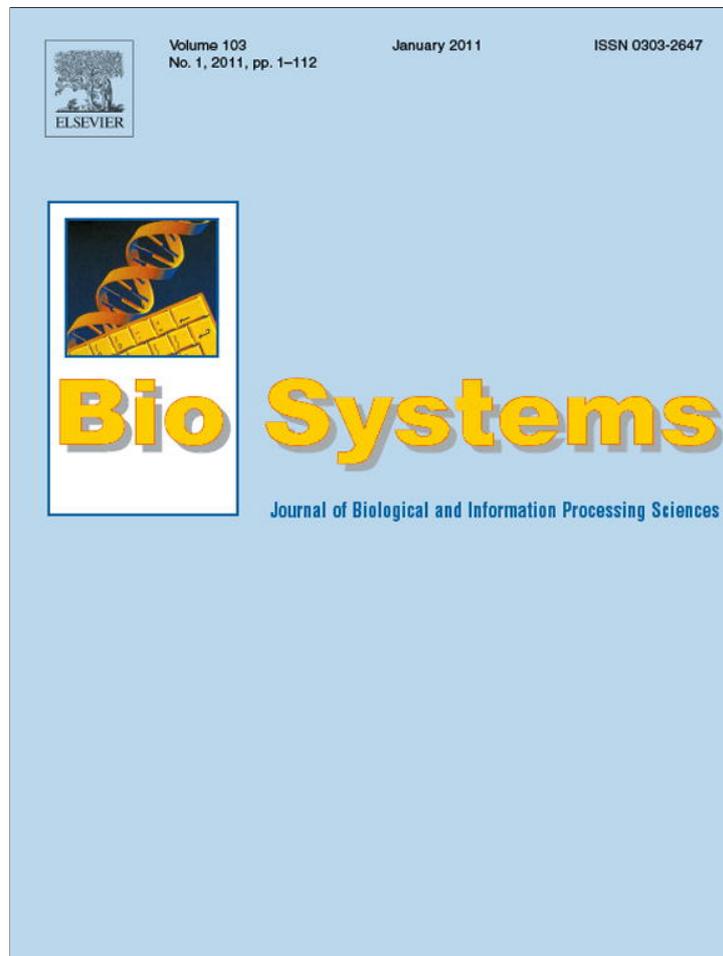


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Research article

Active centrum hypothesis: The origin of chiral homogeneity and the RNA-world

József Garay*

Research Group of Biology the Hungarian Academy of Sciences, Department of Plant Taxonomy and Ecology, L. Eötvös University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary

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ABSTRACT

I propose a hypothesis on the origin of chiral homogeneity of bio-molecules based on chiral catalysis. The first chiral active centre may have formed on the surface of complexes comprising metal ions, amino acids, other coenzymes and oligomers (short RNAs). The complexes must have been dominated by short RNAs capable of self-reproduction with ligation. Most of the first complexes may have catalysed the production of nucleotides. A basic assumption is that such complexes can be assembled from their components almost freely, in a huge variety of combinations. This assumption implies that “a few” components can constitute “a huge” number of active centre types. Moreover, an experiment is proposed to test the performance of such complexes in vitro.

If the complexes were built up freely from their elements, then Darwinian evolution would operate on the assembly mechanism of complexes. For the production of complexes, first their parts had to appear by forming a proper three-dimensional structure. Three possible re-building mechanisms of the proper geometric structure of complexes are proposed. First, the integration of RNA parts of complexes was assisted presumably by a pre-intron. Second, the binding of RNA parts of a complex may give rise to a “polluted” RNA world. Third, the pairing of short RNA parts and their geometric conformation may have been supported by a pre-genetic code.

Finally, an evolutionary step-by-step scenario of the origin of homochirality and a “polluted” RNA world is also introduced based on the proposed combinatorial complex chemistry. Homochirality is evolved by Darwinian selection whenever the efficiency of the reflexive autocatalysis of a dynamical combinatorial library increases with the homochirality of the active centres of reactions cascades and the homochirality of the elements of the dynamical combinatorial library. Moreover, the potential importance of phospholipid membrane is also discussed.

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1. Introduction

Shapiro (2006) assumed that life began among one of the mixture of simple organic molecules that are produced by abiotic processes. In the present paper, logically possible complexes of simple organic molecules are suggested. Wong (2005) and Copley et al. (2007) proposed that a co-evolutionary process existed between genes and metabolism for origin of the RNA world. In the present paper, this kind of co-evolution process is based on the evolution of active centre. A very similar hypothesis with my ideas was proposed by Root-Bernstein (2007). He also assumed that the genetic code and the homochirality evolved simultaneously within a system of interactions involving amino acids, peptides, nucleotide bases and so on. However, he also did not assume evolution of the active centre which is the key issue of the hypothesis presented

here. Furthermore, there are many papers in which the importance of homochirality in the origin of life is emphasized.¹ For instance, Carroll (2009) suggested a new definition of life: “*life is that which self-reproduces homochiral environments.*” There are opinions that chirally active and autocatalytic small organic molecules were relevant in the origin of life (Soai and Kawaski, 2006; Micskei et al., 2006, 2008; Maioli et al., 2007; Fu, 2009). These ideas have importance for my hypothesis in two ways: First, the homochirality of the elements of the active center of the proposed complexes is extremely important. Second, I also think the theory of origin of life without the explanation of the origin of the homochirality of the biomolecules is not complete (Kuhn, 2008). From the point of view of my idea it is more important that, as Tamura and his coworkers (Tamura and Schimmel, 2006; Tamura, 2008a,b) pointed out, aminoacylation of RNA minihelices affords the opportunity to make chiral selection between L- and D-amino acids according to the pre-

* Corresponding author. Tel.: +36 1 381287, fax: +36 1 381288.

E-mail address: garayj@ludens.elte.hu.¹ Without the completeness, only a few papers will be mentioned.

existing chirality of the RNA. Based on this result, it can be assumed that the elements of proposed complexes in this paper were of a chiral character.

One of the major problems of evolutionary biology is to explain the origin of the basic universal biochemical scheme of life (i.e., the information conveyed by the sequence of chiral nucleotides is transformed into the sequence of chiral amino acids through the genetic code). I advocate that the three main components evolved simultaneously (cf., Root-Bernstein, 2007).

My starting point is the hypothesis of the RNA world (Gilbert, 1986; Gesteland and Atkins, 1999; Joyce, 2002), which claims that RNA world is the starting point of life since RNA carries genetic information and has catalytic properties. I shall briefly refer to a few problems and one of the important arguments of the hypothesis of the RNA world which are most important from the viewpoint of this paper. One of the arguments in favour of an RNA world is that most of the recent cofactors have structural relationship to RNA and these cofactors were preserved in all three domains of life (eubacteria, archaea and eukaryotes), thus cofactors seem to be the remnants of an earlier ribozyme metabolism (White, 1976; Benner et al., 1989). Observe that this argument means in essence that the RNA world's active centres have also been inherited to modern enzymes and this kind of active centres appeared in a very early stage of evolution. Unfortunately, the origin of the RNA world has not been explained satisfactorily as yet (see, e.g. James and Ellington, 1995; Joyce and Orgel, 1999; James and Ellington, 1995; Hund and Anet, 2000; Eschenmoser, 1999; Schöning et al., 2000; Szathmáry, 2006). One of the problems of the outset of RNA is the so-called "nucleotide problem": while purine bases could be synthesised by the condensation of HCN (Orro, 1961); "prebiotic" synthesis of pyrimidines was more difficult to achieve (Shapiro, 1999). Moreover, in a pure RNA world there was a trade-off between replication and enzymatic spectrum (Szathmáry, 1992; Szabó et al., 2002). For a more accurate replication few bases are preferred: the smaller the number of base types the lower the chance of errors during replication. In contrast, a wider enzymatic spectrum requires more bases or analogues: more diverse elements allow the formation of more diverse active centres. The catalytic efficiency and spectrum of other ribozymes, however, can be increased by the incorporation of other chemical groups, for instance coenzymes (Szathmáry, 1999; Jadhav and Yarus, 2002a,b). Furthermore, we now know that ribozymes are able to catalyse a very wide range of chemical reactions (Landweber et al., 1998; Unrau and Bartel, 1998; Takagi et al., 2001; Joyce, 2002), but these ribozymes are very large, therefore they are not able to self-replicate.

