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Identification and analysis of a gene that is essential for morphogenesis and prespore cell differentiation in *Dictyostelium*

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SUMMARY

We have identified a gene (*PslA*) that is expressed throughout *Dictyostelium* development and encodes a novel protein that is required for proper aggregation and subsequent cell-type differentiation and morphogenesis. *pslA* null (*pslA*$^-$) cells produce large aggregation streams under conditions in which wild-type cells form discrete aggregates. Tips form along the stream, elongate to produce a finger, and eventually form a terminal structure that lacks a true sorus (spore head). More than half of the cells remain as a mass at the base of the developing fingers. The primary defect in the *pslA*$^-$ strain is the inability to induce prespore cell differentiation. Analyses of gene expression show a complete lack of prespore-specific gene expression and no mature spores are produced. In chimeras with wild-type cells, *pslA*$^-$ cells form the prestalk domain and normal, properly proportioned fruiting bodies can be produced. This indicates that *pslA*$^-$ cells are able to interact with wild-type cells and regulate patterning, even though *pslA*$^-$ cells are unable to express prespore cell-type-specific genes, do not participate in prespore cell differentiation and do not produce *pslA*$^-$ spores in the chimeras. While *pslA*$^-$ cells produce mature, vacuolated stalk cells during multicellular development, *pslA*$^-$ cells are unable to do so in vitro in response to exogenous DIF (a morphogen required for prestalk and stalk cell differentiation). These results indicate that *pslA*$^-$ cells exhibit a defect in the prestalk/stalk cell pathways under these experimental conditions. Our results suggest that PslA's primary function is to regulate prespore cell determination very early in the prespore pathway via a cell-autonomous mechanism, possibly at the time of the initial prestalk/prespore cell-fate decision. Indirect immunofluorescence of myc-tagged PslA localizes the protein to the nucleus, suggesting that PslA may function to control the prespore pathway at the level of transcription.

Key words: *Dictyostelium*, Cell-type differentiation, Gene expression, *PslA*

INTRODUCTION

Understanding the interplay of cell-cell interactions and signal transduction pathways that are involved in regulating cell-type differentiation and morphogenesis is a central goal in developmental biology. The regulation of development in multicellular eukaryotes involves a number of interacting gene products, some of which are highly conserved in evolution. For example, cAMP-dependent protein kinase (PKA) and GSK3 (glycogen synthase kinase 3) have been shown to play critical roles in controlling cell-type-choice decisions in organisms as diverse as *Drosophila*, *Dictyostelium* and vertebrates (Nusse, 1997; Hammerschmidt et al., 1997; Ohlmeyer and Kalderon, 1997; Jiang and Struhl, 1996; Perrimon, 1996; Ingham, 1995; Heasman, 1997; Harwood et al., 1995; Ginsburg and Kimmel, 1997). These pathways are known to be modulated by serpentine, cell-surface receptors, which in some cases are coupled to heterotrimeric G proteins. While the ligands for these receptors can be quite different, the structure of the pathways quite often reveals striking parallels.

A significant amount is understood at a molecular level about these pathways in *Dictyostelium*, *Drosophila*, and vertebrates. In *Dictyostelium*, the formation of the multicellular organism is mediated by extracellular cAMP that interacts with cell-surface, G protein-coupled receptors (cARs). Extracellular cAMP controls the chemotactic aggregation of up to 10^5 cells to form the multicellular organism (Chen et al., 1996; Firtel, 1995, 1996; Van Haastert, 1995). During aggregation, stimulation of the receptors by oscillatory pulses of cAMP results in the periodic activation of adenylyl cyclase and guanylyl cyclase, and the induction of aggregation-stage gene expression, while a continuous flux of cAMP results in the constitutive adaptation of these pathways. Activation of adenylyl cyclase leads to a rise in intracellular cAMP, which activates PKA, and a release of cAMP into the extracellular medium, which relays the chemoattractant signal. Stimulation

of guanylyl cyclase produces a transient rise in cGMP and mediates chemotaxis. Many of the genes encoding the components of the signal transduction pathways that mediate these complex responses have been cloned and disrupted, and their functions elucidated at the physiological and biochemical levels.

As the developing mound forms, levels of extracellular cAMP are thought to rise, resulting in a feedback inhibition of aggregation-stage pathways and the activation of a transcriptional cascade mediated by the transcription factor GBF that leads to cell-type-specific gene expression and morphogenesis (Schnitzler et al., 1994; Abe and Yanagisawa, 1983). The cAMP induction of GBF-activated genes is also controlled through the aggregation-stage receptors cAR1 and cAR3, but in response to continuous cAMP, conditions in which the receptors are fully adapted and are unable to stimulate aggregation-stage pathways (Schnitzler et al., 1995). In contrast to cAMP-mediated pathways that control aggregation, cAMP stimulation of postaggregative and cell-type-specific gene expression through GBF is thought to occur via a G protein-independent pathway (Schnitzler et al., 1995).

Formation of the mound signals the induction of prestalk and prespore cells, the precursor cells to the spores and stalk cells of the terminally differentiated fruiting body. Induction of cell-type-specific gene expression requires GBF activation, the morphogen DIF (Differentiation Inducing Factor) and the function of some of the first wave of postaggregative genes that are induced (e.g. the cell-surface signaling gene product LagC) (Dynes et al., 1994; Williams et al., 1987; Kay and Jermyn, 1983; Schnitzler et al., 1994). The prespore and subclasses of prestalk cells sort and become localized along an anterior/posterior axis with the formation of the first finger (Firtel, 1995; Williams, 1995). In the slug stage, this axis is fully established. The anterior 5% of the slug consists of prestalk AB (pstAB) cells, which are enclosed in an anterior cone within the larger pstA domain. Immediately posterior to the pstA domain is another prestalk subdomain containing pstO cells, which constitute ~10% of the organism (Early et al., 1993). The prespore domain constitutes the posterior ~80% of the slug. Scattered within the prespore domain are a class of prestalk-like cells, anterior-like cells (ALCs), which constitute ~10% of the cells of the organism and have been proposed to be regulatory cells (Firtel, 1995) with many of the properties of pstO cells.

Many of the genes that control spatial patterning and cell-type differentiation in *Dictyostelium* have orthologs or gene family members in higher eukaryotes. These include: PKA, whose activity is required for prespore, spore, and stalk cell differentiation (for review see Firtel, 1995, 1996; Reymond et al., 1995; Williams, 1995), GSK3, which is essential for prespore differentiation (Harwood et al., 1995), a homeobox-containing gene, which controls the proportioning of prestalk and prespore cells (Han and Firtel, 1998), and a STAT transcription factor, which is thought to regulate prestalk and/or stalk cell gene expression (Kawata et al., 1997). GSK3 differentially controls the prestalk and prespore pathways (Harwood et al., 1995; Ginsburg and Kimmel, 1997). As in vertebrates, Li^+, which is an inhibitor of GSK3 activity, affects cell-type differentiation in a manner that is consistent with the phenotype of *gsk3* null cells (Harwood et al., 1995; Ginsburg and Kimmel, 1997; Klein and Melton, 1996). In addition to these factors, other cAR family members, cAR2 and cAR4, are induced in response to rising levels of cAMP and are known to control morphogenesis, cell-type fate decisions and spatial patterning (Ginsburg and Kimmel, 1997; Louis et al., 1994; Saxe et al., 1993).

Whereas a considerable amount is understood about some of the key players that differentially control cell-type proportioning, few genes that are essential for cell-type-specific pathways have been identified. For example, GSK3 is required for prespore differentiation. *gsk3* null cells initially express prespore markers, but the majority of the cells eventually differentiate into pstB cells (Harwood et al., 1995). We undertook a genetic screen to identify genes that are required for prespore cell differentiation. This was accomplished using REMI (Restriction Enzyme-Mediated Integration), which produces mutations by insertion of a vector into the *Dictyostelium* genome (Kuspa and Loomis, 1992). Using this approach, we have identified a gene, presporeless A (*PslA*), which encodes a novel nuclear protein whose function is required for prespore but not prestalk cell differentiation in vivo. *pslA* null (*pslA*$^-$) cells show no detectable expression of prespore-specific markers, while prestalk cell differentiation appears normal. *pslA*$^-$ cells also exhibit an aggregation defect that may be related to the inability of these cells to induce prespore cell differentiation. We suggest that PslA functions after the branch point when prestalk and prespore cell differentiation diverges to regulate a transcriptional event required for prespore cell differentiation.

MATERIALS AND METHODS

Cell and molecular biology

Methods for REMI mutagenesis (Kuspa and Loomis, 1992; Clark et al., 1997), RNA and Southern blot analysis (Dynes et al., 1994; Nellen et al., 1987), transformation (Nellen et al., 1987; Mann et al., 1997), time-lapse videomicroscopy (Ma et al., 1997) and expression and staining of *lac*Z reporters (Mann et al., 1997) have been described previously in detail. Subcellular localization of myc-tagged *PslA* was done as described by Aubry and Firtel (1998) using an anti-myc antibody from Invitrogen (San Diego). Nuclei were identified by staining cells with Hoechst Dye 33258.

The in vitro DIF induction assay was performed as described by Harwood et al. (1995).

Plasmid construction

To construct a *PslA* gene disruption, a Bsr resistance cassette (Lee et al., 1997) was inserted into the *Eco*RV site of the *PslA* cDNA. The DNA was digested with *Spe*I and *Sca*I and transformed into *Dictyostelium* cells selected with 5 μg/ml of Bsr (Sutoh, 1993).

The β-gal expression plasmid was constructed by subcloning the 2.5 kb *Eco*RI-*Hae*III 5′ flanking region containing 306 bp of coding region into the *Eco*RI-*Eco*RV sites of Bluescript (KS) (Stratagene, La Jolla). A *Bgl*II site was created at the *Sma*I site of the vector and the *Bgl*II-*Hin*cII fragment was subcloned into the *Bgl*II-*Bam*HI site of the *lac*Z expression vector pMG20 (Howard et al., 1994; Gamper et al., 1996).

