Enzymatic incorporation of a new base pair into DNA and RNA extends the genetic alphabet

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A new Watson–Crick base pair, with a hydrogen bonding pattern different from that in the A·T and G·C base pairs, is incorporated into duplex DNA and RNA by DNA and RNA polymerases and expands the genetic alphabet from 4 to 6 letters. This expansion could lead to RNAs with greater diversity in functional groups and greater catalytic potential.

LIFE on Earth seems to have passed through several episodes, including one where ribonucleic acid (RNA) was the sole genetically encoded component of biological catalysis[1–5]. Evidence that RNA can act as a catalyst[6–10] supports this view and now influences research in areas as diverse as prebiotic chemistry[11] and virology[12]. Furthermore, models presuming the existence of an RNA world can now rationalize many of the metabolic details of contemporary organisms[13], details which otherwise appear arbitrarily idiosyncratic. One important conclusion from these models is that RNA catalysed many types of reactions before proteins emerged[14].

Even those who find this picture convincing recognize that proteins assumed at some point most of the catalytic roles performed earlier by RNA in the RNA world. This implies that proteins are selectively advantageous as catalysts, and the grounds for this advantage deserve exploration. One difference between the two type of macromolecules is the greater number of building blocks, and accompanying chemical functionalities, available to natural proteins compared with natural RNA molecules. Conversely, the limited functionality present on natural oligonucleotides constrains the chemist attempting to design catalytically active RNA molecules, in particular, RNA molecules that catalyse the template-directed polymerization of RNA.

Additional base pairs could relax these constraints, especially if their hydrogen-bonding patterns differed from those in the A·T and G·C base pairs, as novel hydrogen-bonding schemes would allow additional base pairs to be incorporated enzymatically at specific positions in an RNA molecule[14,15]. If functionalized, such additional bases might also allow the incorporation by transcription of functional groups directly into RNA; the remaining unfunctionalized building blocks could then control secondary structure without introducing over-functionalization and attendant nonspecific catalysis. Although this strategy for increasing the catalytic versatility of RNA has apparently never been pursued by nature, it has special appeal to chemists, who often wish to reproduce the properties of natural systems without precisely duplicating natural chemical structures.

The first problems in implementing this strategy are technological: the design, synthesis and study of new bases that can be incorporated by DNA and RNA polymerases uniquely opposite complementary bases in a template. This technology is needed as RNA molecules are, at present, best obtained by transcription of synthetic DNA using RNA polymerases[16]. Furthermore, it would be desirable if DNA polymerase could also incorporate any newly designed bases selectively in a growing DNA chain, as this would permit the amplification of oligonucleotides containing additional nucleoside 'letters'. We report here a new base pair that meets both of these requirements.

New base pairs

The geometry of the Watson–Crick base pair can accommodate at least six mutually exclusive hydrogen-bonding schemes (Fig. 1). Each is defined by the distribution of hydrogen bonds (donor or acceptor) on the purine and pyrimidine rings. Natural oligonucleotides use only two of these schemes, and one incompletely, as adenine (instead of diaminopurine) is used as the complement for uracil or thymine in natural nucleic acids.

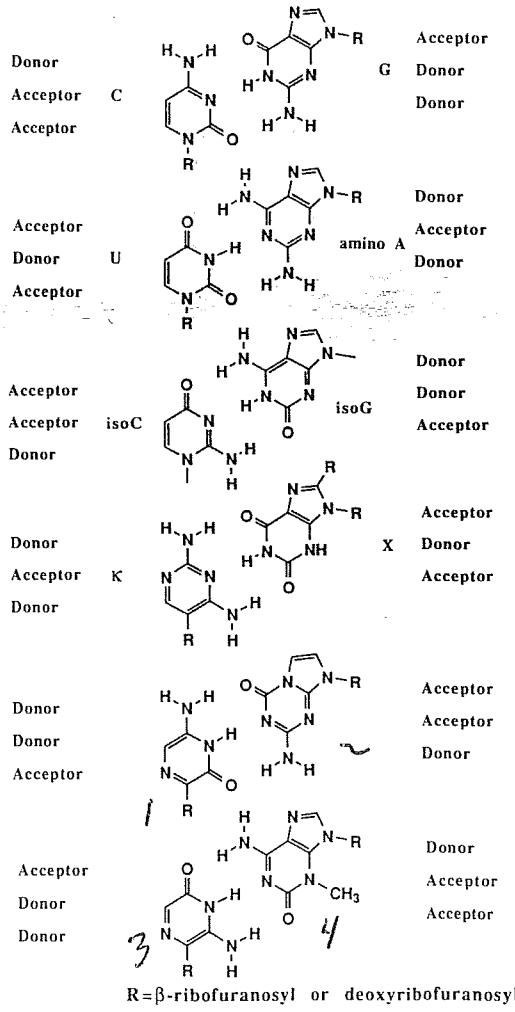


FIG. 1 Independently replicatable base pairs. Base pairs with different, and mutually exclusive, hydrogen-bonding patterns capable of forming Watson–Crick base pairs with three hydrogen bonds. The bases discussed in detail here are κ, X and an analogue of X designated π (shown in Fig. 2*b*) with the same hydrogen bonding pattern as X, but bases on a pyrazolopyrimidine ring system instead of a purine ring system (see text).

