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Biochemistry

Occurrence in *Saccharomyces cerevisiae* of a gene homologous to the cDNA coding for the α subunit of mammalian G proteins

(GTP-binding proteins/signal transduction/ras protein)

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ABSTRACT **From cross-hybridization studies with cDNAs that code for the α subunits of rat brain guanine nucleotide-binding regulatory (G) proteins, we have isolated a gene from yeast *Saccharomyces cerevisiae* encoding an amino acid sequence that is highly homologous to the α subunit of the G protein that mediates inhibition of adenylate cyclase ($G_{i\alpha}$) from rat brain. The gene, tentatively designated as *GPA1*, contains a contiguous, single open reading frame of 1416 nucleotides that codes for a protein of 472 amino acids with a calculated M_r of 54,075. The predicted amino acid sequence of the protein encoded by the *GPA1* gene (tentatively designated as G protein 1α or GP1α) is remarkably homologous to the amino acid sequence of rat brain $G_{i\alpha}$ and the α subunit of the G protein of unknown function ($G_{o\alpha}$); the primary structure of the sites for GTP hydrolysis as well as GTP interaction are nearly identical. The main difference in the molecular sizes of yeast GP1α (472 amino acids) and rat brain $G_{i\alpha}$ (355 amino acids) is due to the presence of a stretch of 110 extra amino acid residues in yeast GP1α, which are inserted near the NH_2-terminal one-third of mammalian $G_{i\alpha}$. From blot-hybridization analysis, the size of the GP1α mRNA was estimated as 1.7 kilobases.**

G proteins are a family of guanine nucleotide-binding proteins that are involved in a variety of receptor-mediated signal transduction systems (1). Thus, G_s and G_i are involved in hormonal stimulation and inhibition, respectively, of adenylate cyclase activity (1, 2), whereas transducin (G_t), which is present predominantly in the retinal rod outer segment, regulates cGMP phosphodiesterase activity (3). Another G protein, G_o, which has recently been reported in several tissues (4–7), may be involved in neuronal responses, but its precise function has not yet been clarified. Furthermore, recent evidence suggests the involvement of G proteins in the activation of phospholipase C (8–12) and the gating of K^+ channels (13).

In a previous report (14), we described the cloning and sequence determination of cDNAs that code for the α subunits of G_s, G_i, and G_o from rat C6 glioma cells. The predicted amino acid sequences of the α subunits of G_s ($G_{s\alpha}$) and G_i ($G_{i\alpha}$) contain 394 and 355 amino acid residues, respectively, whereas the clone of the α subunit of G_o ($G_{o\alpha}$) encodes a sequence of 310 amino acid residues that lack the NH_2 terminus. The amino acid sequence of $G_{s\alpha}$ from rat brain is almost identical (about 99% homologous) to that of bovine adrenal $G_{s\alpha}$ published by Robishaw *et al.* (15) and to that of bovine brain $G_{s\alpha}$ by Nukada *et al.* (16). On the other hand, the sequence of rat brain $G_{i\alpha}$ (14) is about 89% homologous with the bovine brain $G_{i\alpha}$ sequence reported by Nukada *et al.* (17).

Another family of GTP-binding proteins, the ras family, is widely distributed among eukaryotes. This family also consists of several closely related proteins (e.g., those encoded by H-*ras*, K-*ras*, and N-*ras* in mammalian tissues) and is highly conserved among a variety of species including mammals (18–22), *Drosophila* (23), slime molds (24), and the yeasts *Saccharomyces cerevisiae* (25, 26) and *Schizosaccharomyces pombe* (27). In the case of *S. cerevisiae*, genetic evidence suggests that the *RAS2* gene is involved in the activation of adenylate cyclase (28, 29). From this finding, it was speculated that *RAS2* in *S. cerevisiae* is the counterpart of mammalian G_s (28, 30).

However, a remarkable homology between the primary structures of rat and bovine $G_{s\alpha}$ prompted us to search for the presence of G proteins in *S. cerevisiae*. In this paper, we describe the isolation and sequence determination of a gene from *S. cerevisiae*, which codes for a protein that is highly homologous to rat brain $G_{i\alpha}$ and $G_{o\alpha}$. To our knowledge, this is the first demonstration of the occurrence of a G protein in yeast.

