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 also function in proliferation and stress response. Finally, a PKA dependent cascade is involved primarily in metabolic processes.

Our laboratory is studying all of the above cascades, although our main studies in the last years are focused on the extracellular signal-regulated kinase (ERK) cascade. Since ERK activation occurs in response to diverse stimuli, and it can regulate a 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 of resting cells due to interactions with anchoring proteins. Upon activation, ERK1/2 and MEK1/2 are detached from the anchoring proteins and

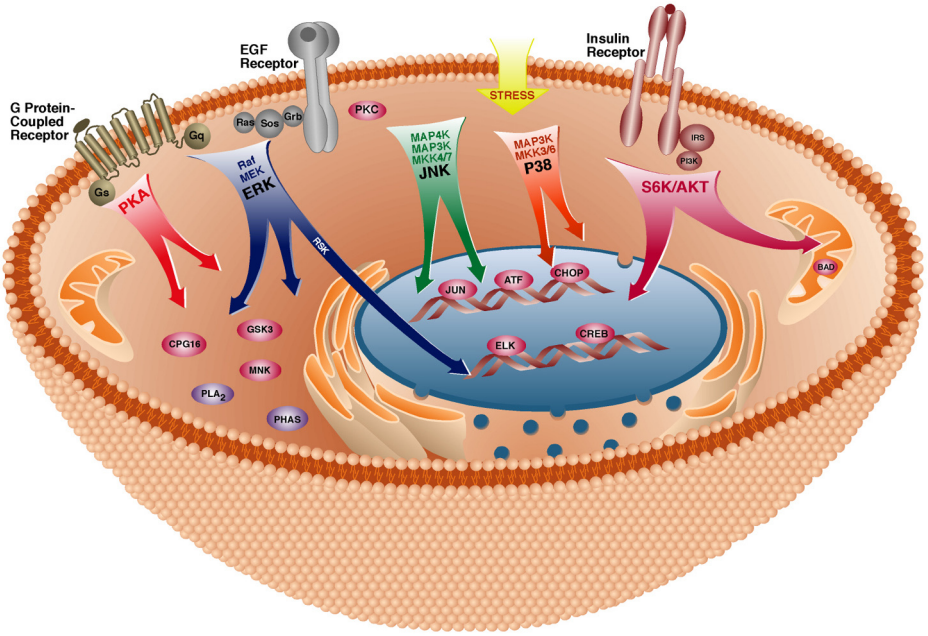

Fig. 1 *Schematic representation of intracellular signaling cascades.*

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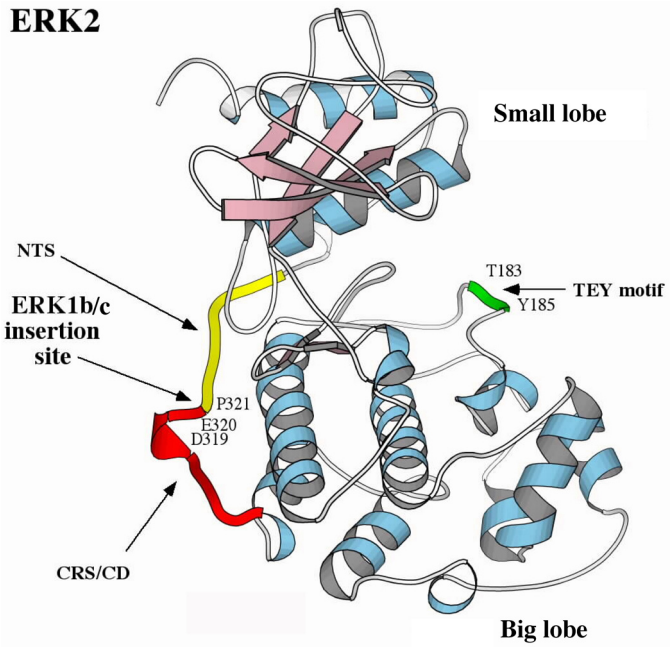

***Fig. 2**. Three-dimensional structure of ERK2 and the site of ERK1b or ERK1c insert.*

translocate into the nucleus. Immediately after entering 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 or estrogen-receptor to the cytosol and inhibit their nuclear activity. (iii) The subcellular localization of ERK5-MEK5 is distinct from other MAPK components as they are constantly localized in the nucleus, and their activity is regulated by modulation in intracellular calcium levels.

