Evolution: Von der Chemie zur Molekulargenetik

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Fallstudien zur naturwissenschaftlichen Erkenntnis

Wien, 08.11.2016

Web-Page für weitere Informationen:

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

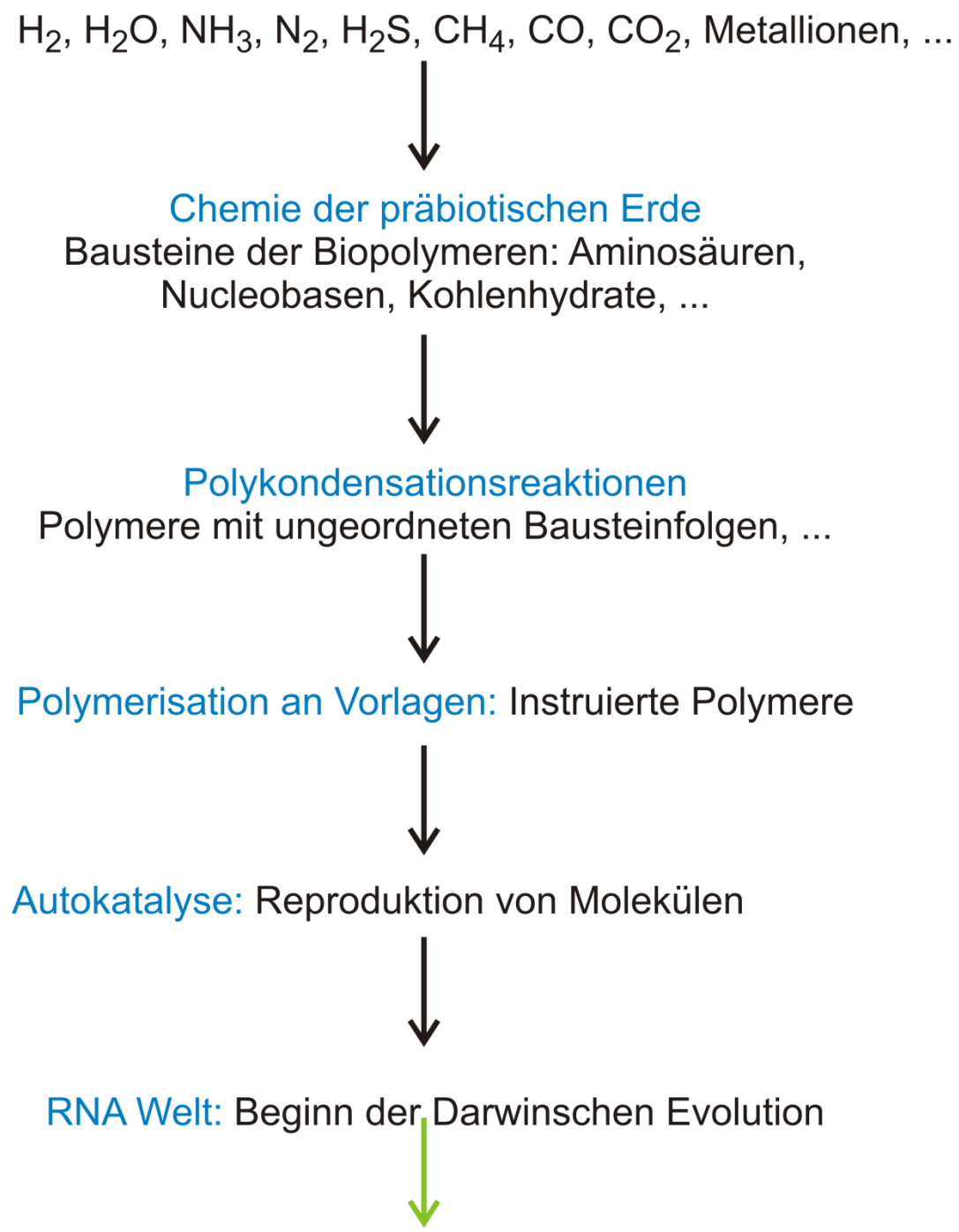
Chemische Evolution

Biologische Evolution

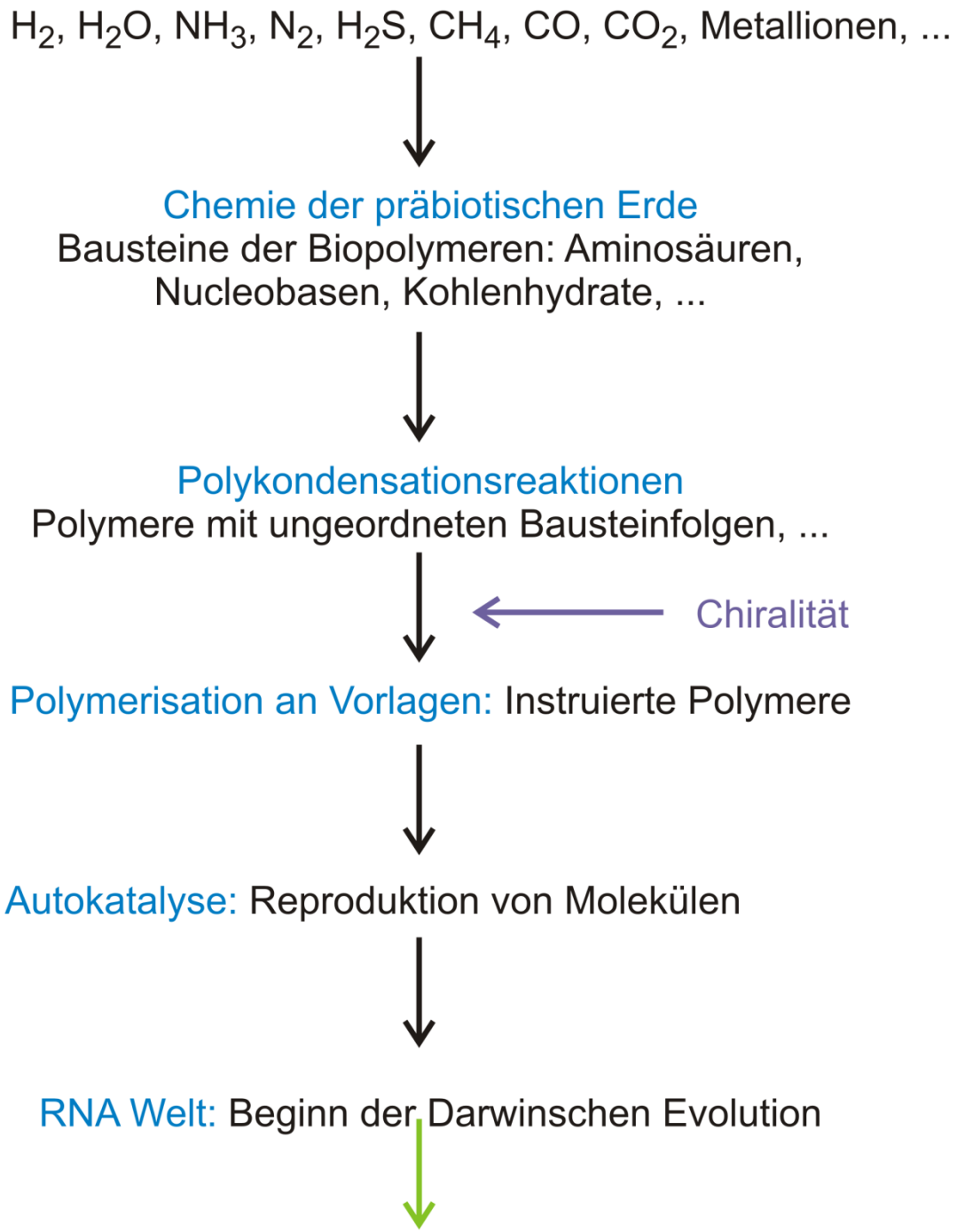
Von kleinen Molekülen zu molekularen Replikatoren

1. Woher kommen die Bausteine des Lebens?
2. Der Ursprung der Chiralität
3. Einfache Metabolismen und Recycling
4. Ribonukleinsäuren – RNA-Welt
5. Desoxyribonukleinsäuren und Proteine

Woher kommen die Bausteine des Lebens?



Präbiotische Chemie:
Von kleinen Molekülen zu molekularen Replikatoren



Präbiotische Chemie:
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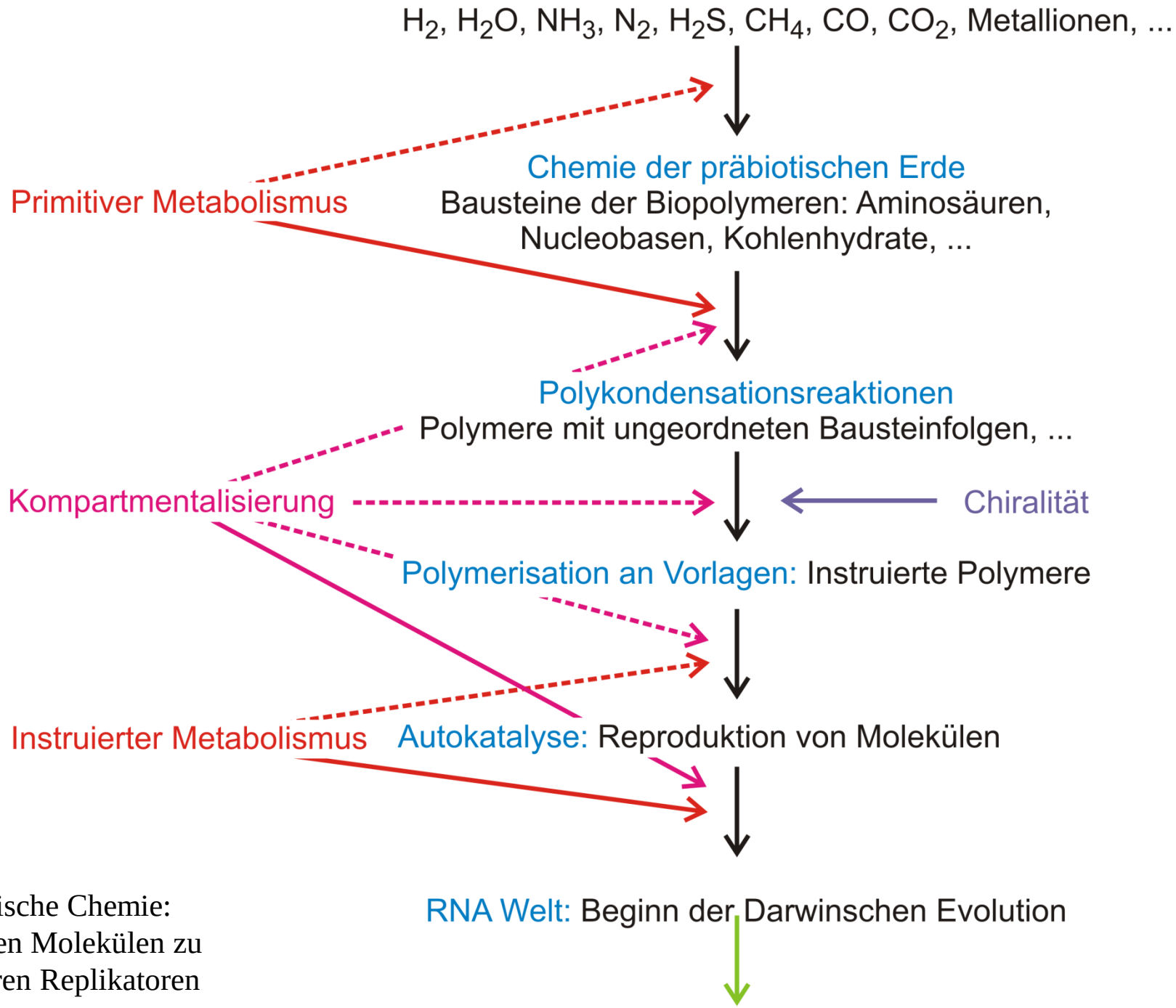
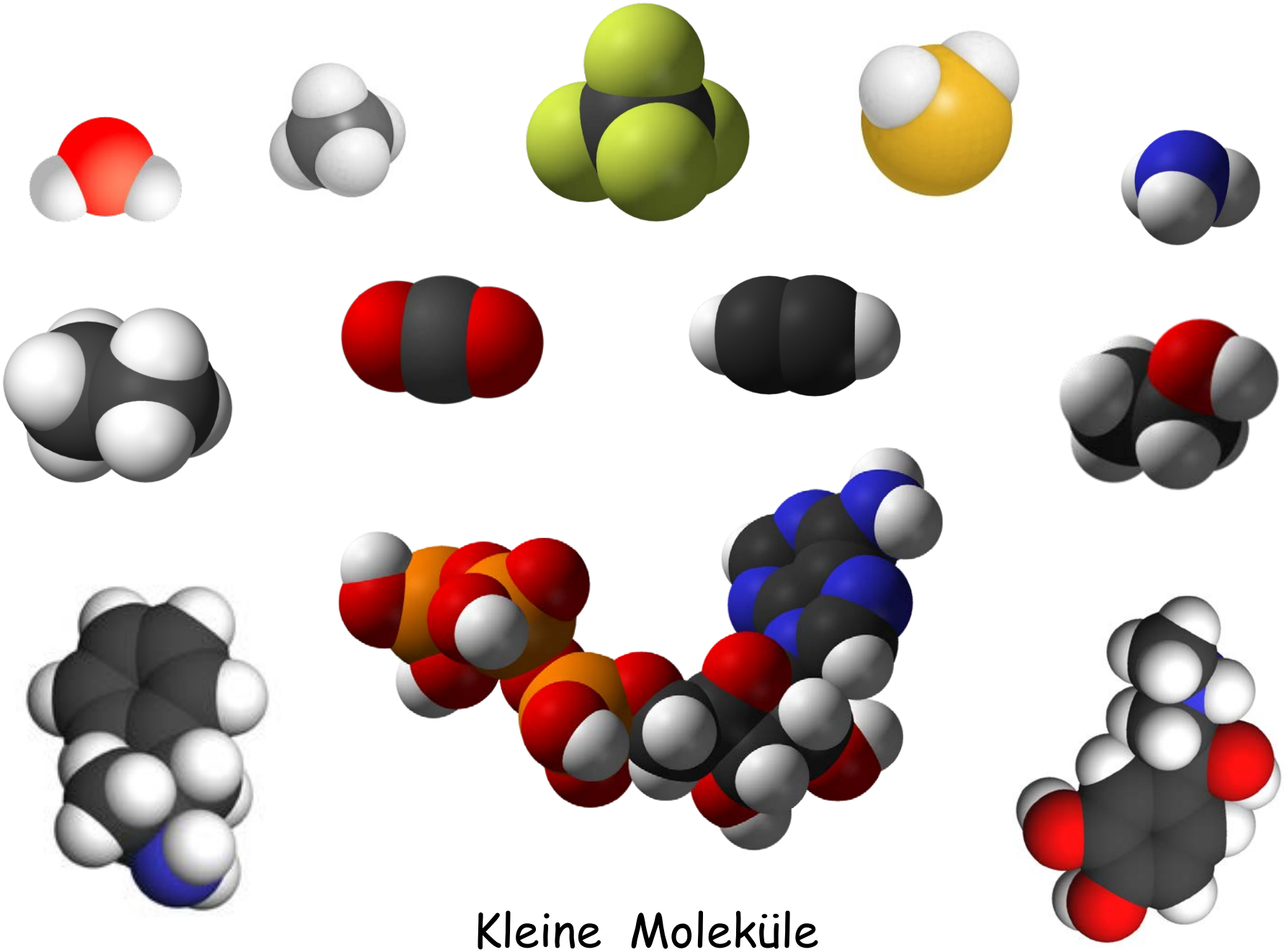


Präbiotische Chemie:
Von kleinen Molekülen zu molekularen Replikatoren

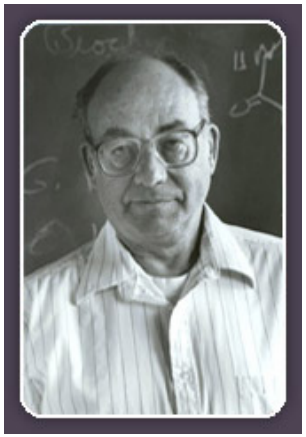


Kleine Moleküle

‚Horsehead‘ nebula in orion contains a huge dark cloud

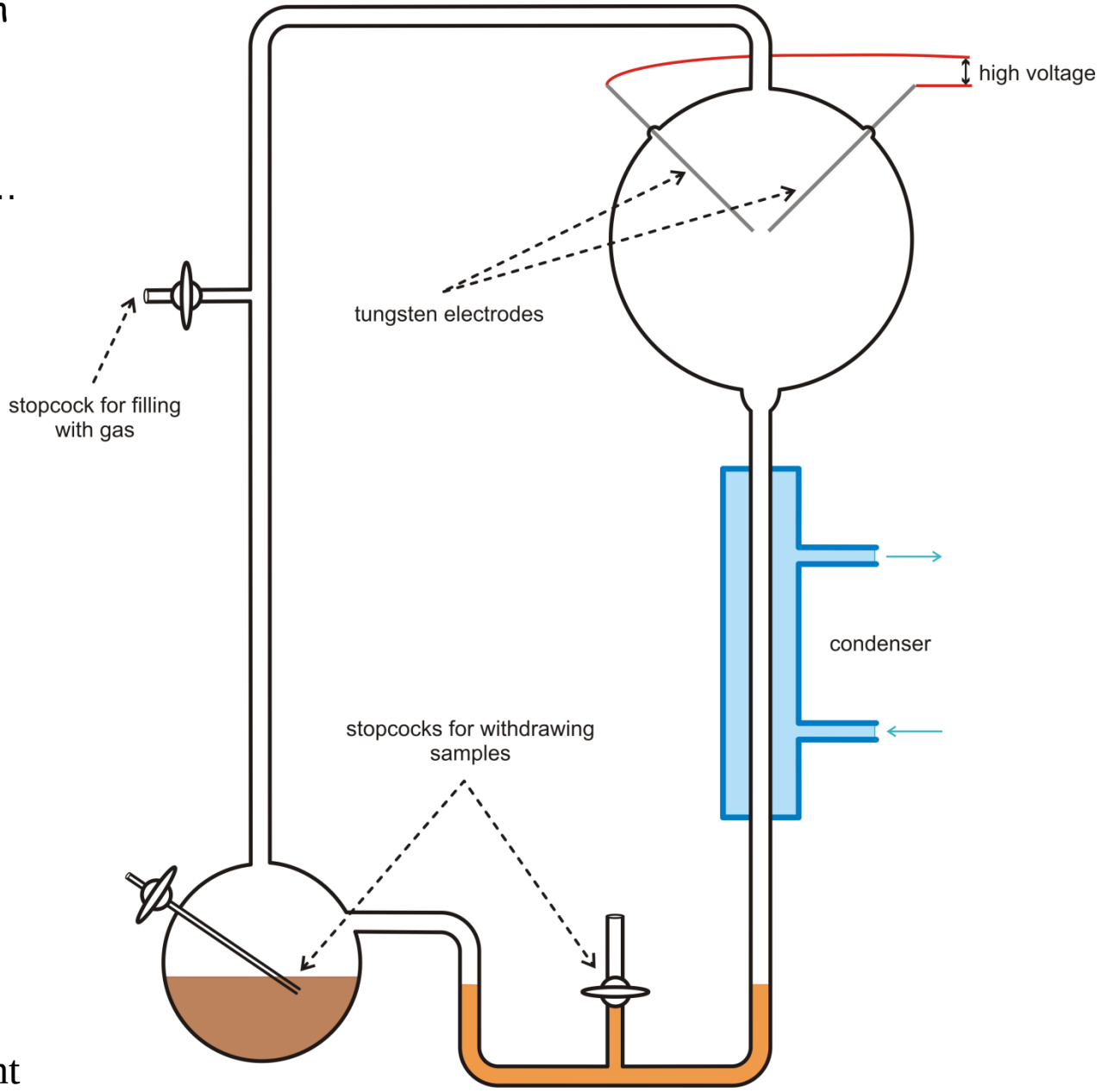
Elektrische Entladung in einer reduzierenden Atmosphäre:

CH_4, CO, NH_3, H_2O, H_2, …

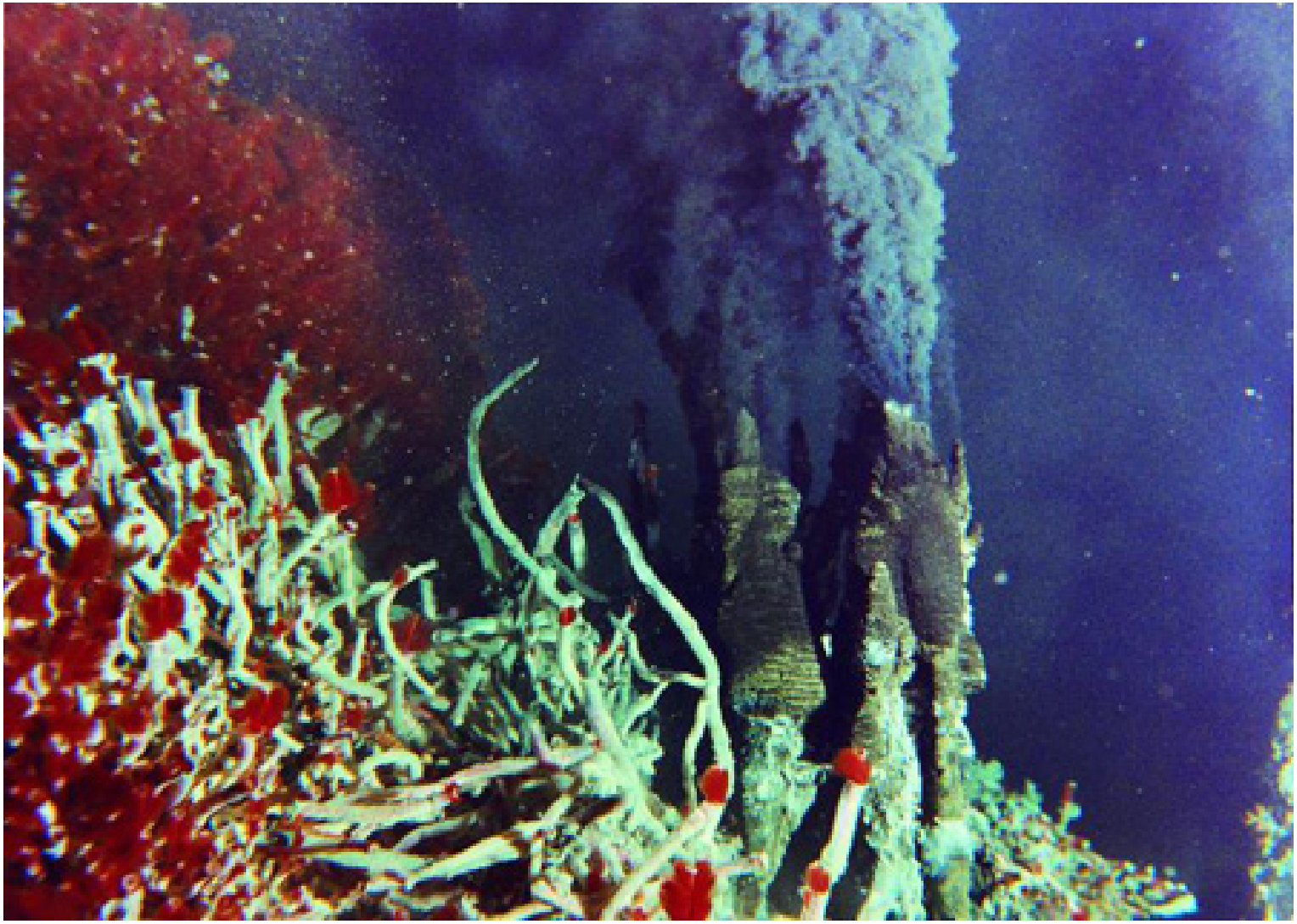


Stanley Miller, 1930 - 2007

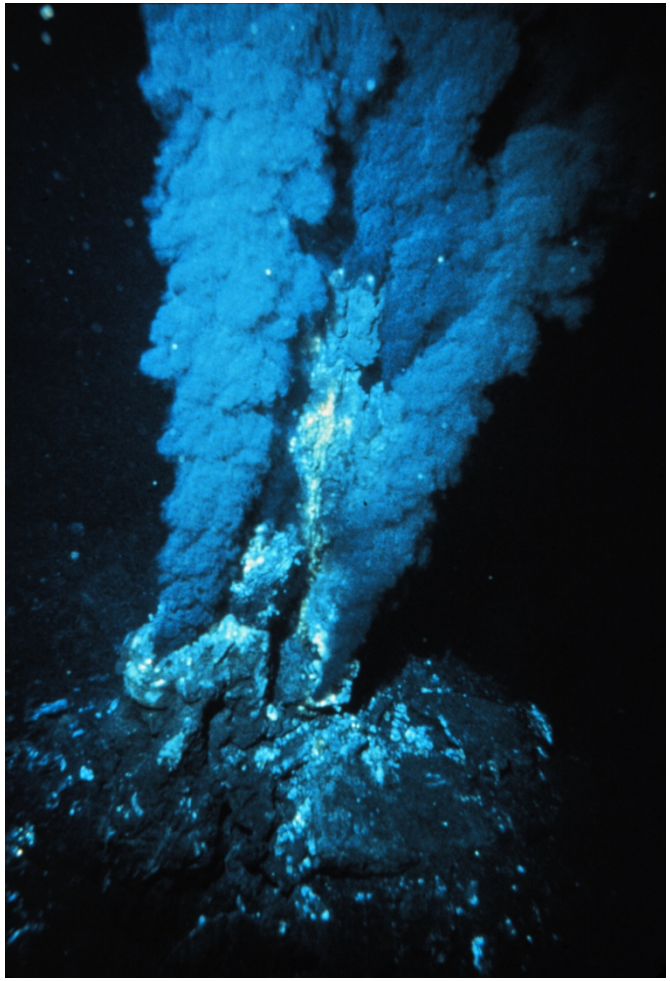
S.L.Miller. 1953. A production of amino acids under possible primitive earth conditions. *Science* **117**:528-529



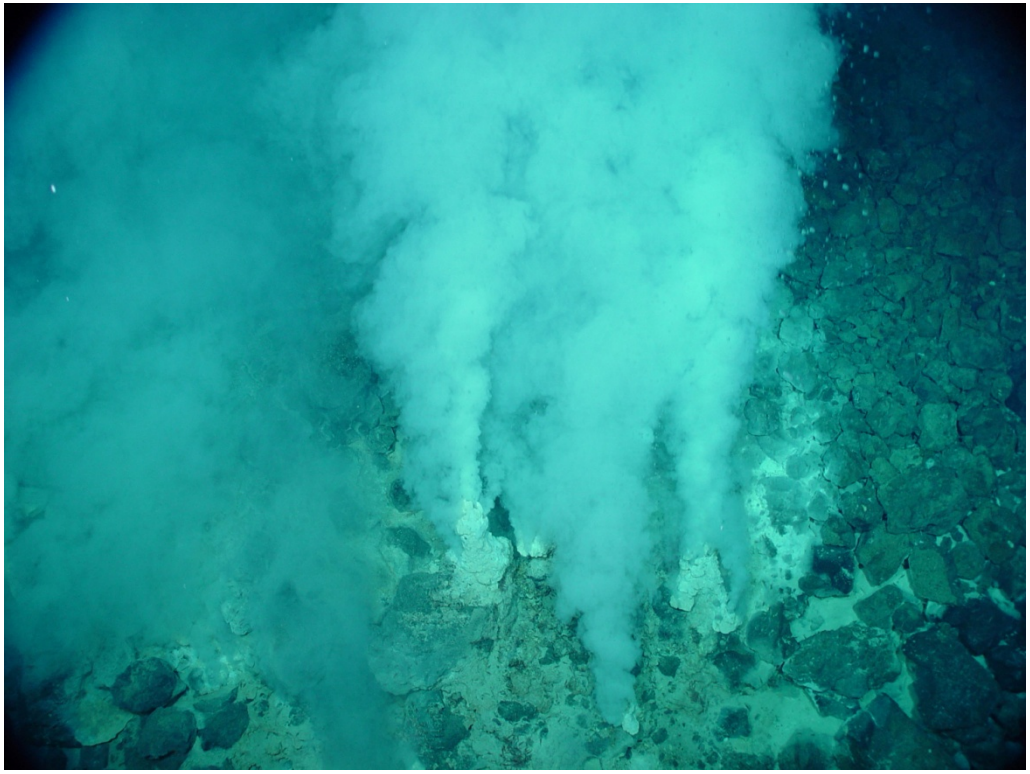
Das Miller-Urey Experiment



A hydrothermal vent



black smoker



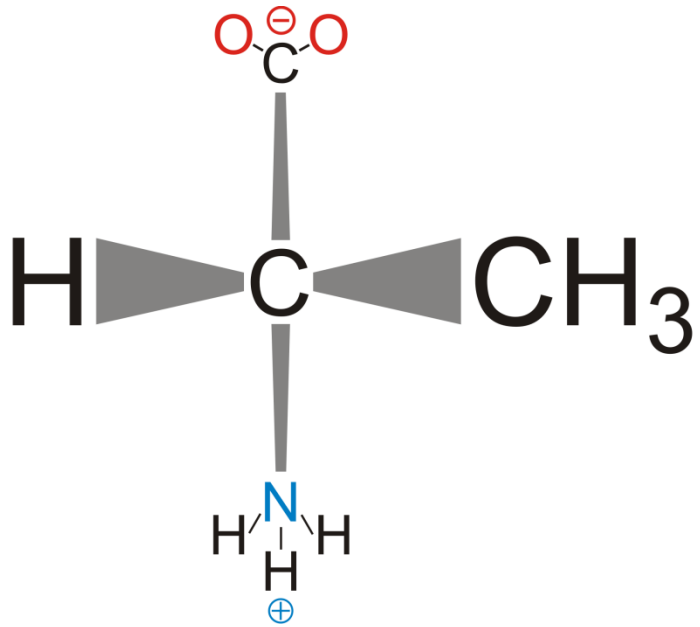
white smoker

Hydrothermale Quellen in der Tiefsee

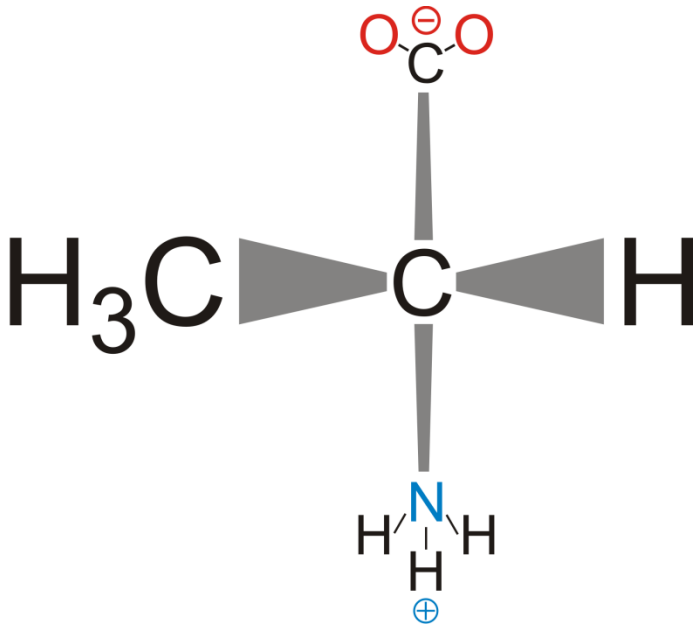
Vorkommen: mid-atlantic ridge, east pacific rise, …
in etwa 3000 m Tiefe

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

Der Ursprung der Chiralität

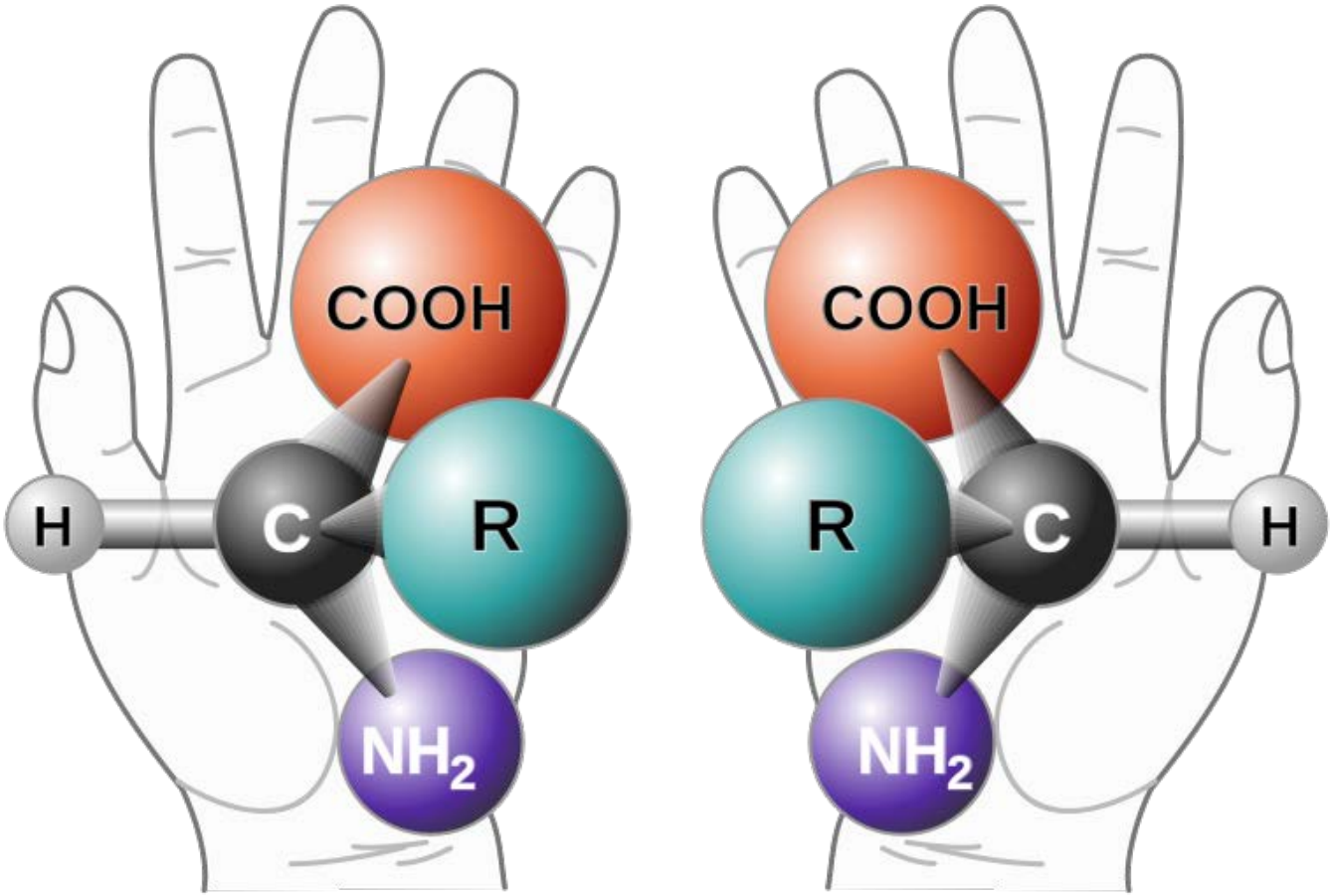


L- (S-) *Alanin*



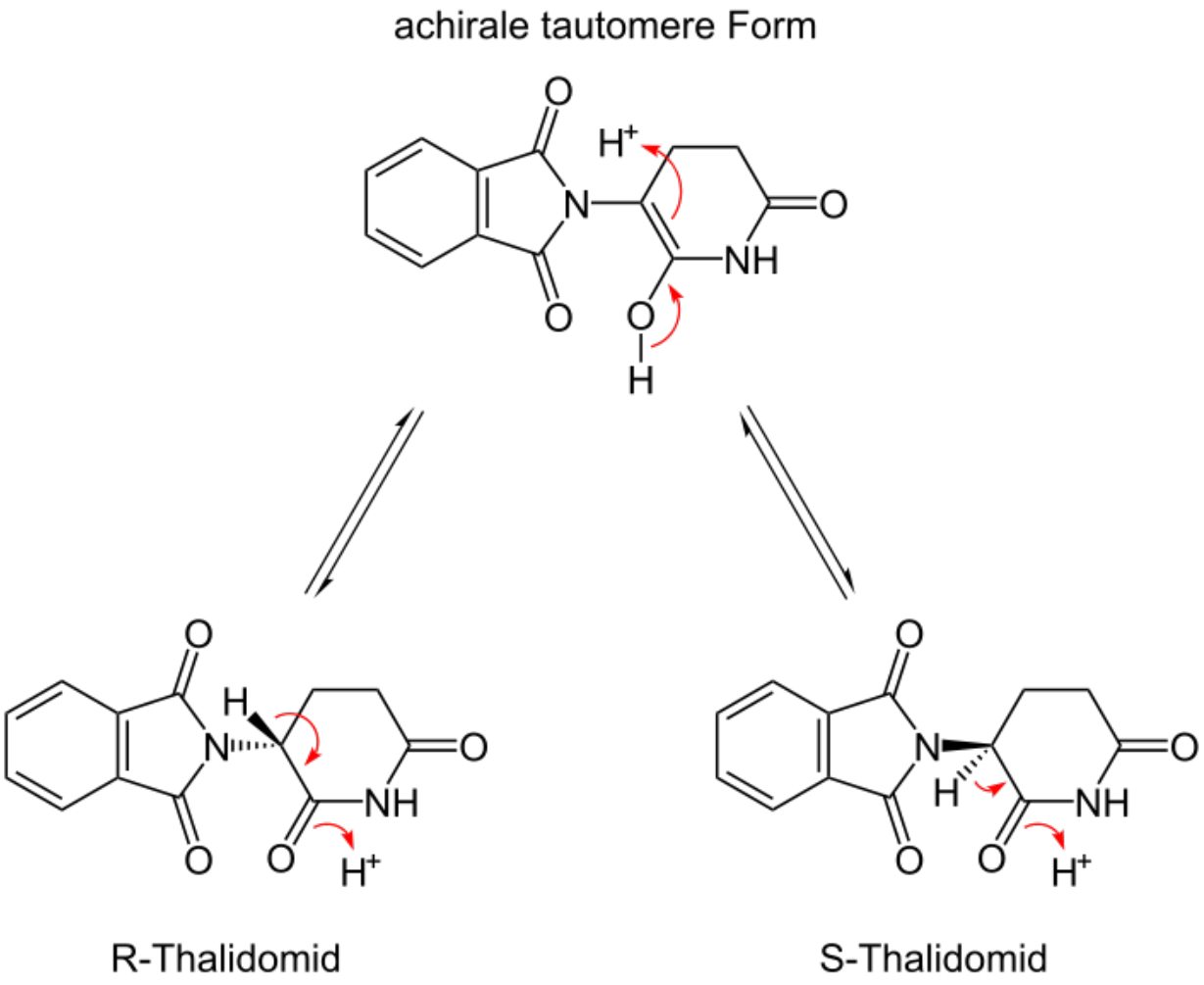
D- (R-) *Alanin*

Die zwei chiralen Formen von *Alanin*



L-Aminosäure **D-Aminosäure**

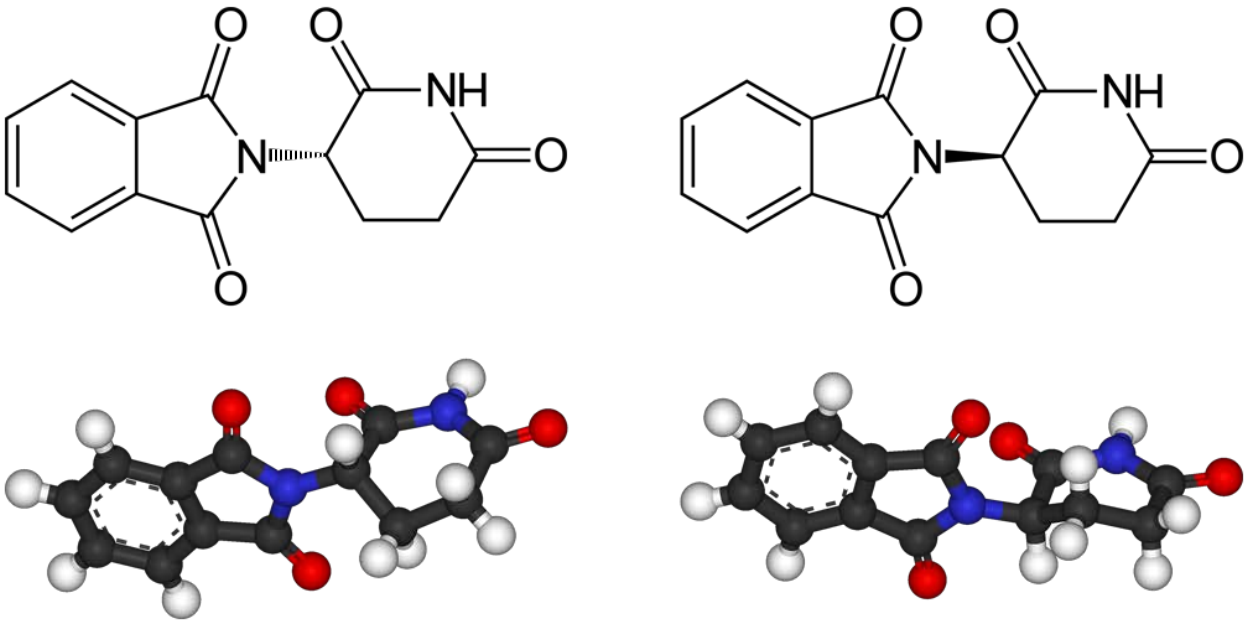
Chiralität der Biomoleküle



Sedativum

teratogene Wirkung

Thalidomid (Contergan)



Sedativum

teratogene Wirkung

Thalidomid (Contergan)

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

Michael Mauksch and
Svetlana Tsogoeva 2007

Reaktionen mit einem etwas erweiterten Frank Mechanismus

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.

