Mutations in the Dictyostelium heterotrimeric G protein α subunit Ga5 alter the kinetics of tip morphogenesis

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SUMMARY

Tip morphogenesis during the Dictyostelium developmental life cycle is a process by which prestalk cells sort to form the anterior region of the multicellular organism. We show that the temporal regulation of this morphological process is dependent on the copy number of the Dictyostelium Ga5 gene. Tip formation is delayed in aggregates of ga5 null mutant cells and accelerated in aggregates overexpressing the Ga5 gene compared to tip formation in wild-type cells. The onset of cell-type-specific gene expression associated with mound formation and tip morphogenesis is also temporally altered in Ga5 mutants. Tip morphogenesis in chimeric organisms of Ga5 mutants and wild-type cells is dependent on the copy number of the Ga5 gene, indicating that Ga5 function plays an integral role in the intercellular signaling of this stage of development. The Ga5 gene encodes a Ga subunit that has 51% identity to the Dictyostelium Ga4 subunit. Like the Ga4 gene, the Ga5 gene is expressed in a subset of cells distributed throughout the multicellular organism, with a distribution that is similar to the anterior-like cell population. Amino acid substitutions in the Ga5 subunit analogous to substitutions altering guanine nucleotide binding and hydrolysis in other Ga subunits had no apparent effect on the rate of tip formation when a single copy of the mutant gene was used to replace the wild-type gene. Overexpression of these mutant Ga5 genes by increased gene dosage resulted in cell death, suggesting that high levels of the altered subunits have detrimental effects during vegetative growth.

Key words: Dictyostelium, G protein, tip morphogenesis, temporal regulation, Ga5, mound formation

INTRODUCTION

Nutrient-deprived Dictyostelium cells aggregate to form a multicellular mound (typically 10⁶ cells) that differentiates to form a migratory slug and then eventually a fruiting body (a mass of spores on top of a stalk) (Loomis, 1982). One of the earliest morphogenic processes of this developmental life cycle is the migration of cells to the top of the multicellular mound to form a tip, which represents the anterior end of the organism (Williams et al., 1989; Traynor et al., 1992; Esch and Firtel, 1991; Early et al., 1993). As the tip forms, the entire aggregate elongates upward to form a finger and then falls to the substratum as a migrating slug or pseudoplasmodium. The cells in the anterior region of the developing organism comprise multiple prestalk cell populations that give rise to the stalk structure and upper cup regions of the fruiting body (Early et al., 1993; Jermyn and Williams, 1991), whereas the majority of cells located in the central and posterior regions of the organism are prespore cells (precursors to the spore mass). In addition to the prestalk and prespore cells, the multicellular organism has anterior-like cells (ALCs) that exist scattered throughout the organism and these cells become localized as part of the lower cup and basal disk of the fruiting body (Williams et al., 1993). ALCs share many characteristics with prestalk cells such as the retention of the dye neutral red and expression of certain classes of developmentally regulated genes. ALCs can be recruited to form new prestalk cells in aggregates that have had their anterior regions surgically removed (Sternfeld and David, 1982; Sternfeld, 1992). In some large aggregates, multiple tips can form and lead to a division of the aggregate into multiple organisms, indicating that tip morphogenesis is also important for controlling the size of the multicellular organism. Moreover, analysis of the function of the heterotrimeric G protein α subunit Go4, which is preferentially expressed in ALCs during the multicellular stages, suggests that ALCs play a cell non-autonomous role in regulating morphogenesis and cell-type differentiation (Hadwiger and Firtel, 1992; Hadwiger et al., 1994).

The role of extracellular cAMP in directing tip morphogenesis was proposed from the initial observations of directed migration of prestalk cells in response to exogenously added extracellular cAMP (Matsukuma and Durston, 1979; Sternfeld and David, 1981). Consistent with these observations, multicellular aggregates overexpressing an extracellular phosphodi-
esterase do not form tips, presumably due to the hydrolysis of extracellular cAMP, and marked prestalk cells sort to the base of the aggregate when mounds are placed on agar containing high levels of cAMP (Trayanor et al., 1992). In addition, cells in which the gene encoding the cell surface cAMP receptor (cAR) cAR2 has been disrupted are developmentally blocked at the stage of tip formation, implying that cAR2 and cAMP are integral components of the signaling mechanisms required for this morphogenetic process (Saxe et al., 1993). However, some car2 null aggregates eventually overcome this block in development after several hours, suggesting that other cARs can facilitate tip formation. In addition to regulating tip formation, extracellular cAMP is the primary signal for aggregation during early stages of development and an important inductive signal for developmental gene expression in the multicellular mound (Devreotes, 1994; Schnitzler et al., 1994, 1995).

