12. D. Moazed and H. F. Noller, *Cell*, in press.
13. D. Moazed, J. M. Robertson, H. F. Noller, *Nature* **334**, 362 (1988).
14. D. Moazed and H. F. Noller, *ibid.* **327**, 389 (1987).
15. ———, *Biochimie* **69**, 879 (1987).
16. D. P. Burma *et al.*, *Arch. Biochem. Biophys.* **239**, 427 (1985).
17. M. Oakes, E. Henderson, A. Scheinman, M. Clark, J. A. Lake, in (*1*), pp. 47–67.
18. G. Stöffler and M. Stöffler-Meilecke, in (*1*), pp. 28–46.
19. M. Boublik *et al.*, in (*1*), pp. 68–86.
20. V. Knauer, R. Hegerl, W. Hoppe, *J. Mol. Biol.* **163**, 409 (1983).
21. S. M. Politz and D. G. Glitz, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1468 (1977).
22. M. R. Trempe, K. Ohgi, D. G. Glitz, *J. Biol. Chem.* **257**, 9822 (1982).
23. H. M. Olson and D. G. Glitz, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3769 (1979).
24. I. N. Shatsky, L. V. Mochalova, M. S. Kojoutiarova, A. A. Bogdanov, V. D. Vasiliev, *J. Mol. Biol.* **133**, 501 (1979).
25. A. G. Evstafieva *et al.*, *EMBO J.* **2**, 799 (1987).
26. P. Gornicki *et al.*, *J. Biol. Chem.* **259**, 10493 (1984).
27. M. S. Capel *et al.*, *Science* **238**, 1403 (1987).
28. C. R. Woese *et al.*, *Nucleic Acids Res.* **8**, 2275 (1980); H. F. Noller and C. R. Woese, *Science* **212**, 403 (1981); C. R. Woese, R. R. Gutell, R. Gupta, H. F. Noller, *Microbiol. Rev.* **47**, 621 (1983); R. R. Gutell, B. Weiser, C. R. Woese, H. F. Noller, *Prog. Nucleic Acid Res. Mol. Biol.* **32**, 155 (1985).
29. P. Stiegler, P. Carbon, J. P. Ebel, C. Ehresmann, *Eur. J. Biochem.* **120**, 487 (1981).
30. R. Brimacombe, J. Atmadja, A. Kyriatsoulis, W. Stiege, in (*1*), pp. 184–202; R. Brimacombe, P. Maly, C. Zwieb, *Prog. Nucleic Acid Res. Mol. Biol.* **28**, 1 (1983).
31. B. J. Van Stolk and H. F. Noller, *J. Mol. Biol.* **180**, 151 (1984).
32. D. Moazed, S. Stern, H. F. Noller, *ibid.* **187**, 399 (1986).
33. H. F. Noller, *Annu. Rev. Biochem.* **53**, 119 (1984).
34. S. Stern, D. Moazed, H. F. Noller, *Methods Enzymol.* **164**, 481 (1988).
35. S. Stern, R. C. Wilson, H. F. Noller, *J. Mol. Biol.* **192**, 101 (1986).
36. S. Stern, L.-M. Changchien, G. R. Craven, H. F. Noller, *ibid.* **200**, 291 (1988).
37. P. Svensson, L.-M. Changchien, G. R. Craven, H. F. Noller, *ibid.*, p. 301.
38. T. Powers, L.-M. Changchien, G. R. Craven, H. F. Noller, *ibid.*, p. 309.
39. S. Stern, T. Powers, L.-M. Changchien, H. F. Noller, *ibid.* **201**, 683 (1988).
40. T. Powers, S. Stern, L.-M. Changchien, H. F. Noller, *ibid.* p. 697.
41. P. Traub and M. Nomura, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 777 (1968).
42. S. Mizushima and M. Nomura, *Nature* **226**, 1214 (1970).
43. W. A. Held, S. Mizushima, M. Nomura, *J. Biol. Chem.* **248**, 5720 (1973).
44. M. Nomura *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **34**, 49 (1969).
45. H. W. Schaup, M. Sogin, C. Woese, C. G. Kurland, *Mol. Gen. Genet.* **114**, 1 (1971); R. A. Zimmermann, A. Muto, P. Fellner, C. Ehresmann, C. Branlant, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1282 (1972); E. Ungewickell, C. Ehresmann, P. Stiegler, R. Garrett, *Nucleic Acids Res.* **2**, 1867 (1975); R. A. Zimmermann *et al.*, *ibid.*, p. 279.
46. R. G. Gregory *et al.*, *J. Mol. Biol.* **178**, 287 (1984).
47. J. Dijk, J. Littlechild, R. A. Garrett, *FEBS Lett.* 77, 295 (1977); M. F. Rhode, S. O'Brien, S. Cooper, K. C. Aune, *Biochemistry* **14**, 1079 (1975).
48. C. Zwieb and R. Brimacombe, *Nucleic Acids Res.* **6**, 1775 (1979); I. Wower and R. Brimacombe, *ibid.* **11**, 1419 (1983); J. Atmadja and R. Brimacombe, *ibid.* **13**, 6919 (1985); J. Atmadja *et al.*, *ibid.* **14**, 659 (1986); W. Stiege, J. Atmadja, M. Zobawa, R. Brimacombe, *J. Mol. Biol.* **191**, 135 (1986); A. Kyriatsoulis *et al.*, *Nucleic Acids Res.* **14**, 1171 (1986); M. Osswald *et al.*, *ibid.* **15**, 3221 (1987); B. Greuer, M. Osswald, R. Brimacombe, G. Stöffler, *ibid.*, p. 3241.
49. S. Stern, B. Weiser, H. F. Noller, *J. Mol. Biol.* **204**, 447 (1988).
50. M. Oakes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 275 (1986).
51. M. Cantrell and G. R. Craven, *J. Mol. Biol.* **115**, 389 (1977); T. G. Fanning, M. Cantrell, C.-Y. T. Shih, G. R. Craven, *Nucleic Acids Res.* **5**, 933 (1978); C. Chang and G. R. Craven, *J. Mol. Biol.* **117**, 401 (1977); I. Fiser, K. H. Scheit, G. Stöffler, E. Kuechler, *FEBS Lett.* **56**, 226 (1975).
52. D. P. Rummel and H. F. Noller, *Nature New Biol.* **245**, 72 (1973); G. Thomas, R. Sweeney, C. Chang, H. F. Noller, *J. Mol. Biol.* **95**, 91 (1975); M. Shimizu and G. R. Craven, *Eur. J. Biochem.* **61**, 307 (1976); L. L. Randall-Hazelbauer and C. G. Kurland, *Mol. Gen. Genet.* **115**, 234 (1972).
53. M. Ozaki, S. Mizushima, M. Nomura, *Nature* **222**, 333 (1969); E. A. Birge and C. G. Kurland, *Science* **166**, 128 (1969).
54. L. Gorini, *Nature New Biol.* **234**, 261 (1971).
55. P. E. Montandon, R. Wagner, E. Stutz, *EMBO J.* **5**, 3705 (1986).
56. J. Davies, W. Gilbert, L. Gorini, *Proc. Natl. Acad. Sci. U.S.A.* **51**, 659 (1964).
57. L. Gorini, in *Ribosomes*, M. Nomura, A. Tissières, P. Lengyel, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1974), pp. 791–804.
58. P. Melancon, C. Lemieux, L. Brakier-Gingras, *Nucleic Acids Res.* **16**, 9631 (1988).
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Research Articles

