

# MAPK signalling: ERK5 versus ERK1/2

Satoko Nishimoto & Eisuke Nishida\*

Kyoto University, Sakyo-ku, Kyoto, Japan

Extracellular-signal-regulated kinase 5 (ERK5) is a member of the mitogen-activated protein kinase (MAPK) family and, similar to ERK1/2, has the Thr–Glu–Tyr (TEY) activation motif. Both ERK5 and ERK1/2 are activated by growth factors and have an important role in the regulation of cell proliferation and cell differentiation. Moreover, both the ERK5 and the ERK1/2 pathways are sensitive to PD98059 and U0126, which are two well-known inhibitors of the ERK pathway. Despite these similarities, recent studies have revealed distinctive features of the ERK5 pathway: ERK5 has a key role in cardiovascular development and neural differentiation; ERK5 nuclear translocation is controlled by its own nuclear localizing and nuclear export activities; and the carboxy-terminal half of ERK5, which follows its kinase catalytic domain, has a unique function.

Keywords: signal transduction; nuclear translocation; growth factor signaling; gene expression; kinase cascade

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## Introduction

The mitogen-activated protein kinase (MAPK) cascade is a highly conserved module that is involved in various cellular functions, including cell proliferation, differentiation and migration. Extracellular stimuli such as growth factors and environmental stresses induce the sequential activation of MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK. At least four members of the MAPK family have been identified—extracellular-signal-regulated kinase 1/2 (ERK1/2), c-Jun-amino-terminal kinase (JNK), p38 and ERK5 (Sturgill & Wu, 1991; Nishida & Gotoh, 1993; Robinson & Cobb, 1997; Davis, 2000; Kyriakis & Avruch, 2001; Wang & Tournier, 2006).

ERK1 and ERK2 are isoforms of the 'classical' MAPK. Both ERK1 and ERK2 (referred to as ERK1/2) are activated by MAP/ERK kinase 1 (MEK1) and MEK2 (referred to as MEK1/2), which are members of the MAPKK family. After stimulation by a variety of mitogens including peptide growth factors, MEK1/2 is activated by MAPKKK-mediated phosphorylation. These MAPKKKs include Raf and Mos. MEK1/2 then phosphorylates threonine and tyrosine residues in the Thr–Glu–Tyr (TEY) sequence of ERK1/2, resulting in the activation of ERK1/2. Activated ERK1/2 phosphorylates many substrates including transcription factors, such as Elk1 and c-Myc, and protein kinases, such as

ribosomal S6 kinase (RSK). Subsequently, immediate early genes, such as c-Fos, are induced. ERK1/2 is therefore an important contributor to cell proliferation (Lewis *et al*, 1998).

ERK5, also known as big MAP kinase 1 (BMK1), is twice the size of other MAPKs (Lee *et al*, 1995; Zhou *et al*, 1995). The amino-terminal half contains the kinase domain, which is similar to that of ERK1/2 and has the TEY activation motif, whereas the carboxy-terminal half is unique. On stimulation, MEKK2 and MEKK3, members of the MAPKKK family, activate MEK5, a specific MAPKK for ERK5, although these two MAPKKKs are associated differently with upstream signalling pathways (Chao *et al*, 1999; Sun *et al*, 2001). Subsequently, MEK5 phosphorylates and activates ERK5, and then the activated ERK5 phosphorylates substrates including myocyte enhancer factor 2 (MEF2; Kato *et al*, 1997). The interaction of MEK5 with MEKK2, MEKK3 or ERK5 is mediated by the PB1 domain of MEK5 (Nakamura & Johnson, 2003; Seyfried *et al*, 2005; Nakamura *et al*, 2006). So far, the function of the MEK5 PB1 domain in the ERK5 signalling pathway is controversial. Seyfried *et al* (2005) proposed that MEKK2 and ERK5 compete for binding to the MEK5 PB1 domain. However, Nakamura *et al* (2006) showed that the PB1 domain functions as a scaffold for a MEKK2–MEK5–ERK5 complex.

## Similarities between the ERK5 and ERK1/2 pathways

ERK5 was initially documented as a MAPK family member that is activated by stress stimuli, as ERK5 was reported to be activated by oxidative stress and hyperosmolarity but not by platelet-derived growth factor (PDGF), a strong stimulus for ERK1/2 (Abe *et al*, 1996). Subsequently, it was shown that ERK5 can be activated in response to serum, one of the well-known activators of ERK1/2 (Kato *et al*, 1997). Nerve growth factor (NGF), another stimulator of ERK1/2, can also increase ERK5 activity (Kamakura *et al*, 1999). Remarkably, PD98059 and U0126, which were identified as MEK1/2-specific inhibitors, also inhibit the MEK5–ERK5 pathway (Kamakura *et al*, 1999; Mody *et al*, 2001). However, the MEK5–ERK5 pathway is less sensitive to PD184352, which is also known as a MEK1/2 inhibitor (Mody *et al*, 2001).

