binding, and given the complementary expression pattern between SAPCD2 and LGN in RPCs, the authors hypothesize that SAPCD2 regulates spindle orientation by interfering with LGN localization. To test this model, the authors overexpressed SAPCD2 in HeLa cells and found that this effectively reduces the abundance of LGN cortical localization in these mitotic cells. Conversely, depletion of SAPCD2 dramatically enhances the cortical localization of LGN. Moreover, the enhancement of LGN cortical localization by Gαi overexpression was suppressed by SAPCD2 overexpression. Remarkably, in *Sapcd2*$^{-/-}$ mutant retina, apical localization of LGN in RPCs increased almost 3-fold compared to the control. All of these observations indicate that SAPCD2 regulates spindle orientation by interfering with the cortical localization of LGN. Future work is needed to test whether SAPCD2 function ultimately influences Dynein localization in RPCs.

What anchors SAPCD2 at the apical cortex in horizontally dividing RPCs is still unclear. Tight junction protein PATJ, which may also interact with SAPCD2 (Chiu et al., 2016), may tether SAPCD2 to the cell cortex. It remains to be tested whether PAR3, identified in the same SAPCD2 immunoprecipitation experiment, provides the asymmetric cue for SPACD2 in RPCs. In addition, it will be of great interest to identify the mechanisms that control the differential localization of SAPCD2 in horizontally and vertically dividing RPCs. Another key regulator and interactor of LGN is Inscuteable (Insc), which is functionally conserved in flies and mammals (Schaefer et al., 2000; Yu et al., 2000; Zigman et al., 2005). Mammalian Insc (mInsc) localizes to the apical side of vertically dividing RPCs and is concentrated at poles and apical cortex in horizontally dividing RPCs (Zigman et al., 2005). Given that Insc and SAPCD2 positively and negatively regulate LGN localization, respectively, future work will be required to determine whether mInsc and SAPCD2 compete for apical localization in RPCs to regulate spindle orientation, ultimately deciding horizontal versus vertical divisions of RPCs.

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Cybernetics, Redux: An Outside-In Strategy for Unraveling Cellular Function

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A new paper in *Science* reveals how repetitive stimulation can identify and help to repair fragilities within a signaling network, while using linear mathematical models inspired by engineering, thereby suggesting how cybernetic methods can be integrated into systems and synthetic biology.

Wendell Lim's laboratory at UCSF has published a report in *Science* in which repetitive pulsing of *Saccharomyces cerevisiae* cells with an osmotic shock revealed what the authors—Amir Mitchell, Ping Wei, and Wendell Lim—described as an "Achilles' heel" in the MAP kinase signaling network (Mitchell et al., 2015). This work builds upon a long tradition of exploiting engineering ideas in biology but also suggests how such methods can be more effectively integrated into modern systems and synthetic biology.

In trying to understand how cells work, there is a strong temptation, in the light of our accumulated molecular understanding, to pull them apart by perturbing individual components at DNA, RNA, or protein level. This "inside-out" strategy has been hugely informative—about components. Engineering offers an alternative "outside-in" strategy, in which a system is interrogated so as to reveal how it works. This offers, in principle, a more integrative approach.

A commonly used interrogation is to vary the frequency of stimulation. A system can be fully reconstructed if its complete frequency response is known. Even the high-frequency response reveals

how many components the system has, without having to identify them. Other forms of interrogation are more specialized, such as asking whether the system's output returns to the same level after a step stimulation, a behavior known as "perfect adaptation." It is a remarkable theorem that a system that perfectly adapts must contain an "integral controller": a component, x, which measures the time integral of the departure of the system's response from its previous level (Yi et al., 2000).

Such results are powerful but have one major drawback for biology. They are only valid (largely) for linear systems. However, if a nonlinear system is at steady state, then an important theorem, due to Hartman and Grobman, tells us that no matter how complicated the system is, its behavior near the steady state can be approximated by a linear system. Hence, it is not unreasonable to expect that the interrogation methods described above can be useful for biological systems, such as homeostatic ones, which are maintained at steady state. How these methods can be extended to systems that are not at steady state remains a challenging problem.

