1. PŘEDNÁŠKA MOL. BIOL.

2009-10

Nucleic acids

Historical view

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The Road to DNA started in Brno

G.J. Mendel

1866

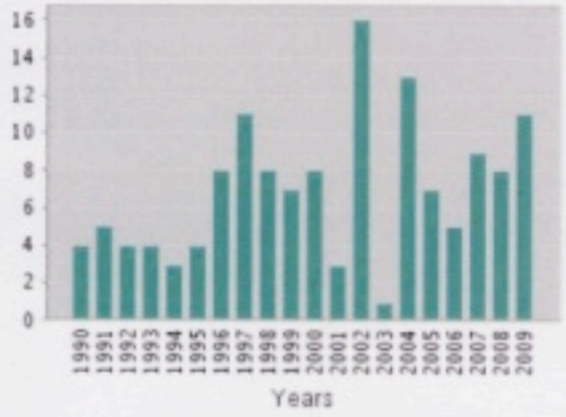
Citation Report Author=(Palecek E)

Timespan=All Years. Databases=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, IC, CCR-EXPANDED.

This report reflects citations to source items indexed within Web of Science. Perform a Cited Reference Search to include citations to items not indexed within Web of Science.

Chemická reaktivita a struktura nukleových kyselin. Lokální struktury DNA stabilizované superhelikálním vinutím; Interakce DNA a bílkovi s povrchy; Interakce DNA-protein;

Published Items in Each Year



The latest 20 years are displayed.
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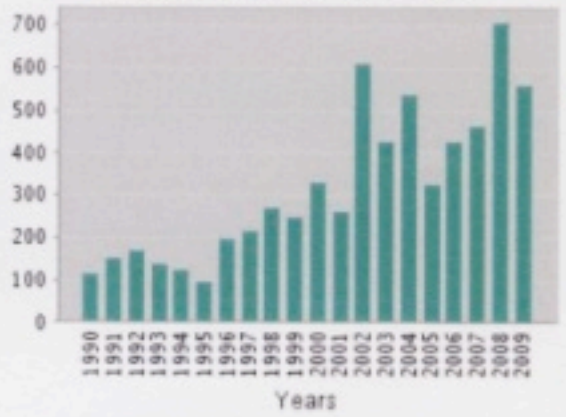

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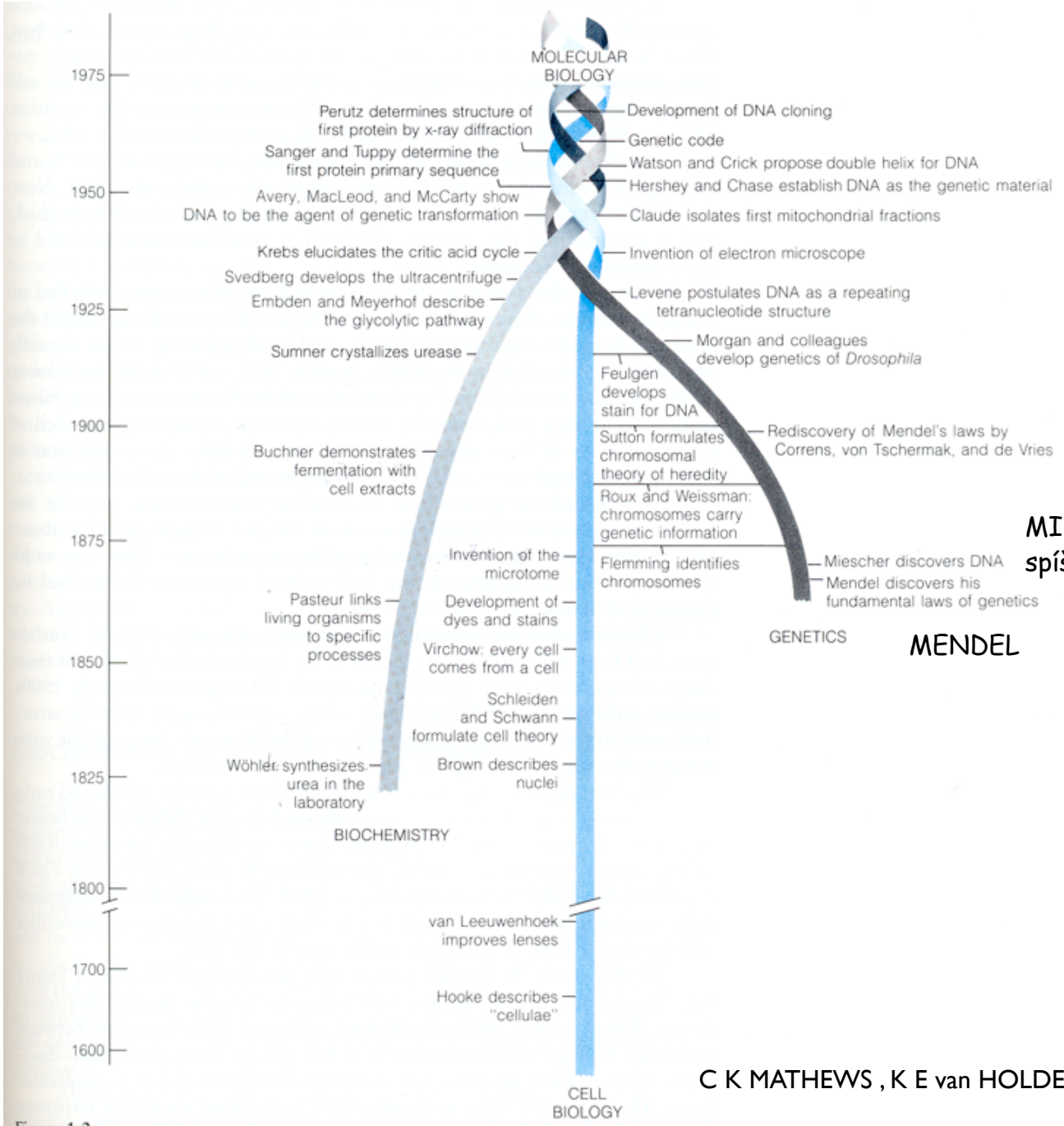
Results found:	264	**264**
Sum of the Times Cited [?] :	8,280	**8280**
View Citing Articles		
View without self-citations		
Average Citations per Item [?] :	31.36	**31.36**
h-index [?] :	52	**52**

Elektrochemie nukleových kyselin a bílkovin; Nádorové supresory, zejména protein p53; Agregace bílkovin v neurodegenerativních chorobách (zejména agregace α-synucleinu v Parkinsonově chorobě)

Results: **264** Page 1 of 27 Go Sort by: Times Cited

Use the checkboxes to remove individual items from this Citation Report or restrict to items processed between 1945-1954 and 2009 Go

	2005	2006	2007	2008	2009	Total	Average Citations per Year
	327	430	464	708	561	8,280	159.23
1. Title: Peptide nucleic acid probes for sequence-specific DNA biosensors Author(s): Wang J, Palecek E, Nielsen PE, et al. Source: **JOURNAL OF THE AMERICAN CHEMICAL SOCIETY** Volume: **118** Issue: **33** Pages: **7667-7670** Published: **AUG 21 1996**	15	11	11	10	13	231	16.50
2. Title: From polarography of DNA to microanalysis with nucleic acid-modified electrodes Author(s): Palecek E Source: **ELECTROANALYSIS** Volume: **8** Issue: **1** Pages: **7-14** Published: **JAN 1996**	6	8	8	24	8	225	16.07
3. Title: Detecting DNA hybridization and damage Author(s): Palecek E, Fojta M Source: **ANALYTICAL CHEMISTRY** Volume: **73** Issue: **3** Pages: **74A-83A** Published: **FEB 1 2001**	20	28	27	28	10	210	23.33
4. Title: LOCAL SUPERCOIL-STABILIZED DNA STRUCTURES Author(s): PALECEK E Source: **CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY** Volume: **26** Issue: **2** Pages: **151-226** Published: **1991**	1	0	2	3	3	178	9.37



MIESCHER?
spíše do biochemie

MENDEL

C K MATHEWS , K E van HOLDE, **BIOCHEMISTRY,** 1990

Figure 1.2
Interweaving of the historical tradition of biochemistry, cell biology, and genetics. These three disciplines, which originally were considered to be quite separate, have become intertwined to yield a true molecular biology, the subject matter of present-day biochemistry.

NUCLEIC ACIDS

Chemical nature and spatial organization
STRUCTURE

F. MIESCHER, TÜBINGEN
1871

Biological function

G. J. MENDEL, BRNO
1866

Timeline of DNA

1865: **Gregor Mendel** discovers through breeding experiments with peas that traits are inherited based on specific laws (later to be termed "Mendel's laws"). By mentioning Elements of Heredity he predicts DNA and genes (published 1866)
1866: Ernst Haeckel proposes that the nucleus contains the factors responsible for the transmission of hereditary traits.
1869: **Friedrich Miescher** isolates DNA/NUCLEIN for the first time.
1871: The first publications describing DNA (nuclein) **by F Miescher, Felix Hoppe-Seyler**, and P. Plosz are printed.

1882: Walther Flemming describes **chromosomes** and examines their behavior during cell division.
1884–1885: Oscar Hertwig, Albrecht von Kölliker, Eduard Strasburger, and August Weismann independently provide evidence that the cell's **nucleus contains the basis for inheritance**.
1889: Richard Altmann renames **nuclein** to **nucleic acid**.
1900: Carl Correns, Hugo de Vries, and Erich von Tschermak **rediscover Mendel's Laws**.

1902: T Boveri and W Sutton postulate that the **heredity units** (called genes as of 1909) are located **on chromosomes**.
1902–1909: A Garrod proposes that **genetic defects** result in the loss of enzymes and hereditary metabolic diseases.
1909: Wilhelm Johannsen uses the word **gene** to describe units of heredity.
1910: T H **Morgan** uses fruit flies (**Drosophila**) as a model to study heredity and finds the **first mutant** with white eyes.
1913: Alfred **Sturtevant** and Thomas Hunt **Morgan** produce the first **genetic linkage map** (for the fruit fly Drosophila).

1928: Frederick **Griffith** postulates that a **transforming principle** permits properties from one type of bacteria (heat-inactivated virulent Streptococcus pneumoniae) to be transferred to another (live nonvirulent Streptococcus pneumoniae).
1929: P **Levene** identifies the **building blocks of DNA,** incl. four bases adenine (A), cytosine (C), guanine (G), thymine (T) .
1941: George **Beadle** and Edward **Tatum** demonstrate that **every gene is responsible for the production of an enzyme**.
1944: Oswald T. **Avery**, Colin **MacLeod**, and Maclyn **McCarty** demonstrate that Griffith's **transforming principle is not a protein, but** rather **DNA**, suggesting that DNA may function as the genetic material

1949: Colette and Roger **Vendrely** and A **Boivin** discover that the nuclei of **germ cells contain half the amount of DNA** that is found in somatic cells. This parallels the reduction in the number of chromosomes during gametogenesis and provides further evidence for the fact that DNA is the genetic material.
1949–1950: Erwin **Chargaff** finds that the DNA base composition varies between species but determines that the bases in DNA are always present in fixed ratios: the **same number of A's as T's and the same number of C's as G's.**
1952: Alfred **Hershey** and Martha **Chase** use viruses (bacteriophage T2) to confirm DNA as the genetic material by demonstrating that during infection viral DNA enters the bacteria while the viral proteins do not and that this DNA can be found in progeny virus particles.

1953: Rosalind **Franklin** and Maurice **Wilkins** use **X-ray analyses** to demonstrate that **DNA** has a regularly repeating **helical structure.**
1953: James **Watson** and Francis **Crick** discover the molecular structure of DNA: a **double helix** in which A always pairs with T, and C always with G.
1956: Arthur **Kornberg** discovers **DNA polymerase**, an enzyme that replicates DNA.
1957: Francis **Crick** proposes the **central dogma** (information in the DNA is translated into proteins through RNA) **1958**: Matthew **Meselson** and Franklin **Stahl** describe how DNA replicates (semiconservative replication).

1960-63: Julius **Marmur** and Paul **Doty** show separation of DNA strands and reformation of DNA double-helical structure – DNA **renaturation/hybridization**
1961–1966: Robert W. **Holley**, Har Gobind **Khorana**, Heinrich **Matthaei**, Marshall W. **Nirenberg**, and colleagues **crack the genetic code**.
1968–1970: Werner **Arber**, Hamilton **Smith**, and Daniel **Nathans** use **restriction enzymes** to cut DNA in specific places for the first time.
1972: **Paul Berg** uses restriction enzymes to create the first piece of **recombinant DNA**.
1977: Frederick **Sanger**, Allan Maxam, and Walter **Gilbert** develop **methods to sequence DNA**.

1982: The first drug (human insulin), based on recombinant DNA, on the market.
1983: Kary **Mullis** invents **PCR** as a method for amplifying DNA in vitro.
1990: Sequencing of the human genome begins.
1995: First complete sequence of the genome of a free-living organism (the bacterium Haemophilus influenzae) is published.
1996: The complete genome sequence of the first eukaryotic organism—the yeast S. cerevisiae—is published.

1998: Complete genome sequence of the first multicellular organism—the nematode worm Caenorhabditis elegans—is published.
1999: Sequence of the **first human chromosome (22)** is published.
2000: The complete sequences of the genomes of the **fruit fly Drosophila** and the **first plant—Arabidopsis**—are published.
2001: The complete sequence of the **human genome** is published.
2002: The complete genome sequence of the first mammalian model organism—the **mouse**—is published.