Finally, the origin of life, especially the origin of RNA world, cannot be disclosed without explaining the origin of chiral homogeneity of biomolecules.² A phenomenon suggesting the extreme importance of homochirality is enantiomeric cross-inhibition: incorporation of a single nucleotide of opposite chirality into a growing chain in template-directed oligomerization is sufficient to terminate the reaction (Joyce, 1989). On the other hand, it is widely accepted that any information-carrying polymer must consist of homochiral and chemically homogeneous units (e.g., Bonner, 1993; Avetisov and Goldanskii, 1996). There are numerous hypotheses for the origin of chiral homogeneity of biomolecules (e.g., Bonner,

1991; Keszthelyi, 1995; Bailey et al., 1998; Cronin and Pizzarello, 1997; Engel and Macko, 1997; Pályi et al., 1999, 2004; Podlech, 2001).

However, none of the hypotheses has become generally accepted so far, since only a slight breaking of chiral symmetry has been demonstrated experimentally (Bonner, 1991). For the amplification of the small enantiomer excess, several mechanisms have been proposed, such as the one based on asymmetric autocatalysis and polymerization (Soai et al., 1990, 1995a,b, 2001; Soai and Niwa, 1992; Joyce and Orgel, 1999; Tamura, 2008a,b). The proposed evolutionary scenario starts from the results of chemistry. The enantioselective homogeneous catalysis is investigated intensively (Consiglio and Waymouth, 1989; Soai and Niwa, 1992; Trost and Van Vranken, 1996), with the conclusion that metal ions and small chiral molecules together possess enantioselective catalytic activity. In our case, it is extremely important that the metal ions and chiral molecules build up chirally self-replicating systems (Soai et al., 1994, 1995a,b).

Accordingly, giving these problems the basic question of this paper is: how did the homochiral RNA world originate?³

In Section 2, a catalytic route is proposed to explain the origin of homochiral biomolecules, supposing that chiral selective active centres appeared on the surface of hypothesized complexes composed mainly of pre-RNA oligomers, bivalent metal ions and amino acids. The basic idea of the proposed complexes and that of the *dynamic combinatorial libraries* (Huc and Lehn, 1997; Klekota and Miller, 1999; Cousins et al., 2000) are identical. Both of them can be viewed as a collection of transient compounds that are reversible assemblies of a collection of building blocks. In these multiplex systems, there is an *n*-to-one adaptive correspondence between a set of components and the target component. Based on these properties of the dynamical combinatorial libraries, it can be hypothesized that it is a "semi-free" complex building. This means that for a "small" number of components, a "huge" number of complexes assemble.

Section 3 examines the question whether the proposed complexes are freely combined from their constituents, and then examines possible mechanisms that can rebuild the proposed complexes such that the active centre remains unchanged.

Another basic assumption of the evolutionary scenario is that the dynamic combinatorial library of the proposed complexes defines a *reflexively catalytic system* (Kauffman, 1986). The main point is that the catalysed reaction network contains positive feedbacks so that it can be considered as a generalized autocatalytic process.

In Section 5, a possible scenario of the origin of homochirality and the evolution of the proposed complexes is outlined. The proposed scenario starts from the hypothesis of mineral surface metabolism (e.g., iron-sulphur world, Wächtershäuser, 1992; Anet, 2004). This two-dimensional world, where molecules and complexes interact locally, is very important from the viewpoints of the chemical and selection processes as well. The proposed scenario gives the missing links between geochemical (e.g., iron-sulphur) and RNA worlds. Finally, the evolutionary importance of lipid membranes is also addressed.

2. A hypothetical pre-biotic chiral active centrum

In recent organisms, chiral biomolecules have been produced by help of chiral active centres. One may assume that at the origin of life the situation was the same, so chiral homogeneity and chiral active centres have emerged together. Now, the question

² Research in the last two decades showed that many chiral biomolecules appear in organisms as both enantiomers. There is, however a systematic preference for one of these (L-amino acids, D-sugars, etc.) but also the minor enantiomers are present and often display a certain biological role. The only exceptions are perhaps D-ribose and D-deoxyribose in RNA and DNA. This prompted participants of the 1st Symposium on Biological Chirality (Serramazzoni, 1998) to vote by large majority for the expression "biological chirality" instead of "biomolecular homochirality" and similar terms. However, one of the main issues of the present paper is the origin of RNA world, and since RNA and DNA are homochiral I use the traditional terms.

³ Bolli et al. (1997) give a partial answer to this question (see Section 5.1).

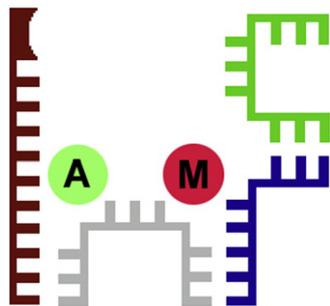


Fig. 1. Around the bivalent metal ion, short RNA molecules build the MAR complex. The RNAs are fixed to each other with base pairing (codon–anticodon relation). The connecting elements of the oligomers of MAR (1–3 pairing bases) not only stabilize the stereo structure of MAR but they also help to assemble MAR complexes. The carrying-RNA contains amino acids. The fixing of the substrate of MAR-complex is based on the polarity of the amino part of RNA.

is how these chiral active centres could appear abiotically? Since the active centres in modern life have amino acids, nucleic acids, coenzymes, ions, etc, there is no reason to exclude the possibility that these molecules were constituents of the earliest active centres as well. On the other hand, the active centre is nothing else than a few chemical groups arranged in a special three-dimensional structure. Since at the outset there were only oligomeric RNAs and small abiotic peptides, the appropriate size was reached through their complexes. I propose the following hypothetical construction of complexes: there is a metal ion surrounded by co-enzymes (for example, amino acids or short peptides, etc.) and short RNAs⁴ (see Fig. 1). To some RNAs, chemical groups such as amino acids are bound (cf., Viladkar, 2002). This kind of a complex will be termed here as the MAR (Metal–Amino–Ribo) complex.⁵ The structure of MAR complexes is stabilised by the hydrogen bonds between nucleotides.

I have to note that Ekland et al. (1995) already emphasised the importance of the RNA complexes: “...even the most complex ribozymes, such as ribonuclease P and the group I and II self-splicing introns could have arisen in one step from long random sequences, and that complex ribozymes may have played an important role early in the RNA world.”

The shortness of oligomers is important for the following reasons:

- **Chirality:** Starting from a racemate, the likelihood of perfect chiral homogeneity is larger when the molecules are short. The chiral homogeneity of oligomers may guarantee that the active centres catalyse stereoselective reactions.
- **Self-replication:** If each oligomer of MAR is fixed by 2–4 bases attached to the MAR-complex, then MAR-complexes, containing 40–50 bases are able to spontaneously dissociate which is the first step in replication. Moreover, the small size of the molecules is very important in the development of a non-enzymatic self-replicating system based on autocatalytic template-directed reactions as well (Sievers and von Kiedrowski, 1994). As far as I know, there is only one example for self-sustained replication of RNA enzymes (Lincoln and Joyce, 2009). However this system contains two enzymes that catalyse each other's synthesis from oligonucleid substrates. But there is a reaction, the cross-catalytic template-directed synthesis of hexadeoxynucleotide derivatives from amino-trideoxynucleotides, involving collec-

⁴ The first catalytic complexes may have been PNA-like polymers, rather than RNA (see Section 5).

