plasms observed in certain persons (*17*). As more oncogenes are isolated and characterized, it may be possible to establish screening procedures to assess individuals at risk for various neoplastic diseases.

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References and Notes

1. C. Shih, B.-Z. Shilo, M. P. Goldfarb, A. Dannenberg, R. A. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5714 (1979); J. G. Krontiris and G. M. Cooper, *ibid.* **78**, 1181 (1981); C. Shih, L. C. Padhy, M. Murray, R. A. Weinberg, *Nature (London)* **290**, 261 (1981); M. J. Murray, B.-Z. Shilo, C. Shih, D. Cowing, H. W. Hsu, R. A. Weinberg, *Cell* **25**, 355 (1981); C. J. Marshall, A. Hall, R. A. Weiss, *Nature (London)* **299**, 171 (1982); S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, S. A. Aaronson, M. Barbacid, *ibid.*, in press; G. M. Cooper, *Science* **217**, 801 (1982); R. A. Weinberg, *Cell* **30**, 3 (1982); M. Perucho, M. Goldfarb, K. Shimizu, C. Lama, J. Fogh, M. Wigler, *ibid.* **27**, 467 (1981).
2. M. A. Lane, A. Sainten, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5185 (1981).
3. S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, K. C. Robbins, M. Barbacid, *ibid.* **79**, 2845 (1982).
4. M. Goldfarb, C. Shimizu, M. Perucho, M. Wigler, *Nature (London)* **296**, 404 (1982); C. Shih and R. A. Weinberg, *Cell* **29**, 161 (1982).
5. C. J. Der, J. G. Krontiris, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3637 (1982); L. F. Parada, C. J. Tabin, C. Shih, R. A. Weinberg, *Nature (London)* **297**, 474 (1982); E. Santos, S. R. Tronick, S. A. Aaronson, S. Pulciani, M. Barbacid, *ibid.* **298**, 343 (1982); E. H. Chang, M. A. Gonda, R. W. Ellis, E. M. Scolnick, D. R. Lowy, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4848 (1982).
6. C. J. Tabin, S. M. Bradley, C. I. Borgmann, R. A. Weinberg, A. G. Papageorge, E. M. Scolnick, R. Dhar, D. R. Lowy, E. H. Chang, *Nature (London)* **300**, 143 (1982); E. P. Reddy, R. K. Reynolds, E. Santos, M. Barbacid, *ibid.*, p. 149; E. Taparowsky, Y. Suard, O. Fasano, K. Shimizu, M. Goldfarb, M. Wigler, *ibid.*, p. 762.
7. R. W. Ellis *et al.*, *ibid.* **292**, 506 (1981); R. W. Ellis, D. R. Lowy, E. M. Scolnick, in *Cell-Derived Oncogenes, Advances in Viral Oncology*, G. Klein, Ed. (Raven, New York, in press), vol. 1.
8. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
9. M. McClelland, *Nucleic Acids Res.* **9**, 5859 (1981).
10. T. W. Sneider, *ibid.* **8**, 3829 (1980); L. H. T. Van Der Ploeg and R. A. Flavell, *Cell* **19**, 947 (1980).
11. M. B. Mann and H. O. Smith, *Nucleic Acids Res.* **4**, 4211 (1977); H. O. Smith, *Science* **205**, 455 (1979).
12. R. A. Makula and R. B. Meager, *Nucleic Acids Res.* **8**, 3125 (1980); V. Cizewski and D. Kuebbing, unpublished observations.
13. G. Wilson, J. Schildkraut, L. Greenough, unpublished observations.
14. G. M. Cooper and P. Neiman, *Nature (London)* **287**, 656 (1980).
15. W. S. Hayward, B. Neel, S. M. Astrin, *ibid.* **290**, 475 (1981).
16. B. Ozanne, T. Wheeler, J. Zack, G. Smith, B. Dale, *ibid.* **299**, 744 (1982).
17. B. S. Schoenberg, in *Persons at High Risk of Cancer*, J. F. Fraumeni, Jr., Ed. (Academic Press, New York, 1975), p. 103.
18. N. Blin and D. W. Stafford, *Nucleic Acids Res.* **3**, 2303 (1976).
19. B. Vogelstein and D. Gillespie, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 615 (1979).
20. P. W. Rigby, B. M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
21. We thank R. Adamson, A. B. Rabson, A. S. Rabson, M. Israel, D. Cosman, M. Kessel, M. König, L. Laimins, G. McKenna, R. Pozzatti, N. Rosenthal, A. Kraft, and F. Worsham for providing us with valuable materials and helpful discussions during the course of these experiments. We also thank M. Priest for preparation of the manuscript.