Intensive searches for components involved in G protein-mediated signal transduction pathways have resulted in the identification of eight Gt subunit genes in Dictyostelium (Devreotes, 1994; Pupillo et al., 1989; Hadwiger et al., 1991; Wu and Devreotes, 1991; Cubitt et al., 1992; Wu et al., 1994). One of these genes, GtG, was identified in a PCR (polymerase chain reaction) screen using degenerate primers to conserved regions of known Gt subunits (Hadwiger et al., 1991). Sequence analysis of the GtG PCR product indicated homology between the GtG subunit and other GtG subunits. The GtG gene was found to be expressed at low levels during growth and early development but at a significantly higher level during multicellular development, similar to the temporal expression pattern of the Dictyostelium GtG4 gene (Hadwiger and Firtel, 1992; Hadwiger et al., 1991; Wu and Devreotes, 1991). In this report, we show that during the multicellular stages of development the GtG gene is preferentially expressed in cells with a spatial pattern similar to that of GtG4-expressing cells and ALCs, and we provide evidence that the GtG subunit plays a functional role in the temporal control of tip morphogenesis.

MATERIALS AND METHODS

Strains and medium

In this study, the following axenic haploid Dictyostelium strains were used: KAx-3 (wild-type), JH10 (thyl::PYR5-6) [described in (Hadwiger and Firtel, 1992)], JH257 (gta5::THY1), JH258 (Gta5HC, contains a high copy number of the GtG expression vector pJH206), and JH262 (strain JH257 with a low copy of the pJH206 construct). All strains used in this study were isogenic to the wild-type strain KAx-3, except at the loci noted, and all strains were grown axenically in HL5 medium or on K. aerogenes unless otherwise noted. Clonal isolates were obtained by plating transformed cell lines in 96-well microtiter plates. Electroporation of DNA into Dictyostelium cells was performed as described previously (Dynes and Firtel, 1989).

DNA constructs

Genomic DNA libraries used to isolate the GtG gene were constructed by digesting genomic DNA with XhoI/BclI or EcoRI only and then ligating size-specific fragments to the vector pHJ81 digested with SalI/BclI or EcoRI only, respectively. pHJ81 was created by inserting an XbaI/BclI linker (5'–CATAGGGGTGATCACCT-3') into the XbaI site of the vector pTT7318U vector (Pharmacia) to create a novel BclI site between duplicated XbaI sites. GtG genomic fragments [XhoI/BclI (2.1 kb) and BclI (1.0 kb)] were joined together in pHJ81 to create the plasmid pHJ198 that contains a contiguous 3.1 kb GtG genomic insert. The GtG expression vector pHJ206 was created from pHJ198 by the insertion of a 2.2 kb EcoRI fragment carrying a pAct6::Neo gene fusion (described in (Hadwiger and Firtel, 1992)) at the EcoRI site of the polylinker to confer resistance to the drug G418. The pHJ206 vector was linearized at the remaining XbaI site in the polylinker region before electroporating into Dictyostelium cells to increase the probability of random integration.

