

# EVOLUTION WITHOUT DIVERGENCE AND MULTIPLE ANCESTORS OF LIFE

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## (Abstract)

Discrepancies among phylogenetic trees based on different biopolymers and morphology, as well as diversity of fundamental features in spite of general belief that most fundamental features are common to all forms of life, led us to propose a hypothesis that the major driving force for evolution is information exchanges among different species, rather than divergence of species resulting from accumulation of point mutations. Mutations are mainly responsible for the changes in the biopolymer sequences in different species. Examples of diversity in biochemical features of living things dealt with include such fundamental features as chirality of the membrane lipids, mechanisms to mature mRNAs, synthesis of aminoacyl-tRNAs, synthesis of isoprenoid precursors, coenzymes, etc. Admitting interspecies information exchanges as the driving force for evolution, the life is not necessarily originated from a single ancestor, but the possibilities of multiple ancestors have to be considered. Several hypotheses for the origin of life, which are consistent with multiple ancestors are presented.

## (Keywords)

evolution without divergence, multiple ancestors of life, interspecies communication, horizontal gene transfer, chirality of membrane lipids, trans-splicing, aminoacyl-tRNA synthetases, phylogenetic trees, phosphonate, CXXC sequence

## 1. Introduction

It is commonly accepted as an established theory that all the living things evolved from a single ancestor, which emerged only once on our planet. The “facts” supporting this theory summarized by Margaret Dayhoff [1] are: (i) all the living things utilize ATP as an energy mediator, (ii) synthesize and breakdown fats, carbohydrates and proteins by similar reactions, (iii) live on proteins made of the same 20 amino acids, (iv) synthesize proteins by the same code system, and (v) utilize the same vitamins and other compounds. This theory naturally leads to a conclusion that two homologous proteins in different species (i.e., orthologous proteins) must have been diverged from their common ancestral protein. This is the basis to construct the phylogenetic tree of living creatures found in leading textbooks in biology and biochemistry [2, 3].

If this “single ancestor theory for the origin of life” is actually the case, all the phylogenetic trees based on any of orthologous protein families must be identical, or must not have any discrepancy even if some trees lack branches due to incomplete survey of orthologous protein families. However, phylogenetic trees based on different protein families are not always identical, and

sometimes they are inconsistent with that based on morphology. This led us to propose a hypothesis that the main driving force for evolution is information exchange (or communication) between different species, rather than divergence of species resulting from accumulation of point mutations [4]. The horizontal gene transfer may result in the morphology, behavior and/or biochemistry of the living creatures. In the present paper some cases in which the most fundamental metabolism is affected probably due to gene transfer between the creatures of different groups will be presented. In this hypothesis, which is schematically represented in Fig. 1, we postulated that all the living things evolved not from a single ancestor, but from multiple ancestors, and that an extensive information exchange among species during evolution makes much of, but not all of essential features of living things in common. Actually, many of fundamental features of living things are by no means in common, which our hypothesis [4, 5] will cope with, but the single ancestor theory [1] will not. Moreover, evidence for interspecies gene transfer is plenty [6-8]. In this paper, we reinforce our hypothesis of “evolution without divergence, or in other words, evolution by interspecies communication,” and discuss possibility of having multiple ancestors.

## 2. Variety of Fundamental Features of Living Things

The fact that most living things have essentially identical pathways for the synthesis of such important molecules as purine and pyrimidine nucleotides is considered to be one of proofs for the single-ancestor theory. However, detailed examination of biochemical features demonstrates that the case of the nucleotide synthesis is rather exceptional, and that many crucial features and processes are by no means in common in living things as shown below.

### 2.1. Chirality of the membrane lipids

Cell membrane is one of the most important constituents of cellular life, of which glycerophospholipids constitute the main components. The glycerophospholipids in bacterial and eucaryal cell membranes are mainly long-chain carboxylate esters of *sn*-glycero-3-phosphate (L-glycero-3-phosphate), whereas glycerophospholipids in archaeal cell membranes are isoprenoid ethers of *sn*-glycero-1-phosphate (D-glycero-3-phosphate) as shown in Fig. 2 [9-11]. It was postulated that Archaea and Bacteria were diverged from a common ancestor before cellular life [11], i.e., surface metabolist without cell [12], since the changeover from one enantiomer to the other of glycerophosphate core in the membrane phospholipids must have been impossible after once

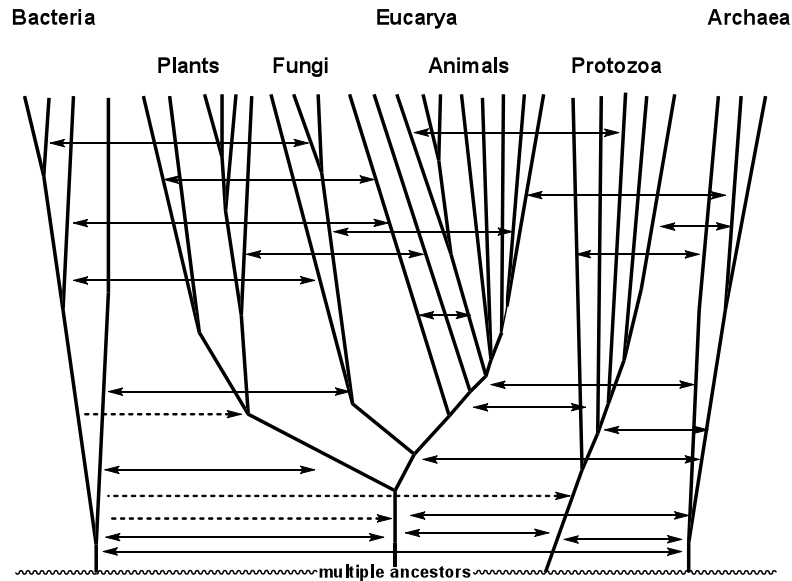


Figure 1 Diagrammatic representation of our hypothesis, “evolution without divergence.” Instead of postulating a single ancestor, this hypothesis assumes possibility of having multiple ancestors (the number of ancestors is not necessarily four as shown). Arrows indicate mutual information exchanges including horizontal gene transfer by viral or bacterial infection, sex, hybridization (two-headed arrows), or symbiosis (dotted arrows). This hypothesis does not deny divergence caused by mutations, but considers that mutual information exchange had major contribution to the evolution.

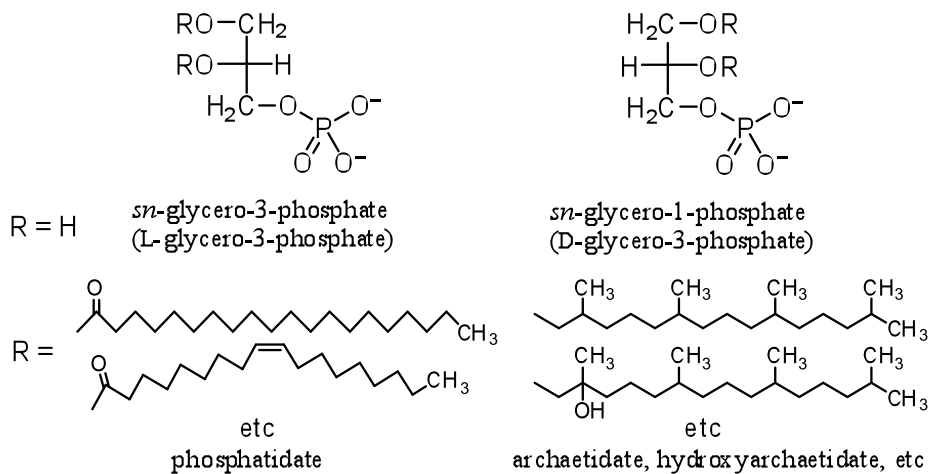


Figure 2. Core compounds of membrane phospholipids in Bacteria and Eucarya (left), and Archaea (right). Only two examples of hydrophobic tails are shown for each group of phospholipids. In membrane lipids, hydrophilic heads such as glycerol, ethanolamine, L-serine, etc are esterified to the phosphate group of phosphatidate (in Bacteria and Eucarya) or archaetidate/hydroxyarchaetidate (in Archaea).

cellular life was established. Rational to suggest the surface metabolists as a common ancestor is the “facts” that most basic biochemical features were shared by Archaea and Bacteria [13]. However, there are a lot of differences in most fundamental features in these Domains, as have been pointed out by Woese et al [14], and as will be described in this paper. Moreover, the surface-metabolist hypothesis [12] does not rule out the possible existence of more than two ancestors to evolve to Archaea and Bacteria (vide post).

## 2.2. Maturation of mRNAs

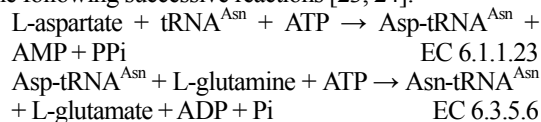
mRNAs play one of the most important roles in “the central dogma of molecular biology.” Prokaryotic mRNAs are polycistronic and matured without splicing, whereas eucaryotic mRNAs are monocistronic and matured only after capping, polyA-tailing, and splicing. Strategy of splicing in most Eucarya is that exons upstream and downstream of each intron are connected by transesterification to release the intron as a lariat, a process called cis-splicing, because two exons to be

connected are located on a single pre-mRNA molecule. However, some eucaryal species including *Trypanosoma* use a different strategy called trans-splicing, in which a capped untranslatable exon called a spliced leader (SL) and an exon containing an ORF are derived from different pre-mRNA molecules. Distribution of the SL-trans-splicing is shown in Fig. 3 [15]. Pre-mRNAs of some species (e.g., *C. elegans*) are transcribed as polycistronic units like those of prokaryotic operons, and are processed by SL-trans-splicing to produce capped monocistronic mRNAs [16]. Different types of trans-splicing were found in various species including *Drosophila* [17-19], rat [20, 21], and even human [22], physiological significance thereof was assigned to produce diversity in its essential proteins at least in case of *Drosophila* [17-19].

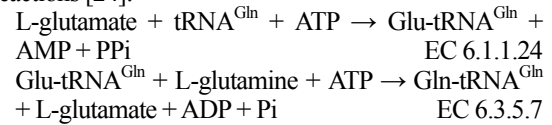
How can we explain the sporadic distribution of trans-splicing based on the single ancestor theory? Is this mechanism invented many times independently, or is it invented in the common ancestor and abandoned randomly in very many unrelated species during evolution? Instead, sporadic distribution of trans-splicing mechanism must have been a result of the interspecies information exchange.

### 2.3. Synthesis of aminoacyl-tRNAs.

Amino-acyl-tRNAs are indispensable materials for the ribosomal protein synthesis encoded by mRNAs. Until early 1990s, it was widely believed that all 20 standard amino acids were connected to their respective tRNAs by ATP-driven two-step reactions catalyzed by their respective aminoacyl-tRNA synthetases (AARSs, EC 6.1.1.class), and that the processes are common to all living things. In late 1990s, some archaeal and bacterial Asn-tRNA<sup>Asn</sup> was found not to be synthesized by Asn-tRNA<sup>Asn</sup> synthetase (EC 6.1.1.22) because of absence of asparagine synthetase, but is synthesized by the following successive reactions [23, 24]:



Likewise, in some Archaea and Bacteria, Gln-tRNA<sup>Gln</sup> is not synthesized by Gln-tRNA<sup>Gln</sup> synthetase (EC 6.1.1.18), but is synthesized by the following successive reactions [24]:



In this non-canonical aminoacylation of tRNAs, “mischarged” aminoacyl residues are prevented from incorporation to protein, a phenomenon reminiscent of the case for L-selenocysteinyl-tRNA<sup>Sec</sup> synthesis [25]. Some Archaea lack Cys-tRNA<sup>Cys</sup> synthetase (EC 6.1.1.16), and Cys-tRNA<sup>Cys</sup> is synthesized by moonlighting of Pro-tRNA<sup>Pro</sup> synthetase [26], or from O-phospho-Ser-tRNA<sup>Cys</sup> [27, 28]. Lys-tRNA<sup>Lys</sup> synthetase (EC 6.1.1.6) belongs to class II AARS in most Bacteria and a few Archaea, but belongs to class I AARS in most Archaea and a few Bacteria [29]. Distribution of these non-canonical routes to synthesize aminoacyl-tRNAs, marked on the phylogenetic tree based on 16S rRNA [30, 31] is shown in Fig. 4.

L-Selenocysteine is regarded as the 21st amino acid to be directly incorporated into protein by ribosomal translation machinery. Likewise, L-asparagine, L-glutamine, and L-cysteine are considered to be the latest members of the 20 established standard amino acids, and their respective AARSs must have been the newest members in the canonical AARS society. Then how did they widen their distribution in living things? Interspecies gene-transfer hypothesis will have no trouble to account for this rather irregular distribution.

### 2.4. Synthesis of isoprenoid precursors

Isoprenoid coenzyme Q is a key electron carrier in the electron-transferring chain to create proton gradient across biomembranes in many living things. Two isoprenoid precursors, isopentenyl diphosphate and dimethylallyl diphosphate, are synthesized by the mevalonate pathway in Archaea and Eucarya, and by MEP pathway in most (but not all) bacterial species, in which 2-C-methyl-D-erythritol 4-phosphate (MEP) is a

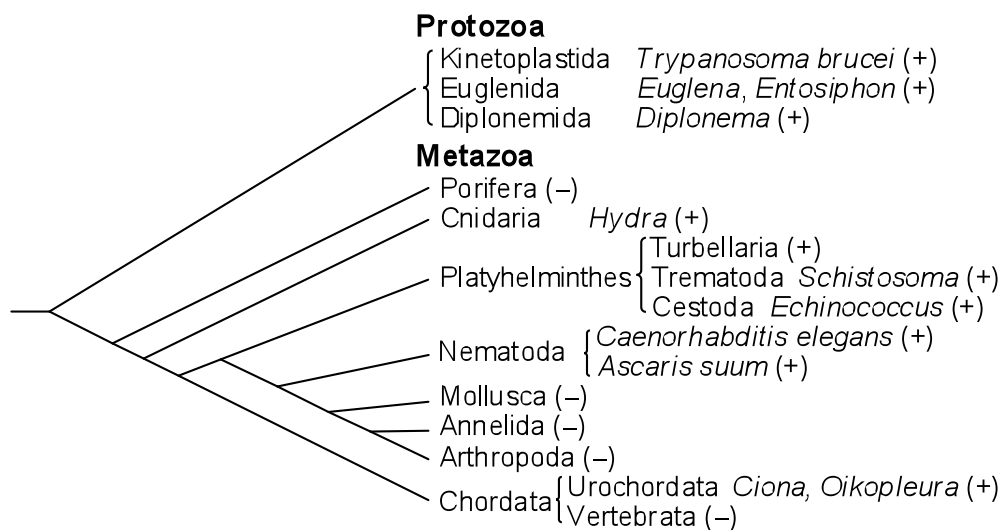


Figure 3. Distribution of SL-trans-splicing species marked on a conventional phylogenetic tree, the branch lengths being arbitrary.

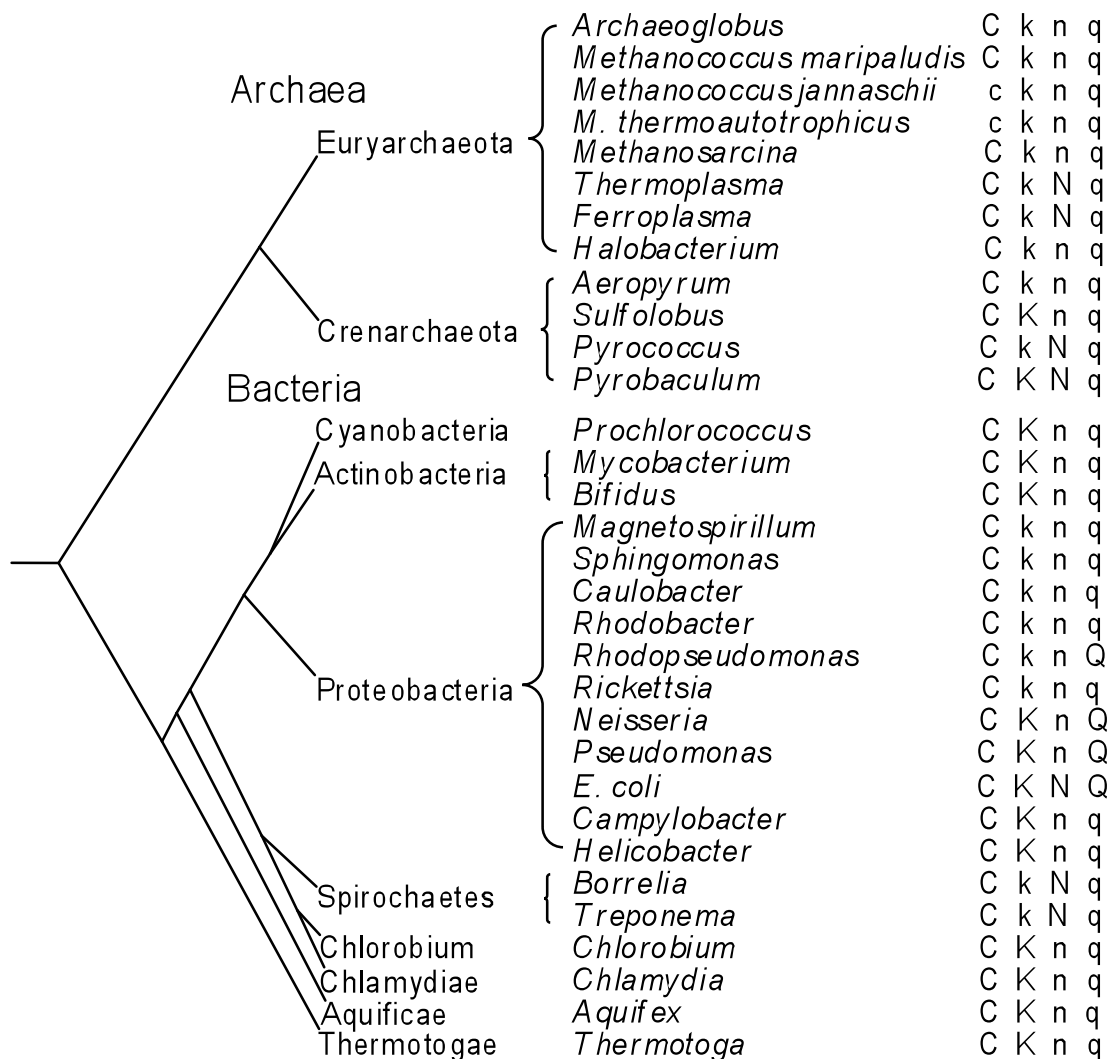


Figure 4. Distribution of canonical and non-canonical pathways to synthesize several amino-acyl-tRNAs, marked on the archaeal and bacterial branches of the phylogenetic tree based on rRNA sequences [30, 31], the branch lengths being arbitrary. Upper-case letters for canonical pathways, and lower-case letters for non-canonical pathways. C and c: Cys-tRNA<sup>Cys</sup> synthesis, K and k, Lys-tRNA<sup>Lys</sup> synthetase (class II being regarded as canonical), N and n, Asn-tRNA<sup>Asn</sup> synthesis, Q and q, Gln-tRNA<sup>Gln</sup> synthesis.

key intermediate as shown in Fig. 5 [32]. The enzyme isopentenyl-diphosphate isomerase (EC 5.3.3.2) is essential in the mevalonate pathway to convert isopentenyl-diphosphate to dimethylallyl diphosphate, but the enzyme is auxiliary in the MEP pathway, because it only fine-tunes the ratio of isopentenyl-diphosphate and dimethylallyl diphosphate (Fig. 5). There are two kinds of the isomerase, type 1 and type 2. Type 2 enzyme differs from type 1 enzyme in sequence, and in requirement of FMN and NADPH cofactors [33].

Distribution of the mevalonate and MEP pathways, as well as two types of isopentenyl-diphosphate isomerases, marked on the phylogenetic tree based on 16S rRNA [30, 31] is shown in Fig. 6.

### 2.5. Additional examples

Variety in fundamental features is not restricted to those described above. There are 3 routes to synthesize heme: two routes to produce 5-aminolevulinic acid, *i.e.*, the well-known route from glycine and succinyl-CoA, and

the route via Glu-tRNA<sup>Glu</sup> in plants and bacteria [34], and a detour to produce coproporphyrinogen III from uroporphyrinogen III, in which two methyl groups (positions 1 and 7) are supplied by *S*-adenosyl-L-methionine [35]. Some bacterial species have polyphosphate-dependent glucokinase [36], and some archaeal species have ADP-dependent kinases in the glycolytic pathway [37, 38]. Some archaeal species use coenzymes different from those used by most Bacteria and Eucarya. For example, tetrahydro-methanopterin substitutes for tetrahydrofolate as a C<sub>1</sub> carrier [39]. 5-Deaza-8-hydroxy-7,8-didemethyl-riboflavin, a redox constituent in archaeal coenzyme F<sub>420</sub>, functions as a cofactor in DNA photolyase (EC 4.1.99.3) of Bacteria *Anacystis nidulans* [40], but not in *E. coli*. Coenzyme M (2-mercaptoethanesulfonate) had been considered to be unique in archaeal methanogens, but was found to be contained in substrates for 2-hydroxypropyl-CoM lyase (EC 4.4.1.23), which is involved in alkene metabolism of some Bacteria [41, 42]. It is amazing why these examples of metabolic

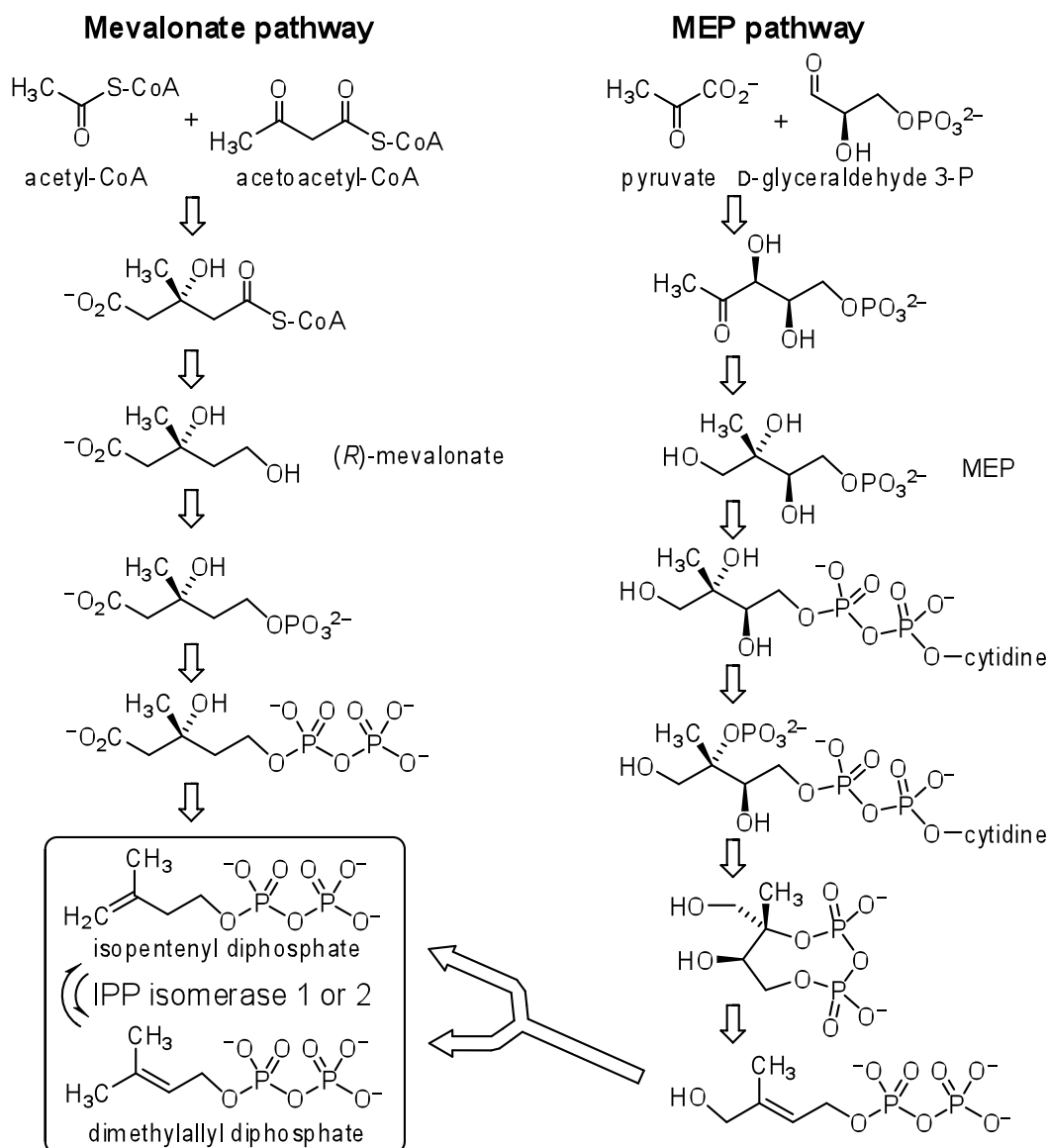


Figure 5. Pathways to synthesize isoprenoid precursors, isopentenyl diphosphate and dimethylallyl diphosphate.

diversity have been neglected when the origin of life is discussed.

### 3. Multiple Ancestors of Life Forms

The idea of the single ancestor as the origin of life may date back to 1933, far before Dayhoff's description [1], when Frederick Hopkins made a comment on the advent of life as "the most improbable and the most significant event in the history of the Universe" (cited in Ref 43). His comment sounds like that the naissance of life is a miracle, and had never happened again on our planet. Dixon and Webb [43] explained the background of his comment as (i) extraordinary biochemical similarity expressed by all living matter (which we presented examples against), (ii) difficulty in building up complex proteins from amino acid mixture assuming the conditions exist to condense amino acid mixture to catalytic polymers, (iii) improbability of forming protein mixture to form a continuous chain

such as we have seen in metabolic maps, signal transduction system, etc, and (iv) difficulty of holding the components of the system together until a cell membrane is formed.

The hypothesis that horizontal gene transfer was the major driving force of evolution, which was first proposed in 1985 [4] and reinforced later [5], explains diversity and sporadic distributions of essential molecules and processes (Actually processes are determined by enzymes and cofactor molecules, these will be referred to as molecules, hereafter). Instead of totally uncontrollable mutations, interspecies gene transfer would bring about a wide spectrum of changes in phenotype from as small as a change in a single molecule to alteration of a total metabolic pathway to generate new species, depending on the extent of gene transfer. Interspecies information exchange at very early stages of evolution, or repeated or wide range exchange would make the resulting molecules distributed widely

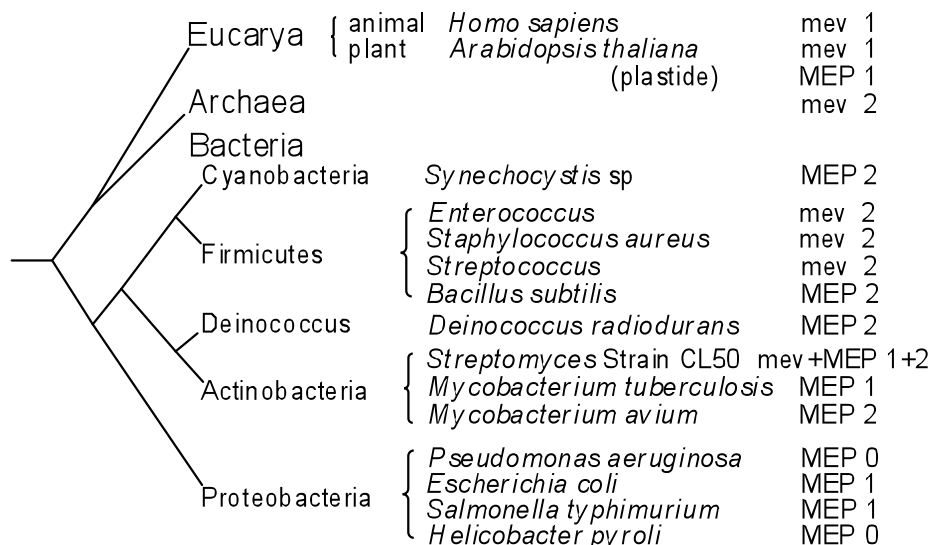


Figure 6. Distribution of the mevalonate pathway and MEP pathway, as well as of two types (type 1, type 2, or absent) of isopentenyl-diphosphate isomerases, marked on the phylogenetic tree based on 16S rRNA [30, 31], the branch lengths being arbitrary.

among living matters, whereas information exchange at later stages, or among limited species would make the resulting molecules distributed sporadically. In this regards, the synthetic routes of nucleotides must have spread at very early stages of the origin of life, whereas the synthetic routes of aminoacyl-tRNAs spread after Archaea and Bacteria had been established, but before the appearance of Eucarya. This conclusion does not conflict with the possible time schedule that aminoacyl-tRNAs must have appeared after the sources of nucleotides were made available.

The ways living things exchange genes are open vertically and horizontally in various directions, namely through sex, hybridization, symbiosis and infection. Gene exchange through sex is efficient even among human beings. Only in 33 generations, namely in 1000 years, theoretical number of ancestors exceeds  $8 \times 10^9$ , which is larger than the world population. Symbiosis and infection might have contributed gene transfer, because the gene exchange between hosts and symbionts could have happened (and will happen) among remotely related organisms. Amoeba, infected with an endosymbiont became to require it as a cytoplasmic component [44]. The Margulis' proposal [45, 46] that eucaryotic organelles originated from symbiont Bacteria, is now widely accepted. Mutations have mainly been responsible for the change in the biopolymer sequences in different species.

Given the interspecies communication as main driving force for evolution, the life is not necessarily originated from a single ancestor, but the possibilities of multiple ancestors have to be considered, provided that Dixon-Webb's problems are to be solved. Some possibilities to solve these problems (ii and iii, vide supra) will be presented.

### 3.1. Hypothesis of conformational stabilization of proteinoids by ligand binding

In the following discussion, proteinoid refers to abiotically produced random polymer of amino acids, whereas protein refers to polypeptide produced by ribosomal translation. It has been argued that if conditions were met to randomly polymerize abiotically produced amino acids, any proteinoids having specific function would have been produced in vanishingly small amount, only to be destroyed by non-specific hydrolytic activities of the co-produced proteinoids, because the condensation products must have been enormously heterogeneous, and most proteinoids had rather hydrolytic activities [47-50]. It is well known, however, that a peptide assumes stable conformation to resist proteolytic degradation if it binds a specific small molecule as a cofactor or a ligand. Two cases of conformational stabilization will be considered here.

Peptides with Cys-X1-X2-Cys sequences: Heme is one of small molecules produced abiotically at the time of chemical evolution [51], and thought to be incorporated in polypeptides either non-covalently or covalently. In covalent hemoprotein such as cytochrome *c*, two mercapto groups of Cys-X1-X2-Cys (CXXC) sequence bind heme by thioether bonds. CXXC sequences also function as binding sites to iron-sulfur cluster in ferredoxins, to Fe(II/III) ion in rubredoxins, and become a redox-active site to make disulfide in thioredoxins. In these redox proteins, the distances between the two sulfur atoms are different in different types of redox proteins as shown in Fig. 7. It was assumed that the distance of the two sulfur atoms of a CXXC sequence in relatively stable local conformation would have selected the cofactor or ligand to bind during the era of chemical evolution. To substantiate this hypothesis, semi-empirical molecular orbital program, MOPAC, was run for CXXC tetrapeptides to see whether the S-S distances in energetically optimized conformers fall in the distances

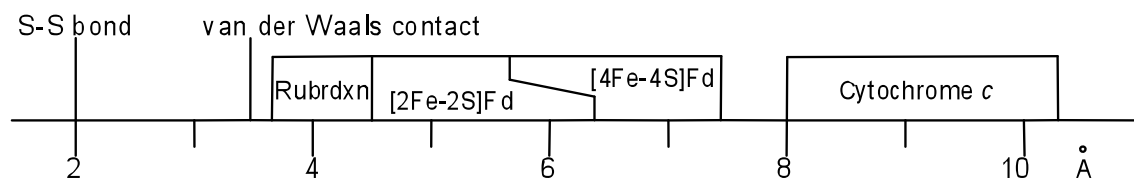


Figure 7. Distances between the two sulfur atoms in Cys-X1-X2-Cys sequences in redox proteins. The S-S distances in the redox proteins, updated from Ref 52, were calculated from the atomic coordinates deposited in the Protein Data Bank.

shown in Fig. 7 [52]. For modern proteins, folding begins with hydrophobic collapse to make a core followed by formation of secondary and tertiary structure to achieve native structure [53]. Prebiotic proteinoids with CXXC sequences would have bound to their respective cofactors or ligands to form cores followed by formation of secondary and tertiary structure, so as to survive proteolytic attacks by co-produced proteinoids to eventually become ancestors of cytochromes *c*, ferredoxins, rubredoxins, etc.

Phosphonates vs phosphate esters: The most important biomolecules are phosphate esters and proteins having affinity thereto. Organic phosphate esters are stable in a time scale of laboratory experiments, but are unstable to be completely hydrolyzed in a much longer time scale during the chemical evolution, and would not have been able to become cores for polypeptide folding. However, organic phosphonates which have stable C-P bonds could have become cores for polypeptide folding, to protect that proteinoids from hydrolytic attack by co-produced proteinoids. Proteinoids without phosphonate ligands would have been destroyed to supply amino acid materials for further synthesis of phosphonate-binding proteinoids. This would have resulted in accumulation of phosphonate-liganding proteinoids. The proteinoids with phosphonate ligands would have had affinity to structurally similar phosphate esters, and would have potential to become enzymes to act on or to produce that phosphate esters. Several proteinoids thus produced would have formed at least parts of a metabolic pathway which involves that phosphate esters. Abiotic production of organic phosphinates (which can be oxidized to phosphonates without C-P bond breakage) was demonstrated by recoil process of ( $n, \gamma$ )reaction of  $^{31}\text{P}$  to produce radioisotope  $^{32}\text{P}$  [54]. Similar recoil reactions were confirmed with ( $n, \gamma$ )reaction of  $^{30}\text{Si}$  in organic soup to produce  $^{31}\text{Si}$  which emits  $\beta$ -ray (half life: 156 min) to become stable  $^{31}\text{P}$  atoms in organic compounds [55]. The amount of organic phosphorus including phosphates, phosphonates, and the reduced derivatives thereof must have been  $3 \times 10^7$  mol during chemical evolution, calculated by the following equation:  $N^* = N\sigma\phi t$ , where  $N^*$  is the amount of  $^{31}\text{Si}$  produced,  $N$ , the amount of  $^{30}\text{Si}$  in the 1 m-depth surface of the earth ( $3.3 \times 10^{17}$  mol),  $\sigma$ , the reaction cross section for thermal neutrons ( $4 \times 10^{-29}$  m<sup>2</sup>),  $\phi$ , the neutron flux 35 billion years ago ( $1 \times 10^4$  m<sup>-2</sup> s<sup>-1</sup>, minimum estimate), and  $t$ , the duration of chemical evolution (assumed to be 0.3

billion years, *i.e.*,  $9.5 \times 10^{15}$  s), and 2.5% of  $^{31}\text{Si}$  produced ( $N^*$ ) was recovered in organic  $^{31}\text{P}$ -compounds after  $\beta$ -decay, followed by recoiling in the organic soup [56]. One might wonder why organic phosphates, instead of phosphonates, play pivotal roles in current life. This is because phosphates are potentially reactive but are kinetically stable, and are able to exist as monoesters, diesters, diphosphates, triphosphates, etc, and that they can be transformed to other molecules without modification of organic moiety. On the other hand, organic phosphonates cannot substitute organic phosphate esters because of their intrinsic stability, *i.e.*, phosphonates played a role as scaffolds for the phosphate-dependent life form to appear. Are the modern phosphonates the metabolic fossil, or new comers after the phosphate-dependent life form was established? Sporadic distribution of ability to synthesize organic phosphonates [57, 58] shown in Fig. 8, as well as the fact that the enzyme to make a C-P bond (phosphoenolpyruvate mutase, EC 5.4.2.9) must have appeared after the establishment of glycolytic pathway to supply phosphoenolpyruvate, would suggest that C-P bond producing activity may have been invented after the phosphate-dependent life form had been established, and widen distribution by gene transfer.

Finally, two hypotheses which do not conflict with the multiple-ancestor-hypothesis for the origin of life, but not necessary the most prevailing, will be briefly presented. One is GADV-protein world hypothesis, or simply GADV hypothesis developed by Ikehara et al [59, 60], and the other, the surface metabolist theory of Wächtershäuser [12].

### 3.2. GADV hypothesis

This hypothesis denies the RNA-world, and insists that the life originated from GADV-proteins, proteinoids with random sequences of only 4 amino acids, glycine (G), alanine (A), aspartate (D), and valine (V). This hypothesis is based in part on the catalytic activities observed for heat-polymerized GADV mixture [59]. Rather simple constituents would minimize the heterogeneity of the polymerized products, and increase the probability of the appearance of “meaningful” proteinoids, which would have paved a way to appear multiple ancestors of life. Although this hypothesis does not suggest the mechanism for growing number of standard amino acids from 4 to 20, if combined with Akabori’s polyglycine hypothesis [61], glycyI residues in the GADV-proteinoids have potential to be modified to seryl residues, which in turn

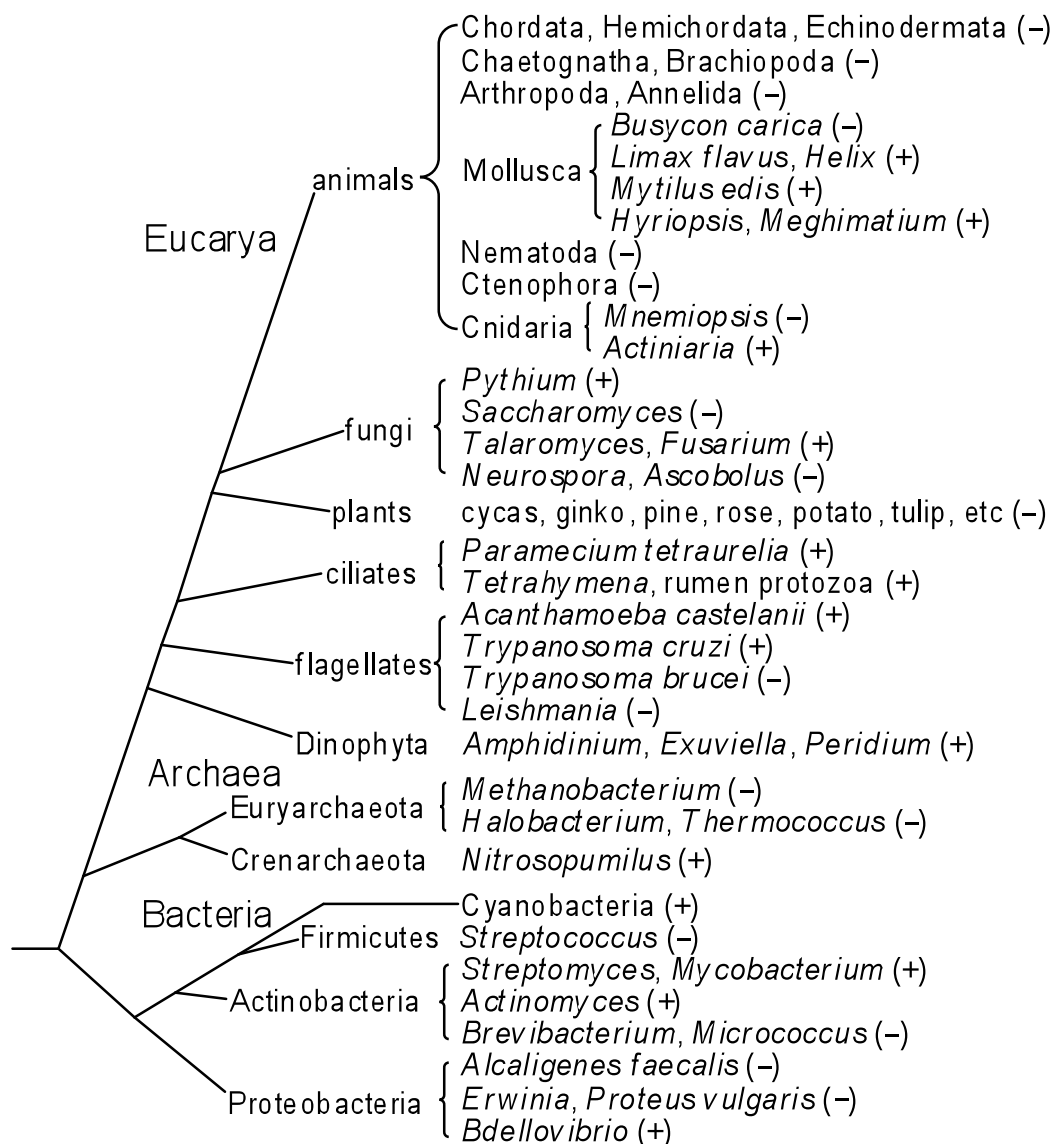


Figure 8. Distribution of the ability to produce C-P bonds, marked on a conventional phylogenetic tree, the branch lengths being arbitrary. C-P compounds include ciliatine (2-aminoethylphosphonate), *N*-methyl derivatives thereof, 3-phosphonoalanine, etc in free, or as components in phosphonolipids.

are eligible to become tyrosyl, histidyl, tryptophanyl residues, etc, by condensation with phenol, imidazole, indole, etc, to result in a complete set of contemporary standard amino acids.

### 3.3. Surface metabolist theory

In this hypothesis [12], the surface metabolists are described as “These organisms are acellular and lack a mechanism for division, yet they can grow. They possess neither enzymes nor a mechanism for translation, but they do have autocatalytic metabolism. They do not have nucleic acids or any other template, yet they have inheritance and selections. Although they can barely be called living, they have a capacity for evolution.” In most hypotheses of the origin of life, polymerization of amino acids and other building blocks in primordial soup was postulated to be driven by local concentration and heating of the soup, or else,

the concentration of the building block molecules must have been to low, and polymerization would have been impossible in a thermodynamic viewpoint. The surface metabolist theory aims at evading this thermodynamic constraint. Linking two molecules to a bigger molecule in surface reaction, loss of the degrees of freedom ( $\Delta S$ ) must be much less compared to three-dimensional solution reaction to make the condensation reaction more feasible. This theory dictates a course of evolution from the surface metabolists to life through cell formation, evolution of genetic machinery, etc. Although this hypothesis was severely criticized by de Duve and Stanley Miller [62], it is worthy to give a glance at this aggressive, revolutionary, and fascinating theory, which does not conflict with the naissance of multiple ancestors.

We presented three possible hypotheses for the multiple ancestors of life forms, the hypothesis of



conformational stabilization of proteinoids, the GADV hypothesis, and the surface metabolist theory. Some of these hypotheses are mutually incompatible, but if we admit appearance of multiple ancestors followed by widespread interspecies communication, multiple ways for the naissance of life may be acceptable.

#### 4. Conclusion

Evidence is presented to suggest that life originated from multiple ancestors, and the major driving force of evolution has been (and will be) interspecies communication. Mutations have mainly been responsible for the changes in sequences of biopolymers in different species. Some hypotheses are presented which are consistent with the multiple ancestors of life forms.

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#### References

- Dayhoff, M. O. Inferences from protein sequence studies, pp. 1-6, in Dayhoff, M. O., Ed., Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Silver Spring, Maryland, 1969.
- Cain, M. L., Damman, H., Lue, R. A. and Yoon, C. K. Discover Biology, 2nd edn., p. 30, Sinauer Associates Inc., Sunderland, MA and W. W. Norton & Company, New York, NY, 2002.
- Voet, D., Voet, J. G. and Pratt, C. W. Fundamentals of Biochemistry 3rd edn., pp. 116-117, John Wiley & Sons, Inc, New York, NY, 2008.
- Tamiya, N. and Yagi, T. Non-divergence theory of evolution: Sequence comparison of some proteins from snakes and bacteria, J. Biochem. 98, 289-303 (1985).
- Tamiya, N. and Yagi, T. Evolution without divergence, IUBMB Life 58, 309-311 (2006).
- Sonea, S. and Panisset, M. A New Bacteriology, p. 22, Jones and Bartlett, Boston, Massachusetts, 1983.
- Bush, R. M. Influenza as a model system for studying the cross-species transfer and evolution of the SARS coronavirus, Philos. Trans. R. Soc. Lond. B359, 1067-1073 (2004).
- Bubanic, I., Najman, S. and Andjelkovic, Z. Origin and evolution of viruses: escaped DNA/RNA sequences as evolutionary accelerators and natural biological weapons, Med. Hypotheses 65, 868-872 (2005).
- Kushwaha, S. C., Kates, M., Sprott, G. D. and Smith, I. C. P. Novel polar lipids from methanogen *Methanospirillum hungatei*: GPI, Biochim. Biophys. Acta 664, 156-173 (1981).
- Nishihara, M. and Koga, Y. Two new phospholipids, hydroxyarchaetidylglycerol and hydroxyarchaetidyl ethanolamine, from Archaea, *Methanosarcina barkeri*, Biochim. Biophys. Acta 1254, 155-160 (1995).
- Koga, Y., Kyuragi, T., Nishihara, M. and Sone, N. Did archaeal and bacterial cells arise independently from noncellular precursors? A hypothesis stating that the advent of membrane phospholipid with enantiomeric glycerophosphate backbones caused the separation of the two lines of descent, J. Mol. Evol. 46, 54-63, erratum in 47, 631 (1998).
- Wächtershäuser, G. Before enzymes and templates: Theory of surface metabolism, Microbiol. Rev. 52, 452-484 (1988).
- Bult, C. J., White, O., Olsen, G. J., Zhou, L., Fleischmann, R. D., Sutton, G. G., Blake, J. A., FitzGerald, L. M., Clayton, R. A., Gocayne, J. D., Kerlavage, A. R., Dougherty, B. A., Tomb, J. F., Adams, M. D., Reich, C. I., Overbeek, R., Kirkness, E. F., Weinstock, K. G., Merrick, J. M., Glodek, A., Scott, J. L., Geoghagen, N. S. and Venter, J. C. Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*, Science 273, 1058-1073 (1996).
- Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., Dyer, T. A., Wolfe, R. S., Balch, W. E., Tanner, R. S., Magrum, L. J., Zablen, L. B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B. J., Stahl, D. A., Luehrs, K. R., Chen, K. N. and Woese, C. R. The phylogeny of prokaryotes, Science 209, 457-463 (1980).
- Mayer, M. G. and Floeter-Winter, L. M. Pre-mRNA trans-splicing: from kinetoplasts to mammals, an easy language for life diversity, Memórias do Instituto Oswaldo Cruz 100, 501-513 (2005).
- Blumenthal, T., Evans, D., Link, C. D., Guffanti, A., Lawson, D., Thierry-Mieg, J., Thierry-Mieg, D., Chiu, W. L., Duke, K., Kiraly, M. and Kim, S. K. A global analysis of *Caenorhabditis elegans* operons, Nature 417, 851-854 (2002).
- Dom, R., Reuter, G. and Loewendorf, A. Transgene analysis proves mRNA trans-splicing at the complex *mod(mdg4)* locus in *Drosophila*, Proc. Natl. Acad. Sci. 98, 9724-9729 (2001).
- Horiuchi, T., Giniger, E. and Aigaki, T. Alternative trans-splicing of constant and variable exons of a *Drosophila* axon guidance gene, *lola*, Genes Dev. 17, 2496-2501 (2003).
- Labrador, M., Mongelard, F., Plata-Rengifo, P., Baxter, E. M., Corces, V. G. and Gerasimova, T. I. Protein encoding by both DNA strands, Nature 409, 1000 (2001).
- Caudevilla, C., Serra, D., Miliar, A., Codony, C., Asins, G., Bach, M. and Hegardt, F. G. Natural trans-splicing in carnitine octanoyltransferase pre-mRNAs in rat liver, Proc. Natl. Acad. Sci. 95, 12185-12190 (1998).
- Frantz, S. A., Thiara, A. S., Lodwick, D., Ng, L. L., Eperon, I. C. and Samani, N. J. Exon repetition in mRNA, Proc. Natl. Acad. Sci. 96, 5400-5405 (1999).
- Li, B. L., Li, X. L., Duan, Z. J., Lee, O., Lin, S., Ma, Z. M., Chang, C. C., Yang, X. Y., Park, J. P., Mohandas, T. K., Noll, W., Chan, L. and Chang, T. Y. Human acyl-CoA:cholesterol acyltransferase-1 (ACAT-1) gene organization and evidence that the 4.3-kilobase ACAT-1 mRNA is produced from two different chromosomes, J. Biol. Chem. 274, 11060-11071 (1999).
- Becker, H. D. and Kern, D. *Thermus thermophilus*: a link in evolution of the tRNA-dependent amino acid amidation pathways, Proc. Natl. Acad. Sci. 95, 12832-12837 (1998).
- Ibba, M. and Söll, D. The renaissance of aminoacyl-tRNA synthesis, EMBO Rep. 2, 382-387 (2001).
- Stadtman, T. C. Selenocysteine, Annu. Rev. Biochem. 65, 83-100 (1996).
- Stathopoulos, C., Jacquin-Becker, C., Becker, H. D., Li, T., Ambrogelly, A., Longman, R. and Söll, D. *Methanococcus jannaschii* prolyl-cysteinylyl-tRNA synthetase possesses overlapping amino acid binding sites, Biochemistry 40, 46-52 (2001).
- Hauenstein, S. I. and Perona, J. J. Redundant synthesis of cysteinyl-tRNA<sup>Cys</sup> in *Methanosarcina mazei*, J. Biol. Chem. 283, 22007-22017 (2008).
- Zhang, C. M., Liu, C., Slater, S. and Hou, Y. M. Aminoacylation of tRNA with phosphoserine for synthesis of cysteinyl-tRNA<sup>Cys</sup>, Nat. Struct. Mol. Biol. 15, 507-514 (2008).
- Ibba, M., Morgan, S., Cumow, A. W., Pridmore, D. R., Voithknecht, U. C., Gardner, W., Lin, W., Woese, C. R. and Söll, D. A euryarchaeal lysyl-tRNA synthetase: resemblance to class I synthetases, Science 278, 1119-1122 (1997).
- Wheeler, M. L., Kandler, O. and Woese, C. R. On the nature of global classification, Proc. Natl. Acad. Sci. 89, 2930-2934 (1992).
- Horiike, T., Miyata, D., Hamada, K., Saruhashi, S., Shinozawa, T., Kumar, S., Chakraborty, R., Komiyama, T. and Tateno, Y. Phylogenetic construction of 17 bacterial phyla by new method and carefully selected orthologs, Gene 429, 59-64 (2009).
- Kuzuyama, T. and Seto, H. Diversity of the biosynthesis of the isoprene units, Nat. Prod. Rep. 20, 171-183 (2003).
- Kaneda, K., Kuzuyama, T., Takagi, M., Hayakawa, Y. and Seto, H. An unusual isopentenyl diphosphate isomerase found in the mevalonate pathway gene cluster from *Streptomyces* sp. strain CL190, Proc. Natl. Acad. Sci. 98, 932-937 (2001).
- Wang, L. Y., Brown, L., Elliott, M. and Elliott, T. Regulation of heme biosynthesis in *Salmonella typhimurium*: activity of glutamyl-tRNA reductase (HemA) is greatly elevated during heme limitation by a mechanism which increases abundance of the protein, J. Bacteriol. 179, 2907-2914 (1997).
- Ishida, T., Yu, L., Akutsu, H., Ozawa, K., Kawanishi, S., Seto, A., Inubushi, T. and Sano, S. A primitive pathway of porphyrin biosynthesis and enzymology in *Desulfovibrio vulgaris*, Proc. Natl. Acad. Sci. 95, 4853-4858 (1998).
- Szymona, M. and Ostrowski, W. Inorganic polyphosphate glucokinase of *Mycobacterium phlei*, Biochim. Biophys. Acta 85,

- 283-295 (1964).
37. Kengen, S. W., Tuininga, J. E., Verhees, C. H., van der Oost, J., Stams, A. J. and de Vos, W. M. ADP-dependent glucokinase and phosphofructokinase from *Pyrococcus furiosus*, *Methods Enzymol.* 331, 41-53 (2001).
  38. Tuininga, J. E., Verhees, C. H., van der Oost, J., Kengen, S. W., Stams, A. J. and de Vos, W. M. Molecular and biochemical characterization of the ADP-dependent phosphofructokinase from the hyperthermophilic archaeon *Pyrococcus furiosus*, *J. Biol. Chem.* 274, 21023-21028 (1999).
  39. Escalante-Semerena, J. C., Rinehart, K. L., Jr. and Wolfe, R. S. Tetrahydromethanopterin, a carbon carrier in methanogenesis, *J. Biol. Chem.* 259, 9447-9455 (1984).
  40. Eker, A. P., Kooiman, P., Hessels, J. K. and Yasui, A. DNA photoreactivating enzyme from the cyanobacterium *Anacystis nidulans*, *J. Biol. Chem.* 265, 8009-8015 (1990).
  41. Allen, J. R., Clark, D. D., Krum, J. G. and Ensign, S. A. A role for coenzyme M (2-mercaptoethanesulfonic acid) in a bacterial pathway of aliphatic epoxide carboxylation, *Proc. Natl. Acad. Sci.* 96, 8432-8437 (1999).
  42. Coleman, N. V. and Spain, J. C. Epoxyalkane: coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of *Mycobacterium* strain JS60, *J. Bacteriol.* 185, 5536-5545 (2003).
  43. Dixon, M. and Webb, E. C. *Enzymes*, pp. 666-670, Longmans, Green and Co, London, 1958.
  44. Jeon, K. W. and Jeon, M. S. Endosymbiosis in amoebae: recently established endosymbionts have become required cytoplasmic components, *J. Cell Physiol.* 89, 337-344 (1976).
  45. Margulis, L. *Symbiosis in Cell Evolution*, W. H. Freeman and Co., San Francisco, CA, 1981.
  46. Margulis, L. Genome acquisition in horizontal gene transfer: symbiogenesis and macromolecular sequence analysis, *Methods Mol. Biol.* 532, 181-191 (2009).
  47. Noguchi, J., Tokura, S., Komai, T., Kokazi, K. and Azuma, T. Studies on the catalytic action of poly- $\alpha$ -amino acids. V. Hydrolyses of *p*-nitrophenyl acetate by poly-L-glutamic acid, poly-L-aspartic acid and the copolymer of L-glutamic acid and L-aspartic acid, *J. Biochem.* 69, 1033-1040 (1971).
  48. Usdin, V. R., Mitz, M. A. and Killos, P. J. Inhibition and reactivation of the catalytic activity of a thermal  $\alpha$ -amino acid copolymer, *Arch. Biochem. Biophys.* 122, 258-261 (1967).
  49. Rohlfsing, D. L. and Fox, S. W. The catalytic activity of thermal polyanhydro- $\alpha$ -amino acids for the hydrolysis of *p*-nitrophenyl acetate: Catalysis by thermal polyamino acids, *Arch. Biochem. Biophys.* 118, 122-126 (1967).
  50. Oshima, T. The catalytic hydrolysis of phosphate ester bonds by thermal polymers of amino acids, *Arch. Biochem. Biophys.* 126, 478-485 (1968).
  51. Hodgson, G. W. and Ponnamperna, C. Prebiotic porphyrin genesis: porphyrins from electric discharge in methane, ammonia, and water vapor, *Proc. Natl. Acad. Sci.* 59, 22-28 (1968).
  52. Shimura, M., Hirota, F. and Yagi, T. Functional sites of cytochrome *c* and other electron carrier proteins: Semi-empirical molecular orbital program PM3 applied to the conformational analysis of Cys-X1-X2-Cys peptides, *Biochimie* 76, 614-621 (1994).
  53. Miranker, A. D. and Dobson, C. M. Collapse and cooperativity in protein folding, *Curr. Opin. Struct. Biol.* 6, 31-42 (1996).
  54. Yagi, T., El-Kinawy, S. A. and Benson, A. A. Phosphorylations and phosphonations of glycerol by recoil atoms, *J. Am. Chem. Soc.* 85, 3462-3465 (1963).
  55. Akaboshi, M., Kawai, K. and Waki, A. Abiological synthesis of adenosine 2'-, 3'- and 5'-monophosphates using recoiled  $^{31}\text{P}$  atoms obtained from the  $\beta$ -decay of  $^{31}\text{Si}$ , *Biochim. Biophys. Acta* 238, 5-7 (1971).
  56. Yagi, T. The role of recoil processes in the non-biological formation of organic phosphorus compounds on the primitive earth. *Tampakushitsu Kakusan Koso (Proteins, Nucleic acids and Enzymes, in Japanese)*, Special Issue for the Origin of Life and Evolution, pp. 109-115 (1972).
  57. Hori, T. and Horiguchi, M, Eds, *Biochemistry of Natural C-P compounds: Aminophosphonates and Phosphonolipids (in Japanese)*, pp. 8-10, Gakkai-Shuppan Center, Tokyo, 1970.
  58. Sarkar, M., Hamilton, C. J. and Fairlamb, A. H. Properties of phosphoenolpyruvate mutase, the first enzyme in the aminoethylphosphonate biosynthetic pathway in *Trypanosoma cruzi*, *J. Biol. Chem.* 278, 22703-22708 (2003).
  59. Oba, T., Fukushima, J., Maruyama, M., Iwamoto, R. and Ikehara, K. Catalytic activities of [GADV]-peptides. Formation and establishment of [GADV]-protein world for the emergence of life, *Orig. Life Evol. Biosph.* 35, 447-460 (2005).
  60. Ikehara, K. and Nihara, Y. Origin and evolutionary process of the genetic code, *Curr. Med. Chem.* 14, 3221-3231 (2007).
  61. Akabori, S., Okawa, K. and Sato, M. Introduction of side chains into polyglycine dispersed on solid surface. I, *Bull. Chem. Soc. Jpn.* 29, 608-611 (1956).
  62. de Duve, C. and Miller, S. L. Two-dimensional life? *Proc. Natl. Acad. Sci.* 88, 10014-10017 (1991).