MAP kinases and cell migration

Cai Huang[1,*], Ken Jacobson[1,2,3] and Michael D. Schaller[1,2,3,‡]

[1]Department of Cell and Developmental Biology, [2]Lineberger Comprehensive Cancer Center and [3]Comprehensive Center for Inflammatory Disorders, University of North Carolina, Chapel Hill, NC 27599-7090, USA

*Present address: Division of Rheumatology, Department of Medicine, University of California San Diego, La Jolla, CA 92093-0726, USA

‡Author for correspondence (e-mail: crispy4@med.unc.edu)

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Summary

Recent studies have demonstrated that mitogen-activated protein kinases (MAPKs), including Jun N-terminus kinase (JNK), p38 and Erk, play crucial roles in cell migration. JNK, for example, regulates cell migration by phosphorylating paxillin, DCX, Jun and microtubule-associated proteins. Studies of p38 show that this MAPK modulates migration by phosphorylating MAPK-activated protein kinase 2/3 (MAPKAP 2/3), which appears to be important for directionality of migration. Erk governs cell movement by phosphorylating myosin light chain kinase (MLCK), calpain or FAK. Thus, the different kinases in the MAPK family all seem able to regulate cell migration but by distinct mechanisms.

Key words: Cell migration, JNK, p38, Erk, Paxillin, Phosphorylation

Introduction

Many extracellular signals converge at a family of serine/threonine protein kinases called mitogen-activated protein kinases (MAPKs) (Johnson and Lapadat, 2002). All MAPKs contain a Thr-x-Tyr motif within the activation loop in the kinase domain. MAPKs are activated through a kinase cascade in which MAPKKKs activate MKKs, which in turn activate MAPKs by phosphorylating the threonine and tyrosine residues within the activation loop. On the basis of the differences in the motifs within their activation loops, the MAPK family can be divided into three groups: extracellular-signal-regulated protein kinase (Erk/MAPK), which has a Thr-Glu-Tyr motif; p38, which has a Thr-Ala-Tyr motif, and Jun N-terminus kinase (JNK), which has a Thr-Pro-Tyr motif. MAPKs play well-known roles in cell proliferation, oncogenesis, differentiation, inflammation and stress responses (Johnson and Lapadat, 2002), but accumulating evidence indicates that this family is also essential for cell migration. Xia and Karin recently reviewed the physiological role of JNK in regulating cell migration during epithelial morphogenesis in *Drosophila* and mice (Xia and Karin, 2004). Here, we summarize the general roles of MAPKs in cell migration, emphasizing their mechanisms of action and recent progress in our understanding of the role of JNK.

JNK signaling

JNK is activated in response to various extracellular stimuli, including tumor necrosis factor (TNF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β) and lysophosphatidic acid, as well as diverse environmental stresses (Derijard et al., 1994; Huang et al., 2003; Kyriakis et al., 1994; Rosette and Karin, 1996; Xia et al., 2000; Yujiri et al., 2000; Zhang et al., 2003). These activate MAPKKKs: such as MEKK1 and MLK, which phosphorylate and activate two MAPKKs, MKK4 and MKK7. In turn, these phosphorylate the threonine and tyrosine residues within the Thr-Pro-Tyr motif in the JNK activation loop. Cytoskeletal proteins, such as tensin and frabin, also participate in JNK activation through unknown mechanisms (Katz et al., 2000; Lee et al., 2003; Umikawa et al., 1999). Interestingly, several signaling pathways that control cell migration, including the Rac, focal adhesion kinase (FAK) and Src pathways, converge at JNK (Almeida et al., 2000; Minden et al., 1995; Oktay et al., 1999). Especially significant in this context is the observation that several targets of the JNK signaling pathway are proteins involved in cell migration.

JNK is essential for cell migration

JNKs are generally thought to play roles in inflammation, differentiation, apoptosis and insulin resistance (Barr and Bogoyevitch, 2001; Ip and Davis, 1998). However, accumulating evidence also implicates the JNK pathway in regulation of cell migration. First, activation of JNK correlates with an increase in cell migration in several cell types, for example, JNK activation is closely associated with cell migration induced by EGF, ephrin B1 and CrkII (Hauck et al., 2001; Huynh-Do et al., 2002; Abassi and Vuori, 2002). Second, the signaling molecules that activate JNK are essential for cell migration. MEK kinase 1, an upstream kinase in the JNK pathway, is essential for cell migration and the developmental process of eyelid closure (Xia et al., 2000; Yujiri et al., 2000). Also, activating JNK by expressing constitutively active MKK4 promotes cell migration in the ECV304 endothelial cell line (Shin et al., 2001). Third, inhibition of JNK by either the chemical inhibitor SP600125 or the dominant-negative mutant JNK1AF, significantly impairs the rate of migration of several different cell types. SP600125 inhibits the migration of fish keratocytes, rat bladder tumor (NBT-II) epithelial cells, Swiss 3T3 fibroblasts, human dermal fibroblasts, mouse embryonic fibroblasts, embryonic stem cells, aortic vascular smooth muscle cells, cortical neurons, epidermal keratinocytes as well

