The *Dictyostelium* MAP kinase ERK2 regulates multiple, independent developmental pathways

Chris Gaskins,¹ Alexandra M. Clark,¹ Laurence Aubry,¹ Jeffrey E. Segall,² and Richard A. Firtel^{1,3}

¹Department of Biology, Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093-0634 USA; ²Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, New York 10461 USA

We showed previously that the MAP kinase ERK2 is essential for aggregation. *erk2* null cells lack cAMP stimulation of adenyl cyclase and thus cannot relay the cAMP chemotactic signal, although the cells chemotax to cAMP (Segall et al. 1995). In this paper we have examined the role of ERK2 in controlling developmental gene expression and morphogenesis during the multicellular stages, making use of a temperature-sensitive ERK2 mutation. Using suspension assays, we show that ERK2 is not essential for aggregation-stage, cAMP pulse-induced gene expression, or for the expression of postaggregative genes, which are induced at the onset of mound formation in response to cAMP in wild-type cells. In contrast, the prespore-specific gene *SP60* is not induced and the prestalk-specific gene *ecmA* is induced but at a significantly reduced level. Chimeric organisms, comprised of wild-type and *erk2* null cells expressing the prestalk-specific *ecmA/lacZ* reporter, show an abnormal spatial patterning, in which *Erk2^{ts}/erk2* cells are excluded from the very anterior prestalk A region. To further examine the function of ERK2 during the multicellular stages, we bypassed the requirement of ERK2 for aggregation by creating an ERK2 temperature-sensitive mutant. *erk2* null cells expressing the ERK2^{ts} mutant develop normally at 20°C and express cell-type-specific genes but do not aggregate at temperatures above 25°C. Using temperature shift experiments, we showed that ERK2 is essential for proper morphogenesis and for the induction and maintenance of prespore but not prestalk gene expression. Our results indicate that ERK2 functions at independent stages during *Dictyostelium* development to control distinct developmental programs: during aggregation, ERK2 is required for the activation of adenyl cyclase and during multicellular development, ERK2 is essential for morphogenesis and cell-type-specific gene expression. Analysis of these results and others supports the conclusion that the requirement of ERK2 for cell-type differentiation is independent of its role in the activation of adenyl cyclase.

[Key Words: *Dictyostelium discoideum*; signal transduction; MAP kinase]

Received August 9, 1995; revised version accepted November 3, 1995.

Upon starvation, *Dictyostelium* amoebae initiate a developmental process that leads to the formation of a multicellular organism comprised of up to 10⁵ cells that forms a fruiting body containing a sorus of spores on a slender stalk held up by a basal disk (Loomis 1982). The formation of the multicellular organism is mediated by the chemotactic responses to extracellular cAMP that binds to cell surface receptors and activates intracellular signaling pathways (Devreotes 1994; Firtel 1995). These pathways include (1) the activation of adenyl cyclase, leading to the secretion of cAMP and the relay of the chemotactic signal; (2) the activation of guanylyl cyclase, which is important for chemotaxis; and (3) the expression of genes (including the cAMP receptor cAR1 and the coupled Gα2 subunit) essential for the aggregation process. These pathways are regulated by oscillatory

pulses of cAMP, which control the sequential activation and adaptation of these pathways, whereas a continuous flux of cAMP results in cells that are continually adapted (Van Haastert 1991; Devreotes 1994; Firtel 1995). Upon the formation of the mound, rising levels of cAMP activate a second signaling pathway that results in the expression and activation of the transcription factor GBF and the induction of postaggregative gene expression and the regulatory cascade that leads to the expression of prestalk- and prespore-specific genes, cell-type differentiation, morphogenesis, and the repression of aggregation-stage gene expression (Abe and Yanagisawa 1983; Schnitzler et al. 1994, 1995; Firtel 1995). In contrast to the cAMP-mediated responses during aggregation, postaggregative gene expression and cell-type differentiation require a high, continuous level of cAMP.

MAP kinases (ERKs) are components of signaling cascades found in all eukaryotes that regulate a diverse set

³Corresponding author.

of pathways, including mating and osmoregulation in yeast, stress-responses in mammalian cells, and cellular and developmental responses to a diverse set of ligands in higher eukaryotes through both seven-span/serpentine and tyrosine kinase receptors (Davis 1993; Errede and Levin 1993; Blumer and Johnson 1994; Hsu and Perimon 1994; Hunter 1995; Karin 1995; Marshall 1995). In many cases, these involve the activation of transcription factors (Errede and Levin 1993; Hunter 1995; Karin 1995; Marshall 1995). Two MAP kinases, ERK1 and ERK2, have been identified in *Dictyostelium*. ERK1 is developmentally regulated, being expressed from one promoter during growth and a second promoter that is induced upon the onset of multicellular development and is preferentially expressed in cells showing characteristics of the anterior-like cell (ALC) population (Gaskins et al. 1994). ERK1 is essential for growth and also plays an important role in regulating multicellular development. ERK2 was identified by a molecular genetic screen for mutants that are unable to aggregate, using a procedure for insertional mutagenesis (REMI) and independently by a PCR screen for genes encoding MAP kinases (Segall et al. 1995). *erk2* null cells do not aggregate and lack the ability to activate adenylyl cyclase in response to extracellular cAMP. *erk2* null cells are, however, capable of forming coaggregates with wild-type cells, but spores are not formed from the *erk2* null cells.

In this paper we report the creation of a temperature-sensitive mutation of ERK2 and its expression in *erk2* null cells. This strain was used, in combination with cell suspension assays and chimeras containing wild-type and *erk2* null cells, to examine the role of ERK2 in gene expression, cell-type differentiation, and morphogenesis during the multicellular stages. Our results indicate that ERK2 is required for proper morphogenesis and prespore cell differentiation. Expression of the prestalk marker *ecmA* is reduced and *erk2* null cells expressing *ecmA/lacZ* show an abnormal spatial distribution in chimeras. Because prespore genes can be induced in cells lacking the adenylyl cyclase ACA (Pitt et al. 1993), which is not activated in *erk2* null cells, our results indicate that ERK2 has multiple, possibly independent functions, including the regulation of signal relay during aggregation, morphogenesis, and cell-type differentiation.

Results

Examination of gene expression in *erk2* null cells

The regulation of expression of genes induced during aggregation and multicellular stages of *Dictyostelium* development can be examined in suspension cultures (Mehdy and Firtel 1985; Mann and Firtel 1987; Berks and Kay 1990). These assays have the advantage that the role of extracellular morphogens and cell-cell contact on gene expression can be directly examined. To determine whether ERK2 was part of the signaling pathway required for the induction of aggregation-stage gene expression in response to pulses of cAMP, we assayed the expression of the gene encoding the cell surface adhesion

molecule contact sites A (*csA/gp80*), which has previously been shown to be induced in response to nanomolar pulses of cAMP (Noegel et al. 1986; Mann and Firtel 1989). As shown in Figure 1, *csA* expression in both wild-type and *erk2* null cells was induced in response to cAMP pulses, but was only minimally induced when the cells were not pulsed with exogenous cAMP. The level of *csA* expression was slightly reduced in the *erk2* null cells when compared to that of the wild-type strain that is isogenic with the *erk2* null cells except for the disruption of the *ERK2* gene.

