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Identification of *Dictyostelium* G_α genes expressed during multicellular development

(GTP-binding protein/signal transduction/polymerase chain reaction)

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ABSTRACT **Guanine nucleotide-binding protein (G protein)-mediated signal transduction constitutes a common mechanism by which cells receive and respond to a diverse set of environmental signals. Many of the signals involved in the developmental life cycle of the slime mold *Dictyostelium* have been postulated to be transduced by such pathways and, in some cases, these pathways have been demonstrated to be dependent on specific G proteins. Using the polymerase chain reaction, we have identified two additional *Dictyostelium* G_α genes, $G_\alpha 4$ and $G_\alpha 5$, that are developmentally regulated. Transcripts from both of these genes are primarily expressed during the multicellular stages of development, suggesting possible roles in cell differentiation or morphogenesis. The entire $G_\alpha 4$ gene was sequenced and found to encode a protein consisting of 345 amino acids. The $G_\alpha 4$ subunit is homologous to other previously identified G_α subunits, including the *Dictyostelium* $G_\alpha 1$ (43% identity) and $G_\alpha 2$ (41% identity) subunits. However, the $G_\alpha 4$ subunit contains some unusual sequence divergences in residues highly conserved among most eukaryotic G_α subunits, suggesting that $G_\alpha 4$ may be a member of another class of G_α subunits.**

Regulatory guanine nucleotide-binding proteins (G proteins) are often involved in the transduction of signals from activated cell-surface receptors to intracellular effectors (for review, see refs. 1–5). The diversity of such signals is reflected in the multitude of different G protein-coupled receptors, suggesting that G proteins represent a common mechanism by which signals are transduced intracellularly. Many of these G protein-mediated signals are essential for proper regulation of cell function, division, and differentiation. Therefore, it is not surprising that some mutant G proteins, like the closely related *ras*-encoded proteins, have now been implicated in the oncogenic growth of certain cell types (6, 7).

G proteins are heterotrimeric, consisting of α, β, and γ subunits, but only the G_α subunit binds and hydrolyzes guanine nucleotides (2, 8). The exchange of GDP for GTP in the G_α subunit is facilitated by ligand-bound receptors, resulting in the dissociation of the G_α from the $G_{\beta\gamma}$ dimer and the activation of downstream effectors. Subsequent hydrolysis of GTP to GDP by the intrinsic GTPase activity of the G_α subunit allows for the reassociation of the G protein subunits. The regions of the G_α subunits that interact with guanine nucleotides are highly conserved and thus useful in the identification of G_α genes (3, 8, 9). The number of G_α genes identified in most organisms is much greater than those identified for G_β and G_γ, which may be due to the relative ease of identification or perhaps to a lower number of G_β and G_γ subunits due to the ability of different G_α subunits to interact with the same $G_{\beta\gamma}$ complex. Some G_α subunits, such as the mammalian G_αs or the G_αt of transducin, appear to be the sole component required for activation of the effector and therefore the primary signal transducer of the heterotrimer (10, 11). Many G_α genes have been identified from a diverse set of eukaryotic organisms (microbes, animals, and plants); however, in most cases relatively little is known about the signal transduction processes in which they function (refs. 9, 12, 13; for review, see ref. 3).

