Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 10566–10570, October 1994
Biochemistry

The G_α subunit $G_\alpha 4$ couples to pterin receptors and identifies a signaling pathway that is essential for multicellular development in *Dictyostelium*

(*Dictyostelium* G_α subunit/folic acid/morphogenesis)

JEFFREY A. HADWIGER*, SUSAN LEE, AND RICHARD A. FIRTEL

Department of Biology, University of California, San Diego, La Jolla, CA 92093-0634

Communicated by J. T. Bonner, June 13, 1994

ABSTRACT **In this paper, we show that the G_α subunit $G_\alpha 4$ couples to pterin receptors and identifies a signaling pathway that is essential for multicellular development in *Dictyostelium*. $G_\alpha 4$ is developmentally regulated, is essential for proper morphogenesis and spore production, and functions cell nonautonomously. We show that $G_\alpha 4$ is coupled to receptors (αFAR) that activate chemotaxis and adenylyl and guanylyl cyclases in response to folate during the early stages of development and to a late class of folate receptors (βFAR) that have different specificities for pterins. $G_\alpha 4$ is preferentially expressed in cells randomly distributed within the aggregate that are a component of the anterior-like cell population, and it is not detectably expressed in prespore cells. Our results suggest that an endogenous factor, possibly a pterin, produced during multicellular development is a requisite signal for multicellular development, acting through $G_\alpha 4$. We propose that the $G_\alpha 4$-expressing cells function as a regulatory cell type controlling prespore cell fate, possibly in response to an endogenous pterin. Our results indicate that $G_\alpha 4$ and $G_\alpha 2$ have parallel functions in mediating cellular responses to folate (pterins) and cAMP, respectively.**

Multicellular development in *Dictyostelium* initiates with the chemotactic aggregation of up to 10^5 cells. As might be expected from this mode of formation of the multicellular organism, development is regulated by extracellular morphogens. These include cAMP, which interacts with guanine nucleotide-binding protein (G protein)-coupled cell surface receptors, and differentiation-inducing factor (DIF), which may interact with a putative intracellular DIF-binding protein (1–5). cAMP plays an essential role as the chemotactic agent controlling aggregation and cell sorting and regulates aggregation-stage and prestalk- and prespore-specific gene expression (1–9). Other factors, including NH_4^+ and adenosine, have also been proposed to regulate various aspects of morphogenesis, possibly by affecting cAMP- and DIF-mediated pathways (10).

Vegetatively growing and starved preaggregation-stage *Dictyostelium* cells exhibit chemotaxis toward bacteria, presumably to locate food. Pan *et al.* (11) identified the chemotactic agent as folic acid. The addition of folate during early development results in the activation of guanylyl and adenylyl cyclases, the rapid association of actin with the cytoskeleton, and chemotactic movement, similar to responses that are elicited by cAMP during aggregation (12–14). Tillinghast and Newell (14) showed that vegetative cells and mound-stage cells differentially exhibit chemotaxis to different folate analogues, suggesting the presence of more than one class of folate receptor (FAR). As has been shown for cAMP, folate is thought to mediate these responses through G protein-coupled receptors (15, 16). Chemotaxis toward cAMP requires the G_α protein subunit $G_\alpha 2$. $g_\alpha 2$ null cells lack cAMP activation of adenylyl and guanylyl cyclases, are incapable of chemotaxis toward cAMP, and do not activate cAMP-mediated gene expression during aggregation (6, 17–19), but they do respond normally to folate (19, 20), indicating that $G_\alpha 2$ does not mediate FAR activation of these processes.

