THE FIDELITY OF TEMPLATE-DIRECTED OLIGONUCLEOTIDE LIGATION AND THE INEVITABILITY OF POLYMERASE FUNCTION

KENNETH D. JAMES and ANDREW D. ELLINGTON
Department of Chemistry, Indiana University and Department of Chemistry and Institute for Cellular and Molecular Biology, University of Texas at Austin, ICMB A4800, 26th & Speedway, University of Texas at Austin, Austin, Texas 78712, U.S.A.

(Received 15 July, 1998)

Abstract. The first living systems may have employed template-directed oligonucleotide ligation for replication. The utility of oligonucleotide ligation as a mechanism for the origin and evolution of life is in part dependent on its fidelity. We have devised a method for evaluating ligation fidelity in which ligation substrates are selected from random sequence libraries. The fidelities of chemical and enzymatic ligation are compared under a variety of conditions. While reaction conditions can be found that promote high fidelity copying, departure from these conditions leads to error-prone copying. In particular, ligation reactions with shorter oligonucleotide substrates are less efficient but more faithful. These results support a model for origins in which there was selective pressure for template-directed oligonucleotide ligation to be gradually supplanted by mononucleotide polymerization.

1. Introduction

It has been suggested that self-replicating oligonucleotides or oligonucleotide analogues may have been the first living systems and may have led to the evolution of an RNA world in which biochemistry and metabolism were based on nucleic acid rather than protein catalysis (Gilbert, 1986; Benner *et al.*, 1989). If so, the prebiotic mechanisms by which oligonucleotides might have replicated themselves are of critical importance to understanding the origin and evolution of life (Sharp, 1985; Orgel, 1992).

Efforts to copy or reproduce oligonucleotide templates by chemically-catalyzed monomer polymerization have been largely unsuccessful, primarily because the different Watson-Crick pairings have wildly different propensities for monomer addition and because the efficiency of monomer addition is relatively low. Moreover, enantiomeric poisoning by non-natural nucleotides present in the primordial soup would likely have doomed any primitive replicators (reviewed in Joyce, 1989; Joyce and Orgel, 1993).

For these reasons, ligation has been proposed as the most likely mechanism for the self-replication of primordial oligonucleotides (reviewed in Ellington, 1993; James and Ellington, 1995; James and Ellington, 1997a). Oligonucleotide liga-

Origins of Life and Evolution of the Biosphere **29:** 375–390, 1999.

tion would have required fewer catalytic steps for the reproduction of a template and would have been relatively immune to enantiomeric poisoning, since oligonucleotides that contained non-natural nucleotides in their interiors could still have formed productive ligation junctions and might have nonetheless been able to carry sequence information between generations (Visscher and Schwartz, 1988; Schneider and Benner, 1990; Bolli *et al*., 1997). Orgel and his co-workers have even demonstrated the commutative transmission of sequence information between oligonucleotides and peptide nucleic acids (Bohler *et al*., 1995; Schmidt *et al*., 1997a, b). Most importantly, template-directed oligonucleotide ligation has been shown to support self-replication under conditions akin to those that may have been present prebiotically. Gunter von Kiedrowski, Leslie Orgel, and their co-workers have demonstrated the faithful and efficient reproduction of oligonucleotide templates by the template-directed ligation of smaller oligonucleotide substrates (Zielinski and Orgel, 1987; von Kiedrowski, 1986; von Kiedrowski *et al*., 1991; Achilles and von Kiedrowski, 1993; Sievers and von Kiedrowski, 1994).

However, most experiments to date have been carried out with defined sequence oligonucleotides, while any primordial oligonucleotides would likely have been heterogeneous in sequence. Therefore, we have carried out experiments to assess whether oligonucleotide templates can be faithfully copied by the ligation of oligonucleotide substrates drawn from random sequence libraries (James and Ellington, 1997b; James and Ellington, submitted). Chemical and enzymatic ligations were compared. While the fidelities of copying were similar, enzymatic ligation enabled the capture of much shorter oligonucleotide substrates than chemical ligation, likely because of the stabilization of template:substrate complexes by the enzyme. These results suggest a model for the evolution of polymerization function in which early replicators pieced together relatively long oligonucleotide substrates while later replicators evolved the ability to stabilize template:substrate complexes and could therefore capture shorter, more abundant oligonucleotide substrates. As this catalytic ability was further refined by evolution, replicators would eventually have been able to use quite short nucleotide substrates, including activated monomers.

2. Results and Discussion

2.1. A MODEL SYSTEM FOR EVALUATING THE FIDELITY OF TEMPLATE-DIRECTED OLIGONUCLEOTIDE LIGATION

A careful assessment of the fidelity of template-directed oligonucleotide ligation is critical to determining whether this mechanism could have served for the origin and evolution of nucleic acid replicators. If template-directed oligonucleotide ligation is generally faithful, then nascent replicators could have passed on their sequence-encoded functions to subsequent molecular generations. However, if template-directed oligonucleotide ligation is error-ridden, then nascent replicators may have been compromised by a high mutational load. We have previously

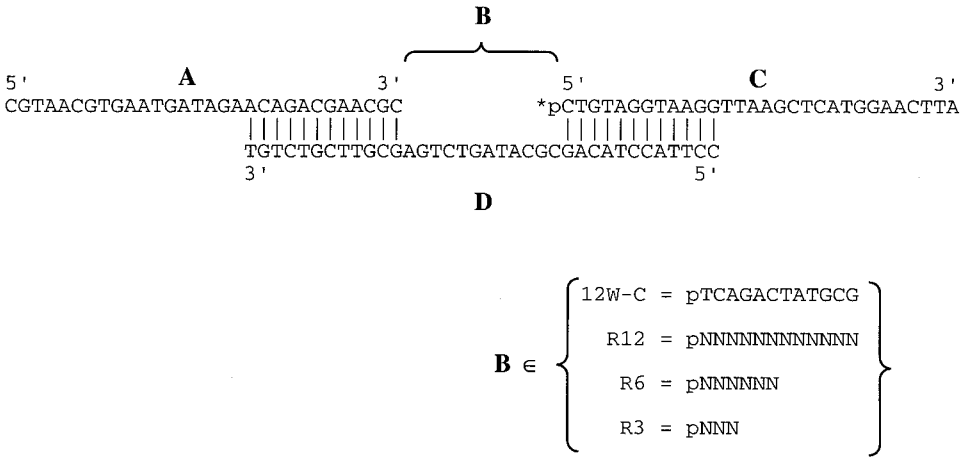

Figure 1. A method for assessing the fidelity of template-directed oligonucleotide ligation. A constant sequence DNA hemiduplex can be formed from oligonucleotides A, C, and D. Random sequence oligonucleotide libraries of twelve (R12), six (R6) or three (R3) residues in length were ligated across from the single-stranded template. Those oligonucleotide substrates (B) that closed the gap between the A and C oligonucleotides could be preferentially amplified by primers specific for A and C. A perfectly paired oligonucleotide substrate (12W-C) was used to evaluate and optimize this method.

evaluated the fidelity of template-directed oligonucleotide ligation catalyzed by organic reagents (James and Ellington, 1997b) and by enzymes (James and Ellington, submitted). The purpose of the current paper is to compare these results and discuss their relevance to the origin of self-replication.

