Spatial and Temporal Expression of the *Dictyostelium discoideum* $G\alpha$ Protein Subunit $G\alpha 2$: Expression of a Dominant Negative Protein Inhibits Proper Prestalk to Stalk Differentiation

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Previous results have shown that the $G\alpha$ protein subunit $G\alpha 2$ is required for aggregation in *Dictyostelium discoideum* and is essential for coupling cell-surface cAMP receptors to downstream effectors in vivo during this stage of development. $G\alpha 2$ expresses at least four distinct transcripts that are differentially regulated during development; two of the transcripts are expressed exclusively in the multicellular stages and their expression is restricted to prestalk cells. We partially dissected the $G\alpha 2$ promoter and identified a component that is expressed exclusively during the multicellular stages using luciferase gene fusions. When this promoter region is coupled to *lacZ*, β -gal expression is restricted to the multicellular stages and localized in prestalk cells with a pattern similar to that of the *ecmA* prestalk-specific promoter. We show that expression in wild-type cells of the $G\alpha 2$ mutant protein [$G\alpha 2$ (G206T)] during the early stages of development blocks aggregation and cAMP-mediated activation of adenylyl cyclase and guanylyl cyclase, suggesting it functions as a dominant negatively active $G\alpha$ subunit. When this mutant $G\alpha$ protein is expressed from the *ecmA* prestalk-specific promoter, abnormal stalk differentiation during culmination is observed. Expression of the mutant $G\alpha 2$ from the *SP60* prespore promoter or wild-type $G\alpha 2$ from either the *ecmA* or the *SP60* promoter results in no detectable phenotype. The results suggest that $G\alpha 2$ plays an essential role during the culmination stage in prestalk cells and may mediate cAMP receptor activation of these processes during multicellular development.

INTRODUCTION

Development of the cellular slime mold *Dictyostelium discoideum* is mediated by cell-cell interactions controlled through cell-cell contact and small molecules, including cAMP, Differentiation Inducing Factor, and folic acid analogues, that function by activating intracellular signal transduction pathways. Over the last few years, we have substantially increased our understanding of the mechanisms by which cAMP, acting as the chemotactic agent, controls the formation of the multicellular aggregate and the induction of genes required for these processes (see Devreotes, 1989; Firtel *et al.*,

1989; Firtel, 1991; Kimmel and Firtel, 1991; van Haastert, 1991; Williams, 1991; for reviews and references). Much of this understanding comes from biochemical and molecular genetic approaches that include cloning of the genes encoding many of the signalling components and the analysis of corresponding null mutations created by gene disruptions. Cyclic AMP, acting through the cell surface receptor cAR1, activates adenylyl cyclase (AC), guanylyl cyclase (GC), phospholipase C (PLC), and Ca^{2+} channels and mediates aggregation-stage gene expression (Europe-Finner and Newell, 1987; van Haastert *et al.*, 1987, 1989; Kesbeke *et al.*, 1988; Mann *et al.*, 1988; Kumagai *et al.*, 1989, 1991; Sun *et al.*, 1990;

Milne and Coukell, 1991; Sun and Devreotes, 1991; Okaichi *et al.*, 1992; Pitt *et al.*, 1992). All of these pathways, with the exception of the activation of Ca^{2+} channels, require the $G\alpha$ protein subunit $G\alpha 2$. Biochemical analysis of $g\alpha 2$ null mutant and strains expressing $G\alpha 2$ proteins carrying specific amino acid substitutions suggests that GC and PLC are directly regulated by $G\alpha 2$. However, AC, which requires $G\alpha 2$ for activation *in vivo*, can be activated *in vitro* by nonhydrolyzable GTP analogues, suggesting that the activation of other G proteins can mediate this activity *in vitro* (Theibert and Devreotes, 1986; Kesebeck *et al.*, 1988; Kumagai *et al.*, 1991; Okaichi *et al.*, 1992). These data and the lack of cAMP activation of AC in a $G\beta$ null mutant *in vitro* strongly suggest that $\beta\gamma$ subunits released from the G protein containing $G\alpha 2$ mediate the activation of AC (Okaichi *et al.*, 1992; Lilly *et al.*, 1993). In mammalian cells, $\beta\gamma$ subunits can significantly enhance the $G\alpha s$ -mediated activation of some isoforms of AC (Tang and Gilman, 1991; Federman *et al.*, 1992). Further studies have indicated that during aggregation, cGMP regulates directed chemotactic movement (Newell and Liu, 1992), whereas cAMP is involved in extracellular signal relay between cells (Devreotes, 1989; Pitt *et al.*, 1992) and intracellularly to mediate signal relay through the activation of cAMP-dependent protein kinase (Mann and Firtel, 1991; Mann, Pitt, Devreotes, and Firtel, unpublished data).

