

Before Enzymes and Templates: Theory of Surface Metabolism

GÜNTER WÄCHTERS HÄUSER

Tal 29, D-8000 Munich 2, Federal Republic of Germany

CENTRAL THESIS: AUTOTROPHIC SURFACE METABOLISTS	452
CHEMICAL PROPERTIES OF A SURFACE METABOLISM.....	453
Surface Bonding	453
Thermodynamics of Surface Metabolism	454
Kinetics of Surface Metabolism	455
Surface-Metabolic Reaction Types	456
MECHANISMS OF EVOLUTION.....	456
First Organism/Environment Dichotomy	456
Evolution by Innovation and Selection	457
Autocatalytic Coenzymes	457
Environment Changes the Surface Organism	459
The Surface Organism Changes Its Environment	459
Changeover to Cellular Mechanisms of Evolution.....	459
A Methodological Rule	460
CELLULAR REVOLUTION	460
Surface-Bonded Isoprenoid Lipids and Membranes	460
Semicellular Organisms	462
Surface Detachment of Coenzymes.....	462
Evolution of Cellular Organisms.....	463
Woese's Three Kingdoms.....	464
FLOW OF ENERGY AND NUTRIENTS	465
First Energy Source for Life.....	465
Minor Nutrients.....	466
Ferredoxins and CoA	467
Cellular Flows of Energy and Nutrients	468
EVOLUTION OF THE GENETIC MACHINERY	469
Tribonucleic Acid (TNA) with Catalytic Imidazole Bases	469
All-Purine TNA	471
Surface-Bonded Ribonucleotides	472
Purine-Ribonucleotide-Derived Coenzymes	472
Earliest Mode of Translation	473
Origin of Transcription and RNA	473
RNA Folding and Ribosomal Translation	474
Advent of Replication and DNA	475
Amino acid Pathways and the Code	476
Folding of Proteins	479
AFTERTHOUGHT ABOUT THE ORIGIN OF LIFE	480
ACKNOWLEDGMENTS.....	480
LITERATURE CITED.....	480

CENTRAL THESIS: AUTOTROPHIC SURFACE METABOLISTS

Woese's three kingdoms (161-166) constitute the deepest branching of the known biosphere. His suggestion to search for a common ancestor has led to the present hypothesis.

It is proposed here that, at an early stage of evolution, there are precursor organisms drastically different from anything we know. 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 an autocatalytic metabolism. They do not have nucleic acids or any other template, yet they have inheritance and selection. Although they can barely be called living, they have a capacity for evolution.

Central to the proposed theory is the idea that life at this early stage is autotrophic (cf. reference 53) and consists of an

autocatalytic metabolism confined to an essentially two-dimensional monomolecular organic layer. These surface organisms (surface metabolists) are anionically bonded to positively charged surfaces (e.g., pyrite) at the interface of hot water. The adherence to the positively charged mineral surface is not the result of adsorption (as suggested by Bernal [6] in his clay theory) but rather of in situ autotrophic growth of anionic constituents acquiring their surface bonding in statu nascendi. Instead of adsorption, the organism is faced with desorption, that is, a selective detachment of its constituents. This means a negative selection favoring higher anionic bonding strength. Large polyanionic constituents with ever stronger surface bonding are automatically selected: first, polyanionic coenzymes, and eventually nucleic acids and polypeptides. The primitive surface metabolists grow by spreading onto vacant surfaces; they reproduce by

reproducing the autocatalytic coenzymes, and they evolve by the environmentally induced ignition of new autocatalytic cycles. The surface metabolists evolve toward higher complexity since the thermodynamic equilibrium in a surface metabolism favors synthesis, not degradation (as would occur in solution). High-energy phosphoanhydride groups are not required for the formation of covalent bonds. Phosphate groups (whose source is taken to be the mineral substrate) have the sole function of surface bonding. The energy for carbon fixation is provided by the redox process of converting ferrous ions and hydrogen sulfide into pyrite, which is not only a waste product but also provides the all-important binding surface for the organic constituents.

The theory makes detailed suggestions as to how the early, essentially two-dimensional surface metabolists evolve by two distinct later stages. The second stage consists of semicellular organisms still supported by a mineral surface, but with an autotrophically grown lipid membrane and an internal broth of detached constituents. In this stage, a membrane metabolism and a cytosol metabolism appear, first as a supplement to and later as a substitute for the aboriginal surface metabolism. Membrane-bound electron transport chains allow the tapping of other redox energy sources and ultimately of light energy. The cytosol metabolism allows the salvaging of detached constituents and of their chemical energy by catabolic processes and the development of modular modes of synthesis that rely upon energy coupling. Eventually, heterotrophy appears as a by-product of the catabolic salvage pathways. The genetic machinery of the cell develops from surface-metabolic precursors with catalytic imidazole residues glycosidically bonded to a polyhemiacetal backbone of (surface-adhering) phosphotrioses. It produces self-folding enzymes which compete with the mineral surface for bonding the metabolic constituents. In this stage evolution becomes double tracked: an evolution of metabolic pathways and one of the bonding surfaces for their constituents. In the third stage the pyrite support is abandoned and true cellular organisms arise which become free to conquer three-dimensional space.

My theory contrasts sharply with the ingenious prebiotic broth theory of Darwin, Oparin, and Haldane (23, 52, 103) for I deny the preexistence of any arsenal of organic building blocks for life (such as amino acids). Rather, I assume that the concentration of dissolved organic constituents in the water phase is negligible (effectively zero). Hence, any process by which a constituent loses its surface bonding is an irreversible loss: there is no readsorption of organic constituents. As soon as a constituent becomes detached, it vanishes into the vast expanses of the water. It never returns.

The prebiotic broth theory has received devastating criticism for being logically paradoxical (11, 135), incompatible with thermodynamics (11, 144, 160), chemically and geochemically implausible (134, 136, 144), discontinuous with biology and biochemistry (160), and experimentally refuted (135, 160). The reason for the tenacity with which it is retained as accepted dogma has been forcefully and clearly stated by Scherer (126): "If this rejection is substantiated, there will remain no scientifically valid model of the self-organization of the first living cells on earth."

I propose this new theory in the hope that it will be a viable alternative to the broth theory. It is sufficiently detailed for ready testing. Also, it is worthy of testing because it satisfies one of Popper's important methodological rules (115): it has far greater explanatory power than its precursors, which have hardly any. In fact, it is not so much a theory about the origin of life as an evolutionary theory of

biochemistry, aiming to serve the student of extant biological chemistry as a gauge to explain what we already know and as a guide to explore what is still unknown.

In a sense, the broth theory has made things too easy for itself by its assumption of ready-made building blocks. Indeed, it is precisely this assumption that has prevented it from adequately addressing the main problems that have given rise to the hypothetical solutions which constitute my theory and notably to the models for the evolution of cellularization, the flow of energy, and the genetic machinery (all addressed in the following sections).

The design of my theory is inspired by the great tradition of research into the biochemical pathways intensely pursued by countless biochemists through more than a century of experimental efforts. That the stark peculiarity of these pathways has resisted evolutionary explanation for so long is due, I believe, to our entanglement in three of the most vicious snares of science: inductivism, reductionism, and determinism. It is for the efforts of Karl R. Popper that we can now try to avoid these snares (114–116, 118–121).

CHEMICAL PROPERTIES OF A SURFACE METABOLISM

Surface Bonding

Before considering any details of the nature of the surface organism and its changeover into cellular forms, it is appropriate to discuss some basic chemical properties of a metabolism in a surface reaction system. I begin with an analysis of the necessary conditions for a strong surface bonding and show that they lead to a self-selection of the large polyanionic biomolecules, well known from extant biochemical pathways.

For the proposed surface metabolism, a strong surface bonding is all important. Yet the bonded constituents should still be capable of some slow lateral two-dimensional migration, since otherwise the number of nearest-neighbor interactions is severely limited. Of all noncovalent bonds which allow such movement, ionic bonding is the strongest, and it decreases with only the second power of the distance. The seemingly large number of possibilities for ionic surface bonding can be reduced by the application of two rules: (i) the postulated ionic bonding of the surface metabolism should satisfy the law of Paneth and Fajans (35, 59), by which the strength of ionic surface bonding increases with the insolubility of the corresponding salt; and (ii) the postulated ionic bonding should be suitable for bonding the ionic constituents of extant biochemistry.

The usual cationic constituents in biochemistry are organic nitrogen bases. These, however, are not known to form insoluble salts. Therefore, we can rule out ionic bonding to negatively charged mineral surfaces. Thus, we can eliminate negative surface charges and especially clays (see references 6 and 108). The mineral surface must have positive charges. These must be due to polyvalent metal ions such as Mg^{2+} , Ca^{2+} , Fe^{2+} , Mn^{2+} , and Zn^{2+} , which form a larger variety of insoluble salts than monovalent metal ions. Transition metal sulfides and notably pyrite (FeS_2) are examples of insoluble minerals with positive surface charges (131).

Accordingly, the organic constituents must be anionic. Acetate ions do not form insoluble salts. This fact may be generalized to the rule that an organic constituent with one single negative charge is not capable of surface bonding that is sufficiently strong, at least as long as the constituent is

hydrophilic. For strong surface bonding, a hydrophilic constituent should have at least one group with at least two negative charges (e.g., $-\text{OPO}_3^{2-}$, $-\text{OP}_2\text{O}_6^{3-}$, or $-\text{OP}_3\text{O}_9^{4-}$) or at least two groups with at least one negative charge (e.g., $-\text{COO}^-$, $-\text{OPO}_3\text{H}^-$, or $-\text{OPO}_3^--\text{O}$). Indeed, the ancient central pathways contain a plethora of such polyanionic surface bonders, while the late-coming secondary and aerobic pathways contain none. This suggests an important classification rule: all polyanionic constituents in the extant pathways are of ancient surface-metabolic origin and all nonpolyanionic constituents are of later post-surface-metabolic origin. It further suggests that the surface-bonding function is the oldest function of the phosphate groups.

All anionically bonded constituents have a certain propensity for depletion by detachment from the surface, through protonation of anionic groups (favored by low pH), hydrolysis of phosphate groups (favored by low and high pH), or decarboxylation (favored by high pH). At a nearly neutral to moderately acidic pH, the propensity for depletion is lowest. Hence, this pH range is an essential condition for the earliest surface metabolism and, indeed, is strictly conserved in all extant forms of life. However, even under neutral conditions, the various surface-bonding constituents have a small but finite differential rate of depletion by detachment. Obviously, any surface metabolism can persist or spread only if the rate of production equals or exceeds the rate of depletion. Any persisting surface metabolism shows continual turnover. This has an important consequence. It constitutes a self-sorting process, which may be considered the earliest mode of selection: a selection of metabolic constituents by dissolution. Weakly bonding constituents give way to stronger bonding ones. In a surface metabolism, large polyanionic structures such as polyanionic peptides and coenzymes, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) have a selective advantage. Such large polyanionic structures, while bonding strongly, can migrate laterally by local detachment without losing their overall cooperative surface bonding.

Thermodynamics of Surface Metabolism

In contrast to a solution reaction system, the thermodynamic equilibria in the proposed surface metabolism tend to favor the formation of large molecules from small ones.

A chemical reaction is possible if the Gibbs free energy of reaction (ΔG) is negative: $0 > \Delta G = \Delta H - T\Delta S$. The enthalpy of reaction (ΔH) depends mainly on the change of bond energy and solvation energy. It is nearly the same in a solution metabolism and in the proposed surface metabolism. The entropy of reaction (ΔS) is a measure of the change in possibility of movement of the constituents of a reaction. Page and Jencks (109) have shown that the change in the degrees of freedom of translational movement and overall rotational movement is most significant. The contribution by internal rotations is small, and the contribution by vibrations is negligible. This fact has the important consequence that the entropy of reaction on a surface is quite different from that in solution, where the splitting of one freely movable molecule into two freely movable molecules is favored by a high gain of mobility equivalent to 6 degrees of freedom (3 translational and 3 rotational). This means that in solution cleavage reactions tend to be thermodynamically favored by this entropy effect. This is a major reason for the implausibility of all versions of the broth theory. To force a solution reaction system in the synthetic direction, highly reactive

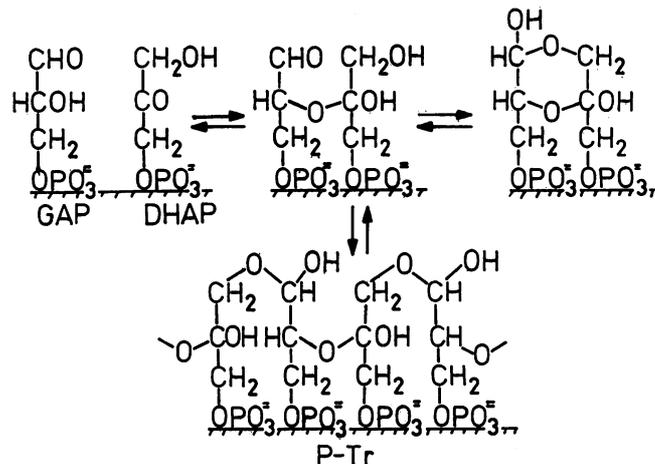


FIG. 1. Evidence that the surface equilibrium favors the conversion of glyceraldehyde phosphate (GAP) and dihydroxyacetone phosphate (DHAP) into long, polyanionically surface-bonded, polyhemiacetal structures. For reasons explained in the text, they are called phosphotribose (P-Tr). A variety of such structures is possible.

functional groups (e.g., phosphoanhydride groups) are required so that a high negative value of ΔH can compensate for a high negative value of ΔS . In the proposed surface metabolism, all constituents are attached to a mineral surface. Therefore, they have no freedom of overall rotation and only 2 df of slow translational migration. This means that a synthesis of large surface-bonded molecules from small ones does not involve a substantial decrease of mobility. Therefore, the proposed surface metabolism favors the formation of large molecular structures from less highly activated functional groups compared with a solution system.

As a first example, we consider phosphorylated sugars. In an aqueous solution, the molecules of phosphotrioses do not unite by forming intermolecular hemiacetal bonds. It is here predicted that, in a surface-bonded state, this unification to larger structures (Fig. 1) will take place because it is thermodynamically favored (just like the intramolecular pyranose and furanose ring formation of sugars). These proposed large structures are polyanionically bonded to the mineral surface and, therefore, have a selective advantage. According to my theory, this explains why phosphorylated sugars play such a pivotal role in the metabolism. They appear in the earliest forms of life as surface-bonded polyhemiacetal structures with a high chemical stability and a high resistance to decay by detachment. These structures are pivotal for the whole theory. They will be shown to form not only the precursor of the nucleic acid backbone but also the breeding ground for the purine bases and the purine-related coenzymes. Many different pathways seem to converge backwards into such structures.

As a second example, consider the thermodynamic condensation equilibrium between amino acids and polypeptides. In a solution system, the equilibrium is wholly on the side of hydrolysis (93), mainly for the following reasons: (i) in peptide bond formation, the reaction enthalpy (ΔH) has a positive value; (ii) in peptide bond formation, water is liberated and in an aqueous solution the mass effect of the water shifts the thermodynamic equilibrium toward hydrolysis; (iii) the reaction entropy is under the influence of two counteractive effects, namely, the unification of two amino

acid molecules to a dipeptide structure (decrease of mobility) and the liberation of water (increase of mobility). This means that the reaction enthalpy cannot be outweighed by the reaction entropy. Turning to a surface reaction system, we find a very different situation. The entropy effect of the unification of the amino acids is insignificant. Therefore, ΔS has a positive value which can, at least partially, compensate for the positive value of ΔH . Also, the formation of surface-bonded polypeptides from surface-bonded amino acids (e.g., aspartic acid, glutamic acid, and phosphoserine) is thermodynamically less unfavorable than in solution. This view is supported by the ready intramolecular formation of carboxypyrrolidone from glutamic acid and carboxypiperidone from 2-amino-adipic acid in hot neutral solution (25). Similarly, the formation of surface-bonded nucleic acid structures is thermodynamically more favorable than in solution.

To sum up these crucial considerations, the reactions among surface-bonded constituents tend to occur without excessive changes in the degree of order as quasi-intramolecular rearrangements within extended surface-bonded structures. They can be close to the thermodynamic equilibrium and yet they favor large molecular structures. The processes of detachment and subsequent decay in solution are, however, irreversible processes (102). This has the important consequence that the surface metabolism can support an evolution of autocatalytic reactions which are inherently synthetic.

Kinetics of Surface Metabolism

I now show that the proposed pre-enzymatic surface metabolism has a high intrinsic reaction selectivity similar to enzymatic reactions and that it requires a high reaction temperature.

In a solution reaction system, the dissolved molecules can move freely. Therefore, a multitude of different approachment paths of two reacting molecules can give rise to a multitude of products and hence a low selectivity. In view of the inverse relationship between reactivity and selectivity (44), the reaction selectivity is particularly low if the reactants have the highly activated groups required for synthesis in solution. In the proposed surface metabolism, all constituents are bonded to the mineral surface at the specific sites of their anionic groups. They have a narrow range of orientation. Chemical reactions between such oriented constituents occur mainly by vibrations and internal rotations. Therefore, the number of possible paths of approach is severely limited. Only those reactions can occur whose transition state is compatible with the requirement of surface bonding. Many reactions which are possible in solution are completely prohibited in a surface metabolism. Those reactions, however, which are permitted by surface bonding will occur with a higher propensity compared with a solution system. The formation of the transition state between two surface-bonded molecules does not involve a significant reduction of mobility, i.e., a low activation entropy. The surface metabolism is characterized by a narrow distribution of reaction possibilities, which is even more pronounced by virtue of the fact that functional groups with a high reactivity (and a low selectivity) are not involved.

In view of this high intrinsic selectivity, the surface metabolism exhibits a number of peculiar kinetic characteristics not found in solution. Three examples with considerable importance for evolution of the biochemical pathways are discussed.

(i) The propensity for a head-to-head reaction between the free ends (heads) of two surface-bonded constituents drops

off rapidly as the distance of the head groups from the surface increases. This is because, in such a reaction of the free ends of the constituents, the transition state (and also the end product) involves an increase of order by the freezing of many internal rotations. With an increase in length of the constituents, this effect increases and, therefore, the probability of a reactive collision between the head groups decreases exponentially. In a solution reaction system, the probability of such a collision does not depend on the length of the reactants.

(ii) Reactions between surface-bonded constituents are quasi-intramolecular. In true intramolecular reactions, five- and six-membered ring structures of transition states and end products are associated with the highest rates of reaction. Larger (medium-sized) rings are strained due to sterical hindrance across the ring. In a surface metabolism with the formation of quasi-rings involving the mineral surface, the situation is quite different, since the ionic surface bonds have no directional limitation.

(iii) Many enzymatic reactions involve the simultaneous association of at least three constituents in the transition state. In a nonenzymatic solution system, all reactions are bimolecular and a trimolecular one is extremely unlikely (102). In a surface metabolism, however, the probability of a trimolecular collision is considerably increased as compared with a solution system.

Turning now to the temperature requirement of a surface metabolism, it should be remembered that two molecules will react only if the kinetic energy of their relative movement is higher than the activation energy. Thus, the reaction velocity depends on the number of molecules in the Boltzmann distribution which satisfy this condition. In the solid state, the molecules can have only vibrational movements with a narrow Boltzmann distribution. Therefore, high temperatures are required for solid-state reactions. In the solution state, the molecules can have rotational and translational movements, which have a very broad Boltzmann distribution so that, even at low temperatures, a large proportion of the molecules have a translational or rotational energy sufficient for reaction (3). Molecules which are bonded to a surface can show only vibrational and internal rotational movements. The Boltzmann distribution of their excitations is expected to be situated between the solid-state condition and the solution condition. On the other hand, the solvation effects of the water phase, which lower the energy of the transition state, are similar, as in the case of the solution reaction system. For these reasons, the reaction temperature of a surface-metabolic reaction is higher than that of a corresponding solution reaction, but lower than the typical reaction temperatures of solid-state reactions. This result is in remarkable agreement with Woese's proposal that life originated under conditions of high temperature (163). At moderately elevated temperatures, the migration of surface-bonding constituents over the surface is accelerated without similarly accelerated losses by detachment, since the activation energy of surface migration is about 10 to 20% of the surface-bonding energy (3). Previous arguments against a thermophilic origin of life (98) based on the heat sensitivity of biomolecules are limited to solution systems. In a surface-bonded state, the propensity for thermal degradation is much lower. It is conceivable that these temperature requirements confine the surface metabolists to a band of temperature in which cellular microorganisms cannot exist. This would mean that the surface metabolists might still live in such regions, unperturbed by cellular forms of

life, and that they could be detected, for example, by staining.

Surface-Metabolic Reaction Types

Having seen how the thermodynamics and kinetics of a surface metabolism differ from those in a solution system, I now show how these facts give rise to some peculiar reaction types, notably, transfer reaction types which are not found in solution and are partly preserved in extant biochemical pathways.

The requirement of anionic groups for surface bonding and of additional functional groups for interconversion make the surface metabolism dependent on a minimum complexity of its constituents with at least two or three functional carbon atoms. On the other hand, the postulated autotrophic nature of the surface organism implies metabolic reactions of small non-surface-bonding units with one carbon atom. Both requirements are compatible if we assume that the small units are indirectly surface bonded by being attached to directly surface-bonded carrier constituents. A surface metabolism with such carrier constituents operates like a bucket brigade. The small units (e.g., units derived from CO_2 , HCOOH , CH_2O , CH_3COOH , CH_3CHO , $\text{CH}_2\text{OH-CHO}$, or NH_3 , but also H^- or e^-) are handed from one surface-bonded carrier to the next, while always remaining indirectly surface bonded.

Next, recall the suggestion (158, 171) that the earliest pre-enzymatic metabolic systems involve classes of reactions (reaction types) rather than specific reactions. For example, the Zn-dependent aldol condensation of surface-bonded glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate should give rise not only to fructose biphosphate, but also to branched surface-bonded bisphosphorylated hexoses such as hamamelose (5), whose involvement in carbon fixation has been suggested (71), and 2,3-bisphosphoglyceric acid, whose involvement in the biosynthesis of pyridoxal phosphate (PLP) (see references 56 and 57) is suspected.

The combination of the two notions of surface-metabolic class reactions and surface-metabolic carrier reactions leads us to the notion of general-purpose shuttles. Each shuttle (e.g., surface-bonded PLP) is specialized for transferring a specific small unit (e.g., NH_3) within a broad class of constituents (e.g., surface-bonded keto and amino acids). Each of these shuttles operates by rapid pivotal motion similar to the Lowe-Ingraham mechanism of enzymatic PLP reactions (88). The central metabolism abounds with such anionic shuttle-implemented transfer reactions. With the emergence of coded enzymes, the former shuttles turn into coenzymes.

The range of action of a surface-bonded carrier or shuttle is greater if its pickup group is at the distal end of a long arm which is attached to a surface-bonding polyanionic moiety and can pivot and reach beyond its nearest neighbors. Such structures are found in the following coenzymes, the number of anionic groups being given in parentheses: coenzyme A (CoA; 3), oxidized nicotinamide adenine dinucleotide (NAD^+ ; 2), NAD^+ phosphate (NADP^+ ; 3), flavin adenine dinucleotide (2), tetrahydrofolic acid (THF; ≥ 3), methanopterin (3), sarcinapterin (4), methanofuran (5), F_{420} (≥ 4), and component B (2). In all of these shuttles with long arms, the number of rotatable links is reduced and the likelihood of reactive collision of the pickup end is increased by rigid structural units within the arm, such as aromatic rings, ribose rings, nonrotatable —CO—NH— bonds, and bulky

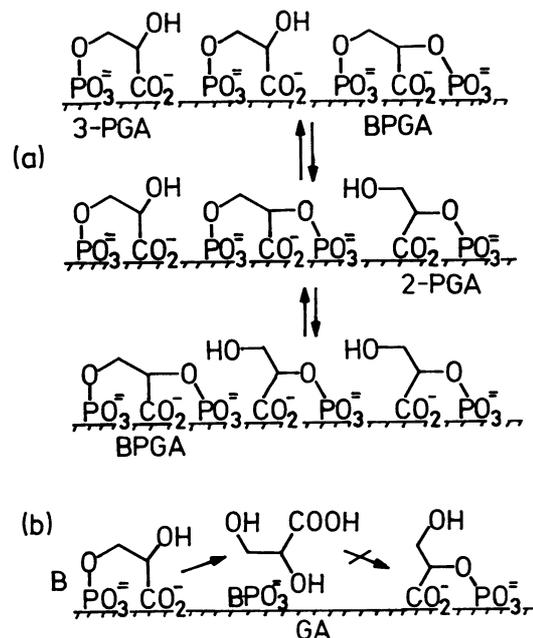


FIG. 2. Transfer of surface-bonding phosphate groups. 3-Phosphoglyceric acid (3-PGA) is converted to 2-phosphoglyceric acid (2-PGA) in (a) a surface-bonding pathway with 2,3-bisphosphoglyceric acid (BPGA) as cofactor or (b) via non-surface-bonding glyceric acid (GA) by an imidazole shuttle (B).

groups with hindered rotation, which are so typical for these coenzymes.

A special transfer situation occurs if a surface-bonding phosphate group is transferred from a first position to a second position of a constituent. In such a reaction, a transfer mechanism (Fig. 2b) would lead to an intermediate without surface bonding and thus to its loss by dissolution. In a mechanism such as that shown in Fig. 2a, this is avoided. It requires initially an additional surface-bonding phosphate group to become attached to the free second position of a molecule of the constituent before the phosphate group in its first position is cleaved and attached to the second position of a further molecule of the constituent, whose phosphate group in the first position is thereafter cleaved and transferred, etc. In extant phosphomutase reactions, this pattern is still conserved, even though in an enzyme reaction system the transfer shown in Fig. 2b would be much simpler. Transfers of surface-bonding phosphate groups may be rampant among the constituents of the surface metabolism, and they are close to equilibrium. This suggests that extant pathways with successive steps of dephosphorylations and rephosphorylations (by adenosine triphosphate [ATP]) are preceded by precursor pathways with transphosphorylations. It is of interest that the recently proposed alternative to the Calvin-Benson cycle (71) suggests such transphosphorylations even for extant carbon fixation.

MECHANISMS OF EVOLUTION

First Organism/Environment Dichotomy

I now show that the proposed surface reaction system can support a form of life in monomolecular layers: true surface organisms engaged in a process of growth and reproduction

and with an inherent propensity for an evolution toward higher complexity.

The proposed surface metabolism has an anisotropic structure. It is extended in the two dimensions of the surface of its mineral support without any clear boundaries and without any individuation by division. But in the direction of the third dimension (normal to the mineral surface), it is limited to the size of a monomolecular layer and its constituents are oriented by the vectorial character of the mineral-water interface. This vectorial interface establishes the physical basis for the earliest and most fundamental organism/environment dichotomy. The "surface organism" is defined as the totality of all surface-bonded organic constituents of the surface metabolism. The "environment" is defined as the totality of the non-surface-bonded molecules in the water phase and the mineral support. These serve the surface organism as sources of inorganic nutrients, and the water phase also serves as the sink for detached products of decay.

A true surface organism is composed of a self-sufficient subset of surface-bonded constituents with the following characteristics. By the uptake of inorganic nutrients (e.g., CO_2) and subsequent rearrangements, the constituents of the self-sufficient subset produce more and more constituents which become surface bonded in *statu nascendi*. This establishes a process of growth which is a process of both spreading onto vacant surfaces and reproducing, as long as the newly grown extensions of the organism contain, again, a self-sufficient subset of surface-bonded constituents. The surface-bonded constituents of the self-sufficient subset are autocatalytic, promoting the production of constituents of their own kind by their organization in reaction cycles. The existence of such autocatalytic cycles is dependent on special chemical constellations, and it is this paucity of possibilities for surface-bonded autocatalysis which establishes the compositional uniformity, uniqueness, and identity of the surface metabolists.

Evolution by Innovation and Selection

I now show that a reproducing surface organism can undergo evolution by the interplay of innovation and selection, even though it has neither nucleic acids nor any template copying.

For understanding this earliest process of evolution, it is important to realize that an autocatalytic surface metabolism produces a diversity of surface-bonded constituents, some of which are not included within autocatalytic reaction cycles but rather are lateral extensions thereof. This tendency to diversification is kept at bay by the self-sorting process of surface detachment. It operates among the surface-bonded constituents (not among competing organisms) and by the process of selective detachment and decay (and not by food shortage). By this self-sorting process, strongly bonding (polyanionic) constituents with chemical stability predominate. Among the weakly bonding or chemically instable constituents, only those can maintain a role in the surface metabolism which have a sufficiently high rate of metabolic turnover, so that their surface-metabolic conversion into chemically more stable and strongly surface-bonded products outruns their decay by detachment and decomposition. This means that all constituents of a surface organism which do not undergo rapid metabolic conversion are characterized not only by chemical inertness, but also by strong polyanionic surface bonding.

The events of surface-metabolic innovation occur by the conversion of preexisting surface-bonded constituents into

novel surface-bonded constituents. Therefore, in contrast to the broth-inspired Horowitz thesis (60) of a retrograde evolution of metabolic pathways, the surface-metabolic pathways are seen as evolving in accordance with Florkin's version (38) of the biogenetic law of Müller and Haeckel (51, 100) as terminal extensions or lateral branchings of preexisting pathways. Most of these novelties are transient and subject to quick removal by decomposition or detachment. Occasionally, however, a novel constituent opens up a novel autocatalytic cycle which becomes grafted onto the preexisting autocatalytic network. Such an autocatalytic novelty, once triggered, constitutes an inheritable change. Metabolic novelties, therefore, do not emerge gradually. They arise after a more or less protracted induction period as sudden saltations from one unique and distinct stage of the surface metabolist to another. Incidentally, seen from the point of view of this scheme of metabolic innovation, the mutations by the mispairing of bases in a replication of surface-bonded nucleic acids (to be addressed later) are but a special and somewhat belated type of inheritable innovation. Today, they may well be all-important. But in the overall history of life, they are neither the primary nor the only cases of inheritable innovation.

Autocatalytic Coenzymes

The evolution of autocatalytic cycles in the surface metabolism is now elucidated, with reference to some of the extant coenzymes which are polyanionic and therefore of great antiquity.

It has been frequently and convincingly argued that some of the coenzymes in extant organisms are vestiges of an early pre-enzymatic metabolism (28, 33, 76-78, 146, 154). The theory of surface metabolism adopts this proposal. Most coenzymes are indeed polyanionic surface bonders (e.g., all of the above-mentioned long-armed shuttles, but also thiamine pyrophosphate (TPP), PLP, siroheme, hemes, and F_{430}). Many of these are produced by biosynthetic pathways which consist largely of surface-bonding constituents. Other coenzymes, e.g., vitamin B_{12} , (bacterio)chlorophylls, and quinones, which themselves are not polyanionic, are derivatives of polyanionic biosynthetic precursors which can often be considered evolutionary precursors with the same or a related catalytic function. Those coenzymes, however, which are neither polyanionic nor synthesized by pathways with polyanionic constituents are *prima facie* considered to be of cellular origin. This holds notably for lipoic acid and for biotin, for which a late origin has been previously proposed for other reasons (147).

The theory of surface-metabolic evolution suggests that all surface-bonding coenzymes originate in the surface metabolism as being not only catalytic for a class of reactions, but also autocatalytic for their own biosynthesis from surface-bonded constituents. The anaerobic pathway to nicotinamide coenzymes [NAD(P)^+] is an instructive example. Figure 3 shows this pathway, which consists strictly of surface bonders in a surface-metabolic representation. The formation of quinolinic acid (QA) from dihydroxyacetone phosphate and aspartic acid (Asp) involves an oxidation and is driven by a high aromatization energy of QA. Later, with the appearance of a metabolic novelty, the ribosylation of QA by means of phosphoribosyl pyrophosphate (PRPP), nicotinic acid mononucleotide arises, which can cycle as a hydride shuttle through hydride acceptor and donor reactions. This means that it can catalyze the hydride transfer between a large variety of surface-bonded constituents. It is

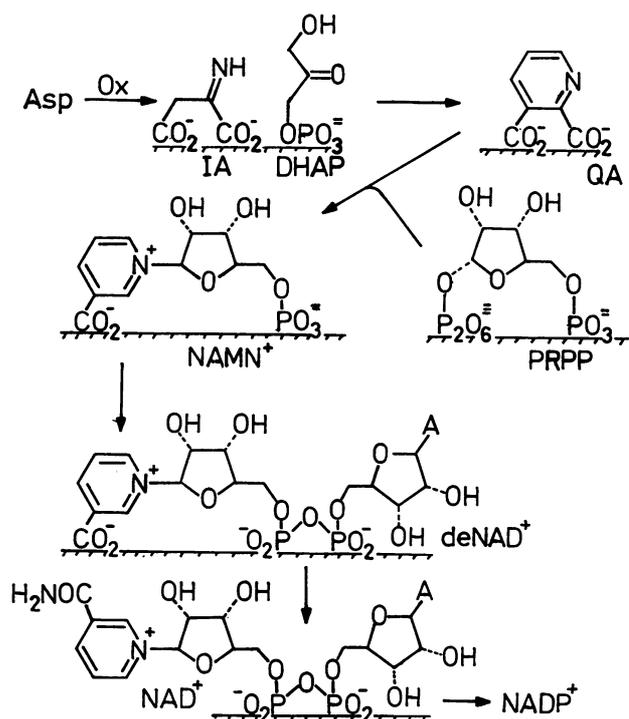


FIG. 3. Surface-metabolic representation of the anaerobic nicotinic amide pathway forming QA from dihydroxyacetone phosphate (DHAP) and Asp via iminoaspartate (IA), where Ox is oxidant. After the advent of PRPP, nicotinic acid mononucleotide (NAMN⁺), desamido-NAD⁺ (deNAD⁺), and NAD⁺, which may be phosphorylated in position 3 (NADP⁺), are formed. They all have coenzyme function.

suggested here that, in the early surface metabolism, it also functions as an autocatalyst by taking over the oxidizer function in the formation of QA and so promotes its own biosynthesis. This closes a novel autocatalytic feedback loop and establishes an inheritable saltatory change of the surface organism. The later extensions of the NAD(P)⁺ pathway serve for modifying its surface bonding (explained below). The involvement of flavin adenine dinucleotide as oxidant in the extant Gholson pathway (101) to QA via iminoaspartate may, therefore, be the result of a belated takeover, and it may be speculated further that the earliest pathway to QA proceeds not via the highly unstable iminoaspartate but rather via dihydro-QA.

Generalizing the above scheme of surface-metabolic coenzyme evolution, predictions of hitherto unknown biosynthetic pathways to surface-bonding coenzymes can be advanced on the basis of the following rules. (i) All constituents in the pathway are surface bonders. (ii) At least one reaction in the surface-metabolic biosynthesis of a coenzyme is of the same type as one of the donor-acceptor reactions of the catalytic cycle of this coenzyme. (iii) A later step in the surface-metabolic biosynthesis of the coenzyme is characterized by a structural modification which lowers the activation energy and/or the free energy difference between the acceptor state and the donor state and thus makes the donor-acceptor reaction faster and/or more readily reversible. (iv) The donor-acceptor cycle of the coenzyme promotes a step in its own biosynthesis. (v) The extant pathway recapitulates its evolution, perhaps with later substitutions of some of its steps.

I now apply these rules to the problem of the biosynthesis

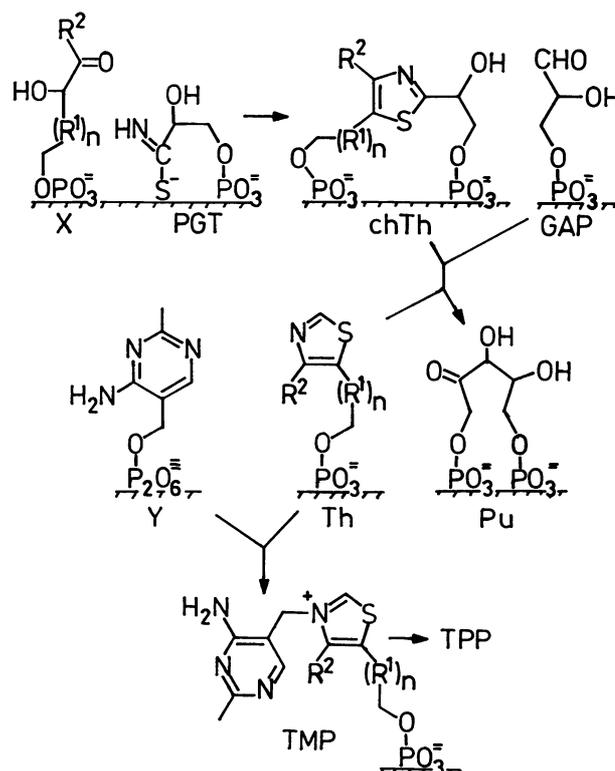


FIG. 4. Hypothetical surface-bonded pathways to TPP, (a) producing a charged thiazole unit (chTh) from phosphoglyceric thioamide (PGT) and a mono- or bis-phosphorylated triose (X: $n = 0$; $R^2 = H$) and converting it with GAP to an uncharged thiazole unit (Th) and a pentulose (Pu). Thiazole combines with the pyrimidine unit (Y) via thiamine monophosphate (TMN) to TPP. A later successor pathway (b) uses the pentulose to produce a phosphorylated C₅ unit (X: $n = 1$; $R^1 = CHOH$; $R^2 = CH_2OH$) as starting material. (c) In a still later pathway, X represents a C₅ unit with $n = 1$, $R^1 = CH_2$, and $R^2 = CH_3$.

of TPP (34, 48, 155). Figure 4 shows an example of a speculative scheme for the evolution of the surface-metabolic biosynthesis of the thiazole unit of TPP, which satisfies the above rules of coenzyme evolution. The earliest pathway produces from unknown surface-bonded C₃ constituents (the shown Hantzsch reaction between phosphoglyceric thioamide and a phosphorylated triose [X] being merely a formal representation) a first-generation thiazole unit charged with a phosphoglycol aldehyde unit. This intermediate has a small but significant propensity to cleave off its phosphoglycol aldehyde unit and to donate it to GAP to produce a bis-phosphorylated surface-bonded pentulose and an uncharged thiazole unit. The later quaternization of thiazole with the surface-bonded pyrimidine unit (Y) or an evolutionary precursor thereof produces a thiamine monophosphate which can now function as an aldehyde shuttle by cycling through acceptor and donor reactions. It is catalytic for a large class of aldehyde transfer reactions and autocatalytic for its own production from charged thiazole. In another evolutionary modification, X is derived from the pentulose, which results again in an autocatalytic effect. Later, in the (semi)cellular stage of evolution, the bis-phosphorylated pentulose is replaced by a C₅ unit which arises by thiamine monophosphate autocatalysis from GAP and the acetaldehyde unit. As one of the last evolutionary events, thiamine monophosphate is phosphorylated to TPP. This pathway invokes only surface bonders, which is consistent with the recent finding that

thiazole may occur in the de novo TPP pathway only in the phosphorylated form while the nonphosphorylated form occurs only in the salvage pathway (64).

Environment Changes the Surface Organism

A change of environment may cause a saltatory change of the organism which truly amounts to an acquisition of inheritable characteristics.

According to Popper's revolutionary cosmology, the propensity theory (120; K. R. Popper, lecture at the World Congress of Philosophy, 1988), propensities for change are always a function of the overall situation. Therefore, all propensities for change of a surface organism, by either the selective detachment of constituents or the emergence of novel constituents of (novel) autocatalytic cycles, are dependent not only on the nature of the surface constituents, but also on the environmental reaction conditions. Any change of the environment, by either temporal fluctuations of the environment or spatial dislocations (or spreading) of the organism, has the effect of changing the organism. It does so not only by changing the propensity distribution of the reproduction and selection of its preexisting constituents, but also by the (transient) increase of the propensity for the appearance of novelties. If such a metabolic novelty is autocatalytic or self-promoting, it has a high propensity to persist even if the environment returns to its former state. In this manner, environmental changes may trigger the acquisition of inheritable metabolic novelties. For example, a temporary increase in the pH of the environment may trigger a reaction which produces a novel surface-bonded nitrogen base, which may in turn function as an autocatalyst for the continued generation of that nitrogen base. Incidentally, a previous existing metabolic pathway may become locally extinguished by a transient change of the environment, but it may spread back from other areas as soon as the local environment returns to the previous state. Occasionally, two novelties, ignited in different local environments, may become united by chemical symbiosis (76). This constitutes a large-scale expansion of the metabolic network, a truly macroevolutionary change. It should be added that, through any of these evolutionary changes by the grafting of novel autocatalytic cycles onto a preexisting metabolic network, some pathways in this network may become permanently extinguished (78).

Upon encountering a number of different environments, the proposed surface organism will grow into a number of differentiated varieties, which may be spatially coherent or separated. Some of these will persist, but others will perish (become extinguished or killed) through a drastic change of the environment (e.g., by the cutoff of food, by burial and suffocation through rapid sedimentation, or by extreme temperature or pH conditions). The first kind of death in the history of life is a death by extinction of a surface-metabolic variety.

The Surface Organism Changes Its Environment

To complete the picture of the surface organism/environment relation, it helps to introduce a distinction. The remote environment (which will be called "surroundings") influences the surface organism without being substantially changed by this organism. The near environment (which will be called "ambience") establishes the reaction milieu close to the surface and is itself strongly influenced by the products of the metabolic process. This means that some of the

feedback cycles involve a change of ambience. Two examples decisive for the evolution of cellular organization and genetic control are treated here in general terms (explained in greater detail later).

(i) After the emergence of pathways leading to lipophilic surface-bonded constituents (e.g., fatty or isoprenoid lipids), the accumulation of these new constituents (waste products of sorts) on the surface is favored by their chemical inertness and low solubility. This accumulation produces a solvent effect by increasing the hydrophobicity and thus lowering the hydrolytic power of the ambience near the surface. This in turn changes the propensity distributions of all surface-metabolic reactions. It lowers the propensity for detachment through hydrolysis and increases the generation of more of these lipophilic constituents.

(ii) The process of nitrogen fixation produces surface-bonded nitrogen compounds (e.g., imidazoles, purines, or amino acids). These are proton acceptors, which have not only a direct catalytic effect through group transfer, acid base catalysis, or sequestered catalytic metal ions, but also an indirect effect by buffering (to some extent) the ambience against fluctuations in pH and thus protecting all constituents by lowering the propensity for detachment through protonation or hydrolysis.

As the organism grows by spreading onto new territories with vacant surfaces, it carries its own favorable ambience (e.g., buffering and lipophilic constituents) along, thus rendering those new territories inhabitable. The surface organism conquers and colonizes the world by turning surroundings into ambience. Therefore, this earliest organism satisfies Popper's characterization of life as searching for a better world (122). Such is the earliest mechanism of evolution, by which the surface organism goes through a long series of metabolic innovations and unfolding biochemical pathways, increasing step by step its chemical versatility and its autonomy from the environment. It is a mechanism of evolution which does not depend on Malthusian population pressure or on competition for or a scarcity of food. This process leads eventually to the two greatest revolutions in the history of life: cellular organization and gene-enzymatic control. Jointly, they amount to the emancipation of life from a surface-bound existence and to the conquest of the third dimension, a new frontier with countless problems on which life has labored ever since.

Changeover to Cellular Mechanisms of Evolution

With the cellular revolution (next section) and genetic control (see later), the mechanism of evolution changes profoundly and assumes the characteristic pattern found for extant cellular forms of life.

In an open surface metabolism, development and evolution are identical. The changeover to a cellular metabolism marks the segregation of ontogenetic and phylogenetic change. In an open surface metabolism, all processes of detachment are processes of elimination, since the detached constituents vanish. The appearance of closed cells gives rise to a radically new mode of selection. It is the selection of whole sets of constituents, of whole cellular units. This gives rise to enzyme control mechanisms which unite all pathways into an integrated control system for optimizing the whole cellular unit. With this step, the mechanism of evolution assumes the familiar bias in the organism/environment dichotomy. The causes of variation are now mostly internal and autonomous. They still occur by the formation of new feedback loops. With the appearance of the genetic

machinery, however, these are more and more restricted to mutations and other changes of the genome. By the same token, the process of selection is dominated more and more by external causes.

In a surface metabolism, the positively charged mineral surface forms a reference surface for the positioning and interaction of surface-bonded anionic constituents. This means a severe limitation of the reaction possibilities. With the emergence of genetically encoded enzymes, this situation changes fundamentally. The enzymes are first still bonded to the surface, but later they fold into three-dimensional structures. Now, and notably after the inclusion of cationic amino acids, the enzymes compete with the mineral surface for bonding the constituents. However, the bonding surfaces of the enzymes are not akin to simple crystal surfaces. They have a three-dimensional, curved configuration and a pattern of selective bonding. Also, the enzymatic bonding surfaces have a variable configuration. The variability is introduced by the possibility of conformational changes. Most important, all of these characteristics can change in the process of evolution due to a variability of the sequences of the nucleic acids and the coded enzymes. This marks a major revolution in the mode of evolution, which can be stated as follows: the precellular mode of evolution is an evolution of surface-metabolic constituents and pathways of constituents. The mineral surface itself is not capable of evolutionary change. After the emergence of cellular organization and genetically encoded enzymes, the mode of evolution is dual tracked. The first track is simply a continuation of the surface-metabolic mode of evolution by the unfolding of the pathways to the constituents. The second track is new. It is the evolution of the new enzymatic bonding surfaces for the constituents evolving on the first track. These new and varying surfaces offer a host of new possibilities for chemical pathways. The decisive importance of pathway possibilities, which are virtual pathways, for the direction of evolution has been overlooked and so has the whole primary evolution to which these virtual pathways essentially belong and which enters into a new stage when the enzymatic surfaces offer themselves. The two tracks of evolution are of course interrelated. The secondary track, evolution of the enzymatic bonding surfaces, enables an enormous increase in possibilities for the first and primary track of evolution, the unfolding of the biochemical pathways. A new nucleic acid sequence, and thus a new bonding surface configuration, which does not correspond to a possible biochemical pathway is without benefit for the whole cellular organism. This means that, among the novel enzymes, only those adapted to a virtual biochemical pathway are retained. Cellular evolution is of a composite nature. It consists of a genomic evolution of sequences grafted onto an older and primary evolution of unfolding biochemical pathways which, like surface bonding, still goes on as in the first days of life.

A Methodological Rule

The surface organism has so far not been found in nature, and it may be extinct. Such extinctions as well as the loss of biochemical pathways are common occurrences in evolution. Evolution theory cannot help but indulge in more or less far-fetched speculations. These, however, should be bridled by methodological discipline. Therefore, I propose a methodological rule: whenever we postulate a precursor (pathway, structure, function, or organism) to have once existed and later presumably disappeared, we are obliged to

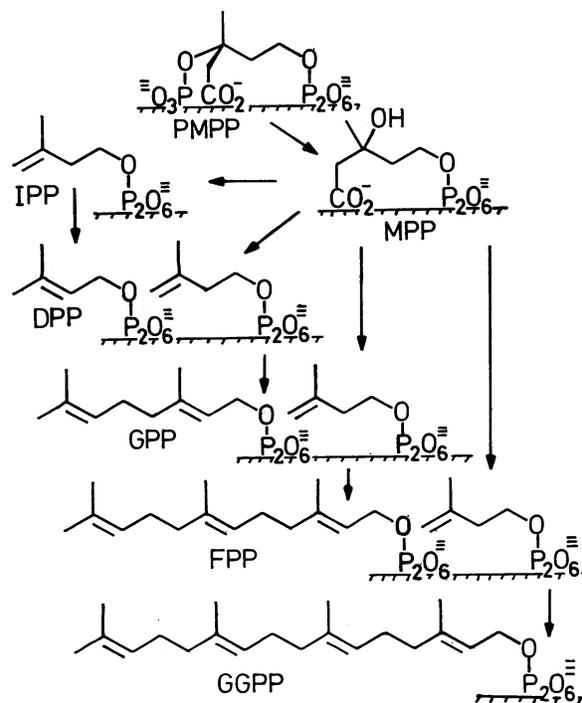


FIG. 5. Surface-bonding chain extension steps in the extant isoprenoid pathway from 3-phosphomevalonate 5-pyrophosphate (PMPP) through mevalonic acid 5-pyrophosphate (MPP), isopentenyl pyrophosphate (IPP), dimethylallyl pyrophosphate (DPP), geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP). A precursor pathway may have monophosphorylated constituents.

demonstrate that (i) it is possible to construct a step-by-step phylogenetic model (for which we do not have to claim validity) which links up the hypothetical precursor with its extant successors, and that such a linkage model is (ii) chemically and thermodynamically plausible, (iii) free of paradoxes, and (iv) preferably sufficiently detailed for experimental tests. In the following sections, it is shown that such linkage models for a changeover of the surface organism to cellular organisms can indeed be constructed. The proposed models are grossly simplified and deficient and at best in need of drastic revision and improvement. It would be preposterous to assume that, with this first attempt, the true historic chain of events could be reinvented. The method used for constructing these explanatory linkage models is an application of Popper's theory of situational logic (112, 113, 116, 117). Within biochemistry, it has been forcefully advocated by H. Kuhn. He combines chemical laws with biochemical facts for a piecemeal reconstruction of biochemical phylogeny (79-81). The method of a pathway-guided construction (which is not reductionistic) has also been advocated by F. Lipmann (87).

CELLULAR REVOLUTION

Surface-Bonded Isoprenoid Lipids and Membranes

The appearance of cellular structures can be seen as a result of the inherent self-sorting tendencies in the evolution of the surface organisms. I begin by showing that surface metabolists produce isoprenoid lipids as by-products which stay surface bonded and accumulate into coherent membranes.

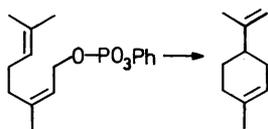


FIG. 6. Intramolecular analog of the quasi-intramolecular chain extension reaction in Fig. 5.

Cell membranes are made of lipids. The universal extant isoprenoid lipid pathway (16) shown in Fig. 5 consists strictly of surface-bonding constituents which must be vestiges of surface-metabolic days. It proceeds through chain extensions by C₅ units from isopentenyl to geranyl, farnesyl, and geranylgeranyl or even geranylfarnesyl pyrophosphate. In this reaction (having a favorable transition state ring size), the free head of a short constituent connects to the foot of another (longer) constituent, severing it from its surface-bonding pyrophosphate group. It cannot be carried out without enzymes *in vitro* as an intermolecular solution reaction. However, in the surface metabolism, this reaction is not intermolecular but quasi-intramolecular, and it is of interest that an intramolecular equivalent (Fig. 6) has been described to occur with high yield in an enzyme-free solution merely by prolonged standing (95). The pathway shown in Fig. 5 begins with 3-phosphomevalonate 5-pyrophosphate, which in extant metabolisms is obtained from non-surface-bonding mevalonic acid. Therefore, this pathway is an enzymatically modified successor of a more ancient precursor pathway in which a surface-bonding group such as the highly reactive phosphate group is attached prior to the removal of surface-bonded CoA (Fig. 7). Incidentally, the oldest pathway operates not with CoA but with H₂S or another CoA precursor.

By invoking Florkin's biochemical version (38) of the biogenetic law, it is assumed that the sequence of the steps in the pathway of Fig. 5 reflects the temporal order of their appearance in evolution. The short surface-bonded isoprenoid lipids are the first to appear. They are all strong surface bonders with a low propensity for detachment, owing to

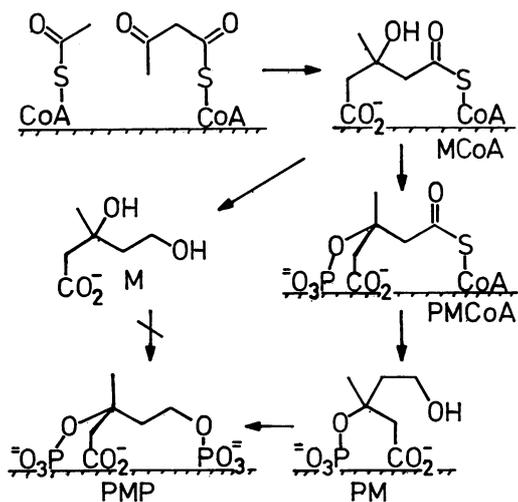


FIG. 7. Extant mevalonic acid pathway (left branch) via non-surface-bonding mevalonic acid (M). In a hypothetical pathway (right branch), all constituents are surface bonding, since mevalonoyl-CoA (MCoA) is converted to 3-phosphomevalonoyl-CoA (PMCoA) and then through 3-phosphomevalonic acid (PM) to 3,5-bis-phosphomevalonic acid (PMP).

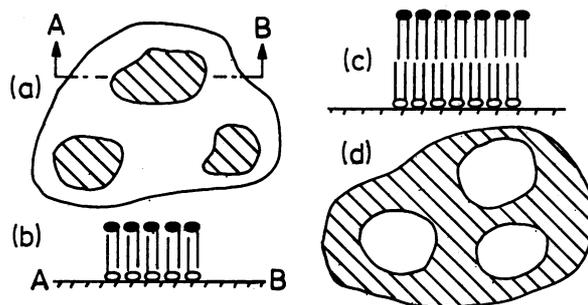


FIG. 8. Surface-bonded membranes. (a) Hydrophobic (hatched) membrane domains within a continuous hydrophilic phase; (b) interdigitated structure; (c) bilayer structure; (d) hydrophilic domains within a continuous hydrophobic membrane phase (hatched). In panels b and c, the white circles represent surface-bonding anionic foot groups, the black circles represent non-surface-bonding head groups, and the lines represent hydrophobic tails.

their lipophilic nature and low water solubility. Therefore, they are highly likely to accumulate on the surface. This accumulation has a lipophilizing effect. The formerly exclusively hydrophilic constituents of the surface metabolism become increasingly mixed with lipophilic constituents which function as a two-dimensional hydrophobic solvent, pushing the water away from the surface. This has important consequences. It reduces the propensity for protonation and hydrolysis, and, therefore, the accumulating lipids protect not only themselves but also the other (hydrophilic) constituents against detachment. Moreover, it promotes a number of new reactions which are not possible in a strictly aqueous milieu. Notably, it tends to push condensation equilibria towards large molecules such as polypeptides and nucleic acids. Most important, it even favors thermodynamically the formation of anhydride bonds, e.g., the condensation of phosphate groups to pyrophosphate or triphosphate groups (as in ATP). The very constituents (the isoprenoids) which cause this change of ambience are themselves among the beneficiaries, since they become equipped with and strongly surface bonded by pyrophosphate groups. Hydrogenation of the double bonds protects these lipids further against hydrolysis and detachment.

With an increasing surface concentration of isoprenoid lipids, notably of those with longer chains, a point will be reached at which a two-dimensional phase separation occurs. Hydrophobic domains of lipids segregate within a continuous hydrophilic phase (Fig. 8a). Compact lipid domains, however, can form only if some of the lipid molecules become detached and inverted in orientation so that their hydrophilic foot groups turn into head groups, which face outwards and form a hydrophilic outer surface in contact with the water phase (Fig. 8b). This reorientation is possible, since the hydrophilic foot groups may be modified and lose their surface-bonding polyanionic character, e.g., by hydrolysis of the phosphate groups or by their substitution by glycerol ether groups. The nonanionic character of the outer head groups has the additional effect of an absence of destabilizing repulsive electrostatic forces. (The anionic foot groups do not have such a destabilizing effect because they are surface bonded.) This explains the universally observed asymmetry of extant cellular membranes, whose inside surface shows a predominance of negatively charged groups while the outside is mainly nonionic. Due to the hydrophobic coherence of the lipid molecules, such a membrane stays surface bonded even if the individual lipid molecule has only a single negative charge.

The first surface-bonded membranes are interdigitated (Fig. 8b), since in phosphoglycerol monoethers (which appear early in the pathway) the hydrophilic end groups have a larger cross section than the hydrophobic tails. Later, with the emergence of phosphoglycerol diethers, both cross sections are about equal and a bilayer membrane (Fig. 8c) with a greater thickness of the lipophilic zone arises.

The formation of coherent surface-bonded membrane domains has the consequence that nonionic and detached wholly lipophilic constituents are caught and accumulated within the membrane. This has three important effects. (i) The wholly lipophilic constituents (e.g., squalene and hopanoids) increase the viscous fluidity in the outer layer of the membrane, while the inner surface-bonded layer has a solid crystal structure. This fluidity allows rapid lateral diffusion in the outer layer. (ii) Long wholly lipophilic constituents (e.g., carotenes) function as tie bars for stabilizing the membrane (124). (iii) Membrane-bound lipophilic compounds with functional groups (e.g., lipophilic proteins, carotenes, quinones, and porphyrins) establish a membrane metabolism and, notably, membrane-bound electron transport chains.

Semicellular Organisms

I now discuss the inherent tendency of the growing surface-bonded lipid membranes to produce semicellular structures with a cytosol metabolism.

With the further accumulation of lipids and the growth of the membrane domains, a point is eventually reached at which a phase inversion occurs. The two-dimensional "oil-in-water" arrangement (Fig. 8a) is inverted to a two-dimensional "water-in-oil" arrangement (Fig. 8d). Now individual hydrophilic metabolic domains are segregated within a continuous hydrophobic membrane domain. This is the first instance of individuation. The hydrophilic domains are under a two-dimensional pressure by the forces of cohesion of the surrounding membrane phase, which keeps the hydrophilic constituents in close lateral proximity. In the further evolution of the isoprenoid lipid membrane, the increasing fluidity, stability, and cohesiveness of the membrane has an important saltatory consequence. This is best understood if we consider that the hydrophilic domains constitute interruptions or holes in the surface-bonded membrane. The removal of these holes by confluence to a completely closed membrane, which forms an overlayer above the hydrophilic domains, is therefore associated with a gain of cohesion energy. This energy gain is connected with a concomitant loss of adhesion energy due to the local surface detachment of the membrane. At a certain point of membrane evolution, the cohesion energy gain outweighs the adhesion energy loss. This may happen after the surface-bonding glycerol moieties are largely modified to groups with a reduced anionic charge repulsion. The membrane then forms a self-supporting continuous blanket covering the hydrophilic domains. Semicellular structures are born (see Fig. 9a).

The water permeability and ion impermeability of lipid membranes cause osmosis by which water tends to accumulate inside the semicellular structure. By continued osmosis (in fresh water), the osmotic pressure will cause a rupture of the membrane, which in these early days is of course not yet equipped with wall reinforcements or osmosis-regulating features. Therefore, the semicellular organisms survive only in water with a high salinity so that the osmolality is the same on both sides of the membrane. This conforms with the fact that many archaebacteria and some eubacteria still require high internal or external salt concentrations or both.

The formation of semicellular structures marks the origin of the cytosol, the first broth in the history of life. It gives rise to a cytosol metabolism. Moreover, the membrane protects the surface metabolism from adverse changes of the environment. It allows the maintenance of a nearly neutral internal pH so that the propensities for surface detachment and hydrolysis of the constituents are reduced. In such conditions, which disfavor indiscriminate rampant detachment and hydrolysis, enzymatic processes for the selective detachment of the constituents and for their subsequent selective hydrolysis and catabolic degradation can appear.

With the appearance of proteins with lipophilic and cationic rests (explained below), the surface reaction pathways can be liberated from their dependence on a mineral surface support by transfer (e.g., retrograde) into either the membrane metabolism or the cytosol metabolism. One by one, the constituents of the surface metabolism are lifted off the surface. This gradual changeover of the surface metabolism to a three-dimensional membrane/cytosol metabolism is made possible by the ever increasing virtuosity of the evolving enzymes. It means a growing independence from the severely restricting mineral surface. This changeover is aided by the emergence of special reactions for the elimination of surface-bonding groups. A most instructive example is offered by the Entner-Doudoroff pathway: in most eubacteria, all of its constituents bear phosphate groups; in halobacteria (145) and clostridia (2), only the last two of its constituents are phosphorylated; and in *Sulfolobus* and *Thermoplasma* species, none of its constituents is phosphorylated (10, 27). This corroborates the antiquated character of these phosphate groups, which may have been even more widespread in the earliest metabolism compared with the extant central pathways.

Surface-bonding carboxylate groups can be removed by decarboxylation or by their conversion to carboxamide groups. Both strategies are displayed in the later stages of many pathways. In the next section, I consider two most instructive examples of the coenzyme pathways. Both pathways reflect the evolutionary sequence proposed here: pre-cellular surface metabolism → semicellular surface/membrane/cytosol metabolism → cellular membrane/cytosol metabolism.

Surface Detachment of Coenzymes

The nicotinic amide pathway (Fig. 3) reflects the tendency for development of a pivotable arm with a functional end by the removal of a surface-bonding group after the previous elongation of the distal end having additional surface-bonding phosphate groups. The adenine (A) group gives this coenzyme an additional indirect surface-anchoring capability via base pairing and/or base stacking with a surface-bonded nucleic acid (explained below). This evolution is driven by the benefits of an increased perimeter of action of the now pivotable catalytic moiety and of a facilitated migration over the surface, which is actually a one-dimensional diffusion along the nucleic acid by alternate loosening of the bonding to the surface and the bonding to the nucleic acid. The conversion of the surface-bonding carboxylic group into a carboxamide group has a windfall profit in the era of semicellular organisms. It facilitates the lifting of this coenzyme off the surface by an enzyme with cationic groups which can compete with the mineral surface for bonding the coenzyme.

It is conspicuous that the metabolism relies on two types of nicotinic amide catalysts, NAD^+ and NADP^+ . The pro-

posed theory provides an explanation. The first proteins capable of lifting coenzymes off the surface are detrimental rather than beneficial, since a catalytic usefulness of a protein-bonded coenzyme cannot yet exist for all of the many (still) surface-bonded substrates of this coenzyme. This suggests that a number of coenzymes in the earliest surface metabolism become obliterated due to lifting. Those coenzymes, however, which happen to occur in the surface metabolism as a whole family of functionally similar coenzymes with different numbers of anchoring groups survive by being lifted off differentially. The weakly bonded family members are lifted off first and become inoperative. The strongly bonded family members stay surface bonded and operative. In the course of time, the protein-bonded coenzymes become operative again. The protein turns into an enzyme. Thereafter, the strongly surface-bonded family members are lifted by (other) proteins. This explains why a larger number of coenzymes (e.g., the pterins) occur as a family of coenzymes with different numbers of anionic groups. It is suggested that this is true generally for all coenzymes that survive the transition from the surface metabolism to the cellular metabolism. The surface-metabolic theory explains another peculiar fact. With few exceptions, NAD^+ and NADP^+ are used noninterchangeably, the first in catabolic and the second in anabolic (biosynthetic) pathways. This is caused by the sequential process of lift-off. NAD^+ with two anionic groups is lifted first and becomes inoperative. The still surface-bonded NADP^+ carries the full burden of surface-metabolic biosynthesis. Next, the water-borne NAD^+ acquires a catalytic function again, but only for the catabolism of substrates which are also water-borne. The final lifting of NADP^+ occurs again in two stages, by which the NADP^+ molecules which are base paired to surface-bonded nucleic acid are the last to be lifted. This happens in conjunction with the transfer of the anabolic pathways into the membrane/cytosol metabolism.

The tetrapyrrol pathway begins with the tetramerization of four molecules of surface-bonded porphobilinogene in a reaction which can occur nonenzymatically even in solution (17). Next, the thermodynamically favored (91) uroporphyrinogene III is formed which sits on the surface with its eight carboxylate legs like a spider. It gives rise to seven important classes of cofactors which differ in the degree of oxidation and in the type of metal ion and which also reflect the changeover from a surface metabolism to a membrane/cytosol metabolism. Siroheme still has eight surface-bonding carboxylate groups. They remain surface bound for a long time. Coenzyme F_{430} is used in the methanogenic energy flow of the archaeobacteria (46, 110). It still has five surface-bonding carboxylate groups, while three others are modified so as to be nonbonding. The pathway to vitamin B_{12} (33, 132), a universal coenzyme, proceeds from cobyrinic acid with seven carboxylate groups by a sequence of reactions in which one carboxylate group after the other is rendered nonbonding by conversion to carboxamide groups. The heme pathway proceeds through coproporphyrinogene still sitting with four carboxylate groups flat on the surface to protoporphyrine with a side-on surface bonding by means of only two carboxylate groups. From here, the pathway radiates into a variety of hemes, which are cofactors of the cytochromes in electron transport chains. They all still have two carboxylate groups at one side, a vestige of their surface-bonding origins. In another branch pathway, restricted to the eubacteria, the last two carboxylate groups are removed and membrane-anchoring farnesyl (C_{15}) or phytol (C_{20}) rests are attached for producing the (bacte-

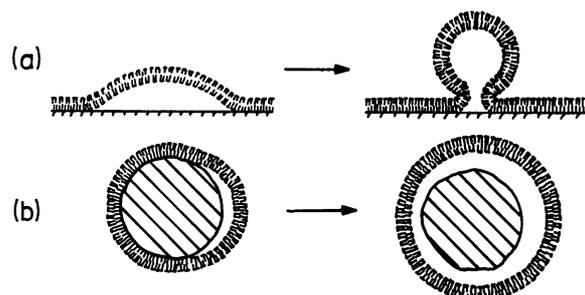


FIG. 9. Cellularization by (a) abstriction and (b) circumdetachment and mineral inclusion.

rio)chlorophylls. It is proposed that siroheme, the hemes, F_{430} , and vitamin B_{12} are all evolutionary successors of surface-bonded functional precursors. Only the (bacterio)chlorophylls appear to be of strictly cellular origin in the eubacterial tree, which speaks for a late arrival of (bacterio)chlorophyll-implemented photosynthesis.

Evolution of Cellular Organisms

The metabolic hybrid stage of the semicellular organisms allows a gradual nondisruptive changeover from a strictly surface-bound metabolism to a strictly cellular metabolism. New features of the membrane/cytosol metabolism can emerge while the surface-metabolic features remain unimpaired. In this way, all multistep biochemical pathways can be liberated from the surface one step at a time. This long semicellular evolution is the breeding ground for all of the basic features with which we are familiar in extant cellular forms of life. The genetic machinery, enzymes, membrane pumps, and electron transport chains are all in place before any true cells devoid of mineral support arise.

I discuss two idealized limiting models of cellularization. First, consider the situation of a large flat surface covered by a coherent lipid membrane. The semicellular structures are distributed over the surface like blisters covered by the lipid membrane overlayer. The generation and accumulation of additional lipid molecules cause an expansion of the membrane of the semicellular structures and, aided by osmosis, the blisters rise. By the same token, as more and more surface-bonded hydrophilic constituents are lifted into the cytosol, the patch of hydrophilic constituents shrinks. Eventually, this process leads to an abstriction of a closed cell (Fig. 9a).

For explaining the second model, begin with the idealized assumption of a mineral grain with a spherical surface completely covered by a partly surface-bonded lipid membrane envelope (Fig. 9b). Therefore, a closed cellular membrane envelope exists before the appearance of a mineral-independent true cell. In every other respect, the situation is the same as in the first model, including the blisterlike semicellular structures covered by the lipid membrane blanket. The formation of a cellular structure is connected with an extension of the lipid biosynthesis pathway. At the end of the lipid pathway, strongly surface-bonding anionic foot groups are converted into nonbonding dipolar ionic or non-ionic groups or into weakly surface-bonding monoanionic groups which allow surface detachment of the membrane. If the rate of these foot group conversions is comparable to the overall rate of lipid synthesis, a cellular structure is formed by abstriction as on a flat surface (Fig. 9a). If, however, the rate of foot group conversion is higher than that of overall

lipid synthesis, the semicellular structure grows larger and larger, until it forms a loose envelope surrounding the entire mineral grain, which stays as a mineral inclusion inside the cell (Fig. 9b). The internal mineral grain disappears much later. This model of circumdetachment and mineral inclusion has a number of remarkable aspects. (i) The transition from a semicellular structure to a true cell is a gradual process, without disruption and without discontinuity. (ii) The mineral grain inside the cell continues to provide a bonding surface for surface-metabolic reactions, even after the formation of a cell envelope. Such a hybrid metabolism could still be at work in undiscovered primitive bacteria. (iii) The mineral grain inside the cell may even grow by the metabolism and facilitate cell division by itself becoming divided between daughter cells.

This process of cellularization establishes true cellular individuation and the physical basis for a more complex notional organism/environment dichotomy. The organism is now of a composite nature, since the inside reaction milieu which includes the internal mineral support is part of the organism and largely controlled and even produced by the organism. The inside mineral support is later fully substituted by an internal membrane/protein support. The outside environment is now the true environment, and it is again subject to the notional distinction between the near environment (ambience), which is influenced by the cellular organism, and the far environment (surroundings), which is not influenced by the organism. Due to the closed nature of cellular organisms, it is now possible that the ambience of a cellular organism contains other distinct cellular organisms. This establishes the physical basis for the notions of organismic interference and the ecological relations of competition, predation, parasitism, and symbiosis.

Woese's Three Kingdoms

I now show how the theory of a transition from a surface metabolism to a true cellular metabolism can help to explain the emergence of Woese's three kingdoms of cellular life: the archaeobacteria, the eubacteria, and the eucaryotes (69, 73, 163).

So far, only one kind of lipid, the isoprenoid ether lipids of archaeobacteria, have been discussed. These are seen as the vanguards of lipophilic metabolism. The intermediates of the chain elongation steps of this pathway (Fig. 5) are all surface-bonded lipids and capable of lipophilizing the mineral surface and forming mineral-supported membranes so that every biosynthetic and evolutionary precursor in this pathway is also a functional precursor. There is, however, another major lipid pathway, the fatty acid lipid pathway. It is composed of a multitude of chain extensions by C_2 increments. This pathway is seen as emerging later and within the isoprenoid semicellular structures for the following reasons. (i) It requires malonyl-CoA, which is produced by the carboxylation of acetyl-CoA. This reaction is dependent on biotin, which must be a late postenzymatic coenzyme as evidenced by its nonanionic structure and its non-surface-bonding biosynthesis. (ii) The growing fatty acid chain stays attached through a thioester group to an acyl carrier protein. Therefore, the biosynthetic and evolutionary precursors of the fatty lipids cannot be their functional precursors, and the final stage of lipid formation is functionally discontinuous. Only after their chain length goes up to the length of the isoprenoid chains (C_{16-18}) do the CoA-bonded fatty acyl rests become functional by forming phosphoglycerol esters capable of membrane formation. It is

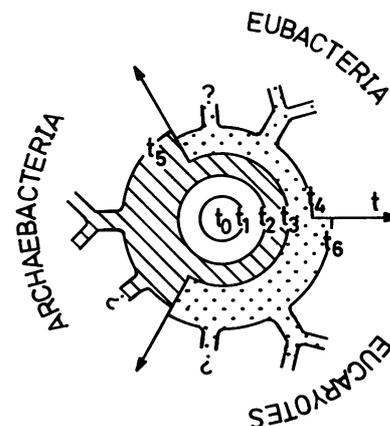


FIG. 10. Kandler-Bernal diagram of the stem of the tree of life. The hatched area represents the semicellular organisms with isoprenoid lipid membranes, and the dotted area represents those with fatty lipid membranes.

proposed that this reaction originates with the assistance of the preexisting isoprenoid lipid membrane.

It is a well-established fact of lipid membrane chemistry (125) that lipids with sufficiently different structures will not mix within one single liquid crystal membrane. So at least in their surface-bonded state, the linear fatty ester lipids and the highly branched isoprenoid ether lipids, which have even sterically different glycerol moieties (75, 82), should have the inherent tendency to segregate into two separate membrane domains. The fatty ester lipids first segregate in the form of patches within a continuous isoprenoid membrane domain. After reaching a certain size, the fatty membrane patches also form blankets above the hydrophilic domains of semicellular structures. In this fashion, the fatty lipid membrane segregation is the evolutionary precursor and also the cause of the first instance of speciation in the history of life. The new species of semicellular fatty lipid organisms arise directly from semicellular isoprenoid lipid organisms. This hypothesis explains why, in extant organisms, these two lipid types never occur in admixture even though within each domain the membranes show a great diversity of lipids. By contrast to the explanation in terms of a semicellular stage, the alternative view of a speciation at the true cellular level has to postulate intermediate stages of cell membranes with isoprenoid-fatty lipid mixtures, and it cannot explain the absence of such lipid mixtures in today's cellular forms.

This semicellular segregation gives rise to the first process of biochemical isolation (Fig. 10). The diagram of Fig. 10 combines O. Kandler's idea of a circular representation of the three cellular kingdoms (70, 72) and C. D. Bernal's idea of a concentric evolution of the central metabolism (7). It is therefore called the Kandler-Bernal diagram. It is oversimplified by failing to reflect the organismal varieties that derive from environmental diversity. The arrow of time t is oriented radially and the origin of life is denoted by t_0 . Major metabolic innovations are marked by concentric circles. The time period between t_0 and t_1 is the era of hydrophilic surface organisms which are at first bonded by carboxylate groups alone (carboxypeds) and later also by phosphate groups (phosphorypeds). The circle t_1 marks the appearance of the first isoprenoid lipid membranes and of a membrane metabolism, t_2 is the advent of semicellular isoprenoid lipid organisms, and t_3 is the segregation of a fatty lipid membrane domain with semicellular fatty lipid organisms. All features

which appear before t_3 are universal for all forms of life. After t_3 , which constitutes a quasi-branch point, the diagram is divided into two sectors which constitute the above-defined two quasi-branches of semicellular organisms. It is a quasi-branch point, since most biochemical features are still shared universally, differences existing at first only with respect to the two incompatible lipid membranes.

For understanding the process of biochemical speciation, we remember that, in the second model, the semicellular organisms are supported by discrete mineral grains. They are in a state of frequent fusion and fission, which prevents individuation by biochemical isolation. With the appearance of a semicellular fatty lipid domain, biochemical isolation sets in by the relative infrequency of fusions between isoprenoid and fatty lipid organisms. Surface-bonded constituents are not any longer easily transferred between the species and tend to remain isolated within their domain. This holds, for example, for the methanogenic cycle (see reference 67) with its peculiar polyanionic coenzymes, which is surface bonded and isolated within the isoprenoid lipid domain. It also holds for the Calvin-Benson cycle or the alternative Kandler cycle (71) with its phosphorylated constituents, which remains isolated after its appearance in the fatty lipid domain. The interspecies transfer of such complex surface-bonded autocatalytic cycles would be inheritable if it could happen, but its occurrence is very unlikely. On the other hand, the transfer of membrane-bonded constituents is still quite likely, but not inheritable. Surface-bonded nucleic acids cannot easily transgress. Their subsequent cytosol-borne versions, however, the protein-bonded nucleic acids, can easily transgress across the domain barrier, and such transgression always constitutes an inheritable acquisition. Most interspecies transfer is of this nature. Of course, some transferred and inheritable genes may have gene products which are inoperative in the host species, notably, if the host species is already devoid of an internal mineral grain, while the operation of the gene product still is dependent on a mineral surface. This means that the first true cellularization by the abandonment of an internal mineral support is a most effective isolation against the infiltration of biochemical (and genetic) innovations which arise in and are still dependent on a mineral-supported metabolism.

These are some of the main principles which result in the peculiar distribution of biochemical features among the three kingdoms of extant organisms. Some are universal, others are found only in one kingdom, and many are found in two of the kingdoms but not in the third. The central features (up to an almost perfected apparatus of translation) are universal, either because they are the invention of the semicellular isoprenoid lipid organisms before the advent of a fatty lipid domain at t_3 or because they transgress across the domain barriers before the first formation of true mineral-independent cells at t_4 . The eubacteria seem to be the first true cellular organisms to become free of an internal mineral support at t_4 . The archaeobacteria are the next to free themselves from the surface at t_5 , and the eucaryotes seem to be the last at t_6 . Such a sequence of events can explain the great similarity between the eucaryotes and the archaeobacteria. It is due to the long mineral surface neighborhood of their semicellular ancestors. The appearance of a nucleus, which later isolates the eucaryotes from the other kingdoms, may well be dependent on both the presence of the mineral support and some special physicochemical properties of the fatty lipid membrane. This would mean that the appearance of a nucleus is impossible in the eubacteria due to the absence of a mineral support and that it is impossible in the

archaeobacteria due to the physicochemical properties of the isoprenoid lipid membranes. The abandonment of the mineral support may occur in any of the three domains more than once (in different environments and at different times). For example, in the isoprenoid domain, a first mineral abandonment may give rise to the branch of the methanogens, while a later, second mineral abandonment may give rise to the branch of the sulfur-dependent thermoacidophiles, which would account for the fact that their similarity to the eucaryotes is greater than that of the methanogens.

The next waves of speciation are caused by the appearance of reinforcements for stabilizing the self-supporting cell envelope. Three basic reinforcement strategies can be discerned which all may get started in the semicellular organisms: (i) the strengthening of the membrane itself by lipid reinforcement; (ii) the advent of an additional outer reinforcement, a coat of mail; and (iii) the advent of an inner reinforcement, a cytoplasmic endoskeleton.

The first strategy is displayed by the extreme thermophiles among the archaeobacteria and among the eubacteria by the thermophilic *Thermotoga maritima* discovered by K. Stetter (62). In both cases, the membranes consist partly of macrocyclic lipids, either macrocyclic bis-glycerol tetraether isoprenoid lipids or macrocyclic bis-glycerol tetraether fatty lipids (26, 62, 82). They function as tie bars for strengthening the bilayer membrane or, if they predominate, they form a strong monolayer membrane. Both types of reinforcements arise perhaps by the same enzyme for tail-to-tail connection which can move or transgress across the membrane domain barrier. They make possible a cellularization at high temperatures. The second strategy is displayed by the murein cell walls of the eubacteria and the somewhat similar pseudomurein cell walls of the methanogenic archaeobacteria (72, 128). It is not unlikely that both go back to a common biochemical precursor which is invented in one of the two domains and later transgresses into the other. Other independently invented versions of this second strategy (outer reinforcements by S-layer proteins, etc.) arise in the archaeobacteria. The third strategy is displayed by the eucaryotes and perhaps by the archaeobacteria (133). It is a strategy of reinforcement by internal chords. It has the important windfall profits of (probably fatty ester lipid-dependent) mechanisms of cell division and phagocytosis of the eucaryotic cells.

The appearance of these different reinforcement strategies is decisive for the branching order close to the stem of the tree of life. Additional cell membrane reinforcements cause additional barriers against the fusion of cells and against the infiltration of alien biochemical features. However, none of these strategies leads to complete isolation as evidenced by gene transfer through viral infections, sexual processes, and endosymbiosis.

FLOW OF ENERGY AND NUTRIENTS

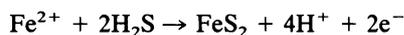
First Energy Source for Life

The surface metabolists are situated as an intermediate stage within an energy flow which is fueled by a specific source of chemical energy and which produces not only the reducing equivalents for carbon fixation, but also the positively charged mineral surface for surface bonding.

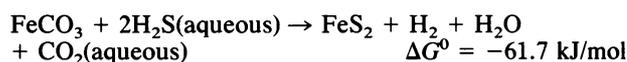
The surface organism is an open-flow system with an input of energy and inorganic nutrients from the surrounding water phase and with an output of detached organic constituents which become dissolved and decomposed in the water phase. The output reactions are irreversible, while the

interconversions of the surface-bonded constituents are close to the thermodynamic equilibrium. Therefore, while the thermodynamics of the overall system favors the formation of small products of decay, the partial thermodynamics on the surface can favor large polyanionically surface-bonded organic constituents.

As to the input reactions, note that in the proposed overall scenario the surface organism is autotrophic, feeding on carbon dioxide (or carbon monoxide). These, however, can only be assimilated by a redox reaction with a source of reducing equivalents. Hydrogen (H_2) can be excluded as the first source of electrons since its reducing potential is not sufficient for reducing CO_2 , CO , or $-COO^-$. It has been suggested elsewhere (150) that a plausible source of electrons for the first organisms is the formation of pyrite from hydrogen sulfide and ferrous ions



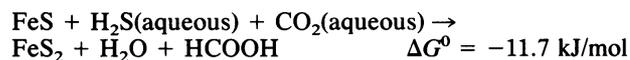
The reaction is driven by the insolubility of pyrite, which does not dissolve even in hot hydrochloric acid. It is highly exergonic as evidenced by the following thermodynamic calculations from the data of free energy of formation (156) for the generation of hydrogen:



For comparison, the standard free energy (ΔG^0) of the endergonic hydrogenation of carbon dioxide is given



By simple summation, it can be seen that the overall reaction of carbon fixation by pyrite formation with a linear electron flow from H_2S to CO_2 has a negative free energy and is therefore thermodynamically feasible



This proposal is in agreement with the knowledge we have of early geochemistry. Hydrogen sulfide is and always has been abundantly available in the exhalations of the earth, and ferrous ions are ubiquitous. Pyrite formation requires anaerobic conditions, which is compatible with the absence of oxygen from the early earth. Pyrite is a ubiquitous mineral. It is found in the oldest sediments and is a faithful companion of kerogen. In the history of the earth, the formation of pyrite with the concomitant origin of life could have commenced with the first formation of liquid water, either within a rapidly forming oceanic body of water (36) or, in Woese's scenario (160), within atmospheric droplets of water condensed around floating dust grains.

It is important to note that pyrite, the end product of the proposed aboriginal energy source of life, is a mineral with a positively charged surface. It shares this property with other heavy-metal sulfides, a fact which has found large-scale industrial application in ore flotation technology (131). On the other hand, the first product of the fixation of carbon dioxide is an anionic carboxylate group. This means that the primary products of carbon fixation do not enter the water phase. They acquire their ionic surface bonding in *statu nascendi*. They accumulate on the growing crystals of pyrite and function as constituents of the subsequent surface-metabolic rearrangements. Therefore, the surface organisms are engaged in the generation of not only more surface-

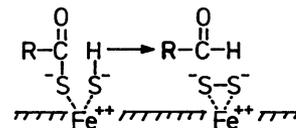


FIG. 11. Schematic mechanism of the reduction of a carboxylic acid by pyrite formation via a thioacid.

bonded organic constituents, but also the mineral support for these constituents by the dumping of pyrite.

The proposed carbon fixation by pyrite formation is thermodynamically possible but mechanistically obscure. A few general characterizations of a possible reaction mechanism can be given, however. From the point of view of an organism, the environment is the source of nutrients and the sink for waste products. From the point of view of the whole system, however, the organism is a catalyst for an electron flow. The very first surface organism can be characterized as a catalyst for speeding up the formation of pyrite by providing a catalytic pathway for the flow of electrons from hydrogen sulfide to carbon dioxide. This means that, without the involvement of a surface organism, the formation of pyrite may be somewhat inhibited. Organisms are also autocatalytic. They catalyze their own reproduction. The automatic formation of surface bonding $-COO^-$ groups by this CO_2 fixation suggests that carboxylate surface bonders may predate the phosphate surface bonders. Their metabolism has been shown to evolve by the compounding of more and more autocatalytic cycles. It is, therefore, suggested that the first pyrite-forming surface organism is based on a redox reaction with a single autocatalytic cycle in which a surface-bonded organic product of CO_2 fixation is catalytic for the formation of pyrite and autocatalytic for its own (re)production.

Hydrogen sulfide is a well-known agent for the reduction of a carbonyl group of diketones by formation of sulfur (92). This reaction seems to be initiated by the nucleophilic attack of H_2S or HS^- at the carbonyl carbon atom, followed by a subsequent nucleophilic substitution of the thiol group by a hydride ion. A similar mechanism of nucleophilic catalysis (Fig. 11) is plausible for CO_2 fixation and carboxylate reduction whereby, however, the much lower reactivities of CO_2 and $-COO^-$ require an augmentation of the reducing power of H_2S by the presence of ferrous ions which scavenge the oxidation products (S_2^{2-}). In such reactions, hydrogen sulfide serves not only as the electron donor, but also as a nucleophilic catalyst. It is of interest that the intermediates in such a reaction, the thiocarboxylate groups ($-COS^-$) are surface bonders relative to the surfaces of pyrite and other heavy-metal sulfides (131).

Minor Nutrients

So far, I have discussed the metabolism of the first surface organism as an autocatalytic reaction involving the elements C, H, O, Fe, and S. Now I turn to the additional nutrients N and P and the trace metal ions. It cannot be excluded that some of these elements are involved already in the very origin of the first surface organism.

Attempts to correlate the distinction between biocatalytic metal ions and nonfunctional or even poisonous metal ions with their abundancies in ocean water (29, 30) have failed. Cupric ions, for example, are as abundant in ocean water as ferrous ions, yet they seem to play no role in anaerobic organisms (63). The present theory suggests a simple answer. In a solution rich in H_2S and under alkaline conditions,

all heavy-metal ions precipitate as insoluble sulfides. The solubility of these sulfides, however, is pH dependent, and in a somewhat acidic H_2S solution, the metal ions are segregated into a first group (Mn^{2+} , Fe^{2+} , Ni^{2+} , Co^{2+} , and Zn^{2+}) whose sulfides stay in solution due to a high-solubility product constant ranging from 1×10^{-11} to 8×10^{-25} and a second group (Cd^{2+} , Pb^{2+} , Cu^{2+} , Ag^+ , Hg^{2+} , Sn^{2+} , As^{3+} , Sb^{3+} , and Bi^{3+}) whose sulfides have extremely low-solubility product constants ranging from 7×10^{-27} to 1×10^{-96} (47). Due to the acidic nature of the exhalations, the first liquid water should have a moderately acidic pH. Under such conditions, metal ions of the first group remain somewhat dissolved in the presence of H_2S and available for catalytic activity in the surface metabolism. The metal ions of the second group, however, are scavenged by sulfide precipitation. They cannot acquire a role in the surface metabolism and turn into universal poisons.

The insolubility of calcium phosphate and the concomitant low abundance of dissolved phosphate ions pose a major difficulty for all versions of the prebiotic broth theory. It is hard to understand how phosphorylated organic compounds can form in a broth scavenged of soluble phosphate by omnipresent calcium ions and how any brothborne first organism could survive for long under conditions of phosphate starvation. In the surface-metabolic scenario, this problem finds a simple solution, if it is assumed that the surface metabolism is attached to a phosphate mineral or to a mineral with adsorbed phosphate ions. The first function of phosphorylation is surface bonding, and for this function dissolved phosphate ions are not required. Other phosphate functions, such as energy coupling by ATP and linkage in nucleic acid backbones, are seen as late-coming windfall profits of the primeval surface-bonding function of the phosphate groups. Incidentally, an Fe^{2+} -phosphate mineral leads necessarily to a liberation of phosphate ions by its conversion to pyrite and it is, therefore, a most promising candidate for tests.

Turning finally to the source of organic nitrogen, it is proposed that, early on, the surface organism is engaged in nitrogen fixation. Archaeobacterial and eubacterial nitrogenases have a high degree of homology (138) which indicates antiquity. They are predominantly anionic and have iron-sulfur centers with or without (14; P. E. Bishop, P. Prekumar, R. D. Joergen, M. R. Jacobson, D. A. Dalton, J. R. Chisnell, and E. D. Wolfinger, in H. Bothe and W. E. Newton, ed., *Proceedings of the 7th International Symposium on N_2 Fixation*, in press) an additional involvement of Mo or V. In the context of the surface-metabolic theory, the aboriginal nitrogen fixation cannot be an ammonia-forming hydrogenation. Since free ammonia is highly water soluble, it instantly vanishes if formed by the precellular surface metabolism. This suggests a nitrogen fixation with a direct formation of constituents with carbon-bonded nitrogen, and perhaps the earliest modes of CO_2 and N_2 fixation originate as a joint process catalyzed by a pyrite-forming iron-sulfur cluster.

Ferredoxins and CoA

The proposed origin of life in a pyrite-driven energy flow can be linked up with two of the most pervasive features of extant metabolism, the universal involvement of ferredoxins and other iron sulfur proteins in most electron transport chains and the central role of cysteinyl rests and CoA in numerous metabolic processes.

Sulfhydryl ions (SH^-) are among the strongest nucleophiles. Therefore, under H_2S -rich conditions, there is a

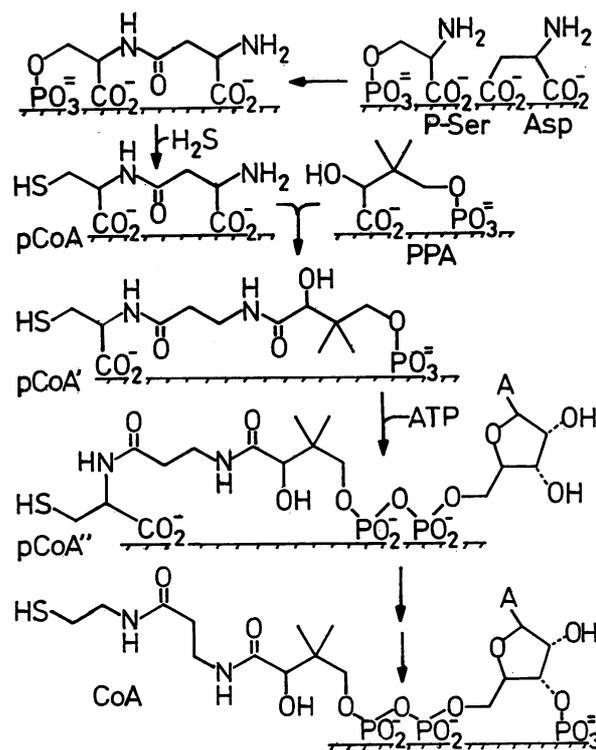


FIG. 12. Hypothetical surface-bonded precursor pathway to CoA from P-Ser, Asp, and phosphopantoic acid (PPA) via proto-CoA compounds pCoA, pCoA', and pCoA''.

certain propensity for the substitution of organic phosphate groups by mercapto groups ($-SH$) (24), which, incidentally, have a surface-bonding capability with respect to heavy-metal sulfides. In this fashion, phosphoserine (P-Ser) is converted to cysteine (Cys) and the phospho-seryl units in surface-bonded oligomeric peptides or isopeptides of P-Ser and Asp are postcondensationally converted into cysteinyl units (Fig. 12). This produces an organic mercapto group which competes with hydrogen sulfide for bonding to ferrous ions. This gives rise to organically bonded iron-sulfur ($Fe-S$) clusters, and with the appearance of anionic oligopeptides with several cysteinyl rests, the first surface-bonded ferredoxins with $Fe-S$ clusters arise. It is remarkable that, to this day, the ferredoxins are short polyanionic polypeptides and that the proposed ancestral sequence of the ferredoxins (43) is made of a set of 11 amino acids which lacks the complicated evolutionary latecomers Met, His, Trp, Tyr, Phe, Leu, Thr, Lys, and Arg. This means that the ancestral ferredoxin sequence is established as a surface-bonded constituent of the surface metabolism and long before the completion of the process of translation. The cysteinyl rests modulate the redox properties of the $Fe-S$ clusters and give rise to redox cycles. In this fashion, ferrous ions and hydrogen sulfide, the sources for pyrite formation, are not only the biosynthetic precursors of the iron-sulfur clusters in ferredoxins, but also their evolutionary functional precursors. In this evolutionary progression, the cycling electron shuttle of the ferredoxins derives from the linear electron flow of pyrite dumping. Later, with the appearance of hydrophobic amino acids, the ferredoxins with $Fe-S$ clusters are lifted off the surface and become cytosol-borne or attached to membranes. This is one of the requisites which prepare the stage for a major changeover of the energy base of life addressed in the next section.

It should be recalled that, in the earliest forms of the surface metabolism, H_2S plays a dual role as an electron source and a nucleophilic agent. In the subsequent evolutionary stages, these two functions of H_2S become segregated. While the redox function is taken over by the ferredoxins, the nucleophilic function is taken over by free cysteinyl groups of surface-bonded (iso)-peptides. In this evolutionary progression, carboxyl activation by thioacid formation is taken over by thioester formation. A variety of such peptides are formed. Much later, their function is taken over by CoA, which arises by a hypothetical precursor pathway such as the one shown in Fig. 12. This evolutionary progression to CoA is driven partly by the selective advantage of an increasing strength of surface bonding through an increasing number of anionic groups and through an additional surface-anchoring capability due to the acquisition of an adenylic moiety capable of base pairing and/or base stacking with a surface-bonded nucleic acid and partly by the selective advantage of an increased perimeter of action by the decarboxylative formation of a long pivotable arm of CoA. It is proposed that the surface metabolism acquires a large family of constituents with CoA-type functions. In the later cellularization and enzymatization of the surface metabolism, some of these precursors (those with peptide structures) become incorporated into the sequences of three-dimensional folded enzymes. They do not turn into coenzymes, but rather directly into enzymes. The surface-bonded CoA-type structures survive the changeover to a cellular metabolism by a conversion of the surface-metabolic pathway to the extant cytosol pathway and either by turning into a true coenzyme (CoA) or by covalent bonding to the seryl rest of a protein (acyl carrier protein). All others become abandoned.

Cellular Flows of Energy and Nutrients

Certain major characteristics of the flow of energy and nutrients in extant organisms can be explained by the transition from an open surface metabolism through semicellular structures to true cellular structures.

Lipid membranes are impermeable not only for inorganic ions, but also for ionic and hydrophilic organic molecules. This poses a serious paradox for all theories of cell formation within a prebiotic broth. In such a scenario, the earliest cellular structures are self-suffocating structures. The present theory is free of such paradoxes, since the nonionic inorganic nutrients H_2O , H_2S , N_2 , CO_2 , CO , and H_2 can pass freely through a lipid membrane. However, the ionic nutrients, notably, phosphate, and ferrous and catalytic metal ions can still be provided by the mineral support of the semicellular structures. This means that the formation of such semicellular structures is not immediately disruptive for nutrition. Later, with the emergence of membrane proteins, the transportation of ferrous (and other) ions through the membrane becomes possible, and the semicellular organisms which still depend on internal mineral support can continue to grow by the internal formation and deposition of pyrite.

The formation of (semi)cellular structures, while not disruptive for nutrition, has most important consequences for the evolution of bioenergetics. Its immediate effect is the formation of a cytosol. Detached organic constituents accumulate and suffer their subsequent reactions within this cellular broth. This marks the beginning of catabolic pathways which characteristically are largely devoid of surface bonders. With this autotrophic catabolism, which arises long

before heterotrophy, two important economizing functions emerge: the salvaging of detached organic constituents (building blocks) and the conservation of energy, which in an open surface metabolism are both wasted. The salvage function leads to a new type of biosynthesis. It is the modular mode of biosynthesis from salvaged building blocks which was mistakenly supposed to explain the origin of life and which, indeed, is typical for the cytosol broth and contrasts with the surface-metabolic mode of biosynthesis by the piecemeal uptake of nutrients and subsequent rearrangements.

The energy conservation function leads to fermentation. It serves as a secondary energy source which merely conserves some of the chemical energy acquired by the primary autotrophic energy source of pyrite formation. In one of these energy-conserving catabolic pathways, phosphoenolpyruvate is converted into pyruvate with a concomitant formation of phosphoanhydrides as in ATP. This marks the origin of fermentative substrate-level phosphorylation. This type of energy conservation gives rise to phosphoanhydride activation. Now the anabolic (biosynthetic) reactions, which are exergonic as long as their constituents remain surface bonded, can become transferred into the cytosol (where they are endergonic) by becoming enzymatically coupled with phosphoanhydride cleavage, first pyrophosphate cleavage and later triphosphate cleavage (irreversible) of ATP and guanosine triphosphate. Moreover, wholly new endergonic modular routes of biosynthesis in the cytosol become possible as a consequence of ATP coupling. This scenario explains why catabolic pathways are not simply the reversal of anabolic pathways. The course of the earliest anabolic pathways is determined by the principles of the surface reaction system, while their earliest catabolic extensions are governed by the laws of solution chemistry.

The evolution of an enzymatic mechanism for coupling endergonic reactions to the exergonic cleavage of phosphoanhydride bonds (ATP) gives rise to a major change in the energy base of life: to the changeover from a reduction solely by the highly reductive Fe^{2+}/H_2S system to an ATP-coupled reduction with the less reductive hydrogen or CO . Without such ATP coupling, the reducing power of hydrogen would be insufficient for the fixation of CO_2 or for the reduction of carboxylate groups. With such coupling, however, the combined exergonic effects of ATP hydrolysis and H_2 oxidation are sufficient for such carbon fixation reactions. Now, NAD(P)H and ferredoxins with a redox potential similar to that of H_2 can also come into play in these reactions. According to a surprising finding by H. Simon, CO has, however, a sufficient reducing power to reduce nonactivated carboxylates (41).

The formation of closed membrane structures has another major effect on bioenergetics. It comes with the establishment of membrane-bound electron transport chains and a mechanism of ATP generation by electron transport phosphorylation. This tertiary source of bioenergy is at first dependent on the hydrogenation of elemental sulfur ($\Delta G^0 = -27.5$ kJ/mol), observed in *Pyrodictium* (140, 142) and *Thermoproteus* (37, 172) species. The available hydrogen is apportioned to (i) direct use as reducing agent of the metabolism and (ii) indirect use for forming ATP (by reaction with sulfur). At first, this sulfur respiration of hydrogen may serve as a supplement for the still operative pyrite-forming energy source, providing it with the required hydrogen sulfide. The pyrite-forming cellular organisms can now venture into areas with hydrogen sulfide starvation but with available hydrogen and sulfur. Later, when the metabolism of these organisms is

no longer dependent on a mineral support, the internal deposition of pyrite is abandoned. Now the cellular organisms can venture into areas with iron starvation, able to live solely on hydrogen and sulfur. This changeover is facilitated by the fact that the redox reaction in the fully enzymatic iron-sulfur clusters is reversible, which allows them to change from a role in the oxidation of H_2S to S_2^{2-} to a role in the reduction of sulfur (through S_2^{2-}) to H_2S .

Next, some H_2 -dependent organisms, e.g., *Archaeoglobus* (141, 143) spp., acquire an independence even from elemental sulfur by a conversion of their electron transport phosphorylation to the use of sulfate ions as terminal electron acceptors ($4\text{H}_2 + \text{H}_2\text{SO}_4 \rightarrow \text{H}_2\text{S} + 4\text{H}_2\text{O}$). This requires merely an extension of the sulfur respiration pathway. Still other H_2 -dependent organisms (autotrophic, of course) acquire an independence from both sulfur and sulfate by the exergonic conversion of CO_2 and H_2 into methane (archaeobacteria) or acetic acid (eubacteria). The methanogens date back to the times when the metabolism still required a support by an internal mineral surface, since most of its coenzymes are polyanionic surface bonders, some with long pivotable arms. They still operate by membrane-bound electron transport phosphorylation (67). With the invention of ferredoxin-dependent reduction with CO (41), other bacteria even become independent from H_2 .

In the evolution of all of these membrane-bound electron transport chains for energy acquisition, the evolution of the tetrapyrrol pathways seems to be of decisive importance. Siroheme, F_{430} , and the various hemes (in cytochromes) arise in conjunction with the unfolding membrane energy generator systems. At the end of this extended system of pathways, the (bacterio)chlorophylls appear. Their lateness is evidenced by their non-surface-bonding structures, their membrane-anchoring lipid chains, and their restriction to the kingdom of eubacteria (and chloroplasts). They open up a totally new quaternary form of energy acquisition by photosynthesis. This new energy source operates not only by the use of a membrane-bound electron transport chain for generating ATP, but also by a light-driven boosting of the reducing power of hydrogen to the redox levels of iron-sulfur proteins. Later, this boosting function is used to boost to the level of NAD(P)^+ the weak reducing potential of H_2S (in the absence of pyrite formation with ferrous ions) and much later even of H_2O . The eubacteria equipped with these new energy generators are now able to venture into hitherto uninhabitable territories devoid of hydrogen (or equivalent reducing agents such as CO).

The evolution of the carbon fixation pathways runs parallel to this evolution of energy sources. The Calvin-Benson cycle is restricted to eubacteria and chloroplasts. It consists, however, strictly of surface-bonding constituents. This means that it emerges in the semicellular ancestors of the eubacteria, perhaps in the form of the Kandler cycle (71). A much older carbon fixation pathway is seen in the reverse or reductive Krebs cycle. It is found in all three kingdoms. It consists strictly of surface-bonding polycarboxylates and operates by the carboxylation of CoA esters. Its origin may go back to the times when CoA was functionally preceded by H_2S , perhaps in the form of a noncyclic portion of the reductive Krebs cycle which is found in certain archaeobacteria (74). It is of interest that ferredoxins are used directly as reducing agents in the reductive Krebs cycle and that the latecoming non-surface-bonding coenzymes (biotin and lipoic acid) arising by non-surface-bonding biosynthetic pathways are not involved.

We have seen that catabolic pathways appear early in

(semi)cellular evolution within strictly autotrophic organisms for salvage purposes. Heterotrophy appears as a wind-fall profit of this preexisting autotrophic catabolism. This conversion from autotrophy to heterotrophy occurs opportunistically whenever biogenic organic compounds are available in the environment. It merely requires the invention of means for importing these organic compounds into the cell. This means that heterotrophy is polyphyletic and inappropriate for the demarcation of major taxons in a natural taxonomy of bacteria, a conclusion supported by Woese's bacterial taxonomy (163) on the basis of 16S ribosomal RNA (rRNA) sequence analysis. It is significant that the oxidative Krebs cycle used by many heterotrophs and which arises partly by a reversal of the direction of reactions requires the non-surface-bonding lipoic acid as cofactor.

With this result, we have reached a complete reversal of the prebiotic broth theory. Heterotrophy is not at the origin of life but arises as a rather late extension of the catabolic salvage pathways which originate in strictly autotrophic organisms.

EVOLUTION OF THE GENETIC MACHINERY

Tribonucleic Acid (TNA) with Catalytic Imidazole Bases

In the preceding sections, a model for the stepwise evolution of cellular organization and bioenergetics has been presented. It assumes a coevolution of more and more sophisticated three-dimensional folded enzymes and of a machinery for the replication and translation of nucleic acids. The theory of a transition from surface organisms to cellular organisms suggests a step-by-step evolution of the genetic machinery and explains some of its most peculiar facets. I shall use the phrase "by an unknown mechanism" several times, but these as yet unknown mechanisms do not seem to be riddles beyond experimental resolution.

In modern versions of the Oparin-Haldane theory, the first "living organisms" are seen as self-replicating "living" RNA molecules (see references 31, 32, 45, 79-81, 104, 105, 107, 152). The "prebiotic broth" is somehow endowed with a content of the purine bases adenine (A) and guanine (G), the pyrimidine bases uracil (U) and cytosine (C), and many other heterocycles, with ribose and many other sugars, and with polyphosphates. These are seen as building blocks that combine by modular modes of synthesis, first to nucleosides and then to phosphorylated nucleotides (e.g., ATP), which somehow polymerize to a large variety of nucleic acids. Selection is seen as beginning with an autocatalytic template-directed polymerization and leading somehow to the exclusive utilization of A, U, G, C, and D-ribose. (Incidentally, Cairns-Smith's theory [11] of replicating clay organisms is an inorganic analogy of organic templating within a prebiotic broth.)

The surface-metabolic theory suggests a radically different course of events. The early surface organisms with autocatalytic metabolic pathways do not depend on any form of template copying. They grow, reproduce, and evolve before the advent of a genetic machinery. A reconstruction of the evolution of the genetic machinery from earliest surface-metabolic precursors can be guided by the study of extant pathways in conjunction with Florkin's version (38) of the biogenetic law and the following four principles of pathway modification: (i) the earliest pathways are strictly surface bonding; (ii) in the earliest pathways, phosphate groups have essentially a surface-bonding function (activated phosphoanhydrides are latecomers); (iii) The earliest pathways

do not operate by a modular mode but rather by a piecemeal buildup through the acquisition of inorganic nutrients and subsequent rearrangements; and (iv) The pathways evolve from linear extension into autocatalytic cycles as has been explained for the coenzymes.

As a point of departure, we assume that, in an early stage of surface-metabolic evolution, surface-bonded phosphorylated C_3 units (notably phospho-trioses [Fig. 1]) arise by an unknown carbon fixation mechanism through thio acid activation. The phosphotrioses accumulate on the surface by forming thermodynamically stable surface-bonded polymer structures (Fig. 1). The covalent backbone consists of intermolecular (quasi-intramolecular) hemiacetal bonds, and the side groups (legs) carry the surface-bonding phosphate groups. These polymers have the selective advantage of polyanionic surface bonding. They are in a state of dynamic equilibrium with rapid rearrangements by the breaking and remaking of hemiacetal bonds. (Of course, a variety of such structures may coexist.) They will be called by the generic name phosphotribose, since their triose units are not only the biosynthetic but also the functional evolutionary precursors of ribose and it is argued that this polyhemiacetal structure is the evolutionary precursor of the backbone of nucleic acids.

Turning now to the bases, the extant nucleic acids contain purine and pyrimidine bases. By application of the principles of modification, it can be shown that the pyrimidines are evolutionary latecomers (149), for the de novo pyrimidine pathway is a modular pathway with the prefabrication of a non-surface-bonding pyrimidine (orotic acid) from a non-surface-bonding precursor (dihydroorotic acid) and subsequent ribosylation. This means that the de novo pyrimidine pathway is a latecomer in the semicellular phase of evolution (perhaps together with the purine and pyrimidine salvage pathways). On the other hand, even the modern de novo purine pathway is not a modular pathway but operates by the piecemeal erection of the purine ring system onto a phosphoribosyl ring. However, the modern de novo purine pathway is a cytosol pathway, with modifications due to the process of lifting off the surface, as evidenced by the requirement for phosphoanhydride activation (PRPP, ATP, and guanosine triphosphate) and the use of non-surface-bonding glycine. Moreover, the modern pathway involves two steps in which C_1 units of the purine ring are introduced by THF, itself a product of a lengthy and certainly late extension of the purine pathway. This overall pattern bespeaks the origin of this modern pathway in an ancient surface-metabolic precursor pathway. The modern de novo purine pathway must have a long history, and I will try to reconstruct its precursor pathway which, in fact, can be traced back to the times of surface metabolism.

In the following hypothetical reconstruction (Fig. 13) of the evolution of the earliest de novo purine pathway, it is assumed that some intermediates are analogous to those in the modern pathway and that the purine ring structure is erected in piecemeal fashion as today, not onto a ribose ring structure but rather onto a surface-bonded phosphotribose structure (Fig. 1).

It is well known that carbonyl compounds form stable Schiff bases with amino compounds. Therefore, the surface-bonded phosphotribose structures function as traps for the incorporation and accumulation of the amino units, which are generated by the earliest process of nitrogen fixation (see preceding section). This process replaces divalent oxygen atoms by trivalent nitrogen atoms, which means that the phosphotribose structure is transformed into a structure

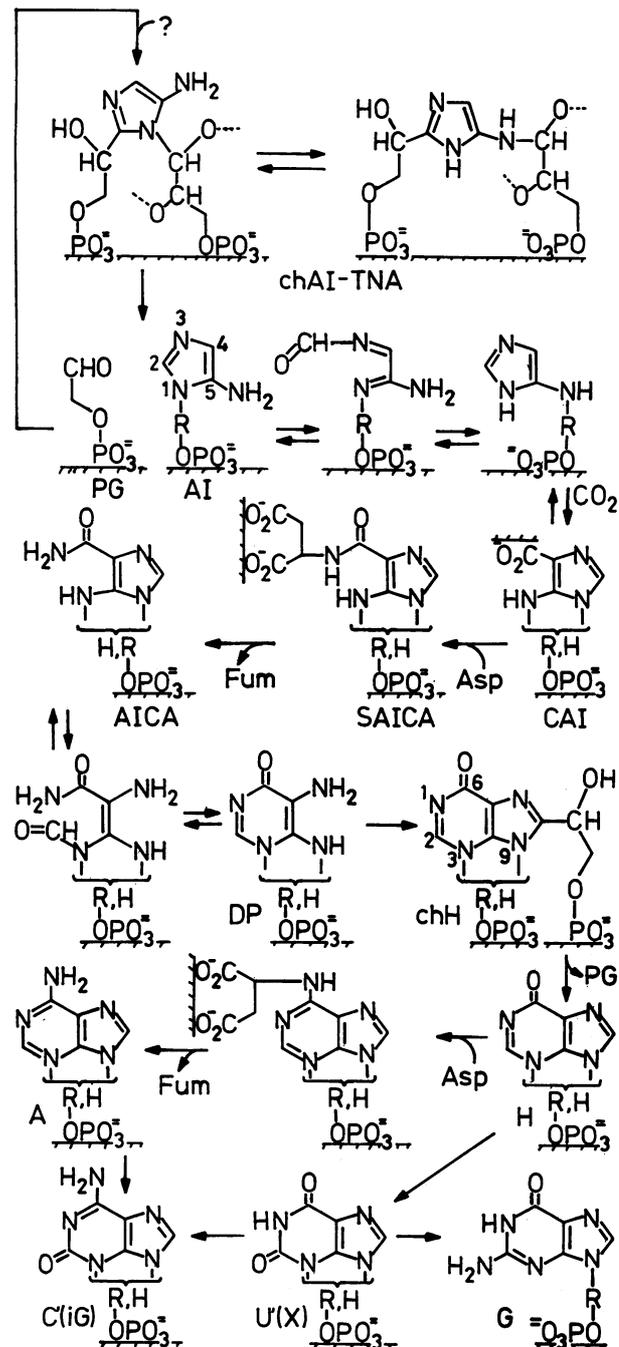


FIG. 13. Hypothetical surface-bonded de novo purine pathway producing 3- and 9-bonded purines. chAI, Charged aminoimidazole base; PG, phosphoglycol aldehyde; CAI, carboxyaminoimidazole; SAICA, succino-aminoimidazole carboxamide; AICA, carboxamide derivative; Fum, fumaric acid; iG, isoguanine.

wherein the nitrogen sites are additional branching sites. The nature of these branches is likely to vary. One of those branch types, the imidazole structure, is pivotal in the purine pathway. Imidazole and thiazole are analogous heterocycles with analogous reactivities. Therefore, we can assume that the first imidazole structures arise by a rearrangement pathway which is a sibling to the formation of charged thiazole structures (Fig. 4). It is further assumed that these imidazole rings become glycosidically bonded onto a phosphotribose in

statu nascendi. Structures of this kind with a phosphotribose backbone and glycosidically bonded imidazole (and later purine) bases will be called TNA. The first one (Fig. 13) has a "charged" aminoimidazole base akin to the charged thiazole in Fig. 4. This charged aminoimidazole base has a low but significant propensity for cleaving off its phosphoglycol aldehyde unit, thereby producing an uncharged aminoimidazole base with a C₁ unit in position 2. The similarity between imidazoles and thiazoles with respect to this reaction type has been demonstrated experimentally (9). The severed phosphoglycol aldehyde unit is recycled and utilized in the formation of a new charged aminoimidazole unit, giving rise to the carbon atoms in positions 4 and 5. (The reaction is driven by the aromatization energy of the imidazole ring.) This means that the imidazole rings arise by autocatalytic rearrangements within a surface-bonded phosphotribose structure. For the next step, we can appeal to the most important and far-sighted results of G. Shaw (22), who, among other things, has shown that 1-cyclohexyl-5-aminoimidazole undergoes a Dimroth rearrangement, a so far little noticed reaction type which, however, plays a decisive role in the surface metabolism and indeed in the origin of life. The same facile Dimroth rearrangement has been more recently shown for the 1-ribosyl-5-aminoimidazole even at neutral pH and room temperature (M. P. Groziak, B. Bhat, and N. J. Leonard, Proc. Natl. Acad. Sci. USA, in press). This means that, in a TNA, the amino-imidazole bases undergo the same rearrangement and are therefore glycosidically bonded by either the endocyclic N-atom or the exocyclic N-atom. Both isomers are interconvertible via an open-chain amidine structure so that both always coexist. A similar rearrangement may occur at the level of charged aminoimidazole. It can be assumed that all later constituents in the pathway are mixtures of isomers which go back to these two *exo/endo* isomers.

In these earliest TNA structures, the bases consist of imidazole rings which do not have a significant base-pairing function. Rather, their functions are strictly catalytic, operating as acid-base catalysts, as group (e.g. phosphate) transfer catalysts, and by sequestering metal ions. Notably in the *exo*-bonded form, they are surface-metabolic functional precursors of the histidyl rests in extant enzymes and they are autocatalytic, promoting some of the earlier steps in their own biosynthesis.

All-Purine TNA

In the further evolution, the (*exo*- and *endo*-bonded) imidazole bases are modified and stabilized in sibling pathways which eventually lead to purine structures. As a first step in this development, the introduction of a surface-bonding carboxyl group by CO₂ addition has been proven by Shaw (21) to proceed nonenzymatically to carboxyaminoimidazole. The subsequent amide formation from carboxyaminoimidazole and Asp does not (as in the modern pathway) require ATP activation, since it occurs in a surface-bonded condition. It produces a succinoylaminoimidazole carboxamide with an increased number of surface-bonding groups. The carboxamide derivative is produced next by cleavage of surface-bonded fumaric acid, as today. In the modern pathway, the C-2 in the purine ring arises by formylation of aminoimidazole carboxamide with a formyl-THF. Thus, this reaction must also have a precursor. We can assume that this precursor reaction involves another Dimroth rearrangement. This time, however (in contrast to the Dimroth rearrangement of aminoimidazole), the imidazole ring is opened

between C-2 and N-3 due to the resonance effect of the carboxamide group. An analog to this ring opening is found in the extant biosynthetic pathway to the pterines (111). After rotation about C-4 to C-5, a diaminopyrimidone is formed. Eventually, a sibling to the first reaction in the pathway produces a charged hypoxanthine ring, the first purine structure in the pathway. The severing of a phosphoglycolaldehyde unit produces a hypoxanthine base which is bonded to the backbone of TNA at either N-3 or N-9.

I now discuss the modification of the purines. Hypoxanthine (H) bonded at N-9 is the first stable product of the pathway, while H bonded at N-3 has an unstable structure and stabilizes by oxidation in position 2. The resulting N-3-bonded xanthine (X) is isoelectronic and isogeometric with uracil (U) and will be denoted by U'. The adenine (A) base is formed by a condensation of Asp in position 6 of the purine ring and subsequent cleavage of fumaric acid as a sibling to the conversion from carboxyaminoimidazole to aminoimidazole carboxamide. The N-9-bonded adenine is a highly resonance-stabilized end product. However, N-3-bonded adenine (94) is not highly stabilized and will undergo oxidation to the resonance-stabilized N-3-bonded isoguanine, which is isogeometric and isoelectronic with cytosine (C) and which will therefore be denoted by C'. Later, by another branch reaction, N-9-bonded H is oxidized to N-9-bonded xanthine. Still later, the amination of N-9-bonded xanthine produces N-9-bonded guanine (G). (Perhaps sibling aminations will convert N3-bonded xanthine to N-3-bonded isoguanine and N-9-bonded xanthine to N-9-bonded isoguanine.)

In this fashion, a variety of all-purine TNA structures come into existence, some purines being bonded at N-9 and others at N-3. The purines, notably the N-3-bonded ones, have catalytic imidazole functions. This TNA structure may be more stable than DNA, since its covalent backbone does not depend on phosphodiester bridge groups which are sensitive to hydrolysis and since reactive hydroxyl groups are absent.

With the formation of these purine bases, an entirely new functional and structural feature emerges. It is the base pairing between the purine bases of two strands of TNA and the base stacking between the purine base pairs. An example of a double-stranded structure with alternating ketotriose and aldotriose units and antiparallel strands is shown in Fig. 14. Such a structure has a high stability due to optimum base pairing and base stacking of the all-purine base pairs. The purine spacing turns out to be ideal for a high stacking energy. Further, the TNA structure is ideal for accommodating the all-purine base pairs (Fig. 15), which had been postulated earlier (149). It was then expected that these base pairs, which do not seem to fit into a standard RNA configuration, require a more accommodating precursor backbone structure. Unexpectedly, the here-postulated TNA precursor can indeed accommodate these base pairs. The conditions of surface bonding, base pairing, and stacking have the effect of forcing an isotactic regularity. This is the origin of optical asymmetry. It is not essential for the early surface metabolism but rather a by-product of surface bonding and base pairing. The highly stabilized double-stranded stereoregular structures have a selective advantage over nonstereoregular or even single-stranded structures, which have a higher propensity for detachment and decay. The double-stranded surface-bonded TNA structure has the character of a ribbon crystal. It accumulates as a surface-metabolic end product in massive amounts and with very high molecular weights.

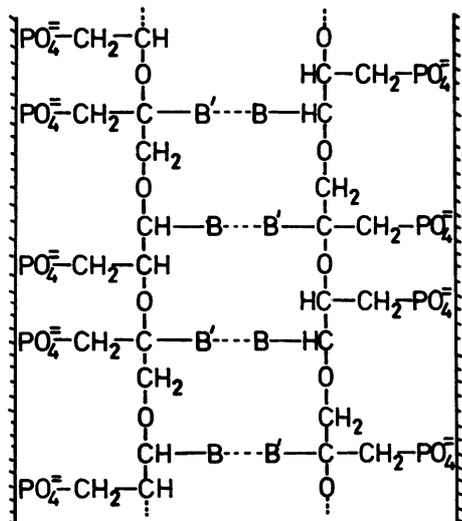


FIG. 14. TNA-TNA ribbon structure in developed representation. Both strands are bonded to the same surface.

Turning now to the base pairs, it is proposed that the purine pathway in conjunction with Florkin's version of the biogenetic law (38) tells us about the actual temporal order in which the purine base pairs make their appearance in the TNA. The first N-9-bonded purine is H and the first stable N-3-bonded purine is U', which arises, as described, by the oxidation of instable N-3-bonded H. Therefore, it is proposed that the earliest double-stranded TNA contains H-U' base pairs (Fig. 15), which are isogeometric and isoelectronic with the extant G-U wobble base pairs (19). Subsequently, the amination with Asp gives rise to N-9-bonded A and N-3-bonded C'. This is the origin of the first base pairs (Fig. 15) with Watson-Crick geometry: H-C' and A-U' (149). Finally, with the advent of N-9-bonded G, the base pair G-C' replaces H-C' (via X-C'), while H-U' is replaced by G-U'. In the extant rRNA and transfer RNA (tRNA) sequences, G-U base pairs, which are the descendants of the H-U' base pairs, are found in crucial and most conservative locations. For example, a single G-U base pair in a tRNA has been proven to be decisive for recognition by a synthetase (61). The G-U base pairs bear witness to the most ancient H-U'-TNA structures. Incidentally, the occurrence of H in the wobble positions of some eucaryotic and eubacterial

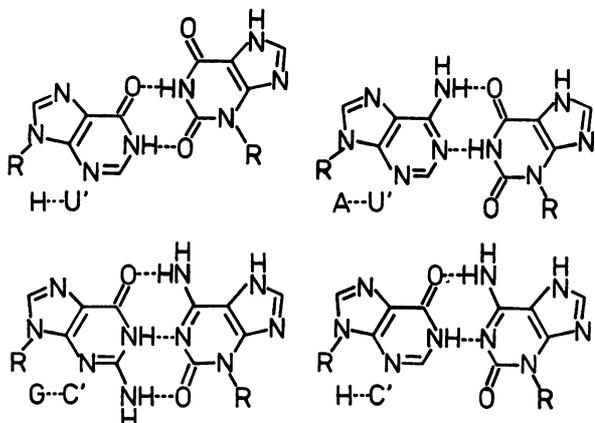


FIG. 15. All-purine base pairs H-U', A-U', H-C', and G-C'.

tRNAs seems to be a remnant from the first TNA rather than due to a late modification. Also, C' and U' can form Hoogsteen base pairs.

Surface-Bonded Ribonucleotides

The presence of ribose in nucleic acids poses a major problem for all versions of the Oparin-Haldane theory (136). A variety of ribose precursors have therefore been proposed (68, 139) which, however, are not more likely to occur in a "prebiotic broth" than ribose. The theory of surface metabolism assumes that surface-bonded phosphoribose units arise sooner or later after the formation of TNA by surface-metabolic pathways akin to the extant ribose biosynthesis. It will be remembered that, in the proposed hypothetical purine pathway, the two C₁ units of the purine ring arise by cleavage of two C₃ units with the concomitant production of two surface-bonded C₂ units. One of these is incorporated into the imidazole ring. The other C₂ unit is united with glyceraldehyde phosphate (GAP) to produce a bis-phosphorylated pentulose (Fig. 4). This by-product of the purine pathway enters a transphosphorylation and isomerization equilibrium in which surface-bonded phosphorylated ribose units arise. These can undergo intramolecular hemiacetal ring formation as well as intermolecular hemiacetal bonding by their free OH groups.

These phosphoribose constituents acquire glycosidically bonded purine bases by the same mechanism as the phosphotribose. This gives rise to surface-bonded ribonucleotides (RNs) which can form base pairs with TNA.

The thiamine pathway (Fig. 4) evolves as a sibling of the de novo purine pathway. With the appearance of quaternized thiazole in thiamine monophosphate and TPP, a highly effective shuttle exists for the interconversion of surface-bonded sugars by C₂ transfer. This leads to the formation of considerable amounts of phospho-ribose. Incidentally, my theory helps to explain a so far unexplained detail of the biosynthesis of the pyrimidine moiety in TPP. This pyrimidine moiety arises in *Escherichia coli* from the aminoimidazole ribotide in the purine pathway: not by a modular biosynthesis but, as shown by Estramareix and Thérissod (34), by an unknown rearrangement in which all carbon and nitrogen atoms of the amino-imidazole and three of the carbon atoms of the very ribose moiety to which the aminoimidazole is attached combine to the pyrimidine ring. Thus, a single surface-bonded TNA polymer structure undergoes a set of sibling rearrangements which produce not only the purines but also the ribose and even the TPP coenzyme for ribose formation (its pyrimidine moiety as well as its thiazole moiety). That only three carbon atoms of the ribose moiety enter into the pyrimidine ring is due to the origin of this pathway in the TNA which happens to have only C₃ units.

Purine-Ribonucleotide-Derived Coenzymes

In the stage of the surface metabolism with surface-bonded all-purine TNA and ribonucleotides, three important pathways arise by purine modification. They are decisive for the later course of evolution.

(i) The pterin coenzymes (THF, methanopterin, and sarcapterin) are strong surface bonders, notably in the form of their polyglutamyl derivatives. The THF pathway (111) is mostly surface bonding. An all-surface-bonding phosphotribose-implemented precursor pathway is postulated. After the changeover to TNA, it is converted to a phosphoribose-

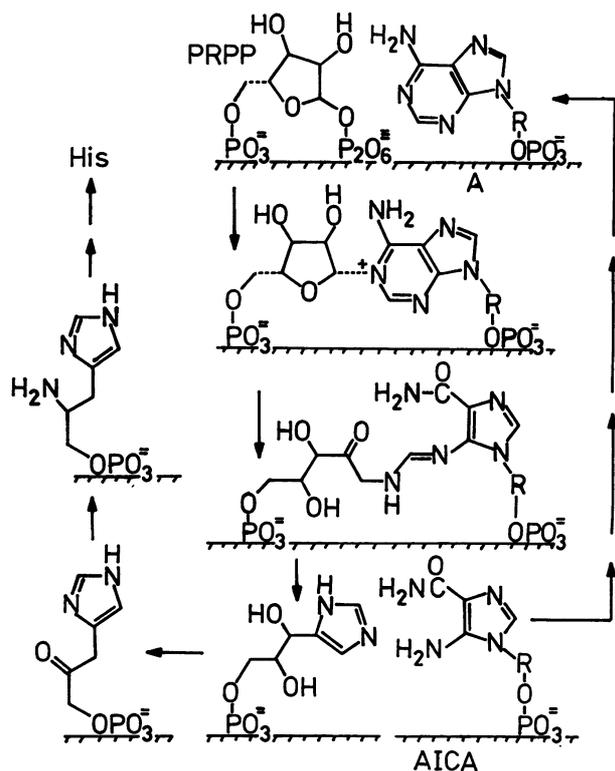


FIG. 16. Surface-bonded representation of the histidine pathway from A and PRPP via autocatalytic aminoimidazole carboxamide (AICA).

implemented pathway. THF is an autocatalytic coenzyme. It catalyzes two steps in the extant *de novo* purine pathway portion of its own biosynthesis.

(ii) The flavine pathway (4) is also an extension of the purine pathway. It arises from an all-surface-bonding precursor pathway, as evidenced by the polyanionic character of flavin adenine dinucleotide, flavin mononucleotide, and perhaps F₄₂₀ (65).

(iii) The histidine pathway (4) is dependent on the previous advent of PRPP (after an accumulation of surface-bonded lipids). PRPP assumes an important function in the transformation from the surface-bonded precursor of the *de novo* purine pathway to its extant cytosol version. At the same time or later, it assumes a role in the *de novo* pyrimidine pathway and in the purine and pyrimidine salvage pathways. The earliest pioneer function of PRPP is perhaps in the all-surface-bonding autocatalytic histidine pathway for producing novel kinds of surface-bonded imidazole catalysts (Fig. 16), which is a precondition for the later elimination of the N3-bonded purines. The three last products of this pathway have catalytic imidazole structures which are not only the surface-bonded functional precursors, but also the biosynthetic precursors of histidine in extant enzymes. They have additional functional groups for anchoring in larger structures.

Earliest Mode of Translation

It is widely accepted that a process of replication predates the process of translation (11, 31, 45, 79–81, 104–107, 152). This view appears to be dictated by the correct assumption that the mechanism of inheritance is a prerequisite for evolution. However, the assumption of a replication of

templates (nucleic acid or clay) as the only possible mechanism for inheritance is quite mistaken. The theory of surface metabolism shows indeed that inheritance by nucleic acid replication is predated by an inheritance of far simpler autocatalytic cycles. Therefore, this theory makes room for an early mechanism of translation which predates replication. With such a sequence of events, the preexisting early process of translation establishes the framework within which the products of an accurate replication can be functionally beneficial. Incidentally, this is a special case of the principle of biochemical evolution (see section, "Mechanisms of Evolution") that inheritable novelties, such as enzymes or genes, are adaptations to virtual pathways. This principle extends Popper's important principle of evolution that the possibility of a functional benefit (preference or aim structure) of an organ predates the organ itself (116).

With the appearance of RNs, a single-stranded TNA comes to bond the RNs by base pairing. This has far-reaching consequences. The RNs come to serve as carriers for other constituents of the surface metabolism, even those which are not surface bonders themselves. The ribose moiety has three positions (2', 3', or 5') available for such charging. By generalizing Crick's adaptor theory (18), it is proposed that TNA serves as a lateral positioning gadget for charged RNs to place the attached constituents in a most favorable relative position for reaction. It also replaces the two-dimensional diffusion of RNs on the surface by their one-dimensional diffusion along a TNA strand (on the surface), a notion inspired by Kuhn's collector strand model (79). Both effects increase the rate of reaction. The RN coenzymes are molecular fossils of this state of evolution (152–154). The adenine base which is widespread in such coenzymes can diffuse freely along a TNA with the bases H and U', since it can pair with both. (Incidentally, A-H base pairs are still found in the wobble position [19], but the notion of an exclusive A-H base pairing in the earliest nucleic acids suggested by L. E. Orgel, as cited by Crick [20], is not tenable, since the A-H base pair is not isogeometric with the Watson-Crick base pairs and therefore violates Crick's principle of continuity [20].)

The adenylated constituents of many biosynthetic pathways, notably of the amino acids and some amino acid precursors, date back to those times. One most important example with far-reaching consequences, however, is the charging of amino acids (and perhaps other constituents) through ester bonding to the ribose moiety of RNs. This leads to the promotion of amide bonds and notably of peptide bonds. Thus, TNA functions as a catalyst for peptide synthesis without translation. (Incidentally, still earlier modes of peptide bond formation may proceed through a thioacid intermediate or through the oxidation of a hemiaminal moiety [—CHOH—NH— → —CO—NH—].) It is of interest that the modern *de novo* purine pathway is composed of several amide bond formations with amino acids. Therefore, the earliest precursor of translation may well be in service of the ancient *de novo* purine pathway. This would make the earliest translation process autocatalytic, the TNA and RNs promoting the synthesis of their bases.

Origin of Transcription and RNA

TNA cannot replicate by modular template copying, since it is not composed of nucleotides which can exist as stable monomers. TNA grows by terminal extensions, and it changes its sequence by purine modifications. With the advent of phosphoribonucleosides, a radically new type of

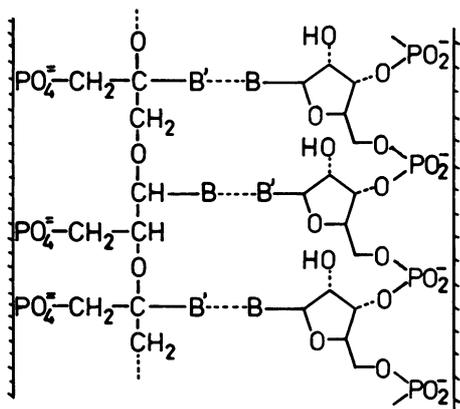


FIG. 17. Surface-bonded TNA-RNA hybrid structure. Both strands are bonded to the same surface.

nucleic acid is formed by (modular) RN polymerization. This occurs because TNA serves as a lateral positioning gadget not only for charged but also, of course, for uncharged RNs, which places them in a relative position favorable for oligomerization by the formation of phosphodiester bridges. Under the entropy conditions of the surface metabolism, this reaction requires less activation than in solution. It is promoted by pyrophosphate groups with phosphoanhydride bonds ($\text{—O—PO}_2\text{—O—PO}_2\text{—O—}$) which are seen as appearing in the stage of the lipophilization of the surface metabolism. This marks the origin of RNAs. The RNA strands can be formed not only by the oligomerization of monoribonucleotide modules but also by the ligation of oligoribonucleotides (see reference 1). Now, notably after the increased rate of ribose formation by TPP catalysis, the TNA-TNA ribbon structures (Fig. 14) are replaced by TNA-RNA hybrid structures (Fig. 17). The RNA strands can be quite long in spite of the 2'-OH groups which favor hydrolysis by an anchimeric effect, since the RNA is stabilized by bonding to the chemically stable TNA. The RNA strand acquires a base sequence complementary to that of the TNA by the base pairs A-U', H-U', H-C'. This is the origin of the first mode of template copying, a TNA-dependent polymerization or transcription. Base mismatches are eliminated by RNA rearrangement. The RNA inherits a high degree of stereoregularity by its transcription on, and its hybridization with, the stereoregular TNA (Fig. 17).

RNA Folding and Ribosomal Translation

RNA bonded to TNA renders this TNA unavailable as a catalytic positioning gadget. Therefore, at first RNA is a TNA repressor or blocker. This situation changes with RNA folding. TNA is stable only when attached to the surface. Detached TNA would suffer hydrolysis of its acetal backbone. Detached RNA, however, can maintain a somewhat stable backbone structure. It can also fold into secondary (helix) and tertiary structures. For these structures, the mineral-water interface has a sorting effect. Nonfolding RNA stays bonded to the surface and to TNA. Only self-folding RNA detaches from its TNA and eventually becomes waterborne. Thus, we no longer ask how the RNA in the cytosol comes to find foldable sequences since only well-folded RNA can enter the cytosol. The formation of folding structures is favored by (i) a high stereoregularity of an all D-ribose structure which the surface-bonded RNA inherits from the tacticity of its TNA template; (ii) a regularity in the

sequence (e.g., alternating H and U') due to TNA regularity; and (iii) hydrogen bonding by the 2'-OH groups of the ribose rings.

With the appearance of self-folding RNA sequences, the process of transcription of surface-bonded TNA becomes differentiated. All TNA segments which transcribe into nonfolding RNAs are repressed by their transcription products. Any TNA segments corresponding to a self-folding RNA are freed from their products of transcription as soon as intramolecular RNA base pairing outrivals hybrid base pairing of RNA with TNA.

The first folded RNAs have hairpin or tRNA-like structures which remain surface bonded due to the presence of anionic groups. These structures can now serve as the adaptors for certain TNA catalytic processes, notably, translation, by replacing RN adaptors. The universal presence of A at the charging end of tRNAs bespeaks the origin of the tRNAs as replacements for A-bearing monoribonucleotide adaptors. The charging end of tRNAs is universally made of the sequence CCA. Therefore, it is proposed that a C'C'A trinucleotide serves as an adaptor in this early mode of translation. It is likely that the bases H and U' on the one side and A and C' on the other side are segregated in separate TNA or RNA segments so that the first true tRNA arises by joining a C'C'A segment to a hairpin pre-tRNA (mainly with bases H and U'), a process conserved in the eucaryotes and archaebacteria (156). Now the C'C'A segment base pairs with a (folded) RNA strand (rRNA) having bases H and U', and the (anticodon) loop of the hairpin structure base pairs with TNA segments having mainly the bases A and C'.

The TNA-tRNA interaction occurs via triplet pairing. The base sequence in TNA is thermodynamically controlled (e.g., a repeating sequence). Therefore, a simple set of two types of tRNAs produces an alternating polypeptide on an alternating TNA sequence. This is the origin of true translation. The polypeptide chain grows while being handed from one surface-bonded and TNA-bonded charged tRNA to the other along the TNA. It will be seen later that this polypeptide becomes surface bonded in *statu nascendi* by surface-bonding amino acid units. From the vantage point of a structurally restrained TNA, the further evolution of translation is driven by the appearance of new bases and amino acids and leads to an explosion of sequence possibilities and to an ever-increasing structural versatility and functional competence of the tRNAs, rRNAs, and the product proteins. This evolution runs parallel to a coevolution of the amino acid pathways and of the genetic code (explained below).

The advent of RNA-dependent translation can be understood if it is appreciated that, in a longer TNA with a variety of bases, only certain segments of the RNA transcript (e.g., having H and U') have a sequence for folding, while other segments (e.g., having A and C') are nonfolding and stay bonded to TNA. However, a folded segment at the leading end promotes the (transient) detachment of the leading end from its TNA and from the surface. Another RNA, notably, a folded rRNA (and later a ribosomal unit), may become attached to this detached segment. This RNA segment competes with the TNA for bonding tRNAs and rRNAs and thus turns into messenger RNA (mRNA). At this point, Woese's reciprocating ratchet mechanism (159) can be invoked. It moves the rRNA (or ribosome) along the mRNA, pulling it progressively off its TNA and off the surface. Additional rRNAs (or ribosomes) become attached to the free end protruding from the first rRNA (or ribosome). In

this fashion, the ribosomes have the effect of freeing protein-coding TNA from blockage by hybridization with its product of transcription. Eventually, the leading end of the mRNA is no longer stabilized by additional ribosomes. It is hydrolyzed, since the thermodynamic equilibrium of dissolved nonfolded RNA is on the side of hydrolysis. This joint process of transcription, translation, and decay, which is conserved in extant bacteria, does not allow for a posttranscriptional modification of mRNA. It can be visualized as moving continuously along the surface-bonded TNA by forming and translating polycistronic mRNA. Therefore, the processes of translation and subsequent decay of mRNA have the effect of freeing the TNA from its hybridization with mRNA and thus preparing it for a subsequent process of renewed transcription. This means that indirectly the tRNA structures at the leading ends of the mRNAs are also promoting transcription. This hypothesis is strikingly similar to an important proposal by Weiner and Maizels (153) that tRNA structures serve as tags for initiating RNA polymerization (actually RNA replication in their proposal; however, see below). Such tags are found today not only in viral RNA genomes, but also as attenuating folding structures at the leading ends of mRNAs and also in a tRNA^{His}-like structure at the *his* mRNA (86) or as tRNAs at *tyrU* and *tyrT* operons of *E. coli* (86).

The earliest ribosome function is attributed to a single RNA akin to the extant primary transcripts of rRNA. This pre-rRNA has two folded subunits (one large and one small) loosely connected by an intervening RNA sequence. It operates by intramolecular dissociation and reassociation of its two interconnected rRNA domains like a pair of pincers. The domains become adapted to each other. The later removal of the intervening sequence merely means a changeover to intermolecular association and dissociation of two separate ribosomal units. This state of affairs still exists in mitochondria. Later, by additional cleavages, the 5S rRNA arises as well as the 5.8S rRNA of eucaryotes, both again inheriting their quaternary association capability from their unitary precursor. The origin of the tRNA-rRNA interaction is seen as arising in a similar manner, as evidenced by the occurrence of tRNAs in primary rRNA transcripts.

With the advent of basic amino acids (explained below), the proteins produced by translation acquire a capability of bonding to RNAs, notably, to rRNA, and they lead to an improvement of the mechanism of translation. This is the origin of the ribosome which is autocatalytic by producing its own proteins. This occurs already at the stage of unitary pre-rRNA as evidenced by the bonding of some ribosomal proteins to the primary transcript of rRNA in the ribosome assembly pathway (86).

We assume that frequently protein-coding nonfolding segments and noncoding but folding segments are intermixed to such an extent that the overall degree of folding in conjunction with the bonding of basic proteins causes the composite primary transcripts to take off and become waterborne as heterogeneous nuclear RNA. These consist, in the early days, of nonfolding exons, self-folding introns, and proteins for aiding liftoff. (Incidentally, in today's introns, the self-folding characteristics are no longer required and, therefore, undetectable.) This heterogeneous nuclear RNA is the transportable form of the primary transcript, the first true mRNA which is properly packaged and protected by the proteins so that it can reach ribosomes, which are far away from the mineral surface and the surface-bonded TNA. They become engaged in a modified process of translation which becomes

the specialty of the eucaryotes and which can later operate outside a nucleus and in the faraway spaces of the cytosol of the eucaryotic cell. It is thus a precondition for the advent of a cell nucleus.

It is very unlikely that the process of base sequence modification of TNA (and later DNA) hits directly upon a sequence which transcribes into an RNA that sits in a pronounced folding energy minimum. It is more likely that any first RNA primary transcript (e.g., that of rRNA) which folds sufficiently strongly to remove itself from its TNA (or DNA) and from the surface will be situated on an energy slope. It will therefore undergo posttranscriptional rearrangements to reach a pronounced energy minimum, e.g., by thermodynamically controlled processes of transesterification and hydrolysis and with the removal of leaders and tails and intervening sequences. These processes are promoted by the free OH groups in the ribose rings (12, 13, 49, 50, 85, 123). The processes of RNA (self-)splicing and RNA folding are siblings.

Later, the folding stability of rRNAs and tRNAs is further increased by the advent of GC' base pairs and by the processes of methylation of 2'-OH groups and posttranscriptional base modifications.

Advent of Replication and DNA

The TNA-TNA and also the TNA-RNA structures and sequences are thermodynamically determined and therefore assumed to have a narrow range of sequence possibilities. Variations of the base sequence of a TNA are perhaps rare, but possible by postcondensational base modifications. The modified sequences are transcribable into RNA but not inheritable. This means a severe limitation of the early process of translation in terms of the reproducible generation of competent enzymes. This situation changes fundamentally with the advent of replication. The first process of replication is an RNA replication. It arises as a derivative of TNA → RNA transcription by a changeover from a TNA template to an RNA template. This type of replication has been most successfully studied by the schools of L. E. Orgel (1, 104–106) (nonenzymatic) and M. Eigen (32) (enzymatic). RNA replication is found in viruses. However, Eigen proved enzymatic de novo RNA synthesis (8). This fact as well as the inherent chemical instability of RNA due to its 2'-OH groups means that the process of RNA replication does not appear to be suitable for supporting an autonomous RNA-based process of evolution. It can, however, play an important role ancillary to the TNA-dependent transcription (supported by the stable TNA) and as a transition to DNA replication.

All arguments for a very late appearance of DNA are inspired by the conditions of a presumptive prebiotic broth. Deoxyribose is unstable, and the formation of deoxyribonucleotides from deoxyribose under broth conditions is inconceivable. The abandonment of the broth theory and the adoption of the theory of surface metabolism make these problems disappear, and it makes room for an early appearance of DNA (see reference 39).

It is assumed that a first catalyst for the removal of 2'-OH groups from RNAs evolves in the era of accumulating surface-bonded lipids (favoring the formation of phosphoanhydrides) since in the extant pathway this reaction occurs at the level of ribonucleoside pyrophosphates. The nature of this catalyst is unknown. It is perhaps related to the recently discovered reductase of the archaebacteria (58). The deoxyribonucleotide pyrophosphates are capable of oligomerization on

an RNA template (reverse transcription) or on a TNA template. The emerging DNA is a replica of the TNA. It has no destabilizing 2'-OH groups and thus a stability higher than that of RNA, similar to that of TNA. Therefore, it can serve as a scaffolding for RNA synthesis, just like TNA. It can also serve as a scaffolding and template for its own production. This is the origin of DNA replication which may be pre-enzymatic (148). The surface-bonded DNA functions as a new coenzyme of sorts. It is catalytic (like its precursor TNA) for the production of RNA, and it is also autocatalytic for its own reproduction. Eventually, the growing and replicating DNA strands replace the TNA which, after its short but decisive role in early evolution, becomes obsolete and disappears.

In a development parallel to the evolution of transcription and replication, the nature of the bases changes. The formation of N-9-bonded G from N-9-bonded H via X changes HU' pairs into GU' pairs and HC' pairs into GC' pairs. This increases the stability of the secondary structures and promotes folding. There are two arguments for an early appearance of GC'. The first is broth inspired and based on the fact that glycine, the most abundant product of Miller-type experiments (96, 97), is coded by GGG. The second assumes that the first nucleic acids require GC pairs with triple hydrogen bonds for stabilizing their secondary structure. Both arguments disappear in the context of the surface-metabolic theory, which denies the existence of a prebiotic broth and sees free glycine as arriving late in the changeover to a cytosol metabolism. Further, the strong polyanionic surface bonding and the strong all-purine stacking confer far more structural stability than the extra hydrogen bond in the GC' pair. This explains why ATP (and not guanosine triphosphate) is the universal energy carrier and why all purine nucleotide coenzymes derive from adenine and not guanine (with the exception of the G-derived pterins and flavins [4, 111] which are, however, latecomers).

After the formation of semicellular structures, the purine salvage pathways appear for salvaging purines which become detached by the depurination processes. This requires PRPP. Several important sibling reactions of PRPP appear. A most decisive one is the (modular) *de novo* pyrimidine pathway. It produces, first, orotidine monophosphate and, later, uridine monophosphate. In the still surface-bonded nucleic acids, both can replace the isoelectronic and isogeometric N-3-bonded xanthine U' without any functional change or selective disadvantage (149). Next, the nucleotide triphosphates appear which provide the possibility of energy coupling for a changeover from exergonic surface-metabolic pathways to endergonic cytosol pathways (based on activation by ATP and to a lesser extent guanosine triphosphate). Now the *de novo* pyrimidine pathway acquires a considerable selective advantage by energy saving, since the formation of uridine monophosphate does not require ATP consumption (merely PRPP and carbamoylphosphate), while the conversion of the surface-bonded *de novo* purine pathway to a cytosol pathway requires a consumption of 4 molecules of ATP in addition to PRPP up to hypoxanthine. Therefore, the pathway to N-3-bonded purines is not transferred into the cytosol. This marks the takeover of the all-purine nucleic acids by purine-pyrimidine nucleic acids. The extension of the *de novo* pyrimidine pathway to C emerges last, as evidenced by the peculiar fact that, in the extant pathway, it occurs at the level of the triphosphates (uridine triphosphate → cytidine triphosphate). With the abandonment of a mineral-supported metabolism, this takeover is permanent and the N-3-bonded purines disappear from the scene. The

purine-pyrimidine nucleic acids have greater structural versatility than their all-purine precursors and a sequence-dependent stacking energy.

Turning finally to DNA folding, the conjecture is proposed that, in the semicellular structures of the second model, the growing DNA strands will eventually encircle the entire mineral grain; under these circumstances the probability of the formation of a closed circular DNA structure is very high. Eventually, a closed annular double-stranded DNA is formed which is stretched around the mineral grain like a hoop. A mineral grain with a diameter of 5 μm (the diameter of many eubacteria) can accommodate such a DNA ring with a total of about 40,000 base pairs with a circumferential base distance of 400 pm. After the formation of a closed double-stranded surface-bonded DNA ring, the process of replication may become coupled with the internal deposition of pyrite as a result of the energy flow and with the process of cell division. With the abandonment of a pyrite-forming energy flow, the dividing cells lose their internal mineral grains and the closed hoop of stretched-out double-stranded DNA frees itself from the requirement of a positively charged mineral support and adopts a new stable conformation. This requires histones or similar positively charged proteins which can compete with the surface for binding to DNA, and it requires the methylation of uracil to thymine (T) for increasing the stacking energy and compensating partly for the loss of stacking energy by the changeover from the N-3-bonded purines to the pyrimidines, the T-U-discriminating repair systems being a later windfall profit.

The most stable conformation of DNA is a right-handed double helix. Compared with such a helix structure, a stretched-out hoop conformation would have a high energy without the bonding to the mandrel of a mineral grain. By the removal of this mineral grain, the double-stranded hoop turns automatically into a double-stranded helix. But for a closed circular hoop, the process of twisting into a positive helix without cleavage of the ring has an automatic topological corollary: a numerically related compensatory formation of negative supercoiling (and partial segments of left-handed Z-DNA).

Amino Acid Pathways and the Code

J. T. Wong's ingenious theory of a coevolution of the genetic code and the amino acid pathways (167-170) can be adapted to the theory proposed here. It can thereby be freed from its difficulties and successfully extended.

According to Wong's theory, a new amino acid arises by side-group modification of a tRNA-bonded precursor amino acid (Wong pathway), and subsequently some of the codons of the precursor amino acid are conquered by the new amino acid. This means that the new amino acid is automatically included in the process of translation. In the context of a surface metabolism, this biosynthetic scheme has the consequence that non-surface-bonding amino acids can become involved in the process of translation before the formation of (semi)cellular structures, since all amino acids and their precursors remain indirectly surface bonded through their attachment to surface-bonded tRNA.

Wong's theory needs some modification because its adherence to the prebiotic broth theory causes some serious trouble, leading to the assumption that the coevolution of codons and tRNA-bonded amino acids begins with those amino acids formed in the Miller experiments (96, 97), notably, Gly and Ala, and which are believed to be contained in the presumptive prebiotic broth. By contrast, it is pro-

posed here that the coevolution of codons and amino acids begins with a class of charging reactions in which surface-bonded amino acids, notably, P-Ser and Asp, become ester bonded to a proto-tRNA and that other surface-bonded amino acids which appear later in the surface metabolism, e.g., glutamic acid (Glu), are included as later participants in this charging reaction. The first charging reaction may proceed through thiocarboxylate formation on a heavy-metal sulfide surface or by phosphate ester cleavage (152, 153).

It is remarkable that P-Ser is found even today being charged onto a tRNA (54) and that it might even be incorporated into proteins by translation (99). Its tRNA has an anticodon for the codon UGA. It may therefore be assumed that P-Ser occupies in the early code the codon family UGN (N = nucleotide) or, more precisely, a precursor codon family (e.g., U'HN) which was later taken over by UGN. To avoid confusion, the following discussion will refer only to the extant table of codons and not to hypothetical precursor codons. The subsequent modifications of P-Ser by Wong pathways are shown in Fig. 18 (the bonding to tRNA not symbolized). Hydrolysis converts P-Ser to Ser, which comes to conquer the two codon families UCN and AGN by single base changes. By nonenzymatic aldol cleavage at elevated temperatures, Ser is converted to glycine (Gly) (98). Later, after the appearance of surface-bonded THF and PLP, the C₁ unit is salvaged in the conversion of Ser to Gly. This reaction leads to a dead end, since Gly is chemically inert. It occupies the codon family GGN by a single base change. Cysteine (Cys) arises from P-Ser by reaction with H₂S (see reference 24), and it inherits its codons from P-Ser. In an extant sibling pathway, selenocysteine (Se-Cys) is formed as Se-Cys-tRNA^{Ser}, perhaps via P-Ser-tRNA (84).

Asp and Glu, both surface bonders, come to share their codons (NAN), because they have a similar structure and because Glu is produced from Asp via a portion of the reductive Krebs cycle. First without assistance and later under the influence of PLP, Asp decarboxylates to alanine (Ala), which has a chemically inert side group and comes to occupy the codon family GCN. A number of amino acids arise by the reduction of the free carboxyl group of Asp and Glu to an aldehyde group. In the case of Glu, this aldehyde reacts immediately by ring closure and further reduction to proline (Pro), which is again chemically inert and comes to occupy the codon family CCN. In the case of Asp, the aldehyde cannot form such a ring. It is reduced to homoserine (hSer), which, however, cannot be incorporated into the code, having a highly reactive hydroxyl group which can undergo intramolecular transesterification. It cleaves itself off its tRNA or, if it has time to enter into the translation process, it leads to the self-termination of translation by cleaving the peptidyl chain off its tRNA. These self-cleavage reactions are exergonic due to the formation of a five-membered ring. A different situation arises if the aldehyde is not converted to hSer but to phosphohomoserine (P-hSer). This amino acid is not self-cleaving. It can therefore enter the code as an analog to P-Ser, with which it may share some codons. Nevertheless, it does not become fixed in the genetic code, since under the influence of PLP it is converted into threonine (Thr) which is sufficiently inert (like Ser) and conquers the codon family ACN.

The appearance of the lipophilic amino acids valine (Val), isoleucine (Ile), and leucine (Leu) is decisive for protein folding and for the formation of membrane-bonded lipophilic proteins. This fact as well as the large size of their codon families indicate their early entrance into the code. Two sibling pathways which even today use identical enzymes

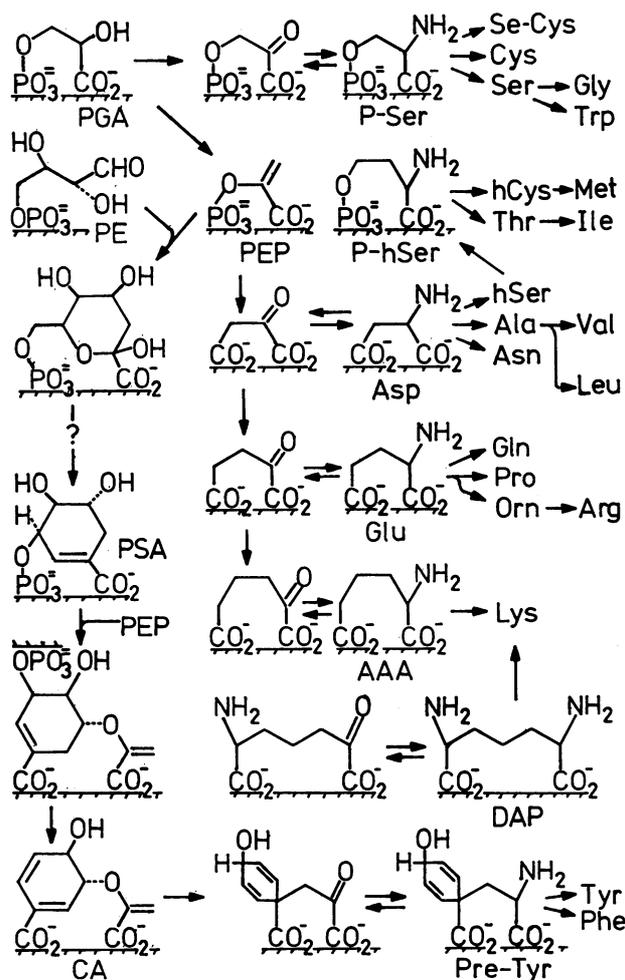


FIG. 18. Amino acid pathways in surface-metabolic representation. The Wong pathways from surface-bonded precursors to the non-surface-bonding amino acids are not shown in detail. It is postulated that an unknown surface-bonded pathway leads from phosphoerythrose (PE) and phosphoenolpyruvate (PEP) to phosphohomoserine (PSA) and chorismic acid (CA) without non-surface-bonding intermediates. DAP, Diaminopimelic acid.

produce Val and Ile. They arise (as Wong pathways) after the appearance of TPP-activated acetic aldehyde as extensions of the Thr and Ala pathways via tRNA-bonded pyruvate and α -keto-butylate. By virtue of the segregation of the pathways to these two different precursors, the sibling extensions Val and Ile come to occupy separate codon families. Val settles in the table of codons next to Ala and Ile is next to Thr. Therefore, their physicochemical similarity does not lead to confusion. Next, Leu appears as a branch of the Wong pathway to Val. It occupies a large codon family next to Val. It is of interest that this pathway is largely a sibling to the portion of the citric acid cycle leading to Glu. Incidentally, pantoic acid (Fig. 12) arises as a sibling of Val, perhaps also tRNA bonded.

The appearance of basic amino acids is of definite importance. They lead to cationic proteins which can compete with the positively charged mineral surface for fettering the anionic constituents and for lifting the pathways off the surface. The Wong pathway to arginine (Arg) arises as a branch of the Wong pathway to Pro. The aldehyde precursor is converted to ornithine (Orn) by transamination with

pyridoxalphosphate. However, an Orn-tRNA is self-destructive by an exergonic intramolecular aminolysis of the ester bond, producing a five-membered ring. If a molecule of Orn-tRNA manages to enter translation, it leads to the cleavage of the peptide chain from its tRNA by the exergonic intramolecular esteraminolysis, i.e., to termination. Some Orn units may manage to become incorporated into the protein chains. Here they will again lead to the self-cleavage of the proteins by an exergonic transamidation. This self-proteinolysis is equivalent to termination.

With the appearance of carbamoylphosphate (for the de novo pyrimidine pathway), ornithine is converted into citrulline before it can undergo self-cleavage. Later, citrulline is converted into arginine by a reaction with Asp, which is a sibling to two reactions in the de novo purine pathway. Arg is inert and conquers several codon families. The discrepancy between the large number of six codons for Arg and the paucity of its occurrence in proteins may be due to the self-cleaving nature of its precursor Orn. With the conversion to Arg, the self-termination and protein self-cleavage functions of Orn are eliminated. They are replaced by proteases, which come to cleave proteins next to Arg or its analog Lys. Theoretically, a sibling to the Arg pathway could be postulated which branches off the aspartic aldehyde in the pathway to Thr and produces tRNA-bonded 2,4-diaminobutyric acid. This, however, is even more reactive than tRNA-bonded ornithine and is not rescued from self-elimination by carbamoylation.

Asparagine (Asn) and glutamine (Gln) arise as sibling products by branches of the pathways to Thr and Pro. Asn and Gln acquire equivalent codon positions to those of Asp and Glu. The formation of Asn and Gln opens a new route to the formation of carboxamides in many pathways [e.g., to the coenzymes NAD(P)⁺ and vitamin B₁₂], and it also assists the lifting of the proteins off the mineral surface. Gln becomes catalytic for amino transfer in many pathways, including the formation of G and C. Incidentally, a Wong pathway from Glu to Gln (while charged onto tRNA) is found universally (42, 129, 157) in eubacteria, in archaeobacteria, and in chloroplasts and mitochondria of eucaryotes (in all of these cases, a Gln-tRNA^{Gln} synthetase is missing). This is a vestige of the oldest translation system. Here it should be remarked that, in the ancient tetrapyrrol pathway, levulinic acid is also produced by a Wong pathway from Glu-tRNA (130).

There are two pathways to lysine (Lys) in the extant metabolisms. Both involve surface-bonding precursor amino acids, and both have mainly surface-bonding constituents. The pathway to 2-aminoadipic acid (AAA) begins with Glu, and it is a homocitric acid sibling to the citric acid pathway to Glu. It may not be a Wong pathway. Due to its great structural similarity to Glu, AAA becomes charged to a tRNA of Glu or Gln. Its subsequent conversion to Lys is a sibling to the formation of Orn. However, Lys does not have self-cleavage properties. Therefore, it becomes incorporated into the code with two codons which were previously occupied by Gln or Orn and which are next to codons of its sibling Arg. AAA does not enter the genetic code since its two codons are taken over by Lys and since it cannot conquer additional neighborhood codons from other amino acids. For the same reason, pipercolic acid which might arise from AAA as a sibling to Pro is barred from entering the now quite crowded code. The second lysine pathway via diaminopimelic acid is used universally in all three kingdoms and is largely surface bonding. It is of interest that diaminopimelic acid is used in bacterial cell walls along with Lys, Orn,

and 2,4-diaminobutyric acid (128). Due to the structural similarity of diaminopimelic acid and AAA, it conquers the tRNA with the same codons as AAA.

Turning now to the aromatic amino acids, it is noticed that the shikimic acid pathway requires as starting materials surface-bonding erythrose phosphate and phosphoenolpyruvate and consists almost exclusively of surface-bonding intermediates (Fig. 18). Only a short section of this pathway involves a non-surface bond, shikimic acid, which then again becomes surface bonding by phosphorylation. Therefore, an older precursor pathway is suggested which produces a surface-bonding phosphorylated shikimic acid directly or in which shikimic acid is anchored to a phosphotribose structure (unless the shikimic acid pathway is, from the start, a mixed-surface cytosol pathway in the semicellular stage). Pretyrosine (pre-Tyr), the first amino acid in this pathway, is surface bonding and structurally similar to Glu. Therefore, it conquers a tRNA from Glu. In two Wong pathways, pre-Tyr turns into tyrosine (Tyr) (66) and phenylalanine (Phe), which conquer closely related codons.

The series of conversions of the side groups of tRNA-bonded amino acids begins nonenzymatically. Later, however, they are assisted by enzymes. A small number of enzyme types is sufficient, since many reactions are siblings. These enzymes acquire a specificity for the tRNAs charged with the substrate amino acids. They evolve, however, in the direction of a looser bonding to the tRNAs charged with the product amino acids, since a strong bonding thereto would block translation. This means that they acquire the capability of distinguishing not only between different tRNAs but also between different aminoacyl rests. In the evolving cytosol metabolism, these enzymes acquire the capability of salvaging detached amino acids from the cytosol by charging them onto their tRNAs. They turn into synthetases. As a typical cytosol reaction, this charging requires phosphoric anhydride activation by adenylation. The diverse origin of the synthetases in diverse precursor enzymes solves the paradox that the synthetases for the same amino acid in different kingdoms have a high homology, while a low homology exists between the synthetases for different amino acids in the same species (127). It is suggested that a certain homology may still exist between a synthetase and an enzyme in a pathway to its amino acid, and also a slightly increased homology may be expected for synthetases for amino acids related by sibling pathways. Much later, the Wong pathways for most amino acids are abandoned and the corresponding salvage pathways take over completely.

The last amino acids enter the code after the appearance of synthetases. Histidine arises by a conversion of the surface-bonded imidazole catalyst histidinol phosphate. The imidazole catalysts function is now found not only on the surface, but also in the enzymes, which acquire their full catalytic capabilities. The tryptophan (Trp) pathway arises as a sibling to part of the histidine pathway and as a derivative of serine.

Methionine (Met) is perhaps the last amino acid to enter the code. In its pathway, P-hSer is converted to homocysteine (hCys) (24) as a sibling to the formation of Cys from P-Ser. Since thioesters have a much higher energy content than esters or amides, hCys cannot undergo exergonic self-cleavage reactions in the stage of hCys-tRNA or in the stage of peptidyl-tRNA or in the stage of proteins. Therefore, it might enter the process of translation and the genetic code and perhaps come to share some codons with its sibling Cys. However, we have so far only considered the possibility of self-cleavage reactions by exergonic ring formation.

We should recall that the mercapto group (SH) has a high nucleophilicity. This fact in conjunction with the formation of a five-membered ring in the transition state has the effect of an intramolecular nucleophilic catalysis of hydrolytic cleavage by anchimeric participation. This means (i) the SH group in hCys is catalytic for the hydrolytic self-destruction of hCys-tRNA; (ii) if hCys manages to enter translation, the SH group catalyzes the hydrolytic cleavage of the peptide chain off its tRNA (self-catalyzed termination); (iii) if hCys manages to enter into the protein chain, the SH group catalyzes the hydrolysis of the protein (autoprolysis). In cases ii and iii, the hCys rest becomes situated at the C-terminal end of the cleaved protein. Here, the SH group has again a catalytic effect whereby the hCys unit cleaves itself off the C-terminal end of the protein. As an alternative, hCys may first cleave the next carboxamide group and remain bonded to the N-terminal end of a peptide chain or to the tRNA and then remove itself. From the foregoing, it should be apparent that the amino acids Cys, hSer, Orn, and 2,4-diaminobutyric acid are all barred by their self-cleavage properties from entering into proteins via translation. Depending on their degree of reactivity, they proceed more or less deeply into the translation process before they cause cleavage. Their codons either remain vacant or are nonsense (stop or start) codons by coding for self-cleavage amino acids. This means that initiation and termination arise from self-cleavage processes. The nonsense codons of Orn, hSer, and hCys are converted into sense codons for Arg, Thr, and Met. Other original nonsense codons are preserved as extant stop codons. The catalytic properties of hCys for the hydrolysis of ester and amide bonds have a peculiar effect. hCys can cause interruption of translation by cleavage of the peptide rest while it stays bonded to its tRNA, which in turn stays attached to the P-site of the ribosome and to the mRNA. It prevents a shift of the reading frame and can become the N-terminal amino acid for the next protein and finally cleaves itself off the N-terminal end of this protein. Rampant cleavages are avoided by the conversions Orn → Arg, hSer → Thr or hCys, and hCys → Met. Their beneficial termination functions are taken over by hydrolytically active termination factors. The initiation function of hCys tRNA is taken over by its biosynthetic successor Met-tRNA. Mechanisms for the de novo establishment of the reading frame appear. Finally, the initiator Met-tRNA becomes formylated in yet another extension, following a Wong pathway to this day (89).

Folding of Proteins

Finally, I show that the theory of a transition from a surface metabolism to a cellular metabolism can explain the appearance of well-folded proteins and the basic protein-folding types.

The earliest peptides are formed without translation (see reference 40) and consist of units with anionic side groups (e.g., Asp, Glu, or P-Ser); their configuration and conformation are determined by their polyanionic surface bonding. By postcondensational modifications, some of the units lose their anionic groups (e.g., P-Ser → Ser or Cys). This endows the surface-bonded polypeptides with metal-ion-binding properties (e.g., ferredoxins) and catalytic properties (e.g., precursors of CoA) and also with greater conformational versatility. The evolving process of translation leads to polypeptides with still greater functional capabilities and structural freedom. The water-mineral interface serves as an automatic filter. Weakly bonding nonanionic polypeptides

become detached and eventually hydrolyzed, while strongly bonding polyanionic polypeptides remain bonded on the surface. The strength of protein surface bonding depends on not only the number of anionic side groups, but also their sequential arrangement with nonbonding ones. The surface bonding is particularly strong if all anionic groups can face the mineral surface. Such sequences are selected. Additional stabilization against detachment occurs by a two-dimensional cross-linking between different strand segments due to hydrogen bonding. This is the origin of surface-bonded antiparallel β -sheets and of loops, like β -turns, which connect the strands of the β -sheets. Alternating sequences of anionic (surface-bonding) and nonanionic units form particularly strongly surface-bonded antiparallel β -sheets. The catalytic activity resides in the β -turn or -loop areas at the fringes of the antiparallel β -sheets.

It has long been noticed that the formation of well-ordered protein structures is dependent on a high regularity of the polypeptide backbone consisting only of L-amino acids with the exclusion of D-amino acids or vice versa and only of α -amino acids with the exclusion of β -amino acids. The evolution of this regularity, while hard to explain with the broth theory, finds a simple explanation in the context of the surface-metabolic theory. β -Amino acids are excluded by the nature of the surface metabolism and the Wong pathways. The stereoregularity (all-L) is imposed by the same forces and by the nature of the two-dimensional β -sheet arrangement on the surface and by the process of translation by means of stereoregular (all-D) surface-bonded nucleic acids.

How the basic protein-folding patterns arise in the course of the evolution of the genetic code and the concomitant surface detachment of the antiparallel β -sheets is now explained. The first amino acids to enter the code are anionic surface bonders. With the expansion of the amino acid pathways and of the code, more and more modified amino acids enter the polypeptides, first the hydrophilic ones (Ser, Cys, Gly, Thr, Ala, Pro, Asn, and Gln), then the hydrophobic ones (Val, Ile, Leu, and Met), and later the aromatic and cationic ones (Phe, Tyr, Trp, Arg, Lys, and His). These become incorporated into the flat surface-bonded antiparallel β -sheets. In the course of this evolution, three different trends occur: (i) the arrival of non-surface-bonding amino acids leading to surface detachment of segments of the antiparallel β -sheets; (ii) the arrival of amino acids which favor the formation of α -helix structures; (iii) the arrival of hydrophobic amino acids which cause the formation of tertiary three-dimensional folding patterns (90) from the secondary structures (from β -sheets and α -helices). The various protein-folding patterns arise from the vantage point of a surface-bonded antiparallel β -sheet by the relative effects of these three trends. In the absence of α -helix formers, the arrival of non-surface-bonding but β -sheet-forming amino acids leads to a detachment of the whole antiparallel β -sheet, which curves into a β -barrel or β -saddle conformation. The accumulation of non-surface-bonding but α -helix-forming amino acids in some of the segments of the antiparallel β -sheets leads to a conversion of these segments into α -helix structures. These lift off the surface but stay anchored at their ends to the flanking strands of the β -sheet. The remaining strands of the β -sheet move together to form again a compact surface-bonding β -sheet underneath the α -helices. A limited number of different β -sheet/ α -helix composites arises depending upon which segments of the antiparallel master β -sheet are converted into α -helices. If every other strand (including loop segments) is converted

into an α -helix, the remaining surface-bonded segments rejoin into a parallel β -sheet underneath the α -helices. The subsequent lifting of the parallel β -sheet produces either a closed α, β -barrel structure (15, 83) (wherein for topological reasons the α -helices are at the outside) or an open structure such as the nucleotide-binding domain of enzymes. If the α -helix structures are formed from two consecutive segments, the new β -sheet is again antiparallel. If three consecutive segments are converted into an α -helix, a parallel β -sheet arises, etc. Various combinations of these β -sheet segment \rightarrow α -helix conversions are possible, including the conversion of only a portion of a strand of the β -sheet and a conversion of all strands into α -helices. This explains the peculiar fact that all folded proteins belong to a limited number of different folding structures. The folding process is thermodynamically controlled. However, not all possible thermodynamically stable folding structures are realized. Only those structures are formed which can be reached from the vantage point of a fully surface-bonded antiparallel β -sheet. All later modifications of the protein sequences and structures and the full virtuosity of their functions are but variations of this simple theme.

Some proteins which are polyanionic and which consist largely of amino acids that are not β -sheet formers, or are even β -breakers, remain surface bonded until folded proteins with basic amino acids can compete with the mineral surface for bonding these acidic proteins. The acidic activator domains of the proteins that stimulate transcription by eucaryotic polymerase II (137) and the acidic proteins of the translocation domain of the ribosome (C. A. Cassiano, A. T. Matheson, and R. R. Trant, Symposium on the Molecular Biology of Archaeobacteria, Victoria, British Columbia, Canada, 1988; C. Newton, L. Schimmin, J. Yee, and P. P. Dennis, Symposium on the Molecular Biology of the Archaeobacteria, Victoria, British Columbia, Canada, 1988) are examples. They arrive relatively late. This is corroborated for the acidic ribosomal proteins by the lateness of their occurrence in the ribosome assembly pathway. Similarly, the acidic nature of the ferredoxins means that they are among the last to lift off the surface, perhaps only with the disappearance of the mineral support, even though the ferredoxins are among the most ancient proteins, as evidenced by their amino acid composition (43).

Quaternary protein associations arise from well-folded tertiary structures of unitary proteins by cleavage into separate subunits. These can dissociate and reassociate. Their mutual affinity is a windfall profit of the evolution of the tertiary structures of their unitary precursors. It is conserved even after the subunits are later translated separately. Again, we have the principle of a late appearance of modular pathways. The universal proteins for chaperoning the quaternary assembly of other protein subunits (55) date back to that time. This is another important example of the general principle that modular modes of synthesis or assembly are evolutionary latecomers and derivatives of precursor rearrangement pathways. Loosely speaking, in biochemical evolution action at a distance is the derivative of action at proximity.

AFTERTHOUGHT ABOUT THE ORIGIN OF LIFE

Cell envelopes are like sophisticated space suits which enable the conquering of strange and previously uninhabitable spaces. All forms of cellular life are occupants of conquered territory, and their evolution is largely driven by this conquering of new environments. This is a special case

of Popper's general insight that organisms that venture into new territories are largely their own makers (122). It means that different forms of life arise in different habitats. This suggests that the earliest stages of life may not only be constructed in our imagination but actually explored in their original dwellings and that the primogenitor of all forms of life may still be tracked down in its earliest habitat. But where is this original homestead of life?

By the various aspects of my theory, it must be a place with liquid water having a nearly neutral pH and high salinity; a place with a high temperature and a high pressure; a place where hydrogen sulfide, carbon dioxide, and nitrogen are pressured into reaction in the presence of ferrous and other catalytic metal ions; a place where hot volcanic exhalations clash with a circulating hydrothermal water flow; a place deep down where a pyrite-forming autocatalyst once gave, and still is giving, birth to life.

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