CATALYSIS AND PREBIOTIC RNA SYNTHESIS

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Abstract. The essential role of catalysis for the origins of life is discussed. The status of the prebiotic synthesis of 2',5'- and 3',5'-linked oligomers of RNA is reviewed. Examples of the role of metal ion and mineral catalysis in RNA oligomer formation are discussed.

There is a growing consensus that the RNA World did not evolve directly from molecules formed by prebiotic processes (Orgel, L. E., 1986; Joyce *et al.*, 1987; Joyce, 1989). The reasoning is that the chemical processes involved in the formation of RNA oligomers are too complex to have occurred on the primitive Earth. It is suggested that the original life was simpler and that it evolved to the RNA world. Indeed, there is evidence to support the hypothesis that it is unlikely that the RNA World evolved from simple prebiotic molecules.

The known problems associated with the spontaneous formation of RNA will be outlined and the need to investigate the possible role of catalysis will be discussed. It is my belief that the limited investigations of the role of catalysis make it impossible to reach a decision concerning the viability of a prebiotic formation of RNA.

Problems with the Prebiotic Formation of RNA – An Overview

One is confronted with the unanswered questions concerning the formation of RNA starting from the first steps of the proposed prebiotic synthesis. There are essentially no problems with the prebiotic synthesis of purines from HCN (Oro and Kimball, 1961, 1962; Sanchez *et al.*, 1967; Ferris *et al.*, 1978), assuming HCN was present on the primitive Earth (Kasting, 1993). Some pyrimidines are also formed from HCN, but less efficiently (Ferris *et al.*, 1978, 1979; Voet and Schwartz, 1982). It has been generally been assumed that the ribose of the RNA nucleotide building blocks was formed by the condensation of formaldehyde in the formose reaction. The low yield of ribose in this reaction (Decker *et al.*, 1982), together with the formation of about twenty other sugars at the same time suggests that it was not possible to generate ribonucleotides from this mixture. The use of minerals to catalyze the conversion of formaldehyde to ribose has so far been unsuccessful (Schwartz and deGraaf, 1993). The yields and selectivity of ribose formation is compounded by an inefficient synthesis of nucleosides in the reaction of purine bases with pure ribose and the absence of any detectable product in the reaction of pyrimidines with pure ribose (Fuller *et al.*, 1972 a, b). It is possible to efficiently phosphorylate nucleosides to nucleotides by heating with phosphate in the dry state

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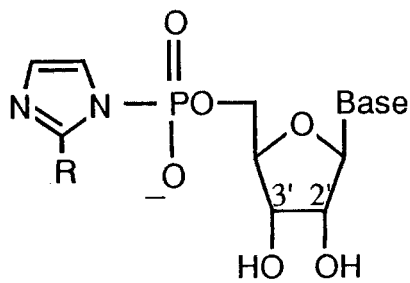
in the presence of urea (Osterberg *et al.*, 1973). This synthesis requires a somewhat specialized set of reaction conditions but the process proceeds in high yield to a mixture of phosphorylated products.

A key part of the RNA World hypothesis is the template-directed synthesis of RNA oligomers. RNA may have been formed autocatalytically from small amounts of prebiotically produced templates (von Kiedroski, 1986; Zielinski and Orgel, 1987). Unfortunately, the template-directed synthesis of the complementary RNA strands is successful in a limited number of instances. Condensation of the phosphorimidazolide derivative of 5'-GMP (**1**, base = guanine) to G oligomers on a poly(C) template proceeds efficiently in the presence of Zn^{2+} to form 3',5'-linked G oligomers (Lohrmann *et al.*, 1980, Inoue and Orgel, 1982). The efficiency of the reaction decreases markedly in the reaction of the phosphorimidazolide of adenosine (**1**, base = adenine) on poly(U) (Sleeper *et al.*, 1979) while it does not proceed at all in the reaction of the phosphorimidazolides of C or U on poly(G) or poly(A) respectively (Orgel, 1986; Stribling and Miller, 1991). Even template directed syntheses using templates containing mixtures of G, C and A do not proceed with the same efficiency and fidelity as those with a poly(C) template (Orgel, 1986).



