Cell

Leading Edge
Review

The Dark Side of Cell Signaling: Positive Roles for Negative Regulators

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Cell signaling is dominated by analyzing positive responses to stimuli. Signal activation is balanced by negative regulators that are generally considered to terminate signaling. Rather than exerting only negative effects, however, many such regulators play important roles in enhancing cell-signaling control. Considering responses downstream of selected cell-surface receptors, we discuss how receptor internalization affects signaling specificity and how rapid kinase/phosphatase and GTP/GDP cycles increase responsiveness and allow kinetic proofreading in receptor signaling. We highlight the blurring of distinctions between positive and negative signals, recasting signal termination as the response to a switch-like transition into a new cellular state.

Introduction

The path of discovery in cell signaling has necessarily focused largely on the activation—or "switching on"—of signaling pathways by activating ligands. Genetic and biochemical approaches have encouraged the conceptual division of large and interconnected cellular signaling networks into sets of linear pathways. Understanding activation of these individual pathways is relatively straightforward—an activating ligand binds to a receptor that transmits the signal inside the cell. In essence, this communication process allows information from the extracellular environment to shape cellular processes. The question then becomes how to put the "brakes" on the response—when and how should the lines of communication be shut down?

Within the cell, propagation of the signal through an integrated network in which multiple different branches interact through positive and negative feedback (and feedforward) loops makes signal termination or deactivation far more complicated than simply flipping a switch to "off." Events that appear to constitute signal termination mechanisms in isolated signaling pathways can actually be very important for propagating signals—or for defining the nature of signals—in a network context. Indeed, the molecular mechanisms that control signal activation and termination are essentially the same, they are just used differently. One illustration of this is seen when two adjacent cells with equivalent differentiation potential are forced to adopt opposite fates through Notch-mediated lateral inhibition (Sancho et al., 2015). The two cells function as one bistable system (Figure 1), and stochastic initial differences between their levels of Notch signaling become amplified. This process leads in turn to complete suppression of the Notch pathway in one cell (the "winner", which also produces more of the ligand, Delta) and elevation of Notch signaling in the other (the "loser"), which downregulates Delta production. Depending on the organismal context, the winner might then differentiate, while the loser remains a stem cell. Molecularly, the same mechanisms that drive Notch signaling in the loser cell are also responsible for terminating Notch signaling in the Delta-producing winner cell (Fior and Henrique, 2009; Sancho et al., 2015).

In this review, we will discuss several negative regulatory processes in cell signaling that are frequently considered to function as mechanisms for signal termination. We will cover responses to a variety of extracellular stimuli, but will focus substantially on receptor tyrosine kinase (RTK) and G protein coupled receptor (GPCR) signaling since it is impossible to be comprehensive and these reflect our own interests. Nonetheless, as will be evident from the additional examples that we discuss, the same basic principles appear to apply in most other signaling systems. Among the most well studied negative regulators in RTK and GPCR signaling are those that reversibly modify proteins and other signaling molecules (e.g., by phosphorylation/dephosphorylation or binding of guanosine triphosphate [GTP] versus guanosine diphosphate [GDP]) or promote receptor internalization. Additional negative regulation arises from induction of inhibitory proteins and microRNAs (miRNAs) in response to signals, constituting apparent negative feedback loops. In many cases, these and other negative regulators keep signals "in check" in the absence of a stimulus, providing local reversibility in the network when signaling inputs are incomplete or partial. Far from terminating signals once they are initiated, however, the negative regulators typically play important roles in defining the nature and quality of the signal. They can also dramatically enhance signaling responsiveness and/or specificity, as discussed below for phosphatases and GTPase-activating proteins (GAPs). The ability of negative signaling regulators to provide local reversibility and/or to stop pulses of signaling activity should be distinguished from actual signal termination, which only occurs once the cell has irreversibly committed to a phenotypic response—be it differentiation, cell-cycle entry, or apoptosis—with the wholesale transcriptional and other changes that ensue. The focus of our discussion in this article will be on the shaping and sensitization of cell signaling responses by the most well-studied negative regulators.

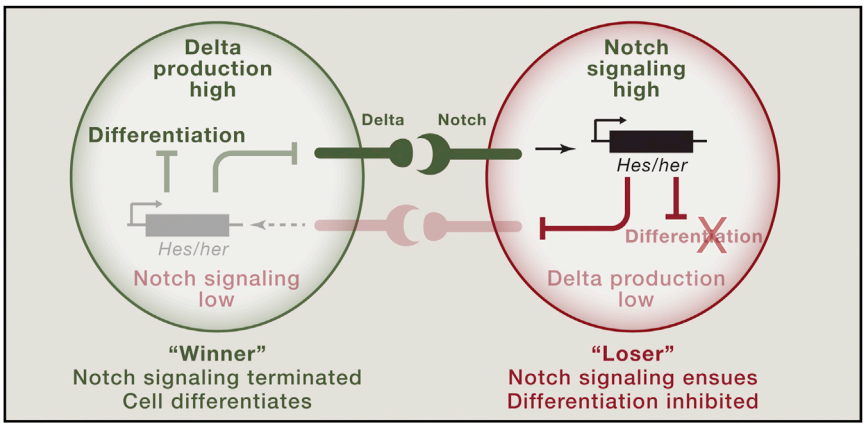

Figure 1. Notch-Mediated Lateral Inhibition
Two adjacent cells with the same fate potential associate and amplify stochastic differences in Notch signaling. Notch signaling in the "loser" cell leads to suppression of Delta production, and the resulting absence of Notch signaling in the "winner" allows increased production of Delta. Stochastic differences between the two cells are thus amplified using the same mechanisms in each cell to define orthogonal fates.

