not show such a signature, except for 1333+2840 which might have a weak one. Three of the other four objects show a very weak (if any) He II BEL, and two of them show a marked decline of the continuum at wavelengths below Lyα (the third does not have the necessary spectral coverage). These three (1314+0116, 1336+1335 and 2212−1759) all show a strong radiative acceleration signature (Figs 1–3). The last object (1243+0121) is a borderline case which shows a drop in the continuum, but also a moderate He II BEL. Its C IV BAL shows what might be a signature of radiative acceleration, but it is not as persuasive as those of the three modelled. The quasar 1120+019 (not in the sample of 72) also meet all the above constraints, and indeed shows a 'ghost' signature (evident in its O VI 1,035-Å BAL; the spectral region of the C IV BAL was not observed[15]).

It is clear that when all the above physical and observational conditions for a radiative-acceleration signature are met, such a signature is indeed observed. Furthermore, the signature is strongly reduced (and in most objects cannot be detected) when even one of these conditions is not satisfied. This correlation is strong evidence that the observed feature is the result of radiative acceleration.

We can estimate the probability that the observed feature occurs randomly in the four objects that we have modelled (including 1120+019). The position of minimum optical depth in the hump is $<300\ km\ s^{-1}$ from its expected position in the four objects that show a clear signature. In addition, the dynamical model requires the minimum optical depth of the hump to be $\gtrsim 0.2$ (this condition is satisfied for the four ghost objects). Using the sample of 72 BAL quasars we find that seven of them satisfy these two criteria. However, we should not take the individual probability to be 7/72, because only 37 of the objects show appreciable absorption in the appropriate velocity range and our first selection criterion bias us toward that group. Therefore, the probability that a given object has a hump that satisfies the position and optical depth criteria is 7/37. The probability that four out of four objects will satisfy these requirements is therefore $\sim 10^{-3}$. Because the hump has to resemble roughly the Lyα BEL the probability of chance occurrence is reduced drastically, but this reduction is not easily quantifiable. We caution that this kind of an *a posteriori* probability analysis may have unintentional biases that can decrease the significance of the probability result. Also, we note that the process for choosing the objects that satisfy our fifth criterion is partially subjective. Another possible caveat is that by selecting objects with strong observed Lyα emission (as evidence for an intrinsically strong Lyα BEL) combined with significant absorption in N V and C IV in the range $-3{,}000$ to $-9{,}000\ km\ s^{-1}$, we may bias the selection toward BALs with intrinsic low optical depth at $\sim -5{,}900\ km\ s^{-1}$. However, we try to compensate for this bias by the second criterion we use to define an object with a strong intrinsic Lyα BEL (strong N V and C IV BELs), which should not have this bias.

Our confidence that the Lyα 'ghost' is evidence for the dominance of radiative acceleration in driving BAL outflows is thus based on the following: the appearance of a clear signature when the dynamical conditions are favourable; its absence when the conditions are unfavourable; the small probability of chance occurrence; and our ability to model both the hump in C IV and the N V-Lyα region with the same dynamical model for individual objects, provided that radiative acceleration is the dominant driving force. We predict that these characteristics will be evident in new samples of BAL quasars, and that the 'ghost' of Lyα will appear in the spectra of a few out of every hundred BAL quasars. This feature should show up as a hump near rest wavelength 1,520 Å in the C IV BAL. □

Received 10 April; accepted 19 July 1995.

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ACKNOWLEDGEMENTS. We thank referees M. Voit and F. Hamann for many useful comments and suggestions. This work was supported in part by NASA and the US NSF.

