bp) (*8*) than the analogous distance in bacteriophage λ (7 bp; Fig. 1). Antitermination activity at 38° to 42°C must require *box*A, since a point mutation in the cloned *box*A sequence restored thermosensitivity to the level observed for the 74-bp *nut*L-mediated antitermination. Specifically, we constructed plasmid pmBAnutL-1, which was identical to pBA74nutL-1, except for a mutated *box*A (CG*G*TCTTA; where the italicized G represents mutation from C to G). The temperature sensitivity of *galK* expression for pmBAnutL-1 was indistinguishable from that for the *box*A-less plasmid p74nutL-1 (Fig. 2). The *box*A module alone had no antitermination activity (Fig. 2, pD553-3).

These results show that *box*A must be a component of the fully functional *nut*L site, but a truncated (*box*A-less) *nut*L sequence still retains a partial antitermination function at 30° to 34°C. At higher temperatures, however, the 74-bp *nut*L is thermosensitive, unlike the complete *nut*L site contained in the 119- or 354-bp sequences (*7*). This thermosensitivity might reflect the partial loss of contact points between the truncated *nut*L site and the proteins participating in the antitermination complex. The present results show that not only is the entire λ genome constructed from modular units, but even an individual recognition site, *nut*, is composed of separate subelements. While the *box*A-less *nut*L sequence displays an imperfect antitermination function, the addition of the *box*A subunit results in improved efficiency and thermal stability of the antiterminator.

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References and Notes

1. J. S. Salstrom and W. Szybalski, *J. Mol. Biol.* **124**, 195 (1978).
2. D. Drahos and W. Szybalski, *Gene* **16**, 261 (1981).
3. K.-C. Luk and W. Szybalski, *ibid.* **20**, 127 (1982).
4. E. R. Olson, E. L. Flamm, D. I. Friedman, *Cell* **31**, 61 (1982).
5. M. E. Gottesman, S. Adhya, A. Das, *J. Mol. Biol.* **140**, 57 (1980).
6. Antitermination by *nut*R in the plasmid system at 42°C was shown first by B. de Crombrugghe, M. Mudry, R. DiLauro, M. Gottesman [*Cell* **18**, 1145 (1979)].
7. N. Hasan and W. Szybalski, in preparation.
8. The plasmid pBA74nutL-1 was constructed by (i) chemical synthesis of the *box*A sequence,

5'-AGCTCGCTCTTACAA
GCGAGAATGTTTCGA

(ii) insertion of *box*A into the Hind III site of pD553 creating pD553-3, (iii) excision of the *nut*L sequence from pDX1 by AluI restriction; (iv) cloning of this *nut*L fragment into the Hind III site of pD553-3 employing Hind III linkers; and (v) sequencing of the resulting pBA74nutL-1 plasmid. The nucleotide sequence of the *box*A-*nut*L core junction in pBA74nutL-1 is

5'-AGCTCGCTCTTACAAAGCTTGCTAAAATT
TCGAGCGAGAATGTTTCGAACGATTTTAA
AAGCCCTGAAGAAGGGCA-3'
TTCGGGACTTCTTCCCGT

(the *box*A and *nut*L core sequences are underlined; see Fig. 1).
9. D. L. Daniels *et al.*, in *Lambda II*, R. W. Hendrix, J. W. Roberts, F. W. Stahl, R. A. Weisberg, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1983).
10. D. Drahos, G. R. Galluppi, M. Caruthers, W. Szybalski, *Gene* **18**, 343 (1982).
11. K. McKenney *et al.*, in *Gene Amplification and Analysis*, J. G. Chirikjian and T. S. Papas, Eds. (Elsevier/North Holland, New York, 1981), vol. 2, pp. 383–415.
12. The plasmid pSPT8 was constructed in two steps: (i) converting the Eco RI site into a Bam HI site by cutting pD571 (*2*) with Eco RI, filling in the ends, and ligating in a Bam HI linker, and (ii) cloning into this Bam HI site the 119-bp *nut*L sequence (Fig. 1) excised with Bam HI from plasmid pNH455 (see below). The plasmid pSPT5 was constructed by ligating the Pst I–Hind III (p_R-74bp*nut*L) fragment of pDE3 (*2*) to the Hind III–Pst I (t_{LI}-*galK*) fragment of pD571 (*2*). The plasmid pSP60 was constructed by insertion in proper orientation of the Hae III–Hinc II (*nut*R-t_{RI}) fragment excised from the λ genome [compare pD129 in (*3*)] into the Eco RI site of pD571 (*2*), with the use of Eco RI linkers. The plasmid pSPT3 was constructed by ligating the Pst I–Hind III fragment of pDE3 (see pSPT5 construction above) with the Hind III–Pst I (*nut*R-t_{RI}-*galK*) fragment of pD129 (*3*). Construction of plasmids pNH206 and pNH455, which contain the 354- and 119-bp *nut*L sequences, respectively (Fig. 1), cloned in a pKO3 derivative (*10, 11*) between Bam HI linkers, will be described elsewhere (*7*).
13. The skillful help of C. Seeliger is greatly appreciated. Supported by the NIH Program Project grant (5-PO1-CA-23076) (W.S.), core grant 5-P30-CA-07175, and NIH training grants CA-09135 (S.W.P.) and CA-09075 (A.L.B. and N.H.)

