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MAPKs in development: insights from *Dictyostelium* signaling pathways

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Abstract

Mitogen activated protein kinases (MAPKs) play important roles in the development of eukaryotic organisms through the regulation of signal transduction pathways stimulated by external signals. MAPK signaling pathways have been associated with the regulation of cell growth, differentiation, and chemotaxis, indicating MAPKs contribute to a diverse set of developmental processes. In most eukaryotes, the diversity of external signals is likely to far exceed the diversity of MAPKs, suggesting that multiple signaling pathways might share MAPKs. Do different signaling pathways converge before MAPK function or can MAPKs maintain signaling specificity through interactions with specific proteins? The genetic and biochemical analysis of MAPK pathways in simple eukaryotes such as *Dictyostelium* offers opportunities to investigate functional specificity of MAPKs in G protein-mediated signal transduction pathways. This review considers the regulation and specificity of MAPK function in pathways that control *Dictyostelium* growth and development.

Keywords

Dictyostelium; ERK1; ERK2; G Protein; MAPK

Introduction

Mitogen activated protein kinases (MAPKs) have been associated with many types of signaling pathways including those pathways activated by G protein-coupled receptors and receptor tyrosine kinases (1–5). A wide range of signals including hormones, chemoattractants, and stress can activate MAPKs through these and possibly other types of receptors to regulate many basic processes such as cell growth, differentiation, proliferation, and survival. Not surprisingly, MAPK signaling pathways have been implicated in a variety of diseases ranging from early onset diseases such as Noonan and Costello syndromes to typically late onset diseases such as cardiac hypertrophy and cancer (6–10). MAPK signaling pathways contribute to the survival of cells through the inhibition of apoptotic mechanisms, including the resistance of cancer cells to anoikis (11,12). In many cases, these diseases result from increased MAPK signaling in one or more tissues but the loss of MAPK signaling can also have dire effects on an organism's health and development. Some MAPKs may also play important roles in the development of more common health issues such as obesity (6,7,13). The prevalence of MAPK signaling in so many pathways has made the investigation of MAPK function an important goal of many research efforts resulting in a large collection of information on different MAPKs from many different eukaryotes. In

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particular, the genetic and biochemical analysis of MAPKs in the development of model organisms has revealed exciting clues about MAPK functional specificity that might provide insight into role of MAPK signaling in normal and disease tissue (2,5,14,15).

ERK regulation and function

The classification of MAPKs into subgroups has been accomplished through sequence and functional comparisons of MAPKs in many different eukaryotes (16). The MAPK subgroup with the greatest distribution among eukaryotes is known as the extracellular-signal regulated kinases (ERKs) but other subgroups p38 and JNK kinases are also found in different eukaryotes (17,18). ERKs are perhaps the best characterized MAPKs and they often function downstream of G protein-coupled receptors. In some eukaryotes such as mammals, ERKs can also function downstream of mitogen or growth factor receptors (receptor tyrosine kinases) but the lack of these receptors in yeast and *Dictyostelium* suggests that the role of ERK signaling in G protein-coupled receptor pathways evolved earlier than their role in receptor tyrosine kinase pathways (1,16,19). Although different receptor types can regulate ERKs, many of the signaling components directly upstream of the ERKs appear to be highly conserved (4).

