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Review

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 that 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*; extracellular signal regulated kinase 1 (ERK1); ERK2; G protein; mitogen activated protein kinase (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 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, 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 can 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 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; Figure 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

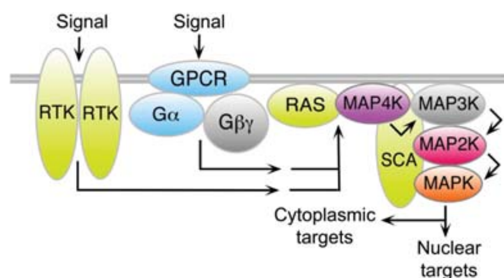

Figure 1 Typical MAPK signaling pathways.
External signals can activate G-protein-coupled receptors or receptor tyrosine kinases leading to the activation of MAPK cascades through several signaling steps. The activated MAPK can phosphorylate both cytoplasmic and nuclear targets to achieve cellular response.

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 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 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/K_{1-3}-$spacer_{2-6}$-L/I-X-L/I), commonly referred to 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 could 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 coactivation has been observed in a wide range of eukaryotes. The coactivation of mammalian ERK1 and ERK2, often referred to as ERK1/2 activation, is common and perhaps partly attributes to the 86% sequence identity between these proteins (33). However, strong sequence similarity is not a necessity for coactivation (Figure 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 coactivation 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 owing 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 crucial role for FUS3 in this response. By contrast, KSS1 but not FUS3 is crucial 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 uncov-

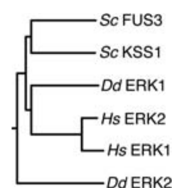

Figure 2 Dendogram of selected ERKs from *Dictyostelium discoideum* (*Dd*), yeast (*Saccharomyces cerevisiae*; *Sc*) and mammals (*Homo sapiens*; *Hs*).
The dendogram was generated at http://clustalw.genome.ed.jp/ by the neighbor-joining method from a ClustalW alignment. Branch lengths reflect sequence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

ered in the gene expression and cell movement during zebrafish development (40, 41). Given the different developmental roles for ERK paralogs, the coactivation 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 two 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 Gα subunits and a single Gβ subunit and Gγ 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 Gα2 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 that 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 crucial 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 almost similar sequence identity (30–40%) with each other and with ERKs in other organisms, suggesting no strong correlations between overall sequence identity and function (Figure 2). However, the *Dictyostelium* ERK2, similar to the mammalian ERK2, has a much more crucial 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 (Figure 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 Gα2 or the Gβ 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 Gα subunits could potentially compensate for the loss of Gα2 and an early report suggested that the Gα4 subunit might be required for this activation (59). However, later studies have indicated that the Gα4 subunit is not required for this activation but this result does not preclude the involvement of other Gα subunits [34, Hadwiger et al., unpublished data]. Explaining ERK2 activation in the

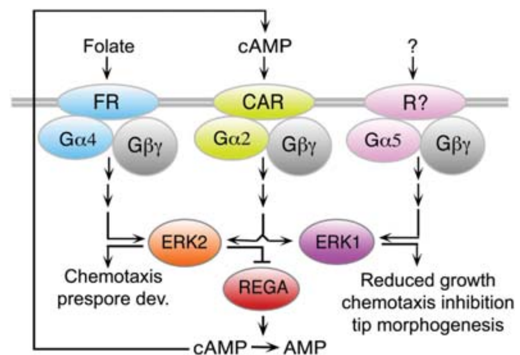

Figure 3 *Dictyostelium* MAPK signaling downstream of three G-protein-mediated signaling pathways.
The cell surface receptors – folate receptor (FR), cAMP receptor (CAR), unknown receptor (R?) – couple to different Gα subunits and can activate one or both ERKs. ERK1 function is required for Gα5 subunit-pathway responses and ERK2 is required for Gα4 subunit-pathways responses. ERK2 can inhibit the cAMP specific phosphodiesterase, REGA, increasing the level of cAMP available for cell aggregation.

