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Morphogen hunting in *Dictyostelium*

ROBERT R. KAY, MARY BERKS and DAVID TRAYNOR

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

Summary

A highly regulative pattern of prestalk and prespore tissue is formed during *Dictyostelium* development, starting from separate amoebae. Potential morphogens controlling this process have been hunted biochemically, using bioassays to monitor activity. All those discovered to date are low MW diffusible compounds: cAMP, adenosine, NH_3 and DIFs 1–3. The DIFs are assayed by their ability to induce isolated amoebae to differentiate into stalk cells and have been identified as a family of chlorinated phenyl alkanones.

The diversification of amoebae into prestalk and prespore cells seems to be brought about by cAMP and DIF-1. cAMP is necessary for both pathways of differentiation but DIF-1 specifically induces the differentiation of prestalk cells while suppressing that of prespores.

When DIF-1 is added to intact slugs, it causes a substantial enlargement of the prestalk tissue at physiological concentrations in the time previously shown to be required for pattern regulation.

DIF-1 is a dynamic molecule and we have found that it is metabolized along a pathway involving at least 8 compounds. Metabolism is developmentally regulated and may be important in producing DIF gradients or other effector molecules from DIF.

Although we almost certainly have some of the central actors, it is difficult to formulate a satisfactory theory of pattern formation in *Dictyostelium* at the moment. We suspect that at least one important actor is missing.

Key words: *Dictyostelium discoideum*, DIF-1, cell differentiation, morphogen.

Introduction

During *Dictyostelium* development, order is literally created out of chaos. A teeming mass of separate amoebae at the start of development is transformed over 24 h into a population of discrete fruiting bodies, each one consisting of a basal disc on the substratum stabilizing a cellular stalk that in turn supports a mass of spores in the air (Raper, 1940; for recent reviews see Loomis, 1982; Gerisch, 1987; Williams, 1988). Because of this life style there can be no spatially organized input of maternal information to the embryo, as is so important in creatures such as *Drosophila*. Instead the patterning process in *Dictyostelium* must rely on the intrinsic properties of the cells and environmental inhomogeneities to produce reference points and morphogen gradients in the aggregate. In this respect *Dictyostelium*, a part-time multicellular organism, resembles mammals, where there is also thought to be little input of maternal positional information to the embryo.

Amputation and grafting experiments suggest that development in the *Dictyostelium* aggregate is organized by long-range signalling (in this context 1–2 mm) and, since the organism lacks intercellular junctions (Johnson *et al.* 1977) and the cells are not electrically coupled (Weijer and Durston, quoted in Loomis, 1982), it seems likely that this signalling proceeds *via* extracellular diffusible molecules. This paper describes first some of the *Dictyostelium* biology that is relevant to pattern formation, then the ways in which potential morphogens have been hunted in this organism (especially the DIFs) and finally what is known of the roles of these molecules in controlling cell differentiation and patterning.

Outline of development

Development is triggered by starvation and thereafter proceeds without external nutrients or essential cell divisions. After a few hours the amoebae start to gather toward collecting centers guided by cAMP signals relayed out from these centers. The number of amoebae gathered into each center is a function of the cell density and the density of signalling centers. In many cases large mounds of around 10^6 cells can form, but invariably these become subdivided into smaller mounds of no more than about 10^5 cells (see later). Each small mound becomes surrounded by a slime sheath, thereby isolating it from its neighbours. As this happens a protruding tip appears on the top of the mound (Fig. 1) and it elongates upwards to form a first finger, which can fall on its side to form the well-known migrating slug. Fate mapping reveals that the anterior 25% of the slug is normally destined to become the

stalk of the fruiting body and that the spores derive from the posterior 75 % (Raper, 1940). At this stage the anterior prestalk and the posterior prespore cells have clearly differentiated from each other. For instance, prespore cells express various components of the eventual spore coat, prepackaged into prespore vesicles (Hohl and Hamamoto, 1969; Maeda and Takeuchi, 1969). By staining these vesicles with antibodies, the prestalk/prespore pattern can readily be visualized (see Fig. 6). Definitive markers for prestalk cells have been much harder to obtain but recently we have isolated two genes whose expression is prestalk-specific and which code for proteins of the extracellular matrix (Williams *et al.* 1987; Jermyn *et al.* 1987). Using markers derived from these genes, an unexpected heterogeneity has been revealed in the prestalk zone of the slug (Jermyn *et al.* 1989; see Williams *et al.* this volume). There appear to be at least 3 types of prestalk cell: pstA cells express pDd63 mRNA and form an anterior cortex of the prestalk zone, pstB cells express pDd56 mRNA and form a central core of the prestalk zone and pstO cells express neither of these markers and form the rear of the prestalk zone. Finally as an added complication there are prestalk-like cells scattered in the prespore zone (Sakai and Takeuchi, 1971; Sternfeld and David, 1981). It is not known whether these cells should be regarded as a fifth cell type or merely as circulating prestalk cells, with a possible role in slug movement (Williams *et al.* 1987). The slug is an arrested stage of development which can persist for days. For this reason of experimental convenience, most work on patterning has focussed on the slug. However, it is now clear from using molecular markers that prestalk and prespore cells first arise earlier, in the mound, at, or shortly