ERK5 can phosphorylate the ERK1/2 substrates, Sap1a, c-Myc and RSK (English *et al*, 1998; Kamakura *et al*, 1999; Pearson *et al*, 2001). However, it has also been reported that low doses of PD184352 block epidermal growth factor (EGF)-induced activation of RSK, indicating that ERK5 is probably not involved in RSK activation (Mody *et al*, 2001). ERK5, as well as ERK1/2, can also induce immediate early genes, such as c-Fos and c-Jun (Kato *et al*, 1997; Kamakura *et al*, 1999).

Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

\*Corresponding author. Tel: +81 75 753 4230; Fax: +81 75 753 4235;

E-mail: L50174@sakura.kudpc.kyoto-u.ac.jp

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ERK5, similar to ERK1/2, has a role in the regulation of EGF-induced cell proliferation, mainly during the G1/S transition (Kato *et al*, 1998). ERK5 is involved in the regulation of cell proliferation in several ways. For example, the phosphorylation of serum and glucocorticoid-inducible kinase (SGK) by ERK5 is required for S-phase entry (Hayashi *et al*, 2001). In addition, ERK5, as well as ERK1/2, is able to drive cyclin D1 expression—a key regulator of the G1/S transition. However, ERK5 and ERK1/2 regulate cyclin D1 expression by different mechanisms: cAMP response element (CRE) is required for induction by ERK5 but not by ERK1/2 (Mulloy *et al*, 2003). It has also been reported that serum-stimulated cyclin D1 expression is inhibited by low doses of PD184352 that specifically inhibit the ERK1/2 pathway (Squires *et al*, 2002).

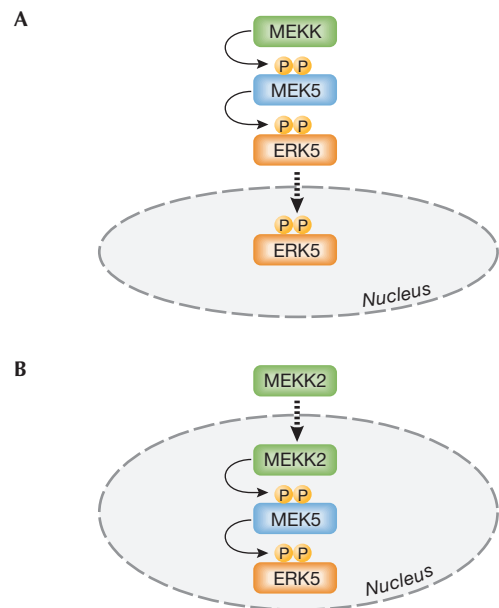
Thus, there are similarities between the ERK5 and the ERK1/2 pathways in their activation modes and function. However, recent studies have also identified some distinctive features of the ERK5 pathway.

### Roles of ERK5 and ERK1/2 *in vivo*

Previous studies with cultured cells have revealed the involvement of ERK5 and ERK1/2 in many cellular responses, including cell proliferation. Recent genetic studies have identified their role *in vivo* in the development of whole organisms. ERK5 is essential for cardiovascular development and neural differentiation, whereas ERK1/2 is important for mesoderm formation.

ERK5-deficient mice die around embryonic day 10.5 because of cardiovascular defects and angiogenic failure in embryonic and extraembryonic tissues (Regan *et al*, 2002; Sohn *et al*, 2002; Yan *et al*, 2003). Similar phenotypic abnormalities are seen in mice with an endothelial-specific conditional knockout of ERK5 and in MEK5-deficient mice (Hayashi *et al*, 2004; Hayashi & Lee, 2004; Wang *et al*, 2005). Endothelial-specific ERK5 knockout mice show cardiovascular defects, whereas cardiomyocyte-specific knockout mice do not, suggesting that ERK5 is required in endothelial cells (Hayashi *et al*, 2004; Hayashi & Lee, 2004). It has also been reported that ERK5 inhibits apoptosis of endothelial cells *in vitro* (Pi *et al*, 2004). Another function of ERK5—the regulation of neural differentiation—has been revealed by a study in *Xenopus laevis* (Nishimoto *et al*, 2005). ERK5 knockdown with antisense morpholino oligonucleotides resulted in the reduction of head structures and the inhibition of neural differentiation. Furthermore, the forced activation of ERK5 promoted neural differentiation. Although these results might be related to the observation that ERK5-deficient mice show severe growth retardation in the head region (Sohn *et al*, 2002; Yan *et al*, 2003), it remains to be shown whether ERK5 regulates neural differentiation in mice. In *Caenorhabditis elegans*, the *sma-5* gene encodes a homologue of ERK5, although it does not contain the TEY activation motif (Watanabe *et al*, 2005). The *sma-5* mutant has a smaller body size than wild-type *C. elegans*.