as Schwann cells (Huang et al., 2003; Javelaud et al., 2003; Kavurma and Khachigian, 2003; Kawauchi et al., 2003; Xia et al., 2000; Yamauchi et al., 2003; Zhang et al., 2003). JNK1AF inhibits the movement of NBT-II epithelial cells, bovine aortic endothelial cells, human umbilical vein endothelial cells and cortical neurons (Huang et al., 2003; Kawauchi et al., 2003; Meadows et al., 2004; Pedram et al., 2001). Fourth, using a gene knockout approach, Javelaud et al., have demonstrated that JNK activity plays a crucial role in the migration of fibroblasts in wound healing assays (Javelaud et al., 2003). It is also required for *Drosophila* dorsal closure, a developmental process dominated by cell migration (Riesgo-Escovar et al., 1996; Sluss et al., 1996). These findings collectively implicate JNK in the control of cell migration in a broad range of cell types and in several developmental processes.

JNK is involved in cytoskeleton reorganization

Recent studies in *Drosophila* embryos have implicated JNK in the control of the actin cytoskeleton. JNK is required for the maturation of actin-nucleating centers, the formation of filopodia and lamellipodia, and cell spreading during dorsal and thorax closure of *Drosophila* (Martin-Blanco et al., 2000; Kaltschmidt et al., 2002). The role of JNK in cytoskeleton reorganization is further supported by the finding that cytoskeletal regulators, such as profilin and cortactin, are upregulated by a constitutively active JNK kinase, and are downregulated by a dominant negative mutant of JNK (Jasper et al., 2001).

JNK has been implicated in regulation of stress fibers in mammalian cells. TGF-β stimulates stress fiber formation in the centers of the MEKK1$^{+/-}$ keratinocytes in a JNK-dependent manner, but causes an accumulation of stress fibers at the edges of the MEKK1$^{-/-}$ cells (Zhang et al., 2003). Thus, inhibition of the JNK pathway seems to cause the formation of stress fibers around cell edges in some cell types, which indicates that JNK might suppress stress fiber formation at these sites in migratory cells. However, JNK activation disrupts stress fibers in fibroblasts (Rennefahrt et al., 2002). These contradictory findings might reflect cell type differences in regulation of the cytoskeleton by JNK. Clearly, these differences and other details of the role of JNK in cytoskeleton reorganization require further investigation.

Active JNK is localized in the cytoplasm as well as the nucleus

After JNK is activated by MKK4 and MKK7, a portion of active JNK enters the nucleus, where it phosphorylates several transcription factors, resulting in an increase in transcriptional activity (Minden et al., 1995; Gupta et al., 1996). However, JNK activated by constitutively active Rac1 accumulates in the apical cytoplasm but not the nucleus of intestinal epithelial cells (Stappenbeck and Gordon, 2001). Also, in cerebellar granule neurons, active JNK is predominantly localized in cytoplasmic compartments (Coffey et al., 2000). Furthermore, active JNK is localized at focal adhesions, and MEKK1, a kinase upstream of JNK, is associated with α-actinin and FAK (Almeida et al., 2000; Christerson et al., 1999; Yujiri et al., 2003). The observations that active JNK is found in cytoplasmic locations provide additional evidence for cytoplasmic functions of JNK, in addition to its established nuclear functions.

JNK substrates and their roles in migration

Along with the various well-known transcription factors and apoptosis-related proteins that are substrates for JNK (Barr and Bogoyevitch, 2001), several cytoskeleton-associated proteins and signaling molecules as well as adaptor proteins have recently been identified as JNK substrates. These include the intermediate filament protein keratin 8 (He et al., 2002), microtubule-associated proteins (MAPs), such as MAP1B, MAP2, DCX and SCG10 (Chang et al., 2003; Gdalyahu et al., 2004; Neidhart et al., 2001), the actin-binding protein spir (Otto et al., 2000), the protein kinase p90RSK (Zhang et al., 2001), and the adaptors insulin receptor substrate 1 (Irs-1), p66ShcA and paxillin (Aguirre et al., 2000; Huang et al., 2003; Le et al., 2001). Of these, paxillin, spir, DCX, MAP1B and MAP2 are probably directly involved in cell migration (Fig. 1).

