Cellular Signal Processing: Out of One, Many

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Cells can make fate decisions in response to information from the environment. In this issue of *Molecular Cell*, Chen et al. (2012) describe how the design of a signal-processing pathway allows a homogenous population of cells to display diverse responses to uniform growth factor cues.

With election season approaching in the United States, voters here will be faced with the task of sifting through a cacophony of competing claims and promises, filtering out the parts most relevant to them, and integrating all of this information in order to make a choice between candidates. Cells face a similar challenge in interpreting multiple environmental signals and translating these complex inputs into a set of well-defined fate choices.

One of the central goals of systems biology is to learn how the architecture of biological regulatory networks enables this information processing. A popular framework for conceptualizing this flow of information envisions an hourglass-shaped design in which multiple signaling pathways impinge on a single integration point that acts as a decision-making node in the network and that in turn leads to multiple effector pathways that mediate cell-fate decisions. In this issue of *Molecular Cell*, Chen et al. (2012) shed light on how the structure of a decision-making hub enables an apparently homogenous starting population of cells to exhibit multiple responses to uniform growth factor cues.

The authors studied the response of the PC12 rat cell line to nerve growth factor (NGF) in culture (Greene and Tischler, 1976). NGF treatment of PC12 cells has previously been shown to stimulate two key signaling pathways, PI3 kinase (PI3K) and Ras, and individual cells treated with NGF can respond by either continuing to proliferate or by ceasing to divide and terminally differentiating into neuronal cells (Huang and Reichardt, 2003). In order to determine what causes PC12 cells to grow or differentiate in response to NGF, the authors collected information on single cells along three axes using live-cell imaging: stimulation of the key Ras target ERK, stimulation of the key PI3K target AKT, and DNA synthesis as a metric of proliferation. Individual cells exhibited relatively large (3- to 4-fold) variations in their level of ERK and AKT activation. Surprisingly, whether a cell grew or differentiated in response to growth factor cues was best explained not by its level of ERK or AKT stimulation individually, but rather by its position in a two-dimensional (2D) map of ERK and AKT activity. A sharp boundary in this map separates proliferating from differentiated cells, suggesting that PC12 cells utilize this 2D map as an information-processing node to integrate relative activation of the two pathways and determine their response to NGF (Figure 1).

Having identified this 2D response map, the authors next turned their attention to deciphering how this sharp boundary is set and what mechanisms allow the population to span this boundary. They did so by means of a genetic screen, generating small interfering RNAs (siRNAs) against a large number of signaling factors and testing the effect of knocking down each component on the ability of PC12 cells to differentiate in response to NGF. By measuring both the relative proportion of proliferating and differentiating cells in the knockdown populations and the levels of ERK and AKT activation in individual cells, Chen et al. were able to distinguish between two kinds of hits from the screen: (1) those that shifted the center of the population further away from the ERK/AKT decision-making boundary, weighting it toward either greater ERK or AKT activity, and (2) those that appeared to shift the position of the boundary itself, resulting in either a higher or lower proportion of proliferating cells at any given level of ERK/AKT activity.

Not surprisingly, a number of cell-cycle regulators were among those factors identified that shifted the position of the decision-making boundary, with knockdown of cyclin D1/D3 having the strongest effect in reducing the number of proliferating cells observed after NGF treatment. Investigating further, the authors found that ERK and AKT activation had opposing effects on cyclin D1 protein stability, implicating this as an important node downstream of the 2D ERK/AKT response map that these two pathways converge on to tip the balance between proliferation and differentiation. As pointed out by the authors, cyclin D1/D3 are likely to be among a number of regulators and pathways that act as downstream effectors of the response map. How these effectors interact to produce all-or-none cell-fate decisions remains a subject for further inquiry.

Factors whose knockdown shifted populations around the ERK/AKT decision-making boundary are likely to act upstream of the response map. Among these, PTEN, a negative regulator of PI3K signaling, was notable for yielding higher levels of AKT activation but also lower ERK activity, suggesting a coupling between these two pathways. Through a combination of localization and biochemical studies, the authors were able to define a negative feedback loop from PI3K to Ras signaling mediated by the Ras GTPase activating protein (RasGAP) Rasa2. Examination of the kinetics of Ras activation led to the discovery of a positive feedback loop in which NGF

treatment causes an upregulation of the NGF receptor TrkA, which introduces a time delay in ERK activation. The net effect of these two opposing feedback loops is to position the center of the activated ERK/AKT vector close to the decision-making boundary, thus maintaining a balance between cell proliferation and differentiation in the population.