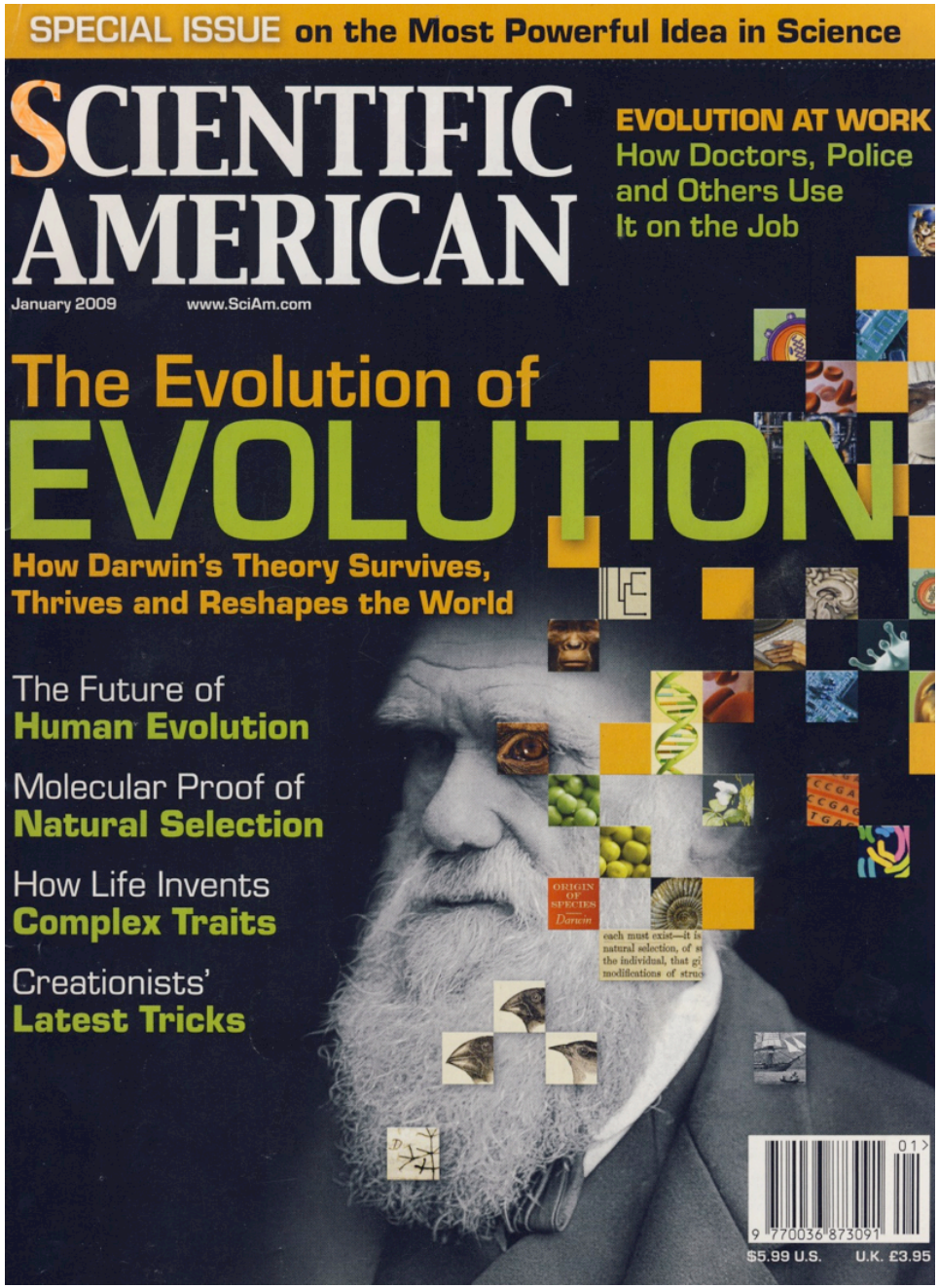
Darwin C. 1859: Book - On the Origin of Species by Means of Natural Selection
Mendel G. 1866
Miescher F. 1871 papers

Charles Darwin - Important claims:
A. Universal Common Descent - Tree of Life - the first one-celled organism, representing the root or trunk of the Tree, gradually developed and changed over many generations into new and more complex forms, representing the branches

B. Natural Selection as a mechanism responsible for the branching pattern
Variations in living forms arise at random
Nature selects the adaptive ones
Adaptive organism survive and reproduce
Inherited adaptations may cause population changes

Darwin understand neither how genetic traits were passed to the progeny nor how the variations arose. He is a founder of Evolution Biology

At present: - Natural Selection as a mechanism for relatively simple processes is fully confirmed
- Universal Common Descent - Tree of Life and the role of natural selection in the origin of species are questioned



EVOLUČNÍ BIOLOGIE

- rychle se vyvíjející vědecká disciplina

vedle ní existuje IDEOLOGIE **EVOLUCIONISMU**

PODLE DARWINISTY M. RUSE NENÍ

BOJ EVOLUCIONISMU S KREACIONISMEM

BOJEM VĚDY S NÁBOŽENSTVÍM ALE

BOJEM NÁBOŽENSTVÍ S NÁBOŽENSTVÍM

M. Ruse, The Evolution-Creation Struggle
HARVARD UNIVERSITY PRESS , 2005

Horizontal gene transfer - cell conglomerate instead of single cell ancestor

On the evolution of cells

Carl R. Woese*

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Contributed by Carl R. Woese, May 3, 2002

A theory for the evolution of cellular organization is presented. The model is based on the (data supported) conjecture that the dynamic of horizontal gene transfer (HGT) is primarily determined by the organization of the recipient cell. Aboriginal cell designs are taken to be simple and loosely organized enough that all cellular componentry can be altered and/or displaced through HGT, making HGT the principal driving force in early cellular evolution. Primitive cells did not carry a stable organismal genealogical trace. Primitive cellular evolution is basically communal. The high level of novelty required to evolve cell designs is a product of communal invention, of the universal HGT field, not intralineage variation. It is the community as a whole, the ecosystem, which evolves. The individual cell designs that evolved in this way are nevertheless fundamentally distinct, because the initial conditions in each case are somewhat different. As a cell design becomes more complex and interconnected a critical point is reached where a more integrated cellular organization emerges, and vertically generated novelty can and does assume greater importance. This critical point is called the "Darwinian Threshold" for the reasons given.

The evolution of modern cells is arguably the most challenging and important problem the field of Biology has ever faced (1, 2). In Darwin's day the problem could hardly be imagined. For much of the 20th century it was intractable. In any case, the problem lay buried in the catch-all rubric "origin of life"—where, because it is a biological not a (bio)chemical problem, it was effectively ignored. Scientific interest in cellular evolution started to pick up once the universal phylogenetic tree, the framework within which the problem had to be addressed, was determined (refs. 3 and 4; Fig. 1). But it was not until microbial genomics arrived on the scene that biologists could actually do much about the problem of cellular evolution.

Initial attempts to frame the issue have typically been in the classical Darwinian mode, and the focus to date has been almost exclusively on modeling the evolution of the eukaryotic cell. The reason, of course, is clear—the appeal of the endosymbiosis concept. Because endosymbiosis has given rise to the chloroplast and mitochondrion, what else could it have done in the more remote past? Biologists have long toyed with an endosymbiotic (or cellular fusion) origin for the eukaryotic nucleus, and even for the entire eukaryotic cell (4–10). These classical explanations have three characteristics: they (*i*) invoke cells that are basically *fully evolved*; (*ii*) evolve the essential eukaryotic cell well *after* its archaeal and bacterial counterparts (as has always been connoted by the term "prokaryote"); and (*iii*) focus attention on eukaryotic cellular evolution, which implies that the evolutions of the "prokaryotic" cell types, the archaeal and bacterial, are of a *different character*—simpler, and, it would seem, less interesting. We cannot expect to explain cellular evolution if we stay locked into the classical Darwinian mode of thinking.

The universal phylogenetic tree in one sense brought classical evolution to culmination. Darwin had said: "The time will come . . . when we shall have very fairly true genealogical trees of each great kingdom of nature" (11). A century later the universal phylogenetic tree based on molecular (rRNA) sequence comparisons did precisely that and went the further, final step to unify all of the "great kingdoms" into one single "empire" (3). The central question posed by the universal tree is the nature of the entity (or state) represented by its root, the fount of all extant life. Herein lies the door to the murky realm of cellular evolution.

Experience teaches that the complex tends to arise from the simple, and biologists have assumed it so in the case of modern cells. But this assumption is usually accompanied by another not so self-evident one: namely that the "organism" represented by the root of the universal tree was equivalent metabolically and in terms of its information processing to a modern cell, in effect was a modern cell. Such an assumption pushes the real *evolution* of modern cells back into an earlier era, which makes the problem not directly addressable through genomics. *That* is not a scientifically acceptable assumption. Unless or until facts dictate otherwise, the possibility must be entertained that some part of cellular evolution *could* have occurred during the period encompassed by the universal phylogenetic tree.

There is evidence, good evidence, to suggest that the basic organization of the cell had not yet completed its evolution at the stage represented by the root of the universal tree. The best of this evidence comes from the three main cellular information-processing systems. Translation was highly developed by that stage: rRNAs, tRNAs, and the (large) elongation factors were by then all basically in near modern form; hence, their universal distributions. Almost all of the tRNA charging systems were in modern form as well (12). But, whereas the majority of ribosomal proteins are universal in distribution, a minority of them is not. A relatively small cadre is specific to the bacteria, a somewhat larger set common and confined to the archaea and eukaryotes, and a few others are uniquely eukaryotic.

Almost all of the universal translational proteins (as well as those in transcription) show what is called the *canonical pattern*, i.e., the bacterial and archaeal versions of the protein are remarkably different from one another, so much so that their difference is distinguished as one of "genre" (12). Except for the aminoacyl-tRNA synthetases the corresponding eukaryotic versions are virtually all of the archaeal genre (12). Why canonical pattern exists is a major unanswered question (3). In the overall it would seem that translation, although highly developed at the root of the universal tree, subsequently underwent idiosyncratic modifications in each of the three major cell types.

Transcription seems to have been rather less developed at the root of the universal tree. The two largest (the catalytic) subunits of the DNA-dependent RNA polymerase, β and β' in bacterial nomenclature, are universal in distribution. But the remaining bacterial subunit (α) is only partially so. Bacterial α exists in two copies in the bacterial polymerase. Its archaeal/eukaryotic counterpart comprises two distinct proteins, each present in single copy in the enzyme and (portions of) each showing homology to (somewhat different) portions of bacterial α and *vice versa* (13). A structural difference of this magnitude must represent at least some functional distinction. The archaeal transcription apparatus also contains additional (smaller) subunits, none of which are found in bacteria but all of which occur in eukaryotes (13). [As in the case of translation, the (three) eukaryotic mechanism(s) contain additional eukaryote-specific small subunits.] Bacterial transcription initiation does not re-

Abbreviations: HGT, horizontal gene transfer; SMA, supramolecular aggregate.

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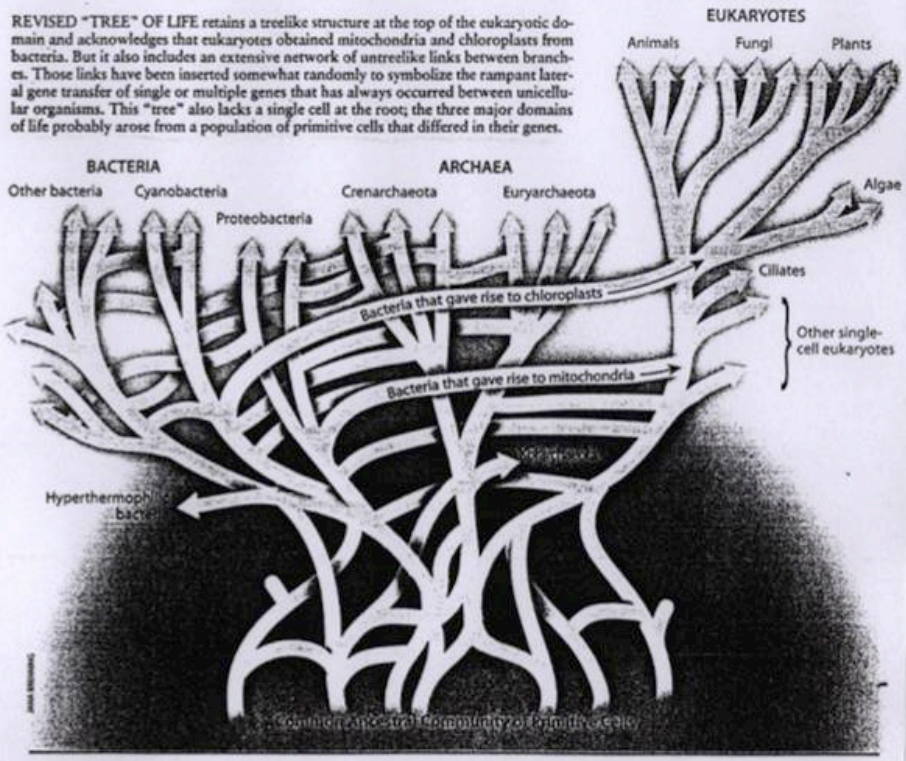
www.pnas.org/cgi/doi/10.1073/pnas.132268999



REVISED "TREE" OF LIFE retains a treelike structure at the top of the eukaryotic domain and acknowledges that eukaryotes obtained mitochondria and chloroplasts from bacteria. But it also includes an extensive network of untreelike links between branches. Those links have been inserted somewhat randomly to symbolize the rampant lateral gene transfer of single or multiple genes that has always occurred between unicellular organisms. This "tree" also lacks a single cell at the root; the three major domains of life probably arose from a population of primitive cells that differed in their genes.

The Author

W. FORD DOOLITTLE, who holds degrees from Harvard and Stanford universities, is professor of biochemistry and molecular biology at Dalhousie University in Halifax, Nova Scotia, and director of the Program in Evolutionary Biology of the Canadian Institute for Advanced Research.

Further Information

- THE UNIVERSAL ANCESTOR. Carl Woese in the *Proceedings of the National Academy of Sciences*, Vol. 95, No. 12, pages 6854–6859; June 9, 1998.
- YOU ARE WHAT YOU EAT: A GENE TRANSFER RACHET COULD ACCOUNT FOR BACTERIAL GENES IN EUKARYOTIC NUCLEAR GENOMES. W. Ford Doolittle in *Trends in Genetics*, Vol. 14, No. 8, pages 307–311; August 1998.
- PHYLOGENETIC CLASSIFICATION AND THE UNIVERSAL TREE. W. Ford Doolittle in *Science*, Vol. 284, pages 2124–2125; June 25, 1999.

ESSAY

Putting the pieces together

Biology's next revolution

The emerging picture of microbes as gene-swapping collectives demands a revision of such concepts as organism, species and evolution itself.

Nigel Goldenfeld and Carl Woese

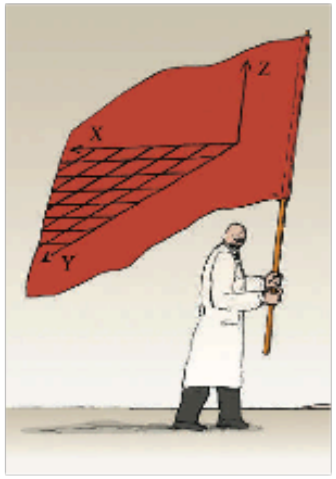
One of the most fundamental patterns of scientific discovery is the revolution in thought that accompanies a new body of data. Satellite-based astronomy has, during the past decade, overthrown our most cherished ideas of cosmology, especially those relating to the size, dynamics and composition of the Universe.

Similarly, the convergence of fresh theoretical ideas in evolution and the coming avalanche of genomic data will profoundly alter our understanding of the biosphere — and is likely to lead to revision of concepts such as species, organism and evolution. Here we explain why we foresee such a dramatic transformation, and why we believe the molecular reductionism that dominated twentieth-century biology will be superseded by an interdisciplinary approach that embraces collective phenomena.

The place to start is horizontal gene transfer (HGT), the non-genealogical transfer of genetic material from one organism to another — such as from one bacterium to another or from viruses to bacteria. Among microbes, HGT is pervasive and powerful — for example, in accelerating the spread of antibiotic resistance. Owing to HGT, it is not a good approximation to regard microbes as organisms dominated by individual characteristics. In fact, their communications by genetic or quorum-sensing channels indicate that microbial behaviour must be understood as predominantly cooperative.

In the wild, microbes form communities, invade biochemical niches and partake in biogeochemical cycles. The available studies strongly indicate that microbes absorb and discard genes as needed, in response to their environment. Rather than discrete genomes, we see a continuum of genomic possibilities, which casts doubt on the validity of the concept of a 'species' when extended into the microbial realm. The uselessness of the species concept is inherent in the recent forays into metagenomics — the study of genomes recovered from natural samples as opposed to clonal cultures. For example, studies of the spatial distribution of rhodopsin genes in marine microbes suggest such genes are 'cosmopolitan', wandering among bacteria (or archaea) as environmental pressures dictate.

Equally exciting is the realization that viruses have a fundamental role in the biosphere, in both immediate and long-term



memory of a community's genetic information, contributing to the system's evolutionary dynamics and stability. This is hinted at, for example, by prophage induction, in which viruses latent in cells can become activated by environmental influences. The ensuing destruction of the cell and viral replication is a potent mechanism for the dispersal of host and viral genes.

