INTRODUCTION

Dictyostelium is a model system for the study of amoeboid cell chemotaxis (for reviews see Van Haastert, 1995; Schleicher et al., 1995; Devreotes, 1994). In the growth phase, the amoebae are chemotactic to folic acid secreted by food bacteria. Upon depletion of the food source, the cells initiate a developmental program that leads to aggregation via chemotaxis to cAMP. Regulation of the actin cortical cytoskeleton is critical for chemotactic responses in Dictyostelium (Schleicher et al., 1995; Condeelis, 1993; Noegel and Luna, 1995). At least 30 families of actin binding proteins have been identified and, in many cases, their interactions with the actin cytoskeleton are altered by molecules implicated in cell signaling (Condeelis, 1993; Theriot, 1994; Janmey, 1994). A variety of biochemical assays have shown that cAMP stimulation generates a transient actin nucleation activity in the cortical cytoskeleton coincident with the first peak of actin polymerization (Hall et al., 1989). Subsequent peaks of actin polymerization occur at later times, together with pseudopod extension. Myosin I and II-mediated contraction aids in optimizing polarization of the cell by suppressing pseudopod formation in inappropriate directions (Ruppel and Spudich, 1995; Manstein, 1993; Jung et al., 1996; Titus et al., 1993). Identifying the mechanisms by which these proteins are regulated is critical to a full understanding of how amoeboid cells move.

The basic mechanisms of signal transduction in Dictyostelium chemotaxis utilize G protein coupled receptors (Soede et al., 1994; Insall et al., 1994). The cAMP receptors CAR1 (cAMP receptor 1) and CAR3 mediate chemotaxis, signal relay, and early gene expression during development. Binding of cAMP to CAR1 stimulates the production of inositol phosphates (IP3), the release of Ca2+ from non-mitochondrial stores, formation of cGMP, actin cytoskeleton rearrangement, and synthesis of cAMP by adenylyl cyclase. Peaks of IP3 formation and actin polymerization can be detected within 5 seconds of cAMP stimulation. The cGMP peak occurs at 10 seconds, while intracellular cAMP reaches a peak at about 60 seconds. Elevation of cGMP is required for chemotactic movement, early gene expression, and other cellular responses. However, a detailed understanding of the intracellular signaling mechanisms leading to chemotactic orientation is still incomplete. The work presented here demonstrates that a member of the ERK/MAP kinase family is involved in this process.

ERK/MAP kinase pathways are under intense study in many systems (Treisman, 1996; Campbell et al., 1995; Cano and Mahadevan, 1995). Chemoattractant stimulation of tyrosine kinases, cytokine receptors and heterotrimeric G protein coupled receptors typically results in the activation of ERK/MAP kinases. MAP kinases belong to the proline-directed family of serine/threonine kinases (Robbins et al., 1994). MAPK activation has been demonstrated to be associated with the regulation of cell proliferation, cell differentiation, and responses to stress. In vitro, MAP kinase substrates include MAP2, MBP, S6 kinases, MAPKAP kinase-2, MEK, Raf, PLA2, and the EGF receptor (Johnson and Vaillancourt, 1994). MAP kinases are found in the cytoplasm, in nuclei, and associated with the cytoskeleton.

Surprisingly, although many chemoattractants activate MAP kinases, determination of the function of MAP kinases in chemotactic responses has been limited. Studies to date have utilized long term assays (Boyden chambers) and correlative studies of chemotactic responses to ligands for receptor
tyrosine kinases. For example, some studies have indicated that activation of MAP kinases alone is not sufficient for chemotactic responses (Chen et al., 1994), and others have demonstrated that some chemoattractants show relatively little activation of MAP kinases (Bornfeldt et al., 1994, 1995a,b).

However, two very recent studies utilizing the MEK inhibitor 98059 suggest a role for MAP kinases in neutrophil chemotaxis (Kuroki and O’Flaherty, 1997) and haptotaxis of highly adherent cells (Klemke et al., 1997). We present work here based on a genetic approach that provides evidence for a role for MAP kinases in amoeboid chemotaxis. In addition, we provide a detailed comparison of cell responses in the presence and absence of MAP kinase activity.