To obtain the desired hydrogen bonding, several of the 'unnatural' base pairs require a carbon–carbon bond joining the ribose ring with the heterocyclic base. Such structures are undoubtedly more difficult to prepare inder prebiotic conditions than N-glycosides, perhaps explaining their absence from the repertoire of natural nucleotides. But such molecules can be obtained by synthetic chemistry which is not subject to prebiotic constraints.

Experiments with *iso*-C and *iso*-G (Fig. 1) have shown that bases containing novel hydrogen-bonding schemes can be incorporated by DNA and RNA polymerases[15]. Chemical considerations, however, suggest that *iso*-C might hydrolyse slowly to U under conditions of DNA synthesis, and that *iso*-G might exist to some extent in a minor tautomeric form complementary to U. Both of these problems were expected to complicate the selective incorporation of the *iso*-C/*iso*-G base pair in oligonucleotides also containing A and T, and complications were indeed observed[15]. Therefore, attention was directed towards developing base pairs (Fig. 1) for which both hydrolysis and tautomeric equilibria might be less problematic.

Selecting new bases κ, X and π

The pyrimidine 3-β-D-ribofuranosyl-(2,6-diaminopyrimidine), trivially designated as κ, presents a donor–acceptor–donor hydrogen bonding pattern to a complementary strand in a duplex structure. The pyrimidine κ was chosen after much work with the analogous pyridine base, 3-β-D-ribofuranosyl-(2,6-diaminopyridine)[17]. We have been unable so far to develop the oligonucleotide chemistry of this pyridine, perhaps because of the ease with which the heterocyclic ring is oxidized. The heterocyclic ring system of κ incorporates an additional nitrogen, rendering the ring more electron deficient than the pyridine system. κ as a deoxyriboside derivative suitable for automated DNA synthesis was readily synthesized from a known precursor by the route shown in Fig. 2*a*. Crystals of the hydrochloride salt were grown from methanol, and a structure obtained by X-ray diffraction will be reported elsewhere.

TABLE 1 Melting temperatures for duplex formation

M	N	T_m [°C]	M	N	T_m [°C]
A	T	40	K	A	22
T	A	37	P	T	29
G	C	38	P	C	19
C	G	42	G	T	21
P	K	35	T	G	26
K	G	18			

The melting temperature T_m for duplexes formed with the general sequence: 5′-CAAA**M**AAAG-3′ and 3′-GTTT**N**TTTC-5′, where **M** and **N** denote bases shown in Table. The melting curves were measured using a Perkin Elmer Lambda 2 UV/VIS spectrophotometer equipped with a Digital Controller thermoelectric temperature programmer. Readings at wavelength λ = 260 nm were taken with a temperature scan rate of 0.5 °C min^{-1}. Profiles of absorbance versus temperature determined at slower heating rates or by increasing the temperature at 1 °C min^{-1} gave identical results. Duplicate measurements were taken and found to yield identical T_m values to within ±0.5 °C. The cell dimensions were 1.2 × 0.6 × 1.9 cm, with a path length of 0.1 cm. The buffer in all cases contained 1 M NaCl, 10 mM sodium phosphate and 0.1 mM EDTA in H_2O at pH 7. The concentration of each oligonucleotide was 50 μM.

Two purine analogues were chosen to complement κ. The first, xanthosine (X), is a natural base available commercially as both the nucleoside and nucleoside triphosphate. However, because of concerns that deoxyxanthosine might undergo depurination, another complementary base, 3-β-D-ribofuranosyl-(1-methyl-pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione), also known as 7-methyl oxoformycin B, and trivially designated here as π, was prepared by the route shown in Fig. 2*b*. In π, the heterocyclic base is joined to the pentose ring by a carbon–carbon bond. A crystal structure of π will also be reported elsewhere.

