Rony Seger
Outhiriaradjou Benard
David Bonfil
Naomie Fitoussi
Yossi Friedman
Tami Hanoch
Dan Michael
Ziv Raviv
Hadara Rubinfeld
Ido Wolf
Yuval Yung
Zhong Yao

The mitogen-activated protein kinase (MAPK) signaling cascades

Department of Biological Regulation

Tel. +972 8 934 3602 Fax. +972 8 934 4116
E.mail: rony.seger@weizmann.ac.il

Intracellular signaling cascades are the main routes of communication between the plasma membrane and regulatory targets in various intracellular compartments. Sequential activation of kinases (protein kinase cascades) is a common mechanism of signal transduction in many cellular processes. During the past decade, several related intracellular signaling cascades have been elucidated, which are collectively known as mitogen-activated protein kinase (MAPK) signaling cascades (Fig 1). Each of these signaling cascades seems to consist of up to five tiers (levels) of protein kinases that sequentially activate each other by phosphorylation. 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; see Fig.1]. 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. In my laboratory we are studying all four MAPK cascades as well as other kinase mediated signaling cascades. However, our main studies in the last four years delt with the first cascade to be elucidated, namely 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 mechanism 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 MAPKs, my group has concentrated on the following:

(a) Mitogenesis and oncogenesis in adherent mammalian cells: 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 (i) the mechanism and importance of nuclear translocation of components of the ERK cascade

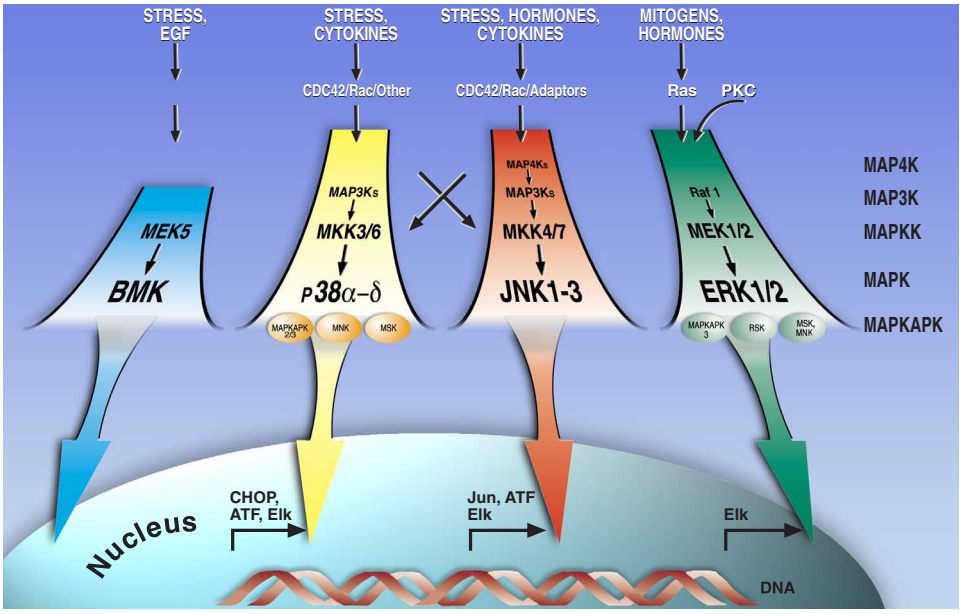

Fig. 1. *The MAPK signaling cascades*

(Fig. 2) (ii) the downstream targets of the ERK cascade, (iii) novel MAPK isoforms (ERK1b, ERK5 and more), and (iv) the mechanism of ERK inactivation.

(b) Signaling by the GnRH receptor in pituitary-derived αT3-1 cells: Although the mechanism of growth factor-induced activation of the MAPKs is well understood, MAPK induction by GPCRs is still not fully elucidated. Using αT3-1 cells, we studied the mechanism of protein Tyr kinases (PTKs) activation by PKC, a key step in Gq signaling towards the MAPK cascades. We also attempted to identify the other components in this signaling cascade.

(c) 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-form of various components of the MAPK cascades, which would allow a simple determination of their activation and inactivation processes both in-vivo and in-vitro.