The accession number for PslA is AF038919.

RESULTS

Identification and molecular analysis of *PslA*

Wild-type cells were mutagenized using REMI [Restriction

Enzyme-Mediated Integration (Kuspa and Loomis, 1992)] and screened for strains that exhibited abnormal morphogenesis during the multicellular stages (see Materials and Methods). Strains carrying mutations that resulted in terminal structures with a deformed sorus (spore head) or no sorus were identified and screened for the expression of prespore-specific genes. *PslA* (presporeless A) was identified in this screen. The insertion vector and flanking sequences were excised from purified genomic DNA from the mutant strain and cloned into *E. coli*. The genomic DNA flanking the vector revealed an ORF. The DNA encoding the ORF was then used to identify a full-length cDNA clone from a λZAP cDNA library. To ensure that the mutant phenotype (see below) is the result of disruption of *PslA*, a second gene disruption construct was made from the cDNA and transformed into *Dictyostelium*. 20 transformants were randomly chosen, some of which exhibited the same phenotype as the original REMI mutant and others that were phenotypically wild-type. Southern blot analysis of these DNA showed a direct correlation between the mutant phenotype and disruption of *PslA* (data not shown). DNA extracted from all clones exhibiting wild-type development showed a normal *PslA* gene restriction pattern. Fig. 1A depicts a map of the *PslA* cDNA and the construct used for the gene disruption.

Fig. 1B shows the derived amino acid sequence of full-length *PslA* cDNA clones. *PslA* encodes a highly hydrophilic protein of 1,272 amino acids. It contains short homopolymer runs, including a run of 11 asparagines and several runs of threonine and serine, which are commonly found in *Dictyostelium* ORFs (Mann and Firtel, 1991; Burki et al., 1991; Pitt et al., 1992). In addition, there is a highly acidic region (residues 374 to 401) in which 18 of 28 amino acids are glutamates or aspartates and a basic region of 16 residues (residues 146-161), which may function as a nuclear localization signal (NLS) (see below). The protein shows no known homologies when GeneBank databases are searched.

The *PslA* promoter was cloned by first recombining an *E. coli* vector into the *PslA* gene. The vector, 2.2 kb of *PslA* 5′ flanking DNA, and part of the coding region were excised and transformed into *E. coli*. Expression of PslA from 2.2 kb of cloned upstream sequences or the *Act15* promoter, which is expressed in all cells, was able to rescue the mutant phenotype (data not shown). No dominant phenotype was observed when PslA was overexpressed in wild-type cells (data not shown).

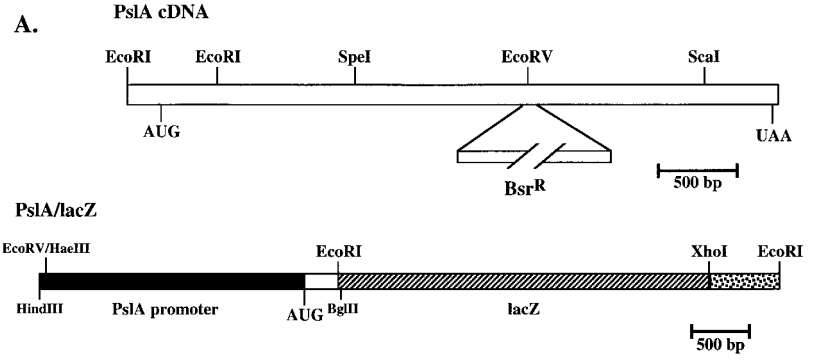
The spatial pattern of *PslA* expression during *Dictyostelium* development was examined by whole-mount β-gal histochemical staining in wild-type cells expressing a *PslA* promoter *lac*Z reporter. Staining is observed in a scattered subset of cells in the mound, first finger and slug that have a distribution similar to that previously observed for anterior-like cells (ALCs) (Fig. 2; Sternfeld and David, 1982). The mature fruiting body revealed strong staining in the upper and lower cups (higher resolution pictures show no detectable staining in spore cells; data not shown), the 'epithelial' layer surrounding the spore mass, and the basal disk, and weaker, scattered staining in the stalk (Fig. 2; data not shown), consistent with previous observations for ALC markers (Jermyn and Williams, 1991; Hadwiger and Firtel, 1992).

PslA protein localizes to the nucleus

To examine the subcellular localization of PslA protein, we expressed PslA protein carrying a myc tag at the C terminus from the *Act15* promoter. Expression of this protein complemented the *pslA*$^-$ phenotype and did not alter development when expressed in wild-type cells (data not shown). Indirect immunofluorescence using an anti-myc antibody indicates that myc-PslA protein is highly enriched in nuclei (Fig. 3A,B), even when it is expressed at high levels. Untransformed cells show no nuclear staining (Fig. 3C,D).

Morphological phenotype of *pslA*$^-$ cells

Fig. 4 shows the developmental morphology of *pslA*$^-$ and wild-type cells. At cell densities >~3×10^6/cm^2, *pslA*$^-$ cells produce very large, elongated aggregation streams compared to those



B.

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MGRMKVIPEFSNYKIRTPIPIFKNELEIEKYIKGAISYIYSENTAKKLELKKRNDEIKEI...60
LNNKNDHEFVEKKIRAIVIQLGDTNLNDRCYLNYLNLYKQPIEFTHGAIMCEKNKYWVAY..120
YASQSLNLDCLNFIIKHKDQFKGLDKDKRIKPIDPKKKISRDLLIGQPITVQCIKEDDLF..180
KFLTVYFNQNGIEFSDSWFSSIVKLIFKGKVYSSDESEKLSTTTTTTTTPNEKYDKKILG..240
EHTVFINKNRDGHLKFSTIPSLLLKISTLMNTGVINEYKQKNYFLETLYNSFPANKHFFN..300
FNEETIQYITNLLLPHLHPLEFKRFNTIPWIIKSKEFIDFIIQHKDFYHLVPSIHKFTKK..360
HENTNLFSASTINDGEEEDDDDDNDVDGNDDDNNKEKVDDTSNKKDSIVKFKDDITIYL..420
NVRQRFLQFSNFELANYFHTKLLECQKESENQINSNIQSINKEIESLSTSSTNTASSTRS..480
KASSNSNQLKKKEDLEQQIIELQSLLKNKYFSNKEIYYLSFLRALKDCDISTIESIDKVV..540
SIDSDQNNNKFQILFQKSFLENQPLKNGISPEFYDFKKVGPSSHPDVYAEGEYDEHDEYD..600
DYQGKLDNHIDIKDSKFTKDENSRDQLFNYLKSSGFKSFTPSIFCYLLELLLLINTEKSN..660
QQFQEIFKPISTTTTTSTTTTTTTTNLDNINEKIFKLSSDLLIHLVHIVDFKKFSIILDS..720
VNLNRKLILEMIDNCKNKLSFQKRIYEFHDVRSKDYQYDINGHFSTNIKFSDAKDILKKL..780
IDENLFPTSKDWIFIYHSLYISILQSTGVTLNNLLEVNKIYEEHEINYPSVLPNHSNYIL..840
DFNNNNNNNNNNNYYGLLNYQPYNNSGNINNESLKNVVSNFVLFYWFHLLDRKLSKLFVR..900
SFSNSNFNEFSSSRLPCLKNSSICRIRSKRHSNTASQIFKSIYELQNFSLLEKYSTFKSY..960
FPEERSYYYNNPSDPFYHPDGSKDFRVLIEEGEFQLAFNHLQLVATKTVSEFPPLTTSRL.1020
FQFITLEDVIELINLTTIKNFDESKDESEQQPLLKMWYGKDVKKCKDWILSCAIAKSRLD.1080
IVDLLLVKDIEYSTLPSTIEFLTGYKIIKSLFSPECDNQTLEYFLTFSNGIVLPSIKQYL.1140
IKDNIATQTKNDVFRVIRHGIGKFELLRTFIPSLGFSSCLIEKMVENRRFETLQYYMEIG.1200
LITNEDLTNQQKDQLKYENDLKHLDWVINLNHKKRVNRNQPTFTPNTNSTTATTATPLLQ.1260
TRSGRTIIPIKK
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Fig. 1. Sequence and maps of *pslA*. (A) Restriction map of *pslA* genomic and cDNA clones is shown. Details of the construct of the *lac*Z reporter are described in the Materials and Methods. (B) Amino acid sequence of the *pslA* ORF is given. The region containing the very acidic domain is boxed. A potential NLS is in bold and underlined. The accession number for PslA is AF038919.

