INTRODUCTION

During asexual development Dictyostelium cells undergo a remarkable transition, from unicellularity to multicellularity. Cells aggregate together in response to pulsatile emissions of cAMP from a signalling centre and form a cylindrical structure called the first finger or standing slug. This behaves like a multicellular organism, in which the constituent cells collaborate to ensure that the fruiting body is formed in an appropriate location. Under the environmental conditions that exist below the surface of the soil or leaf litter (low light levels, high humidity and high ionic strength) the first finger falls on to its side and migrates away as a slug. The slug is phototactic and thermotactic and these sensitivities direct it to the surface where culmination occurs.

The regulator of culmination is ammonia. This is produced in large amounts during development, by cellular catabolism, and experimentally induced depletion of ammonia causes migrating slugs to enter culmination immediately (Schindler and Sussman, 1977). Presumably, arrival at the surface, where there is a high light level, causes the slug to orient upwards off the substratum and so increases the rate of loss of ammonia by diffusion. Ammonia is thought to control entry into culmination by regulating vesicular pH (Davies et al., 1993) but cAMP dependent protein kinase (PKA) is also known to be involved, because expression of a dominant inhibitor of PKA under the control of a prestalk-specific promoter strongly favours slug migration over culmination and prevents stalk cell differentiation (Harwood et al., 1992). Furthermore, when the dominant PKA inhibitor is expressed under control of a prespore-specific promoter development is rendered hypersensitive to the inhibitory effects of ammonia (Hopper et al., 1993). This observation provides strong genetic evidence for a link between ammonia and PKA but the actual mechanisms that couple changes in vesicular pH to PKA are as yet unknown.

In a genetically tractable organism such as Dictyostelium one very powerful approach to tracing a signalling pathway is to isolate mutants. The ‘slugger’ mutants show delayed entry into culmination and, in some cases, they form defective fruiting bodies (Newell and Ross, 1982). The cudA gene encodes a nucleoplasmic protein that is essential for normal culmination. There are no functionally characterised homologues in other organisms but there is a related gene of unknown function in Entamoeba histolytica. The cudA gene is expressed by the prestalk cells that constitute the slug tip (the pstA cells), it is not detectably expressed in the band of prestalk cells that lies behind the tip (the pstO cells) but it is expressed in the prespore cells. This unusual pattern of expression suggests a role on both the stalk and spore pathways of differentiation and cudA− mutant cells are indeed defective in both stalk and spore formation. Furthermore, the slugs formed by cudA− cells continue to migrate under environmental conditions where normal slugs culminate immediately. This aspect of their behaviour can be reversed when the cudA gene is selectively expressed in the pstA cells. This shows that processes occurring in the pstA cells regulate entry into culmination.

Key words: culmination, Dictyostelium, cudA, prestalk cells

**cudA**: a *Dictyostelium* gene with pleiotropic effects on cellular differentiation and slug behaviour

Masashi Fukuzawa, Neil Hopper and Jeffrey Williams*

MRC Laboratory of Molecular Cell Biology and Department of Biology, University College London, Gower St, London WC1E 6BT, UK

*Author for correspondence (e-mail: jeff.williams@ucl.ac.uk)
Experiments in which the tips and bodies of a slug mutant and a normal strain are reciprocally interchanged by grafting show that the tip region dictates entry into culmination (Smith and Williams, 1980). However, the tip is a crudely delineated sub-region of the slug with specific biological properties rather than a defined cell type. Here we analyse the role of specific cell types by directing expression of the cudA gene in different prestalk cell sub-populations in a cudA- background.

