radioimmunoassay based on the Western blot technique, immunoreactivity against the λ CI-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-IIIinfected 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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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 ADPribosylation 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 Go, an abundant GTPbinding protein in brain tissue, have extensive amino acid sequence homology (5). Furthermore, the amino acid sequence of an NH₂-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 λ gt10 library prepared from bovine retinal RNA (6) are shown in Fig. 1A. Approximately 125,000 plaques were screened with the $T_{\alpha}A$ mixed probe and 54 were found to hybridize. These were again screened with the $T_{\alpha}B$ 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 (Lys²⁰⁹-Tyr²³⁰) and of the tetrapeptide that includes the



Fig. 1. (A) Synthetic oligonucleotides used to isolate and confirm the identity of a cDNA clone for the α subunit of transducin. Amino acid sequences of tryptic fragments of T_{α} (5) were selected to synthesize the corre-

sponding oligonucleotides ($T_{\alpha}A$ and $T_{\alpha}B$) representing all possible codon combinations. Specifically for $T_{\alpha}A$, this included 24 molecules, each 17 nucleotides long; for $T_{\alpha}B$, 64 molecules each 17 nucleotides long; and for $T_{\alpha}C$, 1 molecule, 36 nucleotides long. Oligonucleotides were synthesized with an Applied Biosystems model 380A automated DNA synthesizer and purified by gel electrophoresis before use. The bovine retinal cDNA library was screened as described (15). $T_{\alpha}A$ and $T_{\alpha}B$ were hybridized at 35° and 43°C, respectively. The construction of oligonucleotide $T_{\alpha}C$ was based on codons most commonly found in bovine rhodopsin and other eukaryotic proteins. $T_{\alpha}C$ was used as a nucleotide sequencing primer (Fig. 1B) to confirm the identity of a clone (λT_{α} 1) which hybridizes to $T_{\alpha}A$ and $T_{\alpha}B$. (B) Organization and nucleotide sequencing strategy of λT_{α} l cDNA insert. The 0.65-kilobase-pair (kbp) Bam HI, 0.8-kbp Eco RI-Bam HI, and 1.8-kbp Hind III restriction endonuclease fragments of λT_{α} l were subcloned into M13mp10 or 11 and sequencing templates were prepared by standard methods (19). Nucleotide sequences were determined by the dideoxynucleotide chain-termination method (20). Sequences determined with $T_{\alpha}C$ (Fig. 1A) and a universal sequencing primer (Bethesda Research Laboratories) initially served as the basis for the construction of other synthetic oligonucleotide sequencing primers. Symbols used are as follows: thick top line, extent of protein coding region; vertical arrows, sites of tryptic proteolysis of native $T_{\alpha}(5)$; asterisk, tryptic site accessible in T_{α} bound to GDP but not in T_{α} bound to guanosine 5'-(β,γ -imido) triphosphate; numbers, molecular size in kilodaltons of tryptic fragments as determined by gel electrophoresis; triangles, location of amino acid sequences that served as a basis for construction of oligonucleotides shown in Fig. 1A; N and C, amino and carboxyl terminus of T_{α} , respectively; CT and PT, location of amino acids ADP-ribosylated by cholera toxin and pertussis toxin; G, regions predicted to be involved in guanine nucleotide binding and hydrolysis; thick bottom line, extent of cDNA insert; jagged line, Agt10 DNA; horizontal arrows, direction and extent of nucleotide sequences determined using synthetic oligonucleotides as sequencing primers; and dotted horizontal arrows, direction and extent of nucleotide sequences determined with the use of a universal sequencing primer. A scale of nucleotide lengths in kilobase pairs is shown at the bottom. Relevant restriction endonuclease sites are abbreviated as follows: B, Bam HI; E, Eco RI; and H, Hind III.

