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HOMEOTOPIC TRANSFORMATION AND THE ORIGIN OF TRANSLATION

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CONTENTS

I. INTRODUCTION

Is Darwin, with his "warm little pond", the involuntary cause of the main paradigm that plagues most theories of the origin of life with the idea of a prebiotic soup? This is probably true, but only in part, especially because the relationship between water and life is extremely old in our cultures. There have been, indeed, many other views of this major challenge to biological theories. The purpose of this short review* is to emphasize a different paradigm in which the surface of solids is chosen as the primary locus of the origin of living entities.

Many scientists have stressed the fact that life had to start from a mineral environment, had we to propose that it started on Earth. Among them, three major leaders should be considered. Desmond Bernal, who pointed out the importance of clays in mineral catalysis of organic matter (Bernal, 1951); Graham Cairns-Smith, who established clearly that a prebiotic soup would be poisoned by its very capacity to generate a large—much too large—variety of organic compounds, and who proposed the existence of a clay replicating material as predating our organic life (Cairns-Smith, 1982); and, most recently, Günther Wächtershäuser, who insisted on the fact that metabolism, at the surface of solid particles, should be seriously considered as the only possibility for generating life as we know it today (Wächtershäuser, 1988).

What is life? I shall not here summarize the famous little book of Schrödinger, nor endeavour to define the laws of life, but place in the limelight four processes that are intimately associated in all living entities. These are: metabolism, compartmentalization, memory and manipulation. The two former processes are organized by small molecules (a few tens of atoms at most) whereas the two latter—which have been considered as important, almost exclusively, by molecular biologists—are controlled by macromolecules (nucleic acids and proteins). Thus, two spatial scales are at work in living processes. It has been thought to be so difficult to reconcile them that a physicist like Freeman Dyson has even proposed that life originated *twice* (Dyson, 1985)! In this conceptual framework most molecular biologists have simply forgotten to take into account metabolism and compartmentalization as questions posed to all models of the origin of life, and have only

* This review summarizes a lecture at Roscoff (France) in May 1990, for a Jacques Monod conference, devoted to *The Golden Age Revisited*. An extensive account will be found in Antoine Danchin (1990) *Une aurore de pierres. Aux origines de la vie, Le Seuil, Paris.*

considered proteins and nucleic acids. Therefore the recent discovery of ribozymes (Cech and Bass, 1986) has been perceived as allowing us to solve the famous vicious circle, who is the first, the hen or the egg, nucleic acids or proteins? As a consequence life is seen as having originated in an "RNA world" endowed of all kinds of metabolic properties. In this context it seems amusing to extract two quotations expressing the most opposite views. Steven Benner for instance wrote "*arguments that attempt to extrapolate from modern biochemistry back to the origin of life are futile*", (Benner *et al.*, 1987), whereas Günther Wächtershäuser described his own approach as "*a reconstruction of precursor pathways by retrodiction from extant pathways*" (Wächtershäuser, 1990). A major question remains however, for all models involving an RNA world, that of the origin of nucleotides, and—this is not of minor importance either—that of the origin of membranes. This brings us back to the question of metabolism, and to another hen and egg paradox: which is the first, RNA or small precursors? Wächtershäuser's model is meant to solve this issue, by placing metabolism of small molecules at the origin, using the selective power of solid surfaces, without requiring, as Cairns-Smith did, the need for an ancestral mineral *genetic* process.

