Cell-cell adhesion and signal transduction during *Dictyostelium* development

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Summary

The development of the non-metazoan eukaryote *Dictyostelium discoideum* displays many of the features of animal embryogenesis, including regulated cell-cell adhesion. During early development, two proteins, DdCAD-1 and csA, mediate cell-cell adhesion between amoebae as they form a loosely packed multicellular mass. The mechanism governing this process is similar to epithelial sheet sealing in animals. Although cell differentiation can occur in the absence of cell contact, regulated cell-cell adhesion is an important component of *Dictyostelium* morphogenesis, and a third adhesion molecule, gp150, is required for multicellular development past the aggregation stage.

Cell-cell junctions that appear to be adherens junctions form during the late stages of *Dictyostelium* development. Although they are not essential to establish the basic multicellular body plan, these junctions are required to maintain the structural integrity of the fruiting body. The *Dictyostelium* β-catenin homologue Aardvark (Aar) is present in adherens junctions, which are lost in its absence. As in the case of its metazoan counterparts, Aar also has a function in cell signalling and regulates expression of the pre-spore gene *psA*.

It is becoming clear that cell-cell adhesion is an integral part of *Dictyostelium* development. As in animals, cell adhesion molecules have a mechanical function and may also interact with the signal-transduction processes governing morphogenesis.

Key words: Cell adhesion, *Dictyostelium*, Signalling, β-catenin, GSK-3

Introduction

In animals, cell-cell adhesion is responsible for the mechanical forces that regulate cell shape, cell motility and tissue structure. In addition to playing this architectural role, the protein complexes found at points of cell contact are integrated into cellular signalling pathways (Braga and Harwood, 2001). These pathways regulate the creation and remodelling of cell contacts and can also generate their own intracellular signalling activity. Some junctional proteins, such as β-catenin, appear to have separate roles, mediating both cell adhesion and signal transduction. The interface with signal transduction underlies the interaction between cell adhesion, differentiation, proliferation and apotosis. Loss of cell contact not only affects tissue morphogenesis but also is linked with metastasis. There are still major gaps in our understanding of adhesion in animals, and many more adhesion systems remain to be investigated. One approach is to explore the evolutionary origins of cell contact and to examine the features that non-metazoan adhesion systems and their animal counterparts share. One promising organism for this type of study is *Dictyostelium* (Harwood, 2001; Kessin, 2001)

Dictyostelium discoideum is a eukaryote that is related to animals and fungi, a position it shares with Acanthamoebae and the acellular slime moulds, such as *Physarum polycephalum* (Baldauf et al., 2000). It has proved to be a good organism in which to study many cell biological processes, especially cell movement, chemotaxis and phagocytosis (Cardelli, 2001; Firtel and Chung, 2000). It is now apparent that *Dictyostelids* possess signal-transduction pathways that are closely related to those of metazoa. In addition, *Dictyostelium* cells undergo a relatively simple programme of multicellular development, which in many ways resembles animal development. The study of cell adhesion straddles both cell and developmental biology; although at one time a strong area of *Dictyostelium* research, it has had relatively little impact on our understanding of *Dictyostelium* development. We are now entering a renaissance in this area, following the discovery of several *Dictyostelium* adhesion systems that are related to those of animals and involved in a level of developmental complexity that has only become apparent recently.

Cell adhesion during early development

Dictyostelium cells have adopted a strategy for multicellular development that differs from that of metazoa (Fig. 1). In their vegetative stage, *Dictyostelium* cells are single-celled amoebae that feed on bacteria and multiply by binary fission. The striking feature of *Dictyostelium* amoebae is that, when their food source is depleted, they undergo a switch in behaviour to form a fruiting body. This is a highly differentiated multicellular structure composed of spore cells supported by a skeleton of stalk cells that are arranged as a stalk and a basal disc, which anchors the stalk to the substratum. Although the fruiting body possesses only a small number of cell types, its development shows much of the complexity seen in metazoa.

