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Amino acid sequence of the α subunit of transducin deduced from the cDNA sequence

(GTP-binding proteins/signal transduction/ADP-ribosylation sites/ras proteins)

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ABSTRACT **Transducin, a GTP-binding protein involved in phototransduction in the vertebrate retina, belongs to a family of homologous coupling proteins that also includes G_s and G_i, the regulatory proteins of adenylate cyclase. Here we report the cDNA sequence and deduced amino acid sequence of transducin's α subunit (T_α). The cDNA was isolated, by screening with an antibody probe, from a bovine retinal cDNA library in the expression vector λgt11. The 2.2-kilobase cDNA insert hybridized to a single 2.6-kilobase poly(A)$^+$ RNA species present in extracts of bovine retina but not of bovine heart, liver, or brain. The nucleotide sequence of the cDNA revealed an open reading frame long enough to encode the entire 39-kDa T_α polypeptide. The polypeptide sequence deduced from the cDNA would be composed of 350 amino acids and have a molecular weight of 39,971. Portions of the sequence matched reported amino acid sequences of T_α tryptic fragments, including sites specificlly ADP-ribosylated by cholera and pertussis toxins. The predicted sequence also includes four segments, ranging from 11 to 19 residues in length, that exhibit significant homology to sequences of GTP-binding proteins, including the ras proteins of man and yeast and the elongation factors of ribosomal protein synthesis in bacteria, EF-G and EF-Tu. In combination with previous functional studies of tryptic fragments of T_α, the deduced amino acid sequence makes it possible to predict which portions of the polypeptide interact with other molecules involved in retinal phototransduction.**

Transducin, a guanine nucleotide binding protein of retinal rod cells, mediates the activation of a cyclic GMP phosphodiesterase (PDEase) in response to photoexcitation of rhodopsin (1). Each photolyzed rhodopsin (R*) molecule activates hundreds of transducin molecules by catalyzing the exchange of GTP for GDP at transducin's guanine nucleotide binding site. The activated transducin–GTP complex in turn stimulates the hydrolysis of many cGMP molecules by PDEase. Hydrolysis of bound GTP returns transducin to its inactive state.

Transducin contains three subunits, α, β, and γ, whose sizes are 39, 35, and ≈8 kDa, respectively. Transducin exhibits striking structural and functional homologies to the stimulatory (G_s) and inhibitory (G_i) coupling proteins of hormone-sensitive adenylate cyclase. Each protein is a heterotrimer in which the respective subunits exhibit similar amino acid compositions and proteolytic peptide maps (2). In each case, the protein's interaction with an excited signal detector (rhodopsin or hormone receptor) triggers dissociation of the β–γ complex from the α subunit, which then alters activity of the effector molecule (PDEase or adenylate cyclase) (1, 3). After hydrolysis of GTP bound to the α subunit, the β–γ complex recouples the α subunit to the signal detector (4). The α subunits serve as substrates for ADP-ribosylation by cholera toxin (G_s and transducin) (5, 6) and pertussis toxin (G_i and transducin) (7, 8). Each of these toxin-catalyzed covalent modifications characteristically alters the coupling function of the substrate proteins.

Here we report the sequence of a cDNA encoding the entire α subunit of bovine transducin (T_α). The deduced amino acid sequence of T_α elucidates relations between structure and function of T_α and of the family of GTP-binding transmembrane signaling proteins.

MATERIALS AND METHODS

Materials. A bovine retinal λgt10 cDNA library was provided by Jeremy Nathans (Stanford Univ.). Rabbit antiserum raised against the 39-kDa pertussis toxin substrate of bovine brain (9) (antiserum NGA-1) was donated by E. Neer (Harvard Medical School). The expression vector λgt11 and its host strains were the gifts of R. Young and R. Davis (Stanford Univ.). Plasmid vector pUC8 was donated by J. Messing (Univ. of Minnesota).

Antisera. Rabbit antisera were raised by subcutaneous injection of purified (10) T_α or the β–γ subunit complex of transducin ($T_{\beta\gamma}$), emulsified with complete Freund's adjuvant. The anti-T_α serum (F/A-1) had a titer of 1:25,000 against pure T_α in an ELISA and displayed negligible cross-reactivity against other proteins on immunoblots of total retinal homogenate or crude rod outer segments. The anti-$T_{\beta\gamma}$ serum (F/B-1) detected both β and γ but not α subunits of transducin on immunoblots. Antibodies that crossreacted with bacterial or phage proteins were removed from all antisera by adsorption to a lysate of *Escherichia coli* strain BNN97 coupled to Sepharose 4B (11).

