mature T cells with helper phenotype, they may be of practical value for production of various lymphokines.

M. POPOVIC
P. S. SARIN
M. ROBERT-GURROFF
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
V. S. KALYANARAMAN
Department of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland 20895
D. MANN
Division of Cancer Biology and Diagnosis, National Cancer Institute
J. MINOWADA
Department of Immunology, Roswell Park Memorial Institute, Buffalo, New York 14263
R. C. GALLO*
Laboratory of Tumor Cell Biology, National Cancer Institute

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* To whom requests for reprints should be addressed.

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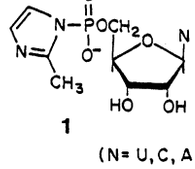
A Nonenzymatic RNA Polymerase Model

Abstract. *Polynucleotide templates containing C (cytidine) as the major component facilitate the synthesis of oligonucleotides from mixtures of the activated mononucleotide derivatives (as indicated by structure* **1** *in the text). A nucleotide is incorporated into oligomeric products if and only if its complement is present in the template. The reaction has a high fidelity and produces products with mean chain lengths of six to ten nucleotides. Bases other than guanosine are incorporated within oligomers or at their 3′ termini, but rarely at their 5′ termini.*

The activated nucleotide 2-MeImpG (*1*) undergoes a template-directed condensation on poly(C) to give predominantly 3′,5′-linked oligo(G)'s in excellent yield (*2*, *3*). Synthesis proceeds in the 5′ to 3′ direction. The mean chain length of the product can be as high as 15, and oligomers with chain length substantially in excess of 30 can be detected. The fidelity of the reaction is high—U, C, and A are discriminated against by factors of 100 to 500, depending on the conditions of the reaction. In collaboration with Frazier and Miles (*4*), we showed that this reaction occurs in a Watson-Crick double helix and yields double-stranded poly(C) · oligo(G) melting above 100°C as the initial product.

The corresponding reactions involving other monomer-homopolymer double helices cannot be carried out for various reasons. Poly(U) forms triple helices rather than double helices with monomeric adenosine derivatives, while poly(G) forms a very stable self-structure that prevents G-C interaction (*5*). Poly(A) does not interact with U derivatives strongly enough to overcome the poor U-U stacking and so does not form helical complexes poly(A) · U of any kind (*5*). It is possible to circumvent these difficulties by using copolymers of C with one or more additional bases as templates. A poly(CU) template cannot form triple-stranded segments if C is present in sufficient amount. Similarly, poly(CG) copolymers cannot form long-interchain G-C self-structure, although they may contain short-intrachain self-structure based on C · G pairing if the G content is high enough. Finally, poly(CA) does interact with U derivatives, since U can stack next to G's even though it will not stack between other U's. In this report we present our findings on the efficiency and fidelity of incorporation of complementary bases on random copolymer templates containing C as a major constituent.



1

(N = U, C, A or G)

All reactions were carried out at 0°C and *p*H 8 and were analyzed after 7 days and again after 21 days (*6*). The reaction mixtures contained 1.2*M* NaCl, 0.22*M* $MgCl_2$, 0.4*M* 2,6-lutidine · HCl buffer, 0.05*M* 2-MeImpU, 0.05*M* 2-MeImpC, 0.05*M* 2-MeImpA, 0.05*M* 2-MeImpG, and an amount of template equivalent to a 0.01*M* solution of total mononucleotides. Tubes were prepared in sets of four, and a different ^{14}C-labeled substrate (0.25 μCi) was added to each of them; for example, ^{14}C-labeled 2-MeImpG was added to the first tube of each set. The final volume in each tube was 20 μl.

