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Multiple α subunits of guanine nucleotide-binding proteins in *Dictyostelium*

(signal transduction/receptor/chemotaxis)

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ABSTRACT **Previous results have shown that chemotaxis and the expression of several classes of genes in *Dictyostelium discoideum* are regulated through a cell surface cAMP receptor interacting with guanine nucleotide-binding proteins (G proteins). We now describe cloning and sequencing of cDNAs encoding two G_α protein subunits from *Dictyostelium*. The derived amino acid sequences show that they are 45% identical to each other and to G_α protein subunits from mammals and yeast. Both cDNAs are complementary to multiple mRNAs that are differentially expressed during development. This evidence and analysis of mutants presented elsewhere suggest that they have distinct physiological functions.**

When deprived of nutrients, *Dictyostelium discoideum* amoebae cease growth and initiate a developmental program. Within a few hours cells, guided by chemotaxis and intercellular signaling, spontaneously aggregate and form a multicellular organism. Cells in specific positions of the multicellular structure differentiate into the stalk and spore cells of a mature fruiting body. The signal molecule is extracellular cAMP that interacts with a cell surface receptor. Present evidence indicates that the cAMP receptor also regulates aggregation stage and cell-type-specific gene expression, cell patterning in the migrating pseudoplasmodium, and morphogenesis during culmination (1–4).

There appear to be two signal transduction pathways that may involve different kinetic classes of the cell surface receptor (5). The first is a "signaling" pathway in which cAMP, presumably binding to the rapidly dissociating class of receptors, results in the activation of adenylate cyclase with the subsequent synthesis and secretion of cAMP from the cell (6). Emitted cAMP can activate receptors on the same cell, creating a positive feedback loop, or on adjacent cells, relaying the signal. This response reversibly adapts and the cycle is repeated with a periodicity of 6–7 min (7, 8).

A second, or "chemotactic," pathway is proposed to be mediated by the slowly dissociating class of cell surface receptors. This pathway is believed to involve activation of phospholipase C with the production of inositol 1,4,5-triphosphate and diacylglycerol (9). Additional responses triggered by extracellular cAMP, such as activation of guanylate cyclase and the transient production of cGMP, actin polymerization, and myosin phosphorylation, also appear to be associated with the chemotactic pathway (10).

Numerous biochemical studies indicate that both signal transduction pathways in *Dictyostelium* involve guanine nucleotide-binding proteins (G proteins). In addition, the primary structure of the cAMP receptor displays seven putative transmembrane domains, a structure identical to other G protein-linked receptors (11). We now report the complete cDNA sequences and deduced amino acid sequences of two distinct G protein α subunits.§ We also show that genes encoding these proteins are expressed at different times in development.

MATERIALS AND METHODS

Oligonucleotide Screening. Oligonucleotides were 5′ end-labeled using T4 polynucleotide kinase (Promega or New England Biolabs) and [γ-^{32}P]ATP (NEN or ICN). *D. discoideum* cDNA libraries were screened with two oligonucleotides at 50°C by the method of Wood *et al.* (12). The sequence of the first oligonucleotide was GG$^{A}_{T}$GC$^{C}_{T}$GG$^{A}_{T}$GA$^{G}_{A}$TC$^{T}_{A}$G-G$^{A}_{T}$AA. The sequence of the second oligonucleotide was GGTGGTCAA$^{A}_{C}$G$^{A}_{T}$TC$^{T}_{A}$GAA$^{A}_{C}$G$^{A}_{T}$AAAAAATGG.

Construction of Plasmids. cDNA inserts obtained from *Eco*RI digestion of phages were subcloned into the *Eco*RI site of pGEM2 or pSP73 (Promega) using standard techniques (13).

RNA Blots. For the development time course of $G_\alpha 1$ and $G_\alpha 2$ expression, *D. discoideum* strain NC-4 cells were grown on SM (15 mM KH_2PO_4/5 mM K_2HPO_4/1% dextrose/1% bactopeptone/0.1% yeast extract/1% agar) plates in association with *Klebsiella aerogenes*, washed, and plated for development. RNA was isolated from cells harvested at specific times in development, sized on denaturing gels, and analyzed by RNA blot hybridization as described (1).

