Ursprung des Lebens und Prinzipien der Evolution

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Institut für Theoretische Chemie, Universität Wien, Austria
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Darwin und Wallace

aus der Sicht der heutigen Biologie

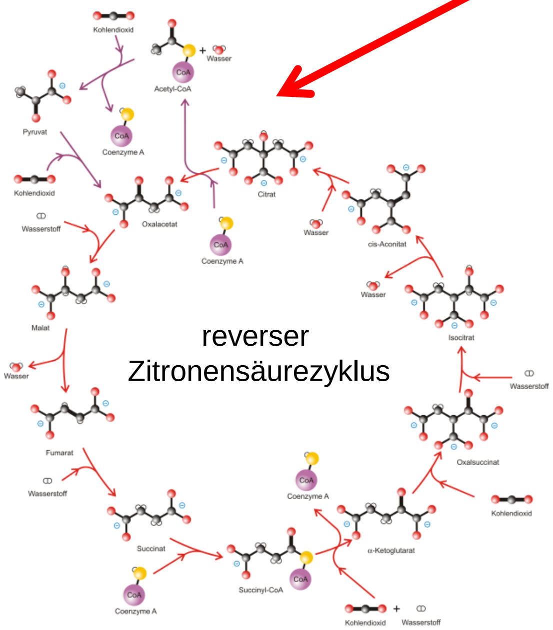
Berlin, 20.– 21.06.2013

Web-Page für weitere Informationen:

http://www.tbi.univie.ac.at/~pks

Prologue

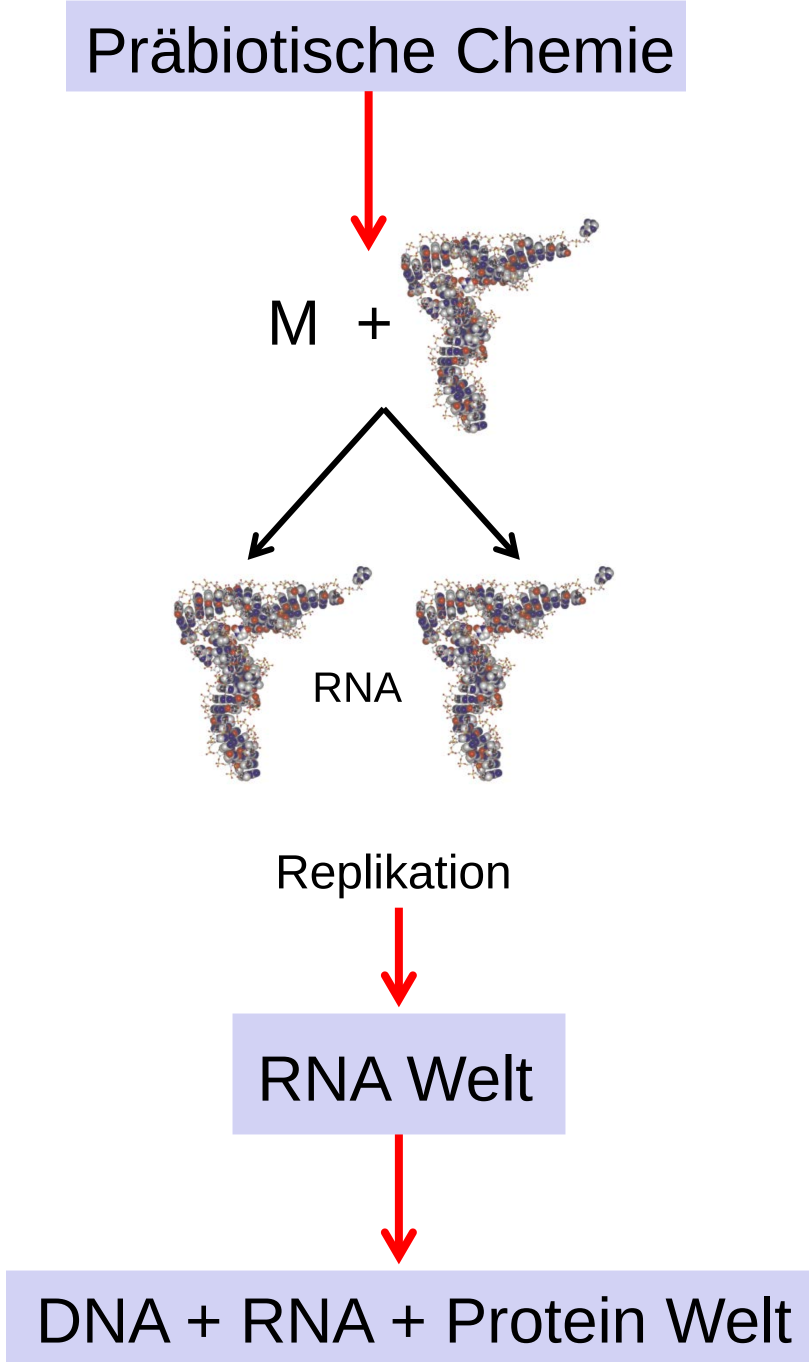
Präbiotische Chemie



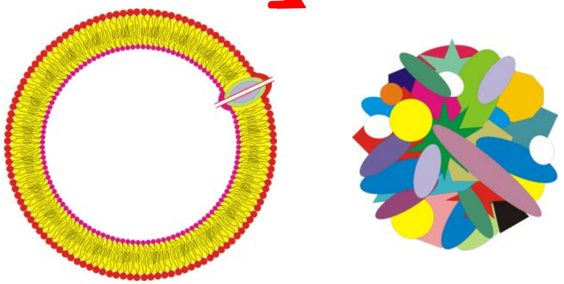
Metabolismus

RNA Welt

DNA + RNA + Protein Welt


Präbiotische Chemie
M +
RNA
Replikation
RNA Welt
DNA + RNA + Protein Welt

Präbiotische Chemie

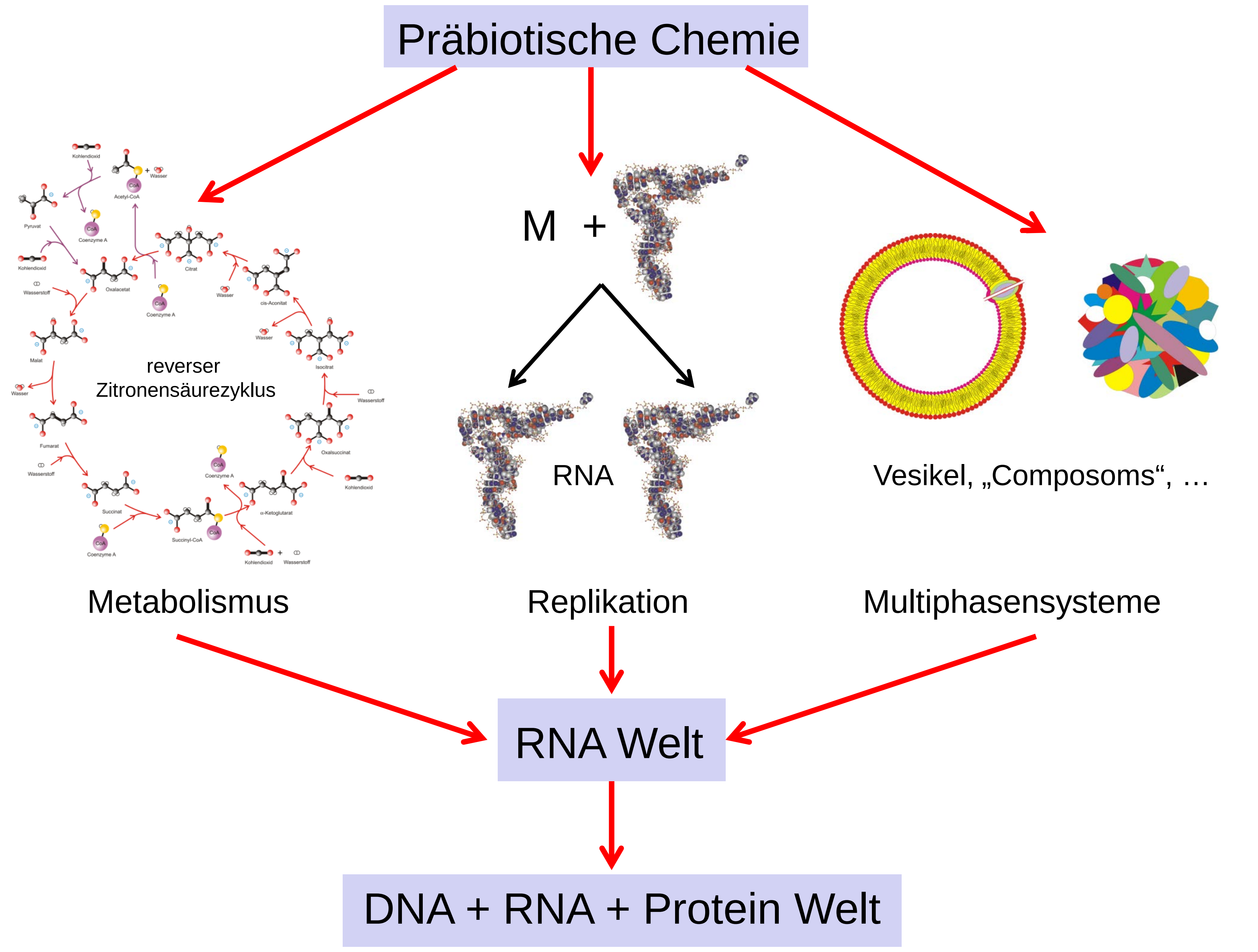


Vesikel, „Composoms“, …

Multiphasensysteme

RNA Welt

DNA + RNA + Protein Welt


Präbiotische Chemie
reverser
Zitronensäurezyklus
M +
RNA
Vesikel, „Composoms“, …
Metabolismus
Replikation
Multiphasensysteme
RNA Welt
DNA + RNA + Protein Welt

J Mol Evol (1997) 44:1–8

JOURNAL OF MOLECULAR EVOLUTION

On the Crucial Stages in the Origin of Animate Matter

Shneior Lifson

Chemical Physics Department, Weizmann Institute of Science, Rehovot 76100, Israel

Received: 29 March 1996 / Accepted: 30 May 1996

Key words: Origin — Animate matter — Autocatalysis — Natural selection — Sequels — Complexity — Metabolism — Cellular organization — Genetic code

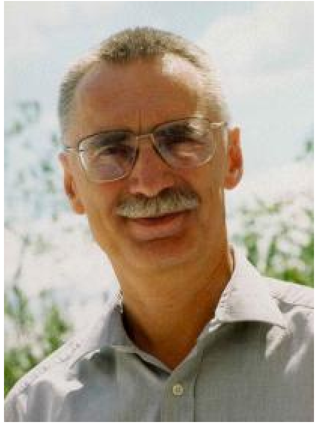
Shneior Lifson, 1914 - 2001

$$A + X \rightarrow 2X \; ; \; X \rightarrow D$$

Here, suffice it to recognize that *adaptation of autocatalysts to their changing environment by incorporating sequels into the autocatalytic process yields a great selective advantage.*

$$A + X \rightarrow 2X \; ; \; X \rightarrow D \; ; \; D \rightarrow A \qquad \leftarrow \quad \text{recycling}$$

Shneior Lifson and the origin of life



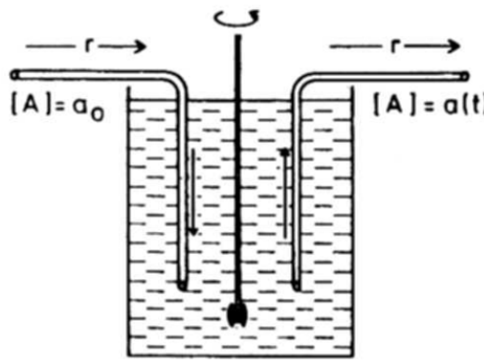
Karl Sigmund, 1945 -

Ber. Bunsenges. Phys. Chem. *89*, 668 – 682 (1985) – © VCH Verlagsgesellschaft mbH, D-6940 Weinheim, 1985.
0005 – 9021/85/0606 – 0668 $ 02.50/0

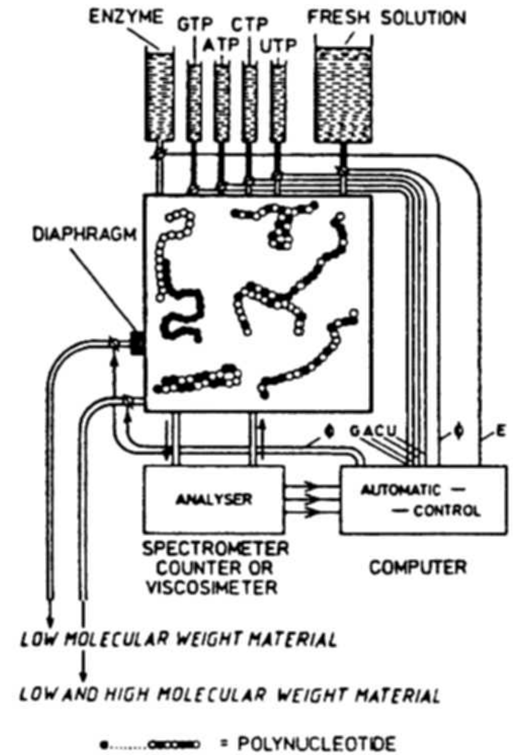
Dynamics of Evolutionary Optimization

Peter Schuster and Karl Sigmund

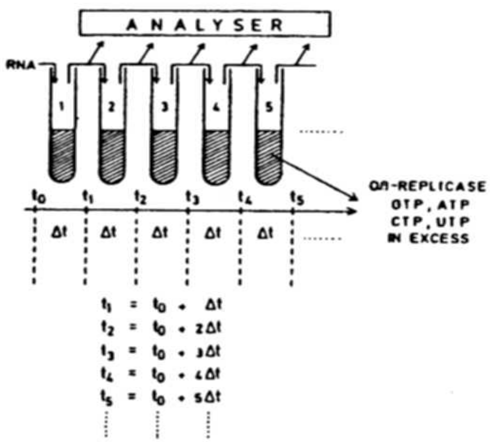
Institut für Theoretische Chemie und Strahlenchemie und Institut für Mathematik der Universität Wien,
Währingerstraße 17, A-1090 Wien, Austria



continuously stirred tank reactor (CSTR)

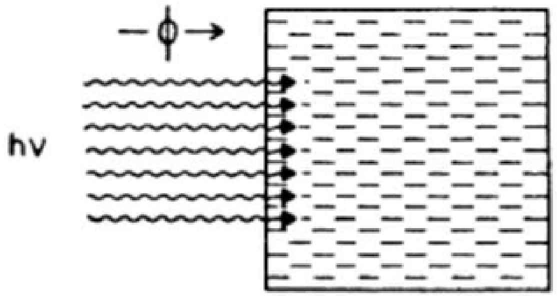


evolution reactor (turbidostat)



serial transfer

Open systems for *in vitro* evolution



$$B \xrightarrow{g^{(\phi)}} A$$

$$A \rightarrow \rightarrow \rightarrow B$$

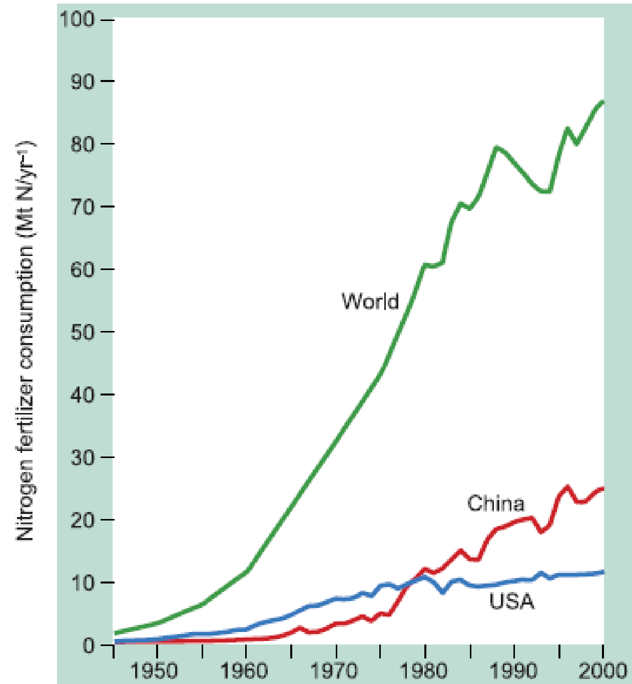
The recycling system. In this open system which is especially suitable for the theoretical study of replication, the energy rich material consumed is renewed by means of an irreversible recycling reaction. This recycling reaction converts the degradation product B into starting material A. As shown in the sketch above the recycling reaction might be represented by a photochemical process $B + h\nu \rightarrow A$. In order to introduce an evolutionary constraint we use here a degradation process: the replicating molecules are converted into energy poor material B. In a realistic system B stands for the nucleoside monophosphates GMP, AMP, CMP and UMP. For further details see [8].

$$A + X \rightarrow 2X\,;\; X \rightarrow B\,;\; B \overset{h\nu,\,\phi}{\rightarrow} A$$

Open systems for *in vitro* evolution

Alexis Madrigal. 2008. How to make fertilizer appear out of thin air.
100 years Haber – Bosch process.

Figure 1. Consumption of nitrogenous fertilizers, 1950–1999. (Plotted from data in refs 2 and 6).



Vaclav Smil. 2002. *Ambio* **31**:126-131

Every fifth nitrogen atom in our body has seen a Haber-Bosch plant from inside at least once!

The importance of recycling in the modern world

1. Vom Ursprung des Lebens
2. Darwinsche Evolution von Molekülen
3. Evolutionäre Biotechnologie
4. Evolutionsexperimente mit Bakterien

1. **Vom Ursprung des Lebens**
2. Darwinsche Evolution von Molekülen
3. Evolutionäre Biotechnologie
4. Evolutionsexperimente mit Bakterien

Kriterien des Lebens

(i) **Vermehrung** und **Vererbung**

(ii) **Mutation** infolge fehlerhafter Reproduktion und Rekombination

(iii) **Stoffwechsel** zur Erzeugung der molekularen Bausteine des Lebens

(iv) **Individualisierung** durch Einschließen in Kompartimente

(v) **Autopoiese** und **Homöostase**

(vi) Organisierte Zellteilung – **Mitose**

(vii) Sexuelle Reproduktion und Reduktions-Zellteilung – **Meiose**

(viii) **Zelldifferenzierung** in Zellen der Keimbahn und somatische Zellen

H_2, H_2O, NH_3, N_2, H_2S, CH_4, CO, CO_2, Metallionen, ...

↓

Chemie der präbiotischen Erde

Bausteine der Biopolymeren: Aminosäuren, Nucleobasen, Kohlenhydrate, ...

↓

Polykondensationsreaktionen

Polymere mit ungeordneten Bausteinfolgen, ...