II. HOMEOTOPIC TRANSFORMATION

In a nutshell, Wächtershäuser's model can be summarized as follows. Appropriate mineral surfaces, carrying an excess of positive charges, can *select* from an aqueous environment molecules that are negatively charged, mainly polycarboxylates and phosphates. These molecules are able to react together, and *only those that persist to bind on the surface* are kept for further chemical evolution. Entropy-driven processes are important because, on a surface, they favour polymerization, especially when it is caused by elimination of a water molecule (this is what usually happens in biological polymerization) (Wächtershäuser, 1988, 1990). Extant metabolism allows us to identify clues suggesting which have been the first steps of such a surface metabolism, stressing the importance of autocatalytic steps (simply because they provide a self-consistent means to stabilize the synthesis of those molecules that are further metabolized). In this model, coenzymes and nucleotides are of major importance; the heart of metabolism is made of triose-phosphates (Wächtershäuser, 1988), and the energy is derived from iron and sulphur, leading to pyrite formation (Wächtershäuser, 1990). At this point it seems interesting to investigate further the fate of solid particles, and to ask for the involvement of some kind of molecule which might replace their action. Cairns-Smith has proposed, precisely that RNA molecules, as polyelectrolytes that could mimic clays, would have been the obvious substitutes for surfaces (Cairns-Smith, 1982). Is it possible to find, in present day RNA molecules, classes that could have played such a role? In 1975, Wong, after having analysed the structure of the "universal" genetic code, proposed that transfer RNA molecules have played the role of a rigid "holder" allowing local modification of substrates (Wong, 1975). Since then, quite a few examples of metabolic alterations involving transfer RNA have been discovered (or rediscovered).

The *in situ* modification of non nucleotide residues on tRNA molecules can be termed *homeotopic modification*, to account for the fact that, often, several different chemical groups can be used to modify a single chemical entity. Two major examples can be given here: amidation of glutamic acid on $tRNA^{gln}$, first described by Wilcox and Nirenberg (Wilcox and Nirenberg, 1968), and recently found in chloroplasts (Schön *et al.*, 1988), and addition of hydrogen selenide on an activated $tRNA^{Secys}$, charged with serine (Leinfelder *et al.*, 1988), for synthesis of proteins containing selenocysteine. Another well-known example of homeotopic modification is the formylation of methionine carried by initiator tRNA in procaryotes. This latter modification is a typical instance of involvement of intermediary metabolism in the control of macromolecular syntheses, as expected if metabolism is historically intimately associated with translation processes (see later) (Danchin, 1973). tRNA is also associated with many other metabolic processes that are not related to translation. For instance it has been observed that charged lysine tRNA is involved in the synthesis of lipids (Nesbitt and Lenarz, 1968), or that charged glutamic tRNA is necessary for synthesis of aminolevulinate in chloroplasts (Schön *et al.*, 1986; Schneegurt and Beale, 1988) or in bacteria (Li *et al.*, 1989).

But charged tRNA molecules can also be required in reactions involving peptide bond

formation. This is the case, for instance, of synthesis of cell wall peptides in Staphylococci or Micrococci (Petit *et al.*, 1968; Roberts *et al.*, 1968; Roberts, 1974) where $tRNA^{ser}$, $tRNA^{thr}$ or $tRNA^{gly}$ are involved. N-modification of proteins by addition of leucine or phenylalanine residues has also been demonstrated in *Escherichia coli* (Leibowitz and Soffer, 1969). Finally, degradation of ubiquitylated proteins requires, at least in some cases, the addition of arginine residues provided by charged $tRNA^{arg}$ (Ferber and Ciechanover, 1987; Kaji, 1976). This corresponds to a specific case of homeotopic modification of proteins.