These ideas entered biology after World War II through Norbert Wiener's "cybernetics," which explored the analogy between machines and organisms (Wiener, 1948). It was cybernetics, and the bioengineering that developed from it, that gave us the analogy between homeostatic systems and engineering control systems like thermostats and autopilots. Cybernetic ideas have been particularly influential in neurophysiology (Stark, 1968; Robinson, 1981)—we still compare the brain to a computer—but their deployment at the molecular level only came with systems biology (Yi et al., 2000; Mettetal et al., 2008; Muzzey et al., 2009). Despite the common principles, the modern manifestation has been largely divorced from its cybernetic roots.

The osmolarity regulation system in yeast is an archetypal cellular homeostat, maintaining turgor pressure in the face of an uncertain environment. The molecular details are sufficiently well understood that Edda Klipp, Stefan Hohmann, and colleagues built an inside-out "comprehensive mathematical description" of the system with 32 molecular components (Klipp et al., 2005), simulation of which yielded agreement with experimental data but offered limited insight into the integrative behavior of the system.

Adopting instead the outside-in, cybernetic approach, Alexander van Oudenaarden's lab determined the frequency response of the yeast osmolarity regulation system (Mettetal et al., 2008). The stimulation consisted of a train of rectangular pulses of constant-amplitude osmotic shock generated by computer-controlled microfluidics. The frequency of the pulse train was altered by changing the period from the start of one pulse to the start of the next, while keeping the pulse width to half the period (Mettetal et al., 2008). The system response was nuclear localization of doubly phosphorylated Hog1 (Hog1-PP), the MAP kinase that becomes activated under osmotic shock. They showed further that the system exhibited perfect adaptation to a step osmotic shock, taking about 25 min at 0.4M KCl to return to its set point (Muzzey et al., 2009).

van Oudenaarden and colleagues also found that, close to the steady-state osmolarity set point, highly reduced linear models, having only two or three variables, could capture certain experimental responses. However, it has remained unclear how informative such reduced models can be in comparison to the detailed model derived from the underlying biochemistry. The tension between detailed and reduced models has been a persistent theme in systems biology (Gunawardena, 2014).

With this in mind, in their *Science* paper, Mitchell et al. found an unexpected feature of the osmolarity frequency response: in the frequency range corresponding to periods of 8 to 16 mins, at an amplitude of 0.4M KCl, the yeast growth rate slows nearly 4-fold (Mitchell et al., 2015). Both higher and lower frequencies lead to faster growth. The authors account for this sensitivity in terms of the perfect adaptation found by van Oudenaarden's group. At high frequencies, Hog1 is not maximally activated before the pulse stops, while at low frequencies, Hog1-PP nuclear localization has time to perfectly adapt back to its normal level. It is only at an intermediate frequency that Hog1-PP can have sustained effect on gene transcription. The authors provide support for this interpretation using a Hog1-GFP construct to measure nuclear localization.

Amazingly, the resulting frequency response data is well fitted by van Oudenaarden et al.'s linear three-variable model with no changes to the parameters (Mitchell et al., 2015). (We are grateful to Amir Mitchell for confirming these points, which are not mentioned in the paper.) We know of no other case in which a model developed in one paper has been informative, as it stands, in answering a different question in another paper.

How does Hog1 nuclear localization relate to cell growth? Mitchell et al. attempt to explain this by transcriptionally monitoring three networks that share upstream signaling components: osmotic shock, filamentous growth, and pheromone response. In response to step osmotic shock, only the first network is activated, but, in response to repetitive pulsing close to the sensitive frequency, osmotic shock and filamentous growth are both hyper-activated (Mitchell et al., 2015). Interestingly, neither stimulation activates the pheromone response network. Mitchell et al. attribute low growth at the sensitive frequency to the inappropriate hyper-activation of osmotic shock and filamentous growth networks and found partial confirmation for this using genetic deletions within these networks (Mitchell et al., 2015).