The system we have used for the assessment of both chemically- and enzymatically-catalyzed oligonucleotide ligation is shown in Figure 1. Oligonucleotides that could form a DNA hemiduplex with a twelve residue single-stranded gap were synthesized and assembled. The hemiduplex was then mixed with random sequence oligonucleotide pools of 3, 6, or 12 residues in length. These pools have complexities of 64, 4096, and 16 777 216 members, respectively. Given the amount of synthetic DNA used in the ligation reactions, all possible sequences should have been present many times in each reaction. Members of the random sequence populations that could bind across from the single-stranded template and be ligated in place were subsequently amplified using primers specific for oligonucleotides A and C. Chemical ligation experiments were carried out with CNBr as a coupling reagent, while enzymatic ligation experiments were carried out with *E. coli* DNA ligase and NAD.

Both chemically- and enzymatically-catalyzed ligation reactions have been shown to be efficient when a perfectly complementary dodecamer is used as a substrate (Figure 2a, lanes 1 and 5; Figure 2b, lanes 5 and 10). The chemically-catalyzed ligation reactions were carried out for one second in the presence of high concentrations (0.5 M) of CNBr (Figure 2a). Time course experiments indicate that

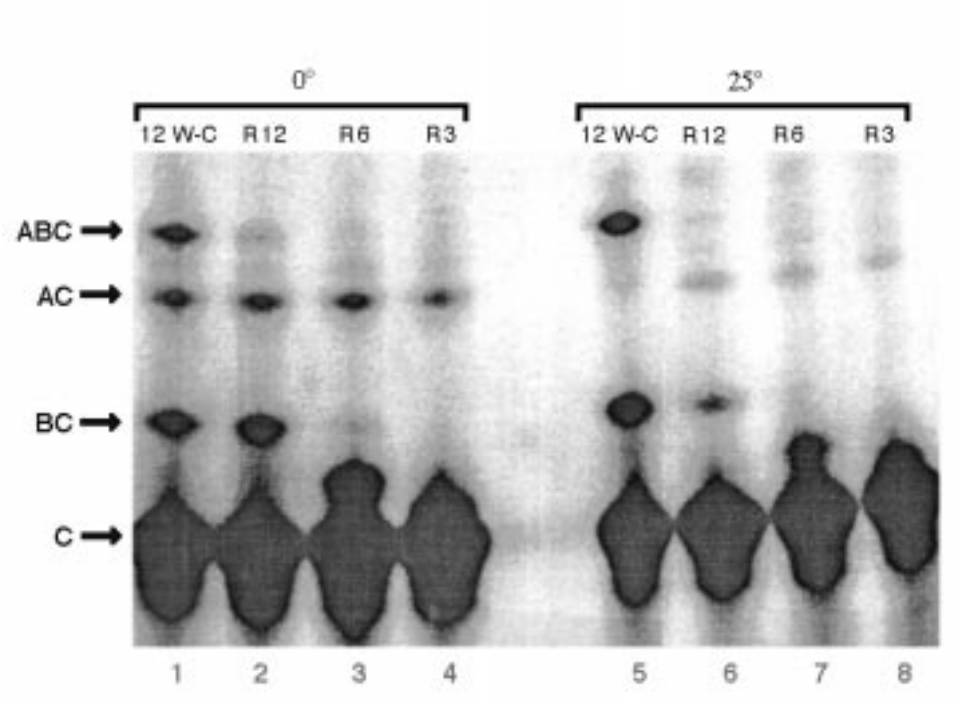

Figure 2a. Evaluation of the method. (a) Gel of chemically-catalyzed ligation. The C oligonucleotide was radiolabeled and ligation reactions were carried out as described in Materials and Methods with either perfectly complementary (12W-C) or pool (R12, R6, R3) oligonucleotide substrates (B) at 0 or 25 °C. The position of C ligated to these substrates (e.g., 'BC') is shown at left. The position of a direct ligation product 'AC' is also shown. (b) Gel of enzymatically-catalyzed ligation. The gel is similar to that shown in (a), except that reactions were carried out for 1 hr or 1 min at 25 °C. The slightly smaller size of the 'ABC' product in the trimer lane at 1 hr is the result of a ligation event between a trimer and the C and D oligonucleotides.

the ligation reaction is largely complete within the first few seconds of addition (data not shown). Reactions were carried out at both 0 and 25 °C to evaluate the effect of annealing temperature on fidelity. In contrast, enzymatically-catalyzed ligation reactions were carried out at 25 °C for either one minute or one hour (Figure 2b; the enzyme ligase would have operated inefficiently at 0 °C and these experiments were not attempted). As can be seen, the ligation product continues to accumulate with time. Thus, the enzymatic ligation experiments allow us to evaluate the effect of a different parameter, annealing time, on fidelity.

As expected, the formation of full-length ligation products was much less efficient with random sequence pools. When CNBr is used as a catalyst, some full-length product is observed with the random sequence dodecamer substrate, but little if any is observed with the random sequence hexamer or trimer substrates (Figure 2a). This is likely because fewer ligation events (2 total) are required to fill the hemiduplex gap with the dodecamer than are required for the hexamer (3 total)

