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The Evolutionary Transition from RNA to DNA in Early Cells

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Summary. The evolution of genetic material can be divided into at least three major phases: first, genomes of "nucleic acid-like" molecules; secondly, genomes of RNA; and finally, double-stranded DNA genomes such as those present in all contemporary cells. Using properties of nucleic acid molecules, we attempt to explain the evolutionary transition from RNA alone as a cellular informational macromolecule prior to the evolution of cell systems based on double-stranded DNA. The idea that ribonucleic acid-based cellular genomes preceded DNA is based on the following: (1) protein synthesis can occur in the absence of DNA but not of RNA; (2) RNA molecules have some catalytic properties; (3) the ubiquity of purine and pyridine nucleotide coenzymes as well as other similar ribonucleotide cofactors in metabolic pathways; and (4) the fact that the biosynthesis of deoxyribonucleotides always proceeds via the enzymatic reduction of ribonucleotides.

The "RNA prior to DNA" hypothesis can be further developed by understanding the selective pressures that led to the biosynthesis of deoxyribose, thymine, and proofreading DNA polymerases. Taken together these observations suggest to us that DNA was selected as an informational molecule in cells to stabilize earlier RNA–protein replicating systems. These arguments include the facts that (1) the 2′-deoxy-containing phosphodiester backbone is more stable in aqueous conditions and in the presence of transition metal ions (such as Zn^{2+}) than its ribo-equivalents; (2) the absence of proofreading activity in RNA polymerases leads to a higher rate of mutation in RNA genomes relative to DNA; (3) information in RNA degrades because of the tendency of cytosine to deaminate to uracil and the lack of a correcting enzyme; and (4) UV irradiation produces a larger number of photochemical changes in RNA molecules relative to double-stranded DNA. The absence of atmospheric UV attenuation during the early Earth environment (Hadean and early Archean) would have imposed an intense selection pressure favoring duplex DNA over other genetic information storage systems.

If RNA preceded DNA as a reservoir of cellular genetic information, then an RNA-replicating oligopeptide must have been one of the earliest protoenzymes from which RNA polymerase presumably evolved. We conclude that RNA polymerases are among the oldest classes of enzymes.

Key words: Primordial genomes — Double-stranded DNA — Early Darwinian evolution — Early Earth environment — RNA versus DNA

Introduction

An intracellular genetic apparatus capable of expressing and, upon reproduction, transmitting to the progeny information capable of undergoing evolutionary change is essential to all contemporary cells

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(Margulis 1981; Hartman et al. 1985). The origin of this ubiquitous genetic apparatus is a major problem in the study of the appearance of life during the Hadean or Archean eons.

The idea that RNA preceded DNA as genetic material has been expressed independently by many authors (e.g., Oparin 1961; Rich 1962; Haldane 1965; Woese 1967; Crick 1968; Orgel 1968; Reanney 1979; Oró and Lazcano 1984; Darnell and Doolittle 1986). However, recent experiments on nonenzymatic template-directed polymerization of activated simple nucleotide analogues (Schwartz and Orgel 1985) and the spontaneous oligomerization of purine-containing deoxynucleoside diphosphates (Schwartz 1986) have been interpreted to suggest that both RNA and DNA were preceded by simple nucleic acid-like polymers (Schwartz 1987). Important evidence argues against the possibility that RNA was the first genetic material (Joyce et al. 1987) including the observations (1) that prebiotic synthesis of ribose yields only minor amounts of this sugar, which is quite unstable on a geological time scale, and (2) that nonenzymatic template-directed polymerization of one activated ribonucleotide enantiomer is inhibited strongly by the presence of the other. Thus, RNA was probably preceded in cell genome evolution by a polymer made of flexible, acyclic, probably prochiral nucleotide analogues of prebiotic origin (Joyce et al. 1987). Thus, although the nature of the polynucleotides used as carriers of genetic information in the earliest forms of life is not known, we assume that the evolution of genetic templates followed this schematic progression: single-stranded RNA → single-stranded DNA → duplex DNA.