The slime mold *Dictyostelium discoideum* provides a good biological system in which genetic and biochemical approaches can be used to study G protein-mediated signal transduction (for review, see refs. 14–16). *Dictyostelium* grow vegetatively as unicellular amoebae that, upon nutrient depletion, aggregate into a multicellular mound. These mounds differentiate into migrating slugs that eventually culminate to form fruiting bodies, consisting of a spore mass upon a stalk. Many of the signals required for the developmental processes, such as cell migration, differentiation, and spacial organization, are known or suspected to be mediated by signal transduction pathways that involve G proteins (refs. 12, 17–22; for review, see ref. 14). One of the best-characterized signals is that of the cAMP signal relay system required for the aggregation phase of development. Starved amoebae respond to extracellular cAMP by initiating a number of cellular responses, including activation of adenylate cyclase, guanylate cyclase, and phospholipase C, developmental gene expression, and chemotactic mobility toward cAMP. These responses are mediated through the G protein believed to contain the α subunit $G_\alpha 2$, which is coupled to a cAMP receptor, presumably cAR1 (18, 23, 24). In addition to this early developmental response, cAMP stimulates many late developmental functions, such as expression of prespore- and prestalk-specific genes and morphogenesis during culmination, suggesting the presence of additional cAMP receptors and G proteins later in development (25–32). Other responses, such as chemotaxis to folic acid during vegetative growth, are also thought to be mediated through G proteins (33, 34). However, the response to folic acid is not dependent on either of the two previously characterized G_α genes ($G_\alpha 1$ or $G_\alpha 2$), suggesting the presence of other G_α genes for these responses (18). Moreover, molecular genetic experiments have suggested that $G_\alpha 1$ is probably not required for cAMP-mediated late responses. Similarly, the temporal pattern of expression of $G_\alpha 2$ also suggests that other G_α subunits are required for controlling cAMP-mediated prestalk and prespore gene expression and morphogenesis during the multicellular stages. We report here the identification of two additional *Dictyostelium* G_α genes isolated by PCR (polymerase chain reaction). Both of these G_α genes are developmentally regulated and are primarily expressed during the multicellular stages of development. One of these genes encodes a G_α subunit with divergence to other G_α subunits in

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specific, highly conserved sites, suggesting that it may represent a member of another class of G_α subunits.

MATERIALS AND METHODS

DNA Sources. *Dictyostelium* genomic DNA used for the PCR studies and for construction of the genomic library was isolated from the *D. discoideum* strain KAx-3 as described by Dynes and Firtel (35). The oligonucleotide primers KWIHCF [OMP19, previously described by Strathmann *et al.* (9)], KWIQCF [CT48, GCGGATCCAA(AG)TGGAT(TCA)CA(AG)T-G(TC)TT], and TCATD [T_α29 (antisense), GGATC-C(GATC)GT(AG)TC(GATC)GT(GATC)GC(AG)CA-(GATC)GT] were used to isolate $G_\alpha 4$ and $G_\alpha 5$ PCR fragments.

PCR. PCR was performed using a Gene AMP kit (Perkin–Elmer/Cetus) under conditions similar to those recommended by the manufacturer: denaturation (94°C), 1 min; anneal (37°C), 2 min; extension (72°C), 3 min; for 35 cycles except that an annealing temperature of 55°C was used in the initial isolation of $G_\alpha 4$ and $G_\alpha 5$ fragments. Analysis of the $G_\alpha 4$ gene from strains NC-4, V-12, HPS400, and KAx-3 and KAx-3 cDNA (original RNA was isolated from cells 16 hr into development) was conducted by PCR using the oligonucleotides JH19 (GTTTGGATCCAGCTGCTAATAATAAC-CATGGTTC) and JH20 (GTTTGTCGACAAATTGCAACT-TATTTCAGCC) (see also Fig. 3).

DNA and RNA Blots. *Dictyostelium* DNA blots were performed as described by Maniatis *et al.* (36), hybridized in a 450 mM sodium chloride/45 mM sodium citrate/8 mM EDTA/0.2% SDS/50% formamide solution at 37°C, and washed in a 15 mM sodium chloride/1.5 mM sodium citrate/0.2% SDS solution at 37°C for high stringency conditions for *Dictyostelium* DNA. Expression of the $G_\alpha 4$ and $G_\alpha 5$ genes was determined by RNA blot analysis as described by Mann and Firtel (37). DNA probes were generated by random primer probe synthesis using a Prime-a-Gene kit (Promega) according to the manufacturer's instructions.

Library Screening and Sequencing. The *Dictyostelium* *Sau*3A partial genomic library was constructed as described by Dynes and Firtel (35) except that pAT153L (38) was used as the vector backbone. Plasmids that contain $G_\alpha 4$ sequences were identified by colony hybridizations as described by Grunstein and Hogness (39). DNA fragments were cloned into phagemid pT7T3U18 or pT7T3U19 (Pharmacia) and sequenced using a T7 DNA polymerase/chain-termination method specified by the Sequenase DNA sequencing kit (United States Biochemical) according to manufacturer's instructions.