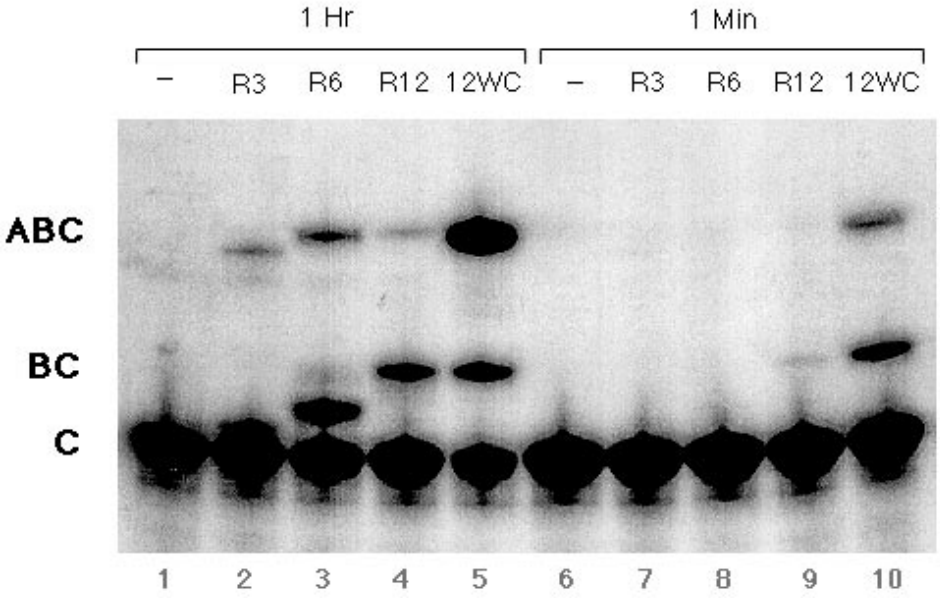

Figure 2b.

or the trimer (5 total). Consonant with this analysis, it is interesting to note that the single, direct ligation of the random sequence dodecamer, hexamer, and trimer substrates to the radiolabelled C oligonucleotide can always be observed ('BC' products in Figure 2a). When ligase is used as a catalyst, more full-length or near full-length product is observed with all three pools than with chemical ligation, especially at longer reaction times. However, there is still less full-length product than with the perfectly paired substrate (Figure 2b). The 'BC' intermediates are again observed.

Despite the fact that the reactions with pools are relatively inefficient, ligation products that fill the single-stranded gap and connect the A and C oligonucleotides can be preferentially amplified using the polymerase chain reaction. Ligation products from all of the reactions shown in Figure 2 were gel-isolated, amplified, and sequenced. To avoid potential cross-contamination, reaction products that were eventually sequenced were never placed on the same gel with reactions that contained the constant sequence B oligonucleotide. Comparison of the results of these experiments should provide insights into the fidelities of template-directed oligonucleotide ligation with different catalysts under different reaction conditions.

2.2. FIDELITIES OF TEMPLATE-DIRECTED OLIGONUCLEOTIDE LIGATION

The sequences of from 18 to 24 different amplified ligation products were typically acquired for each ligation reaction. Since the sequence of the fully-complementary

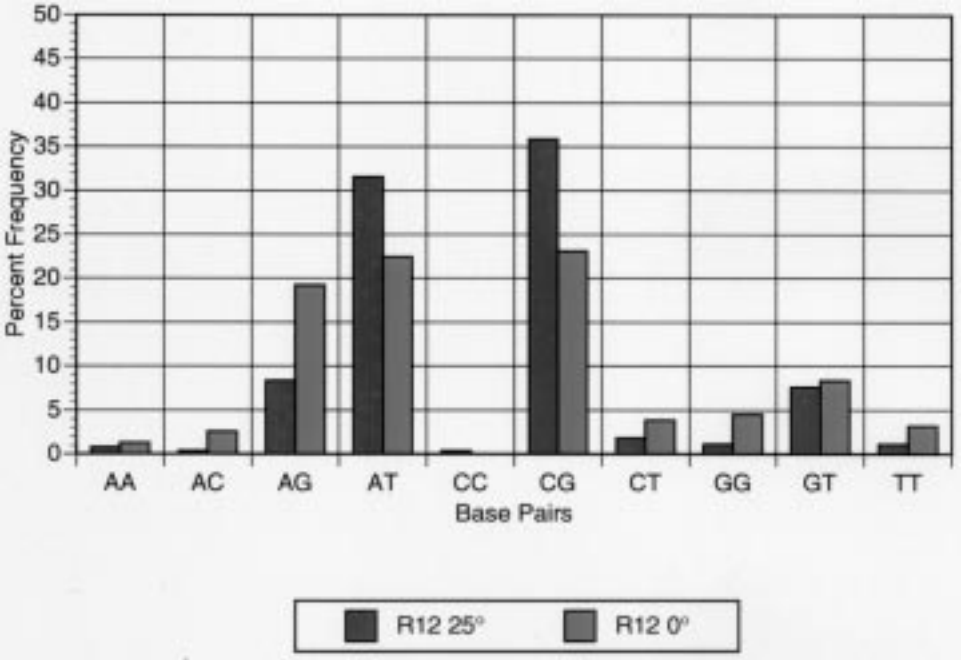

Figure 3a. Fidelity of chemically-catalyzed ligation. Sequences of selected oligonucleotide substrates were determined and compared to the sequence of the perfectly paired (12W-C) substrate. The raw sequence data can be found in James and Ellington (1997b). Different matched (AT, CG) and mis-matched positions were tabulated and are presented here as a percent of total potential pairing positions. Reactions at 25 °C are in black, while reactions at 0 °C are in grey. The numbers represented by the bars do not always add up to 100% because of the presence of deletion variants. The inclusion of the deletion variants in the totals gives a more accurate representation of how frequently (or infrequently) matched positions occurred. (a) Reactions with the dodecamer library. (b) Reactions with the hexamer library. Reactions with the trimer library yielded virtually no full-length ligation products, and the *datum* is therefore not shown.

product was known in advance, the fidelity of the ligation products could be readily determined in comparison. The fidelities of ligation are presented in Figures 3 and 4 (for chemical and enzymatic ligation, respectively) as the proportions of different base-pairings that were observed. Since the single-stranded template region of the DNA hemiduplex contained half A:T pairings and half G:C pairings a perfectly paired oligonucleotide substrate should yield a value of 50% A:T and 50% G:C on the graphs as presented. The total percentages of base-pairings do not always add to 100% because of the presence of deletion variants; for example, many of the oligonucleotide products that gave rise to the data in Figures 3b and 4c were direct ligation events between the A and C oligonucleotides (see also Figure 5). The band corresponding to the direct ligation product is most apparent in Figure 2a; this band was close enough to the full-length ligation product to allow their joint isolation. The deletion variants were included in the tabulation of fidelities because their

