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ERK5 signaling gets XIAPed: a role for ubiquitin in the disassembly of a MAPK cascade

Aileen M Klein & Melanie H Cobb

Mitogen-activated protein kinase (MAPK) cascades are tightly controlled through a series of well-characterized phospho-regulatory events. In this issue, Takeda *et al* (2014) identify the inhibitor of apoptosis protein, XIAP, as a key regulator of ERK5 activation via uncoupling of upstream kinase activity by non-degradative ubiquitination.

See also: **A-N Takeda *et al*** (August 2014)

In eukaryotes, MAPKs are essential mediators of an immense variety of cellular responses to mitogenic, homeostatic, and deleterious stimuli. MAPKs are the terminal enzymes in an architecturally conserved core module of three kinases. MAPKs are regulated immediately upstream by a family of MAPK kinases (MAP2Ks), which themselves are regulated by MAPK kinase kinases (MAP3Ks). In mammals, four canonical MAPK families are recognized: ERK1/2, JNKs, p38s, and ERK5. Signaling specificity, fidelity, and termination are contingent on cell type and state, including expression and localization of substrates and communication between the MAPKs and other signaling pathways that may act to amplify or restrict output. Under physiological circumstances, rapid and robust activation of MAPKs is followed by similarly potent cessation of signaling, a process largely regulated by phosphatases that remove activating phosphorylation, and sometimes by negative feedback phosphorylation disconnecting upstream components (Raman *et al*, 2007).

Cross talk between the MAPKs and other signaling pathways through post-translational modifications other than phosphorylation comprises an additional layer of regulation to fine-tune the amplitude and duration of signaling downstream of MAPK cascades. One mode of coordinated signaling involves modification of the MAPK machinery by covalent linkage of ubiquitin, a small protein that can be added to a substrate to form a single adduct, or in longer chains to form a polyubiquitinated product. Multiple reactive lysines on ubiquitin can be attached, generating a wide range of possible ubiquitin configurations to influence target protein stability and function. Upon discovery, ubiquitination was viewed as a mechanism to signal the degradation of protein targets by the proteasome. More recent work has uncovered a plethora of non-proteolytic functions for ubiquitin in cell signaling (Chen & Sun, 2009). Regulatory ubiquitination plays diverse roles in MAPK signaling, from receptor internalization and recycling to inhibition and degradation of downstream signaling components (reviewed by Nguyen *et al*, 2013).

Previously, the Rajalingam group showed that degradation of c-Raf could be triggered by the inhibitor of apoptosis proteins XIAP and cIAP1/2, which in turn blocked downstream ERK1/2 activation and cell motility in a cell type-dependent manner (Dogan *et al*, 2008). The IAP proteins were initially characterized as inhibitors of caspase-mediated apoptosis. Reports in the last several years have established roles for this family in development and cell migration, particularly XIAP, cIAP-1, and cIAP-2, which possess E3 ubiquitin ligase activity in their C-terminal RING domains (Kenneth & Duckett, 2012).

Setting out to determine whether the IAP proteins could modulate the activity of other MAPK pathways, Takeda and colleagues found that XIAP and cIAP1 impose restrictions on ERK5 activation, surprisingly through a non-degradative ubiquitination mechanism (Takeda *et al*, 2014). ERK5 (also known as Big MAP Kinase, or BMK1) is quite similar to ERK2 with 66% identity in the kinase domain, but possesses a greatly extended C-terminal domain that can modulate its activity and localization (Nithianandarajah-Jones *et al*, 2012). ERK5 is activated by MEK5 (a MAP2K) downstream of MEKK2/3 (MAP3Ks), primarily in response to stress and mitogens, and documented mechanisms to suppress signaling are limited. Knockdown of XIAP and cIAP1 resulted in increased basal and mitogen-induced phosphorylation of ERK5. Biochemical analysis revealed that MEKK2 and MEKK3 were direct ubiquitination substrates of XIAP and cIAP1. MEKK2/3 ubiquitination readily allowed interaction with and phosphorylation of MEK5, but prevented formation of a ternary complex with ERK5. Despite its function as an activator of the JNK pathway, MEKK2 ubiquitination had no observed effect on JNK pathway activation, suggesting pathway specificity of this mechanism. Earlier work by the Nishida group had demonstrated that ERK5 is an essential mediator of myogenesis through its activity toward the Klf family of transcription factors to support a pro-myogenic transcriptional program (Sunadome *et al*, 2011). Consistently, loss of XIAP also enhanced myotube formation and expression of muscle differentiation markers (Takeda *et al*, 2014) (see Fig 1).

Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX, USA. E-mail: melanie.cobb@utsouthwestern.edu
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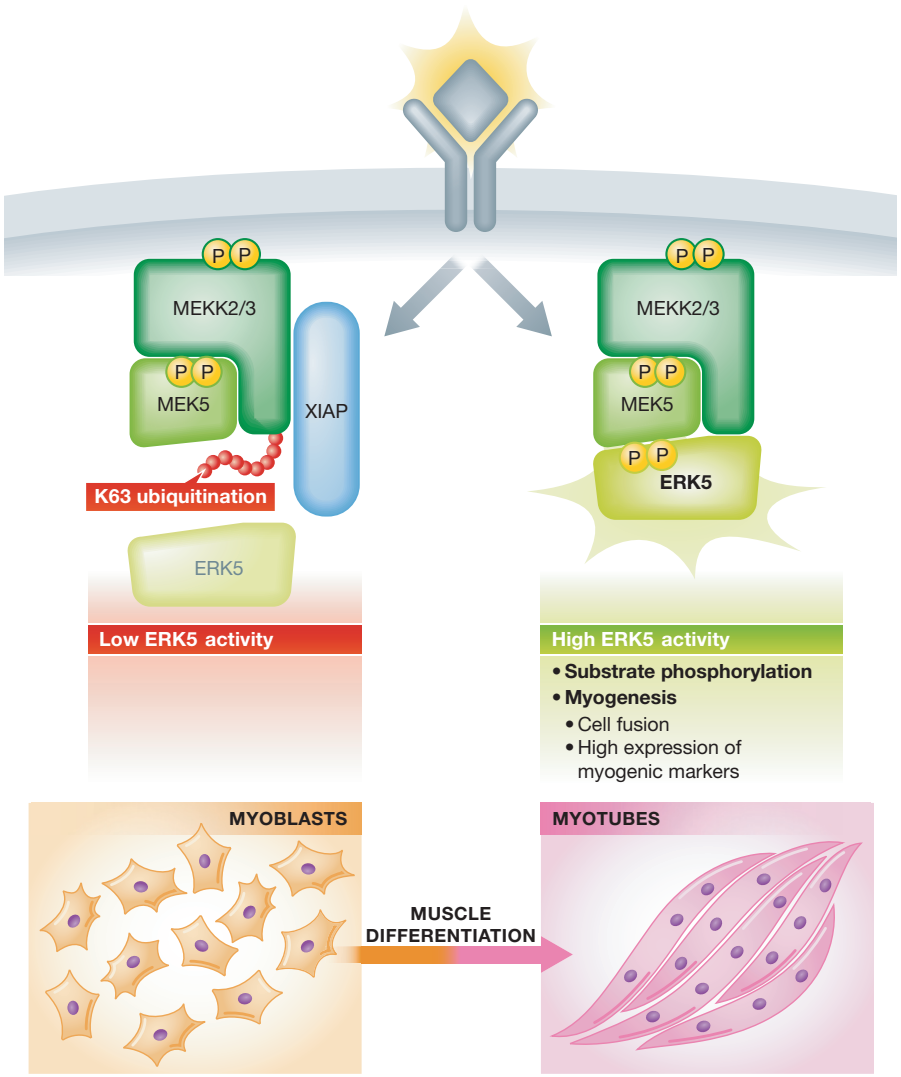

Figure 1. Model of ERK5 pathway activation and inhibition by XIAP-mediated ubiquitination.
See text for details. MEKK2/3, mitogen-activated protein kinase kinase kinase 2/3; MEK5, mitogen-activated protein kinase kinase 5; ERK5, extracellular signal-regulated kinase 5; XIAP, X-linked inhibitor of apoptosis protein.

The discovery of a direct interaction between the IAP family and MAPK cascade components by Takeda and colleagues uncovers a previously unrecognized mechanism to suppress MAPK signaling that involves non-degradative ubiquitination. On a molecular level, it remains unclear how ubiquitination prevents interaction of the MEKK2/3-MEK5 module with ERK5, but not MEK5. Interaction between MEKK2/3 and MEK5 is mediated by heterodimerization of PB1 domains that have a ubiquitin-like structure, and the MEK5 PB1 domain is also important for ERK5 interaction (Sumimoto *et al*, 2007). In addition to possible effects on localization, MEKK2/3 ubiquitination may simply mimic a PB1 domain interface to specifically and elegantly disrupt ERK5 binding.

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