Several factors lead to the hypothesis that a nucleic acid-like genome made a transition to RNA genomes, including (1) the presence of the 2′-OH along polynucleotides; (2) the rigidity of the furanose ring; and (3) the enhanced nucleophilicity of the RNA 2′(3′) cisglycol (Joyce et al. 1987).

This paper reviews the evidence for a second transition: from cell RNA genomes alone to double-stranded DNA in addition to RNA. Here we argue that double-stranded DNA molecules appeared in cells with RNA genomes as a consequence of selection pressures favoring an enhanced stability of the genetic information. We believe the origin and evolution of biosynthetic pathways leading to the reduction of ribose to deoxyribose, the formation of thymine, and the presence of DNA polymerases with proofreading activity occurred subsequent to the origin of life; DNA-based genetic systems were selected for inside cells by Darwinian mechanisms to stabilize earlier systems based on RNA replication, protein catalysis, and double-layered lipid membranes.

RNA Prior to DNA

Early-replicating and catalytic cell systems based on RNA (devoid of DNA) as suggested by Crick (1968) and Orgel (1968) have been discussed extensively elsewhere (Lazcano 1986). The argument includes:

1) Protein biosynthesis can take place in the absence of DNA but not of RNA (Spirin 1986).

2) The existence of viroids (Diener 1982) and RNA viruses (Reanney 1982) indicates that replication systems can use either single- or double-stranded polyribonucleotides to store genetic information.

3) RNA molecules have catalytic properties that include endonuclease (Kruger et al. 1982; Guerrier-Takada et al. 1983), RNA ligase (Zaug and Cech 1985), exonuclease, and RNA polymerase (Zaug and Cech 1986) activities. [For review, see Pace and Marsh (1985).]

4) A number of ribonucleotide cofactors (coenzymes) containing purines, pyrimidines, pyridines, and other nitrogenous bases are ubiquitous and essential to primary metabolism in extant metabolic pathways (White 1976, 1982). Furthermore, several of these coenzymes can be synthesized under prebiotic conditions (Mar et al. 1987).

5) The 2′-OH group of ribose (absent of course in deoxyribose) may be directly involved in a number of phosphorylations (Halmann et al. 1969; Usher 1977) and amino acid condensation reactions (White and Erickson 1981).

6) The biosynthesis of deoxyribonucleotides always proceeds via the enzymatic reduction of ribonucleotides (Sprengel and Follmann 1981; Lammers and Follmann 1983).

All these points support a biotic rather than a prebiotic origin of the DNA system.

Life without DNA

If our analysis is correct, early RNA genomes never coded for a number of proteins involved in DNA replication such as DNA polymerases and DNA primase (Reanney 1982; Lazcano 1986). However, genes coding for RNA replicase and other proteins required for RNA maintenance and replication, as well as the accompanying nucleoside triphosphate metabolism (ATP, GTP), must have been the sine qua non of autopoietic systems of "RNA–protein" cells (Margulis and Guerrero 1987). As in contemporary prokaryotes, some kind of RNA polymerase was involved in tRNA and rRNA synthesis. Replication of the RNA genome and ribosome-mediated translation of RNA into proteins must have occurred within a dynamic lipoprotein membrane. Membranes are the essential prerequisite to Darwinian evolution. Furthermore, they are essential

to RNA-based (or any) life forms because they maintain an internal microenvironment differentiating autopoietic entities from their external milieu (Oró and Lazcano 1987). The presence of lipidic membranes raises, however, the question of the dynamic relationship between the contents of the cell and the external milieu.