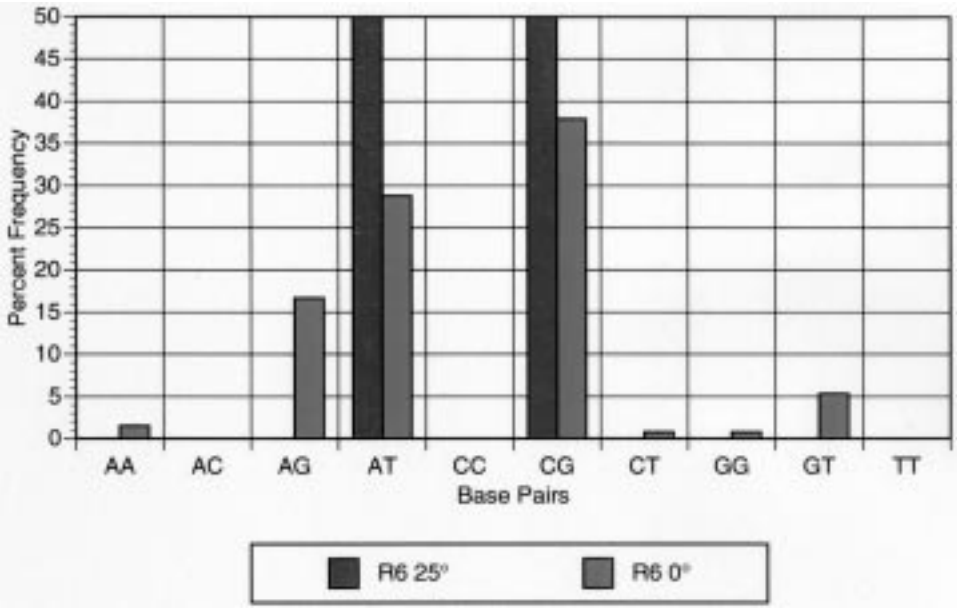

Figure 3b.

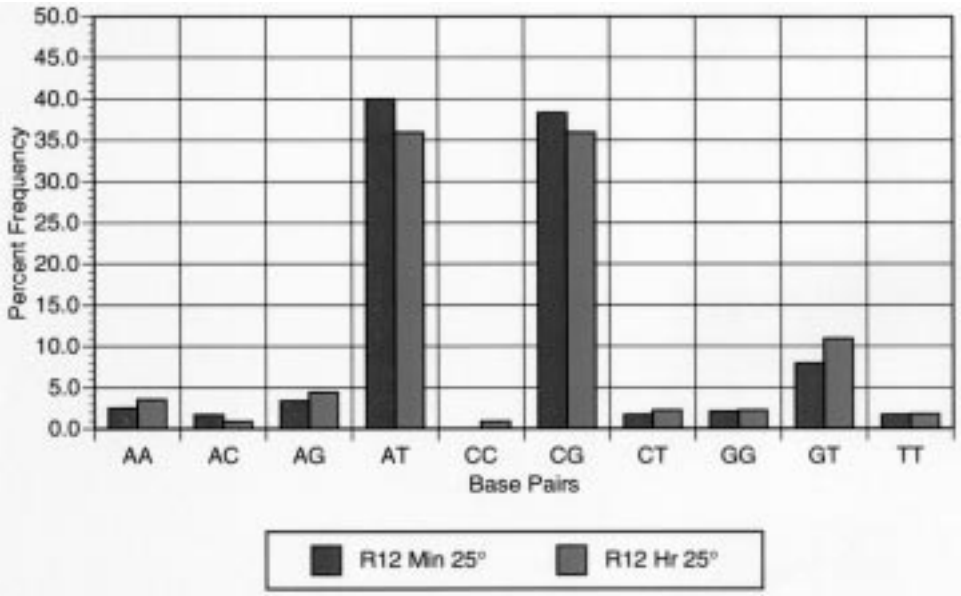

Figure 4a. Fidelity of enzymatically-catalyzed ligation. These graphs are similar to those in Figure 3, except that reactions for one minute are in black, while reactions for one hour are in grey. (a) Reactions with the dodecamer library. (b) Reactions with the hexamer library. (c) Reactions with the trimer library.

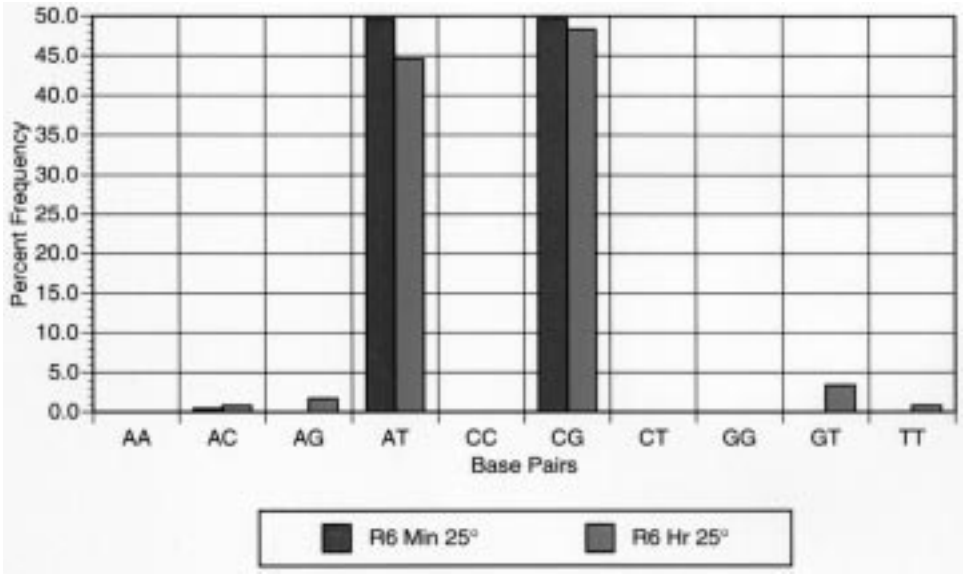

Figure 4b.

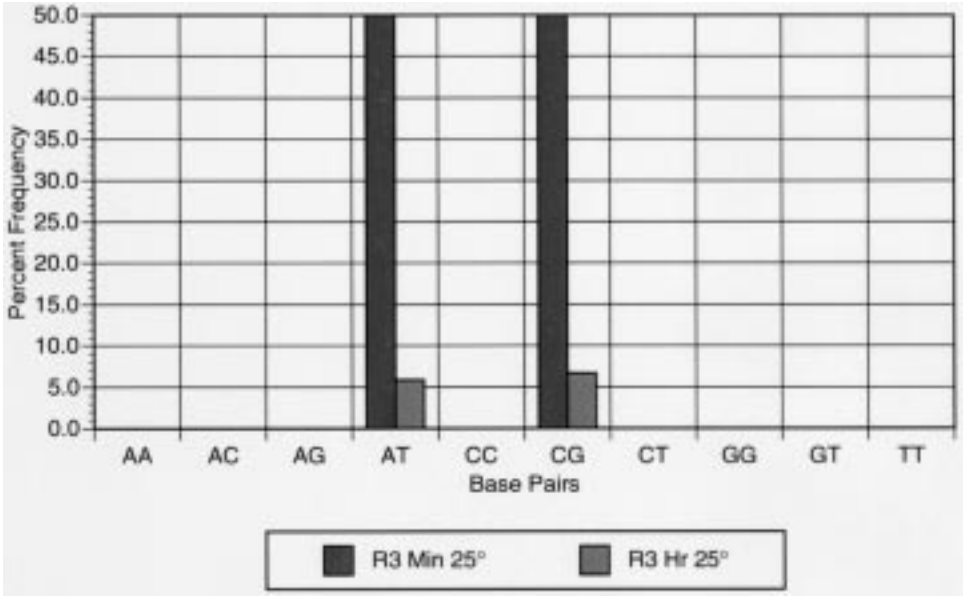

Figure 4c.

