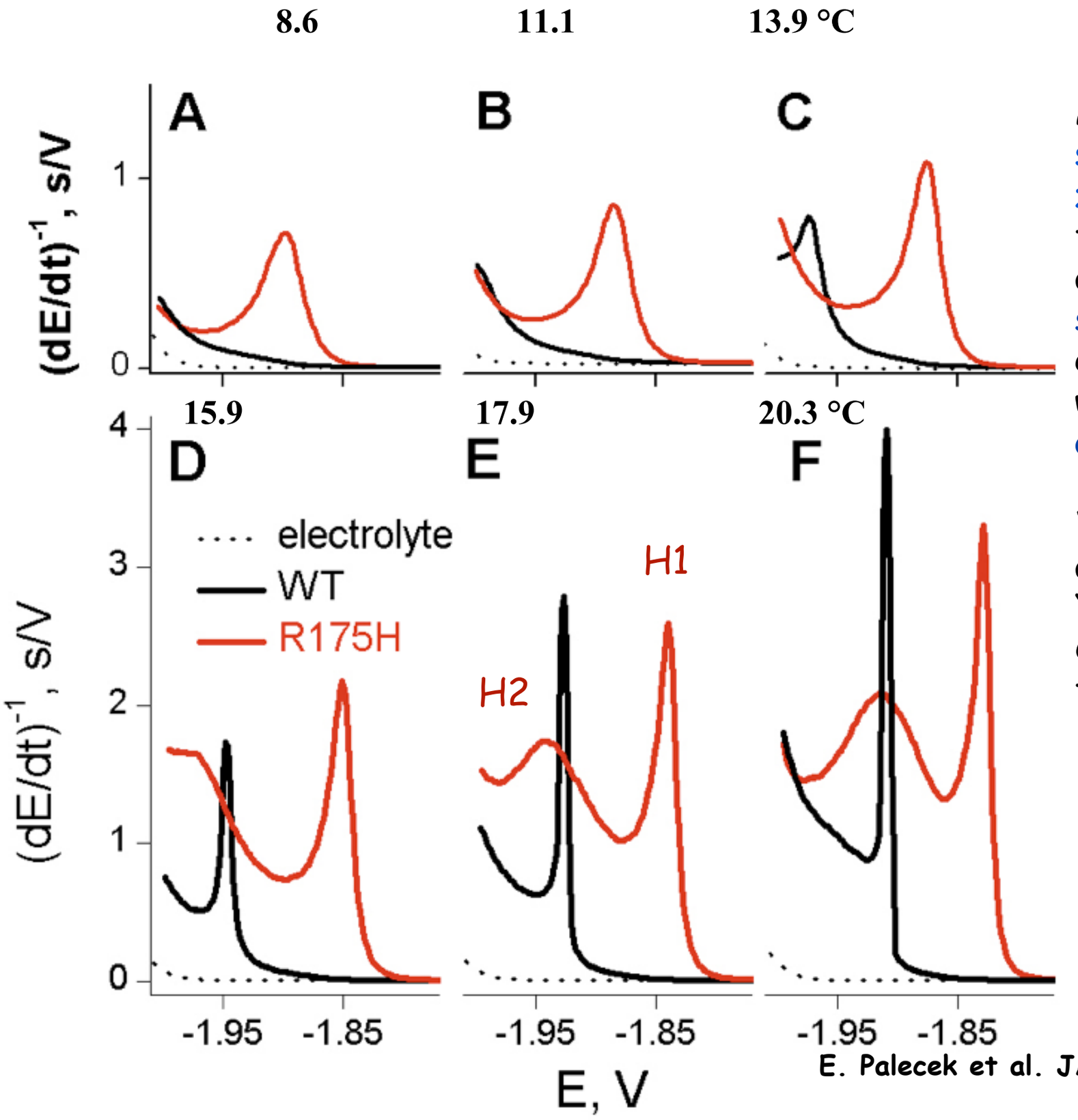
In recent decades, electrochemistry of proteins was limited to relatively small conjugated proteins containing nonprotein redox centers yielding reversible electrochemistry,[7−10] and a majority of proteins were neglected. We have proposed a new electrochemical method for analysis of practically all 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[19,21−23] and relies on constant current chronopotentiometric stripping (CPS, involving very fast potential changes) and mercury-containing electrodes.[13,24] 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**.

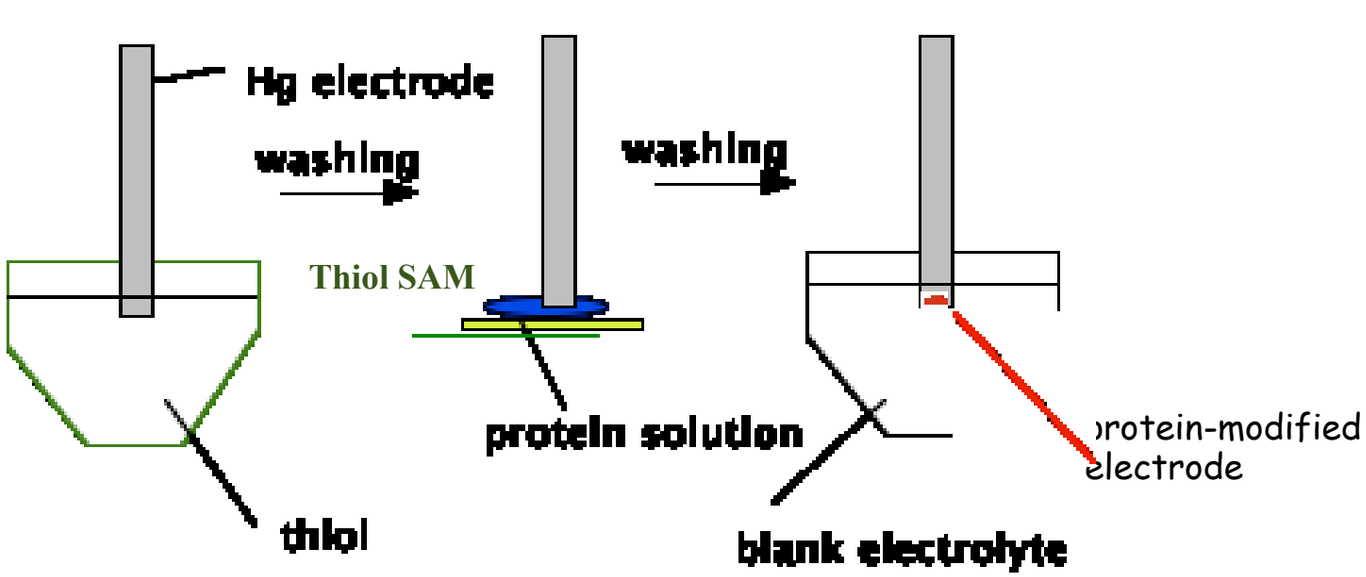
We tested **other mutants** such as V145A, F270L, R273H and Y220C and we always observed CPS **responses different from** the **wt protein**

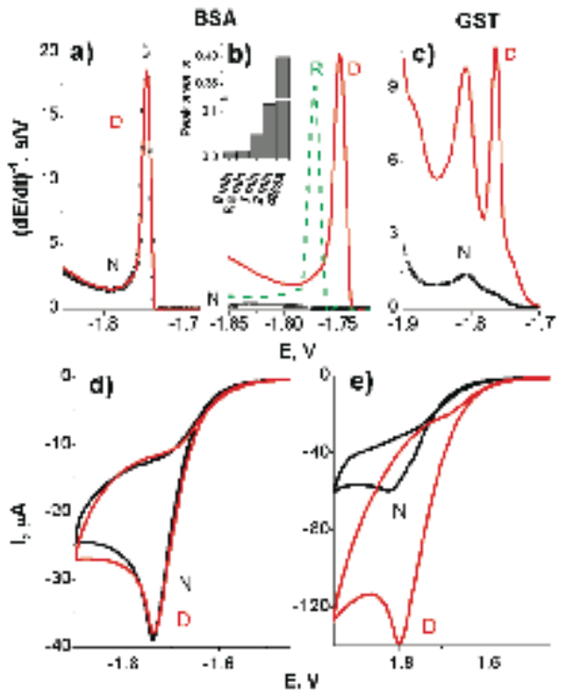
E. Palecek et al. JACS 2011, 133, 7190–7196

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.

Glycoproteins

V přírodě polysacharidy (PS) a oligosacharidy (OLS) vytvářejí velké a dosti odlišné třídy látek vyskytujících se buď volně, nebo vázané na proteiny či lipidy [66]. Díky jejich strukturní flexibilitě, která jim umožňuje nepřeberné množství kombinací vzájemného propojení, jsou bezpochyby ideálními „identifikátory“ v mezimolekulové a mezibuněčné komunikaci. V poslední době se ukazuje, že **většina bílkovin v buňkách savců se vyskytuje právě ve formě glykoproteinů a že jejich glykosylace často hraje důležitou roli ve zdraví i nemoci člověka, a to včetně rakoviny**, u které bývá např. pozorována abnormální glykosylace bílkovin na povrchu nádorových buněk.
V současné době lze pozorovat **zvýšený zájem o nové metody analýzy** PS, OLS a **glykoproteinů.** PS neobsahují redoxní skupiny a byly proto **do nedávna považovány za elektrochemicky inaktivní látky.** Teprve **v r. 2009** bylo zjištěno, že některé **sulfátované PS katalyzují vylučování vodíku** a poskytují CPS signály **na rtuťových elektrodách**. Zcela nedávno bylo zjištěno, daleko i**ntenzivnější signály** tohoto typu poskytuji některé **PS a OLS, obsahující glukosamin** . Vedle toho se ukázalo, že **PS a OLS lze snadno modifikovat komplexy šestimocného osmia s dusíkatými ligandy (Os(VI)L)**, přičemž vzniklé a**dukty jsou elektrochemicky aktivní** (podobně jako výše zmíněné značení mikroRNA). Použití některých ligandů (např. bipyridinu) umožňuje i vznik aduktů, které mohou navíc poskytnout citlivější signály, podmíněné katalytickým vylučováním vodíku. U jiných ligandů (temed) je zase možné stanovení PS a OLS přímo v reakční směsi. Proti některým aduktům PS-Os(VI)L byly generovány vysoce specifické **monoklonální protilátky,** které je možno použít k analýze Os(VI)L-modifikovaných glykanů přímo v glykoproteinech.

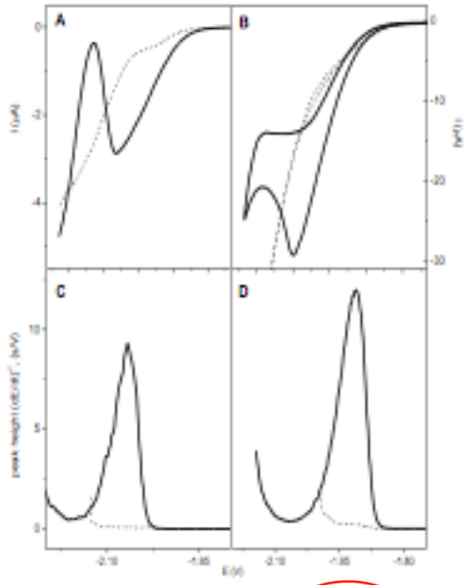


Fig 1

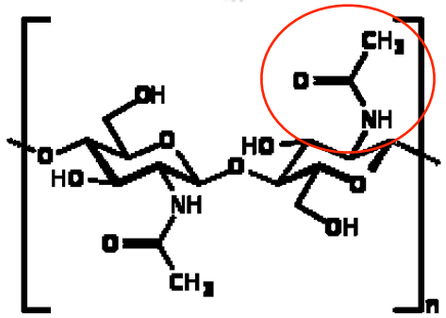
Chitosan - name metody merení

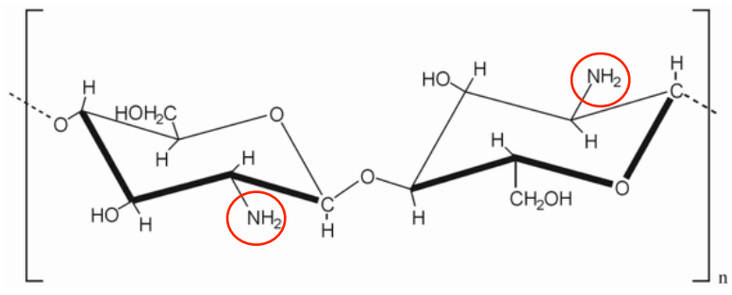
A:SWV, konc. chit.I 10μg/ml, t_A60s, E_A-0.1V, frekv. 20Hz, step 5mV, 0.1M acetate pH5.2,v nadobce, +25°C

