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Regulation of *Dictyostelium* morphogenesis by cAMP-dependent protein kinase

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SUMMARY

During formation of the *Dictyostelium* slug extracellular cAMP signals direct the differentiation of prespore cells and DIF, a chlorinated hexaphenone, induces the differentiation of prestalk cells. At culmination the slug transforms into a fruiting body, composed of a stalk supporting a ball of spores. A dominant inhibitor of cAMP-dependent protein kinase (PKA) expressed under the control of a prestalk-specific promoter blocks the differentiation of prestalk cells into stalk cells. Analysis of a gene specifically expressed in stalk cells suggests that PKA acts to remove a repressor that prevents the premature induction of stalk cell differentiation by DIF during slug migration. PKA is also necessary for the morphogenetic movement of prestalk cells at culmination. Expression of the PKA inhibitor under control of a prespore-specific promoter blocks the accumulation of prespore mRNA sequences and prevents terminal spore cell differentiation. Thus PKA is essential for progression along both pathways of terminal differentiation but with different mechanisms of action. On the stalk cell pathway it acts to regulate the action of DIF while on the spore cell pathway PKA itself seems to act as the inducer of spore cell maturation. Ammonia, the extracellular signal which regulates the entry into culmination, acts by controlling the intracellular concentration of cAMP and thus exerts its effects via PKA. The fact that PKA is necessary for both prespore and spore gene expression leads us to postulate the existence of a signalling mechanism which converts the progressive rise in cAMP concentration during development into discrete, PKA-regulated gene activation events.

1. INTRODUCTION

Extracellular cAMP signals control both the movement and differentiation of cells during the development of the cellular slime mould *Dictyostelium discoideum* (reviewed by Devreotes 1989; Firtel 1989; Kimmel & Firtel 1991). When their bacterial food source is exhausted individual amoebae synthesize and secrete cAMP in a pulsatile fashion, with a periodicity of approximately 7 min. Cells respond to receipt of a pulse of cAMP by moving up the cAMP concentration gradient and by the synthesis and release of cAMP, to relay the signal to cells further out in the aggregation territory (figure 1). Gene cloning studies show there to be several different cAMP receptors each of which differs in its timecourse of accumulation during development (Klein *et al.* 1988; Saxe *et al.* 1991*a*,*b*). They all belong to the family of seven trans-membrane domain receptors, that includes the α pheromone receptor of yeast and the mammalian β-adrenergic receptor. In common with other seven trans-membrane domain receptors, the cAMP receptors couple to G proteins which, in *Dictyostelium*, activate several intracellular enzymes including adenylate cyclase, guanylate cyclase and phospholipase C (Theibert *et al.* 1986; Europe-Finner & Newell 1987; Van Haastert *et al.* 1987; Pupillo *et al.* 1989; and reviewed by Janssens & Van Haastert 1987; Newell *et al.* 1987; Firtel 1989).

During development many new proteins are required, including components of the cAMP signalling system, cell adhesion molecules and various structural proteins, and these new products are regulated in their temporal and spatial patterns of gene expression. Extracellular cAMP signalling acts in some cases to induce, and in others to repress, expression of these different gene products (reviewed by Schaap 1986; Williams *et al.* 1986 Kimmel *et al.* 1991). How is one signalling molecule able to perform so many different functions? One important clue is that the nature of the signal reception system changes, with the several cAMP receptors coupling to multiple G proteins (Hadwiger *et al.* 1991) and with at least two adenylate cyclases (Pitt *et al.* 1992) being expressed at different times during development. Also, the nature of the signal appears to change, because some genes expressed early during development respond only to pulses of cAMP while later expressed genes respond to continuous exposure to a high cAMP concentration (Schaap *et al.* 1986). To understand how extracellular cAMP signalling fulfils its diverse functions, we need to understand the intracellular events that lead to gene activation. In *Dictyostelium discoideum* extracellular cAMP fulfils the function of a hormone but which of the potential second messengers, cGMP, cAMP, calcium ions or diacylglycerol, is the intermediary betwen membrane and nucleus?