Construction and Screening of the λgt11 Retinal cDNA Library. The bovine retinal λgt10 cDNA library, containing 2.5 × 10^5 recombinants (12), was transferred into the expression vector λgt11 to permit screening of the library with antibody probes. The λgt10 library was amplified in Y1073, an Hfl derivative of *E. coli* strain C600 (11). Purified λgt10 DNA from this library was digested with *Eco*RI and size-fractionated by sucrose gradient sedimentation (5–20% sucrose in 10

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Abbreviations: T_α, α subunit of transducin; $T_{\beta\gamma}$, β–γ subunit complex of transducin; PDEase, phosphodiesterase, p[NH]ppG, guanosine 5′-[β,γ-imido]triphosphate; R*, photolyzed rhodopsin; G_s and G_i, stimulatory and inhibitory coupling proteins of hormone-sensitive adenylate cyclase.

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mM Tris Cl, pH 7.2/1 mM EDTA, centrifuged for 10 hr at 38,000 rpm in a Beckman SW41 rotor) to separate λgt10 vector arms from the cDNA inserts. Gradient fractions that contained cDNA inserts ranging in size from 0.1 to 8 kilobases (kb) were pooled and concentrated by ethanol-precipitation.

Aliquots (0.1 μg) of the isolated cDNA were ligated for 14 hr at 12°C with 2.2 μg of λgt11 vector arms (prepared by digestion with *Eco*RI and treatment with calf intestine alkaline phosphatase), packaged *in vitro*, and plated for screening on *E. coli* strain Y1090 (11). Approximately 90% of the packaged phage contained inserts, as shown by insertional inactivation of the phage β-galactosidase gene, whose activity was assessed using the chromogenic indicator 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

The λgt11 library was screened for antigen-producing clones by sequential incubation with anti-T_α serum (F/A-1) at a 1:100 dilution and ^{125}I-labeled protein A, as described by Young and Davis (11).

Subcloning. The insert in the λgt11 phage designated λα2 was inserted into pUC8 to produce the clone designated pα2. pα2 restriction fragments were inserted into M13 mp18 and M13 mp19 for sequencing.

Blot Hybridization Analyses. *Analysis of poly(A)$^+$ RNA.* RNA was isolated as described (13) and poly(A)$^+$ RNA was selected by chromatography on oligo(dT)-cellulose (14). Denatured poly(A)$^+$ RNA was size-fractionated by electrophoresis in 0.8% formaldehyde/agarose gels and transferred to nitrocellulose as described (15). Blots were hybridized with the pα2 probe (labeled by nick-translation) at 42°C in 3× standard saline citrate/50% formamide/5× Denhardt's solution/salmon sperm DNA (200 μg/ml) for 48 hr and then washed three times for 5 min at 20°C in 2× standard saline citrate/0.1% NaDodSO$_4$ and twice for 30 min at 52°C in 0.1× standard saline citrate/0.1% NaDodSO$_4$. Standard saline citrate is 0.15 M NaCl/15 mM sodium citrate, pH 7; Denhardt's solution is 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone.

Southern blot analysis. Genomic DNA was isolated (16) from bovine thymus, digested to completion with restriction endonucleases, and transferred to nitrocellulose by the method of Southern (17). Blots were hybridized and washed as described above for RNA.

DNA Sequencing. Restriction fragments of the cDNA insert were subcloned into M13 vectors and sequenced in both orientations by the dideoxy chain-termination method (18, 19).

RESULTS

cDNA sequences inserted into the unique *Eco*RI restriction site of λgt11, located within the gene coding β-galactosidase, can be induced and expressed in high abundance as a hybrid polypeptide fused to β-galactosidase. If the cDNA encoding T_α is inserted in the proper orientation and in-phase with the β-galactosidase translational reading frame, the hybrid protein should contain antigenic determinants that can be detected by specific antibody to T_α (11).

In the initial screening of 7×10^5 λgt11 recombinants, anti-T_α serum F/A-1 detected nine positive plaques on replicate filters. Of these, however, only a single recombinant (designated λα2) produced a positive signal when rescreened during plaque purification.