It is becoming clear that microorganisms have a remarkable ability to reconstruct their genomes in the face of dire environmental stresses, and that in some cases their collective interactions with viruses may be crucial to this. In such a situation, how valid is the very concept of an organism in isolation? It seems that there is a continuity of energy flux and informational transfer from the genome up through cells, community, virosphere and environment. We would go so far as to suggest that a defining characteristic of life is the strong dependency on flux from the environment — be it of energy, chemicals, metabolites or genes.

Nowhere are the implications of collective phenomena, mediated by HGT, so pervasive and important as in evolution. A computer scientist might term the cell's translational apparatus (used to convert genetic information to proteins) an 'operating system', by which all innovation is communicated and realized. The fundamental role of translation, represented in particular by the genetic code, is shown by the clearly documented optimization of the code. Its special role in any form of life leads to the striking prediction that more powerful early forms of HGT.

Refinement through the horizontal sharing of genetic innovations would have triggered an explosion of genetic novelty, until the level of complexity required a transition to the current era of vertical evolution. Thus, we regard as regrettable the conventional concatenation of Darwin's name with evolution, because other modalities must also be considered.

This is an extraordinary time for biology, because the perspective we have indicated places biology within a context that must necessarily engage other disciplines more strongly aware of the importance of collective phenomena. Questions suggested by the generic energy, information and gene flows to which we have alluded will probably require resolution in the spirit of statistical mechanics and dynamical systems theory. In time, the current approach of post-hoc modelling will be replaced by interplay between quantitative prediction and experimental test, nowadays more characteristic of the physical sciences.

Sometimes, language expresses ignorance rather than knowledge, as in the case of the word 'prokaryote', now superseded by the terms archaea and bacteria. We foresee that in biology, new concepts will require a new language, grounded in mathematics and the discoveries emerging from the data we have highlighted. During an earlier revolution, Antoine Lavoisier observed that scientific progress, like evolution, must overcome a challenge of communication: "We cannot improve the language of any science without at the same time improving the science itself; neither can we, on the other hand, improve a science without improving the language or nomenclature which belongs to it." Biology is about to meet this challenge. ■

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FURTHER READING
Feiguard, N., Martinez, A., Mincer, T. & DeLong, E. *Nature* **439**, 847–850 (2006).
Sullivan, M. et al. *PLoS Biol.* **4**, e234 (2006).
Pedulla, M. et al. *Cell* **113**, 171–182 (2003).
Vetsigian, K., Woese, C. & Goldenfeld, N. *Proc. Natl Acad. Sci. USA* **103**, 10696–10701 (2006).

For other essays in this series, see http://

CONNECTIONS

Thus we regard as regrettable the conventional concatenation of Darwin's name with evolution, because other modalities must also be considered

KEY INGREDIENTS OF THE HUMAN MIND

The four traits below distinguish the human mind from those of animals. Uncovering the origin of the human mind will require explaining how these unique properties came about.

Generative computation enables humans to create a virtually limitless variety of words, concepts and things. The characteristic encompasses two types of operation: recursive and combinatorial. Recursion is the repeated use of a rule to create new expressions. The combinatorial operation is the mixing of discrete elements to engender new ideas.

Promiscuous combination of ideas allows the mingling of different domains of knowledge—such as art, sex, space, causality and friendship—thereby generating new laws, social relationships and technologies.

Mental symbols encode sensory experiences both real and imagined, forming the basis of a rich and complex system of communication. Such symbols can be kept to oneself or expressed to others as words or pictures.

Abstract thought permits contemplation of things beyond what we can see, hear, touch, taste or smell.

THE MIND

BY MARC HAUSER

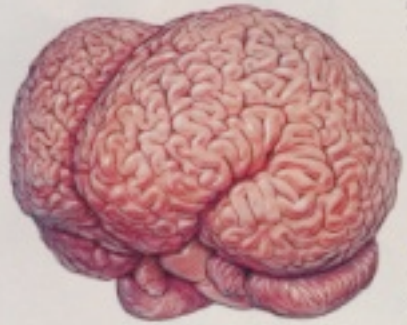
The first step in figuring out how the human mind arose is determining what distinguishes our mental processes from those of other creatures

KEY CONCEPTS

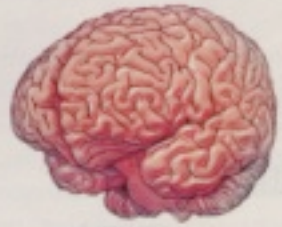
- Charles Darwin argued that a continuity of mind exists between humans and other animals, a view that subsequent scholars have supported.
- But mounting evidence indicates that, in fact, a large mental gap separates us from our fellow creatures. Recently the author identified four unique aspects of human cognition.
- The origin and evolution of these distinctive mental traits remain largely mysterious, but clues are emerging slowly.

LIMITED CLUES

The archaeological record reveals that humans were routinely making art and musical instruments by 35,000 years ago, indicating that they were thinking symbolically by then. But modern scholars have no way of knowing what these long-ago people thought about the symbols they left behind nor how they composed their music. Such artifacts are thus of limited use in piecing together the origins of our unique mental abilities.



Killer whale brain
5,620 grams



Human brain
1,350 grams

Etruscan shrew brain
0.1 gram

SIZING UP THE BRAIN

Humans are smarter than creatures whose brains are larger than ours in absolute terms, such as killer whales, as well as those animals whose brains are larger than ours in relative terms (that is, relative to body size), such as shrews. Thus, size alone does not explain the uniqueness of the human mind.

Sci. Amer. , Sept. 2009

JOHANN GREGOR MENDEL

* 1822 in Hynčice (Moravia, Austro-Hungarian Empire)
+ 1884 in Brno (buried at Central Cemetery in Brno)

discovered through breeding experiments with peas that traits are inherited based on specific laws (later to be termed "Mendel's laws"). By mentioning Elements of Heredity he predicted DNA and genes (published 1866, lecture in Brno 1965)

In the 1950´s Mendelism declared to be a reactionary teaching (LYSENKO, LEPESHINSKAYA)

Mendel statue removed and its destruction ordered
Brno geneticist J. Kříženecký jailed
His pupil V. Orel forced to work manually in industry

1964 attempts to rehabilitate Mendel
Academicians B. Němec (biologist) and F. ŠORM (biochemist, President of the Czechoslovak Academy of Sciences) backed by Soviet Academicians. Dealing between N. Khrushtchov, A. Novotný (President of Czechoslovakia), F. Šorm and biologist J. Pospíšil (later the Party Secretary) resulted in the decision to organize an international conference in 1968 (100 anniversary of publication of Mendel´s paper) in Brno (F. Šorm warned by Novotný that his attempts may result in the end of his career if the action will get out of control). Beginning of Mendel´s Museum in Brno

A milestone not only in the approach of Party and State to Mendel but also a beginning of rehabilitation of SCIENCE against the COMMUNIST IDEOLOGY

Brno Augustinians 1860-62 Abbot C. Napp

Mendel's Medal,
Moravian Museum, Brno

Abbot G. Mendel

Teachers of Brno gymnasium (High School)

G J MENDEL, priest, teacher, scientist and abbot in BRNO

THE STATUE STORY

In 1906 Dr. Hugo Iltis, the gymnasium professor in Brno organized an international collection to build the Mendel's Statue in Brno. Created by a French sculpturer T. Charlemont the Statue was errected at the Mendel Square in 1910

In 1956 Mendel's Statue was ordered by the Regional Authorities to be destroyed. The workers who were supposed to the job decided not to do it because they believed that the statue was nice. Moreover it would be difficult to destroy it.

After February 1948 Soviet „Lysenkism" (T. D. Lysenko 1896-1974) strongly affected biology in Czechoslovakia. After Stalin death (1953) attempts were made by soviet scientists (particularly by physists and chemists) to substitute Lysenko's „materialistic biology" for normal science and by the end of 1950's plans were made to organize in Brno International Mendel Memorial Symposium. In 1962 Lysenko's work was criticized by the Soviet Academy but still in September 1964 N.S. Khrushtchov raised objections against the Mendel Symposium in 1965 in Brno. During his visit in Prague he dealt with the President A. Novotny who finally agreed with the meeting organization after the President of the Academy F. Sorm personally guaranteed that the Symposium will not be politically misused. (F. Sorm was well informed about the activities of the influential Soviet scientist to rehabilitate fully the genetics - Soon after his visit of this country N.S. Khrushtchov was removed from his position).

Before the Symposium the Director of the Institute of Biophysics prof. F. Hercik was entrusted by the Academy to help with the organization of the Mendel International Meeting in Brno. To fulfill his duties he turned to the City Authorities asking to move the Mendel's Statue to the Abbey garden. As his request was ignored he asked his graduate students J. Koudelka and B. Janík to move the Statue from the Abbey yard to the garden. Both fellows were quite strong young men but they found the marble Statue too heavy.

1844 - 1895 Friedrich MIESCHER

1. sdělení v r. 1871

Žák **Hoppe-Seylera** v **Tübingen** se zabýval izolací jaderných komponent (z hnisajících buněk, které získával z tamnější chirurgie). Buňky hydrolyzoval pepsinem-HCl a po třepání s eterem izoloval jádra jako separovanou vrstvu na dně nádoby. Z tohoto materiálu **„nuklein“** - reagoval kysele, rychle se rozpouštěl ve zřeď. louhu a obsahoval velké množství P.

Vysoký obsah P byl považován za velmi pozoruhodný - jediná tehdy známá organická látka obsažená v tkáni - lecitin. Když F.M. předložil práci k publ. shledal ji H.S. tak překvapující, že ji odmítl uveřejnit, dokud ji sám neprověřil.

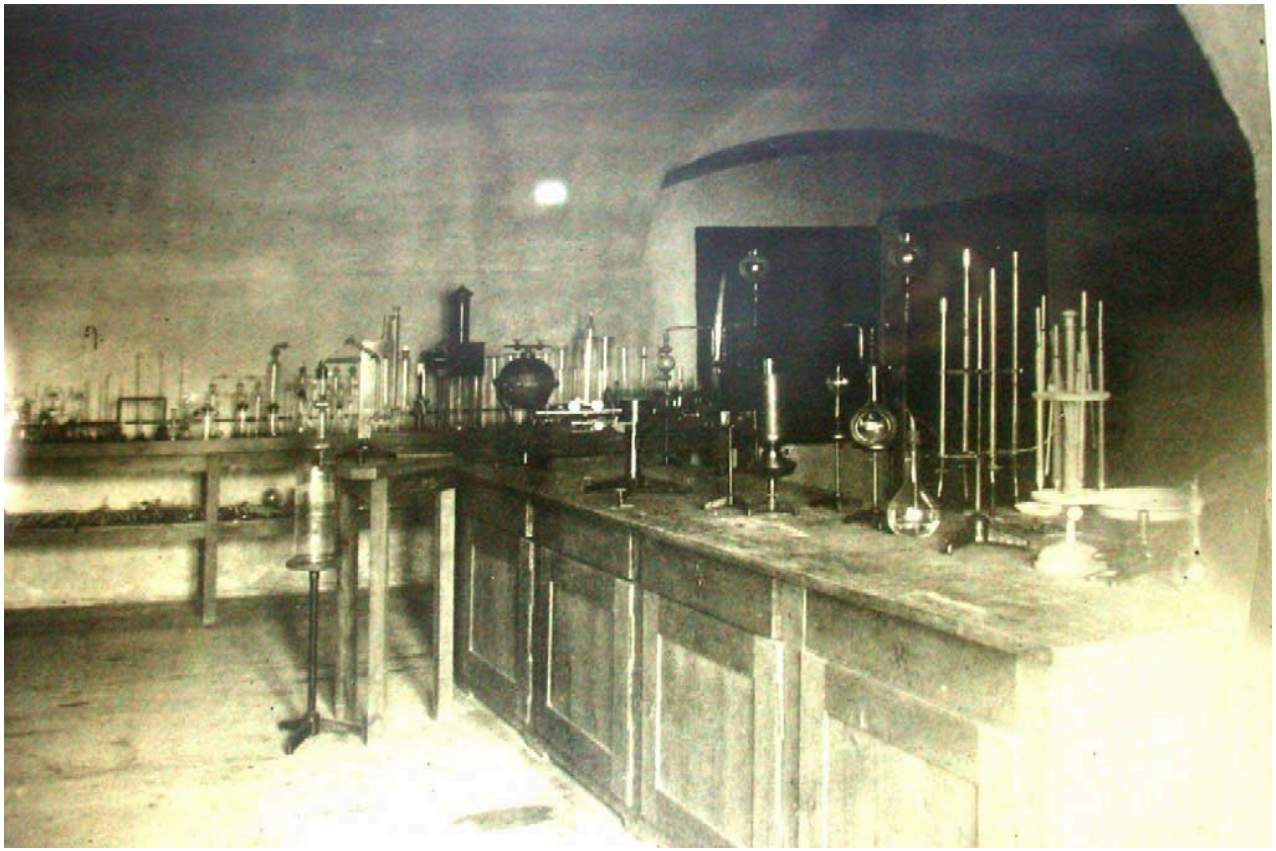
F.M. se pak vrátil do Baselu, kde nalezl **vhodnější materiál k izolaci nukleinu v hlavičkách spermií lososa** - z nich **nuklein o vysoké m.v.** a zásaditý materiál bílkovinné povahy, který nazval **protamin**; obsah P v nukleinu 9,59 %.

Purinové base (**A,G**) objevili **Piccard a Kossel (1874-85) U 1885, Altman** nazval nuklein poprvé **nukleová kyselina**, NK (**nukleinsäure**) (1889); **koncem 19. století** identifikován **T** a vzápětí **C**.

Kolem roku **1930** již známy **DNA** (thymus) a **RNA** (kvasnice) i jejich základní složení. Ve čtyřicátých letech - **DNA v jádře**, **RNA v cytoplazmě a jádře**.

F. Miescher **W. His** **F. Hoppe-Seyler** **A. STRECKER**

Fig. 1. Friedrich Miescher and his mentors. (A) Friedrich Miescher (1844–1895) as a young man. (B) Wilhelm His (1831–1904), Miescher's uncle. His still is famous for his work on the fate of cells and tissues during embryonic development and for his insights into neuroembryology. He, for example, discovered neuroblasts and coined the term bdendriteQ (Finger, 1994; Shepherd, 1991). (C) Felix Hoppe-Seyler (1825–1895), one of the pioneers of physiological chemistry (now biochemistry). Hoppe-Seyler performed seminal work on the properties of proteins, most notably hemoglobin (which he named), introduced the term bproteidQ (which later became bproteinQ), and worked extensively on fermentation and oxidation processes as well as lipid metabolism (Perutz, 1995). He was instrumental in founding Germany's first independent institute for physiological chemistry (in 1884) and in 1877 founded and edited the first journal of biochemistry, the Zeitschrift fu¬r Physiologische Chemie, which still exists today as Biological Chemistry. (D) Adolf Strecker (1822–1871), a leading figure in chemistry in the mid-19th century and professor at the University of Tubingen from 1860 to 1870. Among other achievements, he was the first to synthesize aamino acid (alanine from acetaldehyde via its condensation product with ammonia and hydrogen cyanide) in a reaction known today as Strecker synthesis (Strecker, 1850). (E) Carl Ludwig (1816–1895), a protagonist in the field of physiology in the second half of the 19th century. His focus was the physiology of the nervous system and its sensory organs. In 1869, he founded Leipzig's Physiological Institute.

