Chemistry on the Early Earth

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Germany-Japan Round Table

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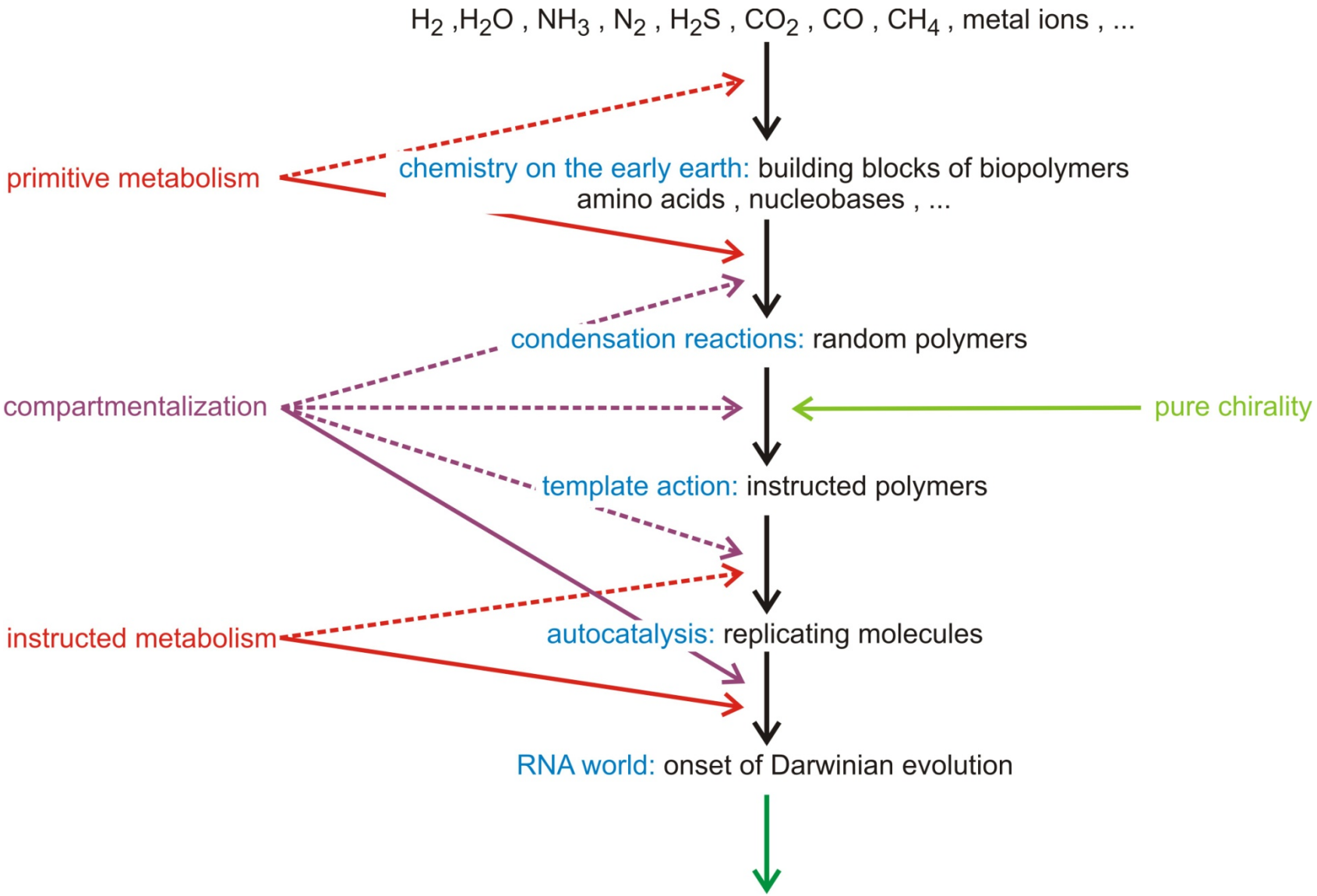
Web-Page for further information:

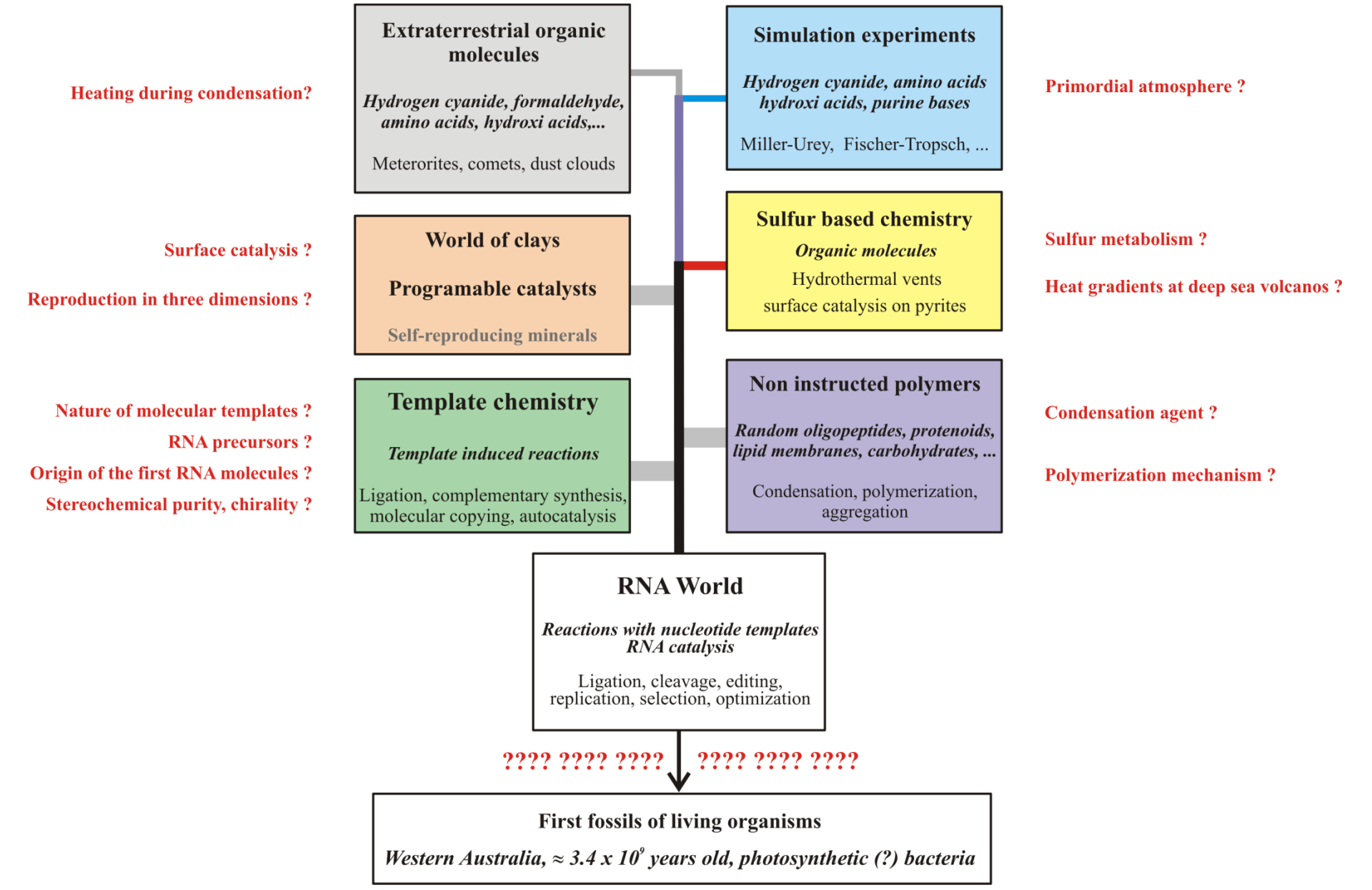
http://www.tbi.univie.ac.at/~pks

1. Prologue
2. Molecular replicators
3. Replication and mutation
4. Perspectives

1. **Prologue**
2. Molecular replicators
3. Replication and mutation
4. Perspectives



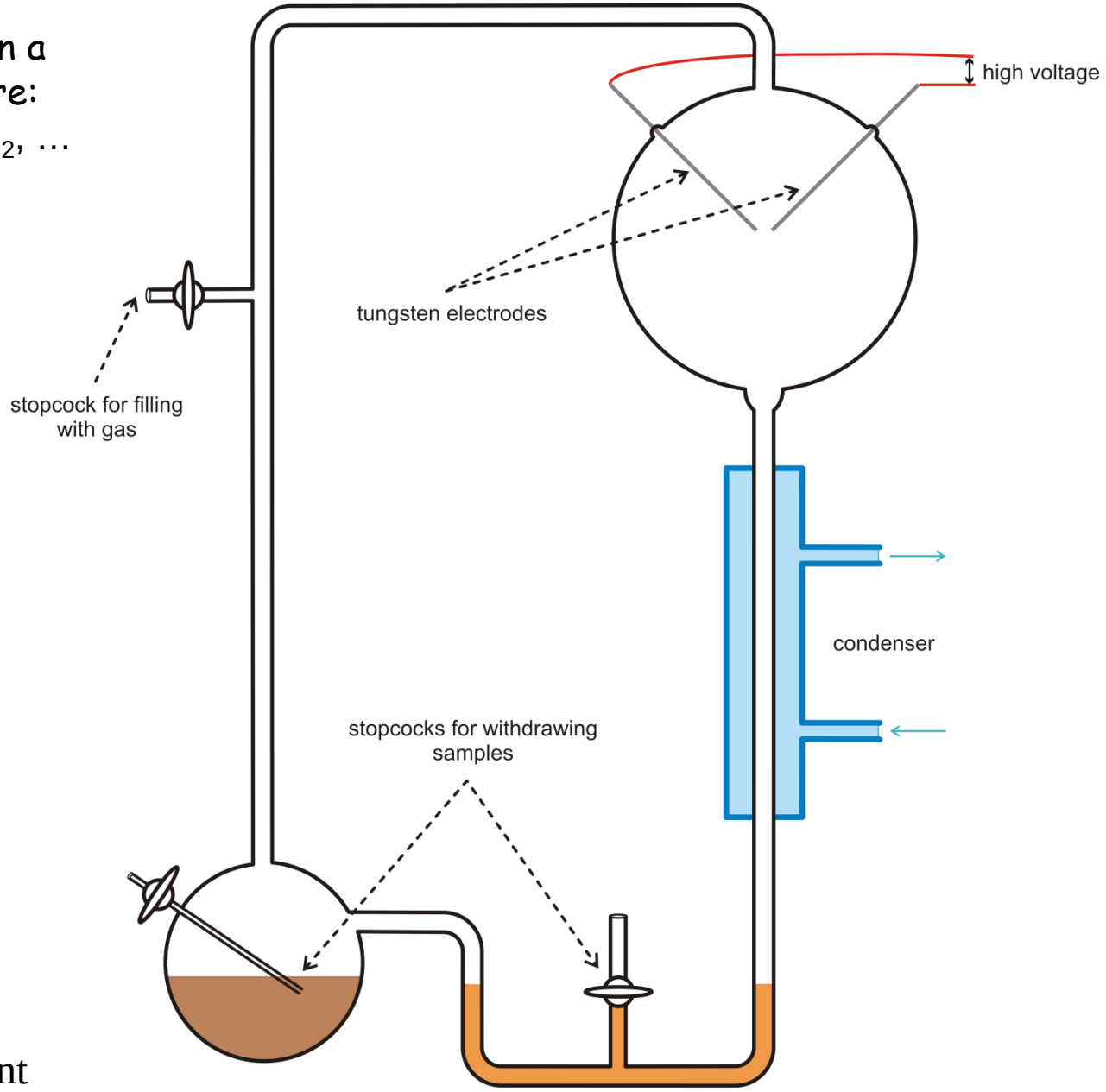
Prebiotic chemistry: From small molecules to molecular replicators


Extraterrestrial organic molecules
Hydrogen cyanide, formaldehyde, amino acids, hydroxi acids,...
Meterorites, comets, dust clouds
Heating during condensation?
Simulation experiments
Hydrogen cyanide, amino acids hydroxi acids, purine bases
Miller-Urey, Fischer-Tropsch, ...
Primordial atmosphere ?
World of clays
Programable catalysts
Self-reproducing minerals
Surface catalysis ?
Reproduction in three dimensions ?
Sulfur based chemistry
Organic molecules
Hydrothermal vents
surface catalysis on pyrites
Sulfur metabolism ?
Heat gradients at deep sea volcanos ?
Template chemistry
Template induced reactions
Ligation, complementary synthesis, molecular copying, autocatalysis
Nature of molecular templates ?
RNA precursors ?
Origin of the first RNA molecules ?
Stereochemical purity, chirality ?
Non instructed polymers
Random oligopeptides, protenoids, lipid membranes, carbohydrates, ...
Condensation, polymerization, aggregation
Condensation agent ?
Polymerization mechanism ?
RNA World
Reactions with nucleotide templates
RNA catalysis
Ligation, cleavage, editing, replication, selection, optimization
???? ???? ????
???? ???? ????
First fossils of living organisms
Western Australia, ≈ 3.4 x 10^9 years old, photosynthetic (?) bacteria

From small molecules to molecular replicators

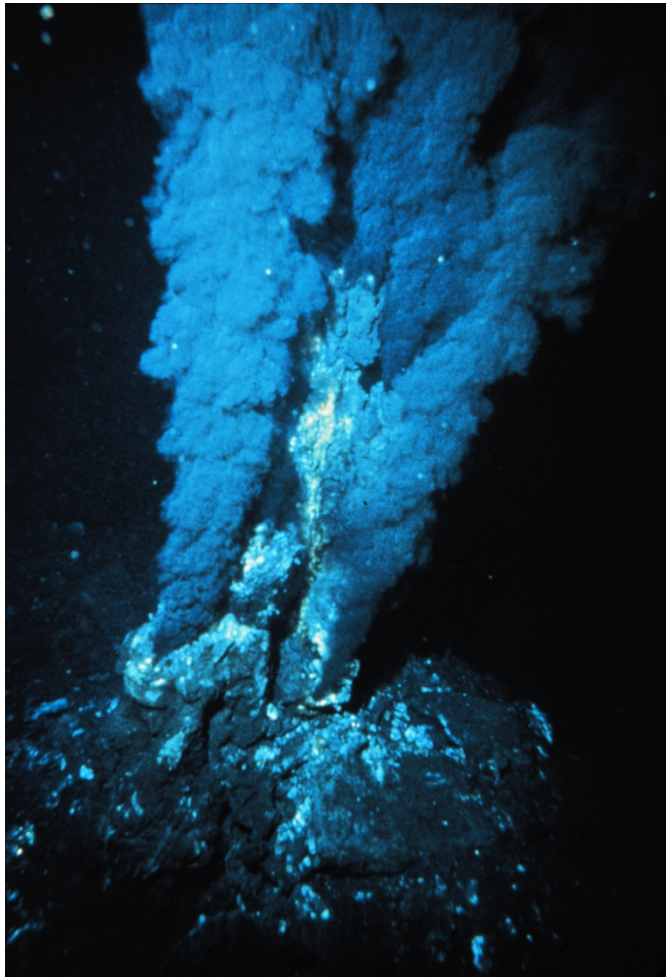
1. **Sources of organic molecules**
2. Origin of chirality
3. Primitive metabolism