To create a disrupted gta5 allele, an EcoRV/XbaI fragment of the GtG gene from pHJ198 was inserted into the same sites of the pBlue-ScriptSK+ vector (Stratagene). A 1.6 kb region containing primarily the GtG open reading frame was PCR amplified from this construct using the oligonucleotide JH129 (5'–GTTTGAATTCCTACACTTACCC-3'), complementary to a sequence 80 bases downstream of the GtG open reading frame and also containing an EcoRI site) and an M13 universal primer (United States Biochemical) was used to generate an 1.6 kb fragment by PCR. This fragment was digested with EcoRI and SalI and then inserted into the same sites of pTT7318U to create the plasmid pHJ213. The GtG open reading frame of this plasmid was disrupted by inserting a 3.0 kb PstI/XbaI fragment containing the THY1 gene into the PstI/SpeI sites of the GtG sequence. The resulting plasmid, pHJ214, was digested with EcoRV and EcoRI and then electroporated into the strain JH10 to create a gta5 gene disruption. Genomic DNA blots probed with the GtG genomic insert of pHJ213 revealed 7 and 0.9 kb BclI fragments and 5.5 and 1.6 kb ClaI fragments for gta5 null cells, compared to the 4 and 0.9 kb BclI fragments and the 6 kb ClaI fragment for wild-type cells. These results are consistent with homologous recombination at the GtG locus.

The pGta5::lacZ gene fusion vector was created by ligating a BamHI linker (12 mer) to the EcoRV site of the GtG genomic fragment. Then a 1 kb SphI/BamHI fragment containing the GtG upstream sequence was inserted into the SphI/BamHI sites of pHJ146 as described (Hadwiger and Firtel, 1992), creating a coding region fusion of the GtG gene and the Escherichia coli lacZ gene. The 2.2 kb EcoRI fragment containing the pAct6::Neo gene fusion was inserted at the EcoRI site to create the plasmid pHJ210. Gta5 point mutations were created by site-directed in vitro mutagenesis as described (Kunkel, 1985). Oligonucleotides 5'–CCTTTTCTGATCTTAGTCACCAACATCC-3' (Gta5Q198I) or 5'–CTGATCTTTGTCAGAACATCTAACATTC-3' (Gta5G196F) were used as primers to mutagenize a 0.3 kb SphI/BclI fragment of the GtG gene. The mutagenized GtG fragments were used to replace wild-type fragments in pHJ230 which consisted of the XhoI/HincII GtG genomic fragment in the SalI/XbaI sites of pTT7318U. The 1.0 kb HindIII/EcoRV fragment of these resulting plasmids was deleted to remove the GtG promoter and initial coding region. A 3.2 kb BamHI fragment containing the Dictyostelium THY1 gene (Dynes and Firtel, 1989) was then inserted into the BamHI site of these vectors to create the vectors pHJ250 (Gta5Q198I) and pHJ252 (Gta5G196F). These vectors and a wild-type control vector pHJ255 (identical to pHJ250 and pHJ252 except for point mutations) were linearized at the SpeI site within the GtG coding region and electroporated into the strain JH10. Transformants with a single copy of these vectors integrated precisely into the SpeI site of the GtG locus were identified by genomic DNA blot analysis. Approximately 40% of the transformants contained the correct integration event. For high-copy integration of GtG point mutant alleles, the BamHI/SpeI fragments of pHJ250 and pHJ252 were used to replace the SpeI/BamHI fragment of pHJ206 to create the vectors pKN1 and pKN2, respectively, which were electroporated into KAx-3 cells.

Analysis of morphology and β-galactosidase activity

Analyses of morphology and β-galactosidase activity during multicellular development were performed on cells grown to mid-log phase
(1-2x10^6 cells/ml) in shaking cultures of HL5 medium. Cells were washed free of medium in 12 mM sodium/potassium phosphate buffer (pH 6.1), resuspended in phosphate buffer at 2x10^7 cells and then spread directly on non-nutrient plates for development. For β-galactosidase staining, cells were developed on nitrocellulose filters resting on top of non-nutrient agar plates and then stained for β-galactosidase activity as described previously (Haberstroh and Firtel, 1990).

Assays of the β-galactosidase activity of developing aggregates were performed by subjecting cells to a freeze/thaw cycle and then incubating with ONPG (o-nitro phenyl-β-D-galactoside) as described (Dingermann et al., 1989).

**DNA and RNA blots**

*Dictyostelium* DNA blots were performed as described (Sambrook et al., 1989) and RNA blots were done as described previously (Mann and Firtel, 1987). DNA probes were generated by random primer probe synthesis using a Promega-Gene kit (Promega) or a Genius kit (Boehringer Mannheim) according to the manufacturers’ instructions.

