Protein Kinase B Gene Homologue pkbR1 Performs One of Its Roles at First Finger Stage of Dictyostelium

Hiroshi Ochiai,1,2,3* Kosuke Takeda,1,† Masashi Fukuzawa,4 Atsushi Kato,1,5,6 Shigeharu Takiya,3,5,6 and Tetsuo Ohmachi2

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan;1 Department of Biochemistry and Molecular Biology, Faculty of Agriculture & Life Science, Hiroasaki University, 3 Bunkyo-cho, Hiroasaki 036-8561, Japan;2 Division of Genome Dynamics, Creative Research Initiative Sousei, Hokkaido University, Sapporo 060-0810, Japan;3 Department of Biology, Faculty of Agriculture & Life Science, Hiroasaki University, 3 Bunkyo-cho, Hiroasaki 036-8561, Japan;4 Division of Functional Genome Science, Department of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan;5 and Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan6

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Dictyostelium discoideum has protein kinases AKT/PKBA and PKB1 that belong to the AGC family of kinases. The protein kinase B-related kinase (PKB1R1) has been studied with emphasis on its role in chemotaxis, but its roles in late development remained obscure. The pkbR1 null mutant stays in the first finger stage for about 16 h or longer. Only a few aggregates continue to the migrating slug stage; however, the slugs immediately go back probably to the previous first finger stage and stay there for approximately 37 h. Finally, the mutant fingers diversify into various multicellular bodies. The expression of the pkbR1 finger protein probably is required for development to the slug stage and to express ecmB, which is first observed in migrating slugs. The mutant also showed no ST-lacZ expression, which is of the earliest step in differentiation to one of the stalk cell subtypes. The pkbR1 null mutant forms a small number of aberrant fruiting bodies, but in the presence of 10% of wild-type amoebae the mutant preferentially forms viable spores, driving the wild type to form nonviable stalk cells. These results suggest that the mutant has defects in a system that changes the physiological dynamics in the prestalk cell region of a finger. We suggest that the arrest of its development is due to the loss of the second wave of expression of a protein kinase A catalytic subunit gene (pkaC) only in the prestalk region of the pkbR1 null mutant.

Dictyostelium discoideum is a social amoeba that feeds on bacteria. When amoebae are deprived of food, less than about 107 cells aggregate into a mound. After several differentiation events, a standing structure, referred to as a first finger, is formed. The anterior region (ca. 20%) is made up of several prestalk cell types, and a larger posterior region (ca. 80%) is composed of prespore cells, within which a few anterior-like cells (ALCs) are interspersed. The expression of these genes is characteristic of prestalk cells. Depending on environmental conditions, a critical decision must be made on whether to initiate the culmination of development and form a mature fruiting body or to fall to the substratum and migrate as a slug, which would seek more favorable conditions to maximize successful spore dispersal.

The start of culmination is initiated by forming a small core of prestalk AB cells in the prestalk A region at the anterior region of the slug. The terms refer to the expression of the extracellular matrix proteins EcmA and EcmB, the first markers identified that distinguished the various subtypes of prestalk cells and their spatial patterning (41). The transition of a subset of the prestalk A cells expressing only ecmA to prestalk AB cells expressing both ecmA and ecmB indicates the induction of culmination and can be visualized with a reporter gene driven by a promoter of ecmB (19, 41) or the more recently identified aslA (acetyl-coenzyme A [CoA] synthetase-like A) gene (36). Prestalk AB cells produce a nascent stalk tube into which surrounding prestalk A cells are recruited, tip cells first, until the tube elongates downward to reach the substratum (15).

Chemotaxis in Dictyostelium is linked to two protein kinase B (PKB) homologues: a phosphatidylinositol 3,4,5-trisphosphate (PIP3)-dependent enzyme, PkbA, which has a PIP3-specific pleckstrin homology (PH) domain at its N terminus, and a PIP3-independent enzyme, PkbR1, which is tied up to the plasma membrane via N-terminal myristoylation (31, 32). When cells are stimulated with cyclic AMP (cAMP) or folate, PkbA and PkbR1 are rapidly phosphorylated within their hydrophobic motifs (HMs) and activation loops (ALs). The phosphorylation of the HMs of the two PKBs is mediated by TorC2 (target of rapamycin complex 2). One of the genetic defects in chemotaxis in D. discoideum was a loss of PianissimoA (piaA), which was later shown to be a subunit of TorC2 (5, 26). In PkbA, the phosphorylation of the AL domain requires the recruitment of the enzyme to the membrane by the transient accumulation of PIP3, while in PkbR1 the phosphorylation of the AL is independent of PIP3, but its regulation is not under-
stood (20). In mammalian cells, PDK1 mediates AL phosphorylation in PKB; in *Dictyostellium* cells, there are two PDK1 homologues, PdkA and PdkB. Recently it was found that these enzymes, mainly PdkA, activate PkbA and PkbR1 and are regarded as key AL kinases (20, 27) that regulate the chemo-tactic response (20). In relation to the processes of spore differentiation, pkbR1 appears to mediate the signal transduction pathway from gamma-aminobutyric acid (GABA), and finally the GABA pathway induces the release of the precursor of the signaling peptide SDF-2 (2). SDF-2 is released by prestalk cells during culmination that stimulates prespore cells to encapsulate.

