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# Systems biology of cellular rhythms: from cacophony to symphony

## Editorial overview

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*This issue is dedicated to the memory of Arthur Winfree, who set in motion the dynamics of the field of quantitative cellular rhythms.*

Biological oscillations are ubiquitous. From the rhythmic behavior of the pumping heart to the firing of neurons and the cycling of intercellular and intracellular signaling, it is clear that a fundamental dynamical principle in biology is oscillation. In the context of gene regulation and cellular signaling, circadian rhythms and the cell cycle reign as 'classical' examples of oscillatory behavior. These systems are often couched in the framework of natural selection, whereby a periodic drive has served as a selection mechanism for the evolution of networks that govern rhythmic cellular responses. Similarly, periodic signals generated by cells may function to organize individual cells into a coordinated function. In the field of signal transduction, dynamical control of signaling mediators may be considered in the framework of information processing to tailor stimulus-specific cellular responses. Indeed, understanding dynamical behavior of molecular networks is a key challenge in the field of systems biology. High throughput approaches have led to the diagrammatic reconstruction of large-scale networks that underlie the complexity of cellular regulation. These efforts have led to the enumeration of small network motifs, and perhaps unsurprisingly, negative feedback is extremely common. As is widely appreciated in engineering and physics, the coupling of such negative feedback with the time delays that are inherent in biological networks naturally leads to oscillatory behavior. From this vantage point, the relevant question may not be whether cellular networks have the capacity to oscillate, but what the underlying mechanistic design principles are that amplify or reduce such oscillatory capacity and how these relate to biological function.

A restricted focus on biological oscillations at the signaling and gene-regulatory levels is still overly broad. However, the dynamics that lead to such network rhythms provides a natural framework for efforts in the emerging area of what can be loosely termed as 'quantitative biology'. Such efforts typically focus on the deduction of quantitative computational models and the development of measurement technologies aimed at characterizing the dynamics of signaling and gene-regulation at the single-cell level.

The articles presented here provide a broad overview of the current understanding of dynamic biological behaviors, with particular focus on the importance of probing and modeling the molecular networks that regulate complex biological functions.

Several recent studies have demonstrated that the dynamical behavior of intracellular signals contains more information than was previously understood. The health of cells and organisms depends on producing an appropriate response to these stimuli, and it is becoming clear that these

responses are tailored to the specific intensity, duration, and temporal program of the perceived signals. As demonstrated by the studies discussed by Behar *et al.*, understanding how cells are able to decode these intricate signals will become an increasingly important undertaking. In contrast to studying the dynamic response of cellular networks to *external perturbations*, the review by Metha *et al.* provides insight into the development of *self-initiated* dynamic behavior in cellular populations. As collective behavior can be observed in a vast range of biological systems, from individual microbial cells to herds of antelopes, it is important to understand how group behavior is organized and sustained. A great deal of recent research has focused on understanding collective behavior. In particular, this review focuses on understanding the molecular origins of oscillatory behavior in cellular populations and highlights the need to develop new experimental techniques to probe the links between macroscopic behavior and underlying microscopic networks.

In recent years great strides have been made in our understanding of endogenous biological rhythms in diverse organisms, largely due to technological advances that have enabled systems approaches. High throughput cell screening and comprehensive transcript, protein, and metabolite profiles have made it possible to move away from reductionist methods to observe a cycling system as a whole.

Transcript profiling was the first such technology to revolutionize the systems-level view of circadian rhythms, as is described in articles on the mammalian (Hogenesch *et al.*) and plant circadian clocks (McClung *et al.*). Improved technologies and reduced costs have made it possible to sample the mammalian transcriptome with greater time resolution, revealing more rhythmic transcripts and oscillations of shorter frequencies than the circadian cycle (Hogenesch *et al.*). In plants transcriptome analyses have emphasized how pervasively the clock controls plant physiology and metabolism (McClung *et al.*). In both of these systems, large-scale analyses of cycling transcripts have pointed to regulatory elements and transcription factors that might emerge only slowly, or not at all, in more classical searches.

High throughput screening has also accelerated the pace of discovery in circadian systems. Perturbation of animal cell reporter systems by chemical or molecular means has demonstrated both the robustness of the circadian clock and its Achilles Heels, the latter providing potential drug targets for clock modulation (Hogenesch *et al.*). In plants the wholesale screening of transcription factors against regulatory DNA elements has allowed researchers to skip laborious mutant hunts and jump quickly to candidate regulators for genes of interest (McClung *et al.*). Mathematical modeling has proposed the existence of factors that function in the plant circadian cycle before their biochemical or genetic description.

Our understanding of other oscillating cellular processes has also benefitted from systems approaches. The refinement of culture conditions for yeast that support 4-h stable oscillations of metabolism has allowed researchers to combine analysis by transcriptomics, metabolomics, and *in vivo* reporters to assemble an unusually comprehensive view of the workings of a cell (Tu *et al.*). The cell cycle in budding and fission yeast is yielding important data on how the feedback loops that control the cell cycle are influenced by internal and external controls that coordinate cell division with cell size and morphologenic checkpoints (Cross *et al.*). The role of coupling of oscillators through a phase-lock mechanism is emerging, in which one oscillator can advance or delay the phase of peripheral oscillators to keep the network coupled.