MATERIALS AND METHODS

Yeast Strain and Media. *S. cerevisiae* haploid strains pep4 (**a**, *ade3, leu1, pep4*) and 106A (α, *arg*) were cultured in a YPD medium (2% polypeptone/1% yeast extract/2% glucose).

Southern and RNA-Hybridization Blot Analysis. Yeast DNA was prepared from the cells of *S. cerevisiae* pep4 essentially as described by Cryer *et al.* (31). Southern blot analysis was performed as described by Southern (32) using low- and high-stringency hybridization conditions. Low-stringency hybridization was carried out at 37°C in 5× NaCl/Cit (1× NaCl/Cit contains 0.15 M NaCl and 15 mM sodium citrate)/20 mM sodium phosphate, pH 7.0/1× Denhardt's solution (0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll)/heat-denatured calf thymus DNA (100 μg/ml)/0.1% $NaDodSO_4$/10% dextran sulfate/20% (vol/vol) formamide. For high-stringency conditions, the incubation temperature was increased to 42°C, and the concentration of formamide was increased to 50% (vol/vol). The filters were washed with 5× NaCl/Cit/0.1% $NaDodSO_4$ at room temperature and then with 0.1× NaCl/Cit/0.1% $NaDodSO_4$ at 37°C.

DNA fragments of the rat G protein cDNAs used as probes for cross-hybridization analysis of yeast genomic DNA were the 1.2-kilobase (kb) *Eco*RI fragment of λGX3 (14), which carries almost the entire rat $G_{o\alpha}$ cDNA, and the 0.7-kb *Eco*RI–*Bam*HI fragment of λGX13 (14), which contains the NH_2-terminal one-third of the rat $G_{i\alpha}$ cDNA. The 1.9-kb *Eco*RI fragment containing the *GPA1* gene was also used as a probe for Southern blot analysis of yeast genomic DNA (see

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Abbreviations: G proteins, guanine nucleotide-binding regulatory proteins; G_s and G_i, G proteins that mediate stimulation and inhibition, respectively, of adenylate cyclase; G_o, a G protein of unknown function; G_t, transducin, a G protein that regulates cGMP phosphodiesterase activity in phototransduction; $G_{s\alpha}$, $G_{i\alpha}$, $G_{o\alpha}$, and $G_{t\alpha}$, α subunits of G_s, G_i, G_o, and G_t, respectively.

Results). ^{32}P-labeled probes were prepared by the primer extension method described by Feinberg and Vogelstein (33).

Yeast RNA for blot-hybridization analysis was prepared as follows. Strain 106A was cultured to logarithmic phase, and spheroplasts were prepared in the same manner as for the preparation of DNA. The spheroplasts were lysed by the addition of diethyl pyrocarbonate and $NaDodSO_4$ to final concentrations of 1% (vol/vol) and 1% (wt/vol), respectively. The mixture was immediately extracted twice with phenol/chloroform (1:1; saturated with 10 mM Tris·HCl, pH 7.4/100 mM NaCl/1 mM EDTA). Two volumes of ethanol were added, and the precipitates were dissolved in 10 mM Tris·HCl, pH 7.4/1 mM EDTA. Poly(A)$^+$ RNA was obtained by oligo(dT)-cellulose column chromatography, and blot-hybridization analysis was performed as described (34).

Screening of the Yeast Genomic Library. The procedure of Hanahan and Meselson (35) was used to screen the yeast genomic library kindly provided by Botstein (36). The hybridization conditions were similar to those of genomic Southern analysis.

DNA Sequence Analysis. Nucleotide sequences were determined by using bacteriophage M13 vectors and the dideoxynucleotide chain-termination method (37).

RESULTS

Presence of Sequences Homologous to cDNAs Coding for the Rat Brain G Protein α Subunits in *S. cerevisiae*. We searched for sequences homologous to cDNAs for mammalian $G_{i\alpha}$ and $G_{o\alpha}$ in *S. cerevisiae* DNA by Southern hybridization analysis. Several bands that hybridized with the ^{32}P-labeled $G_{i\alpha}$ and $G_{o\alpha}$ cDNAs were detected when *S. cerevisiae* DNA was digested with several restriction endonucleases and analyzed by Southern hybridization under low-stringency conditions (data not shown). This suggests the presence of G-protein homologous genes in yeast.

