cAMP signaling in *Dictyostelium*
Complexity of cAMP synthesis, degradation and detection

SHWETA SARAN, MARCEL E. MEIMA, ELISA ALVAREZ-CURTO, KARIN E. WEENING,
DANIEL E. ROZEN and PAULINE SCHAAP*
School of Life Sciences, University of Dundee, MSI/WTB complex, Dundee DD1 5EH, UK

Abstract

cAMP plays a pivotal role in control of cell movement, differentiation and response to stress in all phases of the *Dictyostelium* life cycle. The multitudinous functions of cAMP require precise spatial and temporal control of its production, degradation and detection. Many novel proteins have recently been identified that critically modulate the cAMP signal. We focus in this review on the properties and functions of the three adenylyl cyclases and the three cAMP-phosphodiesterases that are present in *Dictyostelium*, and the network of proteins that regulate the activity of these enzymes. We also briefly discuss the two modes of detection of cAMP.

Functions of cAMP

The evolution of signalling in *Dictyostelium discoideum* has particularly favoured cAMP as jack of all trades. The manifold usages of cAMP are summarized in Figure 1 and can be broadly subdivided into its role as first messenger outside the cell and second messenger inside the cell. Outside, cAMP acts as chemoattractant, which coerces amoebae to convert from solitary predators into gregarious community members (Konijn *et al.*, 1967). Outside, cAMP also acts on gene regulation to induce the alterations in phenotype that are required for chemotactic aggregation and for initiation of prespore differentiation (Gerisch *et al.*, 1975; Kay, 1982; Schaap and Van Driel, 1985). As inhibitor of terminal stalk cell differentiation, cAMP plays a critical role in cell-type choice (Berks and Kay, 1988; Hopper *et al.*, 1993a).

Inside, cAMP triggers initiation of development (Schulkes and Schaap, 1995; Mann *et al.*, 1997), maturation of spores and stalk cells (Harwood *et al.*, 1992; Hopper *et al.*, 1993b; Mann and Firtel, 1993) and maintenance of spore dormancy (Van Es *et al.*, 1996). Inside, cAMP also mediates resistance to osmotic stress (Schuster *et al.*, 1996) and contributes to the process of orientation in chemotactic gradients (Wessels *et al.*, 2000).

cAMP synthesis

Until the early 90's the regulation of cAMP production could only be analysed biochemically. cAMP is transiently synthesized upon stimulation of aggregation competent cells with cAMP. The accumulation of cAMP peaks at 2 min (Devreotes and Steck, 1979) and requires the heterotrimeric G-protein G2 (Kesbeke *et al.*, 1988; Kumagai *et al.*, 1989). In cell lysates, cAMP synthesis requires GTPγS; the cyclase itself is associated with the membrane fraction, but requires a factor from the cytosol to be active (Theibert and Devreotes, 1986). Genetic screens identified a mutant, synag 7, that does not synthesize cAMP and can only aggregate when mixed with wild-type cells. The defective gene was later identified as CRAC (cytosolic regulator of adenylyl cyclase), the essential cytosolic factor for adenylyl cyclase activation (Insall *et al.*, 1994; Lilly and Devreotes, 1994).

The gene for the cAMP-activated adenylyl cyclase *AcaA* was identified by PCR through its homology with the mammalian cyclase domains, and this screen also yielded a second gene, *AcgA*, that was only expressed in spores (Pitt *et al.*, 1992). The third adenylyl cyclase, ACB, was detected biochemically in mutants with a double lesion in *acaA* and *rdeA*, an essential protein for activation of the intracellular cAMP-phosphodiesterase RegA (Chang *et al.*, 1998; Kim *et al.*, 1998a; Thomason *et al.*, 1999). The ACB gene, *AcrA*, was identified from a mutant that was defective in spore maturation (Soderbom *et al.*, 1999).

The catalytic domains of ACA, ACG and ACB classify them as typical eukaryote class III cyclases (Danchin, 1993). This type of cyclase can either be soluble with one or two catalytic domains, membrane-associated with a single transmembrane domain and one catalytic domain or membrane-associated with 12 transmembrane (12TM) domains and two catalytic domains. The catalytic domains are actually half-sites of the catalytic centre and need to form hetero- or homodimers to be active. Optimal juxtaposition of the two domains is usually part of the activation process of the cyclases (Hurley, 1998).
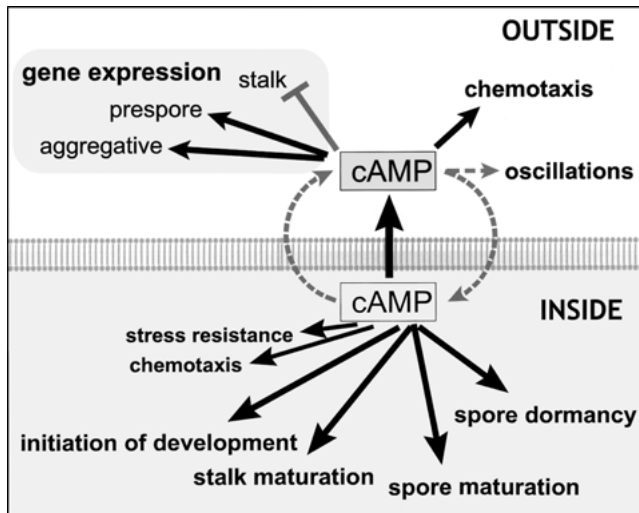
Fig. 1. Functions of intracellular and extracellular cAMP.

Adenylyl cyclase A

ACA is an enzyme with 12TM domains and two cyclase domains. In vertebrates, this type of enzyme is commonly activated by serpentine receptors that couple to heterotrimeric G-proteins. ACA is expressed at high levels during aggregation. During slug formation expression is downregulated at the posterior region and becomes almost exclusively restricted to the tip (Pitt et al., 1992; Verkerke-van Wijk et al., 2001). ACA activity is regulated by a positive and a negative feedback loop, which causes some starving cells to spontaneously produce and secrete cAMP at regular intervals. The positive loop is caused by self-stimulation of ACA by extracellular cAMP, while the negative loop is due to a cAMP-induced adaptation process (Martiel and Goldbeter, 1987; Tang and Othmer, 1994). cAMP-induced cAMP secretion in neighbouring cells causes propagation of the original pulse as a spiral or concentric cAMP wave through the cell population and chemotactic movement towards the signalling source. The regulation of ACA activity is remarkably complex and requires some factors for which there are as yet no counterparts in regulation of the vertebrate 12TM adenylyl cyclases (Figure 2).

The initial steps involve activation of the heterotrimeric G-protein G2 by occupied cAMP receptors and formation of a free $\beta\gamma$ dimer (Lilly et al., 1993; Wu et al., 1995). In analogy to vertebrate systems, where the $\beta\gamma$ dimer directly binds to and activates PI3-kinase (Schwindinger and Robishaw, 2001), the Dictyostelium $\beta\gamma$ dimer is also supposed to activate a PI3-kinase, which converts the membrane lipid phosphatidyl inositol [4, 5] bisphosphate (PIP2) into the CRAC binding site phosphatidyl inositol [3, 4, 5] trisphosphate (PIP3). PIP3 then recruits CRAC from the cytosol to the plasma membrane by binding to its pleckstrin homology domain (Dormann et al., 2002). At this location CRAC can participate in ACA activation. This is not the whole story because ACA activation also requires the small G-protein RasC (Lim et al., 2001), a Ras interacting protein, Rip3 (Lee et al., 1999), the Ras nucleotide exchange factor, RasGEF (Insall et al., 1996), the MAPkinase ERK2 (Segall et al., 1995) and a novel factor Pianissimo (Chen et al., 1997). How these proteins interact with each other and with ACA is not clear.
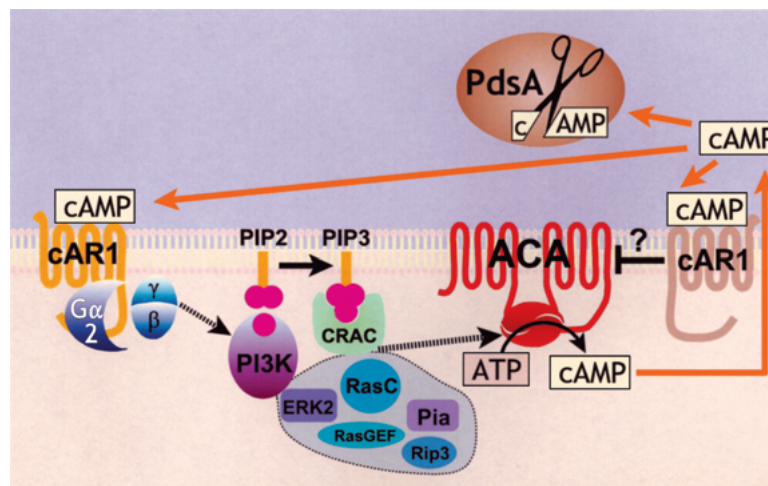