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15 November 1982; revised 27 December 1982

Isolation and Transmission of Human Retrovirus (Human T-Cell Leukemia Virus)

Abstract. *Nine new isolates of human T-cell leukemia-lymphoma virus (HTLV) were obtained from cells of seven patients with malignancies of mature T cells and from two clinically normal relatives of a T-cell leukemia patient. These people were from the United States, Israel, the West Indies, and Japan. The virus was detected in the fresh T cells and was isolated from the established T-cell lines. Each isolate is closely related to the first HTLV isolate, and all the new HTLV isolates were transmitted into normal human T cells obtained from the umbilical cord blood of newborns.*

Human T-cell leukemia-lymphoma virus (HTLV) is a type-C retrovirus that has been isolated from cells of adult patients in the United States with leukemias and lymphomas of mature T cells (*1–4*). This virus was subsequently isolated from adult T-cell leukemia (ATL) patients in Japan (*5, 6*). In this report we describe the isolation of nine new HTLV isolates from people in different parts of the world, show transmission of each of the isolates into human cord blood, and show that HTLV can be isolated not only from patients with aggressive adult T-cell leukemia but also from cases of less aggressive peripheral lymphomas and even from clinically "normal" individuals with abnormal T cells.

The new HTLV-positive T-cell lines were established from four patients in the United States, one in Israel, one in the West Indies, and three members of a family from northwest Japan (Table 1). The last three included a male patient (S.K.) with T-cell leukemia and his parents. His father (H.K.) and mother (T.K.) are clinically healthy, but the mother has abnormal lymphocytes with convoluted nuclei characteristic of leukemic T cells from patients with ATL (*7*). All the lines were derived from the peripheral blood or bone marrow of the patients by using T-cell growth factor (TCGF) as described (*8, 9*). The cells showed a decreased requirement for exogenous TCGF and potential for indefinite growth in vitro. Over 80 percent of the cells were positive for TCGF receptors (reactive with antibody prepared against the activated T cells, anti-TAC) (*10*). All T-cell lines formed rosettes with sheep erythrocytes (E-rosette positive); and they were negative for surface immunoglobulins (IgA, IgG, and IgM), Epstein Barr virus nuclear antigen (EBNA), and terminal deoxynucleotidyl transferase (TdT). In addition, the cell lines reacted with most pan-T monoclonal antibodies (up to 100 percent). No reactions were observed with monoclonal antibodies against B cells (BA-1) and thymus (OKT-6 and NAI/34) T cells. Thus, studies with both conventional and monoclonal antibody T-cell markers indicate that the HTLV-positive cell lines represent mature T cells.

The surface phenotype of these HTLV-producing cell lines was further characterized by using the monoclonal antibodies OKT-4, Leu-3A, OKT-8, and Leu-2A. Five of the seven lines from patients with leukemia consistently exhibited only the "helper-inducer" phenotype (OKT-4$^+$, Leu-3A$^+$) (Table 1). About 20 to 30 percent of the cells of the two remaining cell lines, from P.L. and S.K., also reacted with monoclonal antibodies supposedly typing a "suppressor-cytotoxic phenotype" (OKT-8$^+$, Leu-2A$^+$). Cell lines from H.K. and T.K., the clinically healthy parents of patient S.K., reacted either only with OKT-8 and Leu-2A (H.K.) or, in the case of T.K., exhibited a "double" phenotype (OKT-8$^+$, Leu-2A$^+$, OKT-4$^+$, Leu-3A$^+$). Thus, the data support observations that primary tumor cells of HTLV-positive malignancies are mature T cells with the OKT-4, Leu-3A subtype (*11*). A high percentage of the cells (69 to 92 percent) are TCGF receptor positive as estimated by the direct response of these cells to purified human TCGF (*12*) and by their reactivity with a monoclonal antibody to TCGF receptor (anti-TAC) (*13*). All cell lines are diploid, possess distinct histocompatibility antigen (HLA) profiles, and match the karyotype of the primary donor cells.