Extracellular cAMP, acting through cell surface receptors, is also known to be required for multiple processes during the multicellular stages of development that include the activation of gene expression in prestalk, prespore, and anterior-like cells and cell sorting during tip formation and in slugs (Esch and Firtel, 1991; Kimmel and Firtel, 1991; Seigert and Weijer, 1991, 1992; Williams, 1991; Schaap, 1991; Traynor *et al.*, 1992; Saxe *et al.*, 1993; Louis and Kimmel, personal communication). Genes encoding three additional cAMP receptors, cAR 2–4, have been cloned and shown to be preferentially expressed in the multicellular stages, and disruption of *cAR2* and *cAR4* results in specific morphological defects during the multicellular stages (Saxe *et al.*, 1991, 1993; Johnson *et al.*, 1993; Louis and Kimmel, personal communication). In addition, cAR1, which directly couples to $G\alpha 2$ to regulate aggregation, is also expressed from a specific late promoter during the multicellular stages (Louis and Kimmel, personal communication). Analysis of *car1* null strains in which cAR2 and cAR3 have been expressed shows that $G\alpha 2$ can couple to cARs1–3 (Devreotes, personal communication). To date, eight $G\alpha$ protein subunits have been cloned in *Dictyostelium* ($G\alpha 1$ –8) and all, except $G\alpha 6$ that is expressed in vegetative cells and at the onset of development before aggregation, have been disrupted (Pupillo *et al.*, 1989; Hadwiger *et al.*, 1991, 1992; Kumagai *et al.*, 1991; Wu and Devreotes, 1991; Cubitt *et al.*, 1992; Gaskins, Zhou,

and Firtel, unpublished data; Wu and Devreotes, personal communication). None of these disruptions, other than that of $G\alpha 2$, results in loss of cAR-mediated functions, suggesting the possibility that $G\alpha 2$ may also control cAMP-mediated events through different cARs throughout development or that yet-to-be-identified $G\alpha$ proteins control these functions during the multicellular stages. The requirement of $G\alpha 2$ for cAMP-mediated activation of AC from multiple cAMP receptors also suggests that $G\alpha 2$ may play an essential role during development in addition to controlling chemotaxis and signal relay during aggregation (Pupillo *et al.*, 1992).

Previous studies showed that the $G\alpha 2$ gene expresses multiple transcripts that are differentially regulated during development (Pupillo *et al.*, 1989) (see RESULTS). We describe the patterns of $G\alpha 2$ expression and the isolation and expression of a $G\alpha 2$ late promoter that is preferentially expressed in prestalk cells, as determined by the expression of *lacZ* fusions. Expression from a prestalk-specific promoter of a $G\alpha 2$ mutant subunit, which functions as a dominant negative mutation during aggregation results in aberrant culmination, suggests that $G\alpha 2$ may have a specific function during later morphogenesis.

MATERIALS AND METHODS

Dictyostelium Strains

Cell lines were grown and transformed as previously described (Okaichi *et al.*, 1992). *g\alpha 2* null cells were made by homologous gene disruption of the endogenous $G\alpha 2$ gene and express no $G\alpha 2$ protein (see above).