Different viral (Kamer and Argos 1984) and eubacterial nucleic acid polymerases (Lazcano et al. 1987) share a highly conserved 14-residue segment that consists of an Asp–Asp pair flanked by hydrophobic amino acid residues. This segment may be the active site and/or the nucleic acid recognition region of the nucleic acid polymerases (Kamer and Argos 1984). A sequence rich in hydrophobic amino acids precedes an Asp residue; these sequences may be involved in ATP or inorganic pyrophosphate binding. Proteins displaying these features include eubacterial ATP synthetases, yeast-soluble inorganic pyrophosphates, and other enzymes (Baltcheffsky et al. 1987), suggesting the possibility of a common origin for an early RNA-dependent RNA polymerase and membrane-associated proteins (Lazcano et al. 1987).

What Selection Pressures Led to the Development of Double-Stranded DNA by RNA-Based Autopoietic Systems?

The evolutionary transition from RNA as genomic material to RNA supplemented by double-stranded DNA has not been explained in detail (but see Ferris and Usher 1983). We suggest that the selection pressures for high heritability led to the supplementation of RNA cells by DNA and resulted in the following: (1) greater stability (the 2′-deoxy-containing phosphodiester backbone is more stable in aqueous conditions, and in the presence of transition metal ions such as Zn^{2+}, than its ribo-equivalent); (2) repair (absence of proofreading activity in RNA polymerases leads to higher mutational rate in RNA genomes relative to DNA); (3) resistance to chemical degradation (information in RNA degrades because of the tendency of cytosine to deaminate to uracil; no RNA-dependent enzyme exists capable of reversing this deamination); and (4) UV sensitivity (UV irradiation produces a larger number of photochemical changes in RNA as compared to double-stranded DNA). These arguments are developed below.

Stability to Basic Hydrolysis

The 2′-deoxy-containing phosphodiester of DNA is much more resistant to basic hydrolysis than is its ribo-equivalent (Ferris and Usher 1983). The hydrolysis of RNA proceeds via the formation of intermediate cyclic 2′,3′ phosphonucleosides. The phosphodiester bonds of DNA (that lack the 2′-OH group of ribose required to form the intermediate) are more stable under basic conditions. Several divalent ions catalyze the hydrolysis of the sugar–phosphate backbone of RNA (Butzow and Eichhorn 1965; Eichhorn et al. 1971). Zinc affords a much more rapid reaction than any other metal ions tested. The zinc-induced depolymerization requires the presence of the 2′-hydroxyl. [DNA, in general, is not readily degraded by metal ions (Butzow and Eichhorn 1971).] From an evolutionary point of view these results are important. Zinc ions, used efficiently for template-directed polymerization reactions of activated nucleotides (Bridson et al. 1981), are known to be present in the active site of most DNA and RNA polymerases (Mildvan and Loeb 1979). It is therefore easy to envision the retention of zinc-catalyzed polymerization reactions prior to and after the evolution of the first autopoietic systems.

Existence of DNA Proofreading Enzymes

Phages, bacteria, and many eukaryotes exhibit at least some minor 3′–5′ exonuclease activity in their DNA polymerases, allowing them to edit newly synthesized DNA. Mismatched bases are excised and recopied once a mistake is detected (Loeb and Kunkel 1982). DNA methylation also allows for newly formed strands to be distinguished from parental ones (Fersht 1983; Friedberg 1985). RNA polymerases are not known to possess 3′–5′ exonuclease activity; evidence of error-suppressing proofreading mechanisms in RNA has not been reported (Reanney 1982; Fersht 1983). RNA replication therefore is intrinsically less accurate than DNA replication (Table 1).

The origin of the editing properties of DNA polymerases can only be surmised. They probably succeeded the polymerizing activity of these enzymes. Cells capable of using double-stranded DNA molecules would have been rapidly selected for. These later developed proofreading DNA polymerases. The lack of 3′–5′ exonuclease activity in all RNA polymerases implies that RNA genomes cannot achieve very large sizes without risking their genetic identities. The number of misplaced nucleotides is proportional to the length of the produced RNA (Reanney 1982), an effect that can be observed by the high number of mutations in RNA viruses (Holland et al. 1982; Reanney 1982; Steinhauer and Holland 1987). The segmented genomes found among certain RNA viruses may represent an evolutionary strategy that overcomes the inaccuracy of RNA polymerases (Reanney 1982). During the early evolution of life, selective pressures probably favored DNA supplements as repositories of genetic infor-