↓

Polymerisation an Vorlagen: Instruierte Polymere

↓

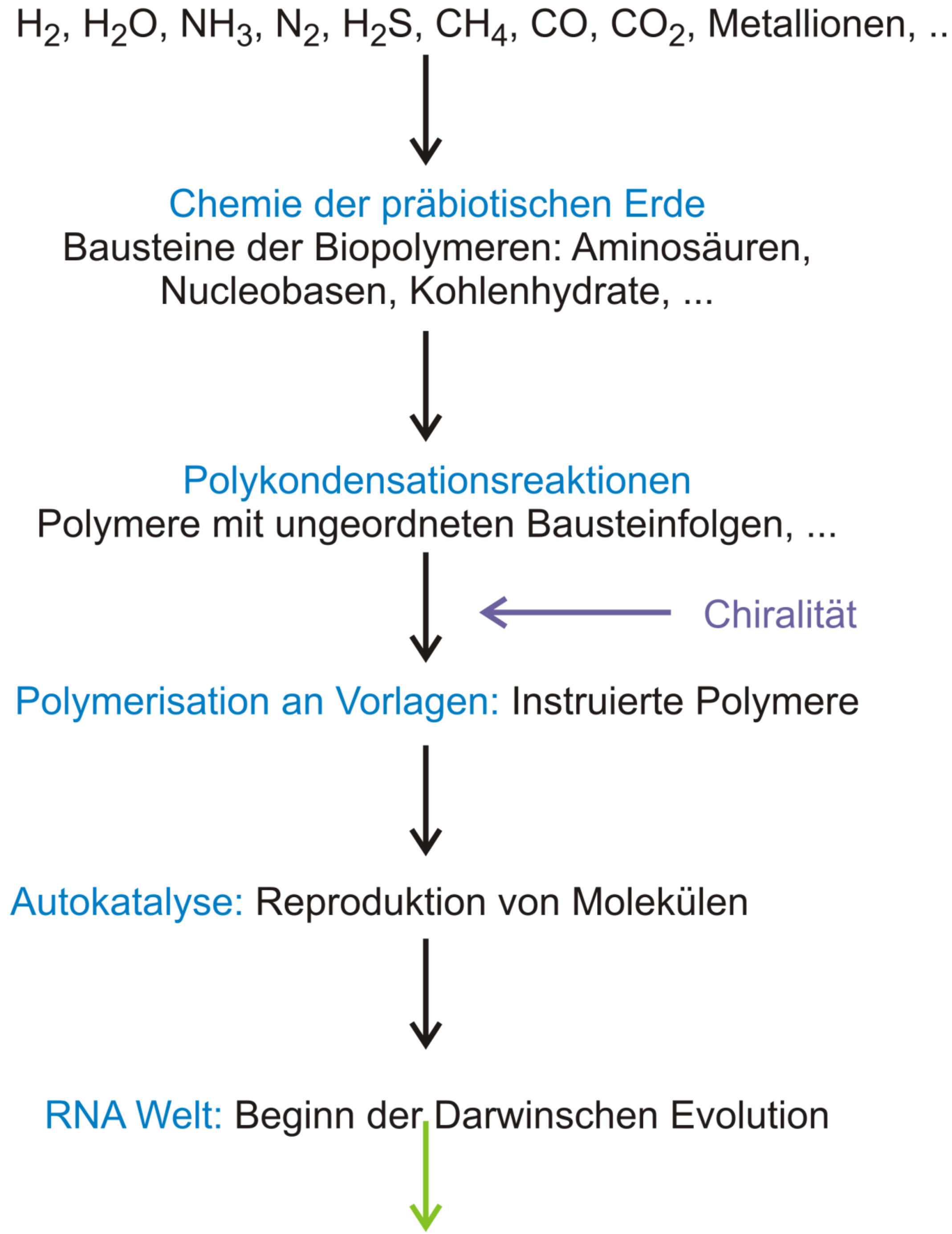
Autokatalyse: Reproduktion von Molekülen

↓

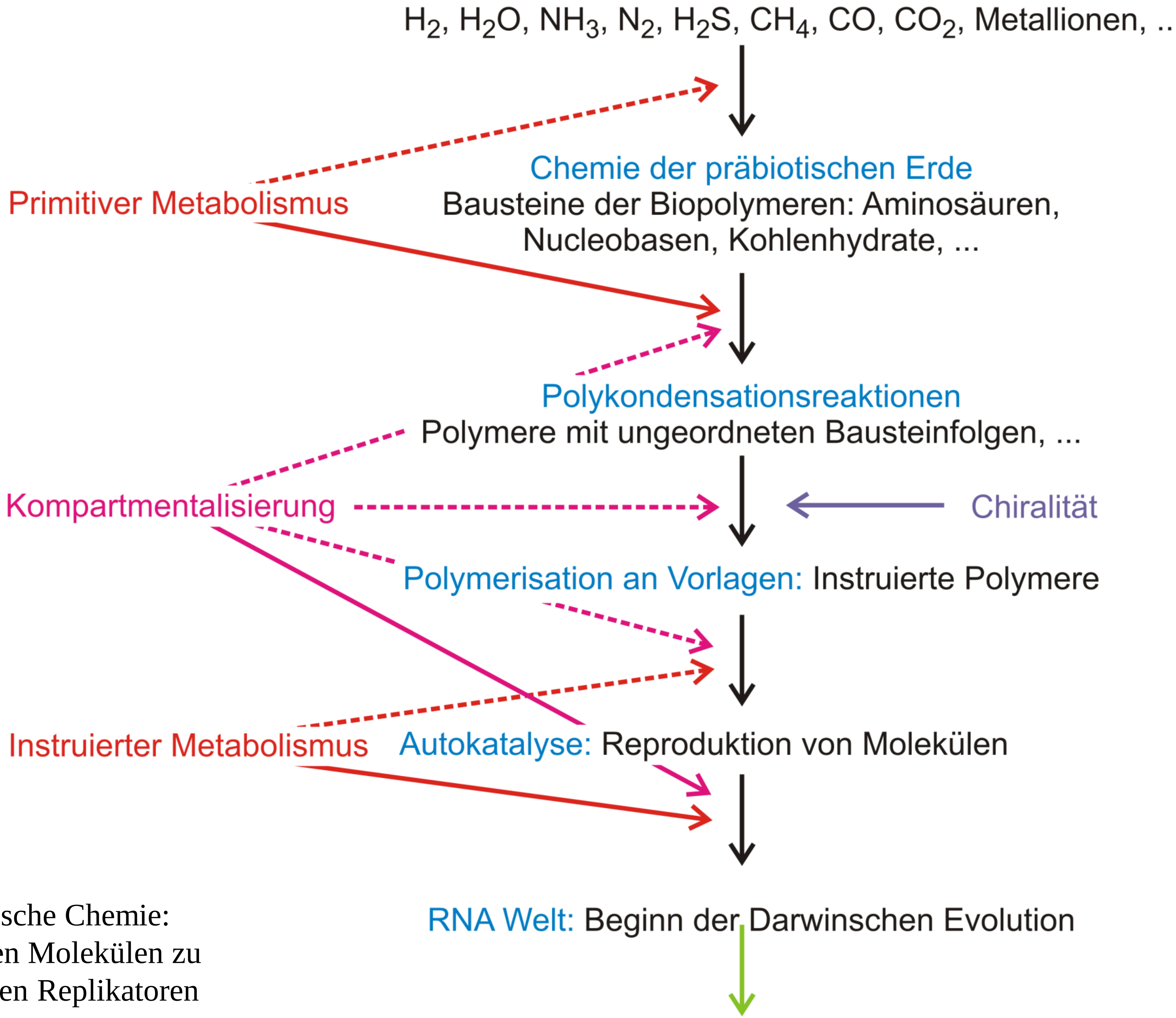
RNA Welt: Beginn der Darwinschen Evolution

↓

Präbiotische Chemie:
Von kleinen Molekülen zu molekularen Replikatoren



Präbiotische Chemie:
Von kleinen Molekülen zu molekularen Replikatoren



Präbiotische Chemie:
Von kleinen Molekülen zu
molekularen Replikatoren

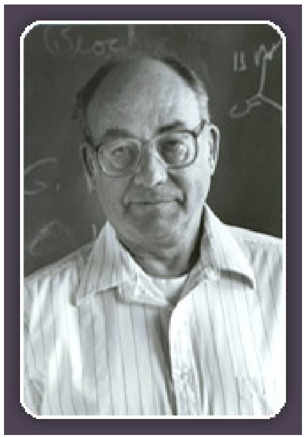
Von kleinen Molekülen zu molekularen Replikatoren:

drei Beispiele

1. **Woher kommen die Bausteine des Lebens?**
2. Der Ursprung der Chiralität
3. Einfache Metabolismen

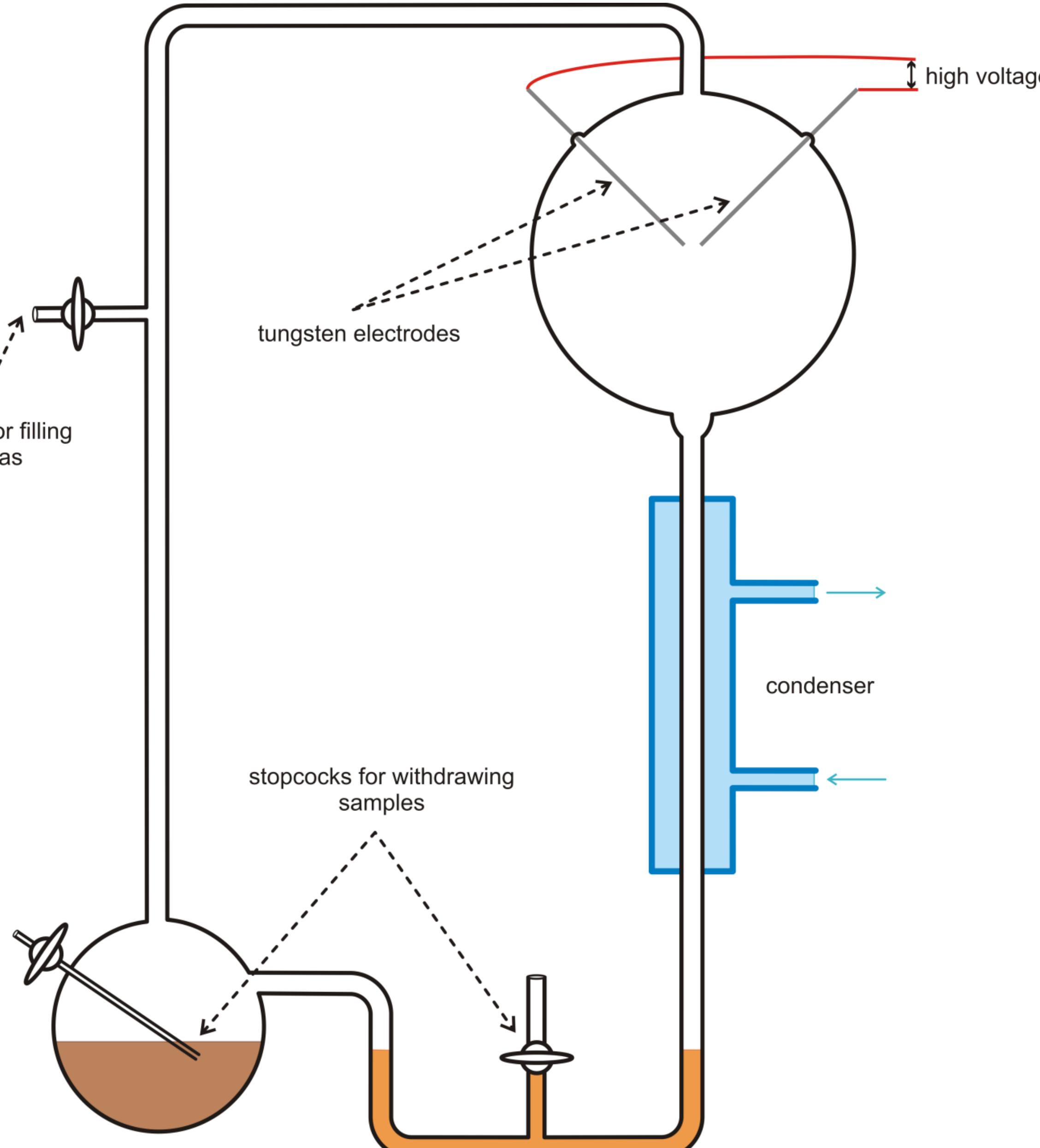
Elektrische Entladung in einer reduzierenden Atmosphäre:

CH_4, CO, NH_3, H_2O, H_2, …

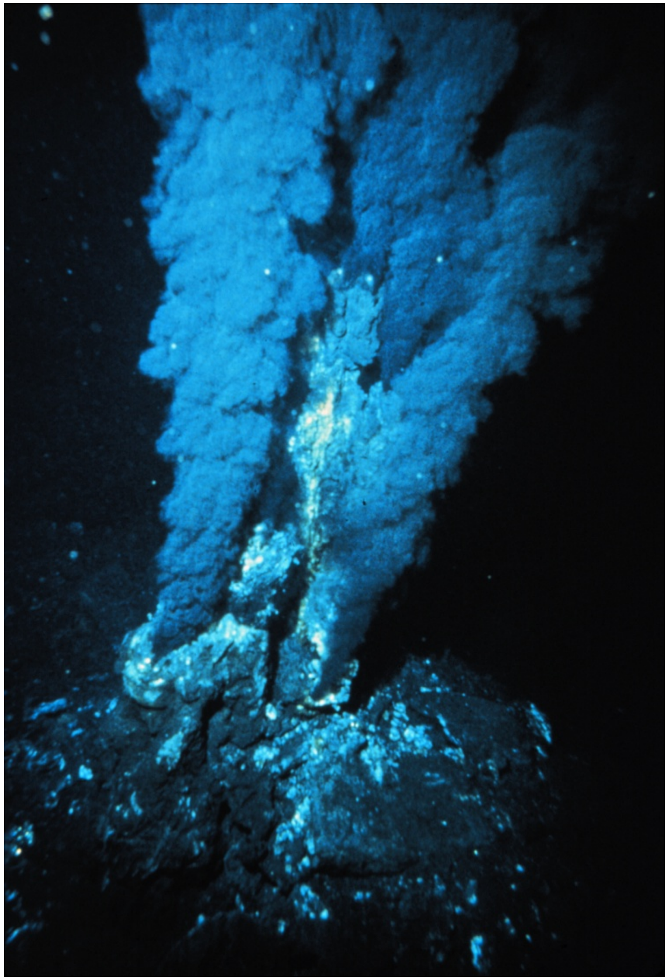


Stanley Miller, 1930 - 2007

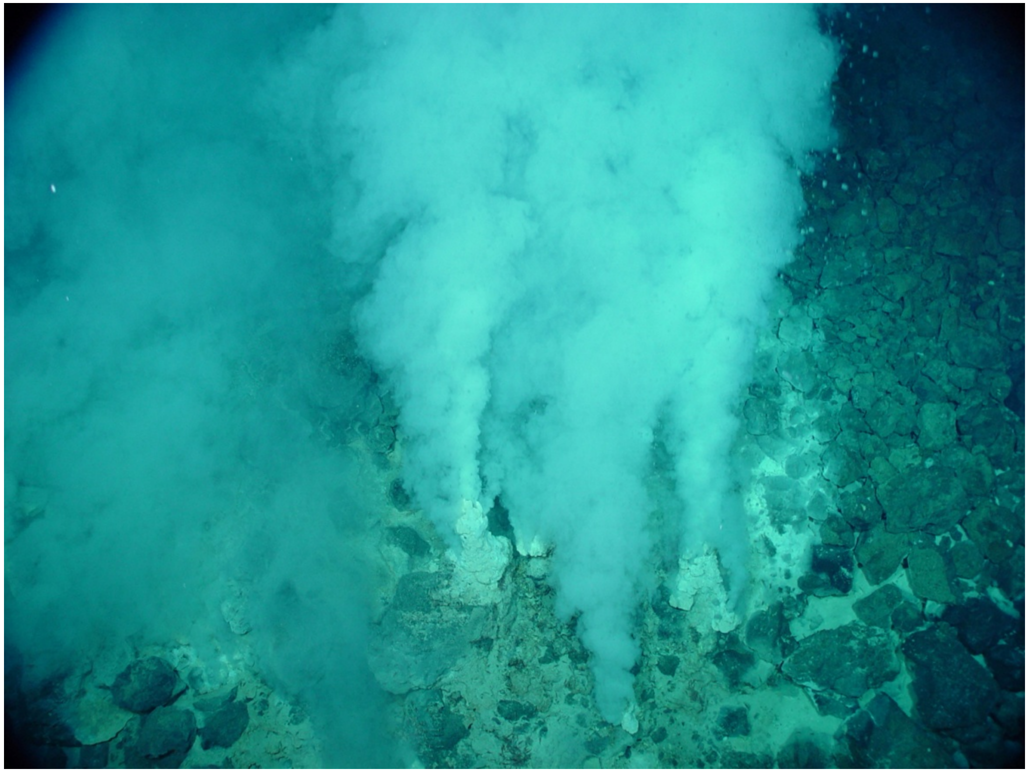
S.L.Miller. 1953. A production of amino acids under possible primitive earth conditions. *Science* **117**:528-529



Das Miller-Urey Experiment



black smoker



white smoker

Hydrothermale Quellen in der Tiefsee

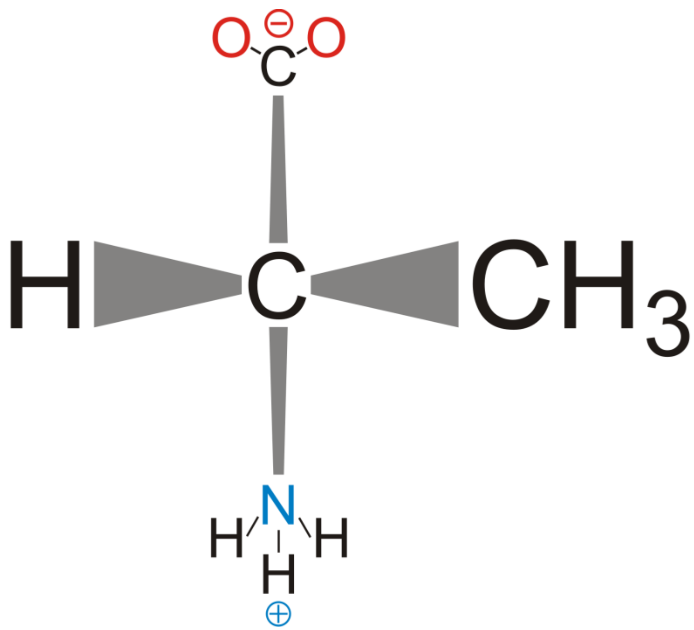
Vorkommen: mid-atlantic ridge, east pacific rise, …
in etwa 3000 m Tiefe