Studies with knockout mice have shown that ERK2 and MEK1, rather than ERK1 and MEK2, are essential for embryonic development: ERK2- or MEK1-deficient mice show defects in development of the placenta, whereas ERK1- or MEK2-deficient mice are viable, fertile and of normal size (Giroux *et al*, 1999; Pages *et al*, 1999; Mazzucchelli *et al*, 2002; Belanger *et al*, 2003; Hatano *et al*, 2003). Recently, it was reported that another line of MEK2-deficient mice lacks mesoderm differentiation, suggesting that ERK2 has a key role in mesoderm formation (Yao *et al*, 2003). Similarly, in *Xenopus*, which seems to have an *ERK2* but not an *ERK1* gene, ERK2 regulates mesoderm formation (Gotoh *et al*, 1995; LaBonne *et al*, 1995;



**Fig 1** | Two mechanisms to transmit the signal from the cytoplasm to the nucleus in the ERK5 pathway. (A) ERK5 and (B) MEK2 can translocate from the cytoplasm to the nucleus on stimulation. ERK, extracellular-signal-regulated kinase; MEKK, MAP/ERK kinase kinase.

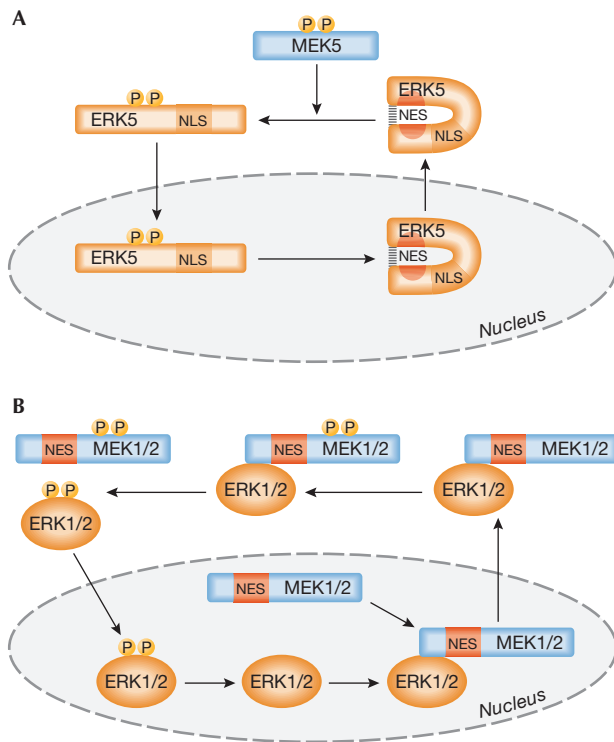
Umbhauer *et al*, 1995). Furthermore, the duration of ERK2 activation regulates the dorsoventral patterning of the mesoderm (H. Hanafusa, K. Matsumoto & E.N., unpublished data). ERK2 is also essential for oocyte maturation and metaphase arrest of unfertilized eggs in *Xenopus* (Gotoh & Nishida, 1995). In addition, it is well known that the Ras/ERK1/2 signalling pathway has a central role in vulval development in *C. elegans* (Sundaram & Han, 1996) and the differentiation of R7 photoreceptor cells in *Drosophila* (Perrimon, 1994).

These observations suggest that ERK5 and ERK1/2 have distinct roles *in vivo*. However, as some redundancy between ERK1 and ERK2 might exist, the production and analyses of ERK1/2 double-knockout mice are needed to understand fully the distinct roles of the ERK1/2 and the ERK5 pathways. Also, ERK5 and ERK1/2 might regulate different stages of neural development, as the ERK1/2 pathway regulates neural induction in *Xenopus* (Pera *et al*, 2003; Kuroda *et al*, 2004) whereas the ERK5 pathway is involved in the regulation of the subsequent neural differentiation (see above; Nishimoto *et al*, 2005).

### Signalling to the nucleus

MAPK pathways control cell proliferation and cell differentiation mainly through the regulation of transcription factors in the nucleus. Thus, to transmit extracellular signals to the nucleus, the terminal component of the MAPK pathways—that is, MAPK—must translocate from the cytoplasm to the nucleus.