ERKs, as well as other MAPKs, are typically found associated with kinase cascades that include MAPK kinases (MAP2Ks such as MEKs) and MAP2K kinases (MAP3Ks such as RAFs). (Fig. 1) (4). The MAP2Ks are dual specificity kinases that phosphorylate the threonine and tyrosine residues of the highly conserved TEY sequence in ERKs (20). This kinase interaction and other upstream kinase interactions are often coordinated through the use of scaffolding proteins, such as the prototype scaffold STE5 in yeast mating responses (2). Many different scaffold proteins have since been identified in other organisms. Scaffold proteins can interact with G proteins or G protein-coupled receptors or function in pathways that lack G proteins (21). The coordination of kinase cascades through scaffolds can accelerate kinase interactions and can also contribute to the specificity of interactions in a given pathway (22). Other upstream signaling components include monomeric G proteins (e.g., RAS), MAP3K kinases (MAP4Ks such as PAKs), and protein kinase C (PKC) and these proteins often connect the MAPK cascades to G protein coupled receptors and receptor tyrosine kinases (2,23). Many of the signaling proteins in these signaling pathways belong to multigene families, allowing for cell-type or tissue specific diversification in the signaling pathways that involve ERK function. In addition to upstream regulators, ERKs might also regulate themselves through autophosphorylation events. A recent study in mice provides evidence that ERKs can transfer the phosphoryl group from the tyrosine residue of the TEY sequence to a highly conserved threonine residue, three residues outside the TEY sequence (24). This phosphorylation mechanism has been associated with long-term ERK activation and cardiac hypertrophy in mice.

ERKs can phosphorylate a wide range of substrates in both the cytoplasm and nucleus and many of these substrates regulate other signaling components or gene expression. Among some of the notable substrates are phosphodiesterases that can be positively or negatively regulated by phosphorylation and then impact cAMP or cGMP-dependent protein kinases (e.g., PKA and PKG) through modulating the levels of cAMP and cGMP (25,26). Protein kinases such as ribosomal S6 kinases (RSKs) in animals and the FAR1 protein kinase in yeast are also known to be ERK substrates (2,27). FAR1 regulates cell growth, cell division or cell polarity to achieve chemotrophic growth during the yeast mating responses. ERKs can also regulate gene expression through either binding or phosphorylating transcription factors, consistent with the movement of ERKs in and out of the nucleus (28,29). ERK substrates typically contain phosphorylation sites that consist of a serine/threonine residue followed by a proline and in some cases a second conserved proline exists two residues

before the phosphorylated residue (P-X-S/T-P) (30). Many ERK substrates also contain MAPK docking sites (R/K₁₋₃-spacer₂₋₆-L/I-X-L/I), commonly referred to as a D-motif, that allows the ERK to be tethered to the substrate. D-motifs are also found on MAP2Ks, phosphatases, or other proteins that may play a role in regulating ERKs (31). Other motifs such as DEF sites (FXF/YP) also contribute to protein interactions with ERKs (32).

ERK specificity

Some extracellular signals are capable of activating two different ERKs simultaneously and such co-activation has been observed in a wide range of eukaryotes. The co-activation of mammalian ERK1 and ERK2, often referred to as ERK1/2 activation, is common and perhaps partly attributed to the 86% sequence identity between these proteins (33). However, strong sequence similarity is not a necessity for co-activation (Fig. 2). The yeast ERKs, FUS3 and KSS1, share 51% sequence identity and both ERKs are activated in response to mating pheromone (2). The *Dictyostelium* ERK1 and ERK2 proteins share only 37% sequence identity and these ERKs are both activated in response to extracellular cAMP (34,35). Do these cases of co-activation represent redundancy in the regulation and function of ERKs or the divergence of a given pathway to generate multiple responses? In yeast, the MAP2K STE7 can phosphorylate both FUS3 and KSS1 in response to mating pheromone but specific interactions with the scaffold STE5 increases the efficiency of FUS3 phosphorylation (36). In addition, FUS3 interacts and phosphorylates the downstream kinase FAR1 much more efficiently than KSS1 due to the specific interaction of FUS3 with the D-motif of FAR1 (37). The loss of FUS3 severely reduces the mating response whereas the loss of KSS1 only has a marginal impact on mating indicating a more critical role for FUS3 in this response. In contrast, KSS1 but not FUS3 is critical for the filamentous growth that results from nutrient limitation indicating that yeast ERKs have different roles in cell fate (2). Different developmental roles for individual ERKs also occur for the more closely related ERK1 and ERK2 in mammals. An ERK2 gene knockout in mice results in death early during embryogenesis whereas an ERK1 gene knockout appears to have only minor effects such as defects in T cell maturation (14,38,39). Distinctions in ERK function have also been uncovered in the gene expression and cell movement during zebrafish development (40,41). Given the different developmental roles for ERK paralogs, the co-activation of ERKs could potentially result in multiple ERK-specific responses.