Postaggregative genes, which are induced as the mound forms, and prestalk- and prespore-specific genes, can be induced in suspension culture in response to high (μ M), continuous levels of cAMP rather than the nm pulses that induce many aggregation-stage genes (Firtel 1995). Postaggregative genes, such as the ones encoding the transcription factor GBF and genes whose expression is enriched in prestalk cells (e.g., *pst-cath/CP2* and *rasD*), are induced in fast-shake suspension culture in the presence of EDTA to help eliminate cell-cell contacts (Mehdy and Firtel 1985; Dynes et al. 1994). Expression of prespore and prestalk cell-type-specific genes (e.g., *SP60/CotC* and *ecmA*, respectively) requires regulatory signals such as stable cell-cell contacts, the *LagC* gene product, and DIF, in addition to the cAMP-mediated signaling pathway, and can be assayed in suspension under slow-shake conditions (Mehdy and Firtel 1985; Williams et al. 1987; Berks and Kay 1990; Haberstroh and Firtel 1990; Dynes et al. 1994; Schnitzler et al. 1994, 1995; Firtel 1995).

As shown previously for wild-type cells, the postaggregative genes *GBF*, *CP2*, and *rasD* were induced in the presence but not the absence of high cAMP levels under fast-shake conditions (Fig. 2A). *GBF* showed a starvation-dependent, low-level induction in the absence of high cAMP observed in 6-hr starved cells, as described previously (Schnitzler et al. 1994). When examined in *erk2* null cells, *GBF* and *rasD* were also induced, but the level of expression was reduced. *CP2* was only minimally expressed under fast-shake conditions, but as in wild-type

Figure 1. Regulation of the pulse-induced gene contact sites A (*csA*) in wild-type and *erk2* null cells. Vegetative cells were harvested, washed, and placed in suspension culture at 5×10^6 cells/ml in MES-PDF buffer (Mann and Firtel 1987). The cells were either pulsed (as designated by P) or not pulsed for the time periods indicated. RNA was isolated, size-fractionated in denaturing gels, and probed for *csA*. Methods are as described previously (Mann and Firtel 1987, 1989).

Figure 2. Regulation of postaggregative and cell-type-specific genes in wild-type and *erk2* null cells. (A) Vegetative cells were washed and placed in suspension culture at 5×10^6 cells/ml and shaken for 6 hr at 150 rpm. Cells were then split and shaken at 250 rpm after the addition of 2 mM EDTA (pH 7.2), either with or without the addition of 300 mM cAMP added every 2 hr. For slow-shake (S), cells were shaken at 125 rpm in the absence of added EDTA with the same additions of cAMP. Cells were harvested after an additional 6 hr of fast (F)- or slow-shake condition. RNA blot hybridization was used to determine the relative expression levels of the RNA. (B) Slow-shake conditions as described in A were used to examine the expression of the prestalk-specific gene *ecmA* and the prespore-specific gene *SP60/CotC*.

cells, this expression was dependent on exogenous cAMP. Under slow-shake conditions, which permit cell-cell contacts and in which small cell agglomerates are formed, *GBF* and *CP2* were induced to similar levels in *erk2* null and wild-type cells in the presence of exogenous cAMP. In wild-type cells, this induction was independent of exogenous cAMP, as cAMP is produced in the agglomerates to a sufficient level to induce these genes (Mehdy and Firtel 1985). As in other strains unable to induce the activation of adenylyl cyclase, such as $\alpha 2$ null cells (Kumagai et al. 1991; Schnitzler et al. 1995), there was no induction of *CP2* nor any increase in the level of *GBF* expression in *erk2* null cells in the absence of exogenous cAMP. In wild-type cells, *rasD* expresses both a 1.2- and a 1.0-kb transcript in slow-shake conditions in the presence of cAMP, with the 1.0-kb transcript being specific for the multicellular stages and the expression level of the 1.2-kb transcript being lower than that observed in fast-shake conditions (Reymond et al. 1984; Esch and Firtel 1991; Esch et al. 1992). In *erk2* null cells, the 1.2-kb transcript was expressed at a slightly higher intensity than in wild-type cells, whereas the 1.0-kb transcript was expressed at a reduced level (Fig. 2A). In both *erk2* null cells and wild-type cells constitutively expressing *GBF* from the *Act15* promoter (Schnitzler et al. 1995), *CP2*, *rasD*, and *LagC* gene expression was induced to a similar level at the onset of development in response to cAMP (data not shown; Schnitzler et al. 1995). The results described here indicate that under conditions that allow cell-cell contacts and in the presence of high cAMP, ERK2 activity was not essential for this class of postaggregative genes.

We also examined the expression of a prestalk- and a prespore-specific gene, *ecmA* and *SP60*, respectively, under slow-shake suspension assays. As seen in Figure 2B, *ecmA* was expressed in the *erk2* null cells, but at a significantly reduced level, whereas *SP60* was not detectably expressed, even on exposures that are $10 \times$ longer than the one shown (data not shown).

Regulation of late gene expression in *erk2* null cells in chimeras with wild-type cells: ERK2 function is cell autonomous

erk2 null cells, as with many aggregation-deficient strains that lack the ability to activate adenylyl cyclase, will coaggregate, and in some cases, proceed through development when mixed with wild-type cells (Darmon et al. 1975; Pereira da Silva et al. 1975; Mann and Firtel 1991; Pitt et al. 1993; Insall et al. 1994a; Soede et al. 1994; Segall et al. 1995). By marking the mutant cells with *lacZ* reporter constructs, one is able to determine if the mutant cells can respond to signals present in coaggregates with wild-type cells and induce cell-type-specific gene expression and if the mutant cells show a normal or abnormal spatial pattern. This method is very sensitive, permitting the detection of expression in a limited number of cells within the population. *erk2* null cells carrying the *ecmA/lacZ*, *rasD/lacZ*, *SP60/lacZ*, or the stalk cell-specific *ecmB489/lacZ* reporter construct were mixed with wild-type cells in a ratio of 1:3 (*erk2* null: wild-type cells). Chimeric organisms were then stained for β -gal expression at different times in development. The anterior $\sim 15\%$ of the slug comprises the prestalk domain and is composed of three compartments as determined by the expression pattern of the two prestalk-specific, DIF-inducible genes *ecmA* and *ecmB* (Jermyn et al. 1989; Early et al. 1993; Williams et al. 1993a,b). In wild-type strains, the very anterior of the slug contains a core of prestalk AB cells surrounded by prestalk A cells. Slightly more posterior, but within the anterior 15% of the slug, is the prestalk O compartment, in which the *ecmA* gene is expressed at a significantly lower level than in prestalk A cells (Early et al. 1993). In addition, both *ecmA* and *ecmB* are expressed in the ALC, a regulatory cell population that is found scattered throughout the slug (Jermyn and Williams 1991; Williams et al. 1993a,b; Hadwiger et al. 1994; Firtel 1995). For a comparison with the results using the mu-

tant *erk2* null cells described below, a cartoon showing the spatial organization at the slug stage and during culmination is presented in Figure 3 (left).