Cloning of the Yeast Gene Homologous to the Rat Brain $G_{i\alpha}$ cDNA. The genomic library of *S. cerevisiae* constructed by Carlson and Botstein (36) by cloning the partial *Sau*3A digests of yeast DNA in vector YEp24 was screened by colony hybridization using the ^{32}P-labeled rat $G_{i\alpha}$ and $G_{o\alpha}$ cDNA probes. Twelve colonies were isolated from screening $\approx 6 \times 10^4$ clones under low-stringency conditions of hybridization. The plasmid DNAs were prepared, digested with restriction endonucleases, and analyzed by Southern hybridization. One of the clones, pMN10, which contains about 12.5 kb of yeast genomic DNA, hybridized with not only the $G_{i\alpha}$ but also with the $G_{o\alpha}$ probes.

The DNA insert of pMN10 was cleaved with several restriction endonucleases and was again analyzed by Southern hybridization using a mixture of rat $G_{i\alpha}$ and $G_{o\alpha}$ cDNAs as the probe. It was found that the yeast sequence homologous to the mammalian $G_{i\alpha}$ and $G_{o\alpha}$ resides within the 1.9-kb *Eco*RI fragment of pMN10. This fragment was then subcloned in the *Eco*RI site of pUC8 to yield pGI1.

Nucleotide Sequence Analysis. The physical restriction map of pGI1, which harbors the yeast G-protein homologous gene (tentatively designated as *GPA1*), is shown in Fig. 1, together with the strategy for DNA sequence determination. The nucleotide sequence (1924 bp) and deduced amino acid sequence are shown in Fig. 2. The DNA sequence contains an open reading frame of 1416 nucleotides coding for a protein of 472 amino acid residues (including the initiator methionine) with a calculated M_r of 54,075. The sequence around the ATG initiator codon (ATAATGG) is a favorable one proposed by Kozak (38)—i.e., a purine in position −3 and a guanosine in position +4. Upstream of the ATG codon, several putative promoter sequences (39) were found (see Fig. 2). The open reading frame ends at the TGA stop codon (positions 1417–1419). Downstream of the stop codon, there is a sequence that agrees well with the consensus sequence for polyadenylylation in yeast (40) (positions 1620–1626).

Comparison of the Amino Acid Sequence of Yeast GP1α with Those of Rat Brain $G_{i\alpha}$ and $G_{o\alpha}$. The deduced amino acid sequence of yeast GP1α (*GPA1*-encoded protein) is highly homologous with those of rat brain $G_{i\alpha}$ and $G_{o\alpha}$. As shown in Fig. 3, the homology is most remarkable in the region of GTP hydrolysis (amino acid residues 43–56), where the amino acid sequence of yeast GP1α is completely identical with rat brain $G_{i\alpha}$. The region responsible for GTP binding (amino acid residues 384–396) was also highly homologous; 12 out of 13 amino acids were identical in yeast GP1α and rat brain $G_{i\alpha}$. Another region of homology was found in amino acid residues 321–336 where a sequence of 16 contiguous amino acids was completely identical in yeast GP1α and rat $G_{i\alpha}$.

Disregarding the sequence of a stretch of 110 amino acids that is unique to yeast GP1α (residues 126–235), the overall homology between yeast GP1α and rat brain $G_{i\alpha}$ is 45% (164 out of 362 amino acids are identical), and the homology is 64% when conservative amino acid replacements are regarded as homologous. The amino acid sequence of rat brain $G_{o\alpha}$ is 43% identical with that of yeast GP1α and is 66% homologous when the conservative amino acid replacements are regarded as homologous. On the other hand, the homologies of the nucleotide sequence of yeast GP1α with that of rat brain $G_{i\alpha}$ and $G_{o\alpha}$ are 54% and 52%, respectively.