III. HOMEOTOPY IN INTERMEDIARY METABOLISM

Are there other, more general, traces of homeotopy in present day metabolism? If one follows the hypotheses of Yčas (Yčas, 1974), more precisely stated by Jensen (Jensen, 1976), that enzyme specificity evolved by recruiting proteins that already existed in catalyzed similar reactions, ancestral metabolic traits should be found in proteins that are grouped as similar in structure (and most probably in amino acid sequence). It follows that in such families one could find traces of the ancestral homeotopic processes. To the present day, only a few such cases have been described, usually by authors who were not aware of comparable work performed in other laboratories. Goncharoff and Nichols, in 1984, discovered that syntheses involving chorismate were performed by enzymes exhibiting a significant degree of similarity; this was the case of enzymes synthesized from genes *papB* (paraminobenzoate synthase) and *trpE* (anthranilate synthase) (Goncharoff and Nichols, 1984). Further work by these authors and others showed that glutamine amido transferase, involved in both reactions, as well as in guanine synthesis (genes *pabA*, *trpG* and *guaA*) was derived from a common ancestor (Kaplan *et al.*, 1985; Zalkin *et al.*, 1985). Very recently a further substantiation of the existence of a primitive amido transferase catalytic domain comes from analysis of human CTP synthetase, where the glutamine amide transfer domain is clearly related to the bacterial counterparts (Yamauchi *et al.*, 1990). In the field of amino acid biosynthesis Parsot, Cohen and their colleagues discovered that many activities involving pyridoxal phosphate were strongly related, in particular in synthesis or degradation of threonine, serine and tryptophane (*thrC*, *dsdA*, *ilvA* and *trpB*), as well as enzymes involved in biosynthesis of methionine (*metB*, *metC* in *E. coli*, as well as counterparts, when known, in yeast) (Parsot, 1986; Parsot *et al.*, 1987). In the same way Schoenlein *et al.* recently identified a significant level of similarity between enzymes responsible for the synthesis of pyridoxal phosphate (*pdxB*) and serine (*serA*) (Schoenlein *et al.*, 1989). This is most revealing in view of Wächtershäuser's proposal of early surface metabolism where triose-phosphates had to play a major role (and this would contradict Benner's dismissal of pyridoxamine as being involved early (Benner *et al.*, 1989)). Finally, we were able to demonstrate that cysteine biosynthesis, in *E. coli*, shares a common ancestor with tryptophan biosynthesis, as derived by homeotopy from serine (Lévy and Danchin, 1988). This, together with the observation that cysteine and tryptophan codons are found in the same box of the genetic code table (in company with the UGA selenocysteine codon, also derived after homeotopic transformation from activated serine), substantiates the hypothesis that serine(-phosphate) was a general precursor of several amino acids synthesis and that tRNA was involved in the process. Preliminary data (Risler and Danchin, unpublished observations) suggest that this could indeed be the case. Another observation, known for a long time but interpreted as anecdotal, the significant binding of charged $tRNA^{leu}$ or $tRNA^{val}$ to *E. coli* threonine deaminase, is well in line with this hypothesis (Singer *et al.*, 1984).

IV. PEPTIDES SYNTHESIZING PEPTIDES

But all this does not tell us directly what could have been the *precursors* of these "holder" nucleic acids that seem to have played an early role in evolution of metabolism. It is clear that nucleotides are, still today, part of many coenzymes, as pointed out by Trémolières (Trémolières, 1980) or, more recently, by Benner (Benner *et al.*, 1987). But it cannot be surmised whether this reflects a trace of older structures rather than a more recent adaptation of long nucleic acid precursors to shorter structures. As stressed by many authors, peptides are in fact far easier to synthesize than nucleic acids. On the other hand, many coenzymes

(e.g. glutathione, pantothenate, folic acid, etc.) are (iso)peptides, or contain peptides, often in their active centre. Is it not possible that (iso)peptides have been precursors not only of most coenzymes, but of nucleotides as well? Many features of extant metabolism could argue in that sense, for amino acids are certainly present in the biosynthesis of purines and pteridines (glutamine, glycine, aspartate and serine, through formyltetrahydrofolate), or pyrimidines (aspartate).

