Review

Developmental decisions in *Dictyostelium discoideum*

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Abstract

Dictyostelium discoideum is an excellent system in which to study developmental decisions. Synchronous development is triggered by starvation and rapidly generates a limited number of cell types. Genetic and image analyses have revealed the elegant intricacies associated with this simple development system. Key signaling pathways identified as regulating cell fate decisions are likely to be conserved with metazoa and are providing insight into differentiation decisions under circumstances where considerable cell movement takes place during development.
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Introduction

The developmental cycle of *Dictyostelium discoideum* shares many features with that of mammals, making it an ideal system in which to study cell fate regulation. Importantly, as in mammals, *Dictyostelium* cell fate remains plastic until late in development and cell movement plays an important role in pattern formation. *Dictyostelium*'s developmental cycle has some unusual features, such as the generation of a mass of cells by aggregation rather than by division, but in other aspects is an excellent system to study developmental processes. The terminal structure consists of a relatively limited number of distinct cell types which are generated in a short period of time. As a haploid eukaryote, the system lends itself to genetic analysis as well as biochemistry and cell biology allowing a multidisciplinary approach to developmental problems. The recent completion of the *Dictyostelium* genome sequence (Eichinger et al., 2005; and accessible with related on-line resources at http://dictybase.org) greatly facilitates such analysis.

The unicellular amoebae which exist when food is plentiful enter a multicellular phase on starvation to form a mound which develops into a fruiting body consisting of a head of spores supported on a stalk of vacuolated cells (Fig. 1A). Spores await dispersal and germination in conditions favorable for amoeboid growth. The multicellular structure is formed by aggregation of individual amoebae which move up a gradient of the chemoattractant cAMP, which is released in a pulsatile fashion by the starving cells. This extracellular cAMP interacts with a seven transmembrane domain receptor, cAR1, which activates a number of signaling pathways in the cell leading to three main responses: the generation of more cAMP which is secreted to relay the signal, the triggering of the chemotactic response to cause the cell to move, and the initiation of changes in gene expression associated with the developmental program. Signaling events downstream of cAR1 associated with chemotaxis have recently been reviewed (Chisholm and Firtel, 2004; Van Haastert and Devreotes, 2004).

Once the multicellular aggregate forms, it has the potential to migrate in the form of a slug, allowing movement to conditions more favorable for culmination (towards heat and light). By this stage, multiple cell types are apparent in the structure and these are arranged in a defined pattern. The prespore cells are found in the rear

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four-fifths of the slug, whereas the prestalk cells are found in the front fifth as well as scattered throughout the posterior portion (anterior-like cells) with some also found at the back of the slug (rearguard cells) (Fig. 1B). These prestalk cells have been sub-divided into a number of distinct cell types dependent on the expression patterns of genes which are

markers of prestalk cell differentiation (Jermyn et al., 1989). PstO cells are found towards the rear of the anterior portion (Early et al., 1993), pstA cells are found at the front of the slug and pstAB cells form a central core (Jermyn and Williams, 1991). The evidence for similar sub-division of prespore cells is somewhat less clear (Haberstroh and Firtel,
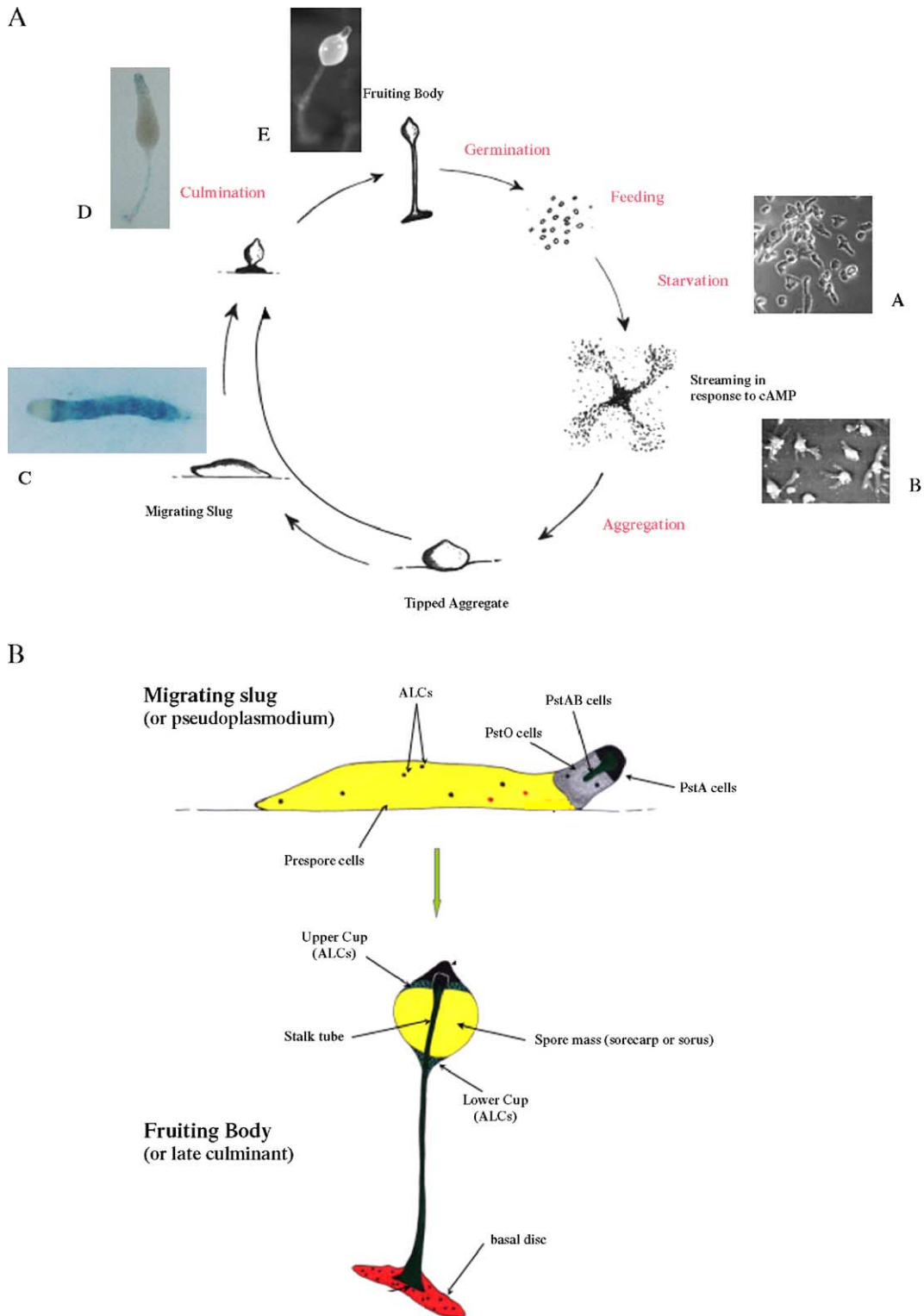
1990; Kibler et al., 2003a). Any structural or functional basis behind the generation of distinct types of prespore cell is not obvious whereas the different prestalk cells go on to generate distinct structures—the stalk itself, the basal disk supporting the stalk, and the upper and lower cup supporting the spore head on top of the stalk (Early et al., 1993).

