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The Duty of an Intracellular Signal: Illuminating Calcium's Role in Transcriptional Control

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An optogenetic approach reveals how cells encode external information in complex patterns of protein activity.

Every cell—whether in a microbial or multicellular context—exists in a complex and changing environment. To deal with their complex surroundings, cells have evolved diverse systems to sense external cues (such as nutrients, stresses, or signals from neighboring cells) and create an internal representation of this information. Decades of elegant genetics and biochemistry have uncovered many of the proteins that play a role in input sensing—for example, the 62 two-component signaling systems in *E. coli* or the 4 mammalian MAP kinase cascades—yet we are still largely in the dark about how they store external information. In this issue of *Cell Systems*, Hannanta-anan and Chow (2016) use optogenetics to disentangle how two properties of calcium signaling affect transcription, revealing that cells are sensitive to the cumulative calcium load and not the frequency of oscillation. Their work showcases how precisely defined, non-natural inputs can be used to dissect the logic of cell signaling.

Calcium signaling is a classic example of complex information storage. During cellular processes like T cell activation or embryogenesis, extracellular inputs induce the periodic release of calcium from intracellular stores, leading to seconds-timescale oscillations in cytosolic calcium levels. For at least 30 years, it has been proposed that the frequency of calcium oscillations could control cell fate (Berridge and Galione, 1988). In this model, the cell's calcium-response circuitry is thought to be wired like a radio: if the calcium signal is played on the right station (oscillation frequency), it will generate a cellular response. Supporting this model, subsequent studies found that certain transcriptional targets are induced to different extents by certain frequencies of calcium (Dolmetsch et al., 1998).

Hannanta-anan and Chow (2016) revisit this model of calcium-induced transcription with fresh insight and powerful new tools. They reasoned that, as the frequency of calcium pulses changes, two other properties may also vary: the fraction of time spent in the calcium-high versus calcium-low state (this is the "duty cycle" of the oscillation) and the amplitude of each calcium spike. Using a mathematical model of calcium-induced transcription, they predicted that varying the duty cycle of the calcium pulses should have a far stronger effect on transcription than the amplitude or frequency.

What makes their approach stand out, though, is their design of a clever experiment to test this prediction (Figure 1). After engineering cells to express a light-gated activator of calcium release (the G-protein-coupled receptor melanopsin), they showed they could apply light pulses to precisely sculpt the profile of intracellular calcium release. With this technique, they varied the frequency of calcium oscillation while holding duty cycle and amplitude constant or varied duty cycle while fixing frequency and amplitude. They went on to show that transcription of the calcium responsive gene NFAT sensitively depends on the duty cycle—and not the frequency—of stimulation.

Studies such as this one suggest that there is much to be learned by measuring cellular responses to precisely defined, non-natural input stimuli. In biochemistry, uncovering mechanism requires systematic variation of each protein concentration. By analogy, we can learn a great deal about how information is stored in cell signaling pathways by systematically varying the inputs we deliver to them. But these studies are only a first step. Once we know which dynamics initiate a response, we are challenged to dissect *how* input discrimination is accomplished.

Experiments like those of Hannanta-anan and Chow (2016) could aid such a search, narrowing the focus to protein networks that serve as integrators of cumulative load, rather than those that respond at a specific resonant frequency. This kind of study would still be limited by an incomplete understanding of which protein networks can perform a given signal processing function, although recent work suggests that it is possible to enumerate the network motifs that share a particular response (Chau et al., 2012). We can thus envision stimulating an intracellular node with different time-varying light inputs, screening for downstream proteins that show an input-selective response and perturbing pathway connections that disrupt known signal processing motifs to test their necessity. In parallel, these studies could provide a fertile ground for theorists to address *why* certain features of a dynamic response (such as cumulative load) might provide better repositories for storing biological information. For instance, cumulative load integration may be an attractive strategy because integrators are especially resistant to error induced by noise.

One of the most exciting aspects of this work is that it is so widely generalizable to other biological systems. Optogenetic inputs are beginning to be available for many other signaling pathways where complex spatial and temporal dynamics have been observed (Wu et al., 2009; Toettcher et al., 2013). As Hannanta-anan and Chow (2016) and others show (Davidson et al., 2013), it is possible to perform these experiments at scale, using multi-well plates with programmable

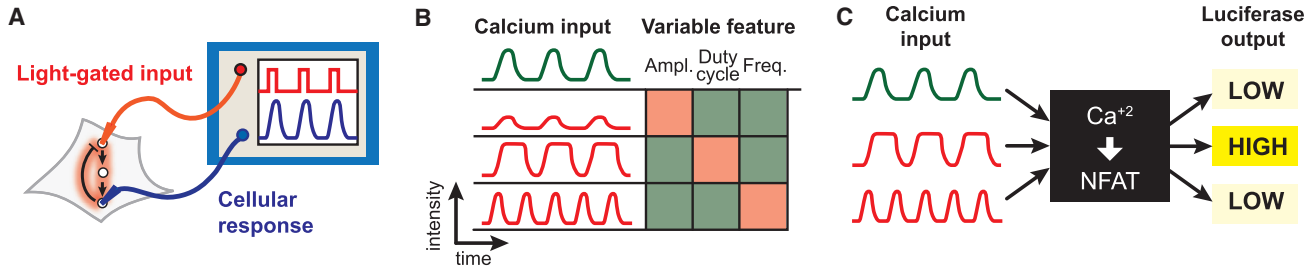

Figure 1. Light-Based Signal Interrogation

(A) By combining precise light inputs (red probe and trace) with downstream biosensors (blue), it possible to deliver complex inputs to an upstream signaling node and measure the magnitude of a response.

(B) Starting with a natural input (green curve), inputs can be designed that vary only a single property at a time (red curves), such as amplitude, frequency, or duty cycle.

(C) In the present study, independently varying the duty cycle or frequency of calcium oscillation has a differential effect on NFAT promoter activity.

light inputs to each well. So far, these devices have been developed within labs or through collaborations between engineers and biologists. Nevertheless, as cellular optogenetics research continues to take off, we can look forward to commercial devices in the formats useful for cell biology—for instance, by combining programmable LEDs with the ubiquitous 96-well plate.

Although this hardware could soon be within the grasp of many, precisely delivering biologically meaningful inputs is still in its infancy and faces many challenges. For example, even optogenetic tools often lack the quantitative accuracy needed to produce a desired level or timescale of protein activity. Feedback control of light delivery could help overcome this challenge by identifying light inputs in real time that can clamp output to a desired value (Toettcher et al., 2011; Olson and Tabor, 2014). Additionally, activating a single intracellular node can be insufficient to specify a cellular response (Chen et al., 2012). If other signals are required in combination, then the answer afforded by stimulating a single pathway may be misleading or unclear.

These challenges notwithstanding, optogenetic control over signaling dynamics could revolutionize our understanding of how cells store their experiences in patterns of protein activity and read them out to initiate responses.

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