G_{olf}: An Olfactory Neuron Specific–G Protein Involved in Odorant Signal Transduction

DAVID T. JONES AND RANDALL R. REED

Biochemical and electrophysiological studies suggest that odorants induce responses in olfactory sensory neurons via an adenylate cyclase cascade mediated by a G protein. An olfactory-specific guanosine triphosphate (GTP)–binding protein α subunit has now been characterized and evidence is presented suggesting that this G protein, termed G_{olf}, mediates olfaction. Messenger RNA that encodes $G_{olf\alpha}$ is expressed in olfactory neuroephithelium but not in six other tissues tested. Moreover, within the olfactory epithelium, $G_{olf\alpha}$ appears to be expressed only by the sensory neurons. Specific antisera were used to localize $G_{olf\alpha}$ protein to the sensory apparatus of the receptor neurons. $G_{olf\alpha}$ shares extensive amino acid identity (88 percent) with the stimulatory G protein, $G_{s\alpha}$. The expression of $G_{olf\alpha}$ in S49 cyc^- kin^- cells, a line deficient in endogenous stimulatory G proteins, demonstrates its capacity to stimulate adenylate cyclase in a heterologous system.

THE VERTEBRATE OLFACTORY SYSTEM IS EXQUISITELY adapted for the detection and recognition of small molecule odorants. For example, olfactory receptor cells can distinguish the subtle differences between chemical stereoisomers and are sensitive to some odorants at airborne concentrations of parts per trillion (*1*, *2*). Olfaction is probably the oldest means of sensory interaction with the external environment. Although our understanding of other sensory transduction systems (that is, vision and audition) is becoming substantial, little is known about the molecular basis of olfaction.

The vertebrate olfactory mucosa contains several million sensory neurons that reside in a psuedostratified columnar epithelium (*3*).

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Each bipolar neuron projects a single unmyelinated axon to the olfactory bulb and a single dendrite to the epithelial surface. Each dendrite terminates in a dendritic knob, which projects specialized cilia into the nasal lumen. The primary events of olfactory signal transduction are believed to occur within these specialized cilia.

Biochemical studies suggest that, for at least some odorants, olfactory signal transduction is mediated by an adenylate cyclase cascade coupled through a stimulatory G protein (*4*, *5*). Components of this cascade appear to be enriched within the sensory cilia (*6*, *7*). In addition, patch-clamp recording studies demonstrate that olfactory cilia have cyclic nucleotide–gated channels that open in response to increases in cyclic nucleotide concentrations (*8*). These observations provide evidence for a model of olfactory signal transduction in which odorants lead to a local increase in cyclic adenosine monophosphate (cyclic AMP) concentration and the subsequent cyclic AMP–dependent depolarization of the sensory neuron.

GTP-binding proteins (G proteins) are heterotrimers ($\alpha\beta\gamma$) that couple membrane-bound receptors to second-messenger enzymes or ion channels (*9*, *10*). It is the α subunit that confers indentity to the multimer and, in most systems, governs the specificity of the interaction with receptor and effector molecules. Accordingly, there are several different types of α subunits including $G_{s\alpha}$ and $G_{i\alpha}$, which respectively stimulate and inhibit adenylate cyclase, $G_{o\alpha}$, a G protein present in relatively large amounts in brain tissue, but with no assigned function, and transducin (T_α), a G protein specific for the visual system. The β and γ subunits copurify as a tightly associated complex that in vitro appears to function interchangeably between different α subunits.

In a previous effort to identify the G protein component or components of olfaction, we used a partially degenerate oligonucleotide directed toward a highly conserved G_α subunit domain to screen a rat olfactory cDNA library (*11*). Five distinct cDNA's encoding $G_{s\alpha}$, $G_{o\alpha}$, and $G_{\alpha 1\text{-}3}$ were identified. Of the proteins encoded by these five clones, only $G_{s\alpha}$ has the capacity to activate adenylate cyclase. Although $G_{s\alpha}$ messenger RNA (mRNA) is

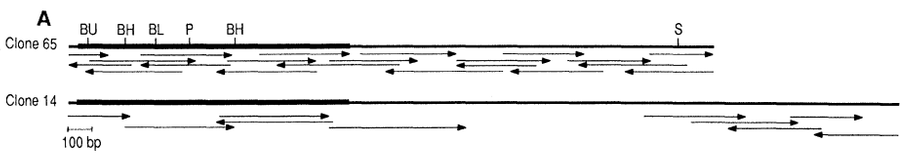

Fig. 1. Rat $G_{olf\alpha}$ cDNA sequence. (**A**) Sequencing strategy for cDNA inserts 14 and 65. The thick lines indicate coding sequence; the arrows indicate the extent and orientation of individual sequencing reactions. Clone 65 was sequenced completely on both strands. The overlap regions between clones 14 and 65 are identical in sequence. BU, Bst UI; BH, Bam HI; BL, Bal I; P, Pvu II; S, Sph I. (**B**) The complete nucleotide sequence of $G_{olf\alpha}$ including the terminal 800 base pairs found in clone 14. The site of polyadenylation in clone 65 is marked. The predicted amino acid sequence is indicated below the nucleotide sequence. A λgt10, rat olfactory cDNA library (*11*) was screened with (i) a mixture of the coding regions of five ^{32}P-labeled (*29*) G_α subunit–encoding cDNA's ($G_{s\alpha}$, $G_{o\alpha}$, and $G_{i\alpha 1\text{-}3}$) (*11*) or (ii) a fully degenerate, ^{32}P–end labeled oligonucleotide (5′-TCAT$^{C}_{T}$TG$^{C}_{T}$TTXAC-ZATXGT; where X is any nucleotide, and Z is G, T, or A). For the cDNA mixture, filters were hybridized under standard conditions [5× SSC (standard saline citrate), 5× Denhardt, 0.1 percent SDS, and salmon sperm DNA (50 μg/ml)] at 50°C and washed at 55°C in 2× SSC, 0.1 percent SDS. Filters were hybridized to the oligonucleotide mixture at 37°C and washed at 50°C as described (*30*). Hybridization with the individual cDNA probes was performed under standard conditions at 65°C, with subsequent washing in 0.1× SSC, 0.1 percent SDS at 68°C. The Eco RI inserts for cDNA's 14 and 65 were subcloned into the Eco RI site of Bluescript (Stratagene) and sequenced with [^{35}S]dATP as described (*31*).

