Our model also implies that the lowermost crust is younger than the crust it underplates. In addition, the newly formed lowermost crust will not necessarily be depleted chemically and could therefore be the source region for large amounts of felsic to intermediate magmas. Thus model ages for the separation of crust-forming material, deduced from rocks derived from this underplated reservoir, will be younger than those from the overlying crust.

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13. Relatively small terranes exposing kyanite-bearing granulites are widespread in Phanerozoic orogenic belts (the Bohemian massif, for example). Such terranes are relatively limited in extent and seem to have been exhumed and cooled rapidly in contrast with many regional granulite terranes. Although such granulite terranes are important in the understanding of Phanerozoic orogenesis, they do not seem representative of large areas of the continental crust and therefore have been excluded from our discussion. In so doing, we recognize that not all granulites form by the same tectonic or geologic processes. Other workers [for example, S. Harley, *Terra Cognita* **8**, 267 (1988)] have emphasized that different kinds of Precambrian regional granulite terranes may form by different tectonic processes. In this report we focus on a significant number of regional terranes that have in common several unifying characteristics that place important constraints on their formation and the formation of related lowermost crustal material.
14. Several suites of crustal xenoliths contain a few members of demonstrably sedimentary origin. Coesite-bearing pelites offer unambiguous evidence that sediments can be buried to great depths [C. Chopin, *Contrib. Mineral. Petrol.* **86**, 107 (1984)]. It is not surprising therefore that a very minor number of metasedimentary xenoliths are found in various suites. It is significant that suites containing an abundance of xenoliths of sedimentary origin yield pressures similar to those of exposed regional granulite terranes. Suites of xenoliths devoid of metasediments, or wherein metasediments comprise only a small number of xenoliths, generally show evidence of distinctly higher pressures of equilibration, as indicated in Table 2.
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Template-Directed Oligomerization Catalyzed by a Polynucleotide Analog

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A pyrophosphate-linked analog of polycytidylic acid has been synthesized and shown to catalyze the oligomerization of the complementary monomer 2′-deoxyguanosine 3′,5′-bisphosphoimidazolide. Analogs of polynucleotides are of interest in studies of the origins of life as possible precursors of the first RNA molecules. These results demonstrate that such molecules are capable of serving as templates for further synthesis.

ENZYMATICALLY SYNTHESIZED POLYcytidylic acid [poly(C)] catalyzes the synthesis of oligoguanylic acids [oligo(G)'s] from an activated form of guanosine 5′-phosphate (*1*). This system has been considered as a possible model for prebiotic replication of RNA. However, the inhibition of the reaction observed when both stereoisomers of the mononucleotide are present is not consistent with this role (*2*). Poly(C) has also been shown to catalyze the oligomerization of both 2′-deoxyguanosine 3′,5′-bisphosphoimidazolide (ImpdGpIm) and an acyclic analog of guanosine not based on ribose (*3–5*). In these latter template-directed reactions, the oligomers produced are linked by pyrophosphate, rather than phosphodiester linkages. Acyclic nucleic acid analogs with pyrophosphate backbones are possible precursors of the first RNA molecules (*6*). It is important, therefore, that these molecules be capable of acting as templates for oligomerization. We have synthesized a pyrophosphate-linked polynucleotide analog based on 2′-deoxycytidine 3′,5′-bisphosphate (pdCp) and now report that this product serves as a catalyst for template-directed oligomerization.

The monomer *N*-4-diphenylacetyl-2′-deoxycytidine 3′-*O*-phosphate 5′-*O*-(*S*-4-methylphenyl)phosphorothioate (structure 1 in Fig. 1A) was synthesized and subjected to oligomerization (*7–12*). In the absence of other nucleophiles, reaction of the activated 5′-phosphate can only occur with a free 3′-phosphate group. After removal of the diphenylacetyl protecting groups (*13*), therefore, the major products expected were the cyclic pyrophosphate (structure 2 in Fig. 1A, produced by intramolecular cyclization of the activated intermediate) and a series of 3′,5′-pyrophosphate–linked oligomers of pdCp (structure 3 in Fig. 1A). The crude products were fractionated, and oligomers were subjected to alkaline phosphatase treat-