Although the PKBs have been extensively analyzed for their role in chemotaxis, their functions during late development are totally unknown; one reason for this may be the incomplete penetrance of the *pkbR1* null mutant in late development. At first, we optimized the developmental conditions for the mutant using a phototactic chamber. Under these conditions the mutant remains at the first finger stage for about 16 h, but in some circles occupied by cells in a droplet (see Materials and Methods), migrating slugs appeared suddenly at about 26 h but disappeared within 20 min. The long-lasting first finger does not show the expression of both the EcmB and ST (stalk) markers at the core region of the anterior prestalk A region. As a possible cause for the developmental arrest of the *pkbR1* mutant, we noticed that new *pkaC* expression at the anterior prestalk region was not initiated at all, while the expression in the wild type was strong. The results do not conflict with earlier findings that the activity of PKA is necessary during the late, multicellular stages of development (14).

**Materials and Methods**

**Strains and culture conditions.** *Dictyostelium discoideum* Ax2 and *pkbR1* null cells were grown in HL5 medium (40) or modified HL5 medium (Bacito proteose peptone, 5 g; casitone peptone [Merek], 2.5 g; Bacto tryptone peptone, 2.5 g; Bacto yeast extract, 5 g; glucose, 10 g; 13 mM sodium phosphate buffer, pH 6.2, per liter). Penicillin and streptomycin were added routinely to the HL5 medium. Fresh cells were set up every 2 weeks from a deep freezer stock (–80°C). For observing cells deposited for the synchronized development of *pkbR1* null cells, at each developmental time point three plates were prepared. On each plate five drops of 50 k cells were placed (5×10^5 to ~8×10^5 cells/ml) were placed on a 13 mM Na^+ agar plate (pH 6.2), and cells in a drop remained within a circle of ca. 15-mm diameter. We refer to an area occupied by a drop as a circle. Accordingly, each observation time point has 15 circles. Finally, the plates were placed in a phototactic chamber (9) to synchronize development. The plates in the chamber were placed in an incubator (21°C) with a 6- or 10-W fluorescent light source (space about 20 cm apart), and photographs were taken at the time intervals shown in the legend to Fig. 1. The cells also were cultured with the following precautions: (i) newly prepared, frozen stocked cells were thawed once in 2 weeks and the number of proliferation cycles was kept to a minimum, and (ii) overgrowth was carefully avoided (~2×10^5 cells/ml). Under these conditions, we could observe slugs migrating forward in unison at 26 h, indicating coordinated development.