absence of the Gβ subunit is more problematic because a second Gβ subunit, at least based on close sequence similarity, does not exist in *Dictyostelium* (46). Although ERK activation downstream of Gβγ dimer signaling is typical in many systems, studies with mammalian cells suggest that Gα subunits can also transduce ERK activation but through pathways that include protein kinase C (1). Therefore, the Gα2 subunit and the Gβγ 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 owing 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 that ERK2 negatively regulates cAMP turnover (Figure 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. Similar to some mammalian phosphodiesterases, REGA contains a D-motif for MAPK docking and an ERK phosphorylation site suggesting that 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 that ERK2 could 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. By 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, similar to 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 s) as ERK2 activation begins to peak (Figure 3) (35). Antibodies that detect the phosphorylated TEY sequence in ERK2 and many other ERKs from other organisms do not recognize ERK1, even through 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 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 under-represented in the extreme anterior of the slug but then *erk1*⁻ cells eventually become more concentrated in the anterior region, suggesting that *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

gα5^{-} cells are delayed in tip morphogenesis, whereas aggregates of cells overexpressing the Gα5 gene have accelerated tip morphogenesis (68). The accelerated tip development associated with increased Gα5 subunit expression is absent in *erk1*^{-} cells, suggesting that ERK1 functions downstream of Gα5 subunit-mediated pathways (Figure 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 that 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. Whereas ERK1 and the Gα5 subunit require each other for some developmental and vegetative phenotypes, *erk1*[-] and *gα5*^{-} cells form very different size aggregates suggesting that these proteins 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 overexpression 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 that these proteins can function in common signaling pathways (Figure 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 be 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). In addition to the receptors, Gα subunits appear to be pathway specific and evidence for this specificity has been provided by a study of chimeric Gα subunits which suggests that specific Gα subunit function goes beyond receptor coupling (71). Putative MAPK docking sites (D-motifs) have been identified in some of the *Dictyostelium* Gα subunits that are crucial for development and chemotaxis, including the Gα2, Gα3, Gα4, Gα5, and the Gα11 subunits (69). All of these subunits except for the Gα4 subunit contain a D-motif near the amino terminus in a region analogous to where a D-motif was discovered in the yeast Gα subunit, Gpa1, used for mating responses. Modeling of *Dictyostelium* Gα subunit structure predicts the amino terminal D-motifs to be near the interface of Gβγ dimer and receptor binding and therefore this site might not be accessible to ERKs unless the Gα subunit is activated and separated from the receptor and Gβγ dimer. The D-motif in the yeast Gpa1 subunit has been shown to be important for Gpa1 interactions with FUS3 in response to 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 that Gpa1 uses the D-motif to retain FUS3 in the cytoplasm (73).

A similar alteration to the amino terminal D-motif of the *Dictyostelium* Gα5 subunit can eliminate some of the phenotypes associated with Gα5 subunit overexpression (69). This includes the detrimental growth effect of the Gα5 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 Gα5 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 Gα5 subunit but the analysis of amino terminal D-motifs in other *Dictyostelium* subunits is expected to provide important insights into function of other Gα subunits during development.

The Gα4 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 a second putative D-motif resides in the Gα5 subunit (63). Modeling studies suggest that this D-motif is exposed even when the Gα subunit is associated with a receptor and Gβγ dimer. A partial alteration of the D-motif in the Gα4 subunit reduces interactions with ERK2 but only has a slight effect on the phosphorylation of ERK2 suggesting that the Gα4 subunit-ERK2 interaction is not crucial for ERK2 activation. The Gα4 subunit with the D-motif alteration rescues many Gα4-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 that morphogenesis in late development might be more sensitive to reduced Gα4 subunit-ERK2 interactions. It is possible that earlier stages of development might be affected by D-motif

alterations that completely disrupt Gα4 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. Although some overlap in ERK activation exists in *Dictyostelium* and other organisms, it is possible that this overlap is not significant with regard to cellular responses and could 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 that 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 because 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 that *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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References