During development intracellular cAMP levels rise (Abe & Yanagisawa 1983; Merkle *et al.* 1984) and expression of a cAMP-dependent protein kinase also increases (Sampson 1977; de Gunzburg & Veron 1982; Leichtling *et al.* 1984; Anjard *et al.* 1993). In contrast to the mammalian enzyme, which is a tetramer containing two regulatory (R) and two catalytic (C) subunits (see Taylor *et al.*, this symposium), *Dictyostelium* PKA is composed of a single R and a single C subunit (Mutzel *et al.* 1987). Both the R and C subunits are present at very low level in growing cells and increase in concentration approximately 20-fold at the time of aggregation (Part *et al.* 1985; de Gunzburg *et al.* 1986; Burki *et al.* 1991; Mann *et al.* 1991; Mann & Firtel 1991). In mammalian cells PKA is a regulator of both cytosolic processes and of gene expression. Previous studies have shown that terminal spore and stalk cell differentiation are induced by 8-bromo cAMP (Kwong *et al.* 1988; Maeda 1988; Kay 1989), a membrane- permeant cAMP analogue which is believed to act intracellularly to activate PKA. We have recently obtained evidence which confirms that intracellular cAMP is the key regulator on both pathways of terminal cellular differentiation and that it functions by activating PKA (Harwood *et al.* 1992; Hopper *et al.* 1993).

During aggregation the cells pile on top of each other to form a hemispherical mound containing up to 100 000 cells (figure 1). Amoebae within the mound then differentiate along one of two pathways. About 80% of the cells become spore precursors and 20% become stalk cell precursors. Prespore and spore cell differentiation are induced by extracellular cAMP and prestalk, and stalk cell differentiation are induced by DIF (figure 2; reviewed by Schaap 1986; Williams 1988), a chlorinated hexaphenone that is produced during *Dictyostelium* development and which is able to divert cells from the spore cell pathway of differentiation into the stalk cell pathway (Town *et al.* 1976; Kay & Jermyn 1983). The prestalk cells arise at apparently random positions within the aggregate and move to the apex of the mound (Williams *et al.* 1989). There a tip is formed that elongates, transforming the hemispherical mound into a cylindrical first finger. Under environmental conditions that are inappropriate for immediate culmination, this topples on to its side to form a slug. The slug migrates to the surface of the soil or leaf litter where the change in environmental conditions triggers culmination. The migratory slug phase is dispensible, so that if conditions are appropriate for fruit formation the first finger enters culmination immediately.

The migratory slug is encased in a matrix, the slime sheath, that is deposited onto the substratum to form the slime trail. The sheath contains an extracellular matrix protein, the EcmA protein, composed of approximately 70 copies of a 24 amino acid repeat (Williams *et al.* 1987; McRobbie *et al.* 1988*a*). The *ecmA* gene is specifically expressed in prestalk cells and is inducible by DIF (Jermyn *et al.* 1987). At culmination the slug sits on end, so that what was the rear of the slug becomes the base of the culminant. Prestalk cells within the tip then undertake an ordered and progressive movement, sometimes likened to a 'reverse fountain', first upwards to the apex and then downwards through the underlying mass of prespore cells. As they move up to the apex they add material to the

