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control and dense nonneuronal cultures, respectively.
17. To determine protein degradation, we exposed cultures to 2 n*M* [^{35}S]methionine (~1000 Ci/mmol) for 24 hours at 37°C and washed and photoinactivated them. The efflux of 10 percent TCA-soluble material into complete growth medium was then monitored for 48 hours at 37°C. To determine protein synthesis, we photoinactivated cultures, washed them, and measured the incorporation of 30 n*M* [^{35}S]methionine (~1000 Ci/mmol, 37°C) into 10 percent TCA-precipitable material immediately or after 24 hours at 37°C. In addition, no difference in the number or morphology of neurons was observed 24 hours after photoinactivation.
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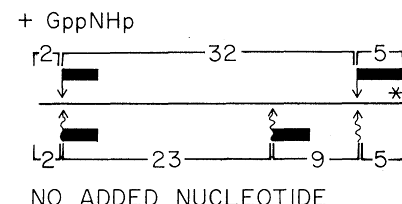
Homologies Between Signal Transducing G Proteins and *ras* Gene Products

Abstract. *The guanosine triphosphate–binding proteins (G proteins) found in a variety of tissues transduce signals generated by ligand binding to cell surface receptors into changes in intracellular metabolism. Amino acid sequences of peptides prepared by partial proteolysis of the α subunit of a bovine brain G protein and the α subunit of rod outer-segment transducin were determined. The two proteins show regions of sequence identity as well as regions of diversity. A portion of the amino-terminal peptide sequence of each protein is highly homologous with the corresponding region in the* ras *protein (a protooncogene product). These similarities suggest that G proteins and* ras *proteins may have analogous functions.*

Cells respond to their environment by means of cell surface receptors that are capable of binding specific ligands. These "signals" are transduced into changes in cellular function and metabolism. One system that mediates the transduction process involves a ubiquitous family of guanosine triphosphate (GTP)–binding proteins (G proteins). These proteins transduce signals generated by ligand interactions with specific cell surface receptors into changes in intracellular levels of cyclic nucleotides (*1*). Different G proteins are found in different kinds of specialized cells. In some types of cells adenylate cyclase is regulated by both a stimulatory G protein (G_s) and an inhibitory protein (G_i) (*2*). In the visual transducing system the G protein analogue, transducin, regulates cyclic guanosine monophosphate concentrations in the rod outer segment by inactivating an inhibitor of a specific phosphodiesterase (*3*). In brain tissue, two G proteins have been found: G_i, and a relatively abundant protein, G_o, whose function is not yet known. The relative ease with which the G_o protein can be purified, however, makes it useful for studies of protein structure (*4*).

G proteins share the following characteristics: They are a complex of three polypeptide subunits termed α, β, and γ. Activated receptors stimulate the α subunit to bind GTP. The α subunit with bound GTP interacts with the effector, for example, adenylate cyclase or the phosphodiesterase inhibitor, and the interaction is terminated when the bound GTP is hydrolyzed to guanosine diphosphate by the α subunit. Finally, the guanosine triphosphatase (GTPase) activity and the receptor coupling properties of the α subunit can be modified by adenosine diphosphate (ADP) ribosylation catalyzed by cholera toxin or pertussis toxin (*5*). The α subunits of the different G proteins appear to be characteristic of each G protein and may be encoded by a family of genes, whereas the β subunit is highly conserved and may be encoded by only one or two genes. For example, the α subunits of G_s, G_i, and transducin have different amino acid compositions, and their peptide maps differ (*6*). However, there is little variation in amino acid composition and peptide maps of the β subunits of these proteins (*6*).

TRANSDUCIN α SUBUNIT:



G_o α SUBUNIT:

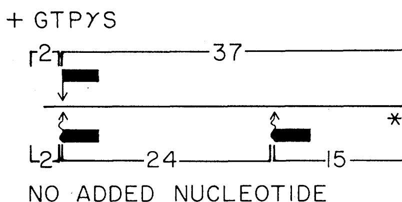


Fig. 1. The distribution of partial proteolytic fragments from the α subunits of transducin and G_o. The black bars indicate the locations on the fragment corresponding to the amino acid sequences that were determined. The wavy arrows represent the cleavage sites in the absence of added nonhydrolyzable guanine nucleotide analog. The G_o was bound to GTPγS (guanosine 5′-[3-*O*-thio]triphosphate) (*4*) and the transducin was bound to GppNHp (guanosine 5′[β,γ-imido]triphosphate) (*18*). The asterisks mark the fragments that were found to be ADP-ribosylated by pertussis toxin. The numbers indicate the size of the fragments in kilodaltons. The amino terminal of the protein is at the left side of the figure.