Paxillin

Paxillin is a focal adhesion adaptor involved in focal-adhesion dynamics and cell migration (Turner, 2000; Schaller, 2001a). It features five copies of a 13-residue 'LD' motif and a proline-

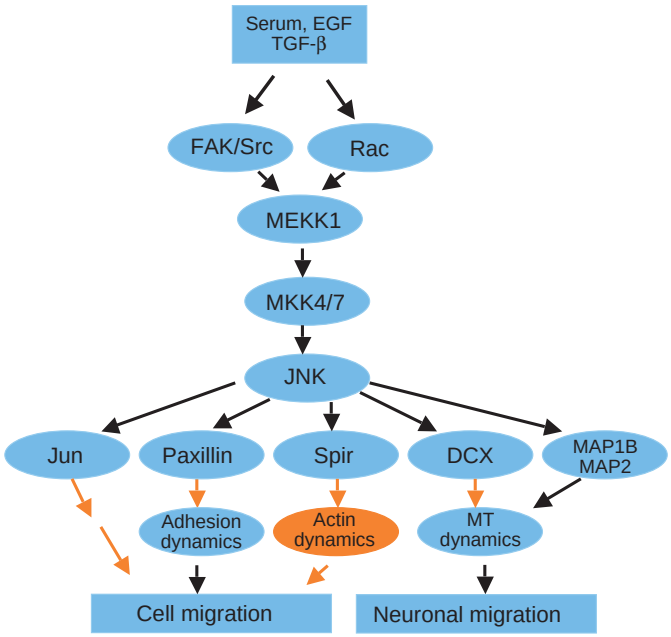

Fig. 1. Signaling pathways for cell migration mediated by JNK. Extracellular stimuli, such as serum, EGF and TGF-β, activate MEKK1 through FAK/Src or Rac. Active MEKK1 phosphorylates and activates MKK4 or MKK7, which then phosphorylates and activates JNK. The activated JNK in turn phosphorylates Jun, paxillin, Spir, DCX and MAPs. Paxillin phosphorylation might facilitate cell adhesion turnover, thus promoting rapid migration of cells. The phosphorylation of DCX, MAP1B and MAP2 might promote microtubule dynamics, thus enhancing neuronal migration. The phosphorylation of Jun is also involved in cell migration, but the mechanism is unknown. Spir phosphorylation might also play a role in actin dynamics and cell migration. MT, microtubule. Orange lines represent speculative pathways.

rich region in the N-terminal half of the protein (Turner and Miller, 1994; Salgia et al., 1995). The C-terminal half contains four LIM domains; this region is thus similar to a double-Zn-finger domain and is involved in protein-protein interactions (Dawid et al., 1998; Turner and Miller, 1994; Salgia et al., 1995). Numerous adhesion and signaling molecules, such as vinculin, integrin α 4 and integrin α9, focal adhesion kinase (FAK), cell adhesion kinase β (CAKβ; also known as Pyk2, RAFTK and CadTK), integrin-linked kinase (ILK), PTP-PEST, PKL (paxillin–kinase linker) and its two relatives, Git1 and Git2, bind to paxillin, largely through LD-motif- or LIM-domain-mediated interactions (Schaller, 2001a).

In addition to tyrosine phosphorylation, paxillin is also phosphorylated at serine residues in many systems in response to adhesion to fibronectin and growth factor stimulation (Bellis et al., 1997; Huang et al., 2003; Liu et al., 2002; Vadlamudi et al., 1999). We recently identified paxillin as a novel JNK substrate (Huang et al., 2003). JNK efficiently phosphorylates paxillin at Ser178 in vitro, and expression of JNK promotes the specific phosphorylation of Ser178 in vivo. Moreover, EGF stimulates phosphorylation of Ser178 in MDA-MB-231 human breast cancer cells and the JNK inhibitor SP600125 blocks this effect. Interestingly, expression of a Ser178 to Ala mutant of paxillin (Pax_{S178A}) significantly inhibits the migration of NBT-II cells, MDA-MB-231 human breast cancer cells, and Chinese hamster ovary (CHO-K1) cells, whereas expression of wild-type paxillin has no obvious effect on cell migration. Furthermore, expression of Pax_{S178A} promotes the formation of focal adhesions and stress fibers in NBT-II cells. Cells expressing wild-type paxillin retain labile close contacts that are associated with rapid cell migration. Considering the crucial role of paxillin in cell migration (Hagel et al., 2002; Liu et al., 1999), this study points to a novel regulatory role played by JNK in cell migration. Understanding exactly how JNK-mediated phosphorylation of paxillin regulates cell migration and adhesions is a direction for future study. We speculate that phosphorylation of paxillin by JNK stimulates paxillin degradation and focal adhesion disassembly, thus promoting cell migration (Huang et al., 2004a). JNK-mediated phosphorylation might instead disrupt the interaction between paxillin and its binding partners. FAK, a direct binding partner and PAK, an indirect binding partner, have been implicated in regulating focal adhesion disassembly (Webb et al., 2004; Zhao et al., 2000). Disruption of such interactions might be an important facet of focal-adhesion turnover.