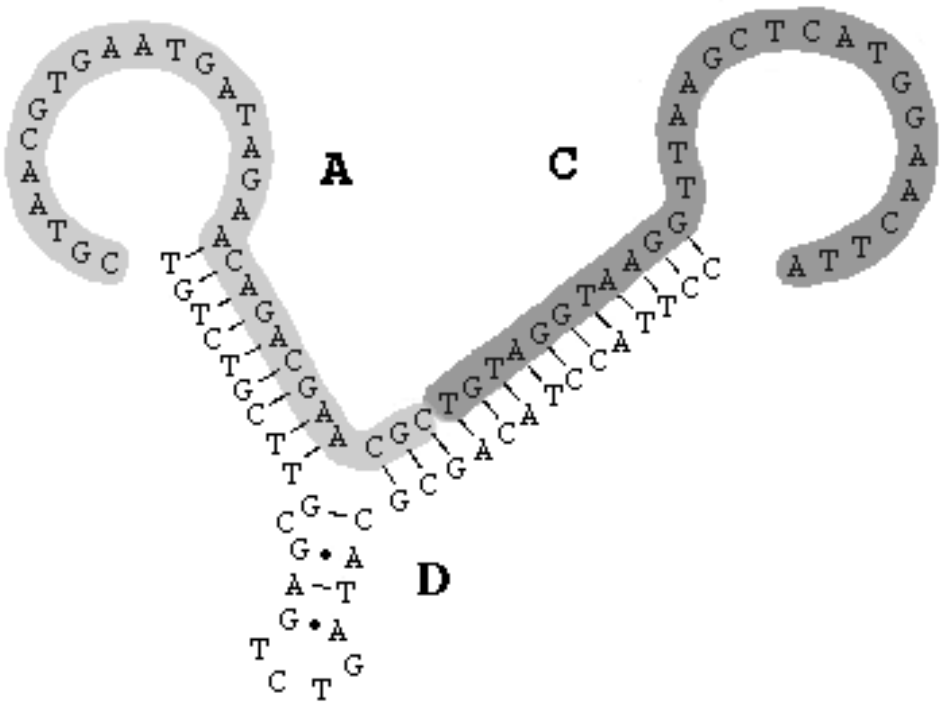

Figure 5. Direct 'AC' ligation product. An alternative conformer that is thought to give rise to the direct ligation of A and C oligonucleotides is shown. The direct ligation event yields amplification products that contain no substrate oligonucleotide insert. This alternative conformer can only form with C oligonucleotides that lack a 3′ terminal cytidine (compare with Figure 1). The A oligonucleotide is shown in light grey, while the C oligonucleotide is shown in dark grey.

presence was obviously germane to evaluating the abilities of oligonucleotides to efficiently and faithfully copy themselves.

The fidelity of chemically-catalyzed ligation was strongly dependent on temperature. At low temperatures (0 °C), mismatches were accepted more readily than at higher temperatures. In particular, A:G pairings become more stable at low temperatures (Patel *et al*., 1984), and the accumulation of predicted A:G base-pairs in captured oligonucleotide substrates was correspondingly observed. At higher temperatures (25 °C), mismatches have a proportionately larger effect on whether or not a given oligonucleotide substrate will be bound by a single-stranded template. In the most faithful chemically-catalyzed ligation reactions, hexamer substrates at 25 °C showed near perfect fidelity (Figure 3b). Greater fidelities were observed with hexamers relative to dodecamers, consistent with the calculated Tm's for the oligonucleotide substrates: duplexes formed by the hexamer substrates should have melted near 25 °C, while duplexes with the dodecamer should have melted at substantially higher temperatures (62 °C). These observed trends in fidelities did not extend to trimer substrates because the trimers did not efficiently anneal and

were not ligated in place at either temperature. Instead, the ligation products that were amplified and sequenced were almost wholly deletion variants.

The fidelity of enzymatically-catalyzed ligation was dependent on time. At longer times, more mismatches and deletions accumulated than at shorter times. In other words, oligonucleotide substrates that were perfectly paired were most quickly fixed in place, while oligonucleotide substrates that were mispaired did not bind as well and could only be haltingly fixed in place. In the most faithful enzymatically-catalyzed ligation reactions, hexamer or trimer substrates ligated for a minute showed near perfect fidelity (Figures 4b, c). Overall, the dependence of fidelity on substrate length was much the same as for chemical ligation, with the exception that the fidelity of trimer ligation improved dramatically. The enhanced recovery of ligated trimers was due almost solely to the presence of the enzyme, as opposed to an increase in the catalytic efficiency of the enzyme relative to CNBr. In fact, at the short times where the greatest fidelities were observed enzymatic ligation is actually less efficient with a perfectly paired dodecamer substrate than chemical ligation. Similarly, the enhanced recovery of ligated trimers cannot be explained by an enzyme-mediated change in substrate conformation or selectivity, since the deletion variants observed during chemical ligation also accumulated during enzymatic ligation. The simplest explanation for the improved fidelity of trimer ligation is that the enzyme non-specifically stabilizes otherwise unstable trimer:template pairings.

2.3. Implications for the origin of self-replicators

While reaction conditions can be found that lead to high copying fidelity for template-directed oligonucleotide ligation, it is instructive that these conditions are relatively narrowly defined: varying the substrates, temperature, or the time of ligation can quickly lead to the accumulation of products that contain multiple mismatches and deletions. The mis-copied products arose either from the insertion of ‘wrong’ oligonucleotides across from the single-stranded DNA template, or from the direct ligation of the A and C oligonucleotides to generate deletion variants. The directly ligated deletion variants likely arose from an alternative conformation of the DNA hemiduplex (James and Ellington, 1997b; James and Ellington, submitted; Figure 5). As was pointed out above, the presence of such deletion variants and the conformation that gave rise to them is as directly relevant to the discussion of fidelity as the insertion of incorrect oligonucleotide substrates. While the alternative conformer might not have been present if a different constant sequence template had been chosen, it is likely that other alternative conformers or competing reactions would have nonetheless been in evidence, just as other alternative conformers or competing reactions would likely have hampered many different primordial replicators. Taken together, these results do not initially appear to bode well for the hypothesis that template-directed oligonucleotide ligation gave rise to the first living systems, especially since the milieu in which the first self-replicating

nucleic acids originated would likely have been extremely heterogeneous in terms of the lengths and sequences of oligonucleotide substrates, the concentrations of mono- and divalent cations, and perhaps even the temperature of the environment.