B

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                                                                    1 GTCTCGCTGGCTCTGGTGCGCCTTCCTACTTGCCTGATCGGAGTGCGAGCCAGCAGGC

 59 ATG GGG TGT TTG GGC AAC AGC AGC AAG ACC GCG GAA GAT CAG GGC GTG GAT GAA AAG GAA CGC CGG GAG GCC AAC AAA AAG ATC GAG AAG
  1 MET Gly Cys Leu Gly Asn Ser Ser Lys Thr Ala Glu Asp Gln Gly Val Asp Glu Lys Glu Arg Arg Glu Ala Asn Lys Lys Ile Glu Lys

149 CAG TTG CAG AAA GAG CGC CTG GCT TAC AAA GCG ACC CAC CGC CTG CTG CTT CTG GGG GCT GGT GAG TCC GGG AAA AGC ACT ATA GTC AAA
 31 Gln Leu Gln Lys Glu Arg Leu Ala Tyr Lys Ala Thr His Arg Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val Lys

239 CAG ATG AGG ATC CTA CAC GTC AAT GGC TTC AAC CCC GAG GAA AAG AAG CAG AAA ATT CTG GAC ATC AGG AAA AAT GTC AAA GAT GCT ATT
 61 Gln MET Arg Ile Leu His Val Asn Gly Phe Asn Pro Glu Glu Lys Lys Gln Lys Ile Leu Asp Ile Arg Lys Asn Val Lys Asp Ala Ile

329 GTG ACA ATC ATT TCA GCA ATG AGT ACC ATA ATA CCT CCA GTT CCA CTG GCC AAC CCT GAG AAC CAG TTT CGG TCA GAT TAC ATC AAG AGC
 91 Val Thr Ile Ile Ser Ala MET Ser Thr Ile Ile Pro Pro Val Pro Leu Ala Asn Pro Glu Asn Gln Phe Arg Ser Asp Tyr Ile Lys Ser

419 ATA GCC CCT ATC ACT GAC TTT GAA TAT TCC CAG GAG TTC TTT GAC CAC GTG AAG AAG CTG TGG GAT GAT GAG GGA GTG AAG GCC TGC TTT
121 Ile Ala Pro Ile Thr Asp Phe Glu Tyr Ser Gln Glu Phe Phe Asp His Val Lys Lys Leu Trp Asp Asp Glu Gly Val Lys Ala Cys Phe

509 GAG AGA TCC AAC GAG TAC CAG CTG ATC GAC TGT GCA CAA TAC TTC CTG GAA AGG ATT GAC AGC GTG AGT CTG GTT GAC TAC ACA CCC ACA
151 Glu Arg Ser Asn Glu Tyr Gln Leu Ile Asp Cys Ala Gln Tyr Phe Leu Glu Arg Ile Asp Ser Val Ser Leu Val Asp Tyr Thr Pro Thr

599 GAC CAG GAC CTA CTC AGA TGC AGA GTG CTG ACA TCA GGG ATC TTT GAG ACA CGA TTC CAA GTG GAC AAA GTG AAC TTT CAC ATG TTT GAC
181 Asp Gln Asp Leu Leu Arg Cys Arg Val Leu Thr Ser Gly Ile Phe Glu Thr Arg Phe Gln Val Asp Lys Val Asn Phe His MET Phe Asp

689 GTT GGA GGC CAG AGG GAT GAG AGA AGA AAA TGG ATC CAG TGT TTT AAC GAT GTC ACT GCC ATC ATC TAT GTG GCA GCC TGC AGC AGC TAC
211 Val Gly Gly Gln Arg Asp Glu Arg Arg Lys Trp Ile Gln Cys Phe Asn Asp Val Thr Ala Ile Ile Tyr Val Ala Ala Cys Ser Ser Tyr

779 AAC ATG GTG ATC CGG GAA GAT AAC AAC ACC AAC AGA CTC CGG GAG TCG CTG GAC CTG TTT GAA AGC ATC TGG AAT AAC AGG TGG TTA CGA
241 Asn MET Val Ile Arg Glu Asp Asn Asn Thr Asn Arg Leu Arg Glu Ser Leu Asp Leu Phe Glu Ser Ile Trp Asn Asn Arg Trp Leu Arg

869 ACC ATT TCC ATC ATC CTG TTC TTG AAC AAA CAA GAT ATG CTG GCA GAA AAA GTC TTG GCC GGG AAG TCA AAA ATT GAA GAC TAT TTC CCG
271 Thr Ile Ser Ile Ile Leu Phe Leu Asn Lys Gln Asp MET Leu Ala Glu Lys Val Leu Ala Gly Lys Ser Lys Ile Glu Asp Tyr Phe Pro

959 GAG TAT GCC AAC TAT ACT GTC CCT GAA GAT GCA ACA CCA GAT GCA GGA GAA GAT CCC AAA GTT ACA AGA GCC AAG TTC TTT ATC CGG GAT
301 Glu Tyr Ala Asn Tyr Thr Val Pro Glu Asp Ala Thr Pro Asp Ala Gly Glu Asp Pro Lys Val Thr Arg Ala Lys Phe Phe Ile Arg Asp

1049 CTG TTC TTG AGG ATC AGC ACA GCC ACG GGT GAT GGC AAA CAT TAC TGC TAC CCT CAC TTC ACC TGC GCC GTG GAC ACA GAG AAC ATC CGC
 331 Leu Phe Leu Arg Ile Ser Thr Ala Thr Gly Asp Gly Lys His Tyr Cys Tyr Pro His Phe Thr Cys Ala Val Asp Thr Glu Asn Ile Arg

1139 AGA GTG TTC AAC GAT TGT CGT GAC ATC ATC CAG AGA ATG CAC CTC AAG CAG TAC GAA CTC TTG TGA GGGCCACCCACCCACCCCTCCTCCTCCTCAC
 361 Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln Arg MET His Leu Lys Gln Tyr Glu Leu Leu END

