The initiation of basal disc formation in *Dictyostelium discoideum* is an early event in culmination

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SUMMARY

We have analysed expression of the *ecmA* and *ecmB* genes of *Dictyostelium* by enzymatic double staining using β-galactosidase and β-glucuronidase reporter gene constructs. Cells expressing the *ecmA* gene first appear as scattered cells at the mound stage of development and we show that this is also true for cells expressing the *ecmB* gene. During tip formation the *ecmA*-expressing cells move to the apex of the mound, while the *ecmB*-expressing cells accumulate in the base. The *ecmB*-expressing cells constitute part of the basal disc if the culminant is formed in situ but are discarded if a migratory slug is formed. During slug migration they are replaced by a band of *ecmB*-expressing cells, situated in the front half of the prespore zone and tightly apposed to the substratum. When culmination is triggered these cells rapidly move to the back half of the prestalk zone, possibly acting as a point of attachment to the substratum. Ultimately, they are joined by cells at the back of the slug, the rearguard cells, to form the basal disc. Thus, contrary to previous belief, basal disc formation is initiated very early during culmination and occurs by the forward movement of cells located in the anterior of the prespore zone.

Key words: *Dictyostelium discoideum*, basal disc, *ecmA* gene, *ecmB* gene, culmination, prestalk cells, β-glucuronidase

INTRODUCTION

Under conditions that are inappropriate for immediate culmination the *Dictyostelium* aggregate forms itself into a migratory slug. This is phototactic and thermotactic and these sensitivities direct it to a suitable site for culmination. The stalk of the mature culminant is composed of dead, highly vacuolated cells and is surrounded by an extracellular matrix, the stalk tube. It tapers from bottom to top and is embedded in a conical structure, the basal disc, which is presumed to help support the culminant. The front one fifth of the slug is composed of cells that will, under normal circumstances, differentiate into stalk cells while the rear four fifths contains predominantly prespore cells. Intermingled with the prespore cells are cells that share the biochemical properties of prestalk cells, the anterior-like cells (ALC; Devine and Loomis, 1985; Sternfeld and David, 1981), and at the extreme posterior of the slug there is a cluster of ALC called the rearguard (Bonner, 1944; Bonner et al., 1955).

When the slug enters culmination the tip becomes arrested in its forward movement and orients itself upwards. The posterior region continues to move forward, so positioning itself underneath the prestalk region. Prestalk cells within the tip then start to synthesize the stalk tube. Cells at the apex move into the stalk tube, where they continue to deposit matrix components and where they ultimately vacuolate and die. The process of stalk elongation continues until all of the prestalk cells differentiate into stalk cells, by which time the spore head stands above the substratum.

Prior to elevation of the nascent spore head the stalk tube embeds itself into a group of cells at the base. These prebasal disc cells have generally been believed to derive from the rearguard cells (Bonner, 1957; Sternfeld, 1992). The ALC undergo a complex pattern of movement at culmination. Some move upwards to form a cup above the spore head, the upper cup, and some move downwards to form the lower cup, below the spore head (Sternfeld and David, 1982; Jermyn and Williams, 1991). This latter population has been proposed also to contribute to the basal disc (Jermyn and Williams, 1991), although there is no direct evidence on this point.

The slug surrounds itself with a matrix known as the slime sheath which it constantly sheds behind it, to form a trail on the substratum. The slime sheath has a chemical structure very similar to the stalk tube (Freeze and Loomis, 1978) and both these matrices contain the EcmA and EcmB extracellular matrix proteins (McRobbie et al., 1988a,b). The patterns of expression of the *ecmA* and *ecmB* genes are complex and have led to the identification of multiple prestalk cell sub-types (Jermyn et al., 1989).