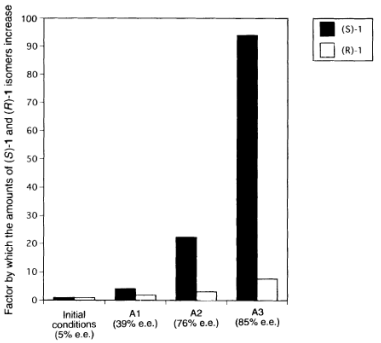


FIG 1. Asymmetric autocatalysis of chiral pyrimidyl alkanol (**1**). Runs A1–3 correspond to Table 1. The enantiomeric excess of (S)-**1** increases from 5 to 89% e.e. without the use of additional chiral auxiliaries. During the reactions (runs A1–3), the (*S*)-**1** increases by a factor of 94 times,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

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

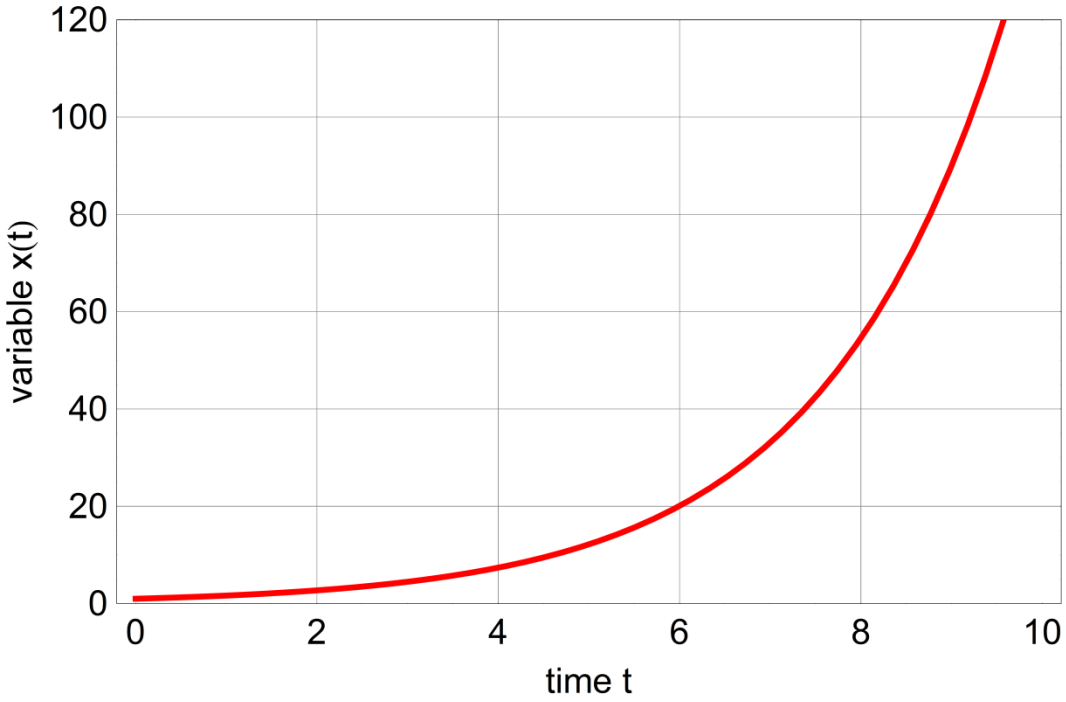
ABSTRACT Spontaneous symmetry breaking in reactive systems, known as a rare physical phenomenon and for the Soai autocatalytic irreversible reaction, might in principle also occur in other, more common asymmetric reactions when the chiral product is capable to promote its formation and an element of "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

Einfache Metabolismen und Recycling

autocatalysis

$$\frac{dx}{dt} = f\,x \quad \Rightarrow \quad x(t) = x(0)\exp(f\,t)$$

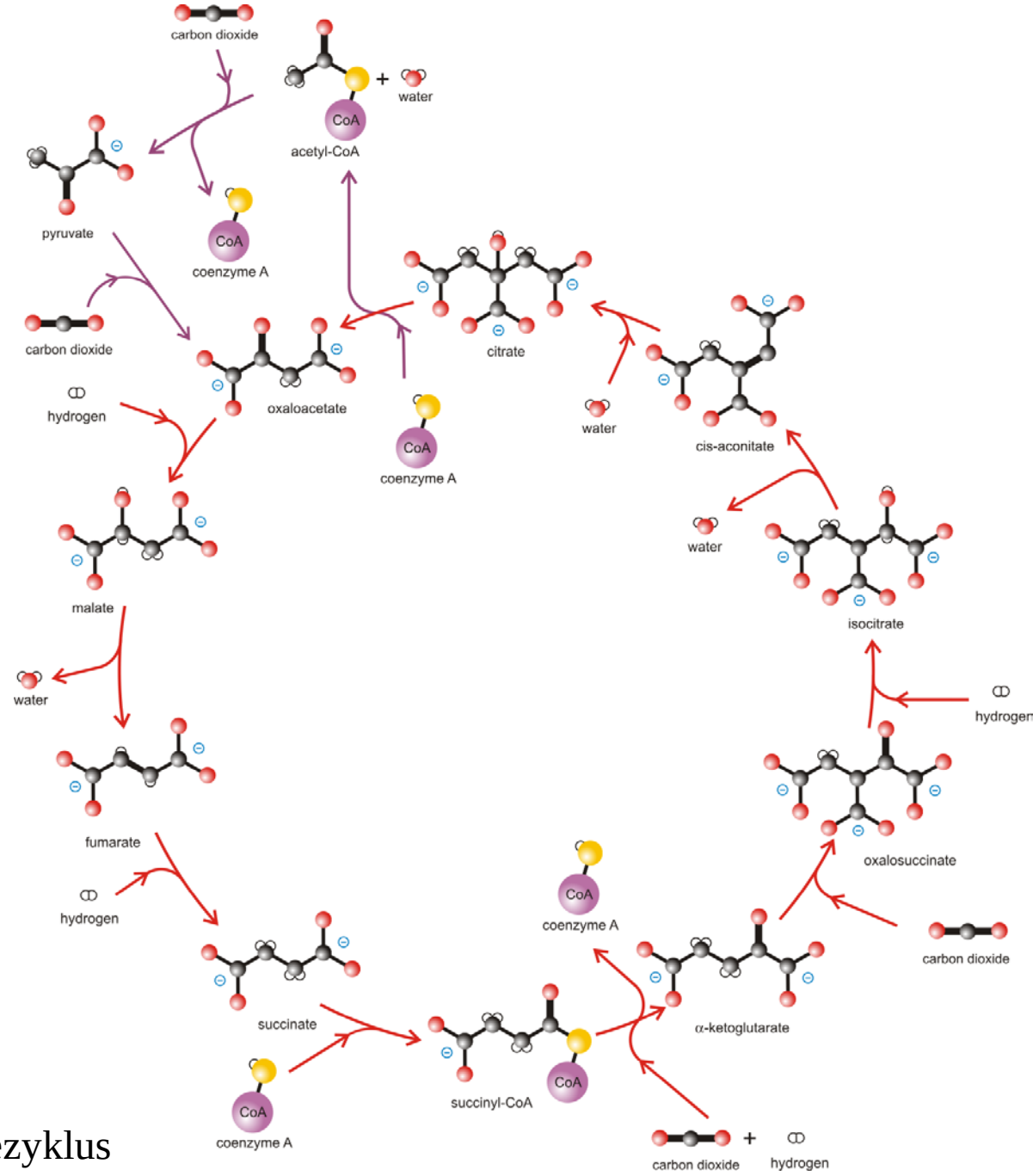


Autocatalysis and exponential growth

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

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

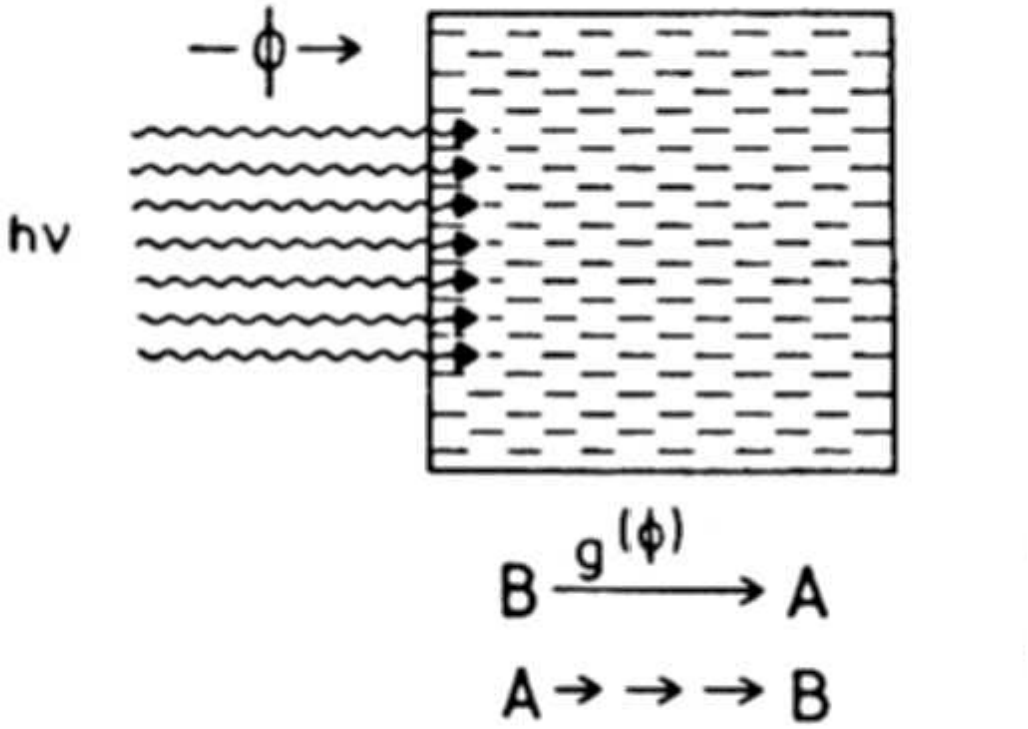
Shneior Lifson, 1914 - 2001

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

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.*

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

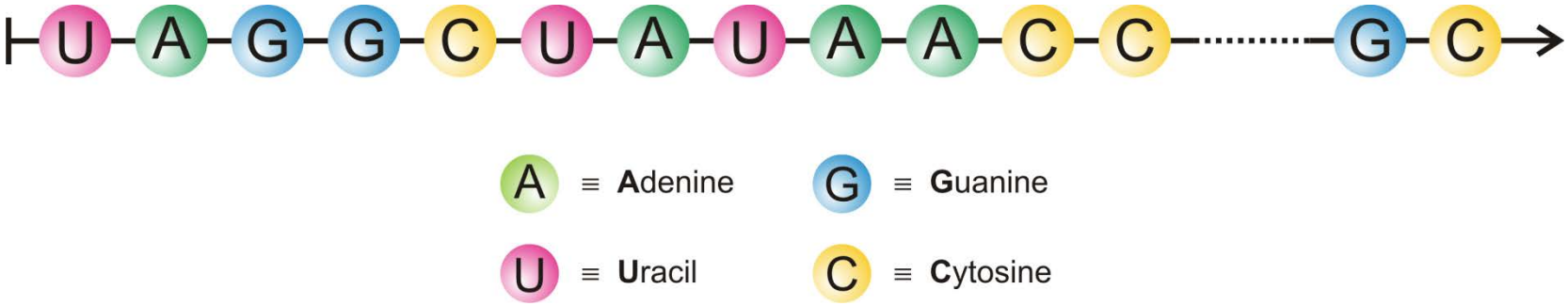
Shneior Lifson and recycling in origin of life models



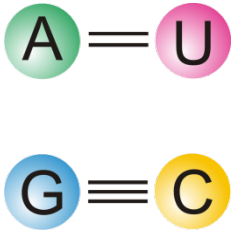
„Los Alamos Bug"

recycling open systems for studying evolution in vitro

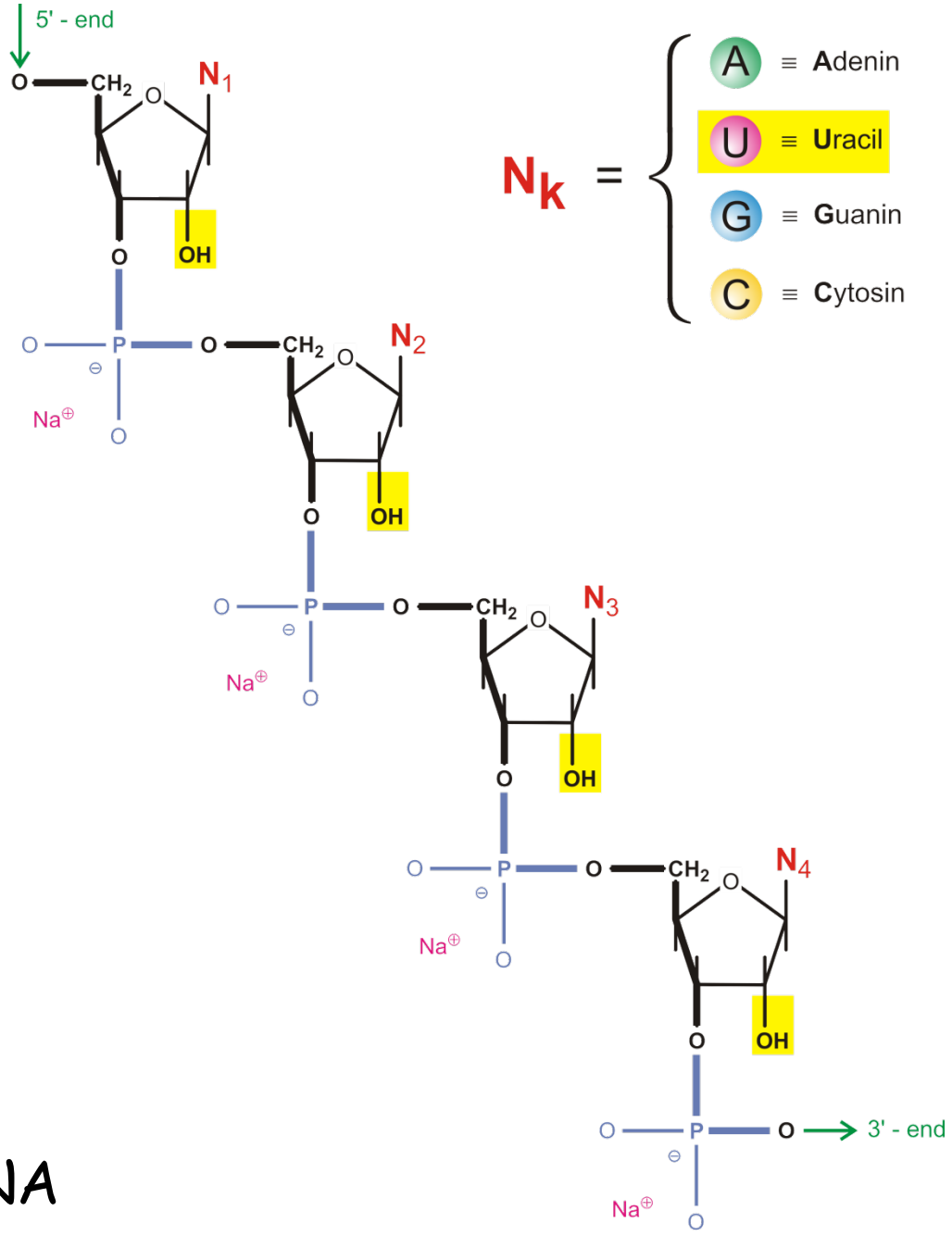
Ribonukleinsäuren – RNA-Welt



Ribonucleinsäure RNA



Nucleotidpaare



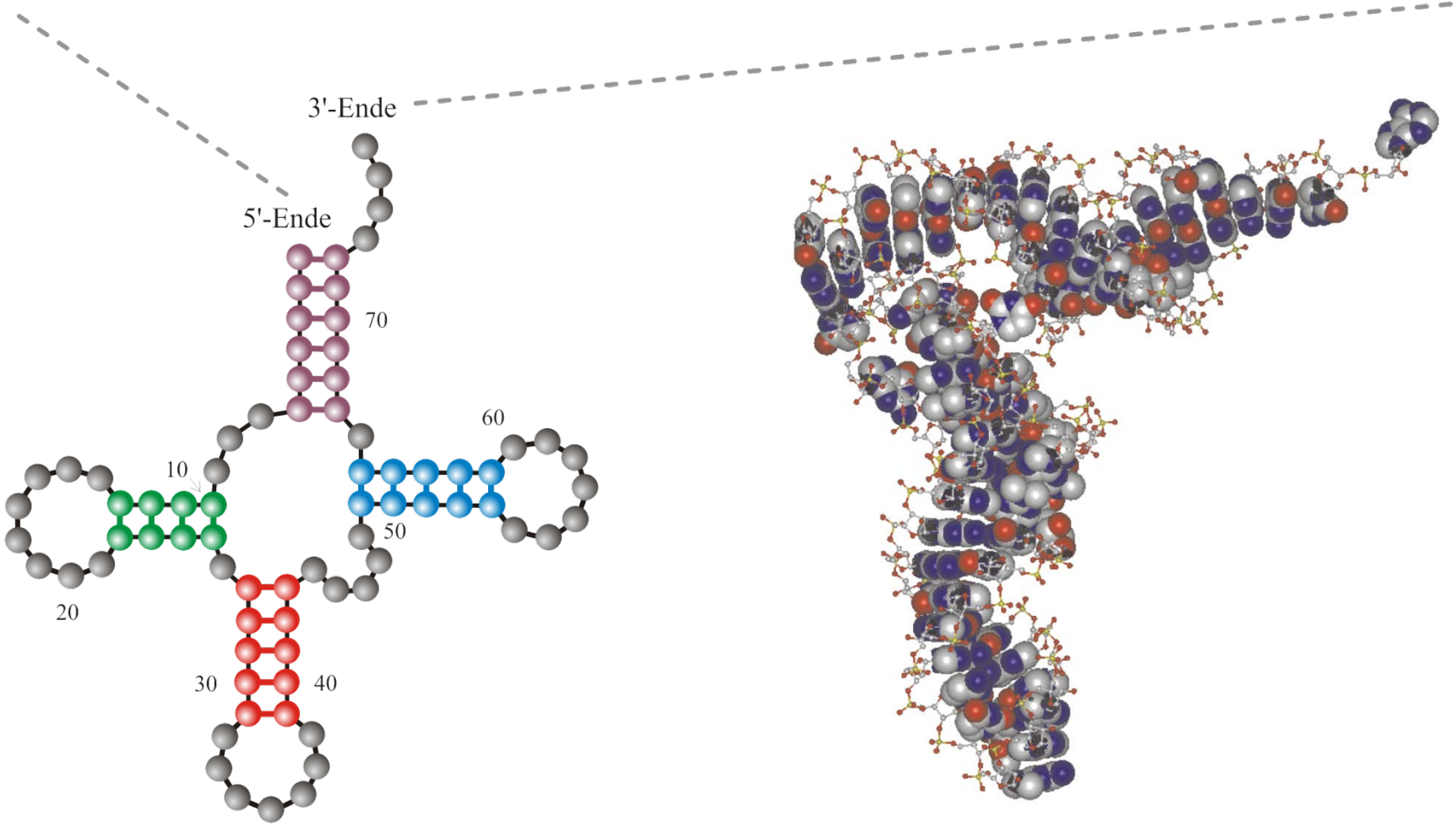
Ribonucleinsäure RNA

Sequenz

5'-Ende

3'-Ende

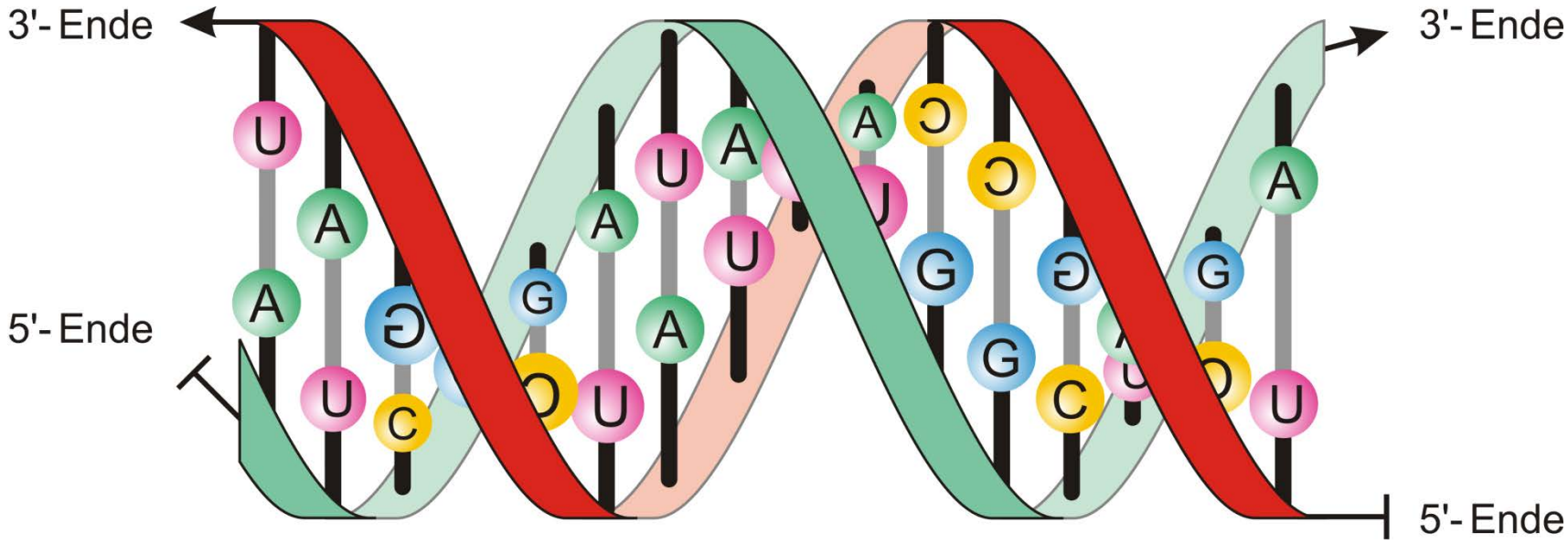
GCGGAUUUAGCUCAGDDGGGAGAGCMCCAGACUGAAYAUCUGGAGMUCCUGUGTPCGAUCCACAGAAUUCGCACCA

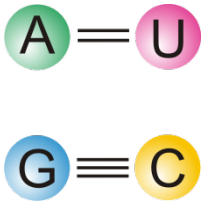


Sekundärstruktur

räumliche Struktur

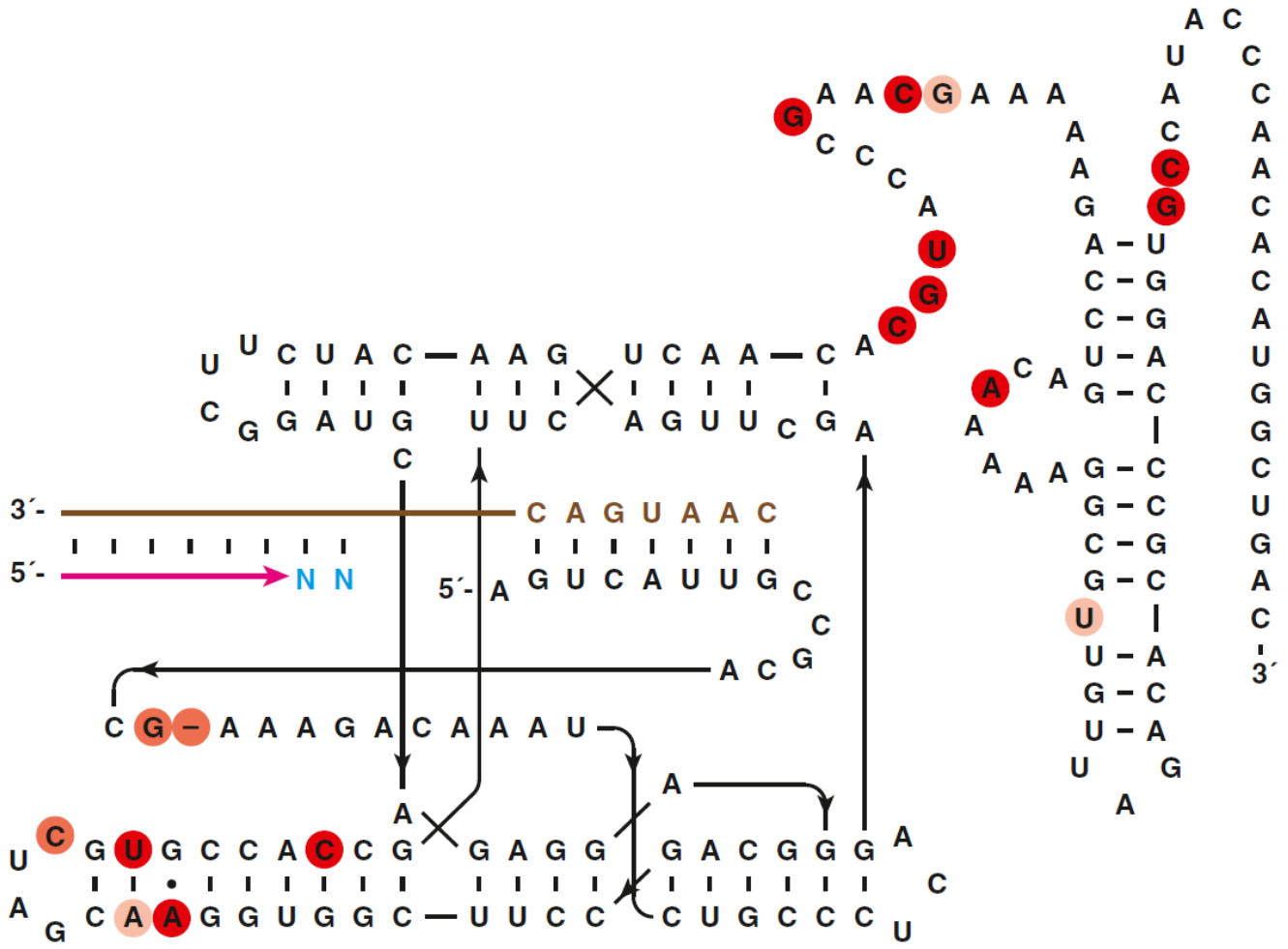
Ribonucleinsäure RNA





„Digitalisierung“ der Chemie durch Basenpaarung

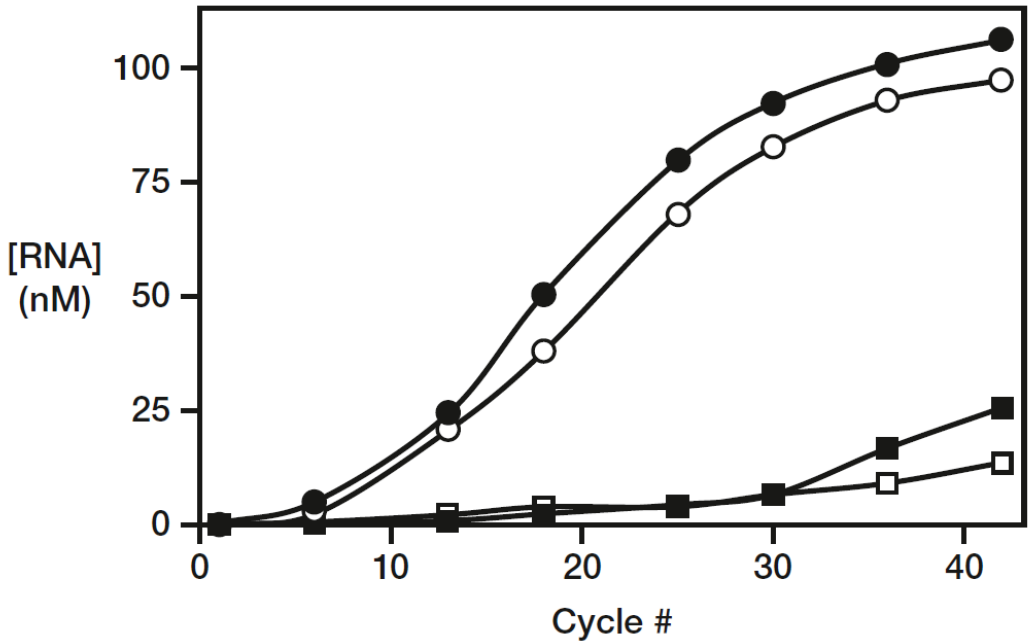
Die A-Form der RNA-Doppelhelix



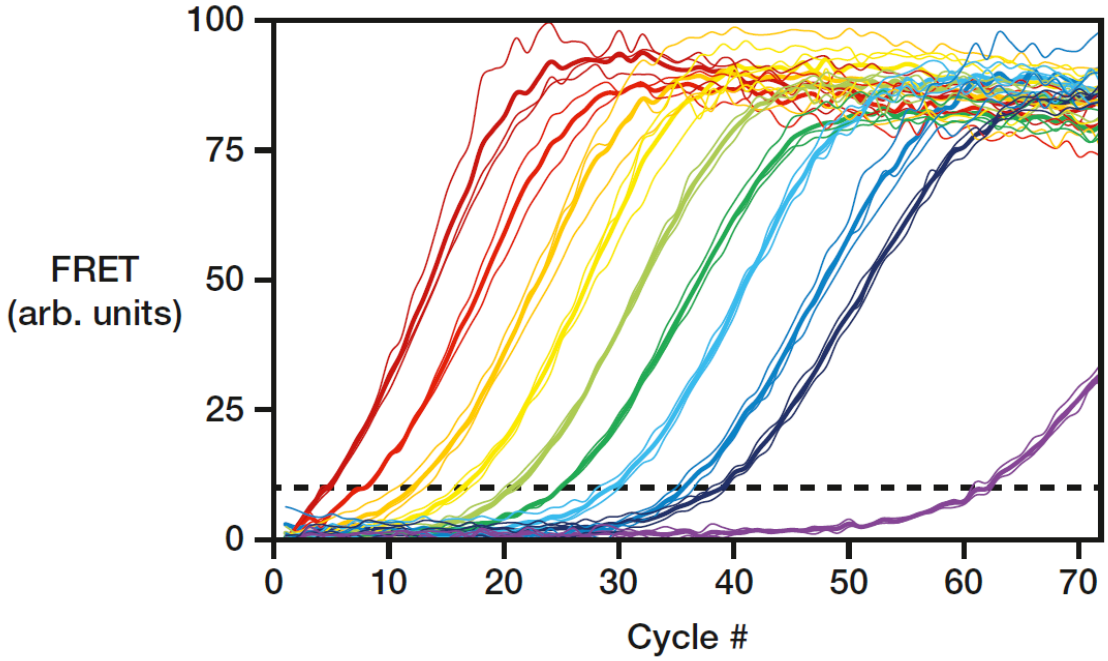
David P. Horning, Gerald F. Joyce. Amplification of RNA by an RNA polymerase ribozyme. Proc.Natl.Acad.Sci.USA 113:9786-9791, 2016