Table 1. Fidelity in replication

Template	Catalyst	Error-rate[a]	References[b]
Poly(C)	Zn^{2+} and activated nucleotides	10^{-1}–10^{-2}	1, 2
RNA	RNA replicases	10^{-3}–10^{-4}	3, 4
Single-strand DNA from phage ϕX 174	DNA polymerase (*E. coli*)	$\sim 5 \times 10^{-7}$	5

Note: overall accuracy achieved by prokaryotes when copying is 10^{-7}–10^{-11} mutrations per base transcribed (Loeb and Loeb 1982)
[a] Error per base transcribed.
[b] References: (1) Bridson et al. 1981; (2) Orgel and Lohrmann 1974; (3) Eigen and Schuster 1977; (4) Springgate and Loeb 1975; (5) Fersht 1979

mation. The character of earlier RNA genomes must have limited strongly not only the fidelity of hereditary transmission of genetic information but the quantity of genetic content as well.

The Stability of Nitrogen Bases

"Double-stranded DNA is more stable to depurination by at least a factor of 10, and this may have been important for the accumulation of DNA" (Miller and Orgel 1974, p. 126). Hydrolytic reactions (deamination, depurination, and depyrimidation) are one to two orders of magnitude higher in single-stranded DNA prepared from rat liver cells than in double-stranded DNA from the same source (Singer and Kusmierek 1982). Similar detailed measurements for RNA molecules have not been made. However, assuming early RNA genomes had single-stranded regions, it can be concluded that their hydrolytic lability would have been higher than that of their 2'-deoxy-equivalents (Ferris and Usher 1983).

Deamination of cytosine into uracil in both RNA and DNA under physiological conditions is considerably faster than the other deamination reactions suffered by purine and pyrimidine bases (Singer and Kusmierek 1982). As a consequence of this deamination the information in RNA tends to degrade. Both the replacement of uracil with thymine in DNA and the removal of uracil from DNA by the correcting enzyme uracil–DNA glycosylase (Kornberg 1980) greatly enhance the stability of information storage in DNA relative to RNA. Clearly, uracil in DNA is not lethal; bacterial mutants are known to persist through several generations with levels of uracil in their DNA as high as one per 100 of thymine (Tye et al. 1978a) and *Escherichia coli* may remain functional with as much as 30% of its thymine replaced by uracil (cf. Kornberg 1980). Nevertheless, at least two enzymes have evolved that prevent the permanent incorporation of uracil into DNA.

One, dUTPase, is a hydrolytic enzyme capable of converting dUTP into dUMP. This enzyme maintains low levels of the endogenous dUTP pool and simultaneously provides the dUMP substrate for thymidylate synthetase (Kornberg 1980). The role of dUTPase in the biosynthesis of dTTP suggests that dUTPase originated to prevent the misinformation of uracil into DNA (Bertani et al. 1963).

The second mechanism, uracil–DNA glycosylase, detects uracil (incorporated in place of thymine or derived from the hydrolysis of cytosine) and removes it by hydrolytic cleavage of the uracil–sugar bonds. This enzyme is present in considerable quantities in both eubacteria and eukaryotes (Kornberg 1980; Lindahl 1982). Free uracil and pyrimidine-free DNA are products of these reactions. The missing pyridimines are usually quickly replaced; nucleotide excision–repair by DNA polymerase follows endonucleolytic incision (Kornberg 1980).

Deamination of cytosine to uracil is common both in vivo and in vitro (Olivera 1978; Shlomai and Kornberg 1978; Tye et al. 1978b). The ubiquity of uracil–DNA glycosylase is an evolutionary adaptation that insured genetic stability (Kornberg 1980). Uracil–DNA glycosylase is highly conserved (Lindahl 1982) and must have evolved after the development of the thymine biosynthetic pathway, i.e., after the appearance of dUTPase. Understandably, an equivalent system never developed among cells that used RNA as informational polymers; because uracil is a natural constituent of RNA, any hypothetical uracil–RNA glycosylase system would be unable to distinguish between the uracil molecules originally present in RNA, from those resulting from deamination of cytosine residues.

