

Synergism in a mitotic signaling network

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ABSTRACT

MAP kinase signal transduction pathways are involved in many cellular processes, such as proliferation, differentiation and apoptosis [1]. Aberrant signaling through the mitotic MAP kinase pathway (Ras-Raf-MEK-ERK) plays a role in the development of cancer [e.g. 2]. Signaling initiates upon ligand-receptor binding at the cell surface and propagates through membrane-bound and soluble cytoplasmic proteins to ERK, which is activated by phosphorylation (to ERK-P). ERK-P stimulates several targets in the nucleus and cytosol to lead to a certain cellular response, e.g. by influencing the expression of a specific set of target genes. The type of response was shown to depend on the duration and strength of the signal [3], i.e. on the dynamics of ERK phosphorylation upon stimulation. This profile is subject to adaptation as a result of several negative feedbacks, which causes the ERK phosphorylation to return to pre-stimulation levels. In CHO cells, this occurred within 20 minutes, regardless of the signal intensity [4]. However, computational studies incorporating the cascade's ultrasensitivity and its inter-pathway connections (e.g. from PKC to Raf) have shown that, in theory, the profile could also show oscillatory behavior or sustained high levels of phosphorylated ERK [5,6]. We wondered whether we could experimentally identify such system properties of this complex network.

We stimulated NRK cells with EGF or the PKC activator PMA and observed a biphasic profile of ERK phosphorylation upon stimulation with EGF. The first (highest) phase peaked after 5 to 7 minutes and returned practically to the baseline at 15 minutes. The second peak started at 20 minutes and, although more variable, was much lower than the first peak but sustained for several hours. After stimulation for 1 hour with various EGF concentrations (range: 0-100 ng/ml) the concentration of ERK-P was measured. The data suggest positive cooperativity in the formation rate of ERK-P with regard to the EGF concentration, with a Hill

coefficient of almost 2. In the presence of 100 nM PMA, we found ERK-P concentrations of 2 to 4 times the sum of the concentrations, obtained after stimulating with EGF or PMA alone. Apparently, the pathways stimulated by EGF or PMA act synergistically in activating Raf. In addition, we observed that also the K_m increased. How downstream gene expression, which in turn governs the cell's decision to divide, is affected by this synergism is subject of further study.

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