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Intracellular signaling cascades

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Intracellular signaling cascades are the main routes of communication between the plasma membrane and regulatory targets in various intracellular compartments. Mitogen-activated protein kinase (MAPK) signaling cascades, which are important in the transmission of many extracellular signals, consist of up to five tiers (levels) of protein kinases that activate each other by phosphorylation (Fig. 1). The four distinct MAPK cascades that are currently known were named according to the subgroup of their MAPK components [the ERK, JNK, SPK (p38MAPK), and BMK (ERK5) cascades. These MAPK cascades cooperate to transmit signals to their intracellular targets and thus to initiate cellular processes such as proliferation, differentiation, development, stress responses and apoptosis. Another important intracellular signaling pathway operates via the lipid kinase PI3K and uses a kinase cascade that includes PDK1, PKB and GSK3 and is known as the PKB cascade. This cascade is thought to be involved primarily in cell survival but can function also in proliferation and stress response. Finally, a PKA-dependent cascade that includes also phosphorylase kinase is known to be involved primarily in metabolic processes.

In my laboratory we are studying all the above cascades although our main studies in the last years dealt with the ERK cascade. Since ERK activation occurs in response to diverse stimuli, key questions in the field are: (i) Are the MAPK cascades directly involved in the transmission of so many signals? (ii) What are the molecular mechanisms by which MAPKs regulate the divergent stimuli-induced processes? (iii) How is the signaling specificity determined? (iv) What is the mechanism by which the multiple stimuli funnel their signals into the MAPK cascades? In order to answer the above questions and to obtain a better view of membrane to nucleus signaling by the MAPK cascades, the group has concentrated on the following issues:

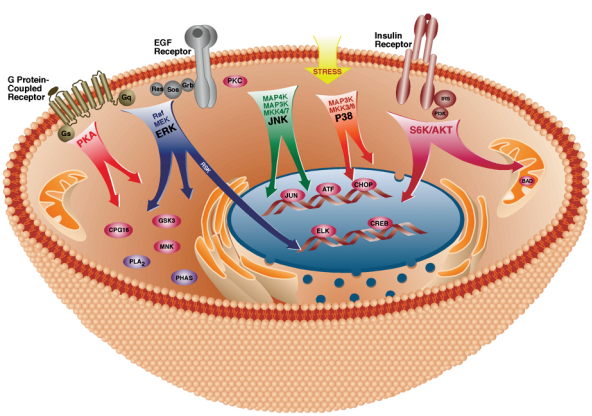

Fig. 1 *Schematic representation of intracellular signaling cascades.*

Subcellular localization of ERK1/2, ERK5 and MEK1/2

Since nuclear translocation of ERK and MEK is an important factor in the transduction of many signals and in the determination of signal specificity, we undertook to study the mechanism of nuclear translocation of components of the ERK cascade. We found that in resting cells ERK1/2, ERK5 and MEK1/2 are localized in the cytosol due to interaction with anchoring proteins and there is also an interaction between ERK1/2 and MEK1/2. Upon activation, ERK1/2 and MEK1/2 are detached from the anchoring proteins and translocate into the nucleus. ERK5 seems to stay in the cytosol under all conditions. The translocation of ERK1/2 and MEK1/2 involves non-regulated, as well as stimulated processes. Upon entering into the nucleus, MEK1/2 are rapidly exported from this location by CRM1 whereas ERK1/2 are retained in the nucleus for 30-180 minutes. During our studies we also identified the regions in ERK and MEK that are responsible for each step of the translocation including cytosolic retention sequence (CRS, also termed CD) and nuclear translocation sequence (NTS) in ERK1/2 (Fig. 2).

Purification, cloning and characterization of ERK1b

In each level of the ERK cascade there are several very similar isoforms. Recently, we cloned a 46 kDa ERK isoform, termed ERK1b that is an alternatively spliced form of ERK1, containing a 26 amino acid insertion between the CRS and NTS of ERK1/2 (Fig. 2). Unlike the uniform pattern of expression of ERK1/2, ERK1b was confined to specific tissues including heart, brain, lung and kidney. Moreover, in Ras transformed Rat1 cells, there was a higher expression of ERK1b, which was also more responsive than ERK1/2 to various extracellular treatments in the transformed cells. We found that a reduced interaction of ERK1b with phosphatases resulted in a reduced sensitivity of ERK1b to dephosphorylation. These results suggest that ERK1b may be required for the transmission of extracellular signals under conditions of persistent stimulation, where induced MAPK phosphatases suppress the activity of ERK1/2.

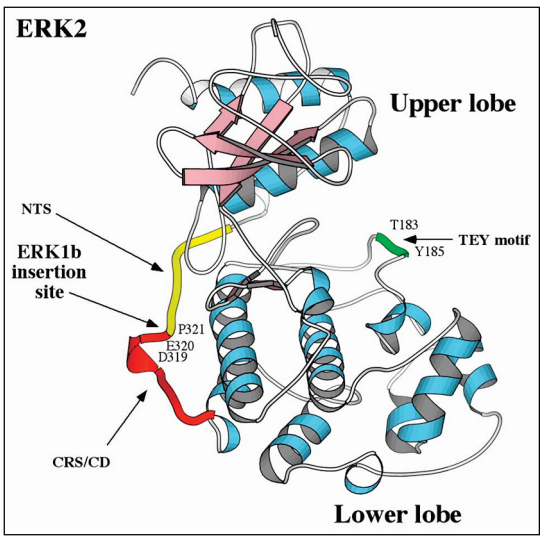

Fig. 2 *Three dimensional structure of ERK2 and the site of ERK1b insert.*

Signaling by the GnRH receptor

Although the mechanism of growth factor-induced activation of the MAPK and PI3K as well as their effect on PKA is well understood, the induction of the cascades by GPCRs is still not fully elucidated. Using αT3-1 and COS7 cells, we found that the activation of the signaling cascades vary between cell lines. In the αT3-1 cells the activation involve PKC, Src and dynamin whereas in the GnRH-receptor-expressing COS7 cells the pathway involves EGF receptor, Src, PI3K and arrestin.

Development of anti-phospho-MAPK antibodies

A major difficulty encountered when studying the ERK signaling cascade was the lack of proper tools to study ERK activity *in-vivo*. This became especially important once we realized that ERK is involved in differentiation and development, which are best examined in whole organisms and might involve only a small number of cells. Therefore, we undertook to develop antibodies directed against the phospho-forms of various components of the different signaling cascades, which would allow a simple determination of their activation and inactivation processes both *in-vivo* and *in-vitro*.

Role of PKA cascade

We investigated the involvement of PKA in gonadotropin and neuronal signaling and found that together with the MAPK cascade PKA is involved in the regulation of StAR expression in granulosa cells. We also found that PKA can be involved in the downregulation of MAPK signals in the brain by activation of the kinase CPG16.

Future directions

Our central long-term objective is to obtain a comprehensive view on intracellular signaling in proliferation and oncogenesis. This will be achieved by studies on: (i) The protein-protein interaction and the subcellular localization of signaling components; (ii) the regulation of nuclear processes by MAPK cascades; (iii) mapping distinct intracellular signaling networks; and (iv) understanding the role of MAPK cascades in cancer. These approaches have, and will continue to allow the elucidation of the key regulators of proliferation and oncogenesis.

Selected Publication

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