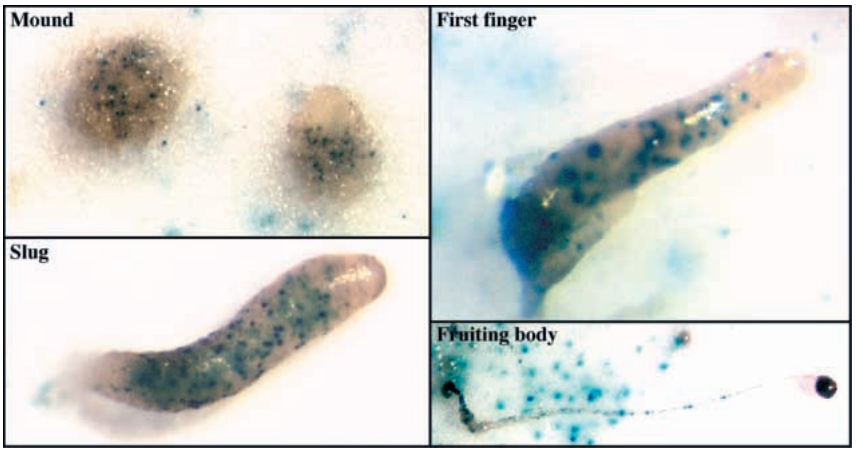

Fig. 2. Spatial patterning of *pslA* promoter expression during multicellular development. Wild-type cells were transformed with *lac*Z driven from the *pslA* promoter, and clones were selected. Multicellular organisms were stained histochemically for β-gal activity and photographed.

produced by wild-type cells and do not form distinct mounds (Fig. 4 compare A and E to B). Tips appear along the aggregation streams that differentiate into small, but separate, multicellular organisms (Fig. 4D,F). Tip and initial finger formation appears normal, although the sizes of the structures are significantly smaller than those of wild-type aggregates. However, ~50% of the cells remain at the base and do not participate in morphogenesis (Fig. 4F). Whereas wild-type cells form migrating slugs (Fig. 4C), *pslA*$^-$ fingers form very few migrating slugs (pseudoplasmodia). Upon culmination, the *pslA*$^-$ organism differentiates into a fruiting body with a very thin, elongated stalk and an extremely small sorus compared to the parental wild-type strain (Fig. 4H,G). When the *pslA*$^-$ fruiting body is examined under higher magnification, the stalk contains vacuolated stalk cells; however, no elliptical spores or spore-like cells are observed (Fig. 5, compare *pslA*$^-$ and wild-type). No mature spores are detected when cells are assayed for heat and detergent resistance (data not shown).

At lower cell densities, *pslA*$^-$ cells form large aggregates that develop multiple tips (data not shown). Time-lapse video phase microscopy of aggregation of *pslA*$^-$ and wild-type cells at $1.5\times10^6/cm^2$ (a density at which the cells are a monolayer with ~95% confluency) shows that both strains initially form similar-sized aggregation domains [Figs 6A (wild-type) and 6B (*pslA*$^-$ cells)]. In wild-type strains, the first signs of the formation of aggregation centers are very small, dark areas within the field of cells (Fig. 6A, 3:40 hour time point). In *pslA*$^-$ aggregation, the centers are larger and more diffuse (Fig. 6B, 3:37 hour time point) but tighten with time. As the *pslA*$^-$ cells move inward by chemotaxis toward the center to form a mound, the domains do not fully separate and eventually fuse (Fig. 6B, last three time points) to produce aggregates that yield multiple tips. In general, *pslA*$^-$ aggregates are larger than those formed by the wild-type parental strain. A larger fraction of the *pslA*$^-$ cells do not participate in aggregate formation compared to wild-type cells as observed by the number of cells left between fully formed aggregates (compare Fig. 6A, 7:00 hour time point to Fig. 6B, 7:09 hour time point). Small population assays of chemotaxis (Konijn and Van Haastert, 1987) to cAMP show no differences between wild-type and *pslA*$^-$ cells (data not shown).

pslA function is essential for prespore cell differentiation

Developmental RNA blots were utilized to screen for possible defects in gene expression in the multicellular stages of *pslA*$^-$ cell development. *pslA* is expressed throughout growth and development with a strong peak of expression at 4 hours, as

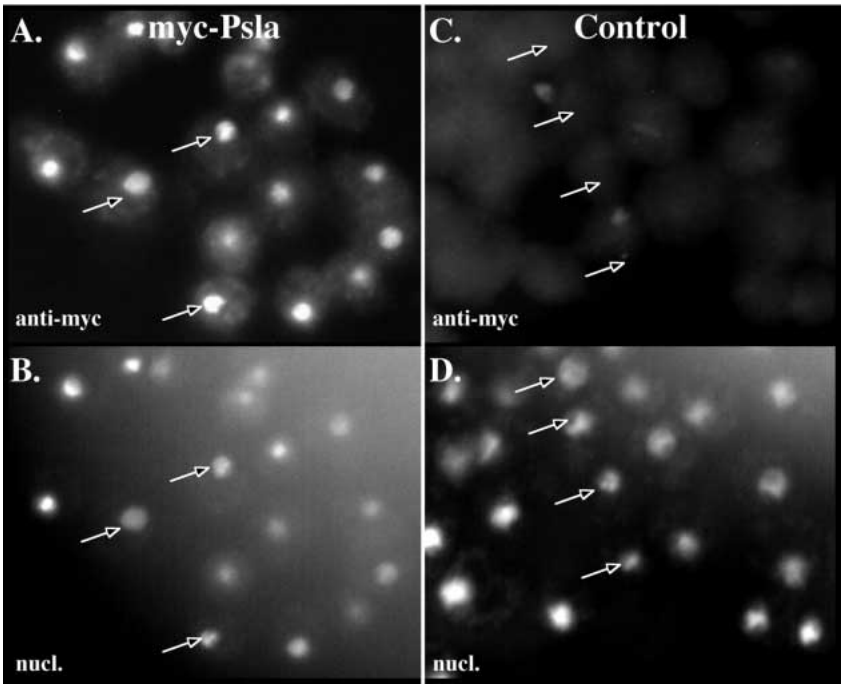

Fig. 3. Nuclear localization of PslA. Wild-type cells expressing myc-tagged PslA from the *Act15* (panel A) or control, untransformed wild-type cells (panel C) were examined by indirect immunofluorescence using a monoclonal anti-myc antibody. The same slides were also stained with Hoechst Dye 33258 to identify nuclei (panel B, myc-PslA-expressing cells, panel D, control cells). Arrows point to equivalent positions in the fields and mark the position of some of the nuclei. Nuclei were stained with Hoechst Dye 33258.

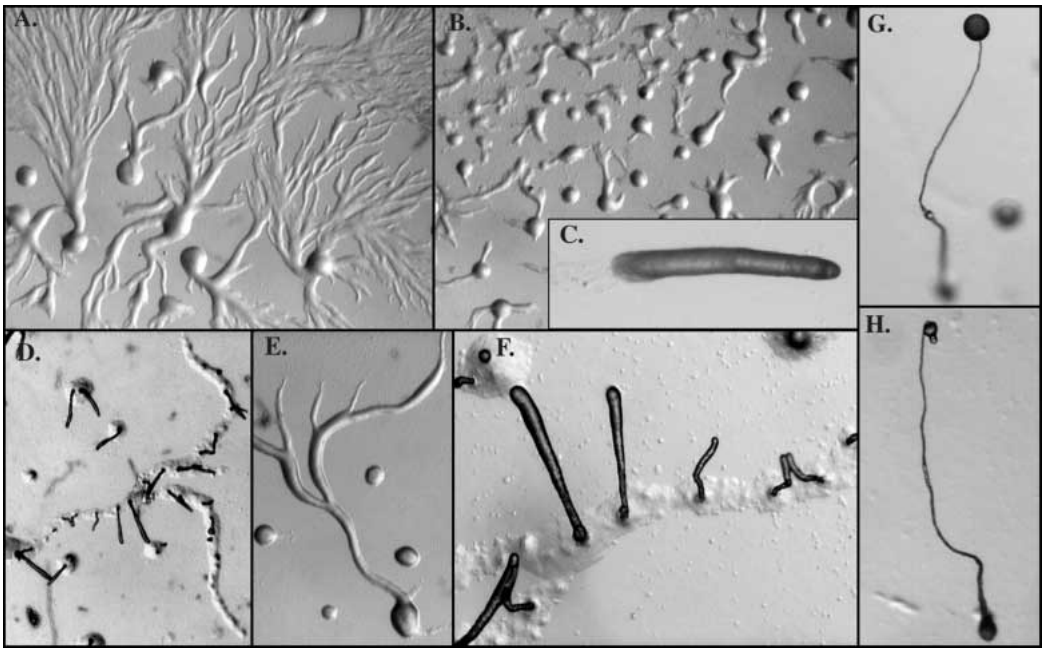

Fig. 4. Developmental morphology of *pslA*⁻ and wild-type cells. Log-phase vegetative cells were washed free of medium and plated on 12 mM Na/KPO_4 (pH 6.2)-containing agar and photographed at the times and stages indicated. (A,E) *pslA*⁻ cells during late aggregations (7 and 9 hours, respectively). (B) Wild-type cells during late aggregation (7 hours). (C) Wild-type slug (16 hours). Magnification is ~5× that of A and B. (D) *pslA*⁻ standing fingers along an arrested aggregation stream (14 hours). (F) Enlargement of a portion of D by 4×. (G) Wild-type fruiting body (26 hours). Magnification is ~2× that in A. (H) *pslA*⁻ fruiting body (26 hours). Magnification is ~5× that in A.

aggregation is initiating (Fig. 7A). As outlined in the Introduction, a proposed rise in extracellular cAMP in the developing mound leads to the activation of a transcriptional cascade, which requires the transcription factor GBF and results in the induction of the postaggregative genes and the subsequent induction of cell-type-specific genes. As shown in Fig. 7A, *pslA*⁻ cells were able to induce a developmental increase in GBF expression with kinetics similar to those observed in wild-type cells. However, the level of GBF transcript accumulation was lower in *pslA*⁻ aggregates. Similarly, expression of the postaggregative gene *LagC*, which is required for cell-type-specific gene expression (Dynes et al., 1994), was induced. The maximal level of expression (at 8 hours) was slightly lower than that observed in wild-type cells. Possibly more significantly, expression at later times was significantly reduced. The prestalk-specific genes *ecmA* and *ecmB* are expressed, although the maximal level of RNA accumulation is lower than that in wild-type cells (data for *ecmB* not shown). In contrast, no expression of the prespore-specific genes *SP60/cotC*, *PspB* or *psA* (D19) was detected (data for *PspB* not shown). We also examined the expression of the gene encoding the heterotrimeric Gα protein subunit Gα4, which is required for cell-type differentiation and morphogenesis (Hadwiger and Firtel, 1992). Gα4 is expressed at low levels in growing cells and its expression is induced starting at the mound stage when it is preferentially expressed in ALCs and functions as a molecular marker for these cells. The level and timing of the expression of *Gα4* are similar to those observed in wild-type cells (data not shown). These results demonstrate that *pslA*⁻ cells are deficient in prespore gene expression.