B: CV, konc. chit.I 10μg/ml, t_A60s, E_A-0.1V, scan rate 100mV/s, 1.scan, step 5mV, 0.1M acetate pH5.2, v nadobce, +25°C

C:CPSA/HMDE, konc. chit.I 10μg/ml, t_A60s, E_A-0.1V, I_{str} -50μA, 0.1M acetate pH5.2, v nadobce, +25°C

D:CPSA/MeSAE, konc. chit.I 15μg/ml, t_A60s, E_a-0.1V, I_{str}-50μA, 0.1M acetate pH5.2, v nadobce, +25°C





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Electrochemistry Communications

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

Short communication

Direct chemical modification and voltammetric detection of glycans in glycoproteins

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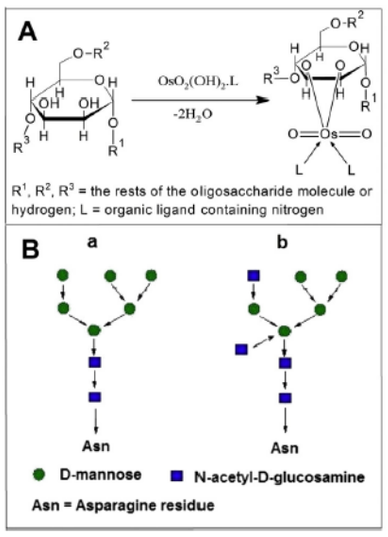
Pyrolytic graphite electrodes
Voltammetry

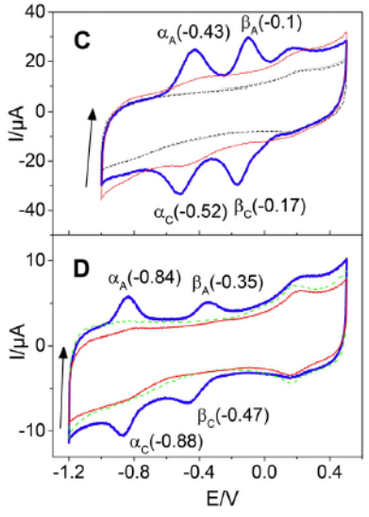
ABSTRACT

Most of the proteins are glycosylated and glycoproteins are involved in physiological and pathological processes. Detection of changes in protein glycosylation is important in early diagnosis of different diseases, including cancer. Among the methods used for the analysis of the carbohydrate components (glycans) of glycoproteins, electrochemical methods were little applied. We propose a modification of glycans directly in glycoproteins using six-valent osmium complexes followed by glycan voltammetric determination at carbon electrodes. The electrochemical responses of two glycoproteins ribonuclease B and avidin as compared to their non-glycosylated counterparts were recorded either directly in the reaction mixture or after a simple purification step. Hundreds of femtomoles of the glycoprotein were sufficient for the analysis.

Chitin **Chitosan**

M. Trefulka, E. Paleček / Electrochemistry Communications 48 (2014) 52–55





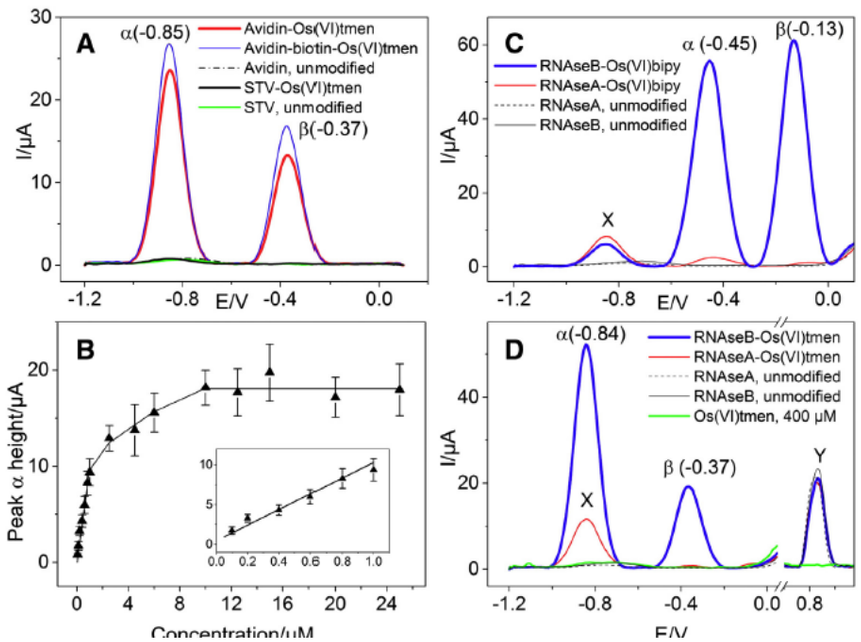


Fig. 1. Modification of glycans in glycoproteins with Os(VI)L. A) Scheme of reaction of mannose-containing OLS or PS with six-valent osmium complexes. B) Examples of N-glycan types occurring (a) in RNaseB and (b) in avidin. C) Adsorptive transfer stripping cyclic voltammetry (AdTS CV) of purified 100 μM Os(VI)bipy-treated RNaseB (blue thick line), and RNaseA (red thin line); untreated RNaseA (black dotted line); RNaseB (black dashed line). D) AdTS CV of unpurified 10 μM avidin–Os(VI)tmen (blue thick line), 10 μM streptavidin (STV)–Os(VI)tmen (red line) and 400 μM free Os(VI)tmen (green dashed line). C, D). The numbers in the parenthesis above the peaks are peak potentials in V. All glycoprotein measurements were done at PGE at full electrode coverage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The Protein Group

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Veronika Vargová
Vlasto Dorčák

....ještě doplněk scientometrie

Index h

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

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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].

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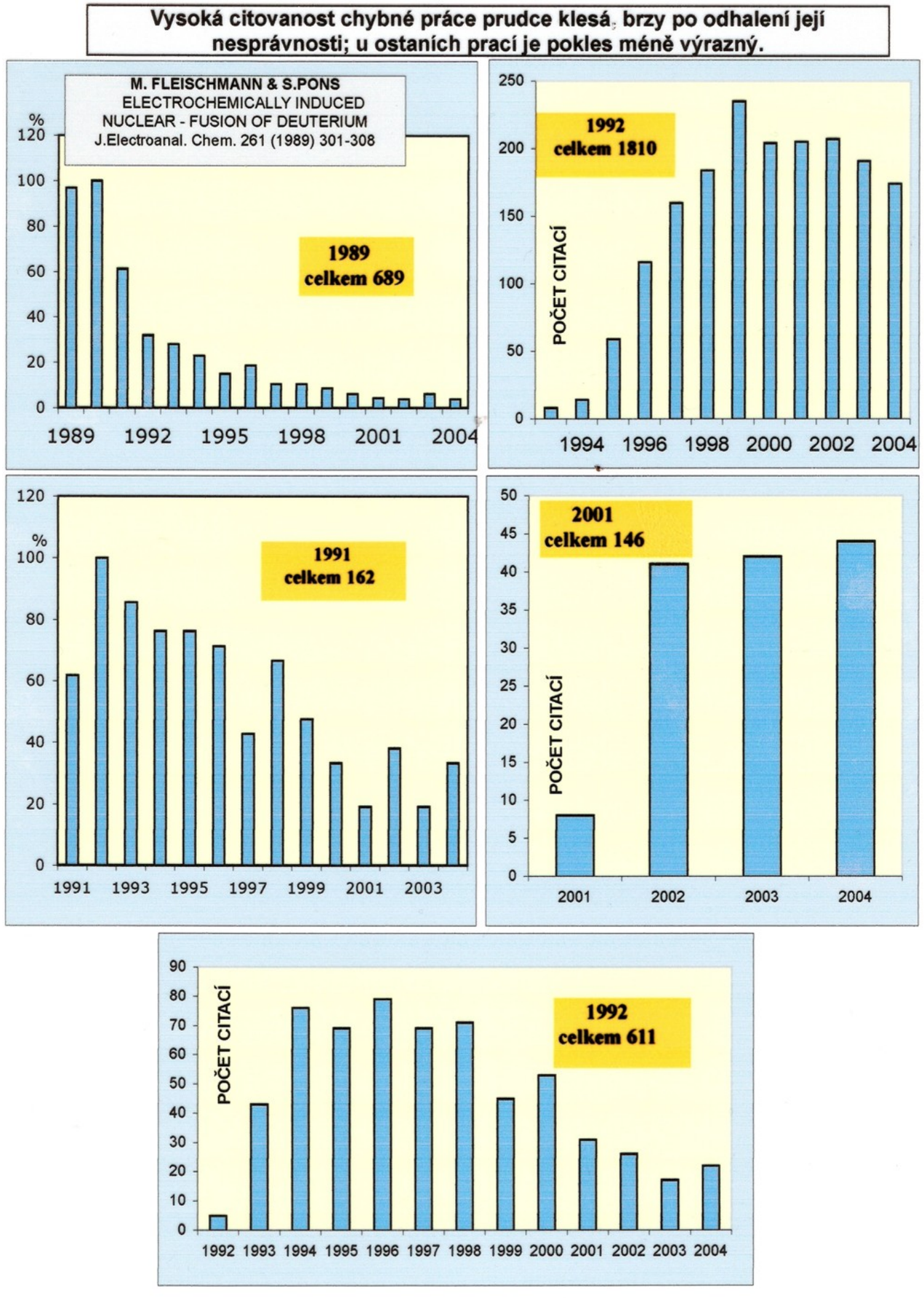

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7. **CSORGO S**, MASON DM
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   Times Cited: 39
8. BURKE MD, **CSORGO S**, HORVATH L
   A CORRECTION TO AND IMPROVEMENT OF STRONG APPROXIMATIONS OF SOME BIOMETRIC ESTIMATES UNDER RANDOM CENSORSHIP
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2. SHORE D, **NASMYTH K**
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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.
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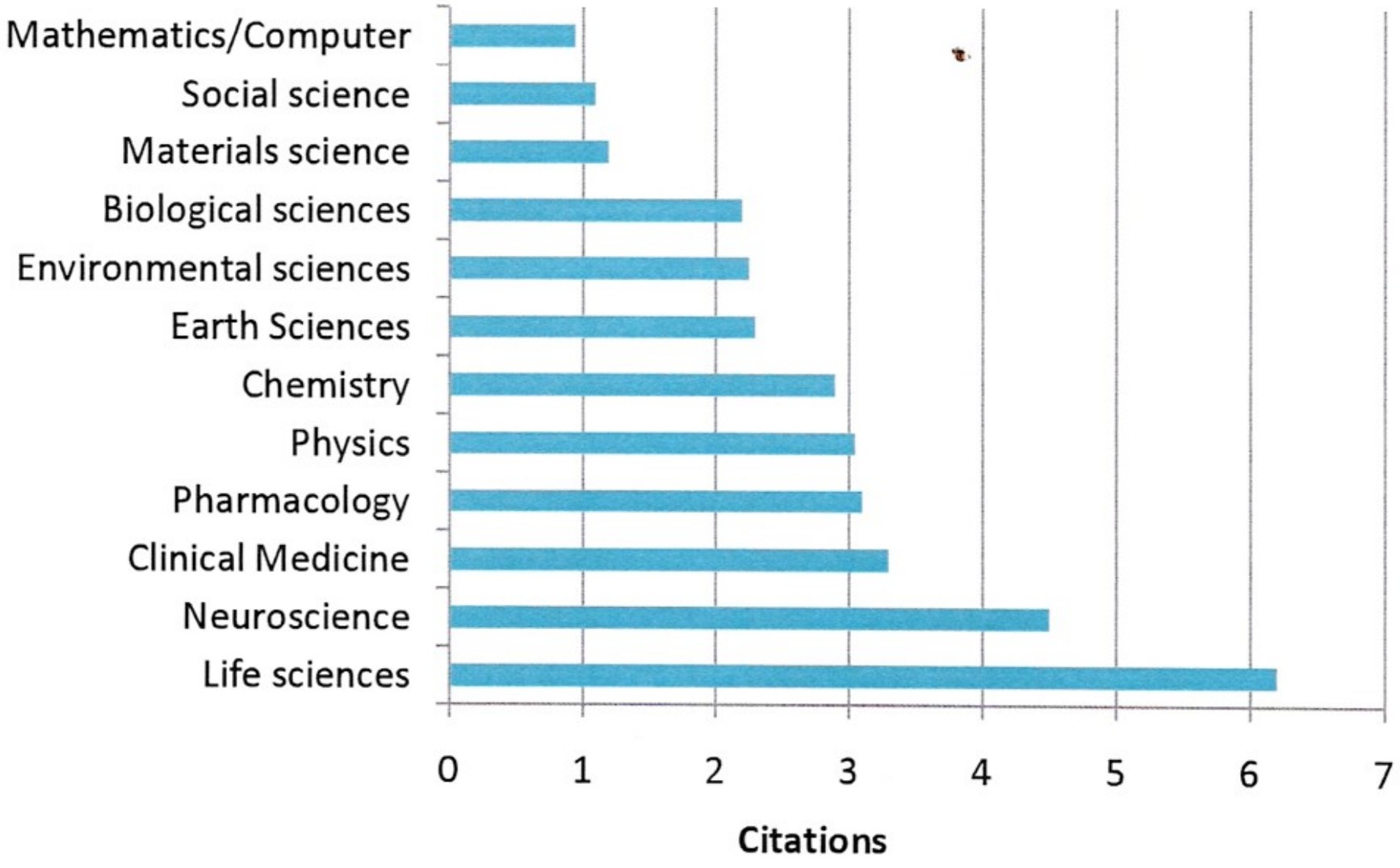