We determined the percentage of total radioactive material incorporated into longer oligomers by carrying out paper chromatography in a mixture of *n*-propanol, ammonia, and water (55:10:35) and measuring, with a scintillation counter, the radioactivity remaining at the origin of the paper (*6*) (Table 1). The yield expected for a completely efficient incorporation of a nucleotide *N* is 0.2 *p*, where *p* is the molecular proportion of *N* in the template; for example, 5 percent incorporation of U on a poly(C_3A) template. We have also calculated the efficiency with which a nucleotide in the template directs the incorporation of its complement into oligomeric products long enough to remain at the origin of our chromatograms. These derived data are also included in Table 1.

To determine the way in which a nucleotide is distributed between 5′-terminal, internal, and 3′-terminal positions, we degraded selected oligomeric products with alkali and analyzed them by electrophoresis (*6*). The results of this analysis are presented in Table 2, together with estimates of the mean chain lengths derived from them.

In our chromatographic system, oligomers four or more units long and rich in G. and other oligomers more than five units long, remain very close to the origin of the chromatograms. The first row

of Table 1 confirms that negligible amounts of material as long as this are formed from a mixture of activated nucleotides in the absence of a template.

The data in the second row confirm that G is incorporated in almost quantitative yield on poly(C) and that the fidelity of this reaction is high. The next three rows confirm that, under the conditions of our experiment, the other homopolymers are not efficient templates. The very small amounts of material incorporated on these homopolymers are possibly attached to the ends of the template strands. Previously reported experiments have shown that poly(U) does facilitate the formation of oligomers of A up to the octamer from ImpA, but the reaction conditions were more favorable (*7*). We can, in fact, detect short oligomers of A, up to at least the tetramer on our paper chromatograms of reaction mixtures containing poly(U), while no comparable amounts of such oligomers are formed on other homopolymers or in the absence of a template.

The data presented in the remainder of Table 1 substantially extend the results of Ninio and Orgel (*8*). They show that random copolymers containing C as the major component facilitate the incorporation of substantial amounts of a base other than G into oligomeric products if and only if the complementary base is present in the template. In this restricted sense, our reaction models an RNA polymerase. We do not know how far the resemblance will extend.

The fidelity of synthesis on random copolymers cannot be determined unambiguously from incorporation data. First, it is not possible to determine whether a base complementary to a component of the template has been incorporated correctly on its complement or erroneously on some other base. This is unfortunate, since the erroneous incorporation of small amounts of G on U by wobble-pairing could never be detected on a polymer rich in C. The absence of U incorporation on poly(CG) templates, however, suggests that wobble-pairing may not be an important source of misincorporation. Second, it is not possible to partition misincorporation between different components of the template. Thus, C misincorporated on a poly(CUA) template could have paired with C, U, or A (or could have attached to chain termini independently of base pairing).

In view of these ambiguities, we can draw only general conclusions from the data in Table 1. The main result is clear—the fidelities of all the reactions, insofar as they can be determined from the incorporation data, are high. The extent of misincorporation of C and A on mixed templates is not significantly different from the extent of misincorporation on poly(C). The misincorporation of U on poly(CU) may be greater than on poly(C), but if it is the effect is very small. Considering the simplicity of the system, the overall fidelity is surprisingly high.

The final proof that some mixed template sequences can be copied accurately must come from data obtained with oligonucleotide templates of known sequence. In the meantime, the incorporation data in Table 1 make it virtually

Table 1. Efficiency of incorporation of 2-MeImpU, 2-MeImpC, 2-MeImpA, and 2-MeImpG on polynucleotide templates. The numbers that are not underlined represent the fraction of the total radioactivity remaining at the origin of the paper chromatograms. Underlined numbers are efficiencies, defined as the ratio of the amount of base incorporated into products to the amount of its complement in the template, expressed as a percentage. To obtain these numbers the corrected incorporation of U, C, or A on a copolymer template was estimated by subtraction of the average incorporation of the base on poly(U), poly(C), poly(A), and poly(G) templates, from the observed incorporation on the relevant copolymer. In the case of G, we subtracted the average incorporation on poly(U), poly(A), and poly(G). The corrected amount of the base incorporated was then compared with the amount of its complement in the template, to obtain the efficiency. Yields shown in parentheses are uncertain because the amount of incorporation on the template is comparable to that in some of the controls.