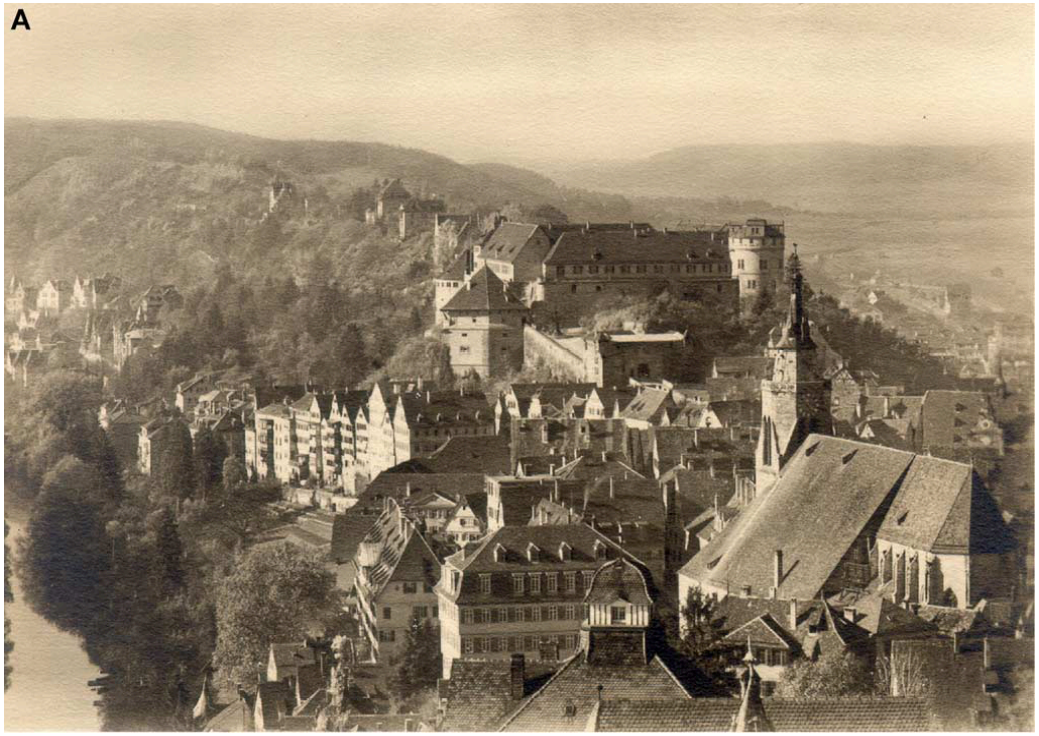


Hoppe-Seyler's laboratory around 1879

Fig. 2. Photograph of Felix Hoppe-Seyler's laboratory around 1879. Prior to becoming the chemical laboratory of Tübingen University in 1823, this room was Tübingen castle's laundry. Here, Hoppe-Seyler had made ground-breaking discoveries regarding the properties of hemoglobin. This achievement was a significant step for later investigations into the properties and functions of this and other proteins. Photography by Paul Sinner, Tübingen.

F. Miescher's laboratory

Fig. 4. The laboratory in the former kitchen of the castle in Tübingen as it was in 1879. It was in this room that Miescher had discovered DNA 10 years earlier. The equipment and fixtures available to Miescher at the time would have been very similar, with a large distillation apparatus in the far corner of the room to produce distilled water and several smaller utensils, such as glass alembics and a glass distillation column on the side board. Photography by Paul Sinner, Tübingen.



Text

Tübingen castle

A, in Miescher's time

B, at present

FIRST PROTOCOL

Before attempting the isolation of cells from the pus on surgical bandages, Miescher took great care to ensure that his source material was fresh and not contaminated. He painstakingly examined it and discarded everything that showed signs of decomposition, either in terms of smell, appearance under the microscope, or by having turned acidic. A great deal of the material he could obtain did not meet these strict requirements (Miescher, 1871d). Those samples that did were subsequently used to isolate leucocytes.

In a first step, Miescher separated the leucocytes from the bandaging material and the serum (Miescher, 1869a, 1871d). This separation posed a problem for Miescher. Solutions of NaCl or a variety of alkaline or alkaline earth salt solutions used to wash the pus resulted in a "slimy swelling" of the cells, which was impossible to process further (His, 1897b). (This "slimy swelling" of the cells was presumably due to high-molecular-weight DNA, which had been extracted from cells that had been damaged.) Only when Miescher tried a dilute solution of sodium sulfate [a mixture of one part cold saturated Glauber's salt (Na2SO4d 10 H2O) solution and nine parts water] to wash the bandages did he manage to successfully isolate distinct leucocytes, which could be filtered out through a sheet to remove the cotton fibers of the bandaging. Miescher subsequently let the washing solution stand for 1–2 h to allow the cells to sediment and inspected the leucocytes microscopically to confirm that they did not show any signs of damage.

Having isolated the cells, Miescher next had to separate the nuclei from the cytoplasm. This had never been achieved before and Miescher had to develop new protocols. He washed the cells by rinsing them several (6–10) times with fresh solutions of diluted (1:1000) hydrochloric acid over a period of several weeks at "wintry temperatures" (which were important to avoid degradation). This procedure removed most of the cells' bprotoplasm,Q leaving behind the nuclei. The residue from this treatment consisted in part of isolated nuclei and of nuclei with only little fragments of cytoplasm left attached. Miescher showed that these nuclei could no longer be stained yellow by iodine solutions, a method commonly used at the time for detecting cytoplasm (Arnold, 1898; Kiernan, 2001).

He then vigorously shook the nuclei for an extended period of time with a mixture of water and ether. This caused the lipids to dissolve in the ether while those nuclei, still attached to cytoplasm, collected at the water/ether interface. By contrast, the clean nuclei without contaminating cytoplasm were retained in the water phase. Miescher filtered these nuclei and examined them under a microscope. He noticed that in this way he could obtain completely pure nuclei with a smooth contour, homogeneous content, sharply defined nucleolus, somewhat smaller in comparison to their original volumes (Miescher, 1871d).

Miescher subsequently extracted the isolated nuclei with alkaline solutions. When adding highly diluted (1:100,000) sodium carbonate to the nuclei, he noticed that they would swell significantly and become translucent. Miescher then isolated a yellow solution of a substance from these nuclei. By adding acetic acid or hydrochloric acid in excess, he could obtain an insoluble, flocculent precipitate (DNA). Miescher noted that he could dissolve the precipitate again by adding alkaline solutions.

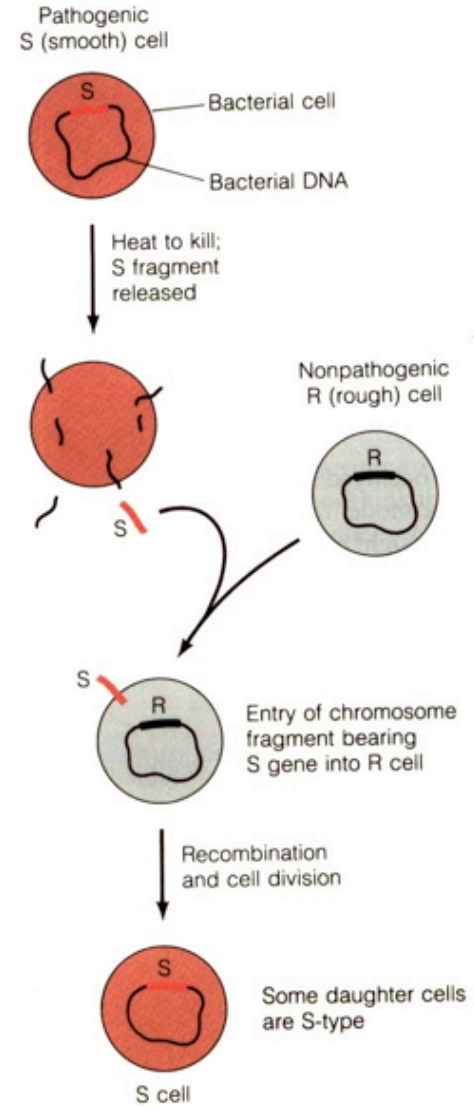
Although this protocol allowed Miescher for the first time to isolate nuclein in appreciable purity and quantities, it was still too little and not pure enough for his subsequent analyses. He consequently improved on this protocol until he established the protocol detailed in Box 2, which enabled him to purify sufficient amounts of nuclein for his first set of experiments on its elementary composition.

M. SECOND PROTOCOL TO ISOLATE DNA

A key concern of Miescher's was to get rid of contaminating proteins, which would have skewed his analyses of the novel substance. "I therefore turned to an agent that was already being used in chemistry with albumin molecules on account of its strong protein-dissolving action, namely, **pepsin** solutions (Miescher, 1871d). Pepsin is a proteolytic enzyme present in the stomach for digesting proteins. Miescher used it to separate the DNA from the proteins of the cells' cytoplasm. He extracted the pepsin for his experiments from pig stomachs by washing the stomachs with a mixture of 10 cc of fuming hydrochloric acid and one liter of water and filtering the resulting solution until it was clear. In contrast to his earlier protocol, Miescher first washed the pus cells (leucocytes) three or four times with warm alcohol to remove lipids. He then let the residual material digest with the pepsin solution between 18 and 24 h at 37–45 C. After only a few hours, a fine gray powdery sediment of isolated nuclei separated from a yellow liquid. Miescher continued the digestion process, changing the pepsin solution twice. After this procedure, a precipitate of nuclei without any attached cytoplasm formed. He shook the sediment several times with ether in order to remove the remaining lipids. Afterwards, he filtered the nuclei and washed them with water until there was no longer any trace of proteins. He described the nuclei isolated in this way as naked. The contours were smooth in some cases or slightly eaten away in others (Miescher, 1871d). Miescher washed the nuclei again several times with warm alcohol and noted that the nuclear mass cleaned in this way exhibited the same chemical behavior as the nuclei isolated with hydrochloric acid. Miescher subsequently extracted the isolated nuclei using the same alkaline extraction protocol he had previously employed on the intact cells (see Box 1) and, when adding an excess of acetic acid or hydrochloric acid to the solution, again obtained a precipitate of nuclein.

Fig. 5. Glass vial containing nuclein isolated from salmon sperm by Friedrich Miescher while working at the University of Basel. The faded label reads Nuclein aus Lachssperma, F. Miescher (Nuclein from salmon sperm, F. Miescher). Possession of the Interfakult-res Institut fqr Biochemie (Interfacultary Institute for Biochemistry), University of Tubingen, Germany; photography by Alfons Renz, University of Tubingen.

Fig. 6. This picture of Friedrich Miescher in his later years is the frontispiece on the inside cover of the two volume collection of Miescher's scientific publications, his letters, lecture manuscripts, and papers published posthumously by Wilhelm His and others (His et al., 1897a,b).



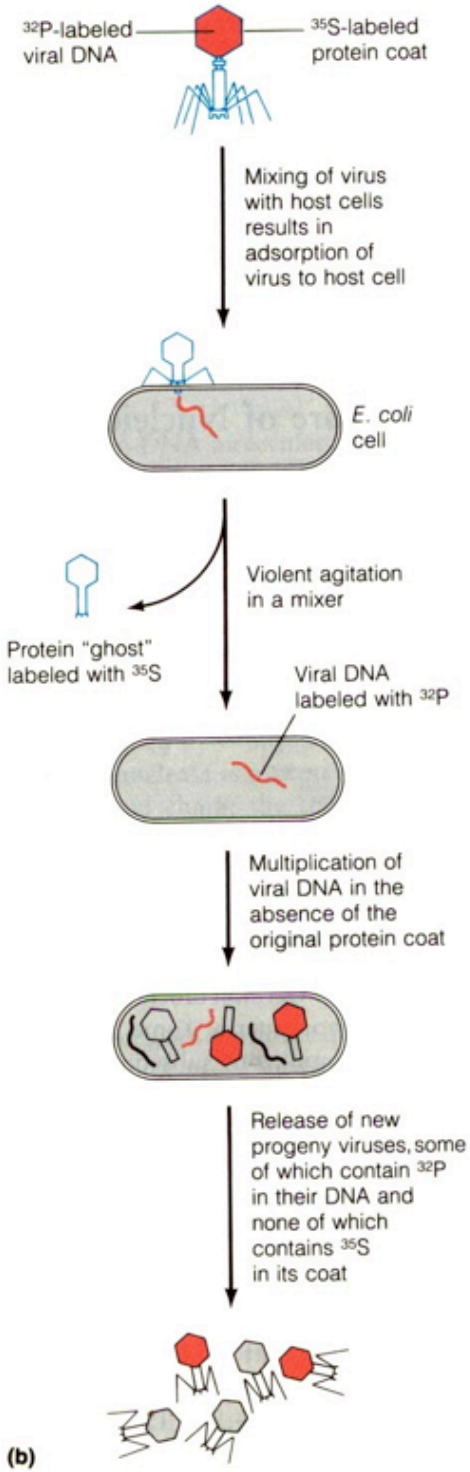


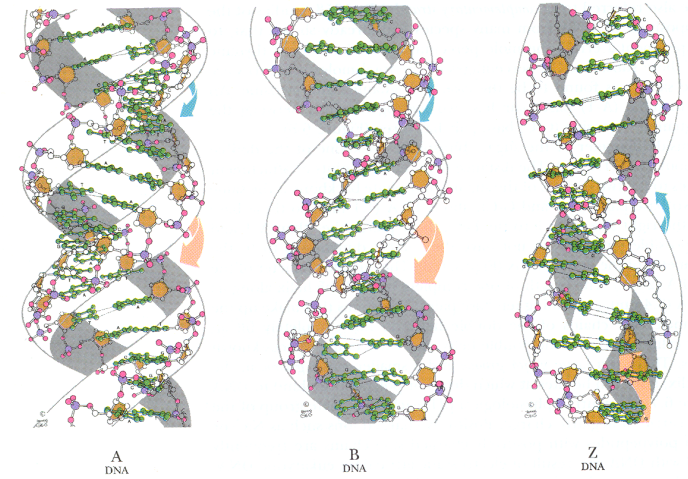
Figure 4.8
Crucial experiments that demonstrated DNA as the genetic substance. (a) The experiment of Avery et al. showing that nonpathogenic pneumococci could be made pathogenic by transfer of DNA from a pathogenic strain. (b) The experiment of Hershey and Chase showing that it is transfer of the DNA from a bacteriophage to a bacterium that gives rise to new bacteriophages.

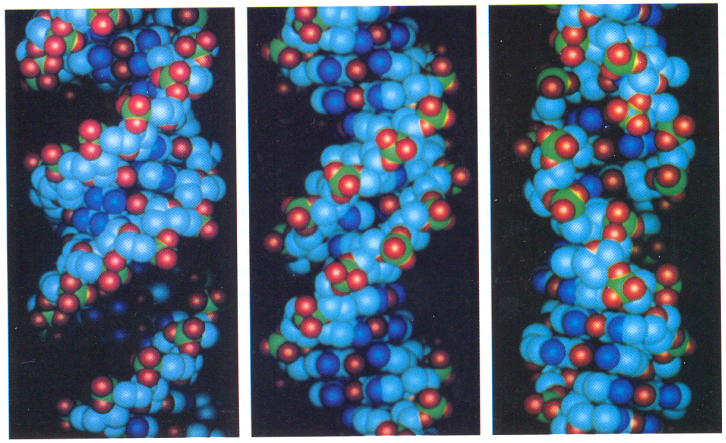
(a) 1944: Oswald T. **Avery**, Colin **MacLeod**, and Maclyn **McCarty** demonstrate that Griffith's **transforming principle is not a protein, but** rather **DNA**, suggesting that DNA may function as the genetic material

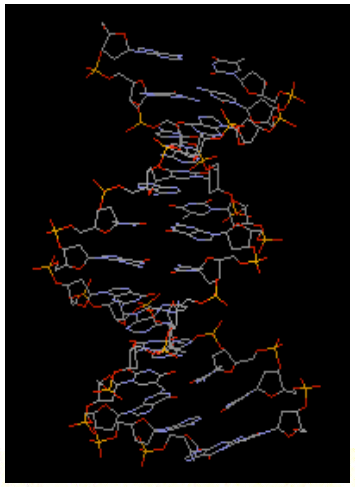
(b) 1952: Alfred **Hershey** and Martha **Chase** use viruses (bacteriophage T2) to confirm DNA as the genetic material by demonstrating that during infection viral DNA enters the bacteria while the viral proteins do not and that this DNA can be found in progeny virus particles.