Electric discharge in a reducing atmosphere:
CH_4, CO, NH_3, H_2O, H_2, …

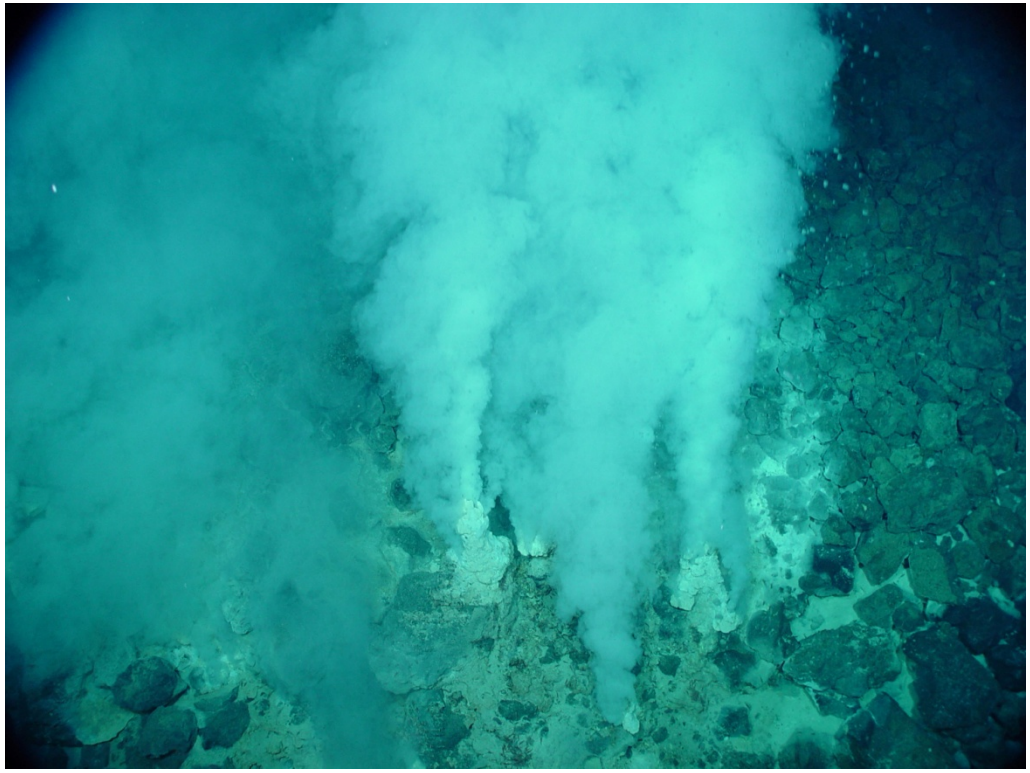


S.L.Miller. 1953. A production of amino acids under possible primitive earth conditions. *Science* **117**:528-529

The Miller-Urey experiment



black smoker

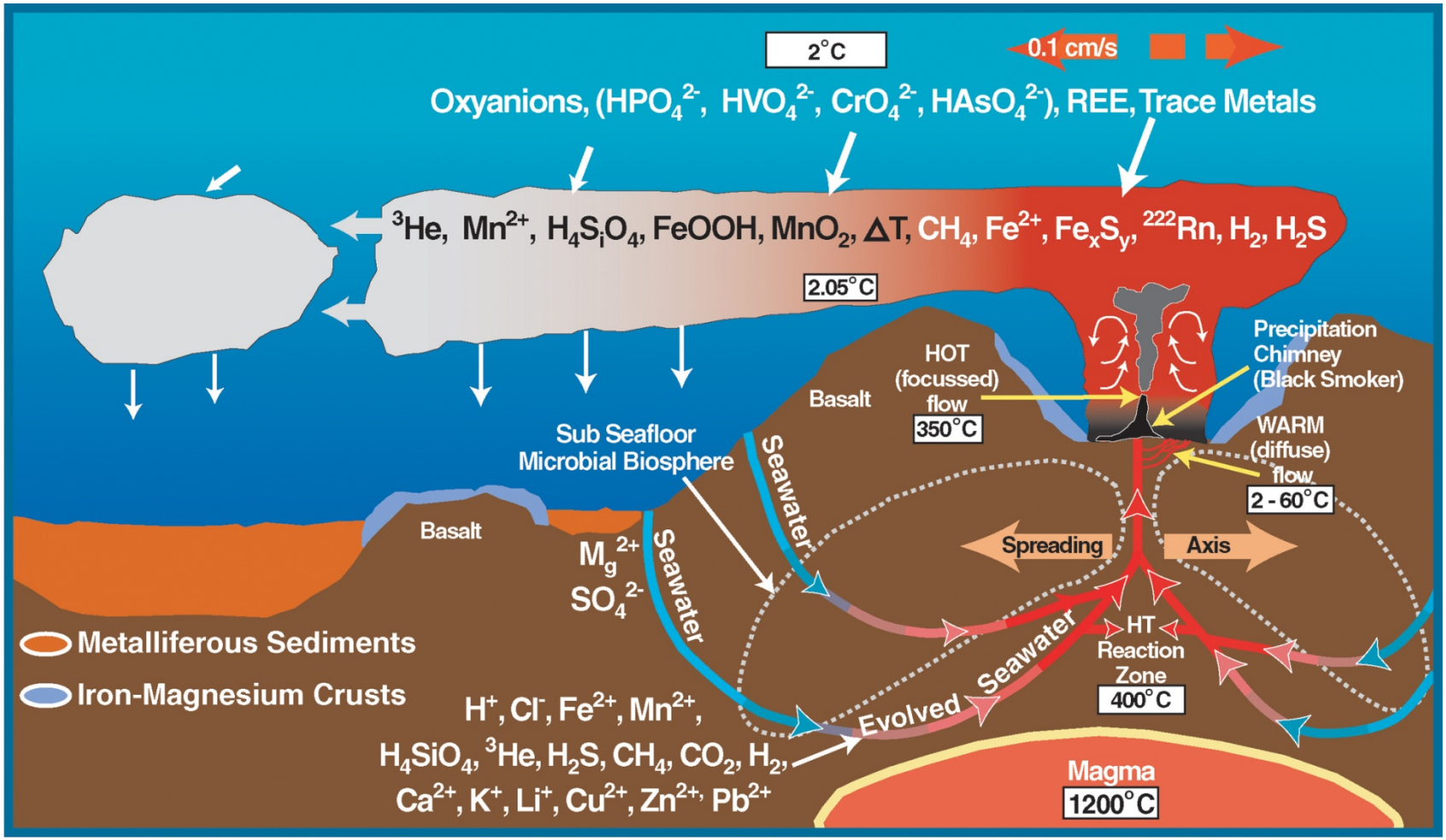


white smoker

Hydrothermal vents in the deap sea

occurrence: mid-atlantic ridge, east pacific rise, …
in about 3000 m depth

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

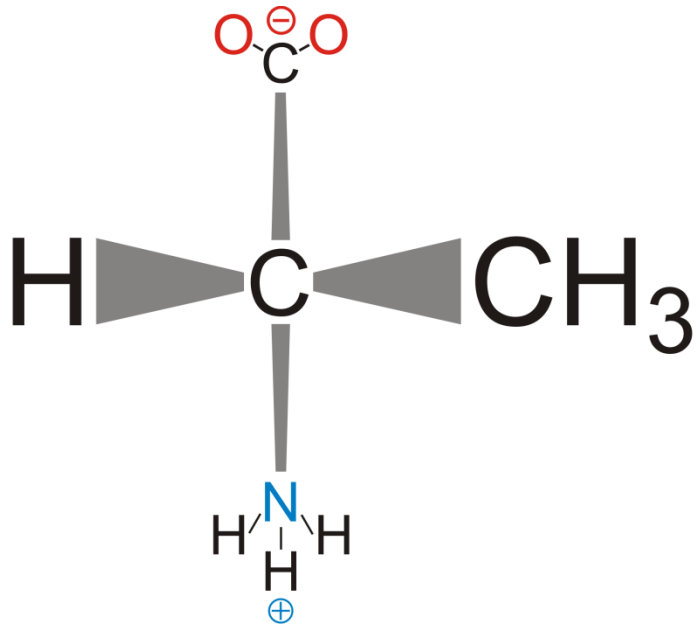


Conditions and materials in and around hydrothermal vents

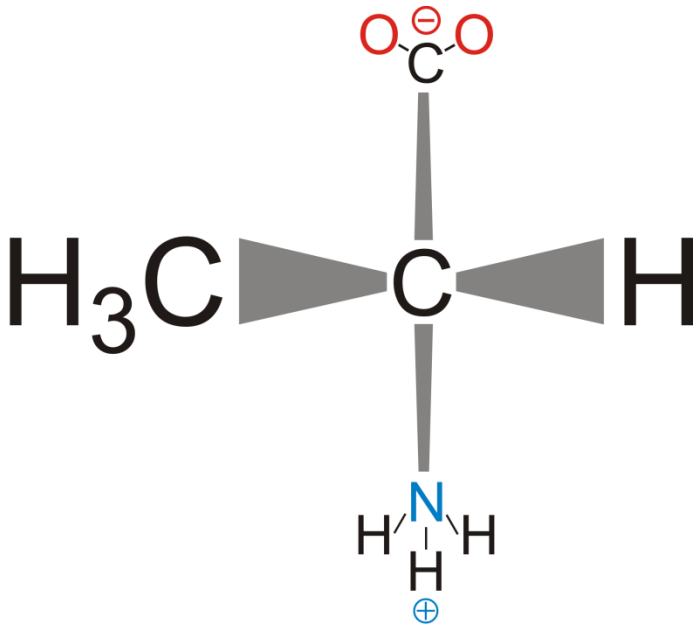
Source: Wikipedia: *Hydrothermal vent*, Nov. 15,2011

From small molecules to molecular replicators

1. Sources of organic molecules
2. **Origin of chirality**
3. Primitive metabolism



L- (S-) alanine



D- (R-) alanine

The two chiral forms of alanine

ON SPONTANEOUS ASYMMETRIC SYNTHESIS

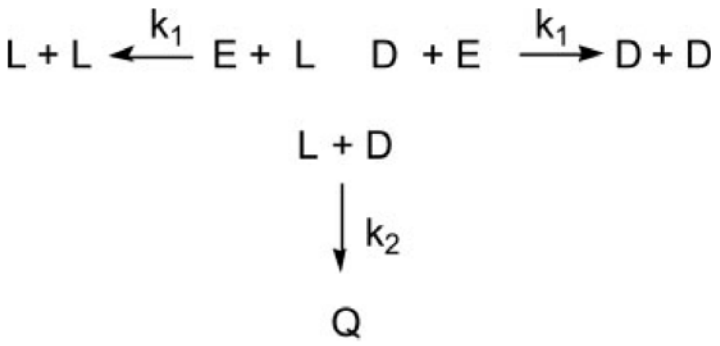
by

F. C. FRANK

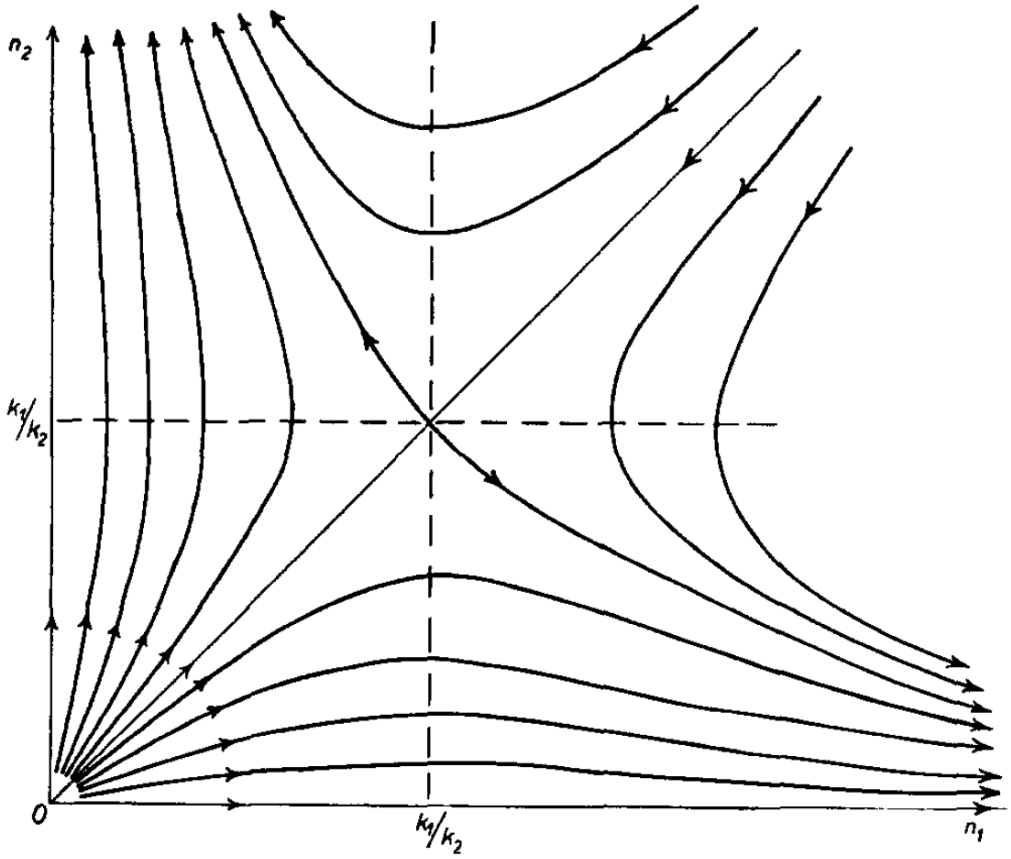
The H. H. Wills Physical Laboratory, University of Bristol (England)

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.

The theoretical prediction of an origin of chirality through autocatalytic asymmetric synthesis by Frederick Charles **Frank** in **1953**



L,**D** ….. the two chiral forms
E ….. achiral substrate
Q ….. inert reaction product



$$\frac{dn_{\mathrm{L}}}{dt} = (k_1 - k_2 n_{\mathrm{D}}) n_{\mathrm{L}}$$

$$\frac{dn_{\mathrm{D}}}{dt} = (k_1 - k_2 n_{\mathrm{L}}) n_{\mathrm{D}}$$

$$\frac{n_{\mathrm{L}}}{n_{\mathrm{D}}} = \frac{n_{0\mathrm{L}}}{n_{0\mathrm{D}}} \exp\left(k_2 (n_{0\mathrm{L}} - n_{0\mathrm{D}}) (e^{k_1 t} - 1)\right)$$

The Frank model of exponential enrichment of one chiral form

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.