1236 TGCTGCCTTTCCTCCCTCTTGACCACCCCATGAGGCAGCATATCCCCCTAGCCTGCGTGTCTGTCCACCCCGAGCCAAGGTAGGGAGTAGTGAGTGTCTAGTGTCATCGGACTGCCGTC
1355 TGTCCTGTCCTAGGTATGCCTATGTGTGACCACCAAGCCTCTGGCTACCTCTGTTCCCCAAGGTTTGGTTCTGTGACTTTGTTTTCACTGGACAAAACAGCCTCCCACCACCAATTTGT
1474 ATCCCCGTATCACCCTCTGGGTGGTACTGCAGTGGATTTTCTCTGGGTGGGAATCTATTTATTCTTTGATGGAATGGTTGATGGGTTGAATCATCAAAGACAGCTTGGTGAACGGGGGA
1593 AATACATTGTTTTCAAGTTATCAAGCATGATCACAAAACTGTCCGGACAGTGCCACGCACTTGGTTTTTCCCTTTATAAAAGGTTGGGCTACCCAGTGTCTGCCCCTTCTGGTCTGCAT
1712 TGGCCCAGGGCTTAAAGCAGATCCACGTCAGCAACAGAAGACGAGGACCTTTTTCAGCCACAGTTGCAATGGCTATAAGCCTTGGGCATTTGGGTTAGAGCACTTCTGGGAGCCTTTTA
1831 CACTTGGGCAGAAAGCCGGCCACATTCACTACTAATGGTGTCCTGTCTATAAACAAGGGGTCCTCTACACCTTCCTGATAGCTGGGCATAGTCCTTCCTGTCTTCTTTCTGTTGTTGCT
1950 TTACCAGACTGTACAGACCACAAGATGTACTACTCTTTTCTGTGGCTACTCCAGAAGCCTCCTTGTAGAGTTCCGTGCCTTGGTGGCGGCTATACCTGTAAGGAAACGTGTCCCTCCTG
2069 TGCCAAGCAGCCTGCAGTCCACACAGCCTGCTGCTTCACAGTGGGGAATCAGTAGTATCTGCCCATCTTAGGATATTTTTATCAGGTTGGCACTTATGGAAGACTCGGTGATTTTAGAA
2188 TCATCCACTTTACCAGCAGTATCTTCCCAACACTGAAACTGTTGCAGCCACCCGCTTTTACTGCTCCAAACCTGAGCTGCTGCACACATTGGGATGACCCGCTTTCAAGGCTTAAATTA
2307 CAATCAATTCTTTTTTCAGGGTTCTAAGGGGTTCCCCCCCCACTCCCCTCTCAAGGTGATCTGTTTTCAATGACCCCTTCAAGTTCTGTAGTCAGGAGCAGGCCAACCATCCCTTAGTTA
2426 AGTTCTACACACAGGCCACCAAACAAGAAACCGGTGGTCAGAGGAAAGCACAGGGGTGTCCATGTTGTATATGGCTGAGGACAATAAGGATGATGTCATCTGAAGATGAAATATACAAA
2545 ACTACTTATTTATTATCTGTCTCTCTATTTGCTAGGGATGAGCCCAGCACCTAGTGCATGCTAGGCAAGCACTCTACAGCTAGTCCATATCCCCAGCCACAACTCCAGCCCTCACAAAA
                                                                  ┌poly-A site (clone 65)
2664 ACCTTTATTTTAAATATAAAGACAAAATAAATGGCTTCTCCTCACATGTGTAAGATCTGCGAATACAACCTCAAGTTATGGTAGGACACCTAGAAAACGACACAGCACATACTGTCTAA
2783 GCCTGAGGCTTTGGGGGTCATTTCTCTTAGGCTTAAAGTTACCTGTAAAGATGTCCCAAGTTAAGTTTCTAACAGTACAGATAGGAAGGAGTGACAGCCTGCAATTCTCCTTATTGTTG
2902 CTTTAAGTTTATTCCGTAAACTGGCATTGTAGAACTCTCTAAAAACTCATCCTGGCCGAGATGTTCTTCCGAGAAAGGGTACAGCACTTTCCCCAGCTCCTCACCACTGTCTTCTGGAA
3021 CCATTCCCTAGCATTGCATAAAACTTCCCTCTGAAGCCAGGCAATGAGGGTGCACACCTTTAATCTCAGCGCTCGGGAAAACAGACAGGCACATCTCAGTGTTCGAGGCCAGCCTGGTC
3140 TACAGAGTGAGTTCCAGGACAACTAGTGCTTCACAGAGAAACCCTGTCTCAAACAAAACAAACTAACTTCTGTCTGAACCAACACATGAAATGACAGCGGCTTTCAGTTCCCCCTGCAC
3259 TCTCAAAGCAAAGCAAGAAGAAAAACTTGCACACACACACACACACACACACACACACACACACGTGTGAGGTTTTATACCACAGTGTGCATTACTACTCTACAGTTCCCTGC
3378 TGACTGAATGACTATTGAAGAGTTTCGTTGGGGCTGAATGTGTAACTCTGACGGAGCACATGCTCAGCATGCGTGAGACCTGAGATCAATCAAGAGAAACGAGATATAAATAAAATCAA
3497 ATCATTAGAAAAGAAAAA 3514
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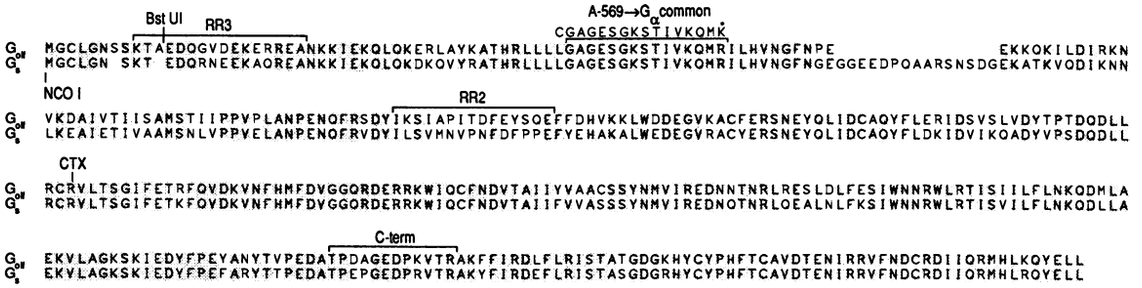

Fig. 2. Alignment of $G_{olf\alpha}$ with the large subunit of $G_{s\alpha}$ (52 kD). Amino acid identities are shaded. The COOH-terminal region, an identifying characteristic of $G_{olf\alpha}$ and $G_{s\alpha}$, is indicated (C-term). The cholera toxin site (CTX) in G_s is indicated. $G_{olf\alpha}$ specific peptides RR2 and RR3 and the G_α common peptide are indicated. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

abundantly expressed in olfactory tissue, we have shown by a neuron depletion technique that most of the $G_{s\alpha}$ mRNA in olfactory tissue is derived from nonneuronal cells (*12*). Therefore, G_s is unlikely to play a role in olfactory signal transduction, which suggests, perhaps, that another G protein may be involved. In an attempt to identify G_α subunit–encoding cDNA species that were not detected in the original library screen, we screened the rat olfactory cDNA library by a more generalized method. We now describe the identification and characterization of a new G_α subunit that is expressed abundantly and exclusively by olfactory sensory neurons. This G_α subunit can activate adenylate cyclase in a heterologous system and is likely to be the G protein component of olfactory signal transduction.

In order to identify G_α subunit–encoding cDNA's that were not detected in our original screen (*11*), we used (i) low stringency hybridization with a combination of the five G_α cDNA species identified previously, and (ii) hybridization with a fully degenerate oligonucleotide directed toward a portion of a highly conserved GTP-binding domain. Altogether 150 hybridizing plaques were identified from a screen of 60,000 independent recombinants. Those clones that represented $G_{s\alpha}$, $G_{o\alpha}$, or $G_{i\alpha 1\text{-}3}$ were revealed by independent hybridization with each of the ^{32}P-labeled G_α cDNA's at high stringency. Elimination of the known G_α cDNA's left a single class of 29 clones that hybridized weakly with $G_{s\alpha}$. This class was detected by both of the above approaches, but was apparently not identified in the original screen because of insufficient homology with the previous oligonucleotide probe. The cDNA inserts from two of these clones (No. 14 and No. 65) were subcloned into a plasmid vector for further analysis.

Comparison of the nucleotide sequences (Fig. 1) revealed that the above-mentioned two inserts are essentially identical and differ only in the utilization of alternative polyadenylation sites. An open reading frame of 381 amino acids encodes a predicted protein (44 kD), which contains the highly conserved guanine nucleotide binding motifs present in all other G proteins (*13*). The location of the initiating methionine was identified by homology to other Gα subunit proteins, the conservation of the purines at positions −3 and +4 important for translation as described by Kozak (*14*), and the lack of any upstream initiation codons.