Compositions: (a) $C_4(G_{0.42}U_{0.42}A_{0.15})$; (b) $C_2(G_{0.49}U_{0.30}A_{0.21})$; (c) $C_{7.4}(U_{0.67}A_{0.33})$; (d) $C_{3.6}(U_{0.69}A_{0.31})$; (e) $C_{2.8}(U_{0.57}A_{0.43})$; (f) $C_2(U_{0.6}A_{0.4})$; and (g) $C_{0.9}(U_{0.55}A_{0.45})$.

Template	2-MeImpN							
	G		C		A		U	
	7d	21d	7d	21d	7d	21d	7d	21d
-	0.08	0.21	0.15	0.14	0.03	0.03	0.03	0.03
C	19.2 96	19.4 97	0.18	0.25	0.10	0.15	0.08	0.13
G	0.15	0.23	0.15	0.14	0.03	0.03	0.03	0.03
U	0.07	0.50	0.13	0.23	0.09	0.11	0.03	0.09
A	0.07	0.18	0.13	0.16	0.03	0.14	0.02	0.04
8:1	15.6 87	15.6 85	1.74 72	1.86 75	0.08	0.17	0.09	0.14
CG 5.4:1	13.2 78	16.8 97	2.32 69	2.51 73	0.09	0.15	0.11	0.17
1.9:1	0.34 (2)	1.64 11	0.20 (0.7)	0.37 (2.4)	0.04	0.08	0.03	0.05
11.3:1	19.1 104	19.9 107	0.18	0.24	0.95 54	1.06 59	0.11	0.16
CU 6.9:1	17.2 98	17.1 96	0.16	0.22	1.44 55	1.52 56	0.11	0.17
2.9:1	14.5 97	14.1 96	0.18	0.22	2.58 49	2.76 53	0.14	0.20
1.3:1	9.41 83	10.4 88	0.18	0.22	2.29 26	2.94 32	0.13	0.21
9.5:1	17.3 95	17.9 97	0.22	0.16	0.11	0.19	1.18 61	1.31 67
5.1:1	15.0 90	16.2 96	0.25	0.36	0.13	0.22	1.57 48	1.79 53
CA 2.9:1	9.46 63	10.8 70	0.21	0.31	0.10	0.18	1.07 21	1.42 27
1.9:1	7.07 53	8.46 62	0.16	0.28	0.10	0.18	0.98 14	1.40 19
0.89:1	1.28 13	2.50 24	0.14	0.20	0.04	0.11	0.18 (1.6)	0.33 (2.9)
CGUA[a]	13.4 83	13.6 83	0.97 51	1.17 60	0.75 42	0.93 51	0.46 72	0.62 95
CGUA[b]	5.47 40	9.16 66	0.51 12	0.94 25	0.30 12	0.68 30	0.24 15	0.49 32
CUA[c]	16.7 94	17.1 95	0.19	0.26	0.82 48	0.88 50	0.51 60	0.60 68
CUA[d]	14.5 92	14.5 91	0.19	0.25	1.23 39	1.30 40	0.64 40	0.80 50
CUA[e]	11.8 80	12.6 84	0.19	0.25	1.38 44	1.49 47	0.59 25	0.77 31
CUA[f]	6.60 49	7.60 55	0.20	0.28	1.35 32	1.68 39	0.47 17	0.69 24
CUA[g]	1.55 16	5.28 54	0.13	0.25	0.30 (4.0)	0.79 13	0.07 (0.7)	0.28 (5.0)

certain that limited regions of each template are copied accurately. They do not establish that extended processive synthesis of the kind that is the rule with DNA and RNA polymerase occurs. However, they strongly suggest that a primitive transcription of informational macromolecules has been achieved for the first time in a nonenzymatic system.