Fig. 2. Schematic of the regulation of adenylyl cyclase A: Binding of cAMP to the serpentine receptor cAR1 induces dissociation of the heterotrimeric G-protein, G2, into its α and $\beta\gamma$ subunits. The $\beta\gamma$ subunits induce activation of phosphatidylinositol-3 kinase (PI3K) in a manner that is not yet understood. PI3K converts phosphatidyl inositol [4, 5] bisphosphate (PIP2) into phosphatidyl inositol [3, 4, 5] trisphosphate (PIP3). PIP3 binds to the pleckstrin homology domain of CRAC, the Cytosolic Regulator of Adenylyl Cyclase and recruits CRAC from the cytosol to the plasma membrane, where it can participate in adenylyl cyclase A (ACA) activation. A set of proteins, including the MAPkinase, ERK2, the small G-protein, RasC, the guanine nucleotide exchange factor, RasGEF, the Ras interacting protein, Rip3, and a novel protein, pianissimo (Pia) are also required for ACA activation. The interactions of these proteins with each other and with CRAC and ACA have not yet been clarified. cAMP produced by ACA is rapidly secreted to further activate ACA in a positive feedback loop. Binding of cAMP to cAR1 blocks ACA activation via a negative feedback loop, that is little understood. Extracellular cAMP is degraded by the phosphodiesterase PdsA, which terminates both loops and returns cells to the basal excitable state.

The adaptation process of ACA has also not been resolved. Mammalian adenylyl cyclases are negatively regulated by inhibitory G-proteins (Hamm, 1998) or the activation process is terminated by receptor desensitization. In the case of serpentine receptors this is usually achieved by phosphorylation of the carboxy-terminal cytoplasmic tail and binding of arrestin (Ferguson and Caron, 1998). cAMP receptors are also phosphorylated during ligand binding (Klein et al., 1985), but this does not terminate G-protein-coupled responses (Kim et al., 1997b). A putative candidate for an inhibitory G-protein, Gα9, was recently identified. Deletion of Gα9 augments signalling centre initiation and subsequent aggregation, which suggests that a functional Gα9 negatively regulates ACA activation. However, periodic signalling still occurs in the Gα9 null cells, which indicates that adaptation through Gα9 cannot be the sole mechanism that terminates cAMP accumulation (Brzostowski et al., 2002).

AcaA null cells show a similar phenotype to the previously identified *synag7* mutant. They show no cAMP-induced cAMP production and can only aggregate in synergy with wild-type cells. They fully differentiate into spores in chimeric fruiting bodies, which implies that ACA provides cAMP for extracellular cAMP signalling, but is not required for intracellular cAMP signalling and activation of PKA-dependent responses (Pitt et al., 1992, 1993).

Oscillatory cAMP signalling not only controls aggregation, but is also considered to coordinate multicellular morphogenesis. The waves of cell movement that are the hallmark of oscillatory chemoattractant secretion have also been observed in slugs and fruiting bodies (Siegert and Weijer, 1992). Slug tips function as classical embryological organizers (Raper, 1940), and most likely do so by acting as pacemakers for cAMP oscillations. This is accentuated by high expression of ACA in slug tips (Verkerke-van Wijk et al., 2001).

Adenylyl cyclase G

The *AcgA* gene is expressed in spores and its ablation has little effect on growth and development to fruiting bodies (Pitt et al., 1992). cAMP levels are high in dormant spores and decrease rapidly when spores are activated for germination. High osmolarity prevents this drop in cAMP levels and inhibits the germination process (Virdy et al., 1999). High osmolarity is maintained in the spore head by the presence of more than 100 mM ammonium phosphate (Cotter et al., 1999). ACG is activated by high osmolarity and its activity blocks the germination process through activation of PKA (Figure 3). In *acgA* null mutants inhibition of spore germination by high osmolarity does not occur (Van Es et al., 1996).

Activation of ACG by high osmolarity requires cellular integrity, but the mechanism is still obscure. Histidine kinases are implicated in sensing osmotic stress in bacteria and fungi (Santos and Shiozaki, 2001) and in
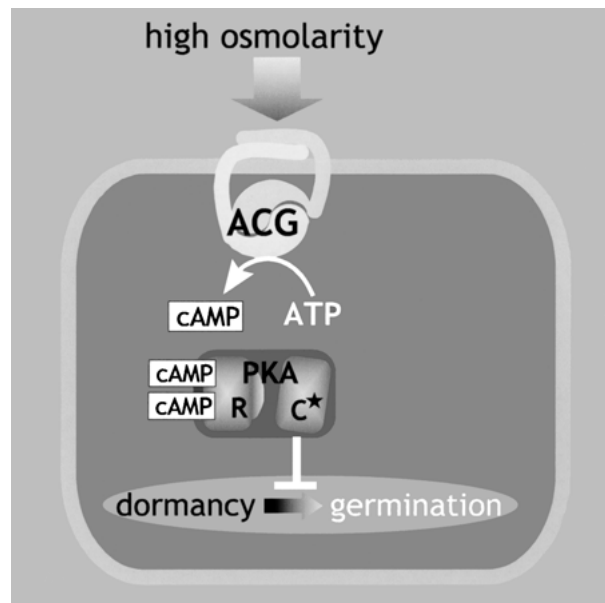

Fig. 3. Regulation of spore germination by high osmolarity: Ambient conditions of high osmolarity in the spore head activate adenylyl cyclase G (ACG) to produce cAMP. cAMP binds to the regulatory subunit (R) of cAMP dependent protein kinase (PKA), causing dissociation and activation of the catalytic subunit (C). Active PKA inhibits the germination process in an unknown manner.

Dictyostelium the histidine kinase DokA mediates osmotic stress sensing. Osmotic stress causes a DokA mediated increase in cAMP levels, which is however not achieved by activation of ACG, but by inhibition of the cAMP-phosphodiesterase RegA (Ott et al., 2000). A relationship between ACG and histidine kinase mediated osmosensing is therefore not obvious. When full length ACG was expressed in yeast, it still showed stimulation by high osmolarity. This almost certainly implies that the osmosensor is intrinsic to the ACG protein (Saran and Schaap, preliminary results).

Adenylyl cyclase B

ACB is as yet the most enigmatic of the three adenylyl cyclases. The ACB gene, *AcrA*, is expressed at low levels during growth; transcripts accumulate to high levels after 4 h of starvation, to remain high up to the fruiting body stage. ACB shows greatest sequence and structural homology to the CyaC adenylyl cyclases from the cyanobacteria *Spirulina platensis* and *Anabena spirulensis* (Soderbom et al., 1999). The cyanobacteria utilized their sodium-bicarbonate saturated sea-water habitat for photosynthesis and are considered to be responsible for transforming the pre-Cambrian carbon-dioxide rich atmosphere into the oxygen-rich atmosphere that allowed the Cambrian explosion of animal life (Ohno, 1997). cAMP is considered to play an important role in stimulating metabolic activity in response to light and carbon dioxide/bicarbonate. The two cyanobacterial adenylyl cyclases are activated by light through a dual component phosphorelay system (Yeh et al., 1997;

Kasahara and Ohmori, 1999) and by bicarbonate through direct activation of the catalytic domain (Chen *et al.*, 2000).

