brane, such as ionic size, activity, affinity to membrane sites, and mobility in the membrane. For example, permeation of Ca^{2+} through the Ca channels in invertebrate muscle membranes is presumed to occur in two steps (*10, 12*): (i) reversible binding of Ca^{2+} to the sites at the membrane, and (ii) passage of Ca^{2+} through the Ca channels, whose conductance is time- and potential-dependent. A comparison of these parameters among the ionic species would provide a new basis for further investigation of the molecular mechanisms of calcium entry through the membrane.

Besides interacting with the Ca channels, some of the permeating cations, such as Sr^{2+}, Ba^{2+}, Mn^{2+}, Cd^{2+}, and Zn^{2+}, seemed to suppress the generation of a potassium current in the muscle membrane. This pharmacological action of the divalent cations also modifies the generation of action potentials in beetle muscle fibers.

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Early Chemical Evolution of Nucleic Acids: A Theoretical Model

Abstract. *Recent experimental work suggests a possible cyclical pathway for early prebiotic oligonucleotide formation that involves (i) dry-state (nontemplate) synthesis of random copolymers with mixed 2',5' and 3',5' bonds, (ii) passage of these oligomers into solution at low temperatures, and (iii) a preferential hydrolysis of the 2',5' bond in any short helices that have formed. This early system could have selected for complementary sequences that were largely 3',5'-linked, but may not have selected efficiently for a single enantiomer of ribose.*

In a recent review (*1*) of theories concerning the origin of life, the assumption was made that the oligonucleotides formed in prebiotic times would have been better able to resist hydrolytic degradation if they could have adopted a helical conformation. A further assumption was made that there existed a nonenzymic mechanism by which a single-stranded RNA molecule could be replicated with a fidelity such that one error was made per 100 bases. Unfortunately, actual attempts to demonstrate template-directed formation of oligoribonucleotides under simulated prebiotic conditions have invariably led (*2, 3*) to the production of a large excess of the unnatural or 2',5' internucleotide bond (*4*). Further, the rate of hydrolysis of this 2',5' bond actually increases when the oligonucleotide of which it is a part forms a right-handed helix (*5*). By contrast, a modest excess of the natural 3',5' bond is formed (*6*) in dry-state polymerization of adenosine 2',3'-phosphate catalyzed by ethylenediamine, but this reaction occurs in the absence of a template. We now report that these recent experimental observations combined with the earlier theory (*1*) show how, under the influence of a daily heating and cooling cycle, a system of oligonucleotide formation could have arisen that selected for (i) the natural 3',5' bond, (ii) longer oligomers, and (iii) complementary sequences. The possible importance in prebiotic chemistry of the natural cycles in temperature and humidity (day and night, tides, seasons) has been explored by others, both in theory (*1*) and through experiment (*7*). In addition, it has been suggested that simulation experiments are more convincing if they are carried out at temperatures that resemble those found on the earth today (*2*), and a recent calculation of the average temperature on the prebiotic earth (about 3.5 to 4 billion years ago) lends support to this suggestion (*8*). The conditions given in Fig. 1 may be taken to represent a semidesert locale that has a reasonably large swing in surface temperature and humidity between day and night. The actual temperatures shown were measured in the Namib Desert (South-West Africa) by Buskirk over a 26-hour period in August 1973 (*9*).