RESULTS

Identification of G_α Genes. A search for additional G_α subunits from *Dictyostelium* was conducted by using a PCR technique similar to that described for identifying further G_α genes from mouse (9). Degenerate oligonucleotides, corresponding to highly conserved regions of G_α subunits, were used as primers for the amplification of *Dictyostelium* genomic DNA, which is suitable for this type of analysis since *Dictyostelium* genes contain relatively few introns and most of these are small [≈100 base pairs (bp)] (40). To avoid isolating previously identified *Dictyostelium* G_α genes ($G_\alpha 1$ and $G_\alpha 2$), one set of oligonucleotides was chosen for the region KWI(H/Q)CF, which is highly conserved among most G_α subunits but slightly divergent in the G_α1 (KWMHCF) and G_α2 (KWLSCF) subunits (23). At an annealing temperature of 55°C, neither $G_\alpha 1$ or $G_\alpha 2$ DNA could be amplified by PCR but fragments 500–600 bp (predicted size for G_α gene fragments) could be amplified from total genomic DNA (data not shown). These fragments were cloned into phagemids and then sequenced. Two of these fragments contained open reading frames that showed 45–51% identity to the corresponding regions of the G_α1 and G_α2 subunits (Fig. 1) and were thus designated as $G_\alpha 4$ and $G_\alpha 5$. To help confirm that $G_\alpha 4$ and $G_\alpha 5$ fragments originated from *Dictyostelium* DNA,

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     *                                    -*-A---          *
Gα1  MGNICGKPELGSPEEIKANQHINSLLKQARSKLEGEIKLLLLGAGESGKSTIAKQMKIIHLNGFNDE EKSSYKTIIYNN   79
Gα2  MG ICASSMEGEKTNTDINLSIE   KERKKKHN EVKLLLLGAGESGKSTISKQMKIIHQSGYSNE ERKEFKPIITRN   74
Gα4  MRFKCFGSEETEQSSKIDK SIE    TDRRKLRKDVKLLLLGPGESGKSTIFKQMKIIQEDGGYSVEELLEYRAFVYSN   75
cs   MG     S            I   L          KLLLLGAGESGKST  KQMKI H  G     E   Y      N

                                                                    *
Gα1  TVGSMRVLVNAAEELKIGISENNKEAA    SRISN    DLGDHFNGVLTAELAQDIKALWADPGIQNTFQRSSEF QLND  152
Gα2  ILDNMRVLLDGMGRLGMTIDPSNSDAAVMIKELTSLQASIVTDCWGELNEDQGKKIKALWTDPGVKQAMRRANEFSTLPD  154
Gα4  CISQMEALLTASAKLNIELEVENKQRAANVLRRTI         GNEPWLLLAADIKHLWEDKGIKETYAQKDKHFQLND  146
cs            A   L I                                    I  LW D G      R  E  QL D

                     *                                  --C-   *                 E
Gα1  SAAYYFDSIDRISQPLYLPSENDVLRSRTKTTGIIETVFEIQNSTFRMVDVGGQRSERKKWMHCFQEVTAVIFCVALSEY  232
Gα2  SAPYFFDSIDRMTSPVYIPTDQDILHTRVMTRGVHETNFEIGKIKFRLVDVGGQRSERKKWLSCFDDVTAVVFCVALSEY  234
Gα4  SAAYFFDNIDRYMREDFVPNEQDVLRCRVRTTGIQESEFTFDKIRLKIVDVGGQRSQRRKWIHCFDCVTAVIFVAAMSDY  226
Gα5                                                              KWIHCFDSVTAVIFCVALSEY
cs   SA Y      R     Y P   D LR R  TTGI ET F      F   DVGGQRSERKKWIHCF  VTA IF  ALS Y

                                        --G--                                    *
Gα1  DLKLYEDDTTNRMQESLKLFKEICNTKWFANTAMILFLNKRDIFSEKITKTPITVCFKEYDGPQTYEGCSEFIKQQFINQ  312
Gα2  DLLLYEDNSTNRMLESLRVFSDVCNS WFVNTPIILFLNKSDLFREKIKHVDLSETFPEYKGGRDYERASNYIKERFWQI  313
Gα4  DQVLREDESVNRTRESLALFKEIVNCDYFKETPIVLFLNKKDLFKEKLKRVPLQSCFSDYTGPNKYKDAAMFIQSQYLAQ  306
Gα5  DQTLREEESQNRMKESLMLFDELLIVIGLEIQHSLYFLNKVDLFREKIRKIDLGDYFPVYPGGLSFDNSTQFINKMFLDL
cs   D  L ED   NRM ES  LF  I N   F  T   LFLNK DLF EK         FP Y G      A       F