The GenBank/EMBL accession number for the Ga5 DNA sequence is #U20806.

**RESULTS**

**Cloning and structure of the Ga5 gene**

Using a previously isolated Ga5 PCR-generated fragment (Hadwiger et al., 1991) as a hybridization probe, the Ga5 gene was isolated as two fragments (2.1 kb XhoI/BclI and 1.0 kb BclI fragments) from genomic DNA libraries (see Materials and Methods). These fragments were combined to make a contiguous 3.1 kb genomic segment that contained the entire Ga5 open reading frame, 1.1 kb of upstream sequence and 0.7 kb of downstream sequence. Sequence analysis revealed this open reading frame encodes 345 amino acids distributed on three exons divided by two small introns (Fig. 1). The predicted primary sequence of the Ga5 subunit is more related to the *Dictyostelium* Ga4 subunit (~51% identical) than any other Gaα subunit from *Dictyostelium* or other organisms. In addition to the identity at the amino acid sequence level, the Ga5 and Ga4 genes both have introns located in analogous positions with respect to the open reading frames (Fig. 2). Both the Ga5 and the Ga4 subunits are more closely related to the members of the Gaα class (Simon et al., 1991) than to any other class of mammalian subunits (data not shown).

**Ga5 mutant strains show temporally aberrant development**

To gain insight into the function of the Ga5 subunit, a ga5 null mutant was created by gene disruption. The *Dictyostelium THY1* gene was inserted into the Ga5 open reading frame at the PstI and SpeI sites of a PCR-generated fragment containing the majority of the Ga5 open reading frame (see Materials and Methods). This construct was electroporated into the thymidine auxotrophic strain JH10 and then prototrophic transformants were selected and screened for disruption of the

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**Fig. 1.** Genomic nucleotide sequence and deduced amino acid sequence of the Ga5 gene. Lower-case sequence represents nontranslated regions. The termination codon is indicated by an asterisk (*). GenBank/EMBL accession number for the Ga5 DNA sequence is #U20806.
Ga5 locus by genomic DNA blot analysis. All ga5 null mutants, as determined by disruption of the Ga5 locus, showed a 3 hour delay in the formation of tip structures on multicellular mounds (Fig. 4). All subsequent developmental stages were also delayed by ~3 hours (data not shown). In addition, the ga5 null mutants formed aggregates, slugs and spore masses that were generally larger than those of wild-type cells. The larger slugs of the ga5 null mutants also had a greater tendency to lose cells from the posterior region compared to wild-type slugs (data not shown). These developmental phenotypes were rescued by the integration of the vector pJH206 (Fig. 3) into the genome at a single or low copy (~2-10 copies) number at loci other than Ga5, indicating that the 3.1 kb genomic fragment contains all the necessary sequences for complementing the ga5 null mutation. However, the integration of many copies (>15) of this Ga5 vector into wild-type or ga5 null mutant cells resulted in the overexpression of the Ga5 gene and aberrant temporal regulation of morphogenesis. Developing aggregates of Ga5HC (Ga5 gene at high copy number) cells had precocious tip formation ~3 hours earlier than wild-type aggregates (Fig. 4) and this morphogenic process was initiated even before the aggregation stage was completed. All subsequent stages of the Ga5HC development were also ~3 hours precocious compared to wild-type development. In contrast to ga5 null cells, Ga5HC cells formed aggregates, slugs and spore masses that tended to be much smaller than the corresponding structures of developing wild-type cells.

To determine whether the aberrant temporal regulation of tip morphogenesis in Ga5 mutant aggregates could be affected by intercellular signaling, Ga5 mutations and wild-type cells were mixed to create chimeric organisms. Aggregates containing equal portions of ga5 null mutant and wild-type cells formed tips at the rate expected for wild-type cells, indicating that cells expressing the Ga5 gene exert a dominant role in the rate of tip formation (Fig. 5). Cells overexpressing the Ga5 gene also exerted a dominant effect on the chimeric organism with respect to tip formation, as aggregates containing mixtures of ga5/Ga5HC or wild-type/Ga5HC cells formed tips at the rate expected for aggregates of only Ga5HC cells, indicating that Ga5 functions cell non-autonomously in regulating tip formation (Fig. 5).