As shown in Figure 3 (right), *ecmA/lacZ*-expressing cells showed a pattern of localization that comprised a subset of cells seen in wild-type strains (Williams et al. 1989, 1993a). Staining was concentrated in the posterior of the developing fingers (possibly rear guard cells) and scattered throughout the body, with a distribution similar to that of anterior-like cells. Strong staining was also observed in the anterior of the second finger. However, this staining appeared to be restricted to a band of cells with a pattern similar to that of prestalk O cells, with no visible staining cells in the more anterior prestalk A domain, suggesting *erk2* null cells cannot form prestalk A cells or enter the very anterior of the slug. During culmination, staining was observed in the upper and lower caps, stalk tube, and basal disk in mature fruiting bodies. To examine prestalk/stalk cell differentiation further, we determined the spatial pattern of expression of *ecmBΔ89*, a deletion of the prestalk-specific *ecmB* promoter, which is expressed during stalk cell differentiation during culmination (Ceccarelli et al. 1991). As can be seen in Figure 3, *erk2* null cells expressing this con-

struct were localized to the stalk, basal disc, and lower cup. In some sorocarps, cells comprising the very papillar region were also stained. When *SP60/lacZ* was examined, no detectable β -gal expression was seen, consistent with the cell suspension assays (data not shown).

In wild-type cells, expression of the *lacZ* reporter from the *rasD* promoter during the multicellular stages is first induced in the late aggregation streams (Esch and Firtel 1991). The β -gal staining cells then sort to the tip and are present in the prestalk A/O region and ALCs during the slug and culminant stages. In the *erk2* null cell:wild-type chimeras, the pattern of staining was very similar to that observed for *ecmA* (data not shown).

The above staining and suspension culture assay results indicated that ERK2 was essential for prespore gene expression and also affected either the distribution or sorting of prestalk A/O and anterior-like cells that express prestalk-specific (*ecmA*) and prestalk-enriched (*rasD*) genes. Previously, the total distribution of *erk2* null cells in chimeras was examined by loading *erk2* null cells with the fluorescent marker CMFDA (Segall et al. 1995). When these cells were mixed with wild-type cells in a 1:10 ratio, a preferential localization of the *erk2* null cells was observed in the anterior of the slug, with very

Figure 3. Spatial patterning of *erk2* null cells in chimeras with wild-type cells. (Left) Cartoon showing the organization in wild-type organisms. [Redrawn from Firtel (1995) and taken from data as referenced in the text.] The drawings were, in part, a gift from J. Williams, (University College, London, UK). *rasD* (data not shown in B) is expressed in prestalk A, prestalk O, and ALCs (Esch and Firtel 1991). (Right) *erk2* null cells carrying either the *ecmA/lacZ* reporter or the *ecmBΔ89/lacZ* reporter were mixed with three parts wild-type cells, allowed to form coaggregates, and then histochemically stained for β -gal expression as described previously. (A,B) *ecmA/lacZ* staining fingers; (C,D) *ecmA* staining during mid (C) and late (D) culmination; (E,F) *ecmBΔ89* staining during mid (E) and late (F) culmination. Labeled arrowheads mark the very anterior prestalk A domain (a), the slightly more posterior prestalk O domain (b), and the rear guard domain (c).

few cells in the posterior, prespore domain. Because of our finding that *ecmA/lacZ*-expressing cells localized to the boundary of the prestalk/prespore domains and the posterior one-third of the finger, we examined the location of *erk2* null cells within chimeras using an *Act15/lacZ* reporter. In the mound and slug stages, the *erk2* null cells expressing *Act15/lacZ* showed a more uniform distribution than observed previously: cells were present in the posterior as well as anterior of the slug, although the very anterior of the slug had few *erk2* null cells (data not shown). These data are consistent with the distribution of *ecmA/lacZ*-expressing cells in different domains of the aggregate in chimeras between wild-type cells and *erk* null cells expressing *ecmA/lacZ*. It was also clear from these experiments, and consistent with previous results, that many of the *erk2* null cells do not effectively participate in the initial formation of the multicellular aggregate. Thus, although *erk2* null cells can chemotax to cAMP (Segall et al. 1995), they did not efficiently coaggregate with wild-type cells. When mixed in a ratio of one part *erk2* null cells to three parts wild-type cells, an estimated 30%–40% of the *erk2* null cells were not found in the multicellular aggregates and remained as free amoebae. In the mature fruiting body, most of the *erk2* null cells were localized in the stalk, upper and lower cups, and the apical papilla of the sorocarp. Few staining cells were located in the sorus, consistent with previous observations of the absence of mature *erk2* null spores in the chimeras (Segall et al. 1995).

Examination of an ERK2-conditional mutation identifies a developmental function for ERK2 during the multicellular stages

To further examine possible functions of ERK2 during the multicellular stages, we needed to bypass the inability of *erk2* null cells to aggregate. To do this, we created a temperature-sensitive (ts) mutation of ERK2 based on the strategy of Hsu and Perrimon (1994). Carr et al. (1989) showed that a temperature-sensitive mutation in the *Schizosaccharomyces pombe* Cdc2 protein resulted from the change of a highly conserved proline residue to a serine. Hsu and Perrimon (1994) created a temperature-sensitive mutation in the *Drosophila* MAP kinase kinase (MEK) by changing the homologous proline to a serine. We created the same mutation in ERK2 (see Table 1) and expressed it in *erk2* null cells (*Erk2^{ts}/erk2* cells).

The *Erk2^{ts}/erk2* cells aggregated and formed mature fruiting bodies indistinguishable from wild-type fruiting bodies at temperatures below 23°C, whereas at temperatures above 25°C, the cells did not aggregate (Fig. 4, left). At 20°C, the timing of development of the *Erk2^{ts}/erk2* cells was delayed, with loose aggregates forming at 11 hr compared to 9 hr for the wild-type cells and mature fruiting bodies forming at 30 hr rather than 26 hr. When cells were developed at 19°C, we also observed a developmental delay, although less than at 20°C. These results suggest that the ERK2^{ts} protein may not have full activity at physiologically relevant temperatures for this organism (data not shown). When cell-type-specific gene expres-

Table 1. The domain containing the conserved proline residue in *S. pombe cdc2*, *Drosophila MEK*, and *Dictyostelium ERK2*

Temperature sensitive mutations	
<i>cdc2</i>	HRDLKPNLLID
Dm-MEK	HRDVKPSNLLVN
Dd-ERK2	HRDLKPSNLLIN
	ts S

See text for details and references.

sion was examined in *Erk2^{ts}/erk2* cells plated for development at 19°C, both *ecmA* and *SP60* were expressed, although *SP60* was expressed at a slightly reduced level and with kinetics that were ~3 hr delayed compared to those in wild-type cells (Fig. 5). When the expression vector carrying the wild-type ERK2 was transformed into *erk2* null cells or wild-type cells, the cells developed normally and produced wild-type fruiting bodies at the temperatures tested (18°C–27°C). Transformation of this vector into wild-type or *erk2* null cells resulted in a several-fold increase in the level of ERK2 protein as determined by Western blot analysis (data not shown). The level of overexpression of ERK2 in wild-type cells observed did not cause an observable phenotype.