RESULTS

Identification of Two Distinct α Subunits. cDNA clones for α subunits of G proteins were obtained by screening libraries prepared from 3.5-, 5-, and 11-hr stages of *Dictyostelium* development. Two oligonucleotide probes, corresponding to the highly conserved putative guanine nucleotide-binding (GGQRSERKKW) and GTPase (GAGESGK) regions of the known mammalian α subunit sequences were designed. These probes included 80–90% of the possible codons by biasing for the highly (A+T)-rich codon usage of *Dictyostelium* (14). An initial collection of 25 cDNAs was obtained from screens of about 250,000 plaques. These separated into two classes defined by the relative intensity of cross-hybridization and the presence or absence of an internal *Eco*RI site (Fig. 1). Further restriction site analysis confirmed the existence of these two classes that were designated $G_\alpha 1$ (with an internal *Eco*RI site) and $G_\alpha 2$ (without an internal *Eco*RI site). The two $G_\alpha 1$ clones tested (RF1 and RF3) weakly cross-hybridized to the $G_\alpha 2$ class, whereas the $G_\alpha 2$ clone tested (FR1) did not cross-hybridize to the $G_\alpha 1$ class. Two distinct size classes were

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Abbreviations: G protein, guanine nucleotide-binding protein; G_o, G protein of unknown function; G_i, inhibitory G protein; G_s, stimulatory G protein; Tr, transducin.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M25060 for $G_\alpha 1$ and M25061 for $G_\alpha 2$).

	RF1-3′	RF3-5′	FR1	KB	EcoR1
RF1	■	■	-	1.2	
FR7	■	■	-	1.2	
FR11	■	■	-	1.2	
AD1	■	■	-	1.0	
AD3	■	■	-	1.0	
AD4	■	■	-	1.2	
RF3	■	■	-	2.4	
RF5	■	■	-	2.4	
FR5	■	■	-	2.4	
F10	■	-	-	2.9	
FR1	-	-	■	2.3	
FR2	-	-	■	1.4	
AD2	♦	♦	■	0.7	
AD5	♦	♦	■	0.8	
AD6	♦	♦	■	1.2	
A1	-	-	■	0.4	
A2	♦	-	■	0.5	
A3	♦	-	■	0.5	
A4	♦	-	■	1.7	
A5	♦	-	■	1.6	
D2	-	♦	■	0.6	
D1	♦	♦	■	0.4	
AD7	♦	-	♦	0.5	

FIG. 1. Isolation of two sets of λ cDNA clones. Several λgt10 and λgt11 libraries were screened with the two oligonucleotides described in *Materials and Methods*. The first oligonucleotide was designated R in Baltimore and D in San Diego. The second oligonucleotide was designated F in Baltimore and A in San Diego. The clone designations indicate the oligonucleotides to which they hybridize. The plaque-purified λ clones were spotted in grids onto lawns of *Escherichia coli* strain LE 392, lifted onto nitrocellulose, and probed with random primed cDNA inserts as indicated. In addition, subcloned inserts were digested with *Eco*RI Southern blotted, and probed with the same random primed inserts. ■, Strong hybridization; ♦, weak hybridization; −, no hybridization above background. Hybridizations were carried out at high stringency.

found repeatedly among the $G_\alpha 1$ clones. RF1, FR7, FR11, AD1, AD3, and AD4 are less than or equal to 1.2 kilobases (kb), whereas RF3, RF5, and FR5 are 2.4 kb.

The nucleotide sequence and the deduced amino acid sequence for $G_\alpha 1$ are shown in Fig. 2. Two clones, RF1 and AD4, were sequenced in both directions. The sequences

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   1                                                                                                GAA TTC CAA AAA AAA AAA CAC AAT CAC AAC TAC TAC AAC AAC
  43 AAC AAC AAC AAC CAA AAA AAT CAA AAA GTC AAA ACA AAT TTA TAA AAT AAA ATA AAA TAA AAT ATA TAT ATA CAT ATA AAA TAA ATA

 130 ATG GGT AAT ATT TGT GGT AAA CCA GAA TTA GGA TCA CCA GAA GAG ATT AAA GCC AAT CAA CAT ATT AAT AGT TTG TTG AAA CAA GCA
   1 MET Gly Asn Ile Cys Gly Lys Pro Glu Leu Gly Ser Pro Glu Glu Ile Lys Ala Asn Gln His Ile Asn Ser Leu Leu Lys Gln Ala

 217 AGA TCT AAA TTA GAG GGT GAA ATT AAA TTA TTA TTG TTA GGA GCA GGT GAA TCA GGT AAA TCA ACA ATC GCC AAA CAA ATG AAG ATT
  30 Arg Ser Lys Leu Glu Gly Glu Ile Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Ala Lys Gln MET Lys Ile