6 September 1984; accepted 3 December 1984

Expression in *Escherichia coli* of Open Reading Frame Gene Segments of HTLV-III

Abstract. *Human T-cell lymphotropic virus type III (HTLV-III), the causative agent of the acquired immune deficiency syndrome (AIDS), was recently isolated and its genomic structure analyzed by DNA cloning methods. In the studies reported here a combined cloning and expression system was used to identify HTLV-III encoded peptides that react immunologically with antibodies in sera from AIDS patients. Cloned HTLV-III DNA was sheared into approximately 500-base-pair fragments and inserted into an "open reading frame" expression vector, pMR100. The inserted DNA was expressed in* Escherichia coli *transformants as a polypeptide fused to the λCI protein at its amino terminus and to β-galactosidase at its carboxyl terminus. Sera from AIDS patients containing antibodies to HTLV-III were then used to screen for immunoreactive fusion proteins. Twenty clones, each specifying a fusion protein strongly reactive with AIDS serum, were identified. DNA sequence analysis indicated that the HTLV-III fragments were derived from the open reading frame DNA segments corresponding to the* gag *and* pol *gene coding regions and also the large open reading frame region* (env-lor) *located near the 3' end of the viral genome.*

The human T-cell lymphotropic virus type III (HTLV-III) has been routinely isolated from patients with the acquired immune deficiency syndrome (AIDS) (*1, 2*). More than 100 isolates have been obtained (*3–5*) and, although genetic variants with different restriction enzyme maps have been observed, serum samples from more than 90 percent of patients with AIDS and pre-AIDS contain antibodies reactive with the prototype HTLV-III isolate, H9/HTLV-III-B2, as demonstrated by Western blot analyses and solid phase immunoassays (*6, 7*). These and other results (*8–10*) suggest that the antibodies to HTLV-III are highly cross-reactive with the different genetic variants and that it may be possible to develop diagnostic assays and, perhaps, a preventive vaccine.

The helper T-cell tropism (*11*), the size of some of the viral proteins (*7, 12*), and serological (*6, 13, 14*) and DNA hybridization studies (*15*) indicate that HTLV-III is related to the leukemia-causing HTLV-I and HTLV-II (*16*). This information and sequence data (*17*) suggest that the HTLV-III RNA genome is similar to those of other retroviruses and contains at least (i) a *gag* gene that encodes the internal structural (nucleocapsid or core) proteins, (ii) a *pol* gene that encodes the reverse transcriptase, and (iii) an *env* gene that encodes the envelope glycoproteins of the virion. In addition, viruses in the HTLV family contain a pX region, which in HTLV-III consists of a coding sequence with one large open reading frame, designated *lor*, located between the *env* gene and the 3′ end of the genome (*18–20*). This *lor* is continuous in the same frame with the *env* and, therefore, is referred to as *env-lor*. It has been suggested that the *lor* is responsible for the unique pathogenic properties of the viruses, namely, the

transforming property of HTLV-I and HTLV-II and the cytolytic property of HTLV-III on helper T cells (*18*).

In a study designed to identify peptides encoded by regions of the HTLV-III genome, open reading frame DNA segments were cloned in an expression vector to generate a library of *Escherichia coli* clones, each expressing a different peptide encoded by a DNA segment of one of the four HTLV-III genes mentioned above. We then determined which of these peptides are reactive with antibodies to HTLV-III in sera from AIDS patients.