We have shown that ERK2 activity is stimulated in response to cAMP using in-gel assays in which the protein is denatured in SDS and then renatured using guanidine hydrochloride (M. Maeda, L. Aubry, R. Insall, C. Gastins, P. Devreotes, and R.A. Firtel, in prep.). When *Erk2^{ts}/erk2* cells were similarly assayed under standard conditions (assay performed at 20°C; Gaskins et al. 1994), no ERK2 activity was observed (data not shown), indicating that the protein is very labile and may be unable to refold properly after severe denaturation.

To determine whether ERK2 function is required for later development, *Erk2^{ts}/erk2* cells were plated for development at 20°C until they formed loose aggregates and were then shifted to 27°C. As is seen in Figure 4A, the development became very abnormal, indicating a specific role for ERK2 in proper morphogenesis. We assayed for the presence of mature heat- and detergent-stable spores and none were found (<0.1% of wild-type). Wild-type cells showed completely normal development at 27°C, forming fruiting bodies that were indistinguishable from those of wild-type cells and having the same number of mature spores in 24–26 hr (data not shown).

To determine whether ERK2 was required at specific stages in development, we plated cells on 12 mM Na/KPO₄ (pH 6.2) agar plates and allowed them to develop for varying times at 19°C. The plates were then shifted to 27°C and either left at 27°C or returned to 19°C after 5 hr. When cells were shifted from 19°C to 27°C at times between early aggregate formation (9 hr) and tipped aggregate (13 hr) for 5 hr and then back to 19°C, cells formed normal fruiting bodies with spores, indicating that the

Figure 4. Phenotypes of *ERK2^{ts}* mutant. (Left) Individual panels are labeled appropriately. In the top three panels, wild-type or *erk2* null cells were allowed to develop at either 20°C or 27°C. In the bottom panels, *erk2* null cells were first allowed to develop at 20°C. (Bottom left panel) cells form mounds at this stage; (bottom right panels) cells were shifted from 20°C to 27°C at the time of loose aggregate formation and allowed to develop. Various morphological phenotypes were observed. Two representative phenotypes are shown. (Right) Developmental phenotypes of cells shifted from permissive to restrictive to permissive temperatures. Cells were plated on Na/KPO₄ agar plates and allowed to develop at either 19°C or 22°C to the times indicated. Cells were then shifted to 27°C and either left there for the indicated times or shifted back to the starting temperature (19°C or 22°C) after 5 hr. Photographs show representative phenotypes. (A) cells developed at 19°C for 17 hr; (B) cells developed at 19°C for 17 hr and transferred to 27°C for 5 hr; (C) cells developed at 19°C for 17 hr and transferred to 27°C for 5 hr and then shifted to 19°C (photograph taken after an additional 12 hr); (D) cells developed at 19°C for 17 hr and transferred to 27°C (photograph taken after an additional 17 hr); (E) cells developed at 22°C for 17 hr and transferred to 27°C for 5 hr (F) cells developed at 22°C for 17 hr and transferred to 27°C (photograph taken after an additional 17 hr); (G) cells developed at 22°C for 22 hr and transferred to 27°C for 5 hr; (H) cells developed at 19°C for 21 hr; (I) cells developed at 19°C for 21 hr and transferred to 27°C for 5 hr; (J) cells developed at 19°C for 21 hr and transferred to 27°C (photograph taken after an additional 17 hr); (K) cells developed at 19°C for 9 hr and transferred to 27°C for 5 hr and then shifted to 19°C (photograph taken after an additional 17 hr).

cells could recover completely for proper morphogenesis and spore formation (K in Fig. 4, right; data for 13 hr not shown). When cells were shifted from 19°C to 27°C at the first finger (17 hr; A in Fig. 4, right) or second finger stage (21 hr; H in Fig. 4, right), morphogenesis was slightly abnormal after 5 hr (B and I, respectively, in Fig. 4, right). If the cells remained at 27°C very aberrant fruiting body-like structures were formed. If the 17- or 21-hr cells were then shifted back to 19°C after 5 hr at 27°C, they formed almost normal fruiting bodies with mature spores, showing only some abnormal stalk morphogenesis (C in Fig. 4, right; data for 21 hr not shown). If the cells are developed at 22°C, the morphology is indistinguishable from that of cells developed at 19°C and normal fruiting bodies are produced (data not shown). How-

ever, if cells are first developed at 22°C rather than 19°C before shifting to 27°C, morphogenesis is significantly more abnormal either after 5 hr at the elevated temperature (e.g., Fig. 4, right, B–G vs. B–H), after extended incubation at 27°C (Fig. 4, right, B–E vs. B–D), or after being shifted back to 19° or 22°C after 5 hr (Fig. 4, right, B–F vs. B–C). This suggests that the presence of some inactive ERK2 or having ERK2 with a lower activity is sufficient to further impair development, and suggests a continual requirement of ERK2 activity for proper morphogenesis.

ERK2 is required for prespore but not prestalk cell-type differentiation

The temperature-sensitive mutation allowed us to deter-

Figure 5. Regulation of cell-type-specific genes in *Erk2^{ts}/erk2* and wild-type cells. *erk2* null cells expressing the *ERK2^{ts}* cells (top two panels; *Erk2^{ts}/erk2*) or wild-type cells (bottom panels; KAx-3) were plated for development on filters at 20°C. Filters were taken at various time points, RNA isolated, size-fractionated, blotted, and probed for expression of *ecmA* or *SP60* by blot hybridization. For wild-type cells, mature fruiting bodies were formed at 26–28 hr in these experiments. *Erk2^{ts}/erk2* cells showed mature fruiting bodies at ~30–32 hr. To obtain a better indication of the relative level of expression of *ecmA* and *SP60* genes in the *erk2* null cells expressing *ERK2^{ts}*, 21-hr samples of wild-type RNA were loaded on the same gel (21WT) for comparison.

