transport of Shiga toxin from endosomes to the Golgi apparatus, with depletion of syntaxin 5 providing the best protection against Shiga toxin (Mallard et al., 2002; Tai et al., 2004; Amessou et al., 2007).

Syntaxin 5 is localized primarily to the side of the Golgi that receives biosynthetic traffic from the ER (i.e., the *cis* side), but its localization extends through the Golgi toward the *trans* side (Figure 1; Hay et al., 1998); hence, syntaxin 5 is likely to function at multiple points between the Golgi and the ER. Therefore, the passage of ricin and Shiga toxin to their destination at the ER may be more sensitive to treatments that affect function and/or localization of syntaxin 5 than the retrieval of, for example, the CIMPR, which is delivered to the *trans*-Golgi.

How do Retro-1 and Retro-2 alter the localization of syntaxin 5? Stechmann et al. show that in cells treated with these small molecules, syntaxin 5 (and to a lesser extent syntaxin 6) relocates from the Golgi apparatus to small vesicles in the cytoplasm, a step that appears to be specific for these SNARE proteins. However, exactly where Retro-1 and Retro-2 act in the retrograde trafficking pathway is still not known. It is also unclear whether the relocalization of syntaxin 5 directly causes the block in endosome-to-Golgi transport of ricin and Shiga toxins or simply results from the inhibition of this pathway. Further, it will be important to determine if and how Retro-1 and Retro-2 affect the ability of these SNARE proteins to drive membrane fusion.

In a key experiment, Stechmann and coworkers show that Retro-2 protects mice from a lethal dose of ricin. To achieve this effect, however, Retro-2 had to be administered prior to ricin exposure, which may preclude the use of Retro-2 in treating individuals already exposed to ricin. Nevertheless, this result suggests that it may be possible to protect against ricin exposure or to treat *Shigella* infection with compounds that selectively block the transport of AB chain toxins in the endosome-to-Golgi pathway. In addition, the toxin inhibitors identified by Stechmann et al. will be useful tools for unraveling the mechanistic details of endosome-to-Golgi transport of both endogenous proteins and toxic intruders.

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The Hidden Rhythms of the Dividing Cell

David O. Morgan[1,*]
[1]Department of Physiology, University of California, San Francisco, CA 94158, USA
*Correspondence: david.morgan@ucsf.edu
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The cell divides in a series of discrete steps that occur in a specific order. Lu and Cross (2010) now propose that cell-cycle events are ordered by a regulatory system in which a master oscillator, based on cyclin-dependent kinases, entrains a series of peripheral oscillators controlling individual events.

Biological rhythms, from the beating of hearts to the flashing of fireflies, are driven by regulatory circuits called oscillators. The frequency of most biological oscillators—the heart rate, for example—can be adjusted by outside signals, allowing coordination of a periodic event with other events or with changes in the environment. Sometimes one oscillator is synchronized with another—as in the well-known case of our body's circadian rhythm, which is entrained to the light-dark cycle of the environment. In this issue of *Cell*, Lu and Cross (2010) take the problem of oscillator control into rich new territory. They provide evidence that a series of oscillators is governed by a single master oscillator to control the rhythms of the cell division cycle.

Orderly progression through the cell cycle is guided by cyclin-dependent kinases (Cdks) in association with oscillating cyclin subunits (Morgan, 2007). We know a great deal about the regulators that generate the ups and downs of Cdk activity, but we have only a minimal understanding of how Cdks trigger cell-cycle events in the correct order. One source of order can be found in "check-

point" mechanisms that delay later events (such as mitosis) when early events (such as DNA replication) are not completed. Another contribution comes from differences in the functional specificity of different cyclins: S phase cyclins are more effective stimulants of DNA replication than M phase cyclins, and so their early expression promotes the correct sequence of events. However, division can occur in some cell types in the absence of checkpoints or cyclin specificity, indicating that other ordering mechanisms must exist.

One potential source of order is described by the "ratchet" model of cell-cycle control, which proposes that Cdk activity does not simply initiate each cell-cycle event but also blocks further progress, such that Cdk inactivation is required for the subsequent event and to reset the cell for the next cycle (Nasmyth, 1996; Stern and Nurse, 1996). This model does not provide a robust explanation for the ordering of S and M phases, but it is consistent with the control of DNA replication: Cdk activity initiates DNA replication but also blocks its reinitiation, so that Cdk inactivation in late mitosis is required to reset replication origins for firing in the next cell cycle.

The omnipotence of the Cdk oscillator has been called into question by experiments showing that some cell-cycle events, such as budding in yeast and centrosome duplication in animals, can occur repeatedly in cells engineered to lack Cdk oscillations (Haase and Reed, 1999; McCleland and O'Farrell, 2008). The clearest example of an autonomous oscillator comes from the work of Haase and colleagues, who observed normal oscillations in cell-cycle-dependent transcriptional programs in budding yeast mutants lacking Cdk activity (Orlando et al., 2008). They proposed that transcriptional control in the cell cycle depends in large part on a Cdk-independent oscillator, which is normally coupled to the Cdk oscillator.