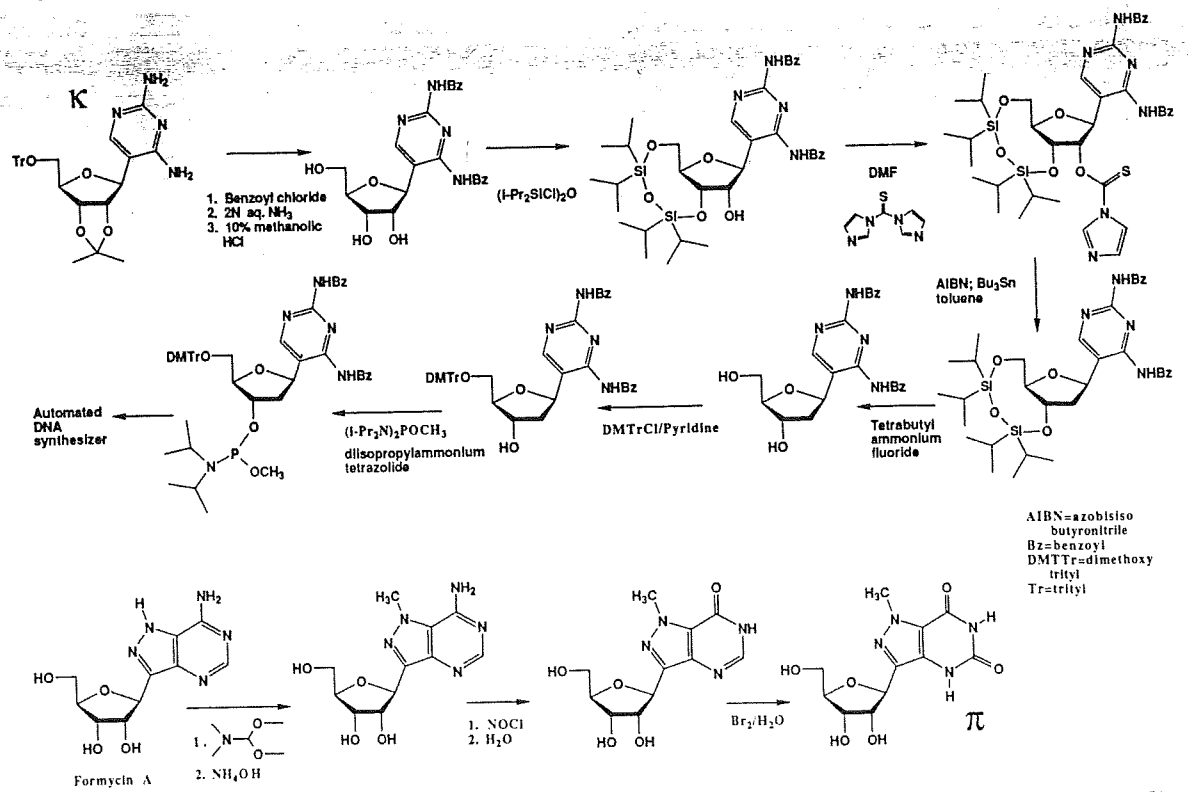


FIG. 2 Synthesis of derivatives of 3-β-D-ribofuranosyl-(2,6-diaminopyrimidine) (κ) and 3-β-D-ribofuranosyl-(1-methyl-pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione) (π). *a*, 3-(2,3-*O*-isopropylidine-5-*O*-trityl-β-D-ribofuranosyl)-(2,6-diaminopyrimidine), prepared by a six-step synthesis[24], was deoxygenated and then converted to a phosphoramidite suitable as a building block for the automated chemical synthesis of DNA (^{31}P NMR doublet at 149.08 and 149.70 p.p.m. downfield from phosphoric acid)[25]. *b*, Formycin A (Calbiochem, as monohydrate) was converted to 3-β-D-ribofuranosyl-(1-methyl-pyrazolo[4,3-d]pyrimidine-5,7(4H,6H)-dione) (π) by methylation and oxidation with nitrosyl chloride and bromine[26].

The κ–π base pair

Physical and chemical characterization of these bases was necessary to evaluate their suitability as replicatable components of a genetic alphabet. Although π exists in the crystal in the desired *anti* conformation, crystallographic studies of the hydrochloride salt of κ found the base in a *syn* conformation (data not shown), which is undesirable for the formation of a Watson–Crick base pair where both nucleoside bases must be in an *anti* conformation). Proton NMR studies were thus undertaken to determine whether κ could form a Watson–Crick base pair with π in solution.

In a solution of a derivative of κ in chloroform, a strong nuclear Overhauser enhancement (NOE) between the proton at C1′ of the ribose ring and the proton at C4 of the heterocyclic ring (Fig. 3) indicated that κ adopts the undesired *syn* conformation when alone in solution. On addition of a protected derivative of the complementary purine nucleoside π, however, this NOE largely disappeared. Furthermore, when both κ and its complement are present, the resonances assigned to the amine protons of κ shift strongly downfield, as does the resonance of π assigned to the nitrogen flanked by the carbonyl groups. These facts together show that κ and π form a standard Watson–Crick base pair in a solution of chloroform.

Stability of duplexes containing the new base pair

To determine the effect of a κ–π on the stability of a DNA duplex, it was introduced synthetically into several oligonucleotides (Applied Biosystems, Table 1). Melting studies showed that duplexes containing a κ–π base pair are only slightly less stable than duplexes containing only natural bases (Table 1). Moreover, duplexes containing the new base pair are considerably more stable than those containing mismatches involving the new bases, which in turn have melting temperatures similar to duplexes containing mismatches of natural bases. The stabilities of various mismatches are consistent with the presumed stability of 'wobble' base pairs, which should be particularly important for the GT and Aκ mismatches. These results indicated that enzymatic incorporation of a new base selectively opposite its complement in a DNA template would be possible, provided that natural DNA and RNA polymerases accepted the new bases.