The predicted protein shares striking amino acid identity with $G_{s\alpha}$ (88 percent) (Fig. 2). $G_{s\alpha}$ exists in multiple forms (45 kD and 52 kD) (*15*) which are produced from the same transcript by alternative splicing of a 45-base exon (*16*). Sequence analysis indicates the novel G_α subunit lacks this alternatively spliced 45-base exon, and we have no evidence that its mRNA undergoes alternative splicing. The predicted protein contains a region identical in size to a 12–amino acid sequence (C-term, Fig. 2) that distinguishes $G_{s\alpha}$ from the other known G_α subunits. Amino acids in this region may play a role in receptor interaction (*17*).

Distribution and identification of $G_{olf\alpha}$. The tissue distribution of the new G_α subunit was determined by RNA blot analysis. In order to avoid cross-hybridization with $G_{s\alpha}$ mRNA, we hybridized RNA blots with probes derived from the 3′ untranslated region. We detected two mRNA species, 2.7 and 3.5 kilobases (kb), that are expressed abundantly and exclusively in olfactory tissue (Fig. 3A). These messages correspond to the two full-length cDNA's described above. On the basis of this olfactory-specific expression, we have named this new G_α subunit $G_{olf\alpha}$.

It was then necessary to ascertain which of the several distinct cell types within the olfactory epithelium express $G_{olf\alpha}$ mRNA. The sensory neurons of the olfactory epithelium degenerate 6 to 8 days after the target tissue, the olfactory bulb, is removed. Specifically, removal of one side of the olfactory bulb leads to ipsilateral degeneration of the receptor neurons within the olfactory mucosa

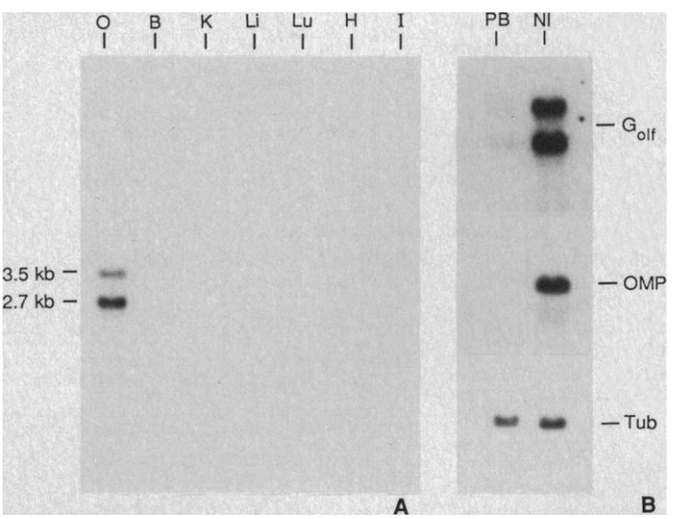

Fig. 3. RNA analyses. (**A**) Expression of $G_{olf\alpha}$ mRNA in various tissues. O, olfactory; B, brain from which the olfactory bulb had been dissected away; K, kidney; Li, liver; Lu, lung; H, heart; and I, intestine. (**B**) Differential RNA blot. Effect of bulbectomy on $G_{olf\alpha}$ mRNA in olfactory tissue. The hybridizing regions from three similar blots are presented. Nl, olfactory RNA isolated from normal animals; PB, olfactory RNA isolated 8 days after bulbectomy; OMP, olfactory marker protein; Tub, tubulin. RNA was isolated, resolved on agarose gels, and transferred to filters (*11*). Each lane contained 10 μg of total RNA. The tissue blot was hybridized to a ^{32}P-labeled (*29*) 3′ untranslated Nae I–Hind III fragment of clone 65 under standard conditions at 42°C with 50 percent formamide and washed in 0.2× SSC, 0.1 percent SDS at 65°C. The differential blot was hybridized at 37°C to a ^{32}P-labeled oligonucleotide derived from the 3′ untranslated region of $G_{olf\alpha}$ (5′ - TGGCTGGGGATATGGACTAGCTGTAGAGTGCTTGCCT-AGCATGCAC) and washed at 45°C in 2× SSC, 0.1 percent SDS. The OMP and tubulin differential blot procedures have been described (*12*). Blots were probed with ribosomal RNA in order to ascertain that equal amounts of total RNA were placed in each lane.

(*18*). We used this neuronal depletion technique to determine whether $G_{olf\alpha}$ mRNA is expressed by the sensory neurons. The mRNA encoding the olfactory marker protein (OMP), an abundant 19-kD protein expressed solely in olfactory neurons (*19*), was at least 20 times lower 8 days after bulbectomy (Fig. 3B). In a similar blot preparation, the $G_{olf\alpha}$ mRNA also decreased dramatically (>20 times less), suggesting that its message was derived from the olfactory neurons (Fig. 3B). These RNA analyses demonstrate that $G_{olf\alpha}$ is expressed abundantly and exclusively in olfactory tissue and that its mRNA is derived primarily, if not exclusively, from the sensory neurons.

The expression of both $G_{s\alpha}$ and $G_{olf\alpha}$ in olfactory tissue and the extreme similarity between these two proteins required the use of an antiserum of high specificity for the detection of $G_{olf\alpha}$. Antisera to peptides specific for $G_{olf\alpha}$ were assayed by probing immunoblots of bacterially expressed $G_{s\alpha}$ or $G_{olf\alpha}$ β-galactosidase fusion proteins. An antiserum (A-569) (*20*) to a peptide common to $G_{s\alpha}$ and $G_{olf\alpha}$ was used to quantify the abundance of each fusion protein. Comparison of the immunoreactivity of the $G_{s\alpha}$ or $G_{olf\alpha}$ fusion proteins with affinity-purified antiserum to $G_{olf\alpha}$ (DJ5.2AP) demonstrated the high specificity of this antiserum (Fig. 4A). Cross-reactivity was undetectable even with larger amounts (100 times) of the $G_{s\alpha}$-fusion protein.

The $G_{olf\alpha}$ antiserum was used to analyze immunoblots of protein extracts derived from the olfactory turbinates of normal (Nl) or bulbectomized (PB) animals. The absence of G_{olf} protein after bulbectomy was parallel to the decrease in mRNA (Fig. 4B). This antiserum was also used to analyze immunoblots of partially purified olfactory sensory cilia (Fig. 4B). $G_{olf\alpha}$ is enriched in these cilia preparations, and this immunoreactivity could be blocked by prior adsorption with free peptide.