Similar to CyaC, the ACB cyclase domain is preceded by a response regulator domain, that is the common target for phosphorelay, and by a histidine kinase domain. The latter domain may not be active in ACB, because it lacks an essential histidine residue for autophosphorylation and is also degenerate in other conserved regions (Soderbom *et al.*, 1999). The AcrA sequence displays two putative hydrophobic transmembrane regions and the ACB enzyme activity is associated with the particulate cell fraction. ACB can readily be measured in cell lysates in the presence of the RegA inhibitor IBMX. In intact cells ACB can only be measured when either RegA or its activator RdeA are absent (Kim *et al.*, 1998a; Meima and Schaap, 1999). ACB differs from most characterized class III ACs in showing higher activity with Mg^{2+}/ATP than with Mn^{2+}/ATP. ACB seems to be active whenever it is expressed and its activity is not influenced by cAMP, GTPγS, DIF, ammonia or bicarbonate (Kim *et al.*, 1998a; Meima and Schaap, unpublished results). Although the tested signals do not exhaust the list of putative regulators of enzyme activity, the apparent constitutive activity of ACB points to a possibility that cAMP production by ACB is largely controlled by RegA activity.

cAMP breakdown

The inactivation of cyclic nucleotides by hydrolysis is often of greater importance for dynamic signalling than their synthesis. For appropriate regulation of second messenger responses, mammalian cells employ no less than 12 different classes of phosphodiesterases, which each contain several members. The carboxyterminal catalytic centre of these phosphodiesterases is conserved, but the enzymes differ in the sequence and structure of the aminoterminal region, which may contain single or multiple regulatory domains that control enzyme activity and anchoring to scaffolding proteins (Houslay and Milligan, 1997; Mehats *et al.*, 2002). This extensive level of complexity is not present in *Dictyostelium*, but five different cyclic nucleotide phosphodiesterases have presently been identified. Two of those, Pde3 and PdeD, are cGMP-specific phosphodiesterases and will only be briefly discussed. PdsA is a dual-specificity phosphodiesterase that hydrolyses cAMP and cGMP at an equal rate. RegA and PdeE are cAMP phosphodiesterases; RegA does not hydrolyse cGMP at all and PdeE does so at a six-fold lower rate than cAMP.

PdsA

The first enzyme to be biochemically identified and subsequently cloned is PdsA. PdsA is expressed in starving cells and targeted both for secretion and for display on the extracellular face of the plasmamembrane (Malchow *et al.*, 1972; Gerisch, 1976). The activity of the secreted form of PdsA is regulated by a secreted inhibitor, PDI (Riedel and Gerisch, 1971; Franke and Kessin, 1981; Wu and Franke, 1990). PDI binds only to the soluble form of PDE and changes its K_M for cAMP from 5 µM to 2 mM (Kessin *et al.*, 1979). Cloning of the PdsA gene revealed that its catalytic domain was unlike that of the mammalian phosphodiesterases, but similar to that of the low-affinity yeast enzyme PDE1 (Nikawa *et al.*, 1987). The *PdsA* gene is regulated by three promoters that control expression during growth, aggregation and multicellular development respectively (Faure *et al.*, 1990). The multicellular (late) promoter element is only active in prestalk cells (Hall *et al.*, 1993). *PdsA* null mutants cannot aggregate (Darmon *et al.*, 1978; Sucgang *et al.*, 1997), but aggregation can be restored by expression of *PdsA* from the aggregative promoter, while restoration of slug and fruiting body formation requires expression from the late promoter (Sucgang *et al.*, 1997). Overexpression of PdsA accelerates the aggregation process, but arrests tip formation and cell differentiation (Faure *et al.*, 1988). Neither ablation nor overexpression of any of the adenylyl cyclases has such profound effects on aggregation and development (Pitt *et al.*, 1992, 1993; Parent and Devreotes, 1996; Soderbom *et al.*, 1999), which emphasizes the crucial role of apposite regulation of hydrolysis of extracellular cAMP.

RegA

The cAMP-specific phosphodiesterase RegA was identified independently by two laboratories as the defective gene in a sporogenous mutant with accelerated development (Shaulsky *et al.*, 1996, 1998; Thomason *et al.*, 1998). A similar phenotype was found in *rdeC* mutants (Abe and Yanagisawa, 1983), which lack a functional PKA regulatory subunit (Simon *et al.*, 1992) and in *rdeA* mutants, which are defective in a phosphotransfer protein (Abe and Yanagisawa, 1983; Chang *et al.*, 1998). RegA is a remarkable hybrid of a bacterial type response regulator and a mammalian type PDE catalytic domain with a K_M for cAMP hydrolysis of 5 µM (Shaulsky *et al.*, 1996; Thomason *et al.*, 1998). *RegA* transcripts appear after 4 h of starvation and thereafter remain present at constant levels. The expression of *RegA* is uniform in young slugs, but becomes localized at the prestalk upper cup region during culmination (Shaulsky *et al.*, 1996; Tsujioka *et al.*, 2001). RegA protein is most abundant during aggregation. However, in contrast to *RegA* mRNA levels, RegA protein levels decrease strongly after slug formation (Thomason *et al.*, 1998). This down-regulation appears to be essential for induction of prestalk and prespore genes and involves two scaffolding proteins, CulA and FbxA/ChtA, that target RegA for ubiquitination and degradation (Mohanty *et al.*, 2001).

Both *regA* and *rdeA* null mutants show elevated cAMP levels, which suggests a causal link between the two. This was demonstrated in an elegant series of experiments by Thomason and coworkers. Eukaryote phosphotransfer systems are initiated by an activated histidine kinase which autophosphorylates itself on a conserved histidine residue and relays the phosphate to a receiver aspartate residue within the same protein (Figure 4). In a series of His–Asp phosphotransfer steps, the phosphate is finally delivered to an aspartate in the response regulator domain of the target protein (Santos and Shiozaki, 2001). The V_{max} of the RegA PDE activity is 20-fold increased by phosphorylation of Asp^{212} in its response regulator region (Thomason *et al.*, 1999). RdeA shows a consensus histidine (His^{65}) in a region of 20 amino-acids that is conserved in histidine phosphotransfer proteins (Chang *et al.*, 1998). Thomason *et al.* (1999) showed that when RdeA was phosphorylated on His^{65} *in vitro*, the phosphate was carried over to the Asp^{212} of RegA, thereby activating the enzyme activity. Conversely, when RegA was phosphorylated on Asp^{212}, it could relay a phosphate to the His^{65} of RdeA. Depending on the relative concentrations of the phosphodonor proteins, the RegA PDE activity can be both positively and negatively regulated by phosphorelay (Thomason *et al.*, 1999). The *Dictyostelium* histidine kinase DhkC may act as a positive regulator of RegA activity (Figure 4A). Similar to *regA* null cells, *dhkC* null cells show rapid develop-

ment, without a migrating slug stage. DkhC is proposed to be activated by ammonia, which induces prolonged slug migration and inhibits terminal differentiation (Singleton *et al.*, 1998). Earlier work indicated that loss of ammonia gas from culminants triggered spore and stalk maturation (Schindler and Sussman, 1977; Wang and Schaap, 1989), supposedly by releasing a block on PKA activity (Harwood *et al.*, 1992; Hopper *et al.*, 1993b). This can now be understood in terms of activation of RegA by ammonia and subsequent inhibition of cAMP accumulation and PKA activation.