Southern Blot Analysis of Yeast Genomic DNA with the Yeast *GPA1* Gene. We have analyzed yeast genomic DNA digested with various restriction endonucleases by using the ^{32}P-labeled 1.9-kb *Eco*RI fragment of pGI1 as a probe. The fragment contains the entire sequence of the yeast *GPA1*

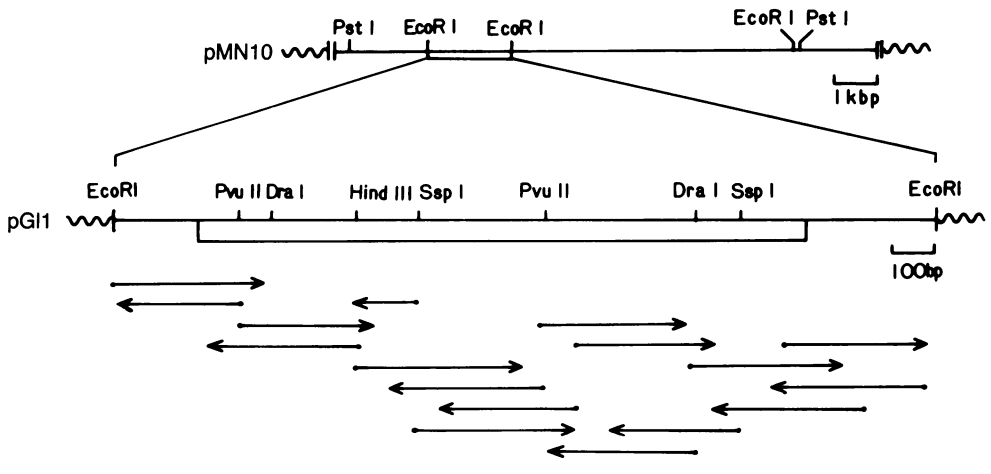

FIG. 1. Restriction map and sequence strategy for the pGI1 plasmid. The wavy and straight lines show vector DNA and inserted yeast DNA, respectively. The amino acid coding region is represented by an open bar. The arrows indicate the direction and extent of the DNA sequence that was determined by the dideoxynucleotide chain-termination method (37).

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                    -201 GAATTCCACCAATTTCTTTACGTTTTATATTATTCGTAATCTTTTGATCTGTTATTCATTTTTTCTTGTCACTCCGTTTCT
-120 AACATTTTTGACCATTTCTAAGACCAAACTGAGTAGAAGCTATTCATACTGTAAATTGGTATTTTAGCATCACATCAATAATCCAGAGGTGTATAAATTGATATATTAAGGTAGGAAATA

   1 ATG GGG TGT ACA GTG AGT ACG CAA ACA ATA GGA GAC GAA AGT GAT CCT TTT CTA CAG AAC AAA AGA GCC AAT GAT GTC ATC GAG CAA TCG
   1 Met Gly Cys Thr Val Ser Thr Gln Thr Ile Gly Asp Glu Ser Asp Pro Phe Leu Gln Asn Lys Arg Ala Asn Asp Val Ile Glu Gln Ser

  91 TTG CAG CTG GAG AAA CAA CGT GAC AAG AAT GAA ATA AAA CTG TTA CTA TTA GGT GCC GGT GAG TCA GGT AAA TCA ACG GTT TTA AAA CAA
  31 Leu Gln Leu Glu Lys Gln Arg Asp Lys Asn Glu Ile Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Val Leu Lys Gln

 181 TTA AAA TTA TTA CAT CAA GGC GGT TTC TCC CAT CAA GAA AGG TTA CAG TAT GCT CAA GTG ATA TGG GCA GAT GCC ATA CAA TCA ATG AAA
  61 Leu Lys Leu Leu His Gln Gly Gly Phe Ser His Gln Glu Arg Leu Gln Tyr Ala Gln Val Ile Trp Ala Asp Ala Ile Gln Ser Met Lys

 271 ATT TTG ATT ATT CAG GCC AGA AAA CTA GGT ATT CAA CTT GAC TGT GAT GAT CCG ATC AAC AAT AAA GAT TTG TTT GCA TGC AAG AGA ATA
  91 Ile Leu Ile Ile Gln Ala Arg Lys Leu Gly Ile Gln Leu Asp Cys Asp Asp Pro Ile Asn Asn Lys Asp Leu Phe Ala Cys Lys Arg Ile

 361 CTG CTA AAG GCT AAA GCT TTA GAT TAT ATC AAC GCC AGT GTT GCC GGT GGT TCT GAT TTT CTA AAT GAT TAT GTA CTG AAG TAC TCA GAA
 121 Leu Leu Lys Ala Lys Ala Leu Asp Tyr Ile Asn Ala Ser Val Ala Gly Gly Ser Asp Phe Leu Asn Asp Tyr Val Leu Lys Tyr Ser Glu