1, R=H, CH_3

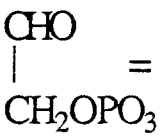
There are additional concerns with the plausibility of the template-directed synthesis scenario. The prebiotic synthesis of the phosphorimidazolides of the 5'-nucleotides (**1**) proceeds from the corresponding nucleoside triphosphate in low yield only in reactions where the humidity is controlled and the imidazole concentration is high (Lohrmann, 1977). The template-directed synthesis of RNA polymers from those oligomers formed in prebiotic simulation experiments has yet to be demonstrated.

Enantiomeric inhibition is yet another problem with the template-directed synthesis reaction. The observation that a template-directed synthesis, in which the template nucleotide are the D-enantiomers, with the phosphorimidazolide of D,L-guanosine is strongly inhibited by the L-enantiomer (Joyce *et al.*, 1984). This finding suggests that it was not possible to have had efficient template-directed syntheses with racemic (D, L) mixtures of nucleotides let alone with the complex

array of nucleotides produced by reaction of bases with the isomeric sugars formed in the formose reaction.

Approaches to the Solution of these Problems

Two approaches have been used to solve the problem of the prebiotic synthesis of ribose: One, the use of different starting materials and two, construction of simpler structures containing both the hydroxyl and phosphomonoester groupings required to form phosphodiester bonds. The reaction of glycol aldehyde phosphate (**2**) with formaldehyde to form 2',5'-ribose diphosphate is much more efficient than the synthesis of ribose from formaldehyde (Drenkard *et al.*, 1990; Wagner *et al.*, 1990; Müller *et al.*, 1990). A problem with this route is there is no prebiotic synthesis of 2-aminopropenitrile, the starting point in this alternative ribose synthetic scheme. This research does demonstrate that efficient prebiotic pathways to ribose may exist from starting materials different from formaldehyde. Efficient ribose syntheses will not solve the problem of enantiomeric inhibition of template-directed synthesis since DL-mixtures are formed.



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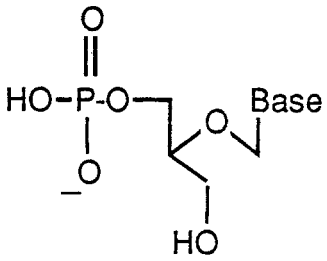
Use of simpler analogs of ribose has been the alternative approach to the prebiotic formation of RNA. It has been suggested that the first RNA consisted of a base attached to an acyclic unit containing one hydroxyl and one phosphate group **3**) (Spach, 1984; Joyce *et al.*, 1987). This basic unit may have formed more readily on the primitive Earth and it would have fewer problems resulting from multiple isomer forms than the prebiotic synthesis of ribose. Unfortunately, compound **3** cyclizes to a six-membered cyclic phosphate more readily than it undergoes polymerization (unpublished research of G. von Kiedrowski cited in Hill *et al.*, 1988). This propensity for cyclization has been noted with a number of phosphates and suggests that it will be possible to form oligomers from only a limited number of phosphate derivatives (Hill *et al.*, 1988; Ferris and Kamaluddin, 1989). The low reactivity of nucleotide analogs containing one hydroxyl group compared to those with the 2',3'-vicinal glycol grouping also suggests that phosphodiester bond formation between these simple analogs may not proceed efficiently (Orgel, 1986; Ferris *et al.*, 1990). It has been possible to circumvent the problems associated with the reactivity of the simple ribose analogs by linking the monomer units with pyrophosphate instead of phosphodiester bonds. Activated dinucleotides (**4**) are used as the starting materials (Schwartz and Orgel, 1985). Poly(C) will direct the synthesis of the complementary G oligomers which contain pyrophosphate



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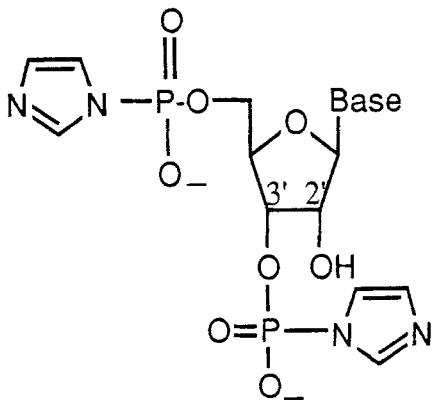
groups (Visscher and Schwartz, 1988). The efficiency of their template-directed pyrophosphate bond formation is lower than that of template-directed phosphodiester bond formation. This is due in part to the ease of conversion of the activated diphosphate monomer to the corresponding cyclic pyrophosphate (Visscher and Schwartz, 1990).