Fragments of HTLV-III DNA derived from λBH-10, a previously constructed recombinant bacteriophage λ (*21*) containing a 9-kb segment of HTLV-III DNA (Fig. 1A), were inserted into the open reading frame (ORF) vector pMR100 (Fig. 2) (*22*). This vector contains a bacterial *lac* promoter DNA segment linked to a second DNA fragment containing a hybrid coding sequence in which the NH_2 terminus (5′ segment) of the λCI gene of λ is fused to an NH_2-terminally deleted *lac*IZ gene (3′ segment). A short linker DNA fragment containing a Sma I cloning site has been inserted between these two fragments in such a manner that a frameshift mutation has been introduced upstream of the *lac*IZ-coding DNA. As a result, pMR100 shows negligible β-galactosidase activity when introduced into cells of the *lac*$^-$ host *E. coli* LG90. The insertion of foreign DNA containing an ORF, in this case the HTLV-III DNA, into the Sma I cloning site can reverse the frameshift mutation if the inserted coding sequence is in the correct reading frame with respect to both the λCI leader sequence and the *lac*IZ gene. Such a three-element fused gene will be expressed as a tripartite fusion protein, having a portion of the λCI protein at the amino terminus, the HTLV-III protein segment in the middle, and the *lac*IZ polypeptide at the COOH terminus. This results in high levels of expression of β-galactosidase activity upon introduction into LG90.

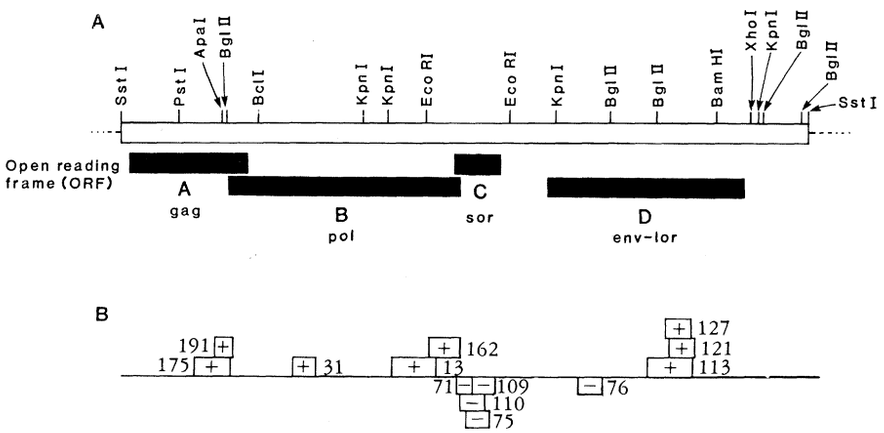

Fig. 1. (A) Restriction map of HTLV-III DNA in λBH-10. (B) Location in HTLV-III genome of DNA inserts in ORF clones. The exact position of each end of the clones is indicated in Table 1. The plus and minus signs indicate that the fusion proteins are or are not, respectively, immunoreactive with sera from AIDS patients, as demonstrated by Western blot analyses.

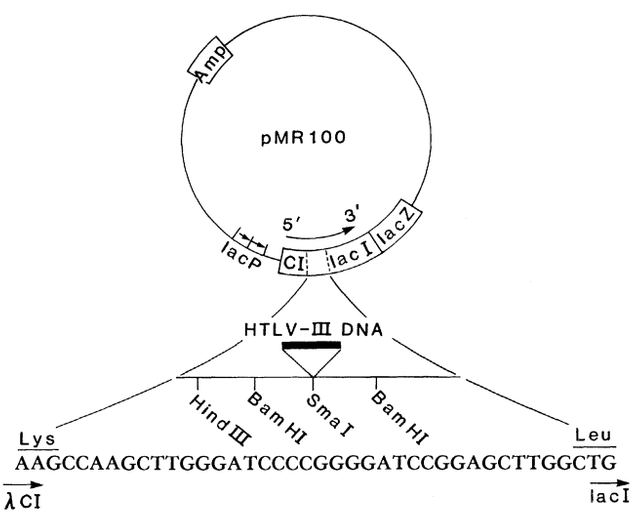

Fig. 2. Construction of the ORF expression vector containing HTLV-III DNA. Ten micrograms of HTLV-III DNA excised from λBH-10 (Fig. 1A) with Sst I were sonicated and end-repaired with T4 DNA polymerase (*27*). DNA fragments of 200 to 600 bp were isolated by gel electrophoresis. The fragments were then ligated to Sma I–cleaved pMR100 with T4 DNA ligase as described (*22*) and used to transform *E. coli* strain LG90 which was then plated on MacConkey agar containing ampicillin (50 μg/ml). *Lac*$^+$ (red) colonies were isolated for further study.