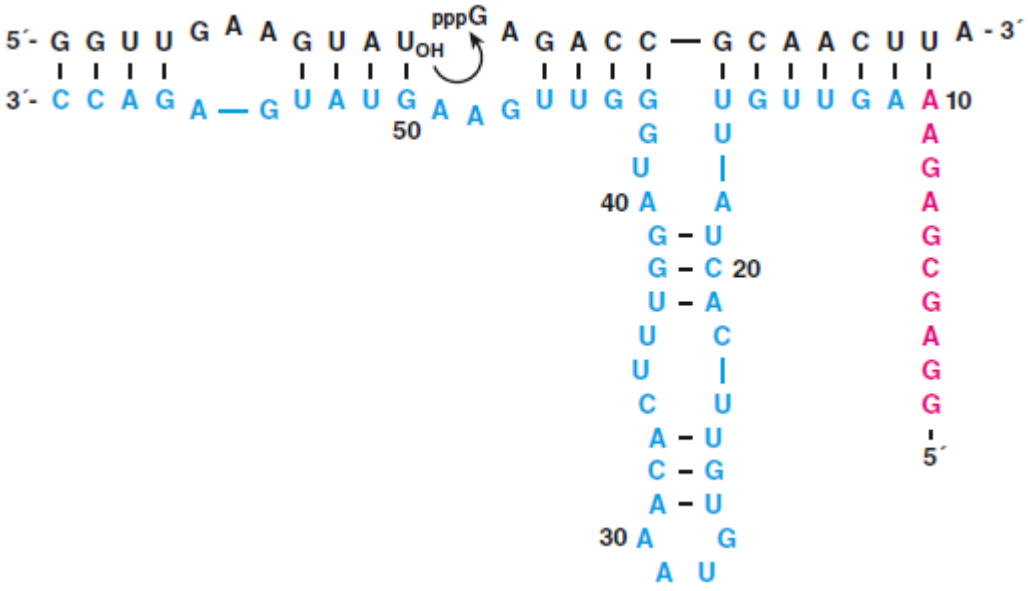
RNA polymerase ribozyme

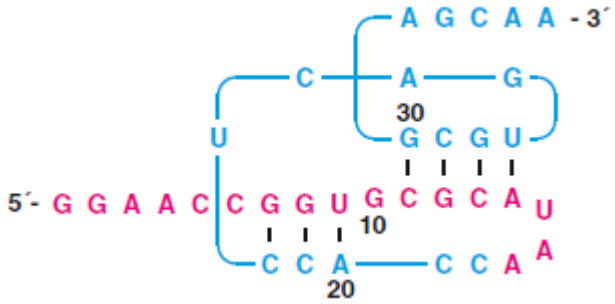
exponential growth of
plus- and minus-strand

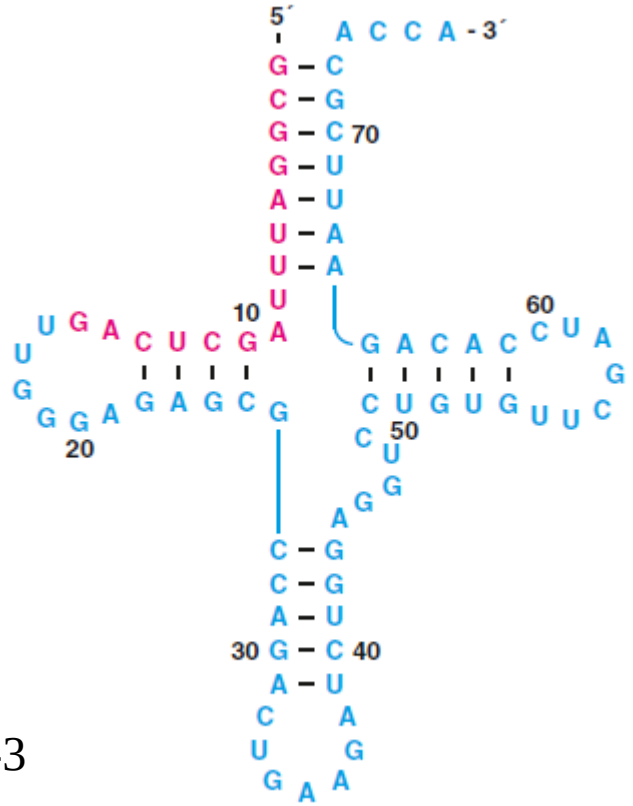


„RNA-PCR“



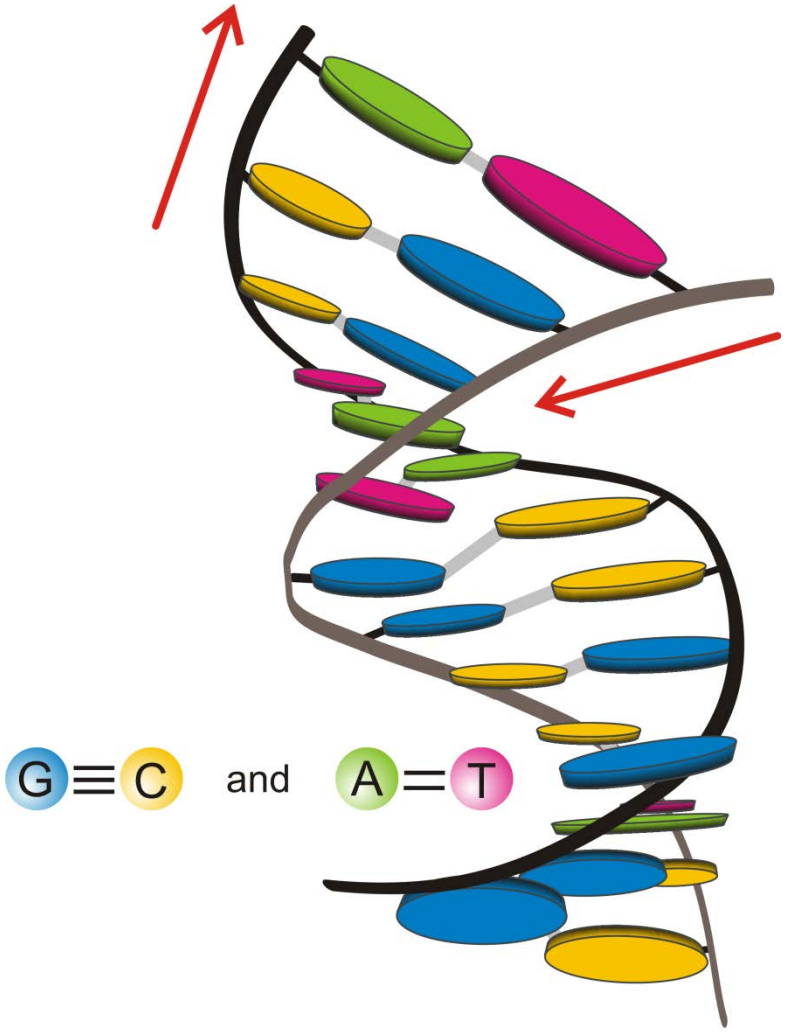


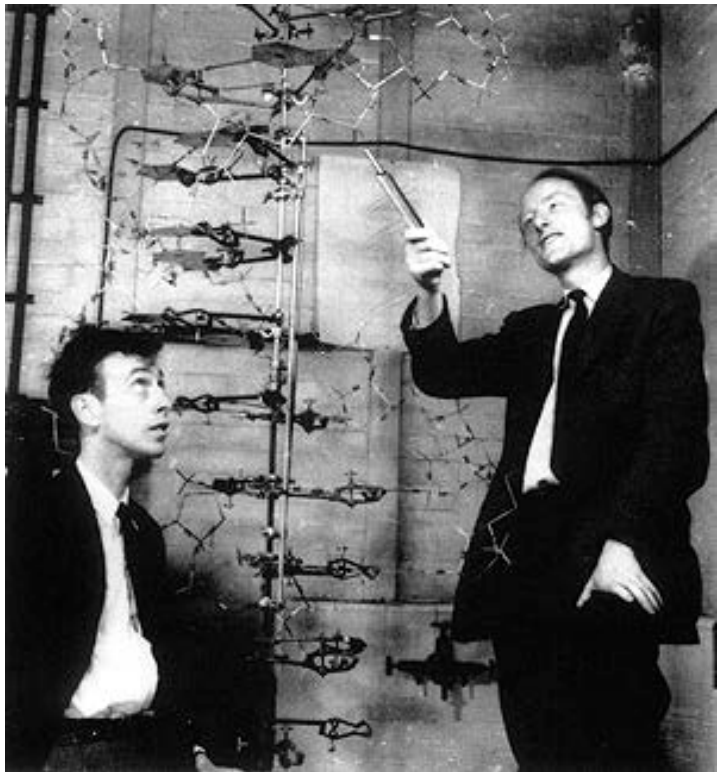




Functional molecules replicated by RNA polymerase 24-3

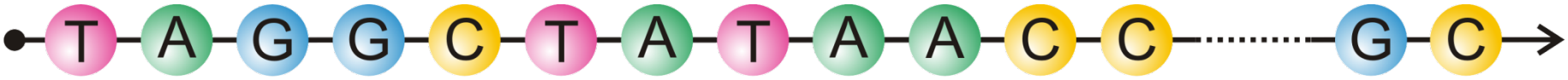
Desoxyribonukleinsäuren und Proteine





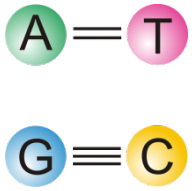
James D. Watson, 1928- , and Francis Crick, 1916-2004,
Nobel Preis 1962

Die dreidimensionale Struktur eines kleinen Stückes der B-DNA

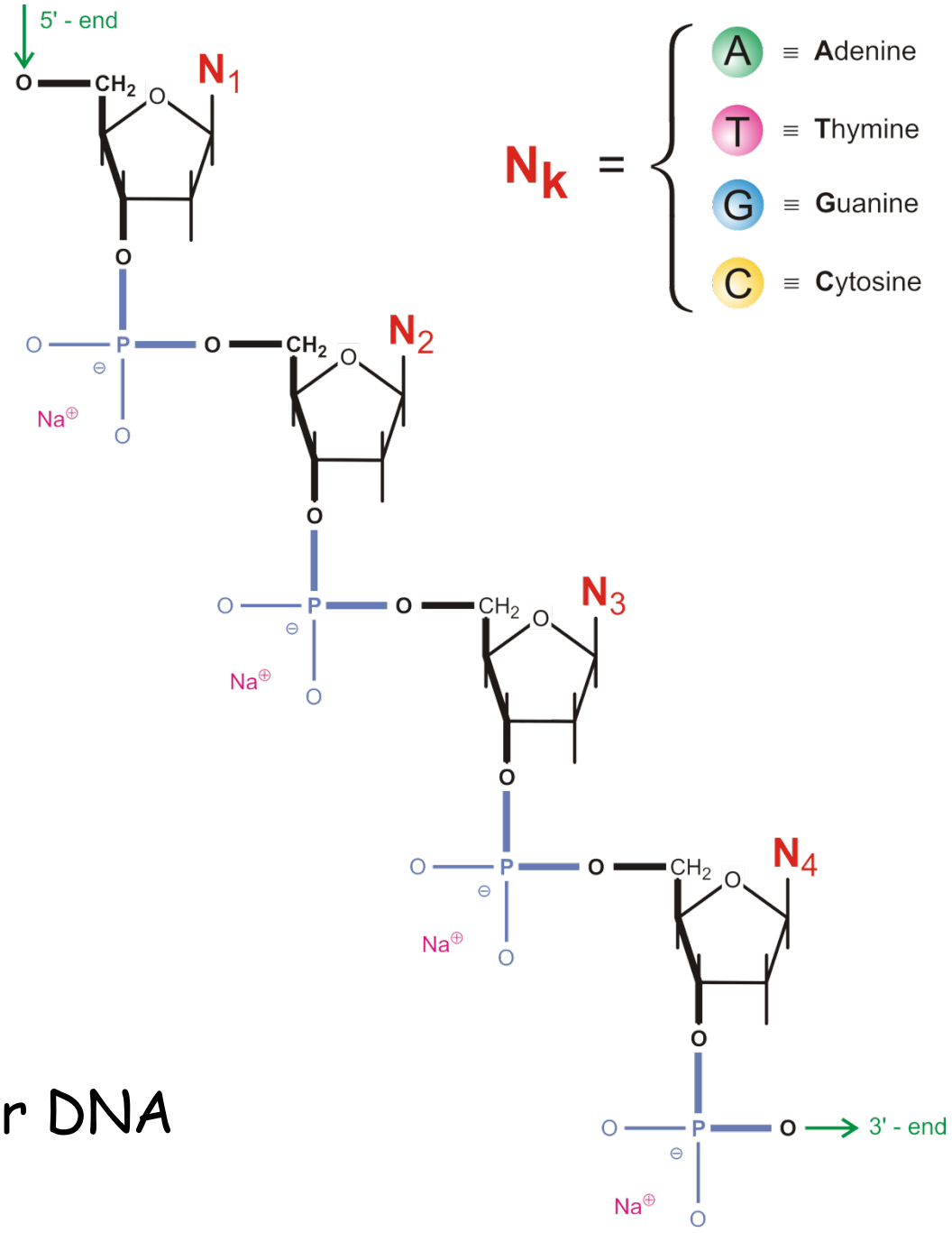


A ≡ **A**denin T ≡ **T**hymin G ≡ **G**uanin C ≡ **C**ytosin

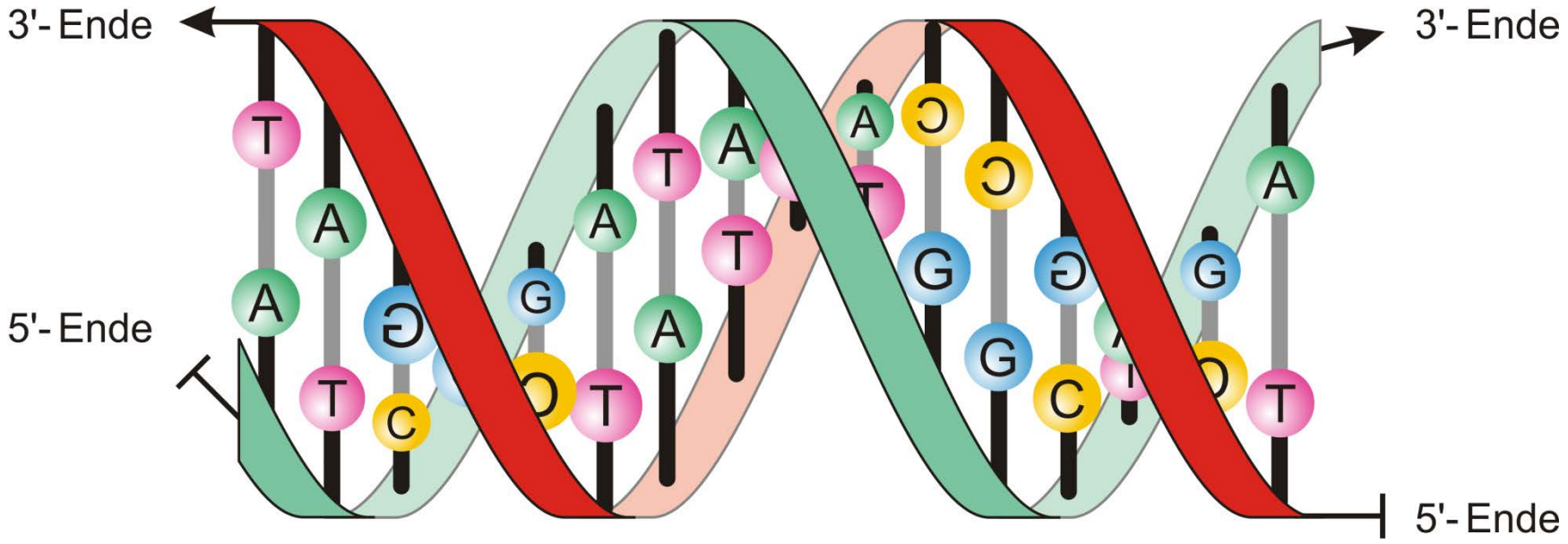
Desoxyribonucleinsäure DNA



Nucleotidpaare

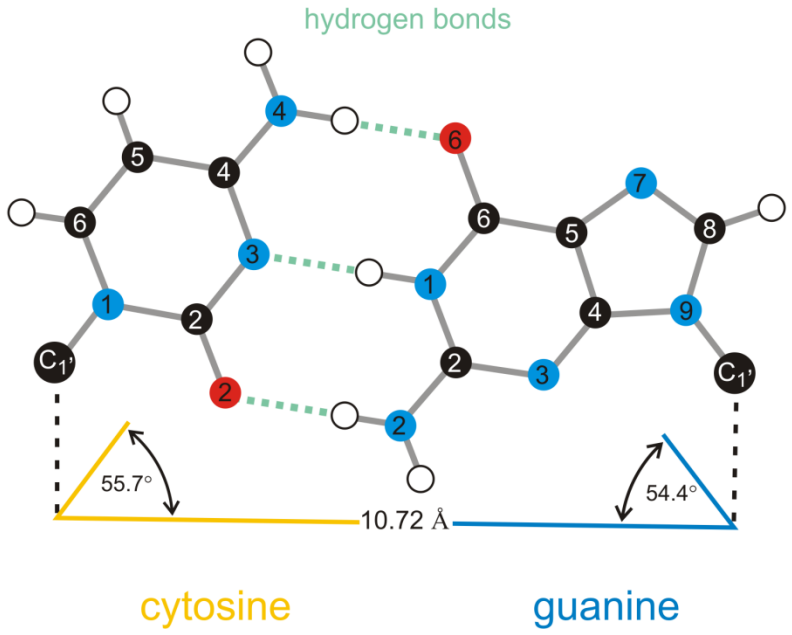


Chemische Formel der DNA



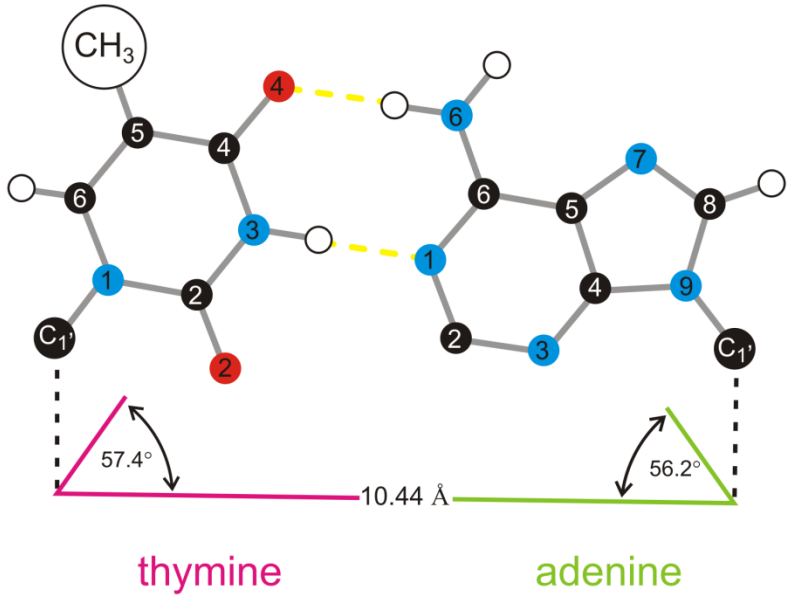
Die B-Form der DNA-Doppelhelix

C ≡ G

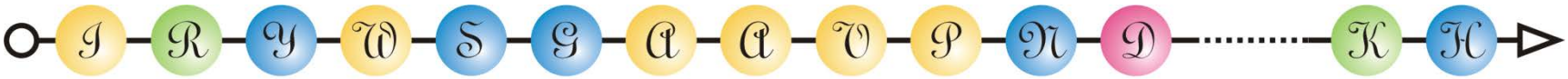


Obwohl die Wechselwirkungen mit G viel stärker sind als alle anderen Wechselwirkungen zwischen Nukleotidbasen, bilden A=T und G≡C gleichberechtigte Basenpaare.

T = A

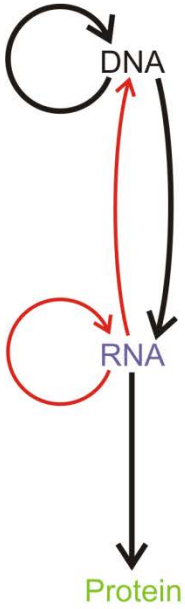


Digitalisierung der Chemie:
The unique assigment of nucleotides in base pairs.

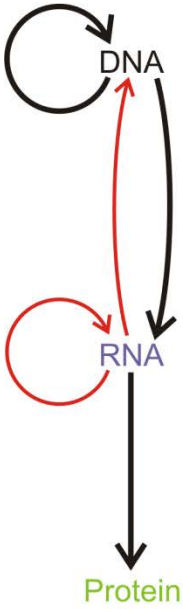


A ≡ **ala**nine	G ≡ **gly**cine	M ≡ **met**hionine	S ≡ **ser**ine
C ≡ **cys**teine	H ≡ **his**tidine	N ≡ **asparagine**	T ≡ **thr**eonine
D ≡ **asp**artic acid	I ≡ **iso**l**e**ucine	P ≡ **pro**line	V ≡ **val**ine
E ≡ **glu**tamic acid	K ≡ **lys**ine	Q ≡ **gl**utamin**e**	W ≡ **tryp**tophane
F ≡ **phe**nyl alanine	L ≡ **leu**cine	R ≡ **arg**inine	Y ≡ **tyr**osine

Protein



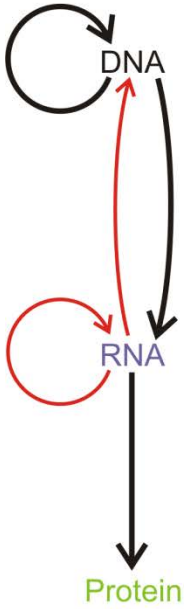
The ‚central dogma' of molecular biology

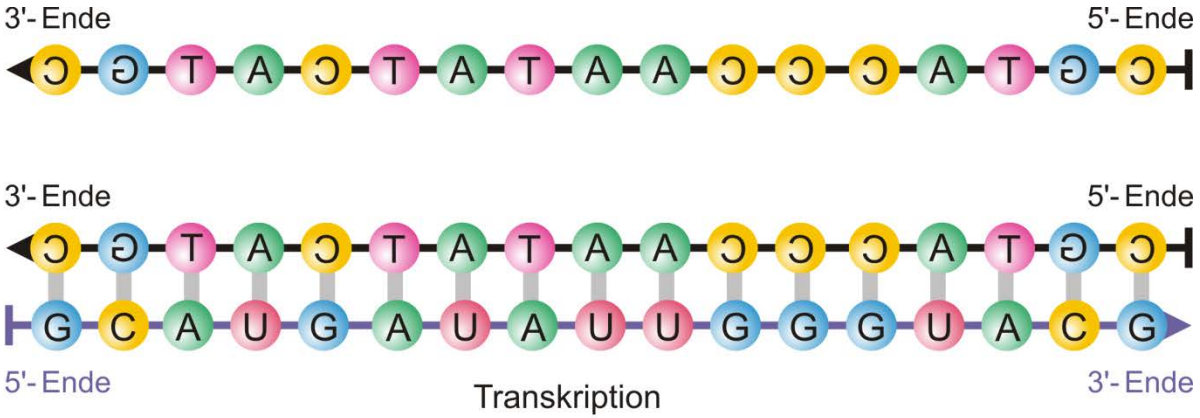


Adenin A Thymin T Uracil U

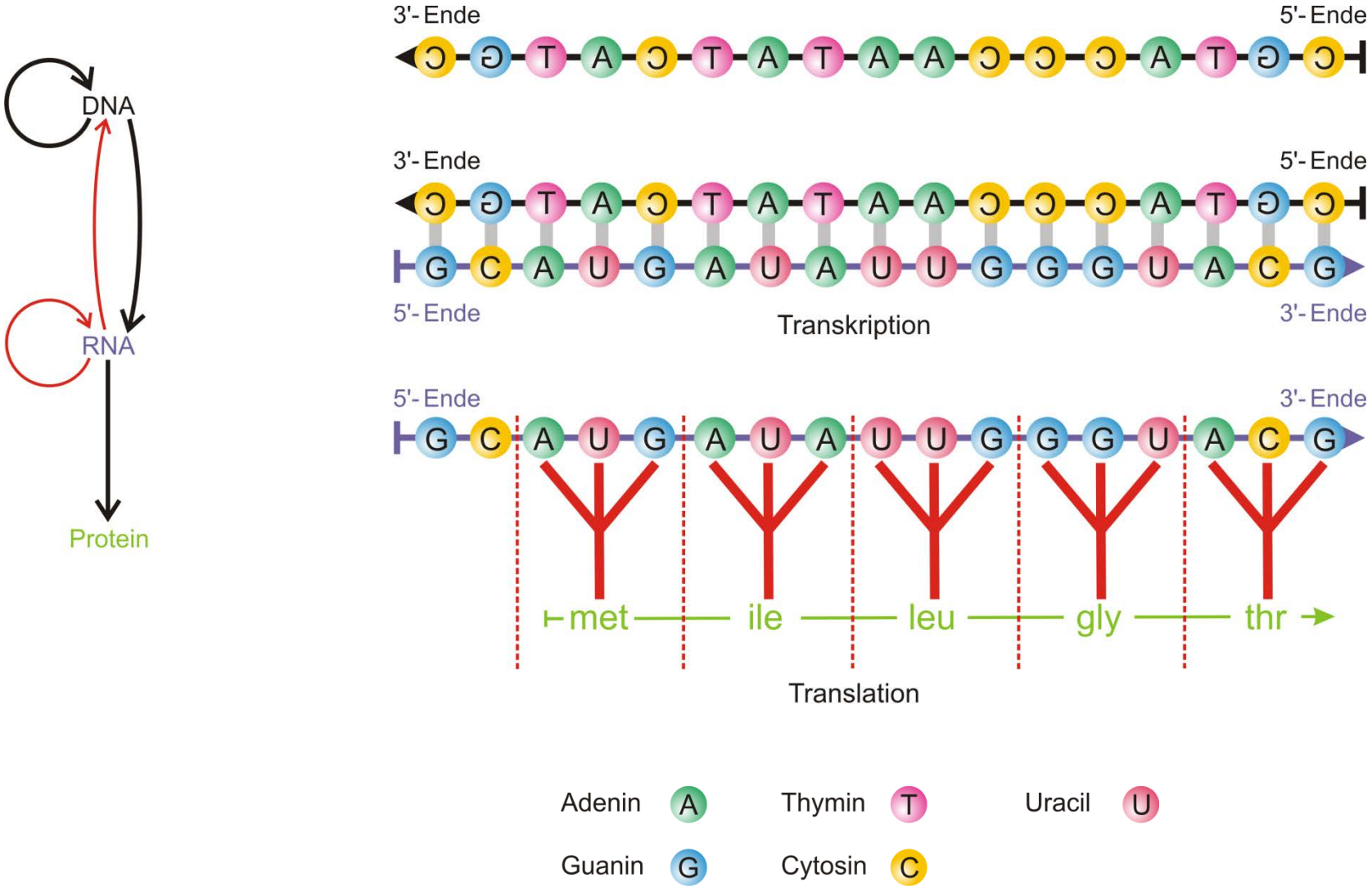
Guanin G Cytosin C

The ‚central dogma' of molecular biology

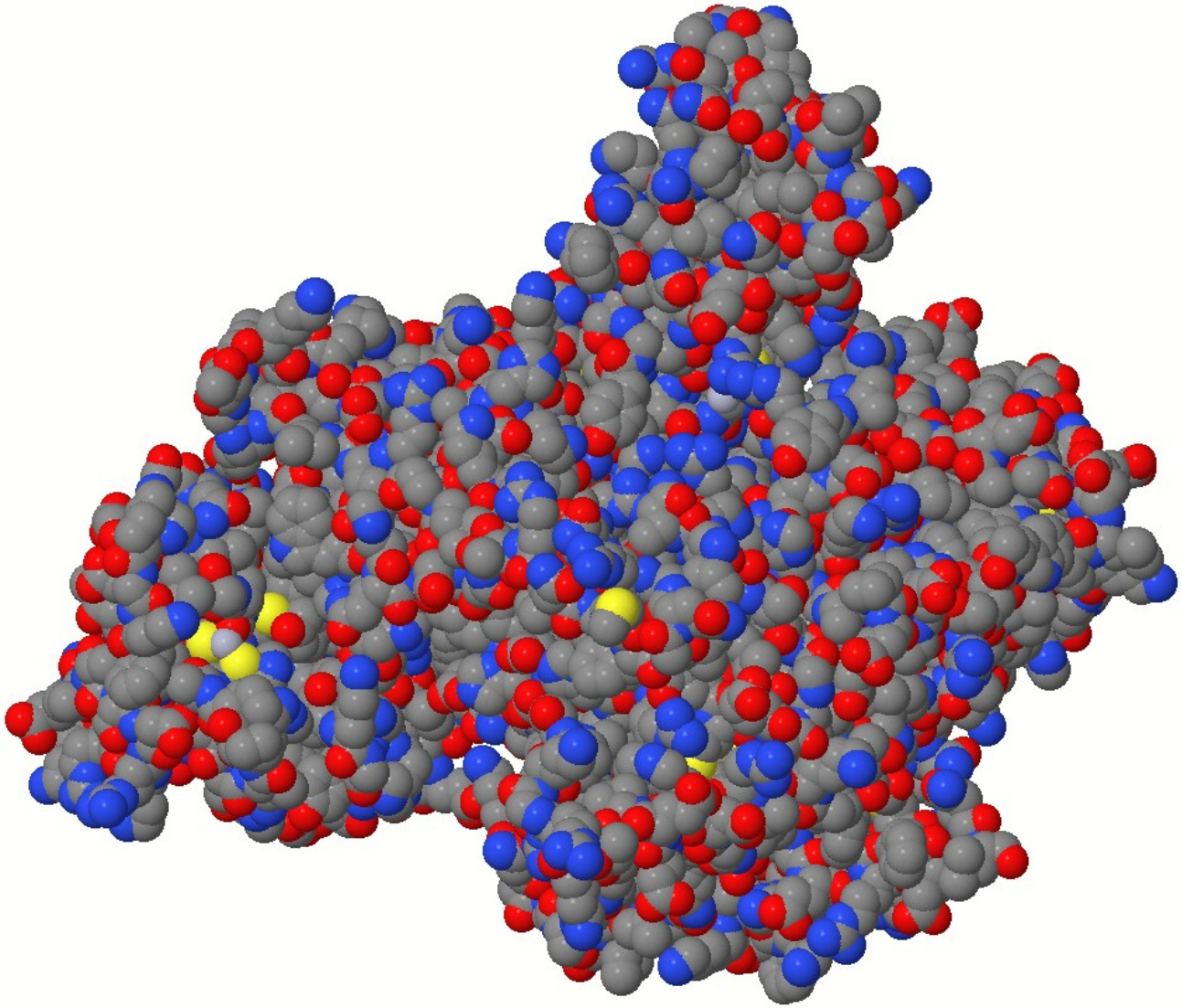




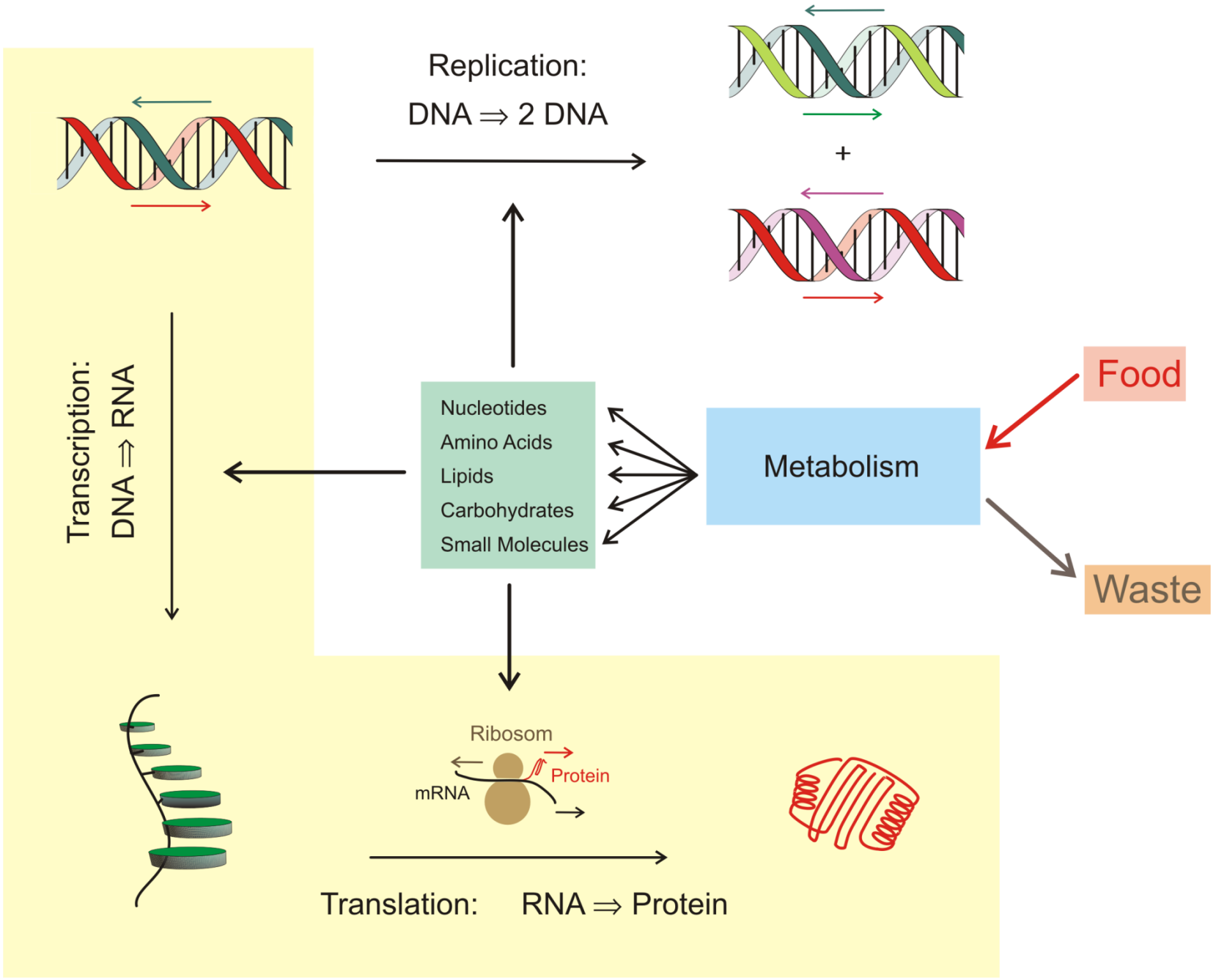
The ‚central dogma' of molecular biology

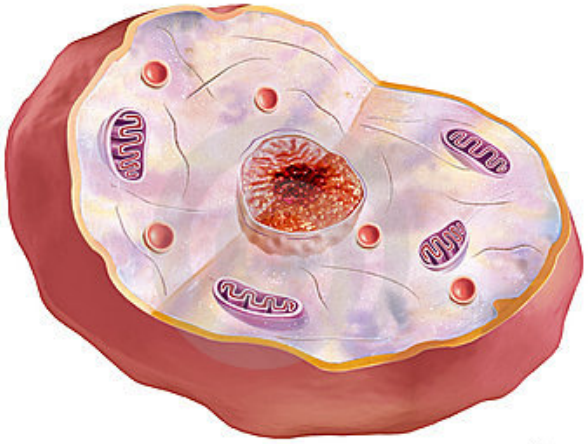


The ‚central dogma' of molecular biology



Transkriptionsenzym: DNA → RNA


Replication:
DNA ⇒ 2 DNA
+
Transcription:
DNA ⇒ RNA
Nucleotides
Amino Acids
Lipids
Carbohydrates
Small Molecules
Metabolism
Food
Waste
Ribosom
Protein
mRNA
Translation: RNA ⇒ Protein



Zelldurchmesser

0,01 mm

DNA-Länge

1 m

Vergrößerung 1 : 100 000

1 m

100 km

die menschliche DNA ist 3 Milliarden Basenpaare lang

Größenverhältnisse

Evolution: Das Prinzip der Biologie

1. Prolog
2. Ein möglichst einfaches Evolutionsmodell
3. Darwinsche Selektion
4. Variation: Rekombination und Mutation
5. Von der Theorie zur Anwendung
6. Evolution zu höherer Komplexität
7. Mutationsschwelle des Überlebens
8. Epilog

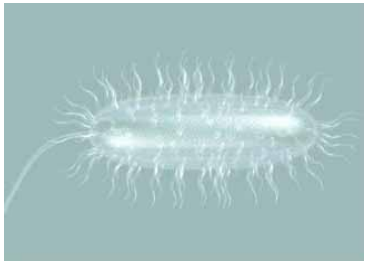
Prolog

Nothing in biology makes sense except in the light of evolution.