The mean chain lengths of the products of all of these reactions lie in the range 6 to 10 (Table 2). In every case, a nucleotide other than G occupies internal positions in oligomeric products 60 to 80 percent of the time, and occurs at the 3′-terminus 20 to 40 percent of the time. Bases other than G rarely occur at the 5′-terminus. This suggests, but does not prove, that oligomer synthesis on random copolymers, like the synthesis of oligo(G)'s on poly(C) (*9*), is initiated by the synthesis of an ImpGpG dimer, which then extends in the 5′→3′ direction by the addition of activated monomers. It has been shown (*10*) that in the reactions on each of the random poly(UC) templates, G and A are distributed in more or less constant ratio among oligomers from 5 to more than 20 nucleotides in length.

It has not proved possible to determine accurately the proportion of 2′,5′ and 3′,5′ linkages formed when bases other than G are incorporated into oligomers. Our preliminary results indicate that 3′,5′ linkages are substantially in excess of 50 percent whenever U, C, or A is incorporated at the 3′-terminus of a chain. When U, C, or A is incorporated internally, we find that the sequence $pG^{3'}p^{5'}X^{3'}p^{5'}G$ is present in substantially more than 40 percent of the product, suggesting again that 3′,5′ linkages are present substantially more than 60 percent of the time. We will need to carry out experiments with much higher levels of radioactivity in order to obtain more accurate data on the regiospecificity of these reactions.

On templates rich in C there is little further change in the amounts of monomers incorporated into oligomers after 7 days (Table 1). However, on templates containing substantial amounts of bases other than C, incorporation increases significantly between 7 and 21 days. Further experiments are needed to determine whether the slower reaction rate on the latter templates is due to the difficulty of forming ImpGpG initiators or due to the slowness of propagation past sequences of "non-C" bases.

Much further experimental work is needed to provide the basis for a detailed description of oligomer synthesis on heteropolymer templates. The following discussion of the template properties characteristic of individual copolymers is therefore tentative.

Poly(CA) templates should provide the simplest picture since they cannot form internal self-structures or wobble-pairs with the monomers. The efficiency of incorporation of both G and U on poly(CA) templates falls steadily as the A content of the template increases. This trend is to be anticipated since the weakness of the stacking forces between U and other bases will lower the stability of the reactive template-oligomer-monomer complex. However, the extent of incorporation of U is surprising, since efforts to incorporate U or T on A-containing templates in other systems have failed (*8*).

We believe that the most likely explanation of the unexpectedly high G:U ratio in the products is "looping-out." It has been demonstrated (*11*) that when a copolymer such as poly(A_8U) is mixed with poly(U), an organized helix is formed in which U pairs with A almost exactly as in a homopolymer poly(A), poly(U) structure, but with intervening nonpairing U bases looped out. Looping-out would certainly occur with poly(CA) if mixed with poly(G), or with long enough oligo(G)'s.

In our system, G is incorporated into oligomers more rapidly than U, because G stacks better than U and occupies the template more effectively. Once long enough oligo(G)'s are formed, they are liable to migrate to new positions, even if a loop-out is involved, since the free-energy of looping-out is not excessive in relation to the thermal energy. Looping out of this kind will slow down the incorporation of U, and facilitate extension of G sequences beyond the length of the runs of C present in the template. We have evidence for unexpectedly long runs of G in our products. At present we are unable to suggest any other equally plausible explanation of the composition of the oligomeric products.

Poly(CU) templates support fairly efficient synthesis over a wide range of polymer compositions. The efficiency of incorporation of G is close to 100 percent for polymers with ratios of C to U from 11.3 to 2.9. The incorporation of A falls only slightly, from 63 to 54 percent over the same range of composition. In this case we can suggest two mechanisms that lead to an incorporation of A which is substantially less than the incorporation of G. Wobble-pairing between G and U could be important if U's in the template are masked by G's in the product. Looping-out is also a possible complication with this as with all other mixed templates. The efficiency of incorporation of A is substantially lower on the 1.3:1 template than on templates with a greater excess of C. Presumably, this template contains substantial runs of U, on which synthesis is relatively slow or inefficient.