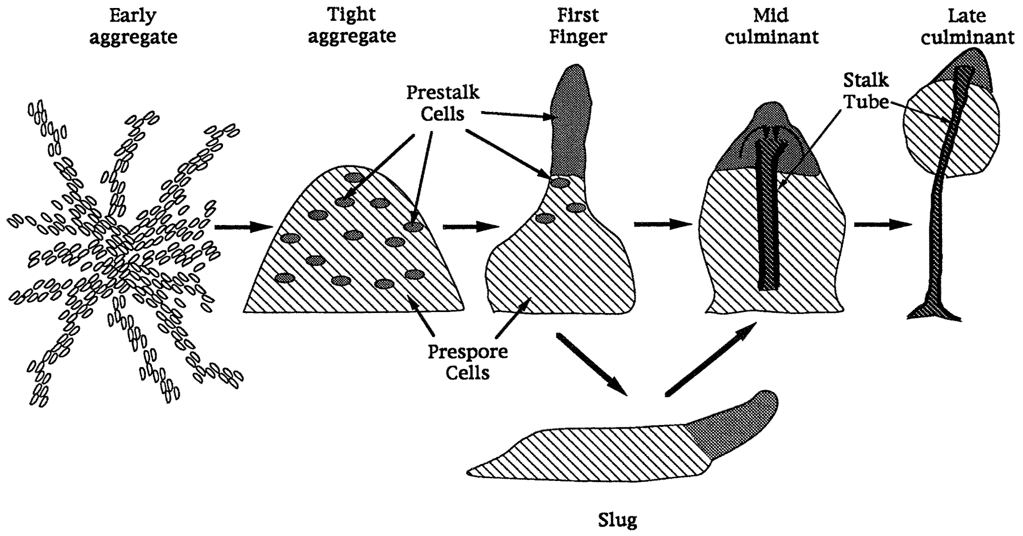

Figure 1. The *Dictyostelium* life cycle. A highly schematic representation of development in which, for clarity, the prestalk cells are grossly enlarged. Depending upon the precise developmental conditions, an aggregate may contain up to 100 000 cells, of which approximately 20% are prestalk cells. The process has been simplified by the omission of anterior like cells (ALC); prestalk-like cells that are present in the rear of the slug and which sort to surround the spore head at culmination (Sternfeld & David 1981, 1982; see figure 3).

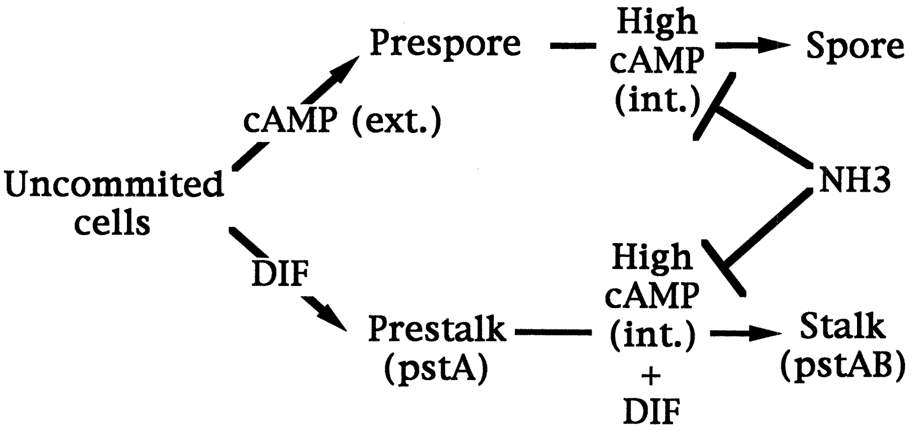

Figure 2. A summary of the proposed roles of cAMP, DIF and ammonia in spore and stalk cell formation. Uncommitted cells at the end of aggregation are proposed to differentiate along the stalk or spore cell pathways, with extracellular cAMP signals (cAMP(ext)) directing prespore differentiation and DIF directing prestalk differentiation. Ammonia represses adenylate cyclase, hence a drop in ammonia at culmination triggers a rise in intracellular cAMP (cAMP(int)) and so induces terminal spore and stalk cell differentiation. On the stalk cell pathway DIF is the inducer and cAMP(int) acts to lift a repression that prevents premature stalk cell differentiation in the migratory slug.

stalk tube, a cylinder of protein and cellulose that comes to encase the stalk cells. On entering the stalk tube they activate expression of the gene encoding an extracellular matrix component, the EcmB protein. (McRobbie *et al.* 1988*b*; Jermyn & Williams 1991). The EcmB protein is composed of approximately forty copies of a 24 amino acid repeat that has an identical consensus sequence to that of the EcmA protein (Ceccarelli *et al.* 1987) and the *ecmB* gene is also inducible by DIF (Jermyn *et al.* 1987).