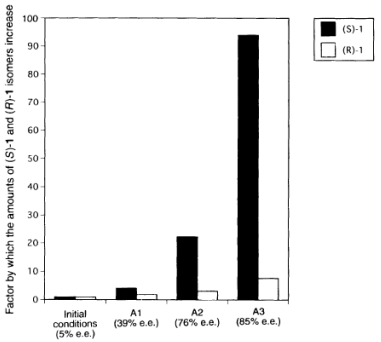


FIG 1. Asymmetric autocatalysis of chiral pyrimidyl alkanol (**1**). Runs A1–3 correspond to Table 1. The enantiomeric excess of (S)-**1** increases from 5 to 89% e.e. without the use of additional chiral auxiliaries. During the reactions (runs A1–3), the (*S*)-**1** increases by a factor of 94 times,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

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

Michael Mauksch and
Svetlana Tsogoeva 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

Reactions following a somewhat extended Frank mechanism

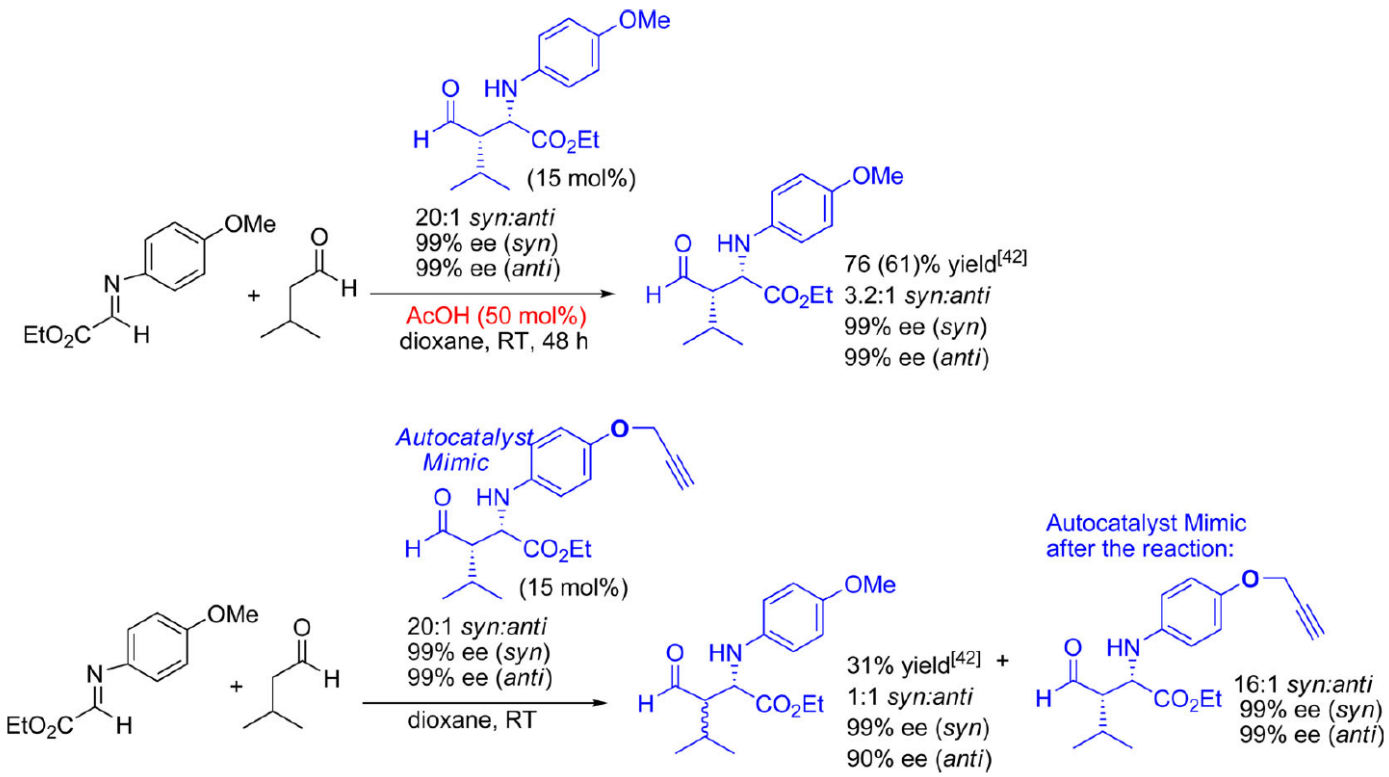


Figure 9 Asymmetric organocatalytic Mannich reaction with autocatalysts and their mimics.

From small molecules to molecular replicators

1. Sources of organic molecules
2. Origin of chirality
3. **Primitive metabolism**

1. Self-organization requires conditions far from equilibrium

2. Avoidance of branching reactions into the vast and inexhaustible space of organic molecules

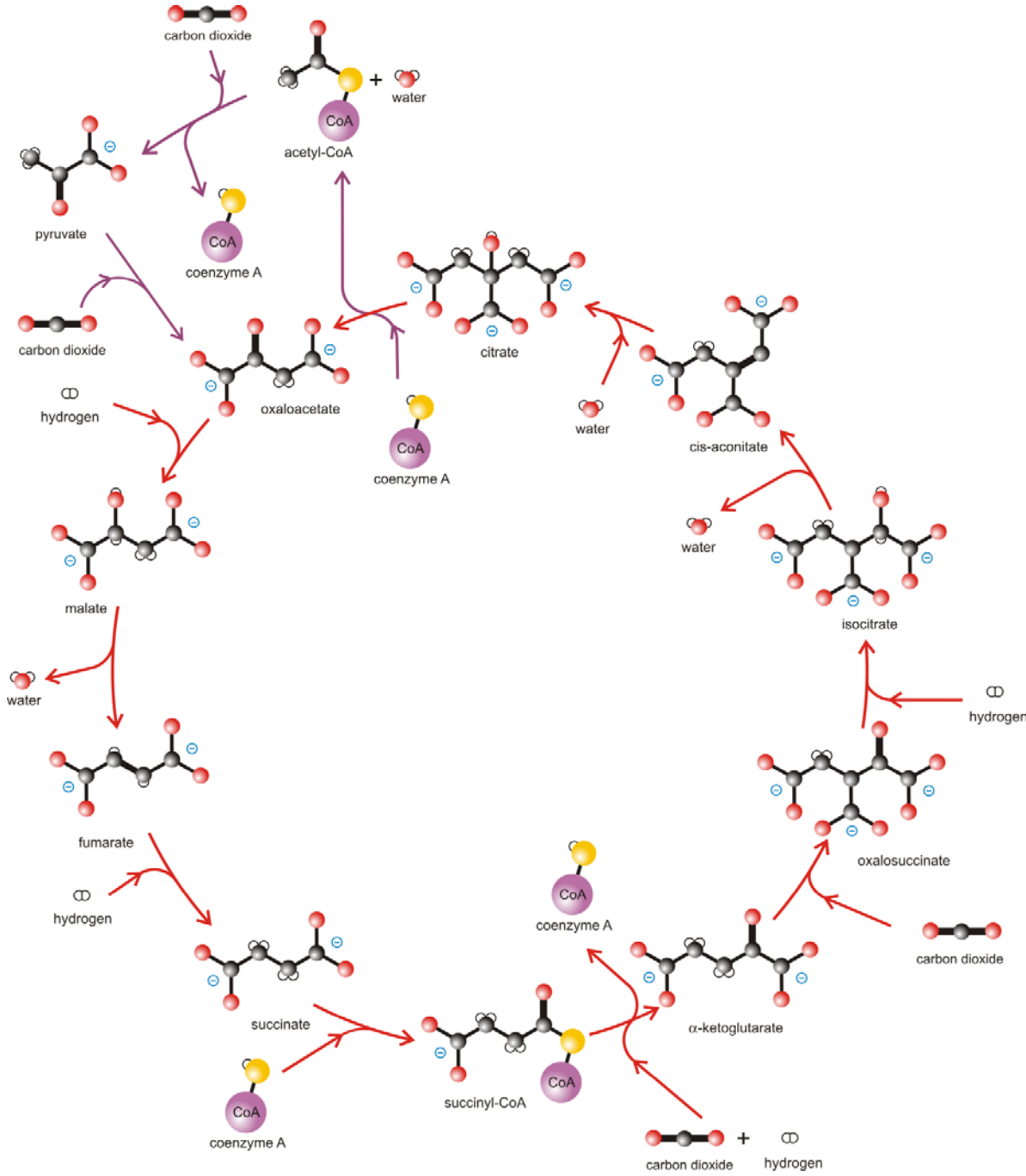
3. Canalizing free energy towards the synthesis of the building blocks of biomolecules

4. Steps towards autotrophy through photosynthesis

Why is a primitive metabolism necessary?

Early metabolism ??

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



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

The reverse citric acid cycle

Leslie E. Orgel, 2008
posthumous publication

OPEN ACCESS Freely available online

PLoS BIOLOGY

Essay

The Implausibility of Metabolic Cycles on the Prebiotic Earth

Leslie E. Orgel*

Cycles occur widely in all branches of chemistry. The definition of a catalyst as an agent that facilitates the conversion of reactants to products without itself being changed almost guarantees that a catalyst can initiate successive "cycles" of the same reaction. Metabolic cycles are different. Strictly, they are by definition restricted to biochemistry. Like catalytic cycles, they too result in repeated conversions of substrates into products, but they involve much more complex sequences of chemical reactions. As far as I am aware, the formose reaction, which converts formaldehyde to a complicated mixture of products, including various sugars [1], is the only known nonenzymatic reaction sequence that is at all similar to a metabolic cycle, although the existence of one or two much simpler cycles has been established or made probable in the literature of prebiotic chemistry [2,3]. The possibility that reactions of hydrogen cyanide (HCN) might form the basis for a complex cyclic organization has been proposed [4], but there is as yet no experimental evidence to support this proposal.

If complex cycles analogous to metabolic cycles could have operated on the primitive Earth, before the appearance of enzymes or other informational polymers, many of the obstacles to the construction of a plausible scenario for the origin of life would disappear. If, for example, a complex system of nonenzymatic cycles could have made nucleotides available for RNA synthesis, many of the problems of prebiotic chemistry would become irrelevant. Perhaps a simpler polymer preceded RNA as the genetic material—for example, a polymer based on a glycerol-phosphate backbone [5] or a phosphoglyceric acid backbone. Could a nonenzymatic "metabolic cycle" have made such compounds available in sufficient purity to facilitate the appearance of a replicating informational polymer?

It must be recognized that assessment of the feasibility of any particular proposed prebiotic cycle must depend on arguments about chemical plausibility, rather than on a decision about logical possibility. Any reaction sequence that is allowed by thermodynamics could, in principle, be realized, given a sufficiently active and specific family of catalysts. Plants synthesize complex alkaloids, such as strychnine, from CO_2, NH_3, and reducing equivalents, so it must, in principle, be possible to achieve these syntheses starting from CO_2, NH_3, and H_2, given a family of sufficiently active and specific prebiotic catalysts. However, few would believe that any assembly of minerals on the primitive Earth is likely to have promoted these syntheses in significant yield. Each proposed metabolic cycle, therefore, must be evaluated in terms of the efficiencies and specificities that would be required of its hypothetical catalysts in order for the cycle to persist. Then arguments based on experimental evidence or chemical plausibility can be used to assess the likelihood that a family of catalysts that is adequate for maintaining the cycle could have existed on the primitive Earth.

The metabolic cycles that have been identified by biochemists are of two kinds: simple cycles and autocatalytic cycles. The citric acid cycle, which brings about the oxidation of acetate to CO_2 with the concomitant synthesis of ATP, and the urea cycle that results in the conversion of toxic NH_3 to relatively harmless urea, are both examples of simple cycles. The initial step of the former cycle is the synthesis of citric acid from oxaloacetic acid and acetyl-CoA. After one turn of the cycle, acetate is completely "burned" to CO_2 as one molecule of oxaloacetate is regenerated. The Calvin dark cycle and the reverse citric acid cycle, both of which result in the fixation of CO_2 into important biochemical intermediates, are examples of autocatalytic cycles. The reverse (reductive) citric acid cycle (Figure 1) is initiated by the splitting of citric acid to give oxaloacetic acid and acetyl-CoA. After one turn of the cycle, two molecules of citric acid are formed, so long as no material is diverted from the cycle. That is why the cycle is described as autocatalytic—each molecule of citric acid introduced into the cycle results, after a turn of the cycle, in the generation of two molecules of citric acid. The proposal that the reverse citric acid cycle operated nonenzymatically on the primitive Earth has been a prominent feature of some scenarios for the origin of life [6–8].

A different kind of autocatalytic cycle, which has no analog in biochemistry, has been hypothesized by Stuart Kauffman to self-organize spontaneously whenever amino acids condense together to form peptides [9]. According to Kauffman, the catalytic properties of some of the members of a random-sequence mixture of peptides guarantee that a cyclic organization will emerge in which a small number of peptides will come to dominate the chemistry of the polymerization reaction. These peptides together with their subsequences will catalyze their own synthesis from monomeric amino acids and will constitute a cycle in which each

Essays articulate a specific perspective on a topic of broad interest to scientists.

Citation: Orgel LE (2008) The implausibility of metabolic cycles on the prebiotic earth. PLoS Biol 6(1): e18. doi:10.1371/journal.pbio.0060018

Leslie E. Orgel, The Salk Institute for Biological Studies, San Diego, California, United States of America. This paper was submitted on behalf of Leslie Orgel, after his death on 27 October 2007, by Gerald Joyce, The Scripps Research Institute, La Jolla, California, United States of America. E-mail: gjoyce@scripps.edu