Source: Wikipedia: *Hydrothermal vent*, Nov. 15,2011

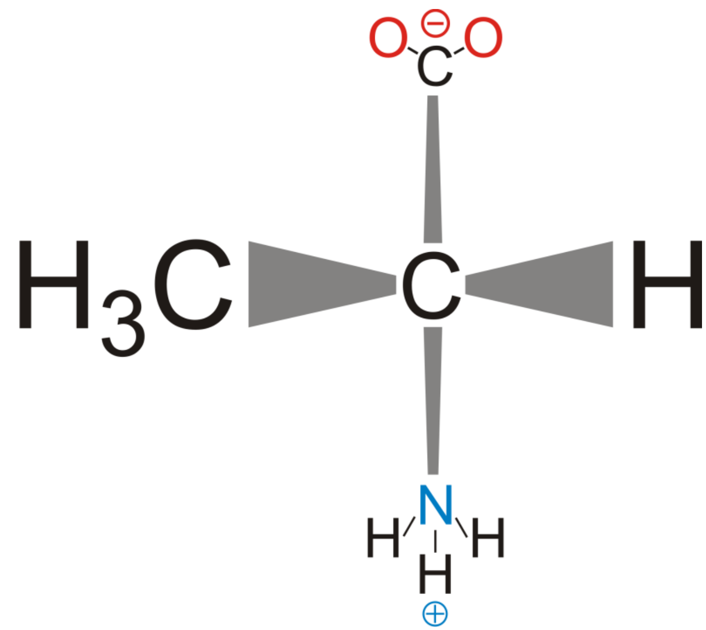
Von kleinen Molekülen zu molekularen Replikatoren:

drei Beispiele

1. Woher kommen die Bausteine des Lebens?
2. **Der Ursprung der Chiralität**
3. Einfache Metabolismen

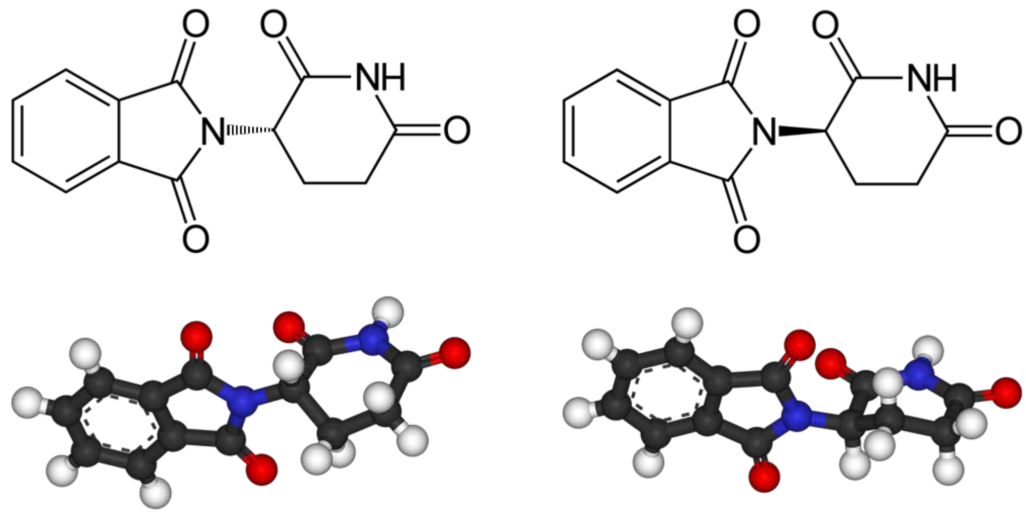


L- (S-) Alanin



D- (R-) Alanin

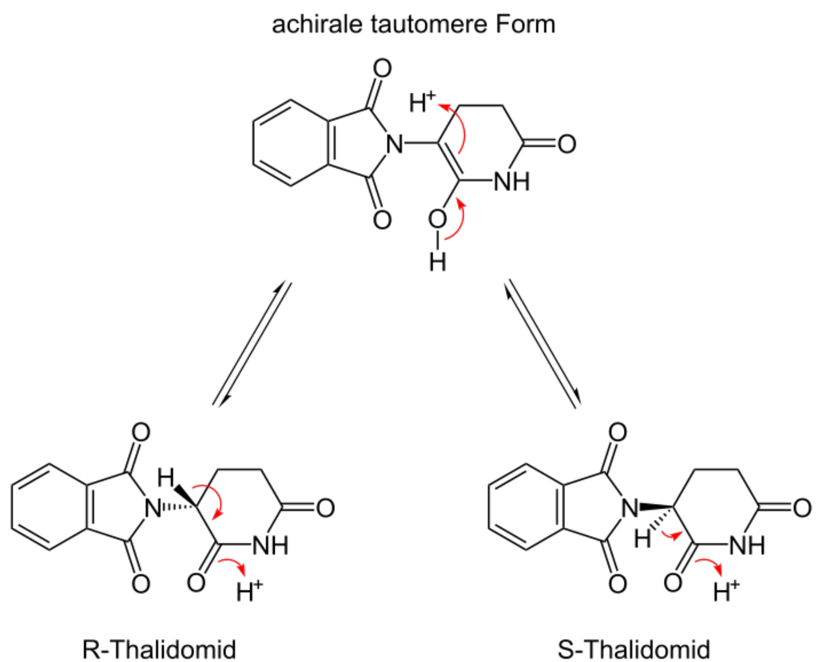
Die zwei chiralen Formen von Alanin



Unterschiedliche Wirkungen von chiralen Formen: Thalidomid als Contergan als Arzneimittel für den Markt zugelassen: 1957 - 1961

Conterganfolgen: Missbildungen bei Kindern vor der Geburt durch S-Thalidomid.

Razemisierung von R-Thalidomid im Körper.



ON SPONTANEOUS ASYMMETRIC SYNTHESIS

by

F. C. FRANK

The H. H. Wills Physical Laboratory, University of Bristol (England)

I am informed by my colleague Professor W. MOORE that there is still widely believed to be a problem of explaining the original "asymmetric synthesis" giving rise to the general optical activity of the chemical substances of living matter. I have long supposed that this was no problem on the basis of a supposition that the initial production of life is a rare event. We may take as the defining property of a living entity the ability to reproduce its own kind. Omitting such simple entities as flames, which are included by such a definition, and confining attention to chemical molecules, the complexity of any having this essential property of life is likely to be great enough to make it highly improbable that it has a centre of symmetry. It is likely, in fact, to contain α-amino acids which are necessarily asymmetric. Then, if the production of living molecules is an infrequent process, compared with the rate of multiplication of living molecules, the whole earth is likely to be extensively populated with the progeny of the first before another appears. In fact they may have so modified the environment by then that no other has a chance of generation. There are, of course, variants of this hypothesis: *e.g.* that a second living molecule is produced before the progeny of the first has colonised the whole earth, and competes successfully with it for nutrient material, "starving", or even "poisoning" the other out of existence. This leads to the same result, and depends essentially on the same initial hypothesis, that spontaneous germination of life is a rare event.

Die theoretische Vorhersage der Erzeugung von Chiralität durch autokatalytische asymmetrische Synthese im Jahre **1953** durch Frederick Charles **Frank**

Kenso Soai, 1950 -

Kenso Soai 1995

Asymmetric autocatalysis and amplification of enantiomeric excess of a chiral molecule

Kenso Soai, Takanori Shibata, Hiroshi Morioka & Kaori Choji

Department of Applied Chemistry, Faculty of Science, Science University of Tokyo, Kagurazaka, Shinjuku-ku, Tokyo 162, Japan

THE homochirality of natural amino acids and sugars remains a puzzle for theories of the chemical origin of life[1–18]. In 1953 Frank[7] proposed a reaction scheme by which a combination of autocatalysis and inhibition in a system of replicating chiral molecules can allow small random fluctuations in an initially racemic mixture to tip the balance to yield almost exclusively one enantiomer. Here we show experimentally that autocatalysis in a chemical reaction can indeed enhance a small initial enantiomeric excess of a chiral molecule. When a 5-pyrimidyl alkanol with a small (2%) enantiomeric excess is treated with diisopropylzinc and pyrimidine-5-carboxaldehyde, it undergoes an autocatalytic reaction to generate more of the alkanol. Because the reaction involves a chiral catalyst generated from the initial alkanol, and because the catalytic step is enantioselective, the enantiomeric excess of the product is enhanced. This process provides a mechanism by which a small initial imbalance in chirality can become overwhelming.

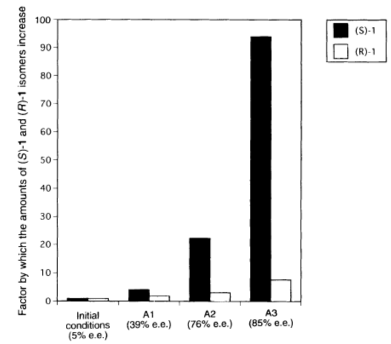


FIG 1. Asymmetric autocatalysis of chiral pyrimidyl alkanol (**1**). Runs A1–3 correspond to Table 1. The enantiomeric excess of (*S*)-**1** increases from 5 to 89% e.e. without the use of additional chiral auxiliaries. During the reactions (runs A1–3), the (*S*)-**1** increases by a factor of 94 times, while (*R*)-**1** increases by a factor of only eight times.

employed as asymmetric autocatalyst, the e.e. of the mixture of catalyst and the product was also 88% (run B5). Thus in series A and B, the low e.e. of (*S*)-**1** was autocatalytically amplified to 88–89%, and the amount of (*S*)-**1** was increased by a factor

Michael Mauksch and Svetlana Tsogoeva 2007

WILEY InterScience DISCOVER SOMETHING GREAT   CHIRALITY 19:816–825 (2007)

Demonstration of Spontaneous Chiral Symmetry Breaking in Asymmetric Mannich and Aldol Reactions

MICHAEL MAUKSCH,* SVETLANA B. TSOGOEVA,*,† SHENGWEI WEI, AND IRINA M. MARTYNOVA

Institute of Organic Chemistry I, University of Erlangen-Nuremberg, Henkestrasse 42, 91052 Erlangen, Germany

ABSTRACT Spontaneous symmetry breaking in reactive systems, known as a rare physical phenomenon and for the Soai autocatalytic irreversible reaction, might in principle also occur in other, more common asymmetric reactions when the chiral product is capable to promote its formation and an element of "nonlinearity" is involved in the reaction scheme. Such phenomena are long sought after in chemistry as a possible explanation for the biological homochirality of biomolecules. We have investigated homogeneous organic stereoselective Mannich and Aldol reactions, in which the product is capable to form H-bridged complexes with the prochiral educt, and found by applying NMR spectroscopy, HPLC analysis, and optical rotation measurements 0.3–50.8% of random product enantiomeric excess under essentially achiral reaction conditions. These findings imply a hitherto overlooked mechanism for spontaneous symmetry breaking and, hence, a novel approach to the problem of absolute asymmetric synthesis and could have also potential significance for the conundrum of homochirality. *Chirality 19:816–825, 2007.*

KEY WORDS: organocatalysis; spontaneous symmetry breaking; asymmetric autocatalysis; Mannich reaction; Aldol reaction; homochirality

Reaktionen mit einem etwas erweiterten Frank Mechanismus

Von kleinen Molekülen zu molekularen Replikatoren:

drei Beispiele

1. Woher kommen die Bausteine des Lebens?
2. Der Ursprung der Chiralität
3. **Einfache Metabolismen**

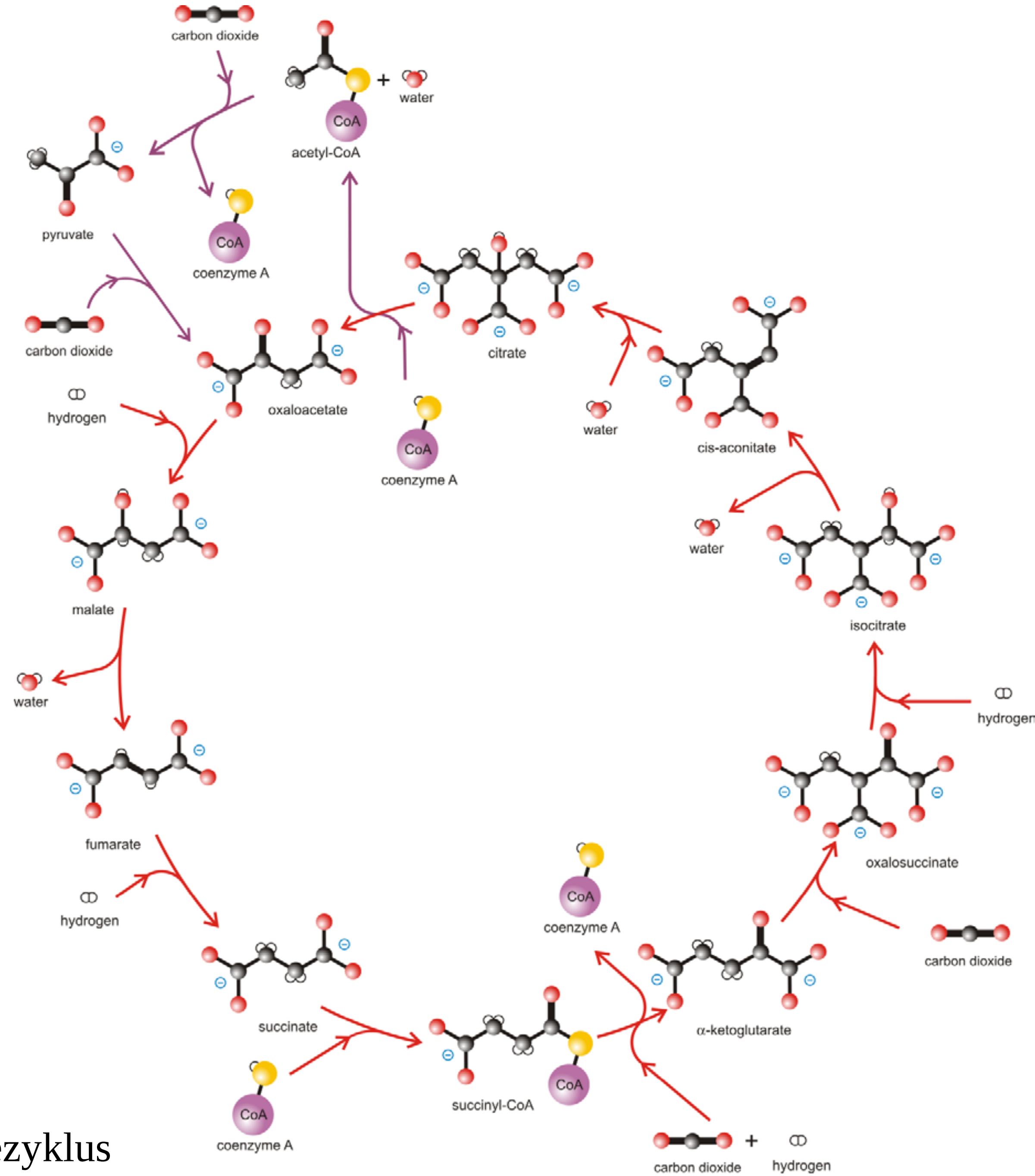
Primitiver Metabolismus??

$$2\,CO_2 + 4\,H_2 \rightleftharpoons CH_3COOH + 2\,H_2O$$

zwölf Teilschritte

G. Wächtershäuser. Before enzymes and templates: Theory of surface metabolism. 1988. *Microbiol. Rev.* **52**:452-484.

Die Umkehrung des Zitronensäurezyklus



1. Vom Ursprung des Lebens

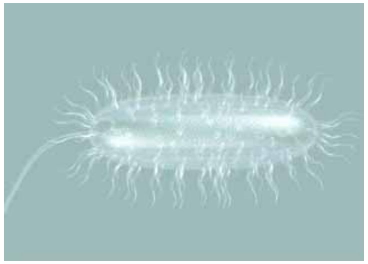
2. **Darwinsche Evolution von Molekülen**