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61 y 11C Phe 1CC Ser ATC	ATU TGI Cys ATU II AA	600 600 600 700 700 700 700 700 700 700	F GAE F Glu A GCC a Ala A CCC C CTC I Leu T CAU F Glu	6 AC1 1 Thr 2 CTC a Leu 1 Phe 6 TTI n Phe	T AAG T Lys T CTC E Leu C CT E Leu	: GCC : GCC : AAC : AAC : AAC : AST : GAL	101 Ser 190 190 190 190 230 230 230 270 270 270 270 270 270 270 270 270 27	GTC Val GA1 GA1 Asp AAG Lys AAG	AAG Lys ATG Met Asp C ATE	GAC Asp GTG Val CTC Leu Arg	TTA Leu CTG Leu TTT Phe	GAG GAG GAG GAG GAG	FIC Phe GAA Glu GAA Glu GIU GIU	CGG Arg GAT Asp AAA Lys CAAA	GAC GAC ASP ATC The ACC ACC ACC ACC ACC ACC ACC ACC ACC AC	Phe GA(G1) AAE Lys	Asp 200 0 GTC 1 Val 240 0 AAA 1 Lys 2 80 0 TAC 2 TAC 2 TAC 2 320	Val Val Asm GTC Val Ser	GI) GI) CGT Arg CAT His CAC His	ATE Met CTC Leu ATE	Glr Glr His AGC Ser Thr	AGA GAG GLU ATT Lle TGT Cys	TCA Ser TGT Cys GCT Ala	CTG CTG Leu TTT Phe ACA Thr	CCA CCA CCA CCA CCA CCA CCA CCA	C CTG S Leu N GAG D Glu	210 210 110 210 110 250 10 10 10 10 10 10 10 10 10 10 10 10 10	C AAC C AAC C AAC C AAC C AAC C AAC C AAC C AAC C AAC	: A60 : Ser : 666 : 61y : 610 : Val	C CAL High C ATA T I I e AAC ASD C AAA L Lys	, TGI 5 Cyr 1 TGI 2 Cys 1 AAC 1 Asn 1 TTI 5 Phe	AAC Asn TCT Ser GTA	CAC CAC His TAT Tyr	G GGA G GAA G G G G GAA G G G G G G G	A GT y Va 5 TTI 5 Phi 6 GA 1 Asy 1 GC/ 0 Al.	C ACI 1 Th 2 TT 2 Pho 4 GC/ 5 A14 4 GT 4 GT	C 16 r Cy 22 t 6 c e A1 26 A 666 a 61 30 t AC L Thi 34	G GCC G GCC Ala G AAT Asn C A GAT C	ALL ACT Thr TAT Tyr ALL 11e	(960) (1080) (1200)
61 y 11C Phe TCC Ser ATC ATC	ATU TGI Cys ATU AA AQ ATU	AT1 10 10 10 10 10 10 10 10 10 10 10 10 10	F GAE F Glu A GCC A Ala A Ala C CTC L Leu T CAL F Glu	G AC1 I Thr C CTC A Leu I Phe G TTT D Phe	T AAG T Lys T C AGU a Ser T CTC E Leu C CT E Leu C CT C CT C	: AAC	101 Ser 190 190 190 190 190 230 230 230 230 270 270 270 270 270 200 200 200 200 20	GTC Val GA1 GA1 Asp AAG Lys CAAC AST	AAG Lys ATG Met GAT Asp C ATE Met	GAC Asp GTG Val CTC Leu AGA Arg CTC	TTA Leu CTG Leu TTT Phe AAAA Lys TTC	AAC Asn GAG Val GAG Glu GAI CASE TAG	FIC Phe GAA Glu Glu Glu TGTC	CGG Arg GAT Asp AAA Lys CAAF	GAC Met GAC Asp ACCA	Phe GA4 G11 AA6 Lys A AT(111	Asp 200 5 ASP 200 5 ASP 200 5 ASP 200 5 ASP 200 5 ASP 240 5 ASP 240 5 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	Val Val Asn GTC Val Ser Ser AA6	GI) GI) CGT Arg CAT His CAC His	ATE ATE Met CTC Leu ATE Met	Glr