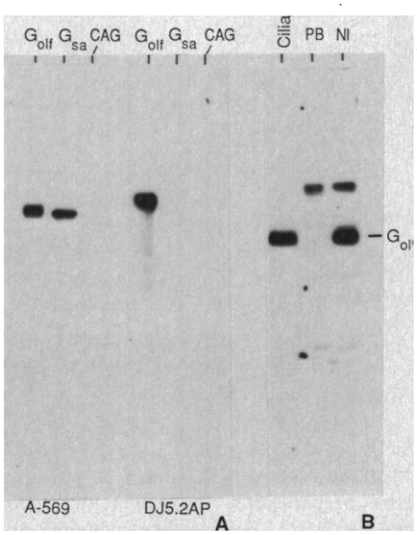

Fig. 4. Immunochemical analyses. (**A**) Immunoblot of bacterially expressed $G_{olf\alpha}$ or $G_{s\alpha}$ β-galactosidase fusion proteins (G_{olf}, G_s) detected with the G_α common antiserum A-569 (1:1000) (*20*) or antiserum DJ5.2AP (1:200), an antiserum to $G_{olf\alpha}$. CAG, control protein derived from the nontransfected CAG–456 host strain. (**B**) Differential immunoblot probed with DJ5.2AP (1:200). Cilia, purified cilia (5 μg per lane); Nl, olfactory protein prepared from normal olfactory turbinates (30 μg per lane); PB, olfactory protein prepared from olfactory turbinates 8 days after bulbectomy (30 μg per lane). Fusion proteins were prepared by ligating the appropriate length Eco RI linker to the Bst UI site of clone 65 (Fig. 2) or the filled-in Nco I site of $G_{s\alpha}$ (45 kD) (*11*) (Fig. 2), subcloning the fragments into the single Eco RI site of pUCX1 (*32*) and transfection into *E. coli* CAG-456 (*33*). The preparation of olfactory protein and the isolation of olfactory cilia were essentially as described (*12, 34*). Two $G_{olf\alpha}$ specific peptides were independently cross-linked to BSA with glutaraldehyde (RR2, CIKSIAPITDFEYSQE) or separately with glutaraldehyde and *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (RR3, CYKTAEDQGVDEKERREA) (*35*), and used to immunize rabbits. Both peptides produced antisera specific to $G_{olf\alpha}$. Antisera DJ5.2 and DJ6.3, to peptide RR3, were affinity-purified (*36*) yielding DJ5.2AP and DJ6.3AP. Both affinity-purified antisera behaved similarly in all analyses. The *E. coli* cell pellets or partially purified protein extracts were resolved by SDS-PAGE and transferred (*12*).

The subcellular distribution of $G_{olf\alpha}$ within particular cell types of the olfactory mucosa was examined. Immunocytochemical analysis localizes $G_{olf\alpha}$ localizes to the sensory neurons. Intense staining is observed along the epithelial surface and in the membrane-dense axon bundles, while the neuronal soma stain very weakly (Fig. 5, A and C). This immunocytochemical staining was specific for $G_{olf\alpha}$ and was blocked when the antiserum had been first absorbed with free peptide (Fig. 5B). The antiserum (DJ6.3AP) used for immunocytochemical analysis does not detect the non-specific 55-kD protein seen in Fig. 4B. The reduced staining in the axon bundles of the treated side is also observed for olfactory marker protein (*12*) and probably results from residual immunoreactivity in the degenerating axon bundles. As would be expected if $G_{olf\alpha}$ expression were confined to the sensory apparatus, the intense epithelial surface (ES) staining disappears after bulbectomy (Fig. 5A). At higher magnification, the intense surface staining can be seen to coincide with the dendritic knobs and collapsed sensory cilia that survived fixation and embedding. These analyses indicated that $G_{olf\alpha}$ is localized to the sensory neurons and suggest that G_{olf} may be enriched in the presumptive sensory structures of these neurons.

Expression of $G_{olf\alpha}$. In order for G_{olf} to play a role in olfaction it must not only be expressed in the sensory neurons but also fulfill at least two biochemical criteria. First, it must couple to odorant receptors, and second, it must be able to stimulate the formation of cyclic AMP. The first criterion cannot be tested in that odorant receptors have not yet been identified. The second criterion can be addressed by expressing $G_{olf\alpha}$ in a murine lymphoma cell line that is deficient in $G_{s\alpha}$. The cell line, S49 cyc^- kin^-, which is refractory to β-adrenergic stimulation because of the lack of $G_{s\alpha}$ (*21*), provides an ideal experimental system in which to measure the efficacy of adenylate cyclase activation by exogeneously introduced G proteins (*22*).

We infected S49 cyc^- kin^- cells with a recombinant retrovirus encoding either $G_{olf\alpha}$ or $G_{s\alpha}$ (45 kD) and isolated clonal derivatives by limiting dilution. Genomic DNA blot analysis confirmed that each stably infected cell line was derived from a single, independent integration event. RNA blot analysis again showed that stimulatory G protein expression in the recombinant cell lines was not the result of reversion of the S49 cyc^- phenotype. Each of the transfected cell lines displays a retroviral genomic transcript and several smaller retroviral transcripts but no detectable message characteristic of the endogenous $G_{s\alpha}$ gene (Fig. 6, A and B). We therefore concluded that the frequency of reversion is insignificant and that $G_{s\alpha}$ or $G_{olf\alpha}$ protein expressed in the transfectants is derived from the retroviral integrant.

Ligand-independent G protein activation can be achieved by the addition of aluminum fluoride (AlF_4^-) to membranes that contain G proteins. Membranes prepared from cell lines that express $G_{s\alpha}$ but not control (cyc^- kin^-) membranes show an increase in adenylate cyclase activity in response to AlF_4^- (Fig. 6C). As with the $G_{s\alpha}$ transfectants, AlF_4^- stimulates adenylate cyclase activity in membranes prepared from $G_{olf\alpha}$ expressing cells. The loading of a nonhydrolyzable GTP analogue, GTP-γ-S, is catalyzed by ligand-bound receptor. This analog stimulates adenylate cyclase activity more efficiently in membranes prepared from lines that express $G_{s\alpha}$ than from cell lines expressing $G_{olf\alpha}$, but it is unclear whether this represents differences in receptor-catalyzed activation of the G protein or G protein interaction with adenylate cyclase. In addition, we have not determined the amount of recombinant G_α protein expressed in these cell lines. Nevertheless, the above data demonstrate that $G_{olf\alpha}$ is capable of stimulating adenylate cyclase activity as required for olfactory signal transduction.

The presence of an abundant, stimulatory G protein confined to the sensory neurons provides strong support for a model of