Defining Signaling Outcomes with Negative Regulators

At its simplest level, a typical profile for a signaling pathway studied in the laboratory might look like the curve shown in Figure 2A, where the stimulus under study (a growth factor in this case) is applied acutely to a cell that was previously starved of that stimulus, and response is monitored over time. Naturally, after the initial "rise" in signal and response, there is a signal decay or "dark side"—as marked in Figure 2A. Studies of this signal-decay phase, along with analyses of desensitization, have revealed a range of negative regulatory events. There is no doubt that these events do terminate the signal monitored in this particular case (as in Figure 2A) and that they can keep the signal "off" in the absence of stimulus, but they also play very important roles in defining the nature of the response.

Activation of the extracellular signal-regulated kinase (Erk) pathway by growth factors provides one of the starkest examples of how differences in the decay phase of a signaling curve can dramatically alter the cellular outcome of receptor activation. Both epidermal growth factor (EGF) and nerve growth factor (NGF) activate RTKs in the neuroendocrine PC12 cell line (EGF receptor and TrkA, respectively), which in turn activate Erk through the Ras pathway. Whereas Erk activation promotes cell proliferation in the case of EGF treatment, the response to NGF is instead terminal differentiation into neuron-like cells (Marshall, 1995). Contrary to initial suspicions that these diametrically opposed outcomes would reflect engagement of distinct signaling pathways by the two growth factor ligands, few qualitative differences could be detected (Chao, 1992). Instead, quantitative differences in the signal decay phases were found to correlate with response (Marshall, 1995). Transient Erk activation in response to EGF leads to cell proliferation (Figure 2B; left), whereas more sustained Erk activation by NGF leads to terminal differentiation (Figure 2B; right). Thus, the nature of the signal-decay phase can define the signaling outcome—although it is important to stress that the phenotypic responses occur many hours after the Erk-signaling response monitored experimentally. The distinction between transient and sustained Erk signaling has been shown to reflect differences in the engagement of feedback and feedforward loops downstream of the EGF and NGF receptors (Ryu et al., 2015; Santos et al., 2007; Sparta et al., 2015). Moreover, the cellular interpretation of the differences in Erk-activation dynamics appears to involve additional layers of signal termination that follow immediate early transcriptional responses (Murphy and Blenis, 2006; Nakakuki et al., 2010). More recent analysis of this same phenomenon over longer periods has shown pulsatile Erk activation following EGF stimulation, with EGF concentration modulating the frequency of the pulses (Albeck et al., 2013). Equivalent pulsatile Erk activation is not seen following NGF activation of TrkA (Sparta et al., 2015), presumably reflecting a different combination of feedback and feedforward effects.

Feedback Loops in Cell Signaling

The shape or pattern and the dynamics of all cell-signaling responses are determined by feedback and feedforward loops. Several excellent reviews have described how such feedback and feedforward loops define the behavior of signaling systems (Alon, 2007; Brandman and Meyer, 2008; Ferrell, 2013; Kholodenko, 2006), and the nature of these signaling-network motifs will not be discussed explicitly here. Key negative feedback mechanisms in EGF receptor signaling include endocytosis, GTPase activation, dephosphorylation, and inhibitory phosphorylation events—marked in red in the simplified network representation shown in Figure 3A (Lemmon and Schlessinger, 2010). Most of these feedback loops are rapid and provide local reversibility, helping to keep the cell "alert" to new signals and switched "off" without them. Several positive feedback loops are also marked in blue in Figure 3A, and a number of possible feedforward loops are evident (Alon, 2007). Together, depending on their relative strengths and timing, these network motifs determine responses at different nodes within the network with a variety of characteristics, including transient, adaptive, sustained, or pulsatile responses that show frequency modulation. Additional positive and negative feedback loops are also shown in Figure 3B, as communicating between elements of a robust "hourglass" or "bow-tie" network (Kitano, 2004). In this representation for RTKs, the "input" (receptor) layer communicates with a set of "core processes" including signaling by MAP kinases, Ras, phosphoinositides, Ca^{2+}, and other kinases, which in turn communicate with an "output" layer defined by changes in transcriptional responses, in epigenetic events, and in others that have longer-term consequences. In general, positive and negative feedbacks that emanate from the output layer of this bow-tie network occur over a longer timescale than those within the input layer or core processes, since they involve transcriptional responses and can contribute to actual termination of cell signaling.