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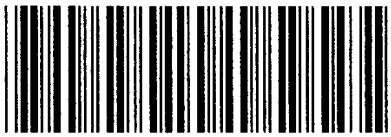

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CHAPTER 64

Signal Transduction by G-Proteins in *Dictyostelium discoideum*

L. Wu, C. Gaskins, R. Gundersen, J.A. Hadwiger, R.L. Johnson, G.S. Pitt, R.A. Firtel, and P.N. Devreotes

A. Introduction

G-protein-linked signal transduction pathways play essential roles during the differentiation process of *Dictyostelium discoideum*, a simple developing eucaryotic organism. These transmembrane signaling systems are essentially the same as those in mammalian cells, and there are simple methods to disrupt genes by homologous recombination and to create cell lines expressing mutant genes. In addition, *Dictyostelium* is easy to grow, and development is synchronous, allowing one to readily obtain 10^{11} cells for biochemical studies. Thus, *Dictyostelium* provides a model system to study G-protein-linked signal transduction.

B. Signal Transduction in *Dictyostelium*

The life cycle of *Dictyostelium* consists of distinct growth and developmental phases. In the developmental phase, triggered by starvation, about 10^5 individual amoebae aggregate to form a multicellular structure. This process is organized by extracellular adenosine 3′,5′-monophosphate (cAMP) that is secreted by cells at aggregation centers. Surrounding cells respond by moving chemotactically toward the signaling cells and by relaying the signal to cells further from the center. The resulting multicellular aggregate undergoes further morphogenesis, in which the signaling system continues to play a role. Cells in the aggregate differentiate into prestalk and prespore cells which eventually form the stalk and spore mass of a fruiting body (Fig. 1). This cell-cell signaling process occurs via cAMP binding to cell surface receptors, which in turn triggers numerous responses (Devreotes 1989; Firtel 1991).

Genes encoding four surface cAMP receptors (cARs), which comprise a family highly related by sequence, have been identified (Klein et al. 1988; Saxe et al. 1991a,b). Each gene is expressed at a different time in development (Fig. 1). cAR1 mRNA is present mainly during early aggregation, although two additional transcripts are induced later in development at much lower levels (Saxe et al. 1991a). cAR3 is expressed next, being induced at late aggregation with maximal expression occurring at the mound stage and continuing through later development at reduced

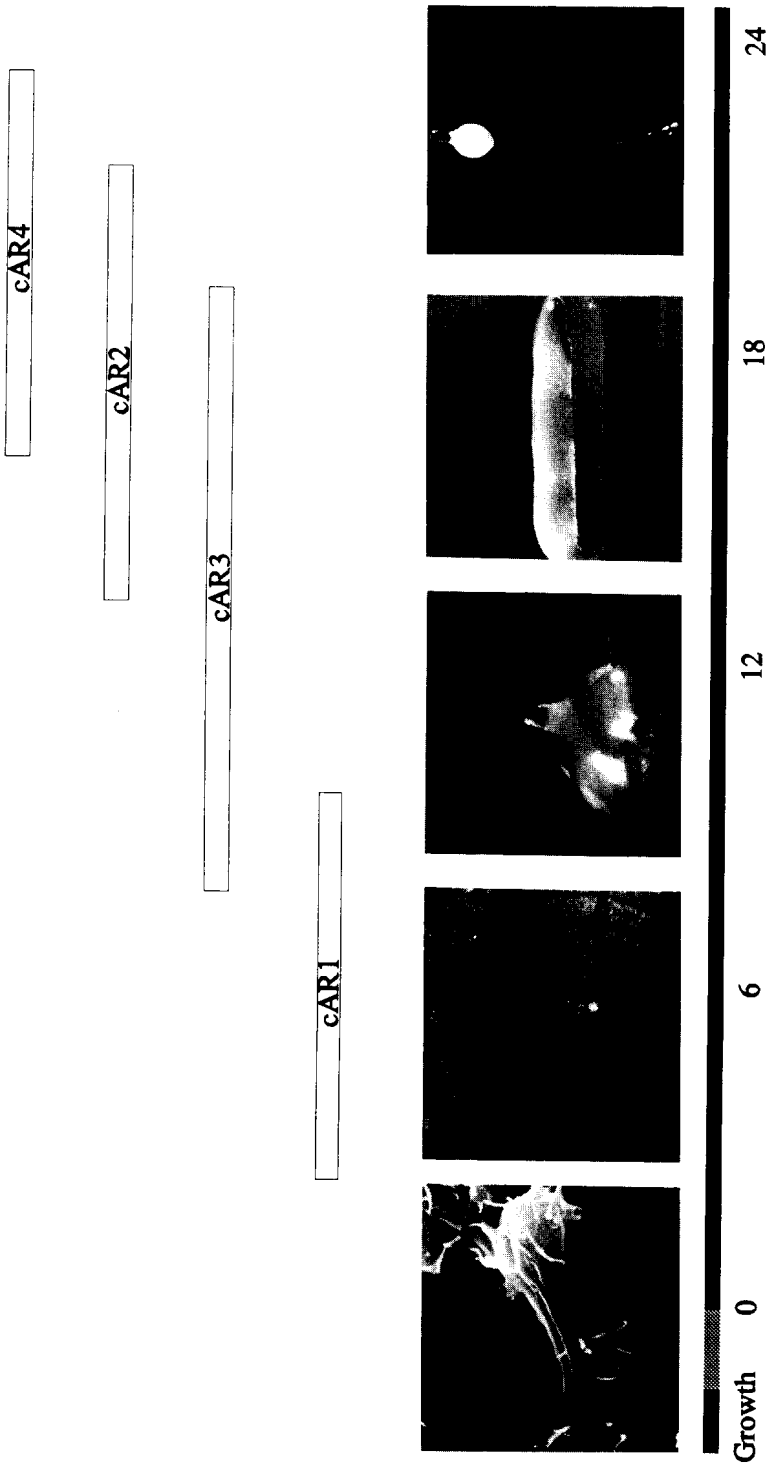

Fig. 1. The prominent stages of *Dictyostelium* development and the expression of cAMP receptors (*cARs*). Five stages of *Dictyostelium* development are shown (*bottom, left to right*: growth, aggregation, mounds, slugs, and fruiting bodies. *Open bars*, developmental expression of the major RNA transcript for each cAR. (SAXE et al. 1991a; KIMMEL, personal communications)