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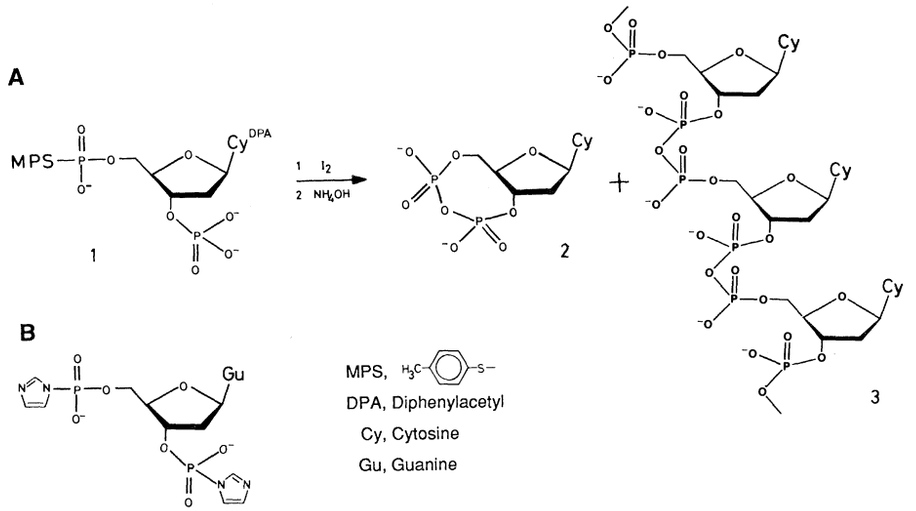

Fig. 1. Structures of monomers and oligomers synthesized. (**A**) Oxidation of monomer (structure 1), followed by hydrolysis, produces cyclic pyrophosphate (structure 2) and oligomers of pdCp (structure 3). (**B**) The structure of the complementary, activated monomer ImpdGpIm.

ment to remove terminal phosphate groups (*14*).

All oligomers with chain lengths of 16 and longer were collected in one fraction to be used as templates. We estimate that the lengths of oligomers in this fraction extended to about 40, with a mean of approximately 20. Analysis of the products by ^{31}P nuclear magnetic resonance (NMR) confirmed the absence of terminal phosphates and showed a resonance peak due only to internal pyrophosphate groups (*15*). Digestion with phosphodiesterase I from venom was used as described (*4*), together with high-performance liquid chromatography (HPLC) on RPC-5 (*1*), to verify an isolated oligomer with a chain length of 8. As expected from previous studies (*3, 4*) on pyrophosphate-linked oligomers of 2′-deoxyguanosine 3′,5′-bisphosphate (pdGp) and 2′-deoxyadenosine 3′,5′-bisphosphate (pdAp), a series of oligomers of lengths 2 to 7 was produced, with ultimate conversion of all oligomers to monomer.

The monomer ImpdGpIm (Fig. 1B) was prepared as described (*4*). We compared the oligomerization of ImpdGpIm in the absence of template with the oligomerization in the presence of oligo(pdCp) (Fig. 2). In the absence of a template, the primary reaction was cyclization of the monomer. Dimer (7%), cyclic dimer (6%), and trimer (2%) were the major oligomers formed. In the presence of oligo(pdCp), the total yield of oligomers increased from 17 to 36%. Much more significant, however, was the formation of product oligomers with chain lengths of at least 9 (longer products are obscured by the presence of the template). The control experiment established that the template is chemically stable under the conditions of the oligomerization. We verified that the products were pyrophosphate-linked by isolating the oligomer with a chain length of 6 from the RPC-5 column and following its sequential degradation by venom phosphodiesterase to yield pdGp. These results with a heterogeneous mixture of relatively short-chain oligo(pdCp) molecules are not dramatic when compared to oligomerization (*4*) in the presence of enzymatically synthesized, high molecular weight poly(C). However, they establish that the substitution of a pyrophosphate-linked backbone for the conventional one does not destroy the ability of the analog to catalyze the synthesis of its complement.

