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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. During the past decade, several intracellular signaling cascades have been elucidated, which operate mainly via a sequential activation of protein kinases (protein kinase cascades), to regulate many cellular processes (Fig. 1). Such a mechanism is used by four mitogen-activated protein kinase (MAPK) signaling cascades, which are important in the transmission of many extracellular signals. Each of these signaling cascades consists of up to five tiers (levels) of protein kinases that activate each other by phosphorylation. These MAPK cascades cooperate to transmit signals to their intracellular targets and thus to initiate cellular processes such as proliferation, differentiation, development, stress response 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 is 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, and it can regulate large number of distinct cellular processes, one of the key questions in the field is the determination of the signaling specificity of the ERK cascade. In other words, what are the mechanisms that allow similar ERK activations to result in such a diverse array of downstream effects? In the past years we concentrated on several such mechanisms, including: (i) Compartmentalization and subcellular localization that may direct ERK signals to distinct targets. (ii) The production of alternatively spliced forms of the ERK with distinct substrate specificities. (iii) Crosstalk with other signaling cascades that may modify the signaling outcome. (iv) Regulation of the ERK cascade by phosphatases, which are the tool that governs the duration of the signal and thereby modulate the repertoire of ERK's targets. Few of our recent findings are:

(a) Subcellular localization: We have previously shown that ERK1/2 and MEK1/2 are localized in the cytosol due to interactions with ancoring proteins that include MEK1/2. Upon activation, ERK1/2 and MEK1/2 are detached from the encoring proteins and translocate into the nucleus. 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. Some of our main findings during this study are: (i) Identification of the regions in ERK1/2 and MEK1/2 that are responsible for each step of the translocation, including cytosolic retention sequence (CRS, also termed CD, Fig. 2). (ii) The cyto-nuclear shuttle of MEK1 may induce an export of nuclear proteins such as PPARγ to the cytosol and inhibit their nuclear activity. (iii)

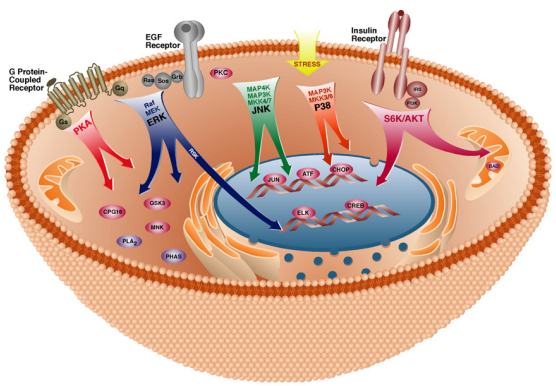

Fig. 1 *Schematic representation of intracellular cascades.*

The subcellular localization of the ERK5-MEK5 is distinct from other MAPK components as they are constantly localized in the nucleus.

(b) Alternatively spliced isoforms of ERKs: The two most abundant isoforms of the ERKs are ERK1 and ERK2, the regulation of which seems to be very similar under all cell lines and conditions examined. We have identified additional forms of ERK that seem to be activated by MEKs but are subjected to a different mode of regulation. One such isoform is the 46 kDa alternatively spliced form of ERK1 that we termed ERK1b, that was identified in mouse and rat. We found that ERK1b has a unique mode of regulation, and under certain conditions its activation is higher than that of ERK1 and ERK2. Interestingly, ERK1b expression is increased upon oncogenic transformation, making it the main isoform of ERK that is responsive to extracellular stimulations. Another isoform that we have identified is ERK1c, which is produced in human and monkey by a similar splicing process as in rat (inclusion of intron 7). However, since the sequence of intron 7 in human and monkey contains a stop codon, ERK1c in these species is a shorter, 42 kDa protein. Interestingly, ERK1c has a unique mode of regulation, distinct from ERK1, ERK2 and ERK1b, and can undergo mono-ubiquitination. In similarity to ERK1b, the amount of this protein is ~10% of ERK1. Finally, we have also identified a 39 kDa ERK1d that is expressed at small levels (~3% of ERK1) mainly in human, and is strongly activated in T cells.

(c) Crosstalk with other signaling cascades: We studied GPCR signaling using the GnRH receptor as a model. Using various GnRH receptor-expressing cell lines, For example, in DU145 cells stimulation of the GnRH receptor may lead to apoptosis via activation of the JNK cascade and inhibition of the PI3K-PKB pathway. In addition, we are also studying the signaling elicited by the plasma factor PEDF. We found that extracellular phosphorylation of this factor can convert its activity from anti-angiogenic to neurotrophic, and this is not mediated by ERK alone, but probably by a combination of several intracellular signaling processes.

The central long-term objective of our study 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. (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.

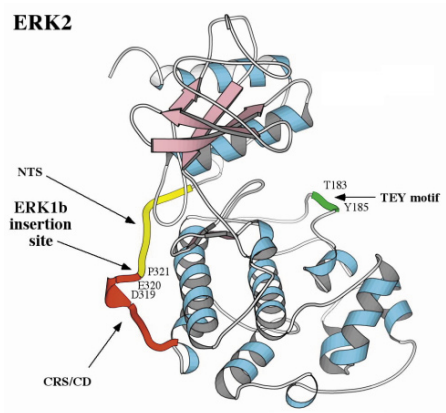

Fig. 2 *Three-dimensional structure of ERK2 and the site of ERK1b insert.*

Selected Publications

Wolf, I., Rubinfeld, H., Yoon, S., Marmor, G. Hanoch, T., and Seger R. (2001) Involvement of the activation loop of ERK in the detachment from cytosolic anchoring. J. Biol. Chem. 276, 24490-97.

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Kraus, S., Benard, O., Naor, Z., and Seger. R. (2003) c-Src is activated by EGF-receptor in a pathway that mediates JNK and ERK activation by GnRH in COS7 cells. J. Biol. Chem. 278, 32618-32630.

Raviv, Z., Kalie, E., and Seger R. (2004) the subcellular localization of components of the ERK5 cascade. J. Cell Sci. 117, 1773-1784.

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