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Příklad analýzy scientometrických dat jednotlivce.

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 oužívat po celém světě pro hodnocení dal-ch oborů.

V našem předešlém článku (viz Vesmír 81,)8, 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-o 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-iéně 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-ıkterizuje 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 opra-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 publikace
ÚLOHA JEDNOTLIVCE

V současné době je připravován systém hodnocení výsledků vědy v AV ČR i na MŠMT, zahrnující scientometriké/bibliometrické hodnocení x kafemelejnek

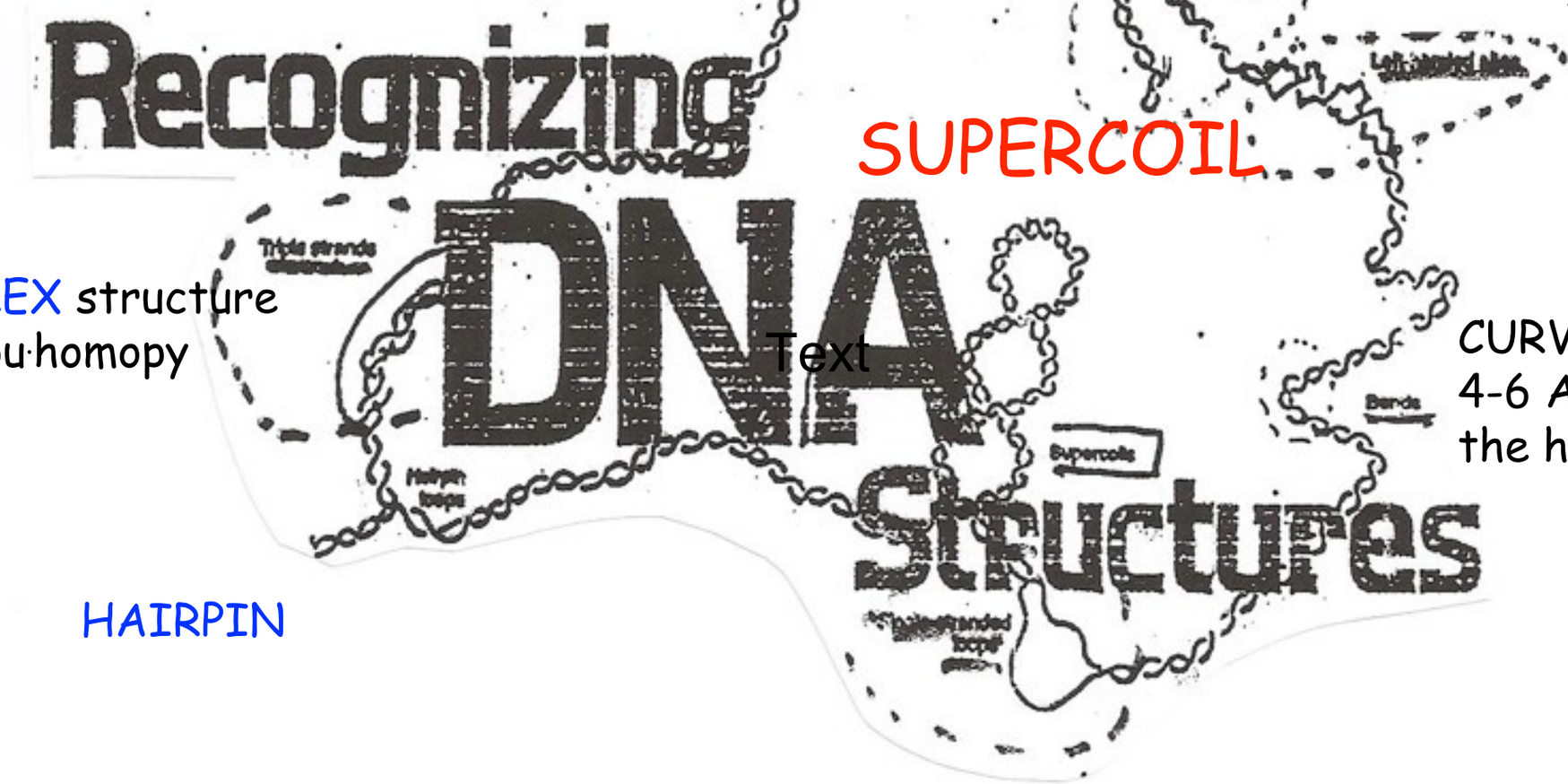
Když si budete vybírat téma své diplomky/dizertace snažte se zjistit jak je váš budoucí školitel ve vědě úspěšný a jak aktuální a zajímavé jsou problémy, které řeší

Již během svého studia můžete dělat vědecké objevy!

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 the helix turns

HAIRPIN

SINGLE-STRANDED region
AT-rich

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

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.

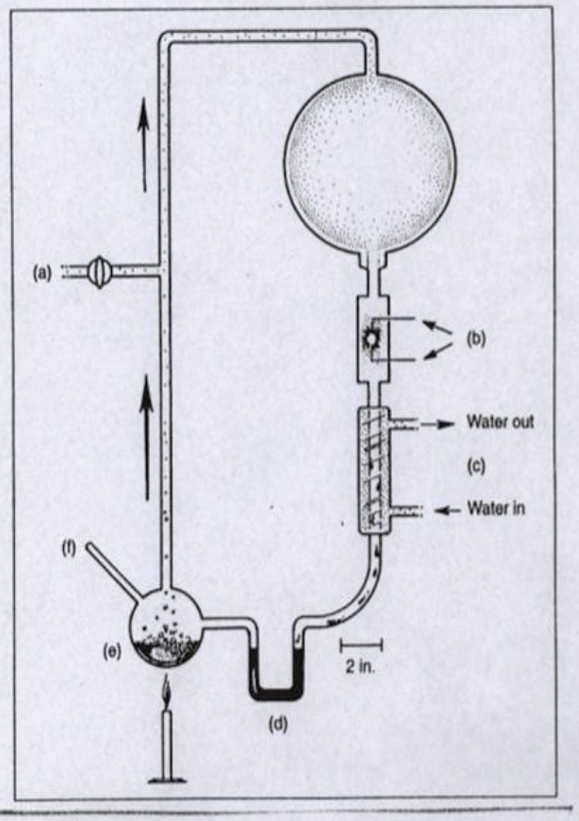
Mám v živé paměti přednášku, kterou přednesl před mnoha lety v Liblicích Harold Urey o vzniku aminokyselin v laboratorních podmínkách, napodobujících podmínky předpokládané na Zemi v době, kdy pravděpodobně vznikl život. Přednáška byla jednoduchá a elegantní a dávala tušit, že během několika málo desetiletí 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

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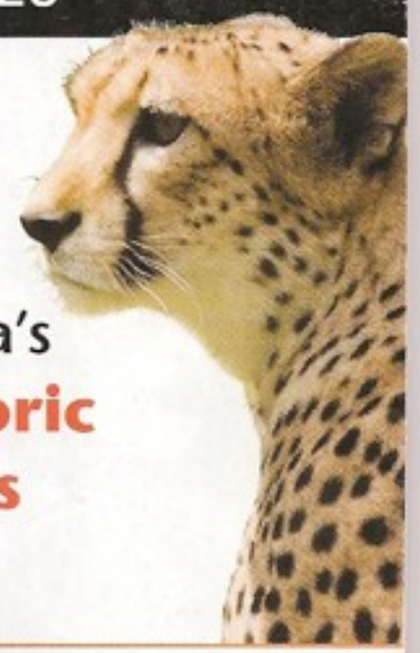
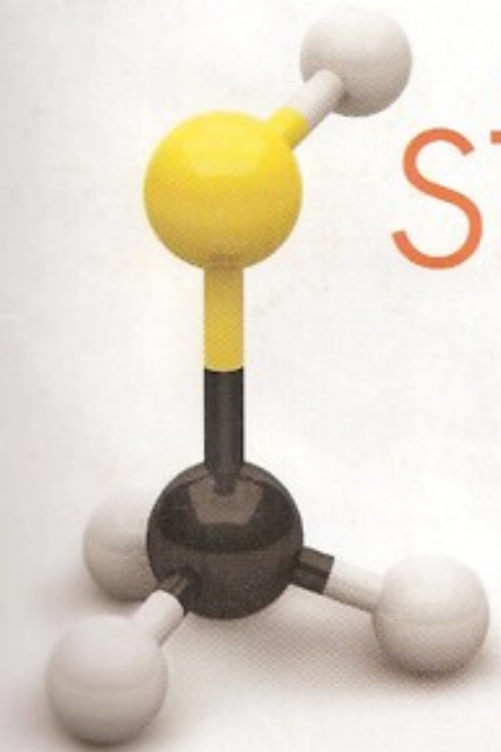


Did this molecule start life?