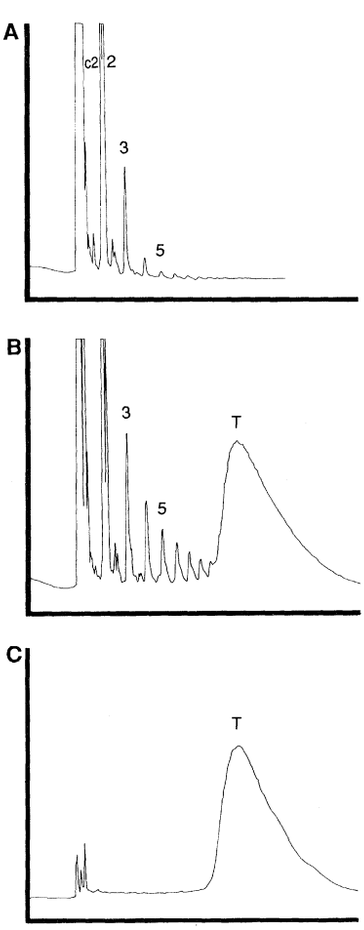

Fig. 2. Oligomerization of ImpdGpIm. (**A**) A solution containing 0.025*M* ImpdGpIm, 0.2*M* $MgCl_2$, 1.0*M* NaCl, and 0.5*M* bis(2-hydroxyethyl)imino-tris(hydroxymethyl) methane-HCl (*p*H 6.5) was incubated for 3 weeks at 0°C. (**B**) Same as (A) but with 0.025*M* (monomer equivalent) oligo(pdCp). (**C**) Same as (B) but without ImpdGpIm. HPLC was on RPC-5 (*1*) in 0.02*M* NaOH with a linear gradient of $NaClO_4$ (0*M* to 0.04*M*, 60 min) at a flow rate of 1.0 ml/min. Before analysis, reactions were stopped and any surviving phosphoimidazolide groups were hydrolyzed to phosphate as described (*4*). Peaks were detected by monitoring absorbance at 254 nm. Oligomers with lengths 2, 3, and 5 are identified on the chromatograms; c2, cyclic dimer; and T, oligo(pdCp) template.

These results have implications for theories on the origins of life. Arguments have been presented that purine and pyrimidine ribosides are highly implausible as prebiotic monomers (*6, 16*). The implausibility is related to the availability and properties of ribose rather than that of the purines and pyrimidines themselves. An acyclic, prochiral monomer based on glycerol bisphosphate and producing pyrophosphate-linked oligomers has been suggested as a possible evolutionary precursor to RNA (*5, 6*). Although an actual demonstration of template-directed synthesis catalyzed by oligomers in which ribose or deoxyribose plays no part remains to be achieved, a step toward that goal has now been taken.

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overall yield (based on deoxycytidine) after purification was about 30%. After evaporation of solvent (with ethanol to decompose TEAB, and repeated coevaporation with pyridine), a saturated solution of the dry product in pyridine was prepared (350 μmol of monomer in pyridine to a total volume of approximately 0.5 ml). Oligomerization was achieved by addition of 3.5 mmol of dry, finely powdered iodine, which activates the phosphorothioate group by oxidation (*10, 12*). The mixture was agitated in an ultrasonic bath for 1 hour and allowed to stand overnight.

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Access to a Messenger RNA Sequence or Its Protein Product Is Not Limited by Tissue or Species Specificity

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RNA amplification with transcript sequencing (RAWTS) is a rapid and sensitive method of direct sequencing that involves complementary DNA synthesis, polymerase chain reaction (PCR) with a primer or primers containing a phage promoter, transcription from the phage promoter, and reverse transcriptase–mediated sequencing. By means of RAWTS, it was possible to sequence each of four tissue-specific human messenger RNAs (blue pigment, factor IX, phenylalanine hydroxylase, and tyrosine hydroxylase) in four cell types examined (white blood cells, liver, K562 erythroleukemia cells, and chorionic villus cells). These results indicate that there is a basal rate of transcription, splicing, and polyadenylation of tissue-specific mRNAs in adult and embryonic tissues. In addition to revealing sequence information, it is possible to generate a desired in vitro translation product by incorporating a translation initiation signal into the appropriate PCR primer. RAWTS can be used to obtain novel mRNA sequence information from other species as illustrated with a segment of the catalytic domain of factor IX. In general, the ability to obtain mRNA sequences rapidly across species boundaries should aid both the study of protein evolution and the identification of sequences crucial for protein structure and function.