The prespore cells produce a surface protein of unknown function called PsA (Barklis & Lodish 1983; Krefft *et al.* 1983; Early *et al.* 1988). Expression of the *pspA* gene is induced by cAMP and repressed by DIF (Barklis & Lodish 1983; Early & Williams 1988). As the prespore cells are lifted up by the stalk at culmination they mature into spores, which are encased in an impermeable protein and cellulose containing coat. Prespore cells contain vacuoles (psvs), that contain spore coat proteins (Devine *et al.* 1983) and which exocytose at culmination to form the spore coat. The PsA (Krefft *et al.* 1983) and psv (Takeuchi 1963; Hayashi & Takeuchi 1976) proteins provide excellent markers of prespore cell differentiation while the *spiA* gene, which encodes a protein important in maintaining spore integrity, is expressed only during terminal spore cell differentiation (Richardson *et al.* 1991; Richardson & Loomis 1992).

We have used these various prestalk and prespore-specific genes (table 1) to investigate the role of PKA in morphogenesis and its interaction with the DIF signalling pathway. The genes have been used both as markers of cellular differentiation and, in some cases, to direct the cell type specific expression of a dominant inhibitor of PKA. This was created by mutating two amino acids within the *Dictyostelium* R subunit, one within each of the two cAMP binding sites, to form an R subunit (Rm) that is unable to bind cAMP and that can therefore irreversibly inactivate the C subunit (Harwood *et al.* 1991). When Rm is expressed under the control of an actin promoter which is active during growth and early development the cells grow normally but development is arrested early during aggregation and cells are defective in cAMP relay (Harwood 1991). When expressed under the control of either the *pspA* or the *ecmA* promoter the Rm protein produces dramatic effects on cellular differentiation.

2. PKA, STALK CELL FORMATION AND THE MORPHOGENETIC MOVEMENT OF PRESTALK CELLS DURING CULMINATION

When the Rm protein is produced under control of the *ecmA* promoter early development appears normal (Harwood *et al.* 1992). Migratory slugs are formed but they differ from normal slugs in that they migrate for a much longer period of time and under conditions which causes wild-type aggregates to omit the migratory slug phase and to culminate *in situ*. When the ecmA-Rm slugs attempt culmination, they adopt a

Table 1. *A summary of the genes used in the studies described in this paper*

(Subsequent to their first description all these genes have been given names that relate to their cell-type specific patterns of gene expression and/or their putative functions. 1, Jermyn *et al.* (1987); 2, Barklis & Lodish (1983); 3, Krefft *et al.* (1983); 4, Early *et al.* (1988); 5, Morrissey *et al.* (1984); 6, Mehdy *et al.* (1983); 7, Fosnaugh & Loomis (1989); 8, Richardson *et al.* (1991).)

gene (and original name)	specificity of expression
ecmA (pDd63)[1]	pstA (prestalk) cells and ALC
ecmB (pDd56)[1]	pstAB (stalk)cells and ALC
pspA (D19)[2,3,4]	prespore cells
cotC(SP60)[5,6,7]	prespore cells
spiA (Dd31)[8]	maturing prespore cells