3. Evolutionäre Biotechnologie

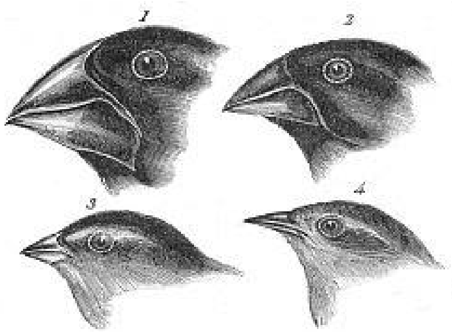
4. Evolutionsexperimente mit Bakterien

Charles Darwin, 1809 - 1882

Voyage on HMS Beagle, 1831 - 1836

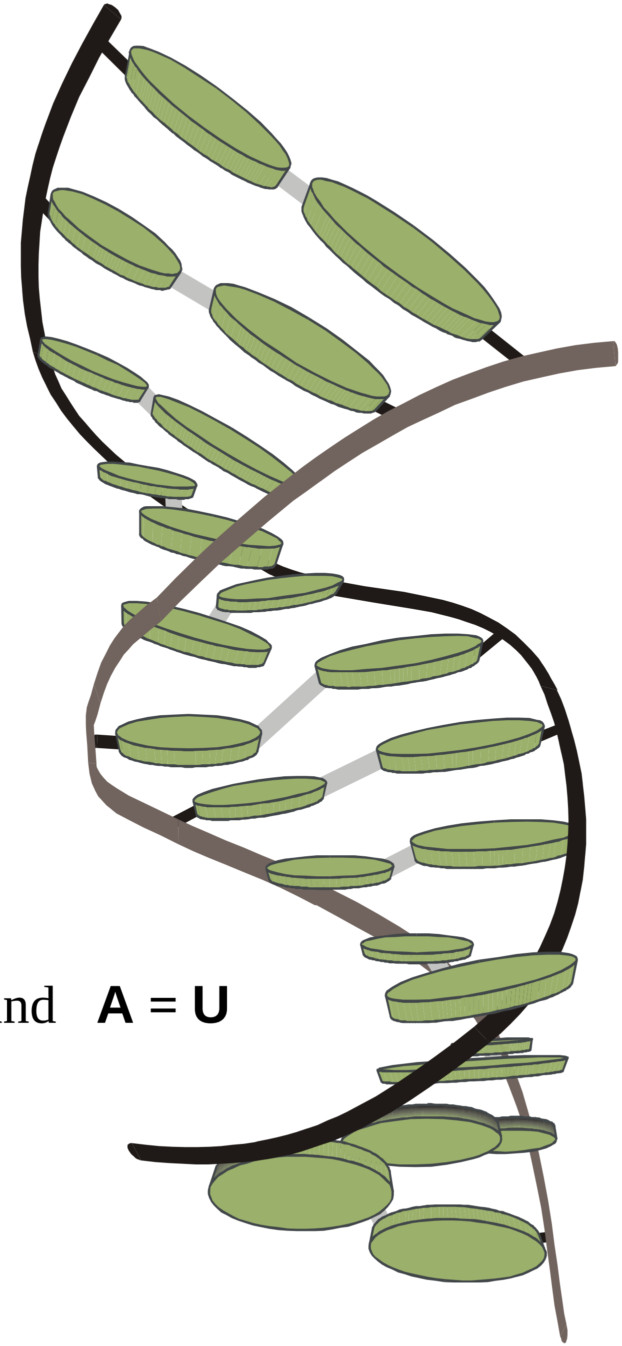


Phänotypen

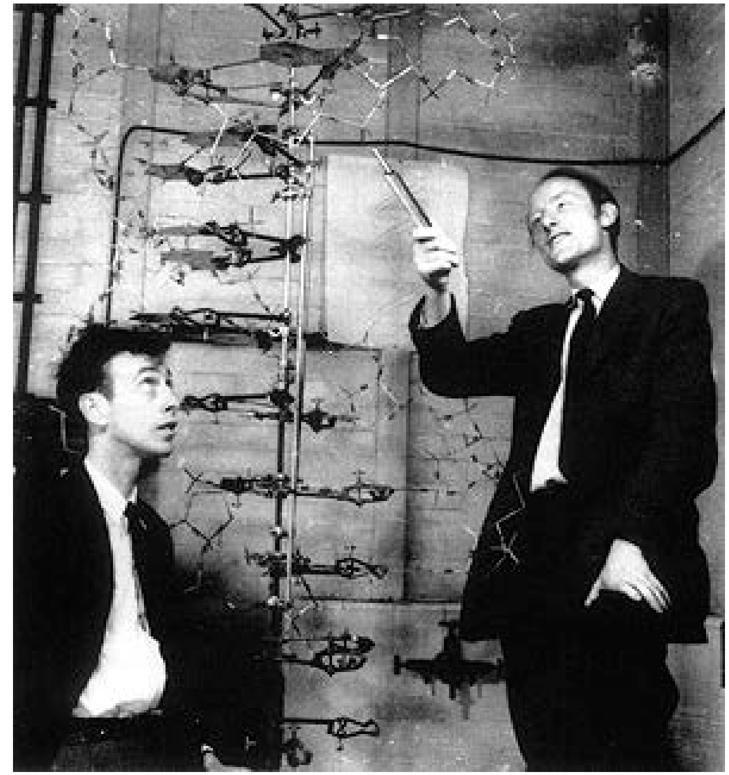


1. Geospiza magnirostris
2. Geospiza fortis
3. Geospiza parvula
4. Certhidea olivacea

Finches from Galapagos Archipelago

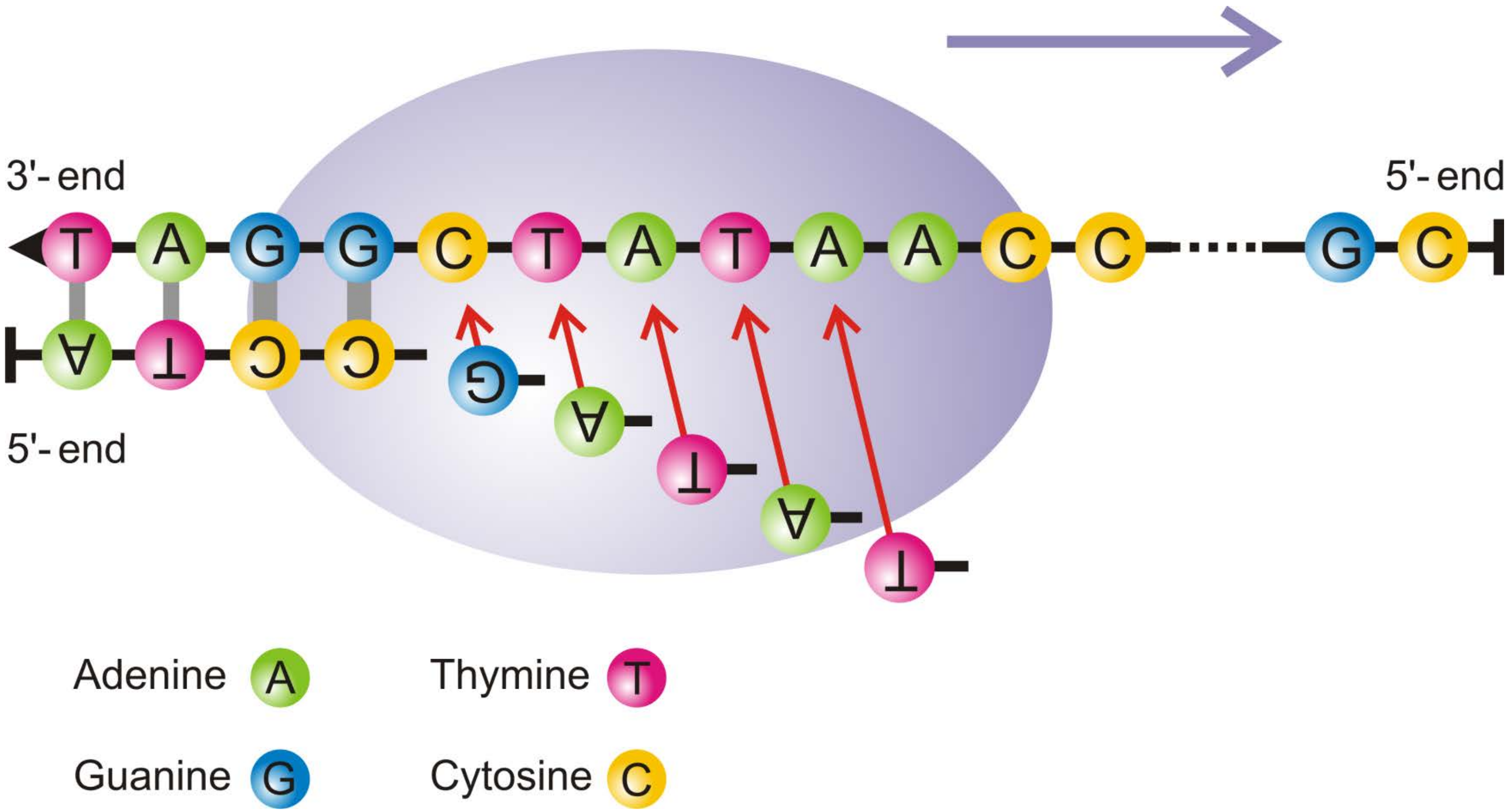


G≡C und **A = U**



James D. Watson, 1928- , and Francis Crick, 1916-2004,
Nobel Preis 1962

Die dreidimensionale Struktur eines kleinen Stückes der B-DNA



Die Replication von DNA mit *Thermophilus aquaticus* Polymerase (PCR)

Die Logik der DNA (oder RNA) Replikation

Drei notwendige Bedingungen für Darwinsche Evolution sind:

1. **Vermehrung** (und Vererbung),
2. **Variation**, und
3. **Selektion**.

Vermehrung führt zu exponentiellem Wachstum, das eine *conditio sine qua non* für Selektion darstellt.

Variation ist ein Nebeneffekt des molekularen Mechanismus der Reproduktion.

Selektion ist eine Konsequenz der endlichen Ressourcen.

Da im Sinne der Optimierung von Fitness durch die Darwinsche Evolution nur Nachkommen gezählt werden, ist sie fast universell gültig.

DOI: 10.1002/anie.200701369

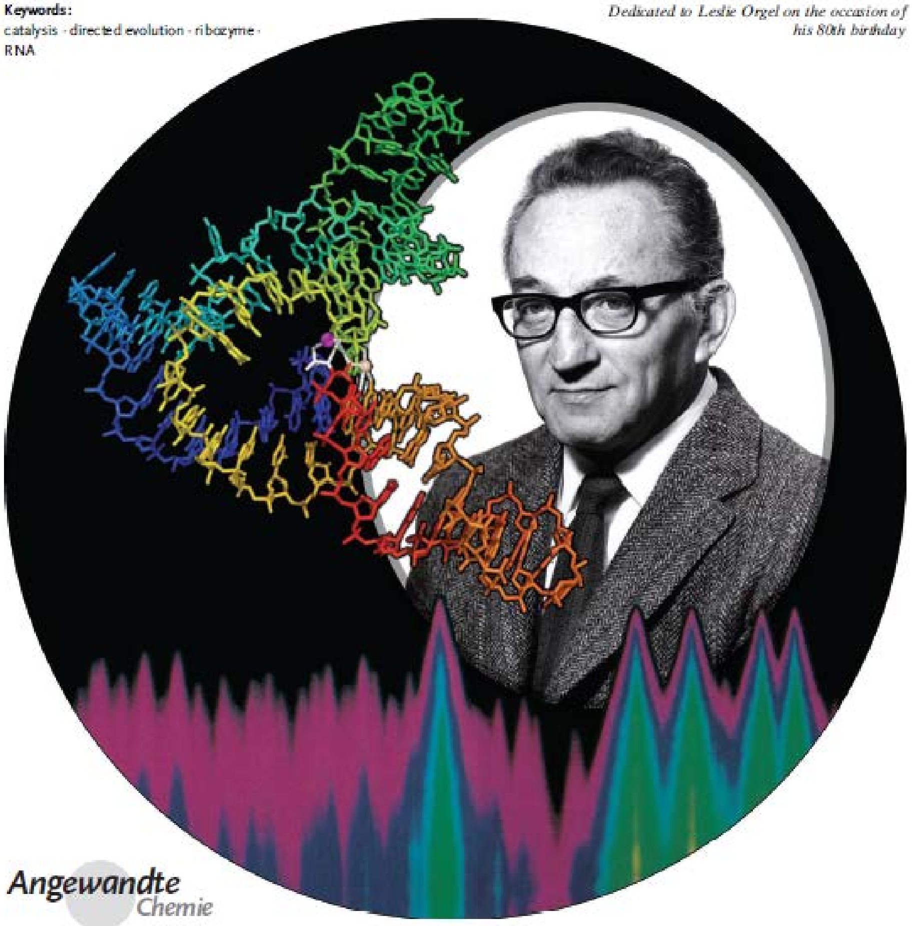
Molecular Evolution

Forty Years of In Vitro Evolution**

Gerald F. Joyce*

Keywords:
catalysis · directed evolution · ribozyme · RNA

Dedicated to Leslie Orgel on the occasion of his 80th birthday



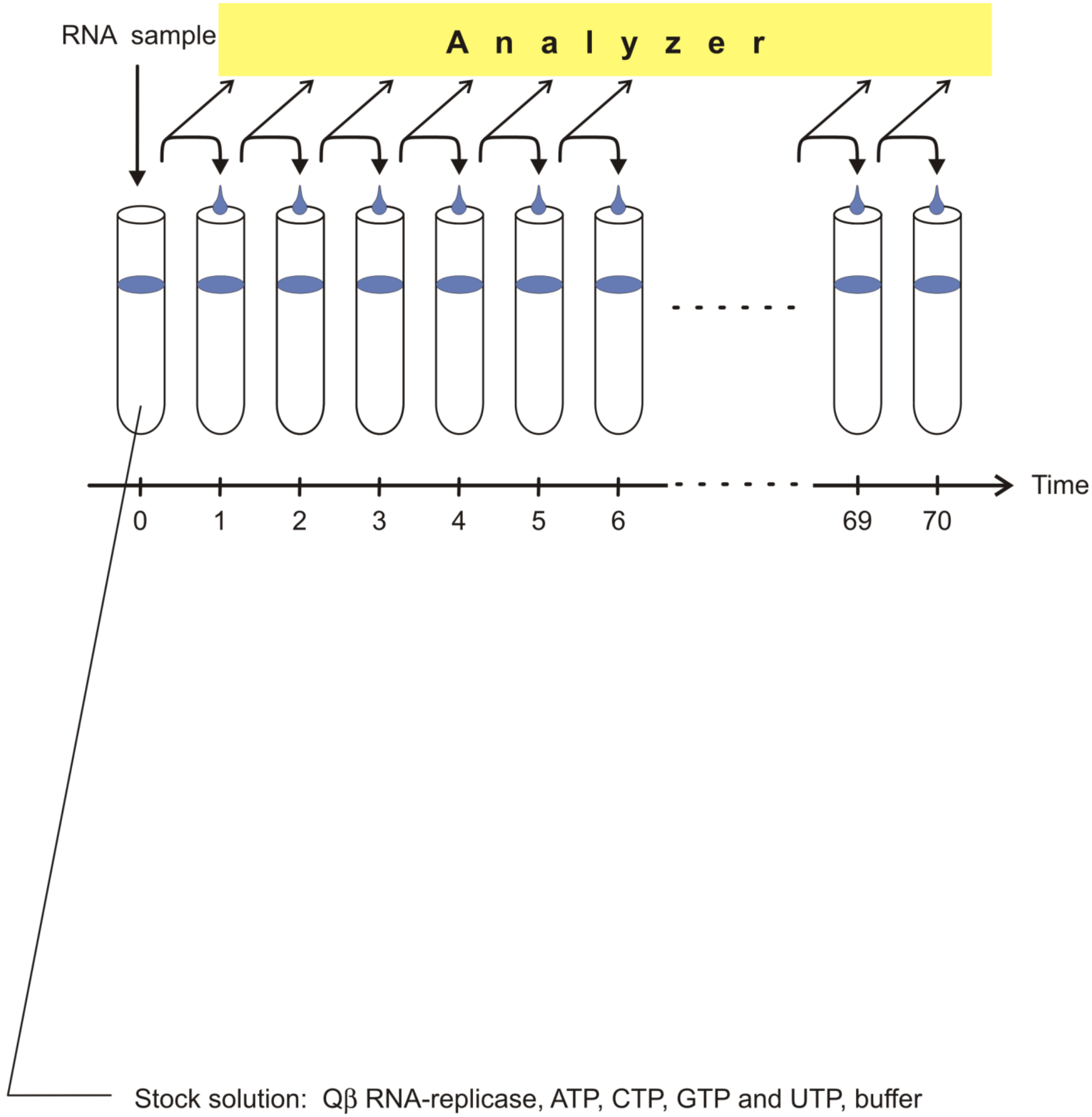
Sol Spiegelman,
1914 - 1983

Angewandte Chemie

Evolution im Reagenzglas:

G.F. Joyce, *Angew.Chem.Int.Ed.* **46** (2007), 6420-6436

The serial transfer technique for *in vitro* evolution



Reproduction of the original figure of the serial transfer experiment with Qβ RNA

D.R.Mills, R,L,Peterson, S.Spiegelman, *An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule*. Proc.Natl.Acad.Sci.USA **58** (1967), 217-224

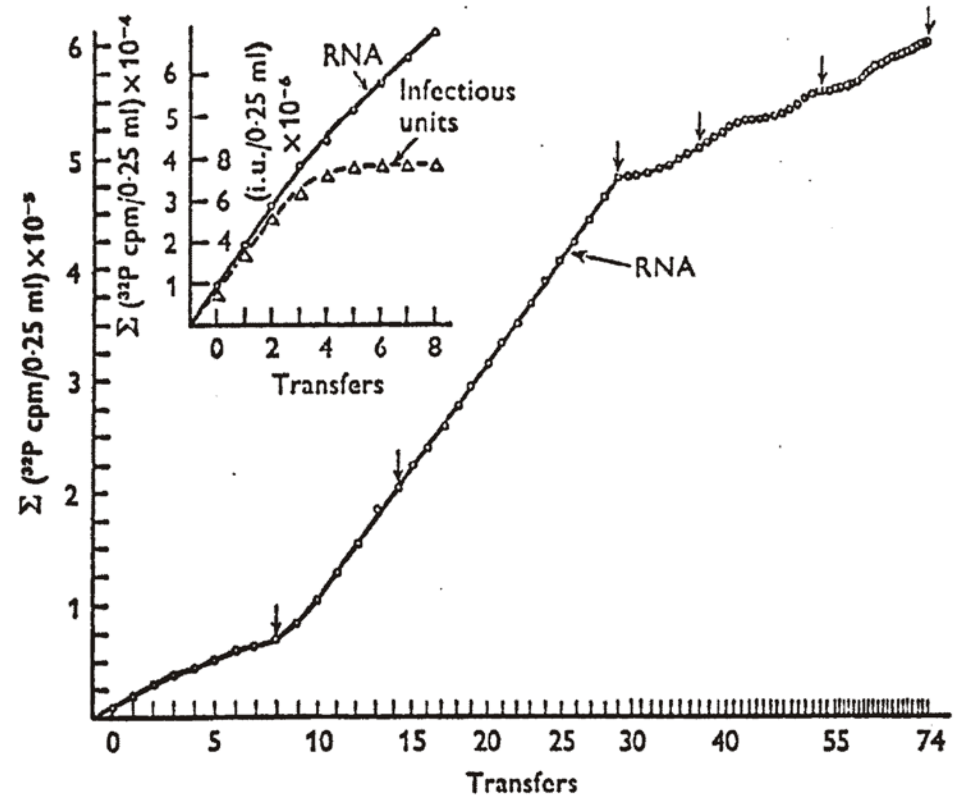
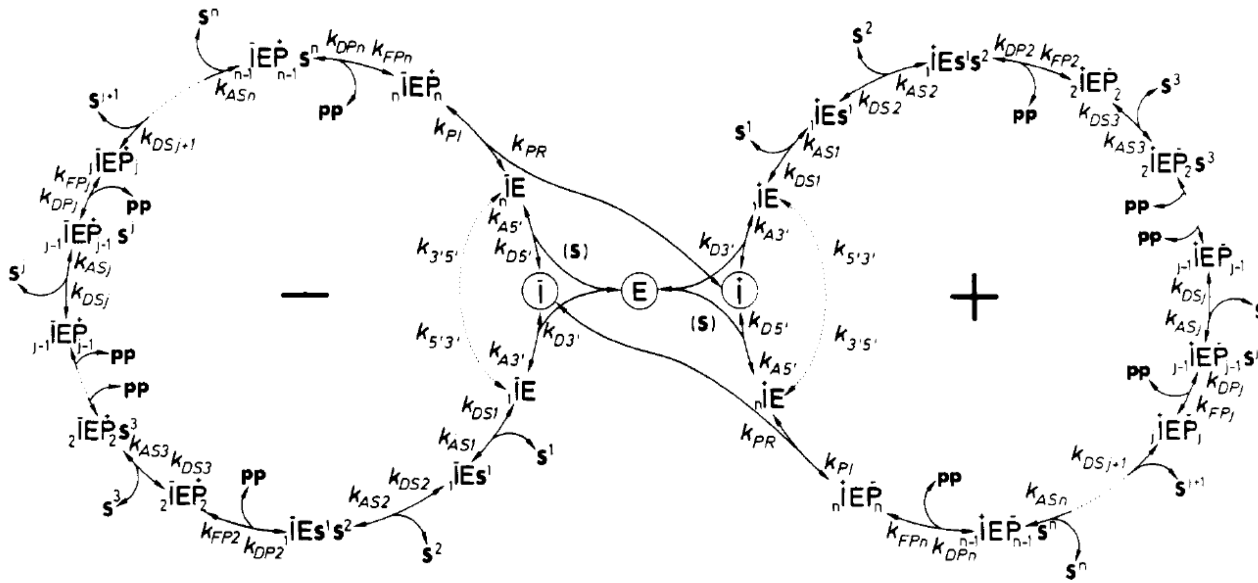
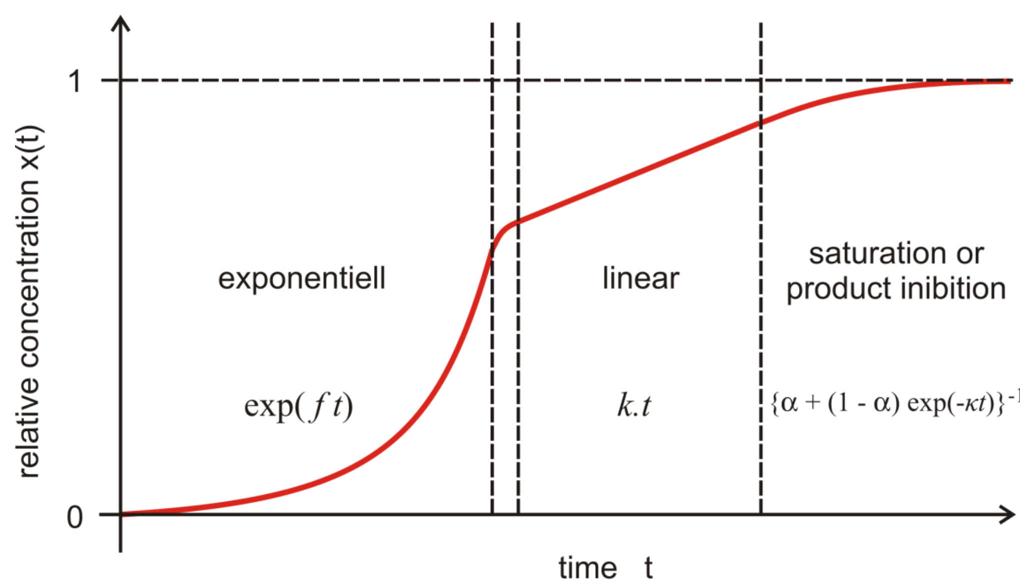


Fig. 9. Serial transfer experiment. Each 0·25 ml standard reaction mixture contained 40 μg of Qβ replicase and ^{32}P-UTP. The first reaction (0 transfer) was initiated by the addition of 0·2 μg ts-1 (temperature-sensitive RNA) and incubated at 35 °C for 20 min, whereupon 0·02 ml was drawn for counting and 0·02 ml was used to prime the second reaction (first transfer), and so on. After the first 13 reactions, the incubation periods were reduced to 15 min (transfers 14–29). Transfers 30–38 were incubated for 10 min. Transfers 39–52 were incubated for 7 min, and transfers 53–74 were incubated for 5 min. The arrows above certain transfers (0, 8, 14, 29, 37, 53, and 73) indicate where 0·001–0·1 ml of product was removed and used to prime reactions for sedimentation analysis on sucrose. The inset examines both infectious and total RNA. The results show that biologically competent RNA ceases to appear after the 4th transfer (Mills *et al.* 1967).