1. Belcheva MM, Coscia CJ. Diversity of G protein-coupled receptor signaling pathways to ERK/MAP kinase. Neurosignals 2002; 11: 34–44.
2. Chen RE, Thorner J. Function and regulation in MAPK signaling pathways: lessons learned from the yeast Saccharomyces cerevisiae. Biochim Biophys Acta 2007; 1773: 1311–40.
3. Pimienta G, Pascual J. Canonical and alternative MAPK signaling. Cell Cycle 2007; 6: 2628–32.
4. Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. Oncogene 2007; 26: 3100–12.
5. Andreasson E, Ellis B. Convergence and specificity in the Arabidopsis MAPK nexus. Trends Plant Sci 2010; 15: 106–13.
6. Aubert J, Belmonte N, Dani C. Role of pathways for signal transducers and activators of transcription, and mitogen-activated protein kinase in adipocyte differentiation. Cell Mol Life Sci 1999; 56: 538–42.
7. Bost F, Aouadi M, Caron L, Binetruy B. The role of MAPKs in adipocyte differentiation and obesity. Biochimie 2005; 87: 51–6.
8. Lawrence MC, Jivan A, Shao C, Duan L, Goad D, Zaganjor E, Osborne J, McGlynn K, Stippec S, Earnest S, Chen W, Cobb MH. The roles of MAPKs in disease. Cell Res 2008; 18: 436–42.
9. Nakamura T, Gulick J, Pratt R, Robbins J. Noonan syndrome is associated with enhanced pERK activity, the repression of which can prevent craniofacial malformations. Proc Natl Acad Sci USA 2009; 106: 15436–41.
10. Tidyman WE, Rauen KA. Noonan, Costello and cardio-facio-cutaneous syndromes: dysregulation of the Ras-MAPK pathway. Expert Rev Mol Med 2008; 10: e37.
11. Howe AK, Aplin AE, Juliano RL. Anchorage-dependent ERK signaling – mechanisms and consequences. Curr Opin Genet Dev 2002; 12: 30–5.
12. Westhoff MA, Fulda S. Adhesion-mediated apoptosis resistance in cancer. Drug Resist Updat 2009; 12: 127–36.
13. Bost F, Aouadi M, Caron L, Even P, Belmonte N, Prot M, Dani C, Hofman P, Pages G, Pouyssegur J, Le Marchand-Brustel Y, Binetruy B. The extracellular signal-regulated kinase isoform ERK1 is specifically required for in vitro and in vivo adipogenesis. Diabetes 2005; 54: 402–11.
14. Pages G, Pouyssegur J. Study of MAPK signaling using knockout mice. Methods Mol Biol 2004; 250: 155–66.
15. Nguyen HN, Raisley B, Hadwiger JA. MAP kinases have different functions in *Dictyostelium* G protein-mediated signaling. Cell Signal 2010; 22: 836–47.
16. Miranda-Saavedra D, Barton GJ. Classification and functional annotation of eukaryotic protein kinases. Proteins 2007; 68: 893–914.
17. Rispail N, Soanes DM, Ant C, Czajkowski R, Grunler A, Huguet R, Perez-Nadales E, Poli A, Sartorel E, Valiante V,