Cells within the front 10% of slug length express the *ecmA* gene at a high level and are called pstA cells. The prestalk cells in the region behind the pstA cells express the *ecmA* gene at a lower level and are called pstO cells. Different parts of the *ecmA* promoter direct gene expression in the pstA and pstO
Fig. 1. (A) The ecmBneoGAL reporter vector. This vector contains the BgII promoter fragment of the ecmB gene (Jermyn et al., 1989) inserted into the pDdGAL17 vector (Harwood and Drury, 1991). (B) The ecmAnucGUS vector. This vector contains the β-glucuronidase gene from *E. coli* (Jefferson, 1986) inserted downstream of a multiple cloning site (MCS), in place of the lacZ gene of ecmA(BgII-)GAL which contains the BgII promoter fragment from ecmA (Jermyn et al., 1989). The resulting ecmAnGUS vector was further modified by the insertion of an oligonucleotide encoding the nuclear localising signal from the SV40 T-antigen (Kalderon et al., 1984), in frame and between the promoter and the reporter. When the resultant ecmAnucGUS construct is introduced into Dictyostelium to give a stable transformant, enzymatic activity is readily detectable by histochemical staining using X-gluc.

Fig. 2. ecmB expression during slug formation. A and B show aggregates derived from cells transformed with the ecmBneoGAL construct. They were fixed and stained with X-gal and then sectioned. In these two panels only, the pstB cells are blue in colour. A shows a longitudinal section of a tight aggregate, while B shows a longitudinal section of an early tipped aggregate. C, D and E show aggregates prepared from cells co-transformed with ecmBneoGAL and ecmAnucGUS that were fixed and stained sequentially for β-glucuronidase and β-galactosidase. pstB cells are red, ecmA-expressing cells are blue and the red cells with blue or purple nuclei are pstAB cells. C shows two standing slugs, D shows a slug just after it commenced migration and E shows an area where aggregation occurred (i.e. the start point of migration of a slug, an area similar to that at the bottom left hand side of D).

sub-types and there are also ALC which utilise pstO-specific promoter elements (Early et al., 1993). These form an inter-changing population with the pstO cells in the prestalk region and are therefore called the pstO/ALC (Abe et al., 1994).
There is another class of ALC, the pstB cells, which express the ecmB gene but not the ecmA gene (Gaskell et al., 1992). There may also be additional classes, because several reporter gene fusion constructs show expression in scattered cells in the rear but no concentration of staining cells in the prestalk region (Hadjiev and Firtel, 1992; Howard et al., 1992; Gaskins et al., 1994; Howard et al., 1994). During culmination pstA cells activate expression of the ecmB gene, just as they enter the stalk tube, and are then termed pstAB cells (Gaskell et al., 1992). Cells within the upper and lower cups also co-express the ecmA and ecmB genes (Jermyn and Williams, 1991), and they also express rasD, another marker of prestalk cell differentiation (Esch and Firtel, 1991).

Previous immunohistochemical studies have shown how the different cell sub-types behave during slug formation, slug migration and culmination (Jermyn and Williams, 1991; Williams et al., 1989). Here we re-investigate these processes using double enzymatic staining with β-galactosidase and β-glucuronidase reporter constructs. EcmA-expressing cells first appear at the loose aggregate stage, where they lie scattered in apparently random positions (Williams et al., 1989). Previously, the ecmB-expressing cells were suggested to arise in the base of the aggregate (Williams et al., 1989) but the increased sensitivity afforded by the β-galactosidase reporter has allowed detection of ecmB-expressing cells at an earlier stage in their differentiation than was possible with immunostaining and we show that they appear in a similar fashion to ecmA-expressing cells, i.e. as scattered cells within the aggregate.

If the slug forms a culminant in situ, the pstB cells form part of the basal disc but, if a migratory slug is formed, they are deposited into the slime trail and left behind as the slug moves forward. They are replaced by ecmB-expressing cells, many of which are clustered in the anterior half of the prespore zone (Jermyn and Williams, 1991). We show, by double staining, that these are pstB cells.

We show that forward movement of the anteriorly located band of pstB cells is one of the very early responses to the signal that triggers culmination and we describe how they come to form part of the basal disc.

**MATERIALS AND METHODS**

**Construction of the GAL and GUS fusion gene vectors**

EcmBneoGAL

This was created by inserting the BglII promoter fragment of the ecmB gene (Jermyn et al., 1989) into pDdGAL-17 (Harwood and Drury, 1990).