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

BY ROBERT SHAPIRO

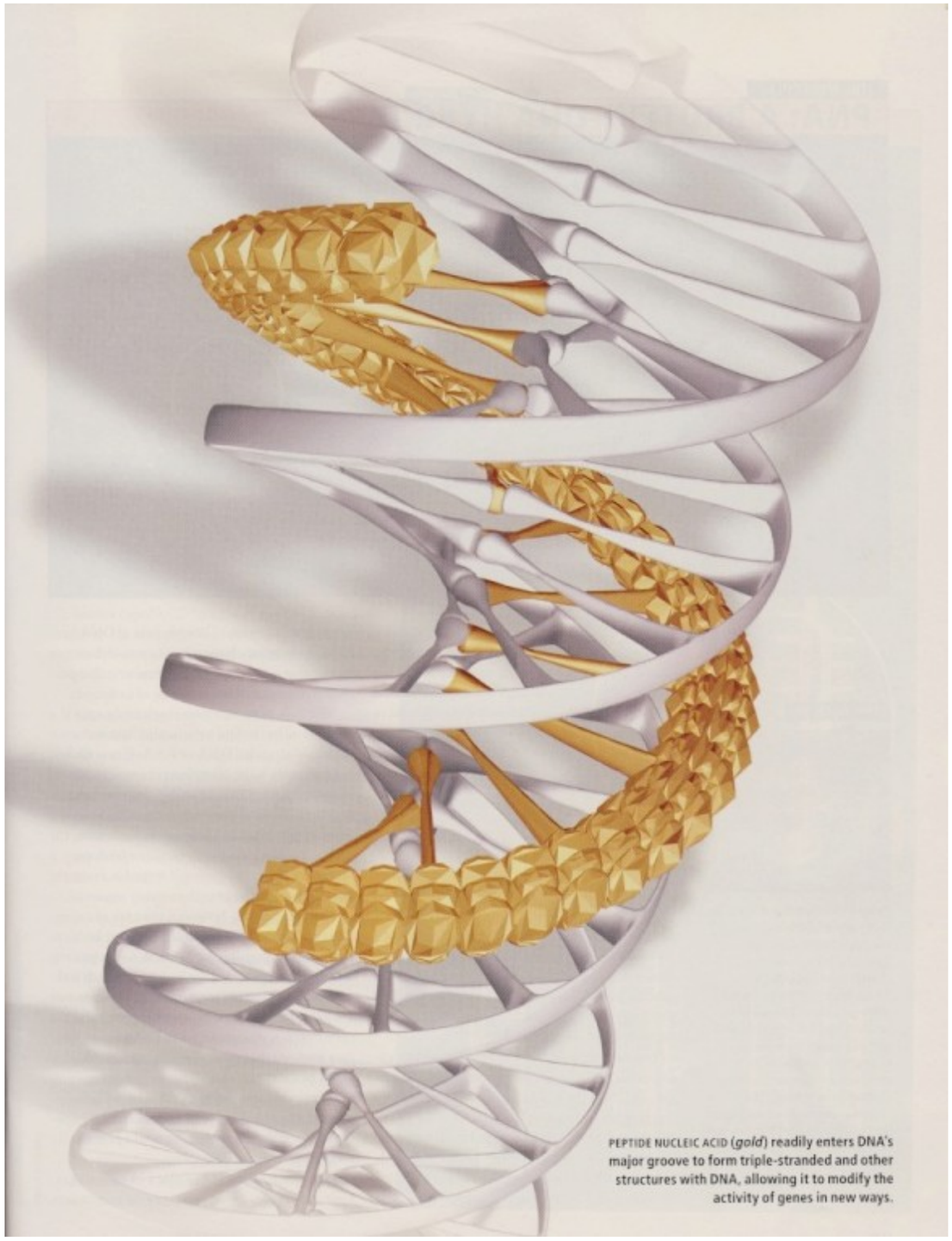
The sudden appearance of a large self-copying molecule such as RNA was exceedingly improbable. Energy-driven networks of small molecules afford better odds as the initiators of life

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.

Peptide Nucleic Acid (PNA)



PEPTIDE NUCLEIC ACID (*gold*) readily enters DNA's major groove to form triple-stranded and other structures with DNA, allowing it to modify the activity of genes in new ways.

Panspermia

RNA First

Metabolism first (2007)

PNA First (2008)

RNA First (again/2009)

Panspermia again and again

Or did life come from another world?

The hypothesis of F. Crick is discussed in November issue of Scientific American 2005.

It is concluded that microorganism could have survived a journey from Mars to Earth

Recent finding of glycine in the comet tail might be considered as support for this alternative

The actual nature of the first organism and the exact circumstances of the origin of life may be forever lost for science.

But research can at least help to understand what is possible

Sci. Amer., September 2009

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CRITICAL REVIEW

Genetics first or metabolism first? The formamide clue†

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

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

Ernesto Di Mauro

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

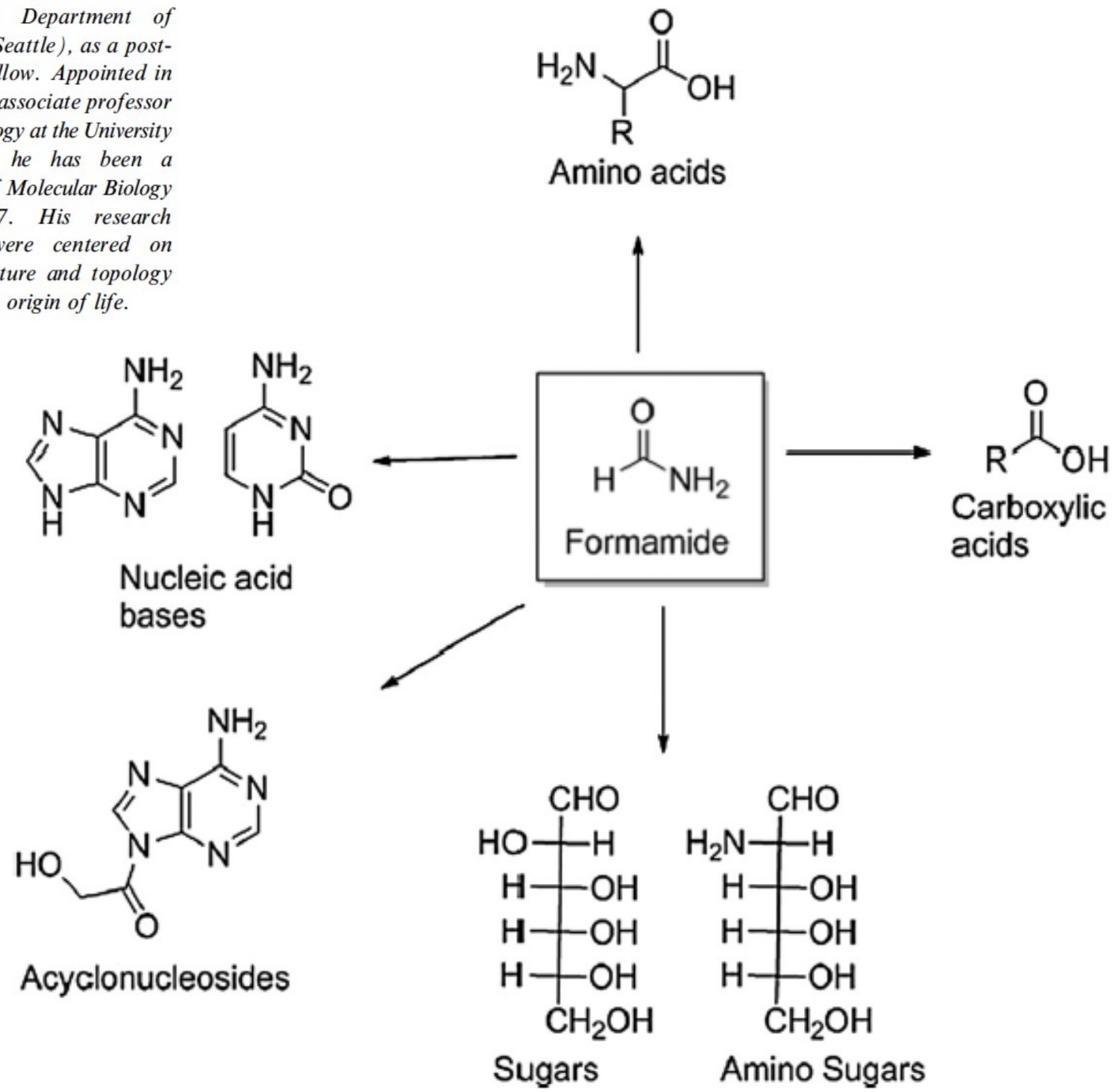


Fig. 1 Syntheses from formamide.

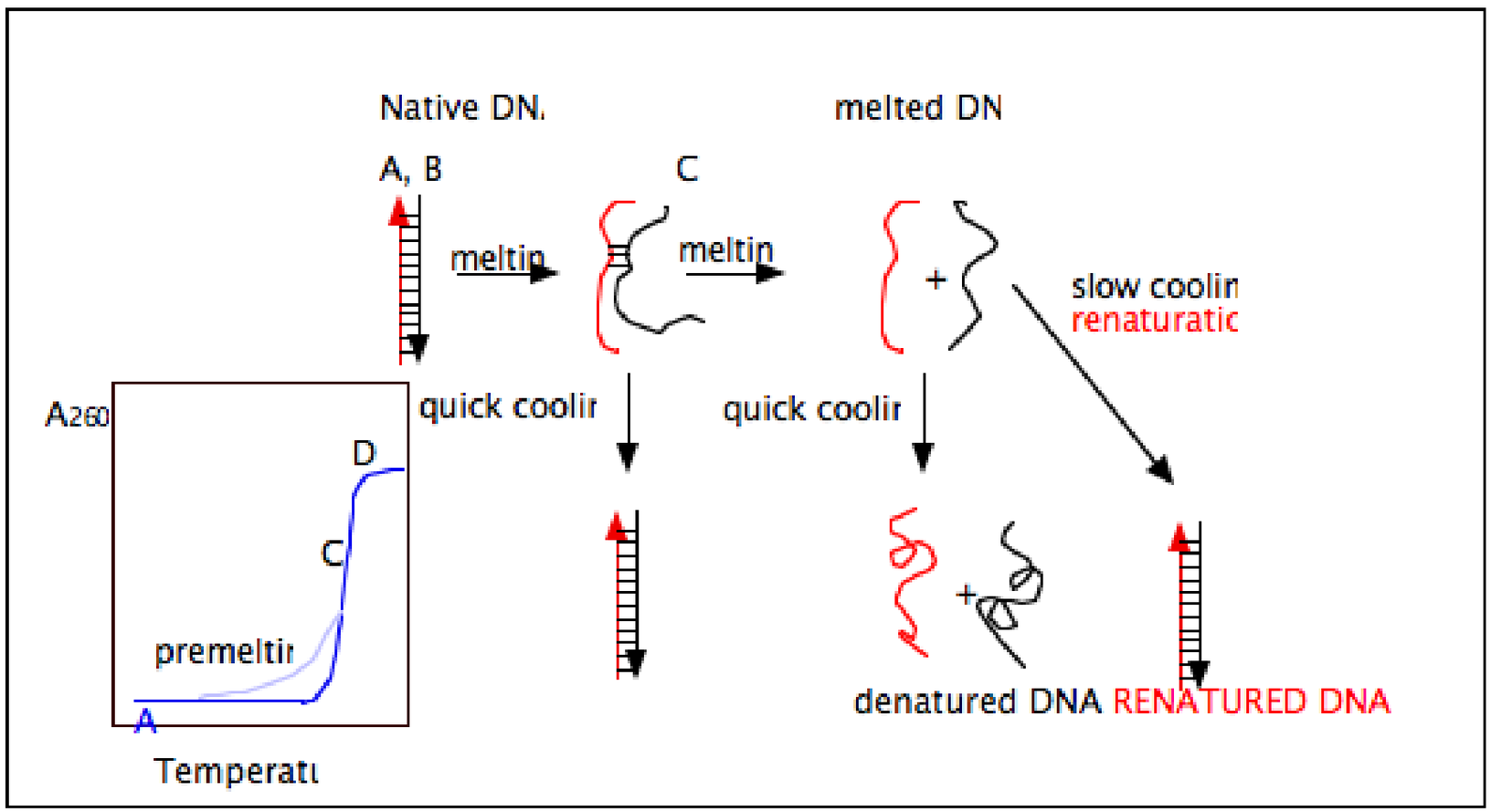
11.1. The limits of the formamide scenario

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

The first riddle is the concentration problem. We have mentioned in Section 2 that the steady state concentration of HCN in the primitive ocean was evaluated to be 4×10^{-12} M at 100 °C, that similar values were reported for NH_2CHO and that even at lower temperatures concentrations were too low to foster biomolecular syntheses in solution. Concentration processes of formamide by eutectics, by absorption onto or into appropriate minerals such as clays, and by evaporation (the boiling point of NH_2CHO being 204 °C), have been studied (see Sections 2 and 3.2.1). Noteworthily, the stability of NH_2CHO towards hydrolysis increases proportionally to its concentration,[399] and efficient prebiotic syntheses from NH_2CHO are operative also in 30% water (v/v).[320] Another possible concentration means as thermophoresis have not yet been sufficiently explored experimentally to indicate novel possible solutions.

Riddles remain

DNA DENATURATION and RENATURATION/HYBRIDIZATION



J. Marmur and P. Doty

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.