Pattern formation

Alternative means of pattern formation during development are used with differing emphasis by different organisms. Both *Drosophila melanogaster* and *Caenorhabditis elegans* rely on asymmetry which exists prior to the first cell division, either laid down as a gradient in the oocyte or defined by the sperm entry point at fertilization. *C. elegans*, in particular, shows restricted plasticity following the first cell division. In the developing *Drosophila* embryo, gradients of different morphogens are used to give each area a specific developmental fate. In contrast, mammalian cells clearly go through a number of cell divisions before cell specification which remains plastic for longer. *Dictyostelium* cells also retain plasticity until late in development.

Like a mass of undifferentiated cells in mammals, a mound of developing *Dictyostelium* cells must establish asymmetry and generate the correct proportions of different cell types. Cells expressing the earliest available markers specific for prespore or prestalk cell fate appear at random locations in the mound and these cells then sort by a process which is likely to involve both differential adhesion and different rates of chemotaxis such that by the slug stage the distinct populations are found in separate locations (Esch and Firtel, 1991; Fosnaugh and Loomis, 1993; Ozaki et al., 1993; Williams et al., 1989; Yoder and Blumberg, 1994). Isolated prestalk and prespore cells show differences in adhesion and in speed of chemotaxis towards cAMP (Early et al., 1995; Clow et al., 2000; Siu et al., 1983; Traynor et al., 1992). These experiments suggest that positional information in the form of a gradient of morphogen is not required for initial cell fate decisions. DIF was originally identified as an activity in conditioned medium which induces stalk cell formation in a low-density monolayer of cells. Purification revealed that there are three related molecules that exhibit DIF activity with DIF-1 being the most active (Kay et al., 1983). The expression of marker constructs driven by a promoter specific for pstA cells is first detectable in cells at the base of the aggregate (Early et al., 1995). As induction of this marker requires around 10-fold higher levels of the prestalk-inducing factor DIF-1 than pstO cells, it is possible that differentiation of the pstA cells is dependent on higher concentrations of DIF-1 at the base of the mound. However, cells move within the mound at a considerable speed (Siegert and Weijer, 1995) and by the time the marker genes have been expressed at sufficiently high levels to allow detection, the cells are distant from the location they were when that expression was induced. In an attempt to resolve this issue, the location of expression of cell-type-specific markers was determined in mounds in which cell movement is blocked (Thompson et al., 2004b). Cells expressing markers specific for pstO and pstA cells arose with normal efficiency, and were both randomly distributed throughout the mound, consistent with positional information not being important in initial cell fate decisions.

If positional information is not important, why do cells start to express markers for one particular pathway and how is the proportioning of different cell types controlled? Cells are predisposed to a particular cell fate dependent on their growth history. Cells grown in media rich in glucose (G+) tend to become spore cells when they co-aggregate with cells grown in the absence of glucose (G−),