The cyclical scheme is shown in Fig. 1, and assumes the existence of ribonucleoside 2'- and 3'-monophosphates (*2*), which, in turn, are converted into the nucleoside 2',3'-phosphates—for example, by reaction with a condensing agent (*10*), or by heating with a catalyst. The cycle starts with the dry-state synthesis of random copolymer oligomers. This occurs at slightly elevated temperatures in the presence of a catalyst (such as imidazole) (*6*) but is not directed by a template, and the ratio of natural to unnatural bonds formed is about 2 : 1. As the sun sets, the surface temperature decreases, and the oligomers go into solution in the dew which forms. At these low temperatures, some of the oligomers will find complementary partners with which they can form short helices (*11*). As the sun rises on the following morning, the solution warms up, and a preferential degradation of the helical 2',5' bonds will occur (*5*). Helical 3',5' bonds will be protected, while nonhelical 2',5' or 3',5' bonds will degrade at an intermediate rate. The slow rise in temperature is important in that a sudden increase would merely melt the helices and not allow the selective degradation of the "unnatural" bonds to occur. The most stable species under these circumstances are long, complementary oligomers that are entirely 3',5'-linked (higher melting temperature, T_m). Helices that contain a number of gaps or nicks from hydrolysis of 2',5' bonds will have lowered thermal stability and a greater tendency to denature as the temperature increases. Eventually, the solution will dry out, and the residue will consist of a mixture of random copolymers of shorter chain length but with an increased fraction of 3',5' bonds than previously. In some of these oligomers, the terminal phosphate will be in the form of the 2' or 3' monoester, and in others as the 2',3'-phosphate (*12*). In order to ensure further coupling between these oligomers, the terminal phosphates should be in the 2',3' form. Reactivation of the 2' and 3' monoesters

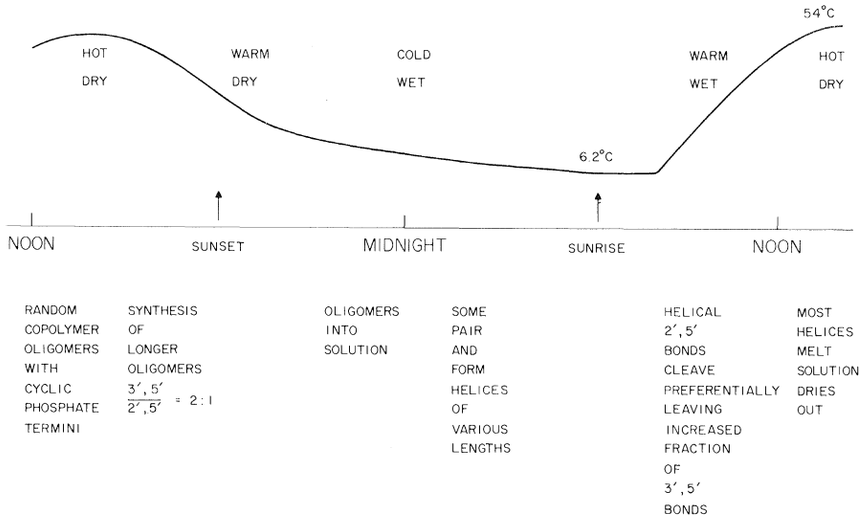

Fig. 1. One possible scheme for the synthesis of oligonucleotides under prebiotic conditions. The surface temperatures shown (continuous line) were measured on a west-facing slope in the Namib desert in August 1973 (*9*).

to give the cyclic phosphate without causing simultaneous degradation of the internucleotide diester linkages is not a serious problem in principle. The 2′ (or 3′) terminal monoesters exist as the dianion at mildly alkaline *p*H, whereas the internal diester bonds are monoanions and are less nucleophilic toward a potential activating reagent. Provided that the rate of renewed dry-state synthesis from these remaining oligomers exceeds the rate of their breakdown, the cycle is completed and conceivably could go on to produce longer complementary oligomers that are largely 3′,5′-linked. An upper limit to the length of the chains appears to be set by (i) the nonzero rate of hydrolysis of the helical 3′,5′ bonds, (ii) the increasing probability, as the chain length increases, that the rate of chain cleavage will exceed the rate of coupling during dry-state synthesis, and (iii) the decreasing probability, as the chain length increases, that random synthesis or coupling will generate complementary sequences. At present, it does not appear possible to calculate the upper limit from the experimental data that are now available.

The above scheme is not a template-directed synthesis, and therefore bypasses the known difficulty of including pyrimidine monomers in such a process (*2*). However, a system capable of direct template synthesis eventually must have arisen, possibly via the aggregation mechanism suggested by Kuhn (*1*). Here the problem is again not insuperable, as Orgel and Lohrman (*2*) have shown that 5′-activated pyrimidine nucleotides can be incorporated in a template-directed synthesis if they are carried to the template as purine-containing oligomers such as pUpG (U, uridine; G, guanosine).