* Deceased

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2. **Molecular replicators**
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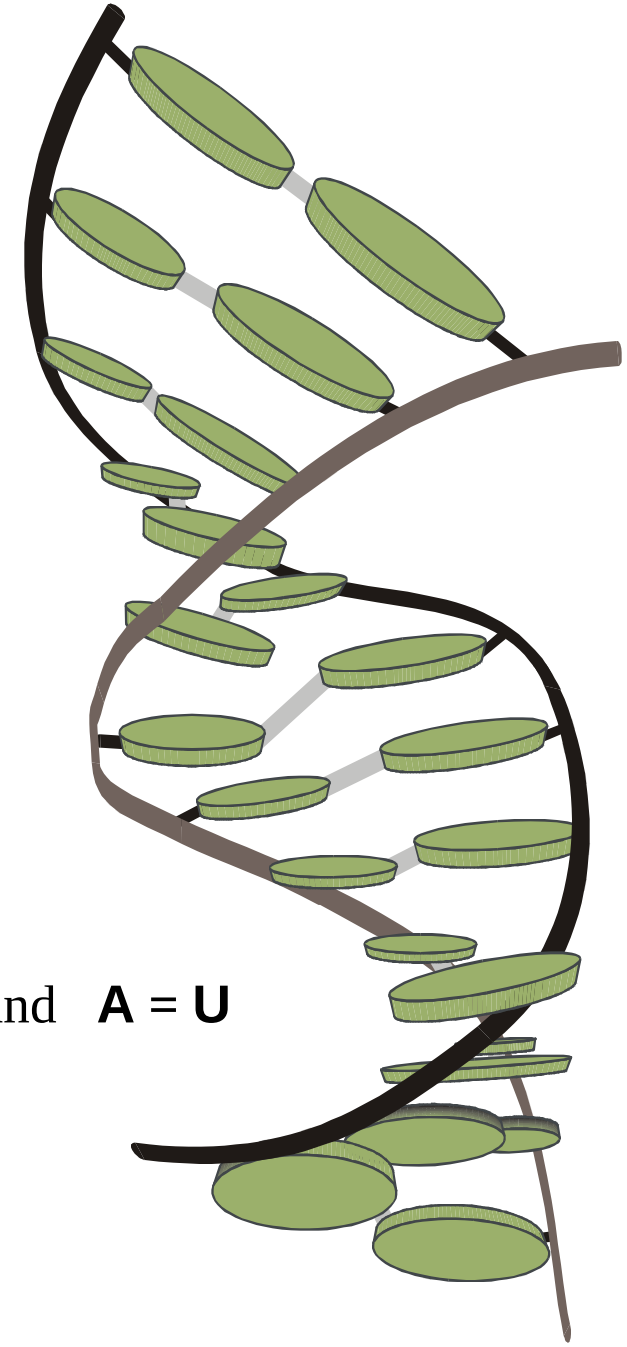
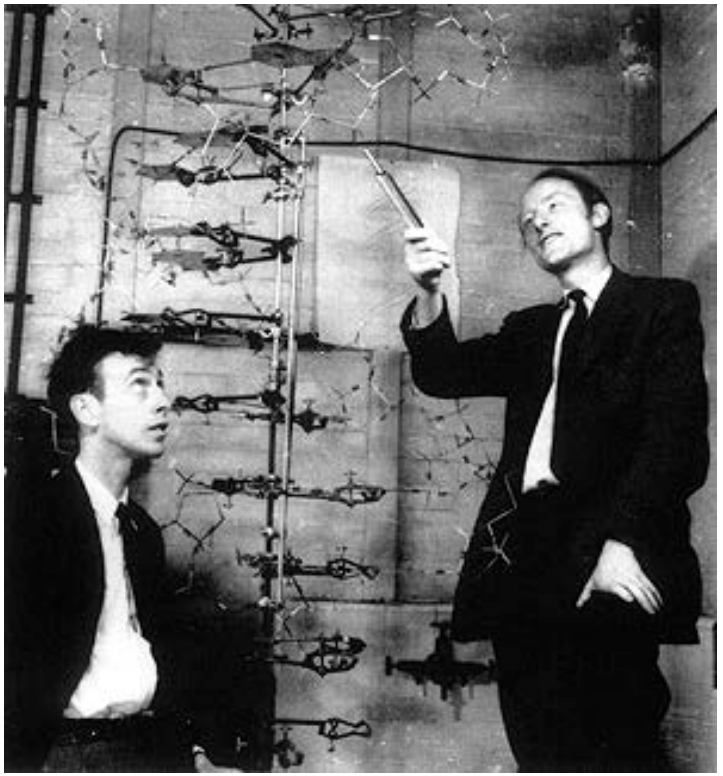
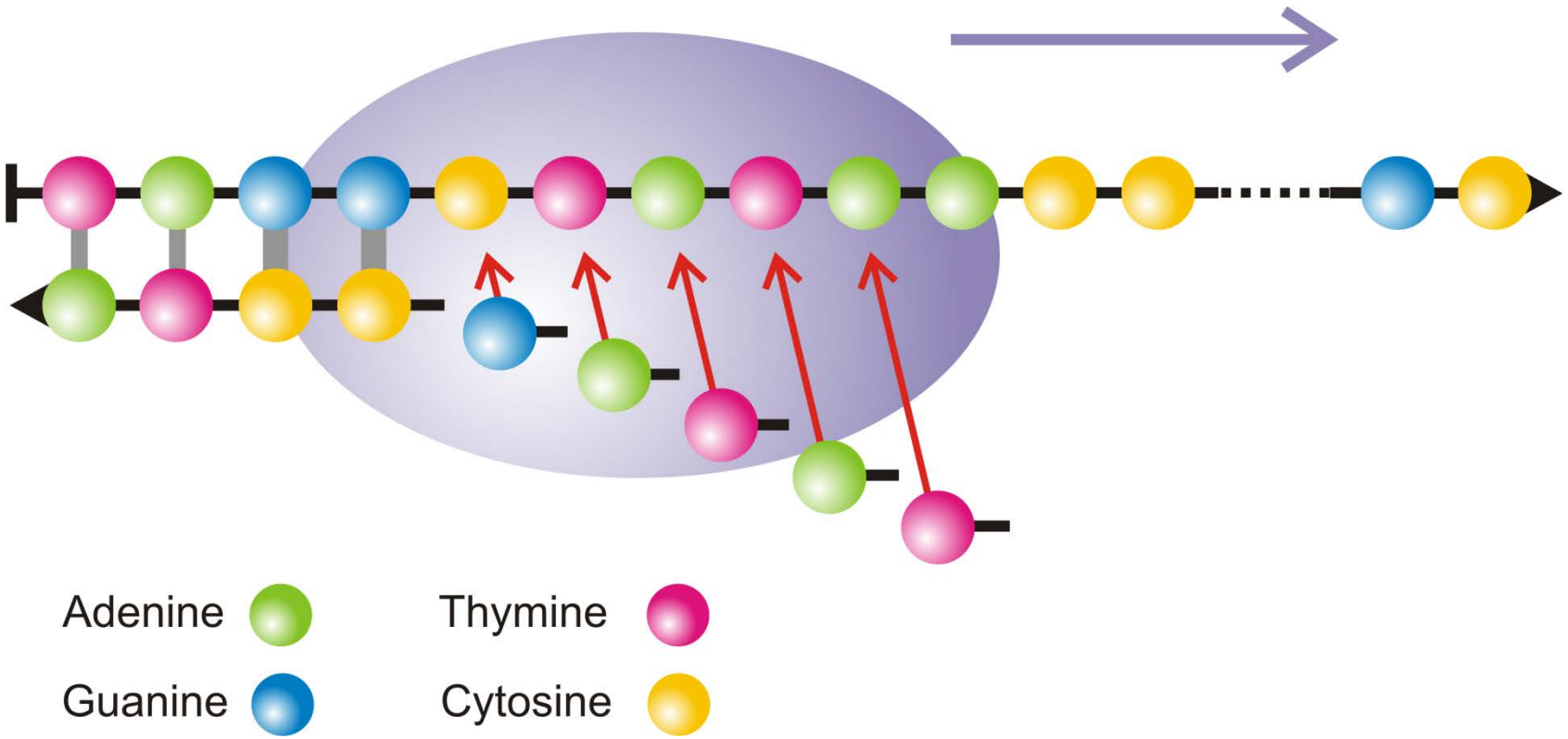


G≡C and **A = U**



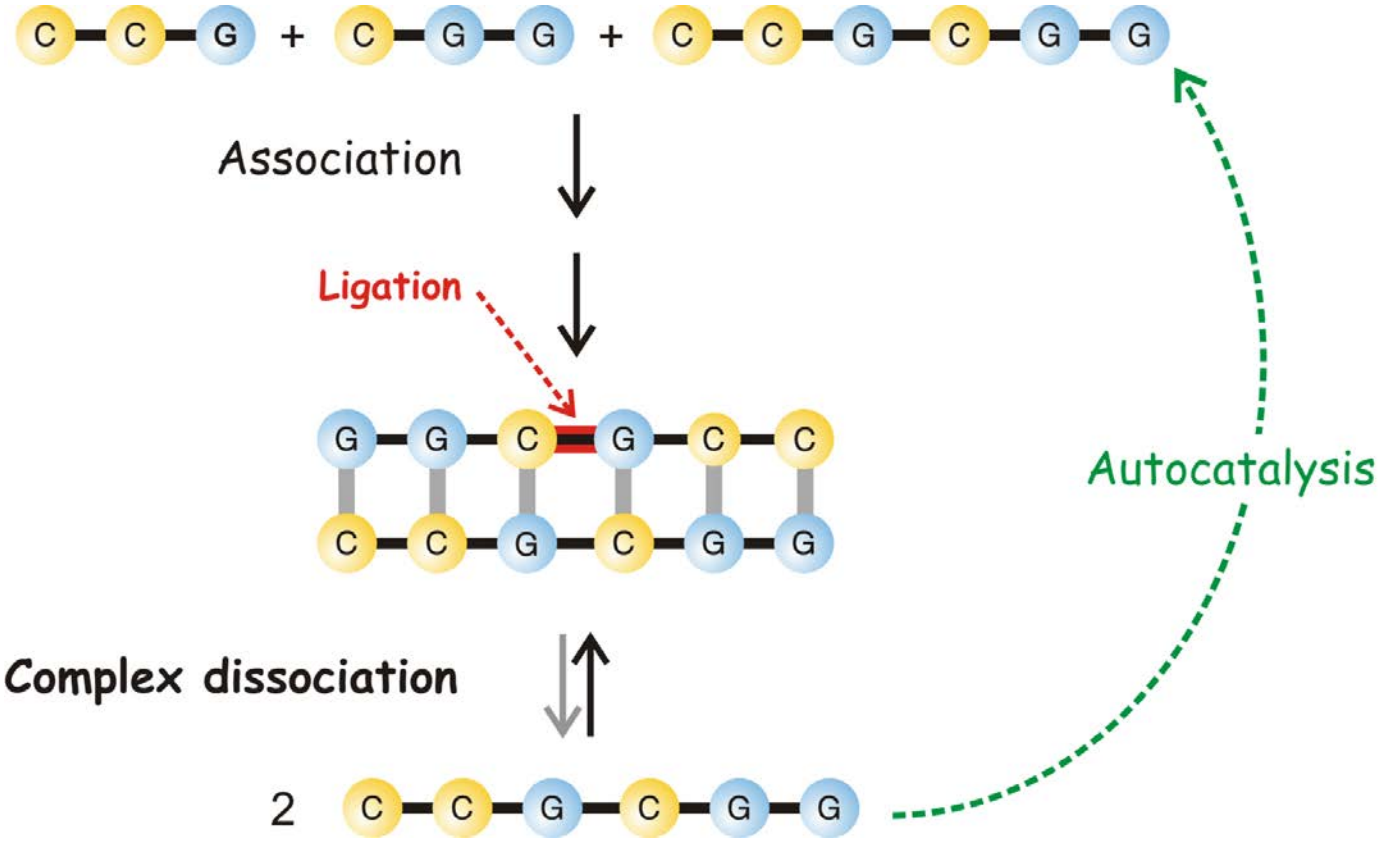
James D. Watson, 1928- , and Francis Crick, 1916-2004,
Nobel Prize 1962

The three-dimensional structure of a short double helical stack of B-DNA



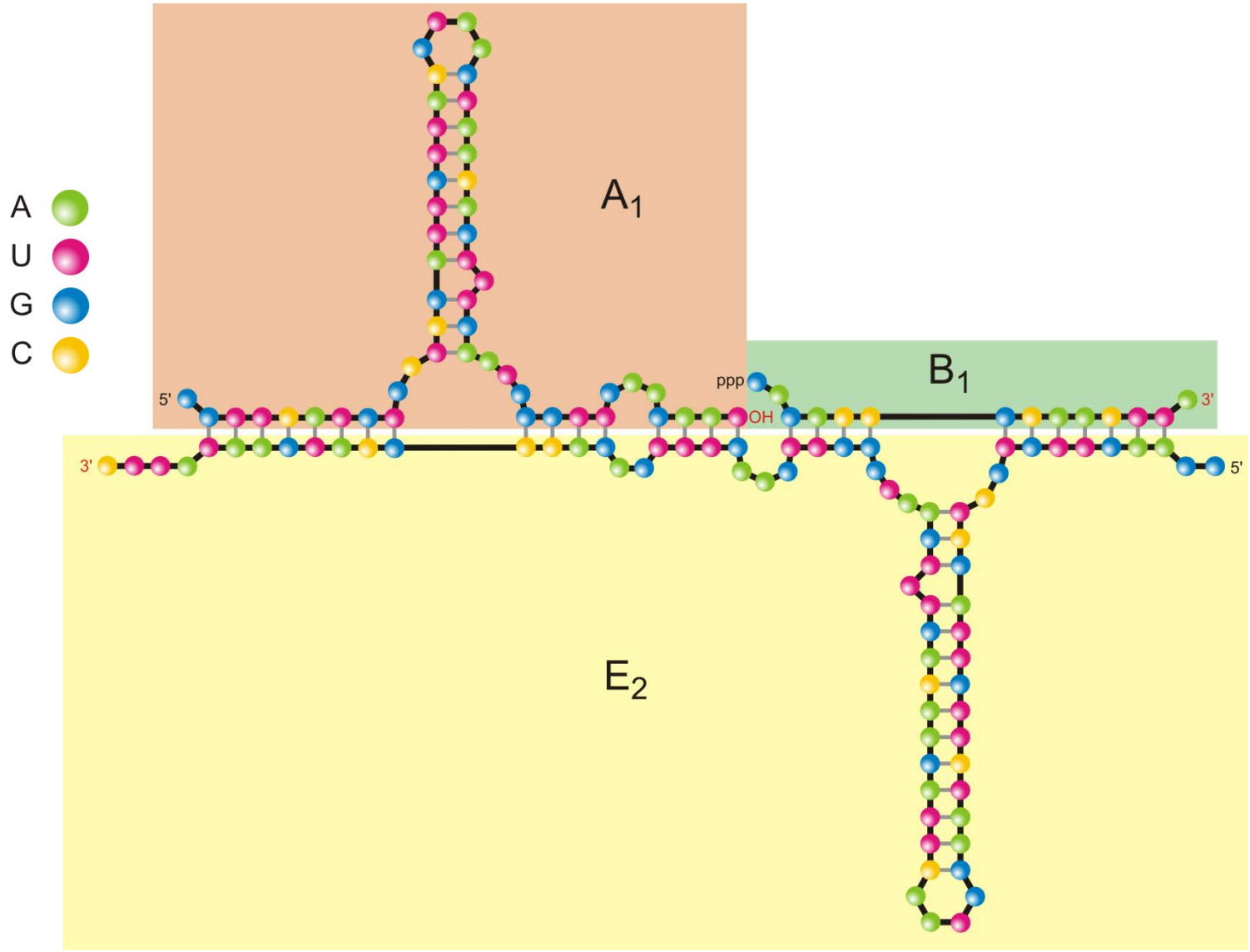
Accuracy of replication: $Q = q_1 \cdot q_2 \cdot q_3 \cdot q_4 \cdot \ldots$

The logics of DNA (or RNA) replication



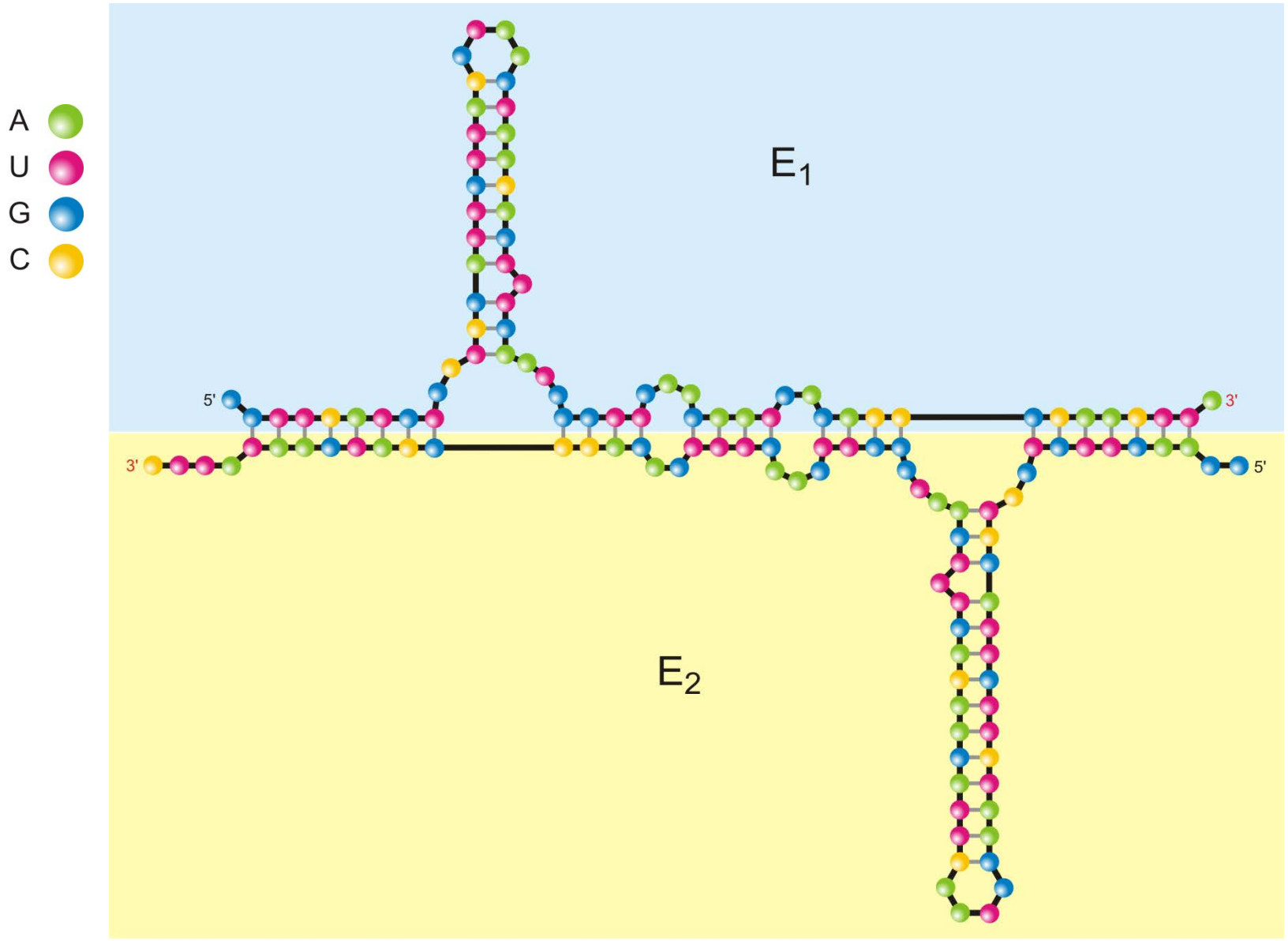
Günter von Kiedrowski. 1986. A self-replication hexanucleotide. *Angew. Chem. Internat. Ed.* **25**:932-935.