In order to understand the nature of the genes that encode G proteins, we have determined amino acid sequences from certain G protein subunits. The complete amino acid sequence of the bovine transducin γ subunit and the nucleotide sequence of the corresponding complementary DNA (cDNA) were determined (*7*). The α and β subunits, however, were refractory to Edman degradation suggesting that the NH_2-terminus was "blocked." In this report we describe partial sequences of proteolytic fragments derived from the α subunits of two G proteins, transducin and G_o.

Tryptic proteolysis of the α subunit of transducin results in the formation of a discrete pattern of peptides, which depends on the time of proteolysis and on the nature of the bound guanine nucleotide (*8*). Similar digestion patterns are obtained with the α subunit of the bovine brain G_o protein (*4*) (Fig. 1). These fragments were purified either by high-performance liquid chromatography (HPLC) gel filtration, by electroelution from polyacrylamide gels, or by reversed phase HPLC. The amino acid sequences of the purified peptides were determined by Edman degradation on an automated gas-liquid-solid phase sequenator.

The order of the tryptic fragments for which partial sequences have been determined is outlined in Fig. 1. The order of the proteolytic fragments shown in Fig. 1 is based upon the following arguments.

1) The initial event in proteolysis of both $G_{o\alpha}$ and T_α is a decrease in size from 39 to 37 kD. The 39-kD subunits are blocked, whereas the 37-kD fragments have free amino termini susceptible to Edman degradation. The initial event in proteolysis of the α subunits is thus the removal of about 10 to 20 amino acids from the amino terminus.

2) The 37-kD fragment of T_α can be digested further to 32-kD and 5-kD fragments. The 32-kD fragment has the same amino terminal sequence as the 37-kD fragment, which indicates that it is derived from the amino terminal portion of the 37-kD fragment.

3) T_α is a substrate for ADP-ribosylation by pertussis toxin and α[^{32}P]NAD. The radioactive ADP-ribose group comigrates with the 5-kD tryptic fragment on sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis gels (results not shown). Manning *et al.* (*5*) determined a sequence of eight amino acids surrounding the pertussis toxin ADP-ribosylation site on transducin. They indicated that this site is probably at the carboxyl terminus of the α subunit. In fact, this amino acid sequence is found at the carboxyl terminus of the 5-kD fragment (underlined sequence in Fig. 2).

4) In the absence of bound nonhydrolyzable GTP analogues, the 32-kD fragment of transducin is further digested to a 23-kD and a 9-kD fragment. The 23-kD fragment has the same amino terminal sequence as the 32-kD fragment. Therefore, the 23-kD fragment is derived from the amino terminal portion of the 32-kD fragment.

5) The 37-kD tryptic fragment of $G_{o\alpha}$ can be cleaved further to 24- and 15-kD fragments in the absence of bound nonhydrolyzable GTP analogues. The 24-kD fragment has the same amino terminal sequence as the 37-kD fragment. Therefore, the 24-kD fragment occupies the amino terminal portion of the 37-kD fragment of $G_{o\alpha}$.

The partial sequences of these fragments are shown in Fig. 2. The 32-kD fragment of transducin is homologous to the corresponding 37-kD fragment derived from G_o. The two sequences are, however, distinctive in a region from 6 to 12 amino acids from the amino terminus of each fragment. The partial sequences of the T_α 9-kD and $G_{o\alpha}$ 15-kD fragments are also similar, but differ at 7 out of 22 positions.

The availability of amino acid sequence data allows us to search for homologies with other known sequences. A short amino acid sequence previously determined for the carboxyl terminus of the α subunit of transducin was homologous to a sequence in the middle of the *ras* gene product (*2*). On further comparison, the partial sequence of the large fragment of the α subunit reported here was found to be similar to the amino terminal sequence of Ras and the Ras-related proteins. The homologies are shown in Fig. 3. The amino acid sequence corresponding to the first exon of the N-*ras* gene has 9 out of 22 amino acids that are identical with both the $G_{o\alpha}$ and the T_α sequences. Furthermore, if we include homologous amino acid replacements in the calculation, for example, leucine for isoleucine or valine, then 13 out of 22 amino acids are homologous.