               --I---
Gα1  NENPKKSIYPHLTCATDTNNILVVFNAVKDIVLNLTLGEAGMIL  356
Gα2  NKTEQKAIYSHITCATDTNNIRVVFEAVKDIIFTQCVMKAGLYS  357
Gα4   GPSPRTIYTHATCAVDTENIKFVFRAVRQTILSQALEHF      345
Gα5  STGSQR IFAHFTCATDT
cs         K IY H TCATDT N   VF AV D I    L   GL
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FIG. 1. Alignment of *Dictyostelium* G_α subunits. Amino acid sequences of G_α1, G_α2, G_α4, and G_α5 (partial sequence; determined from PCR fragment) subunits. $G_\alpha 4$ and $G_\alpha 5$ PCR fragments represent sequence between KWIHCF and TCATDT motifs. Consensus sequence (cs) represents residues conserved >77% among 14 G_α subunits compared: *Dictyostelium* G_α1, G_α2, G_α4, and G_α5, yeast GPA1 and GPA2, rat G_αs, G_αi-1, -2, -3, and G_αo, human G_αz, and bovine rod and cone transducin, where underlined residues indicate complete conservation (3, 12, 41). Residues conserved among all G_α subunits except G_α4 are indicated (*). Regions conserved among different families of G proteins are denoted A, C, E, G, and I as described by Halliday (42).

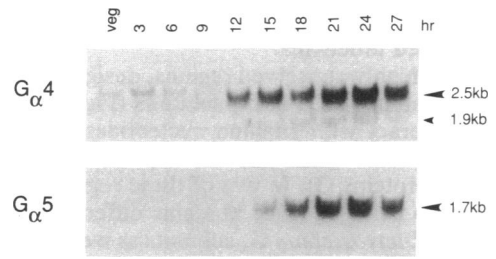

FIG. 2. Developmental expression of $G_\alpha 4$ and $G_\alpha 5$ genes. Total RNA was collected from KAx3 cells during development at times indicated ("veg" represents vegetatively growing cells), subjected to RNA blotting, and probed with $G_\alpha 4$ or $G_\alpha 5$ PCR fragments. Mounds of aggregated cells were observed ≈9 hr and culminants were found at 24 hr. Transcripts [size in kilobases (kb)] are indicated by arrowheads.

these sequences were used to probe a blot of genomic *Dictyostelium* DNA under conditions of high stringency (see *Materials and Methods*). The $G_\alpha 4$ and $G_\alpha 5$ probes detected distinct genomic restriction fragments, suggesting that $G_\alpha 4$ and $G_\alpha 5$ represent *Dictyostelium* single-copy genes (data not shown).

Developmental Expression of $G_\alpha 4$ and $G_\alpha 5$ Genes. Expression of the $G_\alpha 4$ and $G_\alpha 5$ genes during *Dictyostelium* development was monitored by probing RNA blots (Fig. 2). The $G_\alpha 4$ probe detected a major (2.5 kb) and a minor (1.9 kb) transcript that are expressed at a very low level during vegetative growth and early development but at a much higher level starting at the early mound stage (9 hr) and continuing through subsequent stages of development. The length of the major $G_\alpha 4$ transcript is more than twice the size of the $G_\alpha 4$ open reading frame (see below), suggesting that this transcript contains a large untranslated region, such as that found in the *Dictyostelium* $G_\alpha 2$ mRNA (12). A similar developmental pattern of expression was observed for the single 1.7-kb transcript detected by the $G_\alpha 5$ probe, except that the increased level of expression began slightly later starting at the tipped aggregate stage (12 hr).