We have previously shown that the G_α subunit $G_\alpha 4$ is developmentally regulated and required for multicellular development (21, 22). $G_\alpha 4$ is expressed at low levels in vegetative cells. Upon starvation, the transcripts disappear and expression is reinduced at the mound stage to a substantially higher level than that found in vegetative cells. During the multicellular stages, $G_\alpha 4$ expression is primarily restricted to a population of cells with some properties similar to those of anterior-like cells (ALCs), a subpopulation of cells of unknown regulatory function found scattered throughout the posterior prespore domain of the multicellular organism that fate map to the upper and lower caps of the spore mass and the basal disk of the mature fruiting body and express several different gene markers (7, 23–26). $g_\alpha 4$ null cells aggregate normally and form a mound, but development arrests immediately after the first finger stage with an elongated, crenelated anterior region. There is a severe reduction of prespore gene expression and very few spores are produced (<0.1% of wild-type strains). Overexpression of $G_\alpha 4$ from the $G_\alpha 4$ promoter ($G_\alpha 4^{HC}$ cells) also causes aberrant morphological differentiation and spore production is reduced 25- to 30-fold. Chimeric organisms containing equal numbers of $g_\alpha 4$ null cells and either wild-type or $G_\alpha 4^{HC}$ cells undergo multicellular development and produce a mature fruiting body that appears to be essentially wild type (22). These chimeras produce a normal number of wild-type or $G_\alpha 4^{HC}$ spores and also produce $g_\alpha 4$ null spores, although less efficiently than wild-type spores. Moreover, chimeras of $G_\alpha 4^{HC}$ with either $g_\alpha 4$ null or wild-type cells produce a normal level of viable $G_\alpha 4^{HC}$ spores. These results suggest that $G_\alpha 4$ functions, at least in part, cell nonautonomously in controlling an intercellular signaling pathway.

In this study, we examine the functions of $G_\alpha 4$ and show that $G_\alpha 4$ couples to and mediates the responses to two classes of FARs that are differentially expressed during development. Our results suggest that folate or a related pterin is an essential endogenous signaling molecule for multicellular development and that cells expressing $G_\alpha 4$ play an essential regulatory role in controlling morphogenesis and cellular differentiation in this organism.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: G protein, guanine nucleotide-binding protein; ALC, anterior-like cell; FRITC, fluorescein/rhodamine isothiocyanate; β-gal, β-galactosidase; FAR, folate receptor.

*Present address: Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078.

MATERIALS AND METHODS

Cell Lines and Procedures. The growth and construction of wild-type and mutant $G_\alpha 2$ and $G_\alpha 4$ strains have been described (22, 27). To construct a *$G_\alpha 4$/lacZ* expression vector, an *Spe* I linker was inserted immediately 5′ to the ATG translation initiation site after *Exo* III digestion. This was used to replace the *SP60* promoter in the *SP60/lacZ* expression vector (28). Histological staining for *lacZ* expression was performed as described (7).

Neutral Red Staining and Immunofluorescence. For double immunofluorescence, *$G_\alpha 4$/lacZ* cells were developed to the slug stage. Posterior regions were isolated, dissociated in 20 mM EDTA/12 mM sodium/potassium phosphate (PB), pH 6.5, washed, and suspended in PB and then plated in 5 μl onto poly(L-lysine)-coated coverslips. Cells were fixed in cold 100% methanol and then incubated with a rabbit polyclonal antibody against prespore proteins SP60/CotC and SP96/CotA (ref. 29; W. Loomis, personal communication) washed extensively with standard phosphate-buffered saline. The cells were incubated with fluorescein/rhodamine isothiocyanate (FRITC)-labeled goat anti-rabbit IgG (Sigma) and washed. The coverslips were incubated with a mouse monoclonal antibody against β-galactosidase (β-gal) (Promega), washed, and then incubated with FITC-labeled goat anti-mouse antibody (Sigma). In a separate set of experiments, we reversed the anti-rabbit or anti-mouse antisera that were conjugated with FRITC or FITC. Experiments were also performed using FITC-conjugated anti-prespore antibody (30) that was provided by K. Okamoto (Kyoto University). The mouse monoclonal antibody against β-gal followed by FRITC-labeled goat anti-mouse antibody (Sigma) was used to detect *$G_\alpha 4$/lacZ*-expressing cells.

Cells were stained with neutral red as described (23, 31). The posterior region of slugs was isolated, dissociated, and plated on coverslips as described above. Cells were photographed to identify neutral red-stained cells and then histochemically stained for β-gal activity as described by Jermyn and Williams (31).

***In Vivo* Assays.** Adenylyl and guanylyl cyclases were assayed as described (19, 32) with 30 μM folate or monapterin in the presence of 0.33 mM 8-azaguanine (to inhibit deaminase), except in the assays described in Fig. 1, in which 10 mM folate was used. Chemotaxis was assayed as described (32). Strains were assayed as positive for chemotaxis when there was net movement beyond the boundaries of the cell droplets toward the chemoattractant. Movement to folic acid was detected within 4 hr and movement to cAMP was detected within 8 hr.