The cell lines were analyzed for the presence of HTLV by competition radioimmunoprecipitation assay (RIPA)

for the major core protein p24 (*14*), by an indirect immunofluorescence assay (IFA) with highly specific monoclonal antibody for p19 (*15*), and by reverse transcriptase (RT) activity in culture fluids (*16*). HTLV was fully expressed in all established cell lines, and type-C virus particles were detected by electron microscopy, but there was substantial variation in the amounts. For instance, the T-cell line from the mother (T.K.) of patient S.K. had the highest virus titer, whereas the cell line from patient M.J. had relatively low levels of p24 and RT. However, HTLV production fluctuated in the same cell line during cultivation in vitro. All the cell lines contained p19 positive cells (38 to 85 percent of the total cell population) (Table 1).

Serological-epidemiological and nucleic acid hybridization studies have demonstrated that HTLV is exogenous. Possible HTLV transmission was reported when the virus was isolated from cord blood used as a feeder layer during attempts to establish a cell line (MT-2) from an ATL patient in Japan (*5*). Since this cord blood came from an endemic area of southern Japan (Kyushu) where clustering of ATL occurs and where more than 10 percent of healthy individuals have antibodies to HTLV (*17, 18*), it was possible that the recipient cells were already infected.

Our transmission studies with each of the nine new HTLV isolates were carried out on target (recipient) cells that were tested and shown to be negative for HTLV. Donor cells were x-irradiated

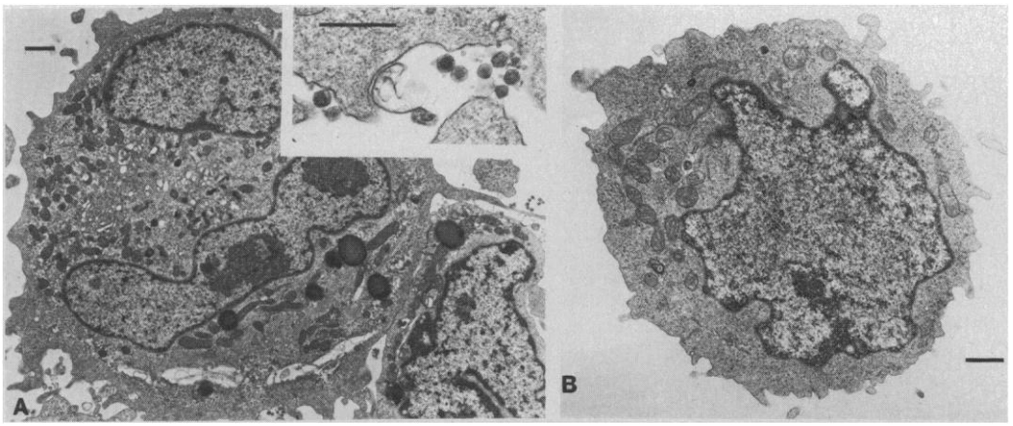

Fig. 1. Electron microscopic examination of HTLV-infected and mitogen-stimulated human umbilical cord blood T cells. (A) Infected C5/MJ cells showing lobulated nucleus and typical type-C virus particles (see insert). (B) Normal human cord blood T cells stimulated with PHA (scale bars, 100 nm).

Table 1. Origin of new HTLV isolates and characteristics of established T-cell lines. The cell lines were derived from peripheral blood (PB) or bone marrow (BM) as follows: MJ from PB of M.J., a 50-year-old white male with cutaneous T-cell lymphoma, mycosis fungoides (CTCL, MF) from Boston, Massachusetts; WA from BM of W.A., a 20-year-old black male with peripheral T-cell lymphoma (PTCL) of a diffuse mixed cell type from Augusta, Georgia; OB from PB of O.B., a 32-year-old black female with PTCL of a diffuse mixed cell type from Manchester, Georgia; PL from PB of P.L., a 29-year-old black female with PTCL (diffuse mixed lymphoma) from Oveido, Florida; UK from PB of U.K., a 45-year-old white male with PTCL (diffuse histiocytic lymphoma) from Jerusalem, Israel; MI from PB of M.I., a 32-year-old black female with T-cell lymphosarcoma cell leukemia (T-LCL) from Granada, West Indies; SK from PB of S.K., a 21-year-old male with adult T-cell leukemia (ATL) from Honshu Island, Japan; TK from PB of T.K., a 45-year-old female (mother of S.K.); and HK from PB of H.K., a 49-year-old male (father of S.K.) from Honshu Island, Japan. N.D., not done.