DhkA and DhkB are putative negative regulators of RegA. In addition to the histidine kinase domain, both proteins harbour putative transmembrane domains and an extracellular putative sensor domain (Zinda and Singleton, 1998; Wang *et al.*, 1999). The proposed ligand for DhkA is SDF-2, a factor that is secreted by prestalk cells to induce the maturation of spores (Anjard *et al.*, 1998; Wang *et al.*, 1999). *dhkA* null mutants are defective in spore maturation and this defect can be suppressed by abrogation of RegA function or constitutive activation of PKA. This suggests that RegA and PKA act downstream of DhkA in a scenario where binding of SDF-2 to DhkA inhibits RegA (Wang *et al.*, 1999) and thereby permits PKA activation and spore maturation (Figure 4A). The proposed ligand for the sensor domain of DhkB is discadenin (Figure 4B), an inhibitor of spore germination that is released in the spore head (Zinda and Singleton, 1998). The spores of
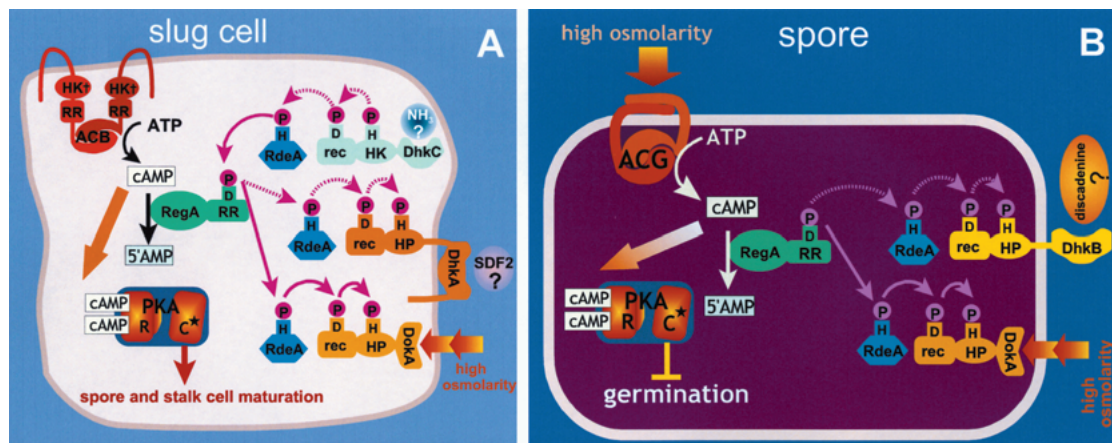
Fig. 4. Network for regulation of terminal differentiation and spore dormancy: (A) In slug cells cAMP is predominantly produced by adenylyl cyclase B, which harbours a single cyclase domain, a response regulator (RR) and a histidine kinase homology domain (HK) that is probably not functional (†). cAMP activates PKA, which will induce the terminal differentiation of spore and stalk cells. cAMP levels are stringently regulated by the cAMP-phosphodiesterase RegA, which is active when phosphorylated on Asp^{212} in its response regulator domain (RR). The phosphorylated state of RegA is controlled by histidine kinases/phosphatases with attached sensor domain. The phosphotransfer protein RdeA acts as intermediate for bidirectional phosphorelay. DhkC functions as a histidine kinase, which is activated by binding of its supposed ligand NH_3 to the sensor domain. DhkC first autophosphorylates itself on a conserved histidine. The phosphate is then relayed to a conserved aspartate in the DhkC receiver domain (rec) and subsequently *via* RdeA to RegA, thereby activating RegA. DhkA and DokA function as histidine phosphatases (HP) when sensing their supposed stimuli SDF-2 and high osmolarity. They reverse the direction of phosphorelay by acting as phosphate sinks and inactivate RegA. (B) In spores cAMP is mainly produced by ACG. RegA also controls cAMP levels at this stage and is negatively regulated by DhkB, which is proposed to act as a histidine phosphatase when bound to discadenine, an inhibitor of spore germination. DokA may act in parallel to ACG at the spore stage to maintain the dormant state under high osmolarity conditions. The phosphate transfers that are indicated by solid arrows in these figures have been confirmed by biochemical methods. The processes indicated by dashed arrows as well as all supposed ligands of the Dhk's are inferred from epistatic relationships, null mutant phenotypes and homologous processes in other organisms.

the *dhkB* null mutant germinate precociously in the spore head. This phenotype can be reversed by constitutive activation of PKA, indicating that also here PKA acts downstream of DhkB (Zinda and Singleton, 1998).

DokA is a third negative regulator of RegA and *dokA* null cells also form fruiting bodies without viable spores. This effect has not yet been pinpointed to either a defect in spore maturation or to precocious germination (Schuster *et al.*, 1996). The dokA genetic lesion also affects general cell physiology, because *dokA* null cells cannot withstand hyperosmolarity and do not show the increase in cAMP levels that wild-type cells show in response to osmotic shock. This increase is due to a DokA-triggered and RdeA-mediated inhibition of RegA activity and DokA was shown to dephosphorylate RdeA *in vitro* (Ott *et al.*, 2000). DokA contains a sensor domain, but no putative transmembrane domains and resides most likely in the cytosol (Schuster *et al.*, 1996). The absence of spores in *dokA* null fruiting bodies can have two causes: (i) high osmolarity acts as on DokA as a additional signal for spore maturation (Figure 4A) or (ii) high osmolarity acts on DokA and ACG in parallel to raise cAMP levels and inhibit (precocious) spore germination (Figure 4B). For all negative regulators of RegA, the ligands of the Dhk sensor domains probably act to revert the histidine kinase into a phosphatase, which turns them into phosphate sinks and revert the phosphorelay reaction (Ott *et al.*, 2000).

The identification of RegA and its interacting factors has provided the molecular framework to understand the signalling networks that regulate terminal differentiation and spore germination in *Dictyostelium*. However, it must be realized that the proposed roles of all histidine kinases/phosphatases have thus far only been inferred from epistatic relationships and that all the proposed ligands have yet to be shown to bind to specific Dhk sensor domains and activate catalytic activity.

In addition to effects on gene regulation, RegA null mutants show defects in oscillatory cAMP signalling and in chemotaxis (Wessels *et al.*, 2000). The signalling defect has been attributed to loss of negative feedback on cAMP production (Laub and Loomis, 1998). The chemotaxis defect is due to a failure to suppress lateral pseudopods, which normally contributes to polarized movement of cells in a chemotactic gradient (Wessels *et al.*, 2000). This points to an as yet undocumented role for intracellular cAMP in chemotaxis.

PdeD and PdeE

Two PDEs were identified by screens of the genomic databanks with consensus motifs for cyclic nucleotide binding domains (Goldberg *et al.*, 2002; Meima *et al.*, 2002; 2003) and named GbpA and GbpB by Goldberg *et al.* (2002) and PdeD and PdeE by Meima and coworkers. They both harbour two cyclic nucleotide binding motifs, preceded by a metallo-β-lactamase domain. Truncated proteins containing solely this motif displayed cyclic nucleotide phosphodiesterase activity

(Meima *et al.*, 2002). The *PdeD/GbpA* gene was found to encode the cGMP-stimulated cGMP phosphodiesterase that is lacking in *stmF* mutants (Ross and Newell, 1981; Bosgraaf *et al.*, 2002; Meima *et al.*, 2002). The second cGMP-specific phosphodiesterase, PDE3 is homologous to the mammalian PDEs (Kuwayama *et al.*, 2001). *PdeE/GbpB* encodes a hitherto unidentified cAMP-stimulated cAMP-phosphodiesterase activity, that shows very pronounced expression during aggregation. PdeE has a low affinity for cAMP as substrate (K_M = 1 mM), but is about four-fold stimulated by cAMP with a K_A of 3 μM. PdeE null mutants aggregate and develop normally. They show a modest augmentation of the cAMP relay response, which suggests that PdeE may contribute to the negative feedback loop of oscillatory cAMP signalling (Meima *et al.*, 2003; Bosgraaf *et al.*, 2002).

cAMP detection

cARs

Extracellular cAMP is detected by G-protein coupled serpentine receptors. Four receptors, in order, cAR1, cAR3, cAR2 and cAR4 are expressed during progressively later stages of development (Klein *et al.*, 1988; Johnson *et al.*, 1993; Saxe III *et al.*, 1993; Louis *et al.*, 1994). The major differences in amino-acid sequence between the receptors are localized in the carboxy-terminal cytoplasmic domain. The receptors show a decrease in affinity which correlates with their timing of expression, with the highest affinity receptor being expressed the earliest (Johnson *et al.*, 1992; Kim *et al.*, 1997a). All four cARs can couple to all investigated cAMP-activated signal transduction pathways, such as chemotaxis, ACA and guanylyl cyclase activation, Ca^{2+} influx and gene regulation. It is assumed that the expression of receptors with lower affinity during multicellular development reflects an adjustment to higher ambient cAMP levels in multicellular structures (Milne and Devreotes, 1993; Verkerke VanWijk *et al.*, 1998; Kim *et al.*, 1998b). Serpentine receptors typically use heterotrimeric G-proteins as intermediates for signal transduction and this is the case for cAR-mediated activation of chemotaxis, ACA, guanylyl cyclase and phospholipase C and for induction of aggregative gene expression. cAR mediated induction of Ca^{2+} influx, ERK2 activation, StatA translocation to the nucleus and prespore gene expression do not require heterotrimeric G-proteins (Milne *et al.*, 1995; Wu *et al.*, 1995; Maeda *et al.*, 1996; Araki *et al.*, 1998; Jin *et al.*, 1998). The immediate targets for cARs in these responses are still obscure.