⁵ MAR bases-pair-complex or MAR bases-pair-supramolecule may be a better name, but both of them are too long.

tive replication of oligonucleotides (Sievers and von Kiedrowski, 1994). If the primordial “living” systems employed a template-directed oligonucleotide ligation for replication, then this ligation was gradually supplanted by mononucleotide polymerization, as suggested by James and Ellington (1999). It is to be noted that a self-replicating ligase ribozyme is redesigned so that it would ligate two substrates (one of these oligomers is a 14-mer) to generate a copy by itself (Paul and Joyce, 2002). In the present context, the template-directed ligation has an overriding importance since the formation of complexes of oligomers is a must for the mechanisms of ligation.

- **Size measurement at the outset:** Supposedly, based on experiments (Orgel, 1998; Joyce, 1989) the first MAR complexes are built by 10–15-mers. On the other hand, it seems that the 30–50-mers size range is considered to be sufficiently long to exhibit a wide enough catalytic spectrum (Joyce and Orgel, 1993; Szostak and Ellington, 1993). The low diversity of RNA side chain, the high charge and the flexibility of its backbone are also expected to limit the catalytic capabilities of RNA (Narlikar and Herschlag, 1997; Illangsekare and Yarus, 1999). MAR-complexes, stabilized by hydrogen bonds, may be large enough so that they might have catalytic activity with a wide chemical spectrum based on their diverse chemical groups.

Two important properties of MAR-complexes are hypothesized here, providing the basis of this paper:

1. **Semi-free MAR-complex building:** The basic 3D-structure of the MAR-complexes depends on the number and length of the RNA pieces and the metal ions. Moreover, the structure of the surface of MAR-complexes should have great plasticity. The diversity of the surface of a MAR-complex can be increased by the exchange of co-enzymes (e.g., metal ions, amino acids) and by minor changes applied to RNAs. That is, MAR-complexes form a dynamic combinatorial library.
2. **MAR-complexes are able to chiral catalysis in a wide range of reactions.** This means that, for instance, from a “small” number of elements a “large” number of MAR-complexes with chiral catalytic activity can be formed. Moreover, MAR-complexes are able to produce either of their constituting elements.

The first hypothesis is supported by the following facts. It is well-known that several catalytic active centres of ribozymes contain a very small motif (see Kazakov and Altman, 1992; Vogtherr and Limmer, 1998). Moreover, there exist ribozymes that are complexes of RNA (e.g. Doudna et al., 1991; Bartel and Szostak, 1993). Furthermore, enzymatically inactive fragments of a ribozyme can form complexes that have enzymatic activity typical of wild-type ribozymes (Guerrier-Takada and Altman, 1992).

The second hypothesis is a “consequence” of the first one. If the structure of the surface of MAR-complexes should have great plasticity and there are an enormously large number of possible MAR-complexes, then a high number of catalytic active centres should be accessible. Especially, those MAR-complexes bear importance which are able to produce the elements of themselves (cf., Doudna et al., 1991).

Now I present some arguments in favour of the theoretical advantage of assuming MAR-complexes.

- **Specificity:** King (1986) noted that the specificity of each reaction step is crucial to the growth of an autocatalytic cycle, naturally in the reflexive autocatalytic system, as well. Thus, the specificity of the chemical reactions is easier to obtain if at the outset there was the same kind of chiral catalytic activity guaranteed by MAR-complexes.

- **Stability:** It is well known that RNA may have been unstable in a hostile prebiotic environment. However, the bivalent metal ions stabilise the RNA structure strongly (Kazakov and Altman, 1992; Landweber, 1999a). On the other hand, the stability of the MAR-complex depends strictly on the number of the hydrogen bonds. From the dynamical properties of MAR-complexes, it is important to check whether two-and-three hydrogen bonds between base pairs is “better” than the one-and-two or three-and-four hydrogen bonds under the chemical circumstances of the outset of life.
- **Synergism:** A wide spectrum of chiral active centres can be created using diverse chemical molecules no matter whether protein or RNA gives the fixing framework of the active centre. It is possible that a MAR-complex is a more selective and more effective catalyst than either of its elements by itself. In one hand, it is well known that the activity of most ribozymes requires, or is greatly stimulated by, the presence of bivalent metal ions (Cech and Golden, 1999; Kazakov and Altman, 1992; Landweber, 1999a,b; Narlikar and Herschlag, 1997; Landweber and Porkovskaya, 1999; Sawai, 2008) since metal ions are essential for folding into the catalytic conformation of RNA (Narlikar and Herschlag, 1997). An important example is that bivalent cations can catalyse template directed oligoribonucleotide ligation as well (Rohatgi et al., 1996). On the other hand, the metal ions are important in the formation and transition-metal complexation in the dynamical combinatorial libraries (Klekota and Miller, 1999). Furthermore, in chiral selectivity there is an important role of bivalent metal ions (see Bonner, 1991; Soai et al., 1994, 1995a,b). Finally, a type of DNA enzyme (desoxyribozyme) uses a histidine cofactor for an RNA cleavage, and the histidine cofactor makes a 10^6 -fold enhancement in the reaction speed (Roth and Breaker, 1998). Moreover, DNA enzyme containing Zn^{2+} and imidazole in its active centre can efficiently cleave an RNA substrate (Santoro et al., 2000).
- **Combinatorics:** I think only combinatorial chemistry can produce a sufficiently high chemical diversity at the outset of life.⁶ There are different combinatorial levels in biochemistry. At the first level, small biomolecules (cf., Dolle, 2002), e.g. amino acids, purines (Eschenmoser, 1999) and oligomers, short peptides etc. are produced. The second level is the combinatorial sequences of macromolecules (e.g., peptides, RNA or DNA). The combinatorial property of the proposed MAR-complex is a connecting link between these above two extremes.
- **Chirality:** The chiral catalytic activity of the MAR-complex guarantees the homo-chirality of the bio-molecules (cf., Soai et al., 1995a,b; Ordoukhanian and Joyce, 2002). (An evolutionary scenario of the origin of homochirality is discussed in Section 5.)
- **Error threshold:** It is well-known that the amount of genetic information (number of nucleotides) that can be maintained selectively was determined by the copying fidelity (the error rate of replication) (Eigen, 1971; Eigen and Schuster, 1977). This means that in the RNA world the ribozymes are too few and inefficient if their length is less than 30, while long sequences cannot be maintained without ribozymes (e.g., Gánti, 1975, 1997, 2004; Maynard Smith, 1979; Joyce and Orgel, 1993; Szostak and Ellington, 1993; Maynard Smith and Szathmáry, 1995; cf. Pool et al., 1999; Scheuring, 2001; Szabó et al., 2002). The MAR-complex is no more than a large “ribozyme” forming a few RNA fragments “polluted” by coenzymes. In MAR-complex world, there are two cases: first, when RNAs were very short (12–15 nucleotides) then

no new RNA sequences could arise due to inaccuracy of self-replication; it could only change the proportions of the different RNAs, since all possible sequences may exist already. Second, when RNAs were longer but still shorter than the error threshold, then the information may have been preserved by selection and the complexes may have played the role of integrator of information in the later steps of evolution (cf. Kuhn and Wasser, 1982). In both cases, the basic problem is transferred to the building up of MAR-complexes.

3. Re-building of complexes

At first glance, the semi-free building assumption prevents that the functions (i.e., active centres) are preserved, since, if the possible number of MAR-complexes is very high (practically “infinite”) while the number of MAR-complexes having catalytic activity is very small (finite), thus the concentration of the functioning MAR-complexes may be arbitrarily small. However, at the outset of MAR-complexes the selection takes place in the rebuilding of MAR-complexes. Rebuilding becomes easier by the base pairing of the RNA parts of MAR complexes. From this perspective, the base pairs were originally selected from rebuilding, however, the template directed ligation supplying RNA units of MAR complexes is also based on base pairing.

