Dictyostelium morphogenesis
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During starvation-induced Dictyostelium development, up to several hundred thousand amoeboid cells aggregate, differentiate and form a fruiting body. The chemotactic movement of the cells is guided by the rising phase of the outward propagating cAMP waves and results in directed periodic movement towards the aggregation centre. In the mound and slug stages of development, cAMP waves continue to play a major role in the coordination of cell movement, cell-type-specific gene expression and morphogenesis; however, in these stages where cells are tightly packed, cell-cell adhesion/contact-dependent signalling mechanisms also play important roles in these processes.

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Current Opinion in Genetics & Development 2004, 14:392–398

This review comes from a themed issue on Pattern formation and developmental mechanisms
Edited by Derek Stemple and Jean-Paul Vincent

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DOI 10.1016/j.gde.2004.06.006

Abbreviations
ACA aggregation stage adenylylcyclase
Dif differentiation inducing factor
PIP3 Phosphatidylinositol(3,4,5)-Phosphate
PkA cAMP dependent protein kinase
RGS regulator of G protein signalling

Introduction
One of the central aims of the study of development is to understand how distinct cellular behaviours (e.g. division, differentiation, apoptosis and movement) are coordinated in space and in time to result in reproducible pattern formation and morphogenesis. Coordination of these cellular behaviours requires extensive communication between cells of different types and cells and their environment. The social amoebae Dictyostelium discoideum, a simple genetically tractable organism situated at the threshold of single and multicellular organisms in the evolutionary tree of life, is well suited for the study of these interactions because its genome has been sequenced and it is amenable to experimental manipulation through targeted gene disruption and replacement [1*]. Dictyostelium cells normally live as single cells in the soil leaf litter where they feed on bacteria and divide by binary fission. Under starvation conditions up to several hundreds of thousands cells aggregate chemotactically to form a multicellular structure (the slug) that, directed by light and temperature gradients, migrates to the soil surface to form a fruiting body. The fruiting body is composed of a stalk supporting a mass of spores. The spores disperse and under suitable conditions germinate to release amoeba, closing the life cycle. I review our understanding of the signalling mechanisms coordinating cellular behaviours responsible for pattern formation and morphogenesis.

The control of cell movement during development
As Dictyostelium development occurs under starvation conditions, only limited cell divisions occur during multicellular development. Morphogenesis primarily results from the arrangement of differentiating cells in a regulative spatial pattern. Key questions are: which signals guide the movement behaviour of thousands of cells during development, and how do movement and differentiation interact?

Aggregation
Starvation induces changes in the gene-expression programme that result in the cells acquiring the ability to produce, secrete and degrade cAMP [2**]. Through the expression of the cAMP receptor, the cells also acquire the ability to respond chemotactically to cAMP gradients. It has emerged that chemotaxis results from the polarisation of the cytoskeletal dynamics persistently along the cAMP gradient. Unstimulated amoeboid cells are changing shape continuously by extension and retraction of pseudopods in all directions resulting in a random walk [4]. In the presence of an external gradient of a chemoattractant such as cAMP, the cells persistently extend successive pseudopods in the direction of rising cAMP concentration while suppressing the extension of lateral pseudopods, which results in an efficient movement up the gradient.

Much current research is directed towards understanding how cells detect cAMP gradients, polarise and move in response to it. Pseudopod extension is driven by actin polymerisation, which provides the driving force for extension and simultaneous disassembly of the myosin thick filaments in the cortex at the site of extension as well as localised delivery of membrane and or proteins to allow extension to occur [5*,6,7**]. Cells also need to pull up their back end and suppress the extension of lateral pseudopods which involves members of the myosin I
family and is dependent on internal cAMP levels \([8^*,9^*]\). To move forward the cells must gain traction from the substrate on which they are moving, which involves the formation of multiple transient (10–20s) actin contact sites that have been shown to transduce traction forces to the substrate \([10^*,11^*]\). It appears that cells may undergo alternating phases of actin-driven extension at the front and myosin-driven contraction at the back \([12^*]\).