**Knockout construct for pkbR1.** To generate a knockout construct for the *pkbR1* gene, we employed a strain that had a mutation in the *pkbR1* region by the introduction of an insertion vector (39). From this *pkbR1*-REMI strain a 1.5-kbp fragment containing *pkbR1* was transferred. The recovered DNA was digested with EcoRI and HindII to remove the Bsr expression cassette and a part of the kinase domain. The recovered DNA was used as a template for inverse PCR so that the sequence between 221 and 412 bp from the start codon of *pkbR1* could be removed. To design inverse PCR primers in outward orientations, the following primer pairs were used: 220 and 201, 5′-TAGATCTCAAATCTGCTGCTGTAGC CGTT-3′; 413 and 432, TAGATCCTGAAGATGAGCCAGGAGG-3′. The oligonucleotides used for amplification contained BglII sites (in italics) that were being washed twice with Z buffer, cells were incubated in a staining solution at 37°C (6). Submerged monolayer culture and chimera experiments. The *pkbR1* null and Ax2 cells (ca. 1×10^6 cells/ml) were incubated using Falcon 24-well tissue culture plates with a stalk medium containing 5 mM cyclic adenosine-3′,5′-monophosphate (22, 23, 24). After 20 h, the medium was removed, and cells were washed twice with stalk medium and incubated with fresh stalk medium containing 100 mM differentiation-inducing factor (DIF-1). After 22 h, cell differentiation was monitored by phase-contrast microscopy. In the synergy experiments, cells from two different strains were washed and mixed, generally at ratios of 1:9 or 9:1, and allowed to develop on 13 mM sodium phosphate-buffered agar plates. In *in situ* hybridization. Whole-mount *in situ* hybridization analyses were performed by a modification of the method described previously (6). Ax2 and *pkbR1* null cells in an Ax2 background were allowed to develop on 13 mM sodium phosphate-buffered agar at 21°C, and the multicellular bodies on a hydrophobic carpet were allowed to float on a water surface by introducing water with a wash bottle along the inner wall of the dish. The multicellular bodies were carefully transferred to coverslips. The coverslips with the attached organisms were fixed, washed, and treated by changing solutions as described previously. Antisense RNA and sense RNA probes were synthesized with the DIG RNA labeling kit (Roche) using T7, T3, or SP6 RNA polymerase according to the manufacturer's instructions.

**Results**

**Development of the pkbR1 null strain.** The development of *pkbR1* null cells varied considerably from wild-type development; consequently, it was difficult to observe and determine the stage where defects became visible. Thus, we first established optimal conditions for cells to develop synchronously. Figure 1 shows that the use of a phototactic chamber allowed the tightly synchronized development of Ax2 and *pkbR1* null cells (e.g., all aggregates are in the Mexican hat stage in Fig. 1A, 12 h). The developmental cycle of the mutant was significantly prolonged (about 36 h), although the mutant cells aggregated and made mounds after 10 h of development (Fig. 1B), similarly to the Ax2 wild type (Fig. 1A). During a period from 12 to 16 h, the mutant showed a balloon-like structure and then proceeded to a rather typical first finger stage (19 to ~23 h). At 26 h, in three out of five circles on one plate within three plates (see Materials and Methods) slugs migrated parallel to each other toward the light source (Fig. 1, 26 h), but they entirely disappeared within 20 min. Instead, structures resembling first fingers were found. These structures persisted for 28 to ~35 h, and we were unable to distinguish them from first fingers. At around 35 h, they diversified into a variety of forms: mounds, fingers, culminates, unusual deformed bodies, and aberrant fruiting bodies, which often formed squat structures with short, bent stalks. The very short period of time during which the mutant was in the migrating slug stage may be correlated with the lack of the expression of *ecmB* (Fig. 2),...
which usually occurs at the core of the apical prestalk A region of the migrating slug. The results suggest that almost all mutant cells were arrested at the first finger stage, and that the mutant lacks a robust mechanism to develop into a slug.

**Lack of expression of prestalk ecmB marker in the pkbR1 mutant.** Since the pkbR1 mutant resulted in arrested development at the finger stage, we thought that the mutant might have some defects in cellular differentiation during late development. Promoters specific to the prestalk A (ecmA), prestalk O (ecmO, data not shown), prestalk AB (ecmB), prestalk AB (ST), and prespore (SP60) regions were used to express the β-galactosidase reporter gene to compare cell type-specific gene expression in the pkbR1 null mutant to that of the wild type (18).

The ecmA and ecmO (data not shown) markers are expressed extensively in the prestalk region of the null mutant (Fig. 2D, E, and F) and wild type (Fig. 2A, B, and C). The ecmB gene first started to be expressed at low levels in the peripheral parts of mounds of both strains (Fig. 2G and J). Later, ecmB in the wild type was expressed extensively in the tip region of the migrating slug (Fig. 2I) and the anterior region of the second finger-like structure (Fig. 2H), whereas ecmB in the mutant was never expressed in the standing finger (Fig. 2K and L). However, the low ecmB expression in the basal disc regions of the mutant (Fig. 2K and L) and wild type (Fig. 2H) seemed to persist at all times. The absolute lack of ecmB expression in the core region of the mutant probably reflected the fact that the mutant finger was unable to develop into the robust migrating slug.