Folate binding was measured with 20 nM [^{3}H]folate in the presence of 0.33 mM 8-azaguanine as described (15, 32). Nonspecific binding was determined by the amount of [^{3}H]folate in cell pellets in the presence of 50 μM unlabeled folate or monapterin. For 1-hr and 6-hr cells, logarithmic-phase vegetative cells were harvested, washed, and resuspended in 17 mM Na_3PO_4 (1×10^7 cells per ml) and shaken for 1 or 6 hr before reharvesting and assaying. For 14-hr cells (tipped aggregates), cells were grown, harvested, and plated on 12 mM PB-containing nonnutrient agar plates (3.5×10^7 cells per 10-cm plate). Cells were developed synchronously for 14 hr. Aggregates were washed off plates in ice-cold 17 mM Na_3PO_4 buffer and centrifuged at 500 rpm for 2 min to harvest aggregates and minimize the pelleting of cells that had not formed aggregates. (Very few cells did not enter aggregates when cells were developed by this method, as determined by microscopic examination of the agar plates at 14 hr.) Aggregates were resuspended and dissociated in ice-cold 20 mM NaEDTA (pH 6.2) and then washed twice in ice-cold 17 mM Na_3PO_4 buffer before proceeding as described above.

RESULTS

$G_\alpha 4$ Is Required for Folate-Mediated Responses. $G_\alpha 4$ mutant cells have different growth rates when grown on nutrient agar plates in association with bacteria but not in axenic medium. *$g_\alpha 4$* null cells grow significantly slower than wild-type cells, whereas *$G_\alpha 4^{HC}$* cells grow faster (data not shown). This phenotype led us to investigate whether *$g_\alpha 4$* null cells are chemotactic toward folic acid. When analyzed in a standard

Table 1. Total amounts of cGMP and cAMP produced by cells

	cGMP levels						cAMP levels in response to folate*		
	In response to folate*			In response to cAMP*					
	pmol per 10^7 cells		-fold	pmol per 10^7 cells		-fold	pmol per 10^7 cells		-fold
Strain	0 sec	10 sec	increase	0 sec	10 sec	increase	0 sec	4 min	increase
Wild type	1.9 ± 0.3	5.1 ± 0.3	2.7 ± 0.3	2.2 ± 0.4	7.5 ± 1.0	3.4 ± 0.4	1.2	12.8 ± 3.3	10.6 ± 3
$g_\alpha 4$ null	2.1 ± 0.2	2.2 ± 0.4	0	2.9 ± 0.7	10.6 ± 1.4	3.7 ± 0.5	0.8	1.6 ± 0.6†	0
$G_\alpha 4^{HC}$	2.2 ± 0.5	10.1 ± 1.8	4.6 ± 0.6	ND	ND		0.5	9.6 ± 2.6	19.2 ± 3.0
$g_\alpha 4$ null:$G_\alpha 4$	2.3 ± 0.3	6.3 ± 0.5	2.7 ± 0.4	2.7 ± 0.5	9.9 ± 1.1	3.7 ± 0.6	0.9	13.2 ± 1.0	14.7 ± 1.5
$g_\alpha 2$ null	2.8 ± 0.4	7.3 ± 0.9	2.6 ± 0.3	2.9 ± 0.3	2.6 ± 0.5	0			
$G_\alpha 2^{HC}$	2.5 ± 0.4	6.4 ± 0.6	2.5 ± 0.4	3.1 ± 0.4	16.8 ± 1.7	5.4 ± 0.6			
	In response to folate‡			In response to monapterin‡					
Wild type									
1 hr	2.9 ± 0.4	8.4 ± 0.6	2.9 ± 0.5	3.1 ± 0.5	3.2 ± 0.4	0			
14 hr	1.0 ± 0.2	3.4 ± 0.3	3.4 ± 0.3	0.9 ± 0.3	1.9 ± 0.3	2.1 ± 0.3			
$g_\alpha 4$ null									
1 hr	2.8 ± 0.5	2.8 ± 0.4	0	3.0 ± 0.3	2.7 ± 0.6	0			
14 hr	0.8 ± 0.3	1.0 ± 0.3	0	0.9 ± 0.2	0.9 ± 0.3	0			

Total amounts of cAMP and cGMP produced by cells were measured by binding assay. Data are an average of three separate experiments, each done in duplicate, except for the assay of cAMP accumulation in the $g_\alpha 4$ complemented strain, for which the results are an average of two experiments. For cGMP assays, time points were taken at 0, 5, 10, 15, 20, and 30 sec. Maximum cGMP accumulation was at 10 sec. For cAMP assays, time points were taken at 0.5, 1, 2, 4, and 8 min. Maximum cAMP accumulation was at 4 min.