DCX

DCX is encoded by the X-linked gene *DCX*, which when mutated results in abnormal cortical lamination in the brain owing to neuronal migration defects (Gleeson et al., 1998). The protein is a MAP that stabilizes microtubules (Horesh et al., 1999). It contains a conserved doublecortin (DC) domain, which interacts with microtubules. Recently, Gdalyahu et al. reported that DCX is associated with both JNK and the JNK-interacting protein JIP (Gdalyahu et al., 2004). Moreover, JNK phosphorylates DCX at Thr331 and Ser334 in vitro and in vivo. Furthermore, the Thr331-Ser334 to Ala mutant of DCX significantly inhibits neurite outgrowth and neuronal migration. These findings implicate JNK-mediated phosphorylation in the control of these processes; however, it is unknown whether phosphorylation affects the ability of DCX to stabilize microtubules or whether this involves a different mechanism.

Spir

Spir is a member of the Wiscott-Aldrich syndrome protein (WASP) homology domain 2 (WH2) family and is involved in actin reorganization (Otto et al., 2000). It is highly conserved between species. The protein contains an acidic domain, a cluster of four WH2 domains, a modified FYVE zinc finger domain and a C-terminal D domain (docking site for MAPK family kinases). The D domain is characterized by a cluster of basic residues followed by an (L/I)x(L/I) motif (Fantz et al., 2001). The WH2 domains bind to monomeric actin, and the D domain constitutes a docking site for JNK or Erk (Otto et al., 2000). In mouse fibroblasts, Spir colocalizes with F-actin and overexpression of Spir induces clustering of F-actin around the nucleus. Spir also colocalizes with JNK and might function to localize JNK.

Spir is phosphorylated in vitro by a JNK2-MKK7 fusion protein that has constitutively active JNK activity (Otto et al., 2000). In NIH 3T3 cells, coexpression of Spir with this mutant induces an electrophoretic mobility shift in Spir that is reversed by phosphatase treatment. An equivalent Erk mutant has little effect (Otto et al., 2000). JNK can therefore probably phosphorylate Spir in vivo. Whether Spir is a physiological substrate for JNK, and, if so, the identity of the phosphorylation site, remain to be determined. Nevertheless, Spir remains an intriguing candidate for a JNK substrate that could function in regulation of the cytoskeleton and cell motility, and further analysis to examine the role of its phosphorylation in cell migration is certainly warranted.

Microtubule-associated proteins

MAPs are a group of proteins that stabilize microtubules, organize them into bundles, and connect them to membranes and intermediate filaments (Maccioni and Cambiazo, 1995). They can be divided into two types: type I MAPs include MAP1A and MAP1B, and type II MAPs consist of MAP2, MAP4 and Tau. The ability of MAPs to modulate microtubule dynamics is regulated by their phosphorylation. For example, phosphorylation of MAP2 by protein kinase A and an embryonic MAP2 kinase decreases its affinity for microtubules (Burns et al., 1984; Lopez and Sheetz, 1995), whereas phosphorylation of MAP4 by Cdc2 kinase abolishes its microtubule-stabilizing activity without affecting its binding to microtubules (Ookata et al., 1995).

Several lines of evidence indicate that MAP2 and MAP1B are substrates for JNK in vitro and in vivo (Chang et al., 2003; Kawauchi et al., 2003). First, MAP2 and MAP1B are phosphorylated by JNK1 in vitro. Second, 2D electrophoretic analysis of MAP2 and MAP1B from $JNK^{-/-}$ brain indicates that the phosphorylation of MAP2 and MAP1B is inhibited in vivo. Third, the phosphorylation of MAP1B is inhibited by the JNK inhibitor SP600125. Thus, these MAPs appear to be bona fide JNK substrates, although the JNK-target sites remain to be determined.

JNK1-mediated phosphorylation of MAP2 and MAP1B seems to be required for maintenance of neuronal microtubules

and neuronal migration (Chang et al., 2003; Kawauchi et al., 2003). MAP2 and MAP1B from JNK$^{-/-}$ mice are hypophosphorylated and exhibit defective microtubule-binding and promotion of microtubule-assembly. Moreover, JNK$^{-/-}$ mice exhibit disorganized neuronal microtubules and progressive degeneration of long nerve fibers (Chang et al., 2003). Treatment of neurons with SP600125 also causes abnormal microtubules (Kawauchi et al., 2003). Because MAPs have many potential JNK-target sites (Ser/Thr-Pro), it is most likely that JNK directly phosphorylates MAPs and enhances their affinity for microtubules, thus promoting microtubule polymerization and neuronal migration. While this is an attractive hypothesis, it remains to be conclusively established that the phenotypes induced by inhibition of JNK signaling are primarily because of defects in phosphorylation of MAP2 and MAP1B.