RECENTLY, METHODS HAVE BEEN described for the direct sequencing of genomic DNA that are based on PCR (*1, 2*). One of these methods, known as genomic amplification with transcript sequencing (GAWTS), incorporates a phage promoter sequence into at least one of the PCR primers (*2*). GAWTS has been modified to allow RNA to be directly sequenced. RAWTS consists of four steps: (i) cDNA synthesis with oligo(dT) or an mRNA-specific oligonucleotide primer, (ii) PCR where one or both oligonucleotides contains a phage promoter attached to a sequence complementary to the region to be amplified, (iii) transcription with a phage promoter, and (iv) reverse transcriptase–mediated dideoxy sequencing of the transcript, which is primed with a nested (internal) oligonucleotide. The incorporation of a phage promoter by PCR has three major advantages: (i) transcription produces a second round of amplification, which obviates the need for purification subsequent to PCR; (ii) transcription can compensate for suboptimal PCR; and (iii) transcription generates a single-stranded template, which in routine practice tends to give a more reproducible sequence than obtained directly from a linear double-stranded PCR product.

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RAWTS is extraordinarily sensitive because it combines the amplification generated by phage transcription with the amplification generated by PCR. To determine whether tissue-specific mRNAs could also be detected, total RNA was isolated from white blood cells, liver, K562 erythroleukemia cells, and cultured chorionic villus cells. The RNA was isolated by lysing the cells in the presence of guanidium-HCl except for the K562 cells, in which lysis occurred into SDS/proteinase K, followed by phenol extraction (*3*). Single-stranded cDNA was made from total RNA by priming reverse transcriptase with oligo(dT). RAWTS was performed on four tissue-specific mRNAs: blue pigment, which is expressed in the retina; factor IX and phenylalanine hydroxylase, which are expressed in the liver; and tyrosine hydroxylase, which is expressed in the brain and adrenal gland. How the primers were chosen for the retina-specific blue pigment mRNA is shown in Fig. 1. The first set of PCR primers were: BP-(T7-29)E5(1453)-44U, which contains the T7 promoter sequence, and BP-E4(1230)-16D [Fig. 1; for an explanation of the notation, see (*4*)]. The primers chosen span at least one intron, so the genomic sequence can be distinguished from that of mRNA. Sequence of the blue pigment mRNA was not obtained from white blood cell RNA even after 40 cycles of PCR. Therefore, we did a second amplification by diluting an aliquot of the first PCR mix 1000-fold in a fresh PCR mix and reamplifying with the same T7 promoter oligonucleotide primer along with E4(1259)-17D. An amplified fragment of predicted size was seen (Fig. 2A). The fragment was transcribed and then sequenced with BP-E4(1293)-17D as the primer for reverse transcriptasse. Intronic sequence was absent, confirming that mRNA and not DNA was the origin of the signal (Fig. 2C). By performing the two rounds of PCR, sequence information could also be obtained from liver, K562, and chorionic villus RNA (Fig. 2A and Table 1). With one

Table 1. Sequencing of tissue-specific human mRNAs. Blue pigment, BP; factor IX, F9; phenylalanine hyroxylase, PH; tyrosine hyroxylase, TH.

Tissue	BP	F9	PH	TH
Blood	+	+	+	+
Liver	+	+	+	+
K562	+	+	+	+
CVS	+	?*	+	+

*Factor IX could not be amplified from cultured chorionic villus cells but this is most likely a consequence of the partial degradation of this RNA (as indicated by the relative intensity of the 28*S* and 18*S* ribosomal RNA species), since the primers utilized for amplification required that more than 2 kb of the mRNA be intact, whereas sequencing of the other mRNAs required that no more than 1.1 kb be intact.