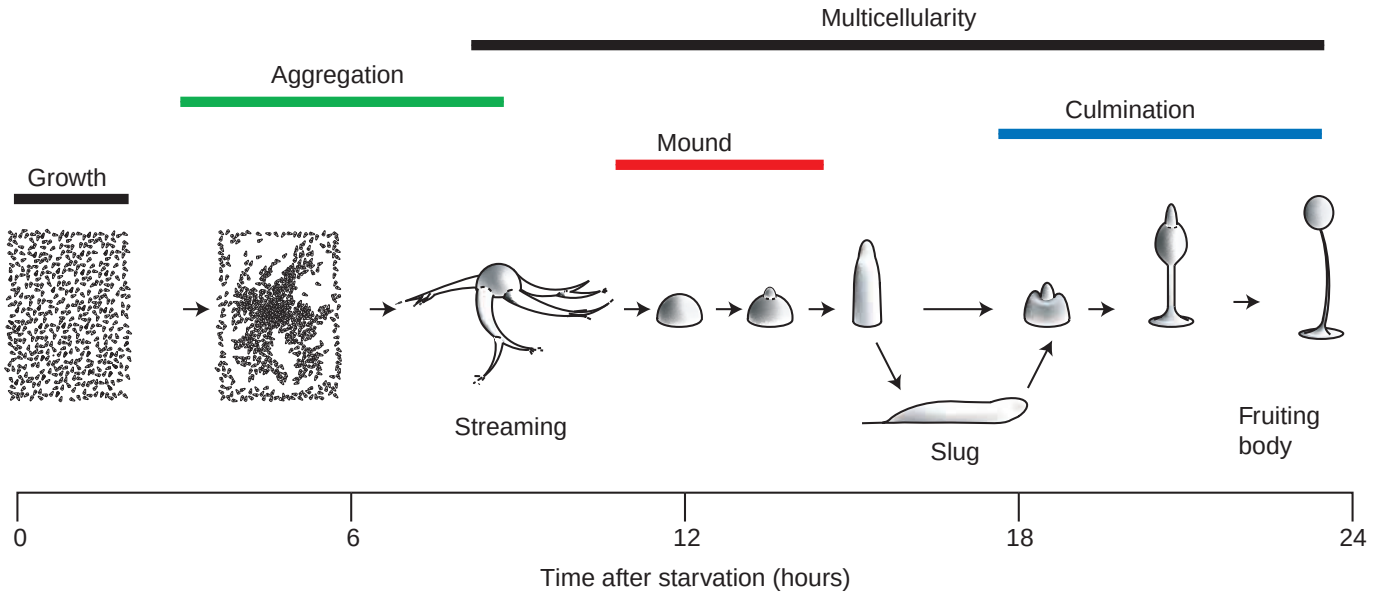

Fig. 1. *Dictyostelium* development. Amoebae proliferate as single cells during the growth phase. Upon starvation, amoebae undergo chemotaxis towards a pulsatile cyclic AMP (cAMP) source. During aggregation, cells coalesce into adherent cell 'streams' that eventually come together to form the mound, the first stage of multicellular development. The mound compacts to form a tight aggregate and then develops a 'tip', which coordinates further development. After extension to form the first finger, the developing structure either immediately forms a fruiting body, the process of culmination or forms a motile slug that migrates to seek conditions favourable for culmination. Scale shows relative timing of development.

There is one fundamental difference: animals develop from a single cell by a combination of growth and differentiation. *Dictyostelium* development, however, requires no growth, and multicellularity is achieved by aggregation of many unicellular amoebae. This greatly simplifies the study of development and provides an easy route to examine cell-cell adhesion through studies of aggregation and cell-surface binding

Dictyostelium cells must first aggregate to form a multicellular mass: the mound. The driving force behind this processes is chemotaxis towards a pulsatile source of extracellular cyclic AMP (cAMP). Initially, amoebae move as individual cells towards the signal. However, as they near the source, and cell density increases, cells coalesce into multicellular streams. These streams move coordinately towards the signalling centre to form a mass of up to 10^5 cells. As this mound forms, cells enter the multicellular stage of development and begin to differentiate into pre-spore and pre-stalk cells, the precursors of spore and stalk cells, respectively (Williams et al., 1989).

It has long been known that cells within streams adhere to each other, and pioneering work in *Dictyostelium* identified several proteins that mediate cell-cell adhesion (Barondes et al., 1982; Gerisch, 1961a; Gerisch, 1968). The first protein known to be expressed is DdCAD-1, also named contact sites B (csB) or gp24 (Beug et al., 1973; Yang et al., 1997). DdCAD-1 is expressed soon after the beginning of starvation and is not seen in cells growing on bacteria. Initially, it is present within the cytoplasm and is only slightly enriched at the plasma membrane. However, as aggregation proceeds, DdCAD-1 redistributes to the external surface of the plasma membrane (Sesaki and Siu, 1996). DdCAD-1-mediated cell adhesion is sensitive to both EDTA and EGTA, which suggests that Ca^{2+} is involved in this process (Garrod, 1972; Gerisch, 1961b). DdCAD-1 has been cloned, and it shares some homology with the extracellular domain of metazoan cadherins (Wong et al., 1996). It appears, however, to have two Ca^{2+}-binding domains. One of these domains may conform to the structure of the Ca^{2+}-binding region seen in cadherin proteins; the second domain is in the C-terminus and has similarity to the EF-hand Ca^{2+}-binding domain. DdCAD-1 also differs from classical animal cadherins by lacking a transmembrane domain, and appears to reach the cell surface through an unconventional route via the contractile vacuole (Sesaki et al., 1997). DdCAD-1 is a soluble protein; however, a substantial portion of secreted DdCAD-1 protein remains associated with the extracellular surface. This association is sensitive to Ca^{2+} chelation, and Siu et al., proposed that the C-terminal Ca^{2+}-binding domain might associate with an unidentified transmembrane linker protein (Siu et al., 1997). Antibodies raised to DdCAD-1 and recombinant DdCAD-1 fusion proteins both block the formation of EDTA-sensitive adhesive contacts between cells (Brar and Siu, 1993; Knecht et al., 1987; Wong et al., 1996). Furthermore, pre-treatment of cells with an anti-DdCAD-1 antibody blocks the binding of labelled recombinant DdCAD-1 to cells, which suggests a homophilic interaction between DdCAD-1 molecules on adjoining cells (Brar and Siu, 1993).

A second homophilic adhesion molecule is expressed during later stages of aggregation. This protein, csA (also known as gp80), has a mass of 54 kDa and is induced by the cAMP pulses that mediate chemotaxis (Faix et al., 1992; Muller and Gerisch, 1978). csA is a globular protein of the immunoglobulin superfamily and has similarity to the neural cell adhesion molecule N-CAM (Noegel et al., 1986; Wong and Siu, 1986). csA is heavily glycosylated, hence its apparent molecular weight of 80 kDa on SDS-PAGE. This modification is lost in the glycosylation mutant *modB* (Gerisch et al., 1985). Loss of glycosylation leaves adhesion unaffected. Unlike cell

contacts involving DdCAD-1, csA-mediated ones are not Ca^{2+} dependent and are insensitive to EGTA and EDTA. The homophilic interaction site has been mapped, and peptides mimicking this region of the protein, as well as anti-csA antibodies, block EDTA-resistant cell-cell adhesion and cell-surface binding of recombinant csA protein (Kamboj et al., 1989; Siu et al., 1987).