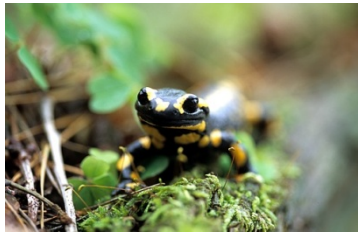
Theodosius Dobzhansky,
1900 – 1975

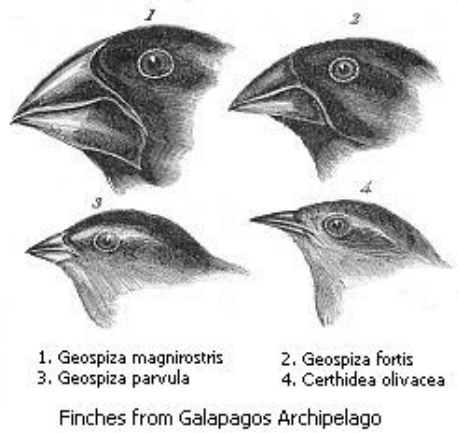
Charles Darwin, 1809 - 1882

Voyage on HMS Beagle, 1831 - 1836



Phänotypen





Finches from Galapagos Archipelago

ON

THE ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

OR THE

PRESERVATION OF FAVOURED RACES IN THE STRUGGLE FOR LIFE.

By CHARLES DARWIN, M.A.,

FELLOW OF THE ROYAL, GEOLOGICAL, LINNÆAN, ETC., SOCIETIES;
AUTHOR OF 'JOURNAL OF RESEARCHES DURING H. M. S. BEAGLE'S VOYAGE ROUND THE WORLD.'

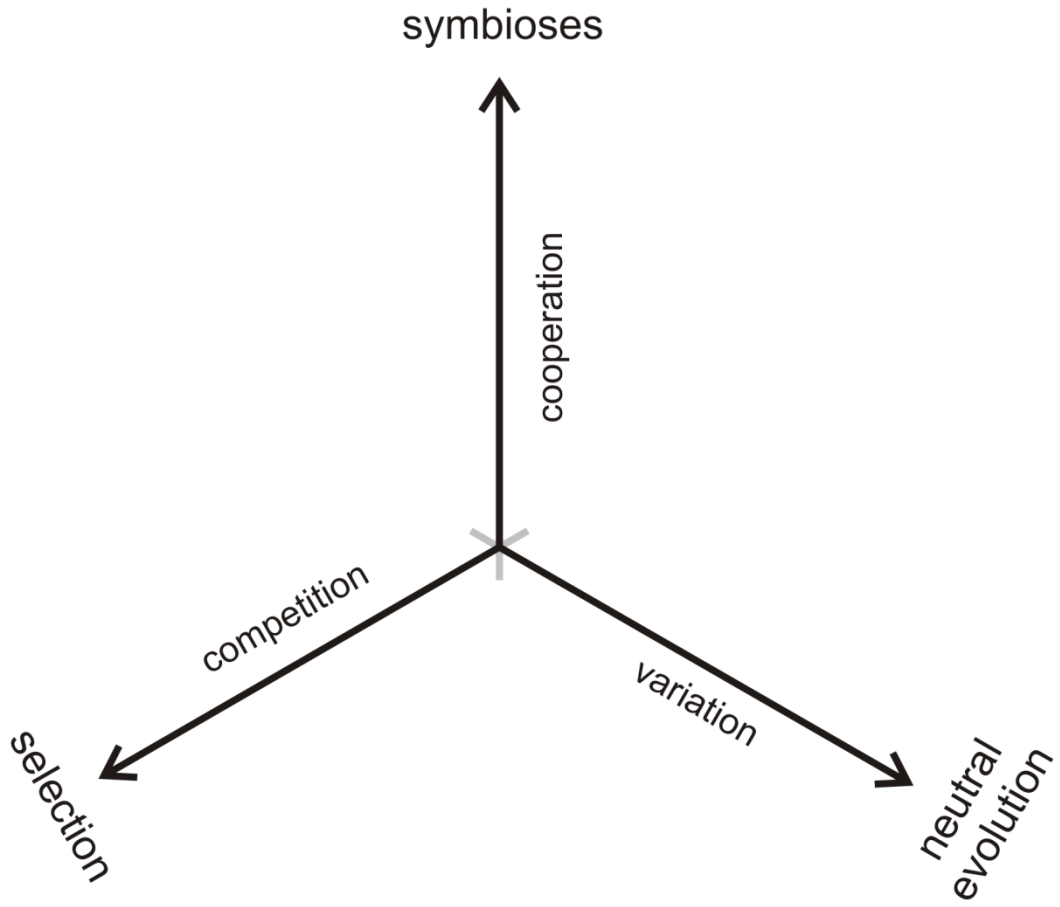
LONDON:
JOHN MURRAY, ALBEMARLE STREET.
1859.

Ein möglichst einfaches Evolutionsmodell

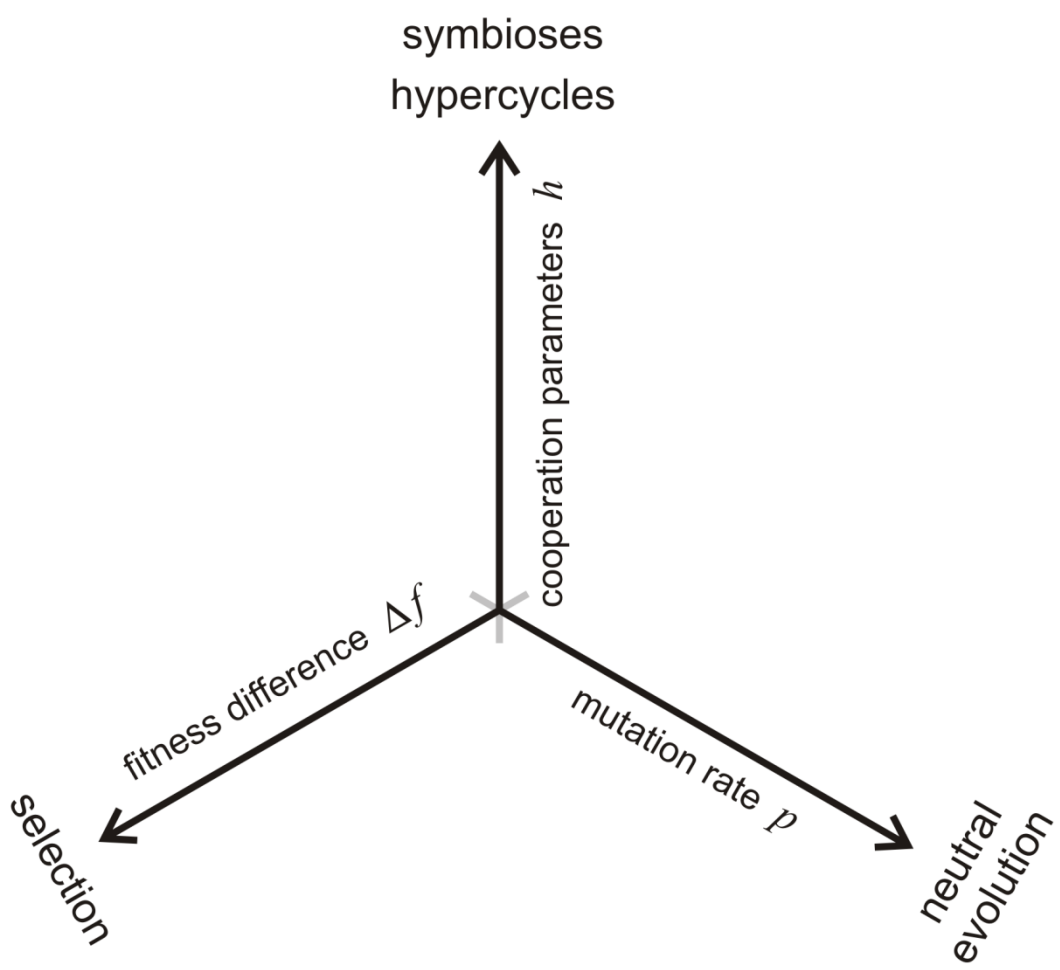
Motto: Occam‘s razor in the twentieth century

Everything should be made as simple as possible, but not simpler.

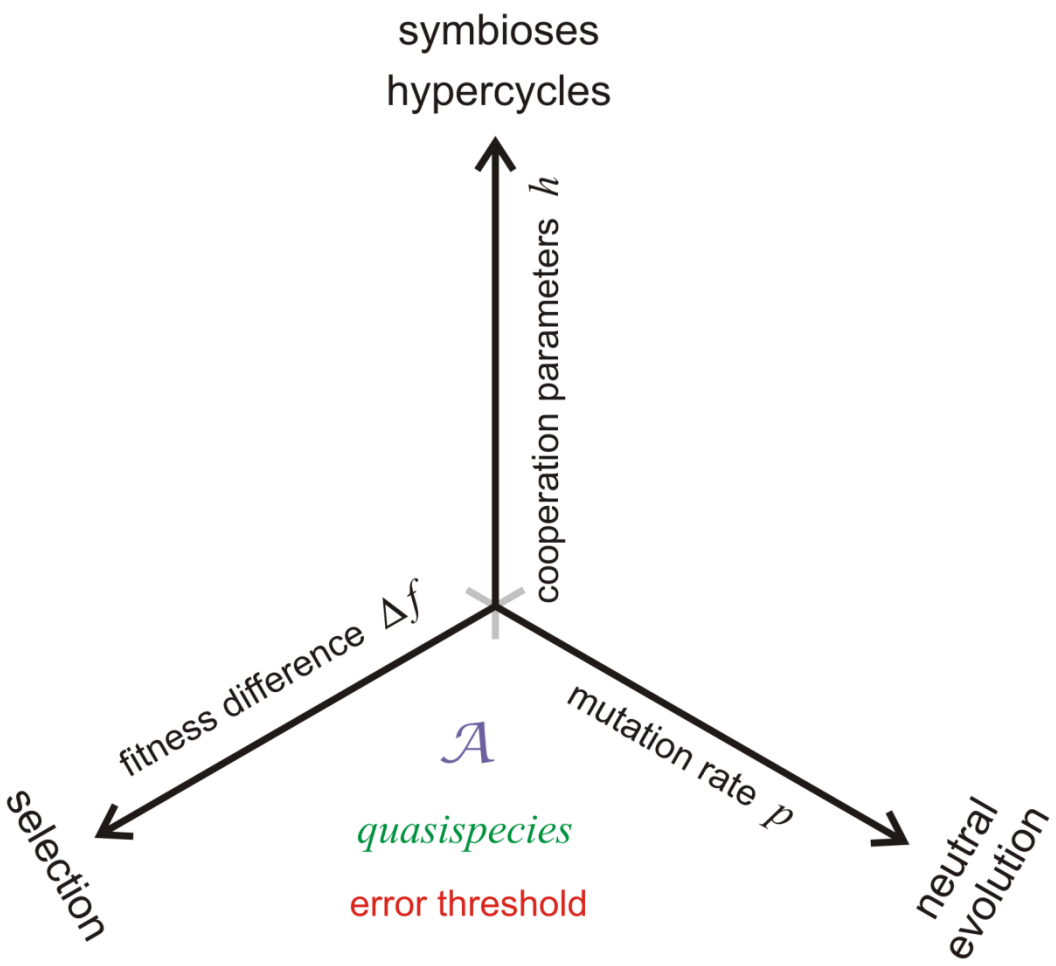
Attributed to Albert Einstein



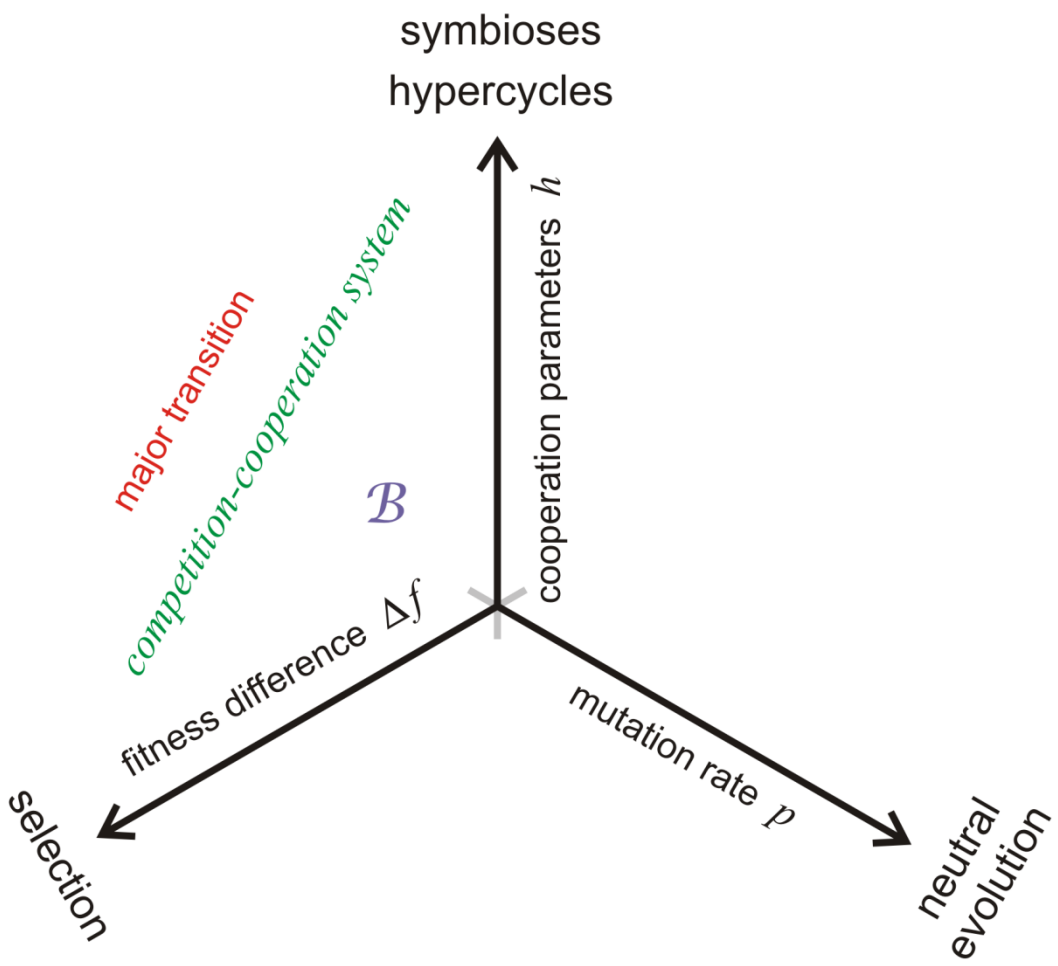
The three major processes driving evolution



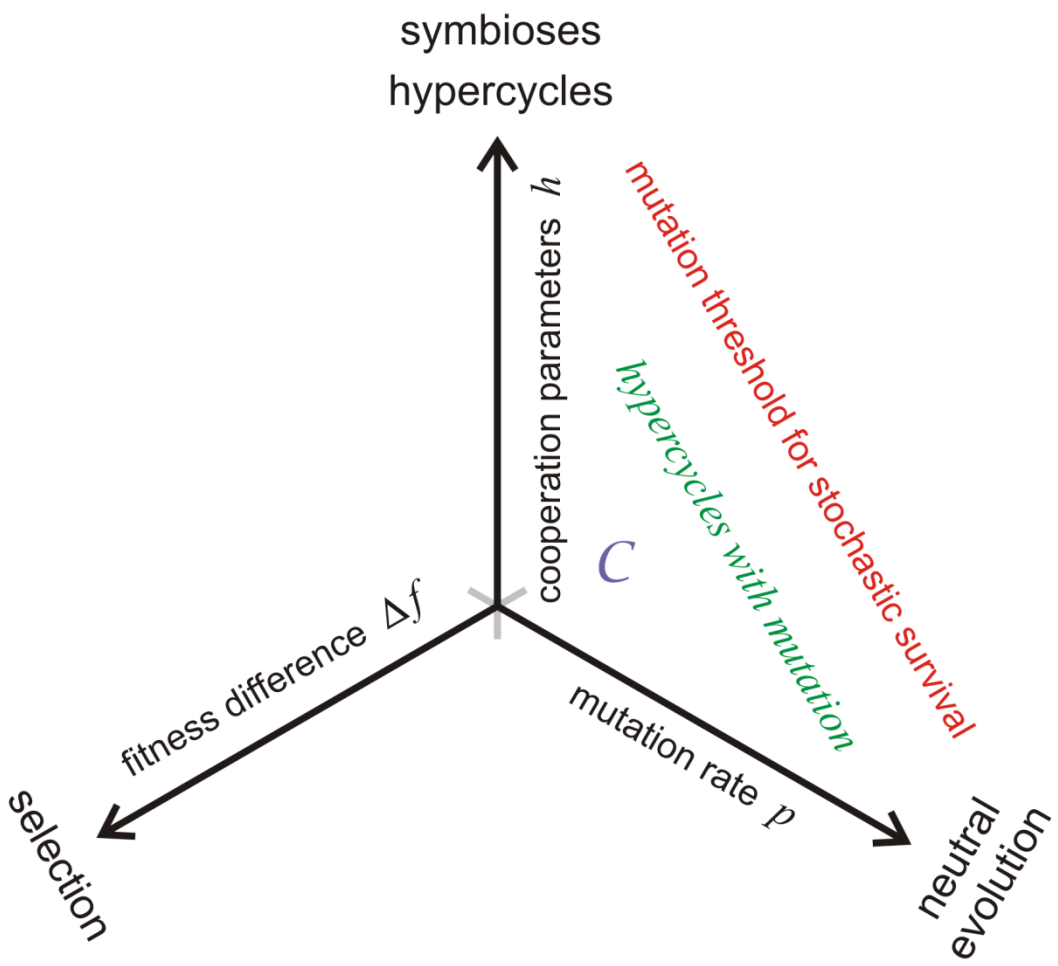
Three internal parameters driving evolution



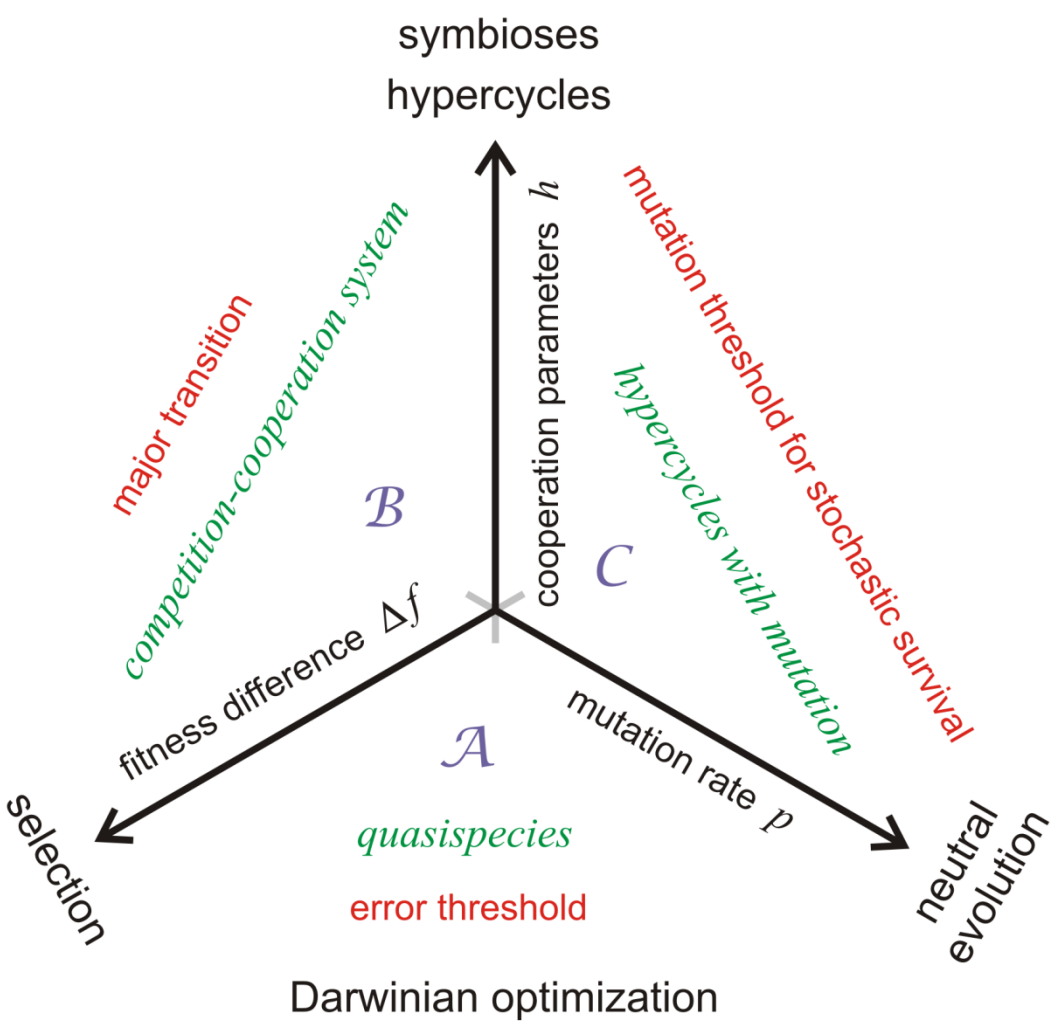
Competition and variation: error threshold



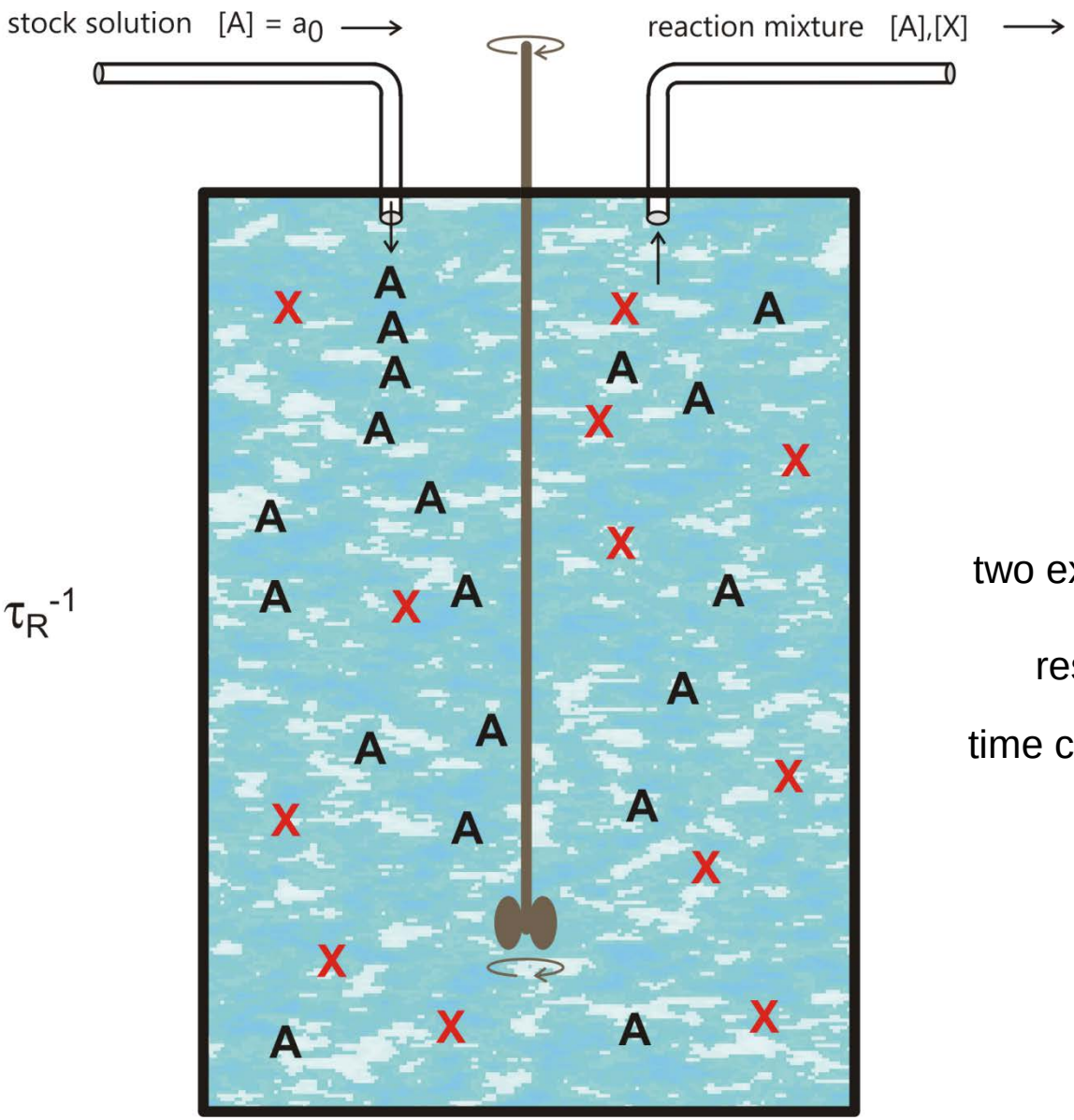
Competition and cooperation: major transition



Cooperation and variation: survival threshold



The minimal model of evolution



flow rate $r = \tau_R^{-1}$

two external parameters:

resources **A** … a_0

time constraint … $\tau_R = r^{-1}$

The continuously fed stirred tank reactor (CFSTR)

Darwinsche Selektion so einfach wie möglich

autocatalysis

$$\frac{dx}{dt} = f\ x \quad \Rightarrow \quad x(t) = x(0)\exp(f\,t)$$

competition

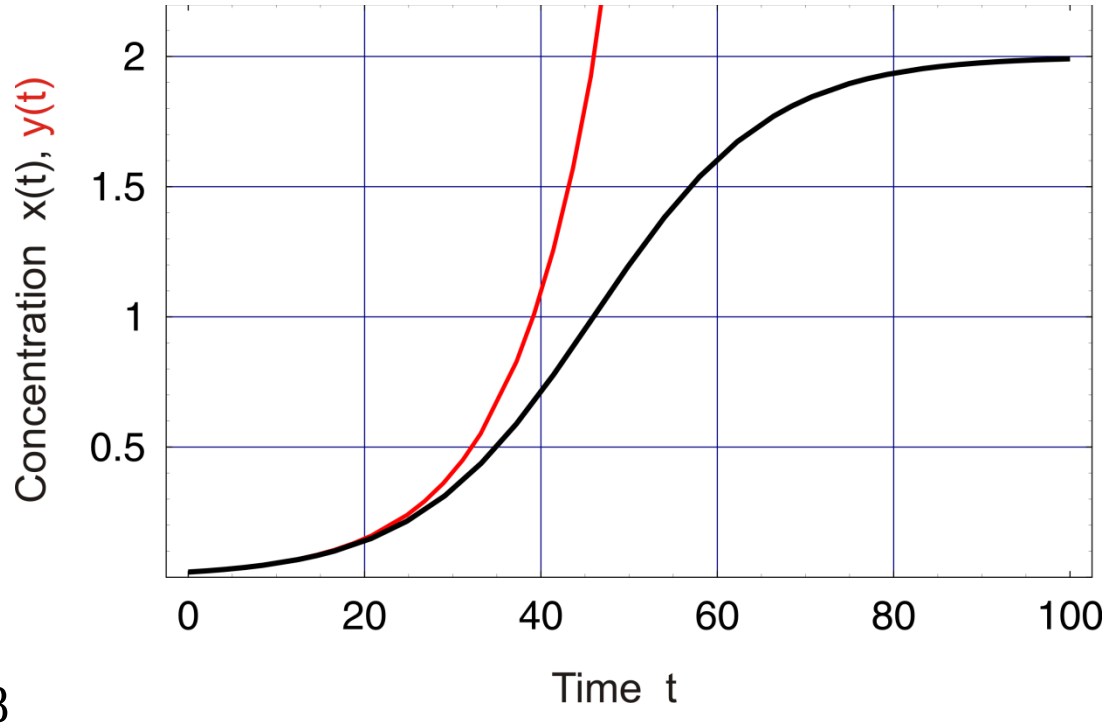
$$\frac{dx_k}{dt} = f_k\ x_k\ ; k = 1,2,\ldots,n$$

$$x_k(t) = x_k(0)\ \exp(f_k\,t)$$

The chemistry and the mathematics of reproduction

Pierre-François Verhulst,
1804-1849

$$\frac{dx}{dt} = r\,x\left(1-\frac{x}{C}\right), \quad x(t) = \frac{x(0)\,C}{x(0)+\left(C-x(0)\right)e^{-r\,t}}$$



The logistic equation, 1828

$$\frac{\mathrm{d}x}{\mathrm{dt}} = r\,x\left(1-\frac{x}{C}\right) \quad \Rightarrow \quad \frac{\mathrm{d}x}{\mathrm{dt}} = r\,x - \frac{x}{C}\,r\,x$$

$$r\,x \equiv \Phi(\mathrm{t}),\, C = 1: \quad \frac{\mathrm{d}x}{\mathrm{dt}} = x\left(r - \Phi\right)$$

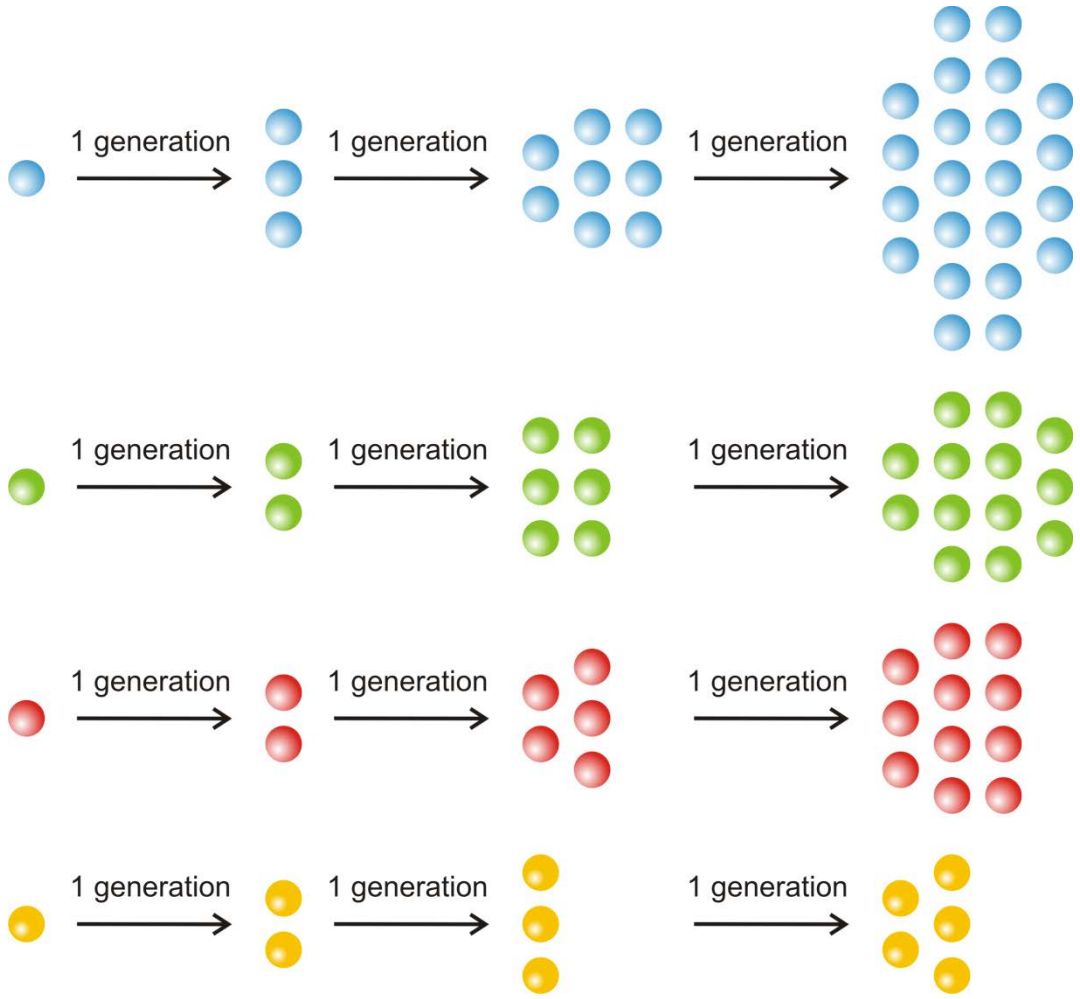
$$\mathrm{X}_1, \mathrm{X}_2, \ldots, \mathrm{X}_n: \quad [\mathrm{X}_i] = x_i; \quad \sum_{i=1}^{n} x_i = C = 1$$

$$\frac{\mathrm{d}x_j}{\mathrm{dt}} = x_j\left(f_j - \sum_{i=1}^{n} f_i\,x_i\right) = x_j\left(f_j - \Phi\right); \quad \Phi = \sum_{i=1}^{n} f_i\,x_i$$

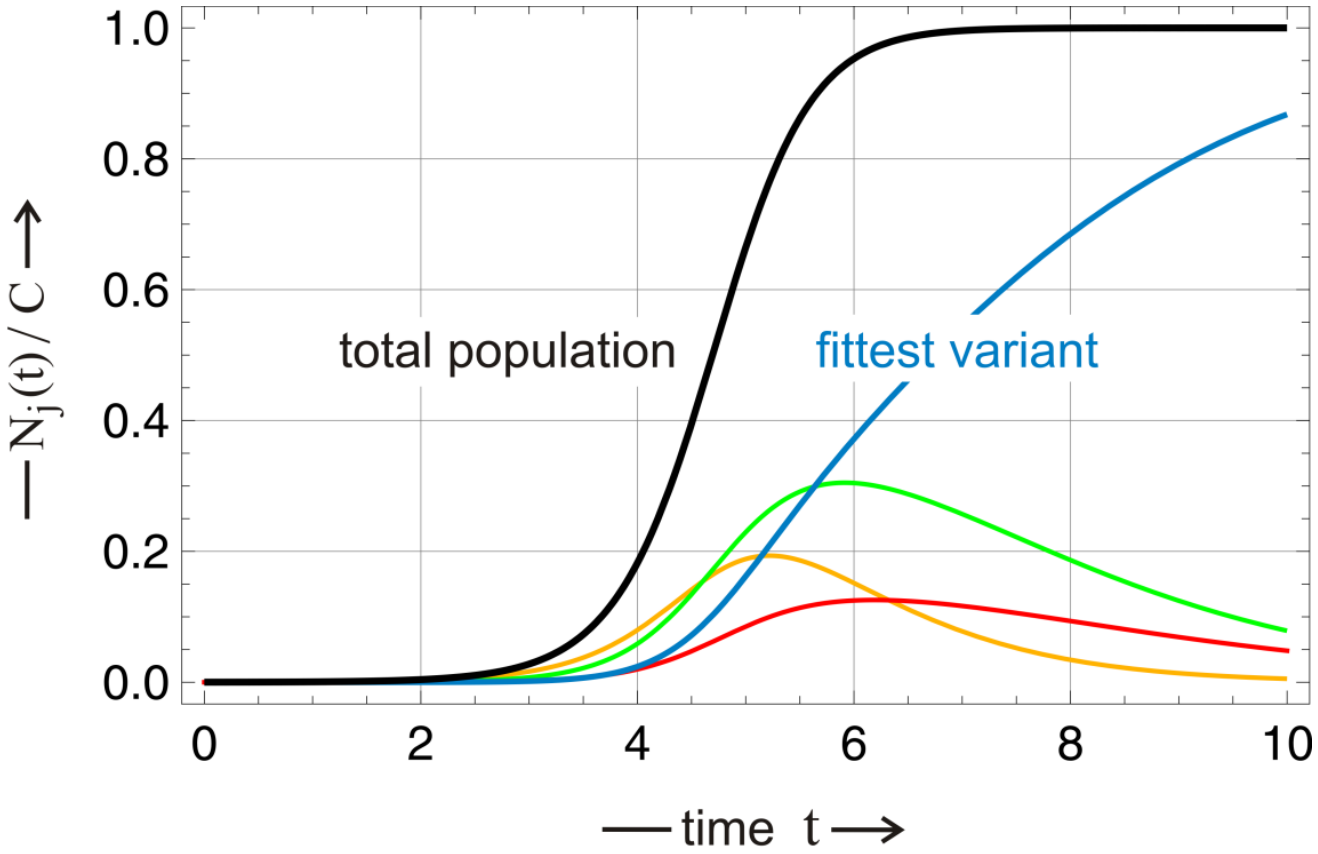
Darwin

$$\frac{\mathrm{d}\Phi}{\mathrm{dt}} = 2\left(< f^2 > - < \bar{f} >^2\right) = 2\,\mathrm{var}\{f\} \geq 0$$

Generalization of the logistic equation to *n* variables yields selection



fitness values: $f_1 = 2.80$, $f_2 = 2.35$, $f_3 = 2.25$, and $f_4 = 1.75$



fitness values: $f_1 = 2.80$, $f_2 = 2.35$, $f_3 = 2.25$, and $f_4 = 1.75$

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.