The reaction on random poly(CG)

Table 2. Mean chain lengths and positions of incorporation in mixed template reactions. Yields of pNp, Np, and N correspond to the nucleoside N in 5′-terminal, internal, and 3′-terminal positions, respectively. The sums of these yields, weighted by the nucleotide abundances, are given as pXp, Xp, and X, respectively.

Compositions: (a) $C_4(G_{0.42}U_{0.42}A_{0.15})$; (b) $C_2(G_{0.49}U_{0.30}A_{0.21})$; and (c) $C_{0.9}(U_{0.55}A_{0.45})$.

Template		Time (day)	pGp:Gp:G	pCp:Cp:C	pAp:Ap:A	pUp:Up:U	pXp:Xp:X	Mean Chain Length
CG	5.4:1	7	17:70:13	4:72:25			15:70:15	6.7
	1.9:1	21	11:74:15	7:61:32			10:73:17	6.3
CU	6.9:1	7	12:79:9		1:70:29		11:78:11	9.1
	2.9:1	7	14:79:8		2:77:20		12:78:10	9.8
	1.3:1	21	10:83:8		2:80:18		8:82:10	10.2
CA	5.1:1	7	12:78:10			3:57:41	11.76:13	7.8
	2.9:1	7	13:76:11			2:65:33	13:76:11	8.9
	1.9:1	7	18:70:12			4:66:30	17:69:14	6.9
	0.89:1	21	13:73:14			4:80:16	12:74:14	7.3
CGUA[a]		21	9:84:7	3:71:26	4:71:25	4:58:29	8:81:11	9.3
CGUA[b]		21	13:79:8	4:71:25	9:67:24	3:63:34	12:76:12	8.3
CUAC[c]		21	12:77:11		5:79:16	10:72:19	11:79:10	9.9

templates leads to an efficient incorporation of both G and C for a 5.4:1 template, but a very low efficiency for a 1.9:1 template. This reduced efficiency is probably due to the extensive and stable self-structure of poly(CG) templates containing a substantial proportion of G.

The few experiments with more complex templates indicate that a wide variety of behaviors is to be anticipated. It is encouraging that only templates containing all four bases facilitate the incorporation of all four bases into product with reasonable efficiency.

We anticipate that even relatively short oligonucleotide sequences will display an enormous diversity in their nonenzymatic template chemistry. Templates will differ in the efficiencies with which they initiate, the rates at which they propagate, the probabilities that they terminate prematurely, and so on. This diversity corresponds, at the level of molecular structure, to the phenotypic differences in fecundity that distinguish organisms, and must make a substantial contribution to the "variation" on which natural selection at the level of molecular replication acts. It seems possible that an extension of our scheme, or of a scheme similar to it, might make it possible to study natural selection in a nonenzymatic system.

The relevance of our results to discussion of the origins of life is necessarily indirect, since it is unlikely that 2-methylimidazole was abundant on the primitive Earth. Nonetheless, the demonstration of a relatively simple reaction in which preformed copolymer templates containing all four of the natural bases direct the synthesis of their complements is significant. It makes it more plausible that the replication of polynucleotides was important at a very early stage in the evolution of life.