csA is anchored to the membrane by a lipid glycan (Sadeghi et al., 1988; Stadler et al., 1989) and, as has been found for other such membrane-linked proteins, associates with a Triton-insoluble floating fraction (TIFF) isolated from the cell membrane of developing cells (Harris et al., 2001). TIFF is rich in sterols and is predicted to form a closely packed liquid-ordered environment. Among the other proteins associated with the TIFF are F-actin, the regulatory myosin light chain kinase and comitin. This suggests a means to link csA to the underlying actinomyosin cytoskeleton. Comitin is a membrane-associated protein that binds to F-actin and intracellular vesicles, and associates with the Golgi (Weiner et al., 1993). TIFF may be transported to the plasma membrane via the Golgi (Heino et al., 2000; Nichols et al., 2001). Anti-csA antibodies cause capping of csA, F-actin, the other TIFF-associated proteins and sterol-rich membrane regions – as visualised by filipin staining (Harris et al., 2001). Regions of csA-mediated cell contact are enriched in sterols and TIFF-associated proteins. In addition, depletion of membrane sterols greatly reduces the ability of wild-type cell contacts to resist high-shear forces. This effect is also seen in cells lacking the *csA* gene.

Cell-cell adhesion mutants

No mutant that lacks the *DdCAD-1* gene has been described; however, blocking DdCAD-1 binding by antibodies and carnitine arrests *Dictyostelium* development (Loomis, 1988; Siu et al., 1992). Similarly, a peptide form of an N-terminal fragment of csA prevents the streaming and morphogenesis of wild-type cells (Kamboj et al., 1989). In contrast, a mutant lacking the *csA* gene exhibits no obvious difference in phenotype when developed under standard laboratory conditions (Harloff et al., 1989), perhaps owing to compensation from other related proteins (see below). However, when cells that lack *csA* are mixed with wild-type cells in cell suspension, both cell types sort out to form strain-specific aggregates (Ponte et al., 1998). This suggests that the relative adhesive strength between neighbouring cells is important for multicellularity. Furthermore, changing the balance of adhesive forces between *csA* mutant cells and their substratum, by developing these cells on soil, leads to their reduced intracellular adhesion and motility. Under these conditions, many *csA* mutant cells fail to enter the multicellular phase of development (Ponte et al., 1998).

The importance of the strength of DdCAD-1- and csA-mediated adhesion during aggregation is also demonstrated by the *smlA* mutant, which forms very small aggregates and hence small fruiting bodies (Brock et al., 1996). *smlA* mutants show reduced expression of DdCAD-1 and csA during streaming and early aggregation (Roisin-Bouffay et al., 2000). This leads to reduced intercellular adhesion and causes *smlA* streams to break up and eventually form aggregates smaller than those of the wild-type. Conversely, *Dictyostelium* prossessing mutations in *countin*, a factor that limits the size of aggregates (Brock and Gomer, 1999), have increased DdCAD-1 expression and cell-cell adhesion during early development. This leads to the formation of giant aggregates and fruiting bodies (Roisin-Bouffay et al., 2000).

Compensatory changes in gene expression have been observed after gene disruption in *Dictyostelium*, as well as in other organisms. This may also be the case with the *csA* mutant, where premature expression of a third adhesion protein, gp150, has been observed. gp150 is normally first expressed at the mound stage (Wang et al., 2000). Interestingly, cells lacking both *csA* and *gp150* genes are still able to stream and aggregate, although EDTA-resistant adhesion is almost totally abolished. A possible fourth adhesive interaction has been reported (Fontana, 1993), and examination of the data from the *Dictyostelium* genome project (Kay and Williams, 1999) indicates the presence of at least one other DdCAD protein and two gp150-related proteins. Nothing more is known about these proteins and their interactions.

Interaction of DdCad-1 and csA

The expression of csA is dependent on DdCAD-1 function and is severely reduced when DdCAD-1-mediated adhesion is blocked by EDTA, carnitine or fast-shaken low-density culture (Desbarats et al., 1994). In the absence of DdCAD-1-mediated cell contact, stimulation with cAMP pulses fails to restore csA expression, which argues that DdCAD-1-mediated cell contact is required for full csA induction. Caution must be taken in interpretation of these observations, because cAMP signalling has also been reported to be stimulated by cell contact; however, this effect is non-specific and can be induced by bacteria or inert latex beads (Fontana and Price, 1988). The specificity of the DdCAD-1-mediated induction has not been fully tested.

The potential link between DdCAD-1 and csA expression is interesting in the context of their interaction in aggregation streams. During early aggregation, before cell contact, DdCAD-1 is not uniformly distributed around the cell periphery and is preferentially localised to membrane protrusions such as filopodia (Sesaki and Siu, 1996). These regions are also enriched with F-actin, although it is not clear how localisation of actin polymerisation and DdCAD-1 is linked. Cells initially contact each other through binding of their adjacent filopodia using DdCAD-1 (Fig. 2A). As neighbouring cells draw their filopodia back into their cell bodies, more extensive membrane contacts are made, and these are also associated with F-actin. This second wave of contacts is made by csA (Choi and Siu, 1987). At the emergence of csA-mediated contacts, DdCAD-1 redistributes to other regions of the membrane (Fig. 2B). This is particularly striking as cells enter the stream, in which cells in the centre are completely surrounded by neighbouring cells through csA-mediated contacts. At the periphery of the stream, cells contact those within the stream through csA but have DdCAD-1 on the membranes that project away from the stream (Sesaki and Siu, 1996).