Overexpressed ERK5 localizes to the cytoplasm in resting cells and translocates to the nucleus when co-expressed with constitutively active MEK5 (Fig 1A; Kato *et al*, 1997). Endogenous ERK5 is cytoplasmic, nuclear or pan-cellular, depending on the cell line (Buschbeck & Ullrich, 2005; Kondoh *et al*, 2006). In human breast carcinoma MCF7 cells and mouse myoblast C2C12 cells, endogenous inactive ERK5



**Fig 2** | Molecular mechanisms to control nucleocytoplasmic transport of ERK5 and ERK1/2. **(A)** ERK5 has a bipartite nuclear localization signal (NLS) in its carboxy-terminal region. In resting cells, the intramolecular interaction between the amino-terminal and the C-terminal halves produces a nuclear export signal (NES) or a domain recognized by cytoplasmic anchor proteins, which has an NES and retains ERK5 in the cytoplasm (not shown). The NES activity might be stronger than the NLS activity. On stimulation, the interaction between the N-terminal and the C-terminal halves is disrupted, and thus the NES activity is abolished. ERK5 then enters the nucleus. **(B)** By contrast, ERK1/2 contains no NLS or NES. In resting cells, MEK1/2, which has an NES, anchors ERK1/2 in the cytoplasm. On stimulation, ERK1/2 dissociates from MEK1/2 and translocates to the nucleus. ERK, extracellular-signal-regulated kinase; MEK, MAP/ERK kinase.

localizes either to the cytoplasm or diffusively throughout the whole cell, and translocates to the nucleus on stimulation (Fig 1A; Esparis-Ogando *et al*, 2002; Kondoh *et al*, 2006). In COS7, HeLa and Rat1 cells, endogenous ERK5 localizes to the nucleus even before stimulation, although the nuclear accumulation of ERK5 is enhanced by the stimulation of HeLa cells (Raviv *et al*, 2004; Buschbeck & Ullrich, 2005; Kondoh *et al*, 2006). In HeLa and Rat1 cells, MEKK2 translocates from the cytoplasm to the nucleus on stimulation (Fig 1B; Raviv *et al*, 2004).

Recently, a mechanism for the nucleocytoplasmic transport of ERK5 has been proposed (Fig 2A; Kondoh *et al*, 2006). ERK5 has nuclear localizing activity in its C-terminal region (Yan *et al*, 2001), owing to a bipartite nuclear localization signal (NLS; Kondoh *et al*, 2006). In addition, ERK5 has nuclear export activity (Raviv *et al*, 2004; Buschbeck & Ullrich, 2005), and the balance between nuclear import and export determines the subcellular localization of ERK5 (Kondoh *et al*, 2006). In quiescent cells, the intramolecular interaction between the N-terminal and the C-terminal halves of

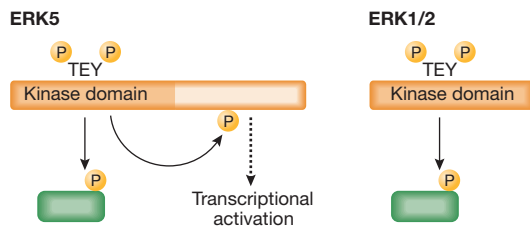
ERK5 forms a region responsible for a CRM1-dependent nuclear export signal (NES). It is possible that the region itself constitutes an NES. However, an NES in ERK5 has not been identified so far. Therefore, it is also possible that the region is responsible for the interaction of ERK5 with a cytoplasmic anchor protein containing an NES. In any case, this NES activity might be stronger than the NLS activity under non-stimulated conditions, meaning that ERK5 is cytoplasmic. On stimulation, ERK5 undergoes activating phosphorylation, and the intramolecular association between the N-terminal and C-terminal halves is dissociated, resulting in the disruption of the nuclear export activity. ERK5 then translocates to the nucleus, as the NLS is constantly active.

ERK1/2 also translocates from the cytoplasm to the nucleus on stimulation but, unlike ERK5, it does not have an obvious NLS or NES. Instead, MEK1/2 has an NES in its N-terminal region and localizes mainly to the cytoplasm (Fukuda *et al*, 1996). In quiescent cells, MEK1/2 retains ERK1/2 in the cytoplasm through direct interaction (Fig 2B; Fukuda *et al*, 1997). On stimulation, ERK1/2 becomes phosphorylated at threonine and tyrosine residues and the latter results in the dissociation of ERK1/2 from MEK1/2. ERK1/2 then translocates to the nucleus by three mechanisms: passive diffusion of a monomer, active transport of a dimer, and direct interaction with the nuclear pore complex (Fig 2B; Khokhlatchev *et al*, 1998; Adachi *et al*, 1999; Matsubayashi *et al*, 2001; Whitehurst *et al*, 2002; Kondoh *et al*, 2005). To export ERK1/2 from the nucleus, MEK1/2 enters the nucleus by passive diffusion (Adachi *et al*, 2000; Yao *et al*, 2001). The nuclear localization of MEK1/2 is also regulated by a stimulus-dependent rapid transport mechanism (Jaaro *et al*, 1997; Yao *et al*, 2001). MKP3, a member of the MAP kinase phosphatase family, also has an NES and anchors ERK1/2 in the cytoplasm under non-stimulated conditions (Karlsson *et al*, 2004).