Isolation of the $G_\alpha 4$ Gene. The PCR fragment of the $G_\alpha 4$ gene was used to probe a *Dictyostelium* genomic library constructed on the vector pAT153L. One of the plasmids isolated in this screen contained a 4.7-kb genomic insert encoding the entire $G_\alpha 4$ open reading frame consisting of 345 amino acids contained in three exons (Fig. 3). The predicted $G_\alpha 4$ subunit showed significant homology to the *Dictyostelium* $G_\alpha 1$ (43% identity) and $G_\alpha 2$ (41% identity) subunits as well as to G_α subunits from other eukaryotes, especially in the regions highly conserved among all known G_α proteins (Fig. 1). However, $G_\alpha 4$ diverges from other G_α subunits in some of the most highly conserved amino acid residues [e.g., R2 (arginine at position 2), P39, Q55, Q137, Q203, in addition to others], some of which are located in putative functional domains. For instance, the highly conserved GAGES box of G_α subunits, thought to be involved in GTP-binding and GTPase activity (43, 44), is altered in the $G_\alpha 4$ (GPGES) subunit (see *Discussion*). The $G_\alpha 4$ subunit has a glutamine at position 55 instead of a histidine residue as in most G_α subunits. This position is located in a highly conserved region near the GAGES sequence and may also be involved in guanine nucleotide interaction. In addition, the $G_\alpha 4$ subunit has a glutamine at position 203 instead of a glutamate residue within the highly conserved GGQRSERKKW region ($G_\alpha 4$-GGQRSQRKKW), a domain required for the intrinsic GTPase activity of G_α subunits (6, 45).

Due to the unusual nature of the GPGES sequence in the $G_\alpha 4$ subunit and the fact that a single nucleotide change can

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AAA AAA TAA AAT AAA ATA AAA TCA AAC AAA CAA TAA CAT ATA CTT TTA ACC TTA AAC AAA TTG CAA CTT ATT TCA GCC TAT TTA AAA TAT   90

ATA TAT AAA TAA ATC AAA AAA ATG AGA TTC AAG TGT TTT GGA TCA GAA GAA ACT GAA CAA AGT TCA AAA ATC GAT AAA AGT ATC GAA ACA  180
                          M   R   F   K   C   F   G   S   E   E   T   E   Q   S   S   K   I   D   K   S   I   E   T    23

GAT AGA AGA AAA CTT AGA AAA GAT GTA AAA TTA TTA TTA TTA GGT CCA GGT GAA TCT GGT AAA TCA ACT ATT TTT AAA CAA ATG AAG ATC  270
 D   R   R   K   L   R   K   D   V   K   L   L   L   L   G   P   G   E   S   G   K   S   T   I   F   K   Q   M   K   I   53

ATT CAA GAA GAT GGT GGT TAT AGT GTT GAA GAA CTT TTA GAA TAT CGT GCA TTC GTT TAT TCA AAT TGT ATC AGT CAA ATG GAA GCA TTA  360
 I   Q   E   D   G   G   Y   S   V   E   E   L   L   E   Y   R   A   F   V   Y   S   N   C   I   S   Q   M   E   A   L   83

TTA ACT GCA TCT GCA AAA TTA AAT ATT GAA TTA GAA GTT GAA AAT AAA▼CAA AGA GCA GCA AAT GTA CTT CGT AGA ACT ATT GGT AAT GAA  450
 L   T   A   S   A   K   L   N   I   E   L   E   V   E   N   K   Q   R   A   A   N   V   L   R   R   T   I   G   N   E  113

CCA TGG TTA TTA TTA GCA GCT GAT ATT AAA CAC CTT TGG GAA GAT AAA GGT ATT AAA GAA ACA TAT GCA CAA AAA GAT AAA CAT TTC CAA  540
 P   W   L   L   L   A   A   D   I   K   H   L   W   E   D   K   G   I   K   E   T   Y   A   Q   K   D   K   H   F   Q  143

TTA AAC GAT TCT GCT GCT TAT▼TTC TTT GAT AAT ATA GAT AGA TAT ATG AGA GAA GAT TTT GTA CCA AAT GAA CAA GAT GTT TTG AGA TGT  630
 L   N   D   S   A   A   Y   F   F   D   N   I   D   R   Y   M   R   E   D   F   V   P   N   E   Q   D   V   L   R   C  173