The re-building of MAR-complexes must be a four-step process. In the first step, the MAR-complex has to disintegrate. After this, the RNA parts become the templates of their complement RNAs. In the third step, the original (“sense”) RNAs are formed by template directed ligation. In the final step, the sense RNA parts are linked in a proper three-dimensional structure. In this step, there are two problematic moments: (1) the appropriate sense RNAs have to be selected to form the new MAR-complexes; and (2) these sense RNA parts have to be assembled into the desired geometrical structure. Three possible solutions of the latter two problems will be presented in the sequel. These solutions do not exclude each other.

1. **Pre-Code:** It seems that the high variability of complexes could be a starting point for the evolution of the genetic code. Suppose that the neighbouring RNA parts of MAR-complexes “recognise” each other, in other words, they form pairs with hydrogen bonds (Fig. 1). Then, the number of possible complexes would decrease. Current genetic code also ensures the precise geometrical structure and the proper composition of the ribosomes.⁷ This idea comes from the hypothesis of “coding coenzyme handles” (Szathmáry, 1993, 1999), which proposes that the amino acid coenzymes of ribozymes could have been fixed by short RNAs.

If the pre-genetic code helped the appropriate building of MAR-complexes, then this process can be further specified by the standardization of the handle-RNA parts of MAR-complexes. (The handle-RNA is a piece of RNA holding the “co-enzymes”; e.g., amino acids or short peptides) in the desired geometric position in the chirally selective active centre of MAR-complexes. If there exists a handle-RNA that possesses co-enzymes having an important role in a few active centres and the same handle-RNA is built in a proper geometrical structure into these active centres, then the number of possible complexes decreases. There is a possibility of this, namely, if the network-RNAs of different complexes are able to fix the same handle-RNA in a good position. (The network-RNA is a piece of RNA that does not hold “co-enzymes” but determines the geometric structure of MAR-complexes.) Finally, it is not impossible that this kind of standardization can lead from handle-RNAs to tRNAs

⁶ I note that the supposed combinatorial process for MAR-complexes is parallel to the study of Yamamoto and Hogeweg (1999). They studied a special resolved model of RNA world, in which primer induced replication, concatenation and random cutting increase the diversity of sequences.

⁷ The tRNAs bind according to the mRNA of the ribosomes during translation. If we take account the different mRNA and semi-finished polypeptide chain we have “practically infinite” different ribosomes as a complex. This supports a semi-free building assumption.

(see Szathmáry, 1999). This means that in this scenario the evolution of ancestor of tRNA started long before the appearance of translation.

The stereochemical match between the code and the coded amino acids is one of three different widely recognised properties in the genetic code (Knight et al., 1999). The stereochemical hypothesis proposes that the origin of the genetic code must have started as a stereochemical interaction between RNAs (either codon or anticodon) and amino acids (Di Giulio, 1997, 1998; Yarus, 1998, 2000, 2002). This stereochemical matching helped the fixation of the free abiogenic amino acid co-enzymes in the active centre of a MAR complex.^{8,9} To check this idea, I propose the following two experiments: First, it may be important to examine whether an abiogenic L-amino acid and the repetitive oligomers of its codon and anticodon (either codon-anticodon-codon-anticodon or anticodon-codon-anticodon-codon or their complex¹⁰) are matching or not. Second, it is also important to check whether a MAR complex containing the above oligomers can fix L-amino acids or not. In both cases, it is most important to test the repetitive oligomers built from adenine, thymine and uracil, and such L-amino acids which are coded by these nucleotids, since the abiogenic origin of cytosine is very problematic (Shapiro, 1999).

The following two assembly mechanisms may have taken place in a later stage of evolution when RNAs became longer, therefore RNA replicas also existed and there may have existed MAR-complexes able to bind amino acids with RNAs.

2. *Ribozymes*: Assume that the chemically independent RNA parts of MAR-complexes are bound to one another (cf., Di Giulio, 2004). Moreover, there is a system of self-sustained replication of RNA enzymes (Lincoln and Joyce, 2009), in which there are two enzymes which catalyse each other's synthesis from oligonucleid substrates. From the point of view of MAR-complexes, it is important that this reactions deal with oligomers. The appearance of ribozymes may have two advantages. First, a large RNA forms a functional complex more easily. Moreover, decreasing the number of RNA parts radically decreases the number of possible MAR-complexes. This possibility may have been important on the set of network-RNAs, since the coenzymes of the carrying-RNAs prevent the bound RNAs from "self-replicating". This process is limited by the following: the single RNA coming from bound RNA parts of MAR-complexes is able to change drastically the secondary structure of the active centre. In this case, its functionality will be lost. Moreover, long RNA needs catalytic help in replication thus the appearance of polymerase must precede that of long RNA. This way, a "polluted RNA world" may have developed, a world "heavily polluted" by metal ions, amino acids, short peptides and other co-enzymes.

3. *Pre-introns*¹¹: Now, suppose that in the world of MAR-complexes an RNA sequence (pre-intron) arises which is able to bind reversibly two RNA parts of the MAR-complexes in the presence of the metal ion of the MAR-complexes. In this case, there is the possibility that this binding may weaken the stability of the MAR-complex. Thus, the bound RNA may leave from the MAR-complex and start replication. The appearance of pre-introns may be able to

synchronise the replication of the RNA parts. On the other hand, if a pre-intron reversibly binds to two RNA parts in the presence of metal ion of the MAR-complexes, then this may help to form further MAR-complexes. The appearance of this kind of pre-intron frees the MAR complex from the ability to disintegrate as well.

Moreover, if the complement RNA of a pre-intron is not able to self-splice from the complement RNA of the RNA parts of a MAR-complex, then this kind of pre-introns further decreases the number of possible MAR-complexes since they would decrease the number of the "nonsense" (complement) RNAs. This kind of pre-intron is also to serve as a starting point of the appearance of the inheritance system since only the large nonsense RNAs code the information without having any other, catalytic, function.

4. MAR-coalitions form reflexively autocatalytic system

The re-building mechanism of a MAR-complex can only guarantee the "inheritance" of active centres while the multiplication of MAR-complexes is based on the replication of their RNA parts by template direct ligation. If a MAR-complex is able to produce any elements of the parts of MAR-complexes or any parts of MAR-complexes, then the selection may only operate on the "coalition" of MAR-complexes, since MAR-complexes are unable to self-replicate. The autocatalytic multiplication of MAR-complexes is possible provided that the MAR-complexes of MAR-coalitions form a reflexively autocatalytic system (Kauffman, 1986), if they are able to catalyse the production of their own components, first of all, their nucleotides (nucleotide bases and sugars) and the chiral elements of the active centres of their own MAR-complexes (see Fig. 2).

In our case, the reflexively autocatalytic MAR-coalition has the following necessary properties: (1) Complexes must be capable to catalyse the formation and cleavage of oligomers bound. (2) An abiotic flux, which synthesises oligomer parts of complexes from the maintained "food set" of nucleotide monomers or short (3–4-mer) oligomers, must be thermodynamically feasible. (3) The reactants must be confined to a sufficiently small volume. (4) The catalysed reactions lead from the "food set" through the synthesis of nucleotide to the oligomer parts of the complexes.

There is another reason for supposing MAR-coalitions. Consider a "parasite" RNA of MAR-complexes,¹² which is a short RNA having a higher self-replication rate. This "parasite" RNA can form inactive complexes but these complexes are able to dissociate as well (this is important for self-replication). If the system is well-mixed then there is the possibility that this kind of RNA destroys that system. Thus, at the outset of life the same kind of effect is needed to decrease the total mixing of the whole system. Now let us recall one theoretical possibility of the first replicators to make a coalition, namely, the hypothetical "primitive pizza" assumes that life originated on a surface rather than in the "primordial soup" (e.g., Wächtershäuser, 1988b, 1992; Maynard Smith and Szathmáry, 1995). The main point is that if reactions and the diffusion processes occur on an absorptive surface and diffusion speed is lower than catalysis reaction speed, then diffusion is able to separate the coalitions (Czárán and Szathmáry, 2000; Könyű et al., 2008), which can guarantee property 3 of reflexively autocatalytic system.¹³

⁸ Observe that in the mechanism with the histidine cofactor enhancing the reaction speed of RNA cleavage by a desoxyribozyme (Roth and Breaker, 1998), some kind of stereochemical match is necessary.