Information relating to the potential enantiomeric specificity of this reaction is sparse. Both the 2′,5′ and 3′,5′ isomers of (L,L)-ApA (A, adenosine) have been found to form a right-handed triple helix with two strands of (D)-polyuridylic acid (*13*). The 2′,5′ isomer seems to resist inclusion in this right-handed helix less strongly than does the 3′,5′ isomer. Indeed the T_m for the helix with (L,L)-2′,5′ ApA is almost equal to that for the similar helix of (D,D)-3′,5′-ApA, while that for (L,L)-3′,5′-ApA is several degrees lower (*13*). I concur with Tazawa *et al.* (*13*) that, as shown by CPK (Corey, Pauling, Koltun) molecular models, there is no obvious stereochemical hindrance to the inclusion of (L)-nucleoside units in a right-handed helix [see (*1*)]. Further, it appears from the models that even when the nucleotides involved are of the L configuration, and are constrained in a right-handed helix, the 2′,5′ bond should again be more labile than the 3′,5′ bond. This prediction is independent of whether the (L)-nucleotide is the 5′ or the 2′(3′) partner to the bond, or, indeed, whether both partners are (L)-nucleotides. The 2′,5′ isomer has the correct geometry for an in-line displacement, the 3′,5′ for an adjacent displacement (*5*). The same argument must, of course, apply to the (D)-nucleotides in a left-handed helix, but at present the only experimental proof of such a difference in hydrolytic stability relates to the all-(D) oligomers in a right-handed helix (*5*). It is not clear to what extent the results of Tazawa *et al.* (*13*) may be applied to random or block copolymers of (L)- and (D)-nucleotides, and more experimental work on these systems is required. However, it seems possible that the inclusion of, for example, (L)-nucleotides in a right-handed helix that is composed predominantly of (D)-nucleotides may tend to lower T_m if the L units are 3′,5′-linked, but have relatively little effect on T_m if they are 2′,5′-linked. In the first case, the helix will melt at a somewhat lower temperature and thus become more susceptible to hydrolysis; in the second case, the helix will be relatively resistant to melting initially, but the helical form will cause a relatively rapid hydrolysis of the 2′,5′ bonds to occur. If this conjecture has any validity, it would mean that a selection for one enantiomer could take place, but more weakly than the selection for the 3′,5′ bond. A stronger selection for one enantiomer may have occurred later with the use of 5′-activated nucleotides (*14*).

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Theta-Sensitive Cell and Erythropoiesis: Identification of a Defect in *W/W^v* Anemic Mice

Abstract. *Nonirradiated mice of the* W/W^v *genotype were injected with normal (+/+) bone marrow cells that had been treated with antiserum to Thy 1.2 and complement (C'). Such bone marrow cells had no effect on the number of macroscopic colonies formed in the spleens of these mice, but did not cure the anemia. The addition of +/+ thymocytes to these bone marrow cells restored their ability to cure the anemia in* W/W^v *mice. These data suggest that a theta-sensitive cell is required in the promotion of differentiation of murine hematopoietic stem cells into erythrocytes, and that there is a deficiency of such a cell in the* W/W^v *mouse.*

Macrocytic anemia in *W/W^v* mice is considered to be an experimental model of hypoplastic anemia in man (*1*). At least one of the mechanisms responsible for this genetically determined disorder carried by WBB6F$_1$ mice is a hereditary stem-cell deficiency (*2*). Thus, bone marrow cells from anemic *W/W^v* animals do not form macroscopic colonies in the spleen of irradiated recipients, but microscopic colonies which are reduced both in number and size (*3*). These *W/W^v* anemic animals are a useful model for experimentation, since they have hematologically normal +/+ littermates. Bone marrow cells from +/+ animals form macroscopic colonies in nonirradiated *W/W^v* recipients (*4*), and the final success of the bone marrow graft may be easily determined by testing the red blood cell values of *W/W^v* recipients (*5*). Anemia in *W/W^v* animals, therefore, provides an experimental system particularly suitable for studies concerning the mechanisms which regulate hematopoiesis.