PKA

PKA plays a very dominant role in regulation of development. The *Dictyostelium* PKA holoenzyme consist of one catalytic (PKA-C) and one regulatory

domain (PKA-R) (Mutzel et al., 1987). This differs from the vertebrate enzymes, which consist of dimers of each domain. Disruption of the *PKA-C* gene or overexpression of a dominant-negative form of *PKA-R* under constitutive or cell-type specific promoters has shown that PKA is required for the growth to development transition (Schulkes and Schaap, 1995; Endl et al., 1996; Mann et al., 1997), the control of developmental timing (Simon et al., 1992), the differentiation of prespore cells (Hopper *et al.*, 1993b), the terminal maturation of spores and stalk cells (Harwood *et al.*, 1992; Mann et al., 1994) and the control of spore germination (Van Es *et al.*, 1996). For most of these responses, the activation of the PKA-C is achieved through binding of cAMP to PKA-R, followed by dissociation of the R–C complex. However, at the onset of starvation PKA-C activity is also controlled by regulation of its translation. During growth, the translational regulator PufA binds to conserved motifs at the 3′ end of the PKA-C transcript, thereby inhibiting PKA-C translation (Souza et al., 1999). Growing cells secrete the prestarvation factor PSF at a constant rate and use the level of accumulated PSF to measure nutrient status relative to cell density (Clarke and Gomer, 1995). The high levels of PSF that accumulate when cells start to starve trigger the transcription of a protein kinase, YakA, which inhibits the function of PufA, allowing PKA-C to accumulate. YakA also induces cell cycle arrest independently from its effects on PufA. The two effects cause the cells to stop growing and initiate multicellular development (Souza et al., 1999).

Recent work shows that YakA can be directly activated by a variety of stress factors, including heat and oxidative stress, which results in growth arrest and PKA activation (Taminato et al., 2002). In combination with earlier work showing that osmotic shock can cause PKA activation both through RegA repression (Ott et al., 2000) and ACG activation (Van Es et al., 1996), these data highlight that *Dictyostelium* development is in essence a stress response.

Future challenges

Gene regulatory pathways

Recent years have seen major advances in understanding the interpretation of cAMP as a chemotactic signal by the cell and the cytoskeletal reorganization that is instrumental for coordinated cell movement. With respect to the control of gene expression by extracellular cAMP our knowledge is still fragmentary. Tagged mutagenesis has proven to be an excellent tool to uncover many novel components of the gene regulatory pathways (Kuspa and Loomis, 1992), but it has not yet been possible to reconstruct a single pathway from cAR activation to gene transcription. Screens for suppressor mutants have been used to great advantage to find interacting partners in signal transduction (Shaulsky

et al., 1996; Bear et al., 1998; Souza et al., 1999) and the use of DNA microarrays may systematically identify all proteins with altered expression in a specific mutant or during a specific gene induction response (Van Driessche et al., 2002). In combination with more classical molecular genetic-, immunological- and biochemical tools to detect protein–protein interactions, these strategies should allow us to find the missing links within the next 10 years.

Targets for PKA and compartmentalization of signalling

Despite the prominent role for PKA at virtually all stages of development, the direct targets are thus far unknown. There is genetic evidence for the regulation by PKA of the transcription factor Myb2 and the nuclear factor CudA, but evidence for direct phosphorylation by PKA is lacking (Fukuzawa et al., 1997; Otsuka and Van-Haastert, 1998). PKA activity has been found both in the cytosol and the nucleus (Woffendin et al., 1986), suggesting compartmentalization of cAMP signalling. In metazoans, PKA is targeted to different subcellular locations by a family of A-kinase anchoring proteins (AKAPs), that bind to the dimerization domain of PKA-R, and to a wide variety of other signalling molecules (Houslay and Milligan, 1997; Feliciello et al., 2001). For example, muscle-selective AKAP forms a signalling module in which the activity of the cAMP-specific phosphodiesterase PDE4D3 is regulated by the anchored PKA holoenzyme (Dodge et al., 2001). In *Dictyostelium*, there is biochemical evidence for the presence of cAMP signalling modules. PKA-R binds to RegA and stimulates its activity at least 18-fold (Shaulsky et al., 1998). RegA only weakly affects cAMP production by ACA, but effectively annihilates the levels of cAMP produced by ACB, suggesting a close proximity between ACB and RegA (Kim et al., 1998a; Meima and Schaap, 1999). It is tempting to suggest the presence of a signalling complex of these enzymes that would allow tight control of cAMP production until the proper signals for terminal differentiation are detected. No AKAPs have been identified in the *Dictyostelium* genome so far and the *Dictyostelium* PKA-R subunit lacks the dimerization domain, that is essential for binding to AKAPs. However, the different vertebrate AKAP families show similarity in secondary structure rather than in protein sequence (Feliciello et al., 2001). Therefore, the presence of functional homologues with different target binding sites in *Dictyostelium* cannot be excluded.

References

Abe K and Yanagisawa K (1983) A new class of rapid developing mutants in *Dictyostelium discoideum*: implications for cyclic AMP metabolism and cell differentiation. Dev Biol **95**: 200–210.

Anjard C, Zeng CJ, Loomis WF and Nellen W (1998) Signal transduction pathways leading to spore differentiation in *Dictyostelium discoideum*. Dev Biol **193**: 146–155.

Araki T, Gamper M, Early A, Fukuzawa M, Abe T, Kawata T, Kim E, Firtel RA and Williams JG (1998) Developmentally and spatially regulated activation of a *Dictyostelium* STAT protein by a serpentine receptor. *EMBO J* **17**: 4018–4028.

Bear JE, Rawls JF and Saxe CL (1998) Scar, a WASP-related protein, isolated as a suppressor of receptor defects in late *Dictyostelium* development. *J Cell Biol* **142**: 1325–1335.

Berks M and Kay RR (1988) Cyclic AMP is an inhibitor of stalk cell differentiation in *Dictyostelium discoideum*. *Dev Biol* **126**: 108–114.

Bosgraaf L, Russcher H, Snippe H, Bader S, Wind I and Van Haastert PJM (2002) Identification and characterization of two unusual cGMP-stimulated phosphodiesterases in Dictyostelium. *Mol Biol Cell* **13**: 3878–3889.

Brzostowski J, Johnson C and Kimmel A (2002) Galpha-mediated inhibition of developmental signal response. *Curr Biol* **12**: 1199–1208.

Chang WT, Thomason PA, Gross JD and Newell PC (1998) Evidence that the RdeA protein is a component of a multistep phosphorelay modulating rate of development in *Dictyostelium*. *EMBO J* **17**: 2809–2816.

Chen Y, Cann MJ, Litvin TN, Iourgenko V, Sinclair ML, Levin LR and Buck J (2000) Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. *Science* **289**: 625–628.

Chen MY, Long Y and Devreotes PN (1997) A novel cytosolic regulator, pianissimo, is required for chemoattractant receptor and G protein-mediated activation of the 12 transmembrane domain adenylyl cyclase in *Dictyostelium*. *Genes Dev* **11**: 3218–3231.