⁹ The importance of stereochemical matching is supported by the fact that only the arginine codons show significant affinity for arginine. Moreover, arginine is a special amino acid, possibly even a late addition to the genetic code (Landweber, 1999b).

¹⁰ These sequences are supported by the presence of codon-anticodon pairs in the acceptor stem of tRNAs (see Rodin et al., 1996).

¹¹ Since recent self-splicing introns are huge RNAs, this rebuilding possibility could arise in a latter stage of evolution.

¹² It is analogous to the case of the parasite of the hypercycle, which can destroy the hypercycle (the mutant is an excellent target of another element of hypercycle, but a poor replicase, see Maynard Smith, 1979).

¹³ It has been shown recently that replicators can coexist and parasites cannot destroy the metabolic network if the primordial soup is assumed to flow chaotically (Károlyi et al., 2002). The reason of similar behaviour in the different system is that mixing is imperfect and replicators are considered as locally interacting individuals.

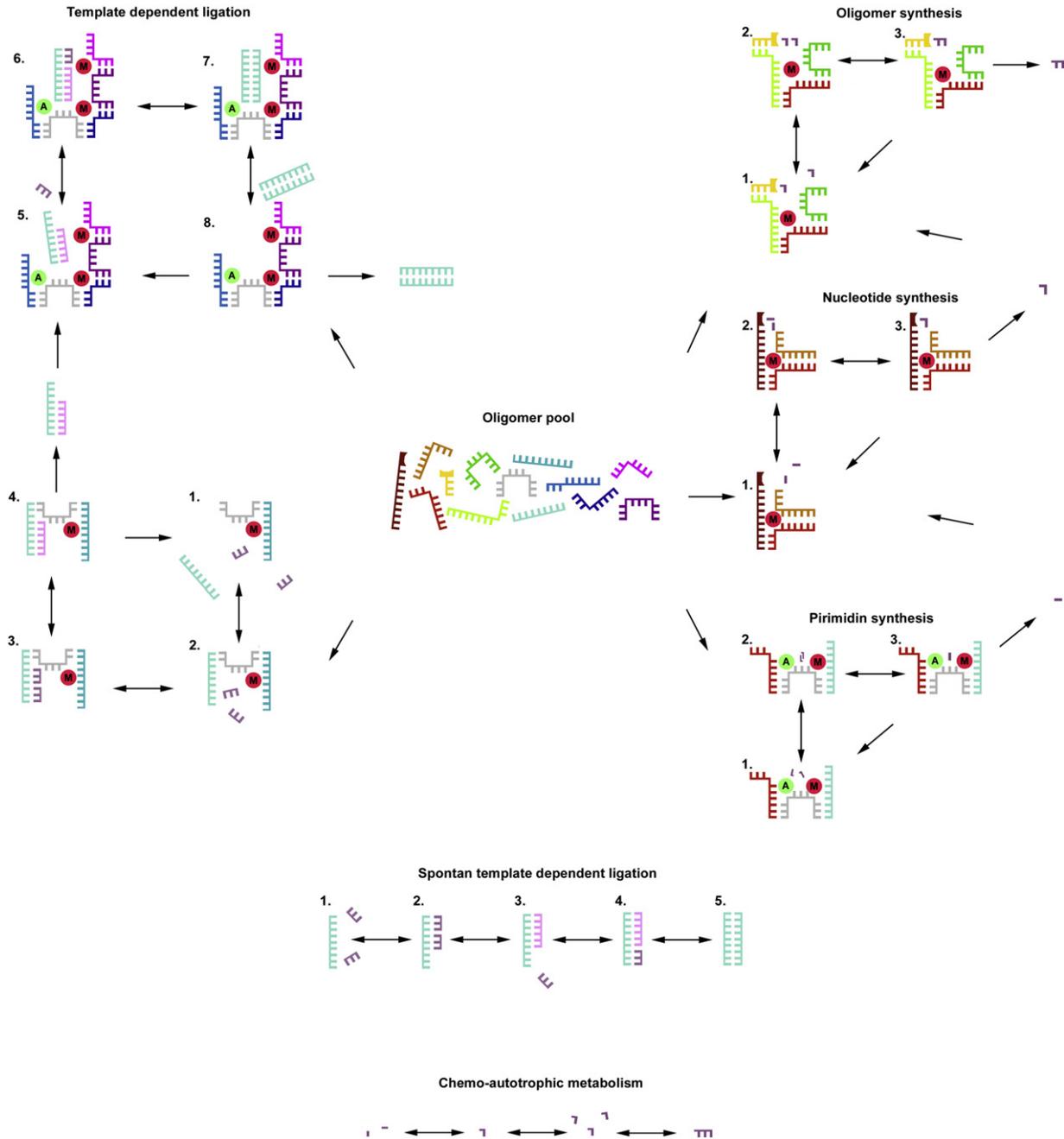


Fig. 2. The reflexive autocatalytic MAR system is built up on the basis of chemo-autotrophic pyrite surface metabolism producing nucleotides, mainly purine, and short oligomers. The initial oligomer pool is produced by spontaneous ligation and non-enzymatic template dependent ligation. The main part of the reflexive autocatalytic MAR system must produce pirimidins, nucleotides, and small oligomers. The first replication system is based on template dependent ligation. Observe the proposed combinatorial property of MAR complexes, namely, a small number of oligomers are able to build up a huge number of MAR complexes, like a Lego.

5. A possible Darwinian scenario for the evolution of mar-complexes

At the outset of the MAR-complex evolution, there are two main problems: the origin of homochirality of its elements and the origin of cytosine. We adapt the hypothesis of iron-sulphur world (Wächtershäuser, 1992) as a starting point, since the chemo-autotrophic surface metabolism provides the molecular basis of MAR-complexes. Moreover, as Wächtershäuser suggested, the homochirality of a given pyrite crystal is expected to be transferred with high enantioselectivity into the organic constituents formed on its surface. Of course, the pyrite surfaces were not absolutely pure, these may have been mixed with (1) other phosphate

minerals, which could activate the monomers (Gao and Orgel, 2000), (2) clay minerals, which could catalyse polymerization of monomers (Ferris and Ertem, 1993; Kawamura and Ferris, 1994; Ferris et al., 1996) and which could preserve RNA from degradation (Franchi and Gallori, 2004), (3) apatite, which might be the other energy resource since it can generate polyphosphate on heating (De Graafe and Schwartz, 2000; Kornberg et al., 1999) and (4) borate which can stabilize ribose (Ricardo et al., 2004). On the other hand, input of molecules came from meteorites and from synthesis in a reducing atmosphere should not be neglected as well (Orgel, 1998).

Furthermore, on the surface of minerals (or lipids see Section 5.3) the chemical reactions are not considered totally mixed. This

two-dimensional world plays crucial role in the chemical and selection processes as well.

5.1. A Darwinian scenario for the origin of homochirality

Here I propose four successive steps for the origin of homochiral MAR coalitions:

1. *Amplification of asymmetry by chiral autocatalytic reactions:* It is well known that chiral autocatalysis in organic reaction, when a small amount of non-racemic product is added at the start, the enantiomer that is initially in excess is produced in a large amount when the reaction is complete (Soai et al., 2001). As Caglioti et al. (2006) emphasized, the statistical nature of the formation of racemates by itself can supply a small perturbation of 50–50%.¹⁴ Moreover, the morphological chirality can induce homochirality if insoluble portions of such crystals are added to reaction mixtures of asymmetric autocatalysis. This phenomenon was experimentally documented for inorganic crystals (e.g. for pyrites, see Soai et al., 1994, 1995a,b, 2001; Wächtershäuser, 1992; Cintas, 2002; and for sodium chlorate and sodium bromate see Sato et al., 2004) and organic crystals (Kawasaki et al., 2008) as well.