Transformants were screened on MacConkey plates to detect individual clones that express β-galactosidase enzymatic activity in situ (*23*). Of the 6000 ampicillin-resistant transformants screened, about 300 were found to express β-galactosidase activity. Colony hybridization with the use of ^{32}P-labeled nick-translated HTLV-III DNA as a probe revealed that all these *lac*$^+$ clones contain HTLV-III DNA. The proteins produced by the *lac*$^+$ clones were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) along with those of the control *lac*$^+$ clone pMR200, which produces a λCI-β-galactosidase fusion protein. The *lac*IZ gene in pMR200 is identical to that in pMR100 except that it has a single base pair deletion which brings it in phase with the λCI gene to produce an active β-galactosidase. By virtue of their very large size, β-galactosidase and its fusion proteins are separated from the bulk of proteins in cell lysates on SDS-polyacrylamide gels and can be easily identified by Coomassie brilliant blue staining (Fig. 3A). Approximately half of the *lac*$^+$ clones containing HTLV-III DNA produce polypeptides that are larger (15,000 to 27,000 daltons) than the λCI-*lac*IZ fusion protein of pMR200 (Fig. 3A, lanes 3, 6, and 7).

These findings are consistent with data showing that the DNA inserts are up to 700 bp long. Of the remaining clones, most showed a stained band with electrophoretic mobility similar to that of the λCI-β-galactosidase fusion protein of pMR200 and a few showed a greater mobility (Fig. 3A, lane 5). This may be due to a small HTLV-III insert, proteolytic degradation or anomalous electrophoretic mobility of the fusion protein, rearrangement of the recombinant plasmid, or internal initiation of translation within the HTLV insert. Similar anomalies have been observed previously with this expression system (*22*). In addition, a few clones show a fusion protein with an apparent molecular weight greater than expected (Fig. 3A, lane 4). This may be due to the presence of either a single larger insert or, as we have demonstrated by sequence analysis of sever-

al clones, multiple smaller inserts. The β-galactosidase fusion proteins account for about 1 to 2 percent of total cellular protein.

The peptides produced by the *lac*$^+$ clones were examined by Western blot analysis (*24*) for immunoreactivity with sera from AIDS patients (Fig. 3B). The recombinant peptides also reacted with antiserum to β-galactosidase, consistent with the proposition that they had the general structure λCI-HTLV-III peptide-*lac*IZ. From the pattern of immunoreactivity of the negative controls pMR100 and pMR200 (Fig. 3B, lanes 1 and 2), which do not contain HTLV-III DNA inserts, it is evident that these sera from AIDS patients contain antibodies reactive with several bacterial proteins of the host *E. coli*. This is not surprising, since human serum commonly contains antibodies to *E. coli*. When sera from AIDS patients was absorbed with Sepharose 4B conjugated with *E. coli* extract, the background immunoreactivity was reduced but not completely eliminated.

Of the 300 *lac*$^+$ clones analyzed, 20 reacted specifically with sera from AIDS patients. The unreactive clones (Fig. 3B, lane 3) probably contain peptides that fold in such a way that they are not reactive with antibodies or correspond to regions of HTLV-III protein molecules that are not immunogenic in AIDS patients. Alternatively, lack of reactivity may be due to the destruction of epitopes by the immunoblotting procedure.

The HTLV-III DNA inserts from the 20 immunoreactive clones were analyzed by DNA sequencing to determine precise sizes and locations on the HTLV-III genome. The HTLV-III genome consists of four ORF segments designated ORF-A, ORF-B, ORF-C, and ORF-D (Fig. 1A) (*17*). ORF-A and ORF-B, which correspond to the coding regions of the *gag* and *pol* genes, are 1.5 kb and 3.0 kb long, respectively. ORF-C is about 0.6 kb long, slightly overlaps with the ORF-B region, and is capable of encoding a polypeptide of 21 kilodaltons (kD). The location of ORF-C and its overlap with the *pol* gene are reminiscent of the structure of the *env* genes in HTLV-I and -II. However, ORF-C, designated the short ORF, *sor*, is too short to code for the entire envelope protein. ORF-D is 2.5 kb long and could encode both a large precursor of the major envelope glycoprotein and another protein derived from the 3′ terminus which may be analogous to the *lor* (pX) products of HTLV-I and -II. This gene region of HTLV-III, designated *env-lor* (*17*), is at least twice as long as the *lor* of HTLV-I and -II and it is unclear whether single or multiple proteins are encoded herein.