 304 ATC CAT TTG AAT GGT TTC AAC GAT GAG GAG AAG TCA TCA TAT AAA ACC ATC ATC TAC AAT AAT ACA GTT GGT TCA ATG CGT GTG TTG
  59 Ile His Leu Asn Gly Phe Asn Asp Glu Glu Lys Ser Ser Tyr Lys Thr Ile Ile Tyr Asn Asn Thr Val Gly Ser MET Arg Val Leu

 391 GTA AAC GCC GCT GAA GAA TTA AAG ATT GGA ATC AGT GAA AAC AAT AAA GAA GCC GCC TCT AGA ATC TCA AAT GAT TTG GGC GAT CAT
  88 Val Asn Ala Ala Glu Glu Leu Lys Ile Gly Ile Ser Glu Asn Asn Lys Glu Ala Ala Ser Arg Ile Ser Asn Asp Leu Gly Asp His

 478 TTC AAT GGT GTG TTG ACT GCA GAG TTG GCA CAA GAT ATT AAA GCC CTT TGG GCA GAT CCA GGT ATT CAA AAT ACC TTC CAA AGA TCT
 117 Phe Asn Gly Val Leu Thr Ala Glu Leu Ala Gln Asp Ile Lys Ala Leu Trp Ala Asp Pro Gly Ile Gln Asn Thr Phe Gln Arg Ser

 565 TCA GAA TTC CAA CTA AAT GAT TCA GCC GCT TAT TAC TTT GAT AGT ATC GAT AGA ATT AGT CAA CCA TTA TAT TTA CCA TCT GAA AAT
 146 Ser Glu Phe Gln Leu Asn Asp Ser Ala Ala Tyr Tyr Phe Asp Ser Ile Asp Arg Ile Ser Gln Pro Leu Tyr Leu Pro Ser Glu Asn

 652 GAT GTT TTA CGT TCA AGA ACT AAA ACA ACT GGT ATC ATT GAA ACA GTT TTT GAA ATT CAA AAT AGT ACA TTT AGA ATG GTT GAT GTT
 175 Asp Val Leu Arg Ser Arg Thr Lys Thr Thr Gly Ile Ile Glu Thr Val Phe Glu Ile Gln Asn Ser Thr Phe Arg MET Val Asp Val

 739 GGT GGT CAA AGA TCA GAA AGA AAG AAA TGG ATG CAT TGT TTC CAA GAA GTT ACA GCA GTT ATC TTT TGT GTT GCC CTT AGT GAA TAT
 204 Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp MET His Cys Phe Gln Glu Val Thr Ala Val Ile Phe Cys Val Ala Leu Ser Glu Tyr

 826 GAT CTT AAA CTT TAT GAA GAT GAT ACT ACA AAT AGA ATG CAA GAG TCA CTT AAA CTC TTT AAA GAA ATA TGT AAC ACC AAA TGG TTT
 233 Asp Leu Lys Leu Tyr Glu Asp Asp Thr Thr Asn Arg MET Gln Glu Ser Leu Lys Leu Phe Lys Glu Ile Cys Asn Thr Lys Trp Phe

 913 GCA AAT ACT GCT ATG ATT CTT TTC TTA AAT AAA AGA GAT ATT TTC TCT GAA AAG ATT ACA AAA ACA CCA ATT ACA GTT TGC TTC AAA
 262 Ala Asn Thr Ala Met Ile Leu Phe Leu Asn Lys Arg Asp Ile Phe Ser Glu Lys Ile Thr Lys Thr Pro Ile Thr Val Cys Phe Lys

1000 GAA TAT GAT GGT CCA CAA ACT TAC GAA GGT TGT TCA GAG TTT ATC AAA CAA CAA TTT ATC AAT CAA AAT GAA AAT CCA AAG AAA TCG
 291 Glu Tyr Asp Gly Pro Gln Thr Tyr Glu Gly Cys Ser Glu Phe Ile Lys Gln Gln Phe Ile Asn Gln Asn Glu Asn Pro Lys Lys Ser

1087 ATC TAC CCA CAT TTA ACT TGT GCC ACT GAT ACT AAT AAT ATC CTT GTT GTA TTC AAT GCT GTC AAA GAT ATT GTA TTA AAT TTA ACT
 320 Ile Tyr Pro His Leu Thr Cys Ala Thr Asp Thr Asn Asn Ile Leu Val Val Phe Asn Ala Val Lys Asp Ile Val Leu Asn Leu Thr