*Cells were starved for 3 hr in phosphate buffer and their activation responses to folic acid were measured as described.

†No significant increase in cAMP accumulation in response to folate.

‡Activation of guanylyl cyclase was measured in 1-hr cells and 14-hr tipped aggregates as described. Relative activation of the 10-sec time point (point of maximum response) is shown.

chemotaxis (32) or spread assay (14), $g_\alpha 4$ null cells did not exhibit chemotaxis toward folic acid (10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M), whereas wild-type cells and $G_\alpha 4^{HC}$ cells exhibited normal chemotaxis (10^{-4}, 10^{-5}, 10^{-6}, but not 10^{-7} M folic acid). $g_\alpha 4$ null cells did exhibit normal chemotaxis to cAMP (10^{-5}, 10^{-6}, but not 10^{-7} M), consistent with their ability to form normal aggregates. When folate activation of guanylyl and adenylyl cyclases was examined at the onset of development when this response was maximal (32), neither pathway was activated in $g_\alpha 4$ null cells, but both responses were present in wild-type and $G_\alpha 4^{HC}$ cells and with the same kinetics as reported previously (32), suggesting that $G_\alpha 4$ is required in activating adenylyl and guanylyl cyclases in response to folate (Table 1; kinetics not shown). $g_\alpha 4$ null mutants complemented with the $G_\alpha 4$ gene at low copy number (22) displayed responses similar to wild-type cells. Previous results showed that $G_\alpha 2$ is essential for the *in vivo* cAMP-mediated activation of adenylyl and guanylyl cyclases (see Introduction). As shown in Table 1, $g_\alpha 4$ null and $G_\alpha 4^{HC}$ cells show normal responses to cAMP, while $g_\alpha 2$ null and $G_\alpha 2^{HC}$ cells show normal responses to folate (data for cAMP stimulation of adenylyl cyclase not shown). In $G_\alpha 4^{HC}$ cells, guanylyl cyclase activity was significantly higher than in wild-type cells, whereas the level of adenylyl cyclase activity in $G_\alpha 4^{HC}$ cells was reproducibly ≈25% lower than that of wild-type cells (Table 1; data not shown). Similar differential effects on the activation of guanylyl and adenylyl cyclases by cAMP are seen in $G_\alpha 2$-overexpressing cells (ref. 27; Table 1).

Two Classes of Developmentally Regulated FARs with Different Binding Specificities. The above results suggest that $G_\alpha 4$ couples to FARs responsible for chemotaxis to bacteria, while the developmental phenotypes of $g_\alpha 4$ null cells indicate a role for $G_\alpha 4$ during multicellular development (22). *Dictyostelium* cells lose their ability to respond to folate during aggregation (33, 34), but folate-mediated chemotaxis is regained as the multicellular organism forms (14). Tillinghast and Newell (14) showed that cells from the multicellular stages orient toward both folate and the pterin monapterin, but monapterin is not capable of eliciting a chemotactic response in growth-stage cells. These results suggested the presence of two classes of pterin receptors or FARs with different agonist specificities. Table 2 presents results of an assay for these two classes of FARs. Growth-stage cells have a class of FARs that is competable with folic acid but not with monapterin, whereas 14-hr cells have two classes of receptors: one class (≈60% of the binding) that is competable with monapterin and a second class that can compete only with folate. We have designated the early class(es) of receptors as α (αFAR) and the receptors that bind monapterin as β (βFAR). The levels of both classes of receptors are very similar in $g_\alpha 4$ null cells compared to wild-type cells (Table 2).

Table 2. Folate binding sites in $g_\alpha 4$ null compared to wild-type cells

	αFAR	βFAR
Wild type		
1 hr	65.0 ± 8.3	0 ± 1.2
14 hr	9.5 ± 4.6	25.8 ± 5.6
$g_\alpha 4$ null		
1 hr	59.4 ± 9.5	0 ± 1.0
14 hr	8.3 ± 5.0	21.7 ± 8.2

Number of folate binding sites on cells at 1 and 14 hr of development was measured as described. Total folate binding sites were determined by total amount of [^{3}H]folate bound minus nonspecific binding. α sites are defined as specific for folate binding that is not competable with monapterin. β sites are specific for folate binding that is competable with monapterin. Results are expressed as no. of binding sites × 10^{-3}.