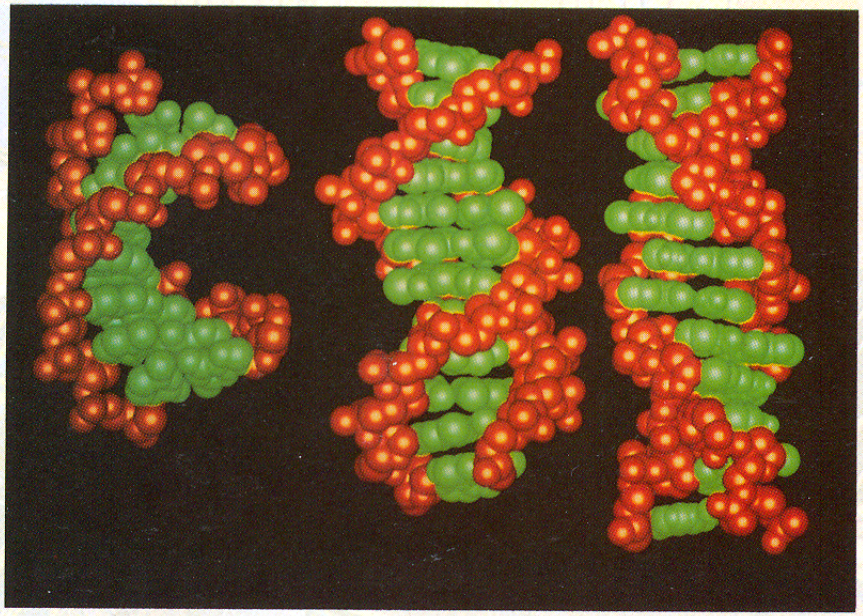
A, B and left-handed Z-DNA as we know them now

How did we arrive to them ?









Double helical conformations of DNA: (left) *A-DNA*, (center) *B-DNA*, (right) *Z-DNA*.

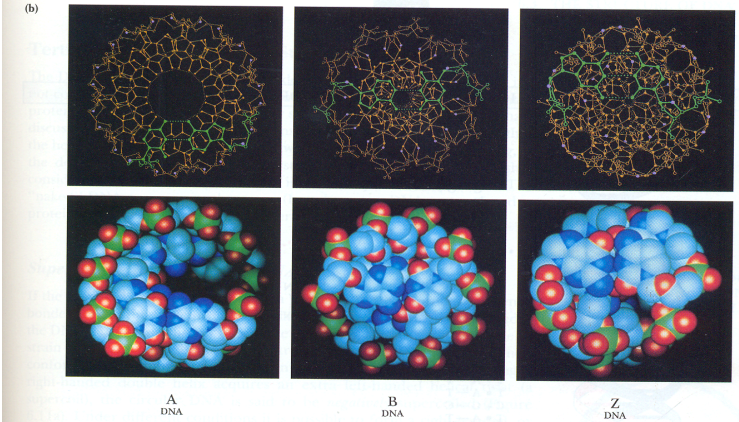
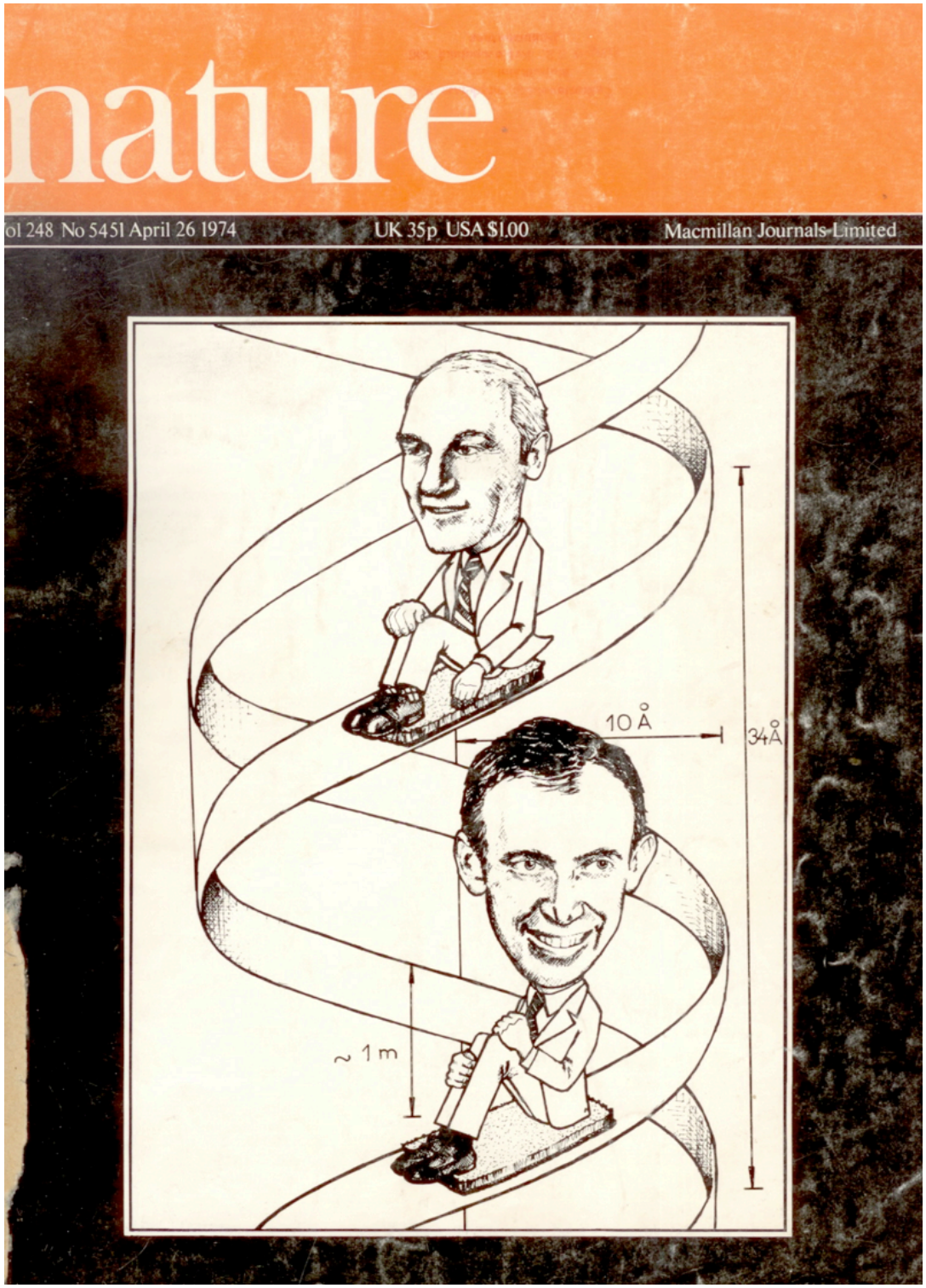


FIGURE 6.9
A comparison of the A, B, and Z forms of DNA. Part (a) shows side views of all



21st Anniversary:

The DNA Double Helix Comes of Age

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

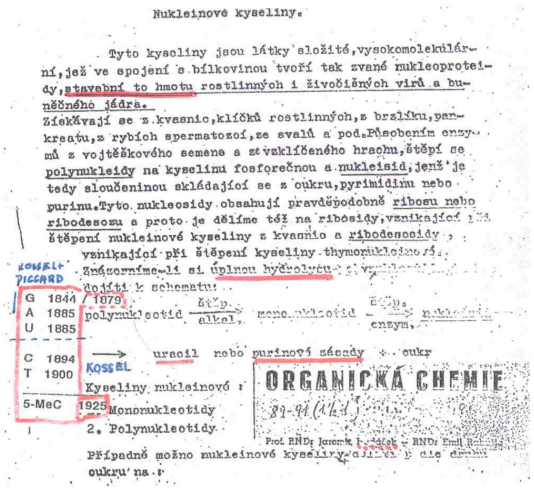
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

1953


Nukleinové kyseliny.

Tyto kyseliny jsou látky složité, vysokomolekulární, jež ve spojení s bílkovinou tvoří tak zvané nukleoproteidy, stavební to hmotu rostlinných i živočišných virů a buněčného jádra.
Získávají se z kvasnic, klíčků rostlinných, z brzlíku, pankreatu, z rybích spermatozoí, ze svalů a pod. Působením enzymů z vojtěškového semene a ze vyklíčeného hrachu, štěpí se polynukleidy na kyselinu fosforečnou a nukleisid, jenž je tedy sloučeninou skládající se z cukru, pyrimidinu nebo purinu. Tyto nukleosidy obsahují pravděpodobně ribosu nebo ribodesosu a proto je dělíme též na ribosidy, vznikající
Štěpení nukleinové kyseliny z kvasnic a ribodesosidy
vznikající při štěpení kyseliny thymonukleové.
Zaměříme-li si úplnou hydrolysu
dojíti k schematu:
G 1844 / 1879
A 1885 polynukleotid — štěp. alkal. — mono nukleotid — enzym.
U 1885
C 1894 → uracil nebo purinové zásady + cukr
T 1900
Kyseliny nukleinové
5-MeC 1925 Mononukleotidy
2. Polynukleotidy
Případně možno nukleinové kyseliny
cukru na

KOSSEL
PICCARD
KOSSEL
ORGANICKÁ CHEMIE

A paragraph dealing with nucleic acids from a text book of Organic Chemistry (in Czech) is shown. Briefly, it says nucleic acids (NA's) form complexes with proteins which are the building blocks of plant and animal viruses and of cell nucleus. Total hydrolysis of NA's proceeds according to the following scheme:

alkaline hydrolysis enzym. digestion

Polynucleotide → mononucleotide → uracil or purine bases

Considering that uracil and adenine were discovered in 1885 and G in 1844 while C in 1894 and T in 1900, our lectures on NA's were up-todate in 1885 but not in 1894

In courses of **Marxism-Leninism** (obligatory to all students)
we were tought that **G. Mendel was a burgeous reactionary pseudoscientist**.
Interestingly there was **not a single chemist** among us **who believed it**.
To my surprise there were some biologists who took this nonsenses seriously

Chargaff's Rules

Tetranucleotide hypothesis originated in 1906: DNA is a "statistical tetranucleotide".
During the 1950´s E. Chargaff showed a number of DNAs, which differ in their base content.
Chargaff´s rules: 1. 6-amino residues = 6-keto-residues; in another expression A+C = G+T;
2. py = pu; C+T = G+A 3. A/T = G/C = 1 (consequence of combining equations 1 and 2)

Watson and Crick (1953) proposed their famous double-helical structure of B-form of DNA on the ground of Chargaff´s rules

- X-ray diffraction of DNA fibers obtained by Maurice Wilkins and Rosalind Franklin
- Construction of molecular models

This structure consists of two antiparallel helical strands. One turn contains 10 residues in every strand, the distance between bases is 3.4 A, the bases are almost perpendicular to the axis, the phosphate group is 9 A from the axis. Bases are specifically paired through hydrogen bonds – AT and GC. The strands are complementary – hydrogen bonds between two strands, the bases are inside the structure. Difference from α-helix in polypeptides. Further forms A and C (besides B): dependence on humidity. The differences are principally in the tilt of bases and in the number of residues per turn, strands are commonly antiparallel, bases are stacked and base pairs located in one plane. It seems that the B-form is the prevalent one in solution as well as in cells and viral particles.

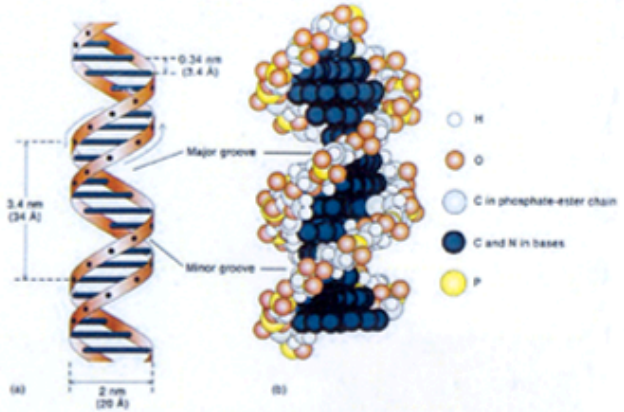
Crick, Watson and Wilkins: Nobel Prize 1962

"The structure is produced like a rabbit out of a hat, with no indication as to how we arrived at it"
F. Crick, NATURE 248(1974) 766- on the occasion of the 21st anniversary of the discovery (commenting their first paper in NATURE). What experimental evidence was available to W+C in 1953?