Whereas the precise function of the Ras proteins is not known, their biochemical properties are similar to the properties of the α subunit of the G proteins. Both Ras and G_α are GTP-binding proteins that are associated with the cell membrane (*9*). Both have GTPase activity and both are substrates for phosphorylation (*10*). Both proteins appear to function by interaction with the cytoplasmic portion of cell surface receptors (*11*). Ras and the α subunit of G protein both seem to be encoded by small families of homologous genes (*12*). The amino acid sequence homologies suggest that these families may be derived from a common ancestor. While we do not know the complete amino acid sequence of the α subunit of transducin, there is sufficient sequence data to indicate that the homology with the Ras protein is concentrated in short regions. These may represent subdomains that share similar functions. It is particularly interesting that the glycine at position 12 is conserved in the G proteins. Amino acid substitutions at this position in the Ras protein correlate with the oncogenic activity of *ras* (*13*) and a significant decrease in GTPase activity (*14*). This short stretch of amino acids may specify the structure of a subdomain that regulates the overall protein conformation. The region of G_α that is homologous to Ras also shows strong homology to a region of several adenosine triphosphatases (*15*). Therefore, it is possible that this site plays a role in the nucleotide triphosphate binding or hydrolytic activity of the Ras and G proteins.

In addition to reflecting the similar biochemical properties of the two proteins, the amino acid homologies also suggest similarities in their physiological functions. The G proteins generally act to transduce signals from receptors that have an immediate effect on cellular metabolism. For example, the activation of

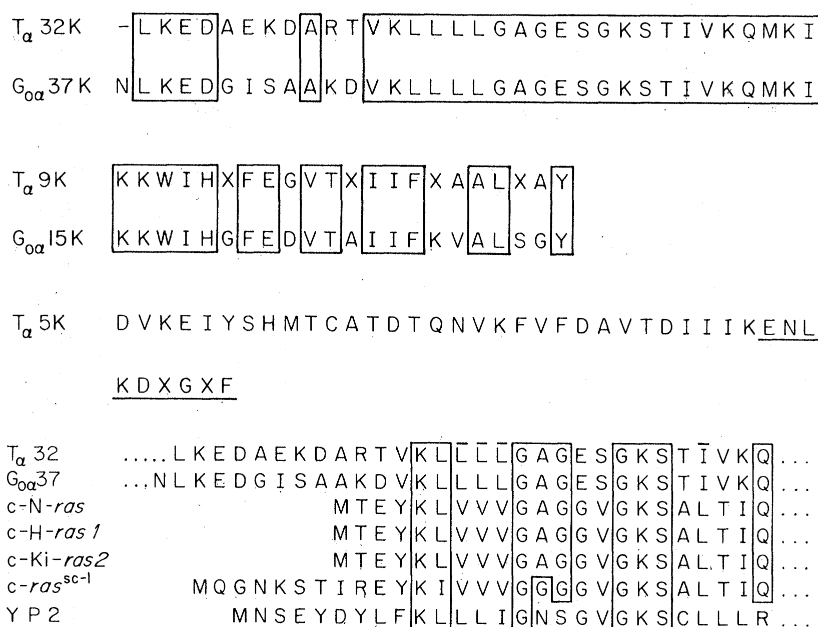


Fig. 2 (top). Partial amino acid sequences of the α subunits of transducin and G_o. Transducin (*18*) and G_o (*4*) were isolated as described. The α subunit of transducin with bound GppNHp was purified by chromatography on heptyl agarose with the use of a gradient of decreasing ammonium sulfate concentration. The α subunit of G_o with bound GTPγS was purified as described (*4*). Proteolysis was done as described (*4, 8*). The proteolytic fragments were isolated either by electroelution from SDS-polyacrylamide electrophoresis gels (*19*), gel filtration on an HPLC column (TSK 3000 SW) in 0.2 percent SDS, 0.2*M* sodium phosphate, *p*H 7.0 or by reversed phase HPLC on an Altex C3 ultrapore column with a gradient of increasing acetonitrile concentration in 0.05 percent trifluoroacetic acid. Automated Edman degradation of the purified fragments was carried out as described (*20*). The boxed sequences indicate identities. The underlined sequence designates the region corresponding to the pertussis toxin ADP-ribosylation site (*5*). This sequence has been deduced from a DNA sequence analysis of a bovine retinal cDNA clone to be ENLKDCGLF (*21*). The aspartic acid residue may be modified posttranslationally to form asparagine before it can be ADP ribosylated or the cysteine residue may be the target for ADP ribosylation. This could explain the previous failure to detect cysteine in the ADP-ribosylated peptide. Single letter abbreviations of 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; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; X, unknown; Y, tyrosine. Fig. 3 (bottom). Comparison of G_α amino terminal with Ras protein (*22*). The boxed sequences indicate identities whereas the dashed lines indicate functional homologies.

transducin by rhodopsin leads to an extremely rapid decrease in cyclic GMP concentrations in the cell which may affect sodium ion permeability in the rod outer segment plasma membrane. The *ras* gene products may be part of a comparable information processing system albeit controlling a different regulatory pathway. There is a growing body of data to suggest that Ras protein mediates signals that regulate cell growth and cell division (*16*). Recent studies have shown that Ras protein is associated with cell surface receptors involved in growth control—for example, the insulin, epidermal growth factor (EGF) (*11*), and possibly transferrin receptors (*17*). The Ras protein may behave like the α subunit of G proteins in that it may cycle through alternative configurations as a result of its association with receptor and guanine nucleotides. Further understanding of the G proteins and the Ras proteins and analysis of the genes that encode them will define in detail the relation between these two gene families.