The timing of late gene expression is altered in Ga5 mutant strains

The correlation between Ga5 function and the temporal regulation of tip morphogenesis was also investigated with respect to developmental gene expression. RNA was isolated from developing Ga5 mutants and wild-type cells at various times after starvation and the level of expression of the prespore gene SP60 and two distinct prestalk genes, emaA and emcB, was

![Fig. 2. Alignment of the Dictyostelium Ga5 and Ga4 subunits. Dashes (-) represent identical amino acid residues. Periods (.) represent gaps created to enhance the alignment. Locations of Ga5 and Ga4 introns indicated by (i). Glycine (G) at position 196 and glutamine (Q) at position 198 are denoted by asterisks (*).](image)

![Fig. 3. Ga5 gene constructs. (A) Map of ga5::THY1 gene disruption. (B) Map of Ga5 expression vector pJH206. Plasmids pKN1 and pKN2 are identical to pJH206 except for specific mutations within the Ga5 coding region. (C) Map of pGa5::lacZ gene fusion vector pJH210. A detailed description of the constructs is presented in Materials and Methods. Ga5 genomic sequences are represented by solid segments. The initiation and termination codons are represented by ATG and TAA respectively. G418r represents the pACT6::Neo gene fusion that confers resistance to the drug G418. Restriction enzymes are shown: BclI (B), ClaI (C), EcoRI (EI), EcoRV (EV), HinclI (H), PstI (P), SpeI (S), XbaI/SpeI junction (X1), XhoISalI junction (X2), XbaI (X3).](image)
analyzed by RNA blot analysis. Using RNA isolated every 6 hours during development, it was clear that the induction of all three genes was delayed in the gα5 null strain as compared to that in the wild-type strain (Fig. 6A). The same blots showed that expression of the two prestalk markers was induced earlier relative to starvation in Ga5HC cells, but these blots could not distinguish differences in the kinetics of expression of the prespore marker between the overexpressors and wild-type cells. To examine the kinetics more accurately, the temporal regulation of cell-type-specific gene expression was examined using RNA isolated from developing cells at 1-hour intervals starting at 9 hours of development. Prespore- and prestalk-specific gene expression was delayed in gα5 null mutants but was precocious in Ga5HC mutants in comparison to the temporal pattern of gene expression in wild-type cells (Fig. 6B). These differences in temporal gene regulation correlated with differences in the temporal regulation of tip morphogenesis. In all three strains, the genes were induced at approximately the same morphological stage and the maximal level of expression of each of the genes was the same.

To further examine the possible intercellular signaling-dependent Gα5 function in controlling the timing of cell-type-specific gene expression, we examined the ability of wild-type and Ga5HC cells to accelerate prespore and prestalk gene expression specifically in gα5 null cells when these cells are developed as chimeras. This was achieved by using gα5 null cells transformed with either a prespore-specific (SP60::lacZ gene fusion) or prestalk-specific (ecmA::lacZ gene fusion) reporter gene. As shown in Fig. 7, SP60::lacZ expression in gα5 null mutant cells was consistently accelerated in the presence of wild-type or Ga5HC cells in chimeric aggregates (Fig. 7). However, the difference in the level of induction between the gα5/Ga5HC chimeras was variable, indicating that parameters other than the level of Gα5 function are important for the levels of induction. Prestalk-specific gene expression (ecmA::lacZ gene fusion) in gα5 null cells was also precociously induced by the presence of wild-type or Ga5HC cells and the level of this induction was consistently greater in gα5/Ga5HC chimeras as compared to gα5/wild-type chimeras, suggesting
Ga5 gene is preferentially expressed in a pattern similar to the distribution of ALCs during the multicellular stages