Clarke M and Gomer RH (1995) PSF and CMF, autocrine factors that regulate gene expression during growth and early development of *Dictyostelium*. *Experientia* **51**: 1124–1134.

Cotter DA, Dunbar AJ, Buconjic SD and Wheldrake JF (1999) Ammonium phosphate in sori of *Dictyostelium discoideum* promotes spore dormancy through stimulation of the osmosensor ACG. *Microbiol* **145**: 1891–1901.

Danchin A (1993) Phylogeny of adenylyl cyclases. *Adv Second Messenger Phosphoprotein Res* **27**: 109–162.

Darmon M, Barra J and Brachet P (1978) The role of phosphodiesterase in aggregation of *Dictyostelium discodeum*. *J Cell Sci* **31**: 233–243.

Devreotes PN and Steck TL (1979) Cyclic 3′5′ AMP relay in *Dictyostelium discoideum*. II Requirements for the initiation and termination of the response. *J Cell Biol* **80**: 300–309.

Dodge KL, Khouangsathiene S, Kapiloff MS, Mouton R, Hill EV, Houslay MD, Langeberg LK and Scott JD (2001) mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module. *Embo J* **20**: 1921–1930.

Dormann D, Weijer G, Parent C, Devreotes P and Weijer C (2002) Visualizing PI3 kinase-mediated cell–cell signaling during *Dictyostelium* development. *Curr Biol* **12**: 1178.

Endl I, Konzok A and Nellen W (1996) Antagonistic effects of signal transduction by intracellular and extracellular cAMP on gene regulation in *Dictyostelium*. *Mol Biol Cell* **7**: 17–24.

Faure M, Franke J, Hall AL, Podgorski GJ and Kessin RH (1990) The cyclic nucleotide phosphodiesterase gene of *Dictyostelium discoideum* contains three promoters specific for growth, aggregation, and late development. *Mol Cell Biol* **10**: 1921–1930.

Faure M, Podgorski GJ, Franke J and Kessin RH (1988) Disruption of *Dictyostelium discoideum* morphogenesis by overproduction of cAMP phosphodiesterase. *Proc Natl Acad Sci USA* **85**: 8076–8080.

Feliciello A, Gottesman ME and Avvedimento EV (2001) The biological functions of A-kinase anchor proteins. *J Mol Biol* **308**: 99–114.

Ferguson SSG and Caron MG (1998) G protein-coupled receptor adaptation mechanisms. *Semin Cell Dev Biol* **9**: 119–127.

Franke J and Kessin RH (1981) The cyclic nucleotide phosphodiesterase inhibitory protein of *Dictyostelium discoideum*. Purification and characterization. *J Biol Chem* **256**: 7628–7637.

Fukuzawa M, Hopper N and Williams J (1997) *CudA*: a *Dictyostelium* gene with pleiotropic effects on cellular differentiation and slug behaviour. *Development* **124**: 2719–2728.

Gerisch G (1976) Extracellular cyclic-AMP phosphodiesterase regulation in agar plate cultures of *Dictyostelium discoideum*. *Cell Differ* **5**: 21–25.

Gerisch G, Fromm H, Huesgen A and Wick U (1975) Control of cell-contact sites by cyclic AMP pulses in differentiating *Dictyostelium* cells. *Nature* **255**: 547–549.

Goldberg JM, Bosgraaf L, Van Haastert PJM and Smith JL (2002) Identification of four candidate cGMP targets in *Dictyostelium*. *Proc Natl Acad Sci USA* **99**: 6749–6754.

Hall AL, Franke J, Faure M and Kessin RH (1993) The role of the cyclic nucleotide phosphodiesterase of *Dictyostelium discoideum* during growth, aggregation, and morphogenesis: overexpression and localization studies with the separate promoters of the pde. *Dev Biol* **157**: 73–84.

Hamm HE (1998) The many faces of G protein signaling. *J Biol Chem* **273**: 669–672.

Harwood AJ, Hopper NA, Simon M-N, Driscoll DM, Veron M and Williams JG (1992) Culmination in *Dictyostelium* is regulated by the cAMP-dependent protein kinase. *Cell* **69**: 615–624.

Hopper NA, Anjard C, Reymond CD and Williams JG (1993a) Induction of terminal differentiation of *Dictyostelium* by cAMP-dependent protein kinase and opposing effects of intracellular and extracellular cAMP on stalk cell differentiation. *Development* **119**: 147–154.

Hopper NA, Harwood AJ, Bouzid S, Véron M and Williams JG (1993b) Activation of the prespore and spore cell pathway of *Dictyostelium* differentiation by cAMP-dependent protein kinase and evidence for its upstream regulation by ammonia. *EMBO J* **12**: 2459–2466.

Houslay MD and Milligan G (1997) Tailoring cAMP-signalling responses through isoform multiplicity. *Trends Biochem Sci* **22**: 217–224.

Hurley JH (1998) The adenylyl and guanylyl cyclase superfamily. *Curr Opin Struct Biol* **8**: 770–777.

Insall RH, Borleis J and Devreotes PN (1996) The aimless RasGEF is required for processing of chemotactic signals through G-protein-coupled receptors in *Dictyostelium*. *Curr Biol* **6**: 719–729.

Insall R, Kuspa A, Lilly PJ, Shaulsky G, Levin LR, Loomis WF and Devreotes P (1994) CRAC, a cytosolic protein containing a pleckstrin homology domain, is required for receptor and G-protein-mediated activation of adenylyl cyclase in *Dictyostelium*. *J Cell Biol* **126**: 1537–1545.

Jin T, Soede RDM, Liu JC, Kimmel AR, Devreotes PN and Schaap P (1998) Temperature-sensitive G beta mutants discriminate between G protein-dependent and -independent signaling mediated by serpentine receptors. *EMBO J* **17**: 5076–5084.

Johnson RL, Saxe III CL, Gollop R, Kimmel AR and Devreotes PN (1993) Identification and targeted gene disruption of cAR3, a cAMP receptor subtype expressed during multicellular stages of *Dictyostelium* development. *Genes Dev* **7**: 273–282.

Johnson RL, Van Haastert PJM, Kimmel AR, Saxe III CL, Jastorff B and Devreotes PN (1992) The cyclic nucleotide specificity of three cAMP receptors in *Dictyostelium*. *J Biol Chem* **267**: 4600–4607.

Kasahara M and Ohmori M (1999) Activation of a cyanobacterial adenylate cyclase, CyaC, by autophosphorylation and a subsequent phosphotransfer reaction. *J Biol Chem* **274**: 15,167–15,172.

Kay RR (1982) cAMP and spore differentiation in *Dictyostelium discoideum*. *Proc Natl Acad Sci USA* **79**: 3228–3231.

Kesbeke F, Snaar-Jagalska BE and Van Haastert PJM (1988) Signal transduction in *Dictyostelium fgd A* mutants with a defective interaction between surface cAMP receptors and a GTP-binding regulatory protein. *J Cell Biol* **107**: 521–528.

Kessin RH, Orlow SJ, Shapiro RI and Franke J (1979) Binding of inhibitor alters kinetic and physical properties of extracellular cyclic AMP phosphodiesterase from *Dictyostelium discoideum*. *Proc Natl Acad Sci USA* **76**: 5450–5454.

Kim JY, Borleis JA and Devreotes PN (1998b) Switching of chemoattractant receptors programs development and morphogenesis in *Dictyostelium*: receptor subtypes activate common responses at different agonist concentrations. *Dev l Biol* **197**: 117–128.

Kim J-Y, Caterina MJ, Milne JLS, Lin KC, Borleis JA and Devreotes PN (1997a) Random mutagenesis of the cAMP chemoattractant receptor cAR1, of *Dictyostelium*. *J Biol Chem* **272**: 2060–2068.

Kim HJ, Chang WT, Meima M, Gross JD and Schaap P (1998a) A novel adenylyl cyclase detected in rapidly developing mutants of *Dictyostelium*. *J Biol Chem* **273**: 30,859–30,862.