We have previously identified a Dictostelium mutant defective in the MAP kinase DdERK2 using REMI (restriction enzyme mediated integration) (Segall et al., 1995). Dderk2−mutant cells are defective in aggregation. The sequence of the DdERK2 gene has 40% identity to human MAP kinases/ERKs and contains residues that are conserved in all kinases as well as the feature motifs of a MAP kinase. In Dderk2−cells, the gene was disrupted by the insertion of a plasmid. As a result, stimulation of cAMP synthesis is blocked, while cGMP synthesis is normal. Those results suggested that the DdERK2 gene product is required for activation of adenyl cyclase. cAMP stimulates transient activation of DdERK2 and multiple signaling pathways are involved in regulation of DdERK2 activity (Knetsch et al., 1996; Maeda et al., 1996). DdERK2 also plays an important role in the regulation of later developmental stages (Gaskins et al., 1996). A second MAP kinase, DdERK1, has been found to be essential for vegetative growth and later cell differentiation in Dictostelium (Gaskins et al., 1994).

We report in this paper that the DdERK2 gene product is important for chemotactic responses in Dictostelium. Dderk2−mutants show reduced chemotactic responses to both folate and cAMP. More detailed analysis reveals altered cell motility and cell morphology. Compared to wild-type cells, Dderk2−mutant cells are relatively rounder and after cAMP stimulation, mutant cells recover more slowly than wild-type cells, forming crown-like structures on the cell surface. Our results suggest that DdERK2 plays an important role in expanding the range of chemoattractant concentrations over which effective chemotaxis can take place by regulating the types of cytoskeletal structures that are generated in response to stimulation with chemoattractants.

MATERIALS AND METHODS

Cells

HS174 and HS175 are Dderk2−mutants derived from HL330 which have been transformed with a vector targeted to the erkb gene (Segall et al., 1995). HS176 is a control transformed line showing wild-type DdERK2 activity (Knetsch et al., 1996; Maeda et al., 1996). DdERK2 also plays an important role in the regulation of later developmental stages (Gaskins et al., 1996). A second MAP kinase, DdERK1, has been found to be essential for vegetative growth and later cell differentiation in Dictostelium (Gaskins et al., 1994).

We report in this paper that the DdERK2 gene product is important for chemotactic responses in Dictostelium. Dderk2−mutants show reduced chemotactic responses to both folate and cAMP. More detailed analysis reveals altered cell motility and cell morphology. Compared to wild-type cells, Dderk2−mutant cells are relatively rounder and after cAMP stimulation, mutant cells recover more slowly than wild-type cells, forming crown-like structures on the cell surface. Our results suggest that DdERK2 plays an important role in expanding the range of chemoattractant concentrations over which effective chemotaxis can take place by regulating the types of cytoskeletal structures that are generated in response to stimulation with chemoattractants.
at 4°C for 10 minutes. Triton X-100 insoluble pellets were resuspended in SDS sample buffer. Particulate fractions were prepared in the same way as described above for actin and myosin. Mouse monoclonal antibody against coronin, 176-306-3, was given by Dr G. Gerisch, secondary antibody was goat anti-mouse IgG from Sigma (A-2304). A duplicate gel was run simultaneously and stained with Coomassie blue and served as loading control. The loading was such that dilution series of the wild-type coronin samples showed a linear response on the western blot (ECL) over a 4-fold range, sufficient to encompass the mutant samples. The western blot films were digitized using a Molecular Dynamics densitometer and quantitated using ImageQuant. Variation in sample loading was normalized by digitizing the Coomassie blue stained gel and dividing the coronin western blot values by the intensity of the region between myosin II heavy chain and 55 kDa (the molecular mass of coronin) in the corresponding lane on the Coomassie blue gel. Whole cell extracts showed equal amounts of coronin in Dderk2− and DdERK2+ cells. However, independent of the normalization procedure, the western blots of Dderk2− cell Triton-insoluble extracts showed a reduced amount of coronin compared to the Triton-insoluble extracts of DdERK2+ cells.

Myosin light chain band shift assay

To detect myosin light chain phosphorylation, the cells were pretreated and stimulated by cAMP in the same way as in the assays for actin and myosin. The cells were lysed by an equal volume of 4× SDS sample buffer for urea/glycerol gels (8% SDS, 230 mM Tris-HCl, pH 6.8). The samples were allowed to cool down on ice after boiling for 5 minutes, and then were mixed with 2× urea sample buffer (9 M urea, 1.5 mM EDTA, 1 mM DTT, 22 mM Tris, 180 mM glycine, pH 8.6) and separated on a urea/glycerol gel (Ostrow et al., 1994; Burns et al., 1995). Myosin light chain was visualized on western blot by antibodies kindly provided by Drs R. Chisholm and J. Smith.