The coupling of the cell division and circadian cycles in a cyanobacterium has been described mathematically and elegantly, producing a model that is more broadly applicable to coupled oscillations of distinct periodicities in diverse organisms (Van Oudenaarden *et al.*). The circadian mechanism in the cyanobacterium *Synechocccus elongatus* is a systems tour de force, in which genetics, transcriptomics, cell biology, modeling, structural biology, and synthetic biology are informing a comprehensive view of how a cell orchestrates its daily events (Dong *et al.*).

The review by Yamada *et al.* presents an overview of the current understanding of *mammalian circadian rhythms* in the context of computational modeling. The authors present a thorough introduction to circadian rhythms, provide insight into the ways in which modeling may advance our understanding of these robust oscillatory systems, and discuss the need to develop tools to help bridge the multiple spatial and temporal scales that these complex systems span. The review by Aubel *et al.* on *synthetic oscillators* presents an overview of the synthetic biology approach to understanding genetic 'clocks'. Over the past decade or so, several successful synthetic systems have been developed that are able to produce and sustain oscillatory behavior. As the designs and approaches have varied, each undertaking has provided new insight into the various network motifs and design criteria that make a robust clock network tick.

The review by Glass *et al.* presents an introduction to the use of logical 'Boolean networks' to provide insight into how the underlying structures of biological systems can determine, or constrain, the dynamics. As systems biology provides increasing information about network structures, and synthetic biology lays the groundwork for understanding how particular network motifs can lead to specific dynamic behaviors, theoretical models can aid

in identifying the defining characteristics of biological processes. This review provides a good introduction to how dynamical models relate the interactions in biological networks to descriptions of their dynamics and can provide a bridge between the structure and function of complex biological systems. Interestingly, the Boolean ON–OFF approach to modeling gene regulation is perhaps the closest in spirit to how experimental biologists often think about fundamental processes such as transcriptional activation or repression.

The success in deconstructing complex networks and producing predictive models to describe their dynamic behavior demonstrates the potential to develop a comprehensive, quantitative understanding of biological systems. As systems and synthetic biology, microfluidic technology, and other novel experimental tools continue to advance, we will have increasing opportunities to observe the temporal response of cellular networks to dynamic environments that mimic natural systems. Using these tools, we can continue to probe network architecture and highlight key features that, while buried deep inside intricate biological networks, are the driving force for fundamental cellular function.

In the signal transduction field, studies of dynamic control have become of increasing interest but the role of rhythmic intracellular signals remains an important open question. Early studies showed that $Ca_2$ ions, the signaling mediator that controls the transcription factor NFAT, oscillate with periods in the order of minutes (*Nature* 1998, 392:933-936), whereas other studies indicated that the duration of stimulus-induced ERK signaling (in the order of hours) seems to be determine whether neuroblastoma PC12 cells proliferate or differentiate (*Cell* 1995, 80:179-185). Recent studies using both experimental and computational tools have focused on the dynamic control of the transcriptional factor NFκB, which is mediated by a variety of negative feedback loops (*J Biol Chem* 2009, 284:5439-43). In this issue, several reviews address the continuing question of the nature of the dynamic control of signaling mediators such as NFκB and the functional relevance of observed dynamic trajectories.

Shankaran and Wiley review the robust oscillatory behavior of the signal transducing kinase ERK, which is controlled by delayed negative feedback loops that exhibit ultra-sensitivity. What is remarkable in this system is that oscillations are stimulus dependent, but do not require sudden perturbations, and are therefore not a form of 'ringing'.

Mengel *et al.* review theoretical studies on the types of oscillatory control that operate in signaling pathways. Rather than address the experimental evidence for the existence or physiological relevance of oscillatory control, they discuss the mechanisms that may underlie oscillatory control in the NFκB and p53 stress response pathways. Cheong and Levchenko take a more agnostic approach and examine the evidence for oscillatory control, considering that some experimental studies may reveal oscillations simply as a byproduct of strong negative feedback control that has evolved to provide rapid adaptation to changing environmental conditions. White and coworkers, whose laboratory first drew attention to periodic peaks of NFκB activity, now observe that NFκB activity peaks are not predictably periodic but may probably be a result of stochastic transcriptional bursts in the synthesis of the short-lived inhibitor. This interpretation ties in with the fact that bursty NFκB activity may also be observed in the absence of an external signal (*PLoS One* 2009, 4:e7163). They consider the view that stochastic NFκB rhythms that exhibit high cell-to-cell variability may in fact be a means to provide for robustness of gene expression within an organ or tissue rather than a means of encoding information about the stimulus.

Finally, Lee and Covert describe the use of microfluidic experimental strategies to address the questions of rhythmic or dynamic control of signaling, as single cell measurements can be made within a highly controlled environment. However, Behar and Hoffmann point out that observing the dynamics of signaling mediators (phenomena) does not adequately address what aspect of such dynamic control is *functionally* important. They point out that to address what feature of a temporal activity profile conveys information about the stimulus to the cellular response, is determined by the signal decoding mechanism, that is for transcription factors, such as NFκB, the regulatory network associated with target gene promoters. Thus in signal transduction research a major focus ought to be the decoding circuitry of intracellular signals. Such a focus may enable us to determine whether stochastic rhythms of signal transducers constitute an exquisitely specific temporal code that specifies distinct cellular responses through a single channel, or allows for tissue robustness via a multitude of cacophonous cellular rhythms.