These studies do not distinguish between potentially different FAR gene products that have similar binding characteristics.

$G_\alpha 4$ Is Required for Late FAR-Mediated Responses. To determine whether βFARs are coupled to intracellular signaling pathways and whether $G_\alpha 4$ is required for these pathways, we examined the ability of monapterin to activate guanylyl cyclase in preaggregation-stage and 14-hr developing cells. As shown in Table 1, in wild-type cells both folate and monapterin activate guanylyl cyclase in 14-hr multicellular aggregates, whereas only folate activates guanylyl cyclase in preaggregation-stage cells, consistent with the absence of βFARs at this stage (see above). However, cells from 14-hr $g_\alpha 4$ null aggregates do not activate guanylyl cyclase in response to either monapterin or folate, although these cells have βFARs. In 14-hr wild-type cells, the response to monapterin is ≈65% of that to folate, consistent with the relative fraction of receptors that bind monapterin versus folate. While the level of response is low (≈2-fold), it is highly reproducible.

Spatial Expression of $G_\alpha 4$ During Multicellular Development. An important issue in understanding the possible cell-nonautonomous function of $G_\alpha 4$ is a more complete

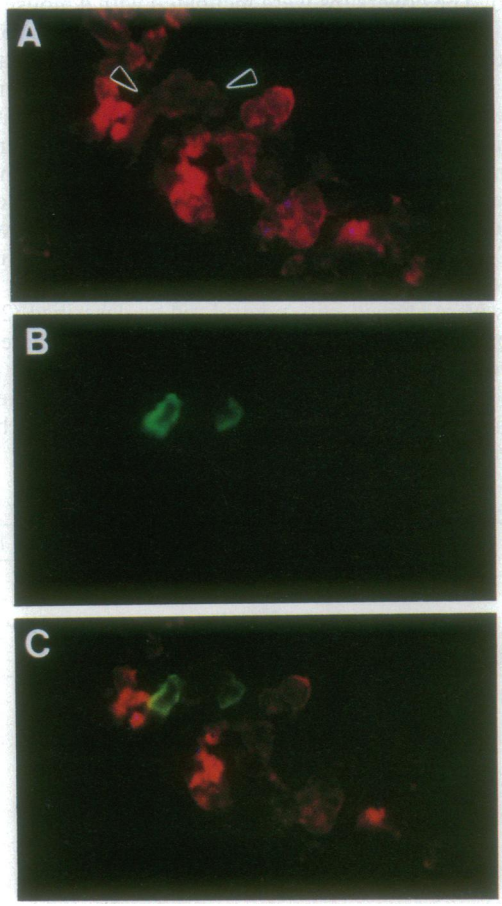

FIG. 1. Identification of $G_\alpha 4$-expressing and prespore cells by double indirect immunofluorescence. The posterior two-thirds of slugs were manually isolated, dissociated, and stained by double indirect immunofluorescence. $G_\alpha 4/lacZ$-expressing cells were identified with a mouse anti-β-gal antibody followed by a fluorescein-labeled goat anti-mouse antibody. Prespore cells were identified with a rabbit antibody to the prespore proteins followed by a rhodamine-labeled goat anti-rabbit antibody. (*A*) Indirect immunofluorescence identification of the prespore cells (rhodamine label). (*B*) Same photographic field but $G_\alpha 4/lacZ$-expressing cells (fluorescein label) are identified. (*C*) Double-exposure photographs of the same photographic field (not a double printing of the first two images), showing both fluorescein- and rhodamine-labeled cells. Arrowheads in *A* identify fluorescein-labeled cells shown in *B*.

understanding of which cells express $G_\alpha 4$ in the multicellular stages. Using a $G_\alpha 4/lacZ$ fusion that produces a more sensitive assay for expression, we show that β-gal staining is restricted to a subpopulation of cells scattered through the slug, with some slugs showing a greater proportion of staining cells near the posterior of the prespore zone (data not shown), consistent with previous results (21). In squashes of the slugs, 5–8% of the cells stain, whereas ≈65% of the cells stain in squashes of slugs expressing *lacZ* from the *SP60* prespore-specific promoter. In the fruiting body, $G_\alpha 4/lacZ$ expression is restricted to the stalk, basal disc, and the upper and lower cap structures; no detectable spore staining is seen in squashed slugs, even after extended incubation. $G_\alpha 4/lacZ$ is strongly expressed in $g_\alpha 4$ null cell aggregates, indicating that $G_\alpha 4$ function is not required for $G_\alpha 4$ expression.

