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NEWS AND VIEWS

Single cell resolution in regulation of gene expression

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Studies on engineered gene networks have brought much insight into the stochastic nature of gene expression and gene regulation (Kaern *et al*, 2005). Using fluorescence imaging methods allowing examination of gene expression in single cells, these studies revealed a high degree of variability between genetically identical cells and suggested that noise in gene regulation and signal propagation is sometimes a dominant factor contributing to this cellular individuality. In fact, such variability may even induce a binary on–off response in single cells (Blake *et al*, 2003). Several recent studies utilize new multicolor fluorescence methods to quantify molecular abundances within individual cells, bringing a new level of resolution to our understanding of the regulation of gene expression. Two recent papers examine the noise in the regulation of gene expression within constructed gene networks in *Escherichia coli*. A third paper, showing the role of stochastic transitions in cellular memory in the *Saccharomyces cerevisiae* galactose regulatory network, provides a timely example of the impact of such stochastic variation in the regulation of endogenous gene networks and in altering physiological states.

The first two studies examine noise within engineered networks in *E. coli* and provide insight into the relationship between input and output signals in gene regulation (Isaacs *et al*, 2005). Michael Elowitz and co-workers (Rosenfeld *et al*, 2005) examine the relationship between the rate at which a gene is expressed and the abundance of a transcription regulatory protein in single cells. The gene regulation function (GRF), which models this relationship, had previously been estimated from population-averaged abundances of transcription factor and protein product. In the current study, the authors engineered a two-step regulatory cascade, and determined the GRF in individual cells by simultaneous and dynamic measurements of these input and output signals with time-lapse microscopy. The measured GRF were found to vary significantly from one cell to another, highlighting how potentially important aspects of cellular regulation might be lost by population averaging. Moreover, the GRF was observed to fluctuate dynamically in individual cells, indicating that variation in biological parameters, stochasticity in gene expression and slowly varying cellular states together limit the accuracy with which transcriptional regulatory networks can transfer signals.

In a study appearing in the same issue, Pedraza and van Oudenaarden (2005) examine how noise propagates through a three-step transcriptional regulatory cascade. By adding inducers at varying concentrations, and measuring the change in the expression of input and output genes, they are able to ask how the abundances of the gene products at different steps in the cascade correlate within single cells. A stochastic model of the cascade was used systematically to interpret the data and to demonstrate that overall cell–cell variability is determined by fluctuations intrinsic to the process of gene expression, noise in regulatory signals and global factors affecting the expression of all genes. Interestingly, transmitted noise, defined by the authors as fluctuations in expression of an upstream regulator that is relayed to the target, was found to account for most of the variation. This suggests that the connections within a network may prove to influence variation more than that arising intrinsically in the process of gene expression.

A third recent study provides an example for just how the variation in gene expression in individual cells may directly influence the determination of physiological states within endogenous pathways. Alexander van Oudenaarden and co-workers (Acar *et al*, 2005) examine the determinants of stability in cellular memory, using as a model system the galactose regulatory pathway in *S. cerevisiae*. Previous studies have shown how constructed signaling networks in *E. coli* can generate bistability (Gardner *et al*, 2000). Networks have also been shown to store memory through the creation of such discrete stable states. Van Oudenaarden and co-workers now present the first study to show how this may occur in an endogenous eukaryotic network, containing complex multiple nested feedback loops.

The authors find that the galactose signaling pathway does create two bistable expression states, regulated by two positive (Gal2p and Gal3p) and one negative (Gal80p) feedback loop. They further found that memory of previous cellular galactose levels is concentration dependent. At either high or low galactose concentrations, signaling was 'history independent', with no stored memory of previous cell concentrations. However, at intermediate levels of galactose, signaling was 'history dependent', showing persistent memory of previous states. In order to understand which elements within the galactose signaling pathway were important for determining this cellular memory, the authors in turn disrupted each of the feedback loops within the network. They found that the Gal3p positive feedback loop was required to generate the two bistable expression states and for memory storage, while the Gal2p positive feedback loop was not essential but did regulate the expression difference between the two states. The negative feedback loop mediated by Gal80p competed with the Gal3p positive feedback loop, reducing memory storage. Interestingly, at low concentrations of Gal80p, there were some conditions under which two stable expression states were

created, although the cell response did not show history dependence. Probing the system further revealed that these cells transition between these two states, destabilizing the storage of memory. In comparison, at higher Gal80p concentrations, there was less fluctuation between these two expression states, trapping cells in either state and thereby tuning the system to persistent memory. These studies suggest that random variation within individual cells may lead to switching between these two states, but that Gal80p acts to reduce the frequency of this switching, effectively providing a safety mechanism to buffer from such random fluctuations.

These studies have provided important tools to examine the regulation of expression within single cells and at individual genes, bringing a welcome degree of quantification to the field. It will be of interest to see how the principles for noise propagation within a network raised by Rosenfeld *et al* and Pedraza *et al* may be generalized when tested with a range of endogenous regulatory pathways. With the example provided by Acar *et al* of how individual cell variation can lead to switching between different expression states and reduce cellular memory, it will also be interesting to see how commonly cell regulatory networks include mechanisms to shield from the effects of stochastic variation in individual cells.

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