This pattern of assembly of adhesive contacts in *Dictyostelium* resembles the sealing of epithelial cell sheets seen during development of *Drosophila* and *Caenorhabditis elegans* (Kiehart et al., 2000; Simske and Hardin, 2001). These processes, known collectively as epiboly, occur as the epithelium migrates around the developing embryo. When the

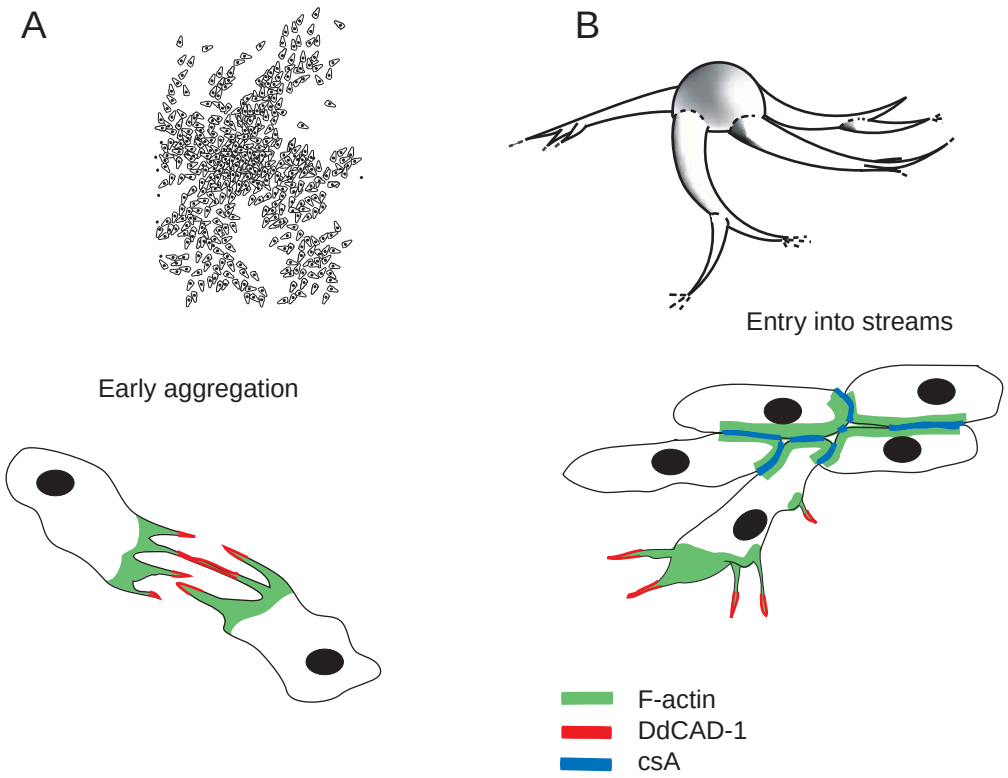

Fig. 2. A model of cell-cell adhesion during aggregation. (A) During early aggregation, cells express DdCAD-1 (red), which is enriched on F-actin-containing filopodia. (F-actin is shown in green.) Cells initially contact make through these filopodia. (B) As cells form and enter the streams, csA (blue) is expressed at the points of contact. DdCAD-1 is relocated to cell surfaces facing away from the streams.

two edges of the advancing cell sheets meet, they seal to make a complete covering. In both species, the cells project filopodia, which extend in front of the moving sheet. In *C. elegans*, when filopodia meet along the contacting cell edges, they promote the assembly of adherens junctions, adhesive contacts mediated by a cadherin-catenin adhesion system (Costa et al., 1998). The closure process involves events similar to those seen in *Dictyostelium*, in which the membranes of streaming cells first make contact through their filopodia and then 'zipper up'. In animals, the epithelial contacts are mechanically more stable than those observed in *Dictyostelium*, where cells remain highly motile throughout multicellular development. However, the processes in *Dictyostelium* and animals both involve common events, such as initiation of contact, rearrangement of the actin cytoskeleton and maturation to form more extensive adhesive contacts.

Cell adhesion and differentiation

Is cell-cell adhesion important for development beyond the mound stage? A simple hypothesis is that adhesion between cells in the mound is required to trigger cell differentiation. Initially, experiments appeared to suggest that this might be the case. Disaggregation of slug cells causes rapid loss of both gene expression (Mehdy et al., 1983) and developmentally regulated enzyme activity (Haribabu et al., 1986). In addition, early experiments indicated that cells starved in high-speed-shaken suspension or in the presence of EDTA do not express developmentally regulated genes (Mehdy et al., 1983; Chisholm et al., 1984). In the majority of these cases, however, post-aggregative genes are induced by the addition of cAMP, a signal molecule present throughout development. This suggests that loss of cell signalling rather than a requirement for cell-cell contact is responsible for the absence of developmentally regulated gene expression in these cases.

Ectopic csA expression in growing cells induces developmentally regulated genes (Faix et al., 1990). In light of the ability of a mutant that lacks csA to develop an apparently wild-type fruiting body under normal conditions, it is unlikely that csA overexpression directly induces development. A more likely explanation is that, owing to the induction of aggregates, cells are excluded from the surrounding growth medium. This leads to starvation, partial entry into development and induction of developmentally regulated genes. Cells overexpressing csA during development do exhibit a degree of aberrant multicellular development, but this is related to excessive adhesion rather than aberrant gene expression (Kamboj et al., 1990; Faix et al., 1992).