MATERIALS AND METHODS

Cell culture, transformation and development

The D. discoideum strain DH1 (a gift from P. Devreotes), a derivative of KA3 in which the pyr5-6 gene is disrupted, was grown at 22°C in HL5 medium (Watts and Ashworth, 1970) supplemented with uracil at 200 μg/ml. AX2 and DH1 cell strains transformed with pyr5-6 vectors were grown in unsupplemented HL5 medium. The lacZ marker constructs ecmAO: lacZ (Jermyn and Williams, 1991), ecmA: lacZ (Early et al., 1993), ecmB: lacZ (Ceccarelli et al., 1991), ST: lacZ (Ceccarelli et al., 1991) and pspA: lacZ (Harwood et al., 1991) were introduced by calcium-phosphate transformation and clones were selected and maintained in 10 μg/ml G418 (geneticin; Gibco, BRL). For development, axenically grown cells (1-5×10⁶ cells/ml) were washed twice in K2 (16.5 mM KH₂PO₄, 3.8 mM K₂HPO₄, pH 6.2) and 2×10⁶ cells were spotted on to either 2% water agar or 2% K₂ agar. Slugs were formed on water agar and harvested immediately after they started migration (normally at 16-18 hours of development). For development on filters, 2×10⁶ cells were spotted onto a nitrocellulose filter (Millipore) supported on a filter pad soaked with LPS buffer (KK2 with 20 mM KH₂PO₄, 3.8 mM K₂HPO₄, pH 6.2) and 2×10⁶ cells were spotted on to either 2% water agar or 2% K₂ agar, which is expressed to yield the N-terminal 326 amino acids of CUD1 (ACUDA). These two expression constructs were transformed into E. coli strain M15 (Qiagen) and the fusion proteins were induced using IPTG. The fusion proteins were purified from bacterial lysates using a nickel affinity column. Mouse monoclonal antibodies were generated against the truncated CUD1 fusion protein (ACUDA), and their specificity was confirmed by western blotting and immunohistochemical staining using both bacterially expressed CUD1 and Dicyostelium cells overexpressing the cudA gene. One of the antibodies, mAb11, was used in all the experiments described.

Western blotting

Total cell lysates were prepared by boiling cells in SDS sample buffer and 5 μg of protein was loaded per lane of a 10% SDS-polyacrylamide gel. Nuclear extracts were prepared as described by Kawata et al. (1996), samples were denatured by boiling in SDS sample buffer and 10 μg was loaded per lane of a 10% SDS-polyacrylamide gel. Proteins were blotted onto Hybond-C filters (Amersham) and incubated sequentially with mAb11 and a 1 in 2000 dilution of goat anti-mouse IgG conjugated with peroxidase (Bio Rad). Signals were detected by chemiluminescence (ECL; Amersham).

Immunohistochemical staining

For individual cell staining, slugs were dissociated and cells were allowed to settle on a glass slide then fixed with methanol for 10 minutes. Whole mounts of slugs were placed onto a coverglass and similarly fixed with methanol. Specimens were incubated with mAb11, followed by FITC-conjugated goat anti-mouse IgG (Sigma).
For double staining, dissociated cell samples were first incubated with mAb11 and then with Texas-Red-conjugated goat anti-mouse IgG (Molecular Probes). After washing they were incubated with mAb17 (an IgM monoclonal antibody that recognises a nuclear-pore component; M. Fukuzawa unpublished results), followed by FITC-conjugated goat anti-mouse IgM (Sigma). False colours (green for Texas Red and red for FITC) were assigned to keep consistency with all the single staining analyses. Samples were visualised with a Leica DMRBE confocal microscope.

**Construction of cudA expression constructs**

A 1.5 kb, 5’ proximal region from the *cudA* gene was amplified from genomic DNA using the primers: 5’-CGGGGATCCATGAATCTAAA- GTCAAAATTTTCAT-3’ and 5’-GCACCTTGGAAATTCATC-3’. The first of these two primers hybridises at the initiation codon (underlined in the oligonucleotide sequence) and the second hybridises at the position of the unique EcoRI site (see Fig. 1A). The amplified product was the size expected from the cDNA sequence, indicating there to be no intron(s) in this region. The insert from cDNA clone #16 was excised and subcloned into the EcoRI/XhoI site of pBlue-scriptII to create pBS16. The 1.5 kb genomic PCR product was then excised with BamHI and EcoRI and inserted into the BamHI/EcoRI site of pBS16 to generate pBScudA. This contains the entire *cudA* coding region and the 3’ non-coding region from cDNA #16. The insert from pBScudA was then excised with BamHI/XhoI and inserted into the BglII/XhoI sites of *ecmA/O:lacZ* (Jermyn and Williams, 1991), *ecmA: lacZ* (Early et al., 1995), *ecmO: lacZ* (Early et al., 1993), *pspA: lacZ* (Harwood et al., 1991) so as to replace the *lacZ* gene and generate *ecmA/O:cudA*, *ecmA:cudA*, *ecmO:cudA* and *pspA:cudA*.