(d) Physiological role of the ERK cascade: The above antibodies and other reagents that we were first to develop, have prompted several collaborations that aimed to study the role of ERK in different tissues and organisms. These studies have contributed largely to the growing appreciation that beside its role in proliferation and growth factor signaling, ERK is involved also in (i) development and differentiation, (ii) morphological changes in cells upon stimulation and (iii) memory process in the central nervous system. These studies led also to identification of new targets of the ERK cascade in specific cells or experimental conditions including (iv) regulation of Syk in Mast cells, (v) glycogen metabolism, (vi) Tau promotor.

In the coming years the research of the group will continue to center around intracellular signaling through the MAPK signaling cascades. 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 regulation of nuclear processes by MAPK cascades, (ii) mapping distinct intracellular signaling networks, and (iii) 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.

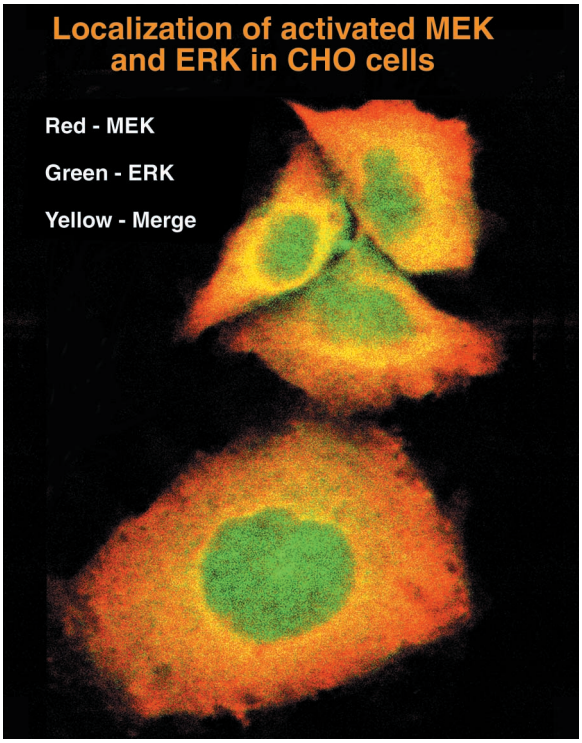

Fig.2. *Staining of ERK and MEK in EGF-stimulated CHO cells. About 50% of the ERK molecules and >10% of the MEK molecules were detected in the nucleus upon stimulation. (with the help of Dr. Eyal Schechter from the Department of Molecular Genetics).*

Selected Publications (out of 41)

Jaaro, H., Rubinfeld, H., Hanoch, T., and Seger, R. (1997) Nuclear translocation of mitogen-activated protein kinase kinase (MEK1). PNAS, 94, 3742-3747.

Yung, Y., *et al.* 1997 Detection of ERK activation by a novel antibody. FEBS Letters 408, 292-296.

Gabay, L., Seger, R., and Shilo B.-Z. (1997) In situ activation pattern of the Drosophila EGF receptor pathway during development. Science 277, 1103-1106.

Levi, N.L., *et al.* (1998) Stimulation of Jun N-terminal kinase (JNK) by gonadotropin-releasing hormone in pituitary αT3-1 cell line is mediated by protein kinase C, c-Src, and CDC42. Mol. Endocrinol. 12, 815-824.

Yao, Z., and Seger, R. (1998) Immunological Detection of Phosphorylation. Current Protocols in Cell Biology. Unit 14.2

Rubinfeld, H., and Seger, R. (1998) Methods in MAPK signaling. Current Protocols in Cell Biology Unit 14.3

Silverman, M.A., *et al.* (1999) CPG16: A novel cAMP-stimulated protein serine/threonine kinase J. Biol. Chem. 274, 2631-2636.

Rubinfeld, H., Hanoch, T., and Seger, R. (1999) Identification of a cytoplasmic-retention sequence in ERK2. J. Biol. Chem. 274, 30349-30352.

Naor, Z. Benard, O. and Seger R. (1999) Activation of MAPK cascades by GnRH. Trends in Endo. In Press

Yung, Y., Yao, Z., Hanoch, T., and Seger, R. (1999) ERK1b - cloning and characterization of a novel ERK isoform. Mol. Cell. Biol., Accepted for publication.