Christof K. Biebricher,
1941-2009





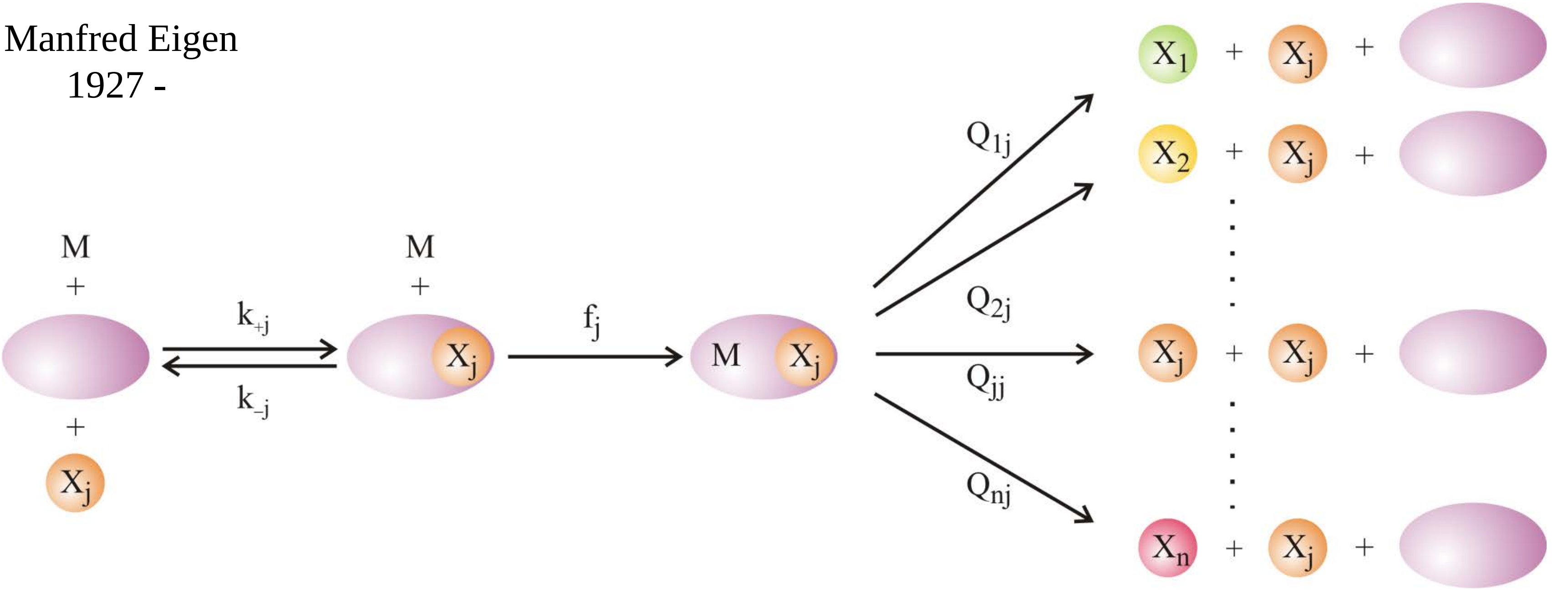
Kinetik der RNA Replikation

C.K. Biebricher, M. Eigen, W.C. Gardiner, Jr.
Biochemistry **22**:2544-2559, 1983

Manfred Eigen
1927 -

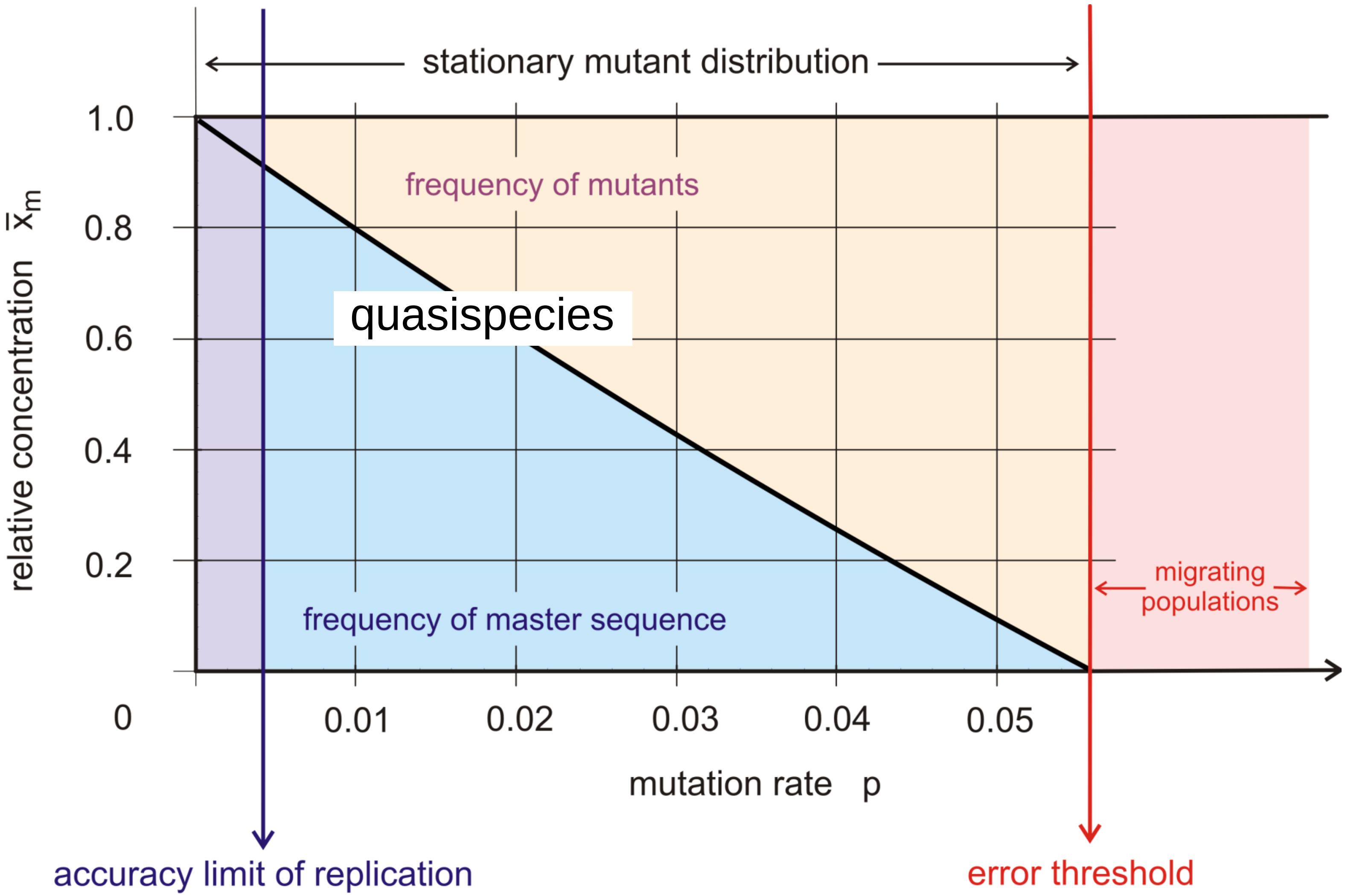
$$\frac{\mathrm{d}x_j}{\mathrm{d}t} = \sum_{i=1}^{n} W_{ji}\, x_i - x_j\, \Phi\,; \quad j = 1, 2, \ldots, n$$

$$\Phi = \sum_{i=1}^{n} f_i\, x_i \Big/ \sum_{i=1}^{n} x_i$$

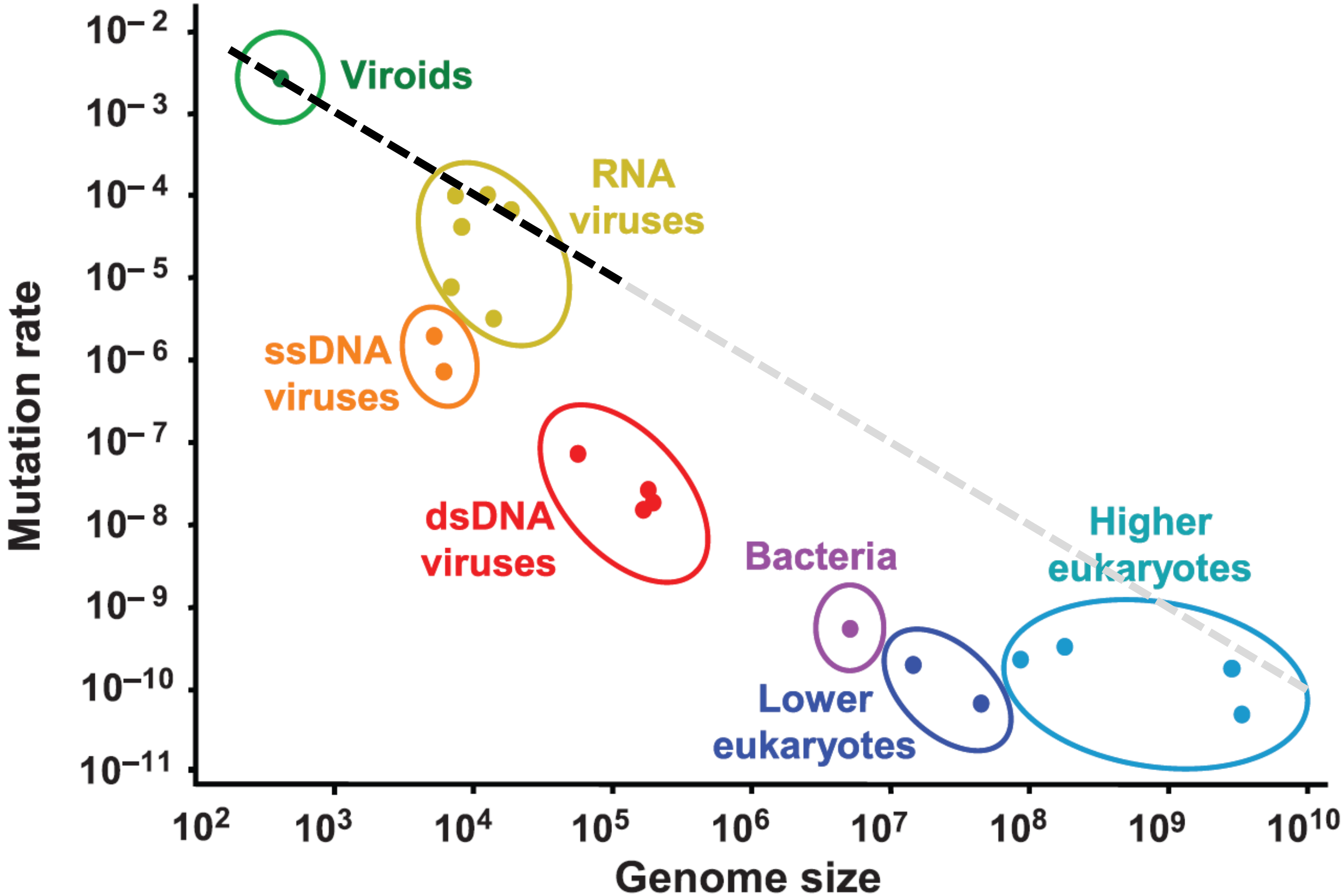


Mutation and (correct) replication as parallel chemical reactions

M. Eigen. 1971. *Naturwissenschaften* 58:465,
M. Eigen & P. Schuster.1977. *Naturwissenschaften* 64:541, 65:7 und 65:341



The error threshold in replication and mutation



Selma Gago, Santiago F. Elena, Ricardo Flores, Rafael Sanjuán. 2009. Extremely high mutation rate of a hammerhead viroid. Science 323:1308.

Mutation rate and genome size

1. Vom Ursprung des Lebens
2. Darwinsche Evolution von Molekülen
3. **Evolutionäre Biotechnologie**
4. Evolutionsexperimente mit Bakterien

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Virus Research
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Preface

Antiviral strategy on the horizon

Error catastrophe had its conceptual origins in the middle of the XXth century, when the consequences of mutations on enzymes involved in protein synthesis, as a theory of aging. In those times biological processes were generally perceived differently from today. Infectious diseases were regarded as a fleeting nuisance which would be eliminated through the use of antibiotics and antiviral agents. Microbial variation, although known in some cases, was not thought to be a significant problem for disease control. Variation in differentiated organisms was seen as resulting essentially from exchanges of genetic material associated with sexual reproduction. The problem was to unveil the mechanisms of inheritance, expression of genetic information and metabolism. Few saw that genetic change is occurring at present in all organisms, and still fewer recognized Darwinian principles as essential to the biology of pathogenic viruses and cells. Population geneticists rarely used bacteria or viruses as experimental systems to define concepts in biological evolution. The extent of genetic polymorphism among individuals of the same biological species came as a surprise when the first results on comparison of electrophoretic mobility of enzymes were obtained. With the advent of in vitro DNA recombination, and rapid nucleic acid sequencing techniques, molecular analyses of genomes reinforced the conclusion of extreme inter-individual genetic variation within the same species. Now, due largely to spectacular progress in comparative genomics, we see cellular DNAs, both prokaryotic and eukaryotic, as highly dynamic. Most cellular processes, including such essential information-bearing and transferring events as genome replication, transcription and translation, are increasingly perceived as inherently inaccurate. Viruses, and in particular RNA viruses, are among the most extreme examples of exploitation of replication inaccuracy for survival.

Error catastrophe, or the loss of meaningful genetic information through excess genetic variation, was formulated in quantitative terms as a consequence of quasispecies theory, which was first developed to explain self-organization and adaptability of primitive replicons in early stages of life. Recently, a conceptual extension of error catastrophe that could be defined as "induced genetic deterioration" has emerged as a possible antiviral strategy. This is the topic of the current special issue of ***Virus Research***.

Few would nowadays doubt that one of the major obstacles for the control of viral disease is short-term adaptability of viral pathogens. Adaptability of viruses follows the same Darwinian principles that have shaped biological evolution over eons, that is, repeated rounds of reproduction with genetic variation, competition and selection, often perturbed by random events such as statistical fluctuations in population size. However, with viruses the consequences of the operation of these very same Darwinian principles are felt within very short times. Short-term evolution (within hours and days) can be also observed with some cellular pathogens, with subsets of normal cells, and cancer cells. The nature of RNA viral pathogens begs for alternative antiviral strategies, and forcing the virus to cross the critical error threshold for maintenance of genetic information is one of them.

The contributions to this volume have been chosen to reflect different lines of evidence (both theoretical and experimental) on which antiviral designs based on genetic deterioration inflicted upon viruses are being constructed. Theoretical studies have explored the copying fidelity conditions that must be fulfilled by any information-bearing replication system for the essential genetic information to be transmitted to progeny. Closely related to the theoretical developments have been numerous experimental studies on quasispecies dynamics and their multiple biological manifestations. The latter can be summarized by saying that RNA viruses, by virtue of existing as mutant spectra rather than defined genetic entities, remarkably expand their potential to overcome selective pressures intended to limit their replication. Indeed, the use of antiviral inhibitors in clinical practice and the design of vaccines for a number of major RNA virus-associated diseases, are currently presided by a sense of uncertainty. Another line of growing research is the enzymology of copying fidelity by viral replicases, aimed at understanding the molecular basis of mutagenic activities. Error catastrophe as a potential new antiviral strategy received an important impulse by the observation that ribavirin (a licensed antiviral nucleoside analogue) may be exerting, in some systems, its antiviral activity through enhanced mutagenesis. This has encouraged investigations on new mutagenic base analogues, some of them used in anticancer chemotherapy. Some chapters summarize these important biochemical studies on cell entry pathways and metabolism of mutagenic agents, that may find new applications as antiviral agents.

doi:10.1016/j.virusres.2004.11.001

This volume intends to be basically a progress report, an introduction to a new avenue of research, and a realistic appraisal of the many issues that remain to be investigated. In this respect, I can envisage (not without many uncertainties) at least three lines of needed research: (i) One on further understanding of quasispecies dynamics in infected individuals to learn more on how to apply combinations of virus-specific mutagens and inhibitors in an effective way, finding synergistic combinations and avoiding antagonistic ones as well as severe clinical side effects. (ii) Another on a deeper understanding of the metabolism of mutagenic agents, in particular base and nucleoside analogues. This includes identification of the transporters that carry them into cells, an understanding of their metabolic processing, intracellular stability and alterations of nucleotide pools, among other issues. (iii) Still another line of needed research is the development of new mutagenic agents specific for viruses, showing no (or limited) toxicity for cells. Some advances may come from links with anticancer research, but others should result from the designs of new molecules, based on the structures of viral polymerases. I really hope that the reader finds this issue not only to be an interesting and useful review of the current situation in the field, but also a stimulating exposure to the major problems to be faced.

The idea to prepare this special issue came as a kind invitation of Ulrich Desselberger, former Editor of ***Virus Research***, and then taken enthusiastically by Luis Enjuanes, recently appointed as Editor of ***Virus Research***. I take this opportunity to thank Ulrich, Luis and the Editor-in-Chief of ***Virus Research***, Brian Mahy, for their continued interest and support to the research on virus evolution over the years.

My thanks go also to the 19 authors who despite their busy schedules have taken time to prepare excellent manuscripts, to Elsevier staff for their prompt responses to my requests, and, last but not least, to Ms. Lucía Horrillo from Centro de Biología Molecular "Severo Ochoa" for her patient dealing with the correspondence with authors and the final organization of the issue.