Immunofluorescence

Starved cells were centrifuged and resuspended in phosphate buffer at 1×10⁶/ml. 40 μl of the starved cells were settled on a 1 cm coverslip in a small Petri dish for 10 minutes. The cells were then fixed with 3.7% formaldehyde and then permeabilized with 0.1% Triton X-100. They were resuspended in PBS, 0.5% BSA and 0.045% gelatin) for 20 minutes. The samples were incubated with monoclonal antibody 176-306-3 for 1 hour in a humidified chamber, followed by washing with PBG for 5× 5 minutes each. The samples were then incubated with preabsorbed trimethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratory, 115-025A-0620), washed with PBG four times and 1× PBS two times, mounted in mounting medium (polyvinyl alcohol with n-propyl gallate) on slides. Coronin was observed with an Olympus IX70 microscope, and images collected with a Photometrics KAF 1400 cooled CCD camera.

RESULTS

We have shown previously that Dderk2− cells are defective in multicellular development, with strongly reduced activation of adenyl cyclase (Segall et al., 1995). Studies with a number of mutants defective in the activation of adenyl cyclase have shown that adenyl cyclase activation is necessary to generate the waves of cAMP that direct cell movement during aggregation, but is not required for the ability of cells to respond to externally imposed gradients (Insall et al., 1994; Pitt et al., 1992; Theibert and Devreotes, 1986). This can be tested in mixtures with wild-type cells, in which the wild-type cells produce the appropriate gradients, and then the ability of the mutant cells to respond can be determined. When this was done using Dderk2− cells, it was found that the efficiency of coaggregation of mutant cells with wild-type cells was reduced (Segall et al., 1995; Gaskins et al., 1996). To determine if the reduced coaggregation of mutant cells with wild-type reflected reduced chemotactic efficiency, we assayed chemotactic responses to folate and cAMP.

Folate chemotactic responses were assayed using the agar well assay (Fig. 1A). Folate was added to a well on an agar plate and cells were placed 3 mm from the well. Diffusion of folate from the well generated a spatial gradient. Most of the wild-type cells (HS176) moved outward in a ring towards the well. As a control for activation of adenyl cyclase, Crac− cells, which are defective in activation of adenyl cyclase but still show activation of DdERK2 (Insall et al., 1994; Aubry et al., 1997), also showed wild-type chemotaxis. On the other hand, Dderk2− cells moved outwards more randomly. The response was quantitated (Fig. 1B) using labeled Dderk2− or wild-type cells mixed with unlabeled wild-type cells in a ratio of 1:10. The unlabeled wild-type cells responded normally, forming a ring moving towards the well. If the labeled cells were also wild-type, they comprised 8.5±2.5% of the cells in the ring, consistent with the original proportion in the population. On the other hand, if the labeled cells were mutant, they formed only 2±1% (HS174) or 2.8±2.3% (HS175) of the cells in the ring, roughly 1/4 that of the wild-type cells. Areas behind the ring showed 10-20% of the cells labeled, indicating that the mutant cells were alive, but not efficiently responding to the chemotactic stimulus. Thus by this assay, the efficiency of folate chemotaxis of Dderk2− cells is about 1/4 of wild-type.

Because chemotactic responses to folate are relatively weak, more detailed studies of chemotactic responses were performed utilizing starved cells and cAMP gradients in a Zigmond chamber (Zigmond, 1977). The cells were placed on a bridge separating two wells, one well containing cAMP, the other only buffer. Diffusion of cAMP across the bridge
generates a gradient to which the cells can then respond. Both wild-type and mutant cells oriented well with 0.1 μM gradients (Table 1). However, wild-type cells (HS176) also showed good chemotaxis with 2 μM cAMP gradients, while Dderk2− cells were strongly defective in 2 μM gradients. During aggregation in streams, external cAMP concentrations may reach the μM range (Tomchik and Devreotes, 1981). Thus, this defect could explain the reduced efficiency of coaggregation. HS174b6 is a Dderk2− cell in which expression of the DdERK2 protein is restored using the actin 6 promoter. HS174b6 cells aggregated and formed fruiting bodies, and showed normal chemotaxis in 2 μM cAMP gradients (Fig. 2). The complementation of the chemotaxis defect confirms that the DdERK2 protein plays a role in the chemotactic responses of Dictyostelium cells. Crac− cells, defective in activation of adenylyl cyclase, showed normal chemotactic responses to 2 μM cAMP, indicating that the defect of Dderk2− cells was not due to a defect in activation of adenylyl cyclase.