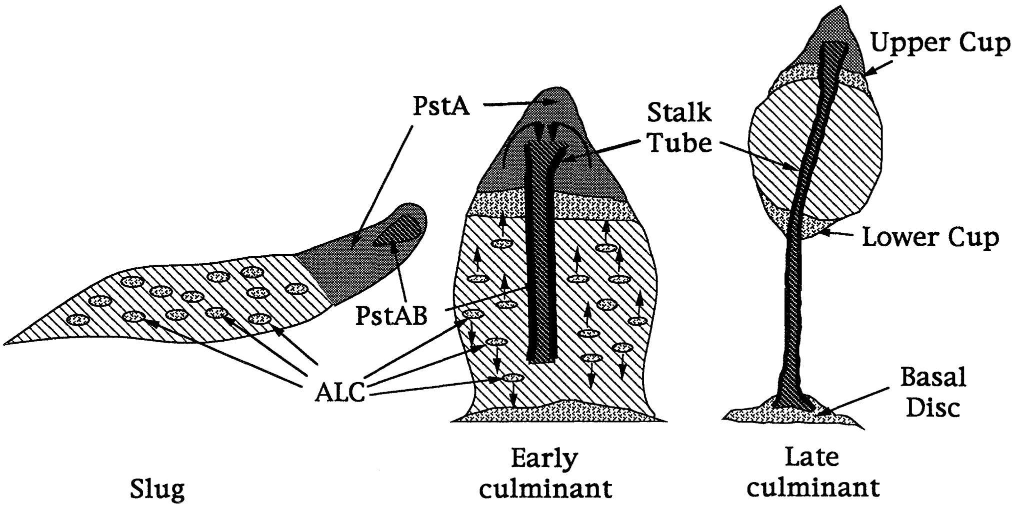

Figure 3. Changes in the pattern of *ecmB* gene expresion at culmination. At culmination the slug tip adopts an upright position. The anterior prestalk cells form the stalk and the anterior-like cells (ALC) form the upper cup (the cells at the prestalk-prespore boundary), the lower cup (the cells below the spore mass) and the outer part of the basal disc. For clarity of presentation, the ALC cells are grossly over-enlarged in this diagram. In reality about 100 000 cells would be present in the entire aggregate and there would be about 15 000 ALC in the prespore region (Sternfeld & David 1982). At culmination the level of *ecmB* gene expression in ALC increases (Jermyn & Williams 1991) and this is directed by sequences distal to the cap site of the promoter (upstream of residue -858; see Ceccarelli *et al.* 1991).

semi-upright position but they become arrested at this stage and eventually form withered, apparently dessicated structures. These structures do not contain a stalk. Thus PKA is required in order that a prestalk cell becomes a stalk cell and we have used the *ecmB* gene to investigate how PKA acts to regulate stalk cell differentiation.

Most of the prestalk cells within the migratory slug express the *ecmA* gene but not the *ecmB* gene and we term them pstA cells (figure 3). As pstA cells pass the entrance to the stalk tube at culmination they activate expression of the *ecmB* gene to become pstAB cells. Migratory slugs contain a core of pstAB cells located at the position where the stalk tube will form at culmination (Jermyn *et al.* 1989). These pstAB cells most probably arise as the result of an abortive attempt at culmination and they are sometimes shed into the slime trail as the slug migrates forward (Sternfeld 1992). At culmination (Jermyn & Williams 1991) expression of the *ecmB* gene is also activated in anterior-like cells (ALC), cells which share many of the properties of prestalk cells but which are scattered through the prespore zone (Sternfeld & David 1981, 1982). During culmination the ALC move to surround the spore head and to form the outer part of the basal disc (Sternfeld & David 1982). DNA sequence elements distal to the cap site of the *ecmB* gene activate its expression in the subset of ALC which move above the spore head (the 'upper cup' (UC) cells), while sequences more proximal to the cap site activate expression in the stalk tube (Ceccarelli *et al.* 1991).

The region of the *ecmB* promoter that directs expression in UC cells is active in ecmA-Rm cells but, as would be expected from the absence of a stalk tube, the region that directs expression in the stalk tube is totally inactive in ecmA-Rm cells (Harwood *et al.* 1992). The latter region contains positively acting sequences, which are capable of directing expression in pstA cells and negatively acting sequences which repress expression until pstA cells have passed into the entrance to the stalk tube and become pstAB cells (Ceccarelli *et al.* 1991; figure 4). We believe that PKA acts to remove the repressor, either by phosphorylating it directly or by activating another kinase. The evidence for this derives from an experiment showing that gene expression directed by the stalk-tube specific part of the *ecmB* gene is DIF inducible in control cells incubated *in vitro* but is not DIF inducible in ecmA-Rm cells (Harwood *et al.* 1992).