EcmAnucGUS

The plasmid pRAJ 275 contains the uidA (GUS) gene of *E. coli* (Jefferson et al., 1986). Two oligonucleotides were synthesised to create, by PCR, a BglII site in the third codon of the translated sequence and a XhoI site beyond the GUS translational stop codon (see Jefferson, 1988). The resulting 1.9 kb product was restricted with BglII and XhoI then ligated into ecmA(BglII)-neoGAL, so as to remove the lacZ gene, and to create ecmAnucGUS.
EcmA(BgII)-neoGAL was created by subcloning the BgII promoter fragment from the ecmA gene (Jermyn et al., 1989) into the BamHI/BgII sites of pDG44-17. A synthetic oligonucleotide, 5'-GATC TCC AAA AAA AAG AAA AGT GGA TGA AGA TCC AG-3', corresponding to the SV40 T-antigen core nuclear localisation signal (Kalderon et al., 1984) was inserted into the novel unique BgII site at the 5' end of GUS sequence in ecmAneoGUS to create the vector ecmAnucGUS.

Growth and transformation of Dictyostelium

Dictyostelium discoideum cells of the strain Ax2 (Watts and Ashworth, 1970) were grown and subjected to DNA mediated transformation as described previously (Nellen et al., 1984; Early and Williams, 1987). The transformed colonies were first selected at 20 g/ml G418 (Gibco) and later at 200 g/ml G418. This was found necessary to maintain a high level of β-glucuronidase expression. Further selection was carried out by cloning on Klebsiella aerogenes lawns (Buhl et al., 1993). Cells were picked from the growing edge of well separated plaques into axenic medium containing 200 µg/ml G418. The plaques were then lifted onto Nitrocellulose 'Extra' filters (Sartorius) and screened sequentially for β-glucuronidase and β-galactosidase activity by a method similar to that described by Buhl et al. (1993), except that the filters were fixed in 0.05% glutaraldehyde as described below and washed in 50 mM NaHPO₄ (pH 7.0) containing 0.2% Tween 20 (Sigma).

Development of transformants

Cells were set up for development and slug formation on 2% water agar plates as described previously (Jermyn and Williams, 1991) and were, in some cases, stained with Neutral red (Sternfeld and David, 1981). Migrating slugs were normally used approximately 24 hours after plating. They were floated off the agar during fixation, and care was taken with culminating stages to ensure that the whole body was immersed during this and subsequent manipulations. Early aggregate stages tended to remain submerged. Dissociated cells were more easily obtained from stages developed on Millipore filters (0.45 µm) supported on K$_{3}$O$_{4}$ (17 mM KHPO₄, pH 6.1) soaked prefilters. Cells were vortexed off the filter, pelleted and resuspended in 20 mM EDTA/20 mM Na/KHPO₄, pH 7.0 (for β-galactosidase staining) or 50 mM NaHPO₄, pH 7.0 (for β-glucuronidase). They were then passed several times through a 25 g gauge needle.

Sections were prepared from stained whole mounts embedded in OCT compound (BDH, UK). 10 µm cryosections were picked onto gelatin-coated slides. Both whole mounts and sections were mounted under coverslips in Gelvatol 20/30 (Monsanto, Mass., USA).

Histochemical assay of β-glucuronidase and β-galactosidase activity

X-gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid) is a substrate for β-glucuronidase which produces a blue product when cleaved. While salmon gal (6-chloro-3-indolyl-beta-D-galactopyranoside), a substrate for β-galactosidase, produces a red precipitate. Initial experiments showed that Dictyostelium co-transformant cells, containing fusion gene constructs that direct co-expression of β-glucuronidase and β-galactosidase, give a purple colour when stained with X-gluc and salmon gal (data not shown). However, production of the purple colour was very inconsistent, suggesting substrate or product inhibition between the two enzymes. We therefore constructed a fusion gene vector for β-glucuronidase, ecmAnucGUS, that contains the nuclear localisation signal from the SV40 virus (Fig. 1B). In co-transformant strains, in which β-glucuronidase is nuclear-localised and β-galactosidase is cytosolic, it is a simple matter to distinguish singly expressing and co-expressing cells. It should be noted, however, that when cells expressing only β-galactosidase are stained for extended periods it can prove difficult to distinguish the resulting intense red from the purple of co-expressing cells.