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Times Cited: 9234

2. **MARMUR J**, DOTY P
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Microbiologist, biochemist and molecular biologist

Julius Marmur – discovered 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)

↓

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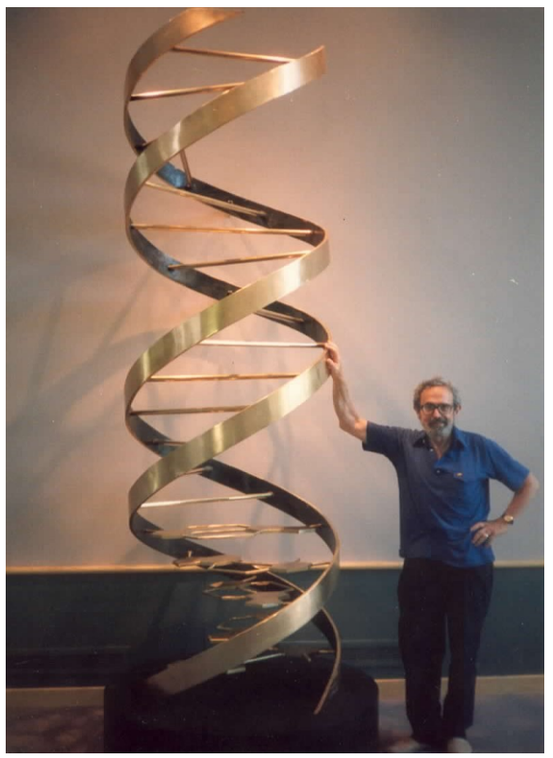
Julius Marmur



1993

The double helix: a personal view

Francis Crick

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

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 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, Brahms[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

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.

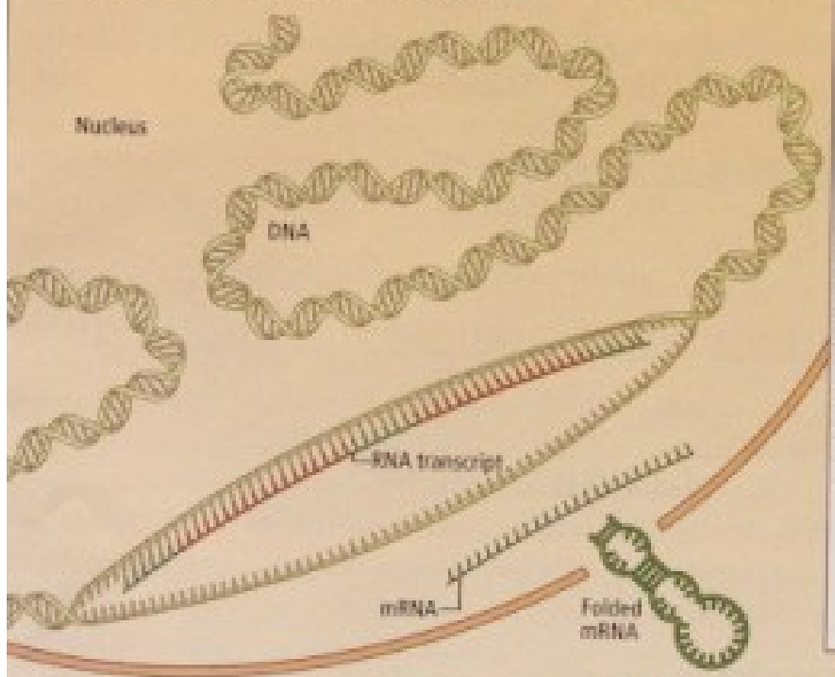
[BASICS]

SILENCE IN THE CODE

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

▼ TRANSCRIPTION AND EDITING

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

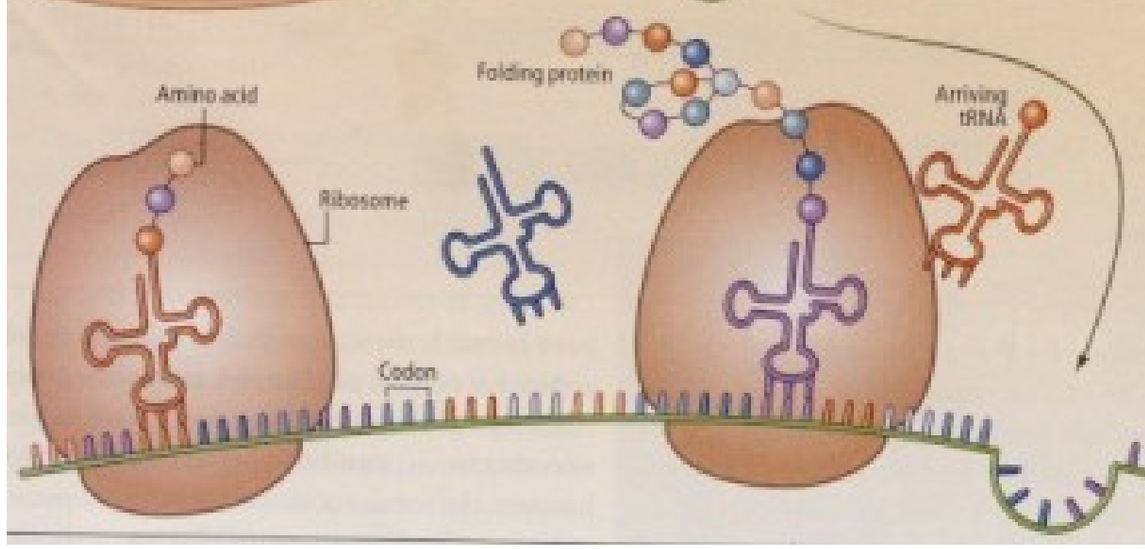


▼ THE CODON–AMINO ACID CODE

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

Second nucleotide position

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



◀ TRANSLATION TO PROTEIN

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


TRANSLATION FROM RNA

MUFFLED MESSAGE

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.

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**

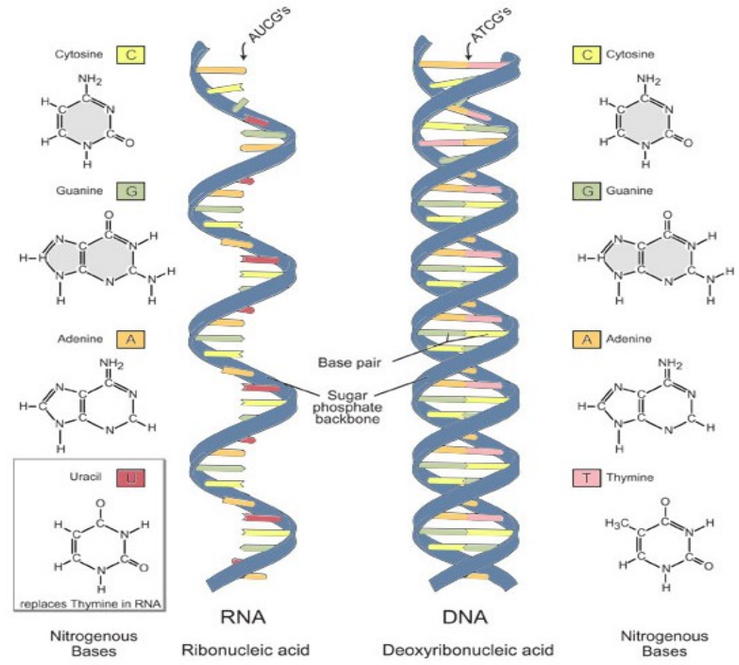
All bases (A, C, G, T, U) yield sparingly soluble compounds with mercury and can be determined at concentration down to 10^{-11}M.
Solid amalgam electrodes can be used instead of the mercury drop electrodes.

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

---

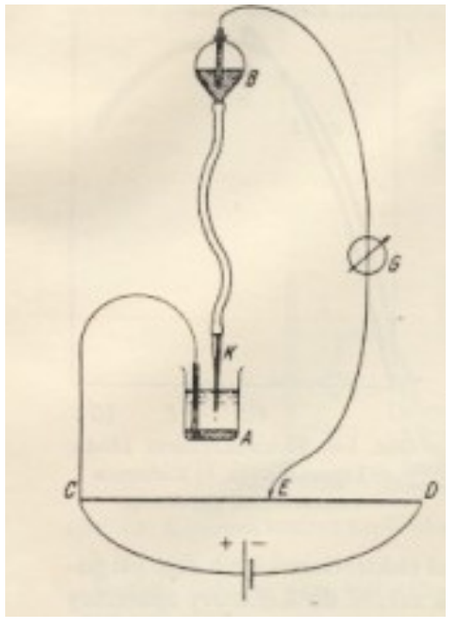


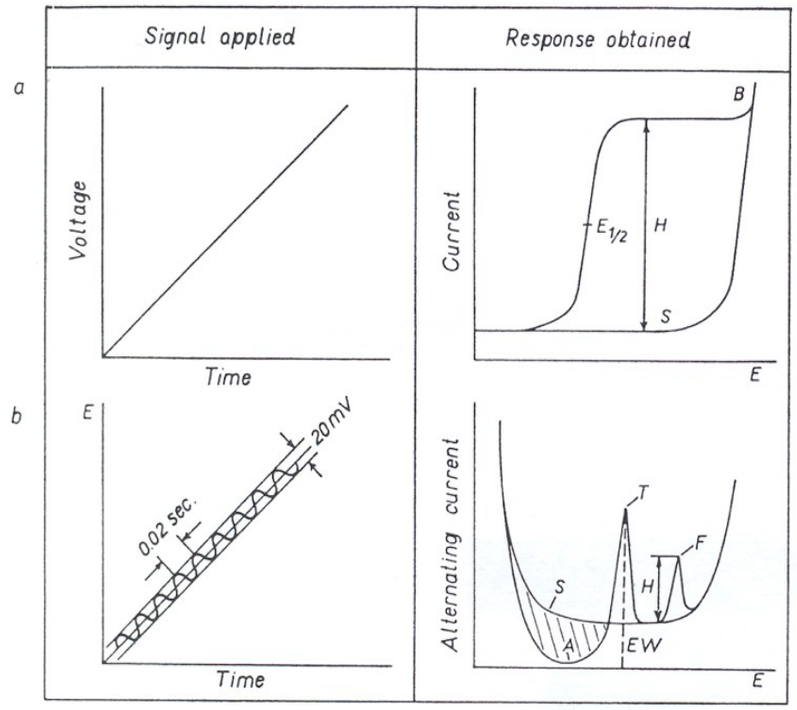
Electroactive Labels can be Introduced in DNA

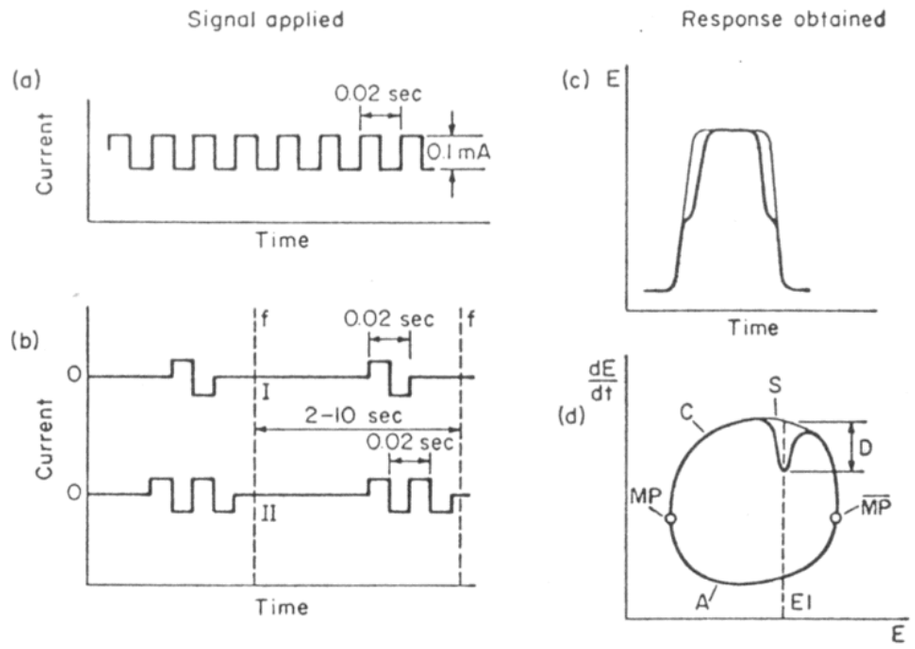
Fojta, M., et al.. (2007): „Multicolor" electrochemical labeling of DNA hybridization probes with osmium tetroxide complexes. Anal. Chem. 79, 1022-1029
Trefulka, M., et al. (2007): Covalent labeling nucleosides, RNA and DNA with VIII- and VI-valent osmium complexes. Electroanal. 19, 1281-1287