Template switching between PNA and RNA oligonucleotides

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THE origin of the RNA world[1] is not easily understood, as effective prebiotic syntheses of the components of RNA, the β-ribofuranoside-5′-phosphates, are hard to envisage[2]. Recognition of this difficulty has led to the proposal[1,3] that other genetic systems, the components of which are more easily formed, may have preceded RNA. This raises the question of how transitions between one genetic system and another could occur. Peptide nucleic acid (PNA) resembles RNA in its ability to form double-helical complexes stabilized by Watson–Crick hydrogen bonding between adenine and thymine and between cytosine and guanine[4–6], but has a backbone that is held together by amide rather than by phosphodiester bonds. Oligonucleotides based on RNA are known to act as templates that catalyse the non-enzymatic synthesis of their complements from activated mononucleotides[7–9], we now show that RNA oligonucleotides facilitate the synthesis of complementary PNA strands and vice versa. This suggests that a transition between different genetic systems can occur without loss of information.

The synthesis of oligo(G)s on oligo(C) templates using guanosine 5′-phosphoro(2-methyl)imidazolide (2-MeImpG; Fig. 1*a*) as substrate has been described in detail[10,11]. We began by comparing the deoxynucleotide 10-mer dC_{10} and PNA-C_{10} (Fig. 1*b*, II) as templates for the oligomerization of 2-MeImpG (Fig. 1*c*, I). The concentrations of reagents, the temperature and the pH were chosen to facilitate comparison with earlier publications[7]. The results are presented in Fig. 2. Both dC_{10} (Fig. 2*b*) and PNA-C_{10} (Fig. 2*c*, *d*) are effective templates for the oligomerization of 2-MeImpG. In both cases the yield of oligomeric products is greatly enhanced relative to that obtained in a template-independent reaction (Fig. 2*a*). However, dC_{10} is a much more efficient template than PNA-C_{10}, as can be seen by comparing Fig. 2*b* with Fig. 2*c*.

A closer examination of the elution profiles shown in Fig. 2 reveals many details concerning the regiospecificity of the reaction. The series of large labelled peaks observed when the template is dC_{10} (Fig. 2*b*) are known to correspond to all 3′-5′-linked oligomers[10,11]. Co-injection with the products obtained

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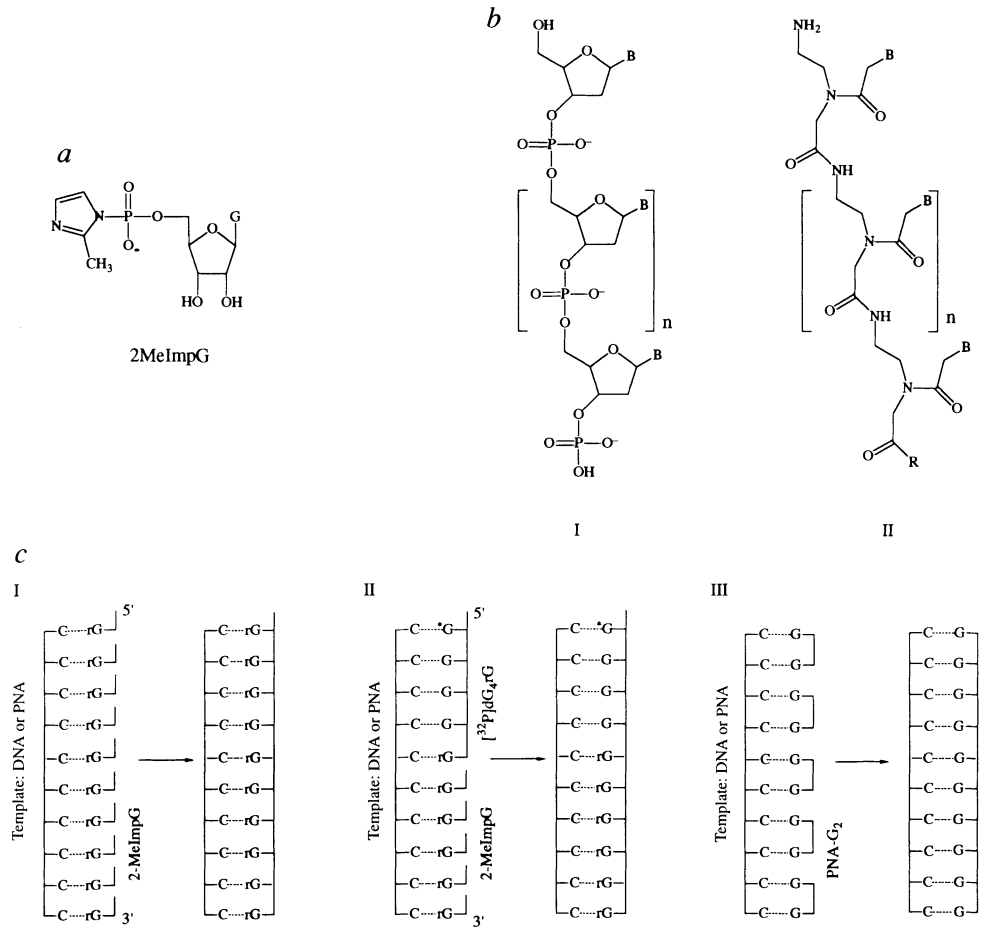