Single cells or multicellular structures were fixed in 50 mM NaHPO₄ (pH 7.0) containing 0.05% (w/v) glutaraldehyde for 10 minutes, and washed twice in 50 mM NaHPO₄ (pH 7.0) for 2 minutes. If both β-glucuronidase and β-galactosidase activity were to be analysed within the same cell or aggregate, the specimen was initially stained for β-glucuronidase, then transferred to β-galactosidase staining buffer. Samples were stained for β-glucuronidase activity (usually overnight at 22°C, but also at 37°C for 6 hours) in 50 mM NaHPO₄ (pH 7.0), 5 mM K$_{3}$Fe(CN)$_{6}$, 5 mM KF(CN)$_{6}$, 2 mM X-glAC CHX (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexylammonium salt; Molecular Probes, Inc., USA). Samples were stained for β-galactosidase activity at 22°C overnight or 37°C (usually for just a few hours) in 60 mM Na$_{2}$HPO₄, 40 mM NaH$_{2}$PO₄, 10 mM KCl, 2 mM MgCl₂, 5 mM K$_{3}$Fe(CN)$_{6}$, 5 mM KF(CN)$_{6}$ containing 2 mM salmon-beta-D-gal (6-chloro-3-indolyl-beta-D-galactopyranoside [Biosynth AG, Switzerland]), or 2 mM X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactoside [Biosynth AG, Switzerland]) if only GAL expression was assayed.

RESULTS

When ecmB-expressing cells first appear they lie scattered within the aggregate

A transformant strain containing the ecmB promoter linked to the lacZ gene (Fig. 1A) was allowed to develop and was analysed histochemically at various stages. Cells expressing the fusion gene first become detectable in large numbers at the tight aggregate stage where they appear to be randomly distributed (Fig. 2A). As was previously shown using immunohistochemical methods, the expressing cells subsequently accumulate in the base of the aggregate, although at the time of tip formation, many ecmB-expressing cells are still located elsewhere (Fig. 2B).

The ecmB-expressing cells in the base of the first finger are lost during migration from the point of aggregation

In order to allow simultaneous analysis of ecmA and ecmB gene expression we made a fusion construct in which the ecmA promoter directs expression of β-glucuronidase (Fig. 1B) and co-transformed it into cells with the construct in which the ecmB promoter directs β-galactosidase expression. For technical reasons (see Materials and Methods), a nuclear localisation signal was incorporated into the β-glucuronidase protein. Using a blue substrate for β-glucuronidase and a red substrate for β-galactosidase it is readily possible to identify cells that singly express, and cells that co-express, the two genes. pstA and pstO cells display a blue nucleus, pstB cells display a red cytoplasm and pstAB cells have a blue nucleus in a red cytoplasm (Fig. 3). Sometimes co-expressing cells appear purple and have no nucleus, presumably indicating that the nucleus lysed liberating the β-galactosidase. It should also be noted that, even in a co-expressing cell, very weak expression of one of the genes may not be noticeable.

The ecmB-expressing cells that eventually accumulate in the aggregate base at the first finger stage (Fig. 2C) are interpreted as being mainly pstB cells. The ecmA expression previously noted in the base of many late tipped aggregates (Williams et al., 1989) cannot be detected within the ecmB-expressing population using the β-glucuronidase reporter. However, over the course of a few hours, the basal cells that are discarded as the slug migrates away from the area of aggregation (Fig. 2D).
become purple showing that they are expressing both *ecmA* and *ecmB* (Fig. 2E). It is not clear whether all the original pstB population is lost in this way, or whether there is a residual population that redistributes once the slug migrates. It is clear that a new population of pstB cells appears in the migrating slug, because the average number of pstB cells per slug increases at least twofold within the first few hours of migration (K. A. J. and J. G. W., unpublished results).

**In the migrating slug most of the pstB cells are situated in the anterior of the prespore zone in a band of basally located cells**

In slugs migrating towards a unidirectional light source there is a scattering of *ecmA*-expressing cells in the prespore region (Fig. 4A; see Fig. 7 for a schematic representation of this and most of the subsequent results). There is a marked asymmetry in their distribution, with most of the cells situated close to the prestalk zone. Most of the pstB cells are localised to a band that lies in the front half of the prespore region, behind the *ecmA*-expressing cells (Fig. 4B). They are squamous in appearance and are predominantly situated on the ventral surface of the slug, immediately adjacent to the substratum (Fig. 4C,D).