We rescreened λα2 with two additional antisera. Antiserum NGA-1, directed against the 39-kDa pertussis toxin substrate of bovine brain (9), detected T_α on immunoblots and also recognized the protein product of λα2. Serum F/B-1, directed against the β–γ complex of transducin, did not.

Detection of a Retinal Specific Poly(A)$^+$ RNA Transcript. Blot hybridization analysis of electrophoretically fractionated poly(A)$^+$ RNA isolated from four bovine tissues (Fig. 1*A*) revealed that radiolabeled subclone pα2 hybridized with a single prominent retinal RNA species ≈2600 nucleotides long. No RNA transcripts were detected in similar extracts of bovine heart, liver, or brain (Fig. 1*A*).

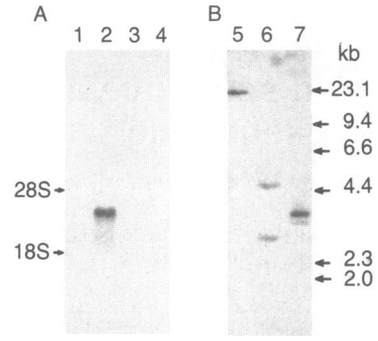

FIG. 1. (*A*) Blot hybridization analysis of poly(A)$^+$ RNA isolated from bovine heart (lane 1), retina (lane 2), liver (lane 3), and brain (lane 4). Each lane contained 1.5 μg of RNA. pα2 cDNA, nick-translated to a specific activity of 3×10^8 cpm/μg, was used to probe the blot. Positions of 28S (4718 nucleotides) and 18S (1874 nucleotides) RNA reference standards are indicated by arrows at left. (*B*) Southern blot hybridization of bovine genomic DNA digested with a 10-fold excess of *Eco*RI (lane 5), *Pst* I (lane 6), and *Bam*HI (lane 7). Nick-translated pα2 cDNA (3×10^8 cpm/μg) was used to probe the blot. Each lane contained 10 μg of DNA. Arrows at right indicate the positions of fragments of *Hin*dIII-digested λ DNA.

Hybridization of α2 cDNA to Genomic DNA. Southern blot analysis of bovine genomic DNA (Fig. 1*B*) showed that radiolabeled pα2 hybridized to a single *Eco*RI fragment (≈20 kb), two *Bam*HI fragments (4.0 and 3.7 kb), and two *Pst* I fragments (5.2 and 3.1 kb). The simplicity of these restriction patterns suggests that the bovine genome contains a single copy of the T_α gene. The gene probably has a size of about 8 kb.

Amino Acid Sequence Deduced from T_α cDNA. Fig. 2 shows the nucleotide sequence of the λα2 cDNA insert. The insert contained only one open reading frame long enough to encode the entire 39-kDa T_α polypeptide. The predicted polypeptide product of the open reading frame contained amino acid sequences perfectly homologous to previously reported sequences of T_α fragments, including partial amino-terminal sequences of tryptic fragments (21) (residues 19–51, 205–226, and 311–350, underlined in Fig. 2) and the tetrapeptide containing the arginine (residue 174) ADP-ribosylated by cholera toxin (22).

The cDNA sequence corresponding to the carboxyl terminus of the T_α polypeptide encodes the amino acid ADP-ribosylated by pertussis toxin (23).

The amino-terminal sequence of T_α has not been determined, because it is not susceptible to Edman degradation and is presumably blocked (21). We assigned the initiation site for synthesis of T_α to the nucleotide triplet ATG at position 65, on the following grounds: (*i*) The nucleotide sequence ACCATG beginning at base 62 conforms with Kozak's observations (24) that the nucleotide triplet AUG serves as the initiation site in all reported eukaryotic mRNA sequences and that the nucleotide sequence immediately preceding the initiation site is usually ACC. (*ii*) The predicted molecular weight of the translated protein, 39,971, agrees closely with the apparent molecular weight of T_α determined by NaDodSO$_4$/PAGE (10). (*iii*) No methionine codons are found for at least 21 codons upstream or for 49 codons downstream from the proposed initiation site. (*iv*) An established site for trypsin cleavage (21) 18 amino acids (Lys-18) downstream from the proposed initiator methionine would generate a 2-kDa peptide fragment; two reports (20, 21) indicate that a peptide fragment of 1–2 kDa is cleaved from the amino