To determine the behavioral defect underlying the reduced chemotactic efficiency of Dderk2− cells, the responses of cells to sudden, uniform increases in cAMP were measured (Segall, 1992; Hall et al., 1989; Varnum and Soll, 1984). Upon exposure to a sudden increase in cAMP concentration, wild-type cells rounded up and stopped moving, but within 5 minutes, in the continued presence of cAMP, wild-type and 174b6 cells began moving, returning to about 50% of the prestimulus speed. Dderk2− cells, on the other hand, stopped moving upon exposure to cAMP and did not recover (Fig. 4A). Dderk2− cells exposed to 0.1 μM cAMP were able to recover (Fig. 4B). Measurements of cell roundness showed also that wild-type and HS174b6 cells were better able to generate an elongated shape (a lower roundness index) than HS174 cells in the presence of high concentrations of cAMP (Fig. 4C). Dderk2− cells were able to maintain a more polarized

Fig. 2. The Dderk2− mutant is defective in chemotactic responses to cAMP in the high concentration range. Cells were assayed in the Zigmond chemotaxis chamber, as described in Materials and Methods, and the chemotactic orientation assayed as the mean cosine of the direction of movement relative to the cAMP gradient. Gradients were from 0 to 2 μM cAMP. Wild-type cells (HS176) and Crac− cells showed normal chemotaxis, but chemotactic responses of Dderk2− cells were dramatically decreased. HS174b6 is HS174 transformed with a DdERK2 expression vector, and showed restored chemotactic responses.
shape in lower concentrations of cAMP (Fig. 4D). Thus there was a correlation between chemotaxis, the ability of cells to repolarize and the ability to recover speed of translocation after exposure to cAMP. Analysis of time-lapse images revealed that although the mutants generated protrusions, those structures tended to develop into the circular structures shown above (Fig. 3B) rather than into pseudopods and net forward cell translocation.

Because the protrusions generated in the Dderk2− cells resembled crowns, we examined the location of coronin, a molecule associated with crown formation (de Hostos et al., 1991). Coronin was enriched in both wild-type and mutant cells near the cell membrane (Fig. 5). In wild-type cells, before stimulation with cAMP, there was a clear polarization of coronin distribution, with coronin most concentrated at leading edges of the cell. Upon stimulation with cAMP, coronin remained enriched at the plasma membrane, but was present over most of the cell periphery, consistent with the loss of cell polarization. After 5-6 minutes of exposure to cAMP, the coronin distribution repolarized, together with a recovery in cell repolarization. In the mutant cells, after cAMP stimulation, the coronin distribution and cell morphology remained unpolarized, and the circular structures showed increased concentrations of coronin. Western blots indicated that roughly half as much coronin was associated with the Triton X-100 insoluble cytoskeleton in Dderk2− cells compared to wild type (Fig. 6). Total cell coronin was equal in mutant and wild-type cells, indicating a redistribution of coronin out of the cytoskeleton in Dderk2− cells. In both wild-type and mutant cells, cAMP stimulation led to a roughly twofold increase in the association of coronin with the cytoskeleton. However, comparison of Dderk2− mutant and wild-type cells showed no significant difference in the kinetics of actin and myosin association with the particulate fraction (Fig. 7). Actin association with the cytoskeleton increased rapidly after stimulation of myosin II association with the Triton-insoluble cytoskeleton may be regulated by DdERK2. The correlation of chemotactic responses could involve regulation of myosin II distribution within the cell. Stimulation with chemoattractants leads to characteristic changes in the association of actin and myosin II with the cytoskeleton. We tested whether this interaction was altered in Dderk2− cells. However, comparison of Dderk2− cells with wild-type cells showed no significant difference in the kinetics of actin and myosin association with the particulate fraction (Fig. 7). Actin associated with the cytoskeleton increased rapidly after addition of chemoattractant, peaking at 5 seconds. Myosin association decreased initially, reaching a minimum 5-10 seconds after stimulation and then increased, reaching a maximum 30 seconds after stimulation and returning to basal values by 120 seconds. The same results were observed for association of actin and myosin with the Triton X-100 insoluble cytoskeleton (data not shown). Thus, the differences in cell polarization minutes after stimulation do not appear to be due to major changes in the early kinetics of actin or myosin association with the cytoskeleton.