ALCs have been defined as cells located in the prespore domain that do not express prespore markers, that stain with neutral red, and that express several different gene markers. While $G_\alpha 4$-expressing cells show an ALC-like spatial pattern, it is necessary to directly show that $G_\alpha 4$-expressing cells stain with neutral red and do not express prespore markers. Fig. 1 presents double indirect immunofluorescence staining of cells from the posterior of $G_\alpha 4/lacZ$-expressing slugs. A mouse anti-β-gal antibody followed by a fluorescein-labeled goat anti-mouse antibody was used to identify $G_\alpha 4/lacZ$-expressing cells and a rabbit antibody that specifically labels the prespore proteins SP60/CotC and SP70/CotB (29) followed by a rhodamine-labeled goat anti-rabbit antibody used to identify prespore cells. As shown, the two antibodies stain different cells, indicating that $G_\alpha 4/lacZ$-expressing cells are not a subpopulation of prespore cells. A similar result was obtained by using the same anti-β-gal antibody followed by a rhodamine-labeled goat anti-mouse antibody and a fluorescein-conjugated general anti-spore coat antibody (ref. 30; data not shown). To determine whether $G_\alpha 4$-expressing cells are a component of the ALC population, we examined whether $G_\alpha 4/lacZ$-expressing cells also stain with neutral red, an ALC marker. When the posterior of $G_\alpha 4/lacZ$-expressing slugs from neutral red-stained organisms are dissociated and plated onto coverslips, 5–8% of the cells are visibly pink under the microscope (data not shown). When the cells on the coverslip are then stained for β-gal expression, ≈5% of the cells stain, and half of these cells are also visibly pink, indicating that $G_\alpha 4/lacZ$-expressing cells are a component of the ALC population (data not shown).

DISCUSSION

We have shown that *Dictyostelium* contains two classes of folic acid binding sites that are differentially expressed during development. The binding specificities of these putative receptors are consistent with the ability of *Dictyostelium* cells to orient toward different folic acid analogues during growth and multicellular development. Our results suggest that $G_\alpha 4$ couples to both classes of receptors. The absence of receptors for monapterin in preaggregation-stage cells, together with the ability of monapterin to activate signal transduction in 14-hr cells, supports a specific role for folate and/or another pterin in the multicellular stages of development and is consistent with previous observations (ref. 14; see Introduction). Interestingly, Segall *et al.* (32) isolated a mutant that cannot exhibit chemotaxis to folate during early development and arrests at the tight mound stage. Their data are consistent with the mutation affecting a protein that interacts with FARs and suggests a possible role for a component of the folate chemotaxis pathway in multicellular development. There is evidence for the use of endogenous pterins in the genus *Dictyostelium*; both *Dictyostelium minutum* (35) and *Dictyostelium lacteum* (12) use a pterin as their chemotactic substance, but there is no previous evidence for the use of pterins to regulate differentiation during the multicellular stages. In *Dictyostelium discoideum*, folate-mediated pathways act both as a foraging response to identify food (bacteria) and to control certain aspects of multicellular differentiation. Since $g_\alpha 4$ null cells express the $G_\alpha 4/lacZ$ construct, the $g_\alpha 4$ null phenotype is probably due not to the inability to differentiate the class of cells that normally express $G_\alpha 4$ but to the inability of these cells to respond to an exogenous signal.

Our experiments show that $G_\alpha 4$-expressing cells are a component of the ALC population within the prespore domain and a subpopulation of the cells in the anterior, prestalk domain. Our results also show that neither prespore nor spore cells detectably express $G_\alpha 4$, suggesting that folate is primarily perceived by a population of nonprespore cells in the multicellular organism. Our results suggest that the βFARs may also be present primarily in this population of cells and directly couple to $G_\alpha 4$. It is possible that $G_\alpha 4$ may also couple to an additional class of receptors that mediate signal transduction pathways in response to a ligand that is distinct from either cAMP or folic acid and mediates the developmental function of $G_\alpha 4$. The requirement for a heterotrimeric G protein in mediating folate responsiveness is consistent with the effects of guanine nucleotides on folate binding and folate stimulation of GTP binding in isolated membranes.