But, as an indispensable self-catalytic step requires, are (iso)peptides involved in synthesis of *peptides*? The example of peptide antibiotics synthesis is a remarkable illustration of such self-referring catalysis. It is known that biosynthesis of tyrocidin or gramicidin derives from formation of peptide bonds, outside the normal framework of the translation machinery. In tyrocidin, for instance, 10 individual amino acid residues are activated by ATP (as they are in translation) but are then transferred to an active SH residue of a protein subunit, forming a thioester bond. Tyrocidin synthesis begins after all 10 sites of the enzyme complex have been esterified with their specific residue. The first three amino acid residues react together sequentially, forming a thioesterified tripeptide. From this step onwards a phosphopantetheine cofactor transports the growing peptide chain on each new residue in turn, using its SH end as a carrier, and forms a new peptide bond following a transthiolation step, until the end of the process is reached when a decapeptide is formed (and finally cyclized). This process is highly reminiscent of synthesis of fatty acids from acetyl coenzyme A (which contains a phosphopantetheine arm as a reactive centre). In this latter process acetyl CoA is first transformed by carboxylation into malonyl CoA using ATP as an energy source. A phosphopantethein arm, bound to a core enzyme makes a succession of transthiolation reactions that lead to decarboxylation of malonate (this is the driving energy source) and condensation of two methylene residues on the growing chain. After six such steps the synthesis is completed, yielding palmitic acid. Thermal agitation supplies the only energy required for positioning of the carrier arm.

Analogy between both processes would only be anecdotal, had it not been discovered recently that, indeed, proteins involved in tyrocidin, gramicidin and fatty acids synthesis share a common ancestor (Krätzchmar *et al.*, 1989). This indicates that their origin is common, and could be very old. Several features of these processes ought to be emphasized: (a) a peptide is able to carry out synthesis of a peptide; (b) the same process permits synthesis of both lipids and peptides; (c) the process requires the presence of active SH groups, essential components of surface metabolism as described by Wächtershäuser; (d) energy is essentially derived from the formation of thioesters (and carboxylation/decarboxylation in the case of fatty acids), and (e) among the amino acids that are used, a basic amino acid residue is present, ornithine, which cannot be incorporated into proteins during translation, because it cannot form stable adducts with tRNA, but which could have been present in early surface metabolism.

If, then, we accept that peptide formation is a very ancient process, synthesis of peptide bond predating translation, it becomes particularly important to assess the hypothesis, that nucleotides could have been derived from peptide containing surface metabolites. Translation would have been a later invention when tRNA molecules, instead of simply offering a general "holding device" for homeotopic transformation, would have been implied in an RNA-mediated process of peptide-bond formation. A large number of examples where peptides can react by intramolecular reaction to form new molecules can be found. This is typical, for instance of the antibiotic nisin or other "lantibiotics", where serine and cysteine react to form lanthionin, a structural analogue of diaminopimelic acid (Schnell, 1988; Banerjee and Hansen, 1988; Buchman *et al.*, 1988). But could not one find in the reverse reaction of the catalysis by GTP cyclohydrolase, which yields pteridine triphosphate from guanosine triphosphate with elimination of a one carbon residue (precisely a residue transported by pteridine containing coenzyme), a model for the straightforward synthesis of *nucleotides*?

$$\text{PteridineTP} + \text{HCOOH} \rightarrow \text{GTP} + \text{H}_2\text{O}$$

This wild speculation would ask for a process permitting synthesis of pteridine from

peptides. Exploration of microbial metabolism might give clues for the plausibility of such processes.

V. CONCLUSION

Wächtershäuser has convincingly proposed that life emerged from a surface metabolism, rather than from a poisonous broth. His approach uses extensively the knowledge of extant intermediary metabolism. If his main contention is right, this means that we are much nearer to the origin than we thought beforehand. Analysis of biosynthetic pathways might therefore provide clues about the original metabolic pathways and processes. Jensen's hypothesis stating that early metabolism evolved through specification of broad range catalytic activities can be appreciated using a comparison of enzyme structures in living cells. Among the most prominent processes are those which use tRNA molecules as carrier for homeotopic transformation of more or less universal precursors. Among such reactions, peptide bond formation could have evolved well before translation. Peptides are therefore placed in the limelight. They could have evolved before complex coenzymes, allowing their synthesis as well as synthesis of nucleotides, which, in turn would have produced RNA carrier molecules, thus solving the egg and hen paradox raised by the generally accepted hypothesis of an ancestral RNA world.

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