 451 AGG TAT GAA ACT AGG AGG CGT GTT CAG AGT ACC GGA CGA GCA AAA GCT GCT TTC GAT GAA GAC GGA AAT ATT TCT AAT GTC AAA AGT GAC
 151 Arg Tyr Glu Thr Arg Arg Arg Val Gln Ser Thr Gly Arg Ala Lys Ala Ala Phe Asp Glu Asp Gly Asn Ile Ser Asn Val Lys Ser Asp

 541 ACT GAC AGA GAT GCT GAA ACG GTG ACG CAA AAT GAG GAT GCT GAT AGA AAC AAC AGT AGT AGA ATT AAC CTA CAG GAT ATT TGC AAG GAC
 181 Thr Asp Arg Asp Ala Glu Thr Val Thr Gln Asn Glu Asp Ala Asp Arg Asn Asn Ser Ser Arg Ile Asn Leu Gln Asp Ile Cys Lys Asp

 631 TTG AAC CAA GAA GGC GAT GAC CAG ATG TTT GTT AGA AAA ACA TCA AGG GAA ATT CAA GGA CAA AAT AGA CGA AAT CTT ATT CAC GAA GAC
 211 Leu Asn Gln Glu Gly Asp Asp Gln Met Phe Val Arg Lys Thr Ser Arg Glu Ile Gln Gly Gln Asn Arg Arg Asn Leu Ile His Glu Asp

 721 ATT GCT AAG GCA ATA AAG CAA CTT TGG AAT AAC GAC AAA GGT ATA AAG CAG TGT TTT GCA CGT TCT AAT GAG TTT CAA TTG GAG GGC TCA
 241 Ile Ala Lys Ala Ile Lys Gln Leu Trp Asn Asn Asp Lys Gly Ile Lys Gln Cys Phe Ala Arg Ser Asn Glu Phe Gln Leu Glu Gly Ser

 811 GCT GCA TAC TAC TTT GAT AAC ATT GAG AAA TTT GCT AGT CCG AAT TAT GTC TGT ACG GAT GAA GAC ATT TTG AAG GGC CGT ATA AAG ACT
 271 Ala Ala Tyr Tyr Phe Asp Asn Ile Glu Lys Phe Ala Ser Pro Asn Tyr Val Cys Thr Asp Glu Asp Ile Leu Lys Gly Arg Ile Lys Thr

 901 ACA GGC ATT ACA GAA ACC GAA TTT AAC ATC GGC TCG TCC AAA TTC AAG GTT CTC GAC GCT GGT GGG CAG CGT TCT GAA CGT AAG AAG TGG
 301 Thr Gly Ile Thr Glu Thr Glu Phe Asn Ile Gly Ser Ser Lys Phe Lys Val Leu Asp Ala Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp

 991 ATT CAT TGT TTC GAA GGA ATT ACA GCA GTT TTA TTT GTT TTA GCA ATG AGT GAA TAC GAC CAG ATG TTG TTT GAG GAT GAA AGA GTG AAC
 331 Ile His Cys Phe Glu Gly Ile Thr Ala Val Leu Phe Val Leu Ala Met Ser Glu Tyr Asp Gln Met Leu Phe Glu Asp Glu Arg Val Asn

1081 AGA ATG CAT GAA TCA ATA ATG CTA TTT GAC ACG TTA TTG AAC TCT AAG TGG TTC AAA GAT ACA CCG TTT ATT TTG TTT TTA AAT AAA ATT
 361 Arg Met His Glu Ser Ile Met Leu Phe Asp Thr Leu Leu Asn Ser Lys Trp Phe Lys Asp Thr Pro Phe Ile Leu Phe Leu Asn Lys Ile

1171 GAT TTG TTC GAG GAA AAG GTA AAA AGC ATG CCC ATA AGA AAG TAC TTT CCT GAT TAC CAG GGA CGT GTC GGC GAT GCA GAA GCG GGT CTA
 391 Asp Leu Phe Glu Glu Lys Val Lys Ser Met Pro Ile Arg Lys Tyr Phe Pro Asp Tyr Gln Gly Arg Val Gly Asp Ala Glu Ala Gly Leu