X-RAY FIBER ANALYSIS OF DNA

represented the main evidence for the Watson-Crick double helix model

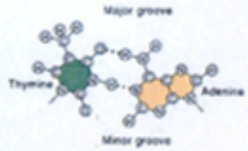
This method enabled analysis of high-molecular DNA, but provided only few basic parameters of the helix, such as



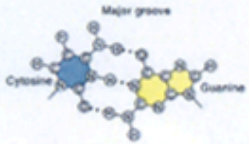
distance between base pairs

number of base residues per turn

Further data were derived from model building considering the laws of structural chemistry



Base pairing from physical-chemical measurements



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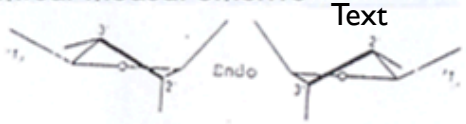
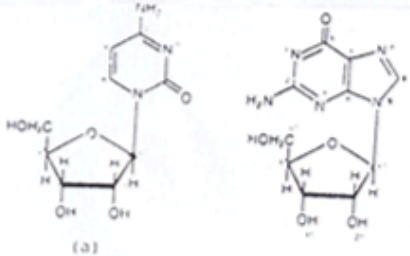
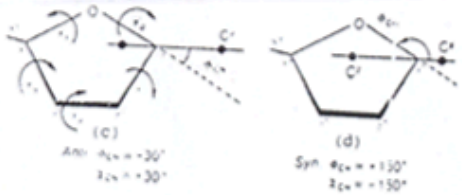


Sugar configuration (PUCKER)

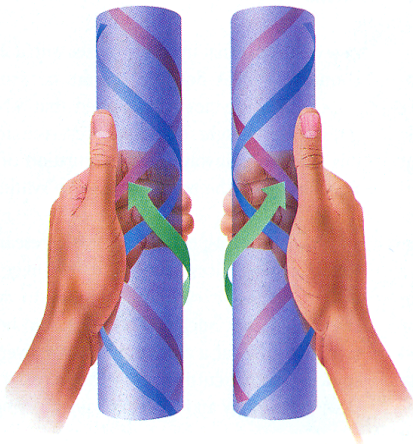


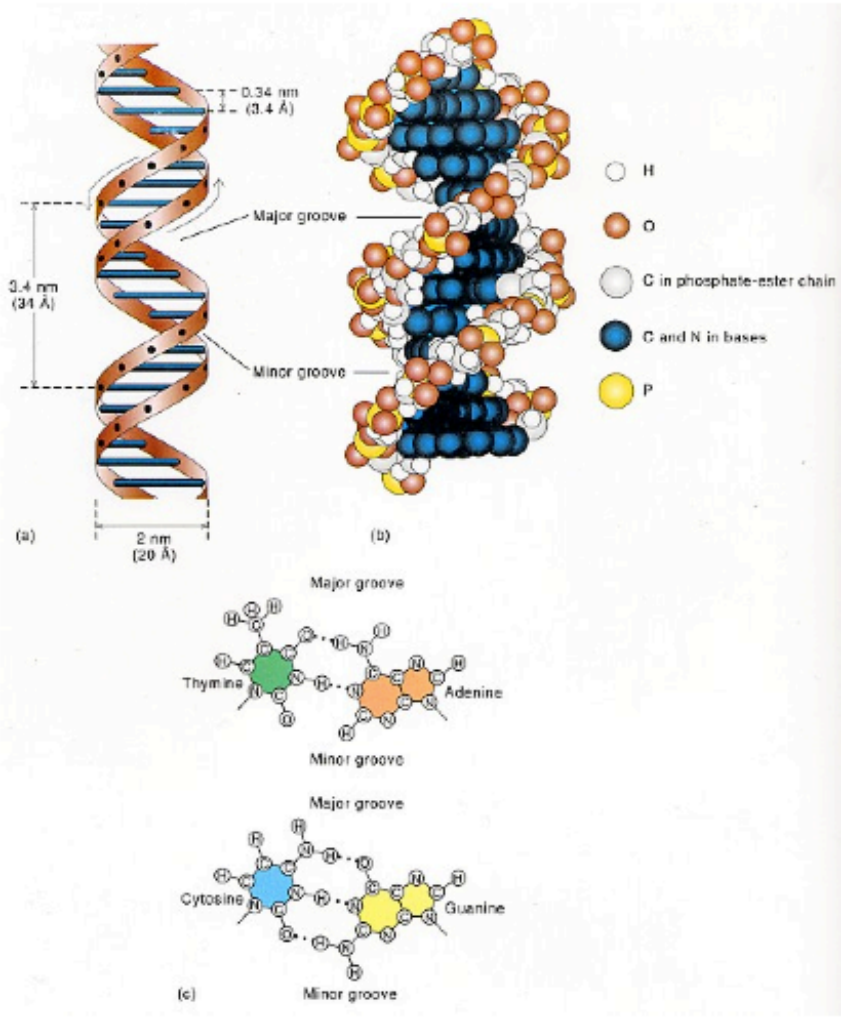
Angles of the glycosidic bonds were fixed within certain limits

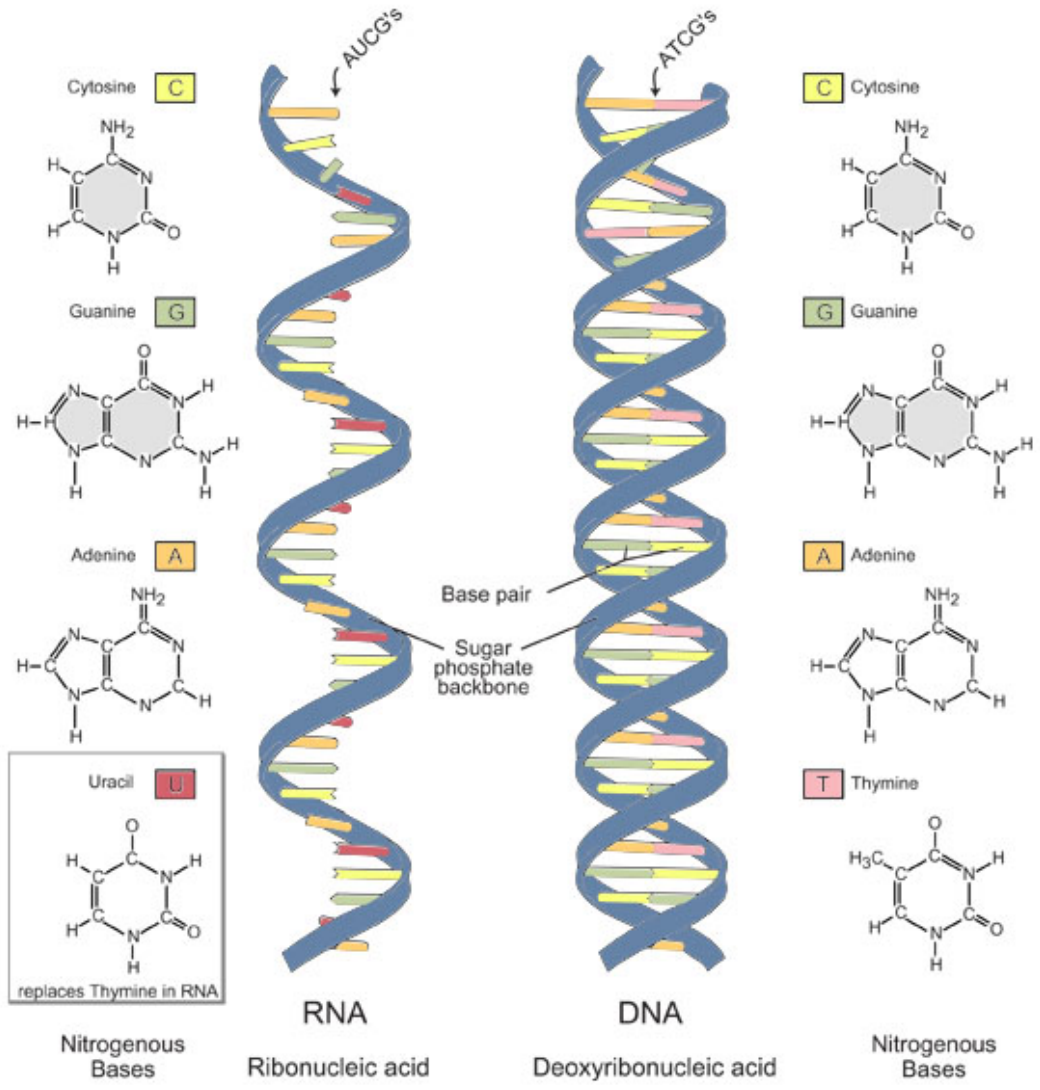


Handedness of the helix

The direction of rotation was guessed and then subjected to testing







DNA is a polyanionic biomacromolecule with bases in its interior and sugar-phosphate backbone on the surface. At neutral pH it carries one negative charge per nucleotide. Below pH 5 and and above pH 9 ionization of bases become important

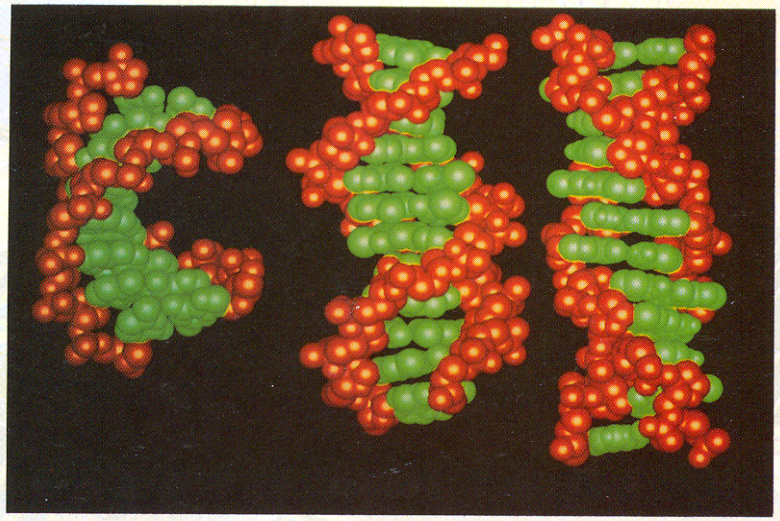
Parameters of DNA structures

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

Helix sense	A-DNA[a] right-handed	B-DNA[a] right-handed	B′-DNA[b] right-handed	Z-DNA[c] left-hande
Base pairs per turn	11	10	10	12 (6 dime
Helix twist (°)	32.7	36.0	34.1, 36.8	−10, −50
Rise per base pair (Å)	2.9	3.4	3.5, 3.3	3.7
Helix pitch (Å)	32	34	34	45
Base pair tilt (°)	13	0	0	−7
P distance from helix axis (Å)	9.5	9.3	9.1	6.9, 8.0
Glycosidic orientation	*anti*	*anti*	*anti*	*anti, syn*
Sugar conformation	C3′-*endo*	Wide range	C2′-*endo*	C2′-*endo*, C3 *endo*[d]

[a] Numerical values for each form were obtained by averaging the global parameters of corresponding double-helix fragments.
[b] B′-DNA values are for a double helix backbone conformation alternating between confor tional states I and II.
[c] The two values given correspond to CpG and GpC steps for the twist and P distance valu to cytosine and guanosine for the others.
[d] Two values correspond to the two conformational states. From Kennard, O. and Hunter, W. *Q. Rev. Biophys.*, 22, 3427, 1989. With permission.

A B Z



Double helical conformations of DNA: (left) *A-DNA*, (center) *B-DNA*, (right) *Z-DNA*.

DNA structures from X-ray crystal analysis

DNA double helix is polymorphic depending on the nucleotide sequence

TABLE 2
Average Helical Parameters for Selected Right-Handed Structures

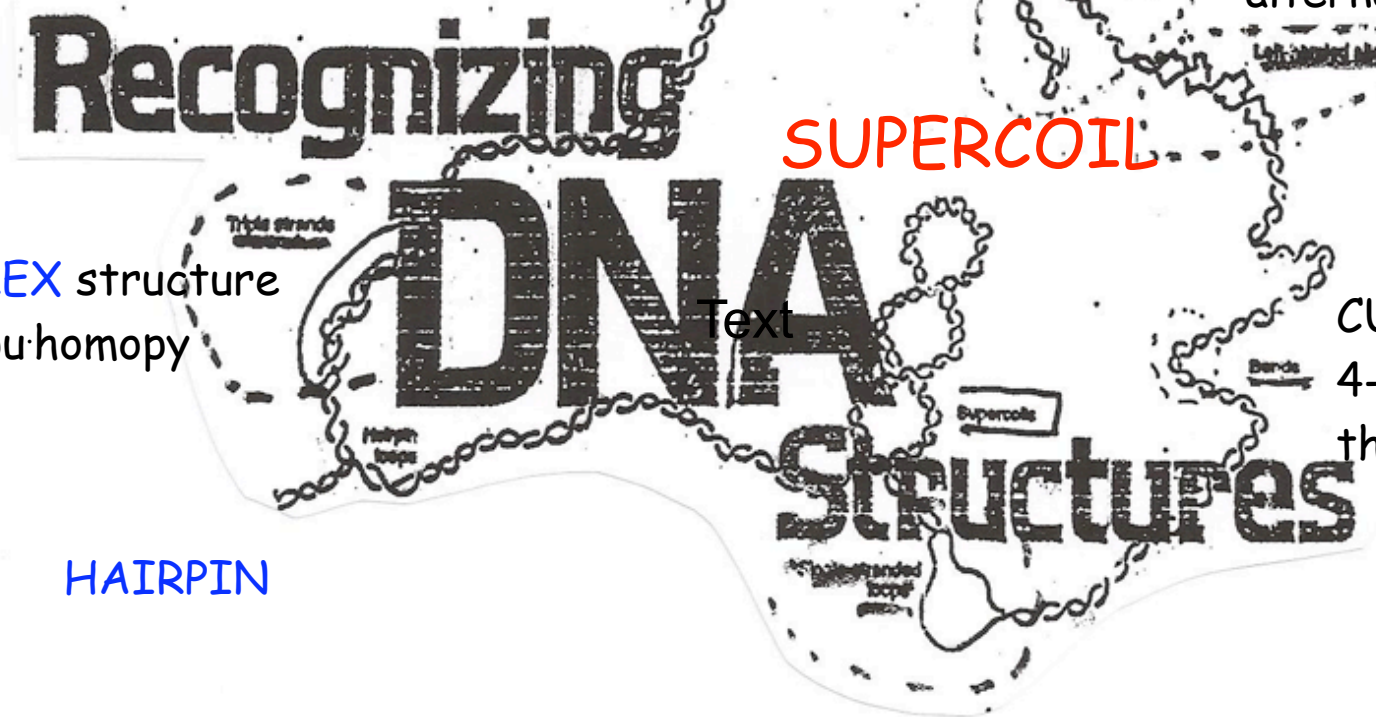
	Helix twist (°)	Rise per base pair (Å)	Base pair tilt (°)	Propeller twist (°)	Groove width (Å) Minor	Groove width (Å) Major	Displacement Da (Å)
A-form							
d(GGTATACC)	32	2.9	13	10	10.2	6.3	4.0
d(GGGCGCCC)	32	3.3	7	12	9.5	10.1	3.7
d(CTCTAGAG)	32	3.1	10	11	8.7	8.0	3.6
r(GCG)d(TATACGC)	33	2.5	19	12	10.2	3.2	4.5
r(UUAUAUAUAUAUAA)	33	2.8	17	19	10.2	3.7	3.6
Fiber A-DNA	33	2.6	22	6	11.0	2.4	4.4
B-form							
d(CGCGAATTCGCG)	36	3.3	2	13	5.3	11.7	−0.2
d(CGCGAATTBrCGCG)	36	3.4	−2	18	4.6	12.2	−0.2
Fiber B-DNA	36	3.4	2	13	6.0	11.4	−0.6

BrC = 5-bronecytosimo.
Adapted from Kennard, O. and Hunter, W. N., *Q. Rev. Biophys.*, 22, 327, 1989. With permission.

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.