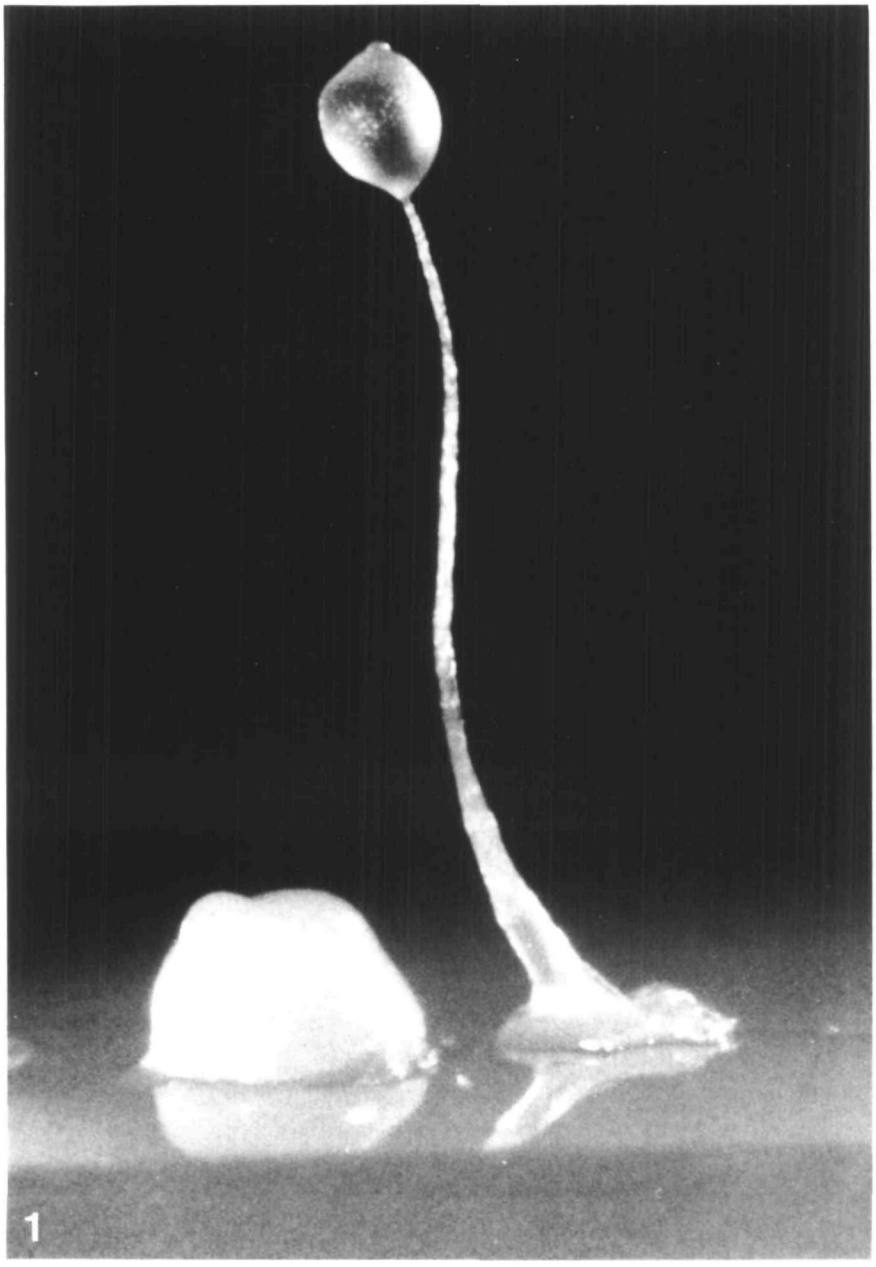

Fig. 1. Tipped mound and mature fruiting body of *Dictyostelium discoideum*. Starvation of *Dictyostelium* triggers development and after a few hours the amoebae aggregate together by chemotaxis to relayed cAMP signals to form a mound of cells. The size of the mound is initially indeterminate but there is an unknown mechanism whereby large mounds are partitioned into aggregates of about 10^5 cells. These aggregates surround themselves in a sort primitive extracellular matrix (the slime) and develop a protruding tip (shown just emerging, left) which thereafter leads the morphogenetic movements. The mound elongates upwards to form the standing slug and this can fall on its side to form the migrating slug which is photo- and thermo-tactic. In suitable environmental conditions, the slug transforms into the mature fruiting body (right) in which a basal disc and stalk support a mass of spores. Depending on strain, the entire process can be completed in 24 h with tips forming at about 11 h. Fruiting bodies can be up to 5 mm tall. Photograph reproduced by permission of The Company of Biologists.

before, the time when a tip forms (Hayashi and Takeuchi, 1976; Takeuchi *et al.* 1978; Krefft *et al.* 1984; Williams *et al.* 1987; Jermyn *et al.* 1987) and it follows that this stage of development is the primary seat of pattern formation. The slug is eventually triggered to transform into the final fruiting body by suitable environmental conditions, such as overhead light. During this process there are complicated movements of the prestalk and prespore tissues, which rival any described in higher embryos, before terminal differentiation into dead vacuolated stalk cells and viable, resistant spores.