1261 AAA TAT TTT GAG AAG ATA TTT TTG AGC TTG AAT AAG ACA AAC AAA CCA ATC TAC GTG AAA CGA ACC TGC GCT ACC GAT ACC CAA ACT ATG
 421 Lys Tyr Phe Glu Lys Ile Phe Leu Ser Leu Asn Lys Thr Asn Lys Pro Ile Tyr Val Lys Arg Thr Cys Ala Thr Asp Thr Gln Thr Met

1351 AAG TTC GTA TTG AGT GCA GTC ACC GAT CTA ATC ATC CAG CAA AAC CTT AAA AAA ATT GGT ATT ATA TGAAGGAACTGTATAATTAAAGTAGTGTTTA
 451 Lys Phe Val Leu Ser Ala Val Thr Asp Leu Ile Ile Gln Gln Asn Leu Lys Lys Ile Gly Ile Ile End 472

1448 GATACGTAAATTCTGTTTCCGAAGATGCAAGAAGGAGCAGCAGCACCAGAAAAAATTACTATTTTTCTTCTCCATTAGAGTCTATGATGGAATGCCAAATGAAAAAGCCATTTTGTTCAA

1568 CAGTTCTTGATCTCGTTAAATCGTTCCGGGTTTTCAATTGAAAAACAAGGGTAATAAAATCGCATGAGAAAAAAAAGGTCCAGACACTTTTTTAGAAGAAAAACTCTTGAAAACAATTGA
1688 TTCTTCTACGTAAGGACAGGGTATTATCTCGAATTC  1723
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FIG. 2. Nucleotide and predicted amino acid sequence of the *GPA1* gene. Numbering of the nucleotide sequence begins at the first nucleotide in the open reading frame. The deduced amino acid sequence is shown below the nucleotide sequence. The putative "TATA" boxes and the polyadenylylation signal are boxed.

gene. Only a single band was detected in each lane even under low-stringency conditions (Fig. 4).

Detection of Transcripts. To see the expression of the yeast *GPA1* gene, we have carried out blot-hybridization analysis of poly(A)$^+$ mRNA prepared from exponentially growing cells of yeast strain 106A by using the ^{32}P-labeled 1.9-kb *Eco*RI fragment of pGI1. As shown in Fig. 5, a single band of about 1.7 kb was detected. This indicates that the *GPA1* gene was transcribed in growing cells.

DISCUSSION

This paper describes the isolation of a gene from *S. cerevisiae* whose predicted amino acid sequence is highly homologous to that of rat brain $G_{i\alpha}$ and $G_{o\alpha}$ (Fig. 3). The sequence is less homologous to that of rat brain $G_{s\alpha}$.

The molecular size of the predicted yeast GP1α (472 amino acids) is considerably larger than rat brain $G_{i\alpha}$ (355 amino acids). The difference in molecular sizes of yeast GP1α and mammalian $G_{i\alpha}$ is mainly due to the presence of a stretch of 110 extra amino acids in yeast GP1α, which is inserted at the NH_2-terminal one-third of the molecule. The nucleotide sequence of this region does not seem to be an intervening sequence since no consensus sequence for splicing signals (42) (i.e., for donor and acceptor sites as well as the TACTAACA sequence) was detected in this region. The inserted sequence of 110 amino acids was analyzed by a computer search for possible homology with known protein sequences compiled in the protein sequence database (43); however, no apparent homology was detected with any of the reported sequences. This sequence resides within the domain of mammalian G proteins, which is assumed to be the site interacting with an amplifier or an effector molecule (44).

Comparison of the predicted amino acid sequence of the yeast GP1α protein with mammalian G proteins (see Fig. 3) reveals a strong conservation in the region of the GTP binding and hydrolysis sites (14). The amino acid residue of mammalian $G_{i\alpha}$, $G_{o\alpha}$, and $G_{t\alpha}$ that is the site of ADP ribosylation by pertussis toxin—i.e., cysteine at the fourth residue from the COOH terminus—has been replaced by isoleucine, which indicates that the yeast GP1α protein is probably refractory to the modification by islet-activating protein. On the other hand, the sequence around Arg-297 of yeast GP1α is highly homologous with the sequence around Arg-201 of rat brain $G_{s\alpha}$, which is the ADP-ribosylation site for cholera toxin.