Why might cells have evolved signal response circuits that allow for divergent responses to common triggers? The positioning of a cell population spanning the decision-making boundary of the ERK/AKT response map allows for a subpopulation of cells to continue proliferating, with a steady siphoning off of cells toward differentiation. This architecture could enable kinetic control of the flow of differentiated and proliferating cells in an in vivo context, as well as provide a mechanism for amplifying the number of differentiated cells derived from a single progenitor. Nongenetic heterogeneity present in the population allows for a diversity of responses to environmental conditions in an isogenic population, similar to mechanisms that have been described for unicellular organisms (Blake et al., 2006; Eldar and Elowitz, 2010; Zhuravel et al., 2010; Balázsi et al., 2011).

The work of Chen et al. raises questions and provides a template for future studies. Among these is the issue of what factors underlie the relatively large variation in ERK and AKT activation between individual cells. The authors postulate that the cumulative effect of stochastic processes acting at upstream regulatory steps generates this variability. However, could metastable interconverting subpopulations, as have been described in other contexts, exist that show differential responses to growth factor treatment (Chambers et al., 2007; Chang et al., 2008; Huang, 2009)? Might biological mechanisms act to ensure a large variation in ERK and AKT activation or to buffer against excessive variation? Elucidation of how biological systems exploit or suppress heterogeneities to generate novel systems properties or enable robust behaviors will add a new dimension to our understanding of the design principles that underlie their function. The approach taken in this work paves the way for higher-dimensional studies of other biological processes, where having the right set of information about the state of individual cells may reduce seemingly intractable decisions to a simple set of parameters that explain their voting preferences.

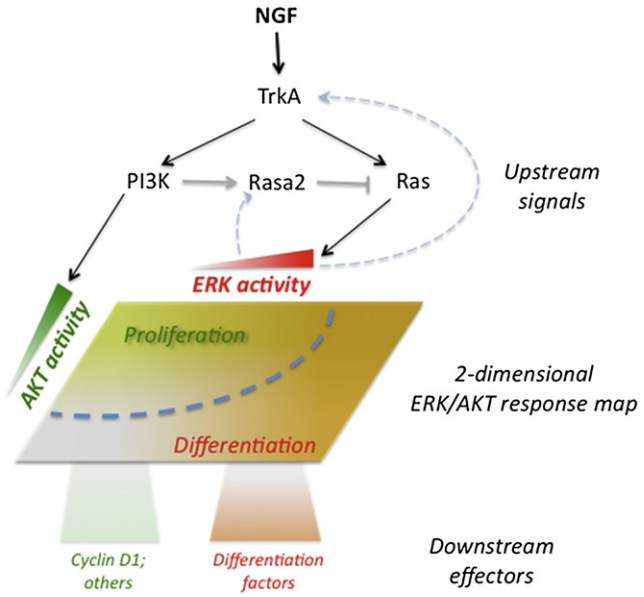

Figure 1. Schematic of a Two-Dimensional Signal-Processing Node that Controls Cell Fate

Treatment of rat PC12 cells with nerve growth factor (NGF) activates the Ras/ERK and PI3K/AKT signaling pathways through the receptor TrkA. Individual cells in a treated population show a gradient of activity levels, and the relative balance of activation of the two pathways determines whether a cell continues to proliferate or differentiates into a neuronal cell. This produces a sharp boundary (dashed blue line) in the two-dimensional ERK/AKT response map that separates proliferating from differentiated cells. Negative feedback (solid gray lines) from PI3K to Ras mediated by Rasa2 acts to position the population close to the decision-making boundary, while positive feedback (dashed gray lines) from NGF acting through its receptor TrkA serves to amplify the long-term signal response. Downstream effectors execute the cell-fate decision mediated by the ERK/AKT response map.

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