Darwins principle is still valid but **nothing** he said about **inheritance**, multiplication and **variation** is correct.

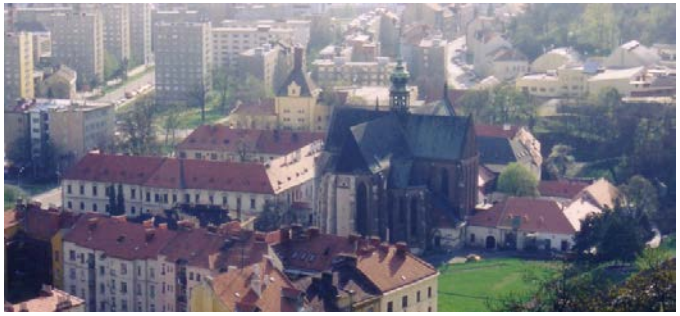
Variation: Rekombination und Mutation

Gregor Mendels Genetik und Rekombination

1. flower color is purple or white
2. flower position is axil or terminal
3. stem length is long or short
4. seed shape is round or wrinkled
5. seed color is yellow or green
6. pod shape is inflated or constricted
7. pod color is yellow or green

Gregor Mendel (1822-1884)

1st experiment	⇒	60 fertilizations on 15 plants
2nd experiment	⇒	58 fertilizations on 10 plants
3rd experiment	⇒	35 fertilizations on 10 plants
4th experiment	⇒	40 fertilizations on 10 plants
5th experiment	⇒	23 fertilizations on 5 plants
6th experiment	⇒	34 fertilizations on 10 plants
7th experiment	⇒	37 fertilizations on 10 plants



Gregor Mendel‘s experiments on plant genetics

Versuche über Pflanzen-Hybriden. *Verhandlungen des naturforschenden Vereines in Brünn* **4**: 3–47, 1866.
Über einige aus künstlicher Befruchtung gewonnenen Hieracium-Bastarde. *Verhandlungen des naturforschenden Vereines in Brünn* **8**: 26–31, 1870.

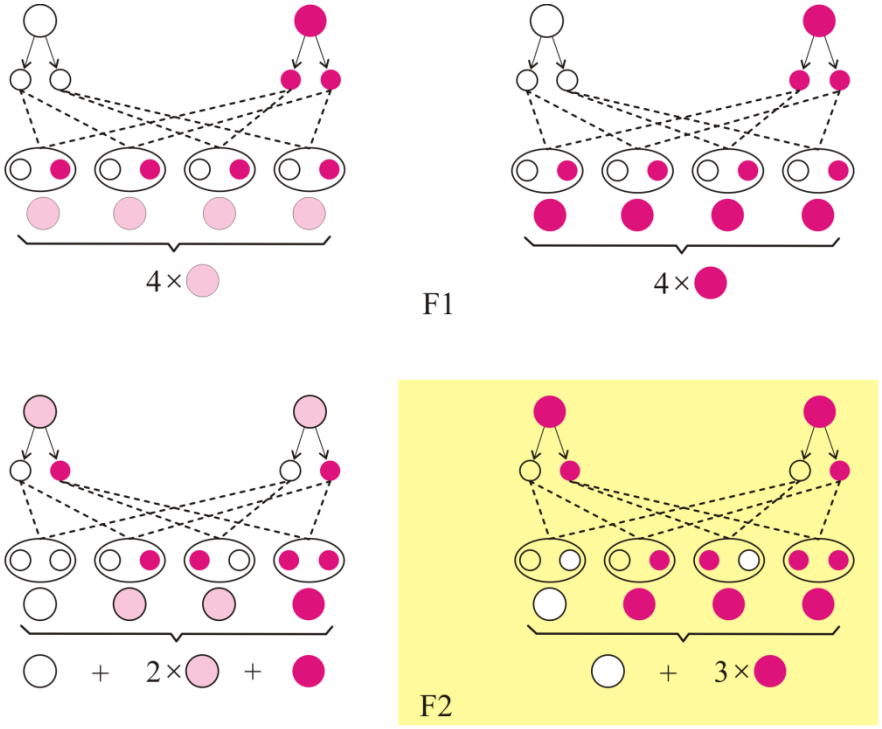
	Experiment 1		Experiment 2	
	Form of Seed		Color of Albumen	
Plants	Round	Angular	Yellow	Green
1	45	12	25	11
2	27	8	32	7
3	24	7	14	5
4	19	10	70	27
5	32	11	24	13
6	26	6	20	6
7	88	24	32	13
8	22	10	44	9
9	28	6	50	14
10	25	7	44	18

- Expt. 1: Form of seed. From 253 hybrids 7324 seeds were obtained in the second trial year. Among them were 5474 round or roundish ones and 1850 angular wrinkled ones. Therefrom the ratio **2.96:1** is deduced.
- Expt. 2: Color of albumen.. 258 plants yielded 8023 seeds, 6022 yellow, and 2001 green; their ratio, therefore, is as **3.01:1**.

Gregor Mendel concluded correctly from his experiments:

1. that the inheritance of each trait is determined by "units" or "factors" that are passed on to descendents unchanged (these units are now called genes)
2. that an individual inherits one such unit from each parent for each trait
3. that a trait may not show up in an individual but can still be passed on to the next generation.

Gregor Mendel‘s experiments on plant genetics

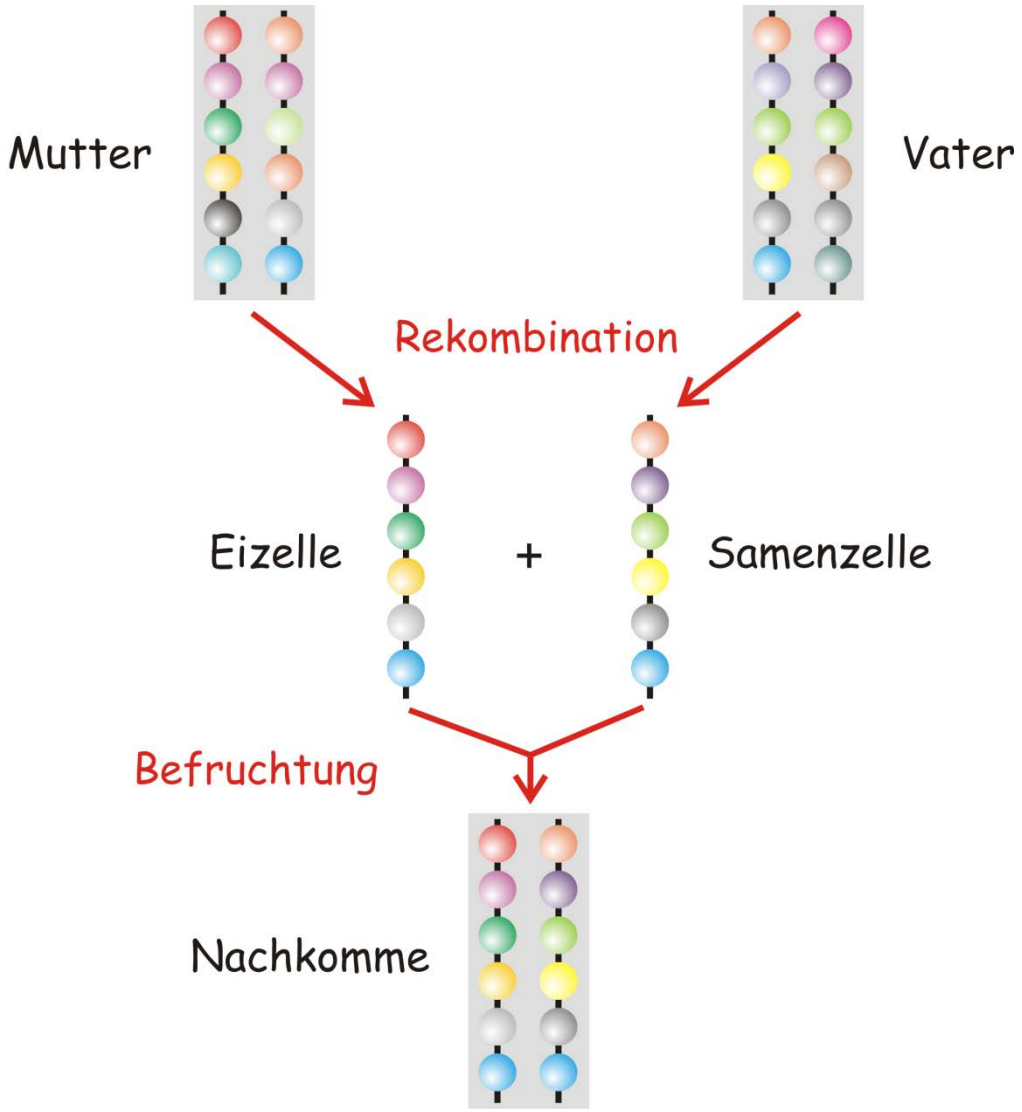


Intermediäres Allelpaar

Dominant/rezessives Allelpaar

Gregor Mendel, 1822-1884

Mendel's rules of inheritance:
white and red colors of flowers



Rekombination in Mendels Genetik

Ronald Fisher (1890-1962)

Allele: $A_1, A_2, \ldots, A_n$

Häufigkeiten: $x_i = [A_i]$; Genotypen: $A_i \cdot A_j$

Fitnesswerte: $a_{ij} = f(A_i \cdot A_j)$, $a_{ij} = a_{ji}$

Mendel

Darwin

$$\frac{dx_j}{dt} = \sum_{i=1}^{n} a_{ji}\, x_i\, x_j - \Phi\, x_j = x_j \left(\sum_{i=1}^{n} a_{ji}\, x_i - \Phi \right), \quad j = 1, 2, \ldots, n$$

$$\text{mit} \quad \Phi(t) = \sum_{j=1}^{n} \sum_{i=1}^{n} a_{ji}\, x_i\, x_j \quad \text{und} \quad \sum_{j=1}^{n} x_j = 1$$

$$\frac{d\Phi}{dt} = 2\left(< \bar{a}^2 > - < \bar{a} >^2 \right) = 2\, \mathrm{var}\{\bar{a}\} \geq 0$$

Ronald Fisher‘s Selektionsgleichung: The genetical theory of natural selection. Oxford, UK, Clarendon Press, 1930.

Ronald Fisher (1890-1962)

J. B. S. Haldane (1892-1964)

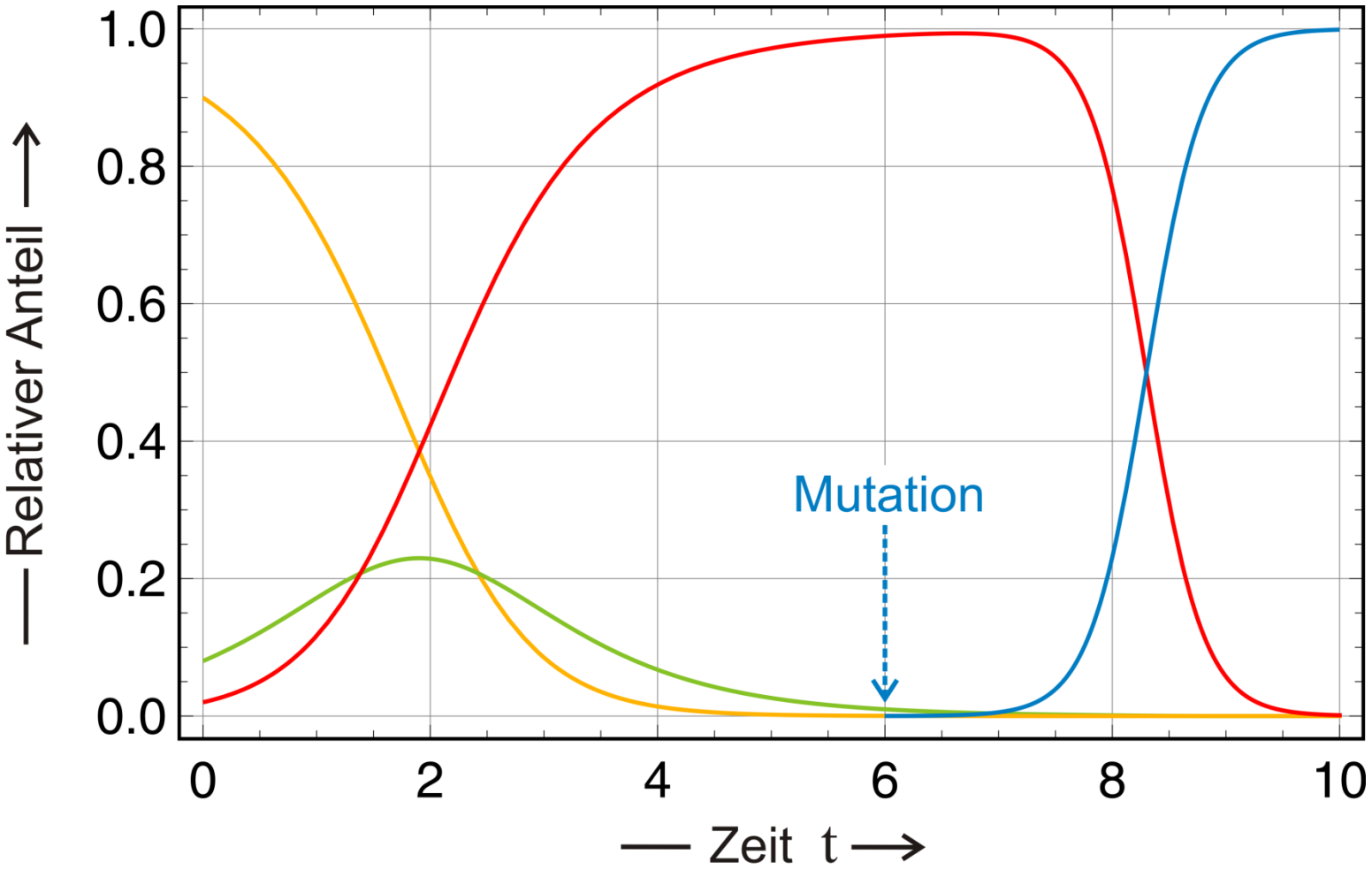
Sewall Wright (1889-1988)

Die drei Begründer der Populationsgenetik

Theodosius Dobzhansky,
1900 – 1975

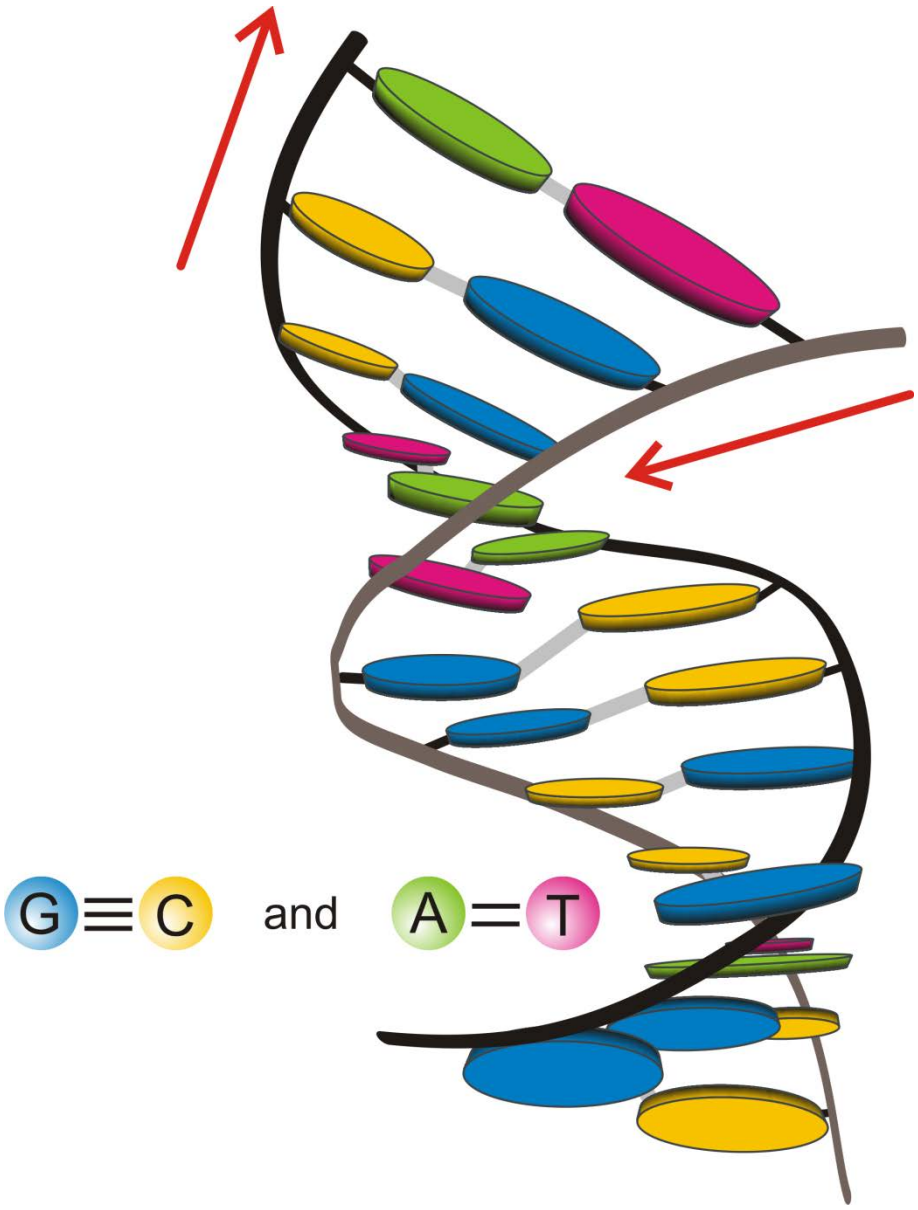
„Genetics is the first biological science which got in the position in which physics has been for many years. One can justifiably speak about such a thing as theoretical mathematical genetics, and experimental genetics, just as in physics. There are some mathematical geniuses who work out what to an ordinary person seems as a fantastic kind of theory. This fantastic kind of theory nevertheless leads to experimentally verifiable prediction, which an experimental physicist then has to test the validity of. Since the times of Wright, Haldane, and Fisher, evolutionary genetics has been in a similar position."

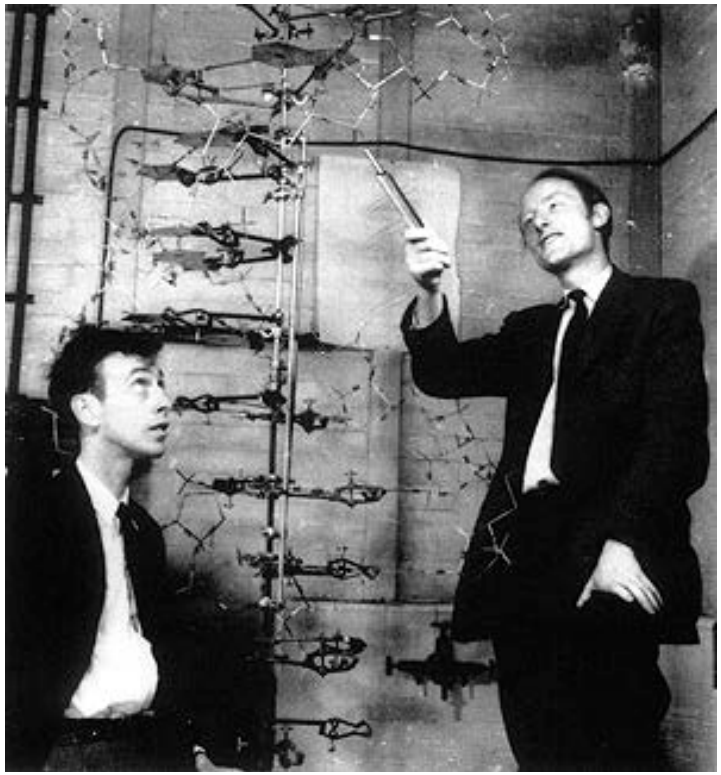
Variation durch Mutation



$f_1 = 1, f_2 = 2, f_3 = 3, f_4 = 7$

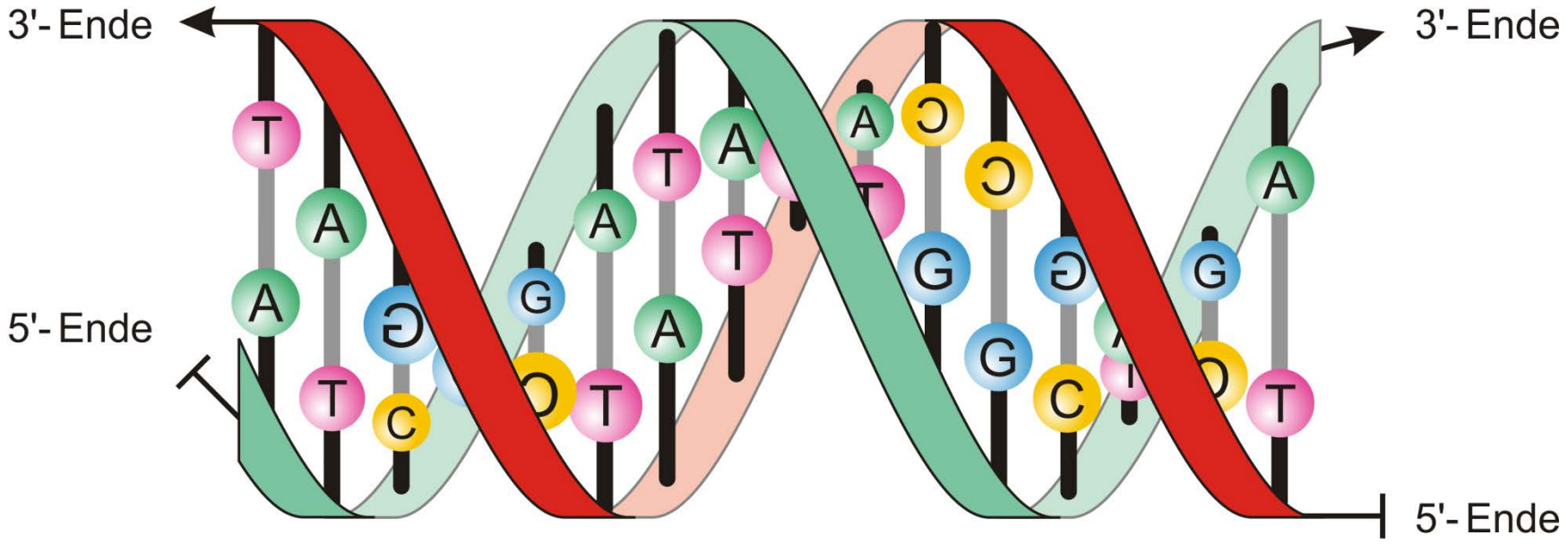
Vor der Entwicklung der Molekularbiologie wurde Mutation als ein "Deus ex Machina" behandelt



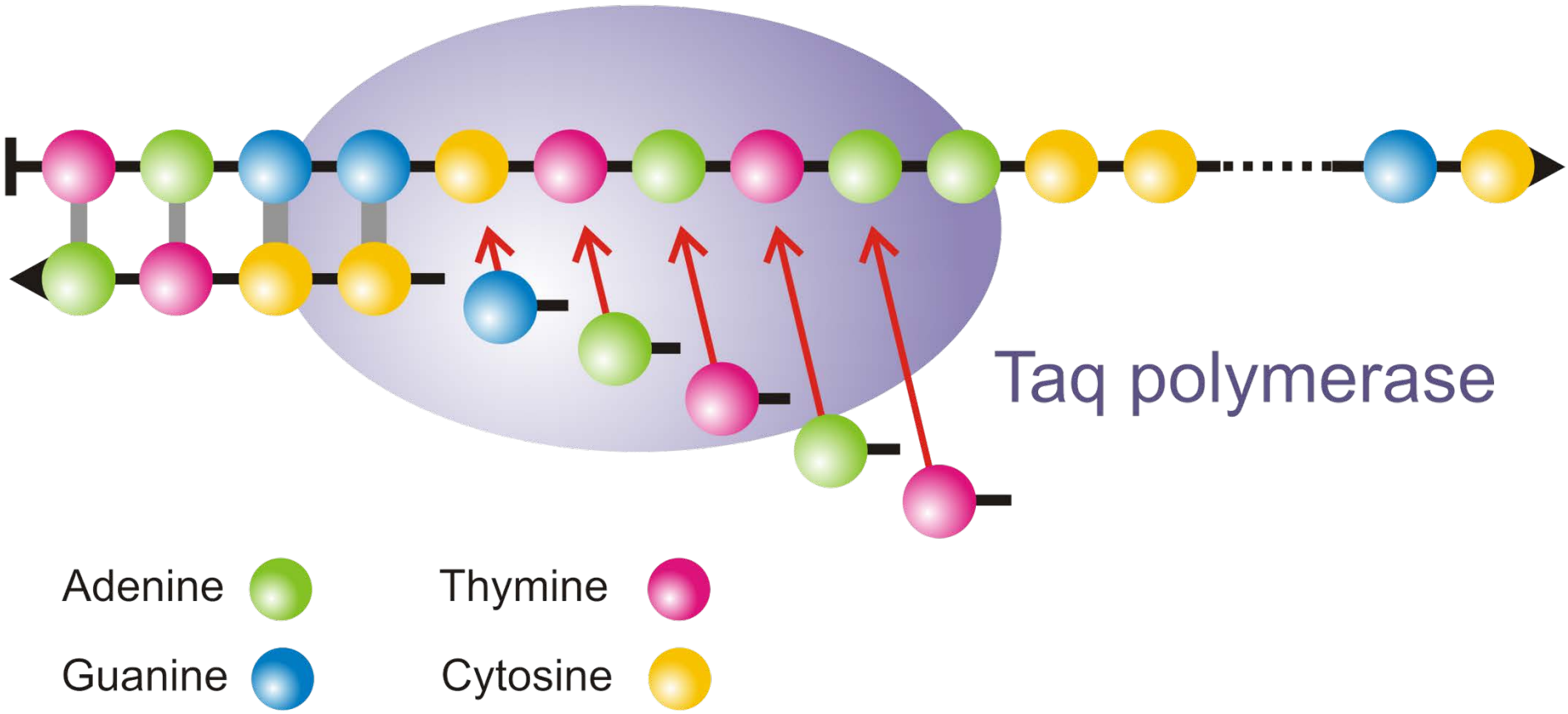


James D. Watson, 1928- , and Francis Crick, 1916-2004,
Nobel Preis 1962

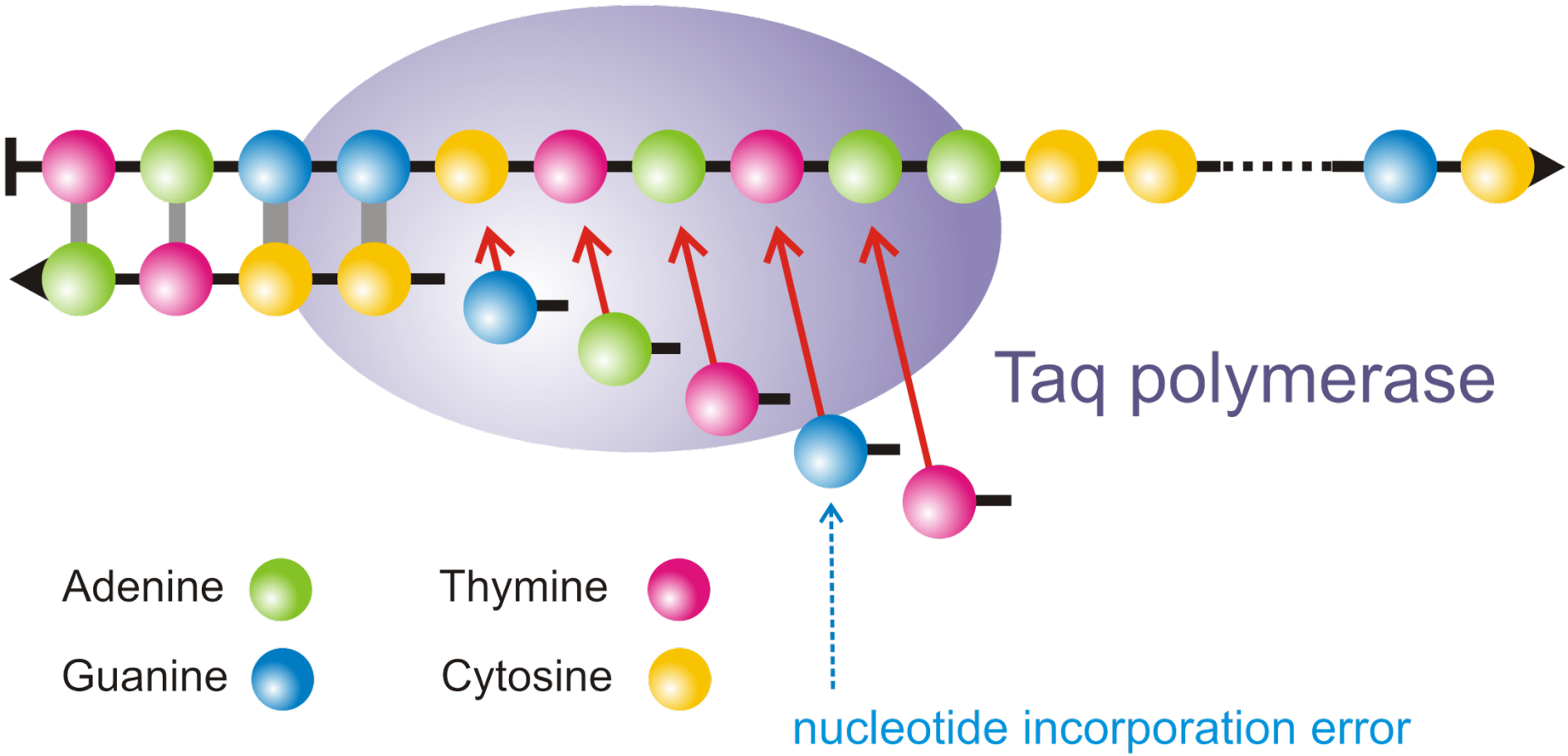
Die dreidimensionale Struktur eines kleinen Stückes der B-DNA



Die B-Form der DNA-Doppelhelix



The logic of DNA (or RNA) replication and mutation



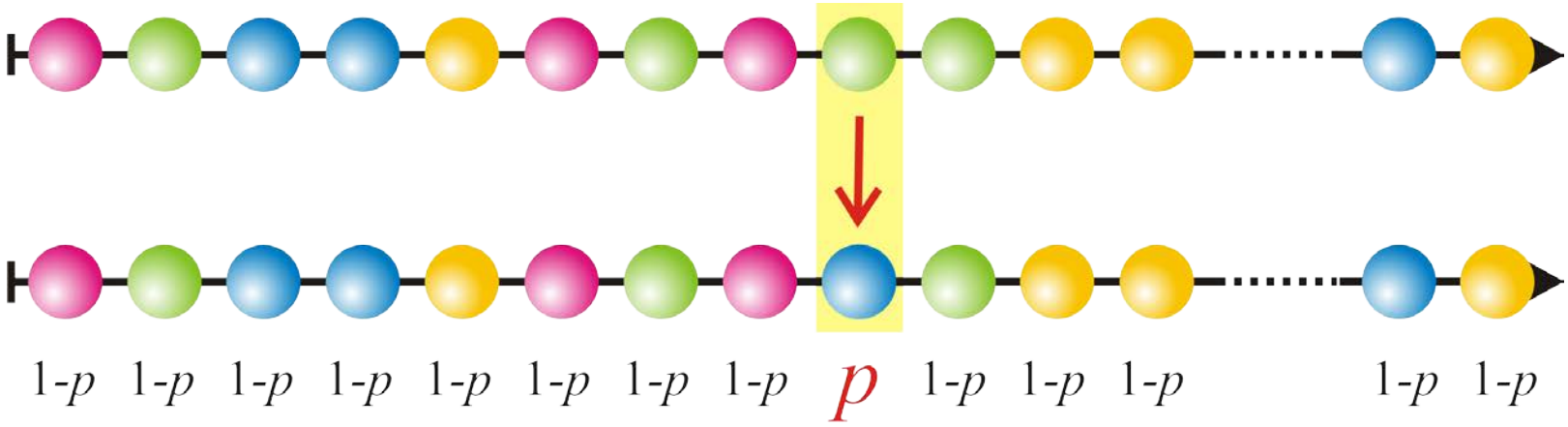
p ..... mutation rate per site and replication

The logic of DNA (or RNA) replication and mutation

$$Q_{ij} = (1-p)^{n-d_{\mathrm{H}}(\mathrm{X}_i,\mathrm{X}_j)}\; p^{d_{\mathrm{H}}(\mathrm{X}_i,\mathrm{X}_j)}$$

p = Fehler- oder Mutationsrate pro Replikation und Nukleotid

n = Kettenlänge und $d_{\mathrm{H}}(\mathrm{X_i},\mathrm{X_j})$ = Hammingabstand



Punktmutation

Von der Theorie zur Anwendung:

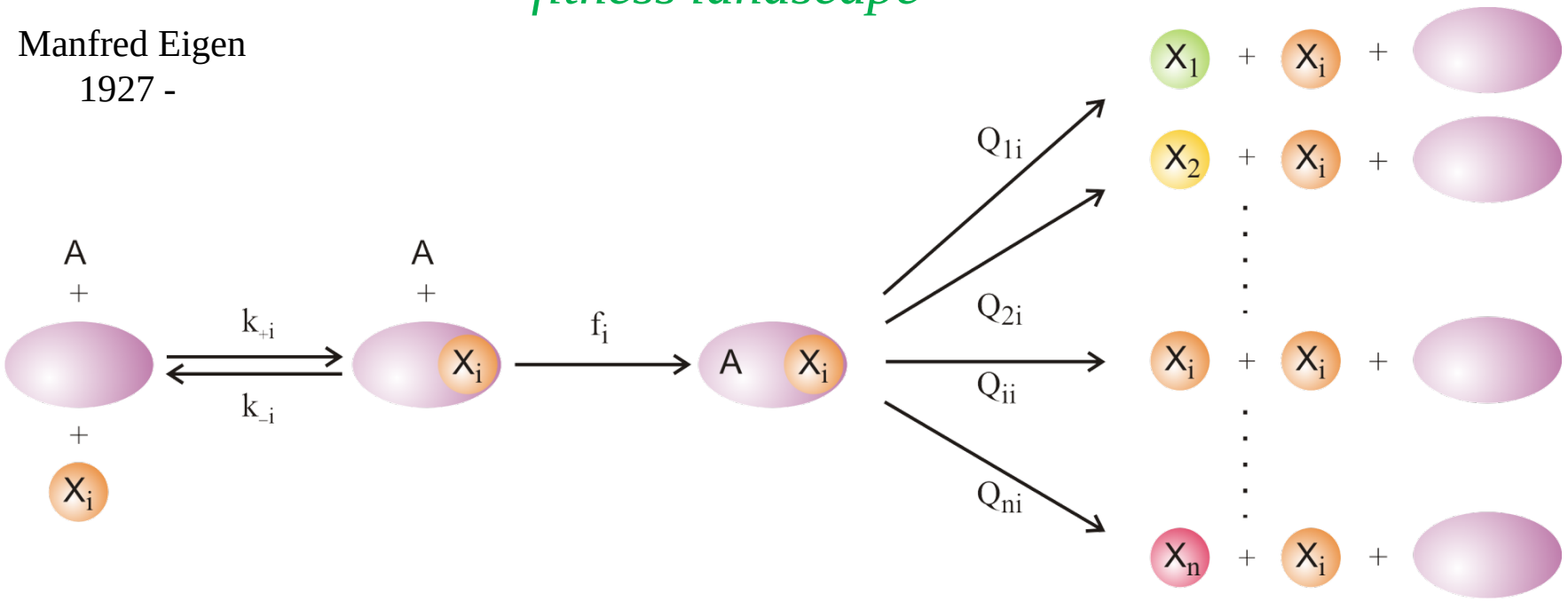
Konkurrenz und Mutation

Manfred Eigen
1927 -

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

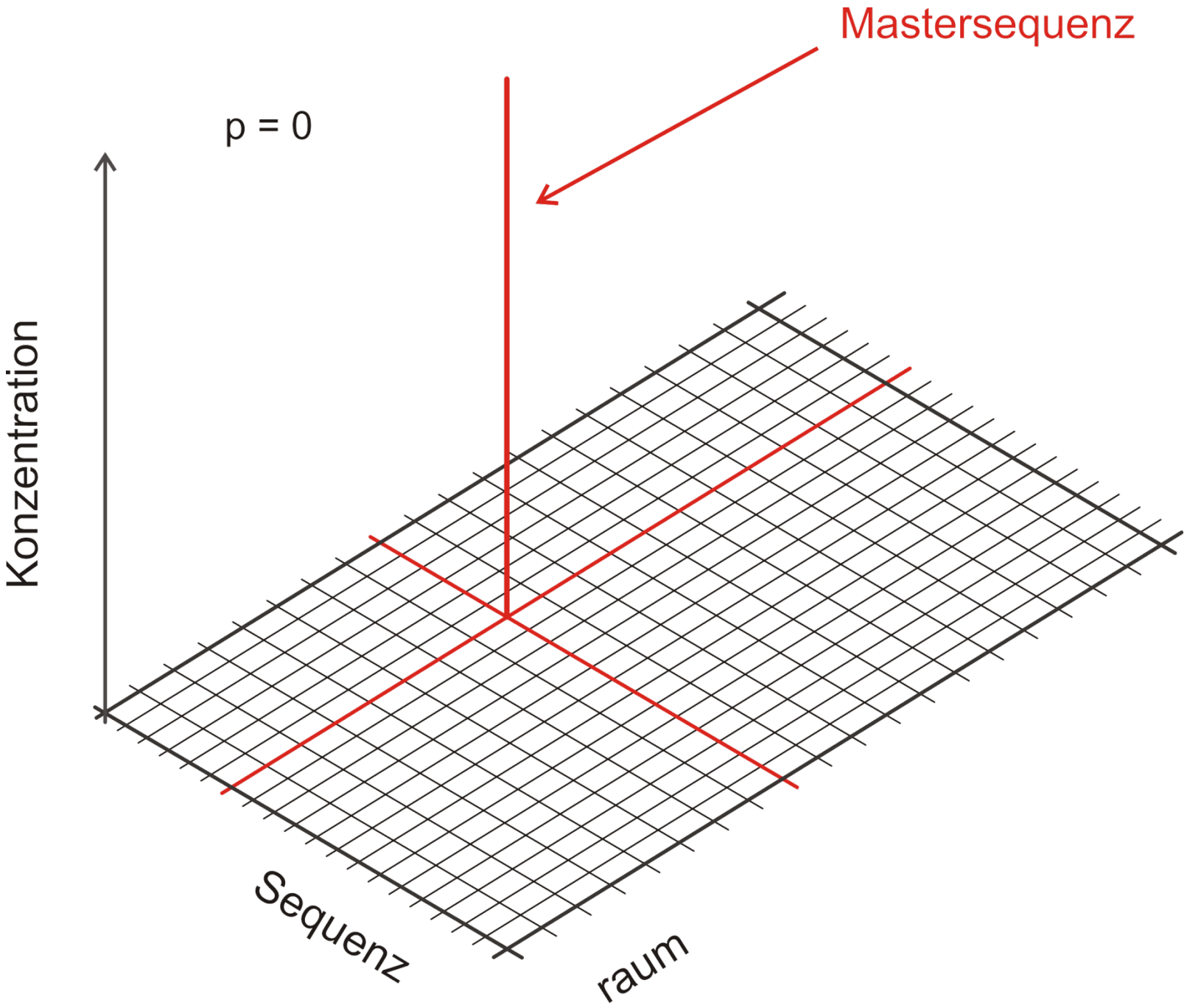
$$W_{ji} = Q_{ji} \cdot f_i\,, \quad \sum_{i=1}^{n} x_i = 1, \quad \Phi = \sum_{i=1}^{n} f_i\, x_i$$