Enzymatic incorporation of a new base into RNA

For enzymatic studies with T7 RNA polymerase[18], κ was complemented by the commercially available xanthosine triphosphate (XTP) instead of π, as xanthosine retains the essential hydrogen-bonding pattern of π, but lacks the methylated pyrazolo ring system that might be considered foreign by the polymerase. A promoter-template including a promoter sequence for T7 RNA polymerase (17 bases) followed by a short oligonucleotide segment (7 bases), the new base κ, 1 additional base, and a final A was synthesized, together with a complementary 18-base primer (Fig. 4). Incorporation of κ into the synthetic DNA templates was verified by digestion of samples of the

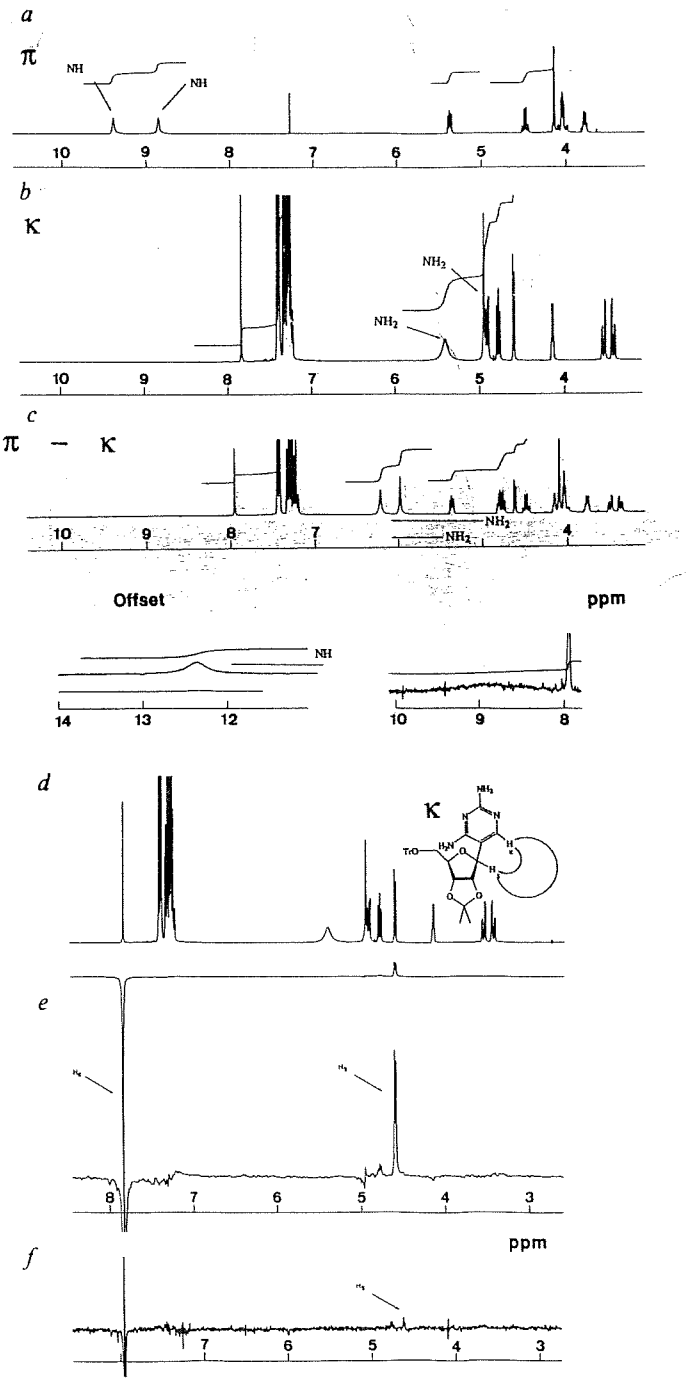
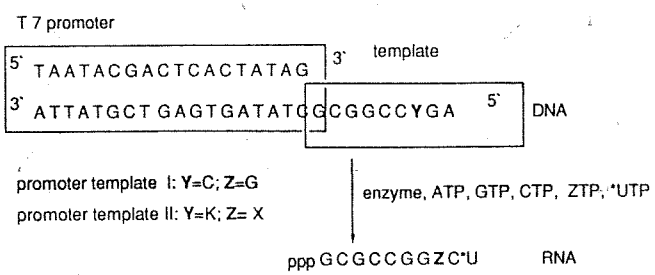