Unfortunately, the difficulties inherent in the hypothesis that template-directed mononucleotide polymerization gave rise to the first living systems also remain. Prebiotic mononucleotides would likely have been present in a variety of chemical and enantiomeric forms, and many of these forms could have been added to growing replicators but would have immediately terminated replication. The seminal experimental example was provided by Joyce and Orgel (1984), who showed that incorporation of a guanosine of the wrong handedness essentially stopped any further elongation on a poly-C template.

Early replicators are thus caught between Charybdis and Scylla: oligonucleotide ligation can in part repair the deficiencies of mononucleotide polymerization, since oligonucleotides can potentially accept one or more chemically or enantiomerically 'unnatural' nucleotides in their interiors yet still be competent for ligation and thus for replication. Mononucleotide polymerization can in part repair the deficiencies of oligonucleotide ligation, since substrate discrimination and fidelity are determined by relatively few hydrogen bond interactions. This seeming paradox provides the basis for a synthesis of the two hypotheses.

2.4. A MODEL FOR THE EVOLUTION OF POLYMERASE FUNCTION

Two results stand out from our comparative inquiries into the fidelity of template-directed oligonucleotide ligation. First, irrespective of the temperature or ligation conditions, the length of the oligonucleotide substrates had a profound effect on the fidelity of replication. Second, enzymatic ligation allowed the almost perfect copying of a template using trimer substrates (at least under some conditions), a feat that was never observed with chemical ligation. These results support a scenario in which early replicators arose via template-directed oligonucleotide ligation, yet eventually evolved and prospered via mononucleotide polymerization (Figure 6).

In this scenario, the initial self-replicators would have been similar to those previously engineered by Orgel, von Kiedrowski, and their colleagues: short oligonucleotide templates that directed the ligation of even shorter oligonucleotide foodstuffs. *Most of these experiments, including our own, have been carried out with DNA. While it is possible that life arose from deoxyribose-based polymers it is generally accepted that the immediate precursors of modern organisms may have had a metabolism based on ribozymes and RNA, the so-called 'RNA world' hypothesis.* The prebiotic synthesis of *RNA* oligonucleotides that could have served *as both template and food* has been resoundingly demonstrated by Ferris and his co-workers (Ding *et al*., 1996; Ertem and Ferris, 1996; Ferris *et al*., 1996). Oligonucleotide replicators that were faster or more efficient would have enjoyed a selective advantage over the course of many generations. Since speed and efficiency are molecular phenotypes that are associated with catalysts, and since oligonucleotide

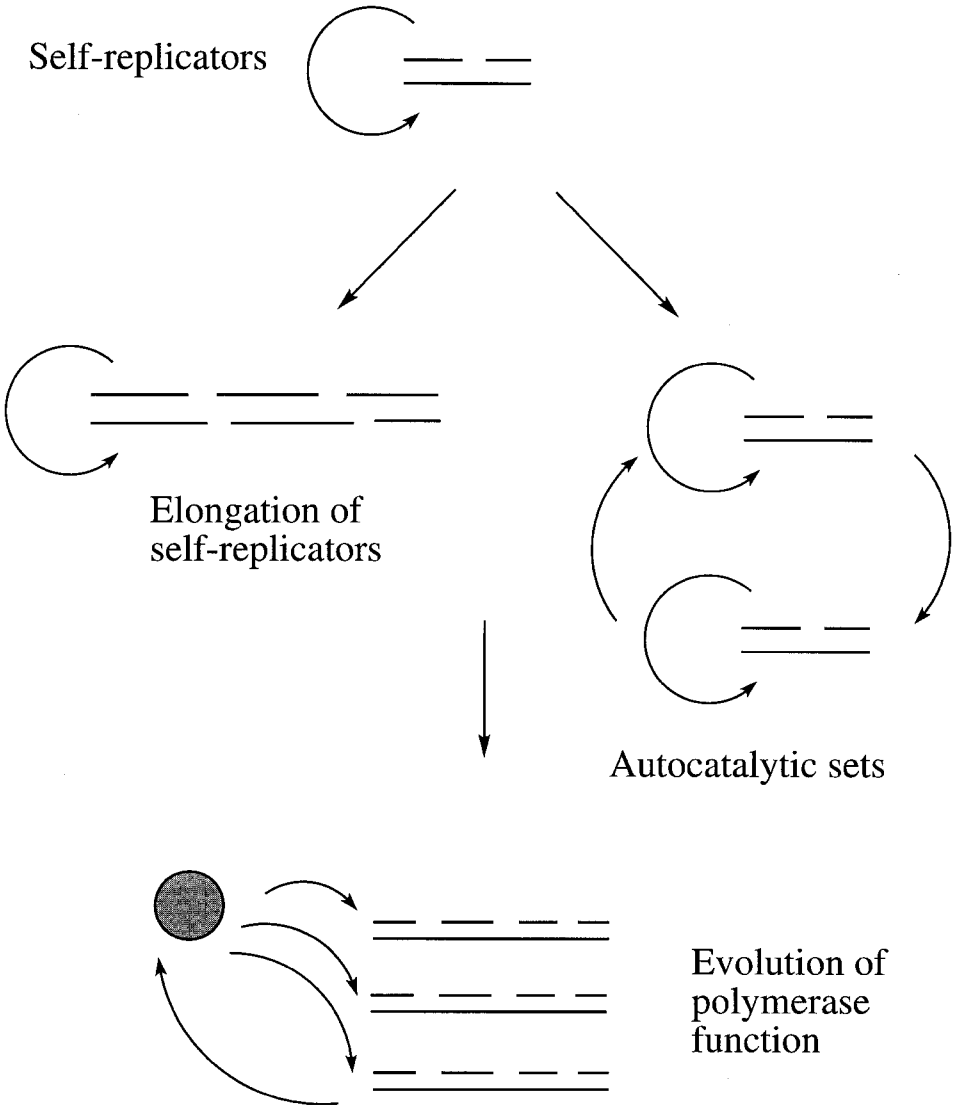

Figure 6. Hypothetical evolution of polymerase function. Self-replicating oligonucleotides similar to those examined by von Kiedrowski (1986) may have arisen prebiotically. These oligonucleotide replicators may have acquired additional, catalytic information and functionality by elongation or through the formation of autocatalytic sets. Our results suggest that there would have been selective pressure for the replicators to grow large and complex enough to stabilize the formation of substrate:template pairings, and therefore to become oligonucleotide or mononucleotide polymerases. Such polymerases could have acted on their own and other templates.

catalysts are more likely to arise from longer oligonucleotides than shorter ones (Sabeti *et al*., 1997), any nascent oligonucleotide replicators would likely have grown and augmented their nascent templating abilities with catalytic functionality. At this stage, longer oligonucleotide replicators could have been assembled from either short or long oligonucleotide substrates. However, as we have observed, template-directed ligation with long oligonucleotide substrates leads to the accumulation of errors, while template-directed ligation with short oligonucleotide substrates results in relatively inefficient replication (see for example Figure 2).