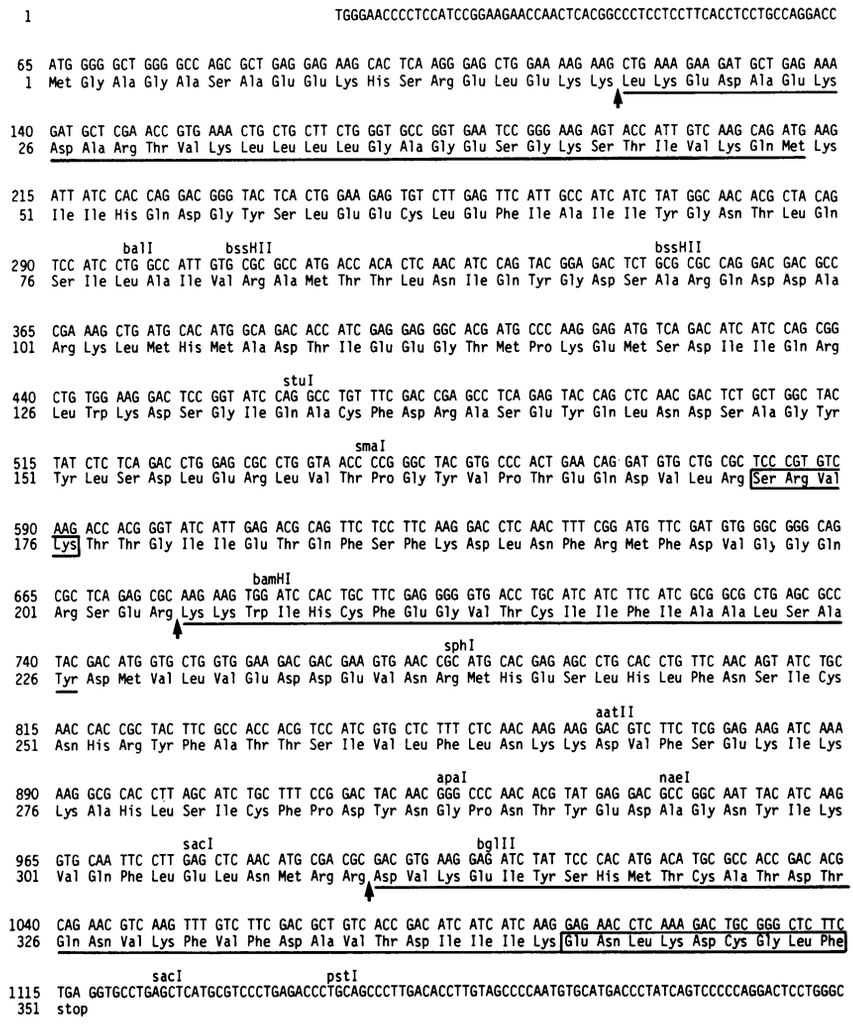

FIG. 2. Nucleotide sequence and predicted amino acid sequence of the T_α cDNA. Arrows indicate sites at which trypsin cleaves the native polypeptide (20) and underlined amino acid sequences are those reported for tryptic fragments of the isolated polypeptide (21). Boxes enclose previously sequenced peptides that include ADP-ribosylation sites for cholera (22) and pertussis (23) toxins. Specific restriction endonuclease cleavage sites are indicated above the nucleotide sequence.

terminus of T_α upon limited digestion with trypsin. We cannot exclude the possibility that one or more amino acids are removed from the amino terminus during posttranslational processing of T_α.

DISCUSSION

Three criteria indicate that the $\lambda\alpha 2$ cDNA insert encodes T_α: (*i*) The predicted amino acid sequence matches reported (21) partial sequences of the isolated polypeptide (Fig. 2). (*ii*) As expected for a protein specifically expressed in the retina, the transcript was detected in extracts of retina but not of heart, liver, or brain (Fig. 1*A*). (*iii*) The deduced amino acid sequence includes sites previously shown to be ADP-ribosylated by both cholera and pertussis toxin in T_α (Fig. 2), whereas the homologous α subunits of G_s and G_i each serve as substrates for only one of the toxins.