Patterning, regulation and gradients

The patterning process in *Dictyostelium* has features that echo those in many other organisms. The regulative properties are perhaps the most extravagant known. Reasonably proportioned fruiting bodies can be constructed consisting of from 10^3 to 10^5 cells (Stenhouse and Williams, 1977). There are even reports of a minute fruiting body consisting of just 7 cells (see Hohl and Raper, 1964). Similarly it is well known that, given time, fragments of slugs can regulate to give properly proportioned fruiting bodies (Raper, 1940; Sampson, 1976). The impression gained from these experiments, that cell-type proportions are regulated by a supracellular signalling system, is confirmed by mixing cells of different physiology and showing that the probability of any cell becoming a spore depends on the nature of the cells it develops with (MacWilliams *et al.* 1985). Though many experimenters have wished for them, giant fruiting bodies have never been reported. There appears to be some mechanism for sub-dividing large aggregates or slugs into smaller ones, of no more than about 10^5 cells. Each entity develops a tip and then behaves independently (Hohl and Raper, 1964; Kopachik, 1982). The sizing mechanism in mounds appears to involve a diffusible tip inhibitor that can penetrate an agar membrane (Kopachik, 1982) and a similar conclusion has been reached from transplantation experiments with slugs (Durston, 1976; MacWilliams, 1982). In these experiments, the frequency with which a standard transplant will form a tip (and hence a separate organism) is measured when it is placed at various positions down a slug. The tip-formation inhibitor gradient thus detected is labile and has its high point at the anterior: the signal probably comes from the tip. When tissue from various positions down a slug is transplanted to a standard site in the host slug, the most anterior tissue is the most likely to form a secondary tip. The tip-activator gradient so revealed is most likely a stable cellular property as it can be measured in small transplants, where a diffusible morphogen would be expected to rapidly equilibrate with the host tissue. The explanation for size-control in terms of gradients of tip inhibitor and activator with high points at the tip is reminiscent of that offered for *Hydra* head formation (Hicklin *et al.* 1973).

Although various schemes can be advanced to explain cell-type proportioning and size-control, it has not been possible to prove any of them by experiments at the organismal level. To do this it is necessary to know about the signalling mechanisms involved, with the first step being the identification of the signal substances themselves.

Morphogen hunting

In *Drosophila* morphogens have been hunted very successfully by molecular genetics. Mutants affecting patterning are isolated, sifted in various ways and the most promising genes cloned by conventional techniques. By good fortune the protein product of a gene such as *bicoid* has turned out to be the morphogen itself, rather than, say, an enzyme that makes the morphogen (Driever and Nüsslein-Volhard, 1988; see St. Johnston *et al.* this volume). This genetic approach is also feasible in *Dictyostelium*, given the ease with which developmental mutants can be isolated, but it has not been used to date. Morphogens have instead been hunted by biochemical means. The key to success is to have a good assay for the morphogen: with this in hand, and a ready supply of material to purify, modern techniques of purification and identification should allow almost any molecule to be identified eventually. The most desirable assay would be one in which some aspect of pattern was affected but in practice less direct, but more convenient, assays have been used. For instance cAMP and adenosine were first implicated at the aggregation stage of development, cAMP as a chemoattractant for amoebae (Konijn *et al.* 1967), adenosine as a factor that reduced the number of spontaneous signalling centers during aggregation (Newell and Ross, 1982). Similarly ammonia was recognised as a factor that promoted the continued migration of slugs (Schindler and Sussman, 1977). Only later were these three compounds implicated in the patterning process.

Our hunt for *Dictyostelium* morphogens has been based on cell differentiation assays, on the assumption that the morphogens must ultimately control the positionally dependent differentiation of prestalk and prespore cells in the mound and later the positionally dependent maturation of prestalk cells as the stalk of the fruiting body is formed. This work started from an observation of John Bonner (1970), who found that cells plated on cAMP–agar remained as a monolayer but nevertheless some of them differentiated into stalk cells. We repeated this experiment with a particularly susceptible strain called V12M2 and also managed to devise conditions in which mature spores differentiated too. Spore formation required either a particular mutation or treatment of the cells with high concentrations of a penetrating cAMP analogue, to overcome a late block to spore maturation in the *in vitro* conditions (Town *et al.* 1976; Kay *et al.* 1978; Kay, 1989). Conditions were modified from those of Bonner, by allowing cells to differentiate as monolayers, submerged in a simple salts solution in tissue culture dishes, and we set out to discover the inducers necessary to make isolated

