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NEWS AND COMMENTARY

Feedback Control

The role of negative feedback in signal transduction control

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It is well known that feedback mechanisms regulate cell metabolism, and accumulating observations indicate that signal transduction events are also controlled by intricate feedback mechanisms. By taking a systems biology approach, in a recent issue of *Nature Genetics* Amit and co-workers provide additional evidence that signal transduction events are controlled by elaborate and well-coordinated feedback mechanisms. Amit *et al*[1] performed a careful kinetic analysis of epidermal growth factor (EGF) receptor signaling by using a panel of phosphospecific antisera to determine activation of specific signaling pathways and carried out microarray analysis to determine mRNA levels. Special attention was given to the regulation of activation of the Erk, JNK and p38 MAP kinase pathways and the induction of immediate early genes (IEGs).

An important initial observation, also made previously by other groups,[2,3] was that inhibition of protein synthesis causes prolonged activation of MAP kinase pathways as well as prolonged induction of IEGs, indicating the presence of transcription-dependent mechanisms to attenuate signaling.

Stimulation of cells by EGF, or other growth factors, causes induction of IEG, such as the transcription factor genes *fos*, *jun* and *EGR1*, with peaks at 20–40 min. These genes drive growth-stimulatory and migratory programs of cells. Amit *et al*[1] recently showed that another set of genes, called delayed early genes (DEGs) are induced soon thereafter, with peaks at 40–240 min after growth factor stimulation. Several of these genes (25/47) were found to encode proteins that downregulate the IEGs by different mechanisms. Examples include NAB2, which binds and inhibits EGR1; FOSL1, which binds and inhibits AP-1 (a heterodimer of members of the Fos and Jun families); JunB, which inhibits activation of Jun; Id2, which inhibits the transcription factor complex TCF; and ATF3, which inhibits AP-1 and NFκB by binding to sequences near their DNA-binding motifs. In addition, ZFP36 was found to be induced; this is a protein that recognizes AU-motifs in the 3′ends of mRNA molecules and causes their degradation. Transcripts with AU-motifs are preponderantly found in early waves of transcription. It is notable that the DEGs outnumber the IEGs, and that several different mechanisms are used by DEGs to inhibit IEGs.

Amit *et al*[1] investigated in particular detail the activation of the kinases in MAP kinase pathways, Erk, JNK and p38. Each of these kinases is activated by phosphorylation by upstream MAP kinase kinases, which in turn are activated by MAP kinase kinase kinases. The kinetics of Erk MAP kinase activation has a major impact on the biological response, as has been demonstrated in the control of growth and differentiation of PC12cells.[4]

The activation of MAP kinases is counteracted by dephosphorylation by a family of MAP kinase phosphatases (also called dual-specificity phosphatases, DUSPs). Amit *et al*[1] found that EGF stimulation caused induction of DUSP3, 4, 6 and 7, that is the same DUSPs that dephosphorylate Erk, which is activated by EGF. In contrast, serum caused induction of DUSP1 and 10, which dephosphorylate p38 and JNK that are activated by serum stimulation. Thus, interestingly, Amit *et al*[1] were able to show specificity in the feedback mechanism, meaning that after attenuation of one signaling pathway, the cell remains responsive to activation of other pathways.

However, feedback mechanisms are not always private. For example, induction of the inhibitory Smad7 serves a negative feedback role in transforming growth factor-β (TGF-β) signaling,[5] and inductions of SOCS proteins inhibits cytokine signaling.[6] As Smad7 is not only induced by TGF-β, but also by interferon-γ, tumor necrosis factor-α (TNFα) and EGF, and as SOCS proteins are induced by a wide array of cytokines and growth factors, these molecules represent mechanisms whereby different signaling pathways can crosstalk with each other. Induction or repression of other DEGs may also provide a mechanism by which different signaling pathways could modulate each other. Moreover, signaling is not always black or white. For example, Amit *et al*[1] found that the Krüppel-like factor 2 inhibits TNFα-induced NFκB transcriptional activity, while activating EGF-induced SP1 activity. Thus, signal transduction is modulated by a context-dependent crosstalk between different signaling pathways.

Some of the IEGs were initially discovered as oncogenes, for example, *fos* and *jun*. Based on the function of DEGs to control IEG activity, one would predict that DEGs could act as tumor-suppressor genes. In line with this prediction, Amit *et al*[1] showed that 18/25 DEGs analyzed were downregulated in different epithelial tumor types. Importantly, the survival of patients with prostate or ovarian tumors with low DEG levels was found to be significantly shorter than that of patients with high levels of DEG. These findings not only emphasize the importance of DEGs in control of signal transduction, but also provide potentially important tools for the diagnosis and prognosis of patients with tumors.

The work by Amit *et al*[1] adds to the emerging picture of different phases of signal transduction (Figure 1). First, there is a phase immediately after initiation of signaling, which is characterized by amplification mechanisms. Examples include inhibition of tyrosine phosphatase

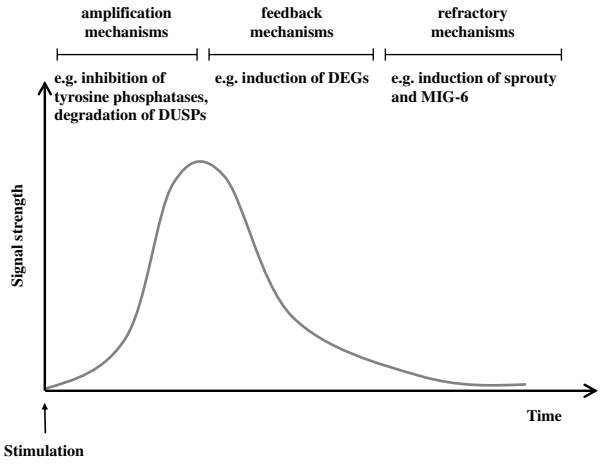

Figure 1 Schematic illustrations of different phases in signal transduction

activity after activation of tyrosine kinase receptors by production of reactive oxygen species[7] and degradation of DUSPs.[8] These mechanisms assure a rapid increase in signal intensity. Thereafter, there is a second phase with an elaborate series of feedback mechanisms to assure that the signal is rapidly attenuated, as thoroughly discussed by Amit and co-workers. In addition, Amit *et al*[1] propose that there is also a third phase during which the cell is refractory to further stimulation, exemplified by Sprouty-2 and Mig6, which negatively regulates EGF stimulation; as these factors are induced later than DEGs, they are not likely to be the part of a signal attenuation mechanism, rather they may be the part of a mechanism to make cells refractory to further stimulation for a certain time period (Figure 1).

Appropriate cellular signaling requires a careful coordination and timing of amplification, attenuation and refractory mechanisms. In addition to the kinetic aspects of signal transduction, also the physical localization of signaling molecules is an important mechanism to regulate signal transduction. At an early stage after receptor activation, many signaling molecules are recruited to the inner leaflet of the plasma membrane, where many of the initial stages of signal transduction occur, by direct or indirect binding to receptors or to membrane phospholipids. At later stages of signal transduction, several signaling components are translocated to the nucleus where they control the transcription of specific genes.

The kinetic and spatial aspects of signal transduction are regulated by transcriptional effects, as well as post-translational modification of signaling molecules, including phosphorylation, ubiquitination, sumoylation, acetylation and so on, which control their activity, stability and subcellular localization. It is likely that we still do not know of all such mechanisms. The combination of methods to monitor signaling events in real time and in living cells and the high-throughput analysis of gene induction and post-translational modifications, which now a days are becoming available, will undoubtedly rapidly advance our understanding of the intricacies of the regulation of signal transduction ■

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