ERK signaling in *Dictyostelium*

Defining ERK function and specificity in signaling pathways can be challenging because of the involvement of these kinases in so many pathways but the analysis of ERKs in organisms such as *Dictyostelium discoideum* can offer some advantages over other complex eukaryotes. The *Dictyostelium* genome encodes only 2 MAPKs, ERK1 and ERK2, and these kinases function to regulate a relatively simple developmental life cycle (42,43). *Dictyostelium* has also been regarded as an important model organism for understanding cell movement and cell differentiation, processes that typically involve with MAPK function. *Dictyostelium* grow as solitary amoebae that search out and feed on bacteria in their environment but when starved, the amoebae aggregate through the relay of an extracellular cAMP signal. The multicellular aggregates undergo several morphological stages including a migratory slug stage. Eventually the slug will form a fruiting body, consisting of a mass of spores supported by a stalk. The differentiation of prestalk and prespore cells begins early in development with many prestalk cells migrating to the top of the mound to form an anterior tip. Prespore cells make up most of the central and posterior regions of the aggregate as it transforms into a migratory slug and then a fruiting body. The use of cell type-specific reporter genes has allowed the identification and tracking of different cell types, including multiple prestalk cell types, throughout the developmental life cycle (44). Many of the

developmental processes are regulated through G protein-mediated signal transduction pathways and at least some of these pathways involve MAPK function.

Unlike mammals, *Dictyostelium* do not possess receptor tyrosine kinases (e.g., growth factor/mitogen receptors) but the *Dictyostelium* genome does encode over 50 putative G protein-coupled receptors that could potentially regulate MAPK activity (45). *Dictyostelium* genome also encodes 12 different G protein $G\alpha$ subunits and a single $G\beta$ and $G\gamma$ subunit, implying that 12 different combinations of heterotrimeric G proteins mediate the transduction of signaling downstream of the receptors (46). The convergence of signaling at the receptor-G protein coupling is supported by the observation that four related receptors CAR1-4, all of which detect extracellular cAMP, can interact with the $G\alpha 2$ G protein (47,48). However, temporal differences in gene expression can allow related receptors to have different developmental roles, such is the case with the CARs (49–53). The two ERKs in *Dictyostelium* are expressed during growth and throughout development suggesting these MAPKs can potentially function downstream of any receptor expressed during growth and/or development (54,55). Substantial progress has been made in identifying receptors, G proteins, and other upstream signaling components that regulate ERK function during both growth and development and the genetic analysis of these regulatory proteins has provided critical information in developing models of signal transduction pathways that operate in *Dictyostelium*.

***Dictyostelium* ERK2 signaling**

Dictyostelium ERK1 and ERK2 were named based on the order of identification and both share roughly similar sequence identity (30–40%) with each other and with ERKs in other organisms suggesting no strong correlations between overall sequence identity and function (Fig. 2). However, the *Dictyostelium* ERK2, like the mammalian ERK2, has a much more critical role in development than its paralog ERK1 (15,35,55). ERK2 is activated in cells responding to either extracellular cAMP or folate, two stimuli that regulate cell fate in very different ways (Fig. 3) (34,56,57). Folate, and related pterin compounds, trigger chemotaxis that allows individual cells to forage for new bacterial food sources whereas cAMP triggers chemotaxis that allows cells to aggregate for multicellular development. The cAMP receptors CAR1 and CAR3 are necessary for cAMP-stimulated phosphorylation and activation of ERK2 but the requirement for heterotrimeric G protein function is questionable (34,58). The loss of either the $G\alpha 2$ or the $G\beta$ subunit reduces but does not eliminate the phosphorylation and activation of ERK2 in response to cAMP suggesting other proteins might contribute to the regulation of ERK2. Other $G\alpha$ subunit could potentially compensate for the loss of $G\alpha 2$ and an early report suggested the $G\alpha 4$ subunit might be required for this activation (59). However, later studies have indicated that the $G\alpha 4$ subunit is not required for this activation but this result does not preclude the involvement of other $G\alpha$ subunits [(34), Hadwiger *et al.*, unpublished data]. Explaining ERK2 activation in the absence of the $G\beta$ subunit is more problematic because a second $G\beta$ subunit, at least based on close sequence similarity, does not exist in *Dictyostelium* (46). While ERK activation downstream of $G\beta\gamma$ dimer signaling is typical in many systems, studies with mammalian cells suggest that $G\alpha$ subunits can also transduce ERK activation but through pathways that include protein kinase C (1). Therefore the $G\alpha 2$ subunit and the $G\beta\gamma$ dimer could potentially activate ERK2 using separate but redundant signaling pathways. The duration of the ERK2 phosphorylation is dependent on the level of extracellular cAMP because excessive cAMP concentrations and the inhibition of extracellular phosphodiesterases both can extend the period of ERK2 activation (58).