Limited digestion with trypsin cleaves native T_α at three sites (20). Comparison of the amino-terminal sequences of three of the four cleavage products (21) with the amino acid sequence deduced from the cDNA indicates that trypsin cuts at Lys-18, Arg-204, and Arg-310 (Fig. 2). To facilitate discussion, the four tryptic peptides are designated P-I through P-IV, in order from the polypeptide's amino terminus (Fig. 3). Fung and Nash (20) designated these peptides, according to their apparent sizes on NaDodSO$_4$/PAGE, as follows: P-I, 1 kDa; P-II, 23 kDa; P-III, 9 kDa; and P-IV, 5 kDa. Functional studies (20) of these four tryptic cleavage products have suggested roles for each in mediating the reversible interactions of T_α with GTP, $T_{\beta\gamma}$, PDEase, and R*. With knowledge of the amino acid sequence, we can now begin a more precise exploration of relations between structure and function of T_α (Fig. 3).

GTP Binding and Hydrolysis. Halliday (25) recently described four regions of conserved amino acid sequence, designated A, C, E, and G, that are common to two families of GTP-binding proteins, the bacterial elongation factors (EF-G and EF-Tu) and the mammalian and yeast ras proteins. T_α contains regions, shaded in Fig. 3, that exhibit significant sequence homology to the same four regions of the ras proteins and the elongation factors (Fig. 4). Homology between sequences corresponding to the A region of *ras* and a peptide fragment of T_α was reported previously (21). The deduced amino acid sequence of the entire polypeptide now reveals homologies to regions C, E, and G as well (Fig. 4). Homologies in these four regions differ in degree; the homology in region C is less convincing than the others. Nonetheless, the four regions appear in the same order in T_α as in the ras proteins and the elongation factors, strongly suggesting that these proteins derive from a common precursor in evolution.

All four regions probably interact with GTP. c-Ha-ras1 mutated at amino acid residue 12 (in region A) hydrolyzes

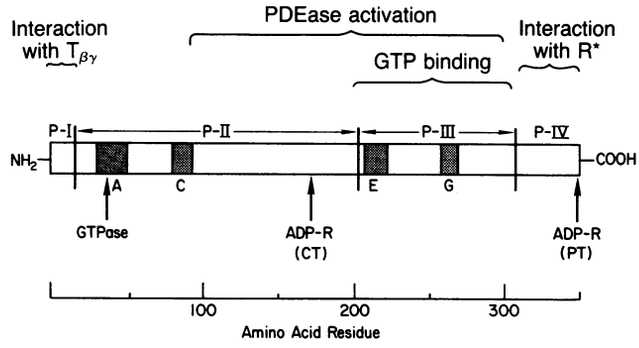

FIG. 3. Schematic diagram of the T_α polypeptide. Tryptic peptides P-I through P-IV are indicated. Shaded areas (denoted A, C, E, and G) represent regions of amino acid sequence that are homologous to regions of the ras proteins and the elongation factors (see Fig. 4). A site analogous to the region that appears to control GTPase activity in the ras proteins and sites for ADP-ribosylation (ADP-R) for cholera and pertussis toxins (CT and PT, respectively) are indicated. Domains of the polypeptide postulated to interact with GTP and other components of the phototransduction system are indicated at the top.

GTP at a decreased rate[g] and biochemical studies of the elongation factors[h] implicate regions C, E, and G as contributing to GTP binding. Similar biochemical observations suggest that tryptic peptides P-II and P-III in T_α, which contain all four regions of homology with the other proteins, contain the guanine nucleotide binding site. Binding to T_α of the hydrolysis-resistant GTP analog guanosine 5'-[β,γ-imido]triphosphate (p[NH]ppG) specifically prevents trypsin from cleaving P-II from P-III (21), at a site closely adjacent to the E region of homology (Fig. 3). After trypsin has removed P-I and P-IV, p[NH]ppG remains bound to the remaining P-II-III polypeptide (20). In addition, binding of p[NH]ppG protects two otherwise accessible sulfhydryl groups on T_α from chemical modification (37); one of these is in P-II, the other in P-III (unpublished results). Finally, an azido derivative of GTP binds specifically to T_α and can be induced by light to bind covalently; after tryptic cleavage of the labeled polypeptide the GTP derivative is found linked to P-III (unpublished results). By analogy, it is likely that regions C, E, and G of T_α contribute to GTP binding and that region A somehow controls hydrolysis of the bound GTP.