Fig. 1. (A) The life cycle of *D. discoideum*. Amoebae feeding on bacteria in the soil exist as single cells but, on starvation, a developmental process is triggered in which the amoebae aggregate to form a mound. Certain cells in the population start to secrete cAMP which interacts with a cell surface receptor on surrounding cells to trigger three responses: chemotaxis towards the source of cAMP (inset A), generation and secretion of more cAMP to relay the signal, and the developmental program. The extracellular cAMP is degraded by extracellular phosphodiesterase so that waves of cAMP travel from the central source. The aggregate or mound of up to 10^5 cells (inset B) develops a tip, which elongates to form a first finger and then can either culminate to form a mature fruiting body where it is, or can topple over to form a migratory or slug phase. Inset C shows a slug stained to show expression of a prespore-specific marker construct in the rear of the slug (see panel B). This slug moves towards sources of heat and light where future spore dispersal or germination is more favorable. Culmination generates a fruiting body in which a mass of spores (approximately 80% of the cells) is supported on a stalk of vacuolated cells (Insets D and E). These spores can remain dormant for long periods of time but germinate when food is available. (B) Structure of the slug and fruiting body. By the slug stage, several different cell types are apparent, defined by gene expression from promoters specific for the different cell types. The rear four-fifths is made up largely of prespore cells with scattered single cells that express markers specific for prestalk cells (anterior-like cells, ALCs). Despite the arrangement, it is clear that there is considerable exchange of cells between the different populations as slugs migrate and cells can switch between alternate fates until late in the developmental process (Abe et al., 1994; Harwood et al., 1991). The prestalk region can be sub-divided into different cell types defined by the expression pattern of prestalk-specific marker constructs (Jermyn et al., 1989). PstO cells (which use particular promoter elements, known as the *ecmO* promoter, to drive expression of the *ecmA* prestalk-specific gene) are found towards the rear of the anterior portion (Early et al., 1993). PstO cells are also defined as a distinct population due to their sorting behavior following transplantation experiments (Buhl and MacWilliams, 1991). PstA cells use an alternative section of the promoter (*ecmA* promoter) to drive expression of the same *ecmA* gene and are found at the front of the slug. PstAB cells express *ecmA* and *ecmB* (another prestalk specific gene) and form a central core (Jermyn and Williams, 1991). Following culmination, the spore head is supported by an upper and lower cup (formed from the ALCs) on top of a slender stalk. The stalk itself is supported on a basal disc. These different structures are derived from the alternative populations of prestalk cells (Early et al., 1995; Jermyn and Williams, 1991).

suggesting that energy reserves may contribute to the difference (Garrod and Ashworth, 1972; Leach et al., 1973; Tasaka and Takeuchi, 1981). The other well-characterized predisposition is the cell cycle phase of the cells at the onset of starvation with cells in mid-late G2 phase showing a prespore tendency (Gomer and Firtel, 1987; McDonald and Durston, 1984; Ohmori and Maeda, 1987; Zimmerman and Weijer, 1993). Links between control of the cell cycle and cell fate are widespread in a number of organisms. For example, in *Drosophila* embryos, localized expression of the phosphatase encoded by the *string* gene or of cyclin E is used to regulate cell cycle progression in groups of cells destined for particular cell fates (Edgar et al., 1994; Knoblich et al., 1994). In *Dictyostelium*, the relationship between cell cycle phase and cell fate has been proposed to be due to cell-cycle-dependent fluctuations in parameters such as cytosolic or vesicular pH or calcium ion (Ca^{2+}) concentrations (Gross et al., 1988; Azhar et al., 1998). Disruption of the *rtoA* gene disturbs the link between cell cycle and cell fate (Wood et al., 1996). The levels of RtoA vary during the cell cycle and its loss alters cell-cycle-dependent parameters such as cytosolic and endosomal pH (Brazill et al., 2000). Disruption of two tandem genes encoding ABC transporters in *rtoA⁻* cells suppresses the alteration in cell type proportioning caused by loss of *rtoA⁻* and restores endosomal pH levels (Brazill et al., 2001). However, strains lacking all three genes still lack the link between cell cycle and cell fate apparent in wild-type cells, suggesting that endosomal pH is not the important parameter linking cell cycle and cell fate.

Upon starvation, populations of cells with differing tendencies show differential rates of expression of genes required for early development (Abe and Maeda, 1994; Huang and Pears, 1999; Wang et al., 1988) which may lead to differences in the ability to sense and respond to extracellular signals. Cells known to have a prestalk tendency (early G2 phase cells or G− cells) show increased sensitivity to differentiate into stalk cells when exposed to DIF-1 in monolayers (Thompson and Kay, 2000a). Therefore, it is proposed that as levels of DIF-1 rise in the mound, those cells with an increased sensitivity will initiate differentiation down the prestalk pathway first (Fig. 2). Similar differences in sensitivity to prespore inducing agents may also be present and components of the cAMP response system are among those known to be differentially expressed.

Extracellular factors influencing cell fate

Two extracellular molecules have been well characterized in *Dictyostelium*, extracellular cAMP and differentiation inducing factor (DIF), a chlorinated hexaphenone. A number of others have been described (e.g., ψ, Kawata et al., 2004; and ammonia, reviewed in Gross, 1994) and a recent report

suggests that a number of polyketides are used as signaling molecules (Serafimidis and Kay, in press).

DIF

The ability of DIF-1 to induce stalk cell differentiation in a low-density monolayer of cells suggested that it may be the major inductive signal responsible for stalk cell formation and this was consistent with the phenotype of a mutant strain HM44 which blocks development at the mound stage and which makes very low levels of DIF, though it does remain DIF-responsive (Kopachik et al., 1983). This strain has allowed DIF-dependent pathways to be studied in the absence of endogenous DIF. Treatment of HM44 cells with DIF leads to induction of a number of genes which are specific to prestalk cells and which have become the classical markers of each of the subtypes of prestalk cell (Williams et al., 1987) (see Fig. 1B). However, the molecular defect or defects associated with HM44 are not defined and any phenotypic changes may be unrelated to the lack of DIF responsiveness. Recently, a defined strain (*dmtA⁻*) which synthesizes very low levels of DIFs 1 and 2 (undetectable and therefore below 5% of wild-type levels) has been generated by disruption of a gene encoding a protein required for DIF synthesis. Characterization of these *dmtA⁻* cells reveals that DIF-1 is essential only for the differentiation of one subset of prestalk cells, the pstO cells as defined by expression from the *ecmO* promoter (Thompson and Kay, 2000b). The factor responsible for induction of pstA cells, and any subtle role for DIF-1 in this process, is unknown (Fig. 3), though higher levels of DIF can induce expression of pstA markers in monolayers. One possibility is that molecules with DIF activity (such as precursors of DIF-1) are still synthesized in developing *dmtA⁻* cells and these may preferentially induce pstA cell differentiation. This would explain the observation that some markers which co-localize with pstO cells in wild-type cells are expressed normally in *dmtA⁻* cells (Maruo et al., 2004) as these genes may be activated by related factors whereas the *ecmO* promoter is not.