The loss of ERK2 function in *Dictyostelium* impairs the ability of cells to generate sufficient cAMP to allow cell aggregation during development (55). The *erk2*[−] cells are capable of

cAMP chemotaxis but they cannot relay the cAMP signal to neighboring cells. However, chemotaxis can be defective at high cAMP concentrations due to the inability of the *erk2*[−] cells to repolarize (60). The *erk2*[−] cells can aggregate with wild-type or other cells capable of generating the extracellular cAMP signal but the majority of *erk2*[−] cells are left behind during the transition to the slug stage, suggesting a requirement for ERK2 function in cell differentiation and movement in the multicellular state (15,55,61). The *erk2*[−] cells retained in the slug appear to be excluded from the prespore region suggesting ERK2 is important for prespore development. Consistent with this cell localization phenotype is the deficiency of prespore specific gene expression in *erk2*[−] cells (61). The inability of *erk2*[−] cells to generate a cAMP signal was initially assumed to be a defect in the activation of adenylyl cyclase but a later study linked ERK2 function with the inhibition of a cAMP-specific phosphodiesterase, REGA, suggesting ERK2 negatively regulates cAMP turnover (Fig. 3) (26). A knockout of the REGA gene was found to suppress aggregation and spore development defects of *erk2*[−] cells and allow cAMP accumulation in response to the extracellular cAMP. Like some mammalian phosphodiesterases, REGA contains a D-motif for MAPK docking and an ERK phosphorylation site suggesting REGA is an ERK2 substrate. Mutations that prevent phosphorylation of this site also prevent cAMP accumulation presumably because ERK2 can no longer inhibit REGA. Increased cAMP accumulation promotes the development of spore cells through the activation of PKA. PKA activity was proposed to negatively regulate ERK2 and adenylyl cyclase through phosphorylation resulting in an oscillatory circuit of cAMP production. However, transient ERK2 activation is observed in cells with low PKA activity and therefore the inactivation of ERK2 must occur by some other mechanism (58). Furthermore, constitutive PKA activity resulting from the expression of a mutant PKA regulatory subunit does not keep ERK2 inactive. In addition to REGA, another putative ERK2 substrate, EPPA, has been identified and loss of this protein leads to the inhibition of cAMP accumulation in response to extracellular cAMP, suggesting ERK2 may have multiple mechanisms to increase cAMP levels (62).

Folate stimulation of *Dictyostelium* leads to a rapid activation of ERK2 and chemotactic movement, a response important for searching out bacteria in the environment (56,57,59,63). The gene(s) encoding folate receptors has not been identified but these receptors transduce their signals through a G protein containing the Gα4 subunit (64). The phosphorylation of ERK2 occurs with kinetics similar to that observed after cAMP stimulation and both chemoattractants modulate cAMP and cGMP production with similar kinetics. In contrast, the Gα4 subunit and Gβ subunit are both essential for the folate activation of ERK2 whereas the Gα2 G protein appears to be nonessential for cAMP activation of ERK2 (56,63). Chemotaxis to folate is mildly reduced in cells lacking ERK2 but absent in cells lacking the Gα4 or Gβ subunit (60,63,65). Likewise, chemotaxis to cAMP requires G proteins (i.e., Gα2 and Gβ subunits) but not ERK2 (60). Therefore ERK2 appears to play only a minor role in both chemotaxis responses. Loss of the Gα4 subunit results in aberrant morphogenesis from the mound to the slug stage and very limited spore production implying that the Gα4 subunit, like ERK2, mediates signaling important for the development of prespore cells (66).