Jun

Another possible mechanism involved in JNK-induced cell migration is the regulation of Jun-activity. Recently, Javelaud et al. demonstrated that expression of JunAA, a Jun mutant in which Ser63 and Ser73 are mutated to alanine residues so that the protein cannot be phosphorylated by JNK, significantly inhibits the migration of fibroblasts in a wound healing assay (Javelaud et al., 2003). These data suggest that JNK-mediated phosphorylation of Jun plays a critical role in cell migration. Although phosphorylation of Jun by JNK is generally thought to increase transcription, transcriptional activity does not seem to be crucial for the migration of fibroblasts in the wound healing assay (Javelaud et al., 2003). Hence, the underlying mechanism is unclear.

p38 signaling

Four isoforms represent the p38 subfamily of MAPKs: p38α, p38β, p38γ and p38δ. The activity of p38 is stimulated by many growth factors, cytokines, and chemotactic substances, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), PDGF, TNF, interleukins, lipopolysaccharide (LPS) and formyl-methionyl-leucyl-phenylalanine (fMLP) (Ono and Han, 2000). The upstream MAPK cascade in this case includes MAPKKKs such as MLK3, DLK and TAK1, which phosphorylate and activate MKK3 and MKK6, which in turn phosphorylate and activate p38 (Herlaar and Brown, 1999).

p38 is involved in cell migration

It is well known that p38 is involved in inflammation, apoptosis, cardiomyocyte hypertrophy and cell differentiation (Ono and Han, 2000). Recently, several studies have suggested it is also involved in the migration of diverse cell types. SB203580 and SB202190, inhibitors of p38, inhibit the migration of smooth muscle cells induced by PDGF, TGFβ and IL-1β (Hedges et al., 1999; Kotlyarov et al., 2002), porcine aortic endothelial cells challenged with PDGF and VEGF (Matsumoto et al., 1999; Rousseau et al., 1997), neutrophils stimulated with fMLP (Heuertz et al., 1999), mast cells treated with stem cell factors and antigen (Ishizuka et al., 2001; Sundstrom et al., 2001), corneal epithelial cells stimulated with hepatocyte growth factor (Sharma et al., 2003), mouse embryonic fibroblasts challenged with PDGF and IL-1 (Kotlyarov et al., 2002), NMuMG and MDA-MB-231 mammary epithelial cells treated with EGF and TGFβ1 (Klekotka et al., 2001; Bakin et al., 2002) and NLT neuronal cells stimulated with Gas6 (encoded by growth arrest-specific gene 6) (Allen et al., 2002). Moreover, p38AF, a dominant negative mutant of p38, also inhibits the migration of smooth muscle cells induced by PDGF, TGFβ and IL-1β (Hedges et al., 1999; Kotlyarov et al., 2002), NMuMG and MDA-MB-231 mammary epithelial cells in response to EGF and TGFβ1 (Klekotka et al., 2001; Bakin et al., 2002) and NLT neuronal cells stimulated with Gas6 (Allen et al., 2002). In addition, p38 is activated by these migration stimulators. Taken together, these findings demonstrate that p38 is involved in growth-factor- and cytokine-induced cell migration.

p38 substrates and their roles in migration

Several protein kinases, such as MAPK-activated protein kinase 2/3 (MAPKAPK 2/3) (McLaughlin et al., 1996), MAPK-interaction protein kinase 1 (also called MNK1) (Fukunaga and Hunter, 1997) and p38-regulated/activated kinase (PRAK) (New et al., 1998), are substrates for p38. Its substrates also include the Na^+/H^+ exchanger 1 (NHE1) (Khaled et al., 2001), cytosolic phospholipase A2 (cPLA2) (Kramer et al., 1996), caldesmon (Goncharova et al., 2002) and paxillin (Huang et al., 2004b), as well as several transcription