CGT GTT AGA ACT ACT GGT ATA CAA GAA TCA GAA TTT ACA TTT GAT AAG ATT AGA TTA AAG ATT GTA GAT GTC GGT GGT CAA AGA TCT CAA  720
 R   V   R   T   T   G   I   Q   E   S   E   F   T   F   D   K   I   R   L   K   I   V   D   V   G   G   Q   R   S   Q  203

AGA AGA AAA TGG ATT CAT TGT TTC GAT TGT GTT ACA GCA GTT ATT TTC GTT GCC GCT ATG AGC GAT TAC GAT CAA GTA CTT AGA GAA GAT  810
 R   R   K   W   I   H   C   F   D   C   V   T   A   V   I   F   V   A   A   M   S   D   Y   D   Q   V   L   R   E   D  233

GAA TCT GTA AAT AGA ACT AGA GAA TCC TTA GCC TTA TTC AAA GAG ATT GTA AAT TGT GAT TAC TTT AAA GAG ACA CCA ATC GTG TTA TTC  900
 E   S   V   N   R   T   R   E   S   L   A   L   F   K   E   I   V   N   C   D   Y   F   K   E   T   P   I   V   L   F  263

CTC AAC AAG AAG GAT CTT TTC AAG GAA AAA TTA AAG AGA GTA CCA CTC CAA TCT TGT TTC TCT GAT TAC ACT GGT CCA AAT AAA TAT AAA  990
 L   N   K   K   D   L   F   K   E   K   L   K   R   V   P   L   Q   S   C   F   S   D   Y   T   G   P   N   K   Y   K  293

GAT GCT GCC ATG TTC ATT CAA TCA CAA TAT CTT GCA CAA GGT CCT TCA CCA CGT ACT ATC TAT ACT CAT GCC ACT TGT GCT GTC GAT ACT 1080
 D   A   A   M   F   I   Q   S   Q   Y   L   A   Q   G   P   S   P   R   T   I   Y   T   H   A   T   C   A   V   D   T  323

GAA AAT ATT AAA TTT GTA TTC AGA GCA GTT AGA CAA ACA ATT TTA TCT CAA GCT TTA GAA CAC TTC TAA AAT AAT CAA TAG TAA TCA AAA 1170
 E   N   I   K   F   V   F   R   A   V   R   Q   T   I   L   S   Q   A   L   E   H   F   *                              345

AAA ACT AAA AAA AAA AAA ATC AAA AAA AAA AAA AAA AAA AAA CAA CAA CCC                                                    1221
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FIG. 3. Genomic nucleotide sequence and deduced amino acid sequence of the $G_\alpha 4$ gene. Locations of intron sequences are indicated (▼). The region between the primer binding sites (underlined) was analyzed from multiple *Dictyostelium* strains to confirm the wild-type sequence (see text). The termination codon is indicated by an asterisk.

result in an alanine to proline transition, it was important to confirm that the $G_\alpha 4$ gene cloned from the genomic library was the wild type and not a fortuitous mutant allele or cloning artifact. Therefore, a fragment containing the amino-terminal third of the $G_\alpha 4$ gene was cloned by PCR from four different strains of *D. discoideum* (KAx-3, NC-4, V-12, and HPS400) as well as a cDNA library constructed from RNA isolated from strain KAx-3 at 16 hr from the start of development (slug stage) in order to confirm the $G_\alpha 4$ sequence. In all cases the sequence from these clones was identical to the original $G_\alpha 4$ genomic fragment except that the cDNA clone lacked the intron sequences as expected (data not shown).

The $G_\alpha 4$ subunit does not contain a myristoylation consensus sequence (MGXXXS) found at the amino terminus of many G_α subunits, suggesting that $G_\alpha 4$ is not myristoylated. Instead, the $G_\alpha 4$ subunit has an unusual amino terminus (MRFKCF) compared to most other G_α subunits. The $G_\alpha 4$, as well as $G_\alpha 1$ and $G_\alpha 2$, subunit also does not have a cysteine four residues from the carboxyl terminus, which in mammalian $G_\alpha i$ subunits is the site for ADP-ribosylation by pertussis toxin (46).