(b) Alternatively spliced isoforms of ERKs: The two most abundant isoforms of the ERKs are ERK1 and ERK2, that are regulated in a very similar manner in all cell lines and under all 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 rodent 46 kDa ERK1b, which has a unique mode of regulation, and its expression is increased upon oncogenic transformation. Another isoform that we have identified is ERK1c, which is produced in primates by a similar splicing process to that of the rodent ERK1b (inclusion of intron 7). ERK1c is a shorter, 42 kDa protein with expression levels of ~12% of ERK1, which is also regulated differently from ERK1 and ERK2. Importantly, we showed that ERK1c has a unique physiological function, as it specifically regulates Golgi fragmentation during mitosis and elevation in cell density. Thus, ERK1c extends the specificity of the Ras-MEK cascade by activating ERK1/2-independent processes. 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 stimulated T cells.

(c) Crosstalk with other signaling cascades: We studied G protein coupled receptor (GPCR) signaling using the gonadotropin-releasing hormone (GnRH) receptor, as a model in various GnRH receptor-expressing cell lines. For example, in DU145 cells stimulation of the GnRH receptor may lead to apoptosis via activation of the c-Jun N-terminal kinase (JNK)/MAPK 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 pigment epithelium-derived factor (PEDF) purified from plasma is a phosphoprotein, which is extracellularly phosphorylated by protein kinase CK2 (CK2), and to a lesser degree, intracellularly, by protein kinase A (PKA). CK2 phosphorylates PEDF on two main residues, Ser24 and Ser114, and PKA phosphorylates PEDF on one residue only, Ser227. The physiological relevance of these phosphorylations was determined by using phosphorylation site mutants. We found that both CK2 and PKA phosphorylations of PEDF markedly affect its physiological function. The fully CK2 phosphorylation site mutant S24,114E abolished PEDF neurotrophic activity, but enhanced its antiangiogenic activity, while the PKA phosphorylation site mutant S227E reduced PEDF antiangiogenic activity.

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 studying (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.

Selected publications

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.

Kraus, S., Levy, G., Hanoch, T., Naor, Z., and Seger, R. (2004) Gonadotropin-releasing hormone induces apoptosis of prostate cancer cells: role of c-Jun NH2-terminal kinase, protein kinase B, and extracellular signal-regulated kinase pathways. Cancer Res. 64, 5736-5744. Selected for the Cancer Research Highlights in Cancer Res. 64, 5525.

Aebersold, D.M., Shaul, Y.D., Yung, Y., Yarom, N., Yao, Z., Hanoch , T., and Seger, R. (2004) ERK1c, a novel 42-kDa ERK demonstrates a unique mode of regulation, localization and function. Mol. Cell Biol., 24, 10000-10015.

Maik-Rachline, G., Shaltiel, S., and Seger, R. (2005) Extracellular phosphorylation converts pigment epithelium-derived factor from a neurotrophic to an antiangiogenic factor. Blood, 105, 670-678.

Yoon, S., and Seger, R. (2006) The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. Growth Factors, 24, 21-44.

Maik-Rachline, G., and Seger, R. (2006) Variable phosphorylation states of pigment epithelium-derived factor differentially regulate its function. Blood, 107, 2745-2752.

Shaul Y.D., and Seger, R. (2006) ERK1c regulates Golgi fragmentation during mitosis. J. Cell Biol., 172, 885-897.

Cohen-Armon, M., Visochek, L. Rosensal, D., Kalal, A., Klein, R. Bendetz-Nezer, S. Yao, Z., and Seger, R. (2006) Activation of PARP-1 by phosphorylated ERK2: A link to histone acetylation. Mol Cell, under revision.

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