Recently much interest has been focused on the structure of G proteins and their function in receptor-mediated transmembrane signaling systems. The cDNA sequences for the α subunits of G_s (14–16), G_i (14, 17), G_o (14), and G_t (45–48) have been determined. The nucleotide and amino acid sequences are highly homologous among these different G proteins, and they are highly conserved among different species. Over 99% homology was observed in the amino acid sequences of rat brain $G_{s\alpha}$ and $G_{s\alpha}$ from bovine brain or

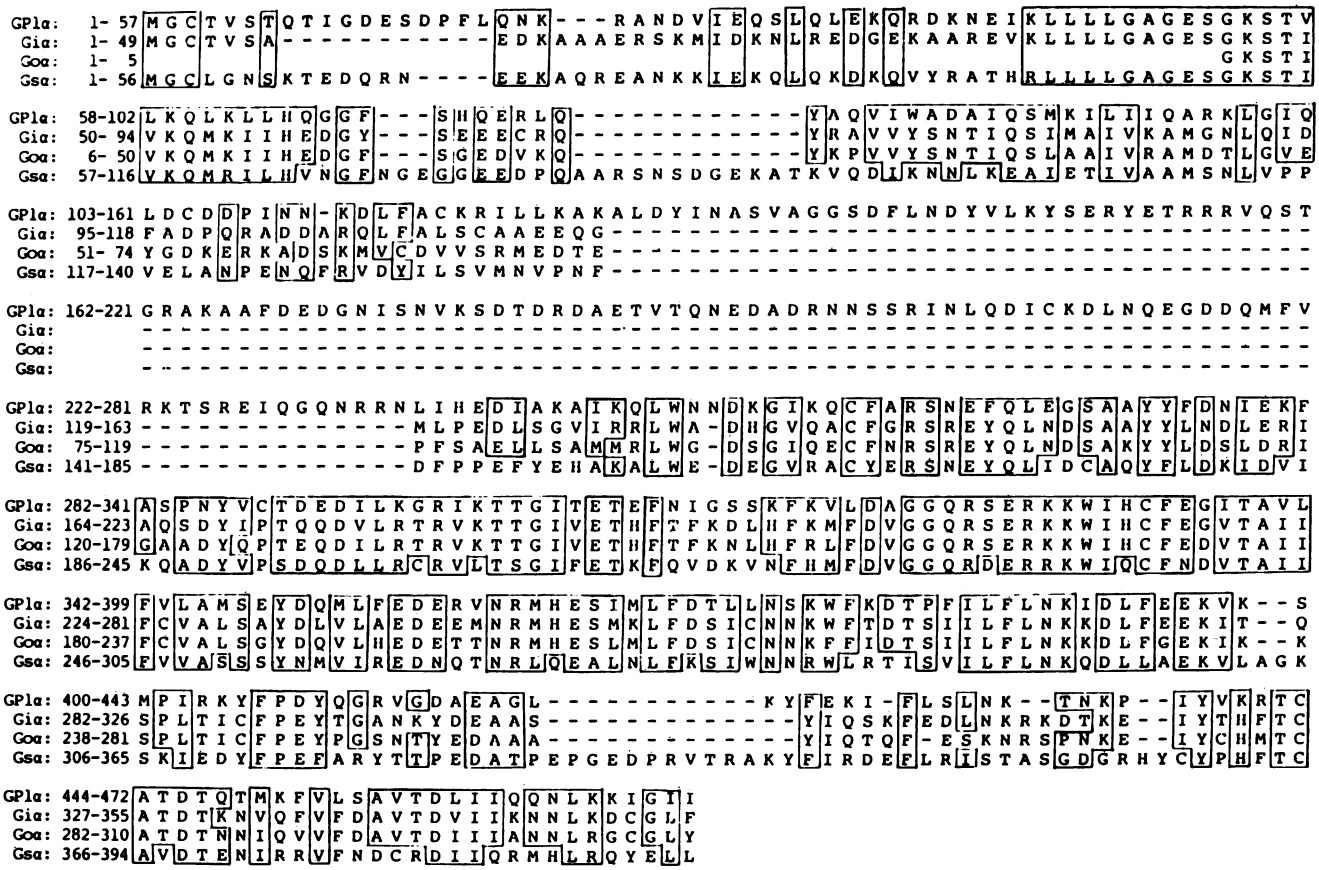

FIG. 3. Alignment of the predicted amino acid sequence of GP1α with those of the α subunits of mammalian G proteins. Sets of identical or conservative residues are enclosed within solid lines. Conservative amino acid substitutions are grouped as follows: C; S, T, P, A, and G; N, D, E, and Q; H, R, and K; M, I, L, and V; and F, Y, and W (41).

adrenal cells (14–16). The remarkable conservation of amino acid sequences of G proteins among different species suggests the ubiquitous occurrence of G proteins.