1174 TTG GGT GAA GCT GGT ATG ATT CTT TAA AGA ATC AAA ACT TGA ACA CAC TAT TCA ATT TTT AAA TAA TAA TAA TAA TAA TAA AAA AAA
 349 Leu Gly Glu Ala Gly MET Ile Leu

1261 AAA AGG AAT TC
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FIG. 2. Nucleotide and deduced amino acid sequence of $G_\alpha 1$. *Eco*RI inserts were subcloned into pSP73 or pGEM2 and sequenced directly with the dideoxynucleotide method using Sequenase.

agreed completely with each other and with partial sequence of FR7 and RF3 (one of the larger class of clones). The longest open reading frame begins at the first AUG at nucleotide 130 and ends at nucleotide 1197. It encodes a polypeptide of 356 amino acids with a calculated molecular mass of 40,621 daltons. The internal *Eco*RI restriction site is at nucleotide 568.

The nucleotide and deduced amino acid sequences for $G_\alpha 2$ are shown in Fig. 3. These were determined by overlapping clones D2 and AD6 and a polymerase chain reaction-generated copy of genomic DNA (15). The clones encompass 1174 base pairs (bp) and a complete coding sequence. The open reading frame begins at the first AUG at nucleotide 72 and ends at nucleotide 1142. It encodes a polypeptide of 357 amino acids with a calculated molecular mass of 41,323 daltons.

Comparison of Primary Sequence of α Subunits. Fig. 4 shows an amino acid sequence comparison between the $G_\alpha 1$ and $G_\alpha 2$ subunits of *Dictyostelium*, yeast GPA1, mammalian G protein of unknown function (G_o), inhibitory G protein (G_i), stimulatory G protein (G_s), and transducin (Tr). $G_\alpha 1$ and $G_\alpha 2$ are 45% identical to each other and 45% identical to mammalian G_i, G_o, and Tr. They are 35% identical to G_s and GPA1 (18, 19). The predicted molecular masses are in the range of mammalian G proteins (39–45 kilodaltons) since the 107 amino acid "insert" found in GPA1 is not present in *Dictyostelium* α subunits.

Five regions of the α subunits are highly identical. Four of these regions have been proposed as the sites for binding GTP based on analogy to c-Ha-*ras* and elongation factor Tu and are designated A, C, E, and G (19). The fifth region near the carboxyl terminus, which we designate region T, has not been previously described.

Region A. This region is located at amino acids 36–52 in $G_\alpha 1$ and 31–47 in $G_\alpha 2$ and is proposed to be the site of GTPase activity. A large portion of this region is 100% identical among all seven α subunits compared in Fig. 4. The sequence GAGESGK, from which one of the oligonucleotides was prepared, is included in the stretch of 100% identity.

Region C. Region C is located at amino acids 201–207 in $G_\alpha 1$ and 203–209 in $G_\alpha 2$. With the exception of the initial position where F is replaced with V, $G_\alpha 1$ and $G_\alpha 2$ are identical to mammalian α subunits. GPA1 has two substitutions in this stretch. The stretch of highest identity among all seven α subunits includes the 3′ half of this proposed site and 6 amino acids on the 3′ side of the C site. This sequence, GGQRSERKKW, is the position of the second oligonucleotide.

Region E. The E region includes amino acids 224–232 in $G_\alpha 1$ and 226–234 in $G_\alpha 2$. The differences between the α subunits in this stretch are clustered in five positions. By comparing the first four positions to $G_\alpha 1$ (amino acids 224, 226, 227, and 229), it is found that yeast differs in all four, Tr and G_s differ in two positions, and $G_\alpha 2$ differs in one position. In the fifth position (amino acid 231 in $G_\alpha 1$ and 233 in $G_\alpha 2$), $G_\alpha 1$, $G_\alpha 2$, and yeast are identical, whereas all of the mammalian α subunits are different.