Interaction with $T_{\beta\gamma}$. Trypsin treatment of p[NH]ppG-bound T_α rapidly removes the P-I polypeptide. The remaining 38-kDa peptide exhibits markedly impaired ability to exchange bound guanine nucleotide or to hydrolyze GTP when reconstituted with R* and intact $T_{\beta\gamma}$ (20). This result implies impaired interaction of the 38-kDa peptide with either R* or $T_{\beta\gamma}$. Recent studies have shown that T_α serves as a substrate for ADP-ribosylation by pertussis toxin only in the presence of $T_{\beta\gamma}$ and that the truncated 38-kDa fragment of T_α cannot serve as a pertussis toxin substrate even in the presence of $T_{\beta\gamma}$ (unpublished work). Taken together, these results strongly suggest that a portion of P-I is required for interaction with $T_{\beta\gamma}$.

[g]Substitution of valine (or several other amino acids) for the normally occurring glycine in position 12 of c-Ha-ras1 markedly decreases the protein's GTPase activity and increases its capacity for oncogenic transformation (31, 32). Interestingly, T_α contains a glycine at the corresponding position (residue 38) in region A (Fig. 4).

[h]Covalent modification of Cys-137 (region G) of elongation factor Tu (33) or Cys-113 (region E) of elongation factor G (34) impairs binding of GTP to these proteins. A photoaffinity analog of GTP binds to a trypsin cleavage product of elongation factor G that includes region E (35). Substitution of threonine for alanine at position 59 in region c of the ras protein leads to GTP-dependent phosphorylation of this residue (31, 36).

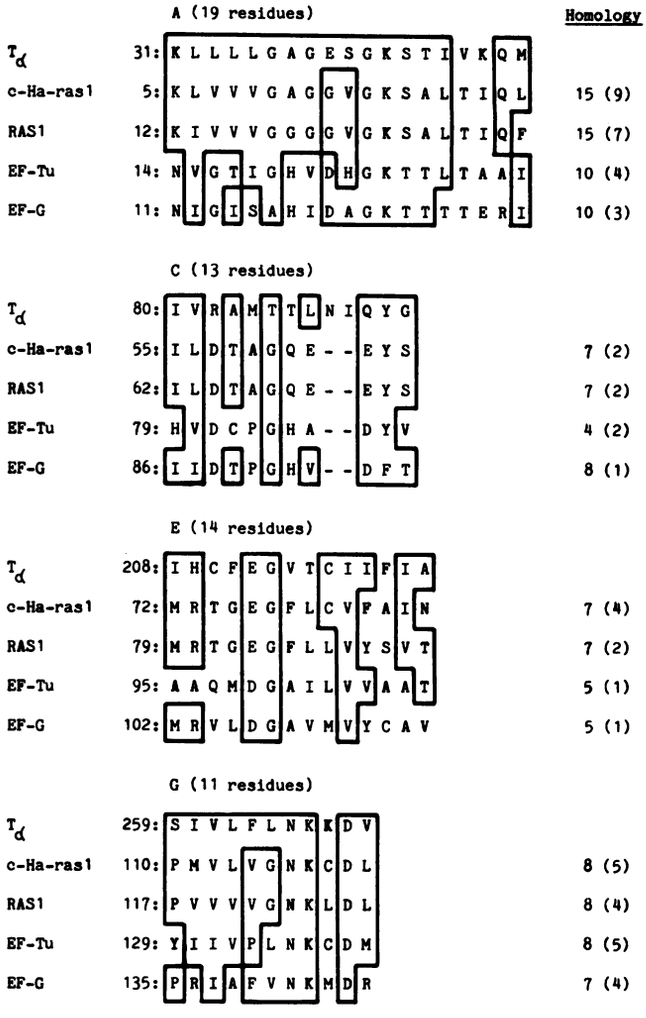

FIG. 4. Amino acid sequences from conserved regions A, C, E, and G of human (c-Ha-ras1) and yeast (RAS1) ras proteins and of bacterial elongation factors EF-Tu and EF-G (25) are aligned with corresponding regions of T_α. Regions of exact homology or conservative Dayhoff substitutions (26) between T_α and any other protein are boxed. Numbers in the column on the right indicate the number of conservative substitutions and, in parentheses, the number of identities, both with respect to T_α. Sequences are taken from the following references: c-Ha-ras1 (27), RAS1 (28), EF-Tu (29), EF-G (30). Single-letter abbreviations for amino acid residues are as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. The following Dayhoff conservative categories (26) were used: C; S,T,P,A,G; N,D,E,Q; H,R,K; M,I,L,V; F,Y,W.