Jaroslav Heyrovský 1890-1967 invented POLAROGRAPHY in 1922

Present electrochemical analysis stems from Heyrovský's polarography







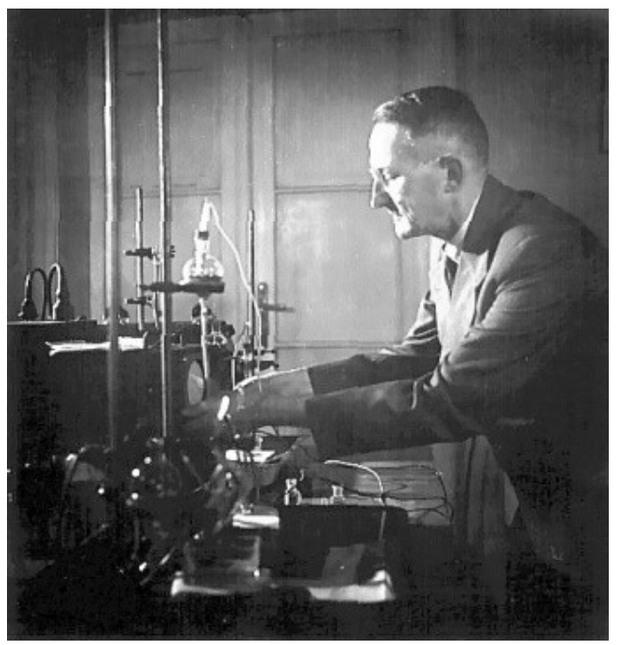
Oscillographic polarography at controlled a.c
(cyclic a.c. chronopotentiometry)

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

Nobel Prize 1959

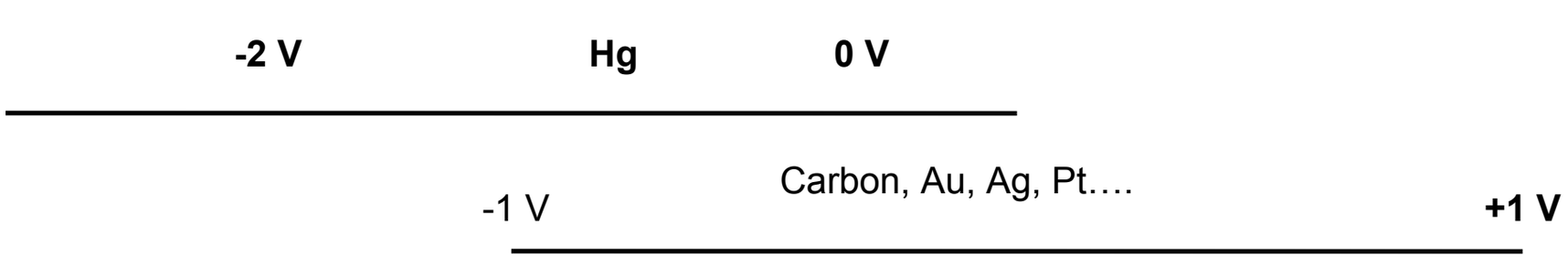
J. Heyrovský

J Heyrovsky S Ochoa A Kornberg



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

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

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

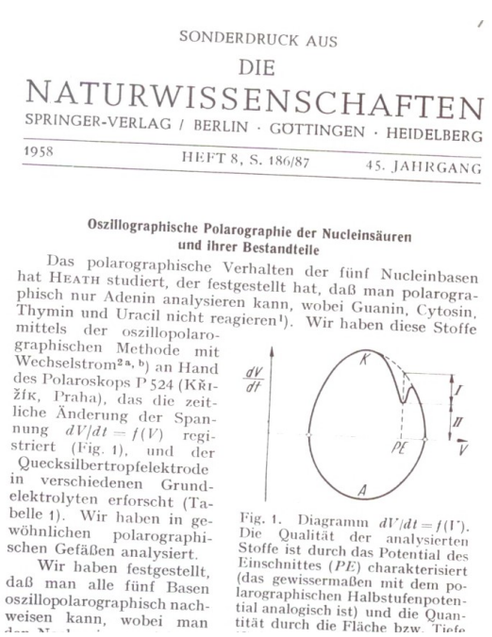
1958: Nucleic acid bases, DNA and RNA are electroactive

1960: Relations between the DNA structure and electrochemical responses

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

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

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



SONDERDRUCK AUS

DIE

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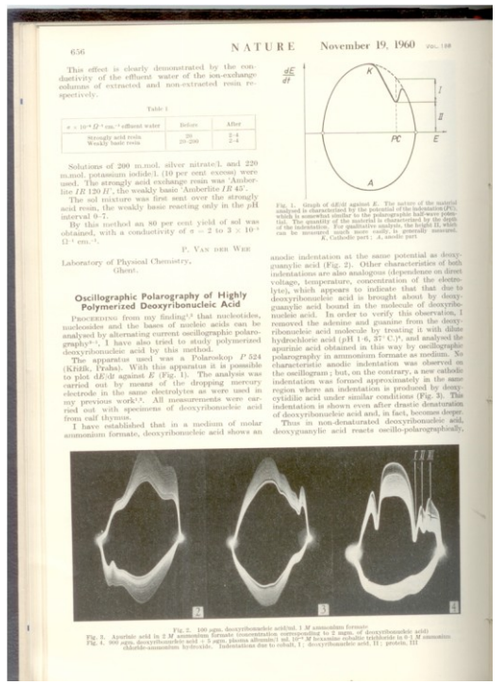
1958 HEFT 8, S. 186/87 45. JAHRGANG

Oszillographische Polarographie der Nucleinsäuren und ihrer Bestandteile

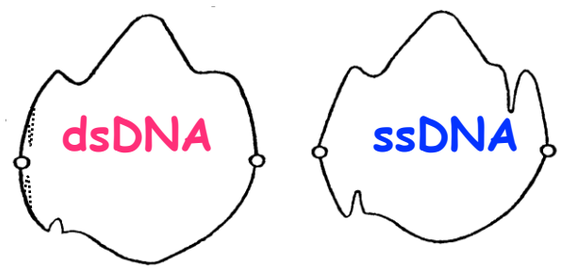
Das polarographische Verhalten der fünf Nucleinbasen hat HEATH studiert, der festgestellt hat, daß man polarographisch nur Adenin analysieren kann, wobei Guanin, Cytosin, Thymin und Uracil nicht reagieren[1]). Wir haben diese Stoffe mittels der oszillopolarographischen Methode mit Wechselstrom[2a, b])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

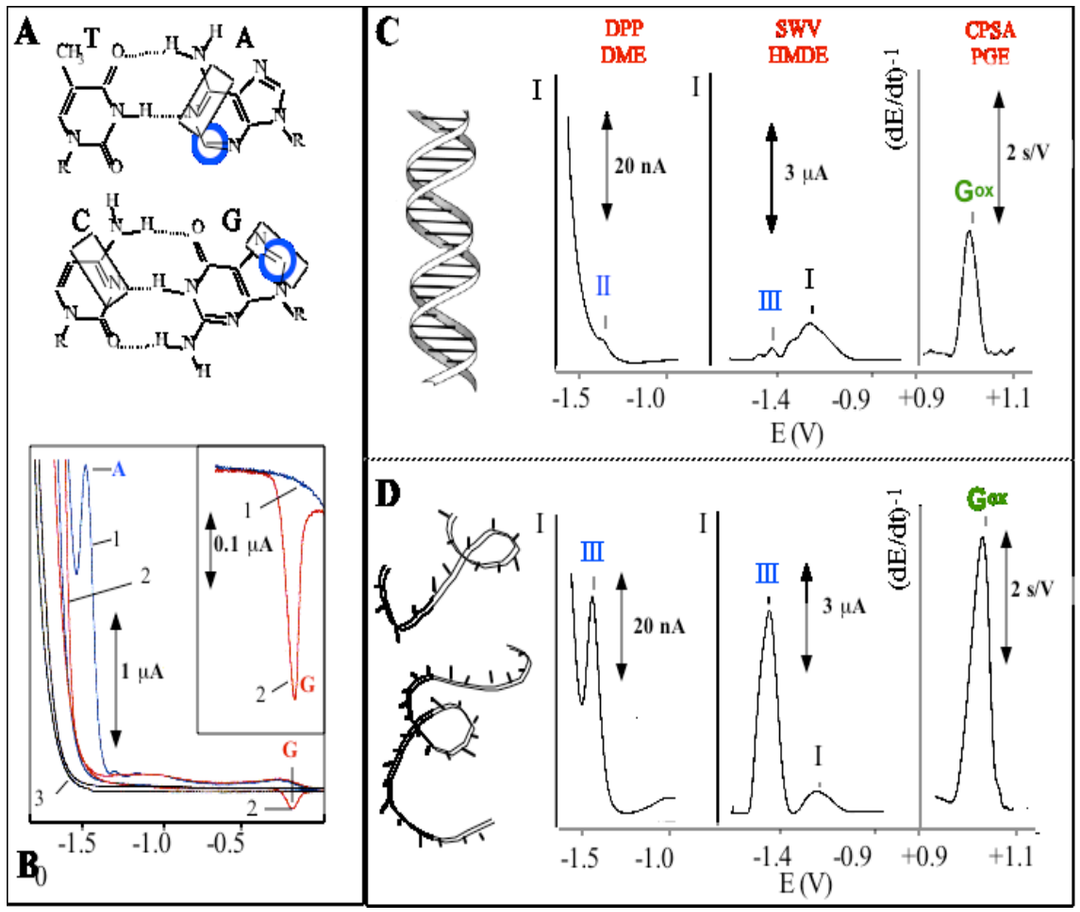
Wir haben festgestellt, daß man alle fünf Basen oszillopolarographisch nachweisen kann, wobei man

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



Oscillographic Polarography of Highly Polymerized Deoxyribonucleic Acid





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

D.c. polarography vs. oscillopolarography (OP)