Vector Constructs

The $G\alpha 2$ coding cassette and $G\alpha 2$ promoter construct were described in Okaichi *et al.* (1992) and are shown in Figure 2. For expression of $G\alpha 2$ from either the *SP60*, *ecmA*, or *Act15* promoters, these promoters were exchanged for the $G\alpha 2$ promoter shown in Figure 2 (see Firtel and Chapman, 1990; Haberstroh and Firtel, 1990; Mann and Firtel, 1993 for details of the *Act15*, *SP60*, and *ecmA* promoter constructs used in these studies, respectively). In all cases, the fusion point for the $G\alpha 2$ coding region and the promoter was at an *Spe* I site in the 5' untranslated region of the promoter cassette and a *Spe* I site that was engineered upstream from the $G\alpha 2$ ATG translation initiation codon. The construction of the *ecmA* promoter containing an *Spe* I linker in the 5' untranslated region is described in Mann and Firtel (unpublished data). The *SP60* promoter is described in Haberstroh and Firtel (1990). The *Act15* promoter cassette is described in Kumagai *et al.* (1989) and has been modified to contain an *Spe* I site immediately 3' to the *Bgl* II site in the Actin 15 5' untranslated region (Dynes and Firtel, personal communication).

RNA Blots and Western Blots

Western blot analysis of $G\alpha 2$ expression was done as described in Gunderson and Devreotes (1990) and Kumagai *et al.* (1989) on plasma membranes using the $G\alpha 2$ -specific anti-peptide antibody described in these papers and generously supplied by P. Devreotes, Johns Hopkins School of Medicine.

Prestalk and prespore cells were isolated on Percoll gradients as described by Ratner and Borth (1983) and were generously supplied

by G. Schnitzler, J. Powell-Coffman, and M. Maeda in the lab of R.A.F. RNA was extracted from developing cells and analyzed by RNA blot hybridization as previously described (Mann and Firtel, 1987).

Biochemical and Histochemical Assays

Assays for cAMP activation of adenylyl cyclase and guanylyl cyclase have also been described previously in Okaichi *et al.* (1992). For assays of cells during later multicellular development, cells were developed on Na-phosphate-containing agar plates. Cells were harvested and dissociated by pipetting the aggregates up and down in 20 mM EDTA (pH 6.1), followed by centrifugation. This was repeated three times, after which the cells were washed in 12 mM Na phosphate pH 6.1. These procedures were carried out at 4°C.

Luciferase and β-galactosidase expression studies were performed as previously described (Howard *et al.*, 1988; Haberstroh and Firtel, 1990; Esch and Firtel, 1991). At least three independent transformations were assayed. The results represent the average of these. Standard errors of the mean are shown.

RESULTS

Analysis of the Cell-Type Specificity of the Gα2 Promoter

Figure 1 shows a more detailed examination of Gα2 transcripts during development than previously published (Pupillo *et al.*, 1989). An ~4-kilobase (kb) mRNA is expressed at low levels in vegetative cells that then decreases in abundance during early development. A major transcript of ~3.5 kb is induced during early development and is present at a maximum level during aggregation, after which the level of the transcript decreases. Two other transcripts of ~2.5 and 2.0 kb are then preferentially expressed during the later multicellular stages (Pupillo *et al.*, 1989).

Figure 1. Developmental expression of Gα2 transcripts. (A) RNA was isolated from cells developed for the times shown and analyzed by RNA blot hybridization as described in MATERIALS AND METHODS. Top and bottom panels represent two exposures of the same RNA blot. The bottom blot is a longer exposure. Veg, log phase vegetatively growing cells; 8 h, aggregation; 16 h, second finger, preculmination. (B) RNA isolated from purified prestalk and prespore cells isolated from migrating slugs (see MATERIALS AND METHODS). Unfractionated RNA from whole slugs. Equal amounts of total cell RNA were run on all lanes. Numbers are used to mark the transcripts. 1, 4 kb; 2, 3.5 kb; 3, 2.5 kb; 4, 2.0 kb.