The strongest objection to the cell contact hypothesis is provided by observations of low-density monolayer cultures. In such experiments, cells are plated at a density low enough for each cell to develop without touching its neighbour. The signals required for cell differentiation are then added exogenously. Amoebae plated in such low-density monolayer cultures can be induced to form cells with the characteristics of both stalk and spore cells (Kay and Trevan, 1981; Town et al., 1976). DIF-1 [(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-1-hexanone (Morris et al., 1987)] is a potent inducer of stalk cells in low-density monolayer culture and induces the transcription of pre-stalk-specific genes in shaking culture (Berks and Kay, 1990; Jermyn et al., 1987; Williams et al., 1987). Spore cells can be induced in low-density monolayer culture by activation of the cAMP-dependent protein kinase (PKA) either by the treatment with 8-Br-cAMP (Kay, 1989) or addition of cAMP to mutants that possess constitutively active PKA (Hopper et

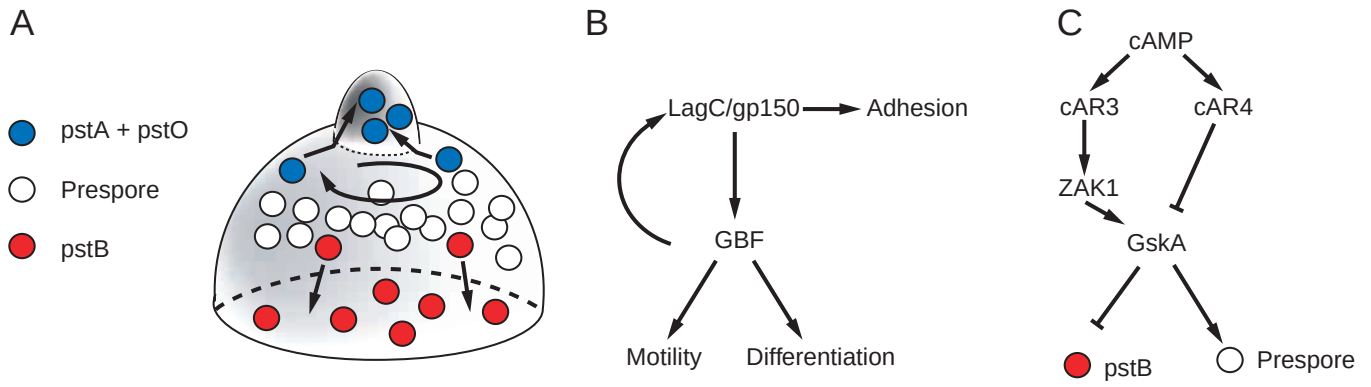

Fig. 3. Morphogenesis in the mound. (A) Within the mound, cell movement switches from directly towards the aggregation centre to rotation perpendicular to the tip axis. In addition, differentiation into the precursor cells of the fruiting body begins. The pstA and pstO cells move into the tip, and pstB cells move to the base. Pre-spore cells occupy the central region of the mound. (B) LagC/gp150 is required for cell-cell adhesion and, through the transcription factor GBF, for the switch in the direction of movement and cell differentiation. G-box-binding factor (GBF) is required for lagC/gp150 expression, which creates a feedback loop. (C) Cell differentiation requires GskA activity. This is regulated by extracellular cAMP through two receptors, cAR3 and cAR4. At low cAMP, cAR3 activates GskA activity through ZAK1; at high concentrations cAR4 inhibits GskA activity. GskA regulates the pstB–pre-spore ratio.

al., 1993; Mann and Firtel, 1993; Simon et al., 1992). These experiments argue that cell contact is not required for differentiation to form the stalk and spore cells – the end point of development. However, before proposing that cell contact is not required for multicellular development, we must consider that cells in culture may receive signal concentrations higher than those encountered during normal development. Furthermore, multicellular development proceeds through a complex process of pattern formation (see below), which could indeed require inputs generated by cell contact.

gp150 and multicellular development

Cell-cell adhesion appears not to be necessary for differentiation in isolated cells. Furthermore, direct visualisation and mathematical modelling (Dormann et al; 2000; Dormann et al., 2001) could explain morphogenesis from mound to fruiting body in terms of differential cell movement and signalling. Several recent observations have demonstrated that cell-cell adhesion is, however, an integral component of multicellular development.

lagC is a mutant that aggregates normally but does not proceed past the early mound stage. At this stage, which is often referred to as the loose aggregate stage, cells are only weakly adherent. Cells lacking *lagC* disaggregate and reaggregate to form small mounds (Dynes et al., 1994). *lagC* encodes gp150 (Geltosky et al., 1976; Wang et al., 2000), a 98 kDa protein that runs on SDS-PAGE with an apparent mass of 150 kDa, owing to glycosylation. Given its predicted transmembrane domain, gp150 is likely to be an integral membrane protein. In contrast to DdCAD-1 and csA, gp150 is a heterophilic cell-surface binding protein, although its ligand is currently not known. gp150 rapidly accumulates at the mound stage, and anti-gp150 antibodies block cell reassociation after experimental disaggregation (Geltosky et al., 1979; Wang et al., 2000). gp150 shows little sequence similarity to any animal protein, although a search of the Pfam database (Bateman et al., 2000) reveals that gp150 has a potential IPT sequence – an immunoglobin-like fold found in mammalian plexin and the MET receptor proteins.

Wild-type cells progress rapidly from a loose aggregate to a tight aggregate, in which the cell mass compacts and the outside of the mound becomes encased within an extracellular matrix known as the slime sheath (Fig. 1). Within the mound, the cells change their morphogenetic behaviour and begin to rotate rapidly around the mound (Fig. 3A). Finally, a ‘tip’ forms on the upper surface of the tight aggregate, which then elongates into a tall, thin finger-like structure, the first finger. This may fall on to its side and migrate along the substratum as a slug. The tip is an organiser that coordinates morphogenesis of the slug and fruiting body. Cells lacking *lagC* fail to establish rotational movement in the mound and have aberrant cAMP signalling (Sukumaran et al., 1998).