FIG. 1 *a*, The structure of guanosine 5′-phosphoro(2-methyl)imidazolide (2-MeImpG). *b*, Chemical structures of DNA (I) and PNA (II), where B corresponds to a nucleobase. The PNA-C_{10} template used in these experiments is derivatized with lysine amide (R is Lys-NH_2) on its carboxyl end[5]. *c*, Schematic representation of template-directed synthesis with I, template C_{10} (DNA or PNA) and substrate 2-MeImpG; II, template C_{10} (DNA or PNA), primer [^{32}P]-dG_4rG and substrate 2-MeImpG; III, template C_{10} (DNA or PNA) and substrate PNA-G_2. The schemes show only one reaction product, the full-length oligomer. A complex mixture of all possible intermediates up to the full-length oligomer is obtained in our experiments.

with a PNA template established that the labelled peaks in Fig. 2*c*, *d* also correspond to all 3′-5′-linked oligomers. When dC_{10} is used as a template, the dimer and trimer are mainly 3′-5′-linked. They are extended to form longer oligomers that are almost exclusively 3′-5′-linked (Fig. 2*b*). With PNA as template the initial synthesis of dimers and trimers yields a complex mixture of products; the 3′-5′-linked isomers are not the major components. However, these and only these 3′-5′-linked oligomers are extended efficiently, and yield longer exclusively 3′-5′-linked products (Fig. 2*c*, *d*).

These observations are most plausibly explained if we suppose that a 3′-5′-linked oligonucleotide primer at least 3 bases long is needed to establish an initial double-helical segment with PNA that is isostructural with a DNA:RNA double helix. Once this segment is formed, elongation proceeds efficiently in the same way as with equivalent DNA:RNA helices. The complexes formed by PNA with monomers and dimers of G have a different or less well-defined structure. This leads to the production of short oligomers with 2′-5′-phosphodiester bonds, pyrophosphate-linked oligomers, and possibly cyclic oligomers. These various oligomers form complexes with PNA that are not isostructural with DNA:RNA helices, and consequently they are not elongated regiospecifically. A detailed interpretation of the data presented in Fig. 2*c*, *d*, provides much evidence for the accumulation of short chain-terminated oligomers at later times in the PNA-catalysed reaction. It is clear, for example, that the 2′-5′-linked trimer accumulates at later times, while the 3′-5′-linked trimer is elongated.

These arguments suggest that the extension of a preformed oligonucleotide primer should proceed efficiently and regiospecifically on a PNA template. Figure 3 shows the results of an experiment in which a [^{32}P]-labelled dG_4rG primer is extended by reaction with 2-MeImpG on a dC_{10} or a PNA-C_{10} template (Fig. 1*c*, II). It is clear from Fig. 3 that dC_{10} (lanes 8–10) is only slightly more efficient than PNA-C_{10} (lanes 5–7) as a template for the primer-extension reaction. The regiospecificity of the reaction was confirmed by hydrolysis of the products of the reaction with ribonuclease T1, an enzyme that cleaves 3′-5′-linked GpG bonds, but does not affect 2′-5′-linked bonds. The almost complete disappearance of product bands from the gel (data not shown) indicates that the oligomers formed on dC_{10} and PNA-C_{10} templates are predominantly 3′-5′-linked.