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Effect of Cell-Cell Interactions on Drug Sensitivity and Growth of Drug-Sensitive and -Resistant Tumor Cells in Spheroids

Abstract. *Multicellular spheroids were grown from mixtures of rat brain tumor cells sensitive (9L) and resistant (R_3) to 1,3-bis(2-chloroethyl)-1-nitrosourea. Percentages of each cell subpopulation in these spheroids were estimated with the sister chromatid exchange assay and were found to be approximately the same as those used to initiate spheroids. Spheroids grown from 9L cells alone had a higher growth rate than spheroids grown from R_3 cells alone. However, the growth rate of mixed-cell spheroids was essentially the same as that of pure 9L spheroids and was independent of the percentages of R_3 cells in mixed-cell spheroids. The sensitivity of 9L cells in mixed-cell spheroids treated with 1,3-bis(2-chloroethyl)-1-nitrosourea, estimated by changes in the number of sister chromatid exchanges per metaphase induced by treatment, decreased as the percentage of R_3 cells increased. These effects are probably the result of an interaction between the two cell subpopulations held in three-dimensional contact, a situation similar to that in tumors in situ. The results suggest why one cell subpopulation of tumors does not become dominant during growth and indicate that interactions between cell subpopulations can influence the sensitivity of one subpopulation to 1,3-bis(2-chloroethyl)-1-nitrosourea.*

Characterization of isolated tumor cell subpopulations has provided important information on the clonal diversity in a tumor and on the basic biology of individual cell subpopulations. However, recent studies (*1*) in which interactions between tumor cell subpopulations were detected suggest that this information may be misleading if attempts are made to discern the behavior of cells in tumors

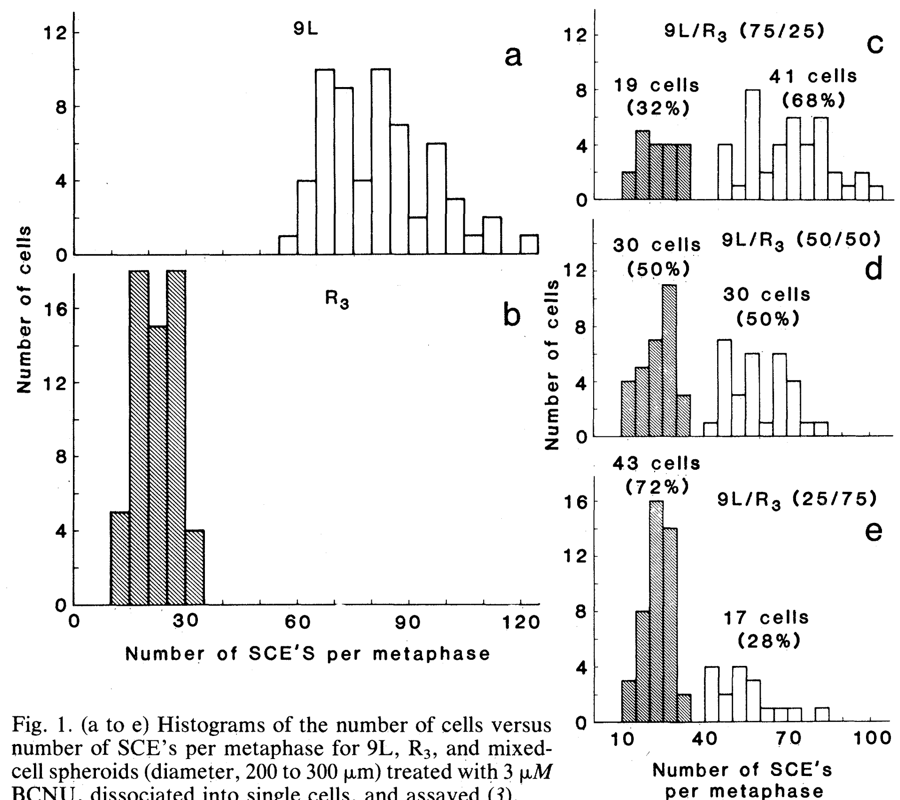

Fig. 1. (a to e) Histograms of the number of cells versus number of SCE's per metaphase for 9L, R_3, and mixed-cell spheroids (diameter, 200 to 300 μm) treated with 3 μ*M* BCNU, dissociated into single cells, and assayed (*3*).