**During culmination the pstB cells move forward and initiate basal disc formation**

When migrating slugs are exposed to overhead light and reduced humidity they enter culmination. Very soon after this transition the band of pstB cells moves forward to enter the pstO region (Fig. 5A,B). There is a detectable reduction of the separation between the pstA and pstB cells within 15 minutes (data not shown) and the process is usually completed within about 30 minutes, although there is slug to slug variation in the speed of this process (Fig. 5A and Table 1).

As the slug rounds up, plan views of whole mounts show the pstB cells now to be concentrated in an incomplete annulus at the base (Fig. 5C,D). These cells remain in their new position as the tip extends upwards (Fig. 5E). Fixation and staining of culminants at various stages in maturation shows that these cells become incorporated into the basal disc, although by the time the prespore mass is lifted off the substratum, pstB cells fill the whole basal area, presumably by recruitment of newly expressing cells (Fig. 5F). The number of non-vacuolated *ecmB*-expressing cells increases approximately twofold between the rounding up of the slug and the lifting of the prespore mass (data not shown).

**Assymetric expression of the *ecmA* and *ecmB* genes in the upper and lower cups**

During culmination the ALC population divides; some cells move up to the prestalk-prespore boundary while others move down to the base. The cells that move upwards form a cup above the spore head while those that move downwards form a cup below the spore head (Sternfeld and David, 1982). Analysis of double transformant slugs reveals a difference between the two cups. In the upper cup, cells expressing the *ecmA* gene and cells expressing the *ecmB* gene are approximately equally represented and there are also cells expressing both genes (Fig. 6A). In contrast, the lower cup is predominantly composed of pstB cells although there are some *ecmA*-expressing cells and some co-expressing cells (Fig. 6B). The basal disc shows a similar staining pattern to the lower cup (Fig. 6C), supporting the idea of a common origin for these two tissues (Jermyn and Williams, 1991).

Since there are co-expressing cells in both cups, we wished to obtain some indication as to whether cells activate expression of both genes while still in the spore head or whether they move as singly expressing cells and activate the other gene upon arrival at the cup, as this may be relevant to their direction of chemotaxis (see Discussion). The cells within the spore head fall into three classes: cells expressing *ecmA*, cells expressing *ecmB* and cells co-expressing both genes (Fig. 6D). Although it is of course impossible to be definitive on this point when using a stable reporter protein, the existence of the latter class makes it seem likely that at least some of the co-expressing cells in the two cups derive from cells that activated expression of both genes prior to moving there.

<table>
<thead>
<tr>
<th>Time</th>
<th>Total number of slugs</th>
<th>Within pstO zone</th>
<th>Behind pstO zone</th>
<th>Unclear</th>
<th>Evenly spread</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>120</td>
<td>15(12.5%)</td>
<td>81(67.5%)</td>
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Slugs were formed from cells co-transformed with *ecmBneoGAL* and *ecmAnucGUS* and induced to culminate as described in Fig. 5. Some of the aggregates were fixed and sequentially stained for β-glucuronidase and β-galactosidase immediately after exposure to overhead light, (t0), while the remainder of the aggregates were fixed after 30 minutes (t30'). After staining, the fields were examined under a dissecting microscope, and the location of the pstB cell band in relation to the pstO zone was determined. In some slugs (‘unclear’) the pstB cells could not be clearly seen beneath the blue pstO cells so these were not scored. In others (‘evenly spread’) the pstB cells were not concentrated in any particular region. The numbers shown are the means from 2 experiments.

**DISCUSSION**

When they first appear during slug formation *ecmA*- and *ecmB*-expressing cells are randomly distributed throughout the aggregate. The prespore cells also differentiate at apparently random positions within the loose aggregate, so that several different cell types arise in an intermingled fashion, with immediate neighbours adopting different patterns of gene expression. How is this possible, given that diffusible extracellular signals direct the differentiation of these different cell types?

One possibility is that there is an inherent pre-disposition to differentiate down one or other of the pathways. There is a considerable body of evidence to show that cells that enter development soon after mitosis tend to become prestalk cells while cells that enter development later in the cell cycle tend to become prespore cells (reviewed by Maeda, 1993). Perhaps the prestalk-disposed population is further sub-divided into cells with a tendency to become *ecmA*-expressing cells and cells that tend to become *ecmB*-expressing cells.