mine whether ERK2 was required at the time of cell-type-specific gene expression or for some early developmental process that was subsequently required for cell-type-specific gene expression. Because the induction of cell-type-specific gene expression is controlled by extracellular cAMP interacting with cell surface receptors and because *erk2* null cells lack cAMP-mediated activation of adenylyl cyclase, it was necessary to perform these studies in suspension, which allowed us to add exogenous cAMP. *Erk2^{ts}/erk2* and wild-type cells were allowed to develop to the loose aggregate stage at 19°C on Na/KPO₄ agar plates, dissociated into single cells, and then stimulated with cAMP at either 19°C or 27°C under slow-shake suspension conditions. As shown in Figure 6, neither strain showed detectable expression of the prespore (*SP60*) or the prestalk (*ecmA*) gene at the time of aggregate dissociation. In the presence of exogenous cAMP, *Erk2^{ts}/erk2* cells induced *ecmA* to a level similar at 19°C or 27°C to that in wild-type cells. At the nonpermissive temperature, *ecmA* expression was completely dependent on exogenous cAMP at the restrictive temperature and no expression was observed in the samples lacking cAMP. The requirement for exogenous cAMP in the *Erk2^{ts}/erk2* strain at the nonpermissive temperature is consistent with the requirement of ERK2 for the activation of adenylyl cyclase. Thus, although the cells formed agglomerates in suspension conditions that allowed wild-type cells to produce cAMP endogenously, the *ERK2^{ts}* protein was not active at the nonpermissive temperature and thus adenylyl cyclase was not activated. In contrast, *SP60* expression was induced in

Erk2^{ts}/erk2 cells at 19°C but not at 27°C, whereas it was induced at both temperatures in wild-type cells. The level of expression at the permissive conditions was lower than that of wild-type cells under the same conditions. These results indicate ERK2 is required for the induction of prespore gene expression and, in effect, distinguishes the effect of ERK2 on adenylyl cyclase and prespore gene expression, suggesting that the temperature sensitivity of cell-type-specific gene expression is restricted to the prespore pathway.

To determine whether ERK2 activity was required for continued expression of prespore genes, *Erk2^{ts}/erk2* cells were developed on agar until the mound stage at 19°C, when *SP60* was induced, and then shifted to 27°C. Time points were taken and *SP60* and *ecmA* RNA levels were assayed (Fig. 7). At this stage, *SP60* transcripts were present at levels approaching those of wild-type cells at early culmination (18 hr), whereas the level of *ecmA* transcripts was still increasing. After being shifted to the restrictive temperature, *SP60* RNA remained high at the 4-hr time point and then fell rapidly. In contrast, *ecmA* transcripts continued to increase for at least 8 hr before decreasing. This subsequent decrease may be attributable to an inhibition in the ability to activate adenylyl cyclase and produce cAMP. The results showed that ERK2 activity is required continuously for *SP60* expression. The difference in the kinetics of decline of *ecmA* and *SP60* suggested that ERK2 activity is required continually for *SP60* expression. When wild-type cells were shifted from 19°C to 27°C at the aggregate stage, prior to

Figure 6. Regulation of cell-type-specific gene expression in *Erk2^{ts}/erk2* cells under restrictive and permissive conditions. *Erk2^{ts}/erk2* cells and wild-type cells were allowed to differentiate at 19°C on agar containing 12 mM Na/K phosphate (pH 6.2). Cells were harvested at the loose mound stage for each strain. The cells were dissociated and split into four aliquots, which were assayed under slow-shake conditions as described in the legend to Fig. 2. Two aliquots each were assayed at 19°C and 27°C. Exogenous cAMP was added to one of these aliquots. RNA was isolated at 6 hr after cells had been placed in suspension.

Figure 7. Effect of temperature shifts on cell-type-specific gene expression. *Erk2^{ts}/erk2* cells were developed on Na/KPO₄ agar plates until the mound stage so that cell-type-specific genes would be induced. A time point for RNA extraction was taken and the remainder of the plates were transferred to 27°C and time points taken every 4 hr. Wild-type cells were developed under the same conditions but to the loose aggregate stage, prior to induction of cell-type-specific genes. A time point for RNA extraction was taken, the remainder of the plates were transferred to 27°C, and time points then taken every 4 hr to examine the effect of 27°C on the induction and continued expression of *ecmA* and *SP60*. (Con) RNA isolated from early culminant (18-hr cells) wild-type cells developed at 21°C used as a marker.

induction of *ecmA* and *SP60*, both genes were induced normally and continued to be expressed (Fig. 7). The level of *ecmA* was higher than that of cells developed at 20°C, whereas *SP60* was similar, indicating that induction and maintenance of cell-type-specific gene expression was not negatively affected at 27°C.

Discussion

MAP kinases have been shown to control multiple regulatory functions mediated through signaling cascades as outlined above. Previously, we showed that *erk2* null cells could not effectively activate adenylyl cyclase and thus were unable to produce and relay the cAMP signal (Segall et al. 1995). However, *erk2* null cells activate guanylyl cyclase, chemotax to cAMP, and form coaggregates with wild-type cells, although we estimate that only two-thirds of the *erk2* null cells participate, suggesting that chemotaxis is not fully wild type. In this paper we examined the functions of the MAP kinase ERK2 in controlling *Dictyostelium* development and showed that ERK2 has a specific cell-autonomous function for cell-type differentiation. Using a temperature-sensitive mutant, we show that ERK2 is required at multiple stages for proper *Dictyostelium* development, including morphogenesis and cell-type-specific gene expression. Furthermore, ERK2 was not essential for the regulation of pulse-induced gene expression during aggregation, suggesting that ERK2 does not function by directly regulating transcription during this stage of development. Other strains that are also unable to activate adenylyl cyclase, including strains in which the gene encoding adenylyl cyclase ACA, CRAC (a regulator of adenylyl cyclase), or the catalytic subunit of PKA has been disrupted, are ag-

gregation deficient. These strains are unable to produce and relay cAMP and, like *erk2* null cells, can chemotax to cAMP, coaggregate with wild-type cells, and express pulse-induced genes in response to cAMP (Mann and Firtel 1991; Pitt et al. 1993; Insall et al. 1994a,b). *aca* null cells and cells lacking the G α subunit G α 2, which is required for the activation of adenylyl and guanylyl cyclases through cAMP receptors (g α 2 null cells), induce prestalk- and prespore-specific genes in response to cAMP and form aberrant structures that contain stalk cells and spores after cAMP treatment and plating on non-nutrient agar (Pitt et al. 1993; Schnitzler et al. 1995). These results suggest that the ability to activate adenylyl cyclase and produce endogenous cAMP is not essential for cell-type differentiation or the expression of prestalk and prespore genes under these conditions (Kumagai et al. 1989, 1991; Pitt et al. 1993; Firtel 1995; Schnitzler et al. 1995). By using chimeric organisms and suspension cultures, we bypassed the inability of *erk2* null cells to activate adenylyl cyclase to examine its possible function in controlling gene expression and cell-type differentiation. We showed that the ability of *erk2* null cells to express cell-type-specific genes was very different from that of *aca* null cells. We therefore conclude that inability of *erk2* null cells to express prespore genes is probably unrelated to the inability of the *erk2* null cells to activate adenylyl cyclase. In addition, we showed that ERK2 activity is required for the continued expression of the prespore gene *SP60* within developing aggregates.

