MAPK signalling: ERK5 versus ERK1/2

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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.

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 MAPKKs 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 MEK2, MEK3 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 MEK2 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).
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., 2003). It has also been reported that serum-stimulated cyclin D1 expression by different mechanisms: cAMP response element (CRE) 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; 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 pancellular, 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...
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 γ1 (PPARγ1) 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).
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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-cellular signal-regulated kinase.

What regulates the transcriptional activation activity of ERK5?
The activity of the C-terminal region of ERK5 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 autophosphorylation of its C-terminal half might be required for the transcriptional activation activity. Therefore, 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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