Characteristic	Patient and cell line identification								
	MJ	WA	OB	PL	UK	MI	SK	TK	HK
Patient									
Geographic origin	United States	United States	United States	United States	Israel	West Indies	Japan	Japan	Japan
Diagnosis	CTCL(MF)	PTCL	PTCL	PTCL	PTCL	T-LCL	ATL	Normal	Normal
Expression of HTLV in cultured cells*									
p24 (ng/mg)	130	80	40	680	1940	1500	170	2700	400
p19 (percent positive cells)	90	80	50	80	70	60	40	50	40
RT (pmole/ml extract)	4	19	3	53	5	17	6	35	15
Virus particles (electron microscopy)	+	+	+	+	+	+	+	+	+
Cultured cell characteristics									
TCGF requirements (units)†	< 0.1	< 0.4	< 0.9	< 0.4	< 0.8	< 0.6	< 0.6	< 0.5	< 0.7
TCGF receptors (TAC)† (percent)	87	92	N.D.	81	89	89	78	69	74
E-rosette† (percent)	95	94	95	79	91	95	90	80	94
EBNA, TdT, IgA, IgG, IgM†	0	0	0	0	0	0	0	0	0
Lymphoid surface phenotype†									
Pan T (T-101; T-28; A-5D; OKT-3; OKT-11) (percent)	50 to 100	80 to 99	75	54 to 95	63 to 95	50 to 95	90 to 95	90 to 95	60 to 95
Thymus (OKT-6; NA1/34) and B cells (BA-1)	0	0	N.D.	0	0	0	0	0	0
OKT-4; Leu-3A (percent)	50 to 60	70 to 90	80	70 to 90	80 to 90	95	50 to 95	18 to 90	0
OKT-8; Leu 2A (percent)	0	0	0	20 to 30	0	0	30	89	42 to 90

*The core protein p24 was detected by RIPA with cell extracts (*14*), and p19 was detected by IFA on fixed cells (*15*); RT activity in culture fluids was assayed as described (*16*). Ten milliliters of the conditioned medium was centrifuged at 90,000*g* for 1 hour. The pellet was resuspended in 300 μl of 50 m*M* tris-HCl (*p*H 7.5), 20 percent glycerol, 0.25 percent Triton ×-100, and 0.25*M* KCl. Ten-microliter samples were assayed with $(dT)_{15} \cdot (A)_n$ (oligodeoxythymidine polyadenylate) in the presence of Mg^{2+} with [^{3}H]dTTP (tritiated deoxythymidine triphosphate) (5000 cpm/pmole) as the radiolabeled substrate. The incorporation of radioactivity is expressed as picomoles of [^{3}H]dTMP (tritiated deoxythymidine monophosphate) incorporated per milliliter of extract. †Values determined by probit analysis; one unit of TCGF is defined as the amount necessary to give 50 percent of maximum [^{3}H]thymidine uptake by normal human T cells from PB in the presence of a standard TCGF preparation. The binding of monoclonal antibody TAC was determined by cytofluorometry with a fluorescence-activated cell sorter (FACS). The phenotypes of the T cells were determined by both IFA and by FACS. Although OKT-4$^+$, Leu-3A$^+$ cells are usually type-specific for a helper-inducer phenotype, exceptions are known.

(6000 R) or treated with mitomycin-C (100 μg/ml for 20 minutes), and then washed and cocultivated with recipient cord blood T cells of opposite sex (male × female and vice versa). After 4 to 5 weeks of cocultivation, the cultures were analyzed for expression of HTLV antigens, extracellular virus, karyotype, and HLA profiles (Table 2).

Virus from the cell line derived from M.J. was transmitted into human umbilical cord blood T cells from four different newborns. On each successive transmission the recipient cord blood T cells became the donor for infection of the new cord blood T cells. Cells of this M.J. line treated with mitomycin C were cocultured with cord blood cells (C1) from a newborn female. The same procedures were used to transmit HTLV from C1 to C2 and from C2 to C3 cord blood cells. Analysis of p19, p24, RT, and electron microscopic examinations showed that HTLV was fully expressed in all three cord blood T-cell lines. These three cell lines exhibited distinct HLA profiles (data not shown), thereby confirming the karyological data. When C3/MJ cord blood T cells with a female karyotype were cocultured with C5 cord blood cells from a male individual, the recipient cells showed male karyotype and HLA profiles (data not shown). Electron micrographs of HTLV-infected cord blood cells C5/MJ and phytohemagglutinin (PHA)-stimulated C5 (control) cells are shown in Fig. 1. The virus-infected cells show a lobulated nucleus (Fig. 1A) and express type-C virus particles (inset) in contrast to the normal cord blood T cells (Fig. 1B). Cocultures of HTLV-negative T cells from peripheral blood of a normal donor with C1 cells or PHA-stimulated C5 cells were consistently negative for p24 and p19.