Alternatively, there may be microenvironments wherein the extracellular signalling conditions differ. This explanation

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Table 1. The location of the band of *ecmB*-expressing cells in slug posteriors during migration, and 30 minutes after the induction of culmination

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Fig. 5. Stages in the culmination of slugs formed from cells co-transformed with ecmBneoGAL and ecmAnucGUS. Slugs were formed on water agar plates and allowed to migrate towards an external light source for at least 8 hours. The plates were then withdrawn and exposed to full overhead light with the lids of the dishes propped open. After 30 minutes, the slugs were fixed and sequentially stained for β-glucuronidase and β-galactosidase. (A) A typical slug, 30 minutes after exposure to overhead light. Compare the position of the pstB cells with that in the slug in Fig. 4B. (B) A high magnification view of the prestalk/prespore boundary of a slug 30 minutes after exposure to overhead light with the ventral surface in the focal plane. Compare the relative positions of the ecmA-expressing and the pstB cells with that in the slug in Fig. 4C. (C) Higher magnification of the prestalk region of a slug 30 minutes after exposure to overhead light with the ventral surface in the focal plane. Note the accumulation of pstB cells at the anterior margins of the prestalk zone. Viewed in other focal planes, it is clear that most of the slug anterior has remained in contact with the substratum, while the tip has re-positioned itself to sit on the anteriodorsal surface of the slug. (D) A plan view of a whole mount of a very early culminant where the aggregate has almost rounded up, i.e. the prespore region has almost come to lie below the tip, which is now raised perpendicular to the substratum. (E) A profile of a whole mount of an early culminant, with its tip to the left hand side. (F) Longitudinal mid-section at the Mexican hat stage. The core of pstAB cells is visible as a reddish-purple region in the tip. At this stage, the pre-basal disc area is largely composed of pstB cells.

Fig. 6. Culminants formed from cells co-transformed with ecmBneoGAL and ecmAnucGUS examined at high magnification. The culminants were gently overlaid with plastic coverslips (Bel-Art/Hoslab, Essex, UK), which were then carefully lifted off for fixing and staining. (A) The papilla region of the spore head from a pre-culminant showing the upper cup region, which contains red (pstB), blue (ecmA-expressing) and purple (pstAB) cells. (B) Lower region of same spore head as in A showing the lower region of the cup, which contains predominantly pstB cells with a scattering of ecmA-expressing and pstB cells. (C) Basal disc from a culminant similar to that shown in A and B. (D) The nascent spore head from a pre-culminant showing co-expressing cells (pstAB cells) within the spore head.
requires that *ecmA* and *ecmB* genes should differ in their responsiveness to these extracellular signals. There is such a heterogeneity, in that both genes are dependent for their expression on the presence of the stalk cell morphogen DIF (Jermyn et al., 1987) but differ in their responses to extracellular cAMP. Expression of the *ecmA* gene is marginally stimulated by cAMP, while expression of the *ecmB* gene is strongly inhibited (Berks and Kay, 1990). Perhaps, therefore, the cells that differentiate as pstB cells are those that perceive a relatively low extracellular cAMP concentration.

The *ecmA*-expressing cells and the *ecmB*-expressing cells display a radical difference in their behaviour during tip formation. The *ecmA*-expressing cells migrate to the apex of the aggregate while the *ecmB*-expressing cells accumulate in the base. *Dictyostelium* cells aggregate in response to pulsatile emissions of cAMP emanating from a signalling centre. It seems that the apex of the aggregate adopts this function during the multicellular stage and that the *ecmA*-expressing cells migrate to cAMP signals emanating from it (Matsukuma and Durston, 1979; Traynor et al., 1992). The downward migration of *ecmB*-expressing cells could be the result of a negative chemotactic response to cAMP or could result from positive chemotaxis to an as yet unidentified signal issuing from the base.

If the slug forms a culminant in situ the *ecmB*-expressing cells become incorporated into the basal disc. If a migratory slug is formed then this population is left behind as the slug moves away, and is replaced from an as yet unidentified population of cells within the slug. The fact that the majority of these cells are contained within a ventrally situated band in the front half of the prespore zone is intriguing. It again implies that the *ecmB*-expressing cells differ from the *ecmA*-expressing cells in their chemotactic behaviour with respect to cAMP. As is the case during tip formation, most pstB cells within the slug are tightly apposed to the substratum. Thus it would appear that cells expressing the *ecmA* gene move away from the substratum while cells expressing the *ecmB* gene move towards the substratum. Presumably, there must also be a signalling mechanism that holds the pstB cells in the front half of the prespore region.