Yang M, Beffa R, Brakhage AA, Gow NA, Kahmann R, Lebrun MH, Lenasi H, Perez-Martin J, Talbot NJ, Wendland J, Di Pietro A. Comparative genomics of MAP kinase and calcium-calcineurin signalling components in plant and human pathogenic fungi. Fungal Genet Biol 2009; 46: 287–98.
18. Ligterink W. MAP kinases in plant signal transduction: how many, and what for? Results Probl Cell Differ 2000; 27: 11–27.
19. Hunter T, Plowman GD. The protein kinases of budding yeast: six score and more. Trends Biochem Sci 1997; 22: 18–22.
20. Chen Z, Gibson TB, Robinson F, Silvestro L, Pearson G, Xu B, Wright A, Vanderbilt C, Cobb MH. MAP kinases. Chem Rev 2001; 101: 2449–76.
21. Dhanasekaran DN, Kashef K, Lee CM, Xu H, Reddy EP. Scaffold proteins of MAP-kinase modules. Oncogene 2007; 26: 3185–202.
22. van Drogen F, Peter M. MAP kinase cascades: scaffolding signal specificity. Curr Biol 2002; 12: R53–5.
23. Goldsmith ZG, Dhanasekaran DN. G protein regulation of MAPK networks. Oncogene 2007; 26: 3122–42.
24. Lorenz K, Schmitt JP, Schmitteckert EM, Lohse MJ. A new type of ERK1/2 autophosphorylation causes cardiac hypertrophy. Nat Med 2009; 15: 75–83.
25. MacKenzie SJ, Baillie GS, McPhee I, Bolger GB, Houslay MD. ERK2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases. The involvement of COOH-terminal docking sites and NH_2-terminal UCR regions. J Biol Chem 2000; 275: 16609–17.
26. Maeda M, Lu S, Shaulsky G, Miyazaki Y, Kuwayama H, Tanaka Y, Kuspa A, Loomis WF. Periodic signaling controlled by an oscillatory circuit that includes protein kinases ERK2 and PKA. Science 2004; 304: 875–8.
27. Anjum R, Blenis J. The RSK family of kinases: emerging roles in cellular signalling. Nat Rev Mol Cell Biol 2008; 9: 747–58.
28. Bardwell AJ, Abdollahi M, Bardwell L. Docking sites on mitogen-activated protein kinase (MAPK) kinases, MAPK phosphatases and the Elk-1 transcription factor compete for MAPK binding and are crucial for enzymic activity. Biochem J 2003; 370: 1077–85.
29. Yang SH, Yates PR, Whitmarsh AJ, Davis RJ, Sharrocks AD. The Elk-1 ETS-domain transcription factor contains a mitogen-activated protein kinase targeting motif. Mol Cell Biol 1998; 18: 710–20.
30. Songyang Z, Lu KP, Kwon YT, Tsai LH, Filhol O, Cochet C, Brickey DA, Soderling TR, Bartleson C, Graves DJ, DeMaggio AJ, Hoekstra MF, Blenis J, Hunter T, Cantley LC. A structural basis for substrate specificities of protein Ser/Thr kinases: primary sequence preference of casein kinases I and II, NIMA, phosphorylase kinase, calmodulin-dependent kinase II, CDK5, and Erk1. Mol Cell Biol 1996; 16: 6486–93.
31. Tanoue T, Nishida E. Molecular recognitions in the MAP kinase cascades. Cell Signal 2003; 15: 455–62.
32. Sheridan DL, Kong Y, Parker SA, Dalby KN, Turk BE. Substrate discrimination among mitogen-activated protein kinases through distinct docking sequence motifs. J Biol Chem 2008; 283: 19511–20.
33. Lloyd AC. Distinct functions for ERKs? J Biol 2006; 5: 13.
34. Maeda M, Aubry L, Insall R, Gaskins C, Devreotes PN, Firtel RA. Seven helix chemoattractant receptors transiently stimulate mitogen-activated protein kinase in *Dictyostelium* – role of heterotrimeric G proteins. J Biol Chem 1996; 271: 3351–4.
35. Sobko A, Ma H, Firtel RA. Regulated SUMOylation and ubiquitination of DdMEK1 is required for proper chemotaxis. Dev Cell 2002; 2: 745–56.