Glr His AGC Ser ACC Thr	AGA GAG GIU ATT Ile TGT Cys	TCA Ser TGT Cys GCT Ala	CTG CTG Leu TTT Phe ACA Thr	CCAC	C CTG C	210 210 110 210 110 250 101 101 330 101	CGT	5 410 5 110 5 AGC 5 Ser 6 666 6 61y 6 61y 7 441	C CAC Hit C ATA C ATA C ATA C ATA C AAA C AAA L Lys TTT	, TGI s Cys cys cys cys AAC Asn TTAA	AAC Asn TCT Ser GTA GCA	GAA	C AAE C AAE S Lys G GAE G GAI C GAI C GAI	A GT y Va G TTI G GA i Asy i GC/ G A1.	C AU 1 Th 2 TT 2 Pho 7 GC/ 5 A1 3 A GT 3 Val	C 16 r Cy 22 1 6Cl 2 A1 26 A 666 a 61 30 1 ACl 1 Thi 34 5 TCl	C AIL S II S GCC a Ala O S GCC a Ala O S GAT A San O O I ATA	ALL ACT Thr TAT Tyr ATT 11e	(960) (1080) (1200) (1320)
61 y 11CC Phe 1CC Ser ATC 11e	ATU TGI Cys ATU IIc AA ATU IIc	GCI GCI GCI GCI GCI GCI GCI GCI GCI GCI	r GAE P Glu A GCC a Ala C CTC L Leu T CAL r Glu S GAA	G AC1 I Thr C CTC I Leu I Phe G TTI I Phe I AAC I ASN	T AAG T Lys T CTO E AGU Ser F CTO E Leu C CT E Leu	; TT1 ; Phe : GCC : A1; : AAC : AAC : AST : AAC	101 Ser 190 190 190 190 190 190 190 190 190 190	GTC Val GA1 Asp AAG Lys AAG AAG AAG AG AG Cys	AAG Lys ATG Met GAT Asp C ATE Met GGA	GAC Asp GTG Val CTC Leu Arg CTC Leu	TTA Leu CTG Leu TTT Phe HC Phe	AAC Asn GAG Val GAG GAG GAG GAG TAG	FIC Phe GAA Glu GAA Glu GAA Glu TGTC	CGG Arg GAT GAT Asp AAA Lys CAAA TCA	GAC Met GAC Asp ATC The ACCA	Phe GA4 G11 AAE Lys	Asp 200 1 Val 240 2 AAA 2 Lys 2 80 2 TAC 2 TAC 2 TAC 2 TAC 2 TAC 2 TAC 2 TAC 2 TAC 2 TAC 2 TAC	Val AA1 Asm GTC Val Ser AA6	GI GI GI GI GI GI GI GI GI GI GI GI GI G	ATE ATE Met CTC Leu ATE Met	Glr Glr His AGC Ser Thr CTA	AGA GAG GIU ATT Ile TGT Cys	TCA Ser TGT Cys GCT Ala	CTG CTG Leu TTT Phe ACA Thr	CCAC CCAC CCAC CCAC CCAC CCAC CCAC CCA	C CTG C C C CTG C	210 210 110 250 111 250 111 117 29 29 00 61n 330 101	GAT GAT GAT GAT Asp O GAT Asp O CGT	C AGC C AGC C Ser GGG G G1y C G1C C Val	C CAL His C ATA I Lie C ATA AAC C AAA L Lys I Lys I TTT	, TGI 5 Cys 7 Cys 7 AAC 1 AAS 1 TTT 1 5 Phe	AAC AAC Asn TCT Ser GTA Val	GAL GAL GAL TAI TAI TYT Phe GAA	G GGA G GIN C AAG S Lys T GAG G G U T GAT	A GT V Va G TTI G GA G GA G GA G GA G GA G A G A G A G	C AU L Th Pho F GC/ D Ali A GT A GT	222 7 Cy 222 7 Cy 22 7 Cy 22 Cy 22 7 Cy 22 7 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 Cy 22 C Cy 22 C Cy 22 C Cy 22 C Cy 22 C Cy 22 C C C C	C AIL S IIe O G GCC A AI A AI A SAT - Asp O A AAA - Asp O A AAA	ATT Lle ACT Thr TAT Tyr ATT Lle TAT	(960) (1080) (1200) (1320)