**RESULTS**

**Isolation of the *cudA*” mutant and structure of the *cudA* gene**

The *cudA*” strain was identified as showing aberrant terminal development and hence was designated a cud (culmination defective) mutant. Genomic DNA was isolated from the *cudA*” strain and two different restriction fragments containing vector DNA and flanking genomic DNA sequences, one bounded by *EcoRI* sites the other by *BclI* sites, were cloned in *E. coli* (Fig. 1A). We showed that the DNA which was isolated was responsible for the mutation by performing homologous gene disruption using the *EcoRI* fragment. About one third of the resultant transformant clones displayed the same phenotype as the original *cudA*” isolate (data not shown). Southern transfer analysis confirmed that they were disruptants of the *cudA* gene (Fig. 1B). We also made *cudA*” disruptants of the strain Ax2 in which an internal deletion was introduced within the *cudA* coding region (Fig. 1A) and, for one of the clones, disruption was also demonstrated by northern transfer analysis (Fig. 1C). In the disruptant there is a great reduction in the apparent level of expression of the *cudA* gene but a faint band of the same size as the *cudA* mRNA is present in the control. This suggests the existence of a related gene.

The *EcoRI* and *BclI* fragments were subcloned into a smaller vector and subjected to DNA sequence analysis. Several cDNA clones were also isolated and the 5’ end of the mRNA was isolated by PCR amplification (Fig. 1A). The complete sequence of the gene is shown in Fig. 2A. The coding region of the gene contains several homopolymer tracts, a typical feature of developmentally regulated *Dictyostelium* genes (Kimmel and Firtel, 1985). It also contains a highly basic region that may act as a nuclear localisation signal (underlined in Fig. 2B). There is only one homologous gene in the databases (Fig. 2C), an *Entamoeba histolytica* gene of unknown function. The *Entamoeba histolytica* protein also contains a putative nuclear localisation signal and it is in the same relative position as in CUDA. The *cudA* gene encodes a 2.5 kb transcript that accumulates during aggregation and then maintains a relatively constant concentration thereafter (Fig. 3).

**CUDA is a nuclear protein**

The subcellular localisation of the CUDA protein was determined by immunochemical staining. A fusion protein was prepared containing CUDA sequences linked to an oligo-histidine tract. The fusion protein was purified from *E. coli* and a mouse monoclonal antibody was prepared against it. On a western blot of *Dictyostelium* proteins from the slug stage the antibody detects a protein of 90×10^3 M_r, the size expected for
the CUDA protein (Fig. 4A). Immunohistochemical staining using cells dissociated from slugs shows localised staining in the majority of cells (Fig. 4B,C). Double staining with an antibody that recognises Dictyostelium antitubulin (underlined) and this may be the nuclear localisation signal. 

The spatial pattern of cudA gene expression suggests a role on both pathways of differentiation

Immunohistochemical staining, using whole mounts of slugs, reveals a novel, and surprisingly complex, pattern of expression for the cudA gene (Fig. 5A). There is staining in the pstA region, staining in the prespore region but little or no staining in the pstO region. The cells from dissociated slugs that showed no staining (Fig. 4B) are presumably, therefore, those that did not aggregate. The persistence of staining data. The maximum concentration of cudA transcripts is present in the pstA zone and there is no detectable signal in the pstO region. There is expression throughout the prespore region but at a somewhat lower level than in the pstA region. At culmination there is strong expression throughout the papilla with an especially high level of staining at the stalk tube.


data

The developmental time course of cudA gene expression. Poly(A)+ RNA was prepared from the indicated stages of development and hybridised with a 32P-labelled cudA probe.