This group of cells most probably corresponds to a ventrally located cluster of prestalk-like cells reported in earlier studies (Elliott et al., 1991) and may also correspond to a similarly located band of cells that display an oscillating, high calcium concentration (Cubitt et al., 1995). It is almost certainly the same neutral red staining, ventral ALC population described by Dormann and co-workers (Dormann et al., 1996). In this study, the authors were able to track the band of living cells and they show them to have a highly dynamic pattern of movement.

At culmination the band of pstB cells rapidly moves forward to accumulate in the pstO region (schematised in Fig. 7). We are confident that this is forward movement, rather than extinction of *ecmB* gene expression in one cohort of cells and activation in more anterior cells, because the β-galactosidase movement is downwards (as was directly observed by Sternfeld and David (1982), using cells stained with a vital dye).

**Mid-culminant.** The stalk tube grows downwards, because of the influx of prestalk cells at its apex, and embeds itself into the prebasal disc. In this, and the previous panel, the structure of the nascent stalk has been simplified. It actually consists of a ‘pathfinder’ region, the core of pstAB cells that were initially present in the tip of the migrating slug, with the newly recruited pstAB cells following behind it (see Sternfeld, 1992).

**Late culminant.** The basal disc now consists of the cells that once formed the rearguard, some pstB cells and some pstAB cells. The latter population in part derives from cells that migrated there as pstAB cells, but may also contain others that moved as pstB cells and activated *ecmA* expression once they reached the basal disc region. There is a similar uncertainty over the band of upper cup cells. It may consist of pstO cells (i.e. the cells in the back half of the prestalk region) that activated *ecmB* gene expression in situ, or it may consist of pstB cells that moved to the upper cup region and then activated *ecmA* gene expression. The in vivo staining studies of Sternfeld and David (1982) support the existence of the latter population but do not exclude the additional existence of the former population. Since there are these uncertainties we have chosen not to show the movement of cells to the upper cup in the central two structures, although this certainly occurs (Sternfeld and David, 1982). Because it is so variable in size and has a similar population of cells types to the basal disc (Fig. 6D), we favour the idea that the lower cup derives from the nascent basal disc. We believe that, as the stalk tube embeds itself into the basal disc and lifts the spore head upwards, a variable number of cells from within the basal disc region are lifted upwards to form the lower cup.

![Fig. 7. Schematic representation of the movements of *ecmA* and *ecmB*-expressing cells during culmination. In this highly diagramatic representation the prespore cells, the rearguard cells and the *ecmA*-expressing cells are shown as blocks of colour while the pstB (red) and pstAB (purple) cells are shown as individual cells. However, for the sake of clarity, only a token number of cells is shown.](image-url)
protein is very stable in Dictyostelium cells (Dettberke et al., 1994). They remain in this anterobasal location where they perhaps play a role in anchoring the slug to the substratum as it adopts an upright posture. They are ultimately joined by the rearguard cells, with which they merge to form the basal disc. (Bonner, 1944; Sternfeld, 1992).

There may also be a contribution to the basal disc from the ALC that migrate downwards within the nascent spore head during culmination. pstB cells are highly enriched within this population relative to the the upper cup, which contains a roughly equal mixture of ecmA-expressing and ecmB-expressing cells. This conforms to the pattern of selective accumulation of ecmA-expressing cells in the apical region, but some of the pstB cells have, contrary to the behaviour observed in slug formation, migrated upwards.

During late culmination, expression of the ecmA gene is activated in the basal disc cells and there are also cells that co-express the ecmA and ecmB genes in the upper and lower cups. Given the differences in migration of cells expressing the ecmA and ecmB genes, one attractive idea is that ecmA-expressing cells move up and ecmB-expressing cells move down, and that both cell types then activate expression of the other marker. However, this is probably not the case, because there are co-expressing cells within the spore head. Clearly there is much more to be learnt about the signals directing these remarkably complex movement patterns.

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REFERENCES


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