

When It Comes to Decisions, Myeloid Progenitors Crave Positive Feedback

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Positive feedback is a ubiquitous feature of networks that establish and maintain cellular decisions. In this issue of *Cell*, Laslo et al. (2006) demonstrate how a feedback loop comprised of two mutual repressors regulates the differentiation of myeloid progenitors into either macrophages or neutrophils.

All blood cell lineages are derived from pluripotent self-renewing hematopoietic stem cells (HSCs) (Orkin, 2000). Because HSCs give rise to many different cell types, the path between undifferentiated

HSCs and differentiated blood cells has many branch points. Progenitor cell populations at these branch points express unique sets of cell-surface receptors poised to respond to subsequent maturation cues. In a

process termed “lineage priming,” progenitors promiscuously express genes characteristic of more than one differentiated blood cell type (Miyamoto and Akashi, 2005). For example, subsets of the genes unique to macrophages and neutrophils are expressed together in their precursor cells, the common myeloid progenitors (CMPs). The successive steps in the maturation of hematopoietic progenitor cells, including CMP differentiation, are thought to involve the upregulation of factors promoting a particular lineage and the repression of factors promoting alternate lineages. However, the precise mechanisms that resolve this mixed-lineage pattern of gene expression are just now beginning to be understood. In this issue, Laslo et al. (2006) provide evidence for a model in which positive feedback between two mutual repressors regulates the differentiation of CMPs into macrophages or neutrophils (Figure 1).

Within the CMP differentiation network, macrophages and neutrophils are cell fates that correspond to two different stable gene-expression steady states. The steady states are stable in that they are robust to the small fluctuations inherent in gene expression and other cellular processes. Unstable steady states, by contrast, are mathematically plausible but not experimentally observable precisely because such stochastic

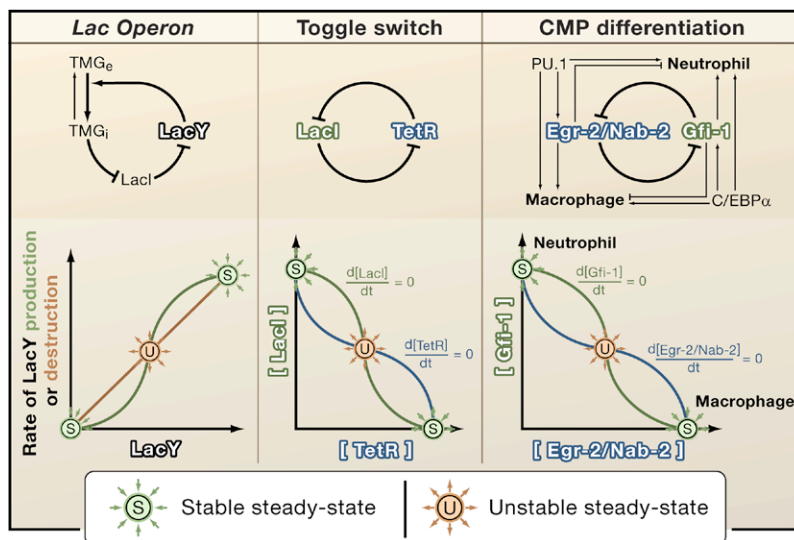


Figure 1. Positive Feedback in Bistable Switches

The top panels show network topology with positive feedback loops in bold, and the bottom panels illustrate the stability of each of the steady states for each system. (Top left) In the *E. coli* network for lactose utilization (Ozbudak et al., 2004), LacY increases the concentration of the intracellular inducer (TMG) via uptake of the extracellular inducer (TMG_e). TMG_i inhibits LacI, which cooperatively represses LacY expression. (Top middle) In a synthetic toggle switch (Gardner et al., 2000), LacI and TetR cooperatively corepress each other. (Top right) In CMP differentiation (Laslo et al., 2006), Gfi-1 and the Egr-2/Nab-2 complex mutually corepress each other via a mechanism that is assumed to be cooperative. (Bottom) Steady states can be graphically represented as the intersection between creation and destruction curves in systems with one state variable (bottom left) or the intersection of nullclines (where the rate of change of a variable with respect to time is zero) in systems with two or more state variables (bottom middle and right). In all three cases shown, cooperative reactions yield sigmoidal curves that give rise to two stable steady states, which are robust to stochastic fluctuations, and one unstable steady state that cannot persist in the presence of even the smallest deviations. If the systems were to lack cooperativity, the respective curves would likely intersect no more than twice, signaling two steady states, where only one is stable.