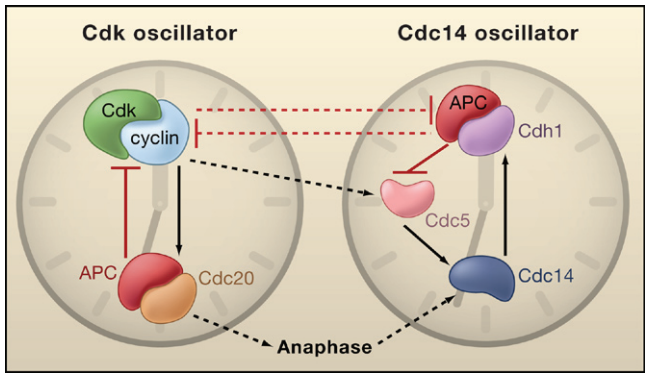

Figure 1. The Master Cdk Oscillator Controls the Cdc14 Oscillator
Oscillations of cyclin-dependent kinase (Cdk) activity (left) are generated in part by a negative feedback loop: mitotic cyclin-Cdk complexes help activate the ubiquitin ligase APC-Cdc20, which eventually triggers cyclin destruction and thus inactivation of Cdk, removing its stimulatory effect and lowering APC-Cdc20 activity to allow cyclin to accumulate again. Similarly, negative feedback provides the basis for an oscillator driving Cdc14 release and activation (right): the protein kinase Cdc5 helps activate Cdc14, which then activates APC-Cdh1. APC-Cdh1 triggers Cdc5 destruction, resulting in Cdc14 inactivation, inhibition of Cdh1, and accumulation of Cdc5. Dashed lines indicate the links that allow control of the Cdc14 oscillator by the Cdk oscillator: most importantly, APC-Cdc20 triggers the onset of anaphase, which acts through various means to promote Cdc14 release; in addition, mitotic cyclin-Cdk influences the frequency of the Cdc14 oscillator by stimulating Cdc5 and by inhibiting APC-Cdh1. The Cdc14 oscillator also influences Cdk activity, in part because APC-Cdh1 triggers cyclin destruction.

Lu and Cross (2010) now take these issues to a new level by uncovering another autonomous cell-cycle oscillator and, most importantly, by using clever experiments and modeling to suggest how multiple independent oscillators might normally be entrained by the master Cdk oscillator. Their work began in studies of a mitotic cyclin mutant that does not undergo the usual degradation in late mitosis. Overexpression of stabilized cyclins has long been known to block late mitotic events, but recent work from the Cross lab (Drapkin et al., 2009) revealed that expression of stable cyclins at physiological levels causes only partial defects in late mitosis despite the presence of constant Cdk activity. The explanation came from studies of the phosphatase Cdc14, which is normally sequestered in the nucleolus but released and activated transiently in anaphase, resulting in some Cdk substrate dephosphorylation even in cells expressing stable cyclin. Remarkably, Lu and Cross now report that cycles of Cdc14 release and resequestration occur repeatedly in cyclin-expressing cells—betraying the existence of a previously unnoticed oscillator controlling Cdc14 localization. They also show that the Cdc14 oscillator, like so many others (including the Cdk oscillator), is based on a negative feedback circuit, the structure of which is suggested by previous studies (Visintin et al., 2008) (Figure 1).

There are multiple molecular connections between the Cdk oscillator and the Cdc14 oscillator (Figure 1), prompting Lu and Cross to suggest that oscillations in Cdk activity normally entrain the Cdc14 oscillator to the same frequency. They use mathematical models to illustrate this concept of "phase-locking" and show how a single master oscillator of some fixed frequency can entrain a series of peripheral oscillators, making them run at the same frequency while generating peak signals at different points in their cycles. If each peripheral oscillator triggers a different cell-cycle event, then the result is an ordered sequence of cell-cycle events, each occurring once per cell cycle.

To test the phase-locking model, Lu and Cross constructed yeast strains in which the amplitude of Cdk oscillations is dampened, either by reducing peak Cdk activity or by raising the level of Cdk activity in the troughs between peaks. In both cases, they observe low frequencies of cells with defects in the order of budding and nucleolar segregation, suggesting that peripheral oscillators had become partially uncoupled as predicted by the phase-locking model. These results do not support a ratchet model, which would predict that changes in Cdk amplitude would cause delays or defects in cell-cycle events but not changes in their order.

Lu and Cross therefore propose that the cell-cycle control system is based on a community of peripheral oscillators under the control of oscillating Cdk activity. These peripheral oscillators are not apparent in a normal cell cycle but reveal themselves in some mutant strains. Some peripheral oscillators might have evolved to depend so completely on the Cdk oscillator that they can no longer be

uncoupled from it; indeed, Lu and Cross argue that such strong coupling to Cdk activity underlies the regulatory circuit driving DNA replication, which is so Cdk dependent that it behaves as a ratchet. It is also clear that peripheral oscillators are not simply downstream targets of the Cdk oscillator but also send signals upstream to influence Cdk activity and function, resulting in a complex two-way relationship between master and servant (Orlando et al., 2008) (Figure 1).

The phase-locking model, like everything in biology, makes particularly good sense in the light of evolution. Lu and Cross speculate that the cell cycle of early eukaryotes depended on multiple autonomous oscillators, each driving a different event with similar frequency. Cdk arrived later in evolution, perhaps starting out as a regulator of one oscillator but eventually assuming control of multiple oscillators to provide more robust centralized control. A single Cdk-cyclin complex might have existed initially, but more effective coordination and timing of events would have become possible with the duplication and specialization of cyclins, together with the evolution of checkpoint controls. Cdks also acquired the ability to directly control hundreds of proteins involved in every aspect of cell division (Holt et al., 2009), expanding their role from that of master controller to include that of micromanager. Even if all these layers of regulation have obscured the original structure of the coupled oscillator, the phase-locking model provides a compelling conceptual basis for understanding the fundamental underpinnings of cell-cycle control.

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