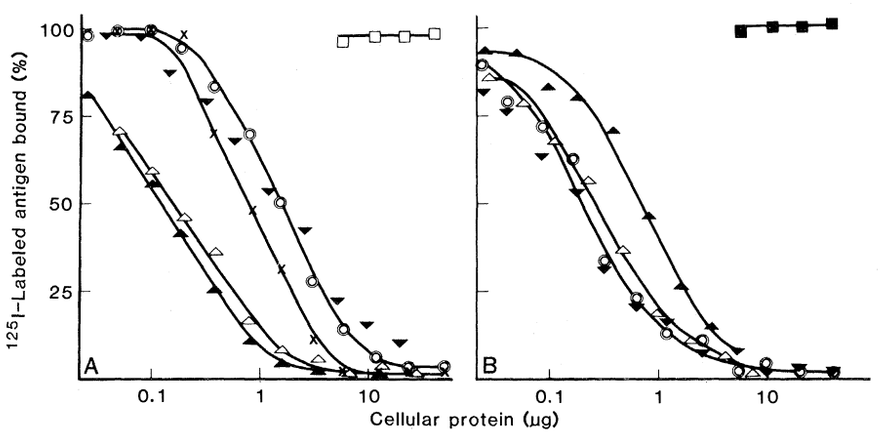

Fig. 2. Homologous competitive radioimmunoprecipitation assay (RIPA) of the major core protein p24 with representative HTLV isolates. Cell extracts of established cell lines were used: (A) from patients C.R. (×), M.J. (○), U.K. (△), M.I. (▲), and S.K. (▼) and from normal human cultured T cells (a negative control) from peripheral blood (□); and (B) from the T-cell lines from cord blood of newborns infected with HTLV isolates C2/MJ (○), C4/UK (△), C21/MI (▲), C8/SK (▼) and normal cord blood T cells (■) (a negative control).

Table 2. Transmission of HTLV into human cord blood T cells by cocultivation with HTLV-positive T-cell lines. N.D., not done; M, male; F, female.

Cocultured cells* (recipient/donor)	Sex chromosome* of fresh and cultured cells		Expression of HTLV proteins*			Electron microscopy
	Recipient	Donor	p19 (percent positive cells)	p24 (ng/mg)	RT (pmole/ml extract)	
			Successive transmission			
C1/MJ	F	M	90	385	3.3	+
C2/MJ	F	F	85	930	N.D.	+
C3/MJ	F	F	95	690	5.1	+
C5/MJ	M	F	90	540	8.1	+
			Individual transmission			
C4/UK	F	M	81	740	34.1	+
C21/MI	M	F	47	235	60.4	+
C6/WA	F	M	53	502	0	−
C91/PL	M	F	71	500	35.6	+
C126/OB	M	F	30	140	N.D.	N.D.
C8/SK	F	M	47	1000	8.7	+
C7/TK	M	F	65	685	84.2	+
C90/HK	F	M	46	500	30.3	+

*The HTLV isolates were transmitted into human cord blood T cells by a modification of the procedure for rescue of avian sarcoma virus genome from nonpermissive cells (*21*). The donor cells were treated with mitomycin-C (100 μg/ml for 20 minutes) or x-irradiation (6000 R), and then cocultured at a ratio of 1:3 or 1:5 with mononuclear cord blood cells and examined for HTLV expression after 4 to 5 weeks in culture. Sex chromosomes and HLA antigens were used as markers for HTLV-infected T cells. Control or PHA- or allogenic (human T cells from peripheral blood)-stimulated recipient cord blood T cells were processed simultaneously and tested for HTLV. Prior to the transmission experiments the cord blood samples were consistently negative for HTLV.

Eight other isolates (Table 2) of HTLV were transmitted into fresh human cord blood T cells resulting in productive infection, with the exception of C6/WA. Karyotype and HLA profiles (data not shown) again consistently matched recipient cord blood T cells. The shape of competition curves in homologous RIPA for p24 were similar for all the new isolates whether the p24 was from cell extracts of HTLV-positive cell lines (Fig. 2A) or from HTLV-infected cord blood T cells (Fig. 2B). These studies show that all the new HTLV isolates are closely related, just like the earlier HTLV isolates ($HTLV_{CR}$, $HTLV_{MB}$) and the two Japanese isolates (MT_1, MT_2) (*17*) but different from the recent new human retrovirus isolated in our laboratory from a hairy cell leukemia (*4*). The results from transmission studies clearly show that various new HTLV isolates can infect and replicate in human cord blood T cells.