FIG. 3 NMR studies of the κ–π base pair in chloroform. The hydroxyl groups of the bases were protected to increase the solubility of the compounds in chloroform and to prevent the hydroxyl groups of the ribose from forming hydrogen bonds with protons on the base. All spectra were recorded at 24 ± 0.01 °C in chloroform (passed through basic alumina immediately before use and kept under nitrogen) on a 400 MHz Bruker NMR spectrophotometer. *a*, Spectrum of a derivative of κ (0.2 M), with resonances assigned to the exchangable hydrogens as indicated. *b*, Spectrum of the derivative of π indicated (0.2 M), with resonances assigned to the exchangable hydrogens indicated. *c*, Spectrum recorded with a 1:1 mixture of the derivatives of κ and π (0.2 M each) showing downfield shift and broadening of the signals assigned to protons involved in the formation of hydrogen bonds (note offset at bottom). In the mixture, shifts greater than 0.8 p.p.m. are observed for the signals of both of the NH_2 protons of κ and one of the amide protons of π (presumably the 6-NH) is shifted by approximately 3 p.p.m. The other amide proton of π is broadened but its chemical shift remains largely unchanged. These shifts indicate a standard Watson–Crick base pair between the two groups. *d*, Spectrum of the derivative of κ (0.2 M) in the *syn* conformation, indicating atoms expected to be close in space. *e*, Spectrum showing nuclear Overhauser enhancement (NOE) of the signal arising from the proton at C-1′ of the ribose ring of κ following irradiation at the frequency of the resonance of the signal assigned to the proton at C-6 of the pyrimidine ring. The large NOE between H-6 and H-1′ indicates that a substantial fraction of κ exists in the *syn* conformation at equilibrium, perhaps due to the formation of an intramolecular hydrogen bond between the 4-amino group and the furanose oxygen. *f*, Spectrum showing the NOE of the signal arising from the proton at C-1′ of the ribose ring following irradiation at the frequency of the resonance of the signal assigned to the proton at C-6 of the pyrimidine ring, when κ is in the presence of an equal concentration of π. In the 1:1 mixture (0.2 M of each nucleoside derivative), the NOE is significantly reduced, suggesting that the average distance between H-4 and H-1′ in the base pair is considerably farther than in κ alone. This indicates that in the base pair with π, κ adopts an *anti* conformation.

template with snake venom phosphodiesterase, hydrolysis of the phosphate from the products with bacterial alkaline phosphatase, and analysis of the resulting nucleosides by HPLC (data not shown). Control templates containing T replacing κ were also prepared by synthesis. Transcription of the primed templates could be detected most simply by the incorporation of radiolabelled UMP (from α-labelled UTP) into a product RNA molecule 10 bases long (the 'full-length product').

When synthetic template 1 was incubated with labelled UTP and various other nucleoside triphosphates, full-length products



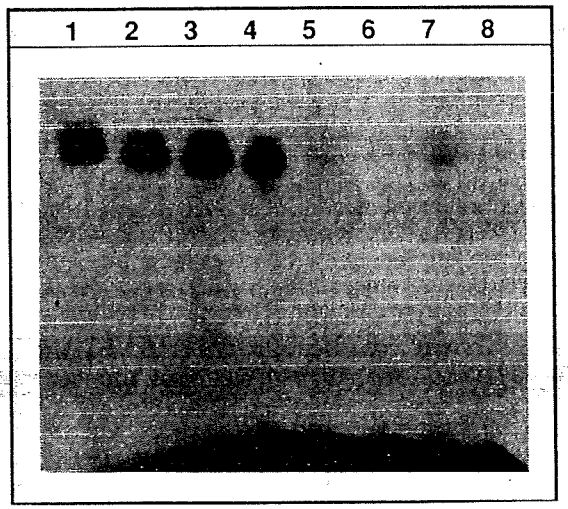


FIG. 4 Runoff transcription with T7 RNA polymerase. The procedure for using synthetic DNA templates to direct the synthesis of short RNA fragments by T7 polymerase was adopted from the literature[17]. Concentrations of single-stranded oligonucleotides were estimated by assuming an average molar extinction coefficient per base of $\varepsilon_{260} = 1 \times 10^4\ M^{-1}$. Double-stranded templates were prepared by heating a 1:1 mixture of complementary single strands in triethyl ammonium bicarbonate (10 mM, pH 7) to 75 °C for 3 min, adding SSC buffer (6 M NaCl, 0.6 M sodium citrate, pH 7, diluted 20-fold), and cooling the solutions slowly to room temperature. They were stored at −20 °C. Standard transcription reactions contained template (4.0 μg ml^{-1}), T7 RNA polymerase (Pharmacia, 140 I.U.), nucleoside triphosphates (Pharmacia, 2.5 mM each), 20 mM $MgCl_2$, spermidine (Fluka, 1 mM), dithiothreitol (Fluka, 5 mM), Tris-HCl buffer (Sigma, 40 mM, pH 8.1), bovine serum albumin (Boehringer Mannheim, 100 μg ml^{-1}), RNAse inhibitor (Boehringer Mannheim, 50 units), Triton X-100 (Sigma, 0.1 %), and radiolabelled nucleotide [α-^{32}P]UTP (Amersham, 3,000 C_i $mmol^{-1}$, 1 μCi) in a total volume of 20 μl. Following the transcription reactions (4 h, 37 °C), the mixture was heated in loading buffer containing 7 M urea and dyes (0.02% bromphenol blue, 0.02% xylene cyanol, Eastman) for 5 min at 70–75 °C, and loaded immediately on an analytical denaturing 20% polyacrylamide gel. The products were separated on a 48 cm (0.6 mm thick) vertical gel apparatus, and the bands located by autoradiography. To determine yields, the product bands were excised, extracted with Protosol (300 μl each, 4 h, 35 °C) and counted in the presence of 2 drops of acetic acid and 9 ml of scintillant with a BETAmatic Kontron Liquid Scintillation counter. Lanes are designated (promoter–template; nucleoside triphosphates present). Lane 1 (I; GTP, CTP, [α-^{32}P]UTP); lane 2 (II; GTP, CTP, [α-^{32}P]UTP, XTP); lane 3 (II; GTP, CTP, [α-^{32}P]UTP, 2 x XTP); lane 4 (II; GTP, CTP, [α-^{32}P]UTP, 5 x XTP); lane 5 (II; GTP, CTP, [α-^{32}P]UTP); lane 6 (I; CTP, [α-^{32}P]UTP); lane 7 (II; GTP, CTP, [α-^{32}P]UTP, ATP); lane 8 (II; CTP, [α-^{32}P]UTP, XTP).