The structure of DIF-1 offers few clues to its mechanism of action. Receptor activity has been identified (Insall and Kay, 1990) but the molecular basis behind this remains elusive. Dissection of promoters of genes whose expression is regulated by DIF-1 led to the discovery of *Dictyostelium* homologues of STAT proteins (signal transducers and activators of transcription) (Kawata et al., 1997). In mammalian cells, these transcription factors have a well-studied role downstream of cytokine receptors. Their activation is triggered by tyrosine phosphorylation by members of the JAK family of tyrosine kinases or by receptors with intrinsic tyrosine kinase activity such as the epidermal growth factor (EGF) receptor. STAT proteins are known to play important roles in development. Mammals express at least seven STAT proteins and mice lacking genes encoding individual STATs have a variety of developmental
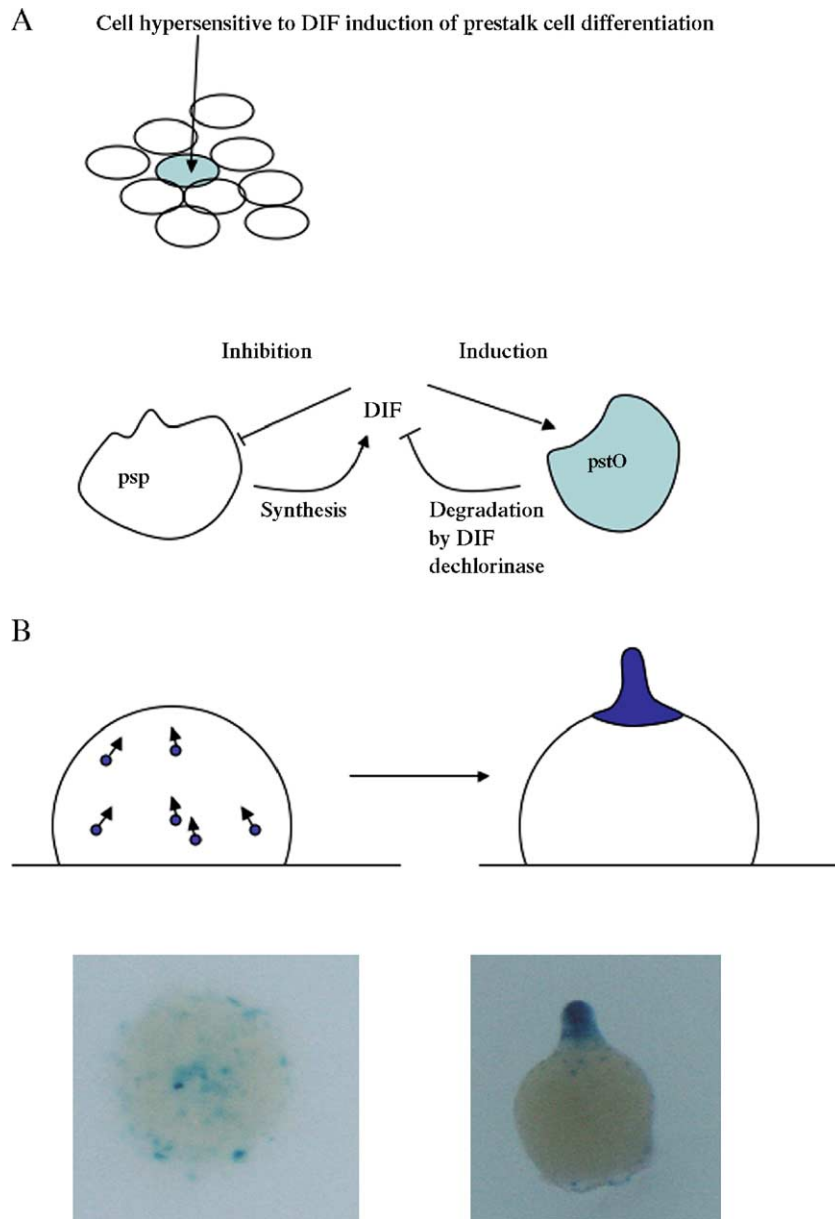
Fig. 2. Cells start to differentiate and then sort to the correct location. (A) As levels of DIF-1 rise in the mound, cells which are hypersensitive to DIF-1, due to cell cycle position or growth history, will preferentially start down the pathway leading to pstO cell differentiation (Thompson and Kay, 2000a). DIF-1 is synthesized by prespore (psp) cells (Kay and Thompson, 2001). PstO cells express DIF dechlorinase which destroys DIF-1 and so lower the concentration of DIF-1 in the local area (Thompson and Kay, 2000b). This presumably acts as a sink of DIF-1 and reduces levels in the local area, reducing the probability of the adjacent cells differentiating down the same pathway, thus influencing the ratio of prestalk to prespore cells. (B) Cells expressing β-galactosidase driven by promoters specific for a particular cell type (e.g., the prestalk specific *ecmA* promoter) are first apparent, randomly scattered in the mound (Early et al., 1995) and then sorted by processes involving differential adhesion and chemotaxis. All the cells in the mound are moving to facilitate this process (Siegert and Weijer, 1995; reviewed in Weijer, 2004).

defects (Levy and Darnell, 2002). For example, loss of Stat5A causes a failure of breast tissue development, and subtle defects in hematopoiesis and regulation of immune responses. The single *Drosophila* STAT, Stat 92E, is required for differentiation of a number of tissue types. The absence of the STAT pathway in single-cell organisms such as yeast is at least consistent with the involvement of this pathway in the extra complexities associated with multicellularity and in cell fate decisions.