The instability of the racemate for autocatalytic dynamics can only explain the local homochiral final state in a single autocatalytic reaction but cannot guarantee the same chirality in all autocatalytic reactions. Chiral autocatalysis must have been very important in the origin of homochirality since it is able to compensate spontaneous racemization, and it can guarantee the chiral homogeneity of the building material of life. Of course, locally those auto-catalytic molecules, which are important in producing RNA, do not necessarily have the same chirality. However, if the surface is large enough then there may exist some local region in which where these molecules have the same chirality. The chiral autocatalytic process maintains locally chirality within a racemic pool.

2. *Surface metabolism produces the first homochiral MAR-complexes:* Let us suppose that on prebiotic mineral surfaces the racemate of sugar or sugar phosphates and the adenine, thymine, uracil and other abiogenic bases formed oligomers with 10–14-mers (Wächtershäuser, 1988a; Liu and Orgel, 1995; Levy and Miller, 1998; Schöning et al., 2000; Eschenmoser, 1999). On the surface of minerals, these pre-RNAs with bivalent metal ions, abiotic racemate amino acids and short peptides make the first MAR-complexes.

Of course, participation of other molecules cannot be excluded from the formation of the first MAR-complex. It is very likely that at the outset there were more than four bases and the recently existing nucleotides are the result of strict selection, since it seems that pyrimidines were missing at the beginning (Wächtershäuser, 1988a, 1992). Moreover, it is very possible that in the first steps RNA-like polymers started the evolution (Levy and Miller, 1998; Eschenmoser, 1999; Hund and Anet, 2000; Nelson et al., 2000). Complexes, building up these RNA-like polymers (e.g. PNA), might produce the first glucose. One possible energy source of ligation was the abiotic pyrophosphate and polyphosphate (Kornberg et al., 1999).

There are two hypothetical processes that can amplify the chirality of oligomers:

◦ *Template directed ligation* can be considered as a chiral autocatalysis, since the stability of the intermediate complex strictly depends on the number of hydrogen bridges between oligomers, and, turns on the chirality of the oligomers. This process also amplifies the local homochirality of oligomers. This agrees with

Bolli et al. (1997) who noted that the chiroselective self-directed oligomerization of oligomers generates sequence libraries consisting of predominantly homochiral oligomers.

◦ The assembly process of MAR complexes strictly depends on the chirality of oligomers. Their stability depends on the number of hydrogen bridges as well, since assembly of a MAR-complex is determined by spherical matching of units. Thus, it can be supposed that homochiral, well-pairing, oligomers form more stable MAR-complexes than “racemic” MAR-complexes, having at least one racemic oligomer. Since on the early Earth the RNA was not stable enough, the easily dissociating “racemic” oligomers of MAR-complexes broke down more frequently. This hypothesis can also be tested easily by the following experiments: In the first one, one can check whether under hostile prebiotic environment MAR-coalitions are more stable than their oligomers. In the second one, one can check whether “homochiral” MAR-complexes are more stable than the “racemic” ones under hostile prebiotic environment.

Observe that in the processes of template directed ligation and assembly of MAR complex, not only the chirality, but the chemical purity of oligomers are also very important. The repetitive and uniform backbone of the oligomers may have relative advantage guaranteeing correct pairing of the bases.

It is also very likely, that locally homochiral but on the average “racemic” active centres were formed in this process. The first MAR complexes with chiral catalysis amplify the starting local chirality in two possible ways: they either produce their own chiral elements or other, non-autocatalytic, chiral molecules, which are precursors of their elements. Of course, here we have locally chiral molecules again but a racemic pool on the average.

• *First reflexively autocatalytic systems:* The main attribute of MAR-coalitions is the ability to form reflexively autocatalytic systems, which had at least four sufficient types of reaction cascades: First one might have catalysed the production of D-glucose, the second one could have produced nucleotides and the third one could be in the outset template independent, nucleic acid polymerases which produced only 2–5 mers. The fourth one could be aminoacyl-oligomer syntheses, which guaranteed sufficient chemical diversity in the active centres.

In a theoretical point of view, the first reflexively autocatalytic system must build up from the molecules produced by abiotic random combinatorial chemistry. The overwhelming majority of molecules producing sufficiently rich combinatorial chemistry of carbon must be chiral. Thus it is very likely that the first reflexively autocatalytic system had to use chiral molecules. The reflexive autocatalytic system is at the same time a generalized autocatalytic system. Thus, I propose, that similarly to the chiral autocatalytic systems, the racemic reflexive autocatalytic systems are also unstable (see e.g. Caglioti et al., 2006). Consequently, any stable reflexive autocatalytic systems can only built up by homochiral chemical cascades or homochiral sub-cycles. In my view, this is the ultimate reason why homochirality is a must for the outset of life no matter what kind of molecules carries the first active centres.

Accordingly, the reflexively autocatalytic MAR-coalitions may evolve only in such places where homochiral chemical sub-cycles or cascades may appear by chance. Those kinds of MAR-coalitions will have a higher “production rate” in which the chiral reaction steps of the two basic cycles (nucleotide and amino acid syntheses) have the same chirality within each cycle but the chirality of the two cycles is not necessarily the same. The homochirality of the nucleotide cascade does also exclude the problem of cross-inhibition. This step is the most important one since here homochiral metabolism, which is supported by active centres, may

¹⁴ A small amount of non-racemic perturbation could come from the universe as well (Bailey et al., 1998; Engel and Macko, 1997).

appear. At the end of this step, coalitions of MAR-complexes were formed, differing mainly in chirality.¹⁵

- *Darwinian selection of MAR-coalitions:* Now let us consider all possible compositions of chiral MAR-coalitions, in which the different autocatalytic reaction cycles may have different chirality. We have two main possibilities for fixing one of chiral composition: The first one is a by-chance process. Since the mineral surface is finite, with or without any chemical advantages of any chiral coalitions, a stochastic process can guarantee that only one coalition is the winner, in general; since all purely homochiral states are absorbent. This means that in a stochastic process only one homochiral state is not lost by chance during neutral evolution (when there is no advantage of any MAR-coalition in static environment). The second possibility is a selection process, if the environment changes, so will the result of evolution process. The different chiral MAR-coalitions compete with each other for their non-chiral elements, e.g., nucleotide bases. Moreover, based on cross-inhibition, different chiral type MAR-coalitions poison each other at the adjoining area. The winning MAR-coalition was either the one that first produced important amino acid co-enzymes or the one that first improved significantly the mechanisms of re-building of the key MAR-complexes having important chiral catalytic activity. These processes which, in theory are analogous to mutations in biology, can improve the competitive ability of MAR coalitions. To sum up Darwinian evolution has fixed one chiral composition.

I do emphasise that the above scenario is based strictly on the two-dimensional surface which implies that the proposed chemical system is not well-mixed. In totally mixed chemical systems, there is a non-zero probability that cross-inhibition also inhibits evolution. I think that at the outset of life a homo-chiral metabolism catalysed by MAR-complexes lacking cytosine existed in which chiral purines (e.g., uracil and 2,6-diaminopurine cf. Reader and Joyce, 2002) were synthesised. This idea comes from Wächtershäuser (1988a, 1992), by putting forward the hypothesis that in the first step only purines built up the RNAs.