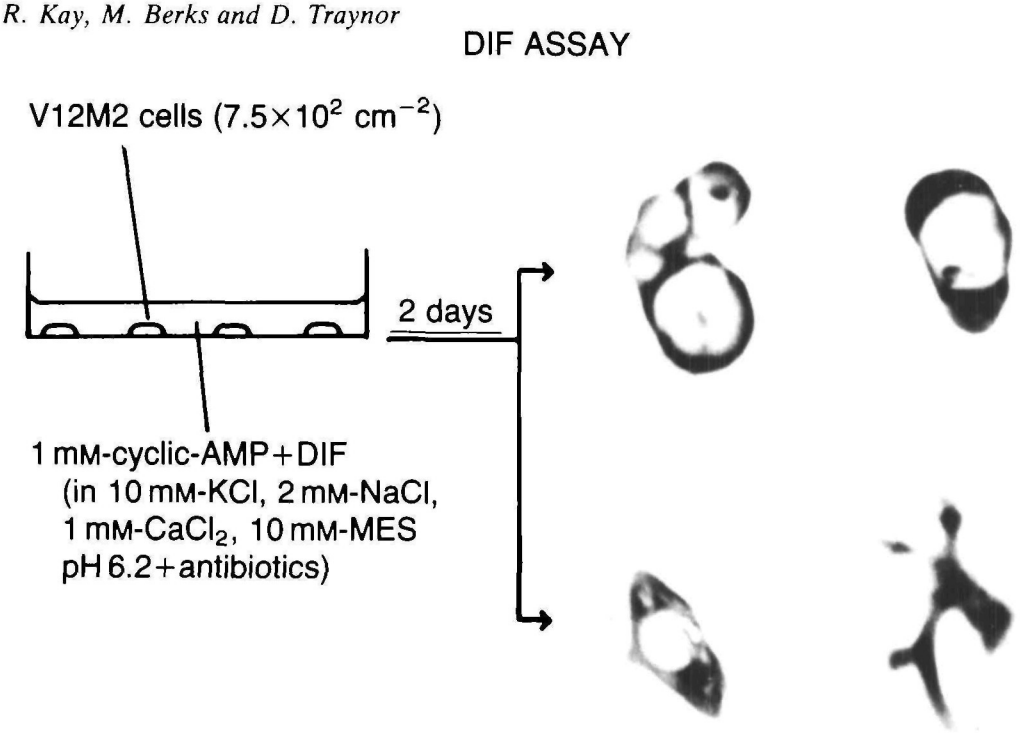

Fig. 2. Bioassay for DIF. The principle of the assay is that cells incubated at low cell density with cAMP in a simple salts solution (in which conditions they accumulate insignificant amounts of DIF) can be induced to form stalk cells by DIF. The proportion of stalk cells is scored by phase-contrast microscopy after 2 days and this gives a measure of the DIF concentration. The fully vacuolated cells (upper panels) are scored as stalk cells, the amoeba and partially vacuolated cell (lower panels) are scored as non-stalk cells. 10^{-10} M-DIF-1 induces approximately 27 % stalk cells in the assay. With a standard 2 ml assay volume 0.1 pmole DIF-1 can be detected.

amoebae differentiate into either stalk cells or spores. Here we concentrate on the DIFs, which specifically induce stalk cell differentiation and have been our major interest.

The DIFs: a new class of effector molecules

The DIFs are assayed by their ability to induce isolated amoebae to differentiate into stalk cells in the presence of cAMP (Fig. 2, Town *et al.* 1976; Town and Stanford, 1978; Brookman *et al.* 1982). At least 5 different DIFs have been resolved by HPLC of the medium collected from developing cells (Kay *et al.* 1983). Of these DIF-1 accounts for about 96 % of the recovered activity, DIF-2 for 3 %, DIF-3 and the others for 1 %. DIF-1 is active in the bioassay at 10^{-11} M and correspondingly minute amounts can be gathered from developing cells: from 2 years of accumulation using 40001 of medium and producing 16 kg of amoebae only about 100 μg of purified DIF-1 was available for identification. Powerful physical techniques such as ^{13}C-n.m.r. were therefore precluded and most information came from mass spectroscopy, with the isomeric possibilities being finally resolved by chemical synthesis. DIF-1 is a phenyl hexanone with di-chloro, di-hydroxy and methoxy substitution of the benzene ring; DIFs 2 and 3 are closely related molecules (Fig. 3; Morris *et al.* 1987, 1988). Chemically the DIFs are quite stable and their solubility

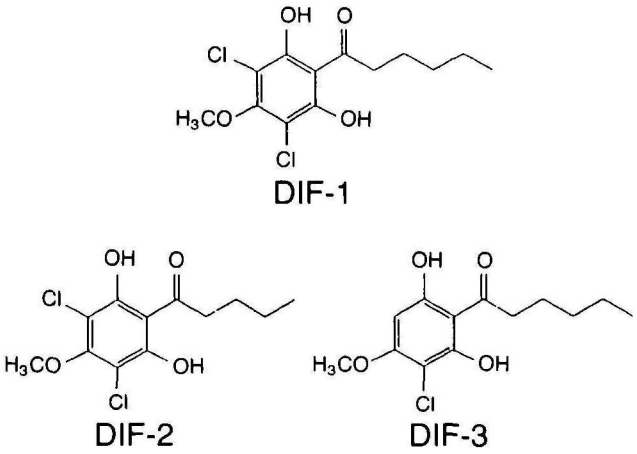

Fig. 3. Chemical structures of DIFs 1, 2, and 3. The structures were established largely by mass spectroscopy from less than 100 μg of purified material in each case.

in both hexane and water suggests that they are membrane permeable, opening the possibility that they have an intracellular receptor in the steroid/thyroid/retinoic acid super-family.

It is not known whether the DIFs and related molecules are unique to slime moulds or could be more widespread in nature. DIF activities have been detected in two other slime mould species, which use different chemoattractants in their aggregation stage (Brookman

et al. 1982), but a systematic search has yet to be made in other organisms. Certainly something as scarce as DIF would have eluded the sort of chemical analysis that has been used to classify major cellular lipids and metabolites in the past.

Role of the DIFs

The main role of the DIFs that we know about at the moment is in controlling cell differentiation during the multicellular stages of development. Most work has concentrated on DIF-1 because it is the most potent and most abundant DIF species in the slug (Masento *et al.* 1988; Brookman *et al.* 1987), but it remains quite possible that DIFs 2 and 3 also have important roles.