Pre-spore and pre-stalk cells form within the mound and sort into different spatial patterns (Fig. 3A). *lagC*-mutant cells fail to express these pre-stalk- and pre-spore-specific genes and exhibit severely deficient spore formation. When mixed with wild-type cells, *lagC* mutant cells form stalk cells but not spores. This suggests that the failure of the *lagC* mutant to proceed through development is in part due to a defect in pre-stalk gene induction and in part due to a failure of prespore cells to respond to a signal for terminal differentiation (Dynes et al., 1994).

The *lagC* phenotype can be rescued by expression of G-box-binding factor (GBF), a transcription factor that is required for the expression of all post-aggregative genes (Sukumaran et al., 1998). The expression of gp150 requires GBF, and gp150 is necessary for GBF expression. This suggests that gp150 acts in a feedback loop to maintain GBF expression (Fig. 3B). Through its genetic interaction with *gbf*, *lagC* is required for cell differentiation and morphogenesis. Presumably, the high level of exogeneously added cAMP is sufficient to bypass the requirement for *lagC* in isolated cell cultures.

Adherens junctions in the fruiting body

Until recently, virtually nothing was known about intercellular

adhesion systems late in *Dictyostelium* development, within the fruiting body. Cells of the first finger and slug are held in a pre-patterned state ready to initiate the morphogenetic transformation into the fruiting body, a process known as culmination (Fig. 4A). The anterior fifth of the slug is composed entirely of pre-stalk cells, whereas the remaining posterior four-fifths contains pre-spore cells and a population of pre-stalk cells known as anterior-like cells (ALC) (Sternfeld and David, 1981; Sternfeld and David, 1982). The anterior of the slug contains the prestalk A and prestalk O (pstA and pstO) cells, which eventually form the stalk (Jermyn et al., 1989). The precursors of the basal disc, the pre-stalk B (pstB) cells, first arise in the mound, where they move to its base (Fig. 3A) (Williams et al., 1989). The slug also contains a population of cells that have differentiated from the pstA cells; these are known as pstAB cells (Fig. 4A). PstAB cells are arranged in an inverted cone shape slightly posterior to the tip, and they mark the position of what becomes the entrance to the stalk tube in the fruiting body (Jermyn et al., 1989). During culmination, the posterior of the slug moves under the tip, and first the pstAB cells and then the pstA and pstO cells pass through the centre of the pre-spore cells, where they differentiate to form the stalk and embed in the basal disc (Jermyn et al., 1989). The stalk elongates through the addition of new stalk cells to its top and raises the spore head as it grows (Fig. 4A). Rapid cryopreservation preparation for electron microscopy has revealed a complex ultrastructure in the developing fruiting body (Fig. 4B) (Grimson et al., 2000).

The stalk is separated from the surrounding pre-stalk cells by a protein and cellulose matrix. This matrix forms a continuous tube, open at the top, into which pre-stalk cells enter and immediately differentiate into stalk cells. Grimson et al. (Grimson et al., 2000) showed that, just below its opening, the stalk tube is compressed to form a constriction (Fig. 4B). Transverse sections through the constriction show that the pre-stalk cells surrounding the stalk are connected by electron-dense junctional complexes that have a morphology similar to metazoan adherens junctions. This similarity is strengthened by the fact that each junction around the stalk is connected within the cells by actin filaments (Fig. 4B).

Metazoan adherens junctions contain transmembrane cadherins, which engage in homophilic interactions with neighbouring cells. Cadherins are connected to the actin cytoskeleton by α- and β-catenin (Angst et al., 2001). A search of the *Dictyostelium* cDNA database (Morio et al., 1998) identified a homologue of β-catenin, Aardvark (Aar). Antiserum raised against Aar protein immunostains the *Dictyostelium* junctional complexes, and disruption of the *aar* gene causes a complete loss of the junctional complex and the stalk-tube constriction. Overexpression of Aar leads to an increase in the number and size of the junctional complexes and an increase in the F-actin content of the junction-containing cells. These observations demonstrate that Aar expression is both necessary and limiting for junction formation. Loss of the junctions does not prevent culmination; however, the resulting fruiting bodies are mechanically unstable, and the majority collapse onto the substratum (Grimson et al., 2000).

A β-catenin homologue required for both adhesion and signalling

In addition to their structural role in adherens junctions, metazoan β-catenin proteins are active in the Wnt-1 signalling pathway. Wnt stimulation leads to an increase in the cellular concentration of β-catenin, which then accumulates in the nucleus where it regulates gene expression (Hulsken and Behrens, 2000). This signal transduction pathway is important in the regulation of cell proliferation and in pattern formation during embryonic development (Dale, 1998).

Although it does not arrest development, loss of Aar causes a reduction in the expression of the pre-spore-specific gene *psA* during *Dictyostelium* development (Grimson et al., 2000). The effect of loss of *aar* on cells developed in shaking culture is more striking: cAMP is no longer able to induce *psA* expression. When Aar is overexpressed, the opposite result is seen: *psA* expression is hyperinduced by cAMP. In monolayer culture, spore-cell formation is reduced, although not completely lost, in the *aar* mutant. These observations argue that Aar has a role in the induction of prespore genes that is independent of cell-cell adhesion. Interestingly, psA is a cell-surface protein that has similarity to csA and animal N-CAM (Early et al., 1988). In mammals, the E-cadherin gene is a direct target of β-catenin signalling (Huber et al., 1996). No role for psA in cell adhesion has been demonstrated, but it is tempting to speculate that Aar, in common with β-catenin, regulates the expression of cell-adhesion molecules required for development.