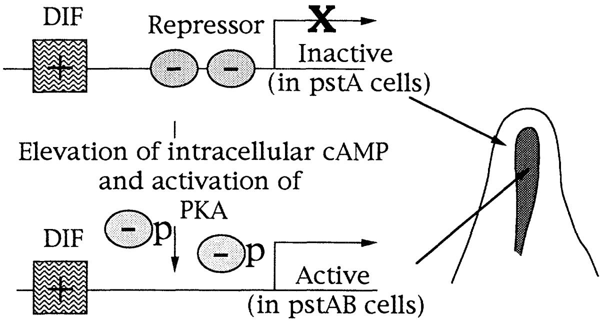

Figure 4. A model for *ecmB* gene regulation. This figure is a simplified representation of the *ecmB* gene showing the sequences proximal to the promoter (downstream of residue -877; see Ceccarelli *et al.* 1991) that direct expression in the stalk tube at culmination, but omitting the sequences that direct expression in ALC. The gene is potentially active in all cells that are exposed to DIF because of the presence of a positively acting region that lies at the end of the DIF signal transduction pathway. However two repressor regions (Ceccarelli *et al.* 1991; A. J. Harwood, A. Early & J. G. Williams, unpublished results) keeps the gene inactive in pstA cells. At culmination, when cAMP levels rise, PKA is activated in cells at the entrance to the stalk tube, the repressor is phosphorylated and hence inactivated and the *ecmB* gene is expressed (Harwood *et al.* 1992).

In addition to its synergistic role with DIF as an activator of stalk cell differentiation, PKA is also necessary for the directed movement of cells to the stalk tube entrance at culmination (Harwood *et al.* 1992). In an experiment, where ecmA-Rm cells are mixed with an equal number of normal cells, a fruiting body is formed which bears a bolus of cells that fail to migrate into the stalk. These are the ecmA-Rm cells which differentiated into prestalk cells and so activated expression of Rm. This observation implicates PKA in some aspect of the reverse fountain movement but we do not yet know whether expression of the Rm protein blocks the cells ability to sense the signal that directs migration to the stalk tube entrance or whether it prevents cells from responding to it.

3. PKA AND SPORE CELL DIFFERENTIATION

When the Rm gene is expressed under control of the promoter of the *pspA* prespore-specific gene apparently normal migratory slugs are formed but analysis of prespore gene expression shows that they are defective in the expression of at least two different prespore genes, *pspA* itself and the *cotC* spore coat protein gene (Hopper *et al.* 1993). The *pspA* gene is expressed early during slug formation but, as the level of pspA-Rm protein rises, the concentration of the *pspA* mRNA decreases dramatically. Similarly, in pspA-Rm cells, the concentration of the *cotC* mRNA sequence decreases after an initial burst of accumulation. We interpret these data to mean that PKA is required for the maintenance of prespore gene expression.

The culminants formed by pspA-Rm cells are normally proportioned but differ radically from wild type fruiting bodies in that the spore head is almost totally transparent. Normal spore heads are opaque because of the presence of caretenoids which accumulate during the maturation of prespore into spore cells. The apical pspA-Rm structures are transparent because the prespore cells remain amoeboid during culmination, i.e. they are blocked in maturation. We have confirmed that they are arrested prior to spore formation by analysing expression of the *spiA* spore-specific marker (Hopper *et al.* 1993). We believe that PKA acts as a direct inducer of spore maturation, because previous studies have shown that a membrane permeant cAMP analogue, 8-bromo cAMP, induces spore cell formation in monolayer, under conditions where untreated cells fail to form spores (Maeda 1988; Kay 1989). In pspA-Rm cells 8-bromo cAMP does not stimulate spore cell maturation nor does it activate expression of the *spiA* gene.

There is also strong genetic support for a role of PKA in spore cell maturation. The rdeC mutants (Abe & Yanagisawa 1983) are accelerated in their development and are sporogenous (Kay 1989), i.e. they will form spores *in vitro* under conditions where wild-type cells will not. In one rdeC strain the R subunit of PKA is not expressed and in another allele there is a point mutation in the pseudo-substrate site (Simon *et al.* 1992), the region of the R subunit that interacts with and inhibits the C subunit. A sporogenous phenotype is also produced by over-expressing the C subunit of PKA under control of an actin promoter (Mann & Firtel 1991; Anjard *et al.* 1992).