We then examined total myosin light chain phosphorylation stimulated by addition of cAMP, utilizing a gel shift assay (Ostrow et al., 1994; Burns et al., 1995). As shown in Fig. 8, upon stimulation with cAMP, myosin light chain in both mutant and wild-type cells showed similar degrees of band shift, reaching a maximum at 30 seconds and returning to basal levels by 6 minutes. These data indicate that chemoattractant-stimulated phosphorylation of myosin light chain can still occur in mutant cells.

However, there was a difference in the localization of myosin II in Dderk2− cells compared to the control. In both mutant and wild-type cells, the majority of myosin II was cytoplasmic. In control cells, the leading front contained polymerized actin and relatively little myosin II. Upon

<table>
<thead>
<tr>
<th>Strain</th>
<th>Speed (μm/minute)</th>
<th>Orientation (cosine)</th>
<th>Turning (deg/10 seconds)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 μM</td>
<td>2 μM</td>
<td>0.1 μM</td>
<td>2 μM</td>
</tr>
<tr>
<td>Dderk2+</td>
<td>7.6±3.0</td>
<td>7.2±2.4</td>
<td>0.50±0.30</td>
<td>0.66±0.28</td>
</tr>
<tr>
<td>Dderk2−</td>
<td>7.4±2.3</td>
<td>3.8±0.88</td>
<td>0.58±0.30</td>
<td>0.11±0.23</td>
</tr>
</tbody>
</table>

Cell chemotaxis was measured on 8 hour starved cells in a Zigmund chamber. The numbers for Dderk2− cells at 2 μM are reduced due to a large number of cells that are so unpolarized that they move too slowly for the measurement program to make accurate measurements. Data are means and standard deviations of the number shown (n).
stimulation with cAMP the cells rounded up and the leading front disappeared, reforming about 4-7 minutes after stimulation. In wild-type cells, after recovery from cAMP stimulation, the leading edge again showed relatively high concentrations of F actin and low concentrations of myosin II (Fig. 9, left side, outlined arrow). In Dderk2− cells (Fig. 9, right side), in the absence of cAMP there was a leading front similar to that in control cells, and the cells’ response to cAMP was similar to control for the first 3 minutes. However, a stable leading front did not appear after 4-7 minutes. Rather, the circular protrusions which formed contained both polymerized actin and myosin II (white arrowheads). As mentioned above, time lapse studies indicated that these structures were less stable than the leading fronts of wild-type cells. The presence

![Fig. 3.](image)

Fig. 3. Cell morphology in the chemoattractant upshift assay. Cells were stimulated and followed by time lapse microscopy. (A) Low magnification view of entire field. The top image in each column shows cell morphology 1 minute before stimulation. Subsequent images in each column are 1, 2, 7, and 10 minutes after stimulation with 1 μM cAMP. Left column: DdERK2+. Right column: Dderk2−. DdERK2+ cells (wild type) showed an elongated morphology before cAMP stimulation; they rounded up after stimulation and returned to the elongated morphology by 7 minutes. Dderk2− cells were not as elongated as the wild-type cells before stimulation; they rounded up upon stimulation and remain rounded even after 10 minutes. Black arrows indicate crowns in Dderk2−, white arrows identify pseudopods in DdERK2+. Scale bar, 20 μm. (B) Higher (∞2.1) magnification view of individual cells in A. Wild-type (HS176) cell shown on left, mutant (HS174) cell shown on right. The interval between images in B is 24 seconds, starting at the top.
MAP kinase function in amoeboid chemotaxis

of myosin II in the protrusions may contribute to this instability by generating contractile forces which result in retraction (Moores et al., 1996). There was not a general alteration of all cytoskeletal elements in that there was not a dramatic difference in microtubule localization between $D_{derk2}^-$ and control cells (data not shown).