were observed in the presence of XTP (Fig. 4). The efficiency of synthesis of full-length product from templates with and without κ was approximately the same, provided that the necessary complementary nucleoside triphosphates were all present in the incubation mixtures. In the absence of XTP, a significant amount of full-length product could be detected only in the presence of ATP, and this at somewhat low concentrations (~24%, measured by scintillation counting of bands cut from the gel). Such a misincorporation presumably occurs through wobble base pairing, and is not infrequent even with natural bases when incorporation experiments are run in incubation mixtures that are missing one component[19].

To determine whether misincorporation of A was diminished by competition with X, experiments were preformed with tritiated XTP (synthesized from tritiated GTP by Demijanov oxidation)[20] and γ-^{32}P-labelled GTP (which is incorporated, with the triphosphate intact, at the 5′ end of the RNA product) together in an incubation mixture varying ratios. Full-length products from an incubation containing a 1:1 molar ratio of [^{3}H]XTP and unlabelled ATP were isolated by gel electrophoresis, the bands excised, and the relative amounts of ^{3}H and ^{32}P determined by scintillation counting. After correction for the specific activities of the starting nucleotides, the misincorporation of adenosine into the product at a XTP:ATP ratio of 1:1 was reduced to ~14%. Infidelity further decreases with increasing ratios of X:A, and probably stems from errors in the action of the polymerase itself[19], rather than from the presence of minor tautomers of the bases.

Incorporation of the κ–X base pair into DNA

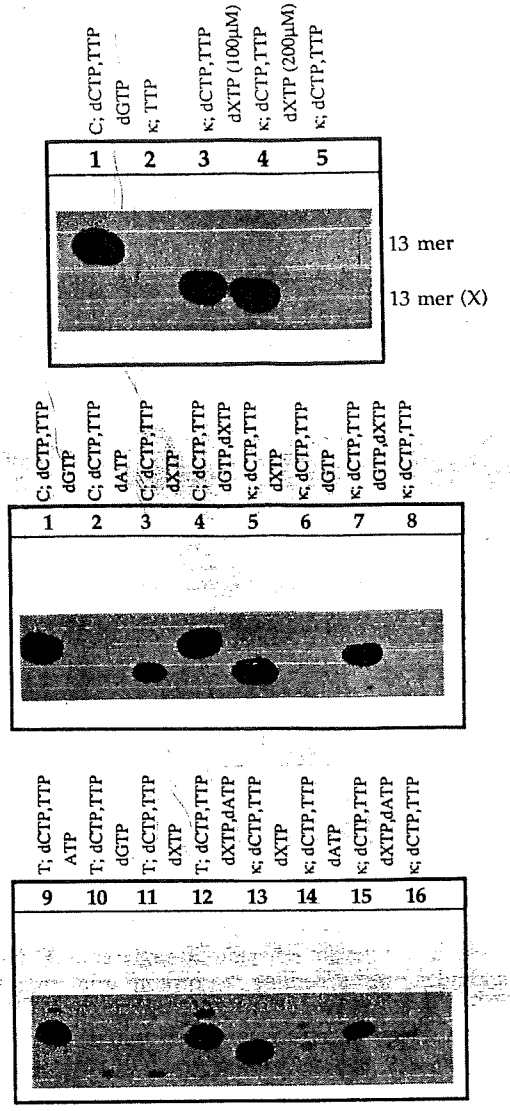
DNA polymerases frequently display higher fidelity than RNA polymerases. To assess the potential for incorporating the κ–X base pair in a replicatable system, the ability of DNA polymerase to incorporate X in a growing DNA product opposite κ in a DNA template was examined. A set of primer-templates (Fig. 5) were prepared containing either κ, C or T (the latter two serving as control templates). Incorporation of κ into the synthetic DNA templates was again verified by digestion of samples of the template with snake venom phosphodiesterase, removal of the phosphate from the products by bacterial alkaline phosphatase, and analysis of the resulting nucleosides by HPLC (data not shown). As before, the last base in the template was a unique A, permitting the detection of full length products most simply by autoradiography following the incorporation of [α^{32}P]TTP.