In normal development, prestalk cells occupy the anterior one-fifth of a migratory slug, while prespore cells hold the posterior four-fifths. The prespore marker SP60 was expressed extensively in the pkbR1 null strain (Fig. 3D, E, and F) as well as in wild-type cells (Fig. 3A, B, and C). Another prespore

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**FIG. 1.** Ax2 (A) and pkbR1 null (B) cells developed in a phototactic chamber in a highly synchronized fashion. The Ax2 and pkbR1 cells were grown in modified HL5 medium. Five drops of 50 μl of suspended cells (≈3.5 × 10⁶ cells/drop) were placed on a nonnutrient agar plate, and the plate was placed in a phototactic chamber. The pkbR1 null cells showed delayed development of around 36 h. At 26 h, some slugs migrated coordinately toward a light source, but the slugs immediately came back to the fingers within 20 min. At 35 to 37 h, the mutant cells begin to exhibit diverse morphology, including various multicellular bodies and a few fruiting bodies.
marker, SpiA mRNA, detected by reverse transcription-PCR (RT-PCR), was expressed later in the pkbR1 null cells (data not shown). The data suggest that the pkbR1 null mutant shows a delay of about 8 h in spore formation.

The explanation of the results obtained with the entire ecmB promoter is complicated by the fact that in wild-type cells, the ecmB gene is distinctly expressed in multiple prestalk and stalk cell types, including the pstAB and pstBA cells (33). The pstAB cells express the ST-lacZ marker. Cells at the base also coexpress the ecmA and ecmB genes, but here the order of gene expression most probably is reversed (therefore termed the pstBA cells) (33). The pstBA cells do not express the ST-lacZ marker. We therefore analyzed the mutant clone by using ST-lacZ, a marker of the earliest possible step in the terminal differentiation of prestalk cells into stalk cells: the transition of pstAB cells from pstA cells. Sequences proximal to the cap site of the ecmB promoter, the ST (stalk) promoter region, drive strong expression in a cell cluster within the stalk tube (i.e., pstAB cells). Before culmination, wild-type cells express the ST-lacZ marker at very low levels, and then rather strong ST-lacZ expression is seen in a small inverted cone of cells just behind the tip of some wild-type slugs (Fig. 3G and

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H), but the ST-lacZ marker is expressed at lower levels in some scattered cells in the pkbR1 slugs (Fig. 3J and K). This expression pattern probably shows the low expression in anterior-like cells (ALC) in the mutant. The ST-lacZ expression pattern is similar to that of ecmB gene expression, i.e., the strong expression at the core region is missing, but the residual expression remains at a very low level (compare Fig. 2J to L to Fig. 3J and K). We suggest that the primary phenotype of the pkbR1 mutant reflects the complete lack of ecmB expression in the core region, that is to say, the most advanced stage achieved by the mutant is the first finger stage.

In chimeras, pkbR1 null cells stay in their original position. Our analyses of differentiation-specific markers led to the assumption that the mutant had defects in progressing to the migrating slug from the standing finger stage. We therefore analyzed the behavior of the mutant cells in chimeras. The pkbR1 null cells constitutively expressing lacZ from an act15 promoter were mixed with the parental Ax2 cells in ratios of 1:9 (Fig. 4A to D) and 9:1 (Fig. 4E to H) and then allowed to develop together. Figure 4 shows that the mutant cells stained in blue were not found in the anterior tip region (Fig. 4B, C, F, and G). We inferred that almost all cells of the mutant congregate at the posterior region, so that the prestalk region was entirely occupied by Ax2 cells; nonlabeled wild-type cells were found only in stalks (Fig. 4D and H). Eventually the pkbR1 null cells were channeled to their spore fate. The pkbR1 null cells

FIG. 3. SP60-lacZ and ST-lacZ expression patterns in Ax2 and pkbR1 null cells. (A to F) SP60-lacZ expression; (G to L) ST-lacZ expression. (A to C and G to I) Ax2; (D to F and J to L) pkbR1 null strain; (A, D) mound; (B, E) tipped mound; (C, F) fingers (note the expanded prespore region in the mutant pkbR1 finger [F] compared to that of the parental strain [C]); (G, H) slug at 17 h; (I) fruiting body at 17 h; (J, K) finger at 20 h (there is very weak dispersed expression, probably in ALC cells, but no ST-expressing cells at the core of the anterior tip of pkbR1 null fingers [compare G and H to J and K]); (L) fruiting body with a short stalk, 40 h (some of the multicellular bodies become fruiting bodies). Black bar, 100 μm.
therefore are predicted to be cheater mutants (7). The unusual behavior of the mutant cells may be ascribed to at least a partial loss of their ability to migrate (31, 21, 27). Second, the abnormal behavior may be due to a reduced ability to differentiate into migrating slugs. In addition, the mutant fingers did not respond to phototactic stimulation at all (data not shown).