TAT TGA ATE CAT AAG AAT GAA TEC ATE CTE CET TGE AAA IGA GTA TGT ATE ATT GEA AET GTE IET CAT TTE GTE TTI TAA AAG EGE GAT AGT TAG EAE AGT TTA AAG AAT GEA GAA CEA (1440)

664 AAT CAG AAG ACC CAG GAT CCA TTA TTG GCT CTG CAA GTT ACT ATT GAT GCA AAA ATG TAA ATA TTT CAT TTG ICT GAG CCT TGA GIC CCI TAT CTA TAA AAI GAA GGT AAT TIC ICT (1560)

ACT ACT TCA CAA GGT TAC TIT AAT GAT CAE AAA CAT AAC TGA AGG CAG GCA CAT AAA AAC TGI GIG GIG ACA CAA AGA AAT CCT ATG TTA AAG GCT CCC ACT AAT (1665)

Fig. 2. Nucleotide sequence of λT_{α} 1 cDNA and deduced amino acid sequence of T_{α} protein. Numbering of the nucleic acid sequence is shown in parentheses. The predicted amino acid sequence is numbered every ten residues. Amino acid sequences from tryptic peptides of T_{α} (5, 8) are underlined. Other symbols and abbreviations are as in Fig. 1B. The insert of λT_{α} 1 cannot be excised with Eco RI even though the cDNA library was constructed with Eco RI linkers. The nucleotide sequence from bases 1666 to 1672, which was GAAATTC instead of GAATTC, explains this anomaly, but the origin of the extra adenine is unknown.

Fig. 3. Hybridization of bovine genomic DNA to T_{α} cDNA. Twenty micrograms of high molecular weight bovine liver DNA was cleaved with Bam HI, fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized to primer extended probes (19) derived from M13 subclones containing the EcoRI-Bam HI fragment corresponding to the amino-terminal two-thirds of T_{α} (N), the Bam HI fragment corresponding to the carboxyl-terminal third of T_{α} (C), or the Hind III fragment that contains the entire coding region (W) (Fig. 1B). The amount of $[\alpha^{32}P]dATP$ (deoxyadenylate) used in the reactions was limited so that a probe of an average length of 1000 bases was made. Therefore, probe W, which begins at base 129, does not extend beyond the last base of probe C at base 1458. Hybridization was at 65°C in 6× SSC (saline sodium citrate), 0.1 percent sodium dodecyl sulfate (SDS), $2 \times$ Denhardt's solution, 50 µg per milliliter sheared salmon testes DNA, and 10 percent dextran sulfate. The filter was washed at 42°C in $0.2 \times$ SSC and 0.1 percent SDS.

cholera toxin ADP-ribosylation site (Ser¹⁷⁷-Lys¹⁸⁰) are identical with the amino acid sequences determined by Edman degradation. The amino acid reported to be ADP-ribosylated by cholera toxin is an arginine in the sequence Ser-Arg-Val-Lys (8). This sequence is found only once, is preceded by an arginine as expected for a tryptic peptide, and is located on the 23-kD tryptic fragment (9). The predicted sequence of the 5-kD tryptic fragment (Asp³¹⁵-Phe³⁵⁴) is also identical with the amino acid sequence as reported (5), except for Cys^{351} and Leu³⁵³, which were not identified. It has been suggested that the target for ADPribosylation by pertussis toxin is an asparagine residue corresponding to position 350 (10). However, the cDNA se-



quence (Fig. 2) predicts an aspartic acid residue at position 350 in the primary translation product. Thus, a modified form of aspartic acid could be the substrate for pertussis toxin. Alternatively, Cys^{351} , which was not previously detected (10), could also be the site of ADPribosylation.