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Catalysis – A Possible Solution?

With few exceptions, most of the experimental approaches described to date do not consider the possible role of catalysis in prebiotic synthesis. This is surprising since contemporary life would cease functioning without catalysis by enzymes and ribozymes. Low energy reaction pathways, often ones which do not occur in the absence of catalysis, proceed on the surface of an enzyme or ribozyme. If present day life requires catalysis, then there is good reason to believe that catalysis was

also required for the formation of RNA and the other complex molecules essential for the origins of life.

What classes of catalyses may have been important for the synthesis of biopolymers on the primitive Earth? In the earliest stages of prebiotic synthesis, soluble metal ions, insoluble metal ion complexes and minerals could have accelerated organic reactions. Their efficiency may have been enhanced or taken over by some of the organic oligomers produced in these catalytic reactions (e.g. Barbier and Brack, 1992).

The catalytic role of metal ions in controlling the regiochemistry of phosphodiester bond formation in template-directed synthesis has already been noted. Calcium or magnesium ion are required for the conversion of ribose and purines to an isomeric mixture of purine nucleosides (Fuller *et al.*, 1972a, b). Metal ions, usually magnesium ion, have an essential role in most reactions involving phosphodiester or pyrophosphate bond formation.

The uranyl ion (UO_2^{2+}) catalyzed formation of mainly 2′,5′-linked oligomers is a dramatic example of metal ion catalysis. Oligomers of A up to the 16 mer and of C up to the 12 mer in length have been detected using phosphorimidazolides of nucleosides as starting materials (Sawai *et al.*, 1989, 1992). Similar but less efficient catalysis has been reported using other metal ions (Sawai, 1976; Sawai and Ohno, 1981).

Mineral catalysis of prebiotic reactions was proposed by Bernal in a lecture given in 1947 (1949) several years before the first report of the synthesis of amino acids in an electric discharge experiment (Miller, 1953). Mineral catalysis has been a popular topic for speculation (Cairns-Smith, 1982; Wächtershäuser, 1988) but very few experiments have provided major advances for the field of prebiotic synthesis. The use of montmorillonite as a catalyst for the conversion of aminoacyladenylates to polypeptide derivatives of 5′-AMP was first reported in 1970 (Paecht-Horowitz *et al.*, 1970). Later it was claimed that a zeolite catalyzed of the synthesis of the starting aminoacyladenylate from an amino acid and ATP (Paecht-Horowitz and Katchalsky, 1973). It has not been possible to reproduce the latter claim but the former report of polypeptide formation from the aminoacyladenylates has been repeated (Warden *et al.*, 1974; Paecht-Horowitz and Eirich, 1988).

A mineral catalyzed catalytic reaction has made a potentially important link between prebiotic chemistry and the RNA World. The observation of the formation of RNA oligomers on montmorillonite (Ferris and Ertem, 1992a, 1992b, 1993) suggests that RNA may have formed directly from mononucleotides on the primitive Earth. Oligomers containing up to ten nucleotides in length have been prepared in pH 8 aqueous solution at room temperature using phosphorimidazolides of nucleosides (**1**) as starting materials. The regiospecificity of 3′,5′-phosphodiester bond formation is about 80% when diadenosine-pyrophosphate ($A^{5'}$ppA) is added to the reaction solution and oligomers linked to $A^{5'}$ppA are obtained. Recent studies have extended these reactions to the formation of uridine and cytidine oligomers (Ferris, Ertem and Ding, 1993).

The successful formation of RNA oligomers in the presence of UO_2^{2+} and montmorillonite demonstrates the potential of metal ion and mineral catalysis in prebiotic synthesis. These reagents initiated a reaction that does not occur in their absence. In addition, these catalysts controlled the regiospecificity of phosphodiester bond formation. The UO_2^{2+} and montmorillonite catalysis is certainly not as specific or efficient as that of contemporary enzymes but it may have been sufficient to be the first step towards the initiation of the RNA World. The search for new catalysts has only just begun – more efficient ones may yet be discovered.