The three published STAT proteins in *Dictyostelium* all lack the transactivation domains found in their mammalian counterparts and so may function as transcriptional inhibitors. DIF-1 induces tyrosine phosphorylation of Dd-STATc and its accumulation in the nucleus. In the slug phase, Dd-STATc accumulates in the nucleus of pstO cells and it is required to repress expression of pstA markers in these cells (Fukuzawa et al., 2001). The realization that Dd-STATc is a DIF-inducible repressor of transcription means that the
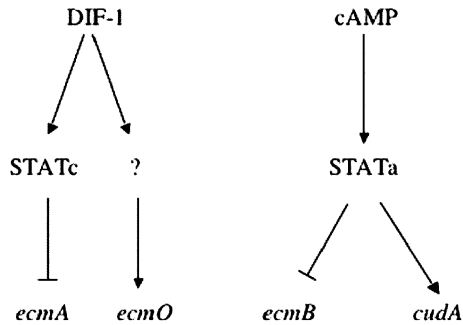
Fig. 3. Signals inducing prestalk marker expression. DIF-1 is essential for differentiation of PstO cells as *dmtA⁻* cells (which synthesize negligible levels of DIF-1) do not express β-galactosidase driven by the *ecmO* promoter region, unless provided with exogenous DIF-1 (Thompson and Kay, 2000b). The pathway responsible for this is not defined, but may involve the transcription factor DimA or a related factor (Thompson et al., 2004a). DIF-1 also can lead to phosphorylation and nuclear translocation of Dd-STATc to repress expression from the *ecmA* promoter. However, DIF-1 is not essential for differentiation of PstA cells and the inducing factor is not known. Extracellular cAMP induces activation of Dd-STATa to repress premature *ecmB* expression in pstO and pstA cells. Thus, Dd-STATa is a repressor which prevents premature stalk cell differentiation. Dd-STATa is also required for activation of expression of the *cudA* gene at the tip of the slug in response to cAMP, and this gene is essential for correct terminal differentiation (Fukuzawa and Williams, 2000). The tip of the slug is known to act in a manner comparable to a mammalian organizer region and is responsible for generation of extracellular cAMP in the slug (Dormann and Weijer, 2001).

factors responsible for activation of prestalk gene expression are still unknown. A promoter element and associated transcription factor required for activation of gene expression in response to DIF-1 has recently been identified (J. Williams, personal communication) and this discovery will facilitate analysis of the signaling pathways regulating expression.

In a complementary approach, a genetic screen for mutant cells which are not responsive to DIF-1 has led to the isolation of a resistant strain, disrupted in the *dimA* gene (Thompson et al., 2004a). The *dimA⁻* strain synthesizes normal levels of DIF-1 but is refractory to DIF-1's ability to induce stalk cell formation and inhibit spore cell induction. The protein encoded by the gene disrupted in the mutant is predicted to be a transcription factor of the bZIP/bRLZ family. It is an attractive idea that this factor acts to induce transcription of prestalk-specific genes in response to DIF-1. However, it is possible that the factor is required for the cells to attain DIF-responsiveness, for example, by being required for expression of components of the DIF-1 response system, such as a receptor. Transcription factors of this family work as dimers so the potential ability to form dimers with different partners might explain the role of DimA in prestalk and prespore cells. Understanding how DIF-responsive transcription factors are regulated will be vital to understanding DIF's importance.

Extracellular cAMP

Nanomolar pulses of extracellular cAMP secreted during aggregation interact with high-affinity cAMP receptors such as cAR1 and co-ordinate early development by acting, for example, as a chemoattractant. Once the aggregate has formed, levels of cAMP rise in the mound to allow interaction with low-affinity receptors such as cAR4. The expression of some prespore-specific genes can be induced by the addition of extracellular cAMP, suggesting that cAMP is a spore-inducing signal (Barklis and Lodish, 1983; Mehdy et al., 1983). Extracellular cAMP also plays a role in regulation of prestalk cell differentiation (Fig. 3). One of the *Dictyostelium* STAT homologues, Dd-STATa, is tyrosine phosphorylated and accumulates in the nucleus in response to cAMP (Mohanty et al., 1999). Dd-STATa null cells are hypersensitive to DIF-1 and the *ecmB* promoter is expressed throughout the prestalk region of slugs, instead of being restricted to certain prestalk subtypes. These results suggest that Dd-STATa functions as a repressor of premature *ecmB* expression in prestalk cells. However, there is also evidence that Dd-STATa can function as an activator of gene transcription. In the tip of the slug, extracellular cAMP, via cAR1 and Dd-STATa induces expression of *cudA* which is essential for normal development (Araki et al., 1998; Fukuzawa and Williams, 2000; Fukuzawa et al., 1997). Localization of this response in the tip is due to restricted expression of the adenylyl cyclase ACA in tip cells (Verkerke-van Wijk et al., 2001).