***Dictyostelium* ERK1 signaling**

ERK1 can be activated in response to extracellular cAMP but the detectable kinase activity rapidly disappears (within ~30 seconds) as ERK2 activation begins to peak (Fig. 3) (35). Antibodies that detect the phosphorylated TEY sequence in ERK2 and many other ERKs from other organisms do not recognize ERK1, even though ERK1 contains the highly conserved TEY sequence (15). Perhaps differences in the residues flanking the TEY sequence must prevent antibody binding to the phosphorylated ERK1 or ERK1 activation

has an atypical phosphorylation pattern. Extracellular cAMP activation of ERK1, but not ERK2, is dependent on MEK1, a MAP2K discovered by sequence similarities to MAP2Ks of other organisms (35,67). Some of the developmental phenotypes of *erk1*[−] cells are similar to those of *mek1*[−] cells supporting the role both proteins in a common signaling pathway. Loss of ERK1 was initially thought to be lethal but subsequent gene knockout attempts resulted in viable *erk1*[−] mutants (15,35). The loss of ERK1 does not appear to alter growth and *erk1*[−] cells can aggregate into mounds that can complete the full developmental cycle (15). However, *erk1*[−] cells precociously form small aggregates when starved at low cell densities as compared to wild-type cells. Many *erk1*[−] aggregates become stalled or completely blocked in tip morphogenesis implying an impairment of prestalk cell development. When *erk1*[−] and wild-type cells are developed as chimeras the *erk1*[−] cells are initially underrepresented in the extreme anterior of the slug but then *erk1*[−] cells eventually become more concentrated in the anterior region, suggesting *erk1*[−] cells have a transient delay in prestalk development.

Somewhat similar to *erk1*[−] mutants, cells lacking the Gα5 subunit have impaired tip morphogenesis. Aggregates of *ga5*[−] cells are delayed in tip morphogenesis whereas aggregates of cells over-expressing the Gα5 gene have accelerated tip morphogenesis (68). The accelerated tip development associated with increased Gα5 subunit expression is absent in *erk1*[−] cells suggesting ERK1 functions downstream of Gα5 subunit-mediated pathways (Fig. 3) (15). This signaling relationship is also supported by an interdependency of ERK1 and the Gα5 subunit for growth phenotypes (15,69). The introduction of ERK1 or Gα5 subunit expression vectors into cells results in very few viable clones unless the cells lack the other gene (i.e., Gα5 or ERK1 gene, respectively), suggesting the detrimental effects on cell growth require the presence of both proteins. The mechanism for these detrimental growth effects remains to be elucidated but could potentially involve a premature entry into the developmental life cycle, resulting in a cessation of nutrient uptake. While ERK1 and the Gα5 subunit require each other for some developmental and vegetative phenotypes, *erk1*[−] and *ga5*[−] cells form very different size aggregates suggesting these protein might also function independently of each other in some pathways.

Impaired cell movement and defects in cell polarity have been reported for *erk1*[−] cells during chemotaxis to cAMP (35). A later study confirmed the loss in directional cell movement but indicated that at least some *erk1*[−] cells can move greater distances in response to the chemoattractants, cAMP and folate (15). Cells carrying an ERK1 expression vector have a slight decrease in folate chemotaxis and this phenotype is dependent on cells having a functional Gα5 subunit. Likewise, the inhibition of folate chemotaxis due to Gα5 subunit over-expression is dependent on ERK1 but not ERK2 function. The requirement of both ERK1 and the Gα5 subunit in the inhibition of chemotaxis, as well as the promotion of tip morphogenesis and growth defects, suggests these proteins can function in common signaling pathways (Fig. 3).