The synthetic primer templates were incubated with the Klenow fragment of DNA polymerase I (*Pol* 1)[21] in the presence of various nucleoside triphosphates, and the products analysed by gel electrophoresis (Fig. 5). κ in the template directed the incorporation of XTP into full-length product. On electrophoresis, the product containing X migrated faster than the analogous products containing G or A, presumably because the xanthine heterocycle carries an additional negative charge under the conditions of the electrophoresis due to its low pK_a of 5.7. Direct evidence for the incorporation of xanthosine was obtained by digestion of the product oligonucleotide, treatment with kinase and electrophoretic analysis.

To measure the relative efficiency as templates of the oligonucleotides containing different bases, product bands from electrophoresis gels were excised and their radioactivity determined by liquid scintillation counting. Templates containing κ were ~70% as efficient at directing the synthesis of full-length product (in the presence of XTP) as those containing only natural bases.

The fidelity of incorporation of X opposite κ was examined by incubating templates containing C, T and κ with purine triphosphates separately and in competition (Fig. 5). As expected, the fidelity of incorporation was considerably higher with DNA polymerase than with T7 RNA polymerase. Essentially no G or A was incorporated by the Klenow fragment of DNA polymerase opposite κ, and essentially no X was incorporated

FIG. 5 Incorporation experiments with DNA polymerase I (Klenow fragment). The standard fill-in reactions contained Tris-HCl buffer (Sigma, 50 mM, pH 7.5), $MgCl_2$ (10 mM), bovine serum albumin (Boehringer Mannheim, 0.10 μg), DTT (Fluka, 1 mM), nucleoside triphosphates (Pharmacia, 100 μM) as specified below, [α-^{32}P]TTP (Amersham, 3,000 Ci/mmol^{-1}, 1 μCi) and Klenow enzyme (Boehringer Mannheim, 1 unit) in a total volume of 20 μl (ref. 20). The mixtures were incubated at 23 °C for 15 min, and then heated in loading buffer containing 7 M urea and dyes (0.02% bromphenol blue and xylene cyanol, Eastman) for 3 min at 70–75 °C, and loaded immediately on an analytical 20% denaturing polyacrylamide gel. The products were separated on a 48 cm (0.6 mm thick) vertical gel apparatus, and the product bands located by autoradiography. To determine yields, the product bands were excised, extracted with Protosol (300 μl each, 4 h, 35 °C) and counted in the presence of 2 drops of acetic acid and 10 ml of scintillant with a BetaMatic Kontron Instrument. The template-primers used had the general formula, 5′-GATTTTGA-3′, and 3′-CTAAAACTGGNGA-5′, where for primer-template I, N=κ; for primer-template II, N=C; and for primer-template III, N=T. Lanes are designated (primer–template; nucleoside triphosphates present) Top gel: lane 1 (II; dCTP, [α-^{32}P]TTP, dGTP); lane 2 (I; [α-^{32}P]TTP); lane 3 (I; dCTP, [α-^{32}P]TTP, dXTP (100 μM)); lane 4 (I; dCTP, [α-^{32}P]TTP, dXTP (200 μM)); lane 5 (I; dCTP, [α-^{32}P]TTP). Middle gel: lane 1 (II; dCTP, [α-^{32}P]TTP, dGTP); lane 2 (II; dCTP, [α-^{32}P]TTP, dATP); lane 3 (II; dCTP, [α-^{32}P]TTP, dXTP); lane 4 (II; dCTP, [α-^{32}P]TTP, dGTP, dXTP; lane 5 (I; dCTP, [α-^{32}P]TTP, dXTP); lane 6 (I; dCTP, [α-^{32}P]TTP, dGTP); lane 7 (I; dCTP, [α-^{32}P]TTP, dGTP, dXTP); lane 8 (I; dCTP, [α-^{32}P]TTP). Bottom gel: lane 9 (III; dCTP, [α-^{32}P]TTP, dATP); lane 10 (III; dCTP, [α-^{32}P]TTP, dGTP); lane 11 (III; dCTP, [α-^{32}P]TTP, dXTP); lane 12 (III; dCTP, [α-^{32}P]TTP, dXTP, dATP); lane 13 (I; dCTP, [α-^{32}P]TTP, dXTP); lane 14 (I; dCTP, [α-^{32}P]TTP, dATP); lane 15 (I; dCTP, [α-^{32}P]TTP, dXTP, dATP); lane 16 (I; dCTP, [α-^{32}P]TTP). The middle and bottom gels are overexposed to allow weak bands (which possibly indicate infidelity) to show clearly. Oligonucleotide products containing X run faster than corresponding products containing A or G, presumably because the xanthine heterocycle (pK_a=5.7) is deprotonated under the conditions of the electrophoresis. The gel 'smiles' significantly; based on the position of the markers, the main band in lane 16 comigrates with other oligonucleotides on the gel containing xanthosine.



opposite T in the template. The only evidence of infidelity was a low level (~5%) of X misincorporated opposite C in the template when GTP was missing from the incubation mixture. This misincorporation was not observed at all when GTP and XTP were present in a 1:1 ratio.