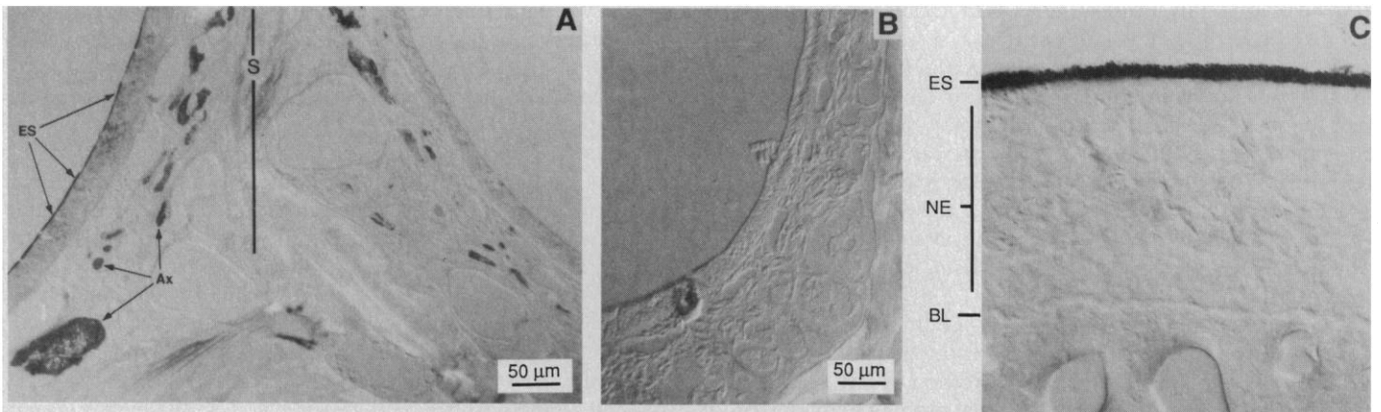

Fig. 5. Immunocytochemistry. (**A**) Olfactory turbinates from a unilaterally bulbectomized animal; the preparation was stained with anitserum DJ6.3AP (1:10). The septum (S) separates the normal side (left) from the neuron-depleted side (right). Ax, axon bundles; ES, epithelial surface; NE, neuroepithelial layer; BL, basal lamina. (**B**) Staining of normal olfactory epithelium with antiserum DJ6.3AP after prior adsorption with peptide RR3. (**C**) High magnification micrograph of epithelial region stained with antiserum DJ6.3AP. Immunohistochemical staining was performed on PLPG-fixed tissue (*12*) and visualized with ABC-HRP (Vector Labs). The standard buffer contained 10 m*M* tris-HCl (*p*H 7.5); 150 m*M* NaCl; 0.05 percent Tween-20; SDS (0.1 percent) was included in incubations with the primary antiserum. Adsorbed antiserum was incubated overnight at 4°C with RR3 peptide at 5 mg/ml.

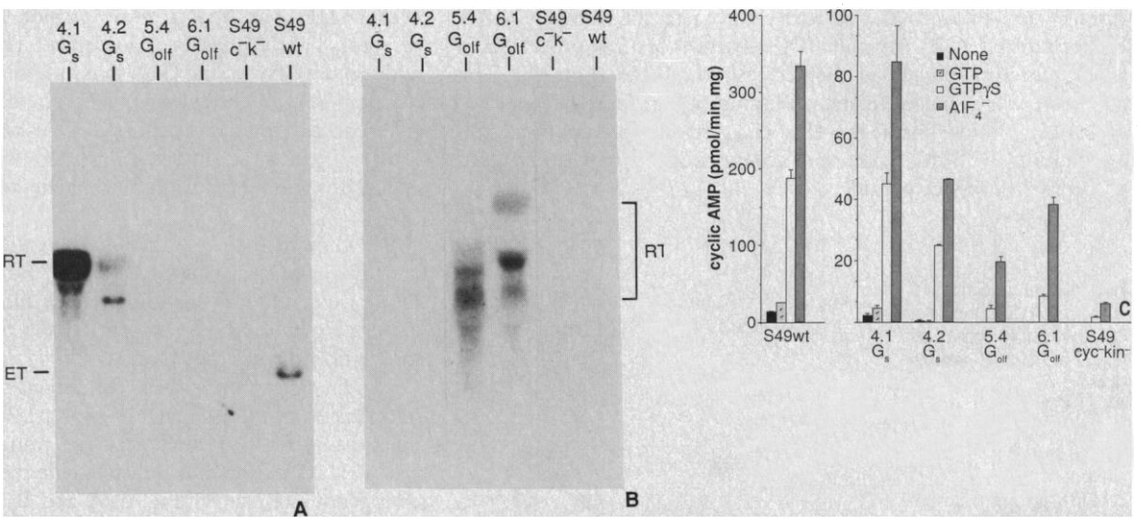

Fig. 6. Analysis of total RNA derived from the retrovirally infected S49 cell lines hybridized to a $G_{s\alpha}$ probe (**A**) or a $G_{olf\alpha}$ probe (**B**). RT, genomic retroviral transcript; ET, endogenous $G_{s\alpha}$ transcript. (**C**) Adenylate cyclase enzyme activity in membranes prepared from retrovirally infected cell lines and control cells. Average values and the corresponding standard deviations of assays run in triplicate are presented. A 1.5-kbp $G_{s\alpha}$ Hind III fragment and a 2.6-kbp Hind III–Sph I $G_{olf\alpha}$ fragment (clone 65), in which the Sph I site was blunted and ligated to a Hind III linker, were subcloned into the single Hind III site of pMV-7 (*37*). These constructions contain the entire protein coding region of each G_α subunit. Wild-type and cyc^- kin^- cells and their infection have been described (*21, 22*). Individual clones were isolated by limiting dilution. Probes for RNA analysis were prepared by ^{32}P-labeling (*29*) of the entire $G_{s\alpha}$ cDNA or the 3′ untranslated Nae I–Hind III fragment of clone 65. Membranes for adenylate cyclase assays were prepared by nitrogen cavitation without purification on sucrose gradients (*38*). Adenlyate cyclase assays (*39*) were performed with 20 μg of membrane protein (Bradford method) and the following additions: none, 50 μ*M* GTP, 50 μ*M* GTP-γ-S, AlF_4^- (20 μ*M* $AlCl_3$ and 10 m*M* NaF). Cell lines 4.1 and 4.2, $G_{s\alpha}$ transfectants; lines 5.4 and 6.1, $G_{olf\alpha}$ transfectants; S49wt, wild-type S49 cells.

olfactory signal transduction in which odorants activate adenylate cyclase via receptors that couple through G_{olf}. Cell-type specific G proteins also occur in the visual system. The retinal rod and cone cells have evolved two G proteins, $T_{\alpha I}$ and $T_{\alpha II}$, which are expressed exclusively in the sensory apparatus of these cells and function in visual signal transduction (*23*). In contrast, the other known mammalian G proteins ($G_{s\alpha}$, $G_{o\alpha}$, $G_{i\alpha 1\text{-}3}$, and $G_{x,z\alpha}$) are each expressed in a variety of tissues (*11, 24, 25*). Analogous in many respects to visual transducin, G_{olf} appears to have evolved for efficient receptor-mediated odorant detection.

Immunocytochemical localization of $G_{olf\alpha}$ to olfactory sensory neurons is appropriate for a G protein involved in odorant signal transduction. We might anticipate the components of the transductory cascade to be enriched in the membrane region with direct access to airborne odorants—the dendritic knobs and the cilia. Immunoblots demonstrate that $G_{olf\alpha}$ is abundant in crude olfactory cilia preparations. Moreover, the intense immunocytochemical staining of the epithelial sufrace with antisera to $G_{olf\alpha}$ or G_β (*6*) is consistent with the enrichment of a functional G protein within the olfactory cilia. The immunocytochemical staining observed in the axon bundles confirms the neuronal localization of G_{olf} and may represent axonal enrichment of G_{olf} or simply the high density of neuronal membranes in these fasciculated bundles. In the absence of specific markers, it is difficult to determine the recovery of G_{olf} in various membrane fractions and the absolute subcellular distribution of G_{olf}.

Several previous reports have invoked a role for a form of G_s in odorant signal transduction. Patients with pseudohypoparathyroidism, a disease believed to be caused by a deficiency in $G_{s\alpha}$, display a tendency toward olfactory dysfunction (*26*). The identification of

G_{olf} suggests that G_s plays a secondary role in the olfactory disorder associated with this disease. Pace and Lancet (7) have identified a protein slightly larger than the 45-kD form of $G_{s\alpha}$, which was abundant in olfactory tissue and could be labeled by cholera toxin. This protein probably corresponds to the olfactory specific G protein $G_{olf\alpha}$, which we have first identified here.