5.2. The evolution of mar-complexes

In the next step, MAR-coalitions were able to produce pyrimidines.¹⁶ From the viewpoint of the origin of cytosine, the existence of the following three ribozymes is extremely important. The first ribozyme catalyses the synthesis of a pyrimidine nucleotide, namely uridine (Unrau and Bartel, 1998). The second one is an RNA ligase ribozyme lacking cytosine and RNA folding and catalysis can be achieved in a three-nucleotide system (Rogers and Joyce, 1999). The third one is a ligase ribozyme, which is composed only by 2,6-diaminopurine and uracil nucleotide, catalyses the template-direct joint of two RNA molecules (Reader and Joyce, 2002). Observe that the existence of these ribozymes gives support to the above mentioned hypothesis of Wächtershäuser (1988a, 1992), so that there may have been MAR-complexes lacking cytosine while are able to synthesise cytosine. The appearance of pyrimidines had three advantages: first, this opened the possibility of accurate replication of the RNA part of MAR-complexes. Second,

the diversity of short RNAs increased dramatically. Third, parallel to the codon/anti-codon pairing, the RNA parts of MAR-complexes were able to “recognise” each other, allowing the re-assembly of MAR-complexes more efficiently.

Those coalitions of MAR-complexes attained relative advantage over the others in which the re-assembly mechanism was more efficient. This was guaranteed by the emergence of MAR-complexes that were able to replicate the short RNA parts of MAR-complexes of the metabolism. Moreover, the re-building mechanism became “standardization”. This means that MAR-complexes, having different function but using the same amino acids in their active centre, were able to “recognise” those potentially repetitive RNA oligomers which fixed the free amino acids in question based on the stereo chemical match at the active centre. The relative advantage of a coalition of those MAR-complexes, which were able to build the amino acids to short RNA and to modify the amino acid part of these molecules, was increased. This increase was possible because new amino acids dramatically increased the efficiency and number of the catalysed reactions of the coalitions of MAR-complexes.

In the next step, after standardization in the MAR-complex word, translation may have evolved according to the following step-by-step scenario. It is reasonable to suppose that two or more amino acids are needed in a very near position in the active centre. Thus some network-RNA must contain adjacent groups fixing the carrying-RNAs. Later on, when the MAR-complexes have enough catalytic spectrum to maintain a longer (40–60-mer) RNA population, MAR-complexes can catalyse the production of activated amino acids and/or the formation of a peptide bond. In this period, a collector RNA-strand and several hairpins form a picket-fence like aggregate (see Kuhn and Wasser, 1982) and this complex may produce short peptides. The first peptides were co-enzymes of the MAR-complexes. In this period, the metabosome¹⁷ (Gibson and Lamond, 1900) could have evolved from the MAR-complexes. The appearance of the pre-translation mechanism had a relative advantage in producing short peptides, which were co-enzymes of MAR-complexes. Moreover, the “replica” MAR complexes allowed that the number of components of the RNA parts of MAR-complexes exceeded 10–12 units. In this situation, the appearance of pre-introns helps re-building the MAR-complexes, if the “replicas” MAR-complex were able to cut the hydrogen bond of RNAs.

5.3. Pre-adaptations before the appearance of pre-cell

It is widely accepted that the appearance of the pre-cell is a very important step in the origin of life (Eigen et al., 1981; Wilson, 1980; Szathmáry and Demeter, 1987; Szathmáry and Maynard Smith, 1997; Szathmáry, 2007; Szostak et al., 2001; Cavalier-Smith, 2001). The membrane glycerol phosphates of all living organisms are not chirally homogeneous¹⁸ (e.g., Peretó et al., 2004). This suggests that the evolution of membrane synthesis finished after the chirality fixed both amino- and nucleic-acid biosynthesis and after the development of fatty acid biosynthesis (Peretó et al., 2004). Based on these assumptions, it is likely that the cell emerged when there was a “symbiosis” between proteins, huge MAR-complexes and lipids. In this section, I propose two pre-adaptive steps before the pre-cell appeared.

¹⁵ Observe that on the surface of the homochiral pyrite crystal homochiral MAR-complex coalitions may have formed more easily since the chirality type of pyrite crystal might have been transferred with high enantioselectivity into the element of the MAR-complexes.

¹⁶ I am stuck with the feeling that the abiogenic absence of cytosine plays an important role in the origin of life. One possibility is that the winning chiral composition of MAR-coalition was able to produce cytosine first.

¹⁷ The “metabosome” is also a complex: a huge framework of RNA with binding sites for “metabolite-caring” adaptor RNAs, which would serve to align the adaptors via complementary base pairing. The adaptor sequences are parallel to the m-RNA and metabolite-caring adaptor RNAs correspond to the t-RNA, see Gibson and Lamond (1900).

¹⁸ Bacterial and archaeal membrane glycerol phosphates are mirror images to each other.

Let us start from the hypothesis that the first phospholipids were synthesized under pre-biotic conditions (Koga et al., 1998; Wächtershäuser, 2003; Désaubry et al., 2003; cf. Segré et al., 1999). The membranogenic molecules would spontaneously be absorbed by the existing membrane (Rashevsky, 1938; Lipowsky, 1991). These phospholipid layers on mineral surface could collect hydrophobic abiotic amino acids, small peptides and RNA molecules (Janas and Yarus, 2003). Moreover, RNA complexes (Vlassov et al., 2001) rapidly bind phospholipid bilayers as well. Another possibility is that MAR-complexes could be anchored by its RNA part which conjugated with hydrophobic amino acids or small peptides. It should be tested in laboratory whether membranes could collect MAR-complexes as well.

At the outset, some protection of the RNA is useful, since chemical reactions, which are not catalysed by active centres but supply chemical compounds for the origin of life, need radicals with high energy. Presence of lipids drastically reduces RNA decomposition induced by radicals generated from pyrite (Cohn et al., 2004). Therefore, lipids could save the MAR-coalitions as well, regardless whether the lipids were coating the mineral surface or encapsulating the RNA.

Furthermore, lipids cannot only collect very diverse molecules, but supply a two dimensional surface for the MAR-coalition. It is very likely that the catalytic activity of this kind of *dynamical combinatorial supramolecular lipid surface* is higher than that of the collected MAR coalitions alone (cf., Segré et al., 1999).

The energy resources were either FeS/H₂S, mineral phosphates or thioester (Weber, 1984) at the outset of life. When these resources were exhausted, membrane would play an important role in making energy, since it can separate two spaces, one is either the surface of mineral or the lumen of vesicula and second one is the ambient water or atmosphere. This hypothesis is confirmed by the following fact, if the membrane is not permeable for H⁺ and cations present, then the growth of fatty acid vesicles by incorporation of additional fatty acids generates a transmembrane pH gradient spontaneously (Chen and Szostak, 2004).

The CO₂ fixation by reduction by H₂ or H₂S was probably one of the first energy producing processes allowing the so-called “ob-cells” to evolve (cf., Maynard Smith and Szathmáry, 1995; Cavalier-Smith, 2001). From the point of view of energy production and of ob-cells, some kind of transport of molecules was important. The minimal transport process was passive diffusion of nutrients (Monnard and Deamer, 2001). On the other hand, RNAs can transport molecules throughout the membranes, for instance an RNA complex can form a selective route through the bilayer for tryptophan (Janas et al., 2004), other RNA complexes can increase the ionic conductance of phospholipids membranes (Khvorova et al., 1999; Vlassov et al., 2001). In this period, translation may appear for the following reasons: First, hydrophobic peptide-anchors could fix better the RNA on the surface of the lipid membrane (cf., Cavalier-Smith, 2001). Second, hydrophobic peptides could help the transport process. Third, peptides can replace the enzymatic function of RNA, separate the information carrying function from the enzymatic function, which opens the way for the evolution of genetic systems (i.e. the number of nucleotides used decrease and simultaneously the genetic code is fixed). During this period, the RNA/protein world evolved with “symbiosis” with lipids. It is well-known that the semi-permeability of membranes (cf., Chen and Szostak, 2004) strictly determines energy production. Thus, the length of linear fatty acid chains (C16–C18), which were made by active centres of RNA/protein world, was optimal for energy production.