The PNA monomers are acyclic amino carboxylic acids that cyclize readily to lactams. Consequently, they are not suitable substrates for template-directed synthesis. Instead we used the PNA dimer G_2 as substrate (Fig. 1*c*, III). We chose 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in the presence of excess-2-methyl imidazole as activating agent. This combination would yield 2-methyl imidazolides of the PNA dimers, carboxylic acid derivatives closely analogous to the activated nucleotides used in the previous experiments. The results of an experiment using dC_{10} as template and PNA-G_2 as substrate are presented in Fig. 4*b*. A series of four product peaks corresponding to PNAs G_4, G_6, G_8 and G_{10} are observed. The retention times of the first three are identical to those of authentic PNAs G_4, G_6, G_8 as shown by co-injection. The combined yield of oligomeric product as estimated from their ultraviolet absorption amounts to 87% of the input of C_{10} template. No substantial peaks corresponding to larger oligomers are detected. Comparison with the results of the non-template reactions (Fig. 4*a*) strongly suggests that G_2 dimers line up on the C_{10} template,

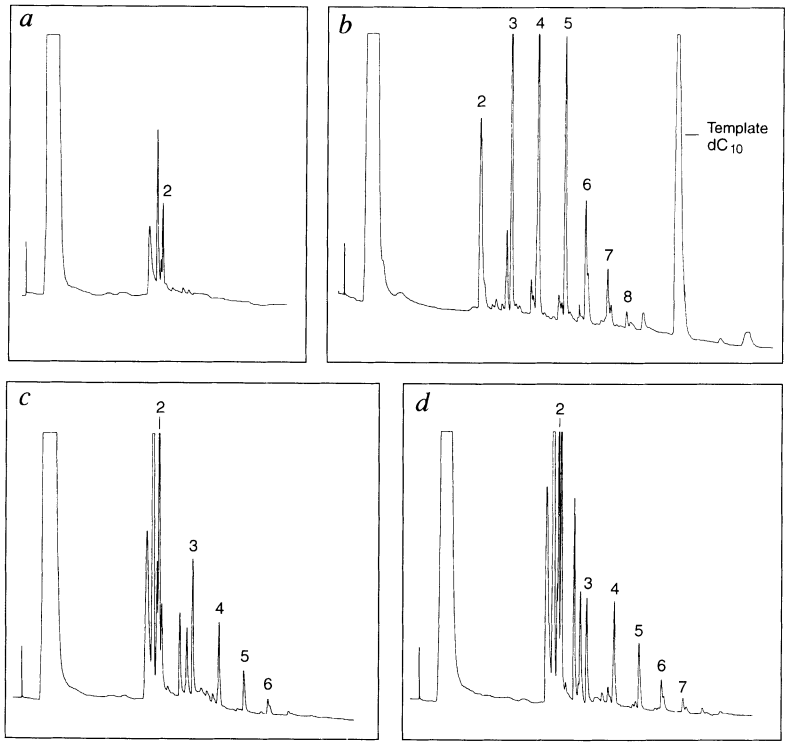

FIG. 2 Oligomerization of guanosine 5′-phosphoro(2-methyl)imidazolide (2-MeImpG) without template (*a*), with dC_{10} as template (*b*), and with PNA-C_{10} as template (*c*, *d*), analysed after 2 days (*a*–*c*) or 6 days (*d*). Reaction conditions: 0 °C; 0.1 M 2-MeImpG; 1.2 M NaCl; 0.2 M $MgCl_2$; 0.005 M template; and 0.2 M 2,6-lutidine buffer (pH 7.9 at room temperature). Reaction solutions were prepared in 0.7 ml Eppendorf tubes in 5 µl volumes. First stock solutions of NaCl and $MgCl_2$ with or without the template were coevaporated to dryness. To start the reaction, the residue was redissolved in a freshly prepared solution of 2-MeImpG in 2,6-lutidine buffer. At appropriate times, 1 µl reaction solution was added to 100 µl of an aqueous solution containing 0.02 M HCl and 0.005 M EDTA. The resulting solution (pH 2.8–3.0) was kept at 37 °C for 24 h to hydrolyse surviving phosphoro-imidazoles and then neutralized with aqueous NaOH. This solution (5 µl) was mixed with 1 ml starting buffer (pH 12) and analysed by high-performance liquid chromatography (HPLC) on RPC-5 as previously described[15]. The reaction products were eluted with a linear gradient of $NaClO_4$ (pH 12, 0–0.06 M, 90 min). The numbered peaks correspond to oligomers with all 3′-5′-linkages.