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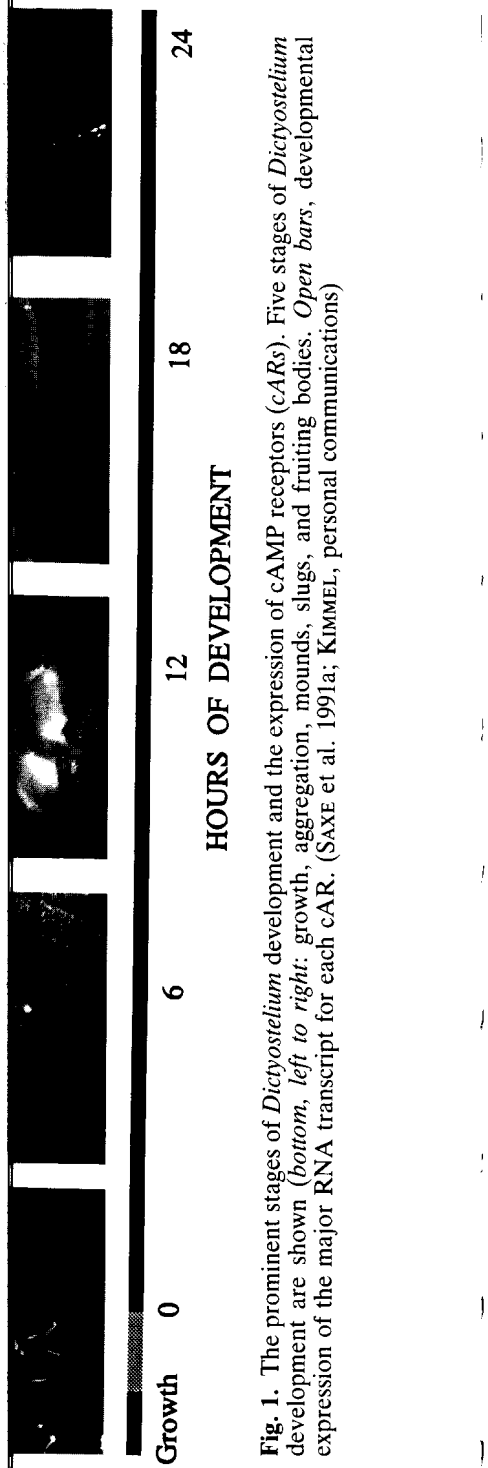

Fig. 1. The prominent stages of *Dictyostelium* development and the expression of cAMP receptors (*cARs*). Five stages of *Dictyostelium* development are shown (*bottom, left to right*: growth, aggregation, mounds, slugs, and fruiting bodies. *Open bars*, developmental expression of the major RNA transcript for each cAR. (SAXE et al. 1991a; KIMMEL, personal communications)

levels. The cAR2 transcript is enriched in prestalk cells and is expressed initially at the mound stage but is present predominantly at the slug stage (SAXE et al. 1991a). cAR4 is expressed lastly, and it appears during the culmination (A. KIMMEL, personal communication).

All of these receptors contain seven putative transmembrane domains, a characteristic of receptors that are linked to G-proteins, such as the β-adrenergic receptor and rhodopsin. It has been shown that cAR1 is needed for normal development (SUN et al. 1990; SUN and DEVREOTES 1991). Cells that lack cAR1 do not aggregate and have almost no detectable surface cAMP binding sites. The expression of early genes in the cAR1-null cells is delayed, and late gene expression is blocked. Preliminary results suggest that the other cARs also serve critical functions in the signaling processes and in controlling development in this organism (JOHNSON et al. 1993; SAXE et al. 1993).

C. Diversity of G-Proteins in *Dictyostelium*

By using oligonucleotides based on the sequences of the conserved GTP-binding domains of G-protein α subunits to screen a *Dictyostelium* cDNA libary or to perform polymerase chain reactions (PCR), genes encoding eight G-protein α subunits have been cloned (PUPILLO et al. 1989; HADWIGER et al. 1991; WU and DEVREOTES 1991; PUPILLO and DEVREOTES, in preparation; WU et al., in preparation; CUBITT et al. 1992). Comparison of the predicted amino acid sequences indicates that the eight G-protein α subunits share 30%–50% identity to each other and to mammalian G-protein α subunits. The eight αsubunits do not fall into any obvious subtypes related to the four α subunit classes, G_s, G_i, G_q, and G_{12} found in higher eucaryotes.