The interaction of cAMP with the cell surface receptor cAR3 leads to an increase in activity of the serine threonine kinase GskA (Plyte et al., 1999). This is the *Dictyostelium* homologue of GSK-3, a kinase implicated in cell fate decisions in a wide range of organisms (Doble and Woodgett, 2003). The activation of GskA is lost in strains lacking the gene encoding the tyrosine kinase Zak1 (Kim et al., 1999). Zak1 phosphorylates GskA on tyrosine residues, leading to its activation. Interaction of cAMP with the related receptor cAR4 leads to activation of a tyrosine phosphatase and subsequent deactivation of GskA (Kim et al., 2002). Thus, extracellular cAMP has opposing effects dependent on relative expression levels or activation of alternative cAR receptors (Fig. 4) and expression of these receptors is known to be enriched in different cell types (Louis et al., 1994; Yu and Saxe, 1996). There is evidence that this antagonistic regulation of GSK-3 signaling pathways leading to alternative cell fate decisions may be a more general phenomenon (Weidinger and Moon, 2003). In the canonical Wnt signaling pathway, interaction of factors with the frizzled class of receptors leads to inhibition of GSK-3 activity and a subsequent stabilization of the transcription co-factor β-catenin. However, other members of the Wnt family act antagonistically on this pathway to inhibit β-catenin activation with opposing consequences for cell fate.

Disruption of the gene encoding GskA in *Dictyostelium* leads to ectopic expression of the *ecmB* gene and cells become refractory to the inhibition of stalk cell induction by cAMP in monolayers (Schilde et al., 2004). Thus, GskA activity regulates the inhibition of premature pstAB cell differentiation. The only characterized substrate of GskA is
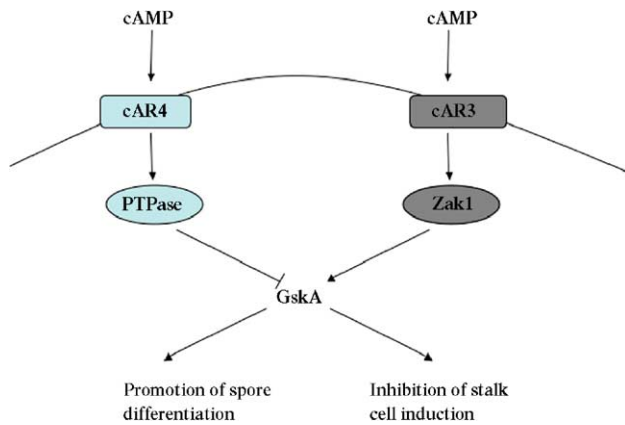
Fig. 4. Conflicting regulation of GskA activity by extracellular cAMP. Extracellular cAMP interacts with the different, though related, cAMP receptors, cAR3 and cAR4. cAR3 promotes activation of the tyrosine kinase activity of the tandem kinase domain protein Zak1 which, in turn, phosphorylates and activates GskA (Kim et al., 1999; Plyte et al., 1999). cAR4, however, leads to activation of tyrosine phosphatase activity and subsequent deactivation of GskA (Kim et al., 2002). GskA has multiple effects at different stages of the life cycle, including inhibition of premature *ecmB* expression associated with stalk cell maturation and increasing the efficiency of spore cell production (Schilde et al., 2004).

Dd-STATa which is phosphorylated in the nucleus to promote nuclear export (Ginger et al., 2000). However, Dd-STATa cannot be the factor responsible for GskA-mediated inhibition of expression from the *ecmB* promoter, as that regulation is preserved in promoters lacking the STAT binding sites. Identifying alternative targets for GskA is an important next step to reveal its role in cell fate decisions.

Intracellular cAMP

As well as a role as an extracellular signaling molecule, it is clear that intracellular cAMP plays an important role in cell differentiation in *Dictyostelium*. cAMP-dependent protein kinase (PKA) is required not only for early developmental events (Harwood et al., 1992a), but also for regulating the transition from slug to mature fruiting body and terminal differentiation of both spore and stalk cells (Harwood et al., 1992b; Hopper et al., 1993a,b). As in other developmental systems, PKA's role seems to be in progression of differentiation rather than in directing choice between alternative fates. Rather than targeting an adenylyl cyclase, it seems that the events of terminal differentiation are controlled via regulation of a cAMP phosphodiesterase, RegA (Shaulsky et al., 1998; Thomason et al., 1998). RegA contains a response regulator domain with a conserved aspartate residue and phosphorylation of this residue leads to an activation of RegA phosphodiesterase activity (Thomason et al., 1999). A number of histidine kinases have been shown to feed into this system, either increasing or decreasing RegA activity with a consequent regulation of PKA activity. The adenylyl cyclase ACB, which is required late in development, contains a response regulator domain

but its activity has not been shown to be regulated (Fig. 5) (Kim et al., 1998; Meima and Schaap, 1999; Soderbom et al., 1999). The data are consistent with ACB working constitutively and intracellular cAMP levels being controlled via RegA. RegA activity can be controlled not only by its response regulator domain but also by phosphorylation by the MAPkinase homologue DdErk2 in early development and by targeted ubiquitinylation and degradation of the protein (Maeda et al., 2004; Mohanty et al., 2001).

Transcription factors implicated in cell fate decisions

As well as the Dd-STAT proteins and DimA already discussed, a number of other transcription factors have been implicated in cell fate decisions. Two transcription factors required for the generation of spore cells are SrfA and StkA (Chang et al., 1996; Escalante and Sastre, 1998). SrfA (a MADS-box transcription factor identified by homology to mammalian Srf) is required for the induction of gene expression at the final stages of spore cell differentiation. *srfA⁻* cells develop fruiting bodies which are relatively normal in appearance. However, the cells found in the heads are not mature spores and there is no induction of genes associated with terminal spore differentiation such as *spiA*. StkA is a transcription factor of the GATA family and *stkA⁻* strains develop into structures which lack any detectable spore heads but contain long twisted stalks. The *stkA⁻* cells show overexpression of both *ecmA* and *ecmB* genes suggesting a general excess of all types of stalk cell, consistent with prespore cells being diverted to a prestalk cell fate.