Stability to Photochemical Degradation

The flux of solar UV radiation at the surface of the early Earth must have been higher than the contemporary level. The lack of substantial amounts of free atmospheric oxygen and the consequent lack of an ozone shield would account for the greater UV flux of the Hadean and early Archean (Lazcano et al. 1983; Schopf 1983). Moreover, calculations based on satellite observations of young T-Tauri stars resembling the young sun (Cantó and Mendoza 1983) suggest that young solar-like stars emit up to 10^4

times more ultraviolet light than the present solar value (Gaustad and Vogel 1982; Canuto et al. 1982, 1983).

Ample paleontological evidence documents the appearance of life by the early Archean, more than 3.5×10^9 years ago (Knoll and Barghoorn 1977; Awramik et al. 1983; Schopf 1983; Schopf and Packer 1987). Such high levels of unattenuated UV radiation led to the early development among prokaryotes of UV protection mechanisms (Margulis et al. 1976; Margulis 1981). Highly sophisticated enzyme-mediated DNA repair mechanisms including photoreactivation, which are found even among obligate anaerobic visible light-insensitive bacteria like *Clostridium sporogenes* (Rambler and Margulis 1980), attest to an early appearance of mechanisms protecting DNA against potentially lethal solar radiation.

Ultraviolet light causes major photochemical changes in nucleic acids. Although the sugars and phosphate groups of both DNA and RNA make insignificant contributions to the absorption spectra at wavelengths greater than 200 nm (Cantor and Schimmel 1980), the purine and pyrimidine moieties of both RNA and DNA show very strong absorption (Shugar 1960). With the exception of uridine, which absorbs in the near-UV at 260 nm rather than at the 268-nm peak of thymidine, the standard deoxyribonucleotides absorb at essentially the same spectra as their corresponding ribo-compounds (Voet et al. 1963).

The two most important types of photoproducts of UV irradiation of nucleic acids are (1) a cyclobutane ring joining two sets of 5,6 double bonds of adjacent pyrimidine residues, and (2) pyrimidine hydrates (Friedberg 1985). UV-irradiation studies of the RNA phage R17 reveal that the major photochemical product is uracil hydrate (Remsen and Cerutti 1972). Of the other photochemical lesions (which include nucleic acid–protein links, breaks in the phosphodiester backbone, and the formation of cross-links in double-stranded DNA), the only reversible lesion is the formation of pyrimidine dimers (Setlow 1968; Friedberg 1985).

The extent of the UV-induced photochemical changes in nucleic acids depends, among other things, on the conformation of the molecule; single- and double-stranded DNA molecules prepared from ϕX 174 have very different UV sensitivities (Cerutti et al. 1965). The quantity of UV-induced thymine dimerization is significantly higher in the single-stranded DNA from *Enterococcus* (McLaren and Shugar 1964). When polyribonucleotides are UV-irradiated, the UV-induced hydration of pyrimidines is significantly suppressed in double-stranded relative to single-stranded molecules (Setlow 1968). The formation of uridine dimers is five times greater in poly(U) as compared with poly(A) : poly(U) copolymers. The hydration of uridylic acid in poly (A): poly(U) duplex is 10 times less than in poly(U) polymers (Pearson and Johns 1966).

The genomes of early cells, before the appearance of DNA, are thought to have been RNA molecules. Presumably, the early cellular RNA genomes contained small regions of short complementary base-pairing double strands, resulting from random matching processes, and probably had long single-stranded regions exposed to photochemical lesioning. Under in vitro conditions, contemporary replication of single-stranded polynucleotides [poly (A,C)] by eubacterial DNA-dependent RNA polymerases is reportedly more efficient than replication of RNA molecules with double-stranded regions (Llaca et al. 1987). In summary, the absence of UV attenuation during the early Earth imposed an intense selection pressure favoring duplex DNA molecules over other possible genetic information storage systems.