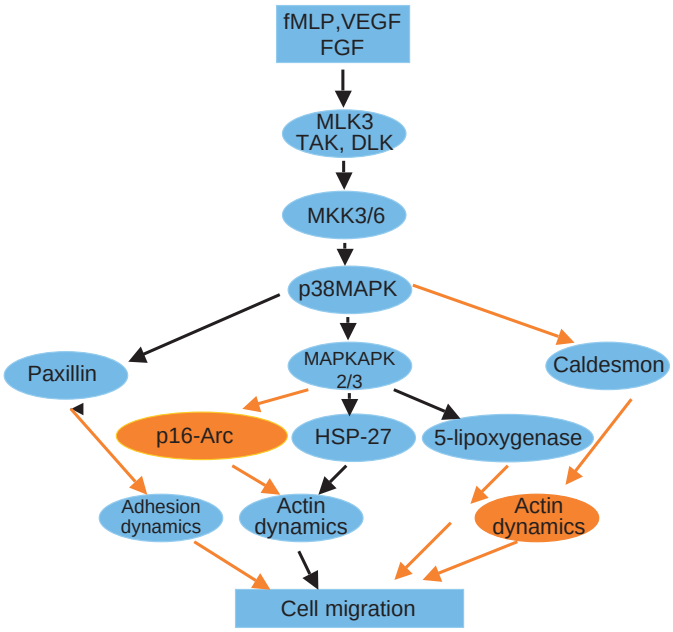

Fig. 2. Signaling pathways for cell migration mediated by p38. Chemotactic factors and growth factors, such as fMLP, VEGF and FGF, activate MKK3/6 via their upstream MAPKKKs, such as MLK3. Activated MKK3/6 phosphorylate and activate p38, which in turn activates MAPKAPK2/3. The active MAPKAPK2/3 then phosphorylate HSP-27, resulting actin reorganization and cell migration. MAPKAPK2/3 also phosphorylates the p16-Arc of the Arp2/3 complex and 5-lipoxygenase, whereas p38 phosphorylates paxillin and caldesmon. These might play roles in cell migration. Orange lines represent speculative pathways.

factors. The roles of MAPKAPK 2/3, paxillin and caldesmon in cell migration are summarized in Fig. 2.

MAPKAPK 2/3

Although the underlying mechanism for p38-induced migration remains to be addressed, activation of MAPKAPK 2/3 by p38 and consequent phosphorylation of heat shock protein 27 (HSP27) is crucial for the migration of smooth muscle and endothelial cells (Hedges et al., 1999; Rousseau et al., 1997). Non-phosphorylated HSP27 inhibits actin polymerization in vitro, whereas the phosphorylated form loses this activity (Benndorf et al., 1994; Miron et al., 1991). Moreover, wild-type HSP27 promotes growth-factor-induced stress-fiber-formation, whereas a non-phosphorylatable mutant inhibits stress fiber formation (Lavoie et al., 1993). However, neutrophils lacking in MAPKAPK 2/3 move faster than normal neutrophils, although they lose their directionality (Hannigan et al., 2001). This indicates that MAPKAPK 2/3 is somehow associated with the directionality of migration. Other p38 substrates may govern migration speed. It has been reported that the p16-Arc subunit of the Arp complex and 5-lipoxygenase are substrates for MAPKAPK-2/3 (Singh et al., 2003; Werz et al., 2000). Further study is required to address the question whether these substrates are involved in the MAPKAPK 2/3-mediated cell migration.

Paxillin and caldesmon

Recently, we have demonstrated that p38 phosphorylates paxillin at Ser83 in vitro and in pheochromocytoma (PC12) cells (Huang et al., 2004b). The p38 pathway is also implicated in regulating focal adhesions, but it is unknown whether phosphorylation of paxillin by p38 is essential for cell migration. Caldesmon is also a potent p38 substrate and phosphorylation of caldesmon by p38 might play a role in urokinase-stimulated smooth muscle cell migration (Goncharova et al., 2002). Although these two proteins are prime candidates for p38 substrates that regulate cell migration, it also possible that other p38 substrates are crucially involved.

Erk/MAPK signaling

The Erk MAPKs are the most extensively studied subfamily of MAPKs. The isoforms – p44 (Erk-1) and p42 (Erk-2) – both contain a Thr-Glu-Tyr motif within the activation loop of the kinase domain, and their activity is stimulated by a wide variety of growth factors and mitogens (Johnson and Lapadat, 2002). These activate the Ras–Raf-1–MEK-1/2–Erk-1/2 signaling module (Seger and Krebs, 1995), in which Ras, activated by extracellular signals, recruits the MAPKKK Raf-1 to the membrane, where it is activated by other protein kinases. Raf-1 specifically phosphorylates and activates the MAPKKs MEK-1 and MEK-2, which are immediately upstream of Erk and phosphorylate the Thr and Tyr residues within the Erk1/2 Thr-Glu-Tyr motif (Seger and Krebs, 1995).