CudA− strains display a strong ‘slugger’ phenotype

The development of cudA− cells appears normal up to and through the first finger stage and the migratory slugs that are formed resemble those of the parental strain (Fig. 6A). When, however, they are allowed to migrate on agar plates in the dark, or towards a low level unidirectional light source, cudA− slugs fail to culminate while DH1 aggregates form culminants within one or two days. When cudA− slugs are placed in overhead light they rear up, as if attempting to culminate (Fig. 6B), but immediately collapse back on to the substratum and continue to migrate for many hours. After about 12 hours of migration they start to deposit very large clumps of cells in the slime trail (Fig. 6C). Eventually they rear up again, finally forming gnarled structures with a small, apparently undifferentiated, cell mass at the top (Fig. 6D,E).
The above behaviour is observed using cells developing on agar under ionic conditions permissive for slug formation but the cudA− strain also shows a ‘slugger’ phenotype under more restrictive conditions. When placed on nitrocellulose filters in high ionic strength buffer the parental strain entirely fails to form migratory slugs and culmination occurs in situ (Fig. 6F). However, the cudA− first fingers go on to form slugs that move away from the site of deposition.

CudA− terminal structures contain neither stalk nor spore cells but a large fraction of the cells express markers of stalk cell differentiation

During culmination in a wild-type strain, cells in the prestalk zone synthesise a cellulose- and protein-containing extracellular matrix called the stalk tube. They do so both while moving upwards, towards the apex of the culmination, and as they change direction and move downwards towards the base. About one half of the cells in the back of the prestalk zone, the pstO cells, give rise to the upper cup, a structure that lies above the nascent spore head (Jermyn, 1996). There are cells that lie scattered in the posterior region of the slug called anterior-like cells (ALC) that share most if not all of the properties of the prestalk cells (Sternfeld and David, 1982; Devine and Loomis, 1985). At culmination some of the ALC differentiate to form the lower cup, a structure that lies below the nascent spore head, and some differentiate to form the basal disc (Jermyn, 1996; Williams et al., 1989).

High power microscopic examination of the terminal cudA− structures, and squashes derived from them, shows there to be no mature spores and no mature stalk cells (data not shown). Also, the cudA− terminal structures entirely fail to express spiA (data not shown), an early marker of spore formation (Richardson et al., 1991). The cells left behind in the slime trail do, however, show the extensive vacuolisation characteristic of stalk cells (Fig. 7A). Furthermore, cells within the terminal structures express an early marker of stalk cell differentiation. The ecmB gene encodes an extracellular matrix protein of the stalk tube. At culmination ecmB is expressed in all cells within the stalk tube, in the basal disc.
Developmental phenotype of \textit{cudA}− cells. (A-E) \textit{cudA}− cells were developed on water agar plates: (A) migrating slugs; (B) the structures that are transiently formed after migrating slugs are exposed to overhead light; (C) slugs that have re-entered migration after exposure to overhead light. Note the large clumps of cells deposited in the slime trail behind the slugs; (D) terminal structures, formed after an extended period of migration and composed of a small cell mass on the top of a withered column of cells. (E) \textit{cudA}− cells were developed on KK2 agar plates containing 4 mM MgSO4 and under these conditions almost all of the cells form part of a withered, stalk-like structure. (F) \textit{cudA}− cells were developed on nitrocellulose filters in parallel with DH1 cells. Development was for 2 days in a dim unidirectional light source. The slime trails that mark the migration paths of the \textit{cudA}− slugs can be seen in the left hand part of the picture.

and in the upper and lower cups (Jermyn and Williams, 1991). The distal part of the \textit{ecmB} promoter (the ST region) directs high level expression in cells within the stalk tube and very low level expression in basal disc cells and lower cup cells (Ceccarelli et al., 1991). Cells transformed with a construct containing the ST region fused to the \textit{lacZ} gene show strong staining precisely as they enter the stalk tube. Hence ST:\textit{lacZ} is a very early marker of commitment to the stalk cell pathway of differentiation. In a migrating slug formed from \textit{cudA}− cells, ST:\textit{lacZ} is expressed in just a few scattered cells, exactly as in normal slugs (Fig. 7B and data not shown) showing that expression of the ST fragment is correctly temporally regulated in \textit{cudA}− cells. However, in the terminal structures formed by \textit{cudA}− cells a large fraction of cells express ST:\textit{lacZ} (Fig. 7C,D; NB because the terminally differentiating cells in these withered structures proved impossible to dissociate, it was not possible to determine the exact fraction of cells expressing the fusion gene).