There are four amino acid differences between the predicted sequence and the reported sequence of the 23-kD tryptic fragment. They are conservative differences and cluster at the amino terminus. Amino acid sequence analysis resulted in identification of Lys²⁴, Glu²⁸, Asp³⁰, and Arg³². The nucleic acid sequence predicts Gin²⁴, Asp²⁸, Glu³⁰, and Lys³². It is possible that these differences do not arise from multiple transducin genes

Fig. 4. Homologies of T_{α} to the protein sequences of translation elongation factors and *ras* oncogenes (21). Homologies were identified with the use of a computer homology matrix program (22) which determines the degree of identity found over an adjustable number of amino acids. (A) Homologies among regions predicted to play a role in guanine nucleotide hydrolysis. (B) Homologies among regions predicted to be involved in guanine nucleotide binding. Sequence identities to T_{α} are boxed and close functional homologies are underlined. Single letter abbreviations used for amino acids are: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.

within an organism but instead represent polymorphisms in the different animals whose tissues were used to prepare transducin protein for sequence determination and poly(A)⁺ RNA (polyadenylated RNA) for cloning. This argument is supported by the hybridization experiment shown in Fig. 3. Digestion of genomic DNA with Bam HI and hybridization with probes made from the NH₂terminal, COOH-terminal, and complete coding regions of the cDNA give a pattern that is only consistent with the presence of a single gene corresponding to T_{α} .

The complete sequence of transducin allows us to compare it with other GTPbinding proteins. Similarities were found in the sequences of mammalian and yeast ras, the T_{α} subunit, and the elongation factors (EF-Tu and EF-1 α). In each of these proteins GTP binding and hydrolysis are central to their function. Homologies between EF-Tu and ras have been previously noted (11). Sequence similarities between T_{α} and the other proteins are primarily clustered in two regions (Fig. 4). One region is at the NH_2 -terminal end starting at Lys^{29} in T_{α} and extending through Lys⁷⁰. There is evidence to suggest that this region is involved in GTP hydrolysis in ras. Mutagenesis resulting in changes in amino acids in this region affect guanosine triphosphatase and oncogenic activity (12). A second region of homology is found toward the COOH-terminal end of the T_a subunit and includes the residues from Ala²⁶⁰ to Lys²⁷⁶. This region may participate in GTP binding. The homologous region in all elongation factors includes an asparagine residue (Asn¹³⁵ in EF-Tu) which has been shown by x-ray crystallographic analysis to form a hydrogen bond with the guanosine moiety of GDP (13). Furthermore, modification of Cys¹³⁷ in EF-Tu eliminates nucleotide binding. In EF-Tu, this region forms part of a six-stranded B sheet structure common to many nucleotide-binding proteins (14).

There is evidence to suggest that the guanyl nucleotide binding site in T_{α} interacts with a region of the protein that is located between amino acids 177 to 208. Thus, for example, bound analogs of GTP block trypsin cleavage at Arg^{208} (7). Furthermore, the ADP ribosylation site at Arg^{178} is only recognized by cholera toxin when T_{α} binds nonhydrolyzable GTP analogs (4). Finally, ADP ribosylation at this site blocks the GTP hydrolysis activity of transducin.

A striking feature that is conserved between T_{α} and *ras* is the presence of a

cysteine residue as the fourth amino acid from the carboxyl terminus. Both the α and the γ (15) subunits of transducin as well as 11 ras gene products that have been examined (16) have a cysteine in this position. This residue is acylated with lipid in ras and is important for ras function (17). Acylation could occur at Cys^{351} in T_{α} . Another possible site for acylation in T_{α} is on Gly². The amino terminus of $T_{\boldsymbol{\alpha}}$ is blocked, and all proteins known to have NH₂-terminal myristic acid blocking groups have glycine as the NH₂-terminal amino acid (18).

The homologies found between ras, elongation factors, and T_{α} reflect the guanine nucleotide binding and hydrolytic activities that are necessary for an alternation between GDP- and GTPbound conformations that determine the nature of the reversible association of these proteins with other macromolecules. The portions of ras and T_{α} that are not directly involved in nucleotide binding or hydrolysis may govern the specificity for interaction with different subcellular components. The availability of the intact cDNA clones makes it possible to overproduce T_{α} and to modify the gene product by mutagenesis. Reconstruction of the G protein-coupling

system in vitro and in vivo will provide an experimental system that may allow us to understand in detail how these proteins function.

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