Much work is directed towards the investigation of the molecular mechanism resulting in the molecular mechanism underlying signal detection and its translation in directed movement, and has been reviewed recently elsewhere \([6,13^*,14^*]\).

Aggregation is caused by periodic cAMP synthesis and secretion by cells in an aggregation centre \([15]\). Detection and amplification of this signal by surrounding cells coupled with desensitisation of the cAMP-producing cells results in the propagation of waves of cAMP from the aggregation centre outward (Figure 1). These waves of cAMP guide the chemotactically moving cells towards the aggregation centre, where they accumulate into a three-dimensional aggregate: the mound \([16,17]\). Initially, the cells move towards the aggregation centre as individuals, but after 10–20 waves have passed they form bifurcating aggregation streams, in which the cells make head-to-tail contacts via calcium-independent adhesion molecules, contact site A and side-to-side contacts via a calcium-dependent contact molecule (Dd cadherin) \([18,19^*]\).

Stream formation is dependent on the localisation of aggregation stage adenylylcyclase (ACA) in the rear of the aggregating cells, resulting in polarised cAMP secretion from the back of the cells \([20^*]\). cAMP wave propagation can be observed indirectly at the population level via the observation of propagating optical density waves that are associated with the periodic surges in cell movement of groups of cells in the direction of the cAMP signal during the rising phase of the cAMP wave, or at the individual cell level by following the localised translocation of PIP3 at the leading edge of the cell \([21^*]\). During aggregation the cAMP signals propagate as target patterns or spiral waves from the aggregation centre outward. Cells stay polarised as long as the cAMP signal is rising in time. However, upon passage of the wave, the chemotactic response adapts, which prevents the cells from turning.

**Figure 1**

Dictyostelium development is controlled by propagating cAMP waves. These waves possess distinct geometries typical for the different stages of development. The cAMP waves are detected indirectly as optical density waves associated with the chemotactic movement of the cells in response to the cAMP waves. Groups of cells moving directly during the rising phase of the waves scatter more light than non-moving, less polarised cells during the falling phase of the waves. (a) Spiral waves typical for the early aggregation phase, when the cells are still in a monolayer on agar. (b) Waves in a streaming aggregate. In the body of the aggregate, multi-armed spiral waves rotates counter clockwise, throwing off individual wavefrons that propagate from the centre outward to the periphery of the aggregate directing inward movement of the cells. (c) Multi-armed waves in a mound. In this image, the waves rotate counter clockwise, directing the clockwise movement of the cells in the mound. (d) A slug migrating from right to left, showing two optical density waves, indicated by arrows, that travel from left to right. These waves direct the migration of the prespore cells in the direction of the tip from right to left.
around and chasing after the cAMP waves once they have passed. Adaptation of the chemotactic pathways, involving depolarisation of the cells may involve activation of a newly discovered RGS-domain containing kinase as well as the activation of PkA [9, 22]. The number of cells in aggregation streams appears to be controlled by the local concentration of a secreted extracellular high molecular weight complex protein complex, counting factor, which through modulation of movement and adhesion may control the numbers of cells that stably migrate in an aggregation stream [23, 24].

**Mound and slug formation**

After the cells have aggregated, they form the hemispherical mound. Mounds are characterised by rotating waves of cAMP that direct the counter-rotational periodic movement of the cells. Cells start to differentiate into prespore and prestalk cells during aggregation, on the basis of physiological biases like nutritional state and cell-cycle position at the time of starvation already present in the population before aggregation [25, 26]. As a result, there is little correlation between the time of arrival in the mound and differentiation fate and the emerging cell types initially form a ‘salt and pepper’ pattern in the mound. Some prestalk cells then sort out to form the tip and the slug tip guides the movement of all other cells thus acting as an organiser. The tip’s action as an organiser can be mimicked by the periodic injection of cAMP pulses of the right frequency and duration [17], suggesting that the tip is a source of periodic cAMP waves, in agreement with the fact that prestalk cells express ACA and the extracellular cAMP phosphodiesterase pdeA [27, 28]. It is not known which signals control tip-cell fate but it is becoming clear that to proceed from the aggregate to the mound stage cell–cell adhesion and/or contact start to play an important role.