ERK specificity in G protein signaling pathways

Phenotypic similarities of *Dictyostelium* ERK and Gα subunit mutants suggest that some G protein signal transduction pathways can specifically utilize ERK1 or ERK2 during growth, development, or chemotactic responses. How can this specificity accomplished, especially when the different signaling pathways use a common Gβγ dimer and possibly other common signaling components downstream of the receptor? Several studies have raised the possibility that receptor activation of ERKs could be in some cases independent of G protein function but no such mechanisms have been described thus far (34,58,70). Besides the receptors, Gα subunits appear to be pathway specific and evidence for this specificity has

been provided by a study of chimeric G α subunits that suggests specific G α subunit function goes beyond receptor coupling (71).

Putative MAPK docking sites (D-motifs) have been identified in some of the *Dictyostelium* G α subunits that are critical for development and chemotaxis, including the G α 2, G α 3, G α 4, G α 5, and the G α 11 subunits (69). All of these subunits except for the G α 4 subunit contain a D-motif near the amino terminus in a region analogous to where a D-motif was discovered in the yeast G α subunit, Gpa1, used for mating responses. Modeling of *Dictyostelium* G α subunit structure predicts the amino terminal D-motifs to be near the interface of G $\beta\gamma$ dimer and receptor binding and therefore this site might not be accessible to ERKs unless the G α subunit is activated and separated from the receptor and G $\beta\gamma$ dimer. The D-motif in the yeast Gpa1 subunit has been shown to be important for Gpa1 interactions with FUS3 in response mating pheromone stimulation (72). This interaction is associated with the activated form of Gpa1, consistent with the predictions of D-motif accessibility. A partial alteration of this D-motif (i.e., replacing the positively charged residues with negatively charged residues) reduces the ability of the Gpa1 subunit to interact with FUS3 and to adapt the responsiveness to mating pheromone (72). The altered D-motif prevents Gpa1 from reducing the nuclear to cytoplasmic ratio of FUS3 during mating responses suggesting Gpa1 uses the D-motif to retain FUS3 in the cytoplasm (73).

A similar alteration to the amino terminal D-motif of the *Dictyostelium* G α 5 subunit can eliminate some of the phenotypes associated with G α 5 subunit over-expression (69). This includes the detrimental growth effect of the G α 5 subunit. The D-motif alteration also prevents the accelerated tip morphogenesis and precocious gene expression during development. These phenotypes of the D-motif alteration are similar to those observed when ERK1 is absent implying that the D-motif might be important for the regulation of ERK1. *In vivo* binding of the G α 5 subunit and ERK1 have not been demonstrated but attempts to show this binding have been hampered by difficulties in expressing epitope-tagged forms of both proteins in the same cells. The amino terminal D-motif analysis has so far been limited to the G α 5 subunit but the analysis of amino terminal D-motifs in other *Dictyostelium* subunits is expected to provide important insights into function of other G α subunits during development.

The G α 4 subunit does not contain a D-motif at the amino terminus but rather has a D-motif located approximately 100 residues away from the amino terminus at a location where there is a second putative D-motif resides in the G α 5 subunit (63). Modeling studies suggest this D-motif is exposed even when the G α subunit is associated with a receptor and G $\beta\gamma$ dimer. A partial alteration of the D-motif in the G α 4 subunit reduces interactions with ERK2 but only has a slight effect on the phosphorylation of ERK2 suggesting the G α 4 subunit-ERK2 interaction is not critical for ERK2 activation. The G α 4 subunit with the D-motif alteration rescues many G α 4-specific functions including the ability to chemotax to folate and the developmental transition from a mound to a slug. However, these slugs are defective in both spore and stalk formation and they terminate development as slugs with a relatively low abundance of spores located near the prespore-prestalk border. This phenotype suggests morphogenesis in late development might be more sensitive to reduced G α 4 subunit-ERK2 interactions. It is possible that earlier stages of development might be affected by D-motif alterations that completely disrupt G α 4 subunit-ERK2 interactions