Table 1. Locations in the HTLV-III genome of ORF clones.

ORF clone number	Coordinates in HTLV-III genome*	
	Left end	Right end
13	3758	4362
31	2486	2808
71	4623	4852
75	4749	5035
76	6260	6603
109	4854	5164
110	4641	4873
113	7077	7716
121	7478	7722
127	7376	7700
162	4229	4617
175	1202	1669
191	1463	1690

*Plasmid DNA from *lac*$^+$ clones was digested with Bam HI and the HTLV-III specific insert (plus 10 bp of flanking vector DNA) was purified by agarose gel electrophoresis and cloned into M13mp19 RFI DNA which had also been digested with Bam HI. The nucleotide sequence at each end of the insert DNA was then determined by means of the "dideoxy" sequencing method, and these sequences were located relative to the known nucleotide sequence of the HTLV-III genome (*17*).

As shown in Fig. 1B, the *lac*$^+$ ORF clones expressing fusion proteins immunoreactive with sera from AIDS patients were located in ORF-A (clones 175 and 191), ORF-B (clones 13, 31, and 162), or ORF-D (clones 113, 121, and 127) and not in ORF-C. The 12 immunoreactive clones not included in Fig. 1B were found to be either duplicates of those that are shown or mosaics containing more than a single fragment of HTLV-III. Since none of the immunoreactive clones mapped to ORF-C, the library of *lac*$^+$ ORF clones was screened by in situ colony hybridization with the use of a ^{32}P-labeled nick-translated probe made from the 1.1-kb Eco RI–Eco RI fragment which spans the entire ORF-C region. Several clones (71, 75, 109, and 110) were isolated and none were immunoreactive. When these clones were sequenced, together they were found to cover 180 of the 203 codons in ORF-C. The function of the *sor* (ORF-C) region remains unclear. Either the *sor* is not expressed at the protein level or the encoded peptide may not be immunogenic in patients with AIDS or pre-AIDS.

Analysis of the ORF structures in HTLV-III leads to the question of which such structure corresponds to the *env* gene. It is possible that the *env-lor* region in HTLV-III contains all or a part of the *env* gene in addition to the presumed *lor* gene. Recent evidence suggests that the *lor* in HTLV-I encodes a 42-kD protein involved in the process of viral activation and transformation (*18–20, 25*). When the lysate of one of the ORF clones (121 in Fig. 1B) was tested against serum samples, from 20 AIDS patients and 12 healthy normal subjects in a strip