**DISCUSSION**

In this report we demonstrate that MAP kinases contribute to optimal chemotactic responses of *Dictyostelium*. Mutants lacking DdERK2 show reduced chemotactic responses to folic acid and high concentrations of cAMP. Our studies using cell mixtures (for folic acid responses) and externally imposed gradients (for cAMP responses) indicate that this is a cell autonomous defect that is independent of the cells’ ability to secrete cAMP. Other mutants specifically defective in cAMP...
synthesis, such as the Crac<sup>-</sup> cells, which are unable to activate adenylyl cyclase (Insall et al., 1994; Pitt et al., 1992; Theibert and Devreotes, 1986), are still responsive to folate and cAMP, thus making it unlikely that the defects we observe are due to defects in cAMP synthesis. In addition, these defects are unlikely to be due to a defect in developmental expression of a specific protein for 3 reasons: (1) the defects are present in responses to both a growth phase chemoattractant (folic acid), and a chemoattractant for developed cells (cAMP); (2) other developmentally regulated genes involved in cAMP chemotaxis such as the cAMP receptor and adenylyl cyclase are expressed; and (3) chemotactic responses to low concentrations of cAMP as well as the initial reduction in speed, changes in initial actin and myosin particulate localization, and activation of guanylyl cyclase are normal (Gaskins et al., 1996; Segall et al., 1995).

A more detailed analysis of cellular responses to stimulation with cAMP revealed that Dd<sup>erk2</sup><sup>-</sup> mutants are unable to recover appropriately after exposure to high concentrations of cAMP. The initial responses of the mutants, including rounding up of the cells and a decrease in translocation speed, are similar to wild type. However, their recovery, including a repolarization of the cell to form an elongated shape with a leading pseudopod and continued cell translocation, is altered. Instead, transient projections form from multiple areas of the cell surface which are then resorbed into the cells. The net result is the formation of multiple protrusions which do not form an active pseudopod for cell movement. This leads to poor cell motility due to the inability to form an organized, polarized cytoskeleton. The defect is most apparent at high concentrations (1 μM), with nearly normal responses occurring to lower concentrations (0.1 μM). The peak concentration of cAMP in streams and aggregates may exceed 1 μM (Tomchik and Devreotes, 1981), consistent with a partial ability of mutant cells to coaggregate with wild-type cells (Segall et al., 1995; Gaskins et al., 1996). This suggests that some aspect of adaptation might be defective in Dd<sup>erk2</sup><sup>-</sup> cells. One form of adaptation might involve phosphorylation of the receptor. However, changes in mobility on SDS gels that reflect phosphorylation of the receptor are normal in Dd<sup>erk2</sup><sup>-</sup> cells. Alternatively, DdERK2 might be critical for modulating cytoskeletal rearrangements in responses to high concentrations of chemoattractant but not critical in cells stimulated by low concentrations. Thus, we examined the cytoskeletal responses of Dd<sup>erk2</sup><sup>-</sup> cells in more detail in order to evaluate the mechanism of action of DdERK2.

Immunofluorescence studies confirmed that atypical structures were formed in response to cAMP. Wild-type cells form F actin-rich pseudopods which contain relatively little myosin II during the extension phase. The projections formed by Dd<sup>erk2</sup><sup>-</sup> mutants contain both F actin and myosin II. The presence of myosin in the cell protrusions along with actin may predispose them to rapid resorption by the cell and inhibit
formation of a stable pseudopod. Coronin was found to be present in both normal pseudopods as well as in the mutant cell protrusions (de Hostos et al., 1991). Coronin in polarized wild-type cells was clearly concentrated in the pseudopod at the front of the cell leading to a highly polarized distribution. In mutant cells after stimulation with cAMP, coronin was concentrated in ruffle or crown-like structures. The amount of coronin present in the Triton-insoluble cytoskeleton was reduced in the mutant.

Coronin has been shown to be involved in both phagocytosis and cell motility (de Hostos et al., 1991; Maniak et al., 1995). Mutants lacking coronin have reduced phagocytic rates and reduced cell speeds. Neither process is completely defective in coronin mutants, and it is possible that coronin may be involved more in the regulation of these phenomena. The Dderk2− mutants showed the formation of multiple ruffle or crown-like structures in responses to high concentrations of cAMP. The initial phases of the formation of phagocytic cups and cell pseudopods are similar: in both cases there are increases in actin polymerization and accumulation of coronin just under the membrane. However, there is then a distinct difference in the further development of these structures correlating with their varied functions: phagocytic cups extend around the object and then form an endocytic vacuole, while cell pseudopods form extensions which can project in the absence of contact with a surface. An important, unresolved issue is how at the molecular level the cell regulates which type of structure is formed. Our results indicate that DdERK2 is important in directing the formation of pseudopods in response to high concentrations of chemoattractant rather than crowns. However, measurements with Dderk2− cells do not show increased phagocytic or pinocytic rates (data not shown), indicating that the crown-like structures are not true phagocytic cups. This is consistent with detailed time lapse viewing of the projections formed by the Dderk2− cells, which indicate that the projections collapse back into the cell without enclosing medium. Thus the loss of DdERK2 alone is not sufficient to direct the formation of functional phagocytic structures. Concomitant localization of additional proteins important for pinocytosis or phagocytosis is likely to be necessary to complete the uptake process.