In this context, it should be noted that the mammalian and yeast ras proteins lack the amino-terminal 19–26 residues that precede the A region of homology in T_α. Because this amino-terminal portion of T_α (peptide P-I) is implicated in the interaction with $T_{\beta\gamma}$, the absence of a corresponding region in the ras proteins suggests that these proteins will not be found to interact with components that correspond to $T_{\beta\gamma}$.

Interaction with PDEase. Prolonged trypsin treatment of p[NH]ppG-bound T_α produces a polypeptide that has lost both P-I and P-IV but that is still capable of stimulating retinal PDEase (20). Present information does not allow us to localize the PDEase-interaction site more precisely. Perhaps this site resides somewhere between regions C and E (see Fig. 3), an intervening segment of 116 residues in T_α, as compared to only 7 intervening residues in c-Ha-ras1, a protein

that does not interact with PDEase (Fig. 4).

Interaction with R*. In the pivotal reaction of phototransduction, R* binds the α–β–γ complex of transducin and catalyzes replacement of bound GDP by GTP. Binding of GTP leads to dissociation of transducin from R* and of the GTP-bound α subunit from β–γ (1). Pertussis toxin-catalyzed ADP-ribosylation of an amino acid near the carboxyl terminus (23) of T_α prevents this pivotal interaction. The covalently modified transducin exhibits markedly reduced light-dependent GTPase activity and affinity for R*, with a resulting reduction in ability to mediate light stimulation of PDEase (8). These results imply that the carboxyl terminus of T_α plays a critical role in interaction with R*.

The amino acid residue that is ADP-ribosylated by pertussis toxin has not been definitively identified. One report (23) indicated that it was an asparagine residue corresponding to position 346 (Fig. 2). In agreement with a subsequent study (21), we have assigned an aspartate residue to this position. It appears unlikely that posttranslational processing converts Asp-346 to an asparagine residue in preparation for subsequent ADP-ribosylation. Of the other amino acids near the carboxyl terminus, Cys-347 seems most likely to be the residue modified by pertussis toxin.

α Subunits of G Proteins. Recent investigations (21, 38, 39) have begun to discern some of the structural features that may underlie functional homologies among the α subunits of transducin and the G proteins. Trypsin cleaves the native form of a 39-kDa pertussis toxin substrate (G_0) from bovine brain into fragments whose sizes (38) and partial amino acid sequences (21) closely resemble those of tryptic fragments of T_α. In addition, the amino-terminal P-I tryptic fragment of T_α may have a counterpart in the α subunit of G_s: When the α subunit of G_s is occupied by p[NH]ppG in pigeon erythrocyte membranes, treatment with trypsin reduces its size from 42 kDa to 41 kDa, and the 41-kDa fragment is then released from the membrane (39). It is reasonable to speculate that the 1-kDa fragment removed by trypsin from the α subunit of G_s is situated at the amino terminus and that its removal prevents association of the remaining 41-kDa protein with the β–γ complex of G_s, by analogy with the postulated role of peptide P-I in T_α.

It should also be noted that all *ras* genes so far reported—including those of yeast (40), *Drosophila* (41), and mammals (summarized in ref. 42)—encode a cysteine at the position four residues from the carboxyl terminus. *In vitro* mutagenesis of a viral *ras* gene showed that replacement of the cysteine at this position by a serine residue prevents oncogenic transformation by the mutant *ras* gene (42). The curious conservation of this residue in both ras and transducin, as well as the covalent modification catalyzed by pertussis toxin, may point to a critical role of these cysteines in the pivotal interaction of transducin and its homologs with R* and other receptors.