These transcription factors lie in different pathways as in *srfA⁻* strains prespore cells accumulate, whereas in *stkA⁻* strains, they are diverted to a stalk cell fate. The phenotype of *stkA⁻* cells is not rescued by 8Br-cAMP (a membrane-permeable cAMP analogue which directly activates PKA) or by overexpression of the catalytic subunit of PKA so StkA lies on a different pathway or downstream of PKA activation. In *srfA⁻* cells, 8Br-cAMP is not able to generate mature spores either but does induce precocious expression of the *srfA* gene in wild-type prespore cells (Escalante and Sastre, 2002). This again puts SrfA downstream of PKA, suggesting PKA may induce expression of spore-specific genes essential for terminal differentiation via induction of SrfA expression. A number of genes whose expression is dependent on SrfA have been identified and expression of some of these is also dependent on StkA (Escalante et al., 2003, 2004; Loughran et al., 2000). Other pathways regulating StkA and SrfA are not known. Cells in which the gene encoding DdCdk8 has been disrupted also fail to generate spores (Lin et al., 2004). This is the *Dictyostelium* orthologue of a kinase known to be involved with transcriptional control by phosphorylation of transcription factors in other systems. Linking the phenotypes of these
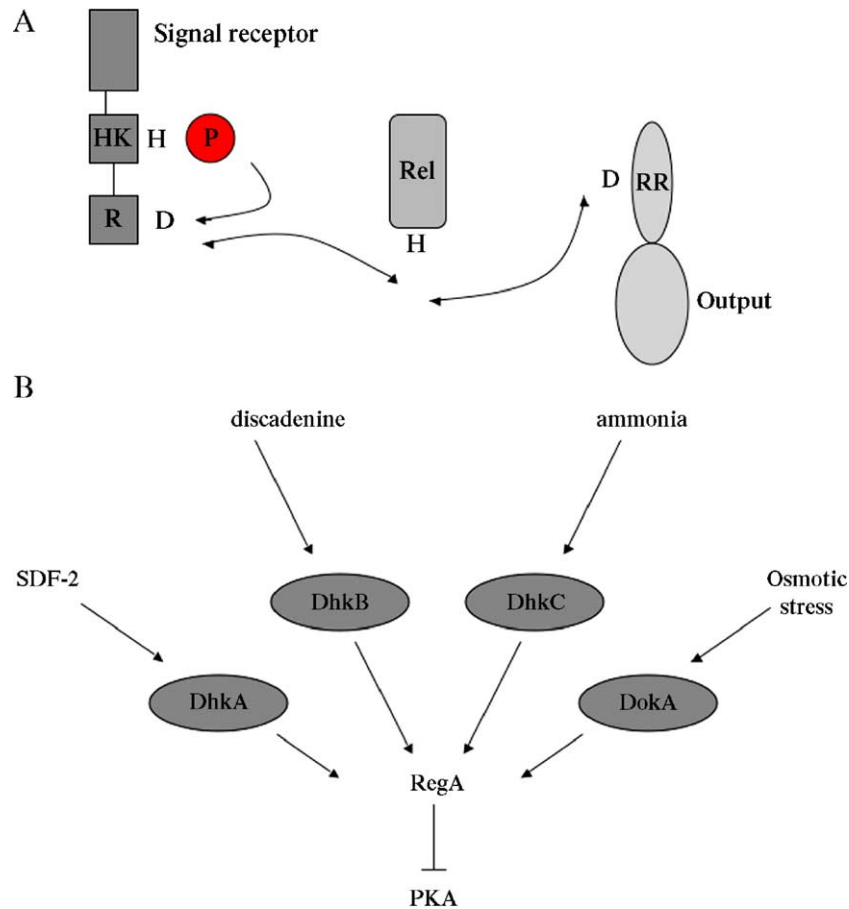
Fig. 5. (A) A typical phosphorelay signal transduction pathway. On activation, for example, by binding a signaling molecule, the histidine kinase (HK) phosphorylates itself on a histidine residue. This phosphate group is then transferred firstly to an aspartate residue in the receiver (R) domain of the same protein. It is then transferred again either directly, or indirectly via a relay protein, to an aspartate residue in the response receiver (RR) domain of a target protein, where it can modulate protein activity. The pathway can work in both directions with the HK acting as a sink to promote dephosphorylation of the RR. The *Dictyostelium* genome (Eichinger et al., 2005) (accessible from http://dictybase.org) contains a number of genes potentially encoding histidine kinases, four of which have been characterized. (B) Regulation of RegA by phosphorelay. The only characterized protein containing an RR domain and regulated by HK activity in *Dictyostelium* is the cAMP phosphodiesterase RegA, where phosphorylation on Asp212 activates the protein (Thomason et al., 1998, 1999). A single relay protein, RdeA, has been characterized. A number of proteins containing HK domains have been shown to modulate histidine phosphorylation of RegA in different circumstances and can act either to promote phosphorylation or dephosphorylation. For example, DokA is thought to act as a phosphatase whereas DhkC is likely to be a kinase (Ott et al., 2000; Singleton et al., 1998). Culmination is triggered by lowering of ammonia levels which signals via the histidine kinase DhkC to RegA (Singleton et al., 1998). SDF2, a factor secreted by prestalk cells to induce terminal maturation of spore cells, acts via the membrane-spanning sensor kinase DhkA, again to regulate RegA activity (Wang et al., 1999; Zinda and Singleton, 1998). At other stages of the life cycle, it is proposed that discadenine interacts with the sensor kinase DhkB to inhibit precocious spore germination by a similar pathway, and DokA leads to changes in intracellular cAMP levels in response to osmotic stress by a similar pathway (Ott et al., 2000; Schuster et al., 1996; Zinda and Singleton, 1998). A number of other sensor kinases have been recognized in the *Dictyostelium* genome and it remains possible that they will all work through regulation of RegA. RegA is thought to be the major intracellular phosphodiesterase responsible for degradation of intracellular cAMP and changes in its activity in a number of circumstances will lead to alterations in cAMP levels and thus in the activity of cAMP-dependent protein kinase (PKA).