Expert Opinion and Outlook

Dictyostelium ERK1 and ERK2 have distinct developmental and chemotactic functions and can operate in different signaling pathways as indicated by the phenotypes of *erk1*[−] and *erk2*[−] mutants. Difference in developmental functions is also the case for FUS3 and KSS1 in

yeast and ERK1 and ERK2 in mammals. The yeast ERKs are important for different pathways but pathway specificity for the mammalian ERK1 and ERK2 is less clear. While some overlap in ERKs activation exists in *Dictyostelium* and other organisms, it is possible that this overlap is not significant with respect to cellular responses and may simply result from structural relatedness among ERKs. Overlap in ERK function might exist but could be difficult to detect in cells that lack only one ERK. Some functional overlap in *Dictyostelium* ERKs is suggested by the ability of ERK2 expression vectors to rescue normal aggregate size for *erk1*[−] cells (15). However, ERK1 expression vectors do not rescue the development of *erk2*[−] cells indicating ERK2 has functions that cannot be provided by ERK1. If both *Dictyostelium* ERKs provide some common function then defects in this function should be apparent in *erk1*[−]*erk2*[−] double mutants. The *erk1*[−]*erk2*[−] double mutant is viable and shows no obvious growth defects indicating that MAPK function is not essential for eukaryotic growth. This observation was not necessarily expected since ERK function has been implicated in the growth of tumor cells (74). However, signaling during the growth of tumor cells might vary significantly from that of normal cells. Interestingly, the sorting of a *erk1*[−]*erk2*[−] double mutant in chimeric organisms is similar to that of *erk2*[−] cells except that the cells retained in the slug localize to a different prestalk cell region than that occupied by *erk2*[−] cells suggesting *erk2*[−] phenotypes are not completely epistatic to *erk1*[−] phenotypes.

The characterization of ERK function in *Dictyostelium* has revealed that ERKs can specifically contribute to G protein-mediated signaling pathways. This specificity does not appear to be tissue or temporally specific as these proteins are expressed throughout development. Rather, ERK specificity is more likely to be accomplished through differential interactions with other signaling components that function in specific pathways. ERK interactions with D-motif-containing proteins are likely to be important for the localization of ERKs to specific signaling complexes in the cytoplasm or nucleus where ERK substrates might be located. Interactions with D-motifs on MAP2Ks, phosphatases, and substrates are all expected to be important for ERK regulation and function but the interactions with D-motifs on Gα subunit might provide a mechanism to tether ERKs to G protein-specific signaling complexes. In addition to D-motifs, other interactions might also contribute to the formation of signaling protein complexes that regulate ERK function to generate specific cellular responses. The identification of other proteins associated with ERKs, using the genetic and biochemical approaches available with *Dictyostelium*, will provide important insights into the role of MAPK function in developmental processes.

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Abbreviations

MAPK	MAP kinase or mitogen-activated protein kinase
MAP2K	MAP kinase kinase
ERK	extracellular-signal regulated kinase
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanine monophosphate

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Figure 1.

Typical MAPK signaling pathways. External signals can activate G protein-coupled receptors or receptor tyrosine kinases leading to the activation of MAPK cascades through several signaling steps. The activated MAPK can phosphorylate both cytoplasmic and nuclear targets to achieve cellular response.

Figure 2. Dendrogram of selected ERKs from *Dictyostelium discoideum* (*Dd*), yeast (*Saccharomyces cerevisiae*; *Sc*) and mammals (*Homo sapiens*; *Hs*). Dendrogram was generated at <http://clustalw.genome.ed.jp/> by the neighbor-joining method from a ClustalW alignment. Branch lengths reflect sequence similarities.

Figure 3.

Dictyostelium MAPK signaling downstream of three G protein-mediated signaling pathways. The cell surface receptors - folate receptor (FR), cAMP receptor (CAR), unknown receptor (R?) - couple to different Gα subunits and can activate one or both ERKs. ERK1 function is required for Gα5 subunit-pathway responses and ERK2 is required for Gα4 subunit-pathway responses. ERK2 can inhibit the cAMP specific phosphodiesterase, REGA, increasing the level of cAMP available for cell aggregation.