Cell-type-specific genes can be induced in suspension cultures in response to DIF and exogenous cAMP (Mehdy and Firtel, 1985; Jermyn et al., 1987). This requires cell-cell contact, which may involve the function of the *Lag*C gene product (Dynes et al., 1994). As shown in Fig. 7B, wild-type cells exhibit a vigorous induction of *SP60* and *ecmA* in cell-suspension conditions in the presence but not the absence of exogenous cAMP. Under these conditions, DIF, which is required for *ecmA* expression, is supplied endogenously by the cells (Jermyn et al., 1987; Williams et al., 1987). *pslA*⁻ cells induce *ecmA* gene expression, although at a reduced level compared to that of wild-type cells; however, no *SP60/cotC*

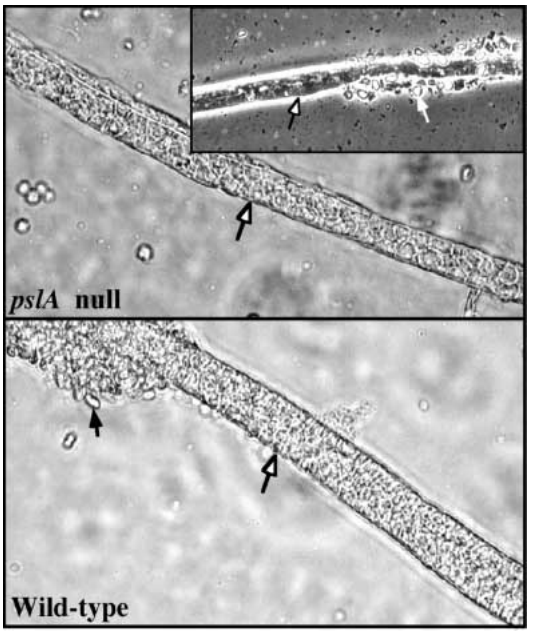

Fig. 5. Structure of the stalk tube and lower portion of the sorus is shown for wild-type and *pslA*⁻ cells as visualized by phase-contrast microscopy. The open arrows point to the stalk containing vacuolated stalk cells. The black arrow in the wild-type panel points to the ellipsoid spores at the base of the sorus. The insert in the *pslA*⁻ panel shows the region at the top of the *pslA*⁻ fruiting body. Note that the cells are not ellipsoid (white arrow) and may represent pstO or ALC derivatives, as no prespore/spore markers are expressed.

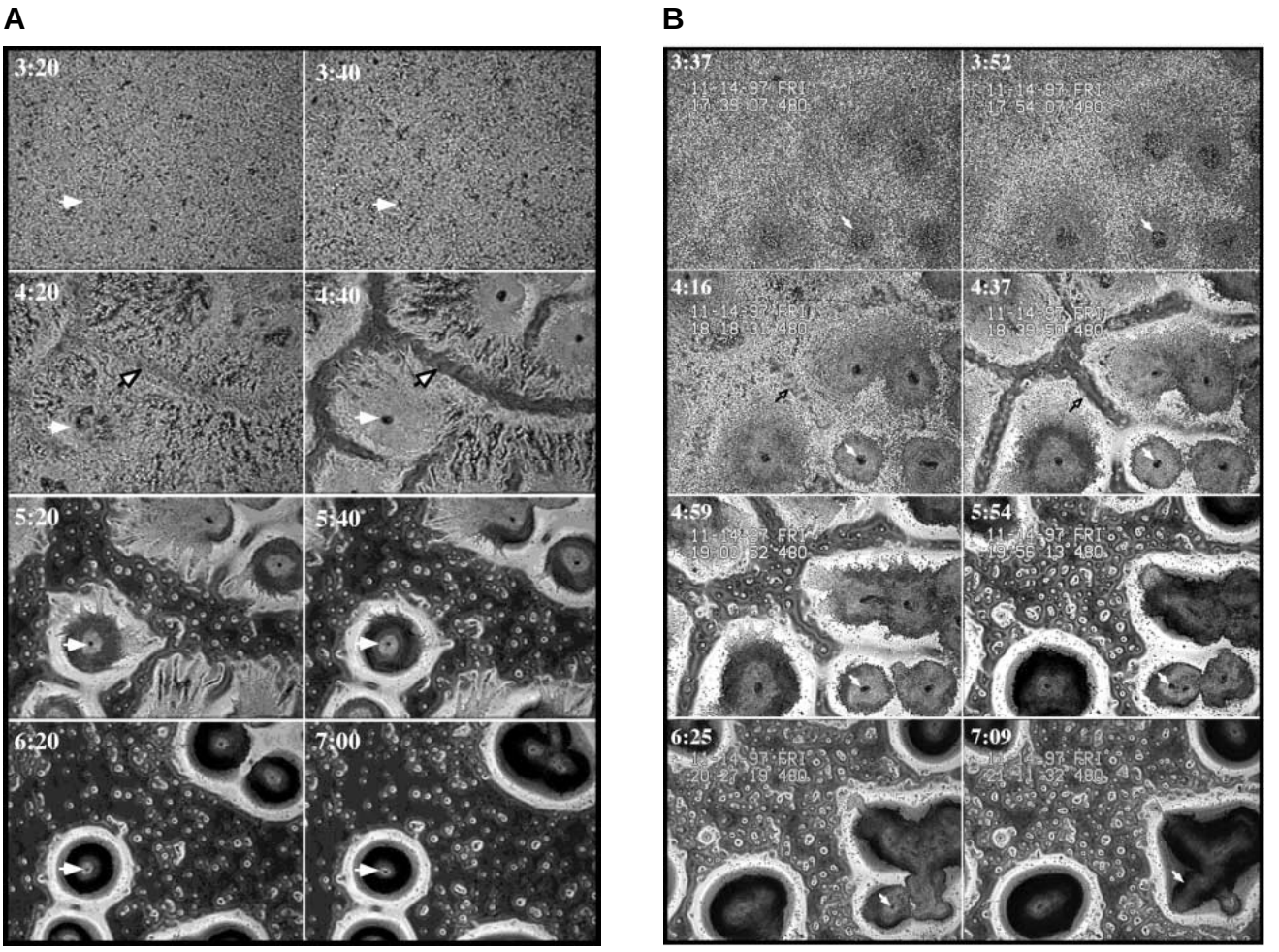

Fig. 6. Time-lapse videomicroscopy of aggregation. (A) Wild-type and (B) *pslA*$^-$ cells were plated as a monolayer on Na/KPO_4-containing agar and examined by time-lapse video phase microscopy as previously described (Ma et al., 1997). Individual frames from the time-lapse videos were captured on a Scion imaging board. The numbers in the upper left-hand corner indicate the time after plating of the cells in hours and minutes. The white arrows point to aggregation centers. The open arrows point to the outside of the forming aggregation domains. Note the fusing of the aggregation domains in *pslA*$^-$ in the lower four panels. Also note that the dark centers of the aggregation domains for *pslA*$^-$ cells are significantly wider than those of wild-type cells throughout the time course.

gene expression is detected in the *pslA*$^-$ cells under these conditions. These results are consistent with an inability of the *pslA*$^-$ cells to express prespore cell-type-specific genes during multicellular development and suggest that the defect is not due to a deficiency in cAMP signaling. Expression of *ecmA* and *SP60* requires common components (e.g. GBF, LagC). The expression of *ecmA* in suspension in *pslA*$^-$ cells suggests that the inability of these cells to express prespore-specific gene is not the result of a deficiency in these common components.

Prespore cell differentiation is known to absolutely require the activity of PKA, which is also required for aggregation (Mann and Firtel, 1991, 1993; Hopper et al., 1993a,b). To determine whether PKA activity may be rate-limiting, the PKA catalytic subunit was constitutively expressed in *pslA*$^-$ cells from the *Act15* promoter. This did not complement either the defect in aggregation or prespore gene expression (data not shown).

Spatial patterning in *pslA*$^-$ organisms

To examine the spatial pattern of cell types in *pslA*$^-$ cells, clonal isolates of *pslA*$^-$ cells transformed with cell-type-specific promoters driving *lac*Z were histologically stained for β-gal activity. *ecmAO*, the full promoter of the *ecmA* gene, drives expression in prestalk A and AB (pstA and AB) cells and to a lesser extent in prestalk O (pstO) cells and ALCs in wild-type strains, with strongest expression in the anterior ~15% of the slug (Early et al., 1993; Jermyn et al., 1987). The staining patterns of the cell-type-specific reporters in wild-type slugs and fruiting bodies are shown in Fig. 8A. In *pslA*$^-$ mounds, *ecmAO/lac*Z is expressed in the center of the mound and the developing tip, similar to observations in wild-type cells (data not shown; Early et al., 1993; Jermyn et al., 1987). However, in *pslA*$^-$ first fingers and slugs, *ecmAO/lac*Z expression extends towards the posterior of the finger and covers >50% of the finger/slug in most organisms (Fig. 8B), indicating that a very large fraction of the first finger and slug is composed of prestalk cells. In the terminal *pslA*$^-$ structure, the stalk and apical region stain (Fig. 8B). No staining is seen in the cells remaining at the base of developing aggregates (data not shown).