Among the G proteins expressed in olfactory tissue, only G_s and G_{olf} have the capacity to activate adenylate cyclase. The mRNA's encoding $G_{s\alpha}$ and $G_{olf\alpha}$ are both abundantly expressed in olfactory tissue. In contrast to the dramatic decrease in $G_{olf\alpha}$ mRNA, the $G_{s\alpha}$ mRNA increases after bulbectomy (*12*) and may be derived primarily from nonneuronal cells. In particular, hormone-responsive secretory glands are abundant in the submucosal layers of the olfactory epithelium. It appears that $G_{olf\alpha}$ mRNA must be abundantly expressed within the sensory neurons since these cells represent only a fraction of the tissue. Whether or not $G_{s\alpha}$ is present within the sensory neurons, olfaction is likely mediated by the more abundant neuronal G protein, G_{olf}.

$G_{olf\alpha}$ and $G_{s\alpha}$ share striking amino acid identity (88 percent) which sets them apart from the other G proteins ($G_{o\alpha}$, $G_{i\alpha 1\text{-}3}$, $G_{x,zd}$, and $T_{\alpha I,II}$) as a separate subclass of G_α subunits. Indeed, on the basis of homology, one might predict the biochemical properties of G_{olf} to be indistinguishable from those of G_s. We have shown that both $G_{s\alpha}$ and $G_{olf\alpha}$ can stimulate adenylate cyclase. The G protein domains responsible for interaction with receptors have not been rigorously determined, but the COOH-terminal region has been implicated in this role (*17*). Both T_α and $G_{i\alpha}$, which are conserved in their COOH-terminal regions, couple to photoactivated rhodopsin. $G_{s\alpha}$, which is divergent from $G_{i\alpha}$ and T_α in this region, couples poorly to photoactivated rhodopsin (*27*). In addition, a $G_{i\alpha}$-$G_{s\alpha}$ chimera in which the COOH-terminal third of $G_{i\alpha}$ has been replaced by $G_{s\alpha}$, interacts efficiently with G_s-coupled adrenergic receptors and poorly with G_i-coupled receptors (*28*). Together, this evidence and the near amino acid identity shared by $G_{s\alpha}$ and G_{olf} in the carboxyl tail imply that they may couple to structurally related receptors. This possibility can be tested directly with the use of transfected S49 cell lines.

It is unclear why the olfactory system evolved a novel G protein that exhibits near identity to the ubiquitous G protein, G_s. Perhaps G_{olf} has different receptor or effector specificities, or alternatively, altered intrinsic kinetic properties that allow it to mediate olfactory signal transduction more efficiently than G_s. Alternatively, the evolution of a separate $G_{s\alpha}$-like gene may facilitate abundant expression of a stimulatory G protein exclusively in olfactory sensory neurons.

REFERENCES AND NOTES

1. M. G. J. Beets, in *Fragrance Chemistry: The Science of Sense and Smell*, E. T. Theimer, Ed. (Academic Press, New York, 1982), p. 93.
2. J. Pevsner, R. R. Trifiletti, S. M. Strittmatter, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3050 (1985).
3. D. Lancet, *Annu. Rev. Neurosci.* **9**, 329 (1986).
4. U. Pace, E. Hanski, Y. Salomon, D. Lancet, *Nature* **316**, 255 (1986).
5. P. Sklar, R. R. H. Anholt, S. H. Snyder, *J. Biol. Chem.* **261**, 15538 (1986).
6. R. R. H. Anholt *et al.*, *Biochemistry* **26**, 788 (1987).
7. U. Pace and D. Lancet, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4947 (1986).
8. T. Nakamura and G. H. Gold, *Nature* **325**, 442 (1987).
9. A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987).
10. L. Stryer and H. R. Bourne, *Annu. Rev. Cell Biol.* **2**, 391 (1986).
11. D. T. Jones and R. R. Reed, *J. Biol. Chem.* **262**, 14241 (1987).
12. D. T. Jones *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **53**, 349 (1989).
13. K. R. Halliday, *J. Cyclic Nucleotide Res.* **9**, 435 (1984).
14. M. Kozak, *Nucleic Acids Res.* **12**, 857 (1984).
15. J. D. Robishaw *et al.*, *J. Biol. Chem.* **261**, 9587 (1986); T. Kozasa, I. Hiroshi, T. Twukamoto, Y. Kaziro, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2081 (1988).
16. P. Bray *et al.*, *ibid.* **83**, 8893 (1986).
17. S. B. Masters, R. M. Stroud, H. R. Bourne, *Protein Eng.* **1**, 47 (1986).
18. G. A. Monti Graziadei and P. P. C. Graziadei, *J. Neurocytol.* **8**, 197 (1979).
19. G. A. Monti Graziadei *et al.*, *J. Histochem. Cytochem.* **25**, 1311 (1977).
20. S. M. Mumby, R. A. Kahn, D. R. Manning, A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 265 (1986).
21. B. A. Harris *et al.*, *Science* **229**, 1274 (1985).
22. K. A. Sullivan *et al.*, *Nature* **330**, 758 (1987).
23. C. L. Lerea *et al.*, *Science* **234**, 77 (1986).
24. M. Matsuoka, H. Itoh, T. Kozasa, Y. Kaziro, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5384 (1988).
25. H. K. W. Fong, K. K. Yoshimoto, P. Eversole-Cire, M. I. Simon, *ibid.*, p. 3066.
26. R. S. Weinstock *et al.*, *Nature* **322**, 635 (1986).
27. R. A. Cerione *et al.*, *J. Biol. Chem.* **260**, 1493 (1985).
28. S. B. Masters *et al. Nature* **241**, 448 (1988).
29. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).
30. W. I. Wood, J. Gitschier, L. A. Lasky, R. M. Lawn, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1585 (1985).
31. F. Sanger, S. Nicklen, A. R. Coulson, *ibid.* **74**, 5463 (1977).
32. The pUCX1 expression vector is a modified form of pUC9. The fusion proteins contain 14 amino acids of the NH_2-terminus of β-galactosidase; P. Machlin and D. Cleveland, unpublished data.
33. T. A. Baker, A. D. Grossman, C. A. Gross, *Proc. Natl. Acad. U.S.A.* **81**, 6779 (1984).
34. C. D. Rhein and R. H. Cagan, *ibid.* **77**, 4412 (1980).
35. N. Green *et al.*, *Cell* **28**, 477 (1982).
36. E. I. Cullen and R. E. Mains, *Mol. Endocrinol.* **1**, 583 (1987).
37. P. T. Kirschmeier, G. M. Housey, M. D. Johnson, A. S. Perkins, I. B. Weinstein, *DNA* **7**, 219 (1988).
38. E. M. Ross, M. E. Maguire, T. W. Sturgill, R. L. Boltonen, A. G. Gilman, *J. Biol. Chem.* **252**, 5761 (1977).
39. Y. Salomon, *Adv. Cyclic Nucleotide Res.* **10**, 35 (1979).

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