An elevated rate of cellulose deposition is another feature of stalk cell differentiation and the \textit{cudA}− structures show dispersed staining with calcofluor, a cellulose-specific reagent (Fig. 7E; NB spore cells also synthesise cellulose but it forms part of their cell walls and no spore cells are present within the \textit{cudA}− structures). Expression of ST:gal and elevated cellulose deposition are both early events in stalk cell differentiation but the \textit{cudA}− terminal structures also express \textit{staB} a gene that is, under some circumstances, a marker of late stalk cell differentiation. When normal slugs enter culmination immediately (e.g. in cells developing on a nitrocellulose filter) the \textit{staB} gene is expressed only in cells in the lower half of the stalk tube, i.e. in vacuolated cells that appear to have completed their differentiation into stalk cells (Robinson and Williams, 1997). The \textit{staB}:\textit{lacZ} construct is expressed throughout the lower part of \textit{cudA}− terminal structures (Fig. 7F).

Thus when \textit{cudA}− slugs attempt to culminate many cells enter the stalk cell pathway of differentiation. They express ST:\textit{lacZ}, \textit{staB}:\textit{lacZ} and cellulose is accumulated. However, they do not vaculate. Interestingly, they also fail to fabricate a stalk tube: there is sometimes an approximately central core of ST:\textit{lacZ}-expressing cells in the anterior region (Fig. 7D) but high power visualisation reveals no trace of a surrounding stalk tube.

Having characterised the terminal \textit{cudA}− structures, we next determined whether there are defects in earlier development using prespore and prestalk-specific markers.

\section*{The CUDA protein is required for normal levels of expression of a sub-set of prespore-specific genes}

The effect of the \textit{cudA}− mutation on those genes that are selectively expressed in prespore cells varies markedly from gene to gene. The \textit{pspA} gene encodes a cell surface protein of unknown function that is present on the surface of prespore cells (Kreft et al., 1983) and the \textit{Dp87} gene encodes a matrix component of the spore mass (Nakao et al., 1994). Analysis by northern transfer shows that the level of expression of both of these genes in \textit{cudA}− cells is two- to three-fold lower than in the control (Fig. 8A) but spatial patterning is normal; in a newly migrating slug derived from \textit{cudA}− cells the \textit{pspA}:\textit{lacZ} fusion gene is expressed in the rear, prespore region and in a few scattered cells in the prestalk region (Fig. 8B). The \textit{cotC} gene encodes a component of the spore coat (Fosnaugh and Loomis, 1989) and its expression is much more severely reduced in a \textit{cudA}− mutant. Expression of \textit{cotC} is approximately ten-fold lower than in control slugs (Fig. 8A) and expression assessed using a \textit{cotC}:\textit{lacZ} construct is also greatly reduced (data not shown).

\section*{In \textit{cudA}− cells expression of several prestalk cell-specific markers appears normal}

The different sub-types of prestalk cell are defined by their patterns of expression of the \textit{ecmA} and \textit{ecmB} genes. The cap-site proximal region in the \textit{ecmA} gene promoter directs expression in the the front half of the prestalk zone, in the pstA cells, and cap-site distal sequences direct expression in the back half of the prestalk zone, in the pstO cells (Early et al., 1993). \textit{EcmA/O}:\textit{lacZ} (a \textit{lacZ} fusion construct containing the entire \textit{ecmA} promoter), \textit{ecmO}:\textit{lacZ} and \textit{ecmA}:\textit{lacZ} constructs are all expressed normally in \textit{cudA}− slugs (Fig. 9). In a newly formed slug the complete \textit{ecmB} gene promoter directs expression in a cone of cells situated at the position in the slug tip where stalk tube formation is initiated at culmination (Jermyn et al., 1989) and there is such a concentration of \textit{ecmB}-expressing cells in the tip of \textit{cudA}− slugs (Fig. 9).
Synergy experiments indicate that cudA− prestalk cells are unable to enter the stalk tube at culmination