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   1                    GA ATT CCG ATT TAT TTA TAA AAC TAT ATA TAT ATA TAT AAT TAT ATA AAA TAA AGA AAA AAA ACT TAA AAA

  72 ATG GGT ATT TGT GCA TCA TCA ATG GAA GGA GAA AAA ACC AAT ACT GAT ATT AAT TTA TCT ATT GAA AAA GAA AGA AAA AAG AAA CAT
   1 MET Gly Ile Cys Ala Ser Ser Met Glu Gly Glu Lys Thr Asn Thr Asp Ile Asn Leu Ser Ile Glu Lys Glu Arg Lys Lys Lys His

 159 AAT GAA GTT AAA TTA TTA TTA CTT GGT GCT GGT GAA TCT GGT AAA TCA ACA ATT TCA AAA CAA ATG AAA ATT ATT CAT CAA AGT GGT
  30 Asn Glu Val Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Ser Lys Gln Met Lys Ile Ile His Gln Ser Gly

 246 TAC AGT AAT GAA GAA AGA AAA GAA TTT AAA CCA ATT ATT ACA AGA AAT ATT CTT GAT AAT ATG AGA GTA TTA TTG GAT GGA ATG GGA
  59 Tyr Ser Asn Glu Glu Arg Lys Glu Phe Lys Pro Ile Ile Thr Arg Asn Ile Leu Asp Asn Met Arg Val Leu Leu Asp Gly Met Gly

 333 AGA CTT GGA ATG ACA ATT GAC CCA AGT AAT TCA GAC GCA GCA GTT ATG ATT AAA GAA TTA ACA TCA TTA CAA GCA TCA ATT GTT ACA
  88 Arg Leu Gly Met Thr Ile Asp Pro Ser Asn Ser Asp Ala Ala Val Met Ile Lys Glu Leu Thr Ser Leu Gln Ala Ser Ile Val Thr

 420 GAT TGT TGG GGA GAA TTA AAT GAA GAT CAA GGT AAA AAG ATA AAA GCC TTA TGG ACA GAC CCA GGT GTC AAA CAG GCA ATG AGA AGA
 117 Asp Cys Trp Gly Glu Leu Asn Glu Asp Gln Gly Lys Lys Ile Lys Ala Leu Trp Thr Asp Pro Gly Val Lys Gln Ala Met Arg Arg

 507 GCA AAT GAA TTT AGT ACA TTA CCA GAT TCA GCT CCA TAT TTC TTT GAT AGT ATA GAT CGT ATG ACA TCA CCA GTT TAT ATT CCA ACT
 146 Ala Asn Glu Phe Ser Thr Leu Pro Asp Ser Ala Pro Tyr Phe Phe Asp Ser Ile Asp Arg Met Thr Ser Pro Val Tyr Ile Pro Thr

 594 GAT CAA GAT ATT TTA CAT ACT CGT GTT ATG ACA AGA GGT GTT CAT GAA ACA AAC TTT GAA ATT GGT AAA ATC AAA TTT AGA TTA GTA
 175 Asp Gln Asp Ile Leu His Thr Arg Val Met Thr Arg Gly Val His Glu Thr Asn Phe Glu Ile Gly Lys Ile Lys Phe Arg Leu Val

 681 GAT GTT GGT GGT CAA CGT TCT GAA AGA AAG AAA TGG TTA TCA TGT TTC GAT GAT GTT ACA GCA GTT GTA TTT TGT GTT GCC TTG TCC
 204 Asp Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Leu Ser Cys Phe Asp Asp Val Thr Ala Val Val Phe Cys Val Ala Leu Ser

 768 GAA TAT GAT TTA TTA TTG TAT GAA GAT AAT TCA ACC AAT CGT ATG TTG GAA AGT TTA CGT GTA TTC AGT GAT GTT TGC AAT AGT TGG
 233 Glu Tyr Asp Leu Leu Leu Tyr Glu Asp Asn Ser Thr Asn Arg Met Leu Glu Ser Leu Arg Val Phe Ser Asp Val Cys Asn Ser Trp

 855 TTT GTA AAT ACT CCA ATC ATT TTA TTC TTA AAC AAA TCT GAT TTA TTC AGA GAG AAA ATC AAA CAT GTT GAT CTC TCT GAA ACT TTC
 262 Phe Val Asn Thr Pro Ile Ile Leu Phe Leu Asn Lys Ser Asp Leu Phe Arg Glu Lys Ile Lys His Val Asp Leu Ser Glu Thr Phe

 942 CCA GAA TAT AAA GGT GGT AGA GAT TAC GAA AGA GCC TCA AAC TAT ATC AAA GAA CGT TTC TGG CAA ATC AAT AAA ACC GAA CAA AAA
 291 Pro Glu Tyr Lys Gly Gly Arg Asp Tyr Glu Arg Ala Ser Asn Tyr Ile Lys Glu Arg Phe Trp Gln Ile Asn Lys Thr Glu Gln Lys