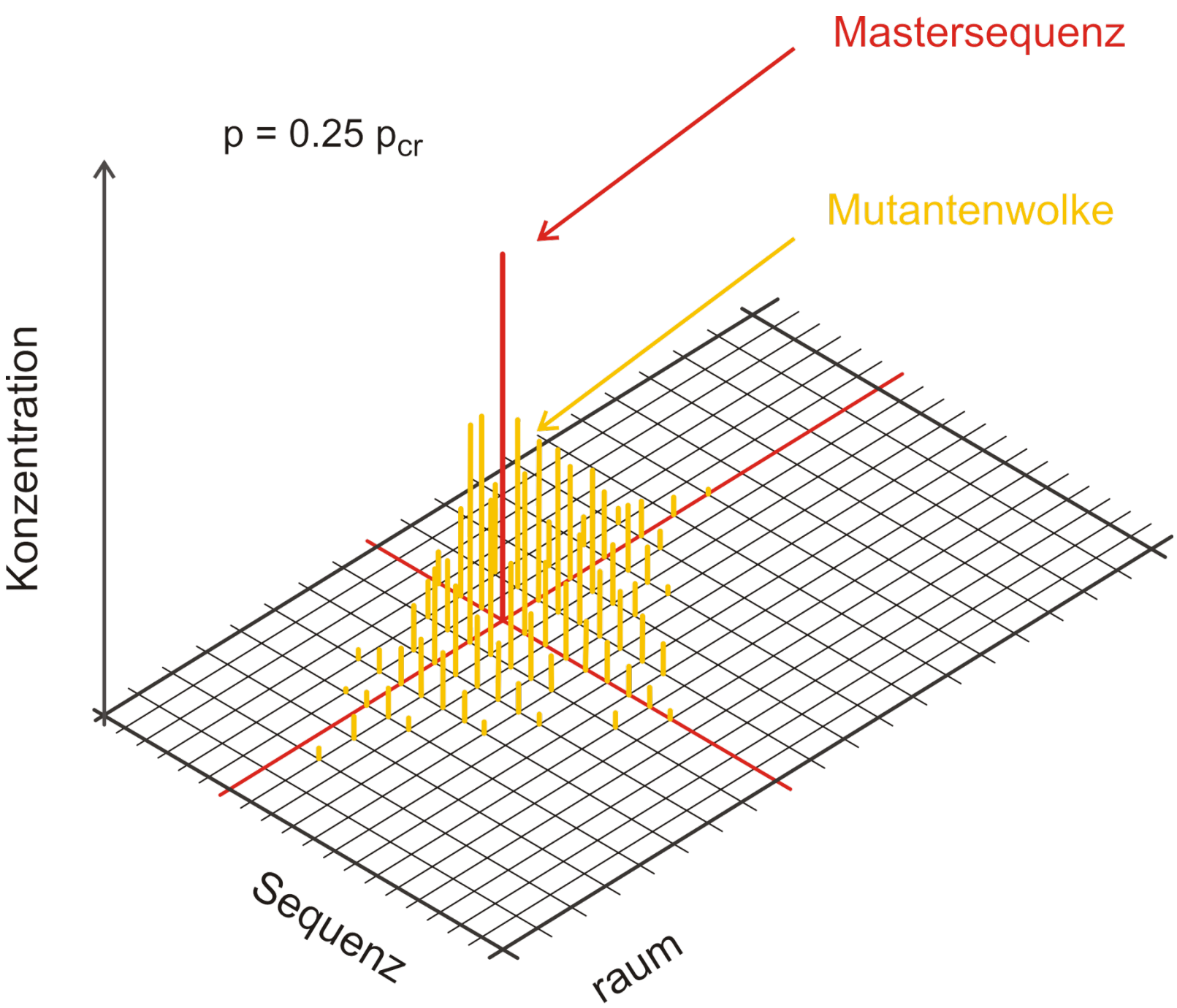
fitness landscape

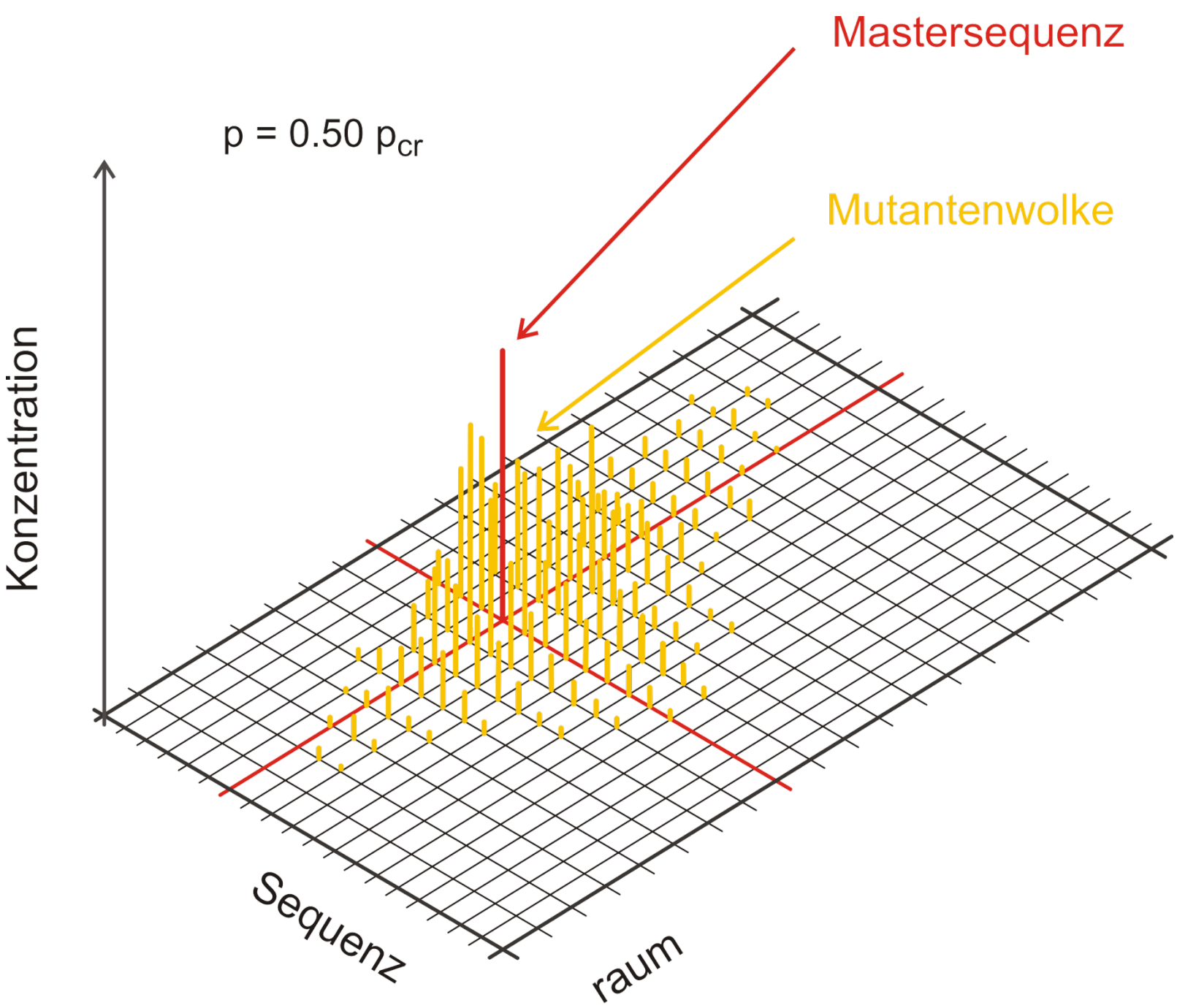


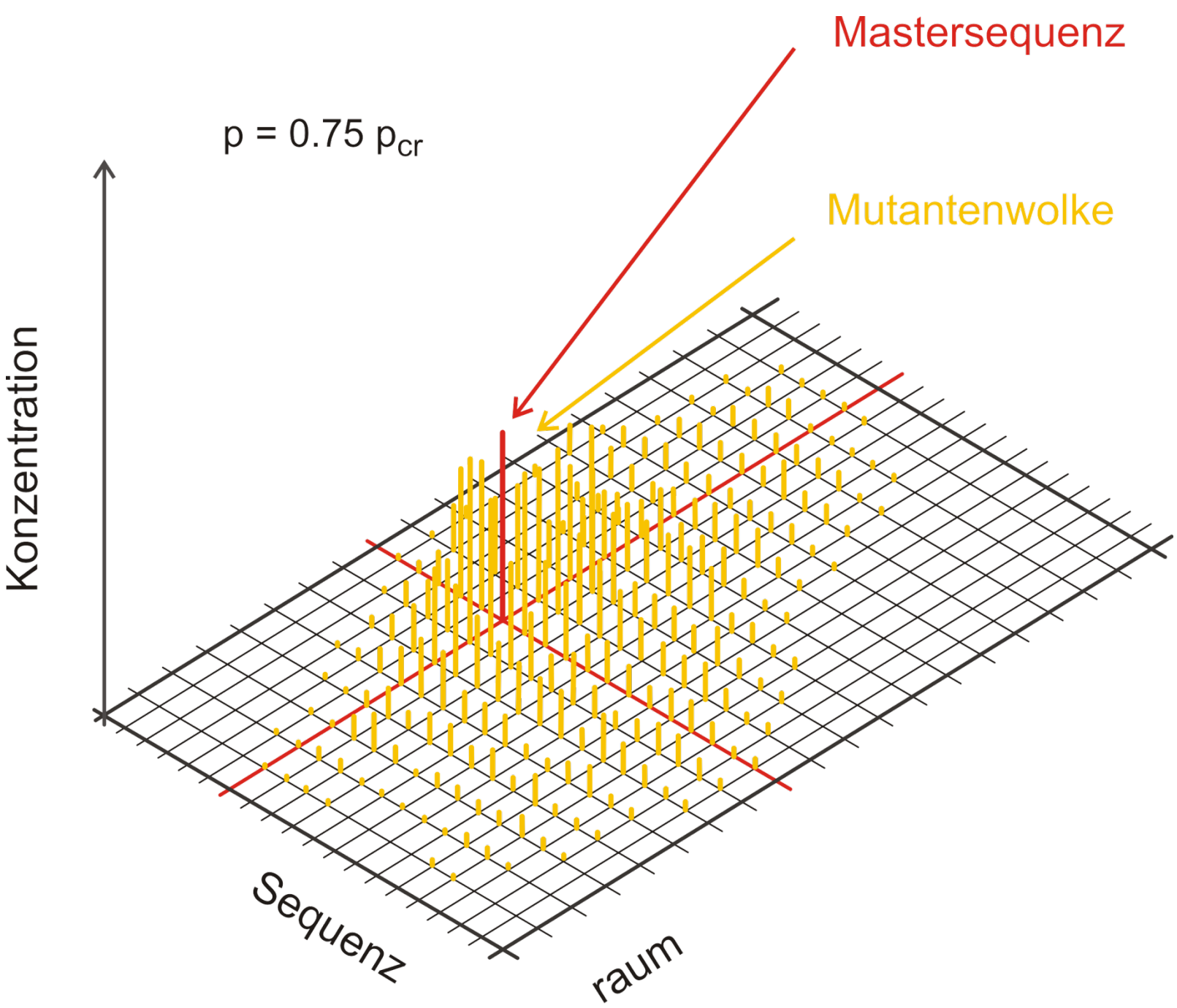
Mutation and (correct) replication as parallel chemical reactions

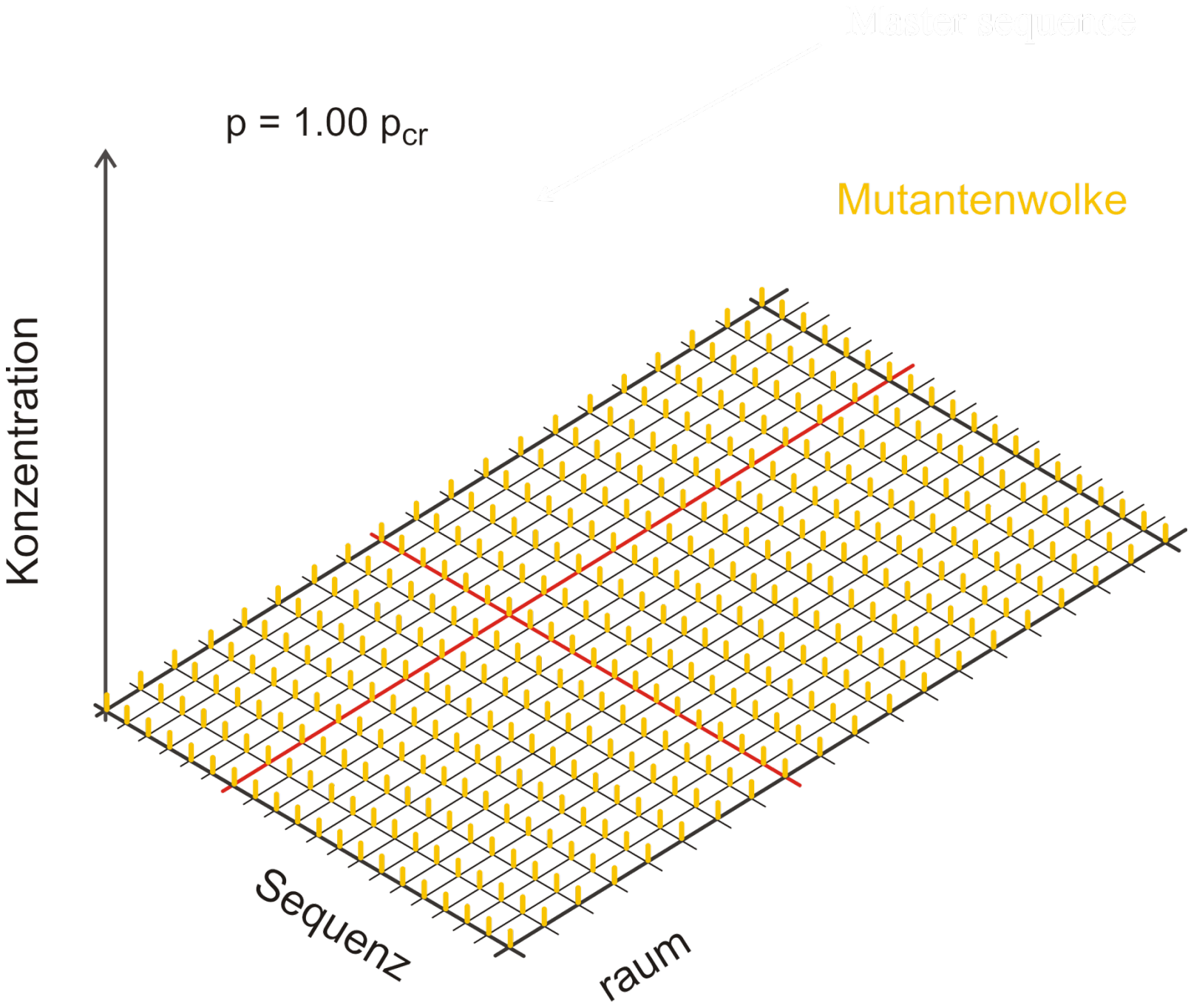
M. Eigen. 1971. *Naturwissenschaften* 58:465,
M. Eigen & P. Schuster.1977. *Naturwissenschaften* 64:541, 65:7 und 65:341


Mastersequenz
p = 0
Konzentration
Sequenz
raum


Mastersequenz
p = 0.25 p_cr
Mutantenwolke
Konzentration
Sequenz
raum


Mastersequenz
p = 0.50 p_{cr}
Mutantenwolke
Konzentration
Sequenz
raum


Mastersequenz
Mutantenwolke
p = 0.75 p_cr
Konzentration
Sequenz
raum


Master sequence
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Mutantenwolke
Konzentration
Sequenz
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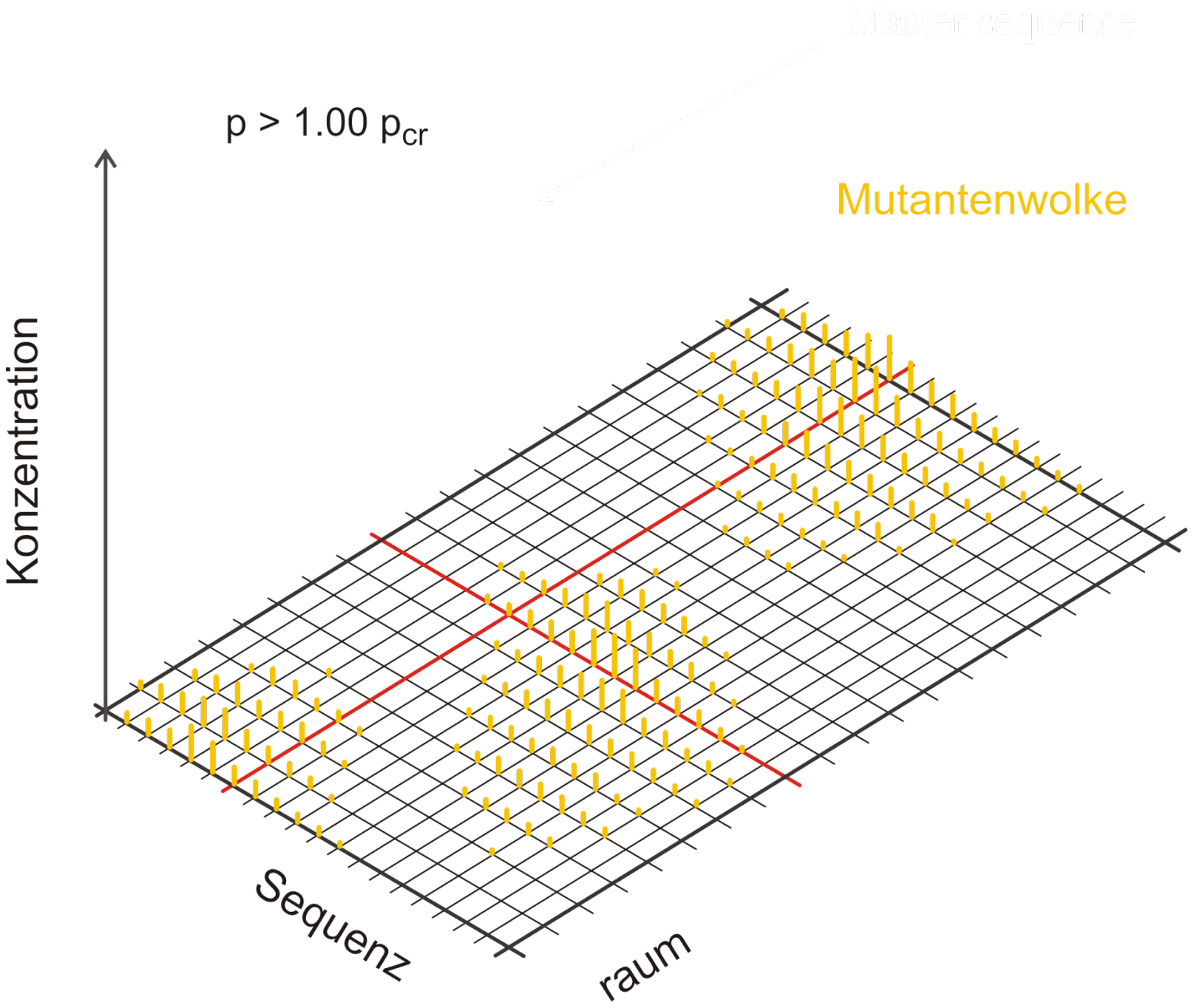
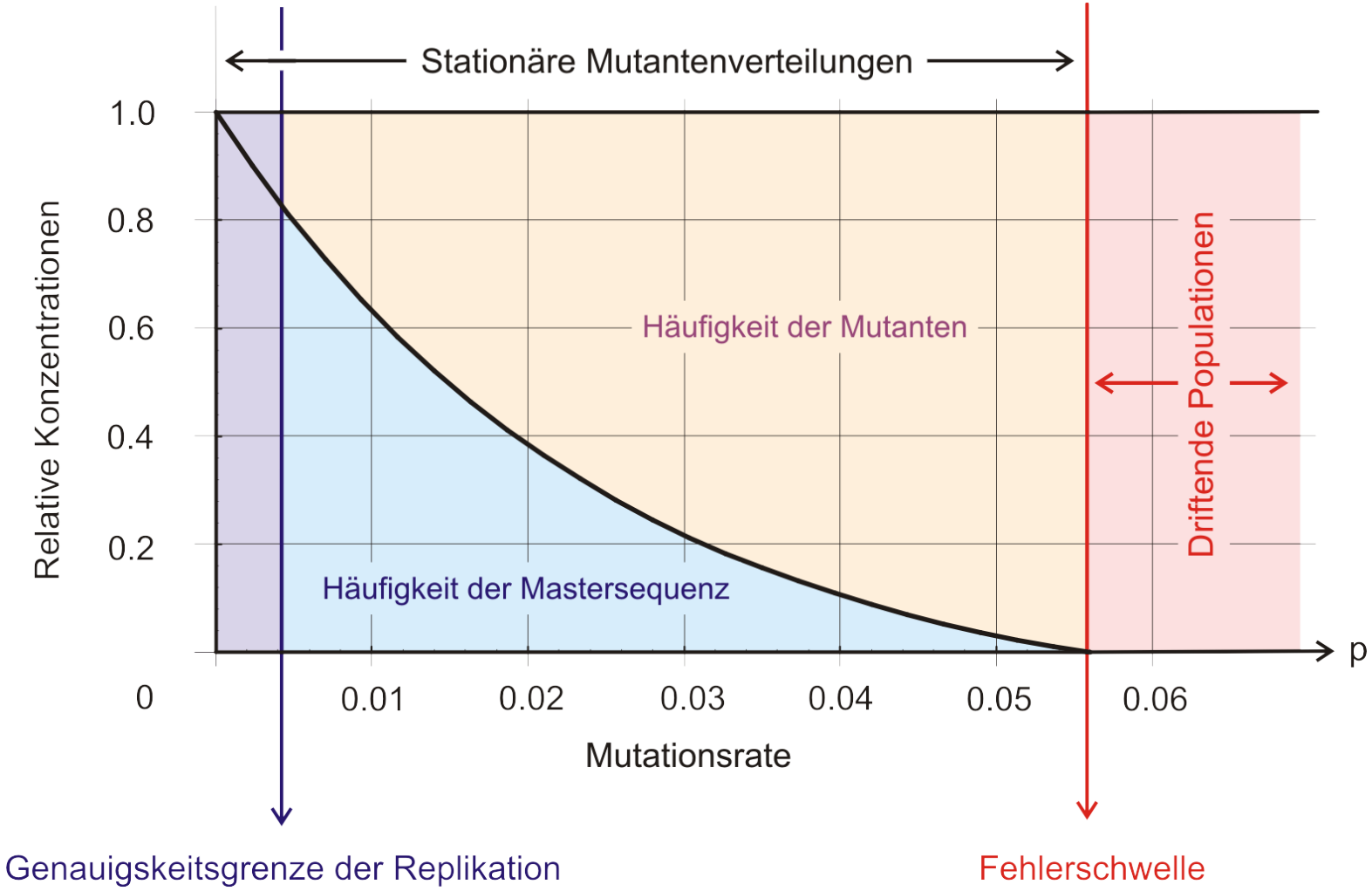
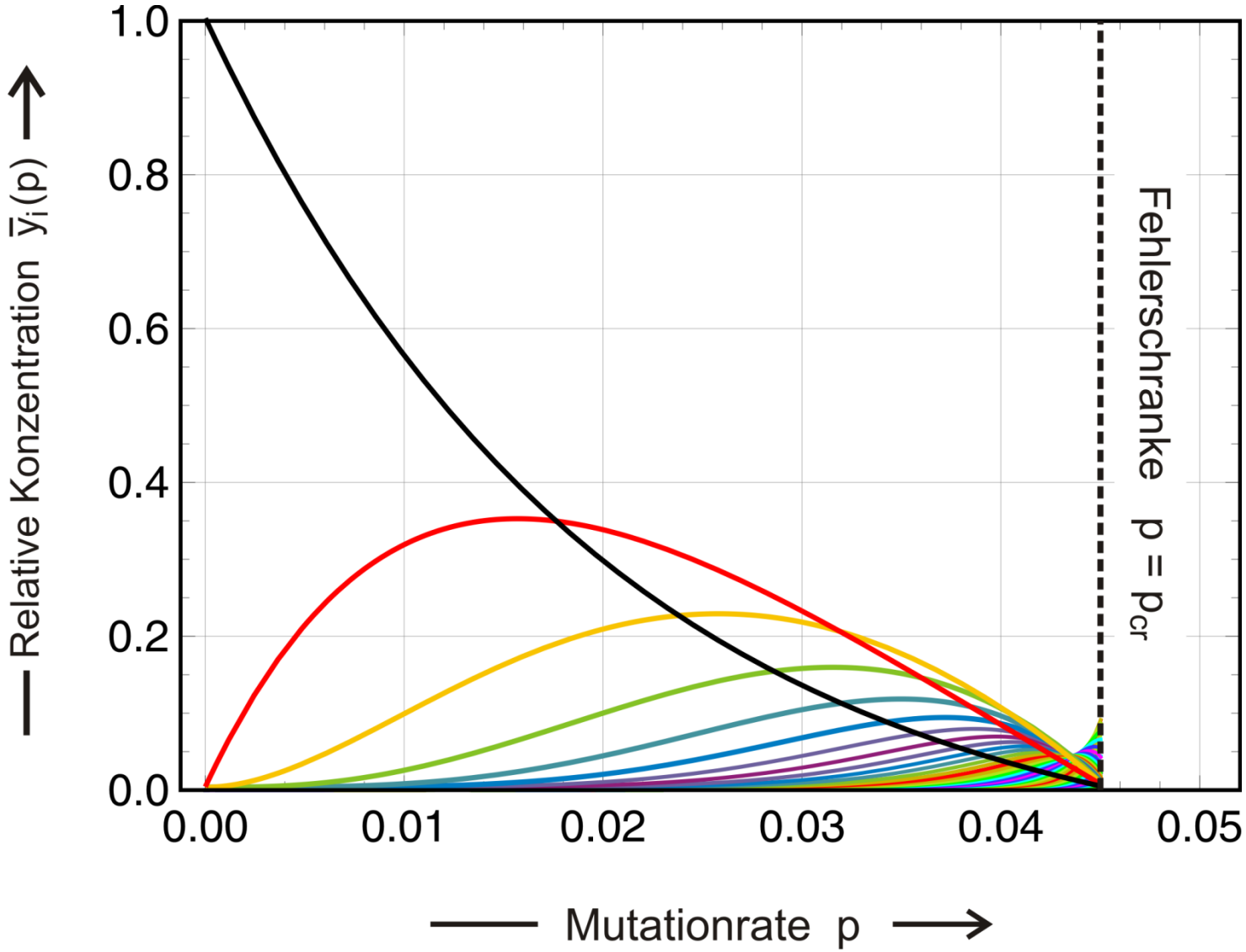
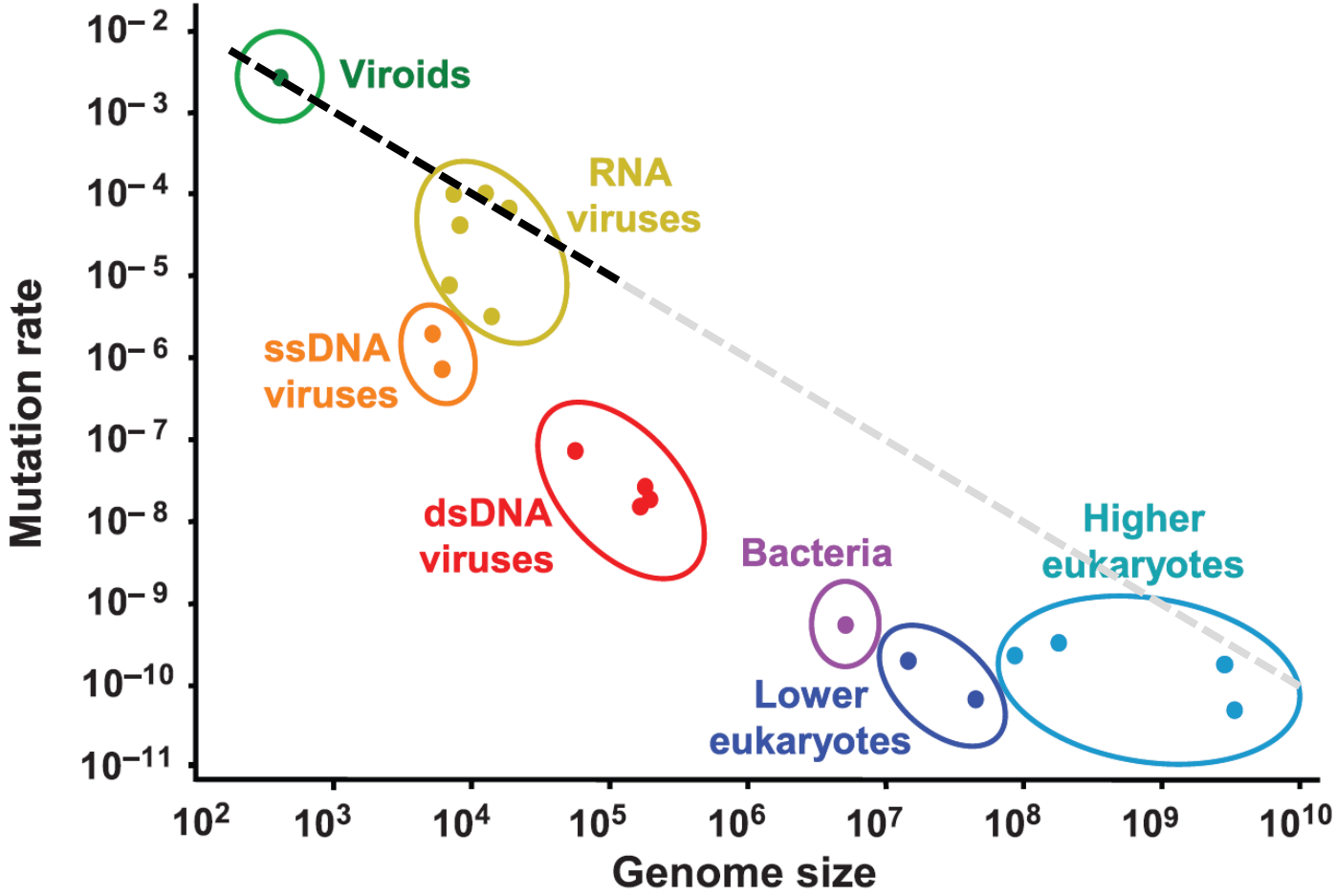

Master sequence
p > 1.00 p_{cr}
Mutantenwolke
Konzentration
Sequenz
raum



Die Fehlerschwelle bei Replikation und Mutation



Die stationäre Mutantenverteilung als Funktion der Mutationsrate p



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

Charles Darwin, 1809-1882

Three necessary conditions for Darwinian evolution are:

1. **Multiplication,**
2. **Variation**, and
3. **Selection**.