DISCUSSION

Identification and analysis of the *Dictyostelium* $G_\alpha 4$ and $G_\alpha 5$ genes suggest the presence of G protein-mediated signal transduction pathways that are important for the development of this organism after cells have aggregated to form multicellular mounds. The previously identified *Dictyostelium* G_α genes, $G_\alpha 1$ and $G_\alpha 2$, are preferentially expressed during growth and the aggregation stage of development. The regulated expression of the $G_\alpha 4$ and $G_\alpha 5$ genes parallels the spacial segregation of prespore and prestalk cells that is initiated early in the mound stage and maintained throughout the remainder of development (38, 47). The temporal pattern of expression suggests that the $G_\alpha 4$ and $G_\alpha 5$ subunits might be involved in the regulation of cell-type-specific developmental gene expression and/or spacial organization of cell types. The molecular mechanisms involved in these processes are not well understood and the identification of the regulatory components, which might include the $G_\alpha 4$ and $G_\alpha 5$ subunits, is a prerequisite for understanding *Dictyostelium* development at this level. The temporal patterns of $G_\alpha 4$ and $G_\alpha 5$ gene expression suggest that the corresponding subunits are not involved in the signal response to folic acid during vegetative growth, which is thought to be mediated by G proteins (33, 34). Therefore, it is likely that *Dictyostelium* contains additional G_α genes, some of which might be specific to vegetative growth and early development.

During the aggregation stage, cAMP induction of early gene expression has been shown to be regulated through cAMP-mediated pathways requiring the $G_\alpha 2$ subunit and the cAMP receptor cAR1 (18, 24). During the multicellular stages, cell-type-specific gene expression has also been suggested to be regulated, in part, by cAMP interacting with cell-surface cAMP receptors (25–32) that might transduce the signals through G proteins containing the $G_\alpha 4$ and/or $G_\alpha 5$ subunits. Recently, genes encoding homologs to the cAMP receptor cAR1 have been identified, all of which contain the seven putative transmembrane domains characteristic of G protein-coupled receptors (48, 49). These cAR1 homologs are preferentially expressed, like $G_\alpha 4$ and $G_\alpha 5$, during the multicellular stages of development (48). It is possible that the $G_\alpha 4$ and $G_\alpha 5$ subunits are coupled to these receptors or that the $G_\alpha 4$ and $G_\alpha 5$ subunits are involved in signal responses to stimuli other than cAMP. Other morphogens of *Dictyostelium* development include differentiation-inducing factor and ammonia (for review, see ref. 50), which are not thought to act through G protein-coupled receptors; however, there might be other unidentified morphogens that do require G protein-mediated processes.

Some of the highly conserved regions, designated A, C, E, G, and I by Halliday (42), of G_α subunits (Figs. 1 and 4) are thought to interact with guanine nucleotides based on the presence of similar regions in other G proteins, such as the family of *ras* proteins (3). In two of these regions, A and C, the $G_\alpha 4$ amino acid sequence contains differences with respect to other *Dictyostelium* G_α subunits as well as to the $G_\alpha s$ and $G_\alpha i$ classes of mammalian subunits, suggesting that the $G_\alpha 4$ subunit may be a member of a different class of G_α subunits with respect to guanine nucleotide interactions. Perhaps the most striking distinction of the $G_\alpha 4$ subunit is the substitution of a proline for an alanine residue at position 39 located in the highly conserved GAGES box within the A region that is thought to be involved in guanine nucleotide binding and hydrolysis (see Introduction). The mammalian $G_\alpha z$ subunit, another G_α subunit reported to have changes in this region (GTSNS; see Fig. 4), has a low guanine nucleotide exchange rate and low GTPase activity compared to other G_α subunits, but the significance of these differences in $G_\alpha z$ function *in vivo* is not yet understood (51). Three recently identified G_α subunits, the rat $G_\alpha 42$ (52) and the mouse $G_\alpha q$ and $G_\alpha 11$ (53), also show divergence in this region (GTGES; see Fig. 4), and biochemical analysis indicates that the rat $G_\alpha 42$ subunit shows unusual guanine nucleotide-binding kinetics (54). Mutations within the GAGES region, specifically a valine substitution for the central glycine, in other G_α subunits and the equivalent glycine in *ras* proteins result in reduced GTPase activity and thus presumably longer activated states (55, 56). Although the hydrophilicity, based on the algorithm of Hopp and Woods (57), of the GPGES region of $G_\alpha 4$ is similar to that of the corresponding regions of other G_α subunits and the proline at position 39 is not predicted to disrupt any α-helices or β-sheets based on models of G_α structure (58, 59), the structural differences of a proline residue might alter guanine nucleotide interactions. $G_\alpha 4$ also shows some amino acid changes in the sequence immediately downstream from the GAGES box. This includes a glutamine for histidine at position 55 and a phenylalanine at position 48. The rat $G_\alpha 42$ and mouse $G_\alpha q$ and $G_\alpha 11$ subunits also contain a phenylalanine at the adjacent position 47. In addition, the divergence of $G_\alpha 4$ from other G_α subunits at position 203 ($G_\alpha 4$ has a glutamine rather than a glutamate residue) within the C region might also affect guanine nucleotide interactions based on the reduced GTPase activity observed for other G_α subunits and *ras* proteins (6, 45). The recent identification of two mammalian G_α subunits that also contain the same GPGES region (T.M.W., unpublished data; ref. 60) suggests that $G_\alpha 4$ and these other G_α subunits might represent a different class of G proteins with respect to guanine nucleotide interactions and function.