and are linked together in a template-directed reaction (Fig. 1*c*, III). Longer PNA sequences which could be formed if product oligomers bridged two template molecules are not observed in this system, as they sometimes are with nucleotide substrates[11].

When we attempted to oligomerize the PNA dimer G_2 on the PNA template C_{10} under our standard conditions we obtained a totally unexpected result: the template inhibited the oligomerization. The effect if not large (Fig. 4*c*), but it is reproducible. This suggests that the PNA-C_{10}:PNA-G_2 double helix has a somewhat different structure from the corresponding RNA:PNA double helix, and that in the PNA structure the amino and activated carboxyl groups of adjacent residues are held in a configuration that is unfavourable for reaction.

Experience with the RNA:RNA and DNA:RNA systems has shown that the success of template-directed reactions with phosphorimidazolide substrates depends on the precise structure of the imidazole moiety[12]. It was only by searching through many imidazole derivatives that we discovered one, 2-MeImpG, that undergoes efficient, regiospecific oligomerization on a poly(C) template. This optimization is likely to hold only for duplexes that adopt the RNA structure; some other imidazole might work better with PNA. We decided, therefore, to repeat the oligomerization reaction of PNA-G_2 using imidazole in place of 2-methylimidazole. The results are presented in Fig. 4*d*, *e*.

Comparison of Fig. 4*c*, *e*, shows that, in the presence of imidazole (*e*) rather than 2-methylimidazole (*c*), PNA-C_{10} does facilitate the synthesis of PNA-G_4 and longer G oligomers. The effect is not dramatic but is readily reproduced. Thus, as in our earlier experiments with RNA templates and substrates, the nature of the imidazole moiety of the activated derivative has a profound influence on the outcome of the reaction. We plan to search a 'library' of imidazoles to see if we can find one that makes possible efficient template-directed ligation reactions of PNAs.

The 'genetic takeover' of the replicating system by another was first discussed by Cairns-Smith. He has argued at length that the first genetic system was a replicating clay, and that it was taken over by a replicating organic polymer[13]. In this and other examples where the structures of the replicating systems are unrelated[3], it is difficult to see how information could be transferred from one system to the other. Our experiments are not relevant to this kind of genetic takeover.

