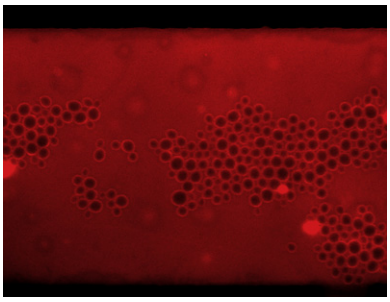
Molecular Biology Select

Cell

Gene networks can act as information-processing modules that regulate cellular metabolism, cell division, and differentiation. New findings in yeast describe how a metabolic gene network behaves in response to a changing nutrient environment. Other recent papers report the design of an engineered gene circuit to screen for antituberculosis drugs, reveal the role of positive feedback in cell cycle transitions, and provide insight into the genetic circuitry underlying the evolution of the nervous system.



Silhouettes of yeast cells highlighted by a red fluorescent tracer used to track changing glucose concentration in a microfluidic growth chamber. Image courtesy of W. L. Peng.

Gene Regulation Goes with the Microfluidic Flow

When faced with an environment that changes unpredictably, what is the best coping strategy? Is it to respond forcefully and rapidly to each perturbation, or is it to take a wait-and-see approach, assessing conditions over time before changing course? And even if the latter is found to be better, what is the optimal period of time over which to make a decision? A recent paper by Bennett et al. (2008) confronts these questions in work describing the regulation of key metabolic genes in the yeast *Saccharomyces cerevisiae*. Using a microfluidic platform, the authors examined the regulation of genes involved in galactose utilization in response to changes in the availability of glucose in their growth media. Only when glucose concentration is low will the yeast switch to using galactose as their primary sugar source. From these efforts, the authors conclude that the gene network for galactose utilization acts as a low-pass filter—meaning that the network is largely insensitive to rapid fluctuations in glucose concentration (such as oscillations with a periodicity of 90 min). Yet, when the change in glucose concentration is slower (for instance, with a periodicity of 3 hr or more), the response of the galactose utilization network closely mirrors the changes in glucose availability. Analysis of a second yeast strain, one that is less sensitive to galactose because of impaired galactose uptake, yielded similar responses, suggesting that the low-pass filter function is an optimized feature of the network as a whole. These findings are relevant to many other systems in that they point out that important network properties might be missed if networks are not analyzed in the context of a dynamic environment. Future work may explore the contributions of individual genetic elements in the network to the robustness of the filter and determine what network parameters set the cutoff frequency for the filter.

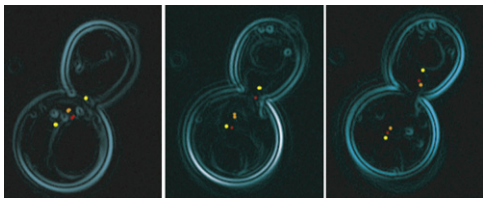
M.R. Bennett et al. (2008). Nature. Published online July 30, 2008. 10.1038/nature.07211.

A Synthetic Circuit Makes Drug Screening Less Circuitous

Given that the bacterium causing tuberculosis *Mycobacterium tuberculosis* is an intracellular pathogen, screening for compounds that both target the pathogen and enter the host cell without having undesired toxic effects can be a cumbersome process. Weber et al. (2008) present a potential shortcut around these problems by engineering a synthetic gene circuit in a human cell line for use in screening potential drugs for efficacy against *M. tuberculosis*. The authors sought to identify compounds that would inhibit EthR, a protein in *M. tuberculosis* that decreases the efficacy of ethionamide treatment, one of the few antibiotics that is effective against multidrug-resistant strains of *M. tuberculosis*. EthR is a repressor protein that blocks expression of EthA, a monooxygenase that converts ethionamide from its prodrug form to an active derivative. Hence, a drug that could bind to EthR and inhibit its repressor function would elevate EthA expression and thereby might increase the effectiveness of ethionamide treatment. Although previous efforts have identified a compound that binds to EthR and induces a conformational change that would impair its repressor function, the hydrophobicity of the compound restricted its bioavailability, presenting a major roadblock in its path from compound to drug. In this new approach, Weber et al. introduced a gene network consisting of (1) an EthR transgene fused to a VP16 transactivation domain and (2) a promoter that contains the EthR-specific operator (O_{ethR}), driving expression of alkaline phosphatase. The enzymatic activity of alkaline phosphatase is easily assessed in high-throughput assays. In this engineered system, a compound that binds to EthR would release the EthR-VP16 fusion protein from the promoter bearing O_{ethR} and lead to a decrease in alkaline phosphatase expression. The authors used this system to identify new compounds that are cell-permeable, noncytotoxic, and active inhibitors of EthR. Of the many compounds identified in the screen, the authors focused additional effort on 2-phenylethyl-butyrate, a compound already licensed as a food additive. They provide evidence that 2-phenylethyl-butyrate has excellent bioavailability in mice, inhibits EthR activity in *M. tuberculosis*, and has a synergistic effect with ethionamide in suppressing the growth of *M. tuberculosis* in culture. Future efforts may establish whether this promising treatment can surpass the remaining hurdles, most critically safety and efficacy in humans, to bolster the depleted arsenal of antibiotics for fighting multidrug-resistant *M. tuberculosis*.