In metazoa, the protein kinase GSK-3 phosphorylates β-catenin, leading to its degradation. Wnt-1 stimulation blocks GSK-3 phosphorylation of β-catenin, allowing β-catenin to build up in the cell. As in metazoa, in *Dictyostelium* the GSK-3 homologue, *gskA*, is critical for regulation of cell fate (Harwood et al., 1995). GskA is required in the mound, where it regulates the ratio of pre-spore to pstB cells (Fig. 3C). A mutant that lacks *gskA* overproduces pstB cells at the expense of the pre-spore cells, producing a fruiting body that has an expanded basal disc but very few spores (Harwood et al., 1995).

Extracellular cAMP activates GskA through the cAMP receptor cAR3 (Plyte et al., 1999) and the tyrosine kinase ZAK1 (Kim et al., 1999). A second receptor, cAR4, appears to negatively regulate GskA, and a mutant that lacks cAR4 has reduced pre-stalk differentiation and increased pre-spore differentiation (Ginsburg and Kimmel, 1997; Louis et al., 1994). This mutant phenotype can be reversed by treatment with lithium, an inhibitor of GSK-3. Given that cAR4 has a lower affinity for cAMP than cAR3 does, this may establish a threshold response in which gskA is inactive at low and high levels of cAMP, but active at intermediate concentrations. Aar has a set of putative GSK-3-phosphorylation sites at its N-terminus (Grimson et al., 2000). Overexpression of Aar in cells lacking *gskA* does not lead to the hyperinduction of *psA* seen in a wild-type background (Grimson et al., 2000). This indicates that GskA activity is required for Aar signalling. Cells lacking cAR4, which have increased GskA activity, also show hyper-induction of pre-spore genes (Louis et al., 1994), which is consistent with *gskA* acting upstream of *aar* in the induction of prespore gene expression. These observations indicate that, as in the case of metazoan β-catenins, Aar possesses both cytoskeletal and signalling roles during development. This argues that both cellular functions of β-catenin proteins evolved before the origin of metazoa. It will be interesting to establish which other eukaryotes possess β-