Despite the relatively low degree of identity among these G-proteins overall, some regions are highly conserved. Figure 2 shows the sequence comparison of the most conserved regions between the *Dictyostelium* Gα subunits and several mammalian Gα subunit subtypes. These sequence motifs are believed to be important for G-protein function. Gα4 and Gα7 have unusual amino acids in region A (. . . GAGESG . . .), which is involved in $\beta\gamma$ release and GTP hydrolysis (SIMON et al. 1991). Gα8, moreover, possesses some very interesting and unusual features. The amino acid sequences of the N-terminal portion (about 75% of the molecule) of Gα8 is similar to other Gα subunits, but its C-terminus portion has an additional 50 amino acids consisting of long stretches of Asn and Ser. Such stretches of repeated sequence have been observed for several cAMP receptors, adenylyl cyclase genes, and the catalytic subunit of cAMP-dependent protein kinase in *Dictyostelium* (JOHNSON et al. 1993; PITT et al. 1992; MANN and FIRTEL 1991), but to our knowledge Gα8 is the first G-protein α subunit identified possessing this motif. Moreover, the

	Region A	Region C	Region G	Region T
Consensus:	KLLLLGAGESGKSTIXKQMK	DVGGQR	LFLNKXD	TCATDT
Gα1:	KLLLLGAGESGKSTIAKQMK	DVGGQR	LFLNKRD	TCATDT
Gα2:	KLLLLGAGESGKSTISKQMK	DVGGQR	LFLNKSD	TCATDT
Gα4:	KLLLLGPGESGKSTIFKQMK	DVGGQR	LFLNKKD	TCAVDT
Gα5:	KLLLLGAGESGKSTIFKQMK	DVGGQR	YFLNKVD	TCAIDT
Gα6:	GAGESGKSTIFKQLK	DVGGQR		
Gα7:	KLLLLGTGDSGKSTVVKQMK	DVAGQR	LFLNKRD	TTATDT
Gα8:	RILLLGAGESGKSTVVKQLK	DVGGQR	LVLNKKD	IAARYK
Gs:	RLLLLGAGESGKSTIVKQMR	DVGGQR	LFLNKQD	TCAVDT
Gi:	KLLLLGAGESGKSTIVKQMK	DVGGQR	LFLNKKD	TCATDT
Gq:	KLLLLGTGESGKSTFIKQMR	DVGGQR	LFLNKKD	TCATDT
G12:	KILLLGAGESGKSTFLKQMR	DVGGQR	LFLNKKD	TTAIDT

Fig. 2. Amino acid sequence comparison of *Dictyostelium* Gα1–Gα8 and mammalian Gα subunits in the most conserved regions. Gα3 sequence is not shown and is cloned by PUPILLO and DEVREOTES (in preparation). The complete sequence of Gα6 has not been determined. The sequences of Gα1 and Gα2 are taken from PUPILLO et al., the sequences of Gα4 and Gα5 are taken from HADWIGER et al. (1991) and HADWIGER and FIRTEL (in preparation), and the sequences of Gs, Gi, Gq, and G12 are taken from SIMON et al. (1991)

well-conserved TCATDT motif of Gα subunits (SIMON et al. 1991) is totally missing in Gα8. It has been suggested that the C-terminal region of the G protein is involved in receptor interactions (SIMON et al. 1991). This suggestion is supported by the observation that modification of the α subunit of the G_i class by pertussis toxin blocks its interaction with receptor, and antibodies or peptides that specifically interact with C-terminal regions, including the TCATDT region, of some of the Gα proteins also block interaction with receptor (DERETIC and HAMM 1987; SULLIVAN et al. 1987; MASTERS et al. 1988). The unusual structure of Gα8 at the C-terminal region may suggest that Gα8 interacts with a structurally different receptor and thus represents a very different class of G-protein superfamily.

Northern blot analyses indicate that each of these genes has a distinct pattern of expression during development of *Dictyostelium* (Fig. 3). Most of these genes hybridize to multiple RNA species that are presumably driven by different promoters. Gα6 is expressed primarily in vegetative cells. Upon starvation, the level of Gα6 mRNA declines rapidly. Gα3 mRNA is detected mainly in growing and very early aggregation stages. Gα1 is expressed at moderate levels in vegetative cells and increases to a maximal level at 10–12 h. Gα2 is expressed at very low levels in vegetative cells. Upon initiation of development, Gα2 RNA levels increase, reaching a maximum level during aggregation and then declining. A second transcript of Gα2 is preferentially expressed late in development in the anterior prestalk region as determined by *lacZ* expression studies (CARREL and FIRTEL, in preparation). Gα8 has a similar expression pattern as Gα2. The expression time course of both Gα2 and Gα8 parallels that of cAR1 during

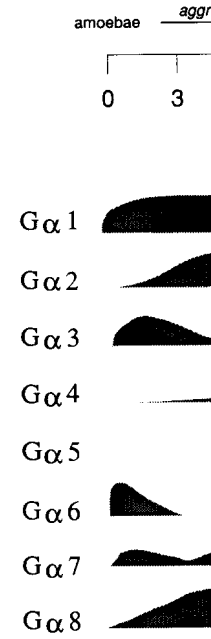

Fig. 3. Developmental expres
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VGGQR	LFLNKSD	TCATDT
VGGQR	LFLNKKD	TCAVDT
VGGQR	YFLNKVD	TCAIDT
VGGQR		
VAGQR	LFLNKRD	TTATDT
VGGQR	LVLNKKD	IAARYK
VGGQR	LFLNKQD	TCAVDT
VGGQR	LFLNKKD	TCATDT
VGGQR	LFLNKKD	TCATDT
VGGQR	LFLNKKD	TTAIDT

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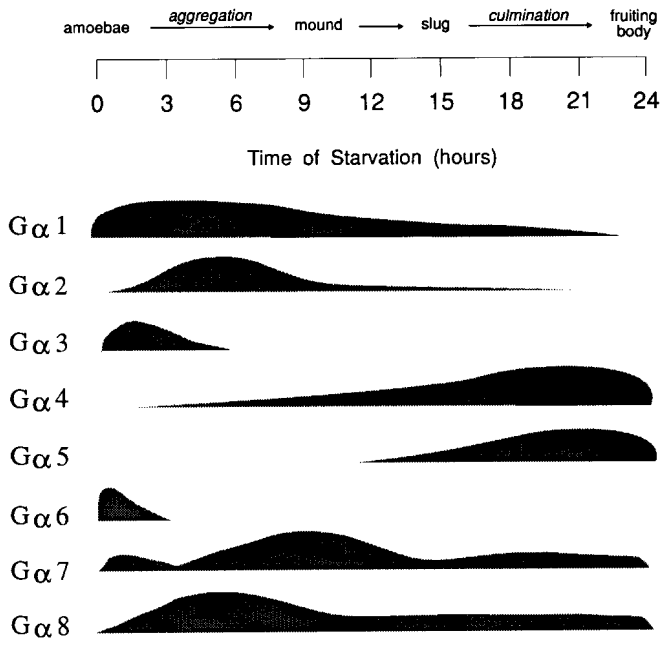