Another family of GTP-binding proteins, the ras family, which may also be involved in biosignal transduction, is also widely distributed and is highly conserved. In yeast *S. cerevisiae* and *Schizosaccharomyces pombe*, the *RAS1* and *RAS2* genes (25, 26) and the *ras1* gene (27), respectively, have been identified as mammalian *ras* homologous genes. (*RAS1* and *RAS2* are *S. cerevisiae* genes homologous to mammalian *ras* while *ras1* represents a *ras* homologous gene of *Schizosaccharomyces pombe*.) Amino acid sequences predicted from the DNA sequences of the yeast *ras* genes show a high level of homology with the protein sequences of the mammalian ras family, but the sizes of the RAS1 (309 amino acids) and RAS2 (322 amino acids) proteins (25) are considerably larger than the mammalian ras proteins (189 amino acids; refs. 18–22).

Initially, it was hoped that the function of mammalian ras proteins would be elucidated by studying the function of the yeast *ras* gene. However, subsequent genetic and biochemical studies on the function of yeast ras proteins have

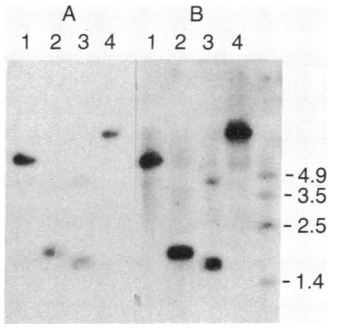

FIG. 4. Southern blot analysis of *S. cerevisiae* DNA. Genomic DNA isolated from *S. cerevisiae* strain pep4 was digested with restriction endonucleases and electrophoresed in a 0.8% agarose gel. DNA was denatured, transferred to a nitrocellulose filter, and hybridized with the ^{32}P-labeled 1.9-kb *Eco*RI fragment of pGI1 under high (*A*)- and low (*B*)-stringency conditions. Each lane contained about 5 μg of DNA. Restriction endonucleases used were *Bgl* II (lanes 1), *Eco*RI (lanes 2), *Hin*dIII (lanes 3), and *Pst* I (lanes 4). Sizes (in kb) of the markers are indicated at the right.

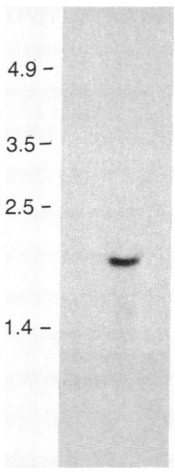

FIG. 5. Blot-hybridization analysis of *S. cerevisiae* poly(A)$^+$ RNA. Poly(A)$^+$ RNA (20 μg) isolated from strain 106A was electrophoresed in a 1.2% formaldehyde/agarose gel. RNA was transferred to a nitrocellulose filter and hybridized with the ^{32}P-labeled 1.9-kb *Eco*RI fragment of the pGI1 plasmid under high-stringency conditions. Sizes (in kb) of the DNA markers are indicated at the left.

revealed that RAS2 is involved in the regulatory mechanism of adenylate cyclase activity (28) in a fashion similar to mammalian $G_{s\alpha}$. These results have led to the supposition that ras proteins in yeast may be counterparts of mammalian G proteins (28, 30).

In view of the strong conservation of the amino acid sequence of $G_{s\alpha}$ in different species, however, we thought that yeast might possess a G protein family in addition to the ras family. This has turned out to be true as evidenced by the isolation of the *GPA1* gene in *S. cerevisiae* that is described in this paper. Our preliminary results suggest that other G proteins may also occur in *S. cerevisiae*. Studies on the function of G proteins in yeast may throw more light on the role of the G and ras proteins in signal transduction in mammalian cells.

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