effects cause deviations that drive the system toward one of the stable steady states. In a bistable system, there can be many unstable steady states, but only two stable steady states, and it is the persistence of these states that forms the basis of cellular memory. The molecular mechanisms and network architectures that can establish and maintain such memory are increasingly well understood. Positive feedback loops often form the core of bistable networks and have recently been the subject of many detailed experimental and theoretical studies in both natural and synthetic systems (Becskei et al., 2001; Isaacs et al., 2003). Even though positive feedback loops are diverse in both the type and number of components they contain, those that give rise to bistability tend to be nonlinear (that is, highly cooperative) in at least one reaction. For example, Ozbudak et al. (2004) observed and characterized bistability in the network of lactose utilization in *E. coli* (Figure 1), where the source of nonlinearity is cooperative repression of LacY via LacI tetramerization. Similarly, Gardner et al. (2000) observed bistability in their study of a synthetic toggle switch in which LacI and TetR act as mutual corepressors (Figure 1); it was the nonlinear, cooperative repression at both promoters that yielded bistability.

Singh and colleagues (Laslo et al., 2006) now describe how nonlinear positive feedback regulates differentiation of CMPs into macrophages or neutrophils (Figure 1). Earlier work from the Singh laboratory suggested that the relative difference in expression of the transcription factors PU.1 and C/EBP α regulates the differentiation of CMPs (Dahl et al., 2003). They found that macrophage differentiation is favored when the level of PU.1 is higher than that of C/EBP α , whereas neutrophil differentiation is favored when the level of C/EBP α is higher than that of PU.1. The simplest model suggests that there is mutual corepression between PU.1 and C/EBP α that

drives CMPs toward one fate or the other. However, both proteins are highly expressed in macrophages and neutrophils, suggesting that other factors regulate lineage specification.

To identify other factors that regulate CMP differentiation, the authors expressed a conditionally active PU.1 fusion protein in cells lacking endogenous PU.1 and performed both genome-wide and single-gene analysis. They found mutual corepression between Egr-2/Nab-2, a complex of genes activated by PU.1, and Gfi-1, a gene activated by C/EBP α . Because both Egr-2 and Gfi-1 are known to promote the expression of genes specific to macrophages and neutrophils, respectively, their corepression may indeed be the basis of a positive feedback loop that promotes and stabilizes a particular cell fate during CMP differentiation.

The authors also present a mathematical model that integrates their new experimental findings about the network circuitry with other available data. It should be noted that few parameters in their model are experimentally measured, and that they assume that the mutual repression between Egr-2/Nab-2 and Gfi-1 is cooperative and nonlinear, thus allowing this mutual corepression loop to give rise to bistability. Yet, the model makes qualitative predictions that are consistent with both their current results and existing experimental data. For instance, when PU.1 and C/EBP α are expressed at low levels as is the case in undifferentiated CMPs, positive feedback between Egr-2/Nab-2 and Gfi-1 is sufficiently weak such that neither is amplified and the mixed-lineage stage persists. In contrast, when PU.1 is expressed at a much higher level than C/EBP α , the mixed-lineage state of gene expression is resolved and the system is monostable, promoting differentiation of the CMP into a macrophage (or, if the ratio is reversed, into a neutrophil). Most intriguingly, the model predicts that for high levels

of PU.1 and C/EBP α , the mutual corepression is strong and the system is bistable as a consequence. Yet, in this case, the mixed-lineage CMP state is unstable (Figure 1). At these high expression levels, a small difference in the concentration of PU.1 and C/EBP α could be sufficient to tip the balance in favor of one cell fate or the other.