Fig. 3. Developmental expression of Gα subunits during *Dictyostelium* development. The relative *width of the bar* indicates the relative level of mRNA detected at the time indicated. *Above*, the time course of the development. The expression pattern of Gα1 and Gα2 is from PUPILLO et al. (1989), of Gα3 from PUPILLO and DEVREOTES (in preparation), of Gα4 and Gα5 from HADWIGER et al. (1991), of Gα6, Gα7, and Gα8 from Wu and DEVREOTES (1991) and CUBITT et al. (1992). See text for a detailed explanation

early development. The level of Gα7 peaks in late aggregation and early mound stages, and declines thereafter. Finally, Gα4 and Gα5 are synthesized predominantly in late development when the multicellular structure is undergoing differentiation, although Gα4 is also expressed at low levels in vegetative cells.

The presence of at least eight G-protein subtypes during development is intriguing. It is unclear why there is such a diversity of G-proteins in the slime mold, and whether they are functionally redundant. The distinct time course of expression of these G-proteins in combination with the fact that the functions of Gα2 and Gα4 cannot be replaced by other G-proteins (see below) suggest that each of these proteins is probably involved in a different signal transduction pathway and thus plays a distinct role.

cDNA encoding for one G-protein β subunit has been isolated (LILLY et al. 1993). The predicted amino acid sequences of Gβ share an extensive degree of identity to its mammalian counterpart, and it is expressed throughout the growth and developmental stages.

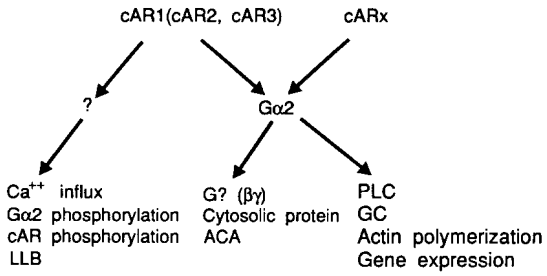

Fig. 4. The proposed model for signal transduction pathways during aggregation. See text for explanation. *cAR*, cAMP receptor; *LLB*, loss of ligand binding; *ACA*, adenylyl cyclase in aggregation; *PLC*, phospholipase C; *GC*, guanylyl cyclase

D. Roles of G-Proteins in Signal Transduction Processes

A number of developmentally defective mutants have been isolated in *Dictyostelium*. In the frigid A (*fgdA*) mutants, the guanine nucleotide effect on cAMP binding is greatly reduced and basal and cAMP-stimulated GTPase activities are lowered (KESBEKE et al. 1988). Molecular cloning of the Gα2 gene indicates that the defective alleles in *fgdA* mutants reside in Gα2 (KUMAGAI et al. 1989). A gene-targeting experiment has generated *gα2*-null cells that display same phenotypes as *fgdA* (KUMAGAI et al. 1991). The studies with these *gα2*-null mutant cells have shown clearly that Gα2, in coupling to a cAMP receptor, plays an important role in signaling and development. A proposed pathway in early aggregation stage of the *Dictyostelium* development is shown in Fig. 4.

The *gα2*-null cells do not aggregate and lack cAMP-mediated activation of adenylyl cyclase, guanylyl cyclase, phosphatidylinositol (PI)-specific phospholipase C (PLC), and regulation of gene expression (KESBEKE et al. 1988; SNAAR-JAGALSKA et al. 1988; KAMAGAI et al. 1991; OKAICHI et al. 1992). They also display a loss of GTP-mediated decrease in receptor affinity for cAMP but have no effect on chemotaxis to folate or folate activation of guanylyl cyclase (KUMAGAI et al. 1991), suggesting that Gα2 is coupled to a cAMP receptor but not to folate receptors. These phenotypes can be rescued by transformation with a vector expressing Gα2, indicating that the defects are caused by the absence of Gα2. It has also been demonstrated that Gα2 is required for actin polymerization (HALL et al. 1989). There are several cAMP receptor-mediated responses, however, that appear to be independent of Gα2 and will be discussed later (see below).

On stimulation of cells with cAMP, Gα2 is phosphorylated on one or more serine residues, resulting in an alteration of its electrophoretic mobility (GUNDERSEN and DEVREOTES 1990). Figure 5 shows a cAMP dose response of the Gα2 mobility shift. Triggered by increased occupancy of the surface

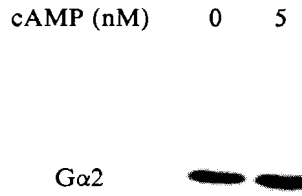

Fig. 5. The cAMP dose respo
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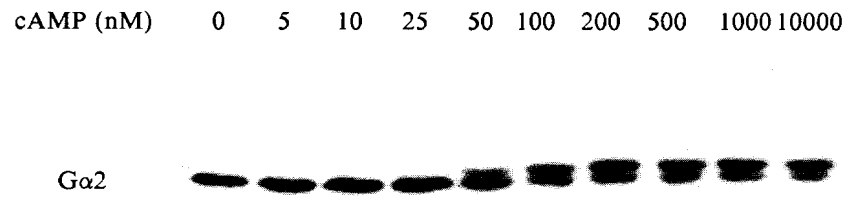