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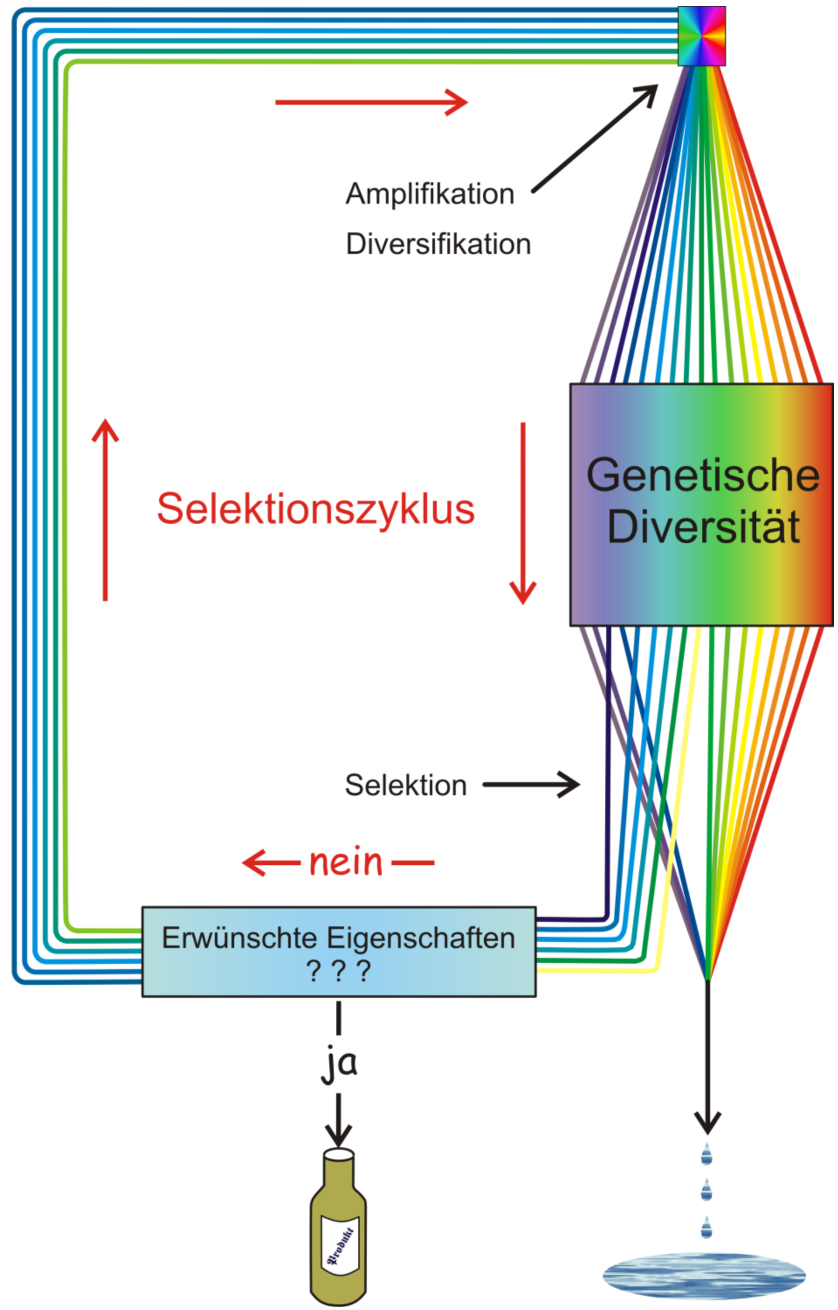
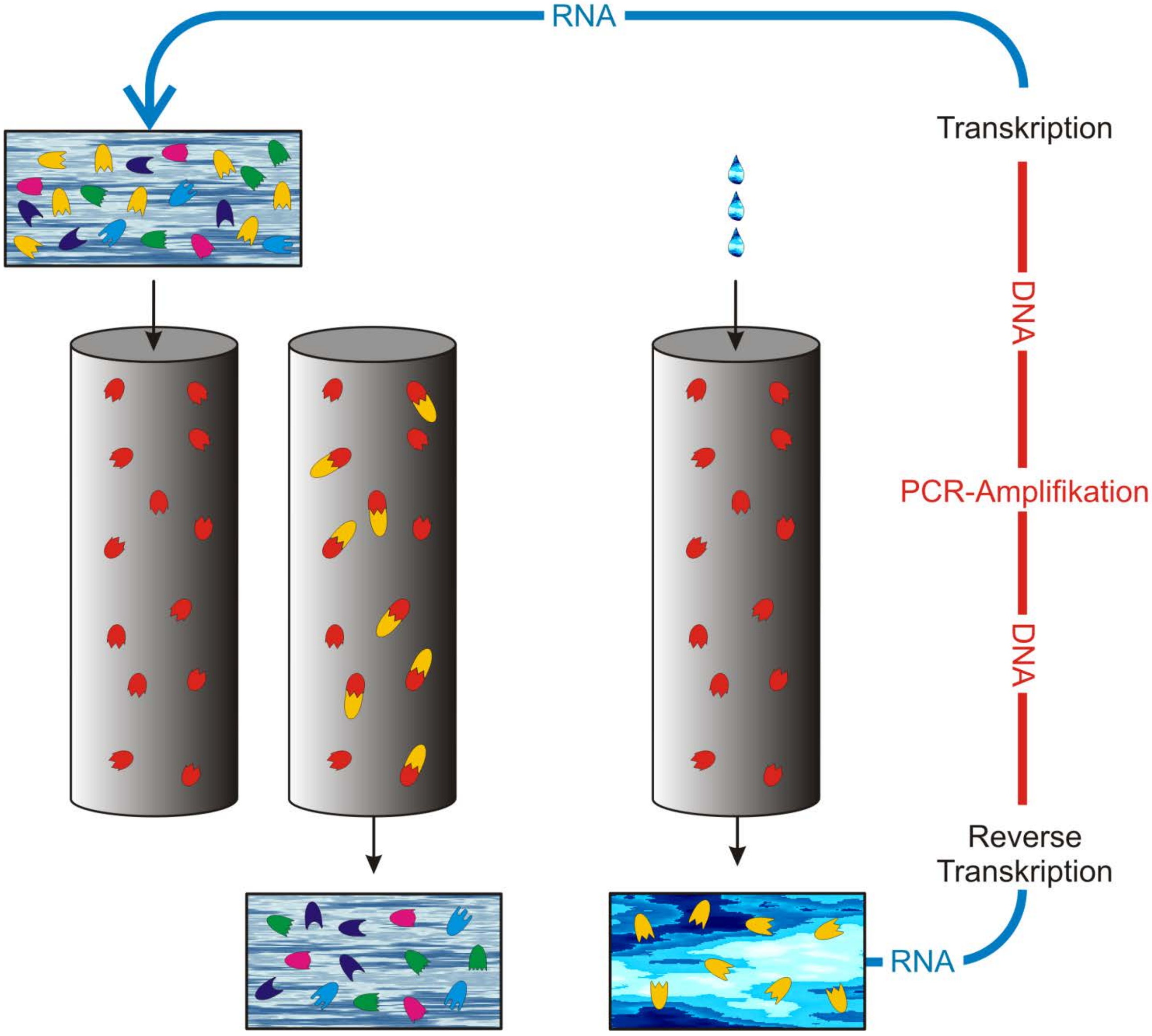
Available online 8 December 2004

Esteban Domingo
1943 -

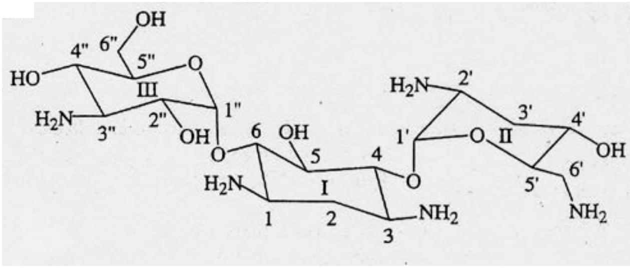
Application of quasispecies theory to the fight against viruses



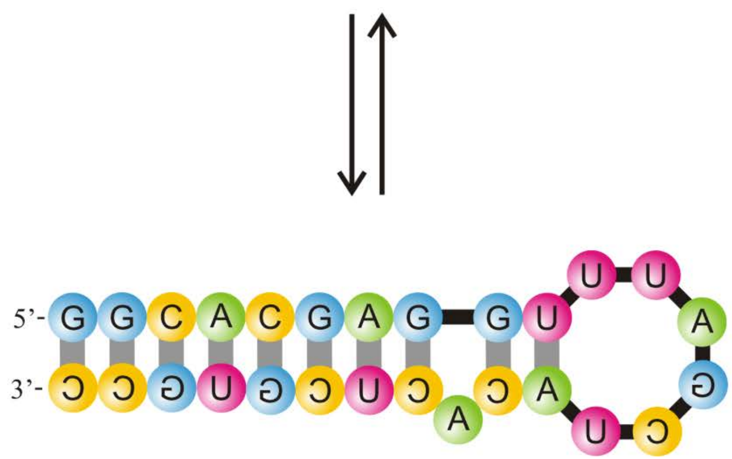
Ein Beispiel für Selektion von Molekülen mit vorbestimmbaren Eigenschaften im Laborexpriment



Die SELEX-Technik zur evolutionären Erzeugung von stark bindenden Molekülen



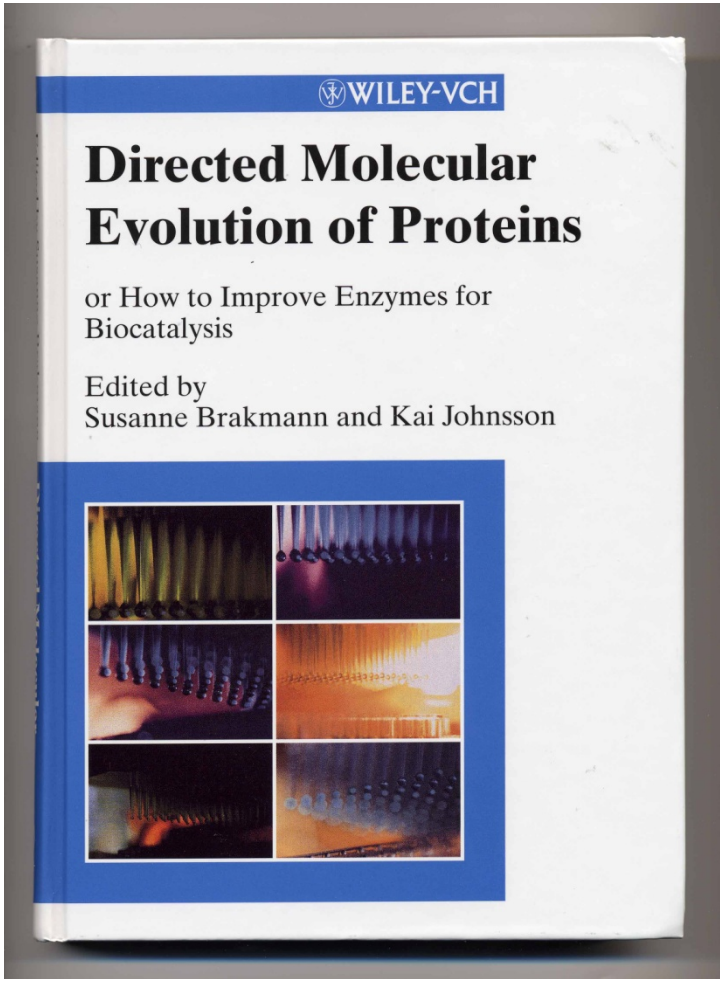
tobramycin

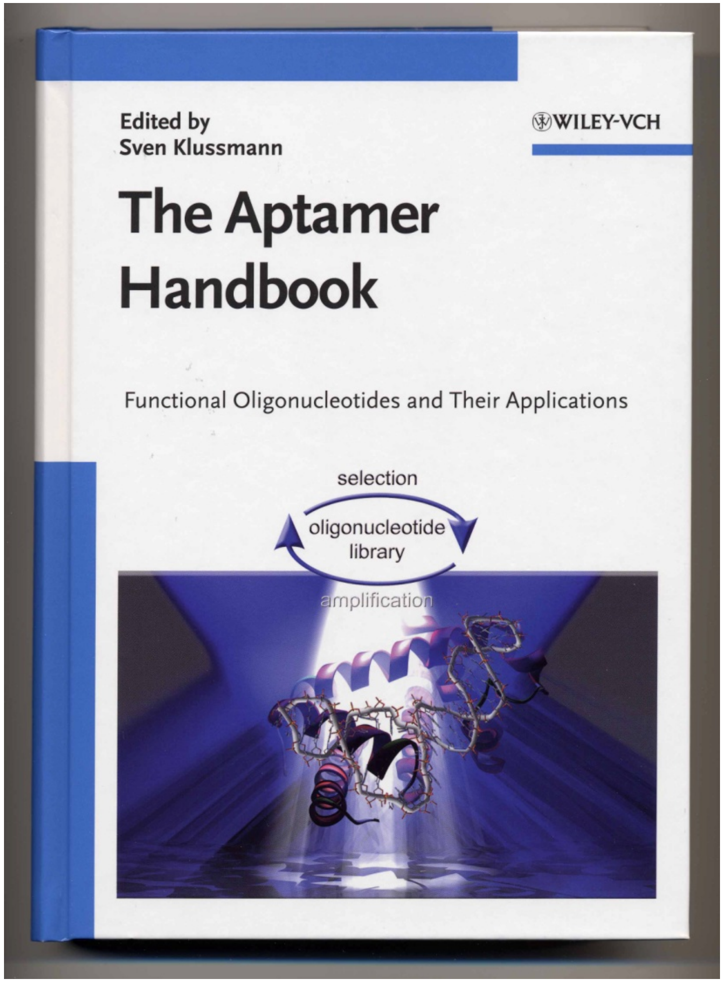


RNA aptamer

Formation of secondary structure of the tobramycin binding RNA aptamer with **K_D = 9 nM**

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, ***Saccharide-RNA recognition in an aminoglycoside antibiotic-RNA aptamer complex.*** Chemistry & Biology **4**:35-50 (1997)





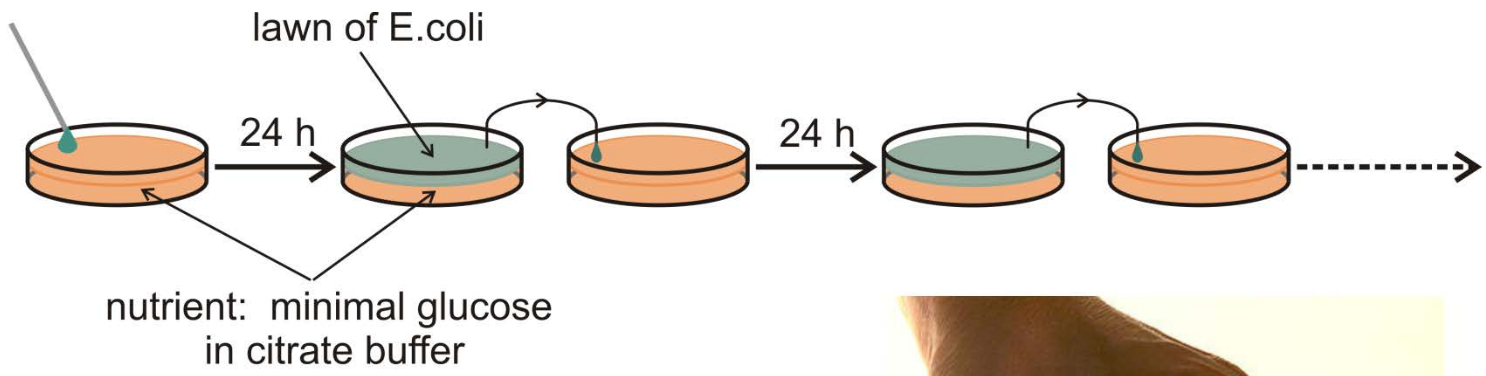
Application of molecular evolution to problems in biotechnology

1. Vom Ursprung des Lebens
2. Darwinsche Evolution von Molekülen
3. Evolutionäre Biotechnologie
4. **Evolutionsexperimente mit Bakterien**

Richard Lenski, 1956 -

Bacterial evolution under controlled conditions: A twenty-five years experiment.

Richard Lenski, University of Michigan, East Lansing



medium supports $\approx 5 \times 10^8$ bacteria

1 day $\approx$ 6.67 generations
1 month $\approx$ 200 generations
1 year $\approx$ 2400 generations

Serial transfer of bacterial cultures in Petri dishes

Bacterial evolution under controlled conditions: A twenty-five years experiment.

Richard Lenski, University of Michigan, East Lansing

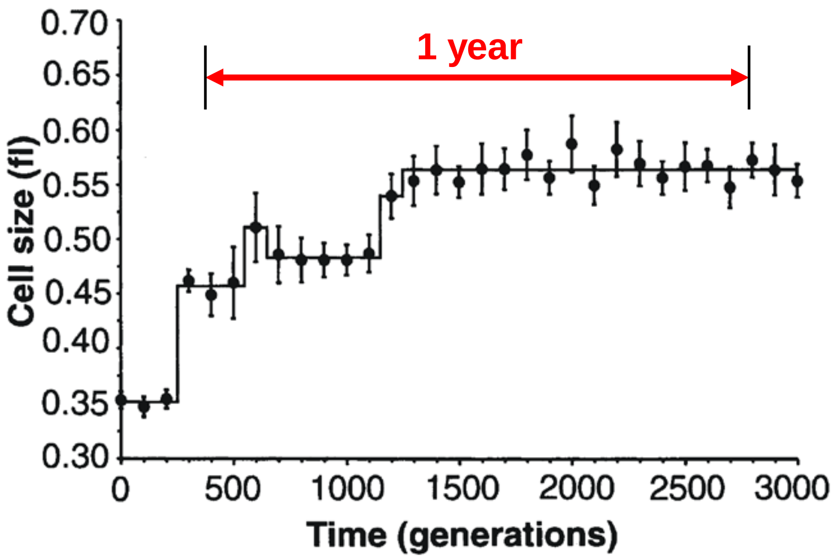


Fig. 1. Change in average cell size (1 fl = 10^{-15} L) in a population of *E. coli* during 3000 generations of experimental evolution. Each point is the mean of 10 replicate assays (22). Error bars indicate 95% confidence intervals. The solid line shows the best fit of a step-function model to these data (Table 1).

Epochal evolution of bacteria in serial transfer experiments under constant conditions

S. F. Elena, V. S. Cooper, R. E. Lenski. *Punctuated evolution caused by selection of rare beneficial mutants*. Science **272** (1996), 1802-1804

The twelve populations of Richard Lenski‘s long time evolution experiment
Enhanced turbidity in population A-3

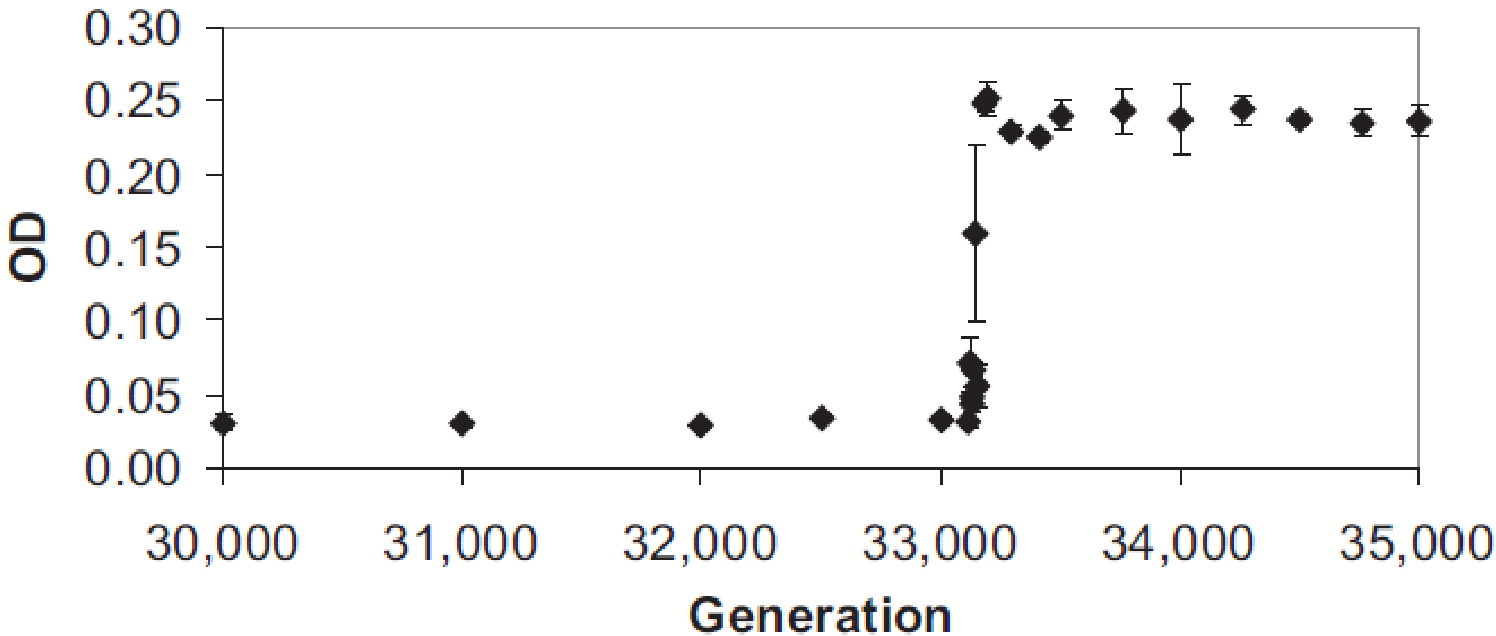


Fig. 1. Population expansion during evolution of the Cit^{+} phenotype. Samples frozen at various times in the history of population Ara-3 were revived, and three DM25 cultures were established for each generation. Optical density (OD) at 420 nm was measured for each culture at 24 h. Error bars show the range of three values measured for each generation.