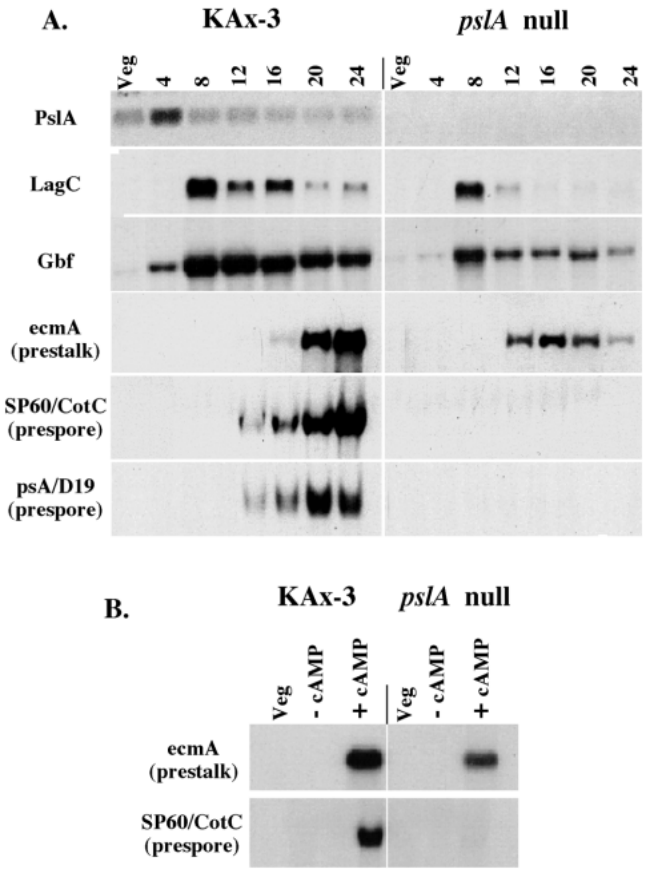

Fig. 7. RNA blot analysis. (A) A developmental RNA time course is shown for wild-type (KAx-3) and *pslA*$^-$ cells. A loose mound formed at 9 hours in these experiments. (B) Induction of prestalk and prespore gene expression in suspension culture. Cells were pulsed for 4 hours with 30 nM cAMP to maximize expression of cAMP receptors and other aggregation-stage genes and then split. One sample was incubated without cAMP and the other with 300 mM cAMP (supplemented every 2 hours with 150 mM cAMP) for 6 hours (Dynes et al., 1994). An RNA blot of extracted RNA is shown.

The pstA-specific reporter *ecmA/lac*Z stains the anterior pstA/AB domain (the anterior ~12% of wild-type slugs, Early et al., 1993). In *pslA*$^-$ slugs, the staining appears even more anteriorly localized and is often restricted to the anterior ~5-10% (Fig. 8B). In the terminal structure, the stalk and very apical region stain (Fig. 8B). In wild-type strains, *ecmO/lac*Z is expressed in ALCs and pstO cells, a ring of cells composing ~10% of the slug that lies between the pstA/AB and prespore domains (Early et al., 1993). In contrast, in the *pslA*$^-$ finger and slug, staining is not localized to such a ring, but is found scattered throughout the slug with a higher fraction of more intensely staining cells in the anterior region normally occupied by pstA cells (Fig. 8B). In the mature structure, scattered staining is seen in the stalk, which normally does not show significant *ecmO/lac*Z staining in wild-type cells, the very apical region, and the basal region of the fruiting body (Fig. 8B). Some of this staining pattern is consistent with *ecmO/lac*Z being expressed in ALCs as well as pstO cells; however, staining of the very anterior of the finger is not observed in wild-type strains. No staining is observed in the cells that do not participate in morphogenesis and are left at the base of the terminal structure (data not shown).

*Gα4/lac*Z, a marker for ALCs (Hadwiger et al., 1994), shows the expected scattered pattern of expression in *pslA*$^-$ cells, except that the staining is significantly stronger in the very anterior of the slug (Fig. 8B). Staining cells are found scattered throughout the terminal structure (Fig. 8B). Only a light background staining is observed in the remaining mound of cells, suggesting that these cells do not differentiate into ALCs.

The prestalk-specific gene *ecmB* is expressed in the very anterior pstAB cells as a centralized cone of cells and, to a lesser degree, ALCs in wild-type and *pslA*$^-$ organisms (Jermyn et al., 1987; see Fig. 8B for *pslA*$^-$ cells; data for wild-type cells is not shown). In the *pslA*$^-$ terminal structure, the developing stalk tube and the apical region stain (Fig. 8B). This apical staining is not observed in wild-type strains (Jermyn et al., 1987). *ecmBΔ89* is a subdomain of the *ecmB* promoter that is expressed only in differentiating stalk cells (Jermyn and Williams, 1991). As expected, no *ecmBΔ89/lac*Z expression is seen in the mound or finger/slug stages of *pslA*$^-$ cells (data not shown). In the terminal structure, the staining pattern is stalk-specific (Fig. 8B).

When the prespore-specific promoter *SP60/cotC* is used to drive *lac*Z, staining is seen in the prespore domain in wild-type slugs and the sorus in fruiting bodies (Fig. 8A). However, no staining is observed in either the *pslA*$^-$ developing finger/slug, the terminal structure or the cells that remain at the base of the developing organism (Fig. 8B; data not shown). This is consistent with the absence of prespore gene expression observed in the RNA blots.

pslA functions cell autonomously

To determine if *pslA* functions cell autonomously to regulate the prespore pathway, *pslA*$^-$ cells carrying one of the various cell-type-specific *lac*Z reporters were mixed with untagged wild-type cells (ratio of 1:3, *pslA*:wild-type cells) and allowed to coaggregate and make chimeric organisms. Under these conditions, fruiting bodies were formed with stalks that are longer than wild-type stalks (compare sorus size to stalk length in Figs 8A and 9). Essentially all *pslA*$^-$ cells participated in development; a large group of cells was not found at the base of developing fingers (Fig. 9). *pslA*$^-$ cells tagged with *ecmAO/lac*Z are found predominantly in the anterior of the slug and form the basal disc, part of the stalk, and the upper and lower cups of mature fruiting bodies (Fig. 9; data not shown). The region of the chimeric slug that expresses strongly *ecmAO/lac*Z represents >20% of the slug, more than is seen in wild-type slugs. Unexpectedly, the very tip of the slug does not stain. This is highly suggestive that the majority of the prestalk domain is composed of *pslA*$^-$ cells rather than a mixture of *pslA*$^-$ and wild-type cells. Our finding is consistent with analysis of chimeras in which the wild-type cells are tagged with *Act15/lac*Z (data not shown). When *ecmO/lac*Z-tagged *pslA*$^-$ cells are mixed with wild-type cells, staining is localized near but not at the point of the developing tip in mounds (data not shown). In slugs, these cells form a collar as they do in wild-type strains, except that the collar is broader, which is consistent with a larger prestalk domain and longer stalk in these organisms. *ecmB*, as expected, becomes localized to the anterior tip and forms components of the stalk basal disc and