Erk/MAPK and cell migration

Erk has been implicated in the migration of numerous cell types. The Erk pathway inhibitors PD98059 and U0126 inhibit the migration of diverse cell types in response to cell matrix proteins, such as fibronectin, vitronectin and collagen (Anand-Apte et al., 1997; Klemke et al., 1997; Webb et al., 2000), growth factors such as VEGF, FGF, EGF, insulin (Eliceiri et al., 1998; Xie et al., 1998; Cheresh et al., 1999; Cho and Klemke, 2000; Lai et al., 2001; Shono et al., 2001) and other stimuli, such as fetal calf serum and urokinase plasminogen activator (uPA) (Nguyen et al., 1999; Degryse et al., 2001; Krueger et al., 2001; Jo et al., 2002; Cuevas et al., 2003). A dominant negative mutant of MEK1 blocks cell migration induced by fibronectin, vitronectin and uPA (Anand-Apte et al., 1997; Nguyen et al., 1999; Webb et al., 2000), and active MEK1 promotes cell migration in several cell types (Klemke et al., 1997; Webb et al., 2000; Krueger et al., 2001; Jo et al., 2002). Moreover, a dominant negative Erk mutant or inhibition of Erk by an antisense strategy also inhibits cell migration (Klemke et al., 1997; Lai et al., 2001). Erk is thus an important factor in the regulation of cell migration.

Erk/MAPK substrates and their roles in migration

Erk phosphorylates serine or threonine residues followed by proline. The most stringent consensus sequence is Pro-Leu-Ser/Thr-Pro (Gonzalez et al., 1991). Identified substrates include several protein kinases, such as p90rsk, MSK1, MNK1/2, myosin light chain kinase (MCLK) and FAK (Deak

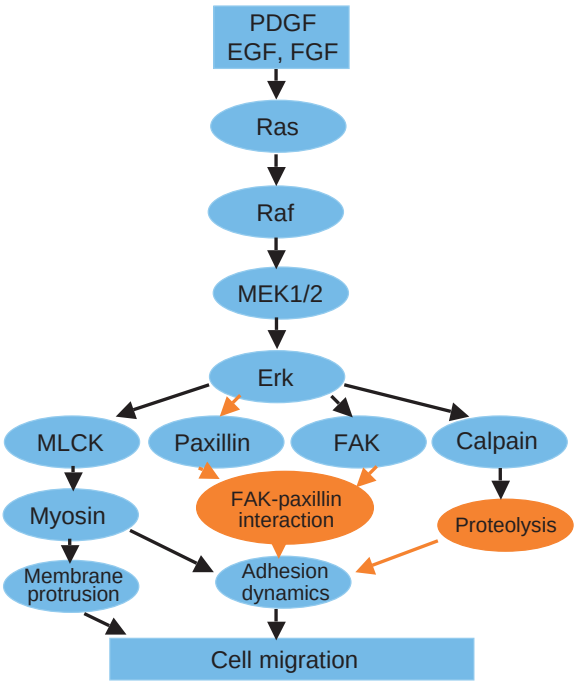

Fig. 3. Signaling pathways for cell migration mediated by Erk. Growth factors, such as PDGF, EGF and FGF, activate Erk through the Ras-Raf-MEK1/2-Erk signaling module. Activated Erk regulates membrane protrusions and focal adhesion turnover via phosphorylating MLCK and promotes focal adhesion disassembly via phosphorylating and activating calpain. Phosphorylation of FAK and paxillin by Erk may regulate focal adhesion dynamics, probably by influencing the paxillin-FAK interaction. Orange lines represent speculative pathways.

et al., 1998; Frodin and Gammeltoft, 1999; Fukunaga and Hunter, 1997; Hunger-Glaser et al., 2003; Klemke et al., 1997; Waskiewicz et al., 1997), the protease calpain (Glading et al., 2004), paxillin (Liu et al., 2002), as well as transcription factors and nuclear proteins. Of these MLCK, calpain, and FAK are most likely to be involved in Erk-mediated cell migration (Fig. 3).

MLCK

Inhibition of the Erk pathway impairs MLCK and MLC phosphorylation and cell migration, and expression of active MEK1 promotes phosphorylation of MLCK and MLC and enhanced cell migration in COS-7, MCF-7 human breast cancer and HT1080 fibrosarcoma cells (Klemke et al., 1997; Nguyen et al., 1999). Moreover, Erk phosphorylates MLCK and causes some increase in MLCK activity (Klemke et al., 1997). MLCK activation might be involved in focal adhesion turnover and membrane protrusion at the front of polarized cells, which are important for cell migration (Totsukawa et al., 2004; Webb et al., 2004).

Calpain

Calpains are a family of Ca^{2+}-activated proteolytic enzymes that are involved in cell migration (Dourdin et al., 2001; Huttenlocher et al., 1997). ERK phosphorylates m-calpain at Ser50 both in vitro and in vivo (Glading et al., 2004). Moreover, Erk-mediated phosphorylation stimulates the activity of m-calpain in the absence of millimolar concentrations of Ca^{2+}. Phosphorylation of m-calpain is required for adhesion turnover and cell migration, because the Ser50 to Ala mutant of m-calpain inhibits cell migration (Glading et al., 2004). m-calpain also associates with the N-terminus of FAK upon Src activation (Carragher et al., 2003). The FAK–m-calpain interaction is involved in targeting m-calpain to focal adhesions, where calpain degrades cytoskeletal proteins and causes adhesion disassembly (Cuevas et al., 2003). This model, in which calpain is recruited to focal adhesions, stimulated by Erk phosphorylation and subsequently degrades cytoskeletal proteins is intriguing; however, the exact molecular mechanism of regulation of motility by Erk and calpain remains to be determined.