Fig. 5. The cAMP dose response of the Gα2 mobility shift (phosphorylation) on SDS-PAGE. Aggregation-competent cells were stimulated with various concentrations of cAMP for 1 min, and proteins were isolated and subjected to immunoblot for Gα2

cAMP receptor, phosphorylation of Gα2 is rapid and transient, coinciding with the time course of activation of physiological responses. The cAMP receptor is essential for Gα2 phosphorylation since cells that do not express the receptor do not phosphorylate Gα2. The site of phosphorylation has been mapped thus far to the N-terminal region of Gα2 (GUNDERSEN and DEVREOTES, unpublished results), which contains 12 serine residues, 4 of which are highly conserved among α subunits. It is unclear what role the phosphorylation of Gα2 plays. Its transient kinetics suggest that it might be involved in the activation of the protein, yet phosphorylation of Gα2 still occurs in certain *fgdA* mutants (R. GUNDERSEN, unpublished results). On the other hand, phosphorylation of Gα2 may affect inherent α subunit functions, such as GTP hydrolysis or binding to the $\beta\gamma$ complex, or it may be important in receptor and/or effector recognition.

Further analysis of the functions of Gα2 has been obtained by expressing Gα2 containing amino acid substitutions in the highly conserved GTP-binding domains (OKAICHI et al. 1992). Two of the mutants analyzed are a G40V change in the GAGES domain and a Q208L change in GGQRS region. The equivalent mutations in *ras* and mammalian Gα subunit Gαs have been shown to substantially reduce the intrinsic GTPase activity of these proteins. The Q227L or R201C in Gαs results in a constitutive, dominant activating phenotype presumably because the protein is "locked" in the on or activating configuration (LANDIS et al. 1989). Expression of Gα2 proteins carrying these mutations in wild-type cells results in an aggregation-deficient phenotype, and the activation of guanylyl cyclase and phospholipase C is almost completely blocked and the activation of adenylyl cyclase is substantially inhibited (Fig. 6). Neither of the mutant proteins is capable of complementing *gα2*-null cells. Overexpression of wild-type Gα2 results in a cAMP-dependent stimulation of a maximum level of guanylyl cyclase activation and an inhibition of the adenylyl cyclase activation (OKAICHI et al. 1992).

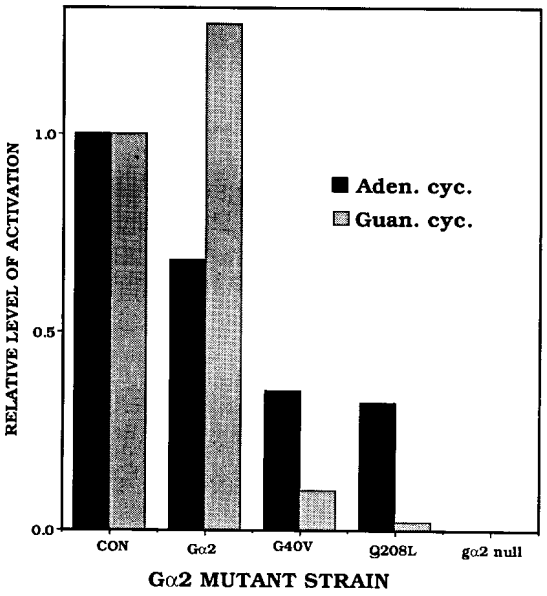

Fig. 6. Effect of amino acid substitutions in Gα2 on the activation of adenylyl cyclase and guanylyl cyclase. Wild-type KAx-3 cells containing expression vectors expressing wild-type Gα2 or Gα2 substitution mutations (G40V and Q208L) were plated on nonnutrient agar. Cells were harvested, and cAMP activation of adenylyl cyclase and guanylyl cyclase was assayed. The maximum levels of activation, as determined by the maximum level of cAMP or cGMP produced, were compared. The value for control KAX-3 cells (CON) was given a value of 1.0. *Gα2*, Cells transformed with wild-type Gα2; *gα2*, *gα2* null cells. In all Gα2 transformants Gα2 protein was over-expressed tenfolds compared to the level found in control cells. See OKAICHI et al. (1992) for details

These results suggest that both the G40V and Q208L mutations have a dominant negative phenotype in vivo, in contrast to the expected dominant activating phenotype seen with similar Gα subunit mutations in other cells. The cAMP receptor-mediated effector pathways that require Gα2, such as adenylyl and guanylyl cyclase, adapt rapidly during persistent cAMP stimulation. Perhaps the activated Gα proteins cause a low-level constitutive activation of these pathways that in turn results in the pathway being constitutively adapted or down-regulated.

As in mammalian cells, adenylyl cyclase activity in *Dictyostelium* is regulated by G-proteins. This suggestion was initially demonstrated by the ability of GTP, Gpp(NH)p (guanyl-5′-yl imidodiphosphate), and GTPγS to activate and GDPβS to inactivate adenylyl cyclase in lysates of aggregation-competent cells (THEIBEIT et al. 1986). Thus, it was predicted that the adenylyl cyclase present during aggregation would closely resemble mammalian adenylyl cyclase. Recently, the gene encoding this adenylyl cyclase, ACA, has been isolated (PITT et al. 1992). Analysis of the predicted

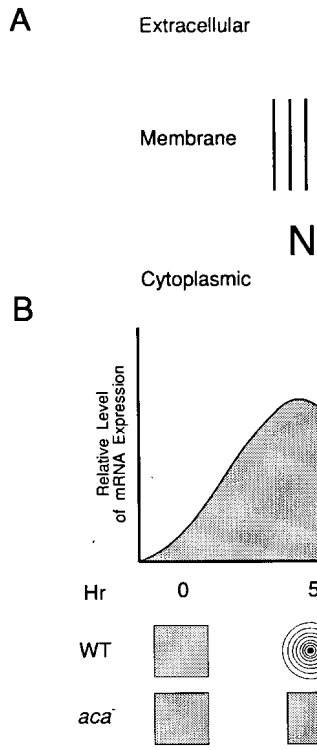

Fig. 7A,B. Topological model cells. **A** Model of ACA. *Vertic* **B** *Top*, the developmentally amount of mRNA expressed phenotype of wild-type and *ac* results in the loss of the capab