Our previous report revealed that the Ga5 gene is expressed at very low levels during vegetative growth and early development, but the expression increases significantly during multicellular development with a temporal pattern similar to that of the Ga4 gene (Hadwiger et al., 1991). To determine the spatial pattern of Ga5 gene expression during the multicellular stages, a segment of the Ga5 gene that included the presumptive promoter (determined by the complementation analysis described earlier) and amino-terminal coding region was fused in-frame with the E. coli lacZ gene (Fig. 3), transformed into wild-type and ga5 null cells, and individual clones were isolated. Wild-type or ga5 null cells carrying the pGa5::lacZ construct expressed β-galactosidase activity in a small subset of cells distributed over the entire organism at all stages of multicellular development (Fig. 9). Although the cells expressing the pGa5::lacZ construct were found in all areas of the multicellular organism, there appeared to be a greater abundance of these cells near the posterior end of slugs. Staining was also observed in the stalk, basal disc and cup regions of the fruiting bodies. This spatial pattern of expression is very similar to the pattern described for Ga4 gene expression (Hadwiger and Firtel, 1992; Hadwiger et al., 1994) and the pattern of ALCs (Jermyn and Williams, 1991; Sternfeld and David, 1982), and is distinct from that of cells expressing prespore- or prestalk-specific genes (Williams et al., 1989; Haberstroh and Firtel, 1990).

Analysis of mutant Ga5 subunits

Mutations that inhibit GTPase activity in G proteins or Ras proteins often result in increased stimulation of downstream effectors and, in some cases, oncogenic transformation (Lyons et al., 1990; Landis et al., 1989; Kalinec et al., 1992; Bourne et al., 1991). A mutation commonly associated with these phenotypic properties is the substitution of a glutamine residue in the highly conserved G3 region (DVGGQR, using nomenclature described in Bourne et al., 1991) with a leucine residue. The analogous Q→L substitution mutation was created in the Ga5 gene by site-directed mutagenesis to give the allele Ga5Q198L (Fig. 2). A plasmid containing this mutant Ga5 allele and also a G418-resistance marker gene (see plasmid pKN1; Fig. 3) was transformed into wild-type cells to examine possible phenotypes of the Ga5Q198L allele. Transformants selected in growth medium containing 10 μg/ml G418 formed colonies but then gradually died, whereas transformants selected with lower concentrations of G418 (2-5 μg/ml) remained viable, suggesting that a high copy number of the plasmid was lethal. As a control, side-by-side transformations using the plasmid pJH206 (a plasmid identical to pKN1 except for the Ga5Q198L mutation) resulted in several hundred high-copy-number transformants that remained viable in medium containing 15 μg/ml G418 and possessed the precocious developmental phenotypes described above. Genomic DNA blots of the viable Ga5Q198L transformants indicated a very low copy number of the Ga5Q198L mutant plasmid (approximately 1-5 copies using the endogenous wild-type Ga5 gene as a reference), consistent with the lower resistance to G418 medium (data not shown). None of these low-copy-number Ga5Q198L transformants displayed altered developmental characteristics. The Ga5Q198L allele was also transformed into
wild-type cells as a truncated gene on a plasmid linearized within the \(G\alpha_5\) coding region so as to direct the integration of the plasmid into the \(G\alpha_5\) locus (Fig. 10). This strategy resulted in approximately 40% of the transformants having a single copy of a full-length \(G\alpha_5\) Q198L gene and a truncated \(G\alpha_5\) gene (no promoter or amino-terminal coding region), as determined by DNA blot analysis. Transformants with this insertional configuration displayed no aberrant developmental phenotypes, suggesting that the \(G\alpha_5\) Q198L allele was capable of complementing the loss of the wild-type \(G\alpha_5\) gene.

In addition to the analysis of the \(G\alpha_5\) Q198L allele, another \(G\alpha_5\) mutant allele, \(G\alpha_5\) G196T, was created by site-directed mutagenesis and assessed for function. The G→T substitution mutation, also in the conserved G3 region of \(G\alpha\) subunits, is analogous to mutations resulting in dominant negative alleles of the mammalian \(G\alpha\) gene (Osawa and Johnson, 1991) and the Dictyostelium \(G\alpha_2\) gene (Okaichi et al., 1992). The \(G\alpha_5\) G196T allele, like the \(G\alpha_5\) Q198L allele, appeared to be lethal at high copy number, as stable transformants contained <10 copies of the plasmid carrying the \(G\alpha_5\) G196T allele (see plasmid pKN2; Fig. 3). None of the viable transformants displayed aberrant tip formation or any other apparent developmental phenotypes. Using the strategy previously described for the single-copy insertion of the \(G\alpha_5\) Q198L allele, a truncated copy of the \(G\alpha_5\) G196T allele was inserted into the \(G\alpha_5\) locus to test the ability of the \(G\alpha_5\) G196T allele to complement for the loss of the wild-type \(G\alpha_5\) gene. All transformants with a single copy of the \(G\alpha_5\) G196T allele and no wild-type allele (determined by genomic DNA blots) displayed no aberrant phenotypes with respect to developmental morphology or kinetics. This ability to complement the wild-type allele suggests that the \(G\alpha_5\) G196T allele can provide sufficient \(G\alpha_5\) function and may not behave as a dominant negative allele.