Kim J-Y, Soede RDM, Schaap P, Valkema R, Borleis JA, Van-Haastert PJM, Devreotes PN and Hereld D (1997b) Phosphorylation of chemoattractant receptors is not essential for chemotaxis or termination of G-protein-mediated responses. *J Biol Chem* **272**: 27,313–27,318.

Klein PS, Sun TJ, Saxe III CL, Kimmel AR, Johnson RL and Devreotes PN (1988) A chemoattractant receptor controls development in *Dictyostelium discoideum*. *Science* **241**: 1467–1472.

Klein P, Theibert A, Fontana D and Devreotes PN (1985) Identification and cyclic AMP-induced modification of the cyclic AMP receptor in *Dictyostelium discoideum*. *J Biol Chem* **260**: 1757–1764.

Konijn TM, Van De Meene JG, Bonner JT and Barkley DS (1967) The acrasin activity of adenosine-3′,5′-cyclic phosphate. *Proc Natl Acad Sci USA* **58**: 1152–1154.

Kumagai A, Pupillo M, Gundersen R, Miake-Lye R, Devreotes PN and Firtel RA (1989) Regulation and function of Galpha protein subunits in *Dictyostelium*. *Cell* **57**: 265–275.

Kuspa A and Loomis WF (1992) Tagging developmental genes in *Dictyostelium* by restriction enzyme-mediated integration of plasmid DNA. *Proc Natl Acad Sci USA* **89**: 8803–8807.

Kuwayama H, Snippe H, Derks M, Roelofs J and Van Haastert PJM (2001) Identification and characterization of DdPDE3, a cGMP-selective phosphodiesterase from *Dictyostelium*. *Biochem J* **353**: 635–644.

Laub MT and Loomis WF (1998) A molecular network that produces spontaneous oscillations in excitable cells of *Dictyostelium*. *Mol Biol Cell* **9**: 3521–3532.

Lee S, Parent CA, Insall R and Firtel RA (1999) A novel Ras-interacting protein required for chemotaxis and cyclic adenosine monophosphate signal relay in *Dictyostelium*. *Mol Biol Cell* **10**: 2829–2845.

Lilly PJ and Devreotes PN (1994) Identification of CRAC, a cytosolic regulator required for guanine nucleotide stimulation of adenylyl cyclase in *Dictyostelium*. *J Biol Chem* **269**: 14,123–14,129.

Lilly P, Wu L, Welker DL and Devreotes PN (1993) A G-protein beta-subunit is essential for *Dictyostelium* development. *Genes Dev* **7**: 986–995.

Lim CJ, Spiegelman GB and Weeks G (2001) RasC is required for optimal activation of adenylyl cyclase and Akt/PKB during aggregation. *EMBO J* **20**: 4490–4499.

Louis JM, Ginsburg GT and Kimmel AR (1994) The cAMP receptor CAR4 regulates axial patterning and cellular differentiation during late development of *Dictyostelium*. *Genes Dev* **8**: 2086–2096.

Maeda M, Aubry L, Insall R, Gaskins C, Devreotes PN and Firtel RA (1996) Seven helix chemoattractant receptors transiently stimulate mitogen-activated protein kinase in *Dictyostelium*. *J Biol Chem* **271**: 3351–3354.

Malchow D, Nagele B, Schwartz H and Gerisch G (1972) Membrane-bound cyclic AMP phosphodiesterase in chemotactically responding cells of *Dictyostelium discoideum*. *Eur J Biochem* **28**: 136–142.

Mann SKO, Brown JM, Briscoe C, Parent C, Pitt G, Devreotes PN and Firtel RA (1997) Role of cAMP-dependent protein kinase in controlling aggregation and postaggregative development in *Dictyostelium*. *Dev Biol* **183**: 208–221.

Mann SKO and Firtel RA (1993) cAMP-dependent protein kinase differentially regulates prestalk and prespore differentiation during *Dictyostelium* development. *Development* **119**: 135–146.

Mann SKO, Richardson DL, Lee S, Kimmel AR and Firtel RA (1994) Expression of cAMP-dependent protein kinase in prespore cells is sufficient to induce spore cell differentiation in *Dictyostelium*. *Proc Natl Acad Sci USA* **91**: 10,561–10,565.

Martiel J-L and Goldbeter A (1987) A model based on receptor desensitization for cyclic AMP signaling in *Dictyostelium* cells. *Biophys J* **52**: 807–828.

Mehats C, Andersen CB, Filopanti M, Jin SLC and Conti M (2002) Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends Endocrinol Metab* **13**: 29–35.

Meima ME, Biondi RM and Schaap P (2002) Identification of a novel type of cGMP phosphodiesterase that is defective in the chemotactive *stmF* mutants. *Mol Biol Cell* **13**: 3870–3877.

Meima ME and Schaap P (1999) Fingerprinting of adenylyl cyclase activities during *Dictyostelium* development indicates a dominant role for adenylyl cyclase B in terminal differentiation. *Dev Biol* **212**: 182–190.

Meima ME, Weening KE and Schaap P (2003) Characterization of a cAMP-stimulated cAMP phosphodiesterase in *Dictyostelium discoideum*. *J Biol Chem* (in press).

Milne JL and Devreotes PN (1993) The surface cyclic AMP receptors, cAR1, cAR2, and cAR3, promote Ca^{2+} influx in *Dictyostelium discoideum* by a Gα2-independent mechanism. *Mol Biol Cell* **4**: 283–292.

Milne JLS, Wu L, Caterina MJ and Devreotes PN (1995) Seven helix cAMP receptors stimulate Ca^{2+} entry in the absence of functional G proteins in *Dictyostelium*. *J Biol Chem* **270**: 5926–5931.

Mohanty S, Lee S, Yadava N, Dealy MJ, Johnson RS and Firtel RA (2001) Regulated protein degradation controls PKA function and cell-type differentiation in *Dictyostelium*. *Genes Dev* **15**: 1435–1448.

Mutzel R, Lacombe M-L, Simon M-N, De Gunzburg J and Veron M (1987) Cloning and cDNA sequence of the regulatory subunit of cAMP-dependent protein kinase from *Dictyostelium discoideum*. *Proc Natl Acad Sci USA* **84**: 6–10.

Nikawa J, Sass P and Wigler M (1987) Cloning and characterization of the low-affinity cyclic AMP phosphodiesterase gene of *Saccharomyces cerevisiae*. *Mol Cell Biol* **7**: 3629–3636.

Ohno S (1997) The reason for as well as the consequence of the Cambrian explosion in animal evolution. *J Mol Evol* **44**: S23–S27.

Otsuka H and Van Haastert PJM (1998) A novel myb homolog initiates *Dictyostelium* development by induction of adenylyl cyclase expression. *Genes Dev* **12**: 1738–1748.

Ott A, Oehme F, Keller H and Schuster SC (2000) Osmotic stress response in *Dictyostelium* is mediated by cAMP. *EMBO J* **19**: 5782–5792.

Parent CA and Devreotes PN (1996) Constitutively active adenylyl cyclase mutant requires neither G proteins nor cytosolic regulators. *J Biol Chem* **271**: 18,333–18,336.

Pitt GS, Brandt R, Lin KC, Devreotes PN and Schaap P (1993) Extracellular cAMP is sufficient to restore developmental gene expression and morphogenesis in *Dictyostelium* cells lacking the aggregation adenylyl cyclase (ACA). *Genes Dev* **7**: 2172–2180.

Pitt GS, Milona N, Borleis J, Lin KC, Reed RR and Devreotes PN (1992) Structurally distinct and stage-specific adenylyl cyclase genes play different roles in *Dictyostelium* development. *Cell* **69**: 305–315.

Raper KB (1940) Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *J Elisha Mitchell Scient Soc* **56**: 241–282.

Riedel V and Gerisch G (1971) Regulation of extracellular cyclic-AMP-phosphodiesterase activity during development of *Dictyostelium discoideum*. *Biochem Biophys Res Commun* **42**: 119–124.

Ross FM and Newell PC (1981) Streamers: chemotactic mutants of *Dictyostelium discoideum* with altered cyclic GMP metabolism. *J Gen Microbiol* **127**: 339–350.