We demonstrated that the postaggregative genes (including *GBF*, *CP2*, and *rasD*) and the prestalk-specific gene *ecmA*, but not the prespore gene *SP60*, were expressed in *erk2* null cells in suspension culture in response to cAMP or when the *erk2* null cells formed a coaggregate with wild-type cells. The level of expression of some of these genes was reduced compared to wild-type cells in suspension cultures in the absence of cell-cell contact, but in slow-shake culture in which cell agglomerates were formed, the level of expression of these genes was similar to that seen in wild-type cells. A reduced level of expression was also seen for *rasD* and *CP2* in strains in which *LagC*, a postaggregative gene whose expression is required for cell-type-specific gene expression, is deleted. It is possible that ERK2 may also have a broad role in modulating postaggregative gene expression but is not absolutely essential, as it is for prespore gene expression. The reduced level of expression of the postaggregative genes in fast-shake conditions (no sustained cell-cell contact) is also seen in cells lacking the putative cell surface protein *LagC* and may be attributable to either a pleiotropic effect of the ERK2 deficiency or the fact that ERK2 is not essential for induction of postaggregative gene expression but is required for full expression.

Examination of the localization of the *ecmA*-expressing *erk2* null cells in chimeras and the level of induction of *ecmA* in *erk2* null cells in suspension culture showed that ERK2 also plays a role in prestalk gene expression and cell patterning. The *erk2* null cells expressing *ecmA* are localized in a domain expected for prestalk O cells as

well as being found in the posterior prespore domain in an ALC pattern. The absence of *erk2* null/*ecmA*-expressing cells within the prestalk A domain in the finger may be a function of the inability to induce the prestalk A component of the *ecmA* promoter at this stage or the exclusion of *erk2* null cells from the anterior by wild-type cells at this stage. The very anterior cells are thought to function as the cAMP oscillator and organizer (for review, see Schaap 1991; Firtel 1995). The inability of *erk2* null cells to activate adenylyl cyclase could thus exclude them from this central organizing function and restrict them to a more posterior domain in the slug. The *ecmB*Δ89-expressing cells in the very anterior of developing culminants (Fig. 3E,F) may be attributable to the localization of anterior-like cells to this region during culmination. Interestingly, *erk2* null cells did express the stalk-specific promoter *ecmB*Δ89, although it is expected that these cells cannot produce endogenous cAMP.

In contrast, no prespore-specific gene expression was observed under any condition. The use of chimeric organisms comprised of various ratios of wild-type and mutant cells allows one to determine whether a mutant is cell nonautonomous. Under such conditions, wild-type cells would supply any required cell-surface or extracellular signals that are lacking in the mutant cells. The inability of *erk2* null cells to express the prespore-specific reporter *SP60/lacZ* in the chimeras or the endogenous *SP60* gene in suspension assays suggests that the requirement of ERK2 function is cell autonomous. This is consistent with the cell suspension assays showing that *Erk2^{ts}/erk2* can induce gene *SP60* under the permissive but not the restrictive conditions after cells are first allowed to form aggregates. Our results with the suspension assays using *Erk2^{ts}/erk2* also distinguished between the ERK2 requirement for the activation of adenylyl cyclase and the induction of prespore gene expression. *ecmA* gene expression required exogenous cAMP to be induced under nonpermissive conditions, in which the ERK2^{ts} protein was presumably inactive and thus adenylyl cyclase was not activated, whereas *SP60* was not induced with or without exogenous cAMP. We presume that ERK2 is present in all cells, at least initially, because during aggregation it is required in all cells for cAMP-mediated activation of adenylyl cyclase and it is expressed during vegetative growth. Our data therefore suggest that ERK2 may be required for the phosphorylation of a protein necessary for the induction of prespore genes but not for the expression of the prestalk marker *ecmA* in at least some prestalk cells.

The morphological abnormalities observed when *Erk2^{ts}/erk2* cells were shifted to the restrictive temperature after mounds formed are very distinct from those observed for other strains in which the cAMP signaling pathways are affected. *aca* null cells, in which the gene encoding adenylyl cyclase ACA is disrupted, and *ga2* null cells, in which the gene encoding the Gα subunit known to be required for cAMP stimulation of adenylyl cyclase in vivo is disrupted, form small, wild-type-looking fruiting bodies if the cells are first given extracellular

cAMP (Pitt et al. 1993; Schnitzler et al. 1995). Moreover, it has been shown for both mutants that extracellular cAMP can stimulate cell-type-specific gene expression and, in the case of *aca* null cells, the cAMP analog 2'dcAMP, which binds very weakly to the PKA-R subunit and thus cannot activate PKA, can also effectively induce development and cell-type-specific gene expression (Pitt et al. 1993; Schnitzler et al. 1995). This analysis suggests that the abnormal morphogenesis observed for *Erk2^{ts}/erk2* cells when shifted to the restrictive temperature may very well be distinct from the ability to efficiently activate adenylyl cyclase. This argues that the mechanism by which ERK2 regulates late gene expression and morphogenesis is, at least in part, distinct from its function in controlling aggregation. Using temperature shift experiments, we show that *Erk2^{ts}/erk2* null cells can recover from a 5-hr shift to the nonpermissive temperature to form normal or almost normal fruiting bodies, depending on the time of the temperature shift. However, when a higher permissive temperature (22°C) was used, the shift to 27°C for 5 hr resulted in more severe phenotypes. Our results suggest that ERK2 may play a continual role in mediating morphogenesis throughout the multicellular stages. The known plasticity of *Dictyostelium* development may explain why cells shifted from and to the lower temperature (19°C) show normal development.

Components of a MAP kinase cascade functioning in two independent regulatory pathways have been seen previously in *Saccharomyces cerevisiae* (Roberts and Fink 1994). In this case, the MEK, MEK kinase, and an upstream kinase (Ste20) are required for both mating and pseudohyphal growth, although the MAP kinases involved in the two pathways appear to be distinct. In *Dictyostelium*, we do not know whether the upstream regulators of the aggregation-stage pathways and those that regulate ERK2 activity in later development are the same. We have shown that cAMP will stimulate ERK2 activation in cells isolated from migrating slugs (Maeda, M., and R.A. Firtel, unpubl.); however, it is possible that the pathway regulating ERK2 activity for the prespore pathway is distinct. We know that ERK2 activity is rapidly stimulated and the activity rapidly adapts upon cAMP stimulation during the aggregation stages (M. Maeda, L. Aubry, R. Insall, C. Gaskins, P. Devreotes, and R. Firtel, in prep.). Prespore gene expression, however, is stimulated by a continuous cAMP flux, a condition that causes only a transient stimulation of the ERK2 during early development. This suggests that distinct pathways may control ERK2 activation in response to cAMP and possibly other stimuli that lead to cell-type differentiation and morphogenesis. Future studies should identify upstream components and determine whether ERK2 functions to directly modify prespore- and/or prestalk-specific transcription factors or functions farther upstream in the regulatory network.