36. Flatauer LJ, Zadeh SF, Bardwell L. Mitogen-activated protein kinases with distinct requirements for Ste5 scaffolding influence signaling specificity in Saccharomyces cerevisiae. Mol Cell Biol 2005; 25: 1793–803.
37. Remenyi A, Good MC, Bhattacharyya RP, Lim WA. The role of docking interactions in mediating signaling input, output, and discrimination in the yeast MAPK network. Mol Cell 2005; 20: 951–62.
38. Pages G, Guerin S, Grall D, Bonino F, Smith A, Anjuere F, Auberger P, Pouyssegur J. Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice. Science 1999; 286: 1374–7.
39. Yao Y, Li W, Wu J, Germann UA, Su MS, Kuida K, Boucher DM. Extracellular signal-regulated kinase 2 is necessary for mesoderm differentiation. Proc Natl Acad Sci USA 2003; 100: 12759–64.
40. Krens SF, Corredor-Adamez M, He S, Snaar-Jagalska BE, Spaink HP. ERK1 and ERK2 MAPK are key regulators of distinct gene sets in zebrafish embryogenesis. BMC Genomics 2008; 9: 196.
41. Krens SF, He S, Lamers GE, Meijer AH, Bakkers J, Schmidt T, Spaink HP, Snaar-Jagalska BE. Distinct functions for ERK1 and ERK2 in cell migration processes during zebrafish gastrulation. Dev Biol 2008; 319: 370–83.
42. Goldberg JM, Manning G, Liu A, Fey P, Pilcher KE, Xu Y, Smith JL. The *Dictyostelium* kinome – analysis of the protein kinases from a simple model organism. PLoS Genet 2006; 2: e38.
43. Loomis WF, editor. The development of *Dictyostelium discoideum*. New York: Academic Press, 1982.
44. Williams JG. Transcriptional regulation of *Dictyostelium* pattern formation. EMBO Rep 2006; 7: 694–8.
45. Eichinger L, Pachebat JA, Glockner G, Rajandream MA, Sucgang R, Berriman M, Song J, Olsen R, Szafranski K, Xu Q, Tunggal B, Kummerfeld S, Madera M, Konfortov BA, Rivero F, Bankier AT, Lehmann R, Hamlin N, Davies R, Gaudet P, Fey P, Pilcher K, Chen G, Saunders D, Sodergren E, Davis P, Kerhornou A, Nie X, Hall N, Anjard C, Hemphill L, Bason N, Farbrother P, Desany B, Just E, Morio T, Rost R, Churcher C, Cooper J, Haydock S, van Driessche N, Cronin A, Goodhead I, Muzny D, Mourier T, Pain A, Lu M, Harper D, Lindsay R, Hauser H, James K, Quiles M, Madan Babu M, Saito T, Buchrieser C, Wardroper A, Felder M, Thangavelu M, Johnson D, Knights A, Loulseged H, Mungall K, Oliver K, Price C, Quail MA, Urushihara H, Hernandez J, Rabbinowitsch E, Steffen D, Sanders M, Ma J, Kohara Y, Sharp S, Simmonds M, Spiegler S, Tivey A, Sugano S, White B, Walker D, Woodward J, Winckler T, Tanaka Y, Shaulsky G, Schleicher M, Weinstock G, Rosenthal A, Cox EC, Chisholm RL, Gibbs R, Loomis WF, Platzer M, Kay RR, Williams J, Dear PH, Noegel AA, Barrell B, Kuspa A. The genome of the social amoeba *Dictyostelium discoideum*. Nature 2005; 435: 43–57.
46. Fey P, Gaudet P, Curk T, Zupan B, Just EM, Basu S, Merchant SN, Bushmanova YA, Shaulsky G, Kibbe WA, Chisholm R. dictyBase – a *Dictyostelium* bioinformatics resource update. Nucleic Acids Res 2009; 37: D515–9.
47. Johnson RL, Van Haastert PJ, Kimmel AR, Saxe CL 3rd, Jastorff B, Devreotes PN. The cyclic nucleotide specificity of three cAMP receptors in *Dictyostelium*. J Biol Chem 1992; 267: 4600–7.
48. Saxe CL 3rd, Johnson R, Devreotes PN, Kimmel AR. Multiple genes for cell surface cAMP receptors in *Dictyostelium discoideum*. Dev Genet 1991; 12: 6–13.