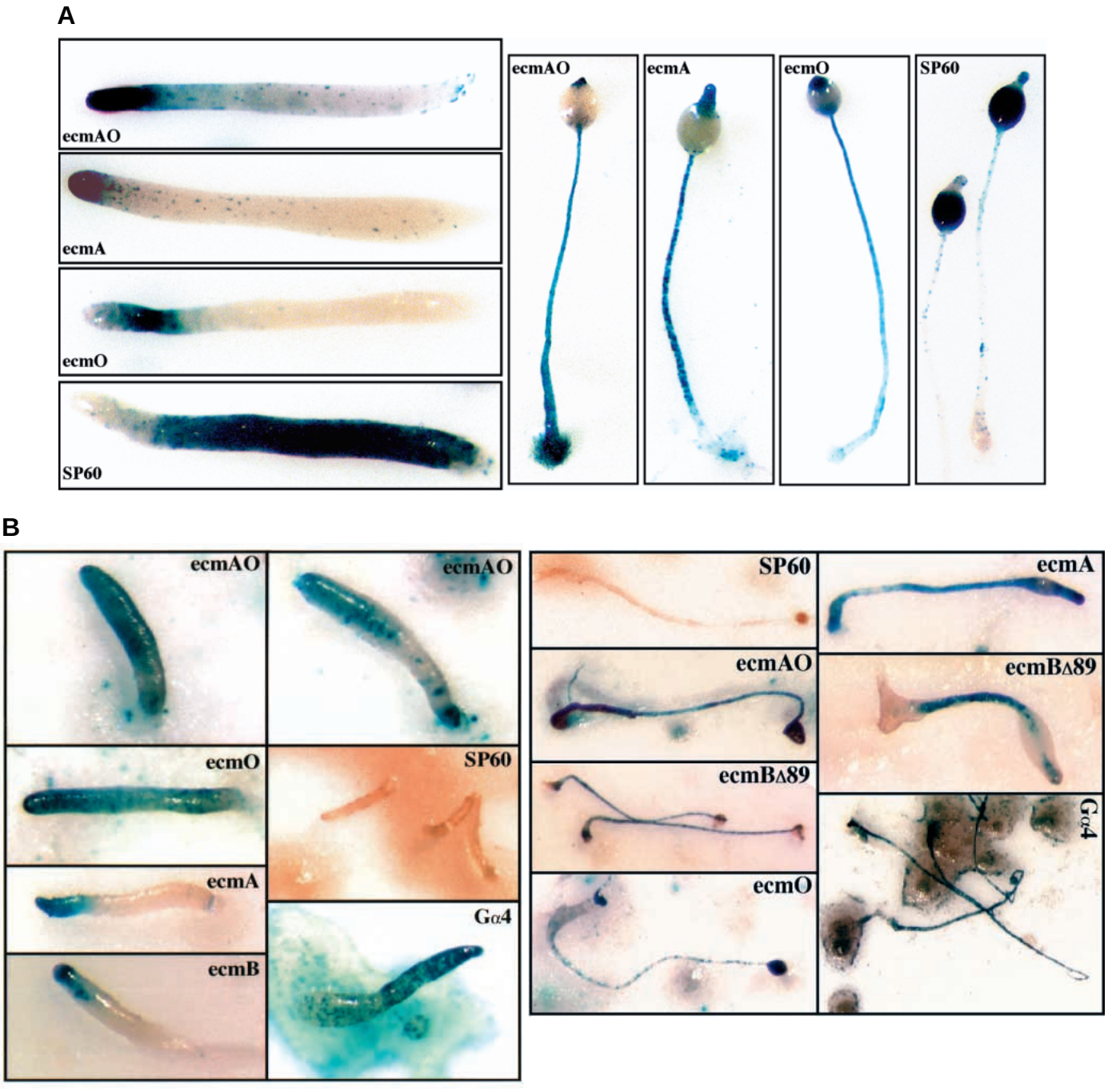

Fig. 8. Spatial patterning in wild-type and *pslA*⁻ cells. (A) Wild-type cells or (B) *pslA*⁻ cells were transformed with the indicated *lac*Z promoter constructs and then stained at the standing finger/slug stage at 16 hours of development or in the final organism at 26 hours of development, except for the panel showing the mid-culminant of *pslA*⁻ cells expressing ecmBΔ89. The cell-type expression specificities for the promoters are: *SP60*, prespore; *ecmA*, pstA; *ecmO*, pstO; ecmAO, strongest in *pstA*, weaker in pstO and ALCs; *ecmB*, pstAB cells, ALCs, pstB; *ecmBΔ89*, stalk-cell-specific; Gα4, ALCs (Early et al., 1993; Hadwiger and Firtel, 1992; Haberstroh and Firtel, 1990; Ceccarelli et al., 1991). See text for details.

the anterior pillae of the mature fruiting body. *ecmBΔ89* is expressed in the developing stalk, as has been seen in wild-type cells. When *SP60/lac*Z-marked *pslA*⁻ cells are mixed with wild-type cells, no expression is observed at any time in development (data not shown). This suggests that the *pslA*⁻ cells' prespore cell defect is cell autonomous.

As a better indication of where the total *pslA*⁻ cells localize in the developing aggregate, *pslA*⁻ cells were marked with *Act15/lac*Z, which is expressed in all cells, and then mixed with three parts of untagged wild-type cells. In the developing mound, stained cells, which mark the positions of the *pslA*⁻ cells, are scattered throughout the mound (data not shown). By the developing finger/slug stage, most of the cells have localized to the anterior ~25% of the slug, with some stained

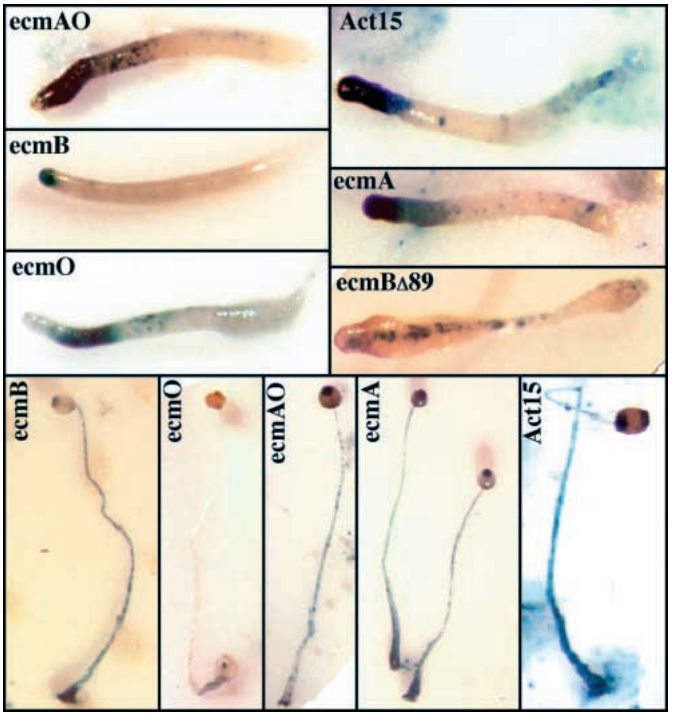

Fig. 9. Spatial patterning of *pslA*⁻ cells in chimeras. *pslA*⁻ cells carrying the indicated *lac*Z reporters were mixed with untagged wild-type cells in a 1:3 ratio (*pslA*⁻ cells:wild-type cells) and allowed to coaggregate, forming chimeric organisms. Organisms were histochemically stained for β-gal activity at the slug (16 hours) or mature fruiting body stage (26 hours), except for *ecmBΔ89*, which does not express in slugs. A midculminant-stage organism is shown, as it demonstrates the formation of the stalk tube during culmination.

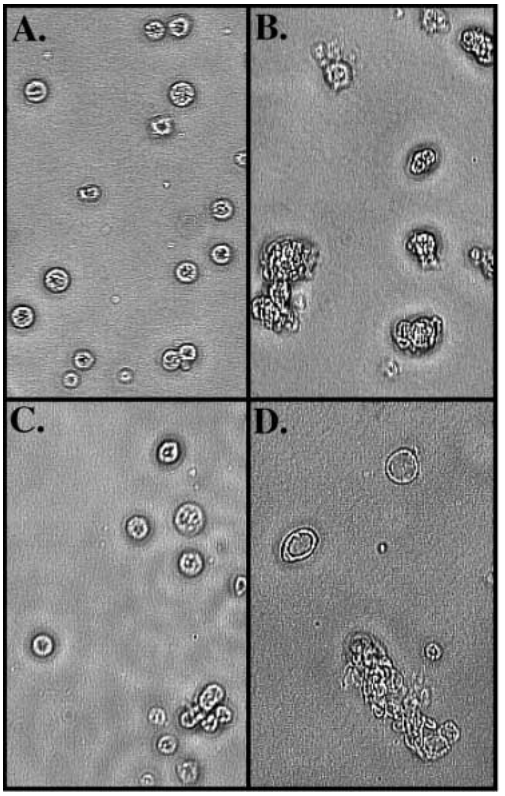

Fig. 10. DIF induction of stalk cell differentiation in vitro. The assay was done as described by Harwood et al. (1995). Cells were incubated for 24 hours in 5 mM cAMP in stalk salt medium. Cells were washed three times with stalk salts and then for an additional 24 hours with 100 nM DIF and with or without cAMP for an additional 24 hours. (A,C) *pslA*⁻ cells with and without cAMP in the presence of DIF, respectively. (B,D) Wild-type cells with and without cAMP in the presence of DIF, respectively.

cells scattered throughout the slug and a few cells localized near the posterior. The size of the anterior *Act15/lac*Z-staining domain is consistent with the size of the combined *ecmA*- and *ecmO*-staining domains and a larger stalk. In the mature fruiting body, the cells are scattered throughout the stalk and in the upper and lower cup regions, but not in the region containing spores (Fig. 9). These results are consistent with our findings using cell-type-specific reporters, which indicate that the *pslA*⁻ cells preferentially localize to the prestalk domains and differentiate into prestalk-derived structures.

pslA⁻ cells do not form stalk cells *in vitro*

To further examine the differential potential of *pslA*⁻ cells, we examined their ability to differentiate into stalk cells in submerged culture in response to DIF. *Dictyostelium* wild-type cells are competent to differentiate into stalk cells in an in vitro submerged culture in response to the morphogen DIF after pretreatment with cAMP (Kay and Jermyn, 1983). The induction of stalk cell differentiation in wild-type cells does not occur if cAMP is left in the culture continuously, a pathway that is mediated through GSK3 (Harwood et al., 1995). As shown in Fig. 10, wild-type cells, but not *pslA*⁻ cells, differentiate into vacuolated stalk cells in submerged culture under standard conditions (Kay and Jermyn, 1983).

DISCUSSION

PslA is a nuclear protein required for prespore cell differentiation

pslA encodes a novel, hydrophilic protein, which localizes to the nucleus. PslA function is absolutely required for prespore cell differentiation. In addition, PslA functions at other steps during *Dictyostelium* development. *pslA*⁻ cells are unable to aggregate efficiently at moderate cell densities and produce fused aggregation domains that develop multiple tips. At higher cell densities, but densities at which aggregation of wild-type cells is still completely normal, long streams are formed that produce tips along the stream. Under both conditions, a subpopulation of the cells produce a finger that eventually forms a fruiting-body-like structure lacking a sorus and spores, with the remainder of the cells remaining at the base. This population of cells does not undergo cell-type differentiation as determined by the lack of expression of prestalk-, prespore-, or ALC-specific markers. We demonstrate that a large fraction of the cells in the finger and subsequent multicellular structures express predominantly prestalk cell markers. The pstO/ALC-specific marker extends through the majority of the slug, suggesting that many of these cells differentiate into pstO cells or ALCs. Staining and the terminal

structure, which lacks a true sorus, are consistent with the conclusion that the majority of the cells that participate in the multicellular structure are prestalk or prestalk-related cells.

It is particularly striking that *pslA*$^-$ cells do not detectably express prespore genes when developed by themselves or in chimeras with wild-type cells. This is not the result of defects in cAMP signaling or PKA expression. Moreover, this appears not to be the result of a reduced expression of essential upstream genes such as GBF and LagC, as *ecmA* expression, which also requires these gene products, is expressed nearly normally. Although *pslA*$^-$ cells do not express prespore markers, not all cells differentiate into prestalk cells, as a significant fraction of the cells remain undifferentiated at the base of the aggregate. This suggests that the prestalk cell populations that form the tip and subsequent structures are developmentally distinct from those that remain at the base. It is possible that the cells that remain at the base may initiate the prespore pathway but are blocked in a step prior to the expression of prespore-specific markers (see below) and are unable to participate in morphogenesis. We suggest that, after the prestalk and prespore pathways diverge, additional steps are required for prespore gene expression and cell-type differentiation and these steps require PslA function. As stated, the expression of *GBF* and *LagC* is reduced in *pslA*$^-$ cells compared to the levels found in wild-type cells. It is possible that the cells remaining at the base of the emerging finger no longer express these genes or express them at a lower level later in development. As the tip of the finger/slug is thought to be the oscillator that functions to initiate the cAMP signal within the organism (Siegert and Weijer, 1995; Dormann et al., 1996; Steinbock et al., 1993), which is then relayed posteriorly, the separation of the base cells from the finger may preclude their receiving proper cAMP signals after the finger emerges.