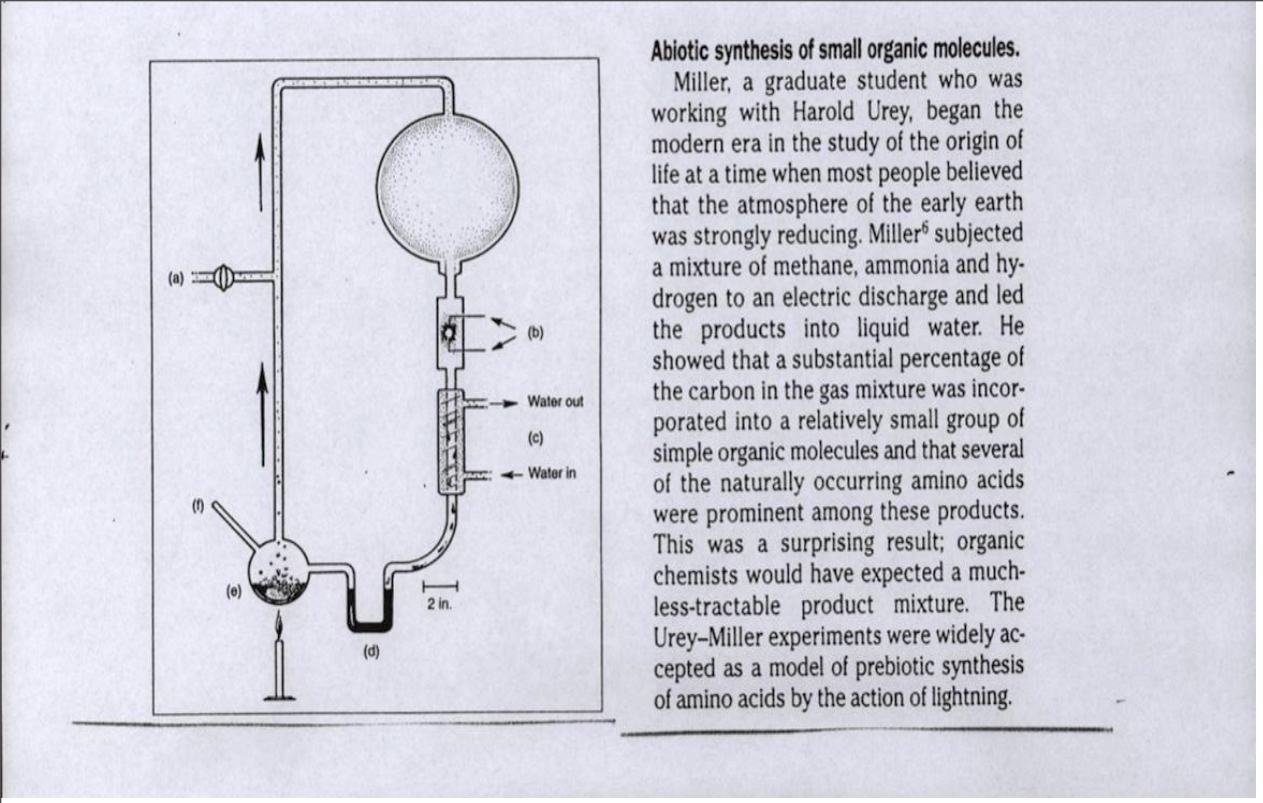
Mám v živé paměti přednášku, kterou přednesl před mnoha lety v Liblicích Harold Urey o vzniku aminokyselin v laboratorních podmínkách, napodobujících podmínky předpokládané na Zemi v době, kdy pravděpodobně vznikl život. Přednáška byla jednoduchá a elegantní a dávala tušit, že během několika málo desetiletí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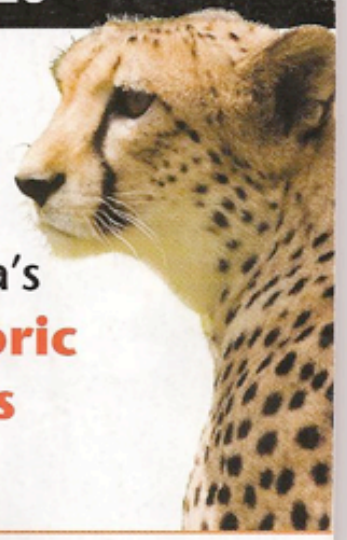


Did this molecule start life?

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 lareate 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

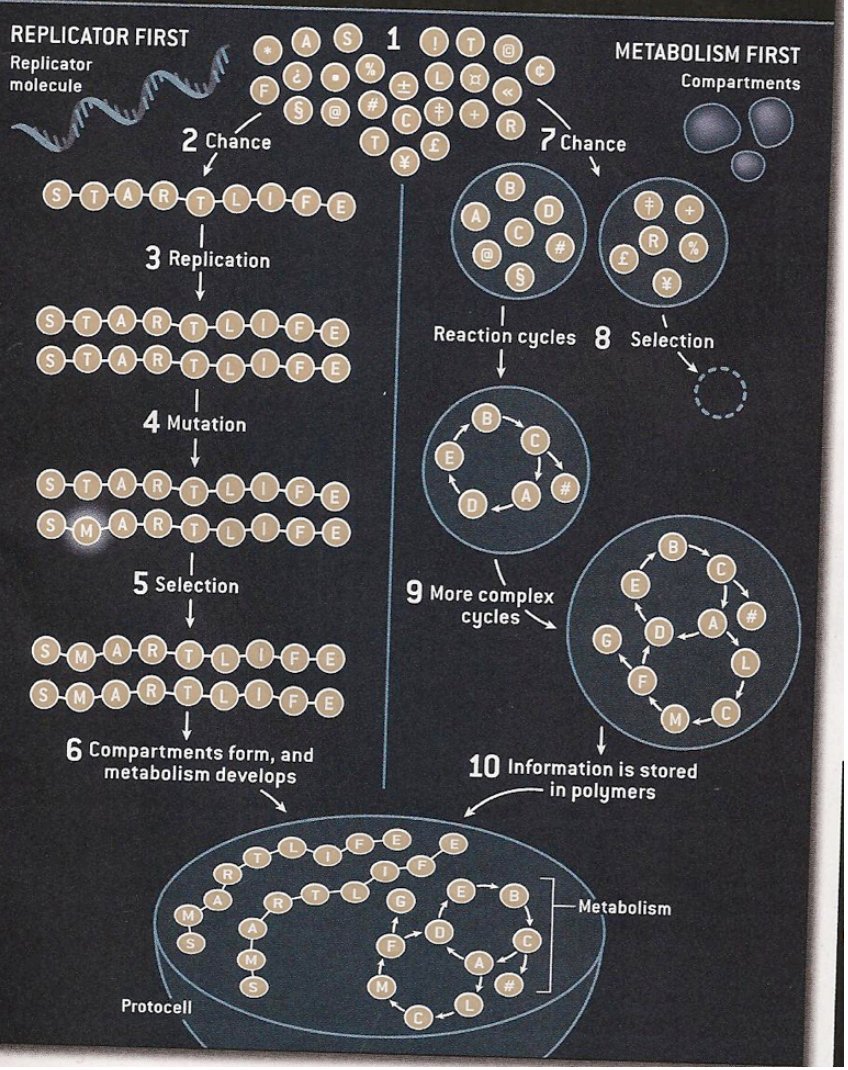
- Theories of how life first originated from nonliving matter fall into two broad classes—replicator first, in which a large molecule capable of replicating (such as RNA) formed by chance, and metabolism first, in which small molecules formed an evolving network of reactions driven by an energy source.
- 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.

REPLICATOR VS. METABOLISM

Scientific theories of the origin of life largely fall into two rival camps: replicator first and metabolism first. Both models must start from molecules formed by nonbiological chemical processes, represented here by balls labeled with symbols (*1*).

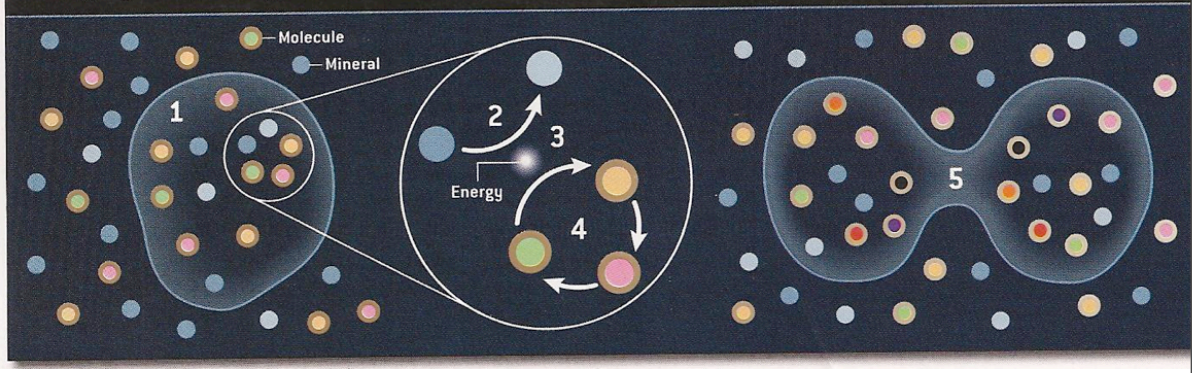
In the replicator-first model, some of these compounds join together in a chain, by chance forming a molecule—perhaps some kind of RNA—capable of reproducing itself (*2*). The molecule makes many copies of itself (*3*), sometimes forming mutant versions that are also capable of replicating (*4*). Mutant replicators that are better adapted to the conditions supplant earlier versions (*5*). Eventually this evolutionary process must lead to the development of compartments (like cells) and metabolism, in which smaller molecules use energy to perform useful processes (*6*).

Metabolism first starts off with the spontaneous formation of compartments (*7*). Some compartments contain mixtures of the starting compounds that undergo cycles of reactions (*8*), which over time become more complicated (*9*). Finally, the system must make the leap to storing information in polymers (*10*).

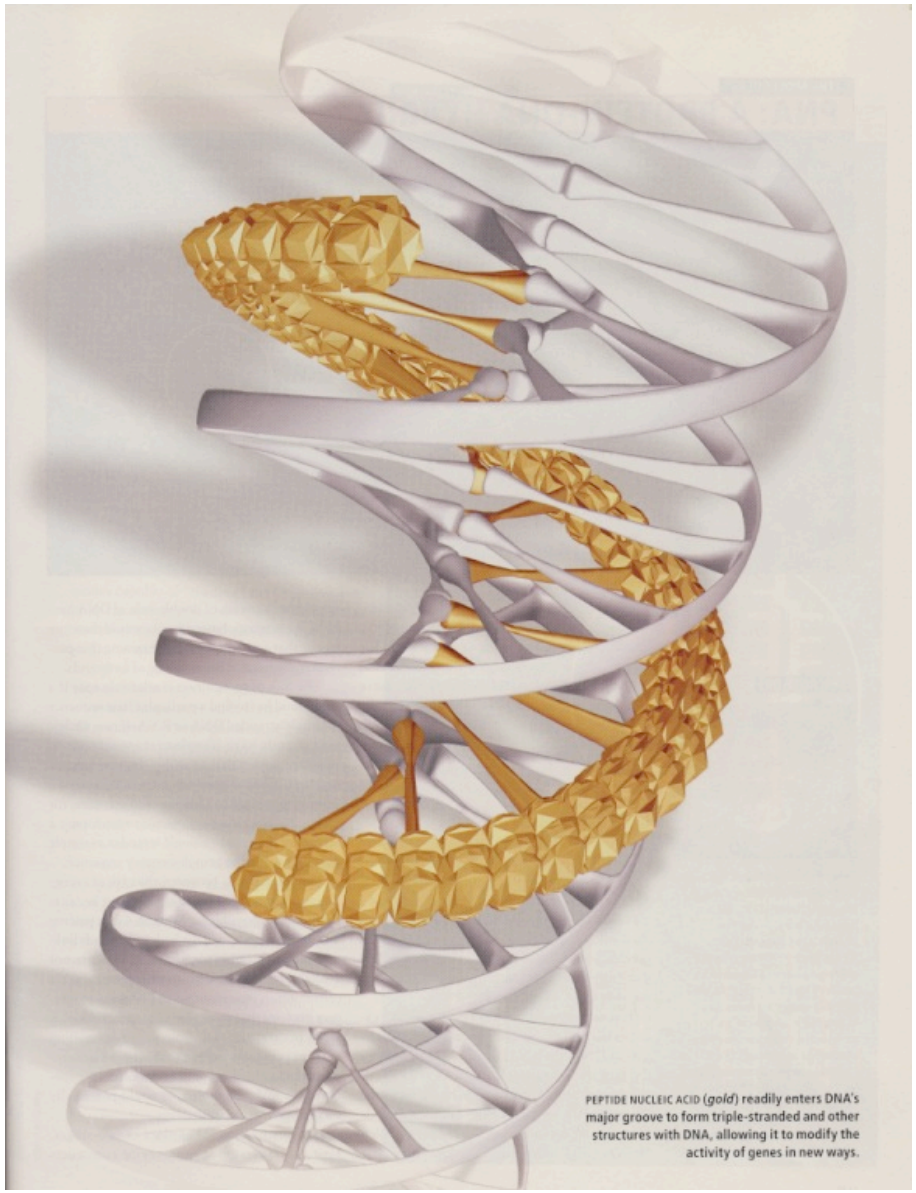


FIVE REQUIREMENTS FOR METABOLISM FIRST

At least five processes must occur for small molecules to achieve a kind of life—here defined as the creation of greater order in localized regions by chemical cycles driven by an energy flow. First, something must create a boundary to separate the living region from the nonliving environment (*1*). A source of energy must be available, here depicted as a mineral (*blue*) undergoing a heat-producing reaction (*2*). The released energy must drive a chemical reaction (*3*). A network of chemical reactions must form and increase in complexity to permit adaptation and evolution (*4*). Finally, the network of reactions must draw material into itself faster than it loses material, and the compartments must reproduce (*5*). No information-storing molecule (such as RNA or DNA) is required; heredity is stored in the identity and concentration of the compounds in the network.



Peptide Nucleic Acid (PNA)

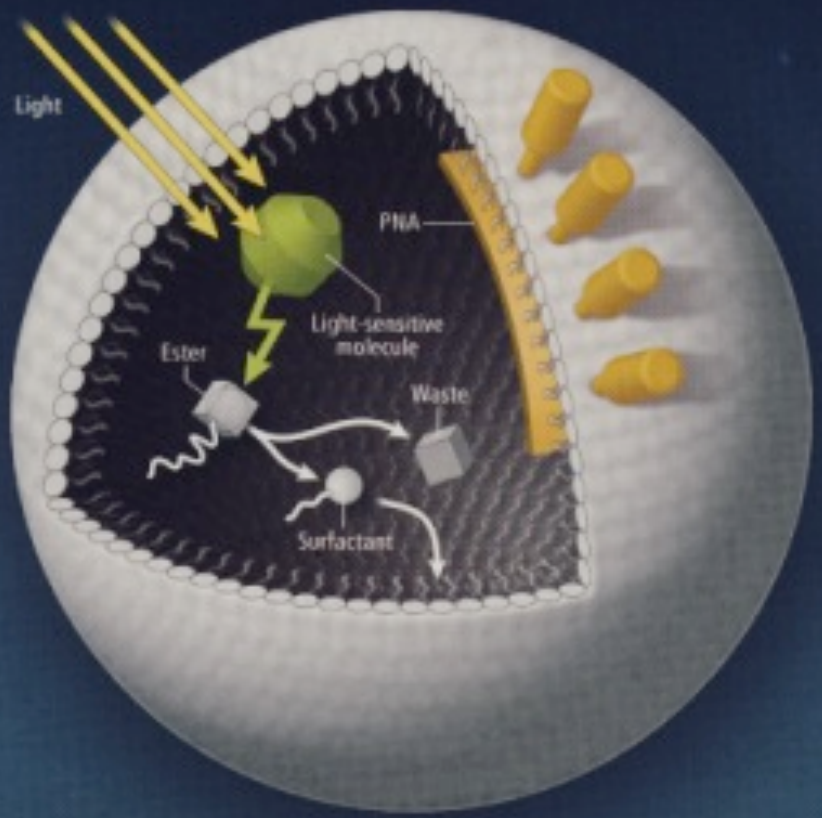


PEPTIDE NUCLEIC ACID (*gold*) readily enters DNA's major groove to form triple-stranded and other structures with DNA, allowing it to modify the activity of genes in new ways.