Autocatalytic template-induced replication



An example of two ribozymes growing exponentially by cross-catalysis.

T.A. Lincoln, G.F. Joyce. 2009. Self-sustained replication of an RNA enzyme. Science 323:1229-1232



An example of two ribozymes growing exponentially by cross-catalysis.

T.A. Lincoln, G.F. Joyce. 2009. Self-sustained replication of an RNA enzyme. Science 323:1229-1232

Three necessary conditions for Darwinian evolution are:

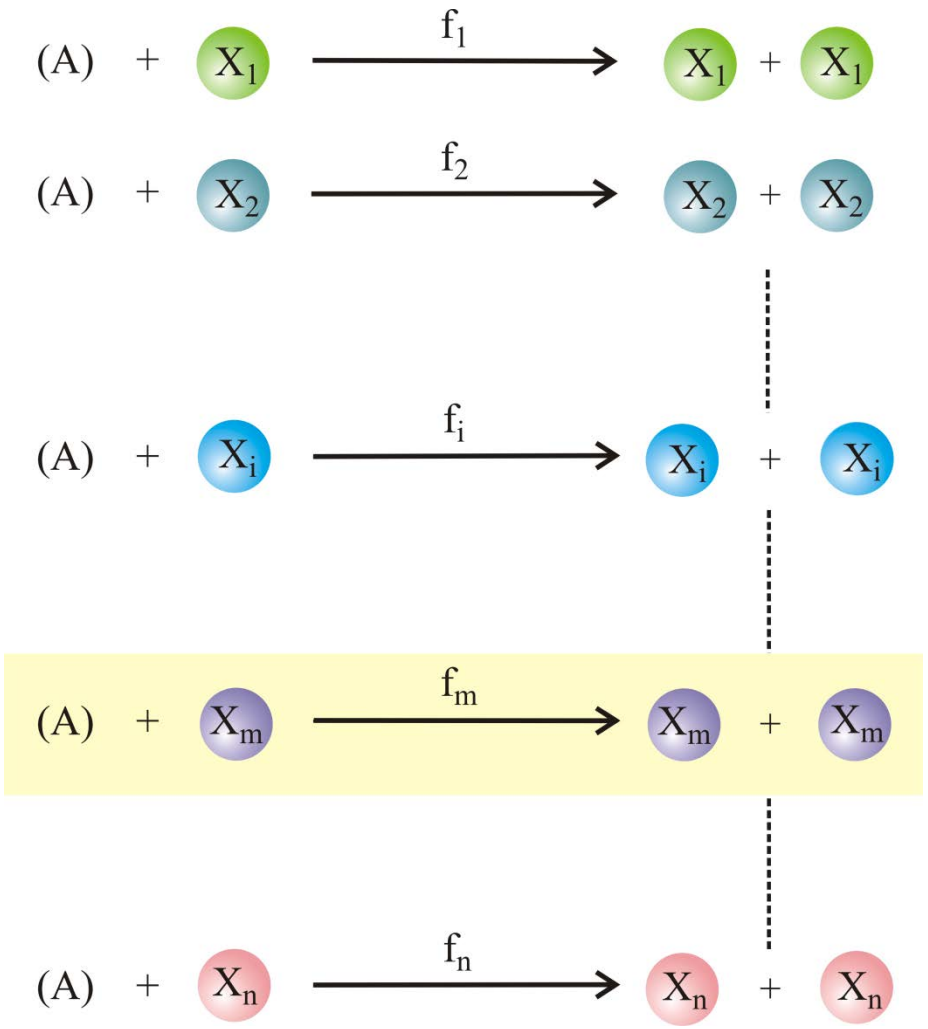
1. **Multiplication,**
2. **Variation**, and
3. **Selection**.

Multiplication leads to exponential growth, which is a *conditio sine qua non* for selection.

Variation is a byproduct of the molecular mechanisms of reproduction.

Selection is a consequence of finite population size.

Darwinian evolution pure is optimizing fitness.

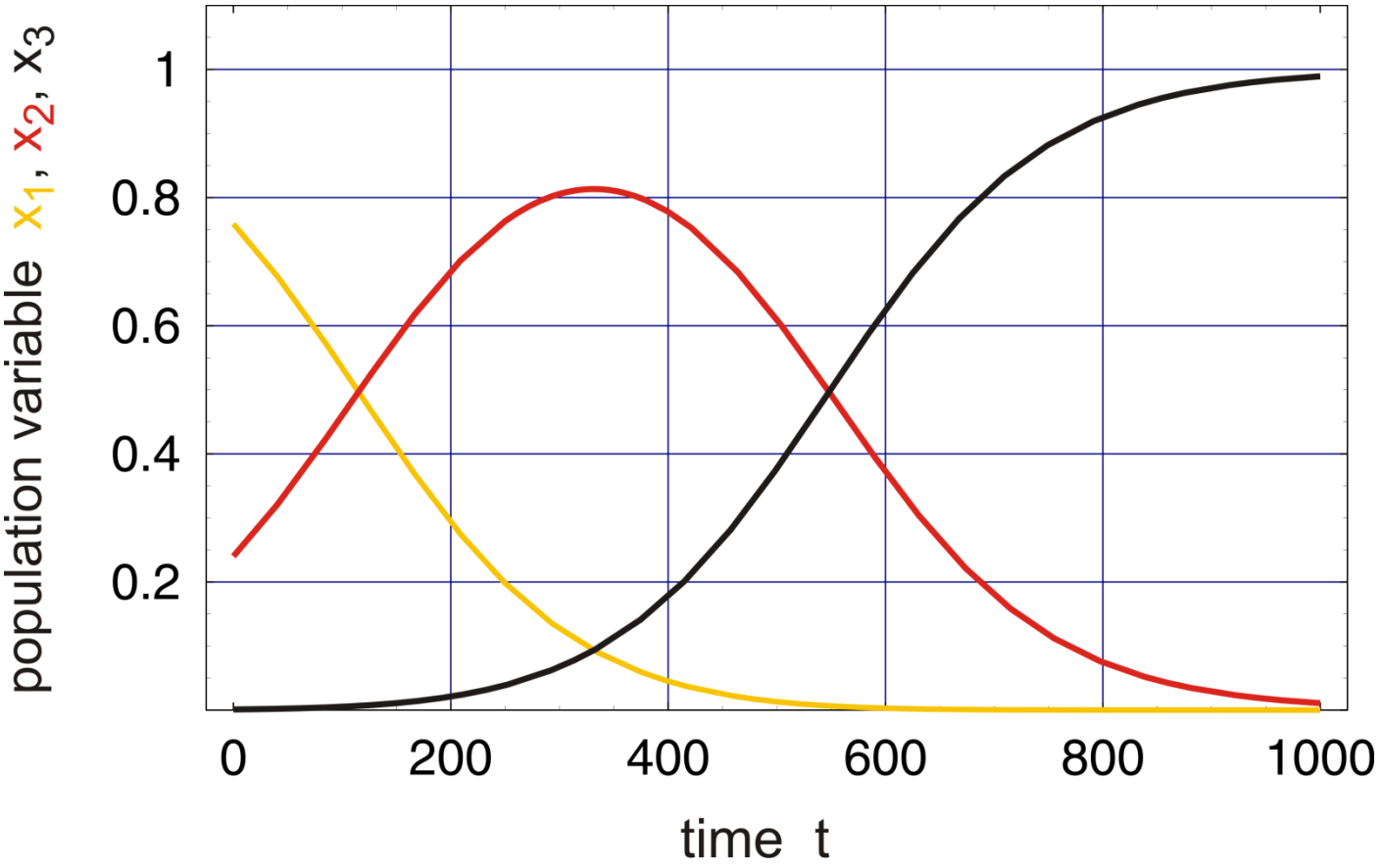


$$x_j(t) = N_j(t) \Big/ \sum_{i=1}^{n} N_i(t)$$

$$f_m = \max\{ f_j ;\ j=1,2,\ldots, n \}$$

$$x_m(t) \to 1 \quad for \quad t \to \infty$$

Reproduction of organisms or replication of molecules as the basis of selection



fitness values: $f_1 = 0.99$, $f_2 = 1.00$, $f_3 = 1.01$

initial conditions: $x_1(0) = 0.759$, $x_2(0) = 0.240$, $x_3(0) = 0.001$

Darwinian selection at constant population size

1. Prologue
2. Molecular replicators
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Sol Spiegelman,
1914 - 1983

Evolution in the test tube:

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

DOI: 10.1002/anie.200701369

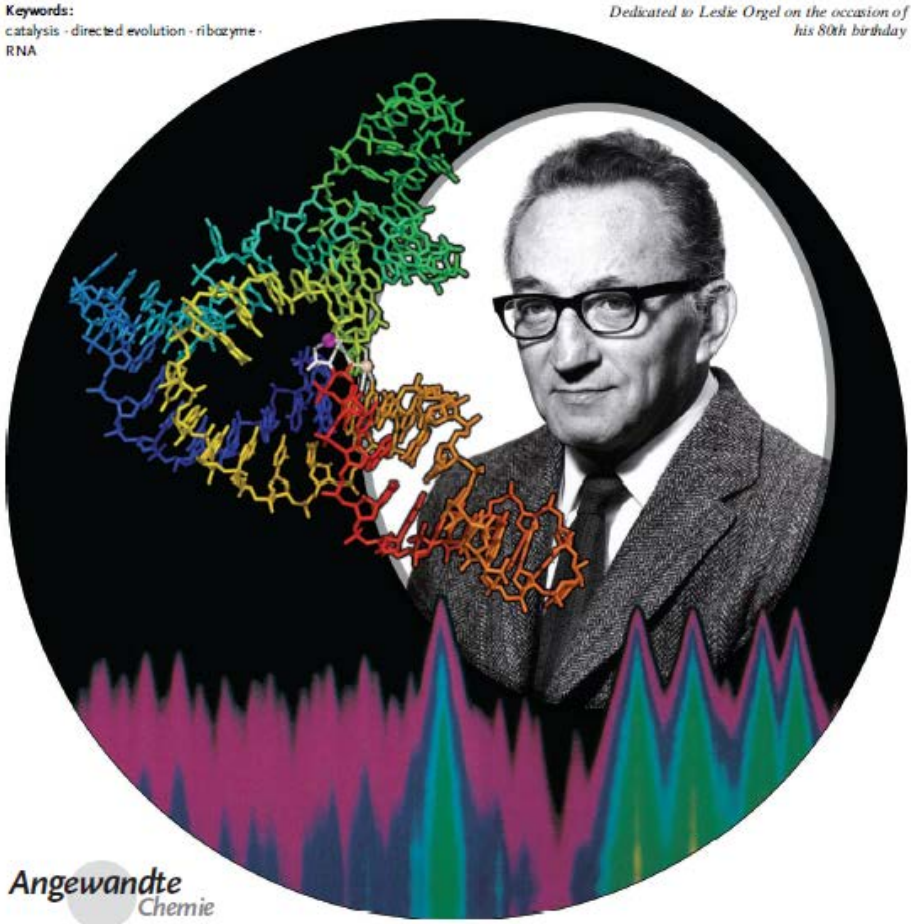
Molecular Evolution

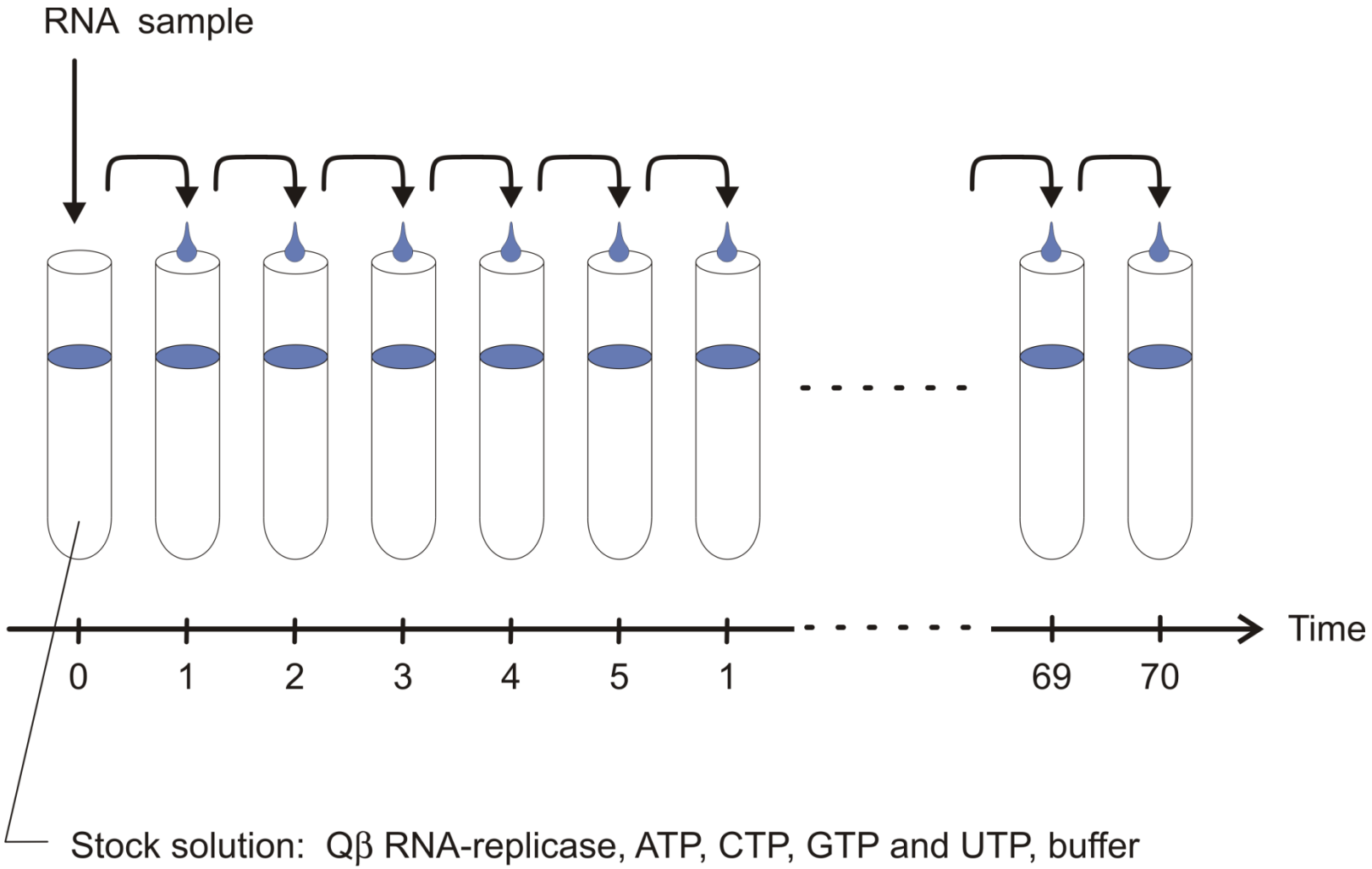
Forty Years of In Vitro Evolution**

*Gerald F. Joyce**

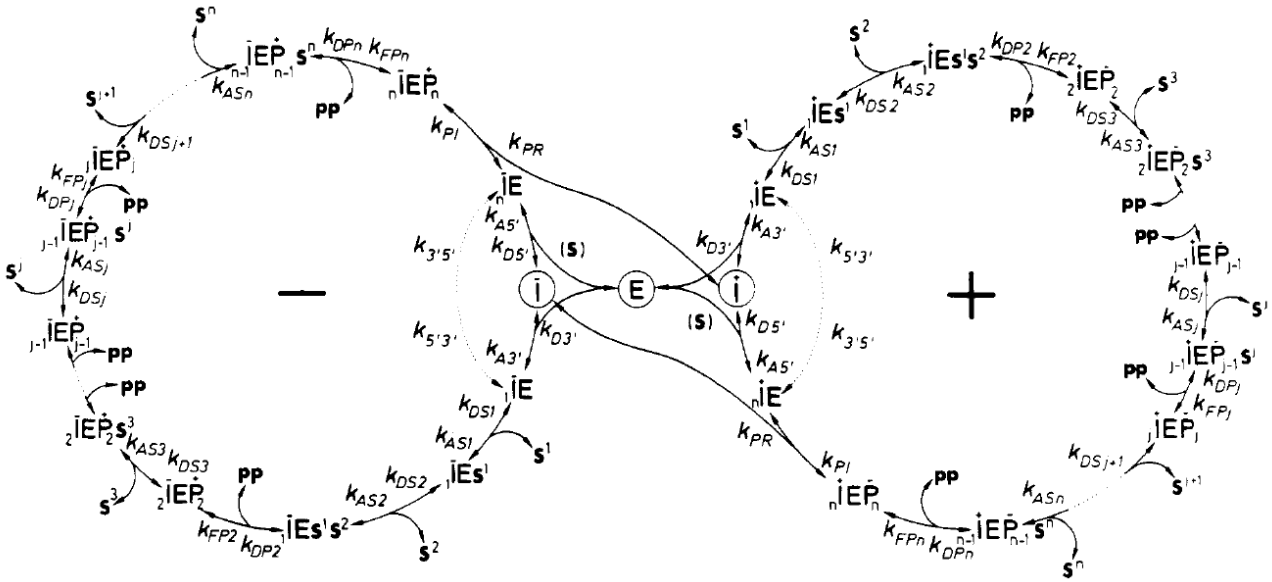
Keywords:
catalysis · directed evolution · ribozyme · RNA

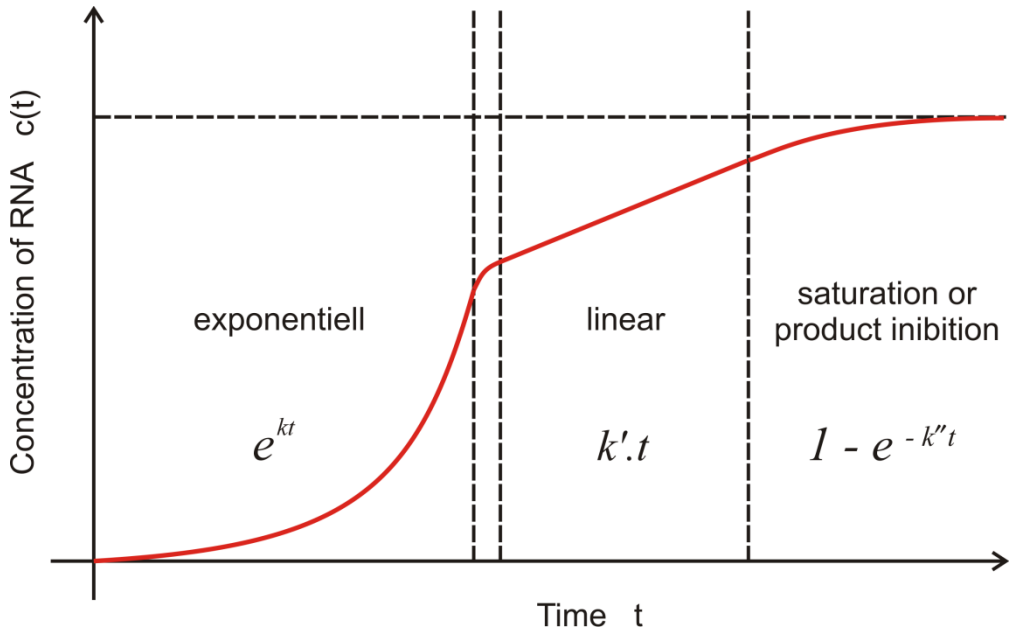
Dedicated to Leslie Orgel on the occasion of his 80th birthday




RNA sample
Time
0
1
2
3
4
5
1
69
70
Stock solution: Qβ RNA-replicase, ATP, CTP, GTP and UTP, buffer

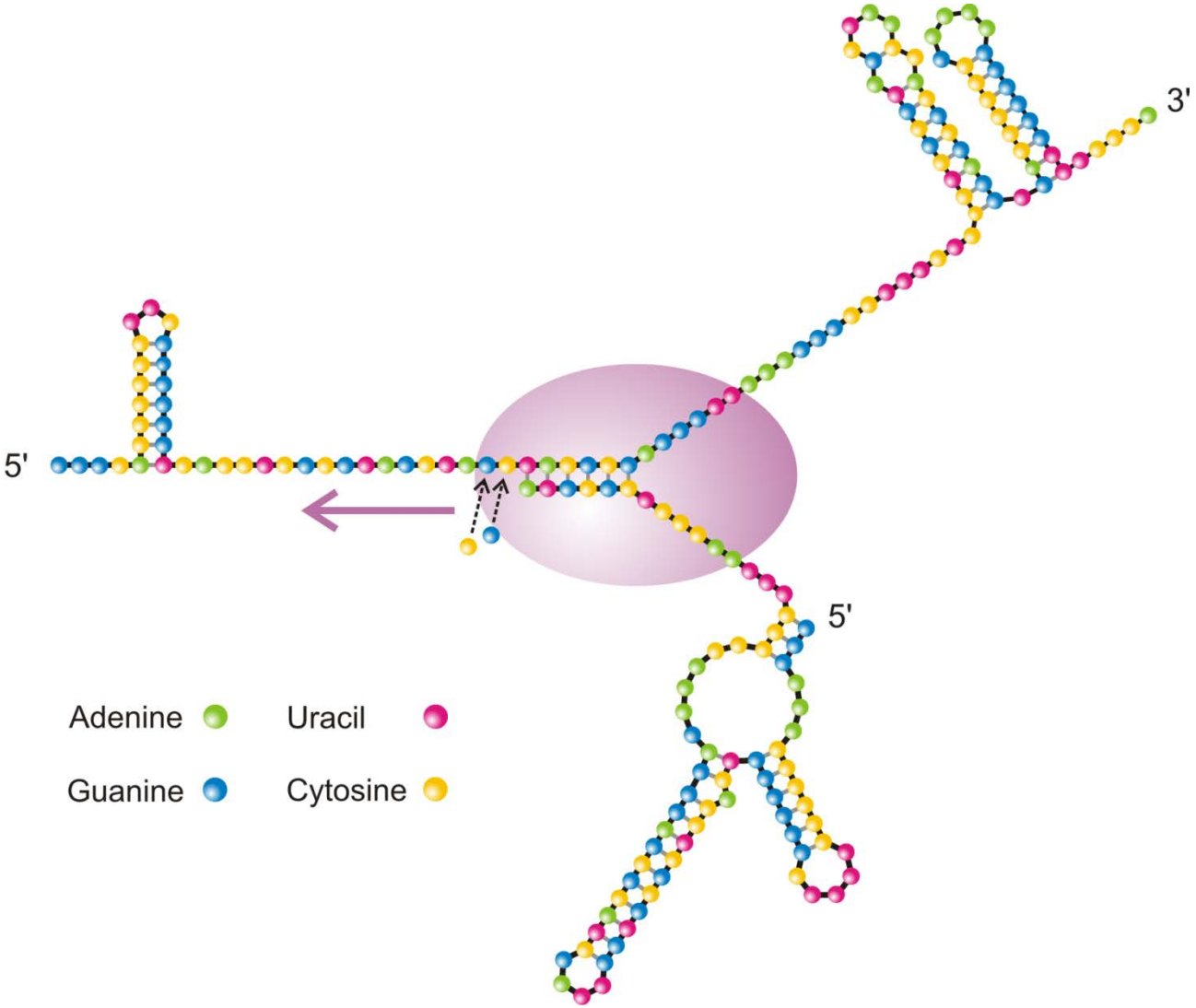
Christof K. Biebricher,
1941-2009

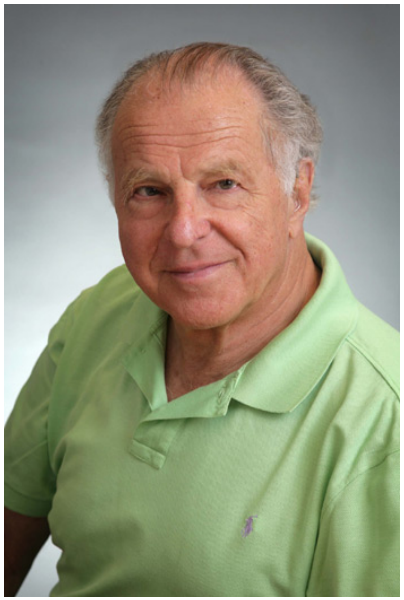




Kinetics of RNA replication

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





Charles Weissmann
1931-

RNA replication by Qβ-replicase

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

DIE NATURWISSENSCHAFTEN

58. Jahrgang, 1971 Heft 10 Oktober

Selforganization of Matter and the Evolution of Biological Macromolecules

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I. Introduction

I.1. „Cause and Effect"

The question about the origin of life often appears as a question about "cause and effect". Physical theories of macroscopic processes usually involve answers to such questions, even if a statistical interpretation is given to the relation between "cause" and "effect". It is mainly due to the nature of this question that many scientists believe that our present physics does not offer any obvious explanation for the existence of life, which even in its simplest forms always appears to be associated with complex macroscopic (i.e. multimolecular) systems, such as the living cell.

As a consequence of the exciting discoveries of "molecular biology", a common version of the above question is: *Which came first, the protein or the nucleic acid?* – a modern variant of the old "chicken-and-the-egg" problem. The term "first" is usually meant to define a causal rather than a temporal relationship, and the words "protein" and "nucleic acid" may be substituted by "function" and "information". The question in this form, when applied to the interplay of nucleic acids and proteins as presently encountered in the living cell, leads ad absurdum, because "function"

* Partly presented as the "Robbins Lectures" at Pomona College, California, in spring 1970.

1971

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The Hypercycle

A Principle of Natural Self-Organization

Part A: Emergence of the Hypercycle

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This paper is the first part of a trilogy, which comprises a detailed study of a special type of functional organization and demonstrates its relevance with respect to the origin and evolution of life. Self-replicative macromolecules, such as RNA or DNA in a suitable environment exhibit a behavior, which we may call Darwinian and which can be formally represented by the concept of the quasi-species. A quasi-species is defined as a given distribution of macromolecular species with closely interrelated sequences, dominated by one or several (degenerate) master copies. External constraints enforce the selection of the best adapted distribution, commonly referred to as the wild-type. Most important for Darwinian behavior are the criteria for internal stability of the quasi-species. If these criteria are violated, the information stored in the nucleotide sequence of the master copy will disintegrate irreversibly leading to an error catastrophy. As a consequence, selection and evolution of RNA or DNA molecules is limited with respect to the amount of information that can be stored in a single replicative unit. An analysis of experimental data regarding RNA and DNA replication at various levels of organization reveals, that a sufficient amount of information for the build up of a translation machinery can be gained only via integration of several different replicative units (or reproductive cycles) through *functional* linkages. A stable functional integration then will raise the system to a new level of organization and thereby enlarge its information capacity considerably. The hypercycle appears to be such a form of organization.

Preview on Part B: The Abstract Hypercycle

The mathematical analysis of dynamical systems using methods of differential topology, yields the result that there is only one type of mechanisms which fulfills the following requirements: The information stored in each single replicative unit (or reproductive cycle) must be maintained, i.e., the respective master copies must compete favorably with their error distributions. Despite their competitive behavior these units must establish a cooperation which includes all functionally integrated species. On the other hand, the cycle as a whole must continue to compete strongly with any other single entity or linked ensemble which does not contribute to its integrated function.
These requirements are crucial for a selection of the best adapted functionally linked ensemble and its evolutive optimization. Only hypercyclic organizations are able to fulfil these requirements. Non-cyclic linkages among the autonomous reproduction cycles, such as chains or branched, tree-like networks are devoid of such properties.
The mathematical methods used for proving these assertions are fixed-point, Lyapunov- and trajectorial analysis in higher-dimensional phase spaces, spanned by the concentration coordinates of the cooperating partners. The self-organizing properties of hypercycles are elucidated, using analytical as well as numerical techniques.

Preview on Part C: The Realistic Hypercycle

A realistic model of a hypercycle relevant with respect to the origin of the genetic code and the translation machinery is presented. It includes the following features referring to natural systems:
1) The hypercycle has a sufficiently simple structure to admit an origination with finite probability under prebiotic conditions.
2) It permits a continuous emergence from closely interrelated (t-RNA-like) precursors, originally being members of a stable RNA quasi-species and having been amplified to a level of higher abundance.
3) The organizational structure and the properties of single functional units of this hypercycle are still reflected in the present genetic code in the translation apparatus of the prokaryotic cell, as well as in certain bacterial viruses.

I. The Paradigm of Unity and Diversity in Evolution

Why do millions of species, plants and animals, exist, while there is only one basic molecular machinery of the cell: one universal genetic code and unique chiralities of the macromolecules?
The geneticists of our day would not hesitate to give an immediate answere to the first part of this question. Diversity of species is the outcome of the tremendous branching process of evolution with its myriads of single steps of reproduction and mutation. It in-

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Molecular Quasi-Species†

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The molecular quasi-species model describes the physicochemical organization of monomers into an ensemble of heteropolymers with combinatorial complexity by ongoing template polymerization. Polynucleotides belong to the simplest class of such molecules. The quasi-species itself represents the stationary distribution of macromolecular sequences maintained by chemical reactions effecting error-prone replication and by transport processes. It is obtained deterministically, by mass-action kinetics, as the dominant eigenvalue of a *value* matrix, **W**, which is derived directly from chemical rate coefficients, but it also exhibits stochastic features, being composed to a significant fraction of unique individual macromolecular sequences. The quasi-species model demonstrates how macromolecular information originates through specific nonequilibrium autocatalytic reactions and thus forms a bridge between reaction kinetics and molecular evolution. Selection and evolutionary optimization appear as new features in physical chemistry. Concentration bias in the production of mutants is a new concept in population genetics, relevant to frequently mutating populations, which is shown to greatly enhance the optimization properties. The present theory relates to asexually replicating ensembles, but this restriction is not essential. A sharp transition is exhibited between a drifting population of essentially random macromolecular sequences and a localized population of close relatives. This transition at a threshold error rate was found to depend on sequence lengths, distributions of selective values, and population sizes. It has been determined generically for complex landscapes and for special cases, and, it was shown to persist generically in the presence of nearly neutral mutants. Replication dynamics has much in common with the equilibrium statistics of complex spin systems: the error threshold is equivalent to a magnetic order–disorder transition. A rational function of the replication accuracy plays the role of temperature. Experimental data obtained from test-tube evolution of polynucleotides and from studies of natural virus populations support the quasi-species model. The error threshold seems to set a limit to the genome lengths of several classes of RNA viruses. In addition, the results are relevant even in eucaryotes where they contribute to the exon–intron debate.