The fact that DIF-1 (together with cAMP) can induce a vegetative amoeba to differentiate into a stalk cell does not necessarily implicate DIF-1 in the patterning process. For instance, DIF-1 could just be stimulating some very early or very late step in the differentiation pathway and not be effective at the time when the prestalk/prespore pattern is laid down. We initially sought to address this problem in several ways: by determining when DIF is made during development, by isolating mutants with reduced DIF levels and examining their phenotypes, by isolating genes whose expression is induced by DIF and examining their regulation and finally by determining the effects of DIF-1 on spore cell differentiation.

The DIFs can be extracted from cells with organic solvents and estimated using the stalk cell bioassay. Growing cells do not contain detectable DIF, there is a small but definite rise during aggregation but the major rise occurs at the end of aggregation when the prestalk/prespore is formed (Fig. 4; Brookman *et al.* 1982; Sobolewski *et al.* 1983). DIF-1 can reach 0.2 μM in the slug (from Brookman *et al.* 1987), which is ample to bring about all the effects to be described in later sections.

Mutants making reduced amounts of DIF, but still responsive to it, were isolated using a stalk cell differentiation assay as the screen. At high density, wild-type cells incubated with cAMP accumulate their own DIF and so are induced to become stalk cells. Mutants impaired in DIF accumulation would not become stalk cells in these conditions but, if no other process was impaired, they should become stalk cells when DIF-1 was added. Three strains producing less than 10% of the peak wild-type levels of DIF but still responsive to it were recognised in this way (Kopachik *et al.* 1983). These mutants had several important properties: they arrested in development as tip-less mounds, suggesting a role for DIF beyond this point, they synergised with wild-type, suggesting a defect in signal production but not reception (as expected from the screen used in their isolation), and they made prespore products but not a prestalk product, suggesting that DIF was required for prestalk but not prespore cell differentiation. Thus the properties of these mutants strongly suggest an essential role for DIF at the time of pattern formation and that

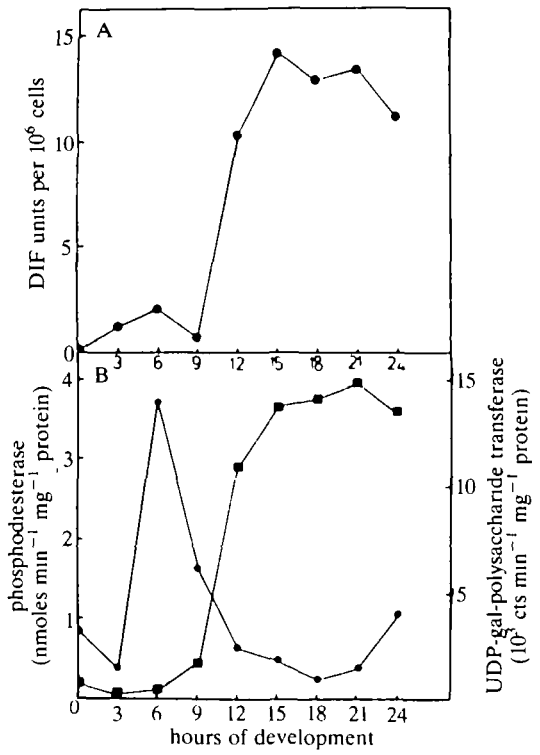

Fig. 4. Developmental regulation of DIF activity. DIF was extracted from developing cells using a standard lipid extraction procedure and assayed with the stalk cell differentiation bioassay (upper panel). There is little if any DIF detectable in growing cells but during aggregation there is a small but definite rise which might reflect a role at this stage of development. However, the major rise in levels occurs during the mound stage of development when the prestalk–prespore pattern is established. For comparison, the developmental regulation of an aggregative (cAMP phosphodiesterase) and a post-aggregative enzyme (glycogen phosphorylase) are also shown (lower panel). Reproduced from Brookman *et al.* (1982), with permission.

this role may be to induce prestalk cell differentiation. There are however a number of caveats. The mutants have not been subjected to an extensive genetic analysis, in no case has their basic lesion been tracked down and we cannot explain why they do make small, but definite, amounts of DIF.

The 'reduced-DIF' mutants allowed us to take the next step of identifying gene products whose expression is induced by DIF-1, by comparing DIF-1-treated and control cells. In this way a number of DIF-1-induced protein spots were recognised on 2D gels (Kopachik *et al.* 1985) and three cDNA species cloned by differential screening (Williams *et al.* 1987; Jermyn *et al.* 1987). The satisfying thing about these experiments was that all of the markers so identified could be shown independently to be prestalk or stalk cell specific. In the case of the pDd56 and pDd63 mRNAs, these markers actually identified a cryptic heterogeneity in the prestalk cell population, as described by Williams *et al.* (this volume).

Finally it could be shown in several ways that DIF-1 repressed prespore and spore differentiation. For in-

stance, wild-type prespore cells are converted to stalk cells under the influence of DIF-1 (Kay and Jermyn, 1983) and expression of prespore markers is repressed (Kopachik *et al.* 1985; Early and Williams, 1988). Likewise monolayer amoebae, which would otherwise become spores, are diverted almost quantitatively to stalk cell differentiation by DIF-1 (Kay and Jermyn, 1983; Kay, 1989).