The data presented here indicate that DdERK2 is involved in the determination as to whether a nascent cell protrusion will develop into a pseudopod in response to stimulation with chemoattractant. DdERK2 activity is maximal 30-60 seconds after stimulation with cAMP (Knetsch et al., 1996; Maeda et al., 1996), while the initiation of protrusions that will develop into pseudopods occurs 2-4 minutes after stimulation. DdERK2 may trigger a cascade of events that results in the decision to form a pseudopod. One possibility is that coronin regulates the decision whether to make a pseudopod or a crown. The interaction of coronin with both the membrane and the Triton-insoluble cytoskeleton may be necessary for formation of a pseudopod. The similarity of coronin to Gbeta subunits of heterotrimeric G proteins may provide a mechanism by which coronin associates with the cell membrane. This association appears to be intact in Dderk2− cells (data not shown). In the Dderk2− cells, there is less coronin binding to the cytoskeleton, suggesting that DdERK2 may regulate the association of coronin with actin, which may be important in forming a pseudopod. In the absence of DdERK2, a default pathway involving formation of crown-like structures may result. Alternatively, phosphorylation of the appropriate substrate by DdERK2 relatively early may already predispose the cytoskeleton to form a polarized structure.

Recent work published by Klemke et al. (1997) suggests...
phosphorylation of myosin light chain kinase is important for haptotactic responses of COS-7 and FG carcinoma cells. Our data indicate that total myosin light chain phosphorylation can still occur in mutant cells. Thus, in *Dictyostelium*, DdERK2 is not required for myosin light chain phosphorylation. Highly adhesive cells such as those analyzed by Klemke et al. contain well-developed focal contacts and stress fibers. These structures are poorly developed or absent in highly motile cells such as *Dictyostelium* and neutrophils. It has recently been shown that myosin contractile activity contributes to the formation of focal contacts and therefore MAP kinase regulation of such structures could be important for appropriate haptotactic movement (Burridge and Chrzanowska-Wodnicka, 1996; Chrzanowska-Wodnicka and Burridge, 1996). In *Dictyostelium*, on the other hand, such structures are effectively absent, and thus the role of MAP kinases in regulating chemotactic responses is likely to be quite different. An alternative target for DdERK2 action in *Dictyostelium* might be coronin, which was originally identified from precipitated actin myosin complexes (de Hostos et al., 1991). The reduced amount of coronin present in the Triton-insoluble cytoskeleton raises the possibility that DdERK2 regulates the distribution of coronin between the cytoskeletal, membrane, and cytoplasmic compartments, and that coronin in turn might affect myosin localization. DdERK2 regulation of coronin localization does not appear to be via direct phosphorylation of coronin (data not shown). We are currently working on the identification of specific DdERK2 substrates in the hope of revealing the mechanism by which DdERK2 enhances pseudopod formation.

In summary, our data demonstrate a role for MAP kinases in chemotactic responses of amoeboid cells. The effect of DdERK2 is to expand the range of chemoattractant concentrations over which strong chemotactic responses can operate. Such a mechanism is critical for allowing cells to maintain high sensitivity to low concentrations of chemoattractant (if aggregation centers or bacterial food sources are far away) while at the same time maintaining the ability to polarize and move when the cell is exposed to high concentrations of chemoattractant such as in an aggregation stream or near the food source.

We thank R. L. Chisholm, J. Condeelis, G. Gerish, and J. Smith for the generous contribution of antibodies and Ying Ping Yu for technical assistance. We thank Michael Cammer and the Analytical Imaging Facility for aid in microscopy. J. Segall is an Established Scientist of the New York City Affiliate of the American Heart Association. This work was funded by NIH GM5 grants 44246.

**REFERENCES**