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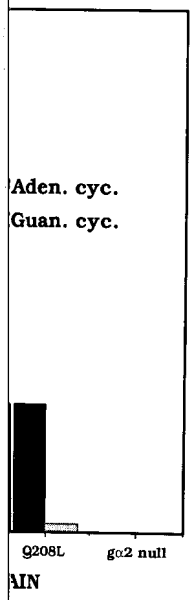

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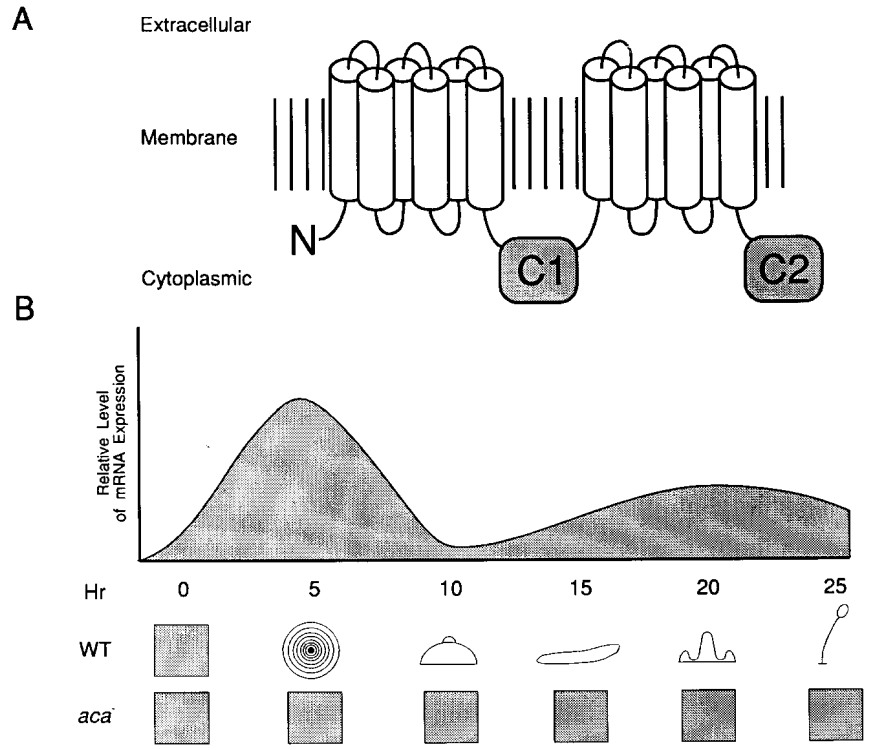

Fig. 7A,B. Topological model and expression of ACA and phenotype of *aca*-null cells. **A** Model of ACA. *Vertical bars*, the plasma membrane; *N*, the amino termini. **B** *Top*, the developmentally regulated expression of ACA, showing the relative amount of mRNA expressed at time indicated; *bottom*, schematic developmental phenotype of wild-type and *aca*-null cells to show that the disruption of ACA locus results in the loss of the capability to aggregate

amino acid sequence demonstrates that the ACA gene product shares significant topological and sequence homology with its mammalian counterparts. These molecules have two large hydrophobic domains, each of which contains six transmembrane spanning domains and two homologous hydrophilic domains (Fig. 7). Sequence homology among mammalian adenylyl cyclases and between mammalian adenylyl cyclase and ACA is highest in the hydrophilic domains. The two hydrophilic domains in ACA are about 50% similar at the amino acid level to each other and to their counterparts within the mammalian adenylyl cyclases (Pitt et al. 1992). ACA is expressed maximumly during the aggregation stage, decreases after aggregation, and is induced again in the later stage of development (Fig. 7). An *aca*-null cell line has been created by gene disruption. These cells have little detectable adenylyl cyclase activity and fail to aggregate (Fig. 7),

demonstrating that ACA is the adenylyl cyclase that coordinates aggregation.

The mechanism by which adenylyl cyclase is activated through cAMP binding to cell surface receptors at the aggregation stage is not understood. As described earlier, Gα2 is required for this function since *gα2*-null cells lack cAMP-stimulated adenylyl cyclase activity in vivo. However, biochemical evidence indicates that guanine nucleotides can regulate adenylyl cyclase in membranes prepared from wild-type cells as well as from cells of *gα2*-null, suggesting that adenylyl cyclase may not be a direct effector of Gα2. Additional experiments have shown that the activation of adenylyl cyclase also requires a cytosolic protein (THEIBERT and DEVREOTES 1986).

There are two possible mechanisms consistent with the known mechanisms of adenylyl cyclase activation in other systems that might explain these data. One possibility is that a G-protein containing an α subunit other than Gα2 is the direct activator of adenylyl cyclase and the Gα2-mediated signaling pathways play a role in its activation. It seems clear that Gα2 directly activates the PI-PLC, and a product of this reaction may lead to activation of adenylyl cyclase. Alternatively, adenylyl cyclase may be activated by $\beta\gamma$ subunits which are released from Gα2. This possibility is consistent with the fact that overexpression of Gα2, which might act as a sink for free $\beta\gamma$ subunits released upon G-protein activation, results in an inhibition of the ability to activate adenylyl cyclase (OKAICHI et al. 1992). In such a model the activation of adenylyl cyclase by GTPγS in *gα2*-null membranes would be mediated through the release of $\beta\gamma$ subunits from other G-proteins, such as those containing Gα1, Gα7, and Gα8, which are known to be preferentially expressed at this time during development. Further in vitro analysis is required to distinguish between these two possibilities as well as other mechanisms.