Mutants defective in the putative single-pass transmembrane contact molecules lagC, lagD and cotC cannot proceed beyond the aggregation stage and are defective in tip formation [29**]. Surprisingly, mutants in some of the newly discovered nine pass transmembrane receptors of the Phag family, shown to be essential for phagocytosis, display severe defects in slug formation and culmination, suggesting a possible role for these receptors in cell–cell adhesion [30]. Sorting of prestalk cells towards the tip requires the invasive movement of prestalk cells through a tightly packed mass of other cells and most likely involves differential cell adhesion. Sorting of prestalk cells is facilitated by secretion of a disintegrin family protein and deletion of this protein results in a delayed sorting and defective cell-type specification, demonstrating that sorting and cell-type specification are tightly interlinked [31].

In slugs, optical density waves can be seen to propagate from the middle of the prestalk zone to the back, reflecting the periodic movement of the cells forward (Figure 1). These optical waves are strictly dependent on the tip. Cells in the tip often rotate perpendicularly to the direction of slug migration, especially when it is lifted from the substrate. In the posterior part of the slug, the cells move forward periodically and all cells move on average with the speed of the whole slug. It has been shown that the assumptions of cAMP wave propagation and chemotaxis in response to these waves is, in principle, sufficient to explain morphogenesis from single-cell via aggregation, stream and mound formation to cell sorting and slug formation. The interactions between cell-signalling and cell movement can be described in a robust way by relatively simple mathematical models. In one of these models the cells are described as a viscous fluid that moves in response to the cAMP waves generated in an excitable manner by the cells. This model is able to explain the formation of aggregation streams, resulting in the formation of a hemispherical mound. With the additional assumptions that the prestalk and prespore cells that differentiate in the mound in a salt and pepper pattern differ in two properties, their ability to relay the cAMP signal and their ability to move chemotactically in response to the cAMP signal it is possible to obtain cell sorting, where the prestalk cells move to the top of the mound form a slug, which falls over and migrates away (Figure 2). It would appear that these processes are sufficient to explain Dictyostelium morphogenesis up to the culmination stage [32**, 33**]. However, the situation is almost certainly more complex because strains lacking the ACA can still form slugs when they over-express the catalytic subunit of protein kinase A. The suggestion is that either there exists an ACA-independent mechanism to produce periodic cAMP signals (e.g. involving cAMP generation by other adenylylcyelas AB and/or ACG and the recently discovered cAMP-stimulated cAMP phosphodiesterase [34]) or that there exists altogether different mechanisms that can control cell movement, such as contact following [35]. The latter mechanism does not, however, explain what directs the movement of the cells in the tip. It is possible that the slime sheath secreted by prestalk cells [36], which surrounds the slug as a ‘stocking’, may keep the cells together and may also give some polarity to the slug but its role, although presumably important, has not yet been investigated in detail.

**Differentiation**

A major goal is to understand the relationship between cell movement and the signals that control differentiation. These signals must be able to maintain the correct proportion of the prespore and prestalk cell types in an environment of extensive cell movement and changes in shape of the slug. In the slug, the different cell types are arranged in a simple axial pattern: pstA (prestalk A) cells in the tip, a band of pstO cells, prespore cells with intermingled anterior-like cells and rearguard cells that...
are precursors of the basal disk in the back of the slug [37]. It seems evident that this requires adaptive signalling dynamics but the signals and the details of their regulation are not understood in detail. cAMP pulses control the expression of aggregation-stage genes necessary for cAMP relay and cell–cell contact and can control prespore cell specific gene expression in later development [2**3]. Prespore cells, in turn, produce DIF, which controls the differentiation of pstO cells [37,38**39]. DIF spreads by simple diffusion from the prespore zone in adjacent regions where it controls the differentiation of prestalk O cells and possibly rear guard cells, in agreement with the fact that Dif-dependent pstO translocation of StatC is seen in both zones [40*]. Cells in the pstA zone express ACA and studies investigating the cAR1-dependent nuclear translocation of the transcription factor statA have shown that cAM P levels are high in the tip, whereas cAMP levels are lower elsewhere in the slug [28,41*], compatible with the idea that all the prestalk cells in the tip and only the anterior-like cells in the posterior part of the slug relay the cAMP signal. It is not known what maintains the expression of ACA in the cells in the tip and in anterior-like cells, but it could involve signalling through newly discovered orphan serpentine receptors, as deletion of these receptors results in defective tip formation [42]. The mechanisms of cell-type proportioning and detailed signal-transduction pathways to cell-type specific gene expression still need to be resolved.