In UV-irradiated poly(U) used as messenger RNA (Grossman 1963) and as a template for in vitro replication (Adman and Grossman 1965), uracil hydrate tends to code as cytosine; cytosine hydrate formed after UV irradiation of poly(C) codes as uracil (Wacker et al. 1964; Ono et al. 1965; Adman and Grossman 1967). These results can be interpreted as equivalent to the changes in specificity of base-pairing due to keto–enol and amino–imino tautomeric shifts in uracil and cytosine, respectively (Lazcano 1986). Under physiological conditions, however, the enol and imino forms are present in only about one in 10^{-4}–10^{-5} (Saenger 1984), and mistakes are rapidly corrected by proofreading DNA polymerases. In a DNA-dependent polymerase system, the photochemical alteration of RNA molecules induced by UV irradiation would be of little significance (Setlow 1968). Yet, early cells with RNA genomes would have sustained very high rates of UV-induced mutations that were irreparable and would have low heritability. Because DNA-repair systems work by duplication of the genetic information contained in the complementary strands of the duplex DNA molecule (Kornberg 1980; Friedberg 1985), the emergence of double-stranded DNA would have stabilized the earlier irreparable RNA systems leading to the selection of mechanisms to correct the damage caused by UV light and, eventually, to bacterial sex (Margulis and Sagan 1986).

Conclusions

In this paper we have argued that the origin and evolution of the biosynthetic pathways leading to the reduction of ribose to deoxyribose, the appear-

ance of thymine, and the presence of DNA polymerases with proofreading activity were not the result of prebiotic processes; rather we claim that Darwinian selective mechanisms, acting on RNA-based cells, led to DNA systems subsequent to the origin of the cells themselves. Double-stranded RNA molecules are very stable and do not separate easily. Thus, it is likely that single-stranded RNA cellular genomes with some regions of short complementary base-pairing double-stranded regions were followed during early cellular evolution by single-stranded DNA genomes, and then by double-stranded DNA genomes. In summary, DNA-based genetic systems would have stabilized earlier systems based on RNA replication, themselves descended from cells with a nucleic acid-like genome (Joyce et al. 1987). If we are correct, researchers carrying out experimental efforts to achieve autopoiesy (Margulis and Sagan 1986; Varela and Maturana 1974) in the laboratory ought not to expect the formation of prebiotic, i.e., nonenzymatic, synthesis of a double-helix DNA or a DNA polymerase any more than they should expect to achieve other complex semes that evolved within living organisms, such as, for example, photosensitive ATP phosphorylation, excitable membranes, oxygenic photosynthesis, or nucleated cells. We do not imply that deoxyribose and thymine were absent in the prebiotic environment. In fact, nonenzymatic synthesis of both compounds has been achieved (Oró 1965). Rather, we suggest that prebiotic chemistry experimentation should focus on the achievements of membrane-bounded systems containing catalyic and autocatalytic RNA or nucleic acid-like polymers (Schwartz and Orgel 1986) with similar properties, capable of self-maintenance in the presence of inorganic ions and those organic compounds such as amino acids, ATP, and others expected to be available under putative prebiotic conditions.

According to the concept developed here, the amplification of ancestral replicase genes must have preceded the appearance of genes coding for proteins involved in DNA synthesis and replication, e.g., DNA primases and DNA polymerases (Lazcano et al. 1987, 1988). This genetic amplification process could have been enhanced by phage and plasmid transfer or gene duplication in the absence of cell division. Replicase activity found in DNA-dependent RNA polymerases supports the concept that they first evolved in polymerization of RNA in the absence of DNA. An early and ubiquitous role for this class of enzymes is suggested, making them ideal for reconstruction of universal (e.g., bacterial, viral, and eukaryotic) phylogenies (Llaca et al. 1987).

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