Materials and methods

Strains

erk2 null cells were obtained by retransforming the recovered

plasmid from the initial REMI mutation (Kuspa and Loomis 1992) into DH1, a uracil auxotroph, selecting for uracil prototrophs, and screening for aggregation-deficient clones. The disruption of the *ERKB* locus was determined by Southern blot analysis. This disrupts the ERK2 open reading frame (ORF) near the carboxyl terminus, and the strain does not produce ERK2 mRNA or ERK2 protein (data not shown). This *erk2* null strain has properties identical to the original REMI mutation (Kuspa and Loomis 1992; Segall et al. 1995). Both this strain and the original *erk2* null strain were used for the experiments. In addition, we created another *erk2* null strain in which the gene was disrupted at the *Bgl*II site in the catalytic domain. These cells also have the same properties. Strain KAx-3, the parental of DH1, or DH1 cells transformed with a Pyr5-6 uracil vector were used for wild-type cells.

Molecular biology, vectors, and cell culture

All experiments were performed using standard molecular biological and cell culture techniques for *Dictyostelium* and have been described previously (Dynes et al. 1994).

The temperature-sensitive mutant was made by site-directed mutagenesis with an oligonucleotide containing the proline to serine amino acid substitution using single-stranded template DNA and the Bio-Rad Muta-Gene phagemid kit. The resultant plasmids were sequenced to identify one carrying the substitution.

The ERK2-complementing vector was made by inserting ~1.5 kb of genomic upstream sequences and the ERK2 ORF up to the *Sph*I site fused in-frame to ERK2 cDNA sequence into a derivative of pATANB43, a Ddp1-based extrachromosomal plasmid (Dynes and Firtel 1989). When transformed into *erk2* null cells, the *erk2* null phenotypes are complemented and cells develop normally. The ERK2^{ts} mutation was expressed in the same vector by replacing the wild-type *ERK2* gene with the *ERK2*^{ts} gene.

Acknowledgments

We thank J. Brown, C. Briscoe, M. Gamper, and W. Loomis for helpful suggestions and critical review of the manuscript. L.A. was supported by a Fondation pour la Recherche Medicale de France fellowship. This work was supported by U.S. Public Health Service grants to R.A.F.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Abe, K. and K. Yanagisawa. 1983. A new class of rapid developing mutants in *Dictyostelium discoideum*: Implications for cyclic AMP metabolism and cell differentiation. *Dev. Biol.* **95**: 200–210.
- Berks, M. and R.R. Kay. 1990. Combinatorial control of cell differentiation by cAMP and DIF-1 during development of *Dictyostelium discoideum*. *Development* **110**: 977–984.
- Blumer, K. and G. Johnson. 1994. Diversity in function and regulation of MAP kinase pathways. *Trends Biochem. Sci.* **19**: 236–240.
- Carr, A.M., S.A. MacNeil, J. Hayles, and P. Nurse. 1989. Molecular cloning and sequence analysis of mutant alleles of the fission yeast *cdc2* protein kinase gene: Implications for *cdc2*⁺ protein structure and function. *Mol. & Gen. Genet.* **218**: 41–49.
- Ceccarelli, A., H. Mahbubani, and J.G. Williams. 1991. Positively and negatively acting signals regulating stalk cell and anterior-like cell differentiation in *Dictyostelium*. *Cell* **65**: 983–989.
- Darmon, M., P. Brachet, and L.H. Pereira Da Silva. 1975. Chemotactic signals induce cell differentiation in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci.* **72**: 3163–3166.
- Davis, R. 1993. The mitogen-activated protein kinase signal transduction pathway. *J. Biol. Chem.* **268**: 14553–14556.
- Devreotes, P.N. 1994. G protein-linked signaling pathways control the developmental program of *Dictyostelium*. *Neuron* **12**: 235–241.
- Dynes, J.L. and R.A. Firtel. 1989. Molecular complementation of a genetic marker in *Dictyostelium* using a genomic DNA library. *Proc. Natl. Acad. Sci.* **86**: 7966–7970.
- Dynes, J., A. Clark, G. Shaulsky, A. Kuspa, W. Loomis, and R. Firtel. 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., M.J. Gaskell, D. Traynor, and J.G. Williams. 1993. The distinct populations of prestalk cells within the tip of the migratory *Dictyostelium* slug with differing fates at culmination. *Development* **118**: 353–362.
- Errede, B. and D. Levin. 1993. A conserved kinase cascade for MAP kinase activation in yeast. *Curr. Opin. Cell Biol.* **5**: 254–260.
- Esch, R.K. and R.A. Firtel. 1991. cAMP and cell sorting control the spatial expression of a developmentally essential cell-type-specific *ras* gene in *Dictyostelium*. *Genes & Dev.* **5**: 9–21.
- Esch, R.K., P.K. Howard, and R.A. Firtel. 1992. Regulation of the *Dictyostelium* cAMP-induced, prestalk-specific *DdrasD* gene: Identification of *cis*-acting elements. *Nucleic Acids Res.* **20**: 1325–1332.
- Firtel, R.A. 1995. Integration of signaling information in controlling cell-fate decisions in *Dictyostelium*. *Genes & Dev.* **9**: 1427–1444.
- Gaskins, C., M. Maeda, and R. Firtel. 1994. Identification and functional analysis of a developmentally regulated extracellular signal-regulated kinase gene in *Dictyostelium discoideum*. *Mol. Cell. Biol.* **14**: 6996–7012.
- Haberstroh, L. and R. A. Firtel. 1990. A spatial gradient of expression of a cAMP-regulated prespore cell type specific gene in *Dictyostelium*. *Genes & Dev.* **4**: 596–612.
- Hadwiger, J., S. Lee, and R. Firtel. 1994. The G α subunit G α 4 couples to pterin receptors and identifies a signaling pathway that is essential for multicellular development in *Dictyostelium*. *Proc. Natl. Acad. Sci.* **91**: 10566–10570.
- Hsu, J. and N. Perrimon. 1994. A temperature-sensitive MEK mutation demonstrates the conservation of the signaling pathways activated by receptor tyrosine kinases. *Genes & Dev.* **8**: 2176–2187.
- Hunter, T. 1995. Protein kinases and phosphatases: The Yin and Yang of protein phosphorylation and signaling. *Cell* **80**: 225–236.
- Insall, R., A. Kuspa, P. Lilly, G. Shaulsky, L. Levin, W. Loomis, and P. Devreotes. 1994a. CRAC, a cytosolic protein containing a pleckstrin homology domain, is required for receptor and G protein-mediated activation of adenylyl cyclase in *Dictyostelium*. *J. Cell Biol.* **126**: 1537–1545.
- Insall, R.H., R.D.M. Soede, P. Schaap, and P.N. Devreotes. 1994b. Two cAMP receptors activate common signaling pathways in *Dictyostelium*. *Mol. Biol. Cell* **5**: 703–711.
- Jermyn, K.A. and J.G. Williams. 1991. An analysis of culmination in *Dictyostelium* using prestalk and stalk-specific cell autonomous markers. *Development* **111**: 779–787.