W. Weber et al. (2008). Proc. Natl. Acad. Sci. USA ***105****, 9994–9998.*

Cell Cycle Spurred On by Positive Feedback



A yeast cell progressing through anaphase, showing mitotic spindle poles (yellow) and loci on the arms of chromosomes IV and V (red and orange, respectively). Image courtesy of L. Holt.

Like a conductor guiding an orchestra from the cacophony of warm up to the first notes of a harmonious symphony, the G1 cyclins direct the coherent regulation of gene expression needed for entry into the cell cycle. New work by Skotheim et al. (2008) reports that positive feedback involving the cyclins Cln1 and Cln2 in the budding yeast *Saccharomyces cerevisiae* ensures that the onset of expression of more that 200 genes in the G1/S regulon is nearly simultaneous. In the absence of either Cln1 or Cln2, synchrony of gene expression of the G1/S regulon is disrupted, and the cells display an increased frequency of failure to bud. Because the study examined individual cells rather than population averages, the authors were able to observe a strong correlation between incoherent gene expression and bud failure. One element of positive feedback proposed by the authors involves the cyclin-mediated phosphorylation of Whi5, a negative regulator of the G1/S regulon and Cln2 expression. Phosphorylation of Whi5 promotes its exit from the nucleus. In the absence of Cln1 and Cln2, the exit of Whi5 from the nucleus is slower and less abrupt, leading to incoherent expression of the G1/S regulon.

An equally dramatic cell cycle transition occurs at anaphase, with the sudden loss of sister chromatid cohesion though the proteolytic cleavage of cohesin by separase. In a related paper, Holt et al. (2008) explore the mechanism underlying this switch-like behavior that is essential for proper chromosome segregation. They show that a positive feedback loop in *S. cerevisiae* regulates the phosphorylation of securin, an inhibitor of separase. In this regulatory system, the cyclin-dependent kinase phosphorylates securin in its destruction box. This inhibits securin ubiquitination and degradation, thereby reducing the rate of separase activation. Feedback comes in the form of the phosphatase Cdc14, which is normally activated by separase. When a small amount of separase activation occurs, Cdc14 is partially activated and feeds back to dephosphorylate securin, thereby triggering more separase activation and thus more Cdc14 activity. The net effect of this regulatory circuit is to make the activation of separase during anaphase more abrupt. If this feedback loop is disrupted in mutants that prevent securin phosphorylation, chromosome disjunction and the regulation of the spindle and kinetochores lack their normal coherence. These two studies reveal the critical importance of positive feedback in temporally coordinating the large number of molecular events associated with cell cycle transitions.

L.J. Holt et al. (2008). Nature ***454****, 353–357.*
J.M. Skotheim et al. (2008). Nature ***454****, 291–296.*

The genome of the sponge *Amphimedon queenslandica* reveals molecular preadaptations for the evolution of complex animal body plans. Image courtesy of B. Degnan.

Neurons Sponge off of a Preexisting Gene Circuitry

Unlike other animals, sponges lack a nervous system. Yet, according to surprising new findings by Richards et al. (2008), sponges nevertheless have the core genetic circuitry required for neurogenesis. The authors examined the newly available genome sequence of the sponge *Amphimedon queenslandica* in a search for orthologs of known neurogenic genes that have been found in animals with nervous systems. They report that *Amphimedon* expresses a basic helix-loop-helix gene, *AmqbHLH1*, that has homology to the transcription factor family that includes *atonal* (*Drosophila*) and *neurogenin* (vertebrates). In other animals, these transcription factors promote neuronal cell fates. Remarkably, injection of *AmqbHLH1* mRNA into frog embryos stimulated ectopic expression of neurogenic genes and led to the formation of ectopic sensory neurons. Similar results were obtained from the transgenic expression of *AmqbHLH1* in the fruit fly wing, where it stimulated neuronal differentiation in the form of ectopic sensory bristles. The authors show that sponges also express a Notch receptor and five Delta ligands—in bilaterians, the Notch-Delta system is a key regulator of neuronal differentiation. In *Amphimedon*, the focal point for the expression of this neurogenic gene circuitry appears to be the globular cells found in the epithelia of sponge larvae. On the basis of the fact that globular cells have protrusions to the external environment and an earlier report showing that elements of the postsynaptic density are found in globular cells, the authors propose that globular cells represent rudimentary sensory cells that are evolutionarily related to neurons. If so, a future challenge will be to identify the signals in the environment that these cells respond to and then to establish how these signals are transmitted to the organism to affect its behavior.

G.S. Richards et al. (2008). Curr. Biol. ***18****, 1156–1161.*

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