As the MAPK substrates are present in both the cytoplasm and the nucleus, the spatial control of MAPKs is essential for the precise regulation of signal transduction. Phosphoprotein enriched in astrocytes 15 (PEA15) and Sef are spatial regulators of ERK1/2 (Formstecher *et al*, 2001; Torii *et al*, 2004; Whitehurst *et al*, 2004). As for ERK5, the muscle-specific A-kinase anchoring protein (mAKAP) complex at the perinuclear membrane anchors ERK5 to inhibit the activity of phosphodiesterase (PDE) 4D3, which hydrolyses cAMP (Dodge-Kafka *et al*, 2005). It is also reported that the activation of ERK5 is disrupted by cAMP (Pearson *et al*, 2006).

### Transcriptional activation activity of ERK5

When MAPKs translocate to the nucleus, they control transcription to elicit the desired cell response. Recently, a unique mechanism of ERK5-mediated transcriptional regulation has been revealed. The C-terminal region of ERK5 contains a unique sequence, and has transcriptional activation activity (Kasler *et al*, 2000; Terasawa *et al*, 2003; Akaike *et al*, 2004). Other MAPKs, including ERK1/2, do not have this long non-catalytic domain (Fig 3). The C-terminal region of ERK5 is required for the maximum activation of MEF2, peroxisome proliferator activated receptor  $\gamma$ 1 (PPAR $\gamma$ 1) and members of the AP1 family, c-fos and Fra1. Activation of the MEK5–ERK5 pathway increases the transcription activity of these factors, whereas it is significantly decreased by the deletion of the C-terminal region of ERK5. Furthermore, the C-terminal half of ERK5 alone has the ability to increase transcription activity (Kasler *et al*, 2000).



**Fig 3** | Hypothetical mechanisms by which ERK5 and ERK1/2 transmit signals to downstream targets. The activating phosphorylation of ERK5 results in the activation of the kinase activity, and ERK5 phosphorylates both downstream target molecules and the carboxy-terminal region of ERK5 itself. The phosphorylated C-terminal region might act as a transcriptional activation activator. Therefore, ERK5 has two mechanisms to transmit signals. By contrast, ERK1/2 might transmit signals only through the phosphorylation of substrates. ERK, extracellular-signal-regulated kinase.

What regulates the transcriptional activation activity of ERK5? The activity of the C-terminal region is inhibited by the N-terminal half of the protein (Kasler *et al*, 2000). Furthermore, the activity is positively regulated by MEK5–ERK5 pathway-mediated phosphorylation (Y. Morimoto, K. Kondoh & E.N., unpublished data). ERK5 autophosphorylation of its C-terminal half might be required for the transcriptional activation activity, as it has been shown that the C-terminal region of ERK5 becomes autophosphorylated (Mody *et al*, 2003). Therefore, phosphorylation of the N-terminal TEY sequence of ERK5 by MEK5 causes the activation of ERK5, resulting in the phosphorylation of downstream target molecules and also autophosphorylation of its C-terminal region. This probably leads to a further increase in the transcription activity of target molecules (Fig 3, left). Future studies should uncover the molecular mechanisms by which the C-terminal region of ERK5 enhances transcription activity.

## Conclusions

The C-terminal region of ERK5, which is unique to ERK5, enables the kinase to increase the transcription activity of target molecules. Therefore, ERK5 is able to transmit signals to downstream molecules in two ways: through either the phosphorylation or the enhancement of the transcription activity of target molecules. It will be interesting to uncover the strategy that determines which of these two mechanisms is used. ERK5 is activated by several extracellular stimuli, such as stress stimuli and growth factors, and has an important role in several cellular responses, such as cell proliferation and cell differentiation. Therefore, ERK5 must transmit signals in a context-dependent manner. This could be achieved, at least in part, by altering the balance between the two mechanisms.

Finally, only a small number of molecules have been identified so far as downstream targets of ERK5. A more complete identification of the downstream factors will be necessary to fully understand the role of ERK5 in signal transduction.