Santos JL and Shiozaki K (2001) Fungal histidine kinases. *Sci STKE* **2001**: RE1.

Saxe III CL, Ginsburg GT, Louis JM, Johnson R, Devreotes PN and Kimmel AR (1993) CAR2, a prestalk cAMP receptor required for normal tip formation and late development of *Dictyostelium discoideum*. *Genes Dev* **7**: 262–272.

Schaap P and Van Driel R (1985) Induction of post-aggregative differentiation in *Dictyostelium discoideum* by cAMP. Evidence for involvement of the cell surface cAMP receptor. *Exp Cell Res* **159**: 388–398.

Schindler J and Sussman M (1977) Ammonia determines the choice of morphogenetic pathways in *Dictyostelium discoideum*. *J Mol Biol* **116**: 161–169.

Schulkes C and Schaap P (1995) cAMP-dependent protein kinase activity is essential for preaggregative gene expression in *Dictyostelium*. *FEBS Lett* **368**: 381–384.

Schuster SC, Noegel AA, Oehme F, Gerisch G and Simon MI (1996) The hybrid histidine kinase DokA is part of the osmotic response system of *Dictyostelium*. *EMBO J* **15**: 3880–3889.

Schwindinger WF and Robishaw JD (2001) Heterotrimeric G-protein βγ-dimers in growth and differentiation. *Oncogene* **20**: 1653–1660.

Segall JE, Kuspa A, Shaulsky G, Ecke M, Maeda M, Gaskins C, Firtel RA and Loomis WF (1995) A MAP kinase necessary for receptor-mediated activation of adenylyl cyclase in *Dictyostelium*. *J Cell Biol* **128**: 405–413.

Shaulsky G, Escalante R and Loomis WF (1996) Developmental signal transduction pathways uncovered by genetic suppressors. *Proc Natl Acad Sci USA* **93**: 15,260–15,265.

Shaulsky G, Fuller D and Loomis WF (1998) A cAMP-phosphodiesterase controls PKA-dependent differentiation. *Development* **125**: 691–699.

Siegert F and Weijer CJ (1992) Three-dimensional scroll waves organize *Dictyostelium* slugs. *Proc Natl Acad Sci USA* **89**: 6433–6437.

Simon M-N, Pelegrini O, Veron M and Kay RR (1992) Mutation of protein kinase A causes heterochronic development of *Dictyostelium*. *Nature* **356**: 171–172.

Singleton CK, Zinda MJ, Mykytka B and Yang P (1998) The histidine kinase DhkC regulates the choice between migrating slugs and terminal differentiation in *Dictyostelium discoideum*. *Dev Biol* **203**: 345–357.

Soderbom F, Anjard C, Iranfar N, Fuller D and Loomis WF (1999) An adenylyl cyclase that functions during late development of *Dictyostelium*. *Development* **126**: 5463–5471.

Souza GM, daSilva AM and Kuspa A (1999) Starvation promotes *Dictyostelium* development by relieving PufA inhibition of PKA translation through the YakA kinase pathway. *Development* **126**: 3263–3274.

Sucgang R, Weijer CJ, Siegert F, Franke J and Kessin RH (1997) Null mutations of the *Dictyostelium* cyclic nucleotide phosphodiesterase gene block chemotactic cell movement in developing aggregates. *Dev Biol* **192**: 181–192.

Taminato A, Bagattini R, Gorjao R, Chen G, Kuspa A and Souza GM (2002) Role for YakA, cAMP, and protein kinase A in regulation of stress responses of *Dictyostelium discoideum* cells. *Mol Biol Cell* **13**: 2266–2275.

Tang Y and Othmer HG (1994) A G protein-based model of adaptation in *Dictyostelium discoideum*. *Math Biosciences* **120**: 25–76.

Theibert A and Devreotes P (1986) Surface receptor-mediated activation of adenylate cyclase in *Dictyostelium*. *J Biol Chem* **261**: 15,121–15,125.

Thomason PA, Traynor D, Cavet G, Chang W-T, Harwood AJ and Kay RR (1998) An intersection of the cAMP/PKA and two-component signal transduction systems in *Dictyostelium*. *EMBO J* **17**: 2838–2845.

Thomason PA, Traynor D, Stock JB and Kay RR (1999) The RdeA–RegA system, a eukaryotic phospho-relay controlling cAMP breakdown. *J Biol Chem* **274**: 27,379–27,384.

Tsujioka M, Yokoyama M, Nishio K, Kuwayama H, Morio T, Katoh M, Urushihara H, Saito T, Ochiai H, Tanaka Y, Takeuchi I and Maeda M (2001) Spatial expression patterns of genes involved in cyclic AMP responses in *Dictyostelium discoideum* development. *Dev Growth Differ* **43**: 275–283.

Van Driessche N, Shaw C, Katoh M, Morio T, Sucgang R, Ibarra M, Kuwayama H, Saito T, Urushihara H, Maeda M, Takeuchi I, Ochiai H, Eaton W, Tollett J, Halter J, Kuspa A, Tanaka Y and Shaulsky G (2002) A transcriptional profile of multicellular development in *Dictyostelium discoideum*. *Development* **129**: 1543–1552.

Van Es S, Virdy KJ, Pitt GS, Meima M, Sands TW, Devreotes PN, Cotter DA and Schaap P (1996) Adenylyl cyclase G, an osmosensor controlling germination of *Dictyostelium* spores. *J Biol Chem* **271**: 23,623–23,625.

Verkerke-van Wijk I, Fukuzawa M, Devreotes PN and Schaap P (2001) Adenylyl cyclase A expression is tip-specific in *Dictyostelium* slugs and directs StatA nuclear translocation and CudA gene expression. *Dev Biol* **234**: 151–160.

Verkerke VanWijk I, Kim JY, Brandt R, Devreotes PN and Schaap P (1998) Functional promiscuity of gene regulation by serpentine receptors in *Dictyostelium discoideum*. *Mol Cell Biol* **18**: 5744–5749.

Virdy KJ, Sands TW, Kopko SH, vanEs S, Meima M, Schaap P and Cotter DA (1999) High cAMP in spores of *Dictyostelium discoideum*: association with spore dormancy and inhibition of germination. *Microbiology* **145**: 1883–1890.

Wang M and Schaap P (1989) Ammonia depletion and DIF trigger stalk cell differentiation in intact *Dictyostelium discoideum* slugs. *Development* **105**: 569–574.

Wang N, Soderbom F, Anjard C, Shaulsky G and Loomis WF (1999) SDF-2 induction of terminal differentiation in *Dictyostelium discoideum* is mediated by the membrane-spanning sensor kinase DhkA. *Mol Cell Biol* **19**: 4750–4756.

Wessels DJ, Zhang H, Reynolds J, Daniels K, Heid P, Lu S, Kuspa A, Shaulsky G, Loomis WF and Soll DR (2000) The internal phosphodiesterase RegA is essential for the suppression of lateral pseudopods during *Dictyostelium* chemotaxis. *Mol Biol Cell* **11**: 2803–2820.

Woffendin C, Chambers TC, Schaller KL, Leichtling BH and Rickenberg HV (1986) Translocation of cAMP-dependent protein kinase to the nucleus during development of *Dictyostelium discoideum*. *Dev Biol* **115**: 1–8.

Wu L and Franke J (1990) A developmentally regulated and cAMP-repressible gene of *Dictyostelium discoideum*: cloning and expression of the gene encoding cyclic nucleotide phosphodiesterase inhibitor. *Gene* **91**: 51–56.

Wu L, Valkema R, Van Haastert PJM and Devreotes PN (1995) The G protein β-subunit is essential for multiple responses to chemoattractants in *Dictyostelium*. *J Cell Biol* **129**: 1667–1675.

Yeh KC, Wu SH, Murphy JT and Lagarias JC (1997) A cyanobacterial phytochrome two-component light sensory system. *Science* **277**: 1505–1508.

Zinda MJ and Singleton CK (1998) The hybrid histidine kinase DhkB regulates spore germination in *Dictyostelium discoideum*. *Dev Biol* **196**: 171–183.