Gα2 appears to be important for many key transmembrane signaling processes. However, it is not required for response to folic acid (see above) and several cAMP-stimulated responses occurring in its absence (Fig. 4), suggesting that other G-proteins might mediate these functions. It has been shown that cAMP receptor-mediated Ca^{2+} uptake is independent of Gα2 (MILNE and COUKELL 1991). Phosphorylation of Gα2, which is mediated by cAR1, seems to be independent of functional Gα2 since the protein can still be phosphorylated in certain *fgdA* alleles. The cAMP-induced phosphorylation of cAMP receptors and the loss of ligand binding, which are components of the desensitization process, are not affected in cells that lack Gα2 protein (VAN HAASTERT et al. 1992).

The discovery of at least eight G-protein α subunits in *Dictyostelium* has provided candidates for the G-proteins that might fulfill these specific roles. The gene-targeting technique provides a powerful tool to investigate the functions of individual G-proteins. Cell lines lacking each subunit have recently been generated and are being analyzed. In the case of Gα1, loss of

Gα1 expression results in
(KUMAGAI et al. 1991), s
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E. Roles of G-Protein and Differentiation

As shown in Fig. 3, both G
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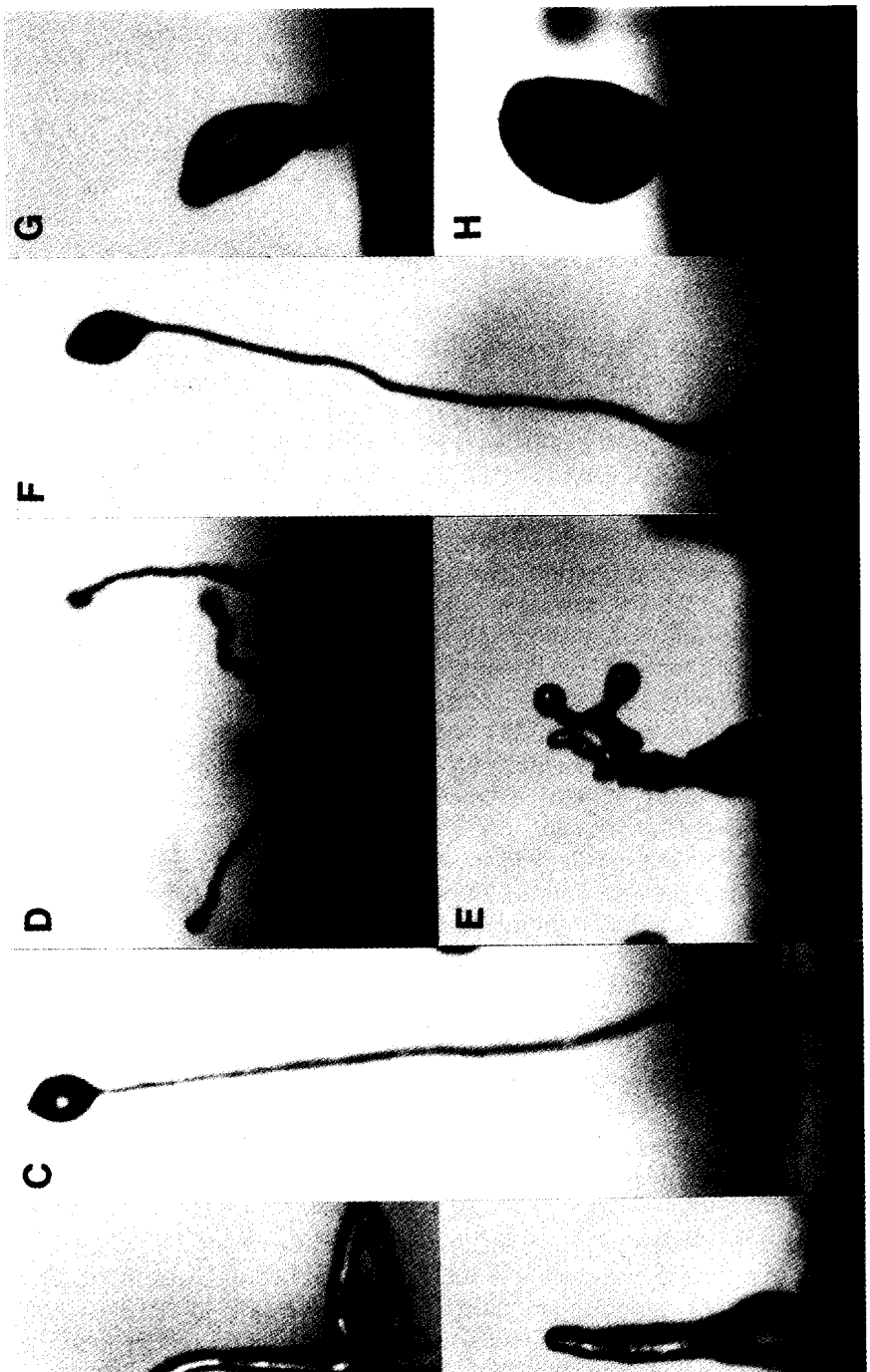
Gα1 expression results in no visible growth or developmental defects (KUMAGAI et al. 1991), suggesting that some of them are functionally redundant or have subtle effects. Thus, it may be necessary to construct cells that are deficient in multiple G-protein α subunits to address the possibility of redundancy.