Our results suggest that $G_\alpha 2$ and $G_\alpha 4$ couple to similar downstream pathways during the earliest stages of development. It has been suggested that $G_\alpha 2$ couples to multiple cell-surface cAMP receptors (cARs) and may regulate many, if not all, G-protein-coupled pathways activated by extracellular cAMP (P. Devreotes, personal communication; ref. 36). We propose that $G_\alpha 4$ plays a role in folate-mediated responses throughout *Dictyostelium* development parallel to the apparent role of $G_\alpha 2$ for cAMP-mediated responses. However, although folate and cAMP can both activate guanylyl cyclase during the multicellular stages, some of the downstream effectors must be different, since cAMP cannot substitute for the $G_\alpha 4$-mediated pathways in $g_\alpha 4$ null cells.

$G_\alpha 4$-Expressing Cells Function as Essential Signaling Cells in Multicellular Development. An important conclusion of the requirement for $G_\alpha 4$ function in folate signaling is that folate or a related pterin (or another factor that functions through a receptor that is coupled to $G_\alpha 4$) functions as a morphogen to regulate morphogenesis and spore production. We suggest that *Dictyostelium* produces a pterin during multicellular development that has greater structural homology to monapterin than to folic acid and that activates βFAR-mediated responses. Whether it is produced by the same cells that express $G_\alpha 4$ or by another cell type is not known. Interestingly, Klein and co-workers have purified a newly discovered pterin, dictyopterin, from vegetative cells that has a structure very similar to monapterin (37). Consistent with our proposal that an endogenous folate or pterin is an essential signaling molecule, we note that *D. discoideum* expresses an extracellular folate deaminase during the multicellular stages (38) that would have the function of clearing the morphogen from the extracellular space or act as a sink for establishing a possible morphogen gradient within the organism. This is similar to the role that extracellular cAMP phosphodiesterase plays during multicellular development (39).

Previous studies of chimeric organisms containing $g_\alpha 4$ null and wild-type cells demonstrated that developmental morphology and spore production can be partially rescued by the presence of wild-type cells, indicating a cell-nonautonomous role for $G_\alpha 4$ function (22). We proposed (22) that prespore/spore differentiation is regulated by an intercellular signal(s) that is absent in $g_\alpha 4$ null aggregates and is generated by $G_\alpha 4$-expressing cells within the slug in wild-type strains. Prespore cell differentiation is thus regulated by both cAMP

acting through cell surface receptors to induce the expression of prespore genes (see Introduction for reviews) and a $G_\alpha 4$-dependent response mediated through the *$G_\alpha 4$*-expressing cells. This cell-nonautonomous role of $G_\alpha 4$ function must occur by way of an intercellular signal(s) that is absent in *$g_\alpha 4$* null aggregates and presumably generated by wild-type *$G_\alpha 4$*-expressing cells in chimeras. Our present results suggest that this proposed intercellular signal(s) is produced in response to the activation of $G_\alpha 4$-coupled receptors, possibly βFAR, present on *$G_\alpha 4$*-expressing cells. The *$G_\alpha 4$*-expressing cells play an essential signaling role in controlling multicellular development. These cells are present in the prespore domain and are thus appropriately localized to function as the proposed signaling cell. Our results are also consistent with previous models in which the proportion of prespore cells is regulated by ALCs (40).

We are indebted to Jeff Segall for helpful discussions; to K. Jermyn and W. Loomis for materials and technical suggestions; to M. Maeda and S. Dharmawardhane for assistance, counseling, and moral support for the neutral red staining and immunofluorescence; and to Sandy Mann for critical reading of the manuscript. This work was supported by U.S. Public Health Service Grant GM37830 to R.A.F.