To determine whether transcripts expressed late in development are differentially expressed in either prestalk or prespore cells, we used Percoll gradients to separate prestalk and prespore cell populations from slugs because of density differences between the two cell populations (Ratner and Borth, 1983). As shown in Figure 1, both the 2.5- and 2.0-kb transcripts are very prestalk-specific. In contrast, the 4- and 3.5-kb transcripts are found in both cell types, although the relative level is slightly higher in prespore cells.

Previously we described the cloning of a 3-kb Gα2 5' flanking region that, when used to express a Gα2-coding cassette, can fully complement a gα2 null strain resulting from a Gα2 gene disruption (Kumagai *et al.*, 1991; Okaichi *et al.*, 1992). To achieve a better understanding of the regulation of the Gα2 promoter, we have fused this region carrying an *Spe* I linker in the Gα2 5' untranslated region immediately upstream from the ATG initiation codon to the luciferase and *lacZ* reporter genes (construct Gα2Xh, Figure 2). The constructs were transformed into wild-type KAx-3 cells, and the temporal and spatial regulation of the Gα2 promoter was examined. As shown in Figure 3, the Gα2Xh/luciferase construct, as determined by assaying luciferase activity, was expressed in vegetative cells and induced during early development. Luciferase activity plateaued and then increased again starting at 15–18 h, suggesting that expression was differentially controlled at several stages during development, consistent with the expression pattern of the endogenous Gα2 transcripts. Compared to several other well characterized promoters examined in our laboratory, *Actin 6* (*Act6*), *Actin 15* (*Act15*), and the prespore-specific promoter *SP60*, the Gα2 promoter was relatively weak, as determined by luciferase specific activity in stable transformants (Ahern *et al.*, 1988; Howard *et al.*, 1988; Haberstroh and Firtel, 1990).

From this and the temporal pattern of expression and sizes of the Gα2 transcripts, we expected that there were several Gα2 promoters, more distal promoters preferentially expressed during growth and aggregation and a more proximal promoter(s) expressed during the multicellular stages. To isolate the late promoter(s), we made 5' deletions of the 3.5-kb Gα2 promoter using convenient restriction sites, fused these to the luciferase coding region, and examined the temporal pattern of expression in stable transformants. Figure 3 shows that construct Gα2Hd (Gα2 promoter deleted to the *Hind*III site ~1 kb upstream from the ATG, see Figure 2B for map) displayed little expression before 6 h of development. Expression of Gα2Hd then increased gradually until 15 h of development after which time the luciferase activity increased rapidly, peaking at 21–24 h, in a pattern similar to that of the increase during late development observed using the complete 3.5-kb region. These results suggested that we separated the late promoter(s) from the vegetative and aggregation-stage (early) promoter(s).

Figure 2. *Ga2* expression vectors. (A) Entire vector is shown. See Okaichi *et al.* (1992) for details. For expression of luciferase or β -galactosidase, the firefly luciferase gene or *lacZ* was used to replace the *Ga2* coding region, respectively. (B) Promoter regions of the *Ga2Xh*, *Ga2Hc*, and *Ga2Hd* constructs carrying the entire *Ga2* promoter (*Ga2Xh*) and those designated *Ga2Hc* and *Ga2Hd*. Constructs are shown with the *Ga2* protein coding region.

To further examine the *Ga2* promoter, we made a deletion to the *HincII* site lying 0.7 kb upstream from the *HindIII* site that contained ~ 1.7 kb of 5' flanking and upstream sequences (*Ga2Hc* promoter, see Figure 2B). As shown in Figure 3A, the *Ga2Hc* promoter showed a pattern of expression similar to that of *Ga2Hd* except that there was a reproducibly slightly higher level of expression during the preaggregation (3 h) and aggregation stages (6 h) of development. These data suggested that the *Ga2Hd* promoter could not drive expression during growth and aggregation but does contain sequences capable of driving complete *Ga2* expression during late development. We were unable to isolate a functional vegetative and/or aggregation-stage-specific promoter(s) by making internal deletions using the restriction sites present in the promoter.