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## REFERENCES

- Abe J, Kusuohara M, Ulevitch RJ, Berk BC, Lee JD (1996) Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. *J Biol Chem* **271**: 16586–16590
- Adachi M, Fukuda M, Nishida E (1999) Two co-existing mechanisms for nuclear import of MAP kinase: passive diffusion of a monomer and active transport of a dimer. *EMBO J* **18**: 5347–5358
- Adachi M, Fukuda M, Nishida E (2000) Nuclear export of MAP kinase (ERK) involves a MAP kinase kinase (MEK)-dependent active transport mechanism. *J Cell Biol* **148**: 849–856
- Akaike M, Che W, Marmarosh NL, Ohta S, Osawa M, Ding B, Berk BC, Yan C, Abe J (2004) The hinge-helix 1 region of peroxisome proliferator-activated receptor  $\gamma$ 1 (PPAR $\gamma$ 1) mediates interaction with extracellular signal-regulated kinase 5 and PPAR $\gamma$ 1 transcriptional activation: involvement in flow-induced PPAR $\gamma$  activation in endothelial cells. *Mol Cell Biol* **24**: 8691–8704
- Belanger LF, Roy S, Tremblay M, Brott B, Steff AM, Mourad W, Hugo P, Erikson R, Charron J (2003) Mek2 is dispensable for mouse growth and development. *Mol Cell Biol* **23**: 4778–4787
- Buschbeck M, Ullrich A (2005) The unique C-terminal tail of the mitogen-activated protein kinase ERK5 regulates its activation and nuclear shuttling. *J Biol Chem* **280**: 2659–2667
- Chao TH, Hayashi M, Tapping RI, Kato Y, Lee JD (1999) MEKK3 directly regulates MEK5 activity as part of the big mitogen-activated protein kinase 1 (BMK1) signaling pathway. *J Biol Chem* **274**: 36035–36038
- Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. *Cell* **103**: 239–252
- Dodge-Kafka KL, Soughayer J, Pare GC, Carlisle Michel JJ, Langeberg LK, Kapiloff MS, Scott JD (2005) The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature* **437**: 574–578
- English JM, Pearson G, Baer R, Cobb MH (1998) Identification of substrates and regulators of the mitogen-activated protein kinase ERK5 using chimeric protein kinases. *J Biol Chem* **273**: 3854–3860
- Esparis-Ogando A, Diaz-Rodriguez E, Montero JC, Yuste L, Crespo P, Pandiella A (2002) Erk5 participates in neuregulin signal transduction and is constitutively active in breast cancer cells overexpressing ErbB2. *Mol Cell Biol* **22**: 270–285
- Formstecher E *et al* (2001) PEA-15 mediates cytoplasmic sequestration of ERK MAP kinase. *Dev Cell* **1**: 239–250
- Fukuda M, Gotoh I, Gotoh Y, Nishida E (1996) Cytoplasmic localization of mitogen-activated protein kinase kinase directed by its NH2-terminal, leucine-rich short amino acid sequence, which acts as a nuclear export signal. *J Biol Chem* **271**: 20024–20028
- Fukuda M, Gotoh Y, Nishida E (1997) Interaction of MAP kinase with MAP kinase kinase: its possible role in the control of nucleocytoplasmic transport of MAP kinase. *EMBO J* **16**: 1901–1908
- Giroux S *et al* (1999) Embryonic death of Mek1-deficient mice reveals a role for this kinase in angiogenesis in the labyrinthine region of the placenta. *Curr Biol* **9**: 369–372
- Gotoh Y, Nishida E (1995) The MAP kinase cascade: its role in *Xenopus* oocytes, eggs and embryos. *Prog Cell Cycle Res* **1**: 287–297
- Gotoh Y, Masuyama N, Suzuki A, Ueno N, Nishida E (1995) Involvement of the MAP kinase cascade in *Xenopus* mesoderm induction. *EMBO J* **14**: 2491–2498
- Hatano N, Mori Y, Oh-hora M, Kosugi A, Fujikawa T, Nakai N, Niwa H, Miyazaki J, Hamaoka T, Ogata M (2003) Essential role for ERK2 mitogen-activated protein kinase in placental development. *Genes Cells* **8**: 847–856
- Hayashi M, Lee JD (2004) Role of the BMK1/ERK5 signaling pathway: lessons from knockout mice. *J Mol Med* **82**: 800–808
- Hayashi M, Tapping RI, Chao TH, Lo JF, King CC, Yang Y, Lee JD (2001) BMK1 mediates growth factor-induced cell proliferation through direct cellular activation of serum and glucocorticoid-inducible kinase. *J Biol Chem* **276**: 8631–8634
- Hayashi M, Kim SW, Imanaka-Yoshida K, Yoshida T, Abel ED, Eliceiri B, Yang Y, Ulevitch RJ, Lee JD (2004) Targeted deletion of BMK1/ERK5 in adult mice perturbs vascular integrity and leads to endothelial failure. *J Clin Invest* **113**: 1138–1148
- Jaaro H, Rubinfeld H, Hanoch T, Seger R (1997) Nuclear translocation of mitogen-activated protein kinase kinase (MEK1) in response to mitogenic stimulation. *Proc Natl Acad Sci USA* **94**: 3742–3747
- Kamakura S, Moriguchi T, Nishida E (1999) Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and