Model calculations of wave propagation and cell movement from aggregation to slug migration using a hydrodynamic model in which the cells are described as a viscous fluid that move in response to propagating cAMP waves generated in an excitable manner by the cells [32**]. The top row depicts the aggregation up to the mound stage. The first image starts with the randomly distributed cells (yellow) that are organised by a spiral wave of cAMP (purple). They form aggregation streams and finally a hemispherical mound [45]. The middle row shows cell sorting and the formation of a slug. The mound consist of two cells types: 20% yellow prestalk cells and 80% green prespore cells. They are initially randomly mixed. The cAMP waves (purple) organise the movement of the cells. The prestalk cells are more excitable and move more strongly in response to a cAMP wave. They move towards the centre of the mound and up to form the tip. The separation of the cells feeds back on the signal propagation resulting in the formation of a twisted scroll wave. This leads to an intercalation of the cells and an upward extension of the slug [46]. The bottom row shows that a slug organised by a scroll wave can move [32**].
The switch from migrating slugs to fruiting body formation (culmination) appears to be controlled by a fall in ammonia concentration. The identification of several ammonia transporters, some of which are expressed in the very tip and when deleted lead to a slugger (slugs that fail to culminate) phenotype, supports the importance of ammonia as a morphogen [43**]. Ammonia signals most likely through the histidine kinase DkhC to the response regulator domain of the internal cAMP phosphodiesterase RegA, which is a major determinant in the control of intracellular cAMP levels [3,44]. High ammonia is expected to result in activation of regA and low internal cAMP levels. A drop in ammonia is expected to result in a rise of intracellular cAMP and stalk-cell differentiation.

Conclusions

Multicellular Dictyostelium morphogenesis results from the chemotactic movement of thousands of differentiating cells coordinated by propagating waves of the chemotactant cAMP produced by these cells. The dynamical interactions between the cAMP waves and the resulting chemotactic movement of the cells is formally sufficient to explain aggregation, stream and mound formation. Slug formation and culmination require the emergence of prespore and several prestalk cell types that show distinct cAMP signalling and movement properties. Only prestalk and anterior-like cells relay the cAMP signals while both prestalk and prespore cells move chemotactically in response to these signals. However, prestalk cells move more effectively than prespore cells, resulting in cell sorting. Many of the molecular details underlying the polarisation response to cAMP gradients and the mechanisms of actual movement, cell-cell contact and differentiation are beginning to be uncovered. It would appear that direct cell-cell contact mediated signalling will play an important role in the modulation of these processes, as is the case in higher organisms. Dictyostelium will thus, besides being a system of choice to investigate the molecular mechanisms underlying cell polarity and chemotaxis, be an excellent model system to investigate the principles underlying multicellular tissue organisation and cell-type proportioning.

Acknowledgements

We apologise to those whose work was not cited due to space constraints. I want to thank Dirk Dormann and Bakhtier Vasiev for their discussions. This work was supported by the Biotechnology and Biological Sciences Research Council and the Wellcome Trust.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
** of outstanding interest

1. Kreppel L, Fey P, Gaudet P, Just E, Kibbe WA, Chisholm RL,

The authors describe the premier site for Dictyostelium resources that includes genome analysis and stock centre information.