To examine the spatial regulation of the *Ga2* promoter, the full-length (*Ga2Xh*) and late *Ga2* (*Ga2Hd*) promoters were separately fused to *lacZ*, and the re-

sulting constructs were transformed into *Dictyostelium*. Cells were developed, and aggregates were histochemically stained at various times during development for β -gal expression. Using the full-length promoter, aggregates showed uniform staining during aggregation and mound formation, suggesting that *Ga2* is expressed in all cells at this stage of development, a result expected from the role of *Ga2* protein in controlling early development. When cells expressing the *Ga2Hd/lacZ* promoter were examined, no expression was seen during the aggregation stages. A few scattered, lightly staining cells were seen at the apical tip of the aggregate. By the slug stage, staining is primarily localized to the anterior of the slug in the prestalk region with staining intensity decreasing toward the center of the prestalk region (Figure 4). Less intense staining was also seen in some cells scattered through the posterior prespore domain, possibly in anterior-like cells, a pattern similar to that of the prestalk promoter *ecmA*. Moreover, like *ecmA*, the spatial expression of the *Ga2* promoter appears to be stronger in the anterior of the prestalk zone (Williams *et al.*, 1989; Jermyn and Williams, 1991).

Possible Function of the *Ga2* Subunit During Late Development

The presence of a developmentally regulated, prestalk-specific promoter for *Ga2* suggested that the *Ga2* subunit may have a specific role during later development. Because *Ga2* is essential for early development, it is difficult to examine *Ga2* function during late development. As expected, expression of *Ga2* from the *Ga2Hd* promoter in *ga2* null cells did not complement *Ga2* function as determined by the absence of aggregation

Figure 3. Expression of luciferase from *Ga2* promoters. Luciferase measurements in cell lysates were performed as described in MATERIALS AND METHODS. Times indicate time of development. 0, log phase vegetative cells. See legend to Figure 1 for details. Xho, full-length *Ga2* promoter; Hinc, *HincII* promoter fragment (see Figure 2); Hd, *HindIII* promoter fragment (see Figure 2).

SPATIAL EXPRESSION OF $G\alpha 2$ LATE PROMOTER

or by the inability of cAMP to activate GC (Figure 5A) or AC in 6-h-starved cells. Two approaches were used to examine possible roles for $G\alpha 2$ in regulating later development. One approach was to express $G\alpha 2$ from an aggregation-stage-specific promoter in $g\alpha 2$ null cells. In this scenario, we expect that cells would aggregate (because they were expressing $G\alpha 2$ during early development). However, because cells would not express $G\alpha 2$ during later development, we might create a late-development-specific $g\alpha 2$ null phenotype. We attempted this approach using the isolated aggregation-stage-specific *cAR1* (*cAR1E*) promoter isolated by A. Kimmel and coworkers (Louis and Kimmel, personal communication), who have shown that expression of this promoter is restricted to the aggregation stage. When a *cAR1E*/ $G\alpha 2$ fusion was expressed in $g\alpha 2$ null cells, the cells aggregated normally and proceeded through development although with kinetics that were slower than those for $g\alpha 2$ null cells complemented with $G\alpha 2$ expressed from the complete $G\alpha 2$ promoter. We expect that there is sufficient $G\alpha 2$ expressed and present in cells carrying these multicopy plasmids so that any postulated $G\alpha 2$ function late in development is also

complemented. Alternatively, the *cAR1E* promoter functioned as expected, and $G\alpha 2$ may not have a specific role in late development.

The second approach was to express a potential dominant negative mutation of $G\alpha 2$ [$G\alpha 2(G206T)$] (glycine at position 206 mutated to a threonine) during late development in wild-type cells, which will also express the endogenous $G\alpha 2$. Overexpression of the dominant negative mutant might be expected to inhibit $G\alpha 2$ function at this stage and potentially lead to an understanding of $G\alpha 2$ function during late development. Expression of an equivalent mutation in the mammalian $G\alpha$ subunit *Gas* produces a dominant negative phenotype in which β -adrenergic receptor-mediated activation of AC in mammalian cells is repressed when the mutant *Gas* protein is expressed at high levels in vivo (Osawa and Johnson, 1991). The mode of action of this mutant subunit is not known, but the phenotype suggests that it may interfere with interaction of the wild-type endogenous *Gas* subunit with the receptor and/or AC (see DISCUSSION).