Thus, the most successful molecular variants would have been those that learned to hold and catalyze the ligation of short oligonucleotide substrates. Along these lines, we believe that the ability of *E. coli* DNA ligase to bind to and stabilize an otherwise unstable binary template:substrate complex is the most likely explanation for the differences in fidelity observed between enzyme and chemical catalysts with trimer substrates. Similarly, we envision that even a short oligonucleotide replicator could have learned to form contacts with the sugar or phosphate backbones of substrates (as a real-world example, see the binding of the hairpin stem P1 by the Tetrahymena ribozyme, Pyle *et al*., 1992). Alternatively, *a catalytic* replicator *could have formed a triple helix with* its substrate (as a real-world example, see the self-cleaving deoxyribozyme selected by Carmi *et al*., 1998). As the length and catalytic capabilities of replicators increased, they may have eventually reached a complexity similar to that of modern designed and selected ribozyme ligases, which are typically several hundred residues in length (Been and Cech, 1988; Bartel and Szostak, 1993) and whose speed can rival that of protein catalysts (Ekland *et al*., 1995). These longer, more sophisticated ribozymes have already been shown to catalyze the template-directed ligation of trimers (Bartel *et al*., 1991) and the template-directed polymerization of monomers (Ekland *et al*., 1996).

In summary, in order to increase fidelity and decrease the possibility of a Malthusian limitation of longer, more complex oligonucleotide substrates, replicators would have increased in size while their foodstuffs decreased in size. Short oligonucleotide templates would have given rise to longer template-directed oligonucleotide ligases which would have in turn have given rise to mononucleotide polymerases similar to the long (and hence prebiotically improbable) ribozyme ligases and polymerases that have been designed or selected from random sequence pools. Although we only have data for nucleic acid oligomers, the scenario we have devised is based on a physico-chemical appreciation of ligation and it is likely that the envisioned progression applies to other replicating polymer systems as well (Visscher and Schwartz, 1988; Lee *et al*., 1996; Bolli *et al*., 1997; Lee *et al*., 1997).

3. Materials and Methods

3.1. Materials and reagents

All oligonucleotides were synthesized on an Applied Biosystems (Foster City, CA) PCR Mate DNA synthesizer. Reagents were purchased from Glen Research (Sterline, VA); columns were purchased from Cruachem (Dulles, VA). Terminal 5′-phosphates (non-radioactive) were added to oligonucleotides during automated synthesis. Oligonucleotides longer than fifteen residues were purified by gel electrophoresis. T4 polynucleotide kinase (New England Biolabs; Beverly, MA) and [γ-32P]ATP (NEN Research Products; Boston, MA) were used to end-label oligonucleotides. DNA ligase from *E. coli* was purchased from New England BioLabs (Beverly, MA); Taq polymerase was purchased from Promega (Madison, WI); Sequenase was purchased from United States Biochemical (Cleveland, OH). Cyanogen bromide was obtained from Aldrich (Milwaukee, WI).

3.2. Chemical ligation reactions

Oligonucleotides were combined in a thin-walled PCR tube in a final volume of 4.5 μL of 0.25 M MES buffer (pH 7.5; adjusted with Et_3N) and 20 mM $MgCl_2$. Oligonucleotides A, C, and D were present at a concentration of 20 μM each. The B oligonucleotides were present in their respective reactions in the following ratios (B:D): 1:1 for 12 W-C, 5:1 for R12, 25:1 for R6, and 125:1 for R3. The reaction mixture was heated to 95 °C for 3 min and cooled to room temperature over 15 min. The solutions were then placed at the desired temperature (0 or 25 °C) and cyanogen bromide (0.5 μL of a 5 M solution in acetonitrile) was added. For the time course reaction shown in Figure 2, the reactions were quenched at times ranging from 1 to 60 sec by the addition of absolute ethanol (100 μL). After centrifugation at 4 °C for 30 min, the supernatant was removed from the precipitated DNA. The pellets were further dried in a vacuum centrifuge and then resuspended in 5 μL water.

3.3. Enzymatic ligation reactions

Oligonucleotides were combined in a thin-walled PCR tube in a final volume of 4.5 μL of 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM dithiothreitol, 26 mM NAD+, and 125 ng bovine serum albumin. Oligonucleotides A, C, and D were present at a concentration of 20 μm each. The B oligonucleotides were present in their respective reactions in the following ratios (B:D): 1:1 for 12 W-C, 5:1 for R12, 25:1 for R6, and 125:1 for R3. The reaction mixture was heated to 95° C for 3 min and cooled to room temperature over 15 min. The solutions were kept at 25 °C and DNA ligase was added. The reactions were quenched at times ranging from 15 sec to 1 hr by the addition of a denaturing dye mix.

3.4. GEL ISOLATION AND PCR AMPLIFICATION

Ligation products were separated from unligated oligonucleotides by gel electrophoresis (12% polyacrylamide; 19:1 mono-: bis-acrylamide). Radioactive bands of appropriate length were cut from the gels and eluted overnight at 37 °C into 0.3 M NaCl. The eluted oligonucleotides were precipitated by addition of three volumes of ethanol followed by centrifugation at 4 °C. The pellets were washed with 70% ethanol, air-dried, and resuspended in 5 μL water. Ligation products that contained both the A and C oligonucleotides were selectively amplified by the polymerase chain reaction: 2 μL of the isolated ligation product were added to a reaction mix that contained 5% acetamide, 0.05% NP-40, 200 μM dNTPs, 500 nM of each primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3. In each thermal cycle, the temperature was held at 94 °C for 30 sec, 45 °C for 30 sec, and 72 °C for 30 sec. From 25 to 28 thermal cycles were typically required to fully amplify ligation products.

3.5. MOLECULAR BIOLOGY METHODS

Double-stranded PCR products were cloned using TA Cloning and Zero Blunt-End Cloning kits by Invitrogen (Carlsbad, CA). Mini-preps of plasmids were performed using a common alkaline lysis protocol. Sequencing was performed using a standard dideoxy method.