Finally, the authors reasoned that if Hog1 reactivation during pulsing could be delayed, the frequency sensitivity might be abolished. They introduced a synthetic negative feedback using OspF, a Type III secretion system phospho-threonine lyase from *Shigella flexneri*, which irreversibly dephosphorylates Hog1-PP, and showed that this feedback mutant, while growing as well as the wild-type under step osmotic shock, grew faster than wild-type under repetitive pulsing at the sensitive frequency (Mitchell et al., 2015). Very interestingly, however, the feedback mutant showed reduced growth under stimulations that were more likely to be encountered in the wild, such as a staircase of increasing steps, as might be experienced under drying conditions. The wild-type system may be the most fit under natural conditions.

In the light of this, Mitchell et al. suggest that sensitivity to repetitive pulses is a side effect of evolution—an Achilles' heel—that has survived through lack of negative selection. What they offer us is a way to identify such fragilities using

only linear models, at least for homeostatic systems near their set points.

Mitchell et al.'s paper is impressive in skipping from cell growth to nuclear localization to gene transcription, without quite tying all the pieces together, and it does exhibit a lack of information about some of the details, as noted above. Nevertheless, they have elegantly shown how the outside-in, cybernetic approach can be combined with the inside-out molecular approach to offer a powerful way to interpret and re-engineer networks, without floundering in the molecular details. Synthetic biologists, including those in the Lim lab (Wu et al., 2015), have already begun re-engineering T cell receptors, with tantalizing implications for cell-based immunotherapy. It is important to understand the fragilities of these systems and to ensure that they are not encountered in the patients on whom such therapies will be tested. Perhaps cybernetics is yet to have its greatest impact on biology.

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When Less Is Better: ER Stress and Beta Cell Proliferation

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Pancreatic β cells synthesize and secrete insulin to increase anabolic metabolism in an organism, and insulin synthesis has long been suspected to inhibit β cell replication. Recently in *Cell Metabolism*, Szabat et al. (2015) present evidence that deletion of *Insulin* genes alleviates ER stress and promotes mature β cell replication.

The β cells in the islets of Langerhans are long-lived cells that secrete the important endocrine hormone insulin. Insulin plays a pivotal role in maintaining metabolic homeostasis, and its action affects major aspects of cellular metabolism, such as glucose absorption and synthesis, lipid synthesis and breakdown, and cellular proliferation and differentiation. Insulin secretion must be tightly regulated to coordinate cellular metabolism with nutrient availability. Dysregulation of insulin synthesis and secretion leads to diabetes, characterized by abnormally high levels of glucose in the blood. Despite the abundant knowledge regarding insulin's biological effects, little is known about the regulation of insulin synthesis and its effect on β cell health. Although characterizing β cells without insulin production poses both theoretical and technical challenges, a pioneering attempt to generate a mouse model with compound insulin gene knockout (*Ins1/2* KO mouse) showed that insulin production is dispensable for both embryogenesis and islet organogenesis (Duvillié et al., 1997). Preliminary observations from this study also suggested that insulin synthesis negatively regulates β cell proliferation. However, whether the hypothesis holds true for mature β cells remained largely un-tested for almost two decades. Reporting recently in *Cell Metabolism*, Szabat et al. (2015) present a breakthrough study showing that normal transcription and translation of insulin suppresses β cell proliferation and induces endoplasmic reticulum (ER) stress-sensing pathways, i.e., the unfolded protein response (UPR), and that relief from ER stress promotes cell proliferation through the AKT-Cyclin D1 axis.

Pancreatic β cells are notoriously resistant to cellular replication, and it has been long postulated that the burden of insulin synthesis inhibits cell cycle re-entry. Although there is evidence supporting the notion that cellular proliferation hampers β cell differentiation (Scharfmann et al., 2014), it is unknown if reducing insulin synthesis can directly promote β cell replication. The inability to test the hypothesis in vivo is mainly due to insulin's pivotal