

STUDYING MAP KINASE SIGNALING WITH A SMALL-MOLECULE INHIBITOR, U0126

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Introduction

To stimulate cells to proliferate, differentiate or undergo apoptosis, extracellular signals need be relayed to the nucleus where they alter the pattern of gene expression. One of the main mechanisms for relaying such signals involves a group of proteins called the mitogen-activated protein kinases (MAPK). These enzymes are proline-directed serine/threonine kinases that are activated by dual phosphorylation of threonine and tyrosine residues in response to diverse extracellular stimuli. Small molecules that inhibit MAPKs are important for understanding cell signaling.

MAPK Pathways

Six MAP kinase signaling pathways have been identified in mammals (ERK1/2, ERK3/4, ERK5, ERK7/8, JNK1/2/3 and p38/ERK6; 1). Despite being complex in nature, these pathways can be divided into three main steps involving the sequential activation of MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK (Figure 1). 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; 2].

ERK1 and ERK2 (ERK1/2) are isoforms of the “classical” MAPK and are activated by MAP/ERK kinase 1 (MEK1) and MEK2 (MEK1/2), which are members of the MAPKK family. ERK1/2 and MEK1/2 are acutely stimulated by growth and differentiation factors in pathways mediated by receptor tyrosine kinases, heterotrimeric G protein-coupled receptors or cytokine receptors, primarily through p21Ras-coupled mechanisms. MEK or ERK activity is upregulated in response to cell stimulation by phosphorylation at residues located within the activation lip of each kinase. In the case of MEK, phosphorylation at two serine residues by upstream protein kinases, Raf-1, c-Mos or MAPK kinase kinase, leads to maximal enzyme activation. Subsequently, MEK1/2 activates ERK1/2 by phosphorylating regulatory threonine and tyrosine residues. Thus, MEKs fall within a relatively rare class of protein kinases with dual specificity toward Ser/Thr and Tyr residues on exogenous substrates (3). 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 (4).

Tools for Studying MAP Kinases

Biochemical reagents that are highly specific and highly selective for individual enzymes in the pathway are essential

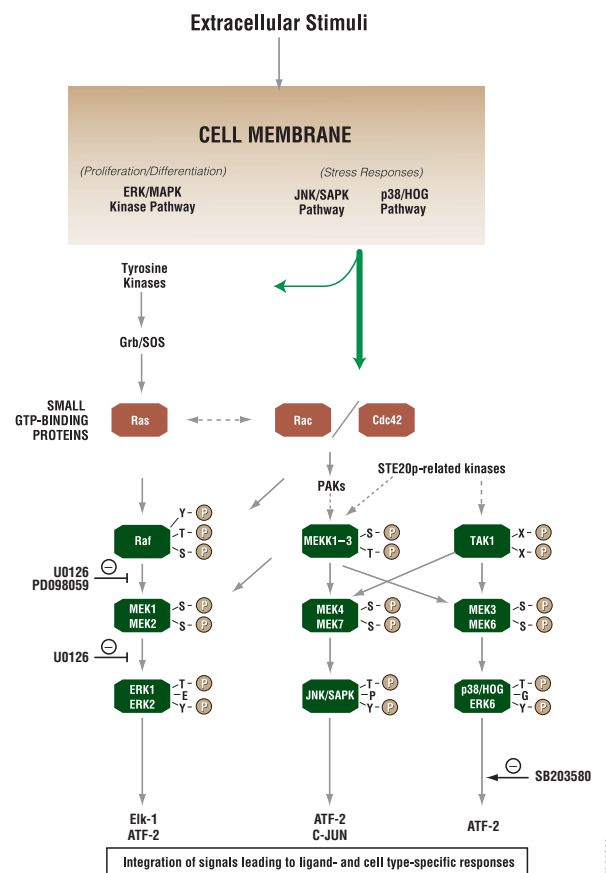


Figure 1. Activation of three different MAPK signaling cascades by different extracellular stimuli. The ERK, JNK and p38 cascades all contain the same series of three kinases. A MEK Kinase (MEKK) phosphorylates and activates a MAP Kinase Kinase (MEK), then MEK phosphorylates and activates a MAP Kinase (MAPK).

to understanding the MAPK cascade. Such reagents have enabled scientists to distinguish among the main MAP kinase pathways and to understand the role of various MAP kinases in cellular regulation by extracellular stimuli. The availability of SB203580, a specific inhibitor of p38, has made it possible to identify effects mediated by or requiring active p38 protein kinase (5). Likewise, another inhibitor, PD098059, which inhibits MEK1/2 activation, has been used to study ERK-mediated responses (6).