E. Roles of G-Proteins in Morphogenesis and Differentiation

As shown in Fig. 3, both Gα4 and Gα5 are expressed at high levels at the time of mound and tip formation following aggregation, implying that they might play a role during this stage of development. The role of Gα4 in development was investigated by creating and examining *gα4*-null mutants and cells that overexpress the Gα4 gene (HADWIGER and FIRTEL 1992). As might be expected from the temporal pattern of expression, the early stages of development appear normal in *gα4*-null cells. They aggregate and form mounds that differentiate into an erect finger morphology similar to wild-types cells (Fig. 8). In contrast to wild-type development in which the finger falls over forming a migrating slug, the apical portion of the tip in the *gα4*-null cells continues to elongate while the basal region remains more rounded. In many cases, the apical projection becomes thinner, while in other cases it falls back on itself, producing a "knotted" structure (Fig. 8). Similarly, the overexpressing strain shows normal mound formation and then produces a very abnormal "fruiting-body-like" structure (Fig. 8). The *gα4*-null cells do not produce mature spores, while the overexpressor cells show a 25-fold reduction in total number of spores produced. The *gα4*-null cells can be complemented with a low copy number Gα4-expression vector, restoring the normal morphological differentiation and the production of mature spores as wild-type cells.

Further insight into the possible role of Gα4 comes from the analysis of the temporal and spatial late gene expression in *gα4*-null cells. Northern blot analysis indicates that the prestalk-specific *ras* gene *DdrasD* is expressed at 50% of wild-type levels, and the prespore-specific protein *SP60* mRNA is reduced to very low levels. Expression of *DdrasD* and *SP60* are preferentially localized to the anterior and more basal regions, respectively, as in wild-type cells, suggesting that the initial spatial patterning of both of these genes is not affected in *gα4*-null cells.

Both cAMP-induced prestalk and prespore-specific genes can also be induced in a shaken suspension of cells, a method that enables one to bypass the effects of a mutation on morphogenesis (MCHDY and FIRTEL 1985). Under these conditions, the induction of the cAMP-inducible prestalk gene *DdrasD* is reduced but that of another cAMP-inducible prestalk gene, *pst-cath/CP2*, is not affected, while the expression of *SP60* is still only induced to a low level. To distinguish cell-autonomous and non-cell-



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F. Conclusions and P

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Fig. 8A–H. Developmental m
type cells. Logarithmically gr
nonnutrient agar. **A** Wild-type
null cells at finger stage (15
stage (>26 h after starvation)
taken at 36 h); **F** *gα4* null cells
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FIRTEL (1992) for details

autonomous function of Gα4, wild-type and mutant strains or two mutant strains are mixed in various proportions and the developmental potential of both cell types examined. When the *gα4*-null cells or the Gα4-overexpressing cells are mixed with wild-type cells, or the *gα4*-null cells are mixed with the overexpressor cells, at a 50:50 ratio, morphologically normal fruiting bodies are observed. The Gα4-overexpressing cells in the chimera with either wild-type cells or *gα4*-null cells produced spores at a level similar to that of wild-type cells. The *gα4*-null cells also produce spores in either chimera, although the level of spore formation was only approximately 3%–4% of that of wild-type cells, indicating that the wild-type and Gα4-overexpressing cells can partially complement *gα4*-null cells. In these chimeras, *gα4*-null cells are found in all of the cell types of the mature fruiting body except that there appear to be a slightly lower level in the spore mass and a slightly higher level in the stalk.

These combined results suggest that Gα4 is essential for proper development during culmination and spore production. The function of Gα4 appears to be non-cell-autonomous because *gα4*-null cells can participate in the formation of a wild-type fruiting body in chimeras with either wild-type or overexpressing cells, and the wild-type cells can partially complement the spore production of *gα4*-null cells. Thus Gα4 may be involved in an extracellular signaling process in which Gα4-producing cells are required for producing either an intercellular soluble signal or a cell-cell surface molecule that directly interacts with downstream cells. Studies with Gα4 promoter/*lacZ* reporter gene indicate that Gα4 is expressed at a detectable level in only a small subpopulation of cells within the multicellular aggregate that are known as anteriorlike cells. Although we cannot exclude the fact that Gα4 may also be expressed at a very low level in prespore cells and possibly also in the general prestalk population as a whole, the expression of Gα4 in anterior-like cells is probably important in controlling both the spatial patterning in *Dictyostelium* multicellular morphogenesis and prespore differentiation.

F. Conclusions and Perspectives

In *Dictyostelium*, genes encoding four surface receptors, eight G-protein α subunits and one β subunit, and an adenylyl cyclase have been identified.

Fig. 8A–H. Developmental morphology of *gα4*-null, Gα4-overexpressor, and wild-type cells. Logarithmically grown cells were washed and plated for development on nonnutrient agar. **A** Wild-type cells at the finger stage (14 h after starvation); **B** *gα4* null cells at finger stage (15 h after starvation); **C** wild-type cells at fruiting-body stage (>26 h after starvation); **D, E** *gα4* cells at final morphological stage (photo taken at 36 h); **F** *gα4* null cells complemented with a low copy of the Gα4 expression vector at the fruiting-body stage (photo taken at 36 h); **G, H** Gα4-overexpressing cells at the final morphological stage (photo taken at 36 h). See HADWIGER and FIRTEL (1992) for details

The rather large diversity of the α subunits indicates that they may be involved in a variety of signal transduction pathways. The characterization of several of these genes, cAR1, Gα2, Gα4, and adenylyl cyclase, has shown that they are essential for the proper development in *Dictyostelium*. Gene-targeting and other genetic and molecular techniques have provided powerful tools to investigate the functions of these proteins. Since the mechanisms of signaling processes in *Dictyostelium* are very similar to those in mammalian cells, the molecular and genetic dissection of these processes will elucidate their possible roles not only in *Dictyostelium* but also in other developmental systems.

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