All three conditions are fulfilled not only by cellular organisms but also by **nucleic acid molecules** – **DNA** or **RNA** – **in** suitable **cell-free experimental assays**:

Darwinian evolution in the test tube

Sol Spiegelman,
1914 - 1983

Evolution im Reagenzglas:

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

G. F. Joyce

DOI: 10.1002/anie.200701369

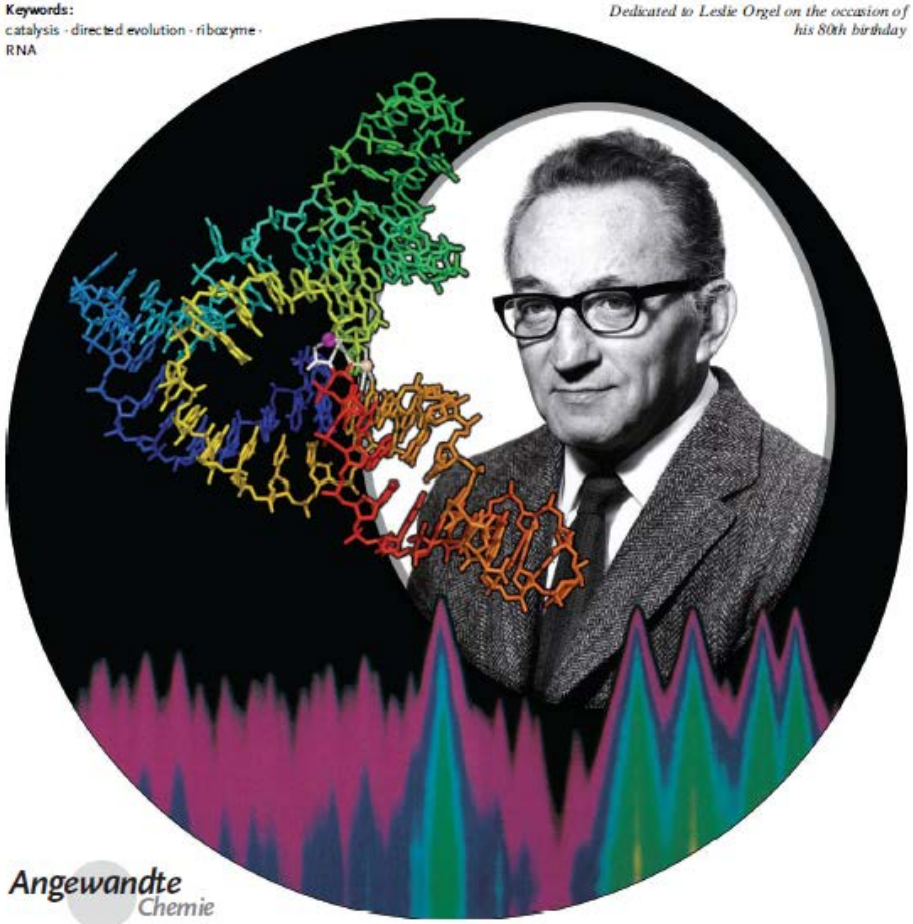
Molecular Evolution

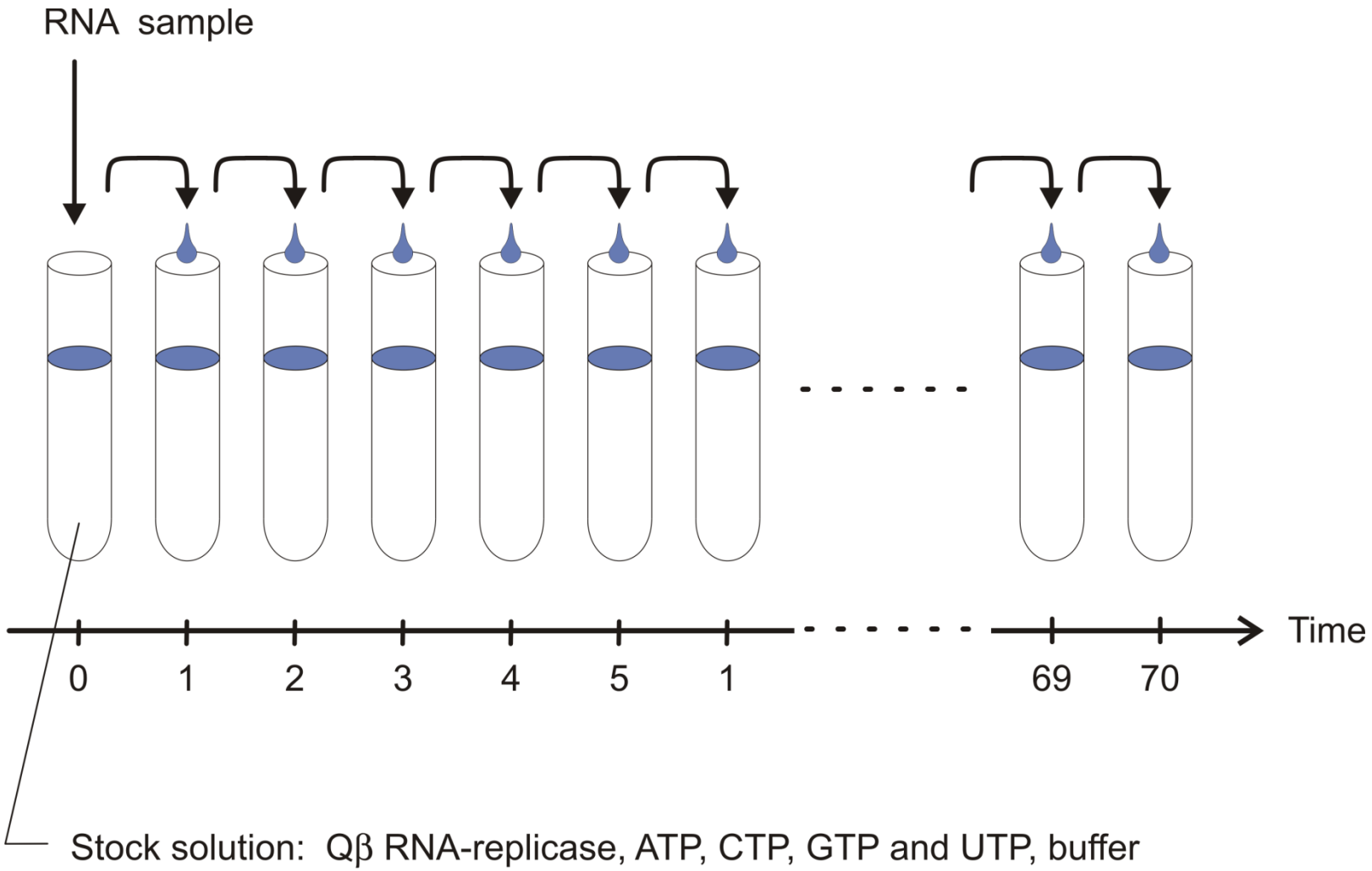
Forty Years of In Vitro Evolution**

*Gerald F. Joyce**

Keywords:
catalysis · directed evolution · ribozyme · RNA

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





Anwendung der Technik des seriellen Transfers zur Evolution von RNA im Reagenzglas

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

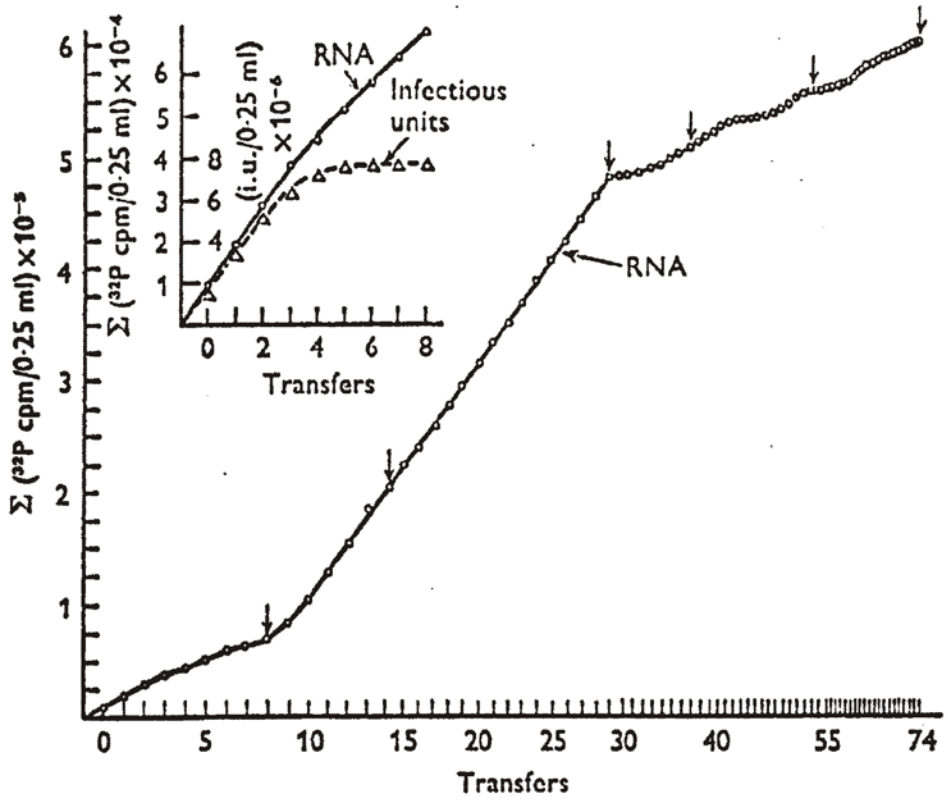
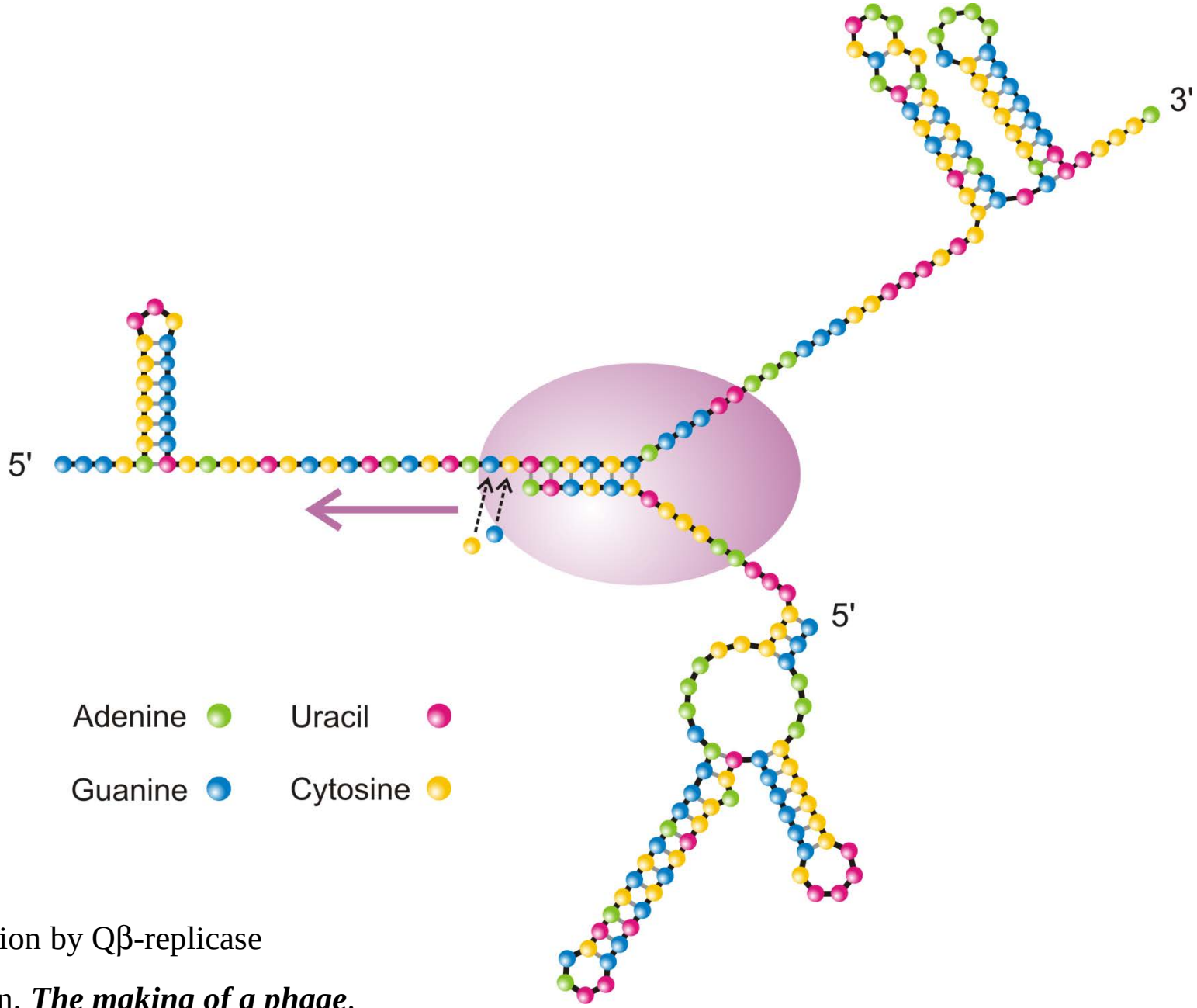


Fig. 9. Serial transfer experiment. Each 0·25 ml standard reaction mixture contained 40 μg of Qβ replicase and ^{32}P-UTP. The first reaction (0 transfer) was initiated by the addition of 0·2 μg ts-1 (temperature-sensitive RNA) and incubated at 35 °C for 20 min, whereupon 0·02 ml was drawn for counting 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).

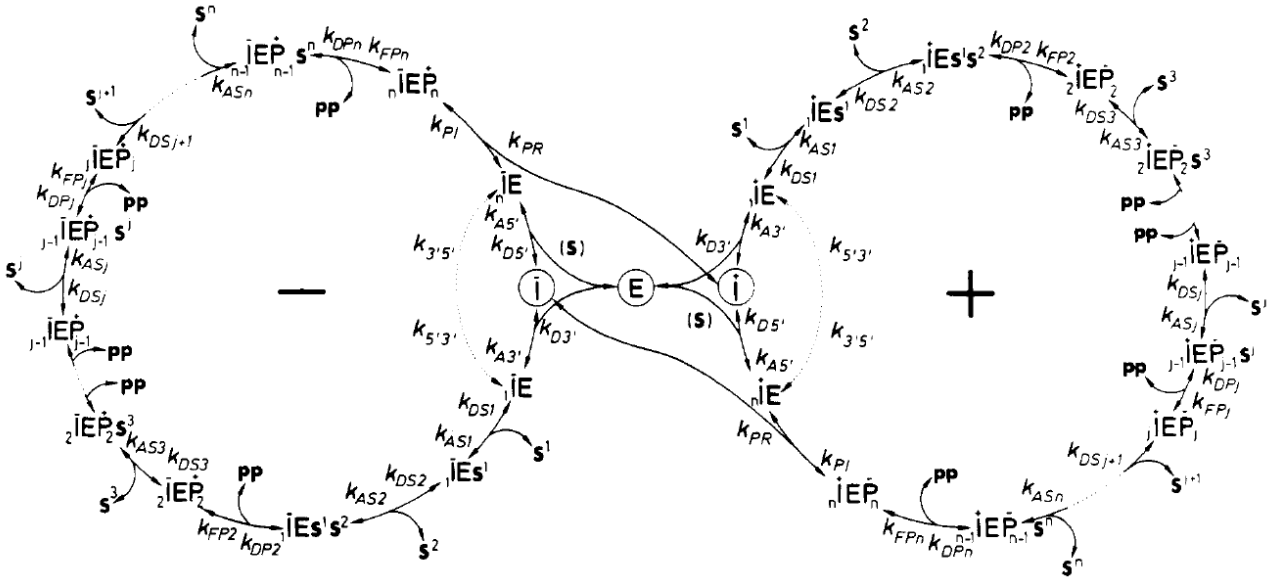
D.R.Mills, R,L,Peterson, S.Spiegelman, *An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule*. Proc.Natl.Acad.Sci.USA **58** (1967), 217-224



RNA replication by Qβ-replicase

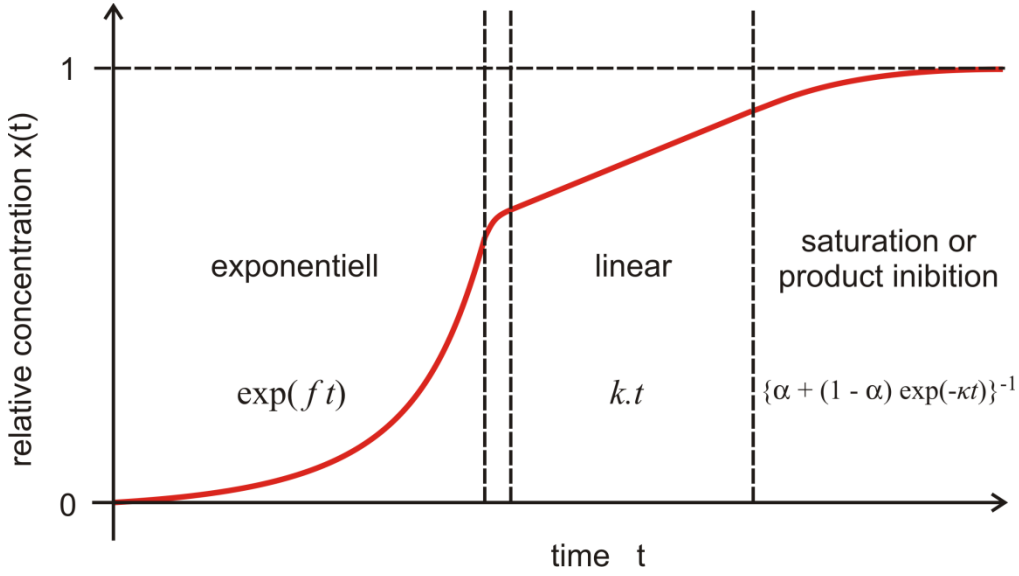
C. Weissmann, ***The making of a phage***.
FEBS Letters **40** (1974), S10-S18

Christof K. Biebricher,
1941-2009

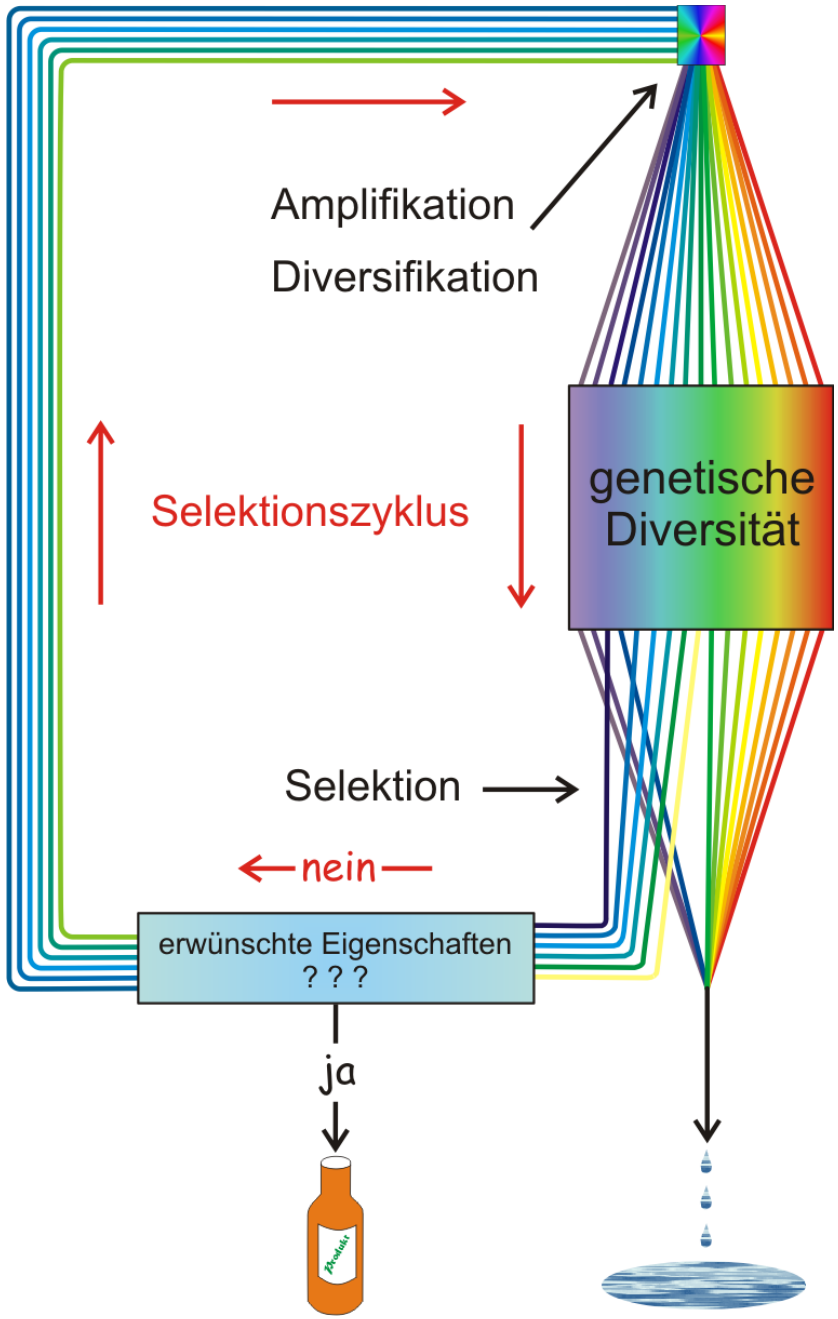


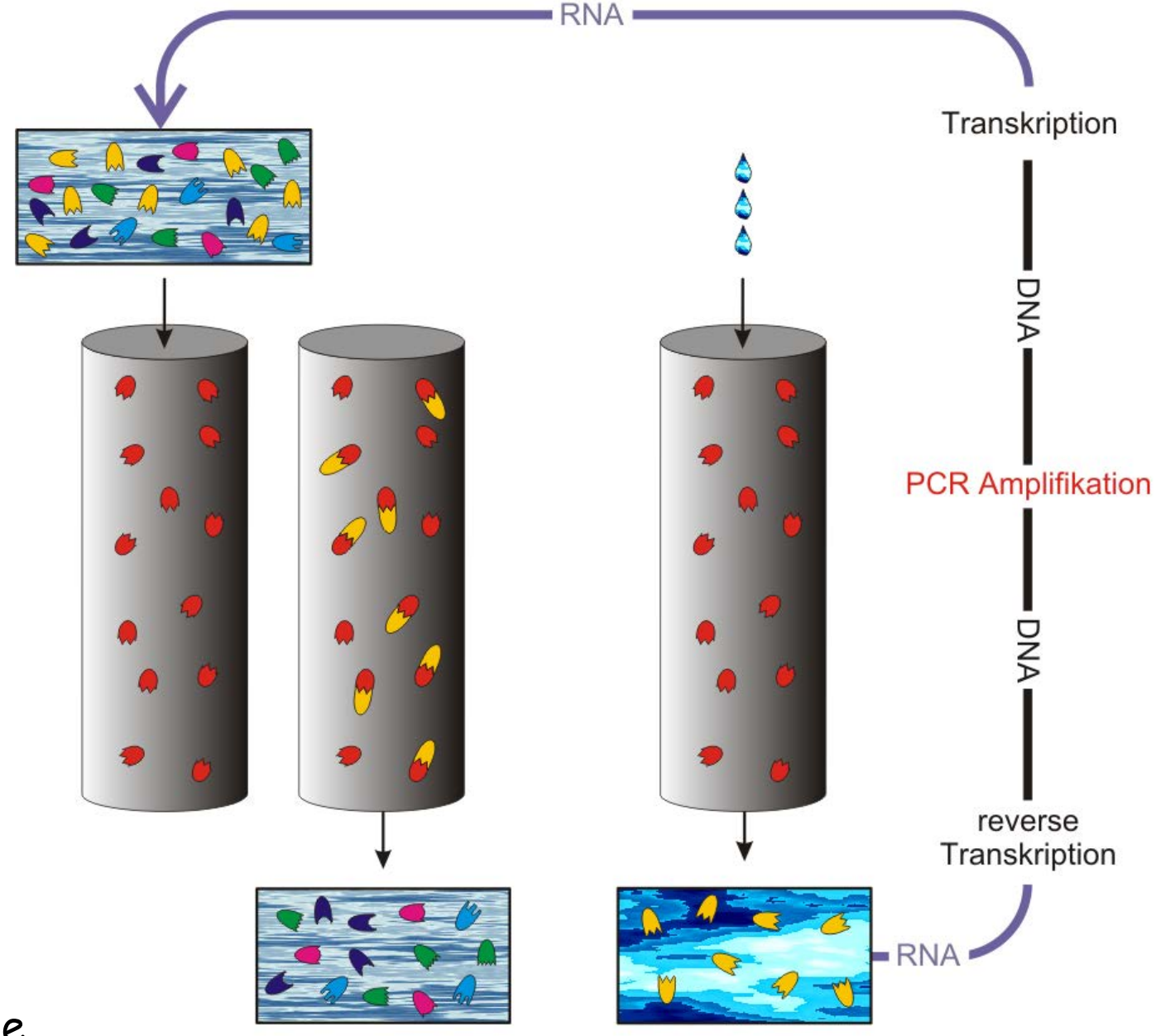
Kinetik der RNA Replikation

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

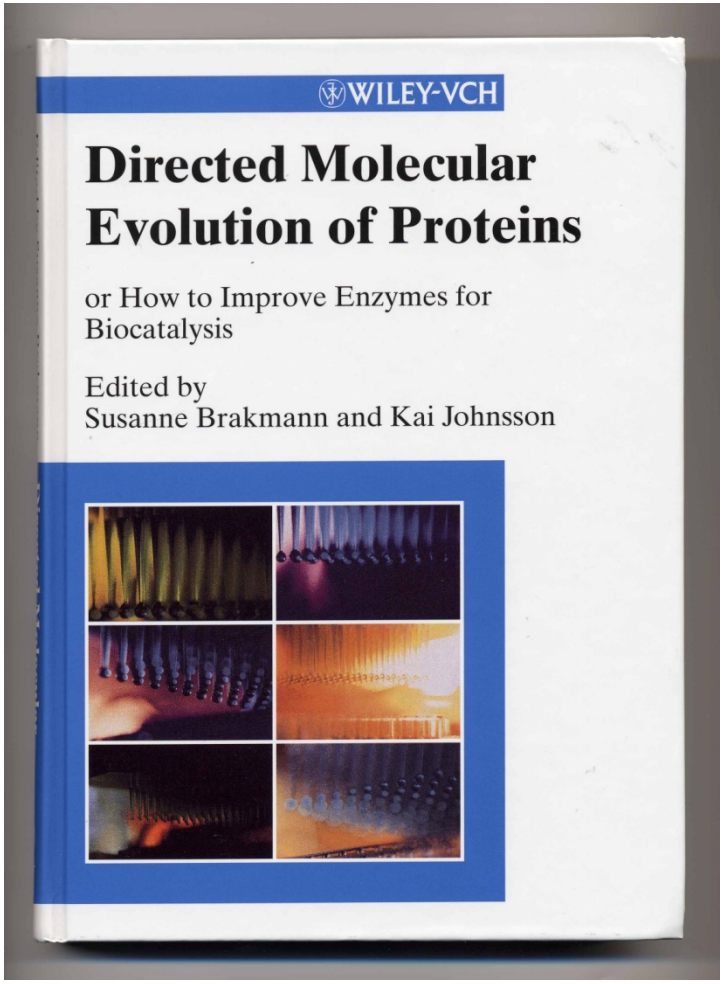


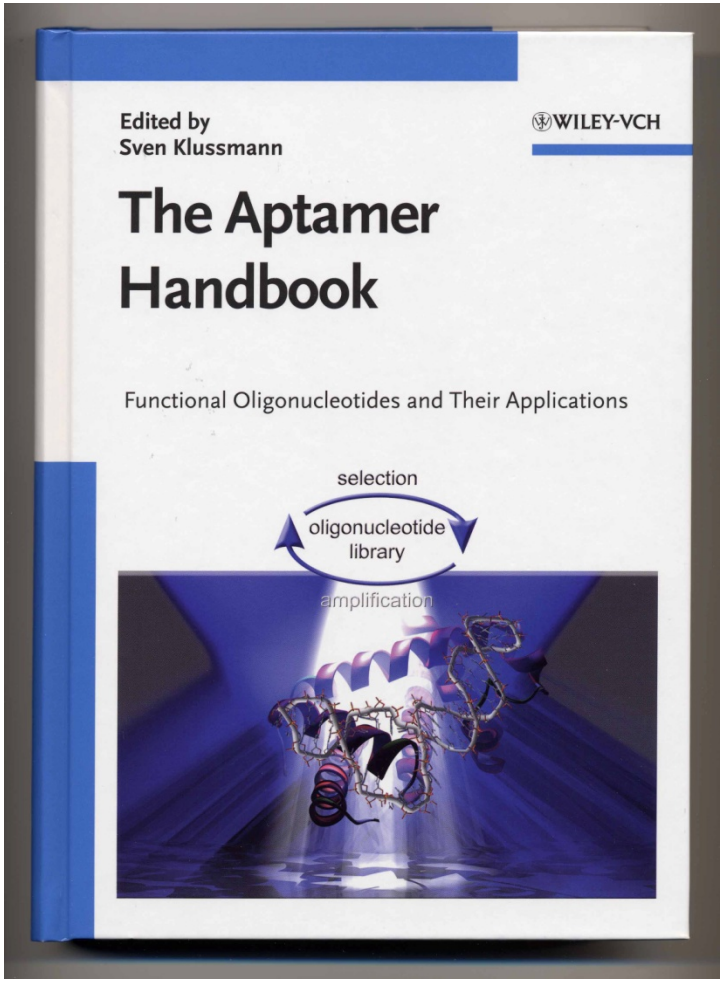
Das Prinzip der evolutionären Biotechnologie



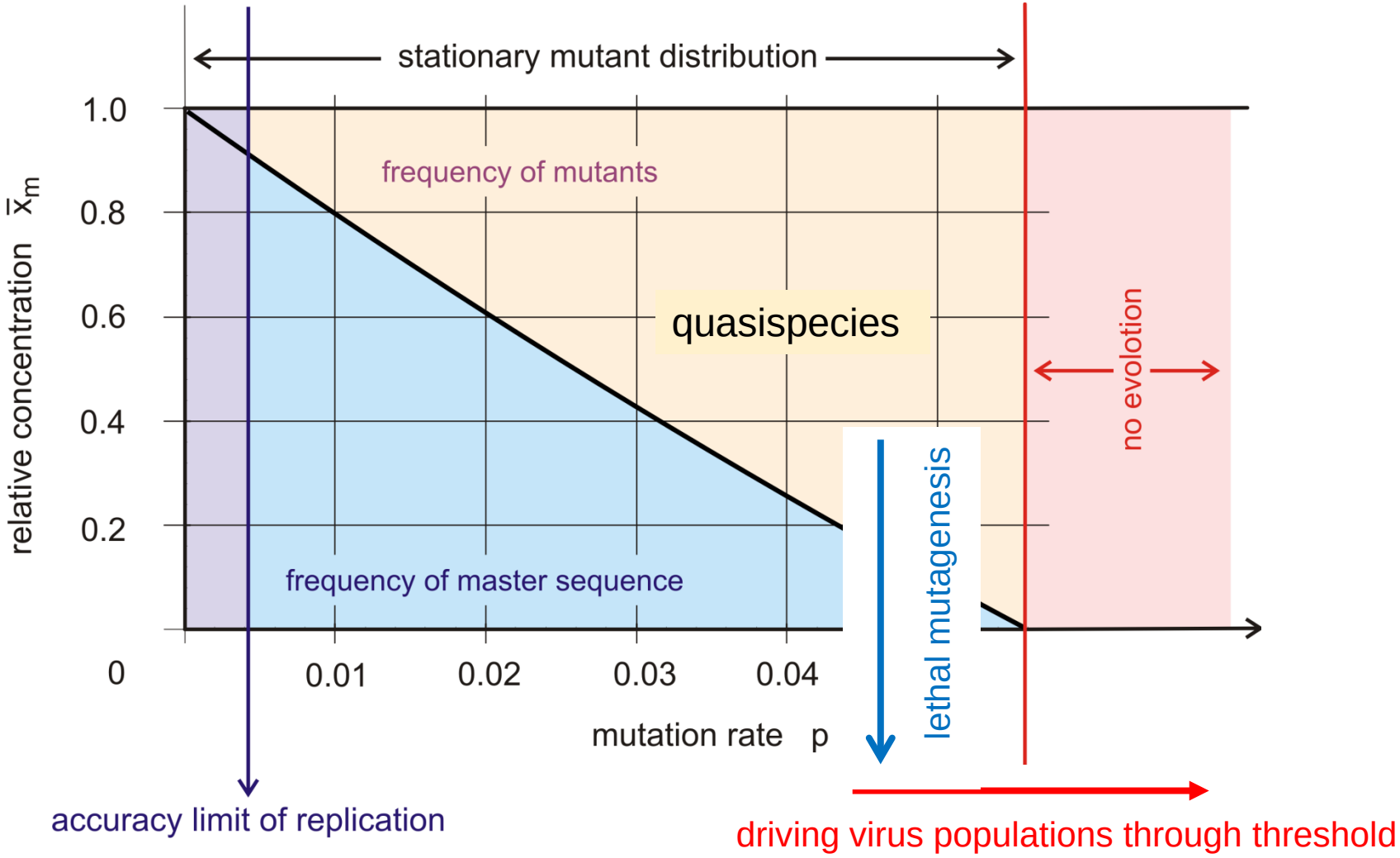


The PCR technique
as a selection tool





Application of molecular evolution to problems in biotechnology



The error threshold in replication

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Virus Research 107 (2005) 115–116
Virus Research
www.elsevier.com/locate/virusres

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.