T. INOUE
L. E. ORGEL
Salk Institute for Biological Studies, San Diego, California 92138

References and Notes

1. Abbreviations: A, adenosine; C, cytidine; G, guanosine; U, uridine; pN(N is A, C, G, or U), nucleoside 5′-phosphate; pNp, 5′-phosphonucleoside 2′(3′)-phosphate; 2-MeImpN, (nucleoside 5′-phosphor)-2-methylimidazolide.
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Gene Reactivation in Erythrocytes: Nuclear Transplantation in Oocytes and Eggs of *Rana*

Abstract. *Adult erythrocyte nuclei of* Rana, *transplanted and incubated in the cytoplasm of maturing oocytes, direct matured oocytes to form swimming tadpoles. These results demonstrate that nuclei of noncycling and terminally differentiated erythrocytes contain the genes to specify tadpole development, and conditioning these nuclei in the cytoplasm of oocytes leads to a widespread reactivation of dormant genes.*

It is now well established that cell specialization is achieved by genetic control. However, it is not known whether the phenotypes of specialized cells are controlled mainly by irreversible alterations in the DNA or by highly stabilized changes in the DNA or chromatin proteins that are potentially reversible. The most rigorous test of this question has been nuclear transplantation in amphibians. Young embryonic nuclei transplanted into enucleated eggs promote the development of the eggs into normal larvae and sexually mature frogs, thus demonstrating genetic totipotency (*1, 2*). Adult nuclei from specialized somatic cell types [lymphocytes (*3*), skin (*4*), and erythroblasts (*5*)] and from germ cells [spermatogonia (*6*)] direct development only to early larval stages and therefore exhibit genetic pluripotency.

Since adult germ cell nuclei by definition are genetically totipotent, but fail to promote normal development of enucleated eggs (*6*), the components in egg cytoplasm apparently have not met the requirements of nuclei from specialized cell types. However, the cytoplasm of oocytes does contain factors capable of transforming oocyte and embryonic nuclei into functional pronuclei (*7*). Therefore, we suggested earlier that these factors might induce nuclei from specialized cells to reveal enhanced genetic potential (*2, 7*).

We now report on the developmental potential of nuclei from noncycling and terminally differentiated adult erythrocytes. Whereas stimulated lymphocytes (*8*), cultured skin cells (*9*), and erythroblasts (*10, 11*) are synthesizing RNA and proteins, erythrocytes are extensively dormant in this respect (*10, 11*). We show that erythrocyte nuclei, after sequential exposure to the cytoplasm of oocytes and eggs, direct the formation of swimming tadpoles. If, however, they are injected directly into eggs, development does not proceed beyond the early gastrula stage. These results demonstrate that (i) adult erythrocyte nuclei contain the genes to specify tadpole development and (ii) conditioning erythrocyte nuclei in the cytoplasm of oocytes leads to a widespread reactivation of dormant genes.

Blood, obtained by cardiac puncture of adult *Rana pipiens* (four females and seven males), was collected in heparinized tubes and centrifuged at 500*g* for 15 minutes. The supernatant and buffy coat were removed, and the erythrocyte pellet was suspended in Ringer solution. Blood smears prepared from these suspensions contained 99.95 percent erythrocytes (range, 99.8 to 100 percent; 1000 cells per frog) (Fig. 1A). The rarely occurring contaminating white cells were identified as nonhemoglobin cells with the Leitz microscope (magnification, ×100) at the time of nuclear transfer. Therefore, only erythrocytes were used for donor cells.

Three to eight cells suspended in Ringer solution were aspirated into a glass micropipette (inner diameter, 25 μm), expelled into distilled water, and immediately aspirated back into the pipette, where the cell membranes ruptured. The nuclei were then injected near the equator into oocytes in the stage of first meiotic metaphase (*7*). Approximately 24 hours later, when the oocytes completed maturation in vitro (18°C), they were activated by being pricked with a glass microneedle and the second black dot, indicative of the egg nucleus at second meiotic metaphase, was removed microsurgically. In another series of experiments, erythrocyte nuclei were injected directly into enucleated eggs (*12*). The type of development from the two series of hosts was compared.

Of the eggs injected with erythrocyte nuclei, 7 percent formed partially cleaved blastulas (Table 1). Most of