Promega also offers the inhibitor U0126 (1,4-diamino-2,3-dicyano-1,4 bis[2-aminophenylthio] butadiene; Figure 1), a chemically synthesized organic, white, solid compound that is soluble in dimethyl sulfoxide (DMSO). U0126 was initially recognized as an inhibitor of cellular AP-1 transactivation in cell-based reporter assays (7). It specifically inhibits MEK1 activity in vitro, as well as activation of ERK1/2 by MEK1/2

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MEK INHIBITOR U0126

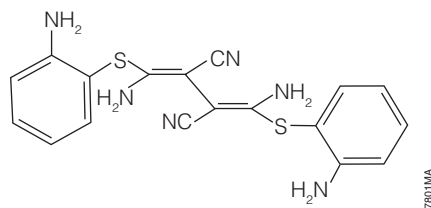


Figure 2. The small molecule MEK1/2 inhibitor U0126.

in vivo. The compound appears to inhibit MEK1 directly in contrast to the widely used inhibitor PD098059, which inhibits MEK1/2 activation indirectly by binding to the inactive enzyme thereby preventing its activation by Raf kinase.

Because of this, PD098059 may not be useful for inhibiting endogenously active MEK1/2. However, U0126 is capable of directly inhibiting activated MEK1 and preventing endogenously active MEK1/2 from phosphorylating and activating ERK1/2 (Figure 1). Therefore, the compound U0126 acts one step further downstream of PD098059 and blocks MEK1/2 activity (8). The inhibitor U0126 thus serves as a useful tool to investigate the effect of various signals on the activation of MAPK in cells and further delineation of the MAPK pathways in cellular regulation.

References

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Ordering Information

Product	Size	Cat.#
MEK Inhibitor U0126	5 mg (5 × 1 mg)	V1121

Peer-Reviewed Articles Citing Use of U0126 Inhibitor

Joo, J.H. and Jetten, A.M. (2008) NF- κ B-dependent transcriptional activation in lung carcinoma cells by farnesol involves p65/RelA(Ser²⁷⁶) phosphorylation via the MEK-MSK1 signaling pathway. *J. Biol. Chem.* **283**, 16391–9.

This article showed that expression of several immune response genes could be induced in lung adenocarcinoma H460 cells by treatment with farnesol and that this induction proceeds through an NF- κ B pathway. To determine which MAPKs were involved in the activation of these genes, the authors used the MEK Inhibitor U0126 and showed that it inhibited the expression of several of the immune response genes as well as specifically inhibited the phosphorylation of p65 on Ser²⁷⁶.

Maekawa, M. *et al.* (2007) Requirement for ERK MAP kinase in mouse preimplantation development. *Development* **134**, 2751–9.

The authors of this study investigated the role of ERK1/2 kinase signaling in the preimplantation developmental events that precede compaction and blastocyst formation (2-cell to 8-cell stages). The MEK Inhibitor U0126 was added to 2-cell stage mouse embryos, and embryos were observed to arrest at the 4-cell stage. The arrest was reversible, but experiments indicated that ERK1/2 signaling was required for M-phase progression early cell cycles during mammalian development.

Case, M. *et al.* (2008) Mutation of genes affecting the RAS pathway is common in childhood acute lymphoblastic leukemia. *Cancer Res.* **68**, 6803–9.

The authors of this study investigated the relationship of somatic mutations that deregulate the RAS-RAF-MEK-ERK pathway and Acute Lymphoblastic Leukemia (ALL) and its progression to relapse in some patients. They show that such mutations are common in ALL and in its recurrence. Furthermore, lymphoblasts from patients with mutations that were associated with upregulation of ERK showed increased cytotoxicity over wild-type controls when treated with MEK Inhibitor U0126, suggesting that specific ERK inhibitors may eventually yield useful therapeutics.

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