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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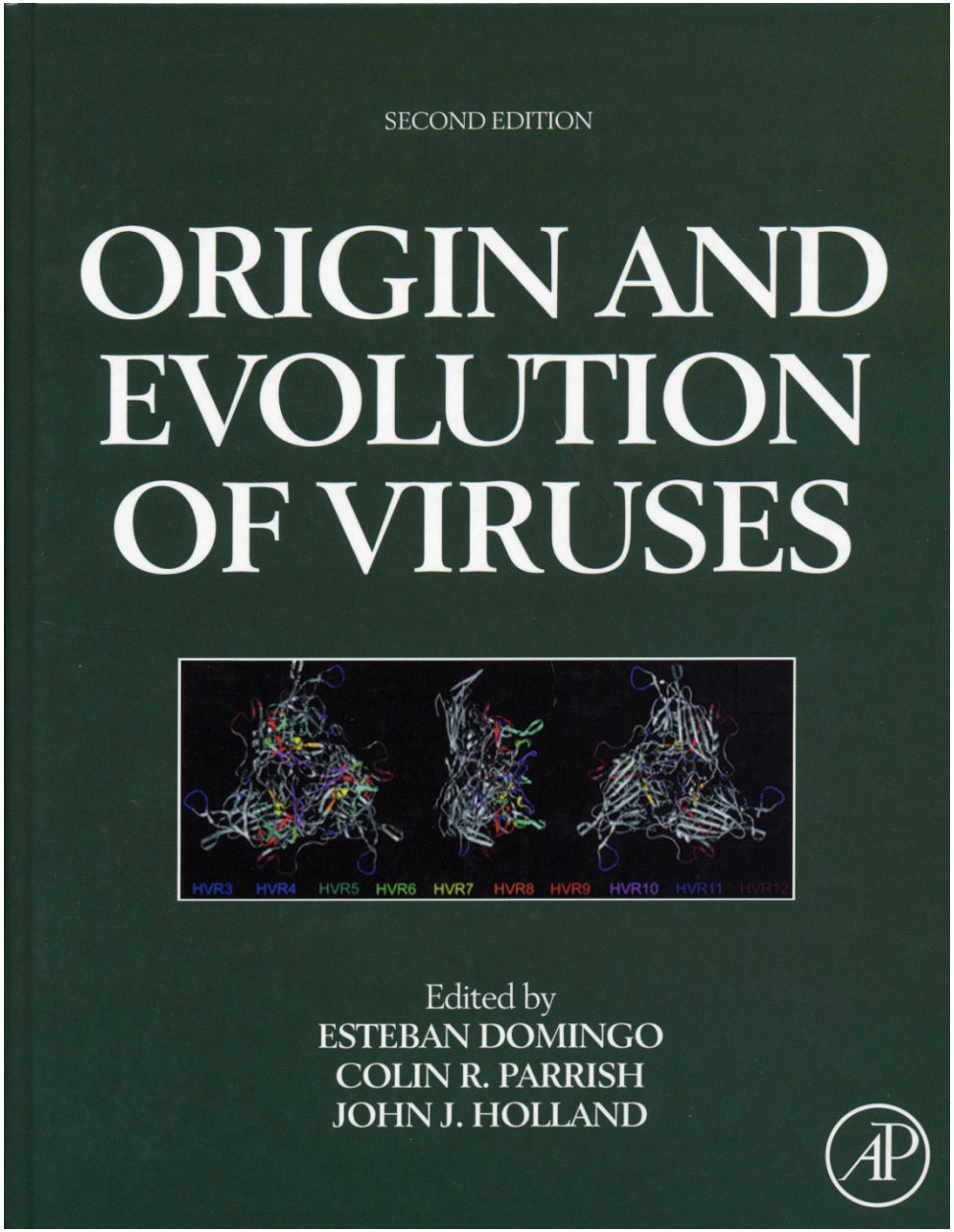
Available online 8 December 2004

doi:10.1016/j.virusres.2004.11.001

Esteban Domingo
1943 -

Application of quasispecies theory to the fight against viruses

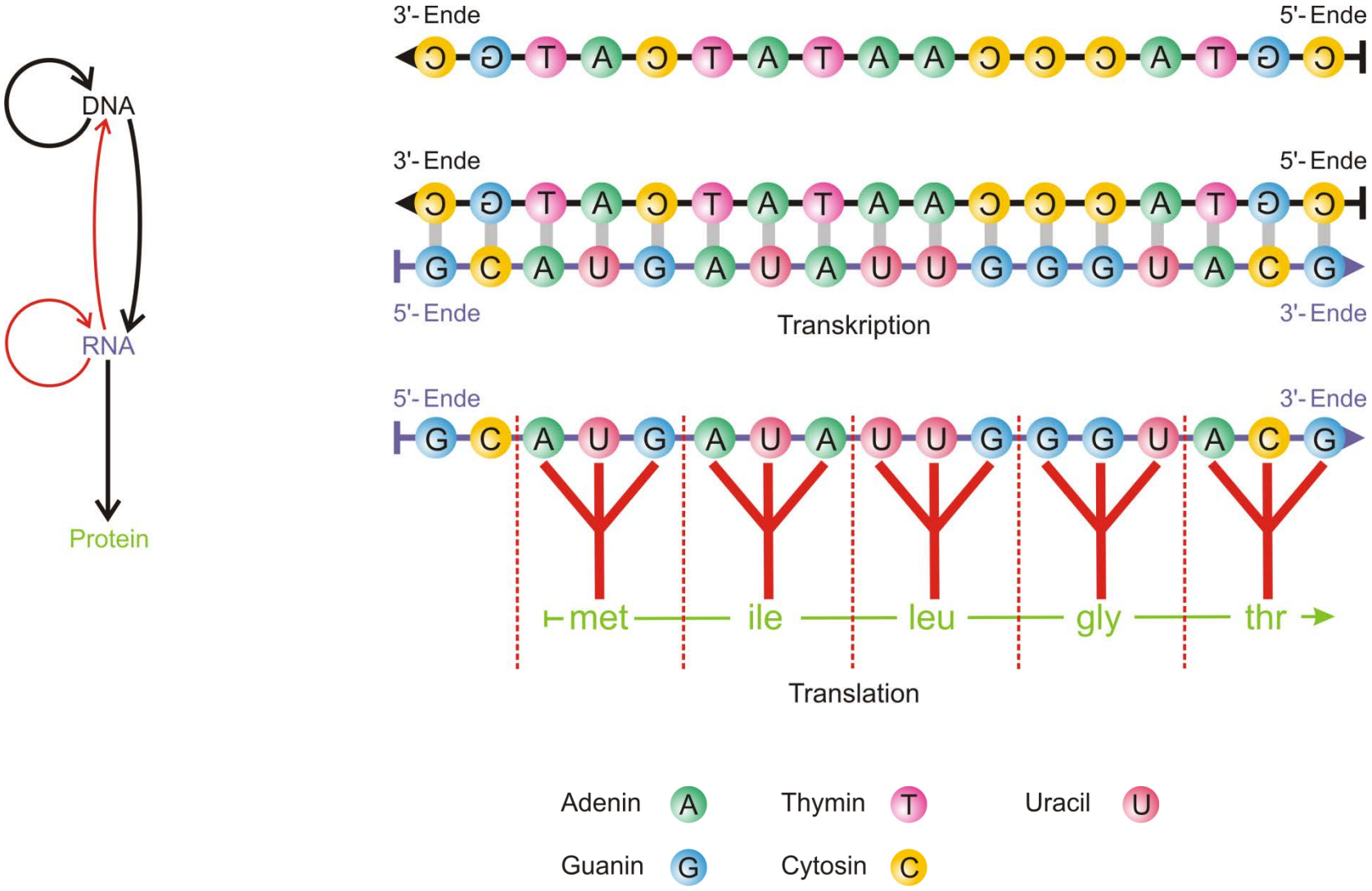
Molecular evolution of viruses



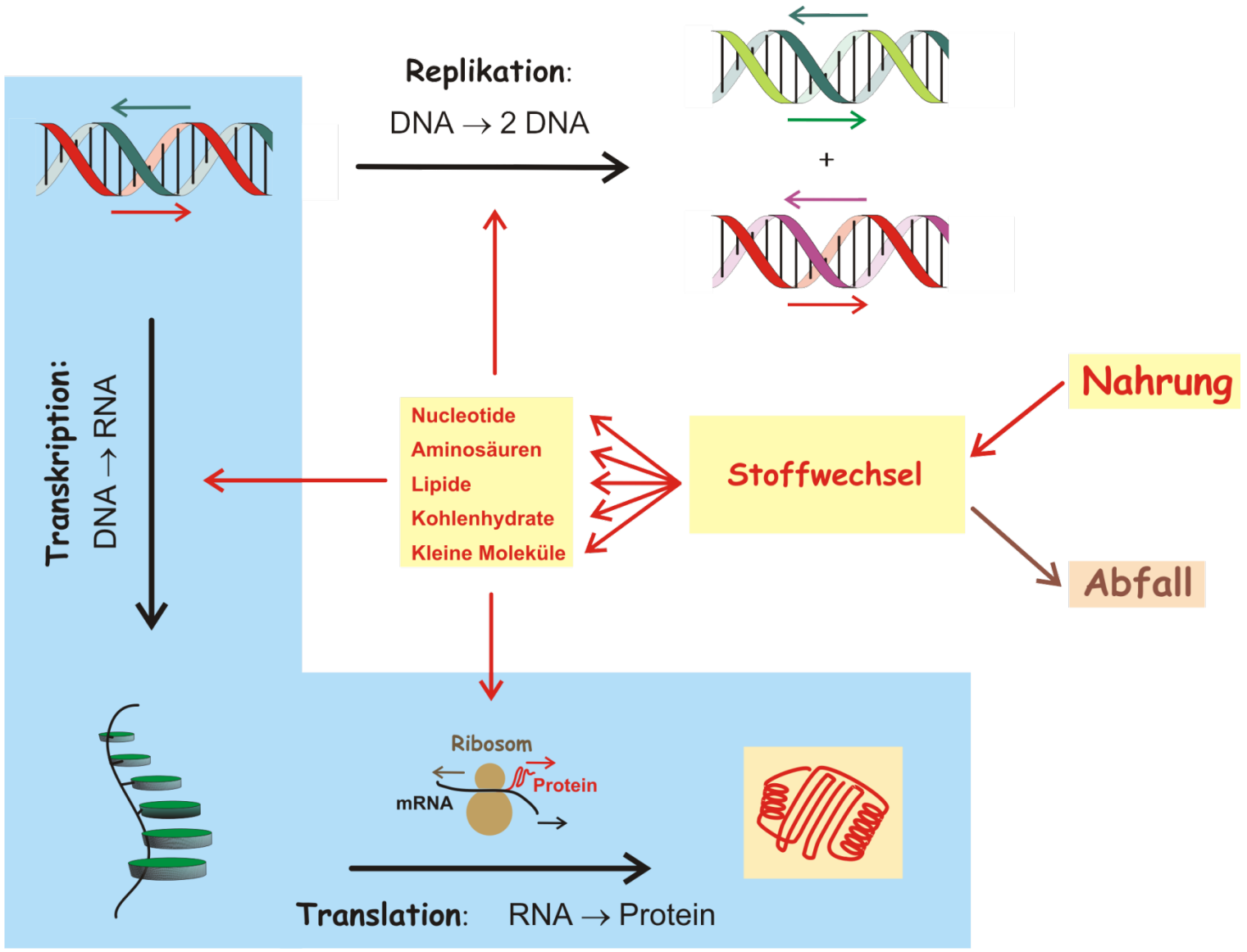
Evolution zu höherer Komplexität

Konkurrenz und Kooperation

Replizierende Moleküle		⇒	Moleküle in Kompartments
	Membranen, organisierte Teilung		
Unabhängige Replikatoren		⇒	Chromosomen
	Molekülverkettung, gemeinsame Replikation		
RNA als Gen und Enzyme		⇒	DNA und Protein
	Genetischer Code, Ribosom		
Prokaryoten		⇒	Eukaryoten
	Zusammenschluß durch Endosymbiose		
Asexuell vermehrende Klone		⇒	Sexuell vermehrende Populationen
	Ursprung der sexuellen Vermehrung		
Protisten		⇒	Pflanzen, Pilze und Tiere
	Zelldifferenzierung und Entwicklung		
Einzeln lebende Individuen		⇒	Tierkolonien
	Entstehung nicht-reproduktiver Kasten		
Primatengesellschaften		⇒	menschliche Gesellschaften
	Sprache, Schrift, Kultur, ...		



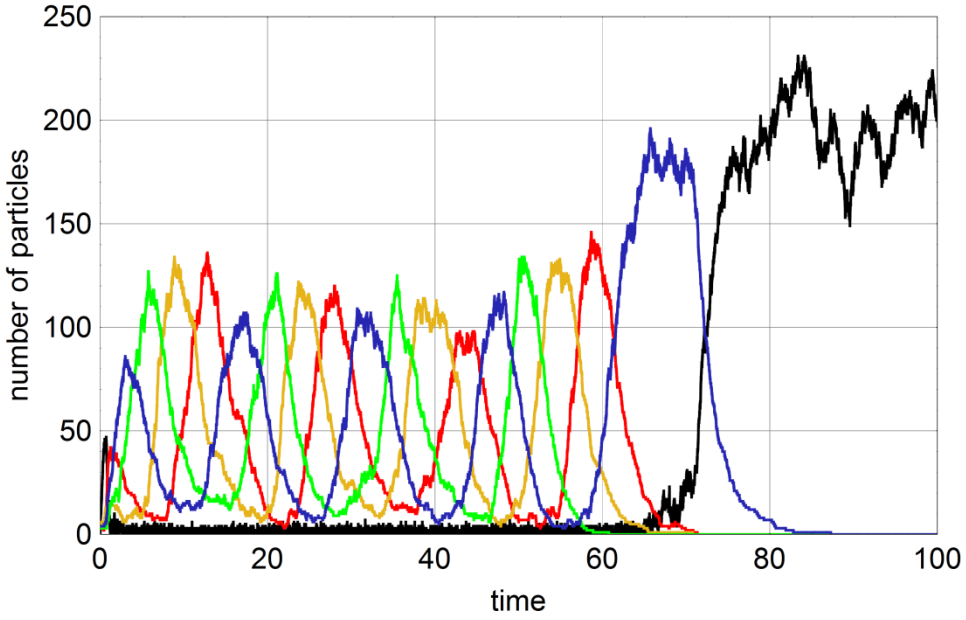
The ‚central dogma' of molecular biology


Replikation:
DNA → 2 DNA
+
Transkription:
DNA → RNA
Nucleotide
Aminosäuren
Lipide
Kohlenhydrate
Kleine Moleküle
Stoffwechsel
Nahrung
Abfall
Ribosom
Protein
mRNA
Translation: RNA → Protein

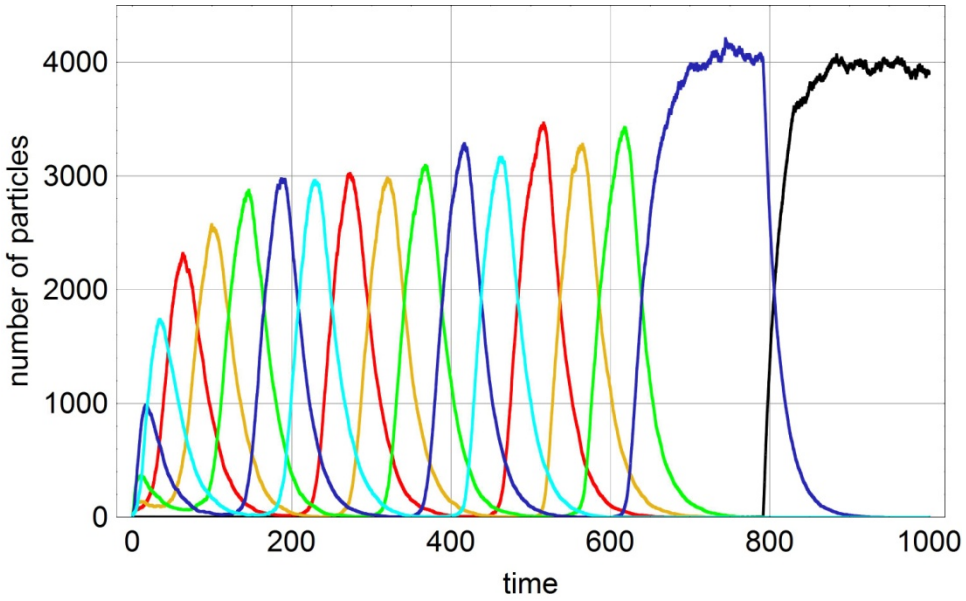
Mutationsschwelle des Überlebens

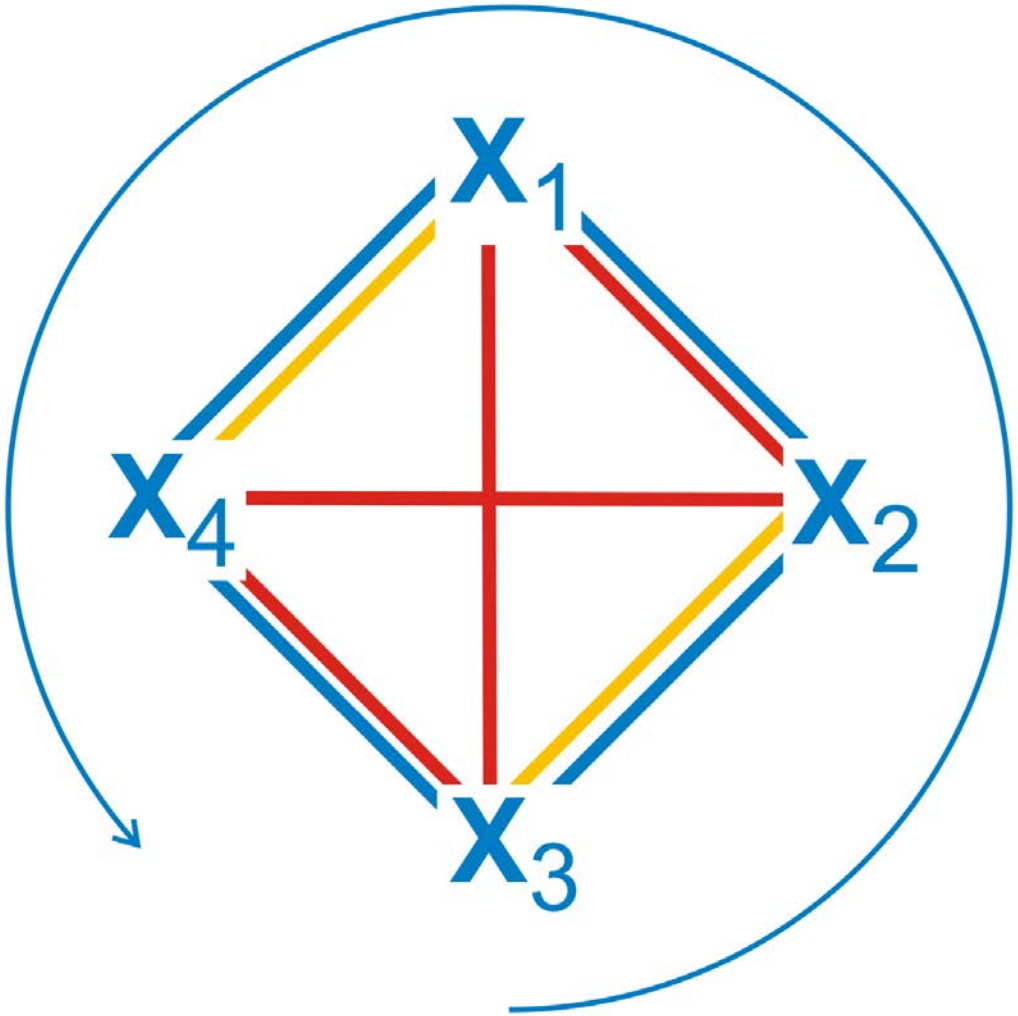
Kooperation und Variation

stochastic hypercycles with $n = 4$

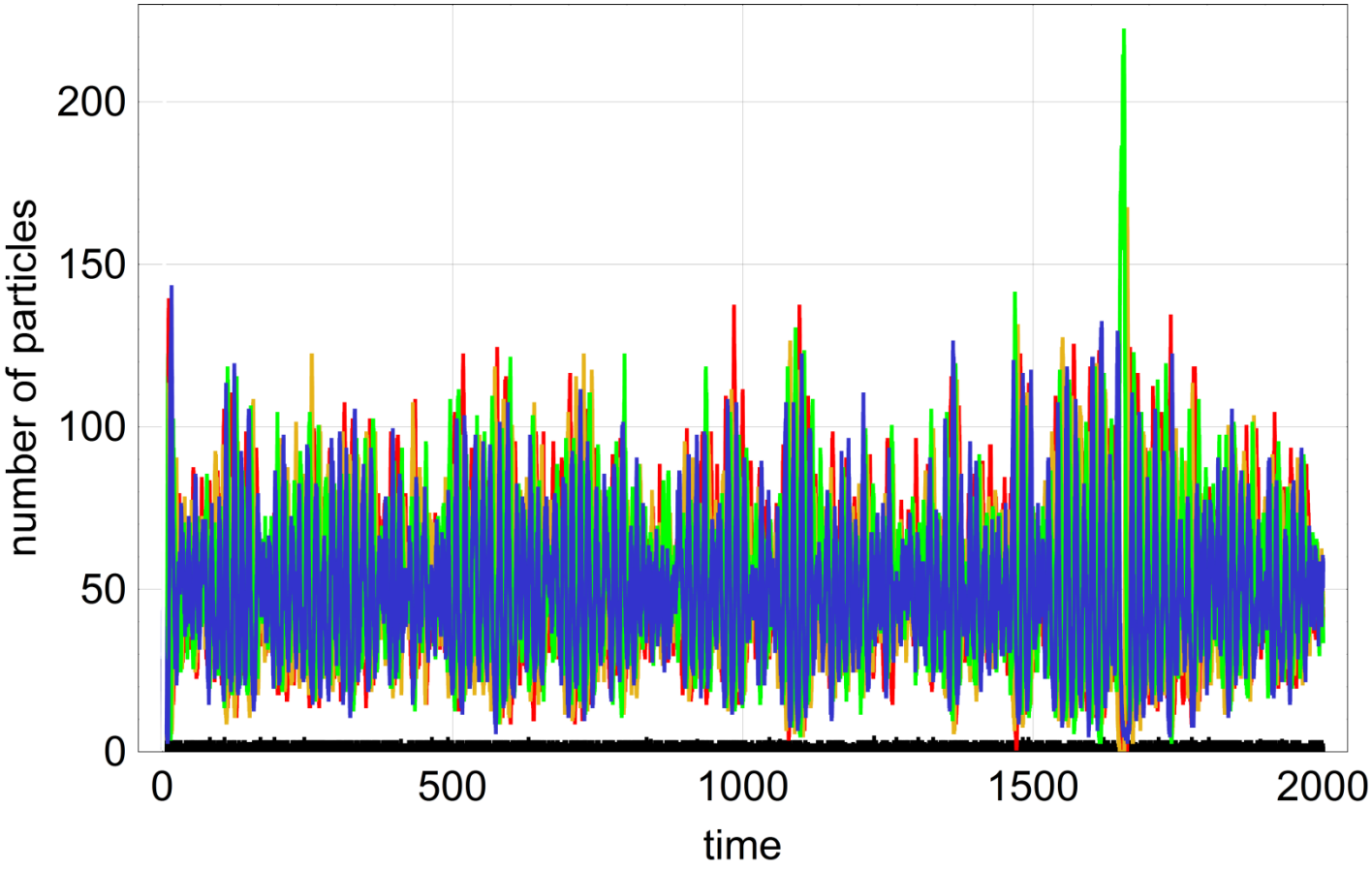


stochastic hypercycles with $n = 5$

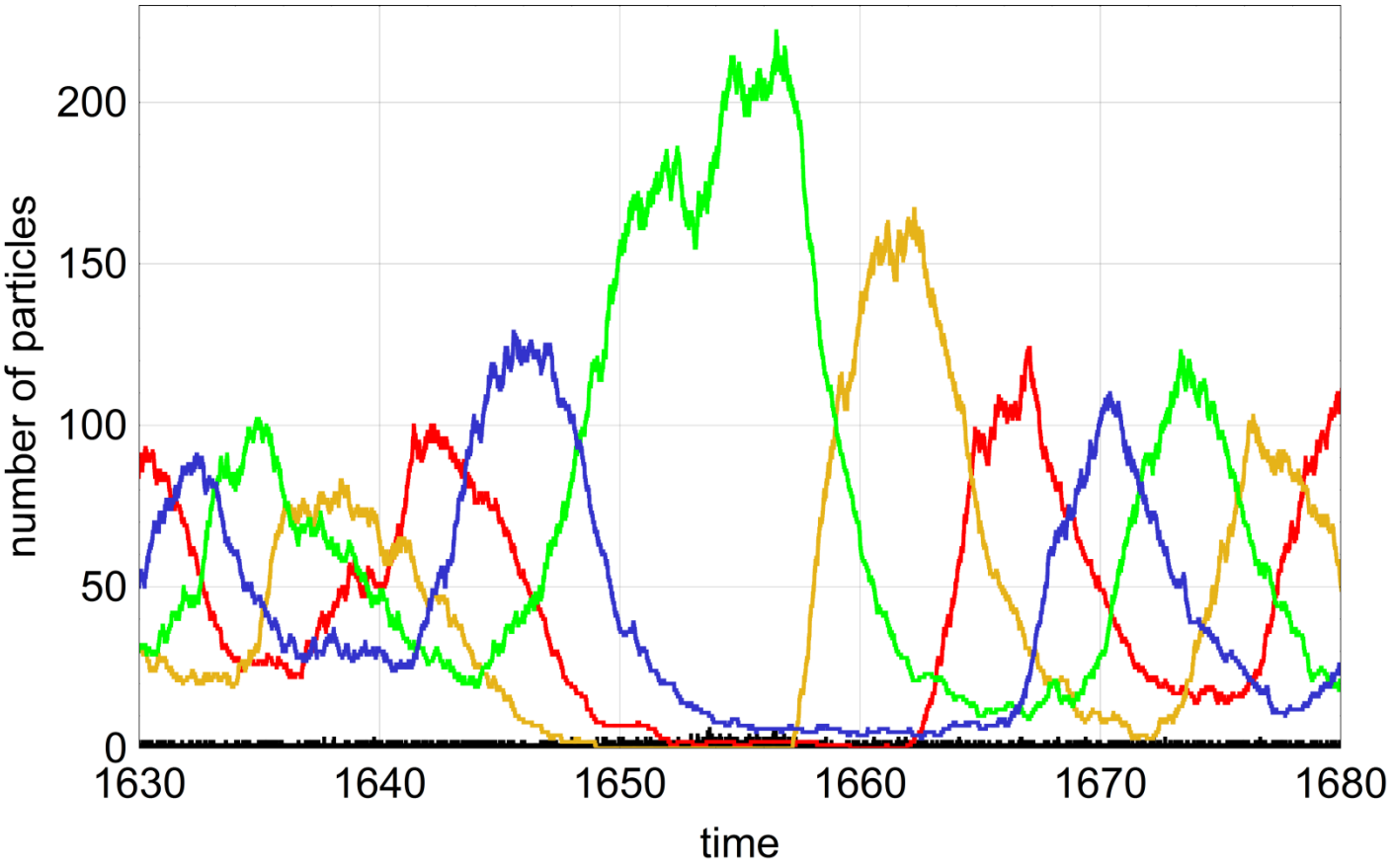




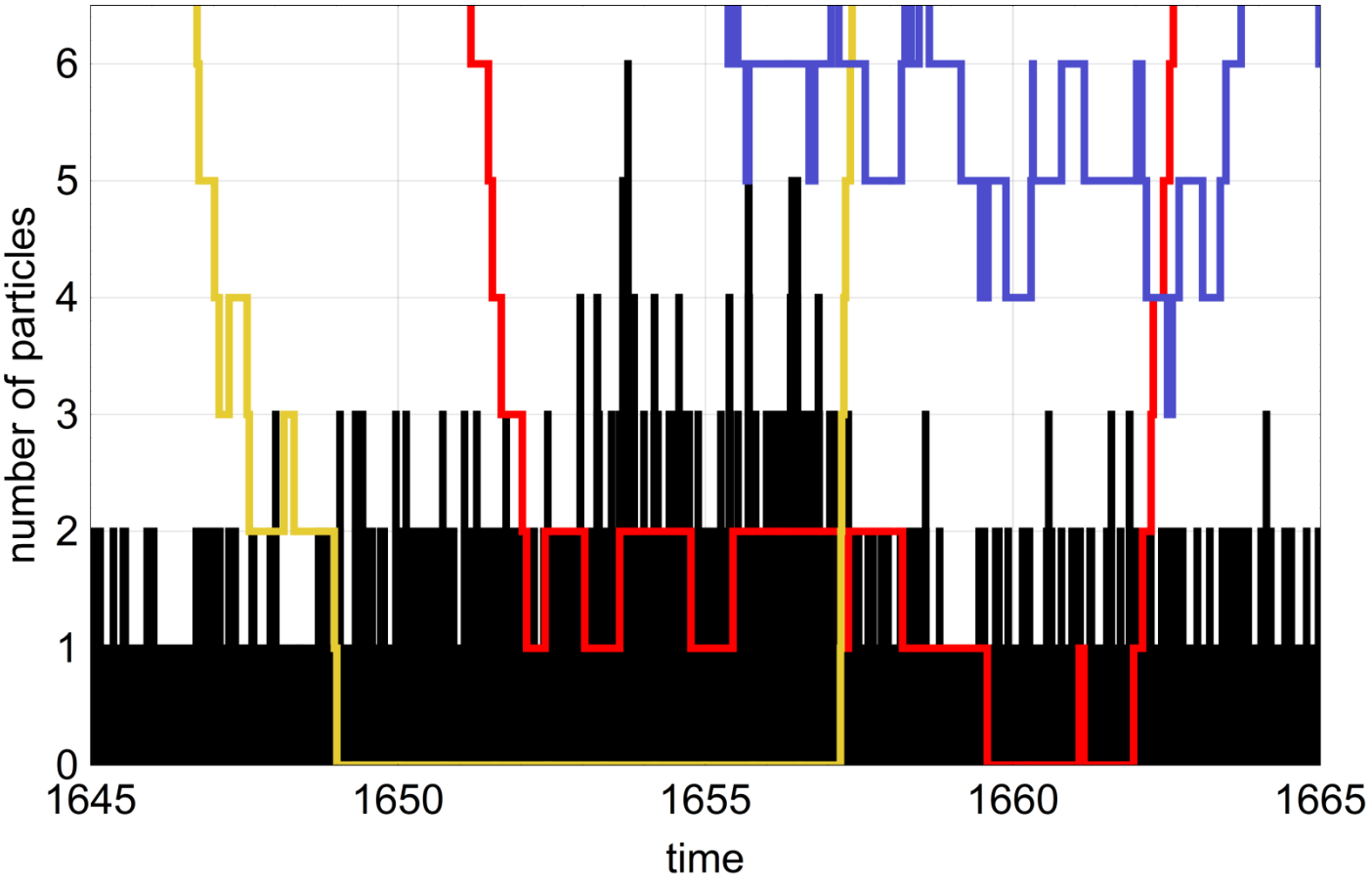
mutation mechanism, N = 4: ‚sequence space‘



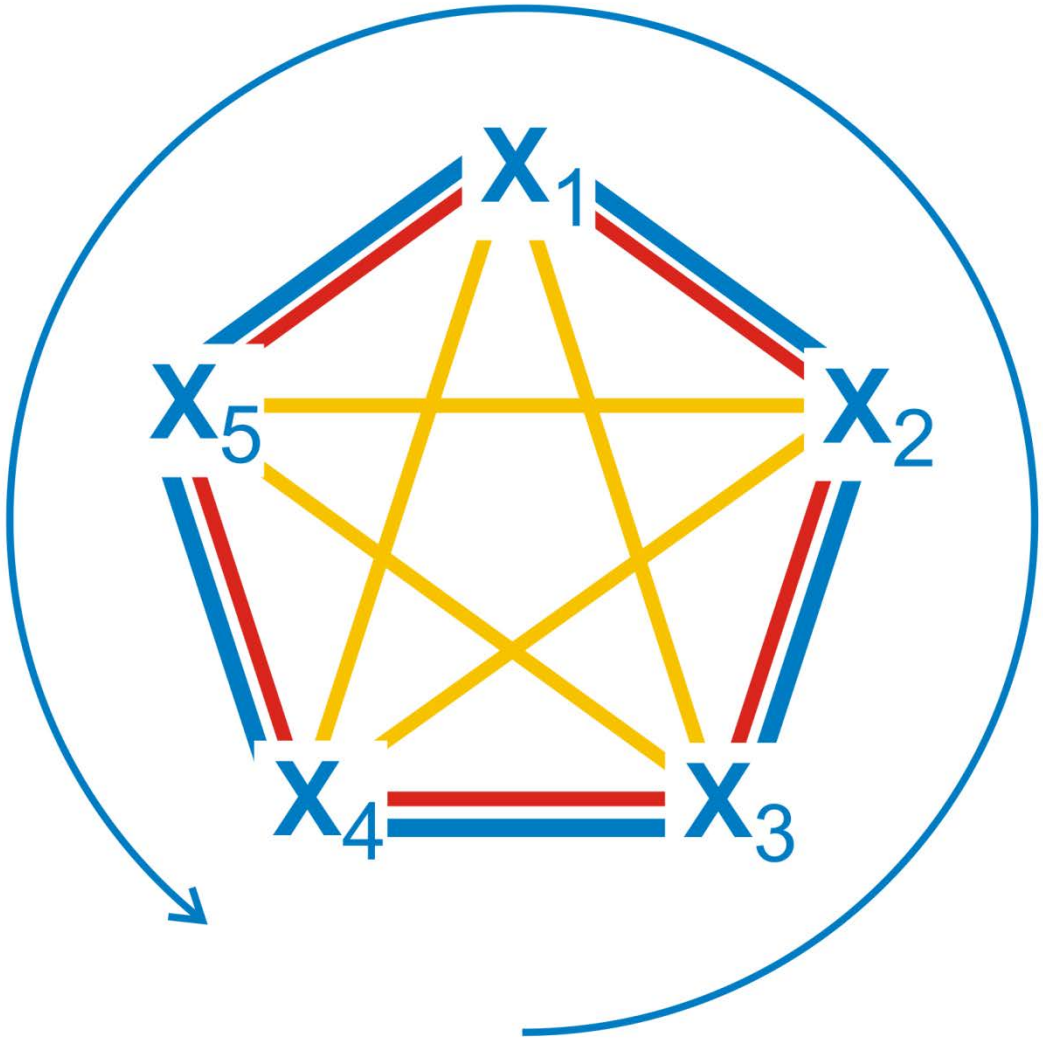
Oscillatory hypercycles:simulation for n=4



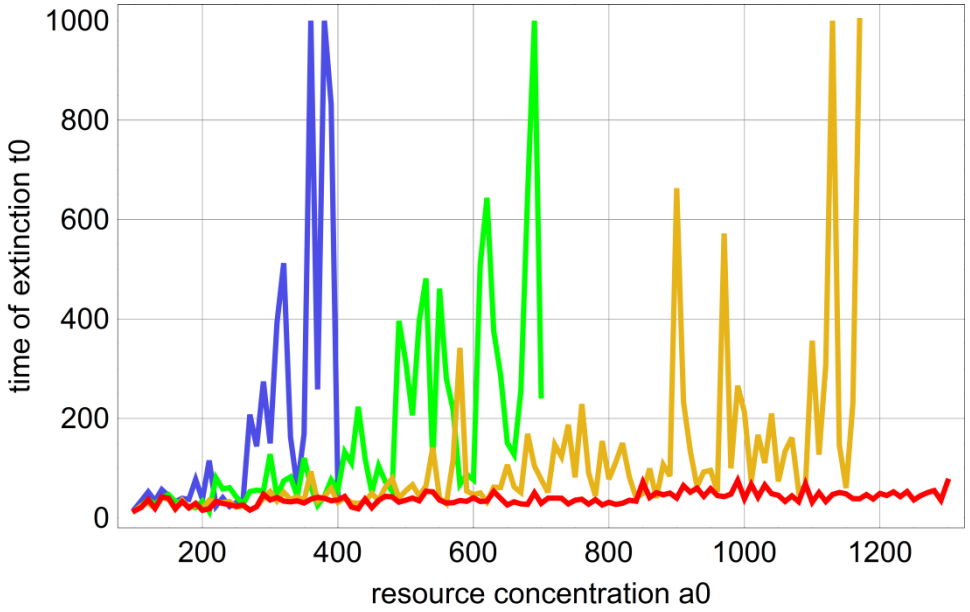
Oscillatory hypercycles:simulation for n=4, enlargement



Oscillatory hypercycles:simulation for n=4, enlargement

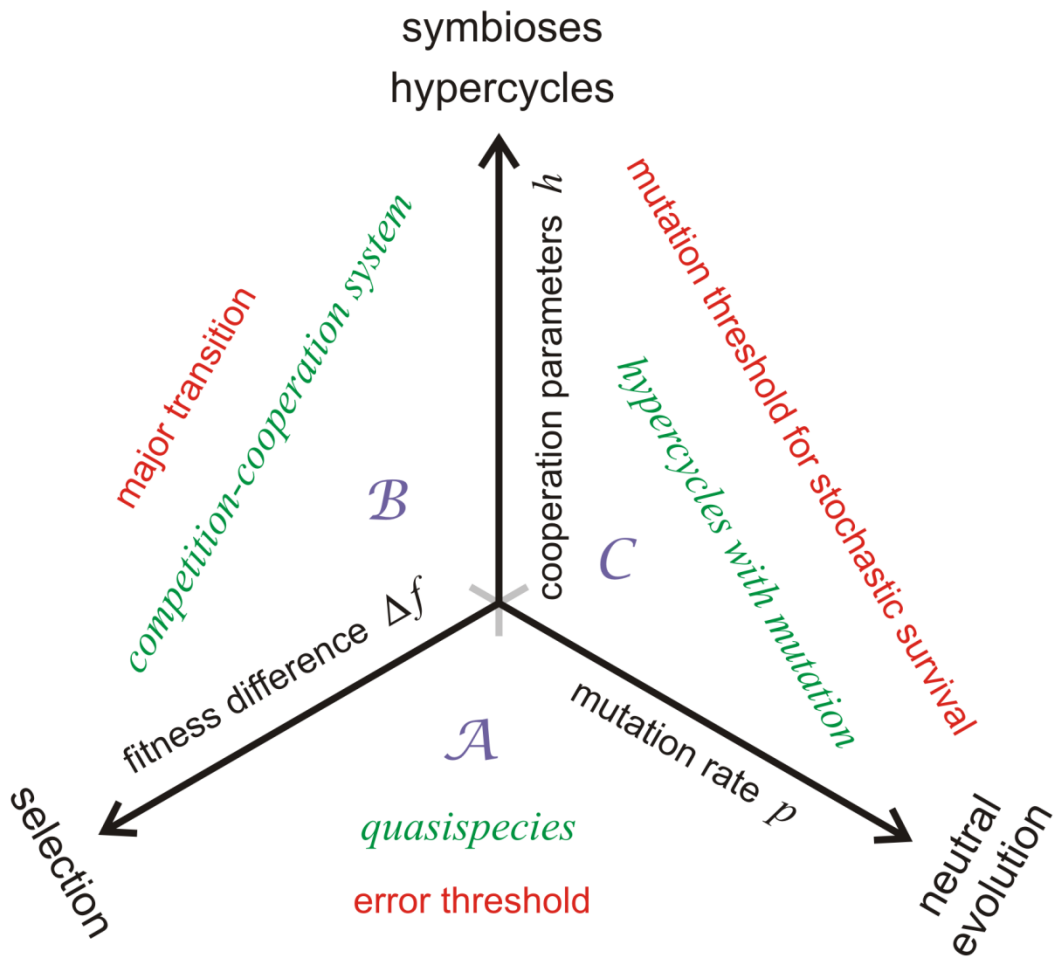


mutation mechanism, N = 5: ‚pentagram‘



mutation rate: p = **0.0000**, p=**0.0005**, p = **0.0010** and p = **0.0020**

Oscillatory hypercycles: simulation for n = 5, ‚pentagram‘



The minimal model of evolution

Epilog

Evolution im Licht der gegenwärtigen Molekulargenetik

1. Die Vorstellungen der konventionellen Genetik müssen hinsichtlich der Genregulation entscheidend erweitert werden.

2. Ein Gen wird im Vielzellerorganismus gewebsspezifisch in mehrere verschiedene Proteine übersetzt.

3. Umwelteinflüsse geben Anlass zu Veränderungen des Genoms, welche einige Generationen lang vererbbar sind.

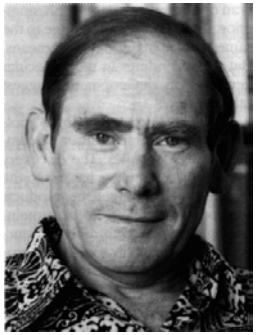
4. Komplexität, Robustheit und Plastizität der Organismen wird erst im Zusammenspiel von Genetik und Epigenetik verstehbar.

Vorteile der molekularen Erforschung des Lebens

1. Komplexe Reproduktionsmechanismen sind erklärbar.

2. Generegulation – basierend auf DNA oder RNA – ist nichts anderes als chemische Kinetik!

3. Epigenetik wird durch die gleichzeitige Betrachtung mehrerer Generationen einfach verstehbar.



Sydney Brenner, 1927 -

What else is epigenetics than a funny form of enzymology ? Each protein, after all, comes from some piece of DNA.

Danke für die Aufmerksamkeit!

Web-Page für weitere Informationen:

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