4. A THRESHOLD MODEL FOR THE REGULATION OF SPORE CELL DIFFERENTIATION BY PKA

PKA regulates cellular differentiation on both the stalk and spore cell pathways (figure 2). On the stalk cell pathway DIF is the driving force that causes cells to become first prestalk cells and then stalk cells. In the migratory slug we believe that a repressor protein acts to prevent premature induction of stalk cell differentiation by DIF and that at culmination PKA lift this repression. On the spore cell pathway PKA is necessary both for prespore and for spore cell differentiation. Although it is impossible to use pspA-Rm to determine whether PKA induces prespore cell differentiation (because pspA-Rm itself must be expressed before it can produce an effect), we have shown that PKA is necessary for the maintenance of prespore cell differentiation. We have recently strengthened this conclusion by showing that, in the absence of PKA, transcription of the *cotC*, prespore-specific mRNA is greatly reduced and several different prespore-specific mRNA transcripts are de-stablized (N. A. Hopper & J. G. Williams, unpublished results). Thus, in contrast to the stalk cell pathway, where DIF is the initial inducer (to form a prestalk cell) and PKA acts to regulate the completion of differentiation (to form a stalk cell), on the spore cell pathway PKA acts first to maintain prespore cell differentiation and then to trigger spore cell maturation. These observations raise two questions. How is PKA activated at culmination and how is PKA able to fulfil functions at two different stages on the spore cell pathway? At culmination there is a rise in the intracellular cAMP concentration (Abe & Yanagisawa 1983; Merkle *et al.* 1984). This presumably activates PKA in prestalk and prespore cells so that they are caused to undergo terminal differentiation. The second question relates to a problem that is addressed elsewhere in this symposium (see article by Smith *et al.*), that of transforming a linear, or semi-linear, rise in the level of an inducer into discrete activation steps.

The problem is best presented by considering expression of the *cotC* prespore-specific gene and the *spiA* spore-specific gene. The *cotC* gene requires PKA for its transcription, so clearly there is sufficient active C subunit in normal prespore cells to allow its expression. The *spiA* gene is inactive in prespore cells. It can be activated in isolated cells by adding 8-bromo cAMP (Richardson *et al.* 1991) and is presumably activated *in vivo* by the rise in intracellular cAMP concentration that occurs at culmination. If there is sufficient PKA activity in the slug to allow the *cotC* gene to be expressed why is there no expression of *spiA*? One simple way in which this could be achieved is shown in figure 5. A transcriptional activator of *spiA* gene expression, that is active on the *spiA* gene only when phosphorylated is proposed to be under control of a phosphatase and a kinase. If the fraction of the

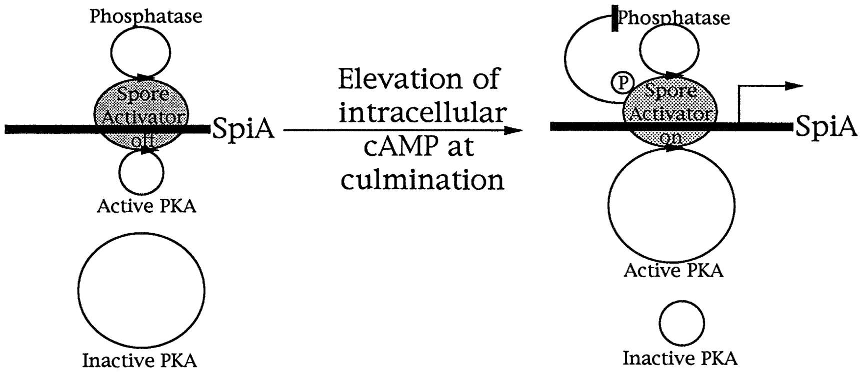

Figure 5. A threshold model for PKA induction of spore cell formation. An activator of the *spiA* gene is proposed to be under the control of PKA and an antagonistic phosphatase. In this representation the size of the circle is meant to correlate with the level of active PKA. In prespore cells, where the concentration of cAMP is relatively low, the activity of the phosphatase exceeds that of PKA so that the activator remains effectively unphosphorylated. In this unphosphorylated state the activator is proposed to be incapable of directing transcription of the spiA gene. At culmination, when cAMP levels rise, additional C subunit is released from the holoenzyme, exceeding the capacity of the phosphatase rise, additional to dephosphorylate the activator. The phosphorylated activator is proposed to have two functions, to induce expression of the *spiA* gene and to in some way repress the activity of the phosphatase so locking the system into an active state.