All of these results strongly indicate that DIF (presumably DIF-1) is the endogenous inducer of prestalk cell differentiation that is active as the prestalk/prespore pattern is generated. It is easy to see that an appropriate gradient of DIF-1 in the aggregate could account for the formation of the prestalk/prespore pattern.

Before taking the patterning problem further, it is important to assess the roles of other *Dictyostelium* signal molecules in controlling cell differentiation. Again we are most interested in signals with a pathway-specific effect, since if these were correctly localized in the aggregate they could help create the prestalk/prespore pattern.

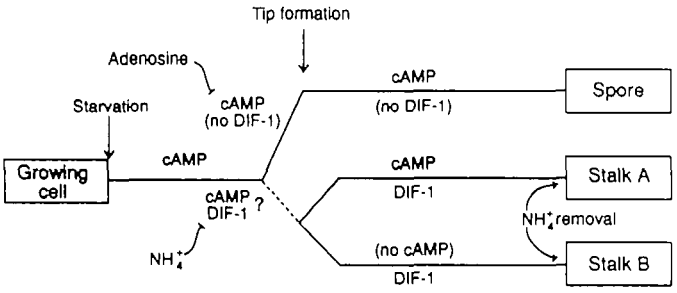

Fig. 5. Control of cell differentiation by diffusible signals. This provides a summary of a large body of work. After starvation the initial differentiation of all cells through aggregation is driven by cAMP signalling. It then appears that cell diversification is brought about by cAMP and DIF-1 acting combinatorially, with DIF-1 being necessary for the differentiation of both types of stalk cell. The dotted line on the stalk lineage indicates an uncertainty as to whether the prestalk A and prestalk B cells share a common prestalk precursor or come directly from aggregative amoebae. Adenosine is envisaged as working by inhibiting cAMP signalling though some direct effect on gene expression cannot be totally excluded. Ammonia antagonises DIF-1 by a mechanism that might involve intracellular pH (see Gross *et al.* 1988; Inouye, 1988).

The origin of prestalk and prespore cells

The signal molecules that have been chemically identified and proposed to play some role in controlling cell differentiation are DIFs 1–3, cAMP, its breakdown product adenosine, and ammonia, a product of cellular catabolism during development. The effects of these compounds on cell differentiation can be examined under two basic circumstances designed either to minimize cell interaction so as to detect direct effects on cell differentiation (single cell and short-term assays) or to permit interaction so as to detect also indirect effects that work by modulating the levels of some other factor such as DIF (high cell density, longer term assays).

A scheme for the regulation of cell-type specific differentiation is given in Fig. 5 and is based on the following points:

1. The basic bifurcation between prestalk and prespore differentiation is made by DIF-1 which directly induces prestalk and inhibits prespore differentiation (last section).

2. cAMP directly induces prespore (Kay *et al.* 1978; Kay, 1979, 1982; Barklis and Lodish, 1983; Mehdy *et al.* 1983) and prestalk A differentiation (provided DIF is present; Berks and Kay unpublished). After the tipped mound stage cAMP represses prestalk B differentiation (Berks and Kay, 1988; and unpublished) and may therefore be responsible for maintaining the distinction between prestalk A and B cells in the slug.

3. Adenosine inhibits prespore cell differentiation (Weijer and Durston, 1985; Schaap and Wang, 1986) probably indirectly by an inhibition of cAMP signalling (Newell and Ross, 1982; Thiebert and Devreotes, 1984).

4. Ammonia may inhibit prestalk cell differentiation (Gross *et al.* 1983; Bradbury and Gross, 1989) by an unknown mechanism and its abrupt removal can trigger stalk cell maturation (Schindler and Sussman, 1977; Wang and Schaap, 1989). probably by causing a drop in intracellular pH (Inouye, 1988).

There are a number of holes in this scheme: it is not clear how the division of prestalk cells into prestalk A and B is first brought about, nor how prestalk O cells (not shown in the diagram) originate, nor how the final maturation of prestalk and prespore cells is controlled. However, Fig. 5 does re-emphasise the central role of DIF-1 in bringing about the basic bifuraction of aggregated amoebae into prestalk and prespore cells. Finally, it is interesting to note that in the slug cAMP and DIF-1 might be acting combinatorily to define cell states: plus cAMP no DIF-1, plus cAMP plus DIF-1 and no cAMP plus DIF-1 each define a distinct cell type (no cAMP no DIF-1 probably corresponds to a state of dedifferentiation toward the aggregative state).

DIF-1 and patterning

Measurement of the distribution of DIF in migrating slugs by dissection, extraction and bioassay raised a puzzle. The gradient is the reverse of that expected (Brookman *et al.* 1987), with the concentration of DIF in the prespore zone (where it is not active) approximately twice that in the prestalk zone (where it is active). There are many possible explanations for the reverse DIF-1 gradient. Two technical doubts are firstly that it was only possible to measure total DIF, not the fraction which is actually active, and secondly that the experiments were performed on slugs, several hours after the primary patterning process in the mound. In this time, as a way of stabilizing the differentiated states, the prespore cells could have become relatively