Discussion

These studies show that although it is present in the crystal and in dilute solutions of chloroform in the *syn* conformation, κ will form a base pair with an appropriate purine nucleoside in organic solvents. Further, templates containing κ direct both T7 RNA polymerase and the Klenow fragment of DNA polymerase 1 to incorporate xanthosine as the complementary purine nucleoside into DNA and RNA products. With DNA polymerase, the incorporation of the new base pair proceeds with high fidelity. Thus, this work demonstrates the feasibility of expanding the genetic alphabet, increasing the number of letters that can be enzymatically incorporated into oligonucleotides by template-directed polymerization.

This expanded genetic alphabet should allow more diversity in the functional groups available to RNA, as synthetic routes to many functionalized bases are described in the literature, and many pyrimidines functionalized at the 5-position are accepted by polymerases[22]. Incorporation of such functionalized bases into RNA should provide RNA molecules with the potential for greatly increased catalytic power, including perhaps RNA molecules that catalyse their own replication.

The new base pair described here should also find use in studies of the structure of biologically important RNA and DNA molecules[23] and protein–nucleic acid interactions. More speculatively, the extra letters in the nucleoside alphabet might eventually be used to expand the genetic code, increasing the number of amino acids that can be incorporated translationally into proteins[24]. □

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1. Gilbert, W. *Nature* **319,** 818 (1986).
2. Orgel, L. E. *J. theor. Biol.* **123,** 127–149 (1986).
3. White, H. B. III *J. molec. Evol.* **7,** 101–104 (1976).
4. Visser, C. M. & Kellogg, R. M. *J. molec. Evol.* **11,** 171–187 (1978).
5. Rich, A. in *Horizons in Biochemistry* 103 (eds Kasha, M. & Pullmann, B.) 103–126 (Academic, New York, 1962).
6. Schram, G., Götsch, H. & Pollmann, W. *Angew. Chem.* **73,** 619 (1961).
7. Usher, D. A. & McHale, A. A. *Proc. natn. Acad. Sci. U.S.A.* **73,** 1149–1153 (1976).
8. Cech, T. R., Zaug, A. J. & Grabowski, P. J. *Cell* **27,** 487–496 (1981).
9. Guerrier-Takada, C. & Altman, S. *Science* **223,** 285–286 (1983).
10. Doudna, J. A. & Szostak, J. W. *Nature* **339,** 519–522 (1989).
11. Joyce, G. F., Schwartz, A. W., Miller, S. L. & Orgel, L. E. *Proc. natn Acad. Sci. U.S.A.* **84,** 4398–4402 (1987).
12. Weiner, A. M. & Maizels, N. *Proc. natn Acad. Sci. U.S.A.* **83,** 7383–7387 (1987).
13. Benner, S. A., Ellington, A. D. & Tauer, A. *Proc. natn Acad. Sci. U.S.A* **86,** 7054–7058 (1989).
14. Benner, S. A. *et al. Cold Spring Harb. Symp. quant. Biol.* **52,** 53–63 (1987).
15. Switzer, C. Y. Moroney, S. E. & Benner, S. A. *J. Am. Chem. Soc.* **111,** 8322–8323 (1989).
16. Uhlenbeck, O. C. *Nature* **328,** 596–600 (1987).
17. Piccirilli, J. A. thesis, Harvard Univ. (1989).
18. Milligan, J. F., Groebe, D. R., Witherell, G. W. & Uhlenbeck, O. C. *Nucleic Acids Res.* **15,** 8783–8798 (1987).
19. Coleman, J. E., Martin, C. T. & Muller, D. K. *Biochemistry* **27,** 3966–3974 (1988).
20. Roy, K. B. & Miles, H. T. *Nucleosides Nucleotides* **2,** 231–242 (1983).
21. Cobianchi, F. & Wilson, S. H. *Meth. Enzym.* **152,** 94–110 (1987).
22. Leary, J. L., Brigati, D. J. & Ward, D. C. *Proc. natn Acad. Sci. U.S.A.* **80,** 4045–4049 (1983).
23. Chen, T. R., Churchill, M. E. A. Tullius, T. D. Kallenbach, N. R. & Seemann, N. C. *Biochemistry* **27,** 6032–6038 (1988).
24. Noren, C. J., Anthony-Cahill, S. J., Griffith, M. C. & Schultz, P. G. *Science* **244,** 182–188 (1989).

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