1. Molecular Selection

Our knowledge of physical and chemical systems is, in a final analysis, based on models derived from repeatable experiments. While none of the classic and rather besieged list of properties rounded up to support the intuition of a distinction between the living and nonliving—metabolism, self-reproduction, irritability, and adaptability, for example—intrinsically limit the application of the scientific method, a determining role by unique *or individual* entities comes into conflict with the requirement of repeatability. Combinatorial variety, such as that in heteropolymers based on even very small numbers of different bases, even just two, readily provides numbers of different entities so enormous that neither consecutive nor parallel physical realization is possible. The physical chemistry of finite systems of such macromolecules must deal with both known regularities and the advent of unique copolymeric sequences. Normally this would present no difficulty in a statistical mechanical analysis of typical behavior, where rare events play no significant role, but with autocatalytic polymerization processes even unique single molecules may be amplified to determine the fate of the entire system. Potentially creative, self-organizing around unique events, the dynamics of this simplest living chemical system is invested with regularities that both allow and limit efficient adaptation. The quasi-species model is a study of these regularities.

The fundamental regularity in living organisms that has invited explanation is adaptation. Why are organisms so well fitted to their environments? At a more chemical level, why are enzymes optimal catalysts? Darwin's theory of natural selection has provided biologists with a framework for the answer to this question. The present model is constructed along Darwinian lines but in terms of specific macromolecules, chemical reactions, and physical processes that make the notion of survival of the fittest precise. Not only does the model give an understanding of the physical limitations of adaptation, but also it provides new insight into the role of chance in the process. For an understanding of the structure of this minimal chemical model it is first necessary to recall the conceptual basis of Darwin's theory.

Darwin recognized that new inheritable adaptive properties were not induced by the environment but arose independently in the production of offspring. Lasting adaptive changes in a population could only come about by natural selection of the heritable traits or *genotype* based on the full characteristics or *phenotype* relevant for producing offspring. A process of chance, i.e., uncorrelated with the developed phenotype, controls changes in the genotype from one generation to the next and generates the diversity necessary for selection. Three factors have probably prevented chemists from gaining a clear insight into these phenomena in the past, despite the discovery of the polymeric nature of the genotype (DNA): the complexity of a minimum replication phenotype, the problem of dealing with a huge number of variants, and the nonequilibrium nature of these ongoing processes.

The formulation of a tractable chemical model based on Darwin's principle may be understood in several steps:

1. The major constituents of the system have to be inherently self-reproductive. Only two classes of molecules are presently

† This is an abridged account of the quasi-species theory that has been submitted in comprehensive form to *Advances in Chemical Physics*.[1]

(1) Eigen, M.; McCaskill, J. S.; Schuster, P. *Adv. Chem. Phys.*, in press.

0022-3654/88/2092-6881$01.50/0

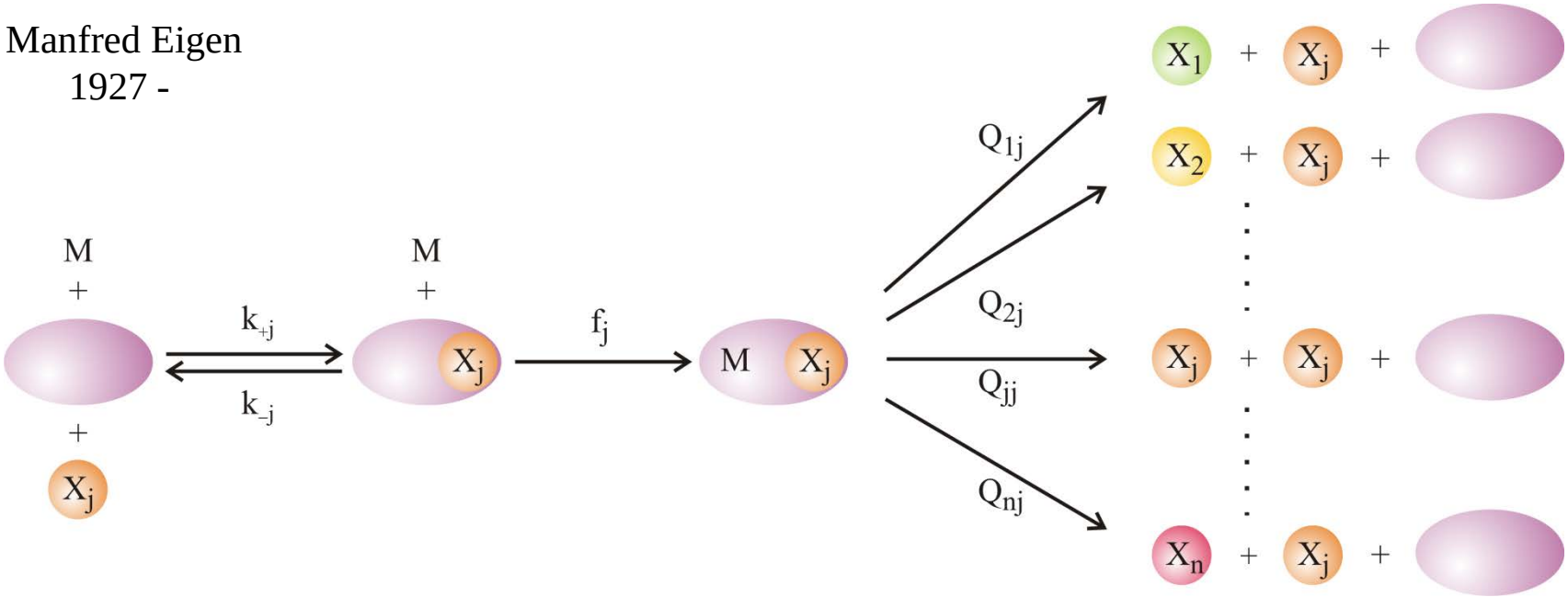
1988

Chemical kinetics of molecular evolution

Manfred Eigen
1927 -

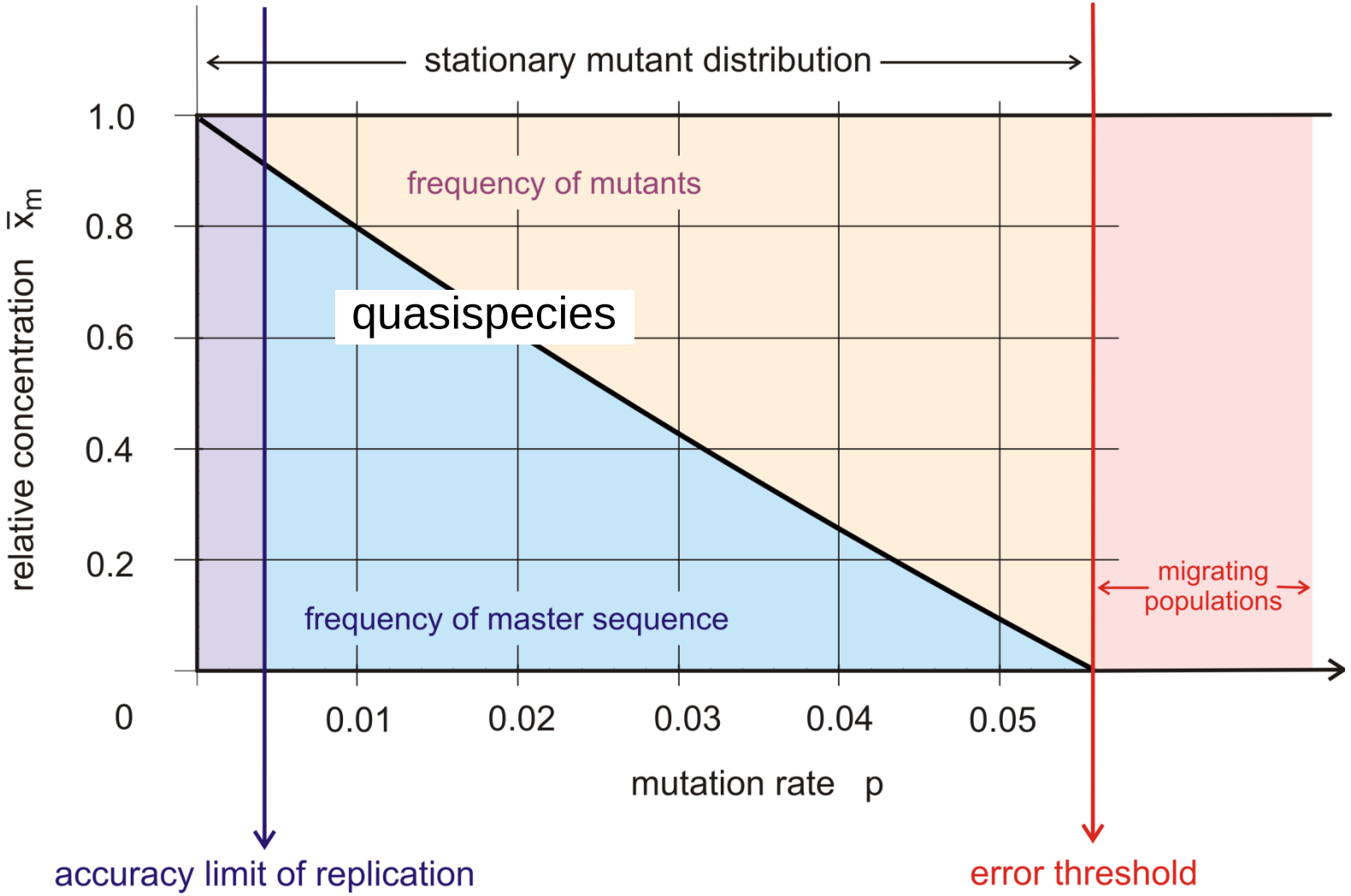
$$\frac{dx_j}{dt} = \sum_{i=1}^{n} W_{ji}\, x_i - x_j\, \Phi\,; \quad j = 1, 2, \ldots, n$$

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



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

p … constant : $n_{\max} \approx \frac{\ln\sigma}{p}$ prebiotic chemistry

n … constant : $p_{\max} \approx \frac{\ln\sigma}{n}$ antiviral strategies

Chain length, replication accuracy and error threshold

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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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Esteban Domingo
1943 -