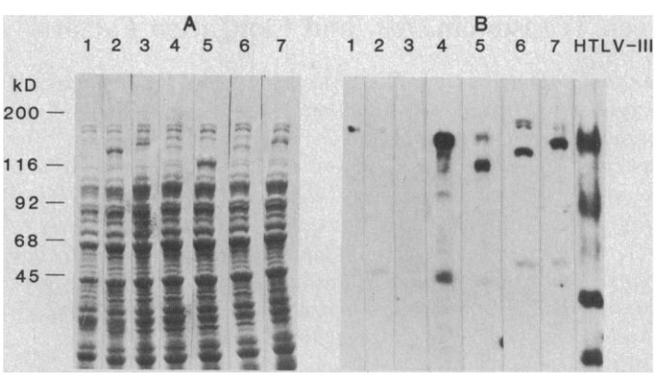

Fig. 3. (A) Gel analysis of λCI–HTLV-III–β-galactosidase fusion proteins. Cells from 1.5-ml cultures [grown in L-broth containing ampicillin (50 μg/ml)] were centrifuged, the pellets were resuspended in 100 μl of 1.2-fold concentrated sample buffer (*26*), and heated at 100°C for 3 minutes. After the samples were sonicated for 5 minutes to reduce the viscosity, 10 to 20 μl were analyzed on 7.5 percent SDS–polyacrylamide gels (*26*) and stained with Coomassie brilliant blue R-250. Lanes: 1, pMR100; 2, pMR200; 3, ORF 109; 4, ORF 4; 5, ORF 191; 6, ORF 121; 7, ORF 127. (B) Immunoreactivity of λCI–HTLV-III–β-galactosidase fusion proteins with sera from AIDS patients. Cells containing the recombinant plasmids were lysed and fractionated by electrophoresis on a 7.5 percent SDS–polyacrylamide gel. The proteins were electrophoretically transferred onto nitrocellulose paper. The nitrocellulose sheet was incubated for 2 hours at 37°C with 5 percent nonfat dry milk, 0.1 percent Antifoam A, 0.1 percent sodium azide in 0.9 percent NaCl (milk buffer) and then for 1 to 2 hours at room temperature with 5 percent normal goat serum in milk buffer. A pool of several well-characterized sera from AIDS patients was added and the nitrocellulose sheet incubated overnight at 4°C. The filter was washed twice with a solution containing 0.5 percent deoxycholic acid, 0.1*M* NaCl, 0.5 percent Triton X-100, 10 m*M* sodium phosphate, and 0.1 m*M* phenylmethylsulfonyl fluoride (*p*H 7.5) (wash buffer) for 30 minutes. After the filter was treated once with milk buffer containing goat serum, ^{125}I-labeled goat antiserum to human immunoglobulin G (1×10^6 cpm/ml) was added and incubation was continued for another 30 minutes at room temperature. The filter was washed three times with wash buffer, 20 minutes each, dried, and autoradiographed. Bands indicate the positions of the immunoreactive proteins.

radioimmunoassay based on the Western blot technique, immunoreactivity against the λCI-HTLV-III-β-galactosidase fusion polypeptide was detected in the serum of 19 of the AIDS patients but none of the normal controls. This indicates that the protein encoded by the portion of the *env-lor* region contained in ORF clone 121 is produced in HTLV-III–infected cells and induces antibody production in most if not all AIDS patients.

These and further studies of the expression of the *env-lor* region in other vector systems and of antibodies to different regions of the fusion polypeptides may lead to the development of useful reagents for studying HTLV-III proteins and for preparing means to diagnose and treat AIDS.

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References and Notes

1. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **224**, 497 (1984).
2. R. C. Gallo *et al.*, *ibid.*, p. 500.
3. P. D. Markham, G. M. Shaw, R. C. Gallo, in *AIDS*, V. T. DeVita, S. Hellman, S. A. Rosenberg, Eds. (Lippincott, Philadelphia, in press).
4. J. E. Groopman *et al.*, *Science* **226**, 447 (1984).
5. D. Zagury *et al.*, *ibid.*, p. 449; D. D. Ho *et al.*, *ibid.*, p. 451.
6. J. Schüpbach *et al.*, *ibid.* **224**, 503 (1984).
7. M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *ibid.*, p. 506.
8. B. Safai *et al.*, *Lancet* **1984-I**, 1458 (1984).
9. H. J. Alter *et al.*, *Science* **226**, 549 (1984).
10. P. M. Feorino *et al.*, *ibid.* **225**, 69 (1984).
11. D. Klatzmann *et al.*, *ibid.*, p. 59.
12. J. Schüpbach, M. G. Sarngadharan, R. C. Gallo, *ibid.* **224**, 607 (1984).
13. M. Essex *et al.*, *ibid.* **220**, 859 (1983).
14. T. H. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
15. S. K. Arya *et al.*, *Science* **225**, 927 (1984).
16. R. C. Gallo, *Cancer Surv.* **3**, 113 (1984).
17. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985).
18. J. G. Sodroski, C. A. Rosen, W. A. Haseltine, *Science* **225**, 381 (1984).
19. T. H. Lee *et al.*, *ibid.* **226**, 57 (1984).
20. D. J. Slamon, K. Shemotohno, M. J. Cline, D. W. Golde, I. S. Y. Chen, *ibid.*, p. 61.
21. B. H. Hahn *et al.*, *Nature (London)* **312**, 166 (1984).
22. M. R. Gray *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6598 (1982).
23. J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982).
24. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
25. W. A. Haseltine *et al.*, *Science* **225**, 419 (1984).
26. U. Laemmli, *Nature (London)* **227**, 680 (1970).
27. P. L. Deininger, *Anal. Biochem.* **129**, 216 (1983).
28. We thank M. Rosbash for the pMR100 and pMR200 vector plasmids, R. Ting for the purified HTLV-III, and V. R. Zurawski, Jr., for valuable discussions.

