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The PHD motif of Map3k1 activates cytokine-dependent MAPK signaling

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We generated a mutation in the gene encoding mitogen-activated protein kinase kinase kinase 1 (*Map3k1*) that results in a protein with an inactive plant homeodomain (PHD). *Map3k1*^{mPHD} cells are defective in cytokine-mediated MAPK signaling. Protein array identified transforming growth factor (TGF-β)-activated kinase 1 binding protein 1 (Tab1) as a PHD substrate. The Map3k1 PHD transfers Lys63-linked poly-ubiquitin onto Tab1 to activate MAPKs.

Mitogen-activated protein kinases (MAPKs) are a family of protein kinases that regulate a multitude of cellular responses including cell death, proliferation, and differentiation.[1-3] MAPKs are regulated by the upstream phosphorylation of their kinases (MAP2Ks), which in turn are regulated by phosphorylation through the MAP2K kinases (MAP3Ks).[3] Of the 19 MAP3Ks present in mammals Map3k1 is unique in containing both a kinase domain and a plant homeodomain (PHD) that shares significant homology to a Really Interesting New Gene (RING) motif, and by these means can regulate both the MAPK signaling pathways and the ubiquitin (Ub) proteasome system.[4-6] To understand the role of the Map3k1 PHD motif *in vivo* we mutated *Map3k1* alleles to ablate the E2 binding region of the Map3k1 PHD motif (*Map3k1*^{mPHD}).[7]

Map3k1^{mPHD} embryonic stem (ES) cells are deficient in c-Jun N-terminal kinase (Jnk) and p38 Mapk activation in response to epidermal growth factor (EGF), transforming growth factor-β (TGF-β), and nocodozole.[7] These findings suggested that the Map3k1 PHD motif has an unexpected and critical role in controlling MAPK activation. Early work using cell lines had previously suggested that the Map3k1 PHD motif silenced MAPK signaling in response to hyperosmotic stress by the transfer of Lys48-linked poly-Ub onto extracellular-signal-regulated kinase 2 (Erk2), leading to its proteasomal degradation.[4]

To better understand the role of the Map3k1 PHD motif as an activator of MAPK signaling pathways we conducted a high-throughput Ub protein array screen of over 9,400 full-length proteins to identify novel substrates for the Mapk31 PHD motif.[7] Out of the screened hits, TGF-β activated kinase 1 binding protein 1 (Tab1) was identified by bioinformatics analysis as a candidate for the regulation of MAPK activation by TGF-β.[7] Indeed, Tab1 is ubiquitinated by Lys63-linked poly-Ub chains in response to TGF-β treatment, and this noncanonical ubiquitination is deficient in *Map3k1*^{mPHD} ES cells.[7] *Tab1*$^{-/-}$ ES cells are also defective in Jnk and p38 activation in response to TGF-β and EGF cytokines.[7]

Following engagement of the EGF receptor or TGF-β receptor (TGFβR) with cytokines, Map3k1 is recruited to the receptor in an inactive state (**Fig. 1**).[7] However, unlike CD40 signaling in B cells, TGFβR signal transduction utilizes tumor necrosis factor receptor associated factor (Traf) 6, rather than Traf2, as an adaptor to mediate MAPK signal transduction.[2,5,7] As Map3k1 becomes activated it binds and acts with Ub-conjugating enzyme E2n (Ube2n): Ub-conjugating enzyme E2 variant 1 (Ube2v1) to transfer Lys63-linked Ub onto Tab1.[7] This Ub signaling complex facilitates Tak1 and Mapk activation.[2,7] Tab2 can also join the Ub signaling complex by binding Lys63-linked poly-Ub chains on Tab1 through its Ub-binding zinc finger motif.[7,8] Thus, the Map3k1 PHD motif facilitates Tak1 and Mapk activation by the noncanonical ubiquitination of Tab1.[7]