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

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

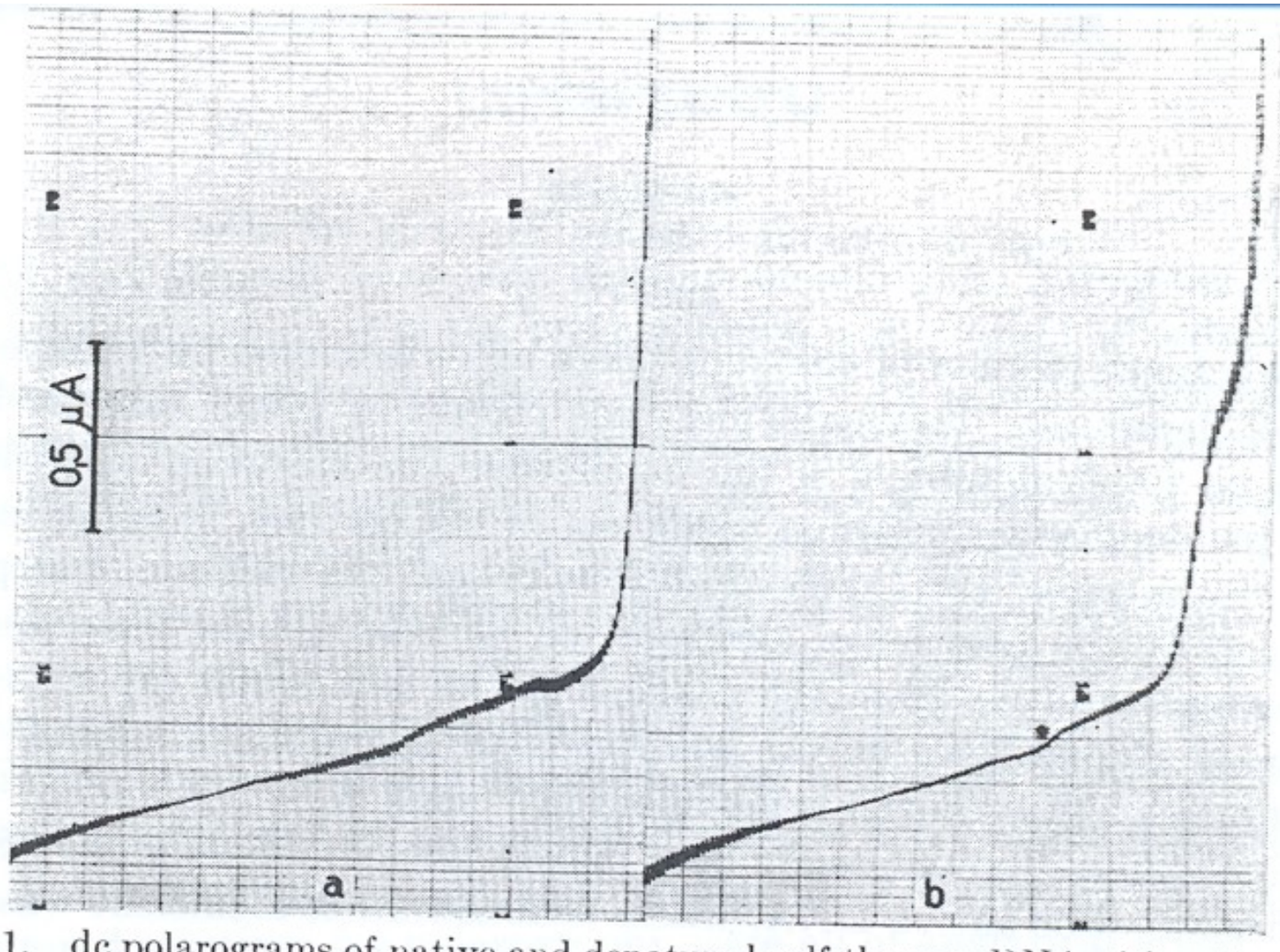


Fig. 1. dc polarograms of native and denatured calf thymus DNA: (*a*) native DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0); (*b*) denatured DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0). DNA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

RENATURATION OF RNA AS DETECTED BY DPP
Time dependence

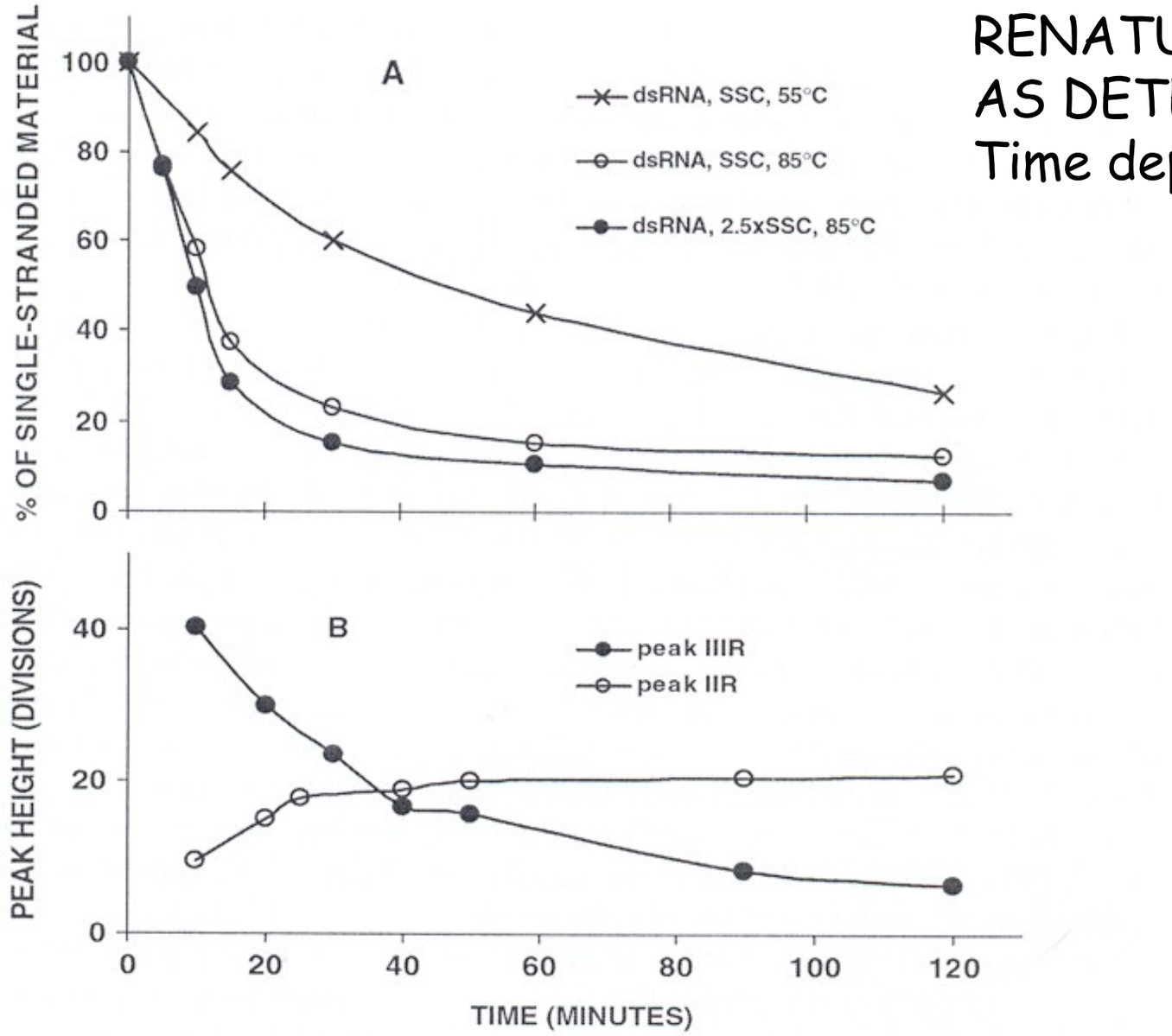


Fig. 10. Time-course of renaturation of phage f2 dsRNA. (A) Thermally denatured ssRNA was incubated (●—●) at 85°C in 2.5 × sodium saline citrate (SSC) or (o—o) at 85°C in SSC, and (x—x) at 55°C. Samples were withdrawn in time intervals given in the graph and quickly cooled. DPP measurements were performed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

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-

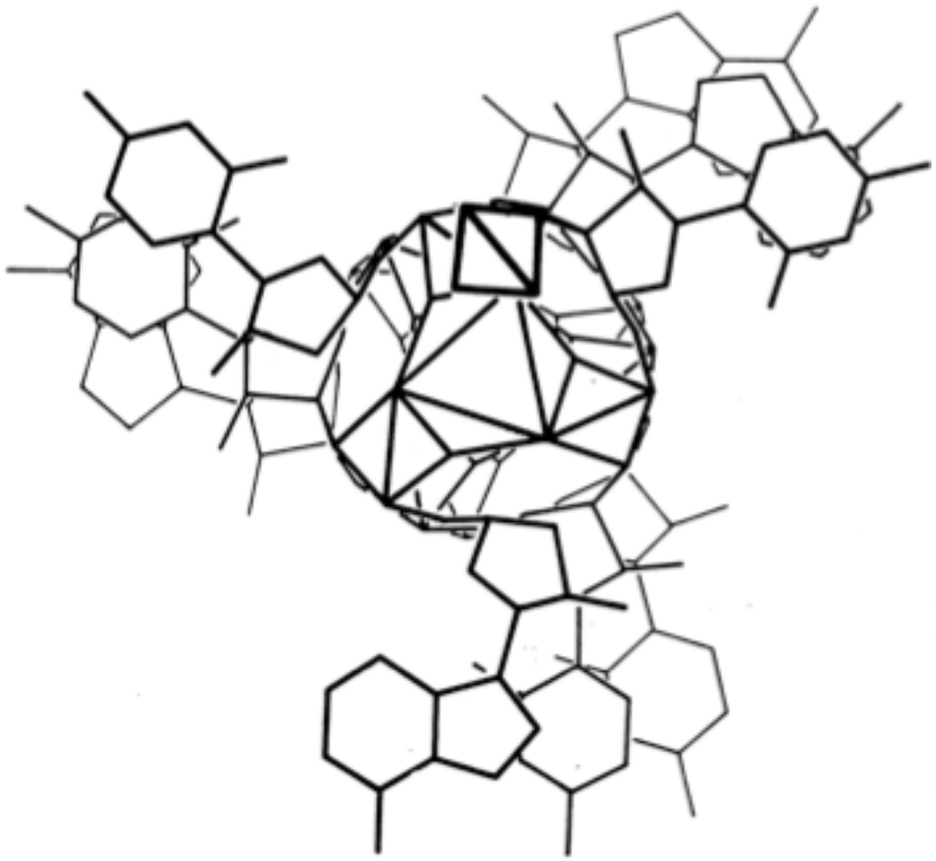


FIGURE 6

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

Triple helix

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

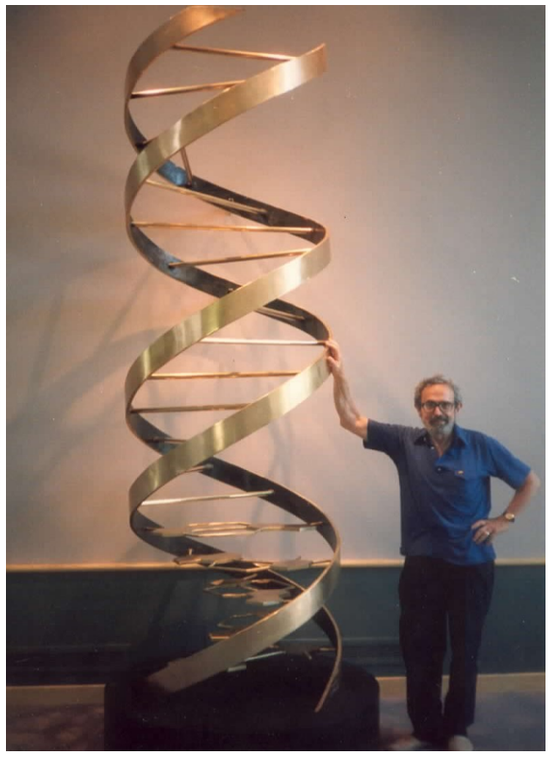
My IFFY story:
If L. PAULING had in his lab an oscillopolarograph in 1952 he would never proposed this structure.
Polarography clearly showed that bases must be hidden in the interior of native DNA molecule and become accessible when DNA is denatured

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

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

I accepted the invitation by Julius Marmur but for more than two years I was not allowed to leave Czechoslovakia. In the meantime JM moved from Harvard to Brandeis Univ. By the end of November 1962 I finally got my exit visa and with Heyrovsky Letter of Reccommendation in my pocket I went to the plane just 24 hours before expiration of my US visa. Before my departure I sent my OP instrument by air to Boston. It arrived after 9 months completely broken.
Instead of OP I had to use ultracentrifuges and microbiological methods.