**DISCUSSION**

The delayed tip morphogenesis and developmental gene expression of \(g\alpha_5\) null mutants suggest that the \(G\alpha_5\) subunit plays an important role in the temporal regulation of these developmental processes. This suggestion is further supported by the precocious tip morphogenesis and gene expression of the \(G\alpha_5\) HC cells. The ability of wild-type or \(G\alpha_5\) HC cells to accelerate the expression of prepsore- and prestalk-specific genes specifically in \(g\alpha_5\) null cells, as determined using \(g\alpha_5\) null cells expressing either SP60::lacZ or ecmA::lacZ, suggests that the \(G\alpha_5\)-mediated signal transduction pathway is important for production rather than reception of an intercellular signal that regulates late developmental gene expression. The dependence of this signal on \(G\alpha_5\) gene-dosage for the induction of prestalk- but not prespore-specific gene expression is consistent with the role of \(G\alpha_5\) function in regulating tip formation, as this process involves the accumulation of prestalk-specific cells on the top of the cellular aggregate. Whether this induction of prestalk-gene expression is due to increased cell differentiation and/or migration has not
been determined but previous studies have indicated that the expression of many cell-type-specific genes (e.g. SP60, ecmA and ecmB) can be initiated before cell sorting, suggesting that cell-type-specific gene expression does not require cell sorting (Esch and Firtel, 1991; Williams et al., 1989; Jermyn and Williams, 1991; Haberstroh and Firtel, 1990; Berks and Kay, 1990). However, the level of cell-type-specific gene expression might depend on the location of a cell within the aggregate. Whether the Go5-dependent intercellular signal directly affects cell differentiation or migration, the cellular aggregate must have alternative but less effective mechanisms to carry out these processes and Go5 appears to play an important role in these processes. Our results also showed that Go5 dosage did not have a significant effect on the spatial patterning of the cells types within chimeric organisms.

The temporal induction and spatial expression pattern of the Go5 gene expression is very similar to that described for the related Dictyostelium Go4 gene (Hadjiger and Firtel, 1992; 1994; Hadwiger et al., 1991; Wu and Devreotes, 1991). Both genes are expressed at low levels before cellular aggregation and then at higher levels during the multicellular stages of development. The higher level of expression during multicellular stages suggests these subunits play important roles in the multicellular stage, which is further supported by the observation that both subunits affect intercellular signaling. However, the very low levels of expression during early development may also play important roles, as suggested by the requirement of the Go4 subunit in preaggregated cells for responses to folic acid (Hadjiger et al., 1994). The spatial expression patterns of the Go4 and Go5 genes suggest neither gene is expressed in prespore or prestalk cells in the multicellular stages, but rather in a subpopulation of ALCs which can function as precursors or regulators of the two major cell types (Jermyn and Williams, 1991; Sternfeld, 1992; Hadwiger and Firtel, 1992; Hadwiger et al., 1994; Sternfeld and David, 1981; Abe et al., 1994). Whether the Go5 gene is expressed in the same subset of cells as the Go4 gene remains to be determined. For our analysis, we have used an upstream regulatory region that we believe contains the full upstream regulatory sequences (see results); however, we cannot exclude that additional sequences may result in additional patterns of expression. While the expression of Go5 is highly enriched in ALCs in the multicellular stages, it is also expressed at earlier stages of development. It is therefore possible that the expression of Go5, while low, during the earlier stages of development may be important in influencing the timing of tip formation. A possible role of Go5 in earlier development and growth is consistent with the lethal effects that we observed when we tried to overexpress certain mutant Go5 proteins. Moreover, since Go5 is expressed during these earlier stages, including vegetative growth, one might expect a low level of Go5 protein to be present in most or all cells of the developing aggregate.