1029 GCA ATC TAT TCT CAT ATC ACT TGT GCC ACC GAT ACA AAT AAT ATT CGT GTC GTT TTT GAA GCT GTA AAA GAT ATT ATT TTC ACT CAA
 320 Ala Ile Tyr Ser His Ile Thr Cys Ala Thr Asp Thr Asn Asn Ile Arg Val Val Phe Glu Ala Val Lys Asp Ile Ile Phe Thr Gln

1116 TGT GTT ATG AAA GCT GGT TTA TAT TCT TAA AAT AAT TAT AAT AAT AAA AAA CGG AAT TC
 349 Cys Val Met Lys Ala Gly Leu Tyr Ser
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FIG. 3. Nucleotide sequence and deduced amino acid sequence of $G_\alpha 2$. *Eco*RI inserts were subcloned into pSP73 or pGEM2 and sequenced directly with the dideoxynucleotide method using Sequenase.

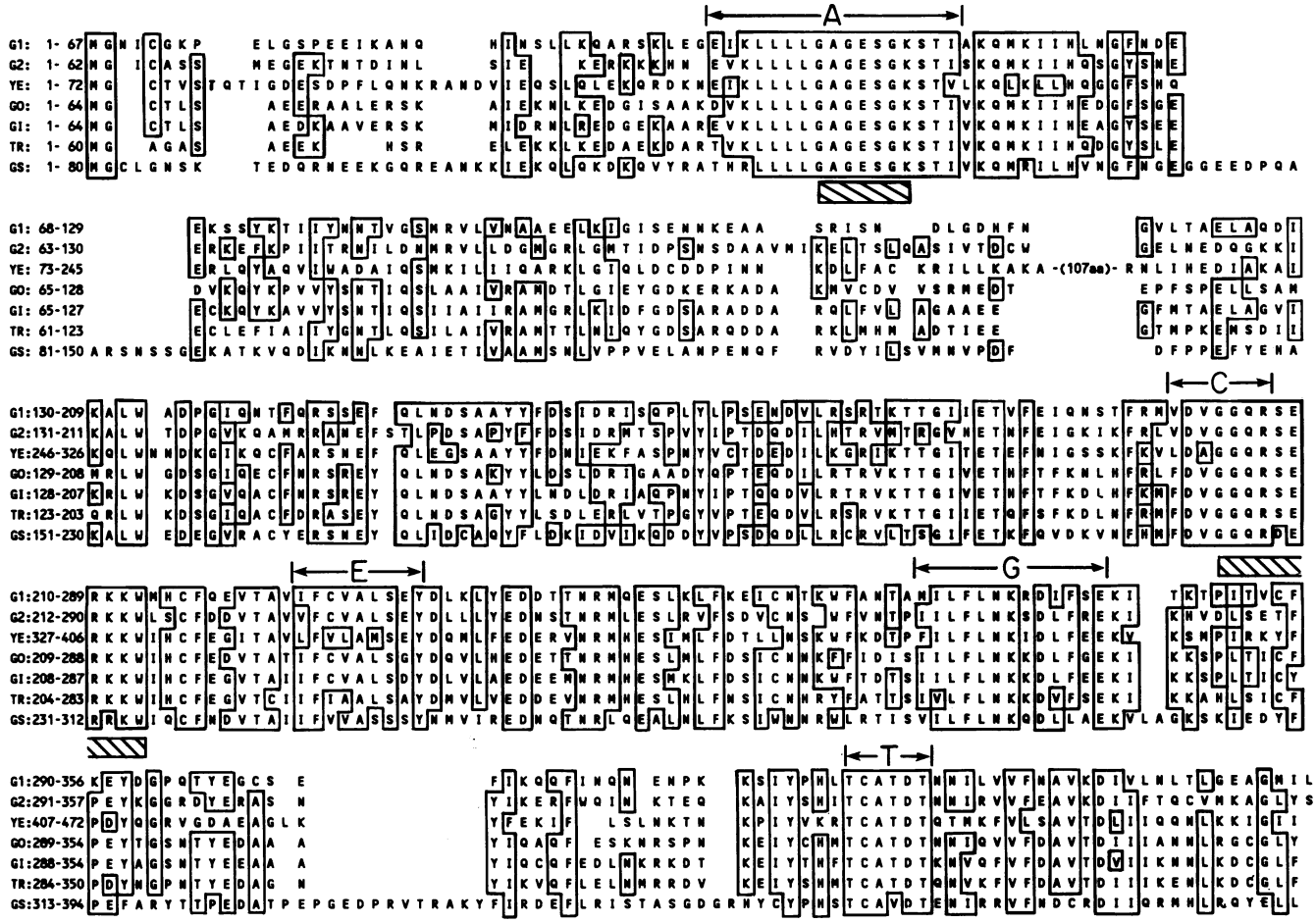

FIG. 4. Comparison of deduced amino acid sequences with those of mammalian G proteins. Amino acid sequences for $G_{\alpha}1$ (G1) and $G_{\alpha}2$ (G2) were compared with those for yeast GPA1 (YE), bovine brain G_o (GO), rat G_{i1} (GI), bovine retinal transducin (TR), and rat G_s (GS). Sequences were aligned with the program of Doolittle and collaborators (16, 17). Positions where either $G_{\alpha}1$ or $G_{\alpha}2$ is identical to at least two other G_{α} proteins are boxed. Hatched bars below the sequence indicate the positions of the oligonucleotide sequences used in the initial screening. Capital letters A, C, E, G, and T indicate regions of the sequence discussed in the text.

Region G. The proposed G region contains residues 266–278 in $G_{\alpha}1$ and 267–279 in $G_{\alpha}2$. Again the nonidentical residues are clustered in a few positions. In positions 273 and 277 of $G_{\alpha}1$, all of the α subunits differ from each other. In the remaining positions, $G_{\alpha}1$, Tr, and G_s have two substitutions and yeast has one.

Region T. The fifth stretch of nearly complete identity is the sequence TCATDT near the carboxyl terminus of the polypeptides. This is located at positions 325–330 in $G_{\alpha}1$ and 326–331 in $G_{\alpha}2$. Only G_s has one substitution in this stretch.