The Search for Catalysis

The succesful search for catalytic metal ions and minerals for the formation of RNA oligomers was the result of 'enlightened' trial and error studies. These studies are described as 'enlightened' because the investigators probably drew on their own chemical intuition, experience, as well as rudimentary data in the literature to choose an array of reagents to test as catalysts. The 'enlightened' approach is just a small step above adding each of the reactants in the chemical stockroom to the reaction mixtures. In each case the discovery and development process was lengthy. There were thirteen years between Sawai's first publication on metal ion catalysis (Sawai and Orgel, 1975) and the report of the formation of the 16 mer polyadenylate (Sawai *et al.*, 1989). In the development of montmorillonite catalysis, there was a six year lapse between the first publication (Ferris and Hagan, 1986) and the report of 10 mers (Ferris and Ertem, 1992a). However, it should be noted that the studies of montmorillonite catalysis were initiated well before 1986 (Ferris *et al.*, 1979). Since there is little information available on metal ion or mineral catalysis of bioorganic reactions, it is likely that the 'enlightened' trial and error approach will be the method of choice in the immediate future.

Key to the use of the trial and error method is the development of a simple assay to evaluate the efficiency of a potential catalyst. The conversion of 3'-AMP to 2',3'-cyclic AMP (Lohrmann and Orgel, 1968) was used to determine the metal ions, minerals and reaction conditions required for the formation of RNA oligomers (Ferris *et al.*, 1984). HPLC analysis provided a rapid quantitative analysis of the extent of conversion of 3'-AMP to 2',3'-cAMP. Failure to use an effective assay for oligonucleotides delayed recognition of the higher molecular weight products (Ferris and Ertem, 1992a). These oligomers were undoubtedly synthesized in 1988 (Kebbekus) but not detected until 1990 using the HPLC procedure of Stribling (1991).

What is needed besides luck and hard work to discover effective catalysts? Kinetic and mechanistic data on the reaction being studied is very helpful in determining the desired properties of an effective catalyst. For example, the kinetic data, studies on the role of Mg^{2+} and pH on the reactivity of the 3'-phosphorimidazolides of nucleosides (Kanavarioti *et al.*, 1989) was very helpful in the understanding of the role of montmorillonite catalysis on oligomer formation (Ferris and Ertem,

1992a).

Data on the binding of reactants and products to metal ions and minerals is also very important in selecting catalysts. There are extensive discussions of the binding of metal ions to nucleotides and other biomolecules (Sigel, 1979–1992). More limited compilations of binding studies with clays (Theng, 1974; Ferris and Ertem, 1989) and even less for other classes of minerals (Gibbs *et al.*, 1980; Holm *et al.*, 1993). Some researchers in the field of prebiotic synthesis have undertaken detailed studies on minerals which may have been important in prebiotic synthesis (Kuma *et al.*, 1989) but much more information of this type is required.

It is important to note the binding of the substrate molecule to potential catalysts is a necessary but not sufficient requirement for catalysis to occur. For example, nucleotides bind efficiently to apatite (Gibbs *et al.*, 1980) or iron oxide hydroxides (Holm *et al.*, 1993) but neither catalyze the formation of oligomers. Theoretical schemes for polymer formation, devised mainly on the binding of monomers to a mineral, assumes catalysis follows directly from binding (Wächtershäuser, 1988). This is an incorrect assumption in many instances. Catalysis is usually the result of proximate positioning to general acid-base groupings on the mineral surface coupled with the proximate orientation of reactive monomer groupings resulting from substrate binding prior to reaction.

In those instances where catalysis has been discovered, it is important to probe the mechanism of metal ion or mineral catalysis. This information is important for the understanding of the particular reaction under investigation because it may suggest other metal ions or minerals to consider as catalysts for the reaction. Mechanistic information also contributes to the general body of information concerning reaction catalysis which will be helpful in predicting catalysts for other prebiotic reactions.

It is important to remember that RNA, its building blocks and related structures also degrade under prebiotic conditions. Consequently, when synthesis is discussed, what is really meant is a synthetic rate that is greater than the rate of decomposition of the reaction product. Decomposition reactions may also be catalyzed. For example, RNA breakdown to mononucleotides and the further decomposition of the mononucleotides is catalyzed by metal ions (Eichhorn and Butzow, 1965; Butzow and Eichhorn, 1965; Breslow and Huang, 1991).

The synthetic processes leading to RNA required energy to form RNA or RNA-like materials which are unstable under primitive earth conditions. One way to drive these reactions to form activated monomers by the use of light, heat (including hydrothermal systems), or high energy particles (electrons, protons and neutrons). The catalyzed reactions of these activated monomers is required for the efficient formation of RNA-like structures.