- characterization of a signaling pathway to the nucleus. *J Biol Chem* **274**: 26563–26571
- Karlsson M, Mathers J, Dickinson RJ, Mandl M, Keyse SM (2004) Both nuclear-cytoplasmic shuttling of the dual specificity phosphatase MKP-3 and its ability to anchor MAP kinase in the cytoplasm are mediated by a conserved nuclear export signal. *J Biol Chem* **279**: 41882–41891
- Kasler HG, Victoria J, Duramad O, Winoto A (2000) ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. *Mol Cell Biol* **20**: 8382–8389
- Kato Y, Kravchenko VV, Tapping RI, Han J, Ulevitch RJ, Lee JD (1997) BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *EMBO J* **16**: 7054–7066
- Kato Y, Tapping RI, Huang S, Watson MH, Ulevitch RJ, Lee JD (1998) Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. *Nature* **395**: 713–716
- Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, Goldsmith E, Cobb MH (1998) Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* **93**: 605–615
- Kondoh K, Torii S, Nishida E (2005) Control of MAP kinase signaling to the nucleus. *Chromosoma* **114**: 86–91
- Kondoh K, Terasawa K, Morimoto H, Nishida E (2006) Regulation of nuclear translocation of extracellular signal-regulated kinase 5 by active nuclear import and export mechanisms. *Mol Cell Biol* **26**: 1679–1690
- Kuroda H, Wessely O, De Robertis EM (2004) Neural induction in *Xenopus*: requirement for ectodermal and endomesodermal signals via Chordin, Noggin,  $\beta$ -Catenin, and Cerberus. *PLoS Biol* **2**: E92
- Kyriakis JM, Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* **81**: 807–869
- LaBonne C, Burke B, Whitman M (1995) Role of MAP kinase in mesoderm induction and axial patterning during *Xenopus* development. *Development* **121**: 1475–1486
- Lee JD, Ulevitch RJ, Han J (1995) Primary structure of BMK1: a new mammalian map kinase. *Biochem Biophys Res Commun* **213**: 715–724
- Lewis TS, Shapiro PS, Ahn NG (1998) Signal transduction through MAP kinase cascades. *Adv Cancer Res* **74**: 49–139
- Matsubayashi Y, Fukuda M, Nishida E (2001) Evidence for existence of a nuclear pore complex-mediated, cytosol-independent pathway of nuclear translocation of ERK MAP kinase in permeabilized cells. *J Biol Chem* **276**: 41755–41760
- Mazzucchelli C *et al* (2002) Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron* **34**: 807–820
- Mody N, Leitch J, Armstrong C, Dixon J, Cohen P (2001) Effects of MAP kinase cascade inhibitors on the MKK5/ERK5 pathway. *FEBS Lett* **502**: 21–24
- Mody N, Campbell DG, Morrice N, Peggie M, Cohen P (2003) An analysis of the phosphorylation and activation of extracellular-signal-regulated protein kinase 5 (ERK5) by mitogen-activated protein kinase kinase 5 (MKK5) *in vitro*. *Biochem J* **372**: 567–575
- Mulloy R, Salinas S, Phillips A, Hipskind RA (2003) Activation of cyclin D1 expression by the ERK5 cascade. *Oncogene* **22**: 5387–5398
- Nakamura K, Johnson GL (2003) PB1 domains of MEKK2 and MEKK3 interact with the MEK5 PB1 domain for activation of the ERK5 pathway. *J Biol Chem* **278**: 36989–36992
- Nakamura K, Uhlík MT, Johnson NL, Hahn KM, Johnson GL (2006) PB1 domain-dependent signaling complex is required for extracellular signal-regulated kinase 5 activation. *Mol Cell Biol* **26**: 2065–2079
- Nishida E, Gotoh Y (1993) The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem Sci* **18**: 128–131
- Nishimoto S, Kusakabe M, Nishida E (2005) Requirement of the MEK5–ERK5 pathway for neural differentiation in *Xenopus* embryonic development. *EMBO Rep* **6**: 1064–1069
- Pages G, Guerin S, Grall D, Bonino F, Smith A, Anjuere F, Auberger P, Pouyssegur J (1999) Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice. *Science* **286**: 1374–1377
- Pearson G, English JM, White MA, Cobb MH (2001) ERK5 and ERK2 cooperate to regulate NF- $\kappa$ B and cell transformation. *J Biol Chem* **276**: 7927–7931
- Pearson GW, Earnest S, Cobb MH (2006) Cyclic AMP selectively uncouples mitogen-activated protein kinase cascades from activating signals. *Mol Cell Biol* **26**: 3039–3047
- Pera EM, Ikeda A, Eivers E, De Robertis EM (2003) Integration of IGF, FGF, and anti-BMP signals via Smad1 phosphorylation in neural induction. *Genes Dev* **17**: 3023–3028