When the $G\alpha 2(G206T)$ subunit was expressed in wild-type cells from the *Actin 15* (*Act15*) promoter,

Figure 5. Activation of adenylyl and guanylyl cyclase in whole cells by cAMP. The levels of cAMP and cGMP production in whole cells in response to cAMP (2'dcAMP for AC activation) in 6-h cells. References to methods are given in MATERIALS AND METHODS. (A) Activation of guanylyl cyclase in *ga2* null cells, *ga2* null cells expressing *Ga2*(G206T) from the *Act15* promoter, *ga2* null cells expressing *Ga2* from the *Ga2Hd* promoter, and wild-type cells (control). *ga2* null cells that express wild-type *Ga2* show a wild-type pattern of activation of adenylyl and guanylyl cyclases (Kumagai *et al.*, 1991; Okaichi *et al.*, 1992). (B) Activation of guanylyl cyclase (left) and adenylyl cyclase (right) in wild-type cells, wild-type cells expressing *Ga2*(G206T) from the *Act15* promoter, and *Ga2*(G206T) from the *ecmA* promoter.

which is preferentially expressed during growth and early development (Knecht *et al.*, 1986; Firtel and Chapman, 1990), or the complete *Ga2* promoter, the cells did not aggregate, indicating that its expression blocked development. When cAMP-activated signaling pathways were examined using the *Act15* promoter to drive *Ga2*(G206T) expression, we found that the cAMP-activation of AC and GC in these cells was substantially inhibited (Figure 5B), suggesting that the *Ga2*(G206T) subunit can function as a dominant negative *Ga* subunit during aggregation. Similar results were obtained using the *Ga2Xh* promoter to express the *Ga2*(G206T) subunit. Previously, we showed that overexpression of the wild-type *Ga2* subunit from either the *Act* or *Ga2Xh* promoter resulted in no visible phenotype (Kumagai *et al.*, 1991; Okaichi *et al.*, 1992). When cAMP-mediated activation of GC and AC cyclases was examined in these cells, we showed that GC activation was enhanced by 30–40%, whereas the activation of AC was suppressed by ~40% (Okaichi *et al.*, 1992). Western blot analysis confirmed that the *Act15* transformed strains expressed a higher level of *Ga2* at these stages than the wild-type cells (Figure 6). Moreover, expression of the mutant *Ga2* subunit in the *ga2* null strain JH104 did not complement the *ga2* null aggregation-deficient phenotype, and these cells did not display cAMP-activation of GC (Figure 5A) or AC. The results suggested that the mutant subunit can function as a dominant negative protein and thus

could possibly be used to inhibit endogenous *Ga2* function during late development.

We initially expressed the *Ga2*(G206T) protein from the *Ga2Hd* promoter. When this was done, we observed no morphological phenotype, possibly because of an insufficient expression of either *Ga2* protein from this relatively weak promoter (see above). Because the *ecmA* promoter has a pattern of spatial expression similar to that of the *Ga2Hd* promoter (Williams, 1989; Ceccarelli *et al.*, 1991; Jermyn and Williams, 1991) and, in our hands, is a substantially stronger promoter, as determined by the relative levels of β -gal expression, we expressed wild-type and the mutant *Ga2* protein from the *ecmA* promoter. The *ecmA/Ga2*(G206T) cells aggregated, and in vivo biochemical analysis of aggregation-competent cells showed normal activation of GC and AC by cAMP (Figure 5B), indicating that the normal *Ga2*-mediated signaling pathways are not detectably affected during aggregation. Moreover, the level of total *Ga2* expression (mutant and wild-type) is similar to that of wild-type cells (Figure 6), suggesting that little *Ga2*(G206T) protein is expressed at this stage from the *ecmA* promoter, as expected from the known temporal and spatial expression pattern of this promoter (see above). The *ecmA/Ga2*(G206T) cells formed slugs normally, but culmination was abnormal and a fruiting body with a very abnormal stalk was produced (Figure 7). The sorus was also abnormal, but mature spores