It is concluded that a serious search for catalytic agents is central to significant progress in the field of origins of life. Metal ions and minerals are prime candidates as catalysts. The search for catalysis should be carried out in conjunction with physical chemical studies on the reaction mechanism with the goal of predicting

other, possibly more effective catalysts for the reaction under investigation. This approach may lead to the discovery of a direct route from prebiotic molecules to RNA oligomers.

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References

Barbier, B. and Brack, A.: 1992, *J. Am. Chem. Soc.* **114**, 3511.
Bernal, J. D.: 1949, *Proc. Soc. London* **62A**, 537.
Breslow, R. and Huang, Deeng-Lih: 1991, *Proc. Natl. Acad. Sci. USA* **88**, 4080.
Butzow, J. J. and Eichhorn, G. L.: 1965, *Biopolymers* **3**, 95.
Cairns-Smith, A. G.: 1982, 'Genetic Takeover and the Minerals Origins of Life', Cambridge University Press, Cambridge.
Decker, P., Schweer, H., and Pohlmann, R.: 1982, *J. Chromatog.* **244**, 281.
Drenkard, S., Ferris, J. P., and Eschenmoser, A.: 1990, *Helv. Chim. Acta* **73**, 1373.
Eichhorn, G. L. and Butzow, J. J.: 1965, *Biopolymers* **3**, 79.
Ferris, J. P.: 1985, *Origins of Life* **16**, 95.
Ferris, J. P. and Ertem, G.: 1989, *Origins Life Evol. Biosphere* **19**, 153.
Ferris, J. P. and Ertem, G.: 1992a, *Science* **257**, 1387.
Ferris, J. P. and Ertem, G.: 1992b, *Origins Life Evol. Biosphere* **22**, 181.
Ferris, J. P. and Ertem, G.: 1993, *Origins Life Evol. Biosphere* **23**, in press.
Ferris, J. P. and Hagan, W. J. Jr.: 1986, *Origins Life Evol. Biosphere* **17**, 69.
Ferris, J. P. and Joshi, P. C.: 1979, *J. Org. Chem.* **44**, 2133.
Ferris, J. P. and Kamaluddin: 1989, *Origins Life Evol. Biosphere* **19**, 609.
Ferris, J. P., Edelson, E. H., Mount, N. M., and Sullivan, A. E.: 1979, *J. Mol. Evol.* **13**, 317.
Ferris, J. P., Ertem, G. E., and Ding, Z.: 1993, unpublished.
Ferris, J. P., Joshi, P. C., Edelson, E. H., and Lawless, J. G.: 1978, *J. Mol. Evol.* **11**, 293.
Ferris, J. P., Kamaluddin and Ertem, G.: 1990, *Origins Life Evol. Biosphere* **20**, 279.
Ferris, J. P., Yanagawa, H., Dudgeon, P. A., Hagan, W. J. Jr., and Mallare, T. E.: 1984, *Origins Life Evol. Biosphere* **15**, 29.
Fuller, W. D., Sanchez, R. A., and Orgel, L.E.: 1972a, *J. Mol. Biol.* **67**, 25.
Fuller, W. D., Sanchez, R. A., and Orgel, L. E.: 1977b, *J. Mol. Evol.* **1**, 249.
Gibbs, D., Lohrmann, R., and Orgel, L. E.: 1980, *J. Mol. Evol.* **15**, 347.
Hill, A. R., DeeNord, L., Orgel, L. E., and Robins, R. K.: 1988, *J. Mol. Evol.* **28**, 170.
Holm, N., Ertem, G., and Ferris, J. P.: 1993, *Origins Life Evol. Biosphere* **23**, 195.
Inoue, T. and Orgel, L. E.: 1982, *J. Mol. Evol.* **162**, 201.
Joyce, G. F., Inoue, T., and Orgel, L. E.: 1984, *J. Mol. Biol.* **176**, 279.
Joyce, G. F., Schwartz, A. W., Miller, S. L., and Orgel, L. E.: 1987, *Proc. Natl. Acad. Sci.* **84**, 4398.
Joyce, G. F.: 1989, *Nature* **338**, 217.
Joyce, G. F., Visser, G. M., Van Boeckel, C. A. A., van Boom, J. H., Orgel, L. E., and Van Westrenen, J.: 1984, *Nature* **310**, 602.
Kanavarioti, A., Bernasconi, C. F., Doodokyan, D. L., and Aberas, D. J.: 1989, *J. Am. Chem. Soc.* **111**, 7247.
Kasting, J.: 1993, *Science* **259**, 920.
Kebbekus, P.: 1988, B.S. Thesis, Rensselaer.

