Hysteresis meets the cell cycle

Mark J. Solomon*

Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, C123 SHM, New Haven, CT 06520-8024

What, you may well ask, does hysteresis have to do with cell cycle progression? The last time most of us heard about hysteresis was in the context of the ferromagnetism that underlies tape players and floppy drives. In general, hysteresis means that it takes more of something to push a system from state A to state B than it does to keep the system in state B. Sha *et al.* (1) report in this issue of PNAS that the cell cycle of *Xenopus* egg extracts exhibits hysteresis in that the amount of cyclin needed to induce entry into mitosis is larger than the amount of cyclin needed to hold the extract in mitosis. This effect creates a nice bistable system with a ratchet to prevent slipping back from mitosis to interphase. This work also represents an excellent marriage of theory and experiment from the labs of John Tyson and Jill Sible, respectively, at Virginia Polytechnic Institute in Blacksburg. Some background, both experimental and theoretical, is necessary before discussing the importance of the current findings.

The eukaryotic cell cycle is driven by sequential activation and inactivation of cyclin-dependent protein kinases (CDKs) (2, 3). The CDK for entry into mitosis is Cdc2. Cdc2 activation requires binding to a regulatory protein (cyclin B) and activating phosphorylation (carried out by CDK-activating kinases, or CAKs). Even in the presence of cyclin and activating phosphorylation, Cdc2 can be inactivated by inhibitory phosphorylations (carried out by the Wee1 and Myt1 protein kinases). Inhibitory phosphorylations are removed by the Cdc25 protein phosphatases, which are the immediate triggers for entry into mitosis. CDKs regulating other cell cycle transitions can in addition be inhibited by direct binding of inhibitory proteins. Mitotic cyclins are subject to ubiquitin-mediated degradation at the end of mitosis by the action of the anaphase-promoting complex (APC; an E3, or ubiquitin ligase, of the ubiquitin system). Extremely important to the proper functioning of the cell cycle are checkpoints that ensure that key cell cycle events are not initiated until prior steps are completed. For example, a DNA replication checkpoint prevents Cdc2 activation until DNA replication is complete and the spindle assembly checkpoint prevents cyclin degradation via the APC until all chromosomes are properly aligned on the metaphase plate.

Many important advances in understanding entry into and exit from mitosis have come from biochemical studies of *Xenopus* egg extracts (2). This "simple" system is obtained by crushing frog eggs in the presence of minimal amounts of buffer. These extracts can undergo multiple rapid cell cycles, monitored either by the morphology of added nuclei or by assays of Cdc2 activity. These cell cycles can be driven by the endogenous synthesis and degradation of cyclin, or, if protein synthesis is inhibited, by the addition and subsequent degradