J Mol Evol (1999) 49:424–431

JOURNAL OF MOLECULAR EVOLUTION

On the Origin of Metabolic Pathways

Antonio Lazcano,[1] Stanley L. Miller[2]

Facultad de Ciencias, Universidad Nacional Autónoma de México, Apartado Postal 70-407, Cd. Universitaria, 04510 México D.F., Mexico
[2] Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92053-0506, USA

Abstract. The heterotrophic theory of the origin of life is the only proposal available with experimental support. This comes from the ease of prebiotic synthesis under strongly reducing conditions. The prebiotic synthesis of organic compounds by reduction of CO_2 to monomers used by the first organisms would also be considered an heterotrophic origin. Autotrophy means that the first organisms biosynthesized their cell constituents as well as assembling them. Prebiotic synthetic pathways are all different from the biosynthetic pathways of the last common ancestor (LCA). The steps leading to the origin of the metabolic pathways are closer to prebiotic chemistry than to those in the LCA. There may have been different biosynthetic routes between the prebiotic and the LCAs that played an early role in metabolism but have disappeared from extant organisms. The semienzymatic theory of the origin of metabolism proposed here is similar to the Horowitz hypothesis but includes the use of compounds leaking from preexisting pathways as well as prebiotic compounds from the environment.

Key words: Last common ancestor — Semienzymatic synthesis — Evolution of metabolism — Heterotrophic origin of life

Introduction

Most origins of life research deals with prebiotic synthesis of small molecules and the origin of replication and catalysis. A heterotrophic origin is generally assumed, but little thought has been given as to how the constituents of the prebiotic soup were used to develop the metabolic pathways. Most discussion on the origin of the pathways is based on extrapolating present metabolic routes to the last common ancestor and beyond. The most extensive example is Wächtershäuser's (1988, 1990, 1992). This process is called "retrodiction" but it is simply a back extrapolation. The biosynthetic pathways in the last common ancestor (LCA) appear to be close to those common to the Bacterial and Archaeal domains.

Figure 1 shows these two approaches. The extrapolation back in time assumes that the origin of the pathways is close to those in the LCA. The word "close" can be taken to mean close in time or close in complexity. The time is easy to define, and it may have been short, 5 to 10 million years (Lazcano and Miller 1994). Complexity is more difficult, and we do not attempt to define it, except to suggest that it is proportional to the amount of genetic information or the number of enzymes and ribozymes. Because of gene duplication, complexity can be acquired very rapidly.

The opposite assumption that the origin of pathways lies close to the origin of life suggests that the pathways arose from the available compounds in the prebiotic soup. Model prebiotic synthesis experiments combined with the organic compounds found in the Murchison meteorite give some idea about the molecules that were available. However, there is no direct geological evidence that these compounds were abundant at the time of the origin of life since the actual prebiotic conditions are not known.

It is our contention that the origin of metabolic pathways lies closer to the origin of life than to the last

Correspondence to: S.L. Miller; *e-mail:* smiller@ucsd.edu

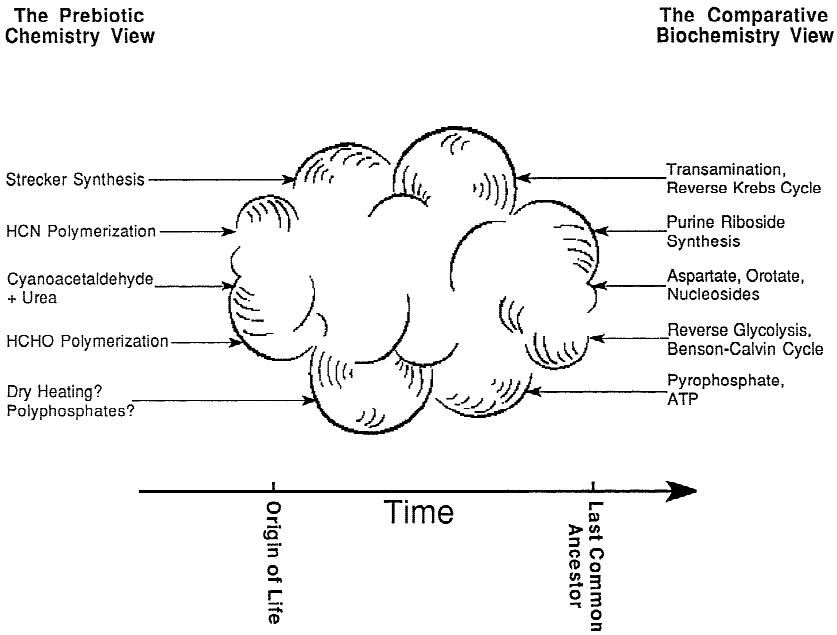

Fig. 1. The cloud model of the relationship between prebiotic chemistry and the emergence of metabolic pathways.

common ancestor (Fig. 1). Of course back-extrapolation from the last common ancestor is a useful guide, but the clues to the emergence of the pathways will, we believe, lie in the chemistry of the constituents of the prebiotic soup.

There are other theories of the origin of the pathways, and we examine them first to see whether any of them is competitive with the heterotrophic hypothesis. We then examine the heterotrophic hypothesis for its limitations and then reformulate this scheme to what we call the semienzymatic origin of the metabolic pathways. This invokes the use of prebiotic compounds as well as metabolites leaking from biosynthetic pathways already in place.

Did Metabolism Precede Replication?

The possibility has been suggested that chemical processes or cycles on the primitive Earth led to complex networks of reactions which in the strict sense can be considered as a form of metabolism. Perhaps the first to suggest such nonorganismal cycles was Ycas (1955). More recently, it has been suggested that life appeared when different systems, some endowed with metabolic cycles and others with replicating abilities, fused and entered into a sort of symbiotic process (Dyson 1982, 1985).

In the same spirit, Kauffman (1993) has suggested that life is the outcome of a process that started with the establishment of complex metabolic networks. These were based on autocatalytic sets of polymers gathered at random and lacking genomic material, which then underwent a phase transition to become a replicating system that lacked genetic material. Further evolution led to the incorporation of DNA. This type of scheme acknowledges the complexity of chemical reactions in the primitive soup. However, regardless of the chemical complexity of the prebiotic environment, life could not have evolved in the absence of a genetic replicating mechanism ensuring the stability and diversification of its information. In other words, if autocatalytic cycles ever existed, they are not competitive with a genetic system.

The problem with these schemes is that there is no known chemical model for them, much less one that is reasonably prebiotic. In any case, such schemes need to explain how a genetic system could arise from such metabolic systems so that Darwinian evolution could take over.

Autotrophic Origin of Life

It is necessary to define what is meant by autotrophy. A prebiotic process which produces amino acids, nucleobases, and other organic compounds that accumulate in the ocean and are used by the first organism is a heterotrophic origin. Most prebiotic syntheses use CH_4 and N_2 or NH_3, but using $CO_2 + H_2 + N_2$, which is efficient in producing amino acids (Schlesinger and Miller 1983), would also be considered an heterotrophic origin. An autotrophic origin usually assumes that the living system made all its constituents from CO_2 and H_2O and does not take them from the environment. However, the carbon source does not have to CO_2, since HCOOH or CH_4 would be equally acceptable.

Despite the apparent success of the heterotrophic hypothesis as indicated by many prebiotic syntheses and the striking correlation with the biochemical compounds found in meteorites, heterotrophy is still being chal-

lenged by proposals for an autotrophic origin of life. These include discussions on primordial photosynthetic life and chemoautotrophic carbon fixation of iron sulfide two-dimensional life (Hartman 1975; Mauzerall 1992; Morowitz 1992; Wächtershäuser 1988, 1990).

An autotrophic theory by Morowitz (1992) proposes charge separation of electrons by light-produced electrochemical potentials which would reduce CO_2 to organic compounds. Potential differences can be produced in model systems, but the voltages obtained are small (Morowitz 1981; Sun and Mauzerall 1996), and in any case reduction of CO_2 has not been demonstrated. Perhaps less demanding reactions, such as reduction of pyruvate to lactate, might be feasible using charge separation potentials.

A different autotrophic theory has been proposed by Wächtershäuser (1988, 1990), based on FeS + H_2S acting as a reducing agent forming FeS_2, supplemented with its proposed strong absorption properties for polymers. There are some attractive features to this theory such as the strong reducing potential of the reaction

$$FeS + H_2S = FeS_2 + 2[H]$$

FeS and H_2S can indeed carry out interesting reductions such as acetylene to ethylene, and thioacetic acid to acetic acid, as well as more complex syntheses (Blöch et al. 1992; Huber and Wächtershäuser 1997):

$$CH_3SH + CO + H_2O \xrightarrow{(Fe,Co,Ni)S} CH_3COOH + H_2S$$

Peptides have also been synthesized on coprecipitated (Ni,Fe)S by activation of amino acids with CO and H_2S or CH_3SH (Huber and Wächtershäuser 1998).

However, the FeS/H_2S system does not seem to be able to reduce CO_2 to amino acids, purines, or pyrimidines, nor can it reduce carboxylic acids to amino acids (Keefe et al. 1995b). In addition, FeS and FeS_2 have not been shown to possess the proposed strong absorption properties needed to make polymers (de Duve and Miller 1992)

In some respects, Wächtershäuser's theory (1988, 1990) is not autotrophic. It is heterotrophic if the organic compounds drift off the FeS_2 particle into the environment and are subsequently taken up by organisms. This is not different from organic compounds produced by electric discharges or bought in by meteorites. If the compounds do not leave the surface but are taken up into the polymers of the pyrite-dependent self-replicating entity, then this would be autotrophic.

In general, autotrophic proposals lack the critical experimental support of processes that reduce CO_2 and produce the required organic compounds. When these prebiotic syntheses can be shown to work, then autotrophic hypotheses can be reconsidered.

Extrapolating Back from Extant Organisms

The availability of complete genomes shows that many enzymes belong to protein families of monophyletic origin, thus providing strong support for the idea of the patchwork assembly of biosynthetic routes and the possibility that early enzymes lacked absolute substrate specificity (Jensen 1976). However, this does not permit the extrapolation to the first pathways, because the first living systems of the RNA or pre-RNA worlds may have lacked proteins altogether.

Backtrack comparisons are hindered by polyphyletic secondary losses, lateral transfers, replacements, redundancies of enzymatic steps, and even of entire metabolic routes that may have been lost. It is also possible there were alternative pathways which no longer exist or remain to be discovered (Zubay 1993; Becerra and Lazcano 1998), or that pathway replacement has taken place, as is the case in fungal lysine biosynthesis from amino adipic acid instead of diaminopimelic acid.

The shikimate biosynthetic pathway might be a case, where it evolved first to make compound X (which could have possibly been inositol), followed by branching out to make phenylalanine, tyrosine, and anthranilic acid, and followed by loss of the branch that made compound X. Finally, it is possible that some enzymes are involved in pathways that may have been used in a different direction in the past, as may have occurred with the Krebs cycle and glycolysis.

Do Metabolic Pathways Resemble Prebiotic Chemistry?

It has been argued that the present metabolic routes are derived from prebiotic chemical pathways and differ essentially by the intervention of enzymes (Degani and Halman 1967; Hartman 1975; de Duve 1991; Morowitz 1992), leading to statements that biochemistry recapitulates prebiotic processes. If this were the case, then it would be relatively simple to trace the origin of a metabolic pathway. A number of attempts have been made to retrace the steps, including pyrrole synthesis using δ-aminolevulinic acid as precursor (Szutka 1966), pyrimidine synthesis from dihydroorotic acid (Yamagata et al. 1990), and purine synthesis from HCN (Oro 1960), but these comparisons are rather forced.

It is clear that the known prebiotic pathways are quite different from the present metabolic routes. For example, amino acid formation is by the Strecker synthesis (Miller 1957), rather than by transamination and the reverse Krebs cycle. The best prebiotic pyrimidine synthesis is from cyanoacetaldehyde (Robertson and Miller 1995), instead of orotic acid (Ferris and Joshi 1979). The prebiotic synthesis of purines is from HCN (Oró 1960) and not from glycine, formate, and NH_3. Only amino imid-

azole carboxamide ribotide in the biosynthetic pathway is similar to the amino imidazole carbonitrile of the prebiotic pathway.

Thus, there is a clear lack of simple continuity between the prebiotic and biosynthetic pathways. However, continuity may be closer in other cases, e.g., branched-chain amino acids (Keefe et al. 1995a). In cases where there was no continuity, we propose the present pathways arose from nonenzymatic reactions of more abundant prebiotic compounds. Thus, purines may have been synthesized first from HCN. When the supply of adenine and guanine was exhausted from the environment, the organisms would of necessity have had to develop a pathway presumably close to the present pathway from glycine. Another possibility is that the organisms first used prebiotic adenine followed by an enzymatic development using HCN and other prebiotic intermediates. This was followed by the present pathway using glycine, formate and NH_3, when HCN from electric discharges was no longer available. It would be useful to work out the details of such processes.

A Reformulation of the Heterotroph Hypothesis

Based on the simplicity and ubiquity of fermentative reactions, Oparin (1938) suggested an heterotrophic origin of life. This fits with the idea of an anaerobic environment and with the prebiotic synthesis of organic compounds. But there needs to be an energy source. Glycolysis was suggested as the first catabolic route, as it was the only well-known fermentation pathway at the time, but it is obvious that it must have been preceded by simpler reactions (Clarke and Elsden 1980; Fothergill-Gilmore and Michels 1992). One such possibility is the fermentation of glycine, an abundant prebiotic compound (Clarke and Elsden 1980),

$$\text{glycine} + \text{NADH} + \text{ADP} + P_i = \text{acetate} + NH_4^+ + \text{NAD}^+ + \text{ATP}$$

This could have been an excellent energy source in both the RNA world and the DNA/protein world. Thus, primordial heterotrophy does not mean glucose fermentation. Any efficient fermentation would do. Heterotrophy means direct uptake of organic compounds from the prebiotic environment. But even fermentations are not required; high-energy compounds such as cyanamide, thioesters, glycine nitrile, and other high-energy compounds would work (de Duve 1991; Lazcano and Miller 1996).

The Horowitz Hypothesis

The first attempt to explain in detail the origin of metabolic pathways was developed by Horowitz (1945) and is frequently refered to as the retrograde hypothesis. This proposal states that the pathways were built up backward a step at a time rather than in the forward direction using intermediates in the prebiotic environments. It should be noted that this scheme is applicable only as long as the prebiotic compounds are available (Horowitz 1945).

The discovery of operons led Horowitz (1965) to restate his original proposal and to argue that the clustering of genes encoding the enzymes of a given pathway could be explained as the outcome of early tandem duplications. The available data do not support this possibility (Lawrence and Roth 1996). Few cases are known in which pairs of homologous genes are adjacent in different prokaryotic chromosomes (Seifert et al. 1997). Analysis of more than 12 completely sequenced microbial genomes has demonstrated not only that homologous genes whose products participate in different metabolic routes are part of different clusters, but gene ordering is extremely labile and tends not to be conserved over large evolutionary distances (Huynen and Bork 1998).

The retrograde hypothesis establishes a clear evolutionary connection between prebiotic chemistry and the development of metabolic pathways and may be invoked to explain some routes. Since homoserine and aspartic acid are prebiotic compounds, they could be mobilized according to the Horowitz hypothesis to yield methionine and threonine after the latter were exhausted from the environment. An additional example would be the photochemical decarboxylation of orotic acid to uracil (Ferris and Joshi 1979; Yamagata et al. 1990), but the orotic pathway to pyrimidines seems to be a minor prebiotic route.

However, the origin of many other biosynthetic routes cannot be understood in terms of their backward development using prebiotic compounds since they involve many unstable intermediates (e.g., ribose, oxaloacetic acid, aminoimidazole carboxylic acid), and their synthesis and accumulation in the prebiotic environment appears unlikely (Canovas et al. 1967; Ornston 1971; Jensen 1976). It has been argued that the Horowitz hypothesis also fails to account for the origin of catabolic pathway regulatory mechanisms, and for the development of biosynthetic routes involving dissimilar reactions (Hegeman and Rosenberg 1970).

According to the retrograde hypothesis, successive steps in metabolic pathways would involve similar chemical reactions, but frequently this is not the case (Clarke 1974). It is easy to overcome such criticism if similar reaction mechanisms are involved (Jeffcoat and Dagley 1973; Clarke 1974). In addition, the enzyme from the duplicated gene can have an entirely different catalytic property (Neidhart et al. 1990), especially if a functional module is acquired from another gene.

If the enzymes catalyzing successive steps in a given metabolic pathway resulted from a series of gene duplication events (Horowitz 1965), then they must share

structural similarities (Hegeman and Rosenberg 1970). The list of known examples confirmed by sequence comparisons is small (Belfaiza et al. 1986; Fani et al. 1995). A possible example may be the set formed by *N*-phosphoribosylanthranilate isomerase, indole-glycerol-3-phosphate synthase, and the α subunit of tryptophan synthase, which catalyze the three sequential steps between phosphoribosyl anthranilate and indole in tryptophan biosynthesis. Three-dimensional structural comparisons of these proteins have shown that they all share an overall eightfold β/α barrel motif and that significant portions of their active sites are superimposable, suggesting a common ancestry (Wilmanns et al. 1991).

The Granick Hypothesis

A less-well known proposal is the development of biosynthetic pathways in the forward direction (Granick 1950, 1957, 1960), where the prebiotic compounds do not play a role. For this to operate it is necessary for each of the intermediates to be useful to the organism, since the development of multiple genes simultaneously in a sequence is too improbable. This might work with heme and chlorophyll as cited by Granick, but problems arise with pathways such as purine and branched chain amino acid syntheses, where the intermediates are of no apparent use. Another example where the Granick proposal has been applied is the development of the isoprene lipid pathway (Ourisson and Nakatani 1994).

A Variation of the Horowitz Hypothesis

A modification of the Horowitz hypothesis is to reverse the direction of the nonenzymatic degradative pathways. The prebiotic compounds on the primitive Earth would decompose and the products would accumulate and be available. Once enzymes developed, these decomposition pathways could be reversed to produce the required metabolite. This has been proposed by Degani and Halmann (1967), who argued that the presence of fructose-6-phosphate, glyceraldehyde-3-phosphate, dihydroxiacetone, and lactic acid as by-products of the nonenzymatic alkaline degradation of glucose-6-phosphate is evidence of ancient, preenzymatic metabolic pathways. However, sugars and their phosphorylated derivatives decompose very rapidly on a geological timescale (Larralde et al. 1995), and glucose-6-phosphate is an unlikely prebiotic compound.

If the first pathways stem from degradative schemes, then the present anabolic pathways are not the oldest ones, and there may have been additional ones no longer extant. This alternative interpretation of the Horowitz hypothesis has been used in an attempt to explain the origin of branched-chain amino acid biosynthesis (Keefe et al. 1995a). Since their biological precursors include β-keto acids which readily undergo irreversible decarboxylations with short half-lives, a retrograde mechanism is unlikely from compounds in the primitive ocean. On the other hand, the oxidative deamination products of valine, isoleucine, and leucine are the stable isobutyric, α-methylbutyric, and isovaleric acids, respectively. The prebiotic availability of these short chain aliphatic acids is indicated by their presence in the 4.6×10^9-year-old Murchison meteorite (Lawless and Yuen 1979). Therefore, the synthesis of branched-chain amino acids via a reductive carboxylation followed by nonenzymatic transamination can be envisoned.

The Patchwork Assembly of Metabolic Pathways

An additional possibility that has been suggested for the development of metabolic pathways is the patchwork hypothesis, according to which biosynthetic routes are the outcome of the serial recruitment of relatively inefficient enzymes endowed with broad catalytic specificity that could react with a wide range of chemically related substrates (Jensen 1976). As demonstrated by whole-genome sequence comparisons, there is a significant percentage of metabolic genes which are the outcome of paralogous duplications described in completely sequenced cellular genomes. This provides strong support for this mechanism, which probably took place both before and after the last common ancestor. However, the patchwork recruitment could operate only after the emergence of protein biosynthesis and the development of enzymes, i.e., after the appearance of the DNA/protein world.

This mechanism could not apply to the earliest pathways because few, if any, enzymes were available. However, it could apply to later pathways, e.g., biosynthesis from polyketides.

Semienzymatic Origin of Metabolic Pathways

In view of the above problems, we wish to propose a different approach to this problem that may be applicable to the origin of some but not all pathways. We assume the following.

(a) There was initially a set of prebiotic compounds available in the primitive ocean. These compounds need to be rather stable or their concentrations could not build up. Thus adenine and guanine are acceptable, but not oxalacetate or glucose.

(b) Compounds due to leakage from existing pathways within cells were also available. The presence of metabolic intermediates is unavoidable because the K_m values are finite. These com-

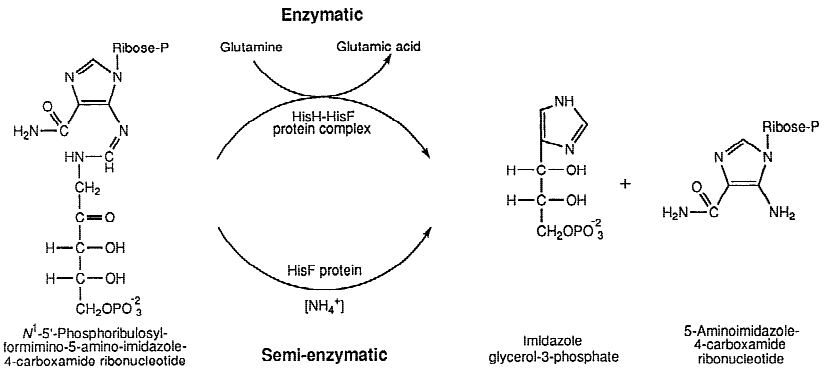

Fig. 2. Enzymatic and nonenzymatic addition of nitrogen to N^1-5′-phosphoribulosylformimino-5-aminoimidazole-4-carboaxamide ribotide to produce imidazole glycerol phosphate (IGP).

pounds need not be particularly stable because they are produced within the cell and used rapidly. Examples are oxalacetate if the Kreb's cycle is available and α-ketoglutarate if there are transamination enzymes.

(c) Existing enzyme types are assumed to be available from gene duplication and they were nonspecific (Jensen 1976). Thus, if there is one NAD-dependent amino acid dehydrogenase, then all amino acid dehydrogenases should be readily formed by gene amplification (e.g., alanine and valine dehydrogenases). It seems likely that having amino acid dehydrogenase would lead rapidly to hydroxy acid dehydrogenases, as well as to other NAD-dependent enzymes. This statement, however, needs to be demonstrated, for example, by showing that enzymes have very small residual activities for reactions distinct from their principal one. Structural comparisons may help in this search.

(d) Starter-type enzymes are assumed to arise by nonenzymatic reactions followed by acquisition of the enzyme. Thus it is easier to acquire an enzyme for a reaction that occurs slowly than for one that does not occur at all. An example is the conversion of chorismate to prephenate and then to phenyl pyruvate, which goes nonenzymatically. Additional examples include (a) photochemical decarboxylation of orotic acid, which yields uracil (Ferris and Joshi, 1979); (b) nonenzymatic synthesis of glutamic acid from α-ketoglutarate, ammonia, and reducing agents (Morowitz et al. 1995); (c) fructose diphosphate aldolase; (d) triose phosphate isomerase; and (e) formaldehyde addition to folic acid.

Most steps in biosynthetic routes are mediated by enzymes, but some occur spontaneously. In other cases the corresponding chemical step can be achieved by changing the reaction conditions and reagents in the absence of the enzyme. An example is the product of the G-type glutamine amidotransferase gene *(hisH)*, which takes part in histidine biosynthesis and catalyzes the reaction shown in Fig. 2.

The reaction adds NH_3 under high ammonia concentrations in the absence of the HisH protein (Martin et al. 1971). Recent experimental evidence has demonstrated prototrophic growth under high ammonia concentrations of a *Klebsiella pneumoniae* strain with a mutated *hisH* gene (Reider et al. 1994). We propose that the reaction first took place with NH_3, followed by the development of HisH, followed in turn by the substitution of glutamine for NH_3 as this compound disappeared from the prebiotic ocean.

An example of how a pathway might have developed is the branched-chain amino acid biosynthesis, written for valine. The pathway is shown in Fig. 3. We assume that it evolved in the DNA/protein world and that valine and isoleucine are depleted from the ocean. In addition, the following are available: (a) pyruvate from alanine dehydrogenase or serine deamination, (b) thiamine or its equivalent, (c) hydroxy acid dehydrogenase, (d) fumarase or β-hydroxy acid dehydratase, and (e) nonspecific transaminases or amino acid dehydrogenases.

The acetolactate synthetase reaction will be catalyzed by thiamine without an enzyme. The acetolactate mutase reaction may proceed without a catalyst, but this remains to be shown. The mutase enzyme carries out both the rearrangement and the reduction, but the hydroxy acid dehydrogenase may have been a separate enzyme when the pathway first operated. Nonspecific enzymes evolved their specificity via gene duplication. The greatest gain would occur when the thiamine and isomerase enzymes developed. The acquisition of regulatory mechanisms would follow.

This scheme should work easily provided the mutase reaction proceeds nonenzymatically or an available enzyme has unsuspected mutase activity. These points are all subject to experiment.

Conclusions

The biosynthetic pathways of the earliest organisms arose after exhaustion of the prebiotic compounds used in the heterotrophic origin of life. It is likely that the first biosynthetic pathways were partially or wholly nonenzymatic. By this we mean that the pathways developed by

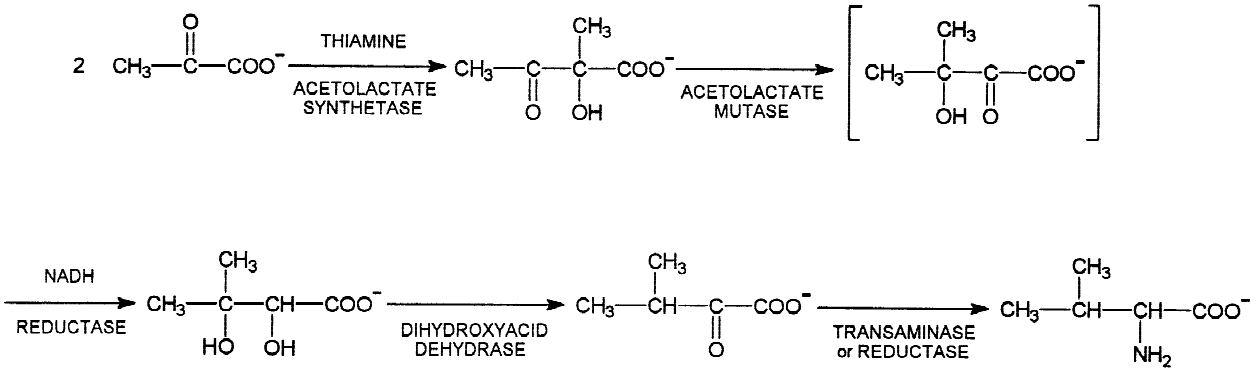

Fig. 3. Semienzymatic synthesis of valine.

using available prebiotic molecules, as well as compounds diffusing off of enzymes within the cell. The enzymes used in the early stages of the pathways were formed by gene duplications giving closely related enzyme activities (e.g., alanine and valine dehydrogenases) and, in some cases, activities that are quite different (e.g., alanine dehydrogenase and lactate dehydrogenase). This process may still be operating in the development of some degradative pathways, e.g., toxins and pollutants.

It is difficult not to accept that this scheme operated at least in part in the origin of metabolic pathways. The problem is that there are many different schemes that can be proposed depending on the available prebiotic compounds and the available enzymes previously evolved. We believe that the historical path can be unraveled on the basis of prebiotic compound syntheses, nonenzymatic reactions of metabolic intermediates, and enzyme homologies. It is also not necessary for all the metabolic pathways to arise in the same manner. Thus some of the earliest pathways may have arisen from the Horowitz scheme, some from the semienzymatic proposal, and later ones from Jensen's enzyme recruitment or Granick's forward direction hypothesis.

Acknowledgments. We thank Dr. Jason Dworkin for Fig. 1. Support was provided by UNAM-DGAPA Project PAPIIT-IN213598 to A.L. and by the NASA Specialized Center for Research and Training in Exobiology (NSCORT) to S.L.M.

References

Becerra A, Lazcano A (1998) The role of gene duplication in the evolution of purine nucleotide salvage pathways. Orig Life Evol Biosph 28:539–553

Belfaiza J, Parsot C, Martel A, et al. (1986) Evolution in biosynthetic pathways: Two enzymes catalyzing consecutive steps in methionine biosynthesis originate from a common ancestor and possess a similar regulatory region. Proc Natl Acad Sci USA 83:867–871

Blöchl E, Keller M, Wächtershäuser G, Stetter KO (1992) Reactions depending on iron sulfide and linking geochemistry with biochemistry. Proc Natl Acad Sci USA 89:8117–8120

Canovas JL, Ornston LN, Stanier RY (1967) Evolutionary significance of metabolic control systems. Science 156:1695–1699

Clarke P (1974) The evolution of enzymes for the utilization of novel substrates. In: Carlile MJ, Skehel JJ (eds) Evolution in the microbial world. Cambridge University Press, Cambridge, pp 113–159

Clarke PH, Elsden SR (1980) The earliest catabolic pathways. J Mol Evol 15:333–338

de Duve, Ch (1991) Blueprint for a cell. Neil Patterson, Burlington, NC

de Duve Ch, Miller SL (1992) Two-dimensional life? Proc Natl Acad Sci USA 88:10014–10017

Degani C, Halmann M (1967) Chemical evolution of carbohydrate metabolism. Nature 216:1207

Dyson F (1982) A model for the origin of life. J Mol Evol 18:344–350

Dyson F (1985) Origins of Life. Cambridge University Press, Cambridge

Fani R, Lió P, Lazcano A (1995) Molecular evolution of the histidine biosynthetic pathway. J Mol Evol 41:760–774

Ferris JP, Joshi PC (1979) Chemical evolution. 33. Photochemical decarboxylation of orotic acid, orotidine, and orotidine 5′-phosphate. J Org Chem 44:2133–2137

Fothergill-Gilmore LA, Michels PAM (1992) Evolution of glycolysis. Prog Biophys Mol Biol 59:105–235

Granick S (1950) The structural and functional relationships between heme and chlorophyll. Harvey Lect 44:220–245

Granick S (1957) Speculations on the origins and evolution of photosynthesis. Ann NY Acad Sci 69:292–308

Granick S (1965) Evolution of heme and chlorophyll. In: Bryson V, Vogel H (eds) In Evolving genes and proteins. Academic Press, New York, pp 67–88

Hartman H (1975) Speculations on the origin and evolution of metabolism. J Mol Evol 4:359–370

Hegeman GD, Rosenberg SL (1970) The evolution of bacterial enzyme systems. Annu Rev Microbiol 24:429–462

Horowitz NH (1945) On the evolution of biochemical synthesis. Proc Natl Acad Sci USA 31:153–157

Horowitz NH (1965) The evolution of biochemical synthesis—Retrospect and prospect. In: Bryson V, Vogel HJ (eds). Evolving genes and proteins. Academic Press, New York, pp 15–23

Huber C, Wächtershäuser G (1997) Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions. Science 276:245–247

Huber C, Wächtershäuser G (1998) Peptides by activation of amino acids with CO on (Ni,Fe)S surfaces: Implications for the origin of life. Science 281:670–672

Huynen MA, Bork P (1998) Measuring genome evolution. Proc Natl Acad Sci USA 95:5849–5856

Jeffcoat R, Dagley S (1973) Bacterial hydrolases and aldolases in evolution. Nature New Biol 241:186–187

Jensen RA (1976) Enzyme recruitment in evolution of new function. Annu Rev Microbiol 30:409–425

Kauffman SA (1993) The origins of order: Self-organization and selection in evolution. Oxford University Press, New York

Keefe AD, Lazcano A, Miller SL (1995a) Evolution of the biosynthesis of the branched-chain amino acids. Orig Life Evol Biosph 25:99–110

Keefe AD, Miller SL, McDonald G, Bada J (1995b) Investigation of the prebiotic synthesis of amino acids and RNA bases from CO_2 using FeS/H_2S as a reducing agent. Proc Natl Acad Sci USA 92: 11904–11906

Larralde R, Robertson MP, Miller SL (1995) Rates of decomposition of ribose and other sugars: Implications for chemical evolution. Proc Natl Acad Sci USA 92:8158–8160

Lawless JG, Yuen GU (1979) Quantification of monocarboxylic acids in the Murchison carbonaceous meteorite. Nature 282:396–398

Lawrence JG, Roth JR (1996) Selfish operons: Horizontal transfer may drive the evolution of gene clusters. Genetics 143:1843–1860

Lazcano A, Miller SL (1994) How long did it take for life to begin and evolve to cyanobacteria? J Mol Evol 39:549–554

Lazcano A, Miller SL (1996) The origin and early evolution of life: Prebiotic chemistry, the pre-RNA world, and time. Cell 85:793–798

Martin RG, Berberich MA, Ames BN, et al. (1971) Enzymes and intermediates of histidine biosynthesis in *Salmonella typhimurium.* Methods Enzymol 17B:3–44

Mauzerall D (1992) Light, iron, Sam Granick, and the origin of life. Photosynth Res 33:163–170

Miller SL (1957) The mechanism of synthesis of amino acids by electric discharges. Biochem Biophys Acta 23:480–489

Morowitz HJ (1981) Phase separation, charge separation, and biogenesis. BioSystems 14:41–47

Morowitz HJ (1992) Beginnings of cellular life: Metabolism recapitulates biogenesis. Yale University Press, New Haven, CT

Morowitz HJ, Peterson E, Chang S (1995) The synthesis of glutamic acid in the absence of enzymes: Implications for biogenesis. Orig Life Evol Biosph 25:395–399

Niedhart DJ, Kenyon, GL, Gertl JA, Petsko GA (1990) Mandelate racemase and muconate lactonizing enzyme are mechanistically distinct and structurally homologous. Nature 347:692–694

Oparin AI (1938) The origin of life. Macmillan, New York

Ornston LN (1971) Regulation of catabolic pathways in *Pseudomonas.* Bacteriol Rev 35:87–116

Oró J (1960) Synthesis of adenine from ammonium cyanide. Biochem Biophys Res Commun 2:407–412

Ourisson G, Nakatani Y (1994) The terpenoid theory of the origin of cellular life: The evolution of terpenoids to cholesterol. Chem Biol 1:11–23

Reider G, Merrick MJ, Castorph H, Kleiner D (1994) Function of *hisF* and *hisH* products in histidine biosynthesis. J Biol Chem 269: 14386–14392

Robertson MP, Miller SL (1995) An efficient prebiotic synthesis of cytosine and uracil. Nature 375:772–774

Schlesinger G, Miller SL (1983) Prebiotic synthesis in atmospheres containing CH_4, CO, and CO_2. I. Amino acids. J Mol Evol 19:376–382

Seifert JL, Martin KA, Abdi F, Widger WR, Fox GE (1997) Conserved gene clusters in bacterial genomes provide further support for the primacy of RNA. J Mol Evol 45:467–472

Sun K, Mauzerall D (1996) A simple light-driven transmembrane proton pump. Proc Natl Acad Sci USA 93:10758–10762

Szutka A (1966) Formation of pyrrolic compounds by ultraviolet irradiation of δ-aminolevulinic acid. Nature 212:491–492

Wächtershäuser G (1988) Before enzymes and templates: A theory of surface metabolism. Microbiol Rev 52:452–484

Wächtershäuser G (1990) Evolution of the first metabolic cycles. Proc Natl Acad Sci USA 87:200–204

Wächtershäuser G (1992) Groundworks for an evolutionary biochemistry: The iron-sulphur world. Prog Biophys Mol Biol 58:85–201

Wilmanns M, Hyde CC, Davies DR, Kirschner K, Jansonius JN (1991) Structural conservation in parallel β/α-barrel enzymes that catalyze three sequential reactions in the pathway of tryptophan biosynthesis. Biochemistry 30:9161–9169

Yamagata Y, Sasaki K, Takaoka O, et al. (1990) Prebiotic synthesis of orotic acid parallel to the biosynthetic pathway. Orig Life Evol Biosph 20:389–399

Ycas M (1955) A note on the origin of life. Proc Natl Acad Sci USA 41:714–716

Ycas M (1974) On the earlier states of the biochemical system. J Theor Biol 44:145–160

Zubay G (1993) To what extent do biochemical pathways mimic prebiotic pathways? Chemtracts Biochem Mol Biol 4:317–323