Differentiation into stalk cells of \textit{pkbR1} null cells by DIF-1.

To determine whether or not the \textit{pkbR1} null cells were able to differentiate in response to DIF signaling, \textit{pkbR1} null and Ax2 cells were incubated under submerged culture conditions containing 5 mM cAMP (22). After 20 h, the medium was removed and the cells were washed and incubated with 100 nM DIF-1 for 22 h. The strains differentiated into stalk cells to similar extents (Fig. 5). The results indicate that the mutant has a sufficient ability to carry out stalk cell differentiation via DIF-1. As described in the next section, the arrest of \textit{pkbR1} fingers may be due to the lack of the second wave of PKA expression only in the prestalk region.

No new \textit{pkaC} transcripts are detected in the prestalk region of \textit{pkbR1} null fingers. To investigate the possibility that the arrest of development of \textit{pkbR1} mutants at the finger stage was

FIG. 4. In chimera, \textit{pkbR1} null cells preferentially sort to the spore region. The results show that \textit{pkbR1} mutant cells localize preferably to the posterior spore region rather than to the stalk region during culmination. \textit{pkbR1} null cells constitutively expressing \textit{lacZ} from an \textit{act15} promoter were mixed with the parental Ax2 cells in ratios of 1:9 (A to D) and 9:1 (E to H). (A, E) Mound; (B, F) tipped aggregate; (C, G) first finger; (D, H) fruiting body. Black bars, 100 μm.

FIG. 5. \textit{pkbR1} null cells are able to differentiate into stalk cells by DIF-1. Ax2 and \textit{pkbR1} null cells were incubated in tissue culture dishes (24-well plate) at a density of $10^5$ cells/cm$^2$ in stalk medium supplemented with 5 mM cAMP. After 20 h, the medium was removed and cells attached on wells were washed with stalk buffer twice and incubated in fresh stalk medium containing 100 nM DIF-1. After 22 h, stalk cell differentiation was scored by phase-contrast microscopy. Results are the averages from three assays.
due to low levels of pkaC expression, we examined its expression levels by in situ hybridization. Figure 6 shows that while some nonspecific staining persisted with a sense RNA probe (Fig. 6A and D), some evenly distributed transcript levels of pkaC were detected with the antisense mRNA probes at both 21 and 34 h. Staining was slightly denser in the prestalk region at 21 h than at 34 h (Fig. 6B, C, E, and F). The new pkaC expression in the anterior region of the pkbR1 null fingers was not detectable compared to that of the Ax2 slugs (Fig. 6H). In Ax2, intense staining was present at the anterior prestalk region (Fig. 6H) compared to that of the sense control (Fig. 6G). In both strains the basal staining persisted throughout both the prestalk and prespore regions. In preliminary analyses, we measured total PKA activities in extracts of the 34-h-developed mutant and 16-h-developed Ax2 cells. The activity of the mutant was similar to that of Ax2. Even though the pkbR1 mutant did not reveal new pkaC transcription only in the prestalk region, the remaining basal level apparently was sufficient to generate some protein product to maintain the finger stage (14, 15, 42). The PkaC activity measurement probably is not sensitive enough to detect the differences in expression that are obvious on the mRNA level.

We conclude that the pkbR1 mutant lacks the second wave of PKA expression only in the prestalk region but certainly has some basal expression through the prespore and prestalk regions.
DISCUSSION

We paid particular attention to a unique developmental phenotype of the pkbR1 null mutant, which remained arrested at the first finger stage and, unlike slugger mutants, did not proceed to the slug stage (34). We used a phototactic chamber for good synchronization of development as shown in Fig. 1, since the cells showed incomplete penetrance in their phenotype.

The use of this method to achieve highly synchronized development made it possible to find that the mutant remained arrested at the first finger stage. In some circles, but not all, almost all slugs were moving in unison toward a light source (Fig. 1B, 26 h). The slugs disappeared within 20 min and apparently returned to the standing finger stage. This surprising result means that the mutant can, at best, transiently proceed to the migrating slug stage, and that the mutation affects the first finger-slug transition. Under normal, not specifically synchronized developmental conditions, this phenotype is barely detectable and difficult to interpret. We suggest that some mutant fingers are able to go on to the slug stage but immediately come back to the first finger and consequently do not express ecmB in the anterior prestalk region of the first fingers.