- Jermyn, K.A., K.T. Duffy, and J.G. Williams. 1989. A new anatomy of the prestalk zone in *Dictyostelium*. *Nature* **340**: 144–146.
- Karin, M. 1995. The regulation of AP-1 activity by mitogen-activated protein kinases. *J. Biol. Chem.* **270**: 16483–16486.
- Kumagai, A., J.A. Hadwiger, M. Pupillo, and R.A. Firtel. 1991. Molecular genetic analysis of two G α protein subunits in *Dictyostelium*. *J. Biol. Chem.* **266**: 1220–1228.
- Kumagai, A., M. Pupillo, R. Gundersen, R. Miake-Lye, P.N. Devreotes, and R.A. Firtel. 1989. Regulation and function of G α protein subunits in *Dictyostelium*. *Cell* **57**: 265–275.
- Kuspa, A. and W.F. Loomis. 1992. Tagging developmental genes in *Dictyostelium* by restriction enzyme-mediated integration of plasmid DNA. *Proc. Natl. Acad. Sci.* **89**: 8803–8807.
- Loomis, W. F., (ed.) 1982. *Development of Dictyostelium discoideum*. Academic Press, New York, NY.
- Mann, S.K. and R.A. Firtel. 1989. Two-phase regulatory pathway controls cAMP receptor-mediated expression of early genes in *Dictyostelium*. *Proc. Natl. Acad. Sci.* **86**: 1924–1928.
- Mann, S.K.O. and R.A. Firtel. 1987. Cyclic AMP regulation of early gene expression in *Dictyostelium discoideum* Mediation via the cell surface cyclic AMP receptor. *Mol. Cell. Biol.* **7**: 458–469.
- . 1991. A developmentally regulated, putative serine/threonine protein kinase is essential for development in *Dictyostelium*. *Mech. Dev.* **35**: 89–102.
- Marshall, C.J. 1995. Specificity of receptor tyrosine kinase signaling: Transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**: 179–185.
- Mehdy, M.C. and R.A. Firtel. 1985. A secreted factor and cyclic AMP jointly regulate cell-type-specific gene expression in *Dictyostelium discoideum*. *Mol. Cell. Biol.* **5**: 705–713.
- Noegel, A., G. Gerisch, J. Stadler, and M. Westphal. 1986. Complete sequence and transcript regulation of a cell adhesion protein from aggregating *Dictyostelium* cells. *EMBO J.* **5**: 1473–1476.
- Pereira da Silva, L.H., M. Darmon, P. Brachet, C. Klein, and P. Barrand. 1975. Induction of cell differentiation by the chemotactic signal in *Dictyostelium discoideum*. *FEBS Proc. Meet.* **40**: 269–277.
- Pitt, G.S., R. Brandt, K.C. Lin, P.N. Devreotes, and P. Schaap. 1993. Extracellular cAMP is sufficient to restore developmental gene expression and morphogenesis in *Dictyostelium* cells lacking the aggregation adenyl cyclase (ACA). *Genes & Dev.* **7**: 2172–2180.
- Reymond, C.D., R.H. Gomer, M.C. Mehdy, and R.A. Firtel. 1984. Developmental regulation of a *Dictyostelium* gene encoding a protein homologous to mammalian *ras* protein. *Cell* **39**: 141–148.
- Roberts, R.L. and G.R. Fink. 1994. Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: Mating and invasive growth. *Genes & Dev.* **8**: 2974–2985.
- Schaap, P. 1991. Intercellular interactions during *Dictyostelium* development. In *Microbial cell-cell interactions* (ed. M. Dworkin), pp. 147–178. American Society for Microbiology, Washington, D.C.
- Schnitzler, G., W. Fischer, and R. Firtel. 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.
- Schnitzler, G.R., C. Briscoe, J.M. Brown, and R.A. Firtel. 1995. Serpentine cAMP receptors may act through a G-protein-independent pathway to induce post-aggregative development in *Dictyostelium*. *Cell* **81**: 737–745.
- Segall, J., A. Kuspa, G. Shaulesky, M. Ecke, M. Maeda, C. Gaskins, R. Firtel, and W. Loomis. 1995. A MAP kinase necessary for receptor-mediated activation of adenyl cyclase in *Dictyostelium*. *J. Cell Biol.* **128**: 405–413.
- Soede, R.D.M., R.H. Insall, P.N. Devreotes, and P. Schaap. 1994. Extracellular cAMP can restore development in *Dictyostelium* cells lacking one, but not two subtypes of early cAMP receptors (cARs): Evidence for involvement of cAR1 in aggregative gene expression. *Development* **120**: 1997–2002.
- Van Haastert, P.J.M. 1991. Transmembrane signal transduction pathways in *Dictyostelium*. In *Advances in second messenger and phosphoprotein research* (ed. P. Greengard and G.A. Robison), pp. 185–226. Academic Press, New York, NY.
- Williams, J., N. Hopper, A. Early, D. Traynor, A. Harwood, T. Abe, M. Simon, and M. Veron. 1993a. Interacting signalling pathways regulating prestalk cell differentiation and movement during the morphogenesis of *Dictyostelium*. *Development* (Suppl.): 1–7.
- Williams, J.G., A. Ceccarelli, S. McRobbie, H. Mahbubani, R.R. Kay, A. Farly, M. Berks, and K.A. Jermyn. 1987. Direct induction of *Dictyostelium* prestalk gene expression by D1F provides evidence that D1F is a morphogen. *Cell* **49**: 185–192.
- Williams, J.G., K.T. Duffy, D.P. Lane, S.J. McRobbie, A.J. Harwood, D. Traynor, R.R. Kay, and K.A. Jermyn. 1989. Origins of the prestalk-prespore pattern in *Dictyostelium* development. *Cell* **59**: 1157–1163.
- Williams, J.G., A.J. Harwood, N.A. Hopper, M.N. Simon, S. Bouzid, and M. Veron. 1993b. Regulation of *Dictyostelium* morphogenesis by cAMP-dependent protein kinase. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **340**: 305–313.

The Dictyostelium MAP kinase ERK2 regulates multiple, independent developmental pathways.

C Gaskins, A M Clark, L Aubry, et al.

Genes Dev. 1996, **10**:

Access the most recent version at doi:[10.1101/gad.10.1.118](https://doi.org/10.1101/gad.10.1.118)

References

This article cites 45 articles, 25 of which can be accessed free at:
<http://genesdev.cshlp.org/content/10/1/118.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).