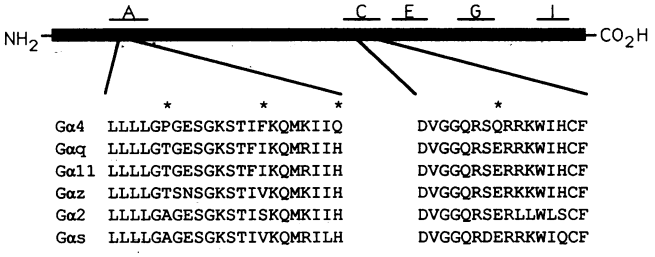

FIG. 4. Map of putative guanine nucleotide-interacting regions A, C, E, G, and I [designated by Halliday (42)] within G_α subunits. Comparisons of A and C regions in the *Dictyostelium* $G_\alpha 4$ and $G_\alpha 2$, mouse $G_\alpha q$ and $G_\alpha 11$, human $G_\alpha z$, and rat $G_\alpha 42$ and $G_\alpha s$ subunits regions are shown below the map. Residues conserved among most G_α subunits except $G_\alpha 4$ are indicated (*).

The relatively low expression of the $G_\alpha 4$ and $G_\alpha 5$ genes during vegetative growth suggests that the corresponding gene products may not be essential during this phase of the life cycle. However, we cannot rule out that these genes might be expressed abundantly in a subset of vegetative cells, possibly at a particular point in the cell cycle, as has been observed for the *Dictyostelium ras* gene *Dd-ras*, which, like the $G_\alpha 4$ and $G_\alpha 5$ genes, is predominantly expressed later in development (47). It is also possible that the expression of the $G_\alpha 4$ and $G_\alpha 5$ genes is regulated by way of the transduction pathway in which they function and, therefore, a low basal level of expression during vegetative growth/early development might be necessary for priming a receptor/ligand-activated autoregulatory induction of their own expression. This type of autoregulation has been observed for the induction of the $G_\alpha 2$ gene (17, 18).

The identification of the $G_\alpha 4$ and $G_\alpha 5$ genes not only provides additional opportunities to study general G protein function but may also define other signal transduction pathways that function during development. The ability to create null mutations by means of gene disruption or to overexpress wild-type or mutant genes in *Dictyostelium* (17, 18) should allow us to determine the function of $G_\alpha 4$ and $G_\alpha 5$ by examining the phenotypes of the resulting strains. Such a genetic analysis has already been initiated with the *Dictyostelium* $G_\alpha 1$ and $G_\alpha 2$ genes (refs. 17, 18; K. Okaichi and R.A.F., unpublished data). It should also be possible to isolate suppressors of recessive and dominant G_α mutations and thus identify other proteins that interact directly or indirectly with the G_α subunits. Genetic analysis with the $G_\alpha 4$ and $G_\alpha 5$ genes may provide us with a greater understanding of the signal transduction pathways involved in the development of *Dictyostelium* as well as other eukaryotes.

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