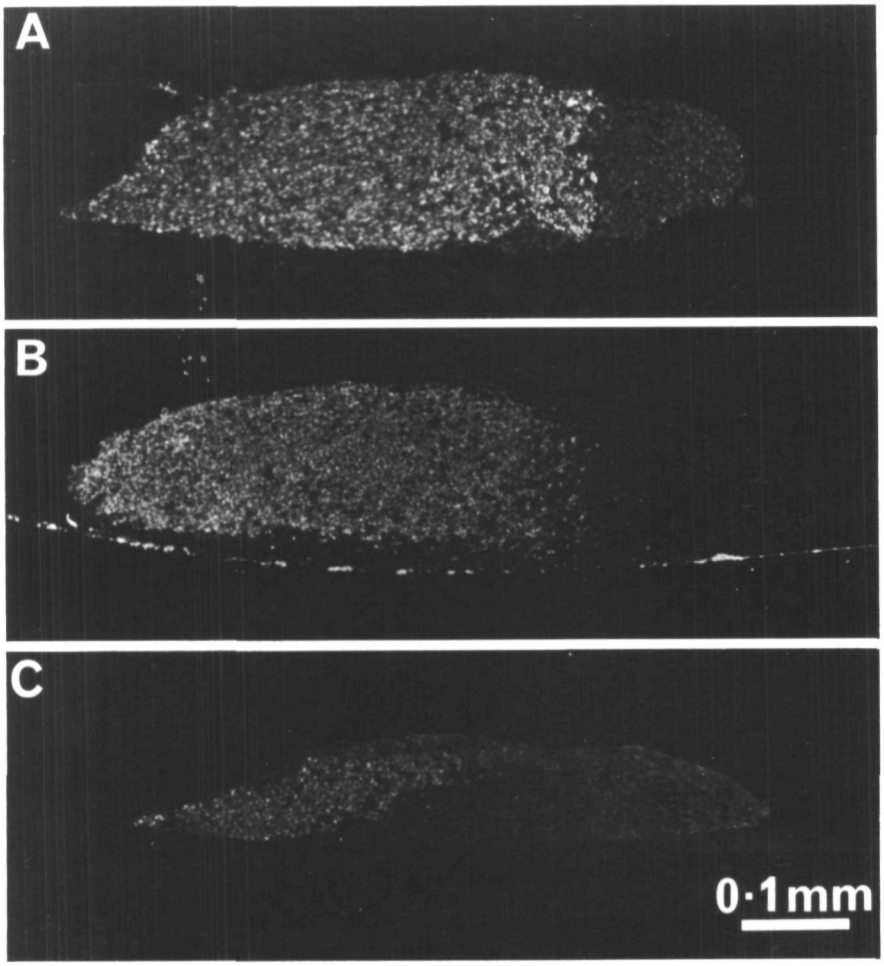

Fig. 6. DIF-1 treatment of slugs causes the prestalk zone to become enlarged. Longitudinal sections of slugs are stained with an antibody recognising prespore cells. (A) Control slug; (B) typical DIF-1-treated slug; (C) extreme example of a DIF-1-treated slug. The effect of DIF-1 is to increase the relative sizes of both the prestalk zone and of the platform zone adjacent to the agar and to blur the prestalk–prespore boundary. Slugs migrating on cellophane were transferred to fresh agar containing 0.2 μM-DIF-1 as indicated. After 4 h slugs were fixed, sectioned and stained by conventional techniques. Reproduced from Kay *et al.* (1988) with permission.

insensitive to DIF-1 and so not be affected by the DIF-1 in the prespore zone. Clearly the dissection experiments need repeating on mounds. Alternatively the results might indicate the involvement of an additional morphogen: either an activator formed from DIF-1 and localized to the prestalk zone (Meinhardt, 1983) or a DIF-1 antagonist in the prespore zone (see below).

In contrast, evidence that DIF-1 does act as a morphogen comes from experiments in which slugs or aggregates were transferred to agar containing DIF-1. The general effect of DIF-1 is to cause enlargement of the prestalk zone and of the 'platform' zone of prestalk cells adjacent to the agar (Fig. 6, Kay *et al.* 1988). Significant changes are produced by 0.1 μM-DIF-1 (Fig. 7). which is less than estimated physiological concentrations in the slug of 0.2 μM. Patterning changes are detectable after 1 h and complete by 4 h of incubation with DIF-1, times comparable to those required for prespore to prestalk conversion in isolated prespore fragments (Sakai, 1973). However, even here there is a complication because the extent of conversion induced by DIF-1 is always limited. In strain V12M2 the proportion of prestalk cells can be increased from 33 % to 53 % but no further (Fig. 7). Other strains, such as X22, behave in a similar way. This lack of responsiveness to DIF-1 of prespore cells in intact slugs is surprising because isolated prespore cells respond readily (Kay and Jermyn, 1983). We do not think the problem is one of DIF-1 penetration; more likely we suggest that there is a DIF-1 antagonist in the slug, produced in response to DIF-1, which limits its effects. To date searches for DIF antagonists have turned up ammonia (Gross *et al.* 1983) and cAMP itself (Berks and Kay, 1988), but neither fits the bill exactly. For instance, neither cAMP nor ammonia (except at very high concentrations) represses the induction of the pDd63 mRNA by DIF-1 (Berks and Kay, unpublished). It may now be necessary to start hunting for a factor that does.