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Sequence of the Alpha Subunit of Photoreceptor G Protein: Homologies Between Transducin, *ras*, and Elongation Factors

Abstract. *A bovine retinal complementary DNA clone encoding the α subunit of transducin (T_α) was isolated with the use of synthetic oligodeoxynucleotides as probes, and the complete nucleotide sequence of the insert was determined. The predicted protein sequence of 354 amino acids includes the known sequences of four tryptic peptides and sequences adjacent to the residues that undergo adenosine diphosphate ribosylation by cholera toxin and pertussis toxin. On the basis of homologies to other proteins, such as the elongation factors of protein synthesis and the* ras *oncogene proteins, regions are identified that are predicted to be acylated and involved in guanine nucleotide binding and hydrolysis. Amino acid sequence similarity between T_α and* ras *is confined to these regions of the molecules.*

An early step in the processing of visual information involves transducin, a protein found in the rod outer segments of photoreceptors. It functions to couple the photolysis of rhodopsin to changes in intracellular levels of cyclic guanosine monophosphate (GMP) (*1*). When photoreceptors are illuminated, transducin interacts with rhodopsin, and, as a result of this interaction, guanosine diphosphate (GDP) bound to the α subunit of transducin (T_α) is exchanged for guanosine triphosphate (GTP). In this form, T_α is able to activate a cyclic GMP phosphodiesterase. The activation is terminated when the intrinsic guanosine triphosphatase activity of T_α hydrolyzes the bound GTP, restoring the transducin-GDP complex and completing the cycle. Transducin is a member of a family of proteins collectively called G proteins (*2*). Other G proteins interact with various hormone and neurotransmitter receptors and couple receptor-ligand binding events to changes in amounts of intracellular "second messenger," such as cyclic adenosine monophosphate and perhaps calcium ion (*3*). The G proteins are also substrates for adenosine diphosphate (ADP)–ribosylation by cholera toxin, pertussis toxin, or both. The ADP-ribosylation of transducin by cholera toxin inhibits GTP hydrolysis and fixes the α subunit in the GTP-bound state, whereas pertussis toxin acts to stabilize the GDP-bound form (*4*).

We have shown that the α subunits of transducin and G_o, an abundant GTP-binding protein in brain tissue, have extensive amino acid sequence homology (*5*). Furthermore, the amino acid sequence of an NH_2-terminal tryptic peptide derived from T_α was shown to have 59 percent homology with a corresponding region of the *ras* gene products. The *ras* gene family encodes guanine nucleotide–binding proteins that may be important in regulating cell growth and oncogenesis. On the basis of the sequence homology and other similarities with G proteins, we suggested that *ras* might also function as a coupling protein to transduce signals from receptors that interact with factors that regulate cell growth. In order to study G protein structure and function and more clearly define similarities and differences between the *ras* and G proteins, we isolated a complementary DNA (cDNA) corresponding to the α subunit of transducin and determined its nucleic acid sequence.

The sequences of the mixtures of oligonucleotides that were used to screen the λgt10 library prepared from bovine retinal RNA (*6*) are shown in Fig. 1A. Approximately 125,000 plaques were screened with the T_αA mixed probe and 54 were found to hybridize. These were again screened with the T_αB probe and one clone ($\lambda T_\alpha 1$) hybridized specifically with both probes. By subcloning three fragments of the insert and using synthetic oligonucleotides as primers, we obtained the complete nucleic acid sequence of the insert. The sequencing strategy is shown in Fig. 1B and the nucleotide sequence and predicted amino acid sequence are shown in Fig. 2. The nucleic acid sequence extends 429 base pairs past the TAG codon signaling the end of the coding region, and no extensive stretch of poly(A) (polyadenylate) sequence was found. There is also a 174-base-pair 5′ untranslated segment.

The open reading frame predicts an amino acid sequence including 354 amino acids. The predicted molecular weight of the protein (40.1 kD), the amino acid composition, and the size of the tryptic fragments agree with recorded values (*5*, *7*). The predicted amino acid sequences of the amino terminus of the 9-kD tryptic fragment (Lys^{209}-Tyr^{230}) and of the tetrapeptide that includes the