We note that *pslA* is expressed at low levels during growth and maximally during aggregation. Thus, PslA protein is probably present in all cells earlier in development. Although *pslA* is expressed predominantly in ALCs and not prespore cells during the multicellular stages, we expect that it functions earlier, at the time of initial prespore cell induction in the mound. We cannot exclude a low level of *pslA* expression in prespore cells.

PslA protein strongly localizes to the nucleus and has a potential NLS and a highly acid domain. We suggest that PslA functions at the level of transcription by controlling the pathway required for prespore gene expression. It is possible that the highly acidic domain could function as a transcriptional activation domain. A transcriptional role for PslA is consistent with our finding that PslA functions autonomously to control prespore cell differentiation. The absence of a dominant, overexpression phenotype when PslA is expressed in all cell types from the *Act15* promoter suggests that *pslA* expression alone is not sufficient to mediate prespore cell differentiation. We expect, therefore, that PslA acts in concert with a cell-type-specific transcription factor or is modified by a signaling pathway in a cell-type-specific way to control the prespore pathway.

Potential position of pslA in the prespore pathway

Previous analyses suggest that there are at least two classes of prespore-specific genes that can be distinguished by the requirement of PKA for their expression (Hopper et al., 1993b). The PKA-independent prespore gene *psA* (D19) appears to be induced earlier than prespore coat protein genes such as *SP60/CotC, SP70/CotB* and *PspB*, which require PKA for expression. Expression of *psA* is observed in aggregation streams, whereas expression of the *CotA, CotB,* and *CotC* class of genes is not (Early and Williams, 1988; Haberstroh and Firtel, 1990; Fosnaugh and Loomis, 1993; Gomer et al., 1986; Krefft et al., 1984). As neither class of prespore genes is detectably expressed in *pslA*$^-$ cells, we suggest that *pslA* is required very early in the prespore pathway.

Our analysis of chimeric organisms indicates that the *pslA*$^-$ prespore cell defect is cell autonomous. Interestingly, when one part of *pslA*$^-$ cells is mixed with three parts of wild-type cells, the vast majority of the *pslA*$^-$ cells participate in multicellular development. These cells predominantly localize to the very anterior of the slug and form the prestalk and stalk components of the multicellular organism, while the wild-type cells in a 1:3 ratio form prespore cells and spores. These results suggest that, in this context, the *pslA*$^-$ and wild-type cells are able to form an organism with prestalk and prespore cells. While proportioning of these chimeras is not perfect (they produce fruiting bodies with a longer stalk), the data suggest that pathways controlling proportioning in slugs function reasonably well in these chimeras. We expect that the fingers that form in organisms composed solely of *pslA*$^-$ cells continue to try to regulate the proportioning of prestalk and prespore cells in this structure but are unable to fully initiate the prespore cell pathway. Regulated conversion of cell types has been reported (Mann and Firtel, 1993; Mann et al., 1994; Shaulsky and Loomis, 1993). In these cases, prestalk cells converted to prespore cells when prespore cells within a slug were driven to differentiate into spore cells through the overexpression of PKA or were poisoned with Ricin A. In both examples, the ratio of prespore cells to prestalk cells decreased, leading to conversion of the some of the prestalk to prespore cells. We expect that a similar regulation is trying to occur in the *pslA*$^-$ cells, which may account for the observation that the posterior part of the finger is not composed solely of cells that express prestalk or ALC markers. This pattern of differentiation, however, is very distinct from that which has been observed for other mutations that affect prespore cell differentiation. *gsk3* null cells (Harwood et al., 1995) initially express prespore markers but then are unable to fully differentiate into prespore cells and, instead, predominantly differentiate into cells expressing the pstB marker *ecmB*.

Our results demonstrate that PslA function is also required during aggregation. It is unclear whether the aggregation-stage defect represents an early component of the defect in the prespore cell differentiation pathway. Although only scattered cells detectably express *PslA/lac*Z early in development, it is probable that all cells may initially express *pslA* at low levels, as the *pslA*$^-$ prespore cell defect is cell autonomous. *pslA* also appears to have a role in stalk cell differentiation that is observed when examined in vitro, but is not detectable when the cells are plated for multicellular development. Whereas wild-type cells are able to respond to sequential signaling by cAMP and DIF to induce stalk cell differentiation in vitro, *pslA*$^-$ cells are unable to do this in single cell culture. It is therefore possible that during differentiation of stalk cells in a multicellular organism, the role of pslA is not essential, but becomes essential under the in vitro conditions that are used to examine DIF function.

In conclusion, our data suggest that PslA is a nuclear protein, possibly a transcription factor, that is essential for prespore cell differentiation and plays a role in other pathways required for multicellular development. Our data indicate that PslA functions early in the prespore pathway. This is distinct from the role of Stalky, a GATA-related transcription factor that is required for spore cell differentiation during later development and not for prespore cell differentiation (Chang et al., 1996). *Stalky* null cells form prespore cells, but at the time of culmination, the prespore cells differentiate into stalk cells rather than spores. *pslA*$^-$ cells appear to segregate into two cell populations at the time of cell-fate decisions: a prestalk cell population and one that does not exhibit any prestalk or prespore cell markers. It is reasonable to assume that this constitutes the prespore cell population that is unable to differentiate further. Thus, PslA protein may be required early in development at the time of the initial cell-fate decision to proceed down the prespore pathway, but after the prestalk and prespore pathways diverge.

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REFERENCES

Abe, K. and Yanagisawa, K. (1983). A new class of rapid developing mutants in *Dictyostelium discoideum*: Implications for cyclic AMP metabolism and cell differentiation. *Dev. Biol.* **95,** 200-210.

Aubry, L and Firtel, R.A. (1998). Spalten, a protein containing Gα-protein-like and PP2C domains, is essential for cell-type differentiation in Dictyostelium. *Genes Dev.*, in press.

Burki, E., Anjard, C., Scholder, J. C. and Reymond, C. D. (1991). Isolation of two genes encoding putative protein kinases regulated during *Dictyostelium discoideum* development. *Gene* **102,** 57-65.

Ceccarelli, A., Mahbubani, H. and Williams, J. G. (1991). Positively and negatively acting signals regulating stalk cell and anterior-like cell differentiation in *Dictyostelium*. *Cell* **65,** 983-989.

Chang, W. T., Newell, P. C. and Gross, J. D. (1996). Identification of the cell fate gene stalky in *Dictyostelium*. *Cell* **87,** 471-481.

Chen, M. Y., Insall, R. H. and Devreotes, P. N. (1996). Signaling through chemoattractant receptors in *Dictyostelium*. *Trends Genet.* **12,** 52-57.

Clark, A., Nomura, A., Mohanty, S. and Firtel, R. A. (1997). A ubiquitin-conjugating enzyme is essential for developmental transitions in *Dictyosteilum*. *Mol. Biol. Cell.* **8,** 1989-2002.

Dormann, D., Siegert, F. and Weijer, C. J. (1996). Analysis of cell movement during the culmination phase of *Dictyostelium* development. *Development* **122,** 761-769.

Dynes, J., Clark, A., Shaulsky, G., Kuspa, A., Loomis, W. and Firtel, R. (1994). LagC is required for cell-cell interactions that are essential for cell-type differentiation in *Dictyostelium*. *Genes Dev.* **8,** 948-958.

Early, A. E., Gaskell, M. J., Traynor, D. and Williams, J. G. (1993). Two distinct populations of prestalk cells within the tip of the migratory *Dictyostelium* slug with differing fates at culmination. *Development* **118,** 353-362.

Early, V. E. and Williams, J. G. (1988). A *Dictyostelium* prespore-specific gene is transcriptionally repressed by DIF in vitro. *Development* **103,** 519-524.

Firtel, R. A. (1995). Integration of signaling information in controlling cell-fate decisions in *Dictyostelium*. *Genes Dev.* **9,** 1427-1444.

Firtel, R. A. (1996). Interacting signaling pathways controlling multicellular development in *Dictyostelium*. *Curr. Opin. Genet. Dev.* **6,** 545-554.

Fosnaugh, K. L. and Loomis, W. F. (1993). Enhancer regions responsible for temporal and cell-type-specific expression of a spore coat gene in *Dictyostelium*. *Dev. Biol.* **157,** 38-48.

Gamper, M., Howard, P. K., Hunter, T. and Firtel, R. A. (1996). Multiple roles of the novel protein tyrosine phosphatase PTP3 during *Dictyostelium* growth and development. *Mol. Cell. Biol.* **16,** 2431-2444.

Ginsburg, G. T. and Kimmel, A. R. (1997). Autonomous and nonautonomous regulation of axis formation by antagonistic signaling via 7-span cAMP receptors and GSK3 in *Dictyostelium*. *Genes Dev.* **11,** 2112-2123.

Gomer, R. H., Datta, S. and Firtel, R. A. (1986). Cellular and subcellular distribution of a cAMP-regulated prestalk protein and prespore protein in *Dictyostelium discoideum*: A study on the ontogeny of prestalk and prespore cells. *J. Cell Biol.* **103,** 1999-2015.

Haberstroh, L. and Firtel, R. A. (1990). A spatial gradient of expresssion of a cAMP-regulated prespore cell type specific gene in *Dictyostelium*. *Genes Dev.* **4,** 596-612.

Hadwiger, J. A. and Firtel, R. A. (1992). Analysis of Gα4, a G-protein subunit required for multicellular development in *Dictyostelium*. *Genes Dev.* **6,** 38-49.