[ALIEN GENES]

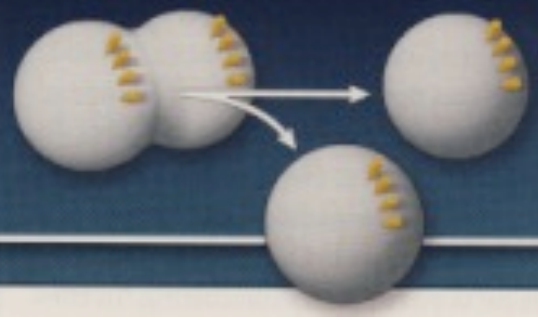
ARTIFICIAL LIFE

Researchers striving to construct new life-forms out of combinations of nonliving chemicals are considering PNA as the genetic (information-carrying) component of their designs because it is simpler and more stable than DNA or RNA. In the proposal shown below, PNA is embedded in the surface of a container that self-assembles out of surfactant molecules. Light-sensitive molecules in the cell power the generation of more surfactant molecules from precursor esters. If the PNA replicates (*top*), the expanding protocell may divide into two similar copies (*bottom*). By self-organizing, metabolizing (exploiting an energy source) and self-replicating, the protocell exhibits some of the hallmarks of life.



The PNA replicates when short, complementary PNA fragments attach to it and it migrates to the protocell's lipid interior, where the fragments join to form a second PNA strand.

When the protocell grows large enough, it becomes unstable and divides in two.



Sci. Amer. Dec. 2008

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

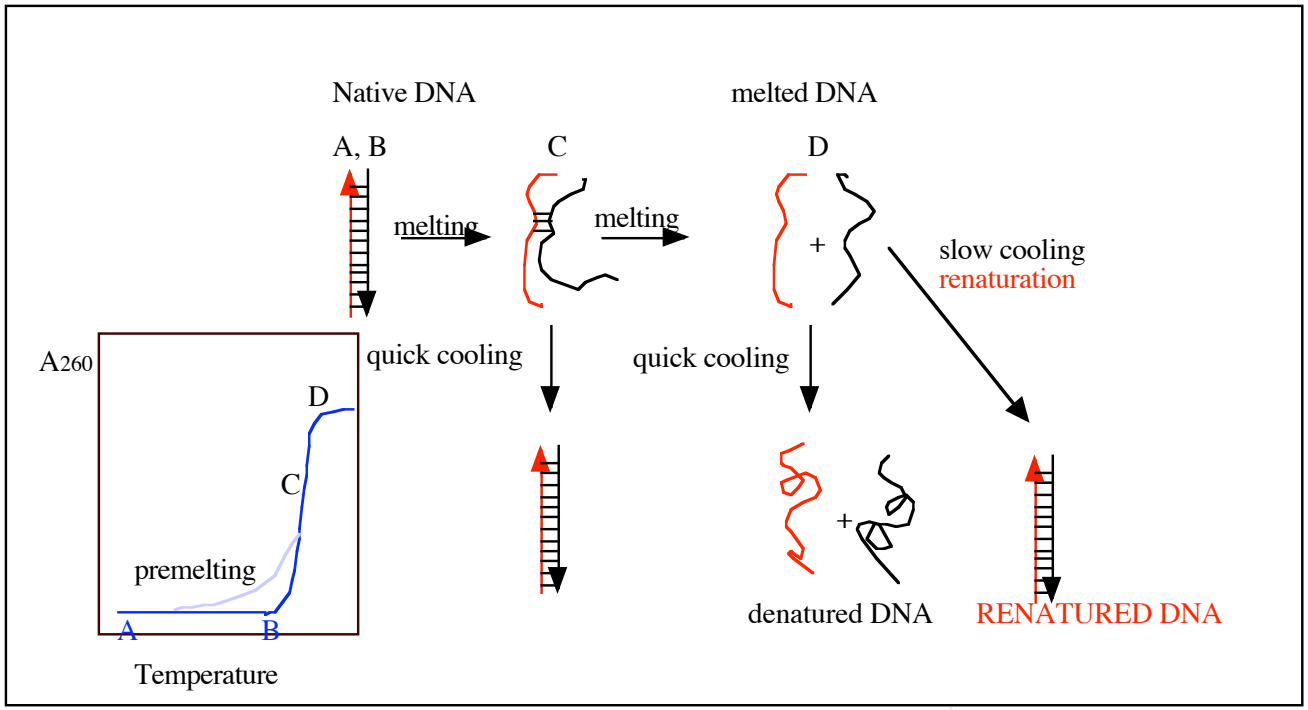
Recent finding of glycine in the comet tail might be considered as support for this alternative

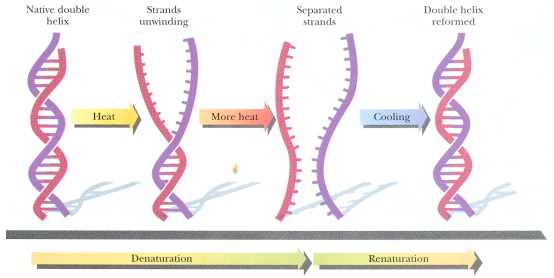
The actual nature of the first organism and the exact circumstances of the origin of life may be forever lost for science.

But research can at least help to understand what is possible

Sci. Amer., September 2009

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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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
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HETEROGENEITY IN DEOXYRIBONUCLEIC ACIDS .1. DEPENDENCE ON COMPOSITION OF THE CONFIGURATIONAL STABILITY OF DEOXYRIBONUCLEIC ACIDS
NATURE 183 (4673): 1427-1429 1959
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Microbiologist, biochemist and molecular biologist

Julius Marmur - dicovered renaturation of DNA

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

Oswald Avery — 1944 - DNA is a genetic material

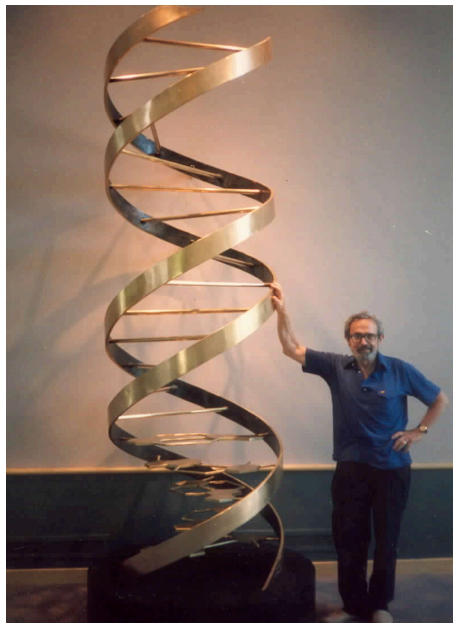
(Rockefeller Institute, New York, NY)

↓

Rollin D. Hotchkiss

↓

Julius Marmur



1993

The double helix: a personal view

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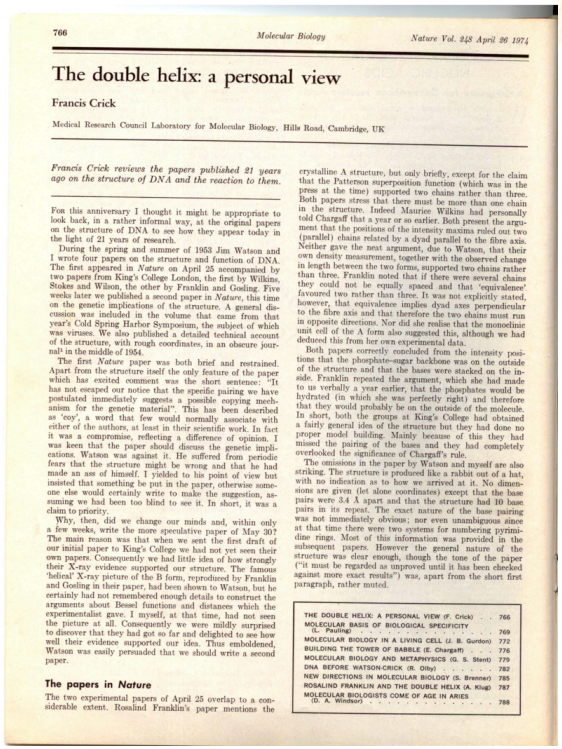

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Francis Crick reviews the papers published 21 years ago on the structure of DNA and the reaction to them.

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

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

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

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

The papers in *Nature*

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

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

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

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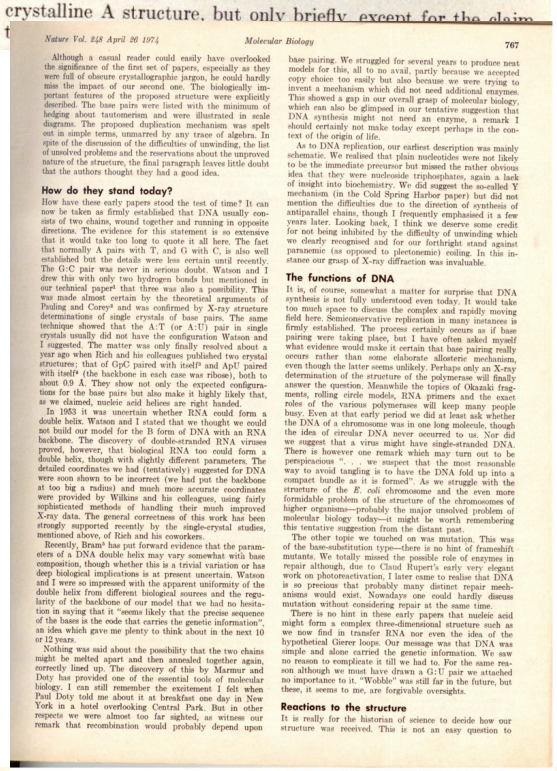

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Although a casual reader could easily have overlooked the significance of the first set of papers, especially as they were full of obscure crystallographic jargon, he could hardly miss the impact of our second one. The biologically important features 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, Brahn[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 cloverleaf 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

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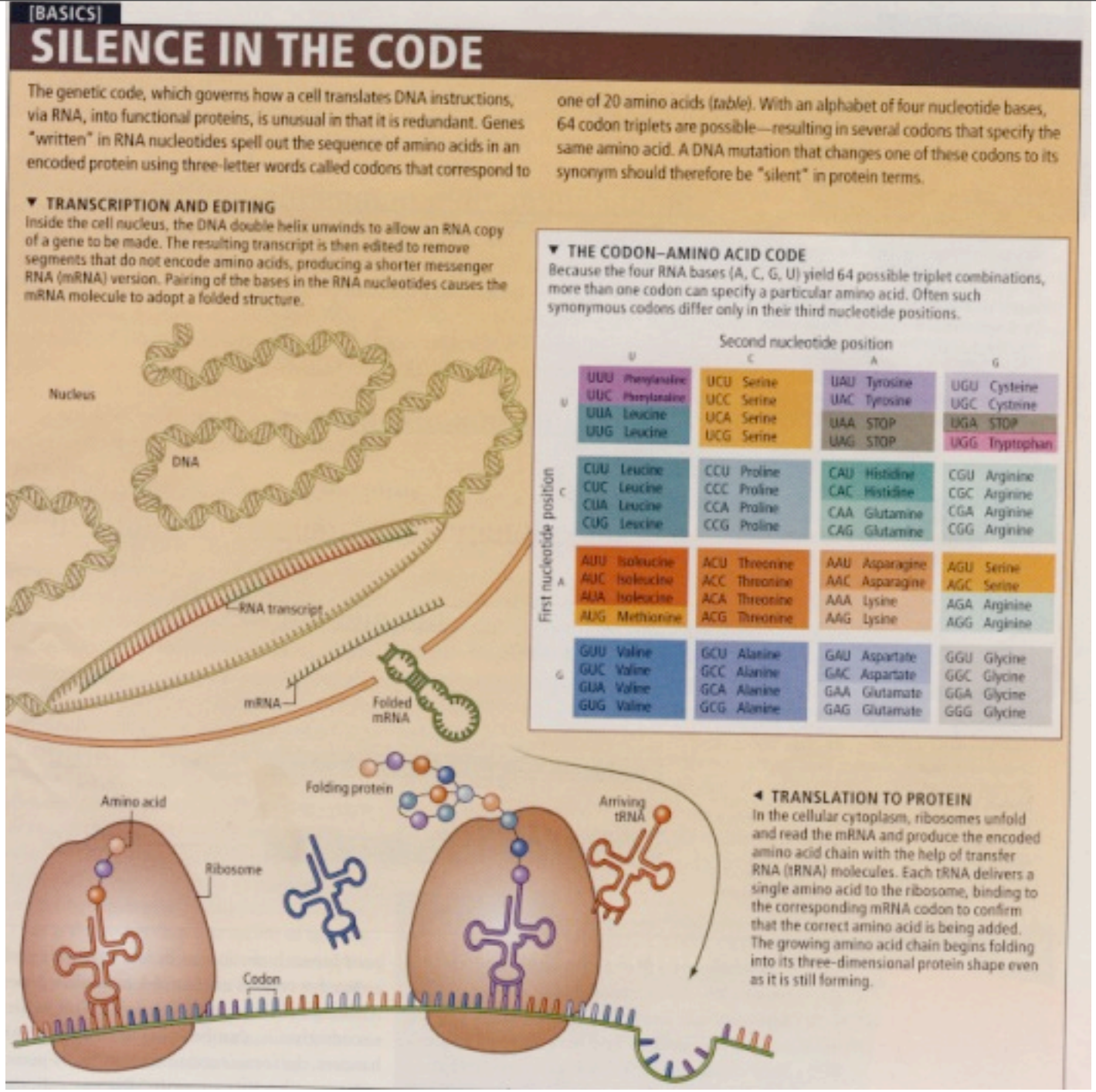
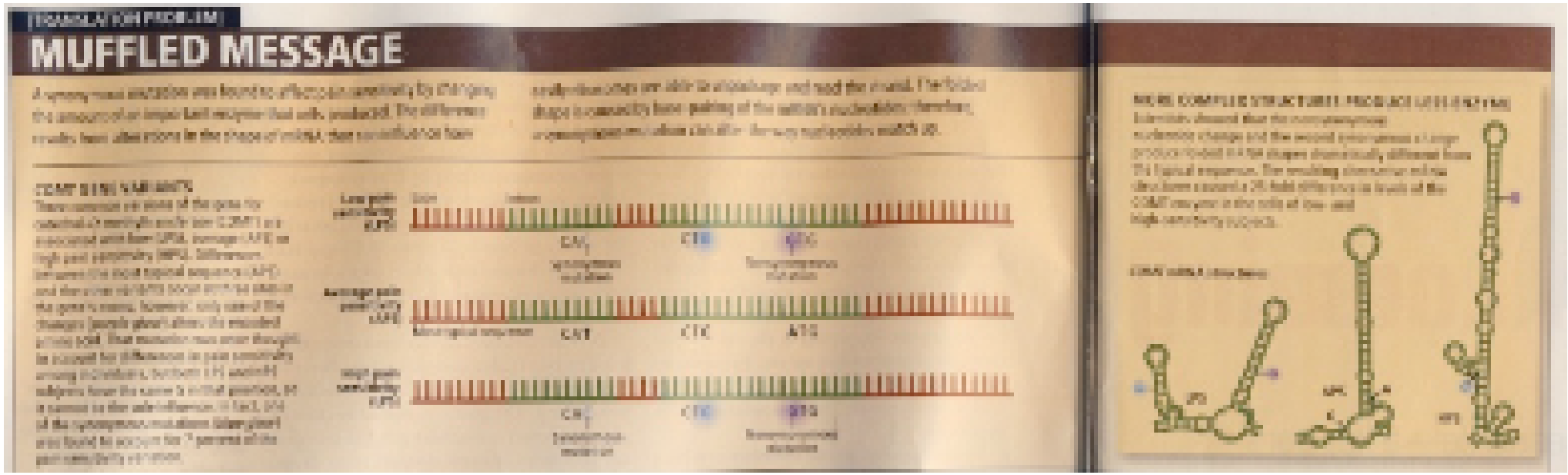
KEY CONCEPTS

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





MUFFLED MESSAGE

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