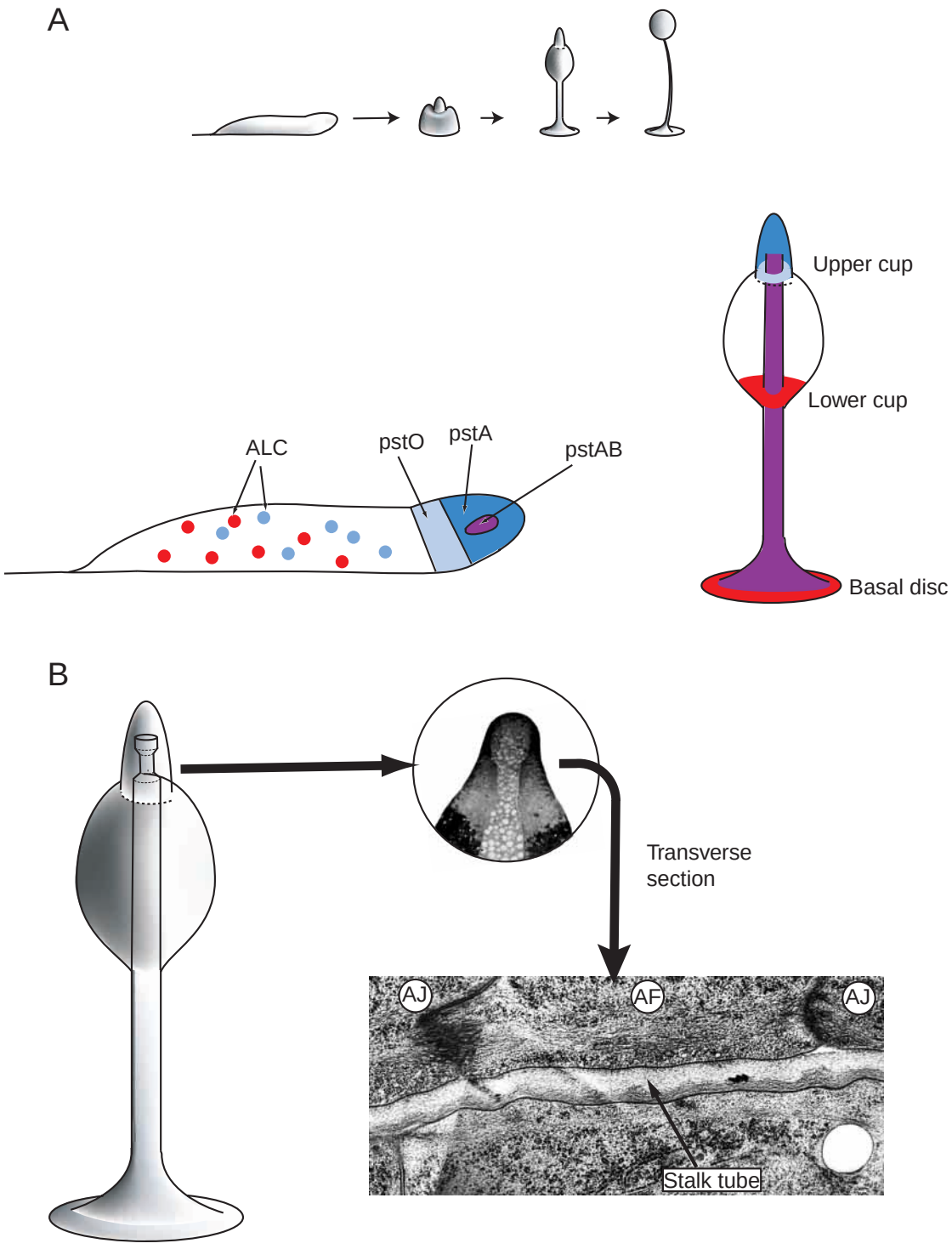

Fig. 4. Formation of the fruiting body. (A) The slug contains a pre-pattern that presages culmination. The pstAB cells form an inverted cone structure within the slug tip. These cells mark the future entrance to the stalk tube and are the first cells to enter the stalk. As culmination proceeds, they embed into the basal disc. When development passes directly from mound to culmination, the basal disc is formed from the pstB population. During slug migration, pstB cells are lost and re-differentiate from the anterior-like cells (ALC). The ALC are also a source of the pstO cells. During culmination, pstO cells differentiate into pstA cells and then into stalk cells within the stalk tube. The upper and lower cup structures form from pstO and pstB populations, respectively. (B) During culmination, the stalk tube forms a constriction just below its entrance. A transverse section of this region shows that the cells surrounding this stalk tube are connected by electron-dense adherens junctions (AJ) and actin filaments (AF). Reprinted by permission from Nature 408:727-731 copyright 2000 Macmillan Magazines Ltd.

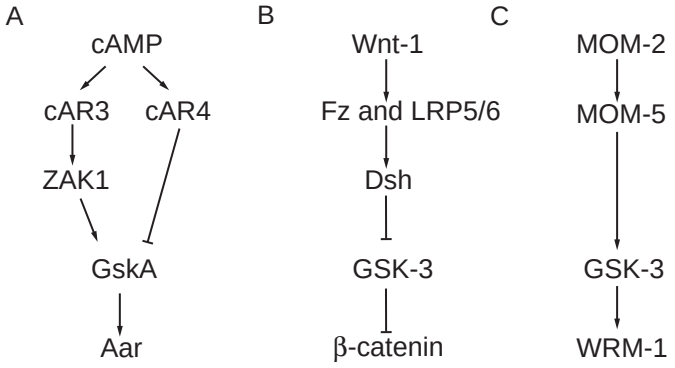

Fig. 5. Comparison of GSK-3/β-catenin signalling in *Dictyostelium* and animals. (A) Extracellular cAMP activates GskA through cAR3/ZAK1 and inhibits it through cAR4. GskA positively regulates the β-catenin homologue, Aar. (B) The extracellular ligand Wnt-1 stimulates the co-receptors Frizzled (Fz) and LRP5 (or LRP6) to inhibit GSK-3 function through Dishevelled (Dsh). GSK-3 negatively regulates β-catenin. (C) In nematodes, the Wnt homologue, MOM-2, acts positively on GSK-3 function via the Fz protein MOM-5. GSK-3 appears to positively regulate the β-catenin homologue WRM-1.

catenin proteins and which functions arose first during evolution.

The Aar signalling pathway in *Dictyostelium* is not identical to that in vertebrates and *Drosophila* (Fig. 5). In *Dictyostelium*, GskA activity is activated by cAR3 and positively regulates Aar to elicit *psA* expression. This contrasts with the negative regulation of GSK-3 by Wnt-1 and the subsequent inhibition of β-catenin degradation seen in vertebrates and flies. However, non-canonical interactions between GSK-3 and β-catenin are not unique to *Dictyostelium*. *C. elegans* possesses three β-catenin homologues, HMP-2, BAR-1 and WRM-1 (Korswagen et al., 2000). HMP-2 has a single, structural role in adherens junctions, and BAR-1 acts within a canonical Wnt pathway. WRM-1 also possesses a signalling role during early embryogenesis, but in this case stimulation by the Wnt homologue MOM-2 acts positively on GSK-3, which then appears to stimulate WRM-1 (Thorpe et al., 2000). This regulation of WRM-1 is analogous to the situation with Aar in *Dictyostelium*. In addition, whereas the canonical Wnt pathway of vertebrates and *Drosophila* activates TCF/LEF transcription factors, WRM-1 appears to derepress the negative effects of a TCF/LEF-related protein, POP-1 (Lin et al., 1995). WRM-1 achieves this by binding to a serine/threonine protein kinase, LIT-1, which phosphorylates POP-1 and may relocalise it to the cytoplasm (Rocheleau et al., 1999; Shin et al., 1999). The mechanism by which Aar regulates transcription is not yet clear, and analysis of its protein binding partners and subcellular distribution will prove valuable.

Concluding remarks

On close inspection, it is clear that cell-cell adhesion is an integral part of *Dictyostelium* development. It is important for morphogenesis and has an influence on gene expression in the multicellular context. *Dictyostelium* cell-cell adhesion is also involved in several processes common to metazoa, and a number of key proteins that function in *Dictyostelium* cell adhesion and signalling share both sequence and functional similarity to their counterparts in animals. In addition to the proteins discussed here, a number of other *Dictyostelium* genes that in animals encode proteins present in adhesion complexes have been cloned. These include two talin homologues and two cortexillins, members of the α-actinin/spectrin superfamily (Faix et al., 1996; Fey and Cox, 1999; Niewohner et al., 1997; Tsujioka et al., 1999), which are required for substrate adhesion and morphogenesis. In addition, a search of the *Dictyostelium* genome sequence database (http://dictybase.org/dictyostelium_genomics.htm) indicates the presence of a number of metavinculins and paxillins. Our knowledge of the roles of these proteins in *Dictyostelium* development is rudimentary at present. It is clear, however, that we have embarked on a journey of rediscovery, and we expect to see further major revelations from the study of *Dictyostelium* cell-cell adhesion in the future.

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