It is reasonable that the development of membrane dependent energy production processes was the most important pre-evolutionary event before the pre-cell appeared. This process

might force the canalization between RNA and protein metabolism, including the fixation of the genetic code.

6. Summary

Replication and metabolism are the essential features of life. In this paper, I proposed that at the outset of life MAR complexes can assemble themselves based on base pairing and that they could contribute to metabolism with their chiral active centres. The main arguments in favour of MAR-complexes are as follows. First, at the very beginning of life small organic molecules (Shapiro, 2006), in the present paper oligomers, existed, so the active centres may have formed with high probability only on the surface of “macro” complexes of oligomers. Second, the length of RNAs strictly limits their self-replication, so short length was important for self-replication of the parts of these MAR-complexes. Third, if the building of MAR-complexes is semi-free then at the beginning of life there may have been a great variability of active centres.

Now the question arises: how to produce the proposed MAR-complexes with chiral active centre in the laboratory? The following process is a slight modification of the selection of ribozymes (Szathmáry, 1984, 1990; Szostak, 1992; Landweber et al., 1998; Landweber, 1999a).

1. Synthesise a stable analogue to the transition state complex of an arbitrary chemical reaction.
2. Make MAR-complexes from short chiral RNA, metal ions, co-enzymes and other chemical groups (e.g., amino acids) bound to RNA.
3. Select, by affinity chromatography, MAR-complexes that bind to the transition stable analogue.
4. Separate the selected MAR-complexes.
5. Analyse the parts of the selected MAR-complexes.
6. Make a small change in the parts of a selected MAR-complex, and form new MAR-complexes. In this step, the process may ramify since the parts of different selected MAR-complexes do not necessarily form functional new MAR-complexes.
7. Carry out repeated cycles of amplification and selection of MAR-complexes.

The active centre hypothesis could be supported by the existence of MAR-complexes, which stereoselectively catalyse the following reactions: any step of the D-glucose or sugar phosphate and the pyrimidine synthesis, RNA-polymerization and the reaction of D-glucose with orotidine monophosphate that eventually produces uracil. A further supporting reaction could be the polypurine reaction with abiogenic L-amino acids. Perhaps the latter reaction could produce parts of complexes being able to catalyse certain steps of pyrimidine synthesis.

In the following, the main logical steps and background theories of the steps of the proposed scenario of the early RNA-world are surveyed. My scenario starts out from the hypothesis of the chemo-autotrophic metabolism on mineral surfaces (cf. *sulphur-iron world* proposed by Wächtershäuser). The chemo-autotrophic metabolism provides combinatorial chemical basis for the outset of MAR-complexes. On the surface of minerals *chiral autocatalysis* can maintain local homochiral states. During this process, all kinds of chiral “compositions” of oligomers may arise but they are locally homochiral on the surface of minerals. *Template directed ligation* was the first “replicating” process and this chiral autocatalytic process can amplify local homochirality. The assembly of MAR-complexes also depends on the number of hydrogen bridges thus also on the chirality of its oligomers. If the MAR-complex is more stable than the oligomers, then the assembly process can also amplify local homochirality. MAR-complexes form a *dynam-*

ical combinatorial library. Based on the combinatorial oligomer complex, assembling the pool of MAR-complexes has a wide spectrum of the chiral active centres. Since these MAR-complexes may produce their chiral elements, there is a chance that a *reflexively autocatalytic system* develops. On the other hand, a *two dimensional surface* of minerals has extreme importance, since MAR-complexes form local reflexive autocatalytic coalitions if diffusion on the surface is slower than the speeds of reactions. These MAR coalitions can be considered as the first units of Darwinian evolution, since these coalitions compete with each other for space and for the non-chiral “food” molecules produced by pyrite metabolisms. The multiplication rate and the efficiency of reflexive autocatalytic MAR coalitions depend on two main factors.

The first one is the re-assembly mechanism of MAR-complexes that have important active centres from the viewpoint of reflexive autocatalytic circles. Of course, replication (based on template directed ligation) of the oligomer parts of these MAR-complexes is a necessary but insufficient condition of the “reproduction” of the MAR-complexes. For the re-assembly mechanism, three possibilities were suggested in this paper: the pre-code, the integration of the network-RNA parts of complexes and the pre-introns, which are self-cleavage RNAs able to bind reversibly two RNA parts of a complex. In essence, the pre-code re-assembly mechanism is the same as Szathmáry's hypothesis on the origin of the genetic code, in the sense that in both scenarios pre-code is evolved from the appropriate re-assembly of the active centres. Furthermore, useful coding precedes translation, because coding, in the form of an unambiguous assignment of amino acids to codons, arises without the need of protein synthesis.

The second factor is the chirality of active centres: I assume that a stable reflexive autocatalytic MAR-coalition must contain a homochiral reaction cascade or homochiral autocatalytic sub-cycles. This is the final reason for the homochirality of life. The MAR-coalitions different chiral composition not only compete for space and essential non-chiral molecules but also poison one another based on cross inhibition. In the end of competition, one of the chiral compositions of MAR-coalitions may fix, for instance, the one that was able to produce cytosine first by a MAR-complex in which cytosine was locking. The appearance of cytosine greatly increases the efficiency of the re-assemble mechanism based on base pairing. And finally, based on the proposed re-assembly mechanisms (the integration of the network-RNA and pre-introns) RNA-word evolves, which is highly “polluted” by amino acids and metal ions.

Possible pre-evolutionary processes between MAR-complexes and lipids are also suggested before the appearance of the pre-cell. Lipid surfaces can collect short abiotic hydrophobic peptides and RNAs as well. It is hard to estimate the evolutionary importance of lipids without knowledge of the catalytic ability of lipid/peptide/RNA supramolecular structures, for which I propose the following experimental scenario:

1. Select an appropriate immuno-globulin against an active centre of an arbitrary chemical reaction (i.e., the building region of desired immuno-globulin is analogous to the transition state complex of the desired chemical reaction).
2. Make a combinatorial lipid/peptide/RNA supramolecular structure: first incubate lipid membrane with hydrophobic peptides with small hydrophilic tails and with random RNA (or MAR-complexes). After that wash out all peptides and RNAs that are not bound to the lipid membrane.
3. Incubate the combinatorial lipid/peptide/RNA supramolecular structure with the selected immuno-globulin.
4. Select molecules that bind to the immuno-globulin (e.g., after the subtraction of lipids, immuno-globulin with bounded molecules can be selected by gel electrophoreses).
5. Separate the selected molecules.
6. Make a small but random change in the parts of selected molecules. Carry out repeated cycles of amplification and selection of lipid/peptide/RNA supramolecular structure.

In my opinion, membranes might have a very important rule in producing energy at the beginning. This was probably the main driving force in forming RNA/protein/lipid “symbiosis”.

The first question in the Introduction concerns the origin of the basic universal biochemical scheme of life. My hypothesis asserts that this scheme evolved from the MAR-complexes, in which chiral amino acids and chiral short RNA were in the active centre, and that re-assembly of MAR-complexes was based on base-pairing. From this assemble started the evolution of the genetic code. From a chemical point of view, the basic idea of this paper is that transition from chemistry to biology is via a dynamical combinatorial library which can form a reflexive auto catalytic system. That is, this library contains elements, which can build (with base-pairing) active centres which catalyse the reactions of the reflexive autocatalytic system. If the efficiency of reflexive autocatalytic activity of a dynamical combinatorial library increases with the homochirality of the active centres of reaction cascades and the homochirality of the elements of the dynamical combinatorial library then Darwinian evolution leads to the homochirality. The crucial part is the set of the active centres which determine the reaction network. This is the reason why I have called my scenario on the origin of life the *active centre hypothesis*.

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