The isolation of HTLV from patients in different parts of the world supports recent serological-epidemiological studies (*18–20*) indicating that this virus is more widespread than previously believed. The isolates from mature T cells of "normal" individuals are of particular interest because these cells exhibited T-cell surface markers that differed somewhat from the HTLV-positive malignant cells. The clinically normal mother (T.K.) of the ATL patient (S.K.) has morphologically abnormal lymphocytes in circulation (resembling T cells from ATL patients) suggesting that she maybe in a preleukemic state (*7*).

Our results demonstrate that all the new HTLV isolates are capable of infecting normal human umbilical cord blood T cells. With the availability of neoplastic T-cell lines from patients as well as HTLV-infected cord blood T cells it will be possible to study in vitro the cellular and molecular mechanisms of neoplastic transformation of human cells by a naturally occurring human retrovirus. In addition, since these new cell lines are

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

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References and Notes

1. B. J. Poiesz, F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980).
2. B. J. Poiesz, F. W. Ruscetti, M. S. Reitz, V. S. Kalyanaraman, R. C. Gallo, *Nature (London)* **294**, 268 (1981).
3. R. C. Gallo, D. Mann, S. Broder, F. W. Ruscetti, M. Maeda, V. S. Kalyanaraman, M. Robert-Guroff, M. S. Reitz, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5680 (1982).
4. V. S. Kalyanaraman, M. G. Sarngadharan, M. Robert-Guroff, I. Miyoshi, D. Blayney, D. Golde, R. C. Gallo, *Science* **218**, 571 (1982).
5. I. Miyoshi, I. Kubonishi, S. Yoshimoto, T. Akagi, Y. Outsuki, Y. Shiraishi, K. Nagata, Y. Hinuma, *Nature (London)* **294**, 770 (1981).
6. M. Yoshida, I. Miyoshi, Y. Hinuma, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2031 (1982).
7. P. S. Sarin *et al.*, *ibid.*, in press.
8. D. A. Morgan, F. W. Ruscetti, R. C. Gallo, *Science* **193**, 1007 (1976).
9. B. J. Poiesz, F. W. Ruscetti, J. W. Mier, A. M. Woods, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6815 (1980).
10. T. Uchiyama, S. Broder, T. Waldemann, *J. Immunol.* **126**, 1393 (1981).
11. D. Catovsky *et al.*, *Lancet* **1982-I**, 639 (1982).
12. M. Popovic, G. Lange-Wantzin, P. S. Sarin, D. Mann, R. C. Gallo, in preparation.
13. T. Waldemann, unpublished results.
14. V. S. Kalyanaraman, M. G. Sarngadharan, B. J. Poeisz, F. W. Ruscetti, R. C. Gallo, *J. Virol.* **38**, 906 (1981).
15. M. Robert-Guroff, F. W. Ruscetti, L. E. Posner, B. J. Poiesz, R. C. Gallo, *J. Exp. Med.* **154**, 1957 (1981).
16. H. M. Rho, B. J. Poiesz, F. W. Ruscetti, R. C. Gallo, *Virology* **112**, 355 (1981).
17. M. Popovic *et al.*, *Nature (London)* **300**, 63 (1982); M. S. Reitz, M. Popovic, B. F. Haynes, M. Clark, R. C. Gallo, *Virology*, in press.
18. V. S. Kalyanaraman, M. G. Sarngadharan, Y. Nakao, Y. Ito, T. Aoki, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1653 (1982).
19. M. Robert-Guroff, Y. Nakao, K. Notake, Y. Ito, A. Sliski, R. C. Gallo, *Science* **215**, 975 (1982).
20. W. A. Blattner *et al.*, *Int. J. Cancer* **30**, 257 (1982).
21. M. Popovic, J. Svoboda, T. Suni, A. Vaheri, J. Pontén, *ibid.* **19**, 834 (1977).
22. We thank B. Kramarsky for help with the electron microscopy, R. Ting for help with chromosome analysis, E. Richardson and A. Woods for technical assistance, C. Murray for HLA typing, and A. Mazzuca for editorial assistance. This work was supported in part by Interagency agreement Y01-CP-00502 with the Uniformed Services University for the Health Services.
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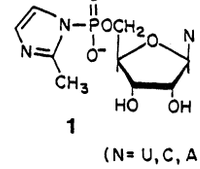
3 November 1982; revised 23 December 1982

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.



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(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