Figure 6. Western blot analysis of Gα2 protein levels. See MATERIALS AND METHODS for details. Times of development and strains are labeled on the figure. Open arrow on the right points to a band of the same molecular weight as Gα2 breakdown product that is sometimes seen in some membrane preparations (Kumagai *et al.*, 1989).

were formed. Western blot analysis showed increased levels of Gα2 cross-reacting protein in these cells at 17 h of development (Figure 6). We then examined the activation of GC in cells from 17-h *ecmA*/Gα2(G206T) early culminants that had been dissociated into single cells (see MATERIALS AND METHODS). We did not observe a reproducible change in the levels of cGMP production, as might be expected because cells expressing the *ecmA* promoter at a high level represent only ~10% of the total cells. Transformants expressing the wild-type Gα2 subunit from the *ecmA* promoter showed a developmental morphology that was indistinguishable from that of wild-type cells. Moreover, cells expressing the mutant or wild-type Gα2 subunit from the *SP60* prespore-specific promoter also showed normal development. The results of these experiments indicate that expression of a dominant negative Gα2 in prestalk cells affects culmination and suggest a specific role of Gα2 in controlling stalk morphogenesis during culmination.

DISCUSSION

From our analysis of strains expressing a high level of the Gα2(G206T) subunit during the aggregation stage, we suggest that Gα2(G206T) acts as a dominant inhibitor of Gα2 function, and we interpret our results using the *ecmA* promoter to suggest that Gα2 has a defined function involved in controlling culmination and stalk morphogenesis. Because overexpressing the wild-type Gα2 subunit did not produce a visible phenotype, we propose that the phenotype of the G206T mutation does not result from pleiotrophic effects on the function of a Gα subunit other than Gα2 (e.g., sequestering βγ subunits). However, the possibility that the phenotype results from interference with a Gα protein containing an α subunit other than Gα2 is not excluded. The luciferase and *lacZ* expression data indicate that the *Gα2Hd* and *Gα2Hd* prestalk-specific promoter are relatively weak,

and we expect that the lack of an observable phenotype when the Gα2(G206T) protein is expressed from the *Gα2Hd* promoter construct may be because of too low an expression of the Gα2(G206T) protein to sufficiently compete with the endogenous Gα2 protein to yield a dominant negative phenotype. When the full-length *Gα2Xh* promoter was tested using luciferase or *lacZ*, we observed developmentally regulated expression during the growth and aggregation stages with little change in expression level during later development. Expression of Gα2(G206T) from this or the *Act15* promoter resulted in an inhibition of aggregation. This promoter analysis suggested that the *Gα2Hd* promoter contains all of the elements essential for the late Gα2 expression and that the addition of more 5' flanking sequences allows aggregation-stage function. When these longer constructs were examined by β-gal staining, we observed a general background staining in most cells, consistent with the expectation that most cells express Gα2 during the aggregation stage.

We do not know the biochemical mechanism of action of the Gα2(G206T) subunit. In the case of the mammalian Gas subunit, Johnson and collaborators showed that a similar CGQRS → TGQRS mutation resulted in the inhibition of receptor-activated AC (Gupta *et al.*, 1991; Osawa and Johnson, 1991). This phenotype could be suppressed by a mutation in the N-terminus of the subunit that increased the rate of GDP/GTP exchange, suggesting that the TGQRS mutation may block receptor/G protein interaction. We think that this is also the most conservative model to explain how the Gα2(G206T) subunit effects the functioning of the endogenous Gα2 protein. Moreover, results from these and a number of other experiments also suggest that in *Dictyostelium*, this mutation probably does not effect downstream pathways by blocking interaction of the