Although prestalk gene expression in cudA− cells appears normal the fact that the cudA gene is expressed in pstA cells and that stalk cells are not formed in the terminal cudA structures suggested that the cudA− prestalk cells might be defective in some other way. We therefore performed synergy experiments with wild-type cells. When marked cudA− cells are mixed with parental cells they become uniformly distributed throughout the slugs that are formed (data not shown). However, at culmination they are almost entirely excluded from the stalk (Fig. 10). They are also under-represented in the nascent spore mass and microscopic examination of squashes of the terminal structures show that they never form spores. The few scattered cells observed in the spore mass are most probably ALC and, consistent with this interpretation, the cudA− cells are greatly enriched in the upper and lower cups (Fig. 10), structures that derive from the ALC population (Jermyn and Williams, 1991).

Selective expression of the cudA gene in the pstA cells of the cudA− mutant reverts the slugger behaviour

In order to further understand the cudA− phenotype we determined the consequences of expressing the cudA gene in different cell types. The cudA− mutant was transformed with fusion constructs containing the cudA coding region under the control of the actin15, pspA, ecmA/O, ecmO, and ecmA promoters. The actin15 gene is expressed during growth and early development (Cohen et al., 1986) and transformation with the actin15:cudA fusion construct reverts the mutant phenotype such that apparently normal mature culminants are formed (data not shown). When cudA− is expressed under the control of the pspA promoter, i.e. in prespore cells, there is no gross difference from the parental cudA− mutant (Fig. 11A). Again, the pspA:cudA-expressing strain forms a withered finger-like structure when it attempts to culminate. Neither spore cells nor stalk cells are present (Table 1).

CudA− transformants expressing ecmA/O:cudA form outwardly normal fruit (Fig. 11B) but close microscopic examination shows that the spore head is semi-transparent. Such a ‘glossy’ spore head is typically observed when spore maturation is repressed and squashes of such structures confirm that very few mature spores are present but that stalk cell differentiation does occur at a high level (Table 1). Those few spores that are present may derive from ecmA/O:cudA-expressing cells that trans-differentiated into prespore cells at some point during development. Expression of the ecmO:cudA construct in cudA− cells yields similar structures to those observed with the ecmA/O:cudA construct (Fig. 11C and Table 1) but with the ecmA:cudA fusion construct the terminal structures comprise a very slender slug-like structure atop a stalk (Fig. 11D). Microscopic examination shows that these latter cells are indeed vacuolated cells, with the typical appearance of stalk cells, inside a stalk tube.

Finally we assessed the slugging behaviour of all four classes of transformant (Table 1). Cells expressing cudA from the pspA promoter show the same, slugger behaviour as cudA− cells. However, strains with the ecmA/O:cudA, ecmO:cudA and ecmA:cudA constructs all migrate on agar for the same approximate time as the parental strain, i.e. the cudA− slugger phenotype is reverted.

DISCUSSION

The sensing system that normally allows a slug to monitor environmental conditions is severely impaired in the cudA− mutant. The mutant slugs migrate on nitro-cellulose filters, a condition that prevents migration in the parental strain, and when kept in darkness on an agar surface they never culminate. In addition to its strong slugger phenotype, the cudA− strain displays a number of other defects.

Upon exposure to overhead light, cudA− slugs make an abortive attempt at culmination but then migrate again for many hours. Later in migration large numbers of stalk cells are left in the slime trail behind cudA− slugs. In wild-type strains such discarded cells are believed to derive from prestalk cells that prematurely initiated terminal stalk cell differentiation, became lost from the back of the slug and then differentiated into stalk cells (Sternfeld, 1992). The large numbers of stalk cells in the trail behind cudA− slugs suggests that premature terminal differentiation of prestalk cells occurs at an elevated rate. This incipient ‘stalkiness’ is consistent with several other features of the cudA− mutant. While there are no mature stalk cells and no stalk tube within the terminally differentiated cudA− structures a large proportion of cells within such structures express ST:lacZ and staB:lacZ: markers of stalk cell differentiation.