Although cAR2 and Go5 are both required for the proper timing of tip formation, they probably function in different signal transduction pathways that independently facilitate tip morphogenesis. cAR2 expression has been proposed to be prestalk-specific (Saxe et al., 1993), whereas we have shown that Go5 is expressed at low level during early development and induced to a higher level in the multicellular stages in which it is preferentially expressed in ALCs. Moreover, tip morphogenesis in car2 null mutant aggregates is delayed significantly longer than go5 null mutant aggregates: car2 cells overexpress prespore-specific genes, whereas go5 cells are delayed in prespore gene expression. The kinetics of tip morphogenesis in the Go5 mutant/wild-type chimeras indicate that Go5 functions cell non-autonomously, presumably by affecting intercellular signaling during tip morphogenesis. Although cAMP appears to be the chemoattractant for prestalk cell migration during tip morphogenesis (Traynor et al., 1992; Matsukuma and Durston, 1979; Sternfeld and David, 1981; Saxe et al., 1993), Go5-expressing cells are not likely to be the source of this cAMP, as these cells are poorly represented in the anterior region where cAMP levels are the highest. However, Go5-expressing cells might modulate cAMP levels throughout the multicellular organism.

The mutations created in the G3 region of the Go5 subunit, Go5Q198E and Go5G715T, do not appear to affect tip morphogenesis, as suggested by the replacement of the wild-type allele with a single copy of the mutant alleles. However, the inability to obtain viable transformants carrying high copy numbers of these alleles suggests that the overproduction of these mutant proteins affects processes essential for vegetative growth. Whether these mutant Go5 subunits affect only the Go5-mediated signal transduction pathway or other pathways remains to be determined. Although Go5 function is not essential for vegetative growth and the expression of the Go5 gene is relatively low before multicellular development, the Go5 signal transduction pathway might be involved in preaggregative functions. This is the case for the Go4 gene, which has a expression pattern similar to that of the Go5 gene.
(Hadwiger et al., 1994). Although mutations analogous to the Q198L mutation have been analyzed in a variety of Ga subunits from different systems, mutations homologous to the G196T substitution in the Ga5 subunit have only been examined in the mammalian Gαs and Dictyostelium Gα2 subunits, for which the downstream effector pathways are understood and thus the effect of a mutant subunit could be evaluated (Osawa and Johnson, 1991; Okaichi et al., 1992). Although the G3 region of all known Gα subunits is highly conserved, it is not clear whether such mutations would function as dominant negative mutations in all Gα subunits. Thus, it is not possible to evaluate the absence of a phenotype in strains in which the wild-type gene was replaced with the Ga5(G196T) allele.

Mutations affecting the temporal regulation of Dictyostelium development have been reported for genes other than the Gα5 gene. These include rapidly developing (rde) mutants, rdeA and rdeC, which result in precocious formation of spore and stalk cells (Sonnewborn et al., 1963; Kessin, 1977; Abe and Yanagisawa, 1983). In the case of rdeC mutants, the defects of spore and stalk cells mature soon after mound formation, resulting in disorganized mounds of stalk cells and spores. The effects of the rdeC mutants have been shown to result in a nonfunctional regulatory subunit of protein kinase A, suggesting that an unregulated catalytic subunit of protein kinase A (PKAcat) stimulates differentiation (Simon et al., 1992; Mann et al., 1992; Anjard et al., 1992). This implication is supported by the accelerated aggregation and spores are formed at the base of these mutants, spores and rdeC mutants, for which the downstream effector pathways are understood and thus the effect of a mutant subunit could be evaluated (Osawa and Johnson, 1991; Okaichi et al., 1992). Although the G3 region of all known Gα subunits is highly conserved, it is not clear whether such mutations would function as dominant negative mutations in all Gα subunits. Thus, it is not possible to evaluate the absence of a phenotype in strains in which the wild-type gene was replaced with the Ga5(G196T) allele.

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