1. Devreotes, P. (1989) *Science* **245,** 1054–1058.
2. Insall, R. & Kay, R. R. (1990) *EMBO J.* **9,** 3323–3328.
3. Firtel, R. A. (1991) *Trends Genet.* **7,** 381–388.
4. Kimmel, A. R. & Firtel, R. A. (1991) *Curr. Opin. Genet. Dev.* **1,** 383–390.
5. Williams, J. G. (1991) *Curr. Opin. Genet. Dev.* **1,** 358–362.
6. Mann, S. K., Pinko, C. & Firtel, R. A. (1988) *Dev. Biol.* **130,** 294–303.
7. Esch, R. K. & Firtel, R. A. (1991) *Genes Dev.* **5,** 9–21.
8. Siegert, F. & Weijer, C. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6433–6437.
9. Traynor, D., Kessin, R. H. & Williams, J. G. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8303–8307.
10. Schaap, P. (1991) in *Microbial Cell-Cell Interactions*, ed. Dworkin, M. (Am. Soc. Microbiol., Washington, DC), pp. 147–176.
11. Pan, P., Hall, E. & Bonner, J. T. (1972) *Nature (London) New Biol.* **237,** 181–182.
12. van Haastert, P., De Wit, R. & Konijn, T. (1982) *Exp. Cell Res.* **140,** 453–458.
13. Wurster, B., Schubiger, K., Wick, U. & Gerisch, G. (1977) *FEBS Lett.* **76,** 141–144.
14. Tillinghast, H. & Newell, P. (1987) *J. Cell Sci.* **87,** 45–55.
15. De Wit, R. & Bulgakov, R. (1985) *FEBS Lett.* **179,** 257–260.
16. De Wit, R. & Bulgakov, R. (1986) *Biochim. Biophys. Acta* **886,** 88–93.
17. Kesbeke, F., Snaar-Jagalska, B. & van Haastert, P. (1988) *J. Cell Biol.* **107,** 521–528.
18. Kumagai, A., Pupillo, M., Gundersen, R., Miake-Lye, R., Devreotes, P. N. & Firtel, R. A. (1989) *Cell* **57,** 265–275.
19. Kumagai, A., Hadwiger, J. A., Pupillo, M. & Firtel, R. A. (1991) *J. Biol. Chem.* **266,** 1220–1228.
20. Kesbeke, F., van Haastert, P. J. M., De Wit, R. J. W. & Snaar-Jagalska, B. E. (1990) *J. Cell Sci.* **96,** 669–673.
21. Hadwiger, J. A., Wilkie, T. M., Strathmann, M. & Firtel, R. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8213–8217.
22. Hadwiger, J. A. & Firtel, R. A. (1992) *Genes Dev.* **6,** 38–49.
23. Sternfeld, J. & David, C. (1982) *Dev. Biol.* **93,** 111–121.
24. Devine, K. & Loomis, W. (1985) *Dev. Biol.* **107,** 364–372.
25. Ceccarelli, A., Mahbubani, H. & Williams, J. G. (1991) *Cell* **65,** 983–989.
26. Gaskell, M. J., Jermyn, K. A., Watts, D. J., Treffry, T. & Williams, J. G. (1992) *Differentiation* **51,** 171–176.
27. Okaichi, K., Cubitt, A. B., Pitt, G. S. & Firtel, R. A. (1992) *Mol. Biol. Cell* **3,** 735–747.
28. Haberstroh, L. & Firtel, R. A. (1990) *Genes Dev.* **4,** 596–612.
29. Devine, K. M., Bergmann, J. E. & Loomis, W. F. (1983) *Dev. Biol.* **99,** 437–446.
30. Takeuchi, I. (1963) *Dev. Biol.* **8,** 1–26.
31. Jermyn, K. A. & Williams, J. G. (1991) *Development (Cambridge, U.K.)* **111,** 779–787.
32. Segall, J. E., Bominaar, A. A., Wallraff, E. & De Wit, R. J. (1988) *J. Cell Sci.* **91,** 479–489.
33. De Wit, R., Bulgakov, R., De Wit, T. & Konijn, T. (1986) *Differentiation* **32,** 192–198.
34. Varnum, B. & Soll, D. (1981) *Differentiation* **18,** 151–160.
35. De Wit, R. & Konijn, T. (1983) *Cell Differ.* **12,** 205–209.
36. Carrel, F., Dharmawardhane, S., Clark, A. M., Powell-Coffman, J. A. & Firtel, R. A. (1994) *Mol. Biol. Cell* **5,** 7–16.
37. Klein, R., Thiery, R. & Tatischeff, I. (1990) *Eur. J. Biochem.* **187,** 665–669.
38. Greiner, R., Jacobskrahnen, D., Mutzel, R., Lichtenberg, B., Wurster, B. & Malchow, D. (1988) *Biol. Chem. Hoppe Seyler* **369,** 825.
39. Franke, J. & Kessin, R. H. (1992) *Cell Signal* **4,** 471–478.
40. MacWilliams, H., Blaschke, A. & Prause, I. (1985) *Cold Spring Harbor Symp. Quant. Biol.* **50,** 779–785.