The cudA− mutant is also defective in prespore gene expression and entirely fails to make spore cells. It seems likely that the defect in spore maturation is a direct result of this failure to undergo normal prespore differentiation: i.e. it is a ‘dependent series effect’. Consistent with this notion, cudA− cells do not express spiA or produce spores when treated with 8 bromo cAMP (M. Fukuzawa and J. G. Williams unpublished results): a membrane permeant cAMP analogue that induces spore cell differentiation in wild-type cells. (Kay, 1989). The defect in prespore differentiation might also explain the pronounced tendency towards the stalk cell pathway of differentiation in the cudA− structures. If prespore to spore differentiation is blocked then the prestalk to stalk cell pathway might be expected to act as the default pathway. It is therefore very important to understand how the CUDA protein functions in prespore-specific gene expression. In this respect, the behaviour of different prespore-specific genes in the cudA− mutant provides a clue.

Studies using a dominant inhibitor of PKA expressed under

Table 1. Phenotypes of cudA-complemented strains

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Slugger</th>
<th>Spore</th>
<th>Stalk</th>
</tr>
</thead>
<tbody>
<tr>
<td>pspA (prespore)</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ecmA/O (prestalk)</td>
<td>No</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>ecmO (prestalk O)</td>
<td>No</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>ecmA (tip)</td>
<td>No</td>
<td>–</td>
<td>++</td>
</tr>
</tbody>
</table>

The symbol ++ shows that there was a level of complementation such that development was equivalent to that of the parental cells and a dash indicates that there was no complementation. The small numbers of spores formed in ecmA/O:cudA and ecmO:cudA transformants (+/-) may be due to transdifferentiation of cudA-expressing prestalk cells into prespore cells.
the control of a prespore-specific promoter, have shown that PKA is required for the transcription of three genes that encode spore coat proteins, \textit{cotA}, \textit{cotB} and \textit{cotC} (Hopper et al., 1995). This is in marked contrast to the behaviour of two other prespore-specific genes, \textit{Dp87} and \textit{pspA} (Hopper and Williams, 1994). Transcription of the \textit{Dp87} gene is only slightly reduced in the presence of the dominant inhibitor and transcription of the \textit{pspA} gene is marginally enhanced. These observations suggest that different signal transduction pathways are utilised by different classes of prespore-specific genes. This conclusion is supported by analysis of the \textit{cudA} mutant, which shows similar extents of repression of the three classes of prespore-specific genes to that observed using the dominant inhibitor construct: i.e. \textit{cotC} > \textit{Dp87} and \textit{pspA}. It also suggests that the CUDA protein may lie in the PKA signalling pathway that directs spore coat protein gene expression.

The \textit{cudA} gene is expressed in prespore cells and this is consistent with a role in the transcription of prespore genes. However, the gene shows an unusually complex pattern of gene expression that suggests that it might have additional functions. The \textit{cudA} mRNA and protein are present within psta cells but are not detectable within the psto cells. The glycogen phosphorylase 2 gene is expressed in both prestalk and prespore cells and its promoter is responsive to DIF, the inducer of prestalk differentiation (Town et al., 1976; Morris et al., 1987; Williams et al., 1987) and to cAMP, the inducer of prespore differentiation (Yin et al., 1994). However, there is to our knowledge, no precedent for a promoter that is active within only one of the two sub-types of \textit{ecmA} expressing prestalk cells and in the prespore cells. Later, at culmination the \textit{cudA} gene is expressed in the psto cells. This occurs both in the subset of psto cells that differentiate into upper cup cells, i.e. those that express the \textit{ecmB} gene prior to entering the stalk tube, and in the sub-set that express the \textit{ecmB} gene only after moving into the stalk tube.