Innovation by mutation in long time evolution of Escherichia coli in constant environment

Z.D. Blount, C.Z. Borland, R.E. Lenski. 2008. Proc.Natl.Acad.Sci.USA 105:7899-7906

Table 1. Summary of replay experiments

Generation	First experiment: Replicates	First experiment: Independent Cit$^+$ mutants	Second experiment: Replicates	Second experiment: Independent Cit$^+$ mutants	Third experiment: Replicates	Third experiment: Independent Cit$^+$ mutants
Ancestor	6	0	10	0	200	0
5,000	—	—	—	—	200	0
10,000	6	0	30	0	200	0
15,000	—	—	—	—	200	0
20,000	6	0	30	0	200	2
25,000	6	0	30	0	200	0
27,000	—	—	—	—	200	2
27,500	6	0	30	0	—	—
28,000	—	—	—	—	200	0
29,000	6	0	30	0	200	0
30,000	6	0	30	0	200	0
30,500	6	1	30	0	—	—
31,000	6	0	30	0	200	1
31,500	6	1	30	0	200	1
32,000	6	0	30	4	200	2
32,500	6	2	30	1	200	0
Totals	72	4	340	5	2,800	8

Contingency of E. coli evolution experiments

Walter Fontana, 1960 -

Evolution *in silico*

W. Fontana, P. Schuster,
Science **280** (1998), 1451-1455

random individuals. The primer pair used for genomic DNA amplification is 5′-TCTCCCTGGATTCT-CATTTA-3′ (forward) and 5′-TCTTTGTCTTCTGT-TCCACC-3′ (reverse). Reactions were performed in 25 μl using 1 unit of Taq DNA polymerase with each primer at 0.4 μM; 200 μM each dATP, dTTP, dGTP, and dCTP; and PCR buffer [10 mM tris-HCl (pH 8.3), 50 mM KCl_2,1.5 mM $MgCl_2$] in a cycle condition of 94°C for 1 min and then 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s followed by 72°C for 6 min. PCR products were purified (Qiagen), digested with Xmn I, and separated in a 2% agarose gel.

32. A nonsense mutation may affect mRNA stability and result in degradation of the transcript [L. Maquat, *Am. J. Hum. Genet.* **59**, 279 (1996)].
33. Data not shown; a dot blot with poly (A)⁺ RNA from 50 human tissues (The Human RNA Master Blot, 7770-1, Clontech Laboratories) was hybridized with a probe from exons 29 to 47 of *MYO15* using the same condition as Northern blot analysis (*13*).
34. Smith–Magenis syndrome (SMS) is due to deletions of 17p11.2 of various sizes, the smallest of which includes *MYO15* and perhaps 20 other genes [(*6*); K-S Chen, L. Potocki, J. R. Lupski, *MRDD Res. Rev.* **2**, 122 (1996)]. *MYO15* expression is easily detected in the pituitary gland (data not shown). Haploinsufficiency for *MYO15* may explain a portion of the SMS phenotype such as short stature. Moreover, a few SMS patients have sensorineural hearing loss, possibly because of a point mutation in *MYO15* in trans to the SMS 17p11.2 deletion.
35. R. A. Fridell, data not shown.
36. K. B. Avraham *et al.*, *Nature Genet.* **11**, 369 (1995); X-Z. Liu *et al.*, *ibid.* **17**, 268 (1997); F. Gibson *et al.*, *Nature* **374**, 62 (1995); D. Weil *et al.*, *ibid.*, p. 60.
37. RNA was extracted from cochlea (membranous labyrinths) obtained from human fetuses at 18 to 22 weeks of development in accordance with guidelines established by the Human Research Committee at the Brigham and Women's Hospital. Only samples without evidence of degradation were pooled for poly (A)⁺ selection over oligo(dT) columns. First-strand cDNA was prepared using an Advantage RT-for-PCR kit (Clontech Laboratories). A portion of the first-strand cDNA (4%) was amplified by PCR with Advantage cDNA polymerase mix (Clontech Laboratories) using human *MYO15*-specific oligonucleotide primers (forward, 5′-GCATGACCTGCCGGCTAAT-GGG-3′; reverse, 5′-CTCACGGCTTCTGCATGGT-GCTCGGCTGGC-3′). Cycling conditions were 40 s at 94°C; 40 s at 66°C (3 cycles), 60°C (5 cycles), and 55°C (29 cycles); and 45 s at 68°C. PCR products were visualized by ethidium bromide staining after fractionation in a 1% agarose gel. A 688-bp PCR product is expected from amplification of the human *MYO15* cDNA. Amplification of human genomic DNA with this primer pair would result in a 2903-bp fragment.
38. We are grateful to the people of Bengkala, Bali, and the two families from India. We thank J. R. Lupski and K.-S. Chen for providing the human chromosome 17 cosmid library. For technical and computational assistance, we thank N. Dietrich, M. Fergusson, A. Gupta, E. Sorbello, R. Torkzadeh, C. Varner, M. Walker, G. Bouffard, and S. Beckstrom-Sternberg (National Institutes of Health Intramural Sequencing Center). We thank J. T. Hinnant, I. N. Arhya, and S. Winata for assistance in Bali, and T. Barber, S. Sullivan, E. Green, D. Drayna, and J. Battey for helpful comments on this manuscript. Supported by the National Institute on Deafness and Other Communication Disorders (NIDCD) (Z01 DC 00035-01 and Z01 DC 00038-01 to T.B.F. and E.R.W. and R01 DC 03402 to C.C.M.), the National Institute of Child Health and Human Development (R01 HD30428 to S.A.C.) and a National Science Foundation Graduate Research Fellowship to F.J.P. This paper is dedicated to J. B. Snow Jr. on his retirement as the Director of the NIDCD.

9 March 1998; accepted 17 April 1998

Continuity in Evolution: On the Nature of Transitions

Walter Fontana and Peter Schuster

To distinguish continuous from discontinuous evolutionary change, a relation of nearness between phenotypes is needed. Such a relation is based on the probability of one phenotype being accessible from another through changes in the genotype. This nearness relation is exemplified by calculating the shape neighborhood of a transfer RNA secondary structure and provides a characterization of discontinuous shape transformations in RNA. The simulation of replicating and mutating RNA populations under selection shows that sudden adaptive progress coincides mostly, but not always, with discontinuous shape transformations. The nature of these transformations illuminates the key role of neutral genetic drift in their realization.

A much-debated issue in evolutionary biology concerns the extent to which the history of life has proceeded gradually or has been punctuated by discontinuous transitions at the level of phenotypes (*1*). Our goal is to make the notion of a discontinuous transition more precise and to understand how it arises in a model of evolutionary adaptation.

We focus on the narrow domain of RNA secondary structure, which is currently the simplest computationally tractable, yet realistic phenotype (*2*). This choice enables the definition and exploration of concepts that may prove useful in a wider context. RNA secondary structures represent a coarse level of analysis compared with the three-dimensional structure at atomic resolution. Yet, secondary structures are empirically well defined and obtain their biophysical and biochemical importance from being a scaffold for the tertiary structure. For the sake of brevity, we shall refer to secondary structures as "shapes." RNA combines in a single molecule both genotype (replicatable sequence) and phenotype (selectable shape), making it ideally suited for in vitro evolution experiments (*3*, *4*).

To generate evolutionary histories, we used a stochastic continuous time model of an RNA population replicating and mutating in a capacity-constrained flow reactor under selection (*5*, *6*). In the laboratory, a goal might be to find an RNA aptamer binding specifically to a molecule (*4*). Although in the experiment the evolutionary end product was unknown, we thought of its shape as being specified implicitly by the imposed selection criterion. Because our intent is to study evolutionary histories rather than end products, we defined a target shape in advance and assumed the replication rate of a sequence to be a function of the similarity between its shape and the target. An actual situation may involve more than one best shape, but this does not affect our conclusions.

An instance representing in its qualitative features all the simulations we performed is shown in Fig. 1A. Starting with identical sequences folding into a random shape, the simulation was stopped when the population became dominated by the target, here a canonical tRNA shape. The black curve traces the average distance to the target (inversely related to fitness) in the population against time. Aside from a short initial phase, the entire history is dominated by steps, that is, flat periods of no apparent adaptive progress, interrupted by sudden approaches toward the target structure (*7*). However, the dominant shapes in the population not only change at these marked events but undergo several fitness-neutral transformations during the periods of no apparent progress. Although discontinuities in the fitness trace are evident, it is entirely unclear when and on the basis of what the series of successive phenotypes itself can be called continuous or discontinuous.

A set of entities is organized into a (topological) space by assigning to each entity a system of neighborhoods. In the present case, there are two kinds of entities: sequences and shapes, which are related by a thermodynamic folding procedure. The set of possible sequences (of fixed length) is naturally organized into a space because point mutations induce a canonical neighborhood. The neighborhood of a sequence consists of all its one-error mutants. The problem is how to organize the set of possible shapes into a space. The issue arises because, in contrast to sequences, there are

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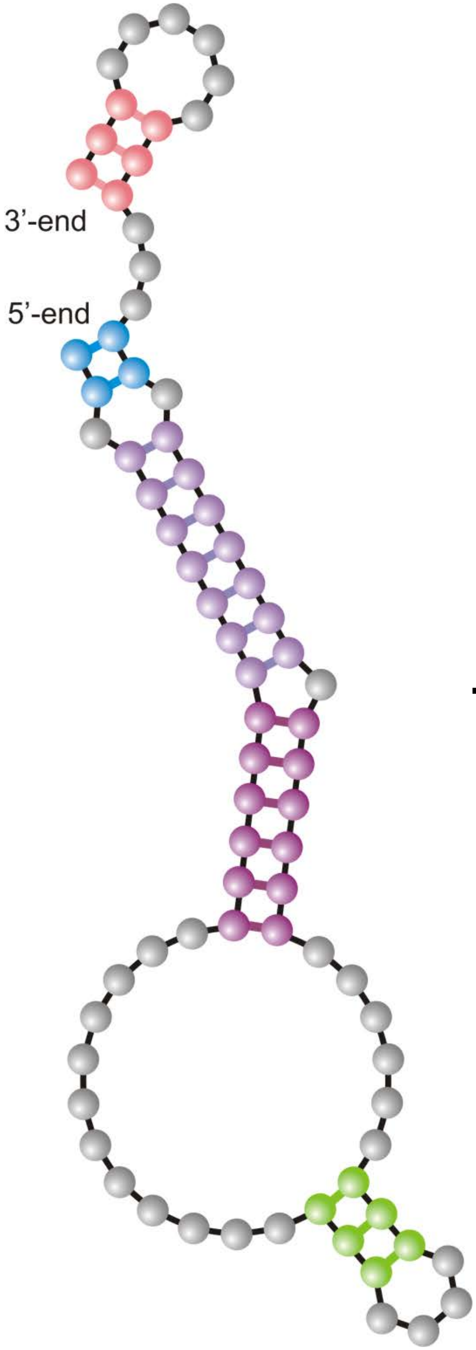
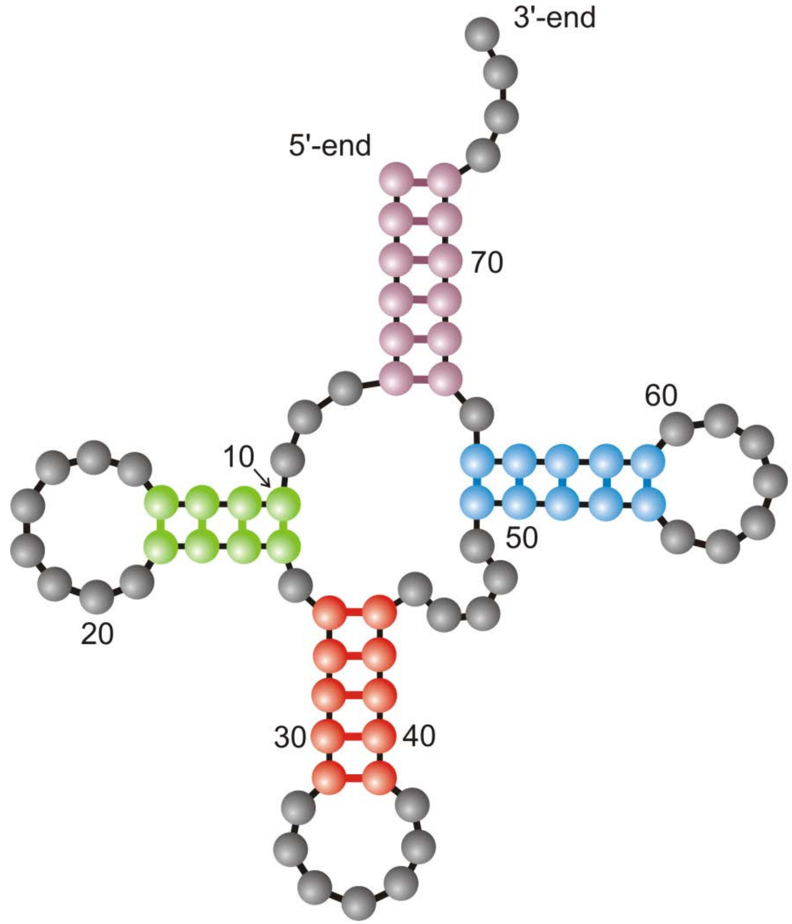
Von kleinen Molekülen zu molekularen Replikatoren:

drei Beispiele

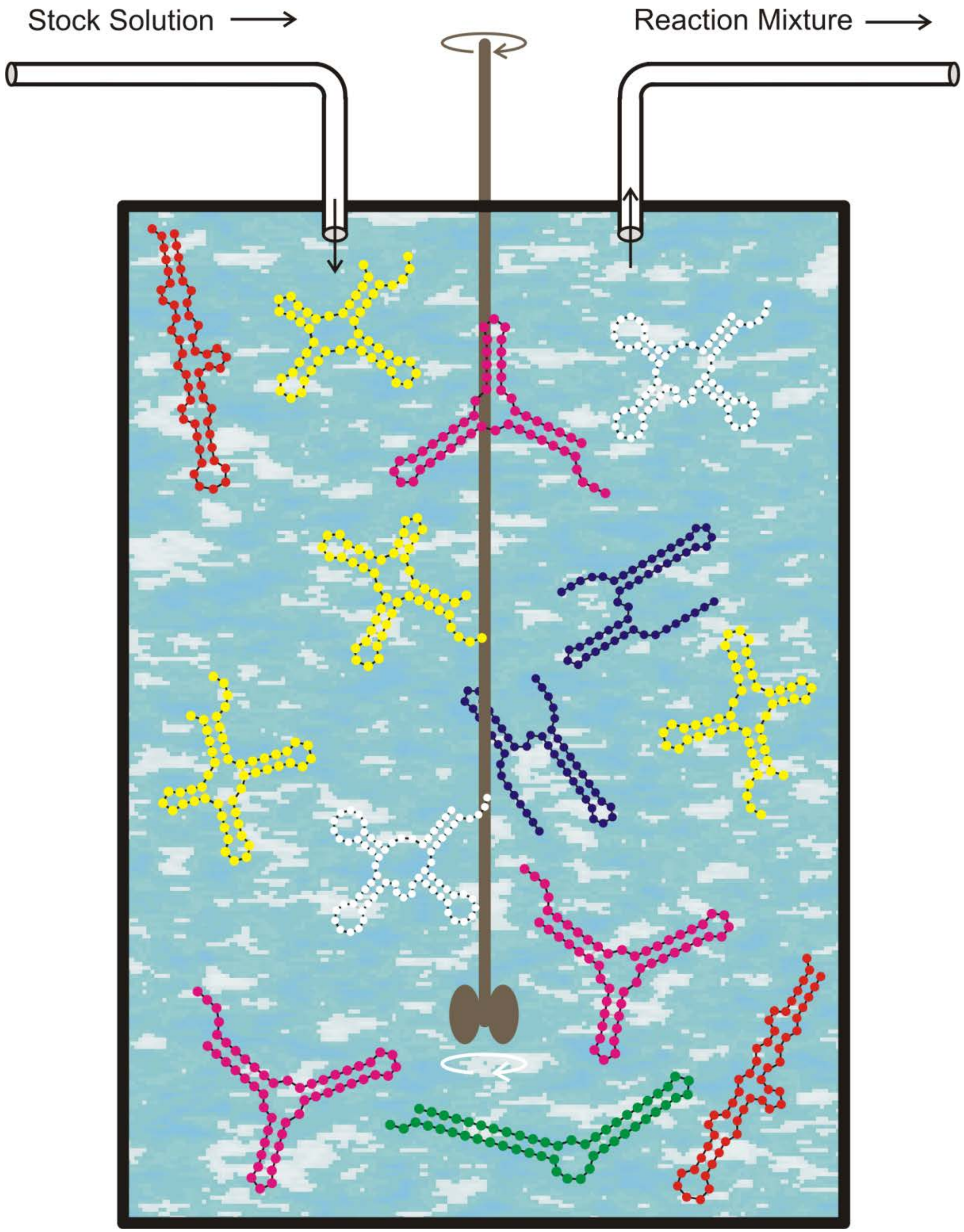
1. **Woher kommen die Bausteine des Lebens?**
2. Der Ursprung der Chiralität
3. Einfache Metabolismen



Structure of
randomly chosen
initial sequence



Phenylalanyl-tRNA as
target structure



Replication rate constant (Fitness):

$$f_k = \gamma \,/\, [\alpha + \Delta d_S^{(k)}]$$

$$\Delta d_S^{(k)} = d_H(S_k, S_\tau)$$

Selection pressure:

The population size,

N = # RNA moleucles,

is determined by the flux:

$$N(t) \approx \overline{N} \pm \sqrt{\overline{N}}$$

Mutation rate:

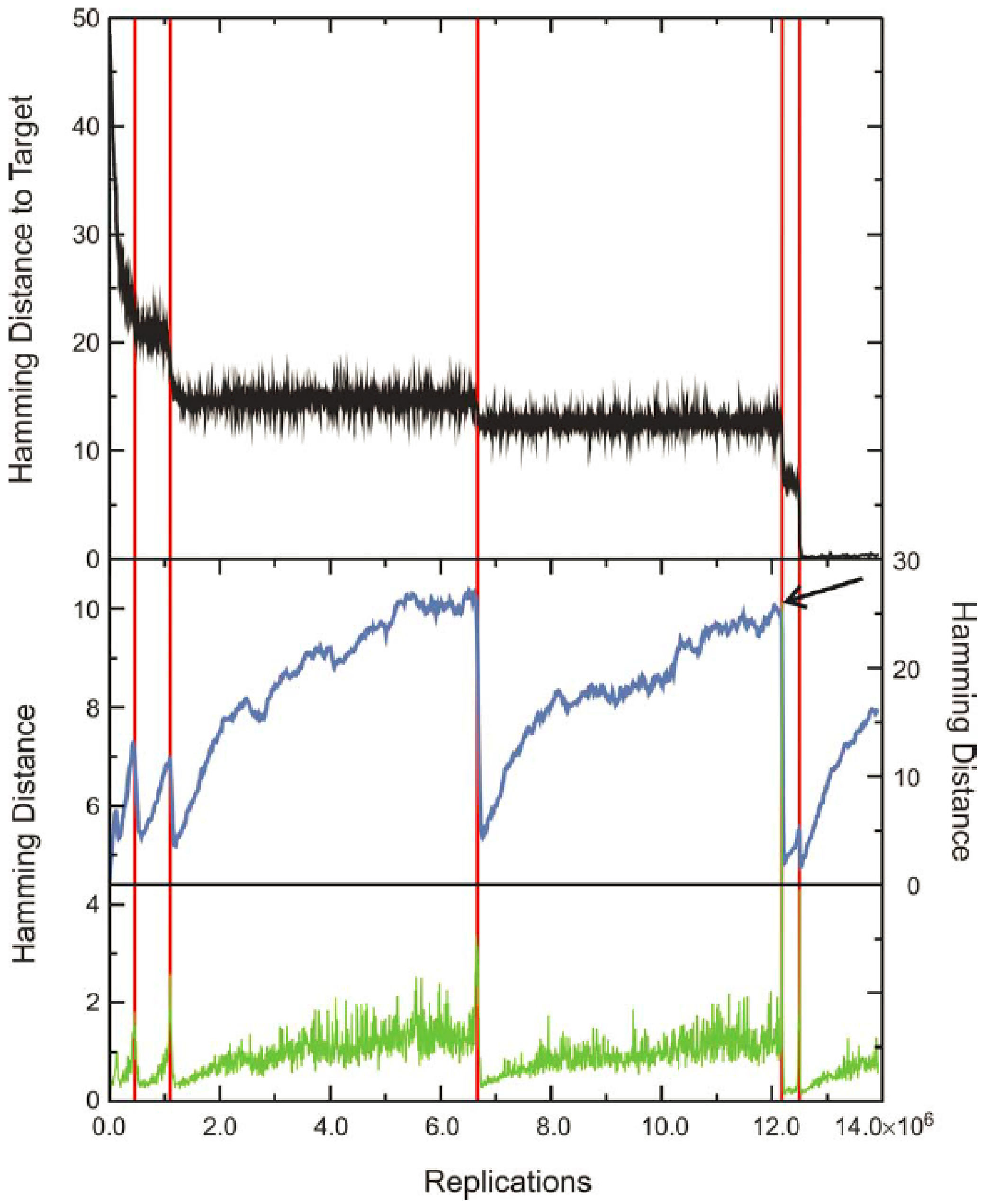
p = 0.001 / Nucleotide × Replication

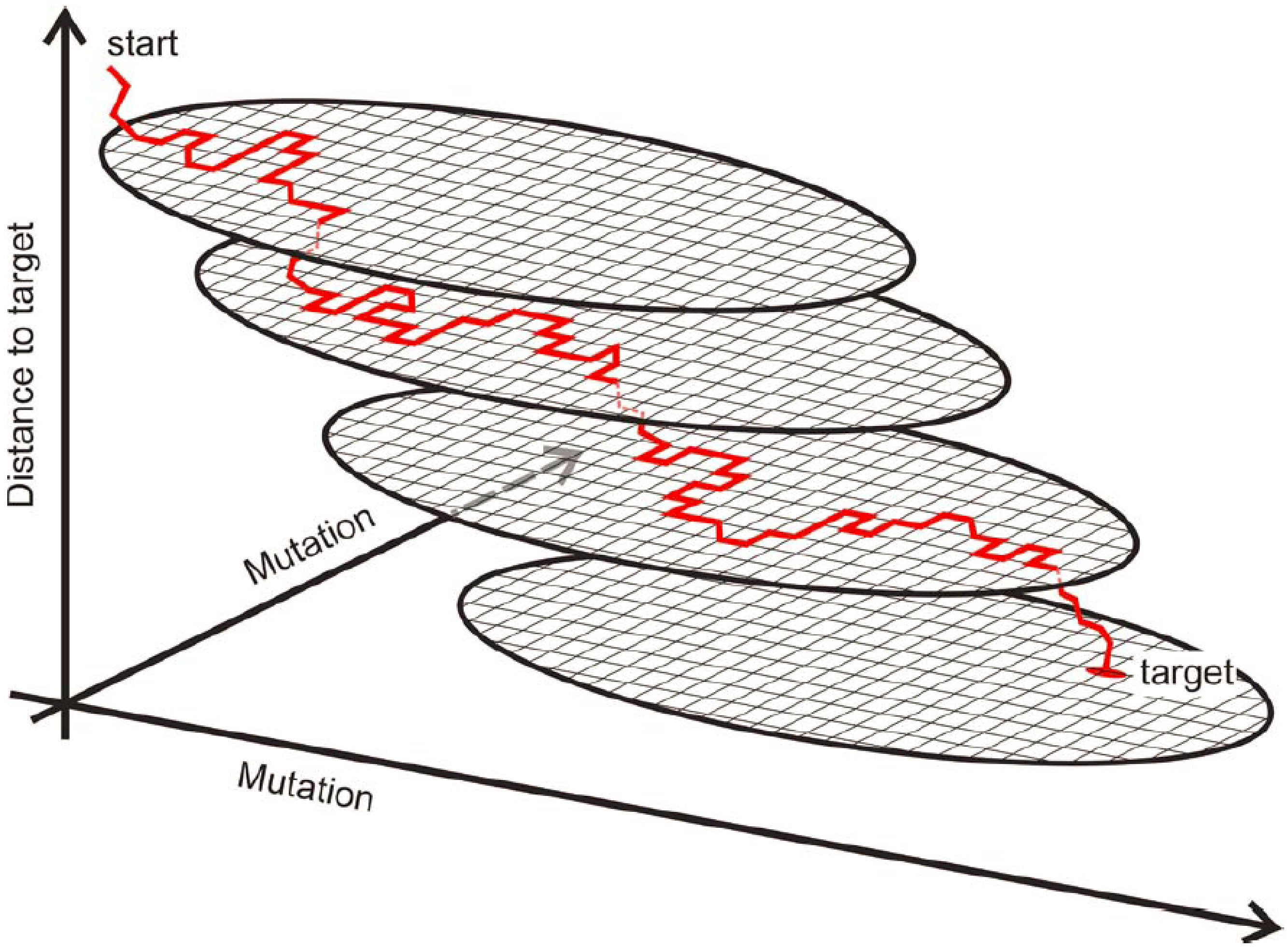
The flow reactor as a device for studying the evolution of molecules ***in vitro*** and ***in silico***.

Evolutionary trajectory

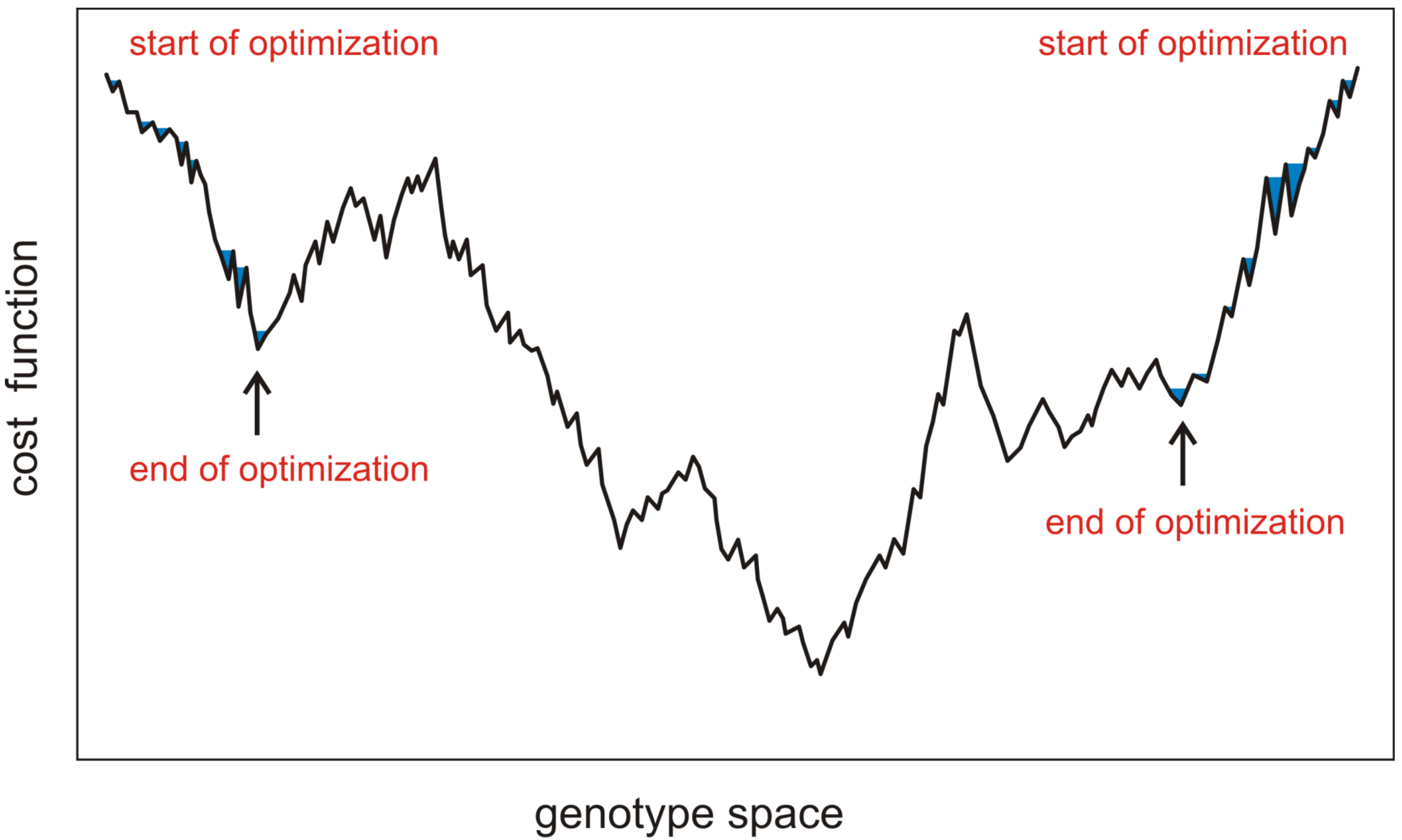
Spreading of the population on neutral networks

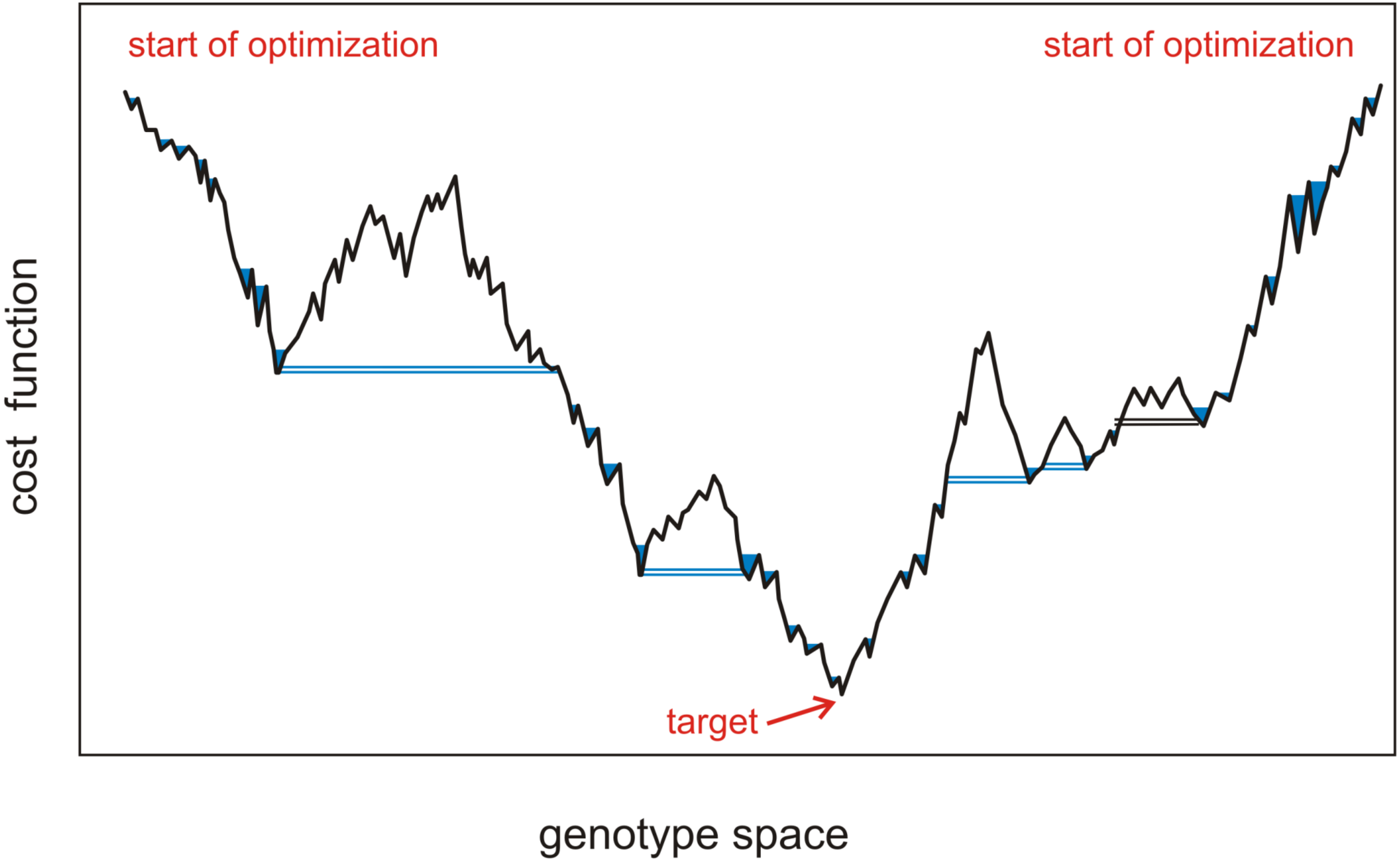
Drift of the population center in sequence space

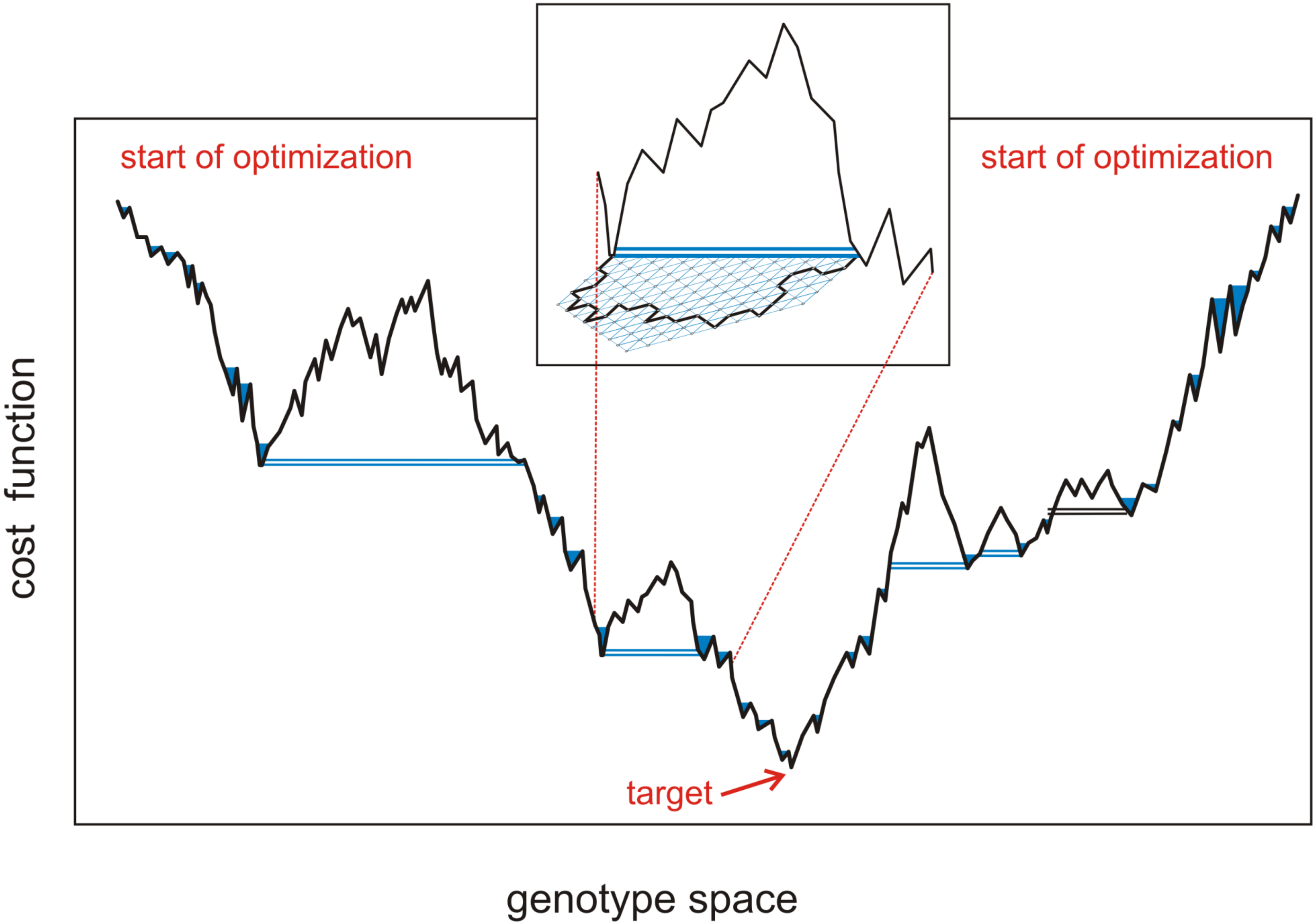




A sketch of optimization on neutral networks


start of optimization
start of optimization
cost function
end of optimization
end of optimization
genotype space


start of optimization
start of optimization
cost function
target
genotype space



start of optimization
start of optimization
cost function
target
genotype space

Danke für die Aufmerksamkeit!

Web-Page für weitere Informationen:

http://www.tbi.univie.ac.at/~pks