Hadwiger, J. A., Lee, S. and Firtel, R. A. (1994). The Gα subunit Gα4 couples to pterin receptors and identifies a signaling pathway that is essential for multicellular development in *Dictyostelium*. *Proc. Natl. Acad. Sci. USA* **91,** 10566-10570.

Hammerschmidt, M., Brook, A. and McMahon, A. P. (1997). The world according to hedgehog. *Trends Genet.* **13,** 14-21.

Han, X. and Firtel, R. A. (1998). The homeobox-containing gene *Wariai* regulates anterior-posterior patterning and cell-type homeostasis in *Dictyostelium*. *Development* **125,** 313-325.

Harwood, A., Plyte, S., Woodgett, J., Strutt, H. and Kay, R. (1995). Glycogen synthetase kinase 3 (GSK-3) regulates cell fate in *Dictyostelium*. *Cell* **80,** 139-148.

Heasman, J. (1997). Patterning the *Xenopus* blastula. *Development* **124,** 4179-4191.

Hopper, N. A., Anjard, C., Reymond, C. D. and Williams, J. G. (1993a). Induction of terminal differentiation of *Dictyostelium* by cAMP-dependent protein kinase and opposing effects of intracellular and extracellular cAMP on stalk cell differentiation. *Development* **119,** 147-154.

Hopper, N. A., Harwood, A. J., Bouzid, S., Veron, M. and Williams, J. G. (1993b). Activation of the prespore and spore cell pathway of *Dictyostelium* differentiation by cAMP-dependent protein kinase and evidence for its upstream regulation by ammonia. *EMBO J.* **12,** 2459-2466.

Howard, P. K., Gamper, M., Hunter, T. and Firtel, R. A. (1994). Regulation by protein-tyrosine phosphatase PTP2 is distinct from that by PTP1 during *Dictyostelium* growth and development. *Mol. Cell. Biol.* **14,** 5154-5164.

Ingham, P. W. (1995). Signalling by hedgehog family proteins in *Drosophila* and vertebrate development. *Curr. Opin. Genet. Dev.* **5,** 492-498.

Jermyn, K. A. and Williams, J. G. (1991). An analysis of culmination in *Dictyostelium* using prestalk and stalk-specific cell autonomous markers. *Development* **111,** 779-787.

Jermyn, K. A., Berks, M., Kay, R. R. and Williams, J. G. (1987). Two distinct classes of prestalk-enriched mRNA sequences in *Dictyostelium discoideum*. *Development* **100,** 745-755.

Jiang, J. and Struhl, G. (1996). Complementary and mutually exclusive activities of decapentaplegic and wingless organize axial patterning during *Drosophila* leg development. *Cell* **86,** 401-409.

Kawata, T., Shevchenko, A., Fukuzawa, M., Jermyn, K. A., Totty, N. F., Zhukovskaya, N. V., Sterling, A. E., Mann, M. and Williams, J. G. (1997). SH2 signaling in a lower eukaryote: A STAT protein that regulates stalk cell differentiation in *Dictyostelium*. *Cell* **89,** 909-916.

Kay, R. R. and Jermyn, K. A. (1983). A possible morphogen controlling differentiation in *Dictyostelium*. *Nature* **303,** 242-244.

Klein, P. S. and Melton, D. A. (1996). A molecular mechanism for the effect of lithium on development. *Proc. Nat. Acad. Sci. USA* **93,** 8455-8459.

Konijn, T. M. and Van Haastert, P. J. M. (1987). Measurement of chemotaxis in *Dictyostelium*. *Meth. Cell Biol.* **28,** 283-298.

Krefft, M., Voet, L., Gregg, J. H., Mairhofer, H. and Williams, K. L. (1984). Evidence that positional information is used to establish the prestalk-prespore pattern in *Dictyostelium discoideum* aggregates. *EMBO J.* **3,** 201-206.

Kuspa, A. and Loomis, W. F. (1992). Tagging developmental genes in *Dictyostelium* by restriction enzyme-mediated integration of plasmid DNA. *Proc. Natl. Acad. Sci. USA* **89,** 8803-8807.

Lee, S., Escalante, R. and Firtel, R. A. (1997). A Ras GAP is essential for cytokinesis and spatial patterning in *Dictyostelium*. *Development* **124,** 983-996.

Louis, J. M., Ginsburg, G. T. and Kimmel, A. R. (1994). The cAMP receptor CAR4 regulates axial patterning and cellular differentiation during late development of *Dictyostelium*. *Genes Dev.* **8,** 2086-2096.

Ma, H., Gamper, M., Parent, C. and Firtel, R. A. (1997). The *Dictyostelium* MAP kinase kinase DdMEK1 regulates chemotaxis and is essential for

chemoattractant-mediated activation of guanylyl cyclase. *EMBO J.* **16,** 4317-4332.

Mann, S. K. O. and Firtel, R. A. (1991). A developmentally regulated, putative serine/threonine protein kinase is essential for development in *Dictyostelium*. *Mech. Dev.* **35,** 89-102.

Mann, S. K. O. and Firtel, R. A. (1993). cAMP-dependent protein kinase differentially regulates prestalk and prespore differentiation during *Dictyostelium* development. *Development* **119,** 135-146.

Mann, S. K. O., Devreotes, P. N., Eliott, S., Jermyn, K., Kuspa, A. F., M., Furukawa, R., Parent, C. A., Segall, J., Shaulsky, G., Vardy, P. H. W., J., Williams, K. L. and Firtel, R. A. (1997). Cell biological, molecular genetic, and biochemical methods used to examine *Dictyostelium*. In *Cell Biology: A Laboratory Handbook*, Second Edition. (ed. J. Celis). pp. 425-459. San Diego: Academic Press.

Mann, S., Richardson, D., Lee, S., Kimmel, A. and Firtel, R. (1994). Expression of cAMP-dependent protein kinase in prespore cells is sufficient to induce spore cell differentiation in *Dictyostelium*. *Proc. Natl. Acad. Sci. USA* **91,** 10561-10565.

Mehdy, M. C. and Firtel, R. A. (1985). A secreted factor and cyclic AMP jointly regulate cell-type-specific gene expression in *Dictyostelium discoideum*. *Mol. Cell. Biol.* **5,** 705-713.

Nellen, W., Datta, S., Reymond, C., Sivertsen, A., Mann, S., Crowley, T. and Firtel, R. A. (1987). Molecular biology in *Dictyostelium*: tools and applications. *Meth. Cell Biol.* **28,** 67-100.

Nusse, R. (1997). A versatile transcriptional effector of *Wingless* signaling. *Cell* **89,** 321-323.

Ohlmeyer, J. T. and Kalderon, D. (1997). Dual pathways for induction of wingless expression by protein kinase A and Hedgehog in *Drosophila* embryos. *Genes Dev.* **11,** 2250-2258.

Perrimon, N. (1996). Serpentine proteins slither into the *Wingless* and *Hedgehog* fields. *Cell* **86,** 513-516.

Pitt, G. S., Milona, N., Borleis, J., Lin, K. C., Reed, R. R. and Devreotes, P. N. (1992). Structurally distinct and stage-specific adenylyl cyclase genes play different roles in *Dictyostelium* development. *Cell* **69,** 305-315.

Reymond, C. D., Schaap, P., Veron, M. and Williams, J. G. (1995). Dual role of cAMP during *Dictyostelium* development. *Experientia* **51,** 1166-1174.

Saxe III, C. L., Ginsburg, G. T., Louis, J. M., Johnson, R., Devreotes, P. N. and Kimmel, A. R. (1993). CAR2, a prestalk cAMP receptor required for normal tip formation and late development of *Dictyostelium discoideum*. *Genes Dev.* **7,** 262-272.

Schnitzler, G. R., Briscoe, C., Brown, J. M. and Firtel, R. A. (1995). Serpentine cAMP receptors may act through a G protein-independent pathway to induce postaggregative development in *Dictyostelium*. *Cell* **81,** 737-745.

Schnitzler, G., Fischer, W. and Firtel, R. (1994). Cloning and characterization of the G-box binding factor, an essential component of the developmental switch between early and late development in *Dictyostelium*. *Genes Dev.* **8,** 502-514.

Shaulsky, G. and Loomis, W. F. (1993). Cell type regulation in response to expression of ricin-A in *Dictyostelium*. *Dev. Biol.* **160,** 85-98.

Siegert, F. and Weijer, C. J. (1995). Spiral and concentric waves organize multicellular *Dictyostelium* mounds. *Curr. Biol.* **5,** 937-943.

Steinbock, O., Siegert, F., Muller, S. C. and Weijer, C. J. (1993). Three-dimensional waves of excitation during *Dictyostelium* morphogenesis. *Proc. Natl. Acad. Sci. USA* **90,** 7332-7335.

Sternfeld, J. and David, C. N. (1982). Fate and regulation of anterior-like cells in *Dictyostelium* slugs. *Dev. Biol.* **93,** 111-118.

Sutoh, K. (1993). A transformation vector for *Dictyostelium discoideum* with a new selectable marker Bsr. *Plasmid* **30,** 150-154.

Van Haastert, P. J. M. (1995). Transduction of the chemotactic cAMP signal across the plasma membrane of *Dictyostelium* cells. *Experientia* **51,** 1144-1154.

Williams, J. (1995). Morphogenesis in *Dictyostelium*: New twists to a not-so-old tale. *Curr. Opin. Genet. Dev.* **5,** 426-431.

Williams, J. G., Ceccarelli, A., McRobbie, S., Mahbubani, H., Kay, R. R., Farly, A., Berks, M. and Jermyn, K. A. (1987). Direct induction of *Dictyostelium* prestalk gene expression by D1F provides evidence that D1F is a morphogen. *Cell* **49,** 185-192.