References

Achilles, T. and von Kiedrowski, G.: 1993, A self-replicating system from three starting materials. *Angew. Chem. Int. Ed. Engl.* **32**, 1198–1201.

Bartel, D. P., Doudna, J. A., Usman, N. and Szostak, J. W.: 1991, Template-directed primer extension catalyzed by the Tetrahymena ribozyme. *Mol. Cell. Biol.* **11**, 3390–3394.

Bartel, D. P. and Szostak, J. W.: 1993) Isolation of new ribozymes from a large pool of random sequences. *Science* **261**, 1411–1418.

Been, M. D. and Cech, T. R.: 1988, RNA as an RNA polymerase: net elongation of an RNA primer catalyzed by the Tetrahymena ribozyme. *Science* **239**, 1412–1416.

Benner, S. A., Ellington, A. D. and Tauer, A.: 1989, Modern metabolism as a palimpsest of the RNA world. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7054–7058.

Bolli, M., Micura, R. and Eschenmoser, A.: 1997, Pyranosyl-RNA:chiroselective self-assembly of base sequences by ligative oligomerization of tetranucleotide-2′, 3′-cyclophosphates: with a commentary concerning the origin of biomolecular homochirality, Chem. Biol. **4**, 309–320.

Bohler, C., Nielsen, P. E. and Orgel, L. E.: 1995, Template switching between PNA and RNA oligonucleotides. *Nature* **376**, 578–581.

Carmi, N., Balkhi, S. R. and Breaker, R. R.: 1998, Cleaving DNA with DNA. *Proc. Natl. Acad. Sci. USA* **95**, 2233–2237.

Ding, P. Z., Kawamura, K. and Ferris, J. P.: 1996, Oligomerization of uridine phosphorimidazolides on montmorillonite: a model for the prebiotic synthesis of RNA on minerals. *Origin Life Evol. Biosph.* **26**, 151–171.

Ekland, E. H. and Bartel, D. P.: 1996, RNA-catalysed RNA polymerization using nucleoside triphosphates. *Nature* **382**, 373–376.

Ekland, E. H., Szostak, J. W. and Bartel, D. P.: 1995, Structurally complex and highly active RNA ligases derived from random RNA sequences. *Science* **269**, 364–370.

Ellington, A. D.: 1993, Experimental testing of theories of an early RNA world. *Methods Enzymol* **224**, 646–664.

Ertem, G. and Ferris, J. P.: 1996, Synthesis of RNA oligomers on heterogeneous templates. *Nature* **379**, 238–240.

Ferris, J. P., Hill, A. R., Liu, R. and Orgel, L. E.: 1996, Synthesis of long prebiotic oligomers on mineral surfaces. *Nature* **381**, 59–61.

Gilbert, W.: 1986, The RNA world, *Nature* **319**, 618.

James, K. D. and Ellington, A. D.: 1995, The search for missing links between self-replicating nucleic acids and the RNA world. *Origins Life Evol. Biosph.* **25**, 515–530.

James, K. D. and Ellington, A. D.: 1997a, Catalysis and the RNA world in The Molecular Origins of Life A. Brack, ed. University of Cambridge Press, Cambridge (in press).

James, K. D. and Ellington, A. D.: 1997b, Surprising fidelity of template-directed, chemical ligation of oligonucleotides. *Chem. Biol.* **4**, 595–610.

James, K. D. and Ellington, A. D.: 1998, The fidelity of template-directed oligonucleotide ligation and its relevance to DNA computation. Submitted to *Nucl. Acids Res*.

Joyce, G. F. *et al*.: 1984, Chiral selection in poly(C)-directed synthesis of oligo(G), *Nature* **310**, 602–604.

Joyce, G. F.: 1989, RNA evolution and the origins of life. *Nature* **338**, 217–224.

Joyce, G. F. and Orgel, L. E.: 1993, *Prospects for understanding the origin of the RNA world in The RNA World*, Gesteland, R. F. and Atkins, J. F., eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. pp. 1–26.

Lee, D. H., Granja, J. R., Martinez, J. A., Severin, K. and Ghadiri, M. R.: 1996, A self-replicating peptide. *Nature* **382**, 525–528.

Lee, D. H., Severin, K., Yokobayashi, Y. and Ghadiri, M. R.: 1997, Emergence of symbiosis in peptide self-replication through a hypercyclic network. *Nature* **390**, 591–594.

Orgel, L. E.: 1992, Molecular replication. *Nature* **358**, 203–209.

Pyle, A. M., Murphy, F. L. and Cech, T. R.: 1992, RNA substrate binding site in the catalytic core of the Tetrahymena ribozyme. *Nature* **358**, 123–128.

Sabeti, P. C., Unrau, P. J. and Bartel, D. P.: 1997, Accessing rare activities from random RNA sequences: the importance of the length of molecules in the starting pool. *Chem. Biol.* **4**, 767–774.

Schmidt, J. G., Nielsen, P. E. and Orgel, L. E.: 1997a, Information transfer from peptide nucleic acids to RNA by template-directed synthesis. *Nucl. Acids Res.* **25**, 4797–4802.

Schmidt, J. G., Nielsen, P. E. and Orgel, L. E.: 1997b, Information transfer from DNA to peptide nucleic acids by template-directed synthesis. *Nucl. Acids Res.* **25**, 4792–4796.

Schneider, K. C. and Benner, S. A.: 1990, Oligonucleotides containing flexible nucleoside analogs. *J. Am. Chem. Soc.* **112**, 453–455.

Sharp, P. A.: 1985, On the origin of RNA splicing and introns. *Cell* **42**, 397–400.

Sievers, D. and von Kiedrowski, G.: 1994, Self-replication of complementary nucleotide-based oligomers. *Nature* **369**, 221–224.

Visscher, J. and Schwartz, A. W.: 1988, Template-directed synthesis of acyclic oligonucleotide analogues. *J. Mol. Evol.* **28**, 3–6.

von Kiedrowski, G.: 1986, A self-replicating hexadeoxynucleotide. *Angew. Chem. Int. Ed. Engl.* **25**, 932–935.

von Kiedrowski, G., Wlotzka, B., Helbing, J., Matzen, M. and Jordan, S.: 1991, Parabolic growth of a self-replicating hexadeoxynucleotide bearing a 3′-5′-phosphoamidate linkage. *Angew. Chem. Int. Ed. Engl.* **30**, 423–426.

Zielinski, W. S. and Orgel, L. E.: 1987, Autocatalytic synthesis of a tetranucleotide analogue. *Nature* **327**, 346–347.