2. Iranfar N, Fuller D, Loomis WF: Genome-wide expression
   analyses of gene regulation during early development of

A comprehensive analysis of expression of ~3000 unique ESTs (expressed sequence tags) during development, showing the specific developmental regulation of 172 genes. 125 genes were expressed in response to extracellular pulses of cAMP. Three genes, cark (cAMP receptor), gbαB (G protein Beta), and pdsA (secreted phosphodiesterase), were consistently expressed early in development in the absence of cAMP pulses, they are necessary to get the cAMP oscillations going. PKA was demonstrated to have a critical role in transducing the cAMP signal to early gene expression. In the absence of constitutive PKA activity, expression of later genes was strictly dependent on ACA in pulsed cells.

3. Sara S, Meima ME, Alvarez-Curto E, Weening KE, Rozen DE,
   Schaap P: cAMP signalling in Dictyostelium. Complexity of
   cAMP synthesis, degradation and detection. J Muscle

   reconstruction and motion analysis of the three-dimensional

5. Thompson CR, Bretscher MS: Cell polarity and locomotion,
   as well as endocytosis, depend on NSF. Development 2002,
   129:4185-4192.

A temperature-sensitive mutant in NSF (NEM sensitive factor), an essential component of membrane transport, shows that NSF is essential for phagocytosis, membrane internalisation and cell movement. The cAMP-induced activin polymerisation (cringe response) is intact in the mutant at the restrictive temperature, but the cells round up and stop translocation, possibly due a defect in generating cell polarity.

6. Merlot S, Firtel RA: Leading the way: directional sensing through
   phosphatidylinositol 3-kinase and other signaling pathways.

   pseudopod dynamics and SCAR activity in Dictyostelium.

This is the first piece of genetic evidence showing that PIR121 is involved in the negative regulation of SCAR, which controls the activity of the Arp2/3 complex and actin polymerisation. PIR121 null mutants show excessive actin polymerisation and continuous splitting of pseudopods implying important roles for SCAR in the control of actin polymerisation during pseudopod formation.

8. Falk DL, Wessels D, Jenkins L, Pham T, Kuhl S, Titus MA, Soll DR:
   Shared, unique and redundant functions of three members of
   the class I myosins (MyoA, MyoB and MyoF) in motility and

This study shows that members of the myosin I class of motor proteins are necessary for suppression of lateral pseudopods and efficient chemotaxis during aggregation in natural waves of cAMP. MyoF in particular is necessary for attaining an elongated shape during chemotaxis.

   Soll DR: Constitutively active protein kinase A disrupts
   motility and chemotaxis in Dictyostelium discoideum.

Cells expressing a constitutively active PKA are incapable of suppressing lateral pseudopods and maintain an ovoid shape, resulting in inefficient movement. They are incapable of moving in response to natural waves of cAMP implying PKA activation in dismantling cell polarity at the peak of the wave.

10. Uchida KS, Yumura S: Dynamics of novel feet of Dictyostelium

The authors show that cells periodically form small, localised and close contacts with the substratum and that these contact points are rich in filamentous actin. By correlating these actin-rich contact points with the local deformations of an elastic substrate on which these cells move, it is shown that these contact points are the places where the cell transmits traction forces necessary for movement to the substrate.

11. Bretschneider T, Diez S, Anderson K, Heuser J, Clarke M,
    Muller-Taubenberger A, Kohler J, Gersch G: Dynamic actin
    patterns and Arp2/3 assembly at the substrate-attached

Using TIRF (total internal reflection fluorescence) microscopy the authors show that cells make short lived (~7-10sec) Arp2/3-rich foci in a myosin-II independent fashion. These high-resolution experiments furthermore

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show that the Arp2/3/foci often induce waves of actin polymerisation propagating through the cell in a wave-like fashion, showing that actin polymerisation machinery operates far from equilibrium. The Arp2/3-rich foci are observed not only in the leading edge but also in the middle of the cell, implying that the actin cytoskeleton is remodelled continuously everywhere in the cell. It appears likely that these foci are the same as the actin-rich contact sites shown in [10] to coincide with the points of transmission of traction forces to the substrate.


This paper shows conclusively that cells undergo alternating cycles of extension and contraction and it correlates these behaviours with the generation of traction forces showing that both extension and contraction at the rear contribute to forward movement.