FAK

FAK is a non-receptor protein tyrosine kinase that localizes at focal adhesions or focal contacts (Schaller, 2001b). It contains a central catalytic domain flanked by an N-terminal band 4.1 homology domain and a C-terminal domain. At the extreme C-terminus is a focal adhesion targeting (FAT) domain, a 140-amino-acid sequence responsible for targeting the kinase to focal adhesions.

Besides being phosphorylated at multiple tyrosine residues, FAK is also phosphorylated at multiple serine residues (including Ser910) in response to physiological stimuli (Hunger-Glaser et al., 2003; Ma et al., 2001). Moreover, Erk phosphorylates FAK at Ser910 both in vitro and in vivo (Hunger-Glaser et al., 2003). Furthermore, the phosphorylation of FAK at Ser910 seems to inhibit its interaction with paxillin. Because FAK and paxillin play a key role in cell migration, it will be very interesting to determine whether the phosphorylation of Ser910 is involved in Erk-mediated cell migration.

Paxillin

Paxillin constitutively associates with MEK, and extracellular stimuli induce the subsequent binding of active Raf and inactive Erk to paxillin, thus mediating Erk activation at focal complexes (Ishibe et al., 2003). The paxillin-FAK interaction is also involved in Erk activation (Subauste et al., 2004). Liu et al. have shown that Erk phosphorylates paxillin both in vitro and in hepatocyte-growth-factor-stimulated epithelial cells, and that paxillin phosphorylation in turn enhances paxillin-FAK association (Liu et al., 2002). However, Hunger-Glaser et al., have reported that Erk-mediated phosphorylation of FAK at Ser910 blocks the interaction of FAK with paxillin (Hunger-Glaser et al., 2003). These intriguing observations suggest sophisticated regulation of the FAK-paxillin complex, in which Erk might initially promote complex-assembly by phosphorylation of paxillin and then promote disassembly by subsequent phosphorylation of FAK. It is possible that Erk-modulated disassembly of the FAK-paxillin complex is involved in focal adhesion disassembly, but its precise role and the mechanism remain to be clarified.

Erk might also participate in cell migration by suppressing the ability of integrins to bind to their extracellular matrix ligands. It is well known that the Ras-Raf-MEK-Erk pathway regulates integrin activation (the affinity of an integrin for its substrate) (Chou et al., 2003; Hughes et al., 1997), although the molecular mechanism remains to be elucidated. Because dynamic integrin activation is required for cell migration (Huttenlocher et al., 1996; Palecek et al., 1997), Erk might also play an important role regulating cell migration, by regulating integrin activation. The implication of these signaling pathways in Erk-dependent motility is intriguing. Erk-dependent activation of specific pathways could regulate motility under particular physiological conditions. Alternatively, coordinated activation of all these pathways by Erk may be crucial for promoting migration.

Concluding remarks

There is clearly evidence implicating the different MAPK family members in cell migration. Cells might employ each MAPK in extracellular-stimulus- and/or cell-type-dependent manners. Alternatively, coordinated regulation of these MAPKs might be required for the control of cell migration. It is interesting to note that to date the regulation of cell motility by each MAPK has been linked to phosphorylation of distinct substrates. This observation supports the model that coordinated regulation of these kinases is important for cell migration. Phosphorylation of paxillin, FAK, calpain, DCX, MAP1B and MAP2, MAPKAPK 2/3, and MLCK by MAPKs might regulate the dynamics of focal adhesion and the reorganization of microtubules and filamentous actin. These play key roles in cell spreading, lamellipodium extension and tail retraction during cell migration. Future studies will undoubtedly focus upon elucidating the precise mechanisms by which distinct MAPKs and their substrates control changes in the cytoskeleton and how these events are coordinated to

elicit cell movement. For example, how does paxillin phosphorylation by JNK regulate dynamics of cell adhesions? Does the phosphorylation change its binding to its partners? Ultimately, these signaling pathways must affect the forces needed to move cells and/or to change adhesive structures. Investigating the precise mechanochemical mechanisms involved is another key target for future investigations. Such studies will involve advanced microscopic tools (Roy et al., 2002) to localize components of the pathways, examine their interactions and mobility, assess their contributions to force production (Jiang et al., 2003) and evaluate effects on overall traction force production (Balaban et al., 2001; Beningo et al., 2001; Munevar et al., 2001; Tan et al., 2003). Through such approaches a more lucid picture of the migratory roles of MAPK cascades should emerge.

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