PKA holoenzyme that is dissociated in prespore cells lies below a threshold value set by the level of the phosphatase, then the activator will be maintained in an inactive state with respect to the *spiA* gene. Hence in the slug the *spiA* gene will be inactive. At culmination, as cAMP levels rise and more of the holoenzyme is dissociated then the activator would become phosphorylated and so rendered active for *spiA* induction. By incorporating the indicated positive feedback loop, whereby phosphorylated activator in some way down-regulated the phosphatase, the system would display both a sharp threshold response to cAMP levels and stability of the active state.

5. REGULATION OF THE ENTRY INTO CULMINATION BY AMMONIA

The trigger for culmination is believed to be a drop in the concentration of ammonia (Schindler & Sussman 1977; Newell & Ross 1982), a gas which is produced in copious amounts during development as the result of catabolism and which also serves to control several other aspects of *Dictyostelium* development (Thadani *et al.* 1977; Bonner *et al.* 1986, 1988, 1989; Feit *et al.* 1990). As the slug reaches the surface of the soil or leaf litter and rears up towards overhead light the rate of ammonia loss is presumed to increase so inducing culmination (Bonner *et al.* 1982). The direct evidence for this model derives from some elegant experiments wherein an enzymic cocktail that depletes ammonia was shown to induce migrating slugs to culminate (Schindler & Sussman 1977). The same workers also showed that ammonia represses the activity of adenylate cyclase and suggested that this might be how ammonia controls culmination (Schindler & Sussman 1979). They proposed that high levels of ammonia in the slug keep intracellular cAMP levels low so that when ammonia levels drop at culmination cAMP levels rise, triggering stalk and spore cell differentiation.

The fact that PKA activity is required for stalk and spore cell differentiation strongly supports a role for intracellular cAMP in regulating terminal differentiation and one phenotypic characteristic of the pspA-Rm slugs adds weight to the notion that ammonia regulates cAMP levels. Ammonia is an inhibitor both of the entry into culmination and, at higher concentration, it also blocks slug formation. PspA-Rm cells are tenfold more sensitive to the inhibitory effect of ammonia than are control slugs (Hopper *et al.* 1993). The fact that a mutation which affects the intracellular cAMP signalling pathway changes the sensitivity of the cells to ammonia provides genetic evidence for an involvement of ammonia in regulating the intracellular cAMP concentration.

6. CONCLUSIONS AND PROSPECTS

Extracellular cAMP, DIF and ammonia are the three best characterized extracellular signals controlling morphogenesis. They act in combination, with PKA acting as an intracellular link between them, to control cell type differentiation. While this much appears clear many questions remain.

During slug formation what mechanisms determine the fraction of cells that will become prestalk, ALC and prespore cells? There is a clear correlation between the phase in the cell cycle at which a cell finds itself when it receives the starvation signal that triggers development and its eventual fate (Macdonald & Durston 1984; Weijer *et al.* 1984; Gomer & Firtel 1987; Ohmori & Maeda 1987). Although this may provide an inbuilt heterogeneity that prejudices cells towards one or other pathway, cell cycle position is not an absloute predictor of cell fate and this is a regulative developmental system; so that if the slug is cut into two both parts can re-form a normal slug. How then do the cells signal to one another to establish and maintain the correct prestalk, ALC and prespore cell ratios during slug formation and migra-

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tion and to re-establish them if the slug is forced to regulate by removal of one or other cell type?

Culmination is a precisely orchestrated process in which prestalk cells first move to the apex of the stalk tube and then start their progression down the stalk cell pathway by activating expression of the ecmB gene. How is this directed movement achieved and is there a positionally localized signal at the entrance to the stalk tube which acts to elevate cAMP levels? Is there, for example, a localized ammonia depletion at the stalk tube entrance or does some other mechanism operate? The answers to all these questions will only be obtained when we have a complete understanding of the intracellular signal transduction systems through which the three extracellular diffusible signals operate.

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