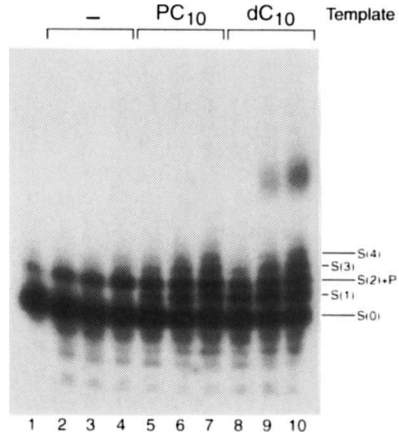

FIG. 3 Primer extension reaction of [^{32}P]-dG_4rG with 2-MeImpG without template (lanes 2–4) with PNA-C_{10} template (lanes 5–7) or with dC_{10} template (lanes 8–10) analysed after 4 h (lanes 2, 5, 8), 1 day (lanes 3, 6, 9) and 2 days (lanes 4, 7, 10) by polyacrylamide gel electrophoresis (PAGE) followed by autoradiography (lane 1 corresponds to the primer). Reaction conditions: 4 °C; 20 nM primer; 2 µM template; 1.2 M NaCl; 0.2 M $MgCl_2$; 0.05 M 2-MeImpG in 0.2 M 2-methylimidazole buffer (pH 8 at 4 °C). S(0) designates the starting material ([^{32}P]-dG_4rG), S(1) the first extension product, S(2) the second extension product, and so on. P designates the pyrophosphate-capped primer, which coelectrophoreses with S(2). Template-directed reactions were prepared in 0.7 ml Eppendorf tubes in 20 µl volumes by coevaporating stock solutions of NaCl, $MgCl_2$ and the primer (about 700,000 c.p.m., 0.2 pmole) to dryness. The residue was redissolved in 10 µl 0.4 M 2-methylimidazole buffer (pH 8 at 4 °C) and 7 µl water. The solution was heated to 95 °C for 10 min and then slowly cooled down, first to room temperature (1 h) and then to 4 °C (30 min). The reaction was initiated by adding 3 µl freshly prepared solution of 0.33 M 2-MeImpG. The tubes were then kept at 4 °C. Sampling: at given times, 2 µl reaction solution were added to 100 µl aqueous solution containing 0.01 M EDTA and 0.02 M HCl. The resulting solution (pH 4) was kept at 37 °C for 2 h to hydrolyse excess 2-MeImpG and then neutralized with 2 µl 1M NaOH. Samples (20 µl) of this solution were evaporated to dryness, redissolved in 5 µl water and 15 µl loading buffer. Before loading on the gel the solution was heated to 95 °C and cooled quickly to 0 °C.