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1. Stryer, L. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48**, 841–852.
2. Manning, D. R. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 7059–7063.
3. Gilman, A. G. (1984) *Cell* **36**, 577–579.
4. Fung, B. K.-K. (1983) *J. Biol. Chem.* **258**, 10495–10502.
5. Cassel, D. & Selinger, Z. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3307–3311.
6. Abood, M. E., Hurley, J. B., Pappone, M.-C., Bourne, H. R. & Stryer, L. (1982) *J. Biol. Chem.* **257**, 10540–10543.
7. Bokoch, G. M., Katada, T., Northup, J. K., Hewlett, E. L. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 2072–2075.
8. Van Dop, C., Yamanaka, G., Steinberg, F., Sekura, R. D., Manclark, C. R., Stryer, L. & Bourne, H. R. (1984) *J. Biol. Chem.* **259**, 23–26.
9. Neer, E. J., Lok, J. M. & Wolf, L. G. (1984) *J. Biol. Chem.* **259**, 14222–14229.
10. Fung, B. K.-K., Hurley, J. B. & Stryer, L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 152–156.
11. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
12. Nathans, J. & Hogness, D. S. (1983) *Cell* **34**, 807–814.
13. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
14. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
15. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
16. Blin, N. & Stafford, D. W. (1976) *Nucleic Acid Res.* **3**, 2303–2309.
17. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
18. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
19. Messing, J., Crea, R. & Seeburg, P. (1984) *Nucleic Acids Res.* **9**, 309–321.
20. Fung, B. K.-K. & Nash, C. R. (1983) *J. Biol. Chem.* **258**, 10503–10510.
21. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D. & Gilman, A. G. (1984) *Science* **226**, 860–862.
22. Van Dop, C., Tsubokawa, M., Bourne, H. R. & Ramachandran, J. (1984) *J. Biol. Chem.* **259**, 696–698.
23. Manning, D. R., Fraser, B. A., Kahn, R. A. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 749–756.
24. Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–873.
25. Halliday, K. (1984) *J. Cyclic Nucleotide Res.* **9**, 435–448.
26. Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. (1978) *Atlas Protein Sequence Struct.* **5**, 345–352.
27. Capon, D., Chen, E., Levinson, A., Seeburg, P. & Goeddel, D. (1983) *Nature (London)* **302**, 33–37.
28. DeFeo-Jones, D., Scolnick, E. M., Koller, R. & Dhar, R. (1983) *Nature (London)* **306**, 707–709.
29. Laursen, R. A., L'Italien, J. J., Nugarkatti, S. & Miller, D. L. (1981) *J. Biol. Chem.* **256**, 8102–8109.
30. Ovchinnikov, Y. A., Alakhov, Y. B., Bundulis, Y. P., Bundille, M. A., Dovgas, N. V., Kozeon, V. P., Motuz, L. P. & Vinokurov, L. M. (1982) *FEBS Lett.* **139**, 130–135.
31. McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984) *Nature (London)* **310**, 644–649.
32. Seeburg, P. H., Colby, W. W., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984) *Nature (London)* **312**, 71–75.
33. Alakhov, Y. B., Motuz, L. P., Stengrevics, O. A. & Ovchinnikov, Y. A. (1978) *FEBS Letts.* **85**, 287–290.
34. Kaziro, Y. (1978) *Biochim. Biophys. Acta* **505**, 95–127.
35. Girshovich, A. S., Bochkareva, E. S., Pozdnyakov, V. A. & Ovchinnikov, Y. A. (1978) *FEBS Letts.* **85**, 283–286.
36. Shih, T. Y., Papageorge, A. G., Stokes, P. E., Weeks, M. O. & Scolnick, E. M. (1980) *Nature (London)* **287**, 686–691.
37. Ho, Y.-K. & Fung, B. K.-K. (1984) *J. Biol. Chem.* **259**, 6694–6699.
38. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806–13813.
39. Hudson, T. H., Roeber, J. F. & Johnson, G. L. (1981) *J. Biol. Chem.* **256**, 1459–1465.
40. Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Strathern, J., Broach, J. & Wigler, M. (1984) *Cell* **36**, 607–612.
41. Neuman-Silberberg, F. S., Schejter, E., Hoffmann, F. M. & Shilo, B.-Z. (1984) *Cell* **37**, 1027–1033.
42. Willumsen, B. M., Norris, K., Papageorge, A. G., Hubbert, N. L. & Lowy, D. R. (1984) *EMBO J.* **3**, 2581–2585.