strains may provide valuable insight into the signaling pathways involved in regulating StkA and SrfA.

Genetic interactions

Stalk and spore cell differentiation are inter-linked at a number of levels. Factors produced by one cell type are required for differentiation of the other, thus ensuring that the two cell types proceed through development co-ordinately. This interdependency precludes the differentia-

tion of one cell type in mutant strains lacking at least initial differentiation down the alternative pathway. For example, DIF-1 is produced by prespore cells to induce pstO cell differentiation and SDF-2 is produced by prestalk cells and induces terminal differentiation of prespore cells (Anjard et al., 1998; Kay and Thompson, 2001). The *tag* genes are predicted to encode proteins containing an N-terminal domain with serine protease activity and a C-terminal domain homologous to the ABC family of membrane transporters (Good et al., 2003; Shaulsky et al., 1995). These proteins thus have the potential to be involved in

generation of an extracellular signaling peptide by cleavage of the peptide from a precursor and transport into the extracellular environment and one possible substrate for these transporters is SDF-2. Disruption of the *tagA* gene causes defects in prespore cell differentiation whereas disruption of *tagB* suggests a role in stalk cell differentiation. However, the phenotypes are complex with both cell autonomous and non-autonomous effects on cell fate suggesting that not only does the secreted peptide influence surrounding cells but that retention of the unprocessed precursor may act to inhibit differentiation. Any role for ABC transporter-driven peptide secretion in mammals is not clear although there is evidence that such proteins are required for the maintenance of stem cell populations (Johnstone et al., 2000). In *Arabidopsis*, one transporter of this family has been shown to influence the transition from cell growth to differentiation in the root (Gaedeke et al., 2001).

Transcription factors can also have pleiotropic roles in cell fate determination. *DimA⁻* cells do not respond to DIF-1 by becoming pstO cells and so it would be predicted that, in chimeric mixes with wild-type cells, *dimA⁻* cells would be over-represented in the spore cell population. At the slug stage, this bias in fates appears to be holding true, but the bias is lost by the time of terminal differentiation (Foster et al., 2004). This suggests that the protein encoded by the *dimA* gene has an alternative role in regulating the balance between cell types at a later stage of development. Other regulatory proteins have been shown to have both a positive and negative role in differentiation of one cell type at different stages. For example, cells deficient in Dd-STATa are hypersensitive to DIF-1 induction of prestalk cells in monolayer but give rise to no mature stalk cells during multicellular differentiation (Mohanty et al., 1999). The simplest interpretation is that Dd-STATa has an inhibitory effect on prestalk induction but is required for stalk cell differentiation at a later stage.

Future

The advent of methods for targeted gene disruption and for random insertional mutagenesis followed by phenotypic screening has hugely increased the library of gene products required for different aspects of *Dictyostelium* development (Kuspa and Loomis, 1992). Signal pathways, however, rarely work in isolation as linear chains but interact to form networks. These allow multiple signals to be integrated and the responses co-ordinated. The existence of such networking is already evident at many levels in the control of *Dictyostelium* development. For example, promoter analysis of genes expressed in a cell-type-specific manner has frequently revealed complexity as different promoter elements drive expression in alternative cell types. Correct expression patterns involve interaction of both positive and negative factors downstream of different signaling pathways. Networking is certain to exist at multiple levels of signaling pathways.

The challenge now is to understand the interactions in these networks. The development of methods to generate strains in which multiple genes have been disrupted will facilitate the analysis of genetic interactions between redundant and linked pathways, and allow the identification of suppressor mutations. *Dictyostelium* genetics has suffered from a paucity of suitable selectable markers to facilitate generation of multiple disruptions. The development of the cre-lox recombinase system in *Dictyostelium* allows re-use of the available markers (Faix et al., 2004) and allows generation of strains lacking multiple genes. Similarly, parasexual genetics can be used to generate strains with multiple disruptions and also partial diploids (King and Insall, 2003). Microarray analysis of gene expression patterns in mutant strains enables similarities in patterns to be used to group genes which generate overlapping patterns and allow epistasis analysis (Kibler et al., 2003a,b; Van Driessche et al., 2005; Zupan et al., 2003). Similarly, co-regulated genes can be clustered to define multiple end-points of pathways (Iranfar et al., 2001; Van Driessche et al., 2002, 2005). This identifies similarities between previously unlinked signaling molecules. Advances in proteomic technology allow the consequences (both transcriptional and post-transcriptional) of gene disruption to be determined and compared between strains, in order to identify shared and distinct end-points to signaling pathways (Secko et al., 2004; L.S., A. Harwood, J. Williams, C.P., unpublished data). All of these methodological advances will advance our understanding of the complexity of signaling networks involved in regulating development in *Dictyostelium*.

Acknowledgments

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