- Perrimon N (1994) Signalling pathways initiated by receptor protein tyrosine kinases in *Drosophila*. *Curr Opin Cell Biol* **6**: 260–266
- Pi X, Yan C, Berk BC (2004) Big mitogen-activated protein kinase (BMK1)/ERK5 protects endothelial cells from apoptosis. *Circ Res* **94**: 362–369
- Raviv Z, Kalie E, Seger R (2004) MEK5 and ERK5 are localized in the nuclei of resting as well as stimulated cells, while MEK2 translocates from the cytosol to the nucleus upon stimulation. *J Cell Sci* **117**: 1773–1784
- Regan CP, Li W, Boucher DM, Spatz S, Su MS, Kuida K (2002) Erk5 null mice display multiple extraembryonic vascular and embryonic cardiovascular defects. *Proc Natl Acad Sci USA* **99**: 9248–9253
- Robinson MJ, Cobb MH (1997) Mitogen-activated protein kinase pathways. *Curr Opin Cell Biol* **9**: 180–186
- Seyfried J, Wang X, Kharebava G, Tournier C (2005) A novel mitogen-activated protein kinase docking site in the N terminus of MEK5 $\alpha$  organizes the components of the extracellular signal-regulated kinase 5 signaling pathway. *Mol Cell Biol* **25**: 9820–9828
- Sohn SJ, Sarvis BK, Cado D, Winoto A (2002) ERK5 MAPK regulates embryonic angiogenesis and acts as a hypoxia-sensitive repressor of vascular endothelial growth factor expression. *J Biol Chem* **277**: 43344–43351
- Squires MS, Nixon PM, Cook SJ (2002) Cell-cycle arrest by PD184352 requires inhibition of extracellular signal-regulated kinases (ERK) 1/2 but not ERK5/BMK1. *Biochem J* **366**: 673–680
- Sturgill TW, Wu J (1991) Recent progress in characterization of protein kinase cascades for phosphorylation of ribosomal protein S6. *Biochim Biophys Acta* **1092**: 350–357
- Sun W, Kesavan K, Schaefer BC, Garrington TP, Ware M, Johnson NL, Gelfand EW, Johnson GL (2001) MEK2 associates with the adapter protein Lad/RIBP and regulates the MEK5–BMK1/ERK5 pathway. *J Biol Chem* **276**: 5093–5100
- Sundaram M, Han M (1996) Control and integration of cell signaling pathways during *C. elegans* vulval development. *Bioessays* **18**: 473–480
- Terasawa K, Okazaki K, Nishida E (2003) Regulation of c-Fos and Fra-1 by the MEK5–ERK5 pathway. *Genes Cells* **8**: 263–273
- Torii S, Kusakabe M, Yamamoto T, Maekawa M, Nishida E (2004) Sef is a spatial regulator for Ras/MAP kinase signaling. *Dev Cell* **7**: 33–44
- Umbhauer M, Marshall CJ, Mason CS, Old RW, Smith JC (1995) Mesoderm induction in *Xenopus* caused by activation of MAP kinase. *Nature* **376**: 58–62
- Wang X, Tournier C (2006) Regulation of cellular functions by the ERK5 signalling pathway. *Cell Signal* **18**: 753–760
- Wang X *et al* (2005) Targeted deletion of mek5 causes early embryonic death and defects in the extracellular signal-regulated kinase 5/myocyte enhancer factor 2 cell survival pathway. *Mol Cell Biol* **25**: 336–345
- Watanabe N, Nagamatsu Y, Gengyo-Ando K, Mitani S, Ohshima Y (2005) Control of body size by SMA-5, a homolog of MAP kinase BMK1/ERK5, in *C. elegans*. *Development* **132**: 3175–3184
- Whitehurst AW, Wilsbacher JL, You Y, Luby-Phelps K, Moore MS, Cobb MH (2002) ERK2 enters the nucleus by a carrier-independent mechanism. *Proc Natl Acad Sci USA* **99**: 7496–7501
- Whitehurst AW, Robinson FL, Moore MS, Cobb MH (2004) The death effector domain protein PEA-15 prevents nuclear entry of ERK2 by inhibiting required interactions. *J Biol Chem* **279**: 12840–12847
- Yan C, Luo H, Lee JD, Abe J, Berk BC (2001) Molecular cloning of mouse ERK5/BMK1 splice variants and characterization of ERK5 functional domains. *J Biol Chem* **276**: 10870–10878
- Yan L, Carr J, Ashby PR, Murry-Tait V, Thompson C, Arthur JS (2003) Knockout of ERK5 causes multiple defects in placental and embryonic development. *BMC Dev Biol* **3**: 11
- Yao Y, Li W, Wu J, Germann UA, Su MS, Kuida K, Boucher DM (2003) Extracellular signal-regulated kinase 2 is necessary for mesoderm differentiation. *Proc Natl Acad Sci USA* **100**: 12759–12764
- Yao Z, Flash I, Raviv Z, Yung Y, Asscher Y, Pleban S, Seger R (2001) Non-regulated and stimulated mechanisms cooperate in the nuclear accumulation of MEK1. *Oncogene* **20**: 7588–7596
- Zhou G, Bao ZQ, Dixon JE (1995) Components of a new human protein kinase signal transduction pathway. *J Biol Chem* **270**: 12665–12669