Several studies indicate that the thymus gland exerts a stimulatory effect on erythropoiesis. The addition of 10^6 or more thymocytes to bone marrow cells from transfused into irradiated recipients increased the number of macroscopic colonies (*6*). This cannot be explained by the presence in the thymus of cells forming colonies, since there is only one such cell present in about 100×10^6 thymus cells (*7*). On the other hand, thymectomy of neonatal mice caused anemia and reduced both the total number of bone marrow cells and the number of colony-forming cells present in the bone marrow (*8*). These observations may indicate that while the hematopoietic stem cell, as measured by the assay for colony-forming units in the spleen (CFU-S), is directly responsible for a hematopoietic self-renewal potential, its proliferation, differentiation, and maturation are regulated by lymphoid cells derived from the thymus.

We have performed studies which suggest that the presence of a theta-sensitive cell is required in normal bone marrow, thymus, and spleen for the promotion of normal differentiation of hematopoietic stem cells along the erythroid pathway, and that there is a deficiency of such a cell in the *W/W^v* mouse.

We used WBB6F$_1$ adult male (*W/W^v* and +/+) mice (Jackson Laboratory), that were produced by breeding WB/ReJ-*W*/+ and C57B1/6J-*W^v*/+ parents. Both of these parental strains possess the Thy 1.2 antigen. Bone marrow cells were obtained from the femurs and tibiae of these mice by gentle flushing with medium (RPMI-1640). Thymocytes and spleen cells were obtained by teasing these tissues with a rubber policeman into the medium. Antiserum to Thy 1.2 antigen was made by the method of Reif and Allen (*9*) by immunizing AKR mice with CBA/J thymocytes.

Bone marrow cells were treated with antiserum to Thy 1.2 as follows. The bone marrow cells (10^7) were incubated with 1 ml of a 1 : 30 dilution of antiserum in medium for 20 minutes at 4°C. The unbound antiserum to Thy 1.2 was removed by washing with medium, and the cells were resuspended in 1 ml of a 1 : 8 dilution of rabbit complement (C'), and the suspension was incubated at 37°C for 30 minutes. The cells were washed twice with medium and counted. In control experiments, the bone marrow or spleen cells were treated with normal serum from AKR mice (NMS) and C'. Cell viability was determined by the trypan-blue dye exclusion technique. Viability of the bone marrow cells after treatment with either antiserum to Thy 1.2 plus C' or NMS plus C' was between 90 and 95 percent (*10*). Viability of the spleen cells was about 60 percent after treatment with antiserum to Thy 1.2 plus C' compared to 90 percent after treatment with NMS and C'. Dead cells were eliminated by the Ficoll-Hypaque centrifugation technique, and cell suspensions used for injections consisted of 95 percent viable cells.

We used *W/W^v* mice as recipients throughout these experiments. The mice were divided into groups, with five mice per group. Each mouse was injected by way of the tail vein with appropriately treated cell preparations. The number of macroscopic colonies formed by transfused bone marrow cells in nonirradiated *W/W^v* recipients was determined on day 7 after the transplantation according to the method of Till and McCulloch (*11*). Mice were bled every 10 days by way of a retro-orbital sinus for hematocrit and red blood cell (RBC) determinations by routine methods.

In the first series of experiments, we depleted the +/+ bone marrow or +/+ spleen cells of theta-sensitive lymphoid cells by treatment with antiserum to Thy 1.2 in the presence of complement, and used such preparations for the correction of *W/W^v* anemia. Injection of 10^7 +/+ bone marrow or spleen cells treated with NMS and C' was equally active in improving the blood values of *W/W^v* recipients (Table 1). In marked contrast, injection of either +/+ bone marrow or +/+ spleen cells treated with antiserum to Thy 1.2 serum and C' failed to produce an increase in the blood values. As expected, control groups receiving either *W/W^v* bone marrow or spleen cells treated either with antiserum to Thy 1.2 and C' or with NMS and C' failed to show an increase in the hematocrit values and RBC counts in *W/W^v* recipients (data not shown). Table 1 shows data of one representative experiment. The whole experiment was repeated six times and gave similar results.

Although antiserum to Thy 1.2 prepared and used for treating cells in vitro as above has been shown previously (*12*) not to affect cells forming macroscopic colonies in the spleen, we argued that the most likely explanation for the observed effect with our preparation of antiserum