Kuma, K., Paplawsky, W., Gedulin, B., and Arrhenius, G.: 1989, *Origins Life Evol. Biosphere* **19**, 573.
Lohrmann, R.: 1977, *J. Mol. Evol.* **10**, 137.
Lohrmann, R. and Orgel, L. E.: 1968, *Science* **161**, 64.
Lohrmann, R. E., Bridson, P. K., and Orgel, L. E.: 1980, *Science* **208**, 1464.
Miller, S. L.: 1953, *Science* **117**, 528.
Müller, D., Pitsch, S., Kittaka, A., Wagner, E., Wintner, C., and Eschenmoser, A.: 1990, *Helv. Chim. Acta* **73**, 1410.
Orgel, L. E.: 1986, *J. Theor. Biol.* **123**, 127.
Oro, J. and Kimball, A. P.: 1961, *Arch. Biochem. Biophys.* **94**, 217.
Oro, J. and Kimball, A. P.: 1962, *Arch. Biochem. Biophys.* **96**, 293.
Osterberg, R., Orgel, L. E., and Lohrmann, R.: 1973, *J. Mol. Evol.* **2**, 231.
Paecht-Horowitz, M. and Eirich, F. R.: 1988, *Origins Life Evol. Biosphere* **18**, 359.
Paecht-Horowitz, M. and Katchalsky, A.: 1973, *J. Mol. Evol.* **2**, 91.
Paecht-Horowitz, M., Berger, J., and Katchalsky, A.: 1970, *Nature* **228**, 636.
Sanchez, R. A., Ferris, J. P., and Orgel, L. E.: 1967, *J. Mol. Biol.* **30**, 223.
Sawai, H. and Ohno, M.: 1981, *Chem. Pharm. Bull.* **29**, 2237.
Sawai, H. and Orgel, L. E.: 1975, *J. Am. Chem. Soc.* **97**, 3532.
Sawai, H., Higa, K., and Kuroda, K.: 1992, *J. Chem. Soc. Perkin I*, 505.
Sawai, H., Kuroda, K., and Hojo, T.: 1989, *Bull. Chem. Soc. Jpn.* **62**, 2018.
Sawai, H.: 1976, *J. Am. Chem. Soc.* **98**, 7037.
Schwartz, A. E. and deGraaf, R. M.: 1993, *J. Mol. Evol.* **36**, 101.
Schwartz, A. E. and Orgel, L. E.: 1985, *Science* **228**, 585.
Sigel, H. (ed.): 1973–1992, 'Metal Ions in Biological Systems', Marcel Dekker, N.Y.
Sleeper, H. L., Lohrmann, R., and Orgel, L. E.: 1979, *J. Mol. Evol.* **13**, 203.
Spach, G.: 1984, *Origins Life Evol. Biosphere* **14**, 433.
Stribling, R.: 1991, *J. Chromatogr.* **338**, 474.
Stribling, R. and Miller, S. L.: 1991, *J. Mol. Evol.* **32**, 289.
Theng, B. K. G.: 1974, 'The Chemistry of Clay-Organic Reactions', Wiley, New York.
Visscher, J. and Schwartz, A. W.: 1988, *J. Mol. Evol.* **28**, 3.
Visscher, J. and Schwartz, A. W.: 1990, *J. Mol. Evol.* **31**, 163.
Voet, A. B. and Schwartz, A. W.: 1982, *Origins Life Evol. Biosphere* **12**, 45.
von Kiedroswki, G.: 1986, *Angew. Chim. int. Edn. Engl.* **25**, 932.
Wagner, E., Xiang, Yi-Bin, Baumann, K., Gück, J., and Eschenmoser, A.: 1990, *Helv. Chim. Acta* **73**, 1391.
Warden, J. T., McCullough, J. J., Lemmon, R. M., and Calvin, M.: 1974, *J. Mol. Evol.* **4**, 189.
Wächtershäuser, G.: 1988, *Microbiological Reviews* **52**, 452.
Zielinski, W. S. and Orgel, L. E.: 1987, *Nature* **327**, 346.