DIF-1 metabolism

It is important to understand DIF-1 metabolism for several reasons. First, this is likely to be an important way in which DIF-1 levels are regulated in the aggregate and provide a target for control of DIF-1 levels by other morphogens. Second, an understanding of how DIF-1 is metabolised should allow us to manipulate DIF-1 levels in the aggregate and thereby learn more of its role in development. Finally, it is possible that some metabolites may turn out to be morphogens in their own right: possibilities include a short-range activator as suggested by Meinhardt (1983) or the inhibitor mentioned above.

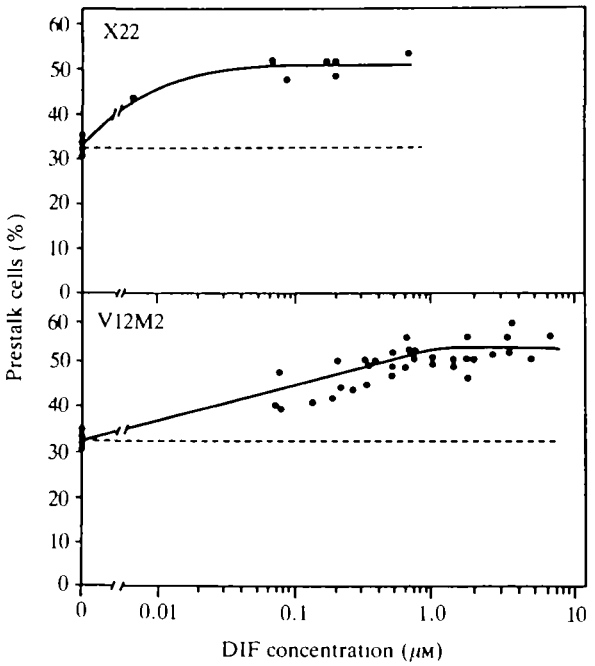

Fig. 7. Dose dependence of patterning changes caused by DIF-1. Slugs of strain V12M2 or X22, migrating on cellophane, were transferred to agar containing various concentrations of DIF-1. After a further 4–6 h, the slugs were harvested, disaggregated to single cells and stained with an antibody against prespore cells (Takeuchi, 1963) and the proportion of non-staining (=prestalk) cells determined.

Radioactive DIF-1, with ^{3}H or ^{14}C label of the ring methoxy group was synthesised chemically and fed to cells. DIF-1 metabolites were then recovered from the medium or cells and examined by reverse-phase HPLC or TLC. It was immediately apparent that DIF-1 is metabolized in a surprisingly complicated way and we have so far recognised at least 8 significant metabolites (Traynor and Kay, unpublished). By following the flow of counts from one compound to another and by determining the fate of purified intermediates refed to cells, we have been able to deduce a tentative pathway for DIF-1 metabolism, in which the first component is largely cell-associated and the other metabolites are found mainly in the medium. Little is yet known of their chemical nature.

DIF metabolism is developmentally regulated, reaching a maximum at about the mound stage, when DIF-1 levels are rapidly rising and the prestalk/prespore pattern is being established. At this stage or in the slug the cellular DIF-1 metabolizing capacity is such that DIF-1 must be rapidly metabolized with a half-life of only a few minutes. Thus DIF-1 is probably a dynamic molecule that is made and degraded rapidly.

Most of the DIF-1 metabolites are active in the stalk cell induction bioassay, but none has more than 5 % of the specific activity of DIF-1. To date none synergise with DIF-1 or inhibits its activity. Thus so far none of the metabolites is a candidate morphogen in its own right: what, if anything, do all these compounds do?

Prospects

The study of pattern formation in *Dictyostelium* is at a tantalizing stage, where candidate morphogens have been identified but a coherent scheme for their action is still lacking. We cannot yet explain how the prestalk/prespore pattern is generated, how a large aggregate is partitioned into discrete entities or the nature of the tip inhibitory gradient emanating from the tip. Several factors impede, or have until recently impeded, progress in this area. At a conceptual level, too great an emphasis has in the past been devoted to patterning in the migrating slug. This is several hours removed from the mound stage, which is the primary seat of patterning, and in this time cell differentiation could complicate the analysis. Perhaps the greatest technical problem here, and in other organisms using small, diffusible morphogens, is that of visualizing morphogen gradients. Even with suitable antibodies it is unlikely that the fixation procedures used to reveal the distribution of a large morphogen like the bicoid protein would be of any use for cAMP or DIF-1. An additional problem is that we probably do not have the complete inventory of *Dictyostelium* morphogens: the nature of the tip inhibitor is obscure (though it could be adenosine; Schaap and Wang, 1986) and we have been forced here to postulate the existence of a new DIF-antagonist. Perhaps some of the DIF metabolites or other factors recently described will complete the story (Gibson and Hames, 1988; Kumagai and Okamato, 1986; Mehdy and Firtel, 1985)?

Thus the main consequence of knowing the structure of DIF-1 seems not to have been an immediate understanding of the biological processes in which it participates, but instead the ability to do more and better experiments. DIF-1, which was once scarce, can now be synthesised chemically along with radioactive DIF-1 and various analogues. Work on DIF-1 has led to the identification of new markers for prestalk cells, which in turn have revealed the existence of a new anatomy in the prestalk zone. It is clear that the basic diversification of developing amoebae toward stalk or spore cell differentiation can be brought about by DIF-1. With this rate of progress and because *Dictyostelium* is now such a favourable organism to work with, one must be optimistic of obtaining quite soon a working outline of the patterning process.

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