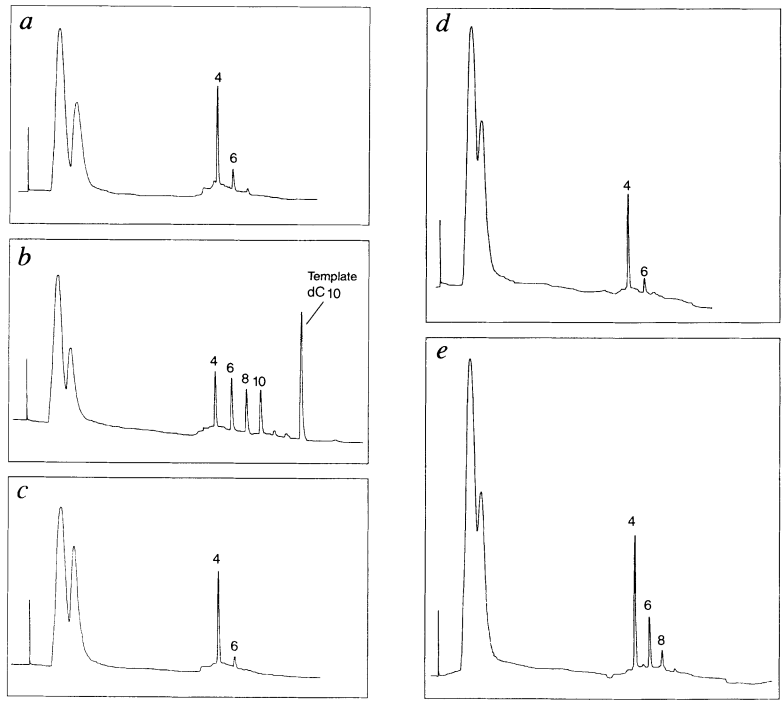

FIG. 4 Oligomerization of PNA-G_2 without template (*a*, *d*), with dC_{10} template (*b*) or PNA-C_{10} template (*c*, *e*) in the presence of 2-methyl imidazole buffer (*a*–*c*) or imidazole buffer (*d*, *e*), analysed after 6 h. Reaction conditions: 25 °C; 5 mM PNA-G_2; 0.5 mM template; 0.2 M EDC; 0.4 M buffer (2-methylimidazole or imidazole; pH 7 at 25 °C). Reaction solutions were prepared as described in Fig. 3 by coevaporating a stock solution of PNA-G_2 with or without the template. The reaction was initiated by redissolving the residue in 5 μl freshly prepared solution of 0.2 M EDC in buffer. HPLC analysis: 1 μl reaction solution was added to 1 ml starting buffer (pH 12) and heated to 95 °C for 15 min. The solution (60 μl) was then analysed by HPLC on RPC-5 (see Fig. 2). The reaction products were eluted with starting buffer for 5 min, followed by a linear gradient of $NaClO_4$ (pH 12; 0–0.08 M in 35 min). The numbered peaks correspond to PNA-G oligomers of the designated length.

A fundamentally different mechanism for genetic takeover has also been suggested[2]. It is proposed that a preformed molecule of the ancestral genetic system acted as a template for the incorporation of the monomers of the second system, without loss of sequence information. Our experiments are a first step towards the demonstration that takeovers of this kind can occur. A PNA oligomer C_{10} acts as a template for the regioselective oligomerization of 2-MeImpG while a DNA sequence dC_{10} acts as template for the oligomerization of the PNA oligomer G_2. Many further experiments will be needed to determine the range of sequences that act as efficient templates. It is also important to find out whether heteropolymeric chains containing RNA and PNA monomers can be generated and, if they can, whether they act as templates.

PNA is an achiral molecule so it must catalyse the oligomerization of activated D- and L-nucleotides with equal efficiency. When short PNA oligomers are complexed with racemic nucleotides, it is likely that double helices will form which contain only D- or only L-nucleotides. Template-directed synthesis might then yield a mixture of homochiral L-oligomers and homochiral D-oligomers from a racemic substrate. When longer PNA templates are used, analogous reactions should lead to block-copolymers of all D- and all L-RNA sequences. However, enantiomeric cross-inhibition[14] may well limit the efficiency of these processes. The use of PNA as a 'chemical mirror' is also a theoretical possibility. A D-oligonucleotide of defined sequence could be used as a template for the synthesis of PNA. The PNA in turn could be used to synthesize an L-oligonucleotide with the same sequence as the original D-oligonucleotide. □

Received 1 May; accepted 18 July 1995.

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ACKNOWLEDGEMENTS. This work was supported by grants from NSCORT/EXOBIOLOGY, the National Aeronautics and Space Administration, and the Danish National Research Foundation. We thank A. R. Hill Jr for technical assistance, H. F. Hansen for synthesizing the PNA G-dimer, and S. Bailey for manuscript preparation.

Polymer microstructures formed by moulding in capillaries

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THE formation of patterned structures on micrometre-length scales is essential for the fabrication of many electronic, optical and mechanical devices[1]. Patterning technologies are well established for semiconductors and metals, but are relatively undeveloped for organic polymers (with the notable exception of the specialized polymers used in photolithography[1]). Polymeric replicas of some structures have been formed by filling them with monomers which are subsequently polymerized[2–5]. But these procedures have important limitations, in that they usually involve the destruction of the template structure, or the resulting structures are not sufficiently regular for most applications. Here we describe a general moulding procedure which does not suffer from these limitations. For the mould we use the continuous network of channels formed when a substrate and a patterned elastomeric master are placed in intimate contact. A low-viscosity polymer precursor is placed in contact with the network, which then fills spontaneously by capillary action. After cross-linking the precursor, the master is removed (and can be reused), leaving a patterned polymer layer; depending on the choice of substrate, patterned free-standing films can be similarly produced.

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