On the other hand, our results indicated that the pkbR1 null cells did not display increased pkaC mRNA only in the prestalk region, since the second wave of pkaC expression did not occur. The fact that the pkbR1 null cells were able to develop at least until the first finger stage suggests strongly that not all transcripts of the pkaC gene in the prestalk region were lost, because it is known that pkaC null strains cannot aggregate at all (29) and PKA activity seems to control the expression of many postaggregative genes (10, 11, 16, 30). We therefore ascribed the developmental arrest of the pkbR1 null mutant to the lack of the second wave of pkaC expression only in the prestalk A region. Still, we could not explain why the pkbR1 null mutant arrested at the first finger stage, although many slugger mutants remained at the slug stage for a long time (1, 17, 37, 12).

The null fingers did not express any ecmB at the core region of the anterior prestalk region, although they did express the ecmB gene in the rare fruiting bodies that were formed later. Previously, Harwood et al. (14) made transformants with a construct where the ecmA promoter drives a modified, dominant-negative PKA regulatory subunit (ecmA-Rm) (14). The transformants produced seemingly normal slugs that failed to culminate (15). In these slugs, ecmB expression at the core of the prestalk A region is strongly reduced, suggesting that PKA plays a role during the slug stage in the induction of the ecmB gene. These findings are similar to the results obtained for the pkbR1 null cells; the development of both the ecmA-Rm transformants and the pkbR1 null mutant was arrested at later developmental stages, and the strains did not express the ecmB marker at the prestalk A region, except for some very weakly stained cells in the ecmA-Rm.

To obtain visual cues for the relationship between pkaC reduction and the loss of ecmB expression (15), we analyzed pkaC gene expression by in situ hybridization and by analyzing PKA activity in the extracts of the mutant and Ax2 cells. The pkbR1 null cells showed no increase of the pkaC transcripts in the prestalk region of the finger stage, but total cell extracts had basal activity similar to that of Ax2 cells. Hence, the cause for the developmental arrest of the mutant could be ascribed to the lack of new pkaC expression at the prestalk A region. However, a difference between pkbR1 null and ecmA-Rm transformants should be noted: while the former does not go into slug stage primarily, and thereby never expresses ecmB, the latter makes slugs that do not express the ecmB marker.

The regulation of ecmB expression in the slug has been extensively investigated. Genes expressed in the core region of slugs seem to be variably regulated at later stages (25, 26, 28). The ecmB gene was never expressed in the pkbR1 null mutant and the mutant arrested the standing finger stage, probably because transition to the slug stage was impaired. In contrast, almost no ecmB was expressed in the core region of amicC mutant cells, but this mutant still developed into slugs (25). The as1A gene was expressed in the core region of the wild type (36), and the null mutant developed normally. The behavior of these mutants differs from that of the pkbR1 knockout. The difference is explained by the fact that the pkbR1 mutant did not proceed to the slug stage. The arrest may be ascribed to the lack of some protein phosphorylation by the pkbR1 kinase. Some protein may be essential for developing to the slug stage but not for the elongation of the stalk tube. The lack of ecmB expression in pkbR1 knockout thus may be indirect, in that the cells never acquire the prerequisites to proceed to the developmental stage where ecmB can be expressed.

In our preliminary experiments, pkbR1 null slugs showed a continued expression of the acaA gene (data not shown), which is essential to produce extracellular cAMP (35). This suggests that excess extracellular cAMP in the multicellular structure of the mutant and stalk differentiation is inhibited by extracellular cAMP (3). Hopper et al. (16) reported that extracellular cAMP also repressed ST-gal expression in cells with the pkaC gene controlled by a prestalk-specific promoter. Apparently, extracellular cAMP exerts an inhibitory effect on stalk cell differentiation.

We conclude that the pkbR1 null strain arrested just before the slug stage, although a small number of cells may go on to the slug stage but go back immediately. This is very different from slugger mutants. The phenotype of the mutant is unique, and the characteristic arrest at the first finger stage can be exploited to dissect the developmental phases of Dictyostelium. To identify primary causes of the developmental arrest of the pkbR1 null fingers, we should find the underlying conditions that affect the phenotype of the mutant. There are at least two transcriptional irregularities detected in the mutant; we will examine how the lack of cell type-specific pkaC transcription in the slug is modulated in the pkbR1 null cells and/or how extracellular cAMP exerts its inhibitory effect on stalk cell differentiation.

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