Julius Marmur 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

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

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

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

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

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

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

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

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

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Early evidence of DNA Premelting and Polymorphy of the DNA Double Helix

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

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

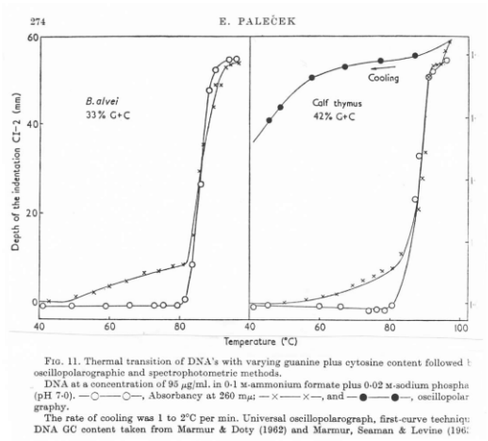


FIG. 11. Thermal transition of DNA's with varying guanine plus cytosine content followed b oscillopolarographic and spectrophotometric methods.
DNA at a concentration of 95 μg/ml. in 0·1 M-ammonium formate plus 0·02 M-sodium phospha (pH 7·0). —○——○—, Absorbancy at 260 mμ; —×——×—, and —●——●—, oscillopolar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

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

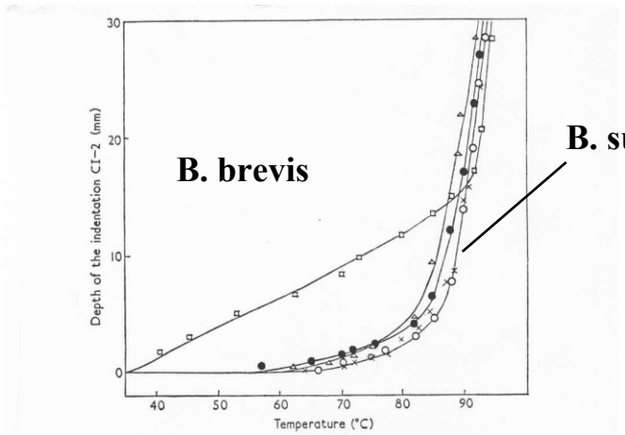
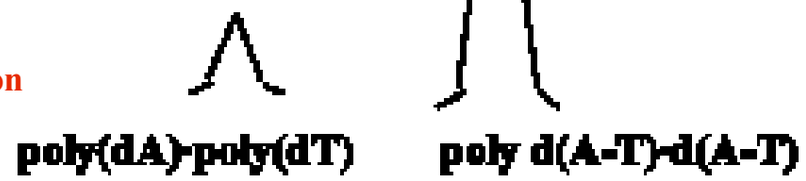


FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 μg/ml. in 0·25 M-ammonium formate plus 0·025 M-sodium phosphate (pH 7·0).
—●——●—, *B. subtilis* 168; —×——×—, *B. natto*; —○——○—, *B. subtilis* var. *niger*; —△——△—, *B. subtilis* var. *aterrimus*;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

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



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

What the people said

Before 1980
No doubt that this electrochemistry must produce artifacts because we know well that the DNA double helix has a unique structure INDEPENDENT of the nucleotide SEQUENCE

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

1976

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

Premelting Changes in DNA Conformation

E. PALEČEK

6. POLYMORPHY OF DNA SECONDARY STRUCTURE

On the basis of the preceding discussion, a schematic picture of the structure of natural linear DNA in solution under physiological conditions (e.g., at 36°C, moderate ionic strength, and pH 7) can be drawn. We can assume that the double-helical structure of the very long (A + T)-rich regions differs from the structure of the major part of the molecule and that some of the (A + T)-rich segments are open (Fig. 20). An open ds-structure can be assumed in the region of chain termini and/or in the vicinity of ss-breaks and other anomalies in the DNA primary structure. The exact changes in the open ds-regions will depend on the nucleotide sequence as well as on the chemical nature of the anomaly. Most of the molecule will exhibit an average Watson–Crick B-structure with local deviations given by the nucleotide sequence. Elevating the temperature in the premelting region (Fig. 20) is likely to lead to the opening of other regions and, eventually, to expansion of the existing distorted ds-regions and to further structural changes. Thus the course of the conformational changes as a function of temperature (premelting) will be determined by the distribution of the nucleotide sequences and anomalies in the primary structure, and may have an almost continuous character.

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

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

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

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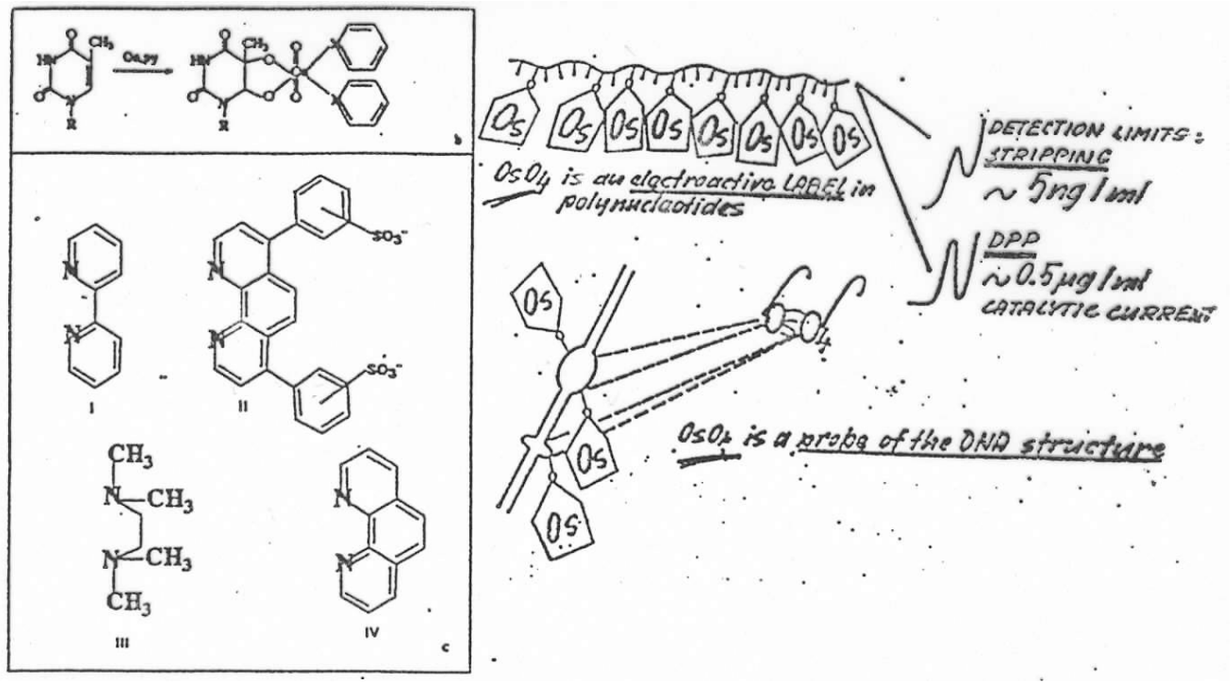
Electroactive labels can be introduced in nucleic acids

Os(VIII)L complexes are sensitive to the DNA structure (CHEMICAL PROBES OF THE DNA STRUCTURE)
they react with single-stranded and distorted but NOT with intact double-stranded DNA in vitro and in cells



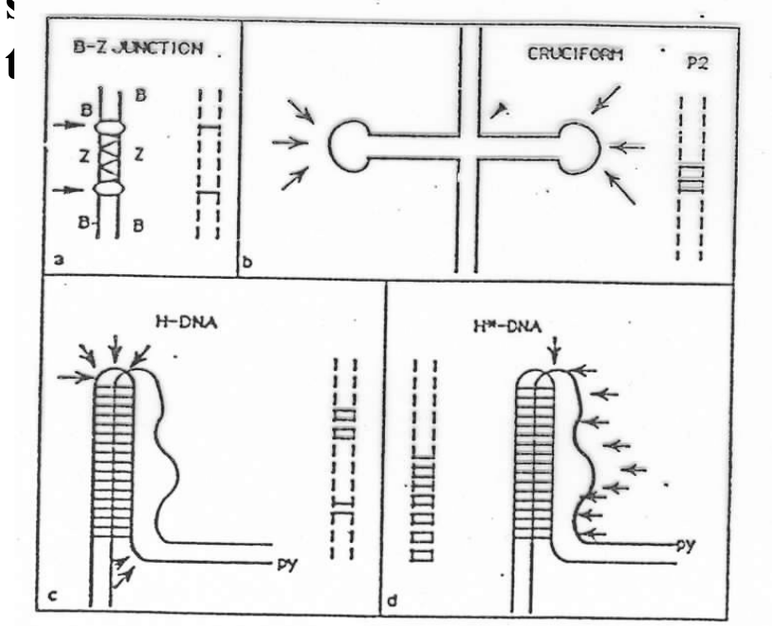
In the beginning of the 1980's Os,L complexes were the first electroactive labels covalently bound to DNA. These complexes produced catalytic signals at Hg electrodes allowing determination of DNA at subnanomolar concentrations

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

Local Supercoil-Stabilized DNA Structures

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

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



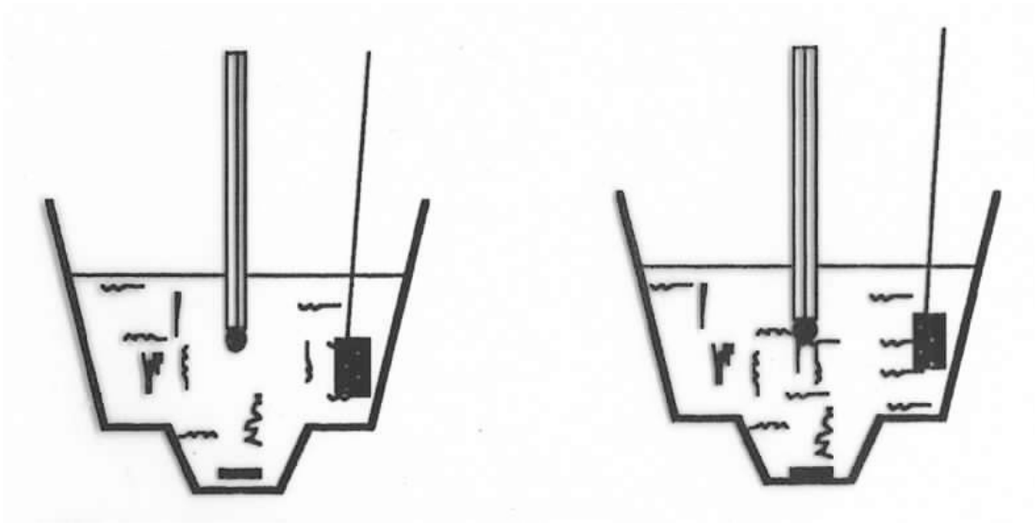
These methods yielded information about the distorted and single-stranded regions in the DNA double helix at single-nucleotide resolution. DNA probed both in vitro and

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

By EMIL PALEČEK

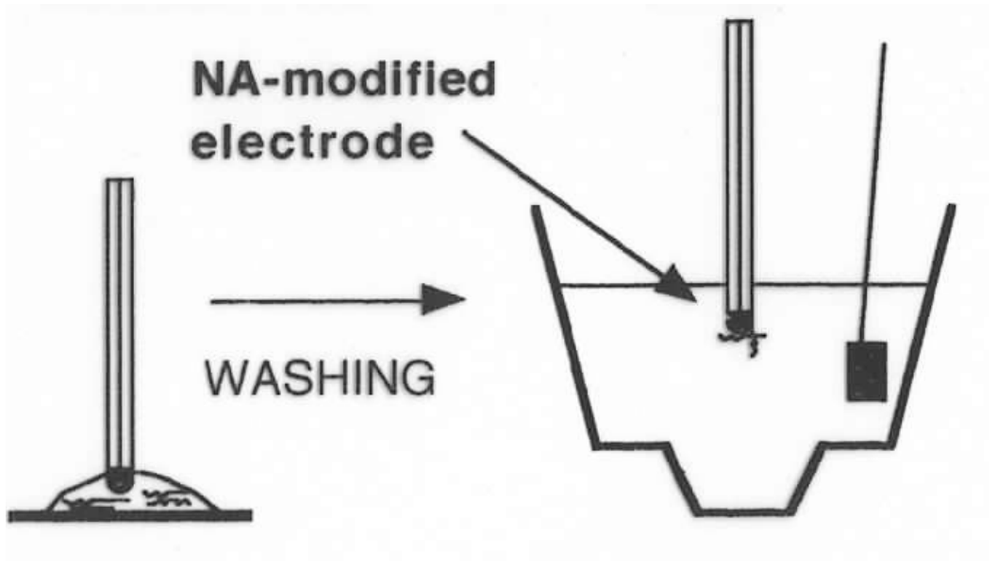
METHODS IN ENZYMOLOGY, VOL. 212

ADSORPTIVE STRIPPING



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

ADSORPTIVE TRANSFER STRIPPING



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

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

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

AdTSV has many advatages over conventional voltammetry of NAs:

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