Although *Map3k1*^{mPHD} ES cells have an aberrant global gene expression signature they are normal in their pluripotency.[7] Instead, *Map3k1*^{mPHD} and *Tab1*$^{-/-}$ ES cells are deficient in differentiation and are aberrant in the neuroectoderm, the germ layer that is important in the formation of the nervous system, and the mesoderm, the middle layer of germ cells in the developing embryo, as they form embryoid bodies.[7] *Map3k1*^{mPHD} embryos suffer from early lethality and die within a few days.[7] Aberrant regulation of MAPK signaling and the Ub-proteasome

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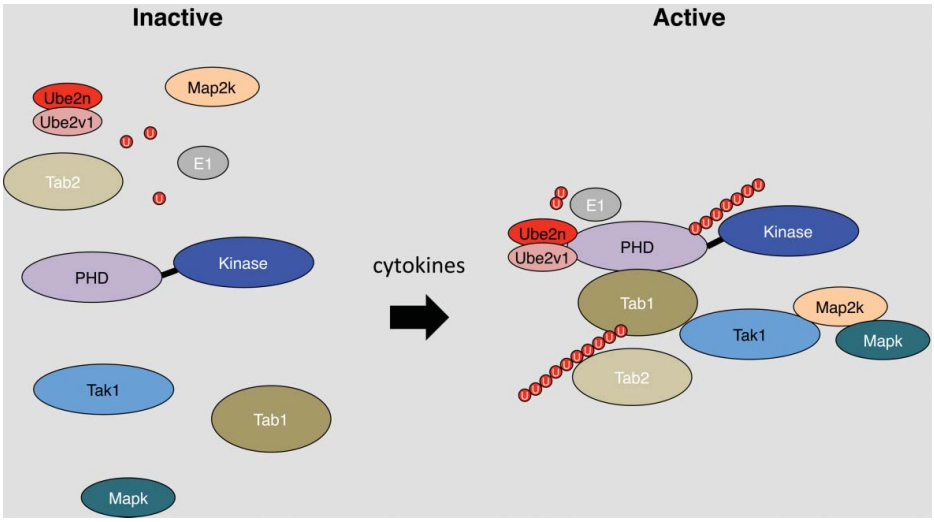

Figure 1. Map3k1 initiates Jnk and p38 Mapk activation in response to cytokines via its PHD motif. Following treatment with the cytokine TGF-β, the Map3k1 PHD motif binds and then, in concert with E1 and Ube2n:Ube2v1, transfers Lys63-linked poly-Ub onto Tab1 to enhance activation of Tak1, Map2k, and Mapk. Tab2 can then be recruited into the Ub signaling complex by its zinc finger motif. Jnk, c-Jun N-terminal kinase; Map3k1, mitogen-activated protein kinase kinase kinase 1; MAPK, mitogen activated protein kinase; PHD, plant homeodomain, Tab1, Tak1 binding protein; Tak1, TGF-β activated kinase 1; TGF-β, transforming growth factor-β, Ube2n, ubiquitin (Ub)-conjugating enzyme E2n; Ube2v1, Ub-conjugating enzyme E2 variant 1.

system both provide a likely explanation for the more severe phenotype of *Map3k1*^{mPHD} mice relative to *Map3k1* kinase-deficient (*Map3k1*$^{\Delta KD}$) mice.[7,9] *Map3k1*$^{mPHD/+}$ mice are viable and display defective B-cell development, Itch phosphorylation in T cells, cardiac tissue, and gonadal development.[7] *Map3k1*^{mPHD} and *Tab1*$^{-/-}$ stem cells are also defective in tumorigenesis when transplanted into an immunodeficient mouse strain, forming tumors of lower size and mass and with altered tissue composition.[7] Overall, our research findings reveal a novel signaling mechanism through which the Map3k1 PHD motif and the Ub-proteasome system activate MAPKs from cytokine receptors, and also demonstrate the critical importance of the Map3k1 PHD motif in mammalian biology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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