Two areas in which the α subunits diverge significantly from each other are the amino-terminal 30–35 amino acids and the region between the A and the C sites. In $G_{\alpha}1$ and $G_{\alpha}2$ this second divergent area includes residues 62–197 and 57–199, respectively. This area can be subdivided into two regions with different degrees of identity. The 5′ half (roughly amino acids 62–129 in $G_{\alpha}1$ and 52–130 in $G_{\alpha}2$) contains very few identities among the α subunits. However, the 3′ half (amino acids 130–197 in $G_{\alpha}1$ and 131–199 in $G_{\alpha}2$) contains patches where several of the α subunits are identical.

Both $G_{\alpha}1$ and $G_{\alpha}2$ contain the arginine (position 180 in $G_{\alpha}1$ and 182 in $G_{\alpha}2$), which, in mammalian G_s, is the site for ADP-ribosylation by cholera toxin (20). Neither α subunit has a cysteine as the fourth residue from the carboxyl terminus, which is the consensus site for ADP-ribosylation by pertussis toxin (21).

Developmental Expression of $G_{\alpha}1$ and $G_{\alpha}2$. RNA isolated from wild-type *Dictyostelium* cells (strain NC-4) was probed with $G_{\alpha}1$ and $G_{\alpha}2$ cDNA (Fig. 5). $G_{\alpha}1$ hybridizes to multiple mRNAs. The predominant species of 1.7 kb is expressed at moderate levels in vegetative cells and increases to a maximal level at 10–12 hr, corresponding to the time of loose aggregate formation in these experiments. Thereafter, RNA levels rapidly decrease. The 2.6-kb species has a similar pattern of regulation. The 2.2-kb RNA is preferentially expressed in multicellular aggregates.

$G_{\alpha}2$ also hybridizes to multiple RNA bands having different patterns of expression. The predominant 2.7-kb RNA is not expressed or expressed at very low levels in vegetative cells. Upon initiation of development, RNA levels increase, reaching a maximum level during aggregation and then declining. The 2.9-kb mRNA is present briefly very early in development. The two other mRNAs (1.9 kb and 2.3 kb) appear in sequence during the multicellular aggregate stage.

Genomic Structure of α Subunits. Genomic mapping and isolation of genomic clones suggest that there is a single $G_{\alpha}1$ gene and a single $G_{\alpha}2$ gene (data not shown; ref. 22). This suggests that the multiple transcripts with dramatically different developmental time courses result from alternate splicing events or nested promoters.