49. Johnson RL, Saxe CL 3rd, Gollop R, Kimmel AR, Devreotes PN. Identification and targeted gene disruption of cAR3, a cAMP receptor subtype expressed during multicellular stages of *Dictyostelium* development. Genes Dev 1993; 7: 273–82.
50. Louis JM, Ginsburg GT, Kimmel AR. The cAMP receptor CAR4 regulates axial patterning and cellular differentiation during late development of *Dictyostelium*. Genes Dev 1994; 8: 2086–96.
51. Saxe CL 3rd, Ginsburg GT, Louis JM, Johnson R, Devreotes PN, Kimmel AR. CAR2, a prestalk cAMP receptor required for normal tip formation and late development of *Dictyostelium discoideum*. Genes Dev 1993; 7: 262–72.
52. Sun TJ, Devreotes PN. Gene targeting of the aggregation stage cAMP receptor cAR1 in *Dictyostelium*. Genes Dev 1991; 5: 572–82.
53. Yu Y, Saxe CL 3rd. Differential distribution of cAMP receptors cAR2 and cAR3 during *Dictyostelium* development. Dev Biol 1996; 173: 353–6.
54. Gaskins C, Maeda M, Firtel RA. Identification and functional analysis of a developmentally regulated extracellular signal-regulated kinase gene in *Dictyostelium discoideum*. Mol Cell Biol 1994; 14: 6996–7012.
55. Segall JE, Kuspa A, Shaulsky G, Ecke M, Maeda M, Gaskins C, Firtel RA, Loomis WF. A MAP kinase necessary for receptor-mediated activation of adenylyl cyclase in *Dictyostelium*. J Cell Biol 1995; 128: 405–13.
56. Maeda M, Firtel RA. Activation of the mitogen-activated protein kinase ERK2 by the chemoattractant folic acid in *Dictyostelium*. J Biol Chem 1997; 272: 23690–5.
57. Kosaka C, Pears CJ. Chemoattractants induce tyrosine phosphorylation of ERK2 in *Dictyostelium discoideum* by diverse signalling pathways. Biochem J 1997; 324: 347–52.
58. Brzostowski JA, Kimmel AR. Nonadaptive regulation of ERK2 in *Dictyostelium*: implications for mechanisms of cAMP relay. Mol Biol Cell 2006; 17: 4220–7.
59. Knetsch ML, Epskamp SJ, Schenk PW, Wang Y, Segall JE, Snaar-Jagalska BE. Dual role of cAMP and involvement of both G-proteins and ras in regulation of ERK2 in *Dictyostelium discoideum*. EMBO J 1996; 15: 3361–8.
60. Wang Y, Liu J, Segall JE. MAP kinase function in amoeboid chemotaxis. J Cell Sci 1998; 111: 373–83.
61. Gaskins C, Clark AM, Aubry L, Segall JE, Firtel RA. The *Dictyostelium* MAP kinase ERK2 regulates multiple, independent developmental pathways. Genes Dev 1996; 10: 118–28.
62. Chen S, Segall JE. EppA, a putative substrate of DdERK2, regulates cyclic AMP relay and chemotaxis in *Dictyostelium discoideum*. Eukaryot Cell 2006; 5: 1136–46.
63. Nguyen HN, Hadwiger JA. The Gα4 G protein subunit interacts with the MAP kinase ERK2 using a D-motif that regulates developmental morphogenesis in *Dictyostelium*. Dev Biol 2009; 335: 385–95.
64. Hadwiger JA, Lee S, Firtel RA. The G alpha subunit Gα4 couples to pterin receptors and identifies a signaling pathway that is essential for multicellular development in *Dictyostelium*. Proc Natl Acad Sci USA 1994; 91: 10566–70.
65. Wu L, Valkema R, Van Haastert PJ, Devreotes PN. The G protein β subunit is essential for multiple responses to chemoattractants in *Dictyostelium*. J Cell Biol 1995; 129: 1667–75.
66. Hadwiger JA, Firtel RA. Analysis of Gα4, a G-protein subunit required for multicellular development in *Dictyostelium*. Genes Dev 1992; 6: 38–49.
67. Ma H, Gamper M, Parent C, Firtel RA. The *Dictyostelium* MAP kinase kinase DdMEK1 regulates chemotaxis and is essential for chemoattractant-mediated activation of guanylyl cyclase. EMBO J 1997; 16: 4317–32.
68. Hadwiger JH, Natarajan K, Firtel RA. Mutations in the *Dictyostelium* heterotrimeric G protein α subunit Gα5 alter the kinetics of tip morphogenesis. Development 1996; 122: 1215–24.
69. Raisley B, Nguyen HN, Hadwiger JA. Gα5 subunit-mediated signalling requires a D-motif and the MAPK ERK1 in *Dictyostelium*. Microbiology (Reading) 2010; 156: 789–97.
70. Brzostowski JA, Kimmel AR. Signaling at zero G: G-protein-independent functions for 7-TM receptors. Trends Biochem Sci 2001; 26: 291–7.
71. Hadwiger JA. Developmental morphology and chemotactic responses are dependent on Gα subunit specificity in *Dictyostelium*. Dev Biol 2007; 312: 1–12.
72. Metodiev MV, Matheos D, Rose MD, Stone DE. Regulation of MAPK function by direct interaction with the mating-specific Gα in yeast. Science 2002; 296: 1483–6.
73. Blackwell E, Kim HJ, Stone DE. The pheromone-induced nuclear accumulation of the Fus3 MAPK in yeast depends on its phosphorylation state and on Dig1 and Dig2. BMC Cell Biol 2007; 8: 44.
74. Bessard A, Fremin C, Ezan F, Fautrel A, Gailhouste L, Baffet G. RNAi-mediated ERK2 knockdown inhibits growth of tumor cells in vitro and in vivo. Oncogene 2008; 27: 5315–25.