The fact that the CUDA protein is located within the nucleoplasm in both psta and prespore cells suggests that it functions as a transcriptional regulator in both cell types. There is, however, no recognisable DNA binding domain. If, therefore, it is a transcriptional regulator it must contain a novel form of DNA binding domain or it must function by interacting with other transcription factors. The notion that CUDA may be a transcriptional regulator is consistent with the very low level of expression of the \textit{cotC} gene in \textit{cudA}-- slugs but we have no direct evidence that it acts as a transcriptional regulator in prestalk cells. The \textit{ecmA}:lacZ, \textit{ecmO}:lacZ, \textit{ecmB}:lacZ and \textit{ST}:lacZ constructs are all expressed. There could be subtle differences in the level of expression that, because of variation in copy number of the fusion genes would be difficult to estimate (to minimise this problem we use pooled populations derived from large numbers of transformant clones), but there is definitely not the major reduction observed for \textit{cotC}.

Fig. 7. Analysis of cellular differentiation within \textit{cudA}-- structures. (A) High power (×40) view of cells within the slime trail behind a \textit{cudA}-- slug. The cells show the vacuolated appearance typical of mature stalk cells. (B-D) Structures derived from cells transformed with the stalk marker \textit{ST}-lacZ: (B) slugs, (C) a terminal structure, (D) the anterior part of a terminal structure. (E) A terminal structure stained with calcofluor, a cellulose-specific reagent. (F) A terminal structure derived from \textit{cudA}-- cells transformed with \textit{stab}:lacZ, a late stalk marker.

Fig. 8. Analyses of prespore gene expression in \textit{cudA}-- slugs. (A) Total RNA was prepared from migrating slugs derived from \textit{cudA}-- cells and control, DH1 cells, and expressions of three prespore-specific genes (\textit{psA}, \textit{Dp87} and \textit{cotC}) was analysed by northern transfer. The filters were reprobed using a probe for \textit{IG7}, a gene which is expressed at a constant level during development (Jermyn et al., 1987). (B) Expression of pspA:lacZ in a \textit{cudA}-- slug.
Although we are unable to detect a defect in their gene expression the prestalk cells are clearly abnormal in that they cannot form a stalk tube when developing alone, they cannot enter the stalk tube when synergised with wild-type cells and they cannot differentiate into mature stalk cells unless they are shed from the aggregate into the slime trail. Further evidence for a defect in the prestalk cells of the cudA mutant is revealed when the CUDA protein is expressed under the control of different cell type-specific promoter fragments. When CUDA is expressed under the control of a prespore-specific promoter there is very little effect. The transformants display a strong slugger phenotype and form terminal structures that closely resemble the parental, cudA- mutant. The failure to form spores even though the defect in prespore cells is, presumably, corrected is consistent with previous studies where a defect in prestalk to stalk differentiation prevented spore maturation (Harwood et al., 1992; Shaulsky and Loomis, 1993). It may reflect the existence of a signal that passes from the prestalk cells to the prespore cells and induces their differentiation.

When the CUDA protein is expressed under the control of the ecmA/O prestalk-specific promoter, the slugger behaviour is corrected and outwardly normal looking fruit are formed but spore maturation does not occur. The same result is observed when the CUDA protein is expressed under the control of the ecmO sub-fragment of the promoter and this presumably reflects the fact that there is a continuous process of transdifferentiation of pstO cells into pstA cells during slug migration (Abe et al., 1994), so that, with increasing time of slug migration, expression of an ecmO:cudA construct becomes functionally equivalent to expression of an ecmA/O:cudA construct.

Expression of cudA under the ecmA coding region under the control of various cell type-specific promoters. CudA- cells were transformed with the cudA coding region under the control of various cell type-specific promoters. Cloned transformants were developed on water agar for 2 days. (A) pspA:cudA. (B) ecmA/O:cudA. (C) ecmO:cudA. (D) ecmA:cudA.
expression is confined only to pstA cells accords well with results from grafting experiments, between a slugger mutant and a normal strain, which showed that the tip region dictates slugging behaviour (Smith and Williams, 1980). However, our result extends the previous observation because grafting experiments can give only an estimate of the physical location of the tip. The demonstration that the defect in entry into culmination is corrected when cudA is expressed in pstA cells indicates that the functionally defined tip is composed of cells that utilise the ecmA-specific part of the ecma/O promoter, i.e. that they are likely to be pstA cells or some, as yet undetected, sub-set of the pstA cells.

This work was supported by BBSRC grant CAD05617.

REFERENCES


(Accepted 24 April 1997)