DISCUSSION

D. discoideum has at least two G protein α subunits, designated $G_{\alpha}1$ and $G_{\alpha}2$. They are closely related to the mammalian G protein α subunits, being most similar in amino acid

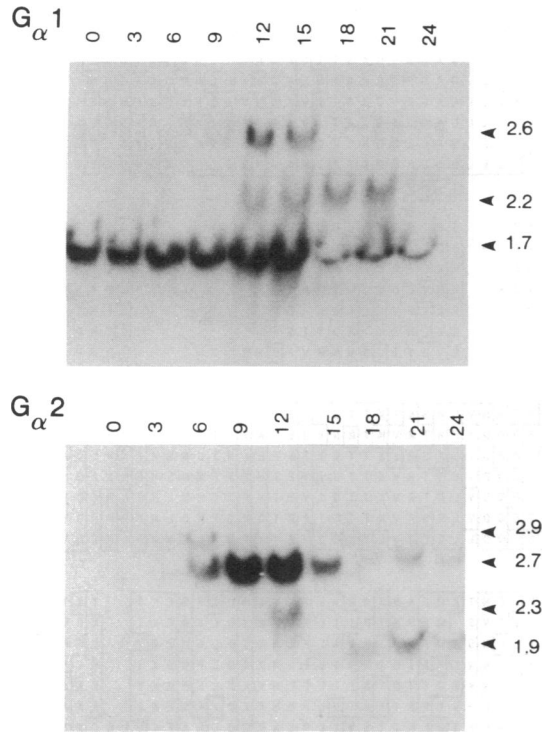

FIG. 5. Developmental regulation of G_α mRNAs. RNA samples isolated from wild-type NC-4 cells developed on filters and harvested at the times indicated were probed with $G_\alpha 1$ or $G_\alpha 2$ cDNA inserts. "0" represents RNA isolated from vegetative cells. In this developmental time course, visible aggregation initiated at ≈7–8 hr, streams were visible at ≈8–9 hr, loose aggregates were formed by 12 hr, and early culminants were found by 21 hr. Numbers on the right indicate the size of the RNAs in kb.

sequence to $G_{\alpha i}$. Both subunits are potential substrates for ADP-ribosylation by cholera toxin. No physiological effects of cholera toxin on *D. discoideum* have been found, although ADP-ribose acceptors have been reported (23). Although pertussis toxin exerts its effects most frequently on G_i-like G proteins, such as $G_\alpha 1$ and $G_\alpha 2$, neither sequence contains the cysteine, which is the consensus site for ADP-ribosylation. However, biochemical effects of pertussis toxin on signaling in *D. discoideum* have been reported, suggesting that there may be at least one more G_α subunit (24).

The previously unidentified T region (325–330) appears to be important since it is completely identical in all α subunits. The carboxyl terminus of α subunits has been implicated in receptor binding based on antibody blocking experiments (25). A recent report demonstrates that a peptide that encompassed this sequence blocks binding of Tr to rhodopsin (26). The most critical residue for the blocking effect was cysteine 321, which is the C of TCATDT.

The A region is flanked by highly divergent areas (amino acids 3–35 and 60–125). The amino terminus of α subunits has been implicated in $\beta\gamma$ subunits binding based on reconstitution assays with proteolytically cleaved Tr (27). At least two mammalian β subunits and two γ subunits have been identified (28). Neer and Clapham (28) suggest that different combinations of β and γ may be functionally different. If this is the case, the site of $\beta\gamma$ interaction with α may be expected to be a divergent area. The stretch 60–125 has been proposed to confer effector specificity. However, a chimeric α subunit containing the amino half of murine $G_{\alpha i2}$ and the carboxyl half of murine $G_{\alpha s}$ stimulated adenylate cyclase (29). Either the effector interaction site is not contained in this portion of the molecule or only part of the site is contained in this region. In addition, the stretch 60–125 corresponds to residues 73–245 in GPA1, which includes the 107 amino acid insert not found in *Dictyostelium* or mammalian α subunits.

Both $G_\alpha 1$ and $G_\alpha 2$ probes are complementary to multiple, developmentally expressed RNAs, but DNA blot data suggest that both are encoded by single genes. At present, we do not know if the multiple mRNAs result from differential splicing or multiple transcription initiation sites. It is possible that some of the less abundant mRNA species complementary to these probes could be the products of other related G_α protein genes.

Two lines of evidence strongly suggest that $G_\alpha 1$ and $G_\alpha 2$ have distinct functions. (*i*) $G_\alpha 1$ and $G_\alpha 2$ are as divergent from each other as they are from the α subunits of other organisms. (*ii*) The mRNAs encoding these proteins have very different developmental patterns of expression. We have examined the regulation and function of $G_\alpha 1$ and $G_\alpha 2$ during growth and development (22). Our results indicate that the two proteins have distinct functions and may regulate different signal transduction systems.

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