Though the predictions of the model are qualitatively consistent with the reported data, further exploration of the experimental system coupled with refinement of the quantitative model will offer even greater insight into the mechanisms of CMP differentiation. For instance, the authors can manipulate expression of C/EBP α (Dahl et al., 2003) and take advantage of their control over the activity of the inducible PU.1 fusion protein to acquire better quantitative estimates of system parameters. Such measurements would make model predictions more accurate and may also suggest a new behavior of the system currently obscured by parameters that have been assumed. For example, quantitative analysis of the bistable yeast galactose network has shown that perturbation of certain system parameters leads to increased stochastic switching between states (Acar et al., 2005). However, except in artificial circumstances (for example, in which a gene promoting an alternate fate is greatly overexpressed), the resolution of lineage priming is thought to be irreversible. Progenitor cells commit to one of their potential paths and do not switch to different lineages or revert to less differentiated progenitor cell types. Such stability is clearly important for immune cells, which receive a transient lineage-specific differentiation stimulus in the bone marrow but then spend days or years living in the bloodstream or other tissues. It would be very exciting if the authors' model, like the yeast galactose network, is able to predict conditions that are experimentally achievable in which macrophages and neu-

trophs could switch cell fates, or possibly even revert to the less-differentiated CMP state.

The work of Laslo et al. (2006) represents a significant advance in understanding the molecular mechanisms that regulate CMP differentiation and provides further evidence for the ubiquity of positive feedback in the regulation of cellular decisions and memory. Although it is not the first molecular characterization of mutual corepression in the context of hematopoiesis (Cantor and Orkin, 2001), it is also unlikely to be the last. Irreversible resolu-

tion of lineage priming appears to be a common feature of blood cell differentiation and may in fact be a general feature of other developmental processes as well.

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A Transcriptional Logic for Nuclear Reprogramming

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Limitations on a differentiated cell's pluripotency can be erased by nuclear transfer or by fusion with embryonic stem cells, but attempts to recapitulate this process of nuclear reprogramming by molecular means have failed. In this issue of *Cell*, Takahashi and Yamanaka (2006) take a rational approach to identifying a suite of embryonic transcription factors whose overexpression restores pluripotency to adult somatic cells.

The phenomenon of nuclear reprogramming was first demonstrated in the context of somatic cell nuclear transfer experiments. These experiments showed that the developmental state of a nucleus from an adult somatic cell can be reprogrammed upon its transfer into an unfertilized oocyte. Such a strategy can result in the generation of cloned embryos with the potential to develop into another entire animal, such as Dolly the sheep (Wilmut et al., 1997). Although cloning experiments were, and still are, inefficient, they provide definitive proof that pluripotency

can be restored to the nucleus of a terminally differentiated cell. Subsequently, cell fusion experiments in which adult somatic cells are fused with mouse embryonic germ cells, mouse embryonic stem (ES) cells, or human ES cells have shown that these pluripotent cells also harbor reprogramming activities (Cowan et al., 2005; Tada et al., 2001). These findings demonstrate the biological reality of nuclear reprogramming, yet the nature and identity of the molecules in an oocyte or pluripotent cell that constitute this activity have remained elusive.

In this issue of *Cell*, Takahashi and Yamanaka (2006) take a significant step toward delineating the minimal set of factors required to confer the developmental potential of an ES cell onto a terminally differentiated somatic cell. Leveraging the knowledge that ES cells have reprogramming capabilities, the authors reasoned that forcing the expression of ES cell-specific genes, particularly transcription factors, in somatic cells might induce them to take on a more embryonic character. In order to assay for reprogramming, mouse fibroblasts were generated that