A clear review describing rivalising hypotheses for cell polarisation.


A review of current molecular insight in the mechanisms underlying coordination of events at the leading and trailing edges of the cell during chemotactic movement.


The cadA gene encodes the Ca2+-dependent cell adhesion molecule DdCdA-1, one of the major calcium-dependent adhesion molecules. It is expressed soon after the initiation of development in Dictyostelium. Cells that lack DdCdA-1 are able to complete development and form fruiting bodies, but they make abnormal slugs, culmination is delayed by ~6 hours, and the yield of spores is reduced by ~50%. The proportion of prestalk cells in cadA-4 slugs showed a 2.5-fold increase over the parental strain. This indicates that, in addition to cell–cell adhesion, DdCdA-1 plays a role in cell-type proportioning and pattern formation.


Kriebel et al. show that Dictyostelium cells, like neutrophils, polarise in response to uniform stimulation of cAMP, which is normally produced by ACA. Furthermore, it shows that the posterior localisation of ACA in aggregating cells is necessary for efficient stream formation most likely because it leads to localised end-to-end signalling between cells in aggregation streams.


In this paper, we show that cells in aggregation streams and mounds show periodic PI3P production at the leading edge and that this correlates with a surge in forward movement. Cells in slugs, however, appear to be polarised permanently in the direction of slug migration and this polarisation depends on close range cell–cell signalling or direct cell–cell contact.


The authors show that three single-pass transmembrane putative calcium-independent adhesion molecules comC, LagC and lagD are essential for the transition of development from the late aggregate to the mature mound stage. Evidence is presented that these genes fall into a regulatory network in which comC inhibits lagC and activates lagD expression, lagC and lagD are mutuially inductive, and the cell adhesion gene lagC is the terminal node in this signaling network.


We have shown through the development of a physico-chemical model that cAMP wave propagation by preprostalk and anterior-like cells and chemotactic movement of both prestalk and prespore cells are sufficient to explain slug formation and migration. In this model, the prestalk and prespore tissues are described as two distinct incompressible viscous fluids, that move in response to cAMP waves, produced and propagated by the preprostalk cells. By varying the dynamics of CAMP signaling, the chemotactic responsiveness of the prestalk and prespore cells and the cell–cell interactions as described by changes in tissue viscosity, it is possible to analyse their influence on slug migration and shape.


See annotation [32*].


A bZIP/bRLZ transcription factor is here identified as a central regulator of all known Dif responses and the DimA null mutant phenocopies the DmtA, Dif null mutant, in that it does not make PatO cells.


Shows that Dif directs tyrosine phosphorylation and nuclear accumulation of StatC (signal transducer and activator of transcription). The nuclear accumulation of StatC is controlled by the inhibition of StatC nuclear export, as shown via cytoplasmic photobleaching of StatC-GFP in living cells. The paper furthermore shows that StatC functions as a transcriptional activator in a stress-response pathway.


We show by monitoring the nuclear translocation of StatA, which is dependent on the level of extracellular cAMP, that slug tips are regions of high extracellular cAMP. The absence of nuclear translocation of StatA in prespore cells is the result of ambient levels of extracellular cAMP in the prespore zone being too low to cause nuclear translocation of StatA. An increase in extracellular cAMP concentration by injection of cAMP in the extracellular space in the prespore region of the slug results in rapid translocation of StatA in prespore cells. These findings are in agreement with findings described in [28], where extracellular cAMP was manipulated by ectopic expression of the aggregation stage adenylcyclase (ACA) in prespore cells, resulting in nuclear translocation of StatA in prespore cells. These results are compatible with the idea that prespore and anterior-like cells relay the cAMP signal and prespore cells do not.


Three ammonium transporters (amtA–C) are identified in Dictyostelium, which may play a role in ammonia signaling, in addition to physically altering the levels of ammonia within cells. AmtC is expressed in the most anterior tip of fingers and slugs, corresponding to cells that mediate ammonia’s effect on the choice between slug migration and culmination. Indeed, amtC null cells have a slugger phenotype suggesting that it may play a role in this choice.