Figure 7. Developmental phenotype of the wild-type strain KAx-3 and the wild-type strain KAx-3 expressing Gα2(G206T) from the *ecmA* promoter. (A) Wild-type cells. (B) *ecmA*/Gα2(G206T) cells.

endogenous $G\alpha 2$ subunit with the effector. For example, we showed a complete inhibition of the activation of receptor activation AC, although published results suggest that AC activation may not require $G\alpha 2$ (van Haastert, *et al.*, 1987; Okaichi, *et al.*, 1992).

Our results using the $G\alpha 2(G206T)$ mutant protein point to a specific function for $G\alpha 2$ during later development in prestalk cells during stalk differentiation. Presently, we do not know if the phenotype we observe is equivalent to a complete or partial lack of $G\alpha 2$ function in prestalk cells during culmination. Although the $G\alpha 2Hd$ promoter appears to have the same spatial and temporal pattern of expression as *ecmA*, it is possible that there are some subtle differences between the prestalk-specific component of the $G\alpha 2$ promoter and *ecmA*. Considering that extracellular cAMP controls prespore gene expression (see INTRODUCTION), we are surprised that expression of the mutant $G\alpha 2$ protein from the *SP60* prespore promoter, a very strong promoter, showed no phenotype, suggesting that $G\alpha 2$ does not have an essential role in these cells at a time when the *SP60* promoter is fully functional. It is possible that the level of endogenous $G\alpha 2$ is too high in prespore cells to be sufficiently inhibited to produce a phenotype, that another, yet to be identified $G\alpha$ subunit fulfills this role in these cells, or that any role $G\alpha 2$ may play in regulating prespore differentiation is complete before the level of the $G\alpha 2(G206T)$ protein rises to a sufficient level to block the function of the endogenous protein.

We note that $G\alpha 2$ has been shown to couple to $cAR1-3$ and that the late $cAR1$ promoter, $cAR2$, and $cAR4$ have all been shown to be preferentially expressed in prestalk cells (see INTRODUCTION) (Louis and Kimmel, personal communication). It is possible that $G\alpha 2$ may be coupled to all of these late cARs during later development and differentially control the downstream effects of each cAR. This would be of interest because disruptions of $cAR2$ and $cAR4$, which are expressed respectively at the tip and culmination stages, have distinct phenotypes (Saxe *et al.*, 1993; Kimmel, personal communication). GC can be activated by cAMP during culmination (Kesbeke *et al.*, 1986; Schaap, 1991), and our laboratory has reported a rapid increase in 1,2-diacylglycerol upon the initiation of culmination (Cubitt *et al.*, 1993). Presently, we do not know if either of these pathways is affected in *ecmA/G\alpha 2(G206T)* cells or if they are, whether either accounts for the aberrant morphological phenotype or whether they are altered in *car2* or *car4* null strains. At present, there is insufficient information on the functions regulated by either of these cARs or by $G\alpha 2$ that may result in the mutant phenotype during culmination. We also cannot exclude the possibility that $G\alpha 2$ may couple to receptors in addition to or other than cARs in vivo and, if so, whether these receptors may regulate culmination through $G\alpha 2$. Although, as mentioned above, we cannot exclude that

the observed phenotype results from interference with the function of a $G\alpha$ subunit other than $G\alpha 2$, we presently think that this is not the case. We note that genes encoding eight $G\alpha$ subunits have been cloned, and the seven that are expressed in later development have been disrupted. None of these have a phenotype similar to that of the $G\alpha 2(G206T)$ expressed from the *ecmA* promoter (see INTRODUCTION) (Hadwiger and Firtel, 1992; Wu, Gaskins, Zhou, Firtel, and Devreotes, unpublished data). Identification of the effectors lying downstream from $cAR2$, $cAR4$, and $G\alpha 2$ during later development and their preferential activation in different cell types will be essential for fully understanding the function of $G\alpha 2$ during culmination.

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