Application of quasispecies theory to the fight against viruses

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Elsevier Biomedical Press

SELF-REPLICATION WITH ERRORS

A MODEL FOR POLYNUCLEOTIDE REPLICATION **

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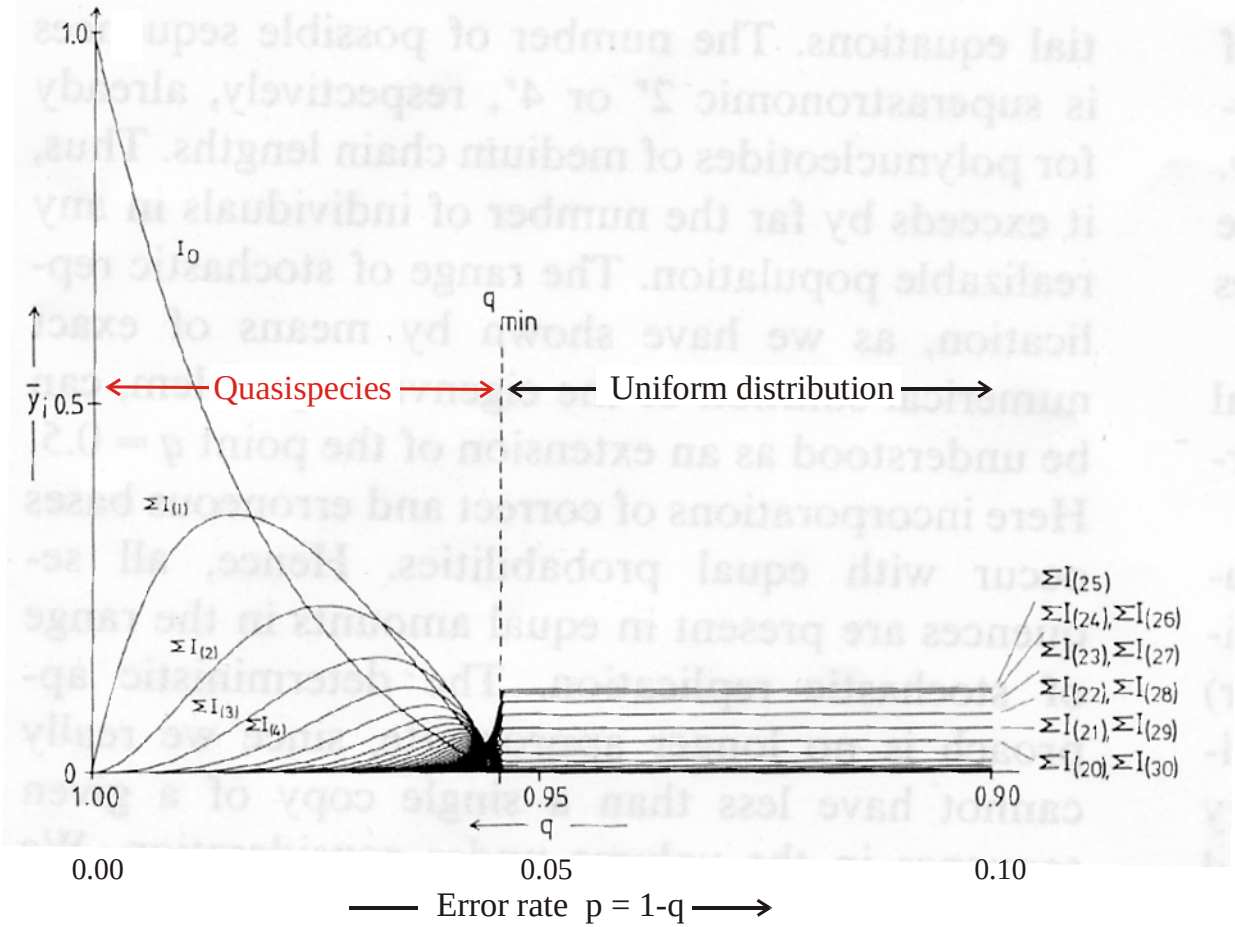
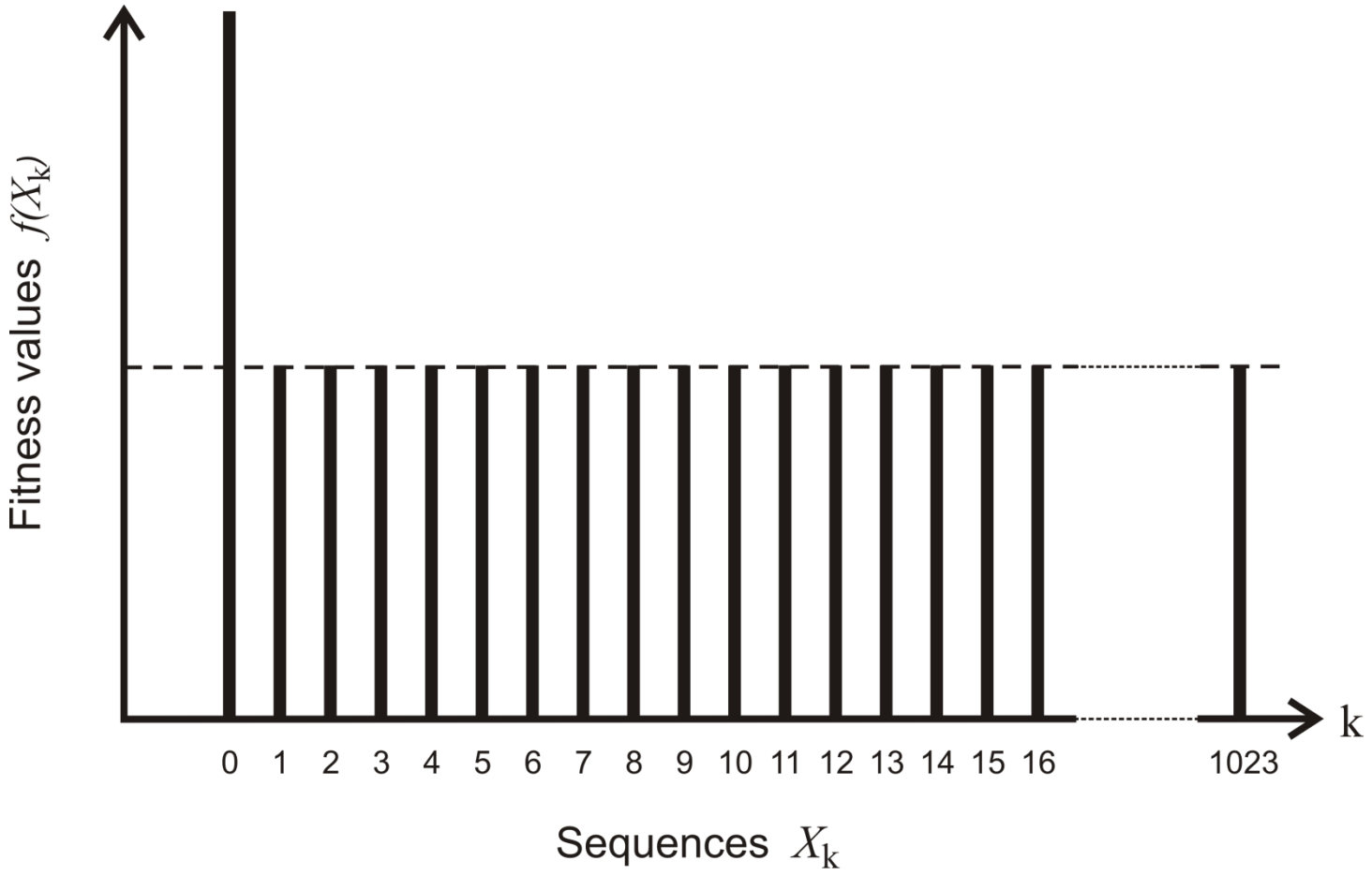
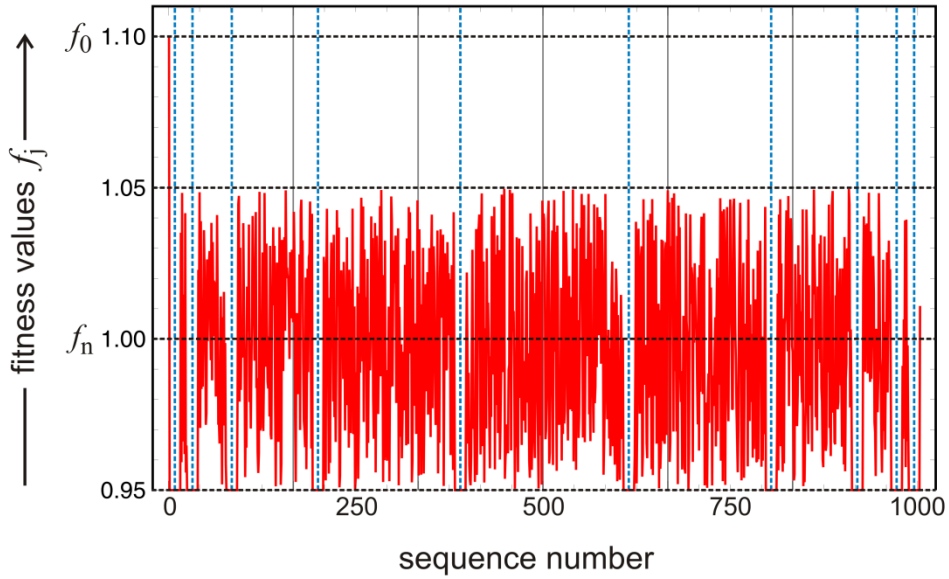


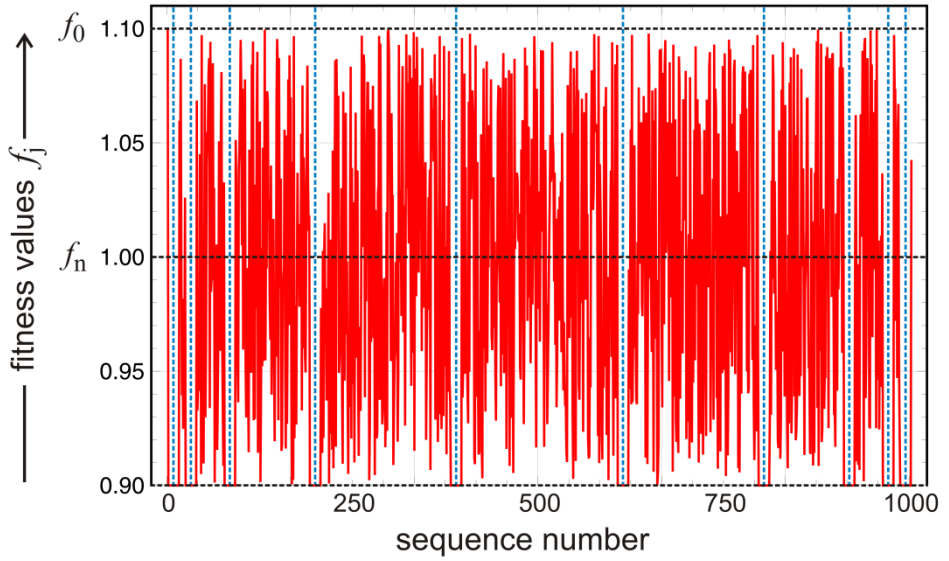
Stationary population or quasispecies as a function of the mutation or error rate *p*



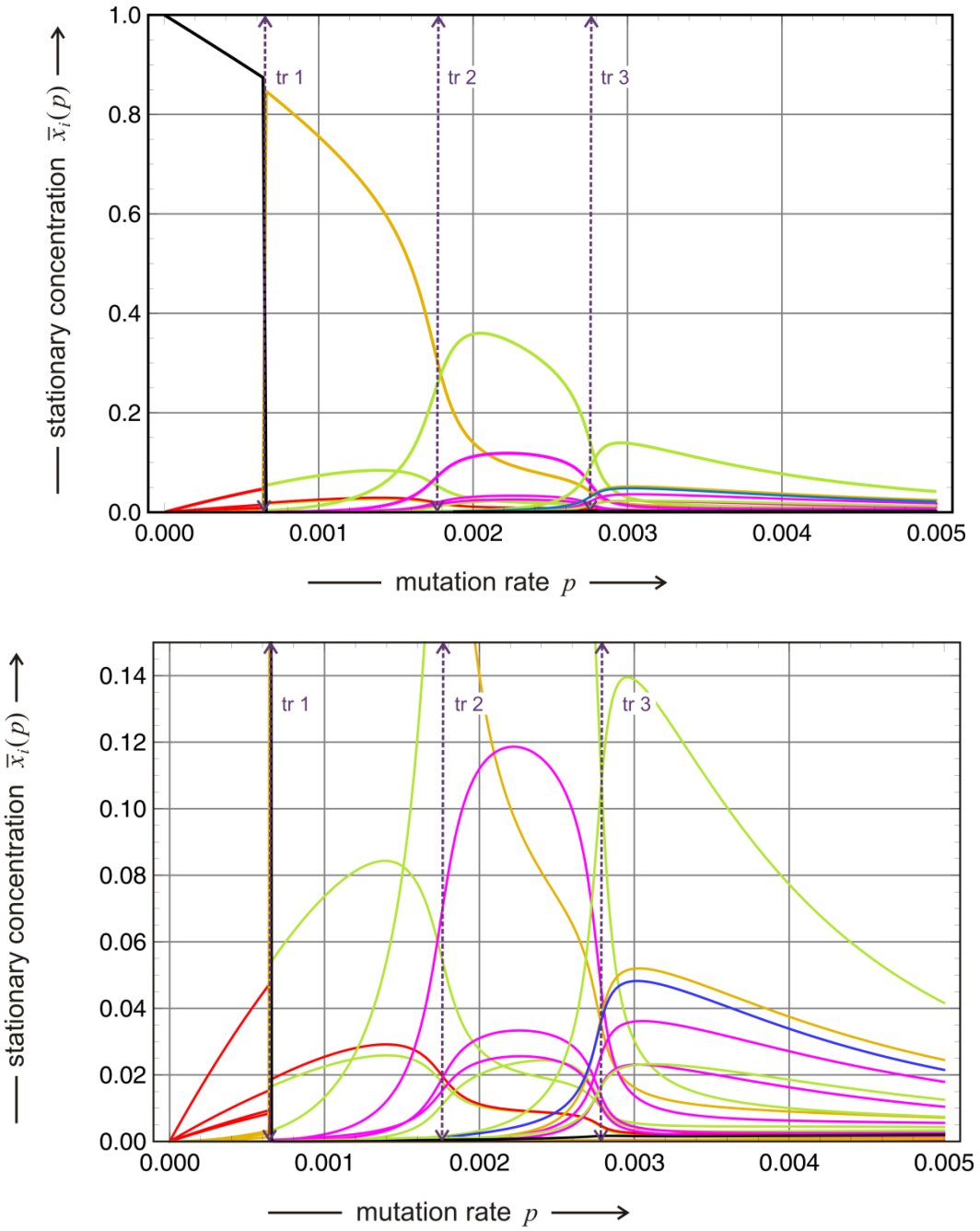
The single peak model landscape for all sequences with chain lengths n = 10

„Realistic“ fitness landscapes
with scattered fitness values

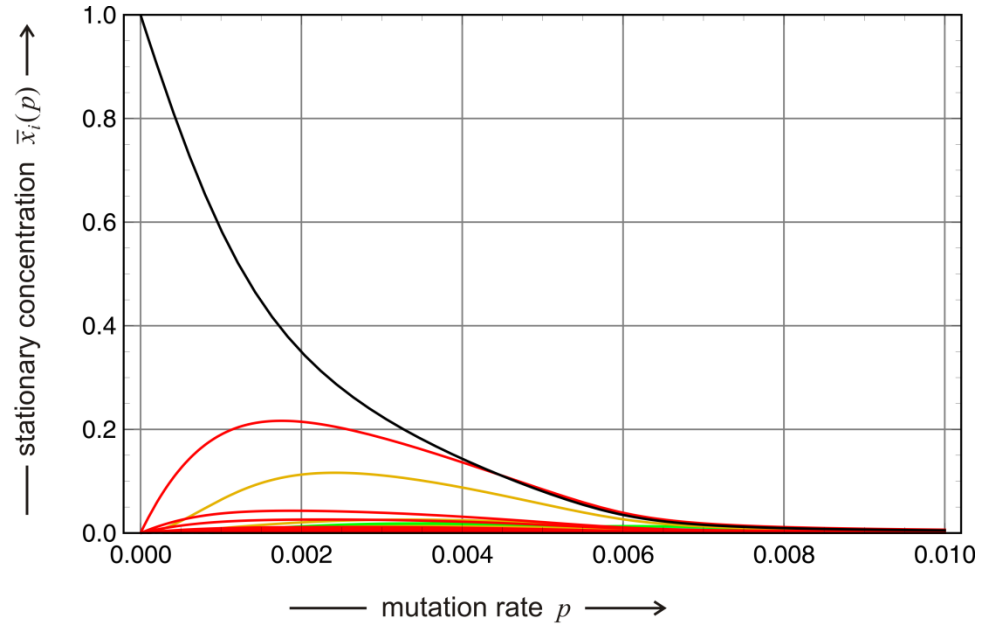


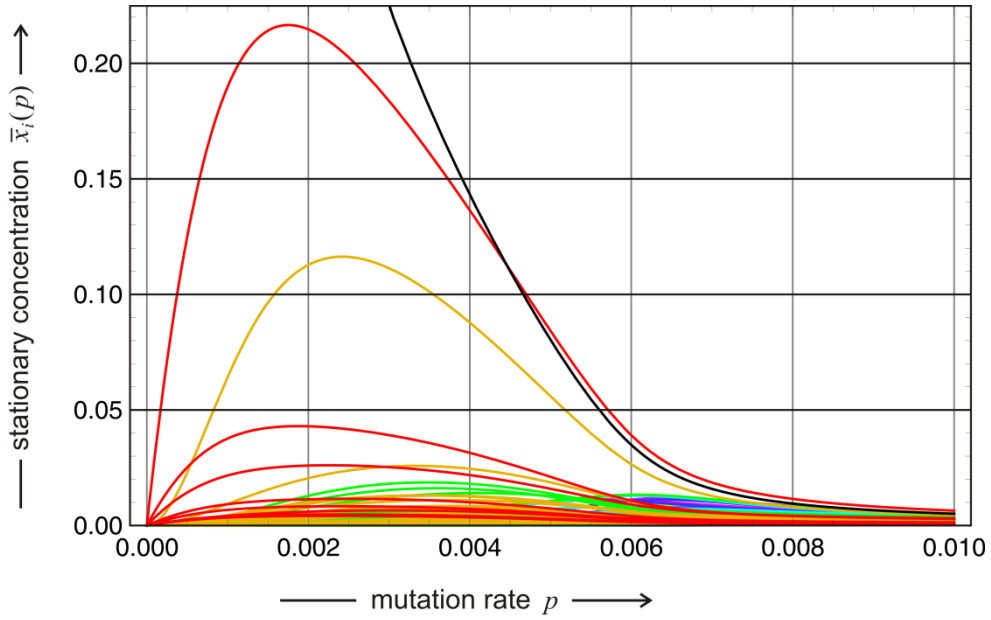


Quasispecies with
phase transitions



Strong quasispecies





1. Prologue
2. Molecular replicators
3. Replication and mutation
4. **Perspectives**

1. ‚**Origin of Life**' is **not** an established area of research with a **generally accepted methodology**.

2. There are **many open questions**, which **require further research**.

3. An answer to the question whether or not a **common** primitive **core metabolism** has preceded the origin of biomolecules is of crucial importance.

4. The role of **compartmentalization** and the **origin of the biological cell** is still a burning unsolved problem.

5. Although the question how life began on earth is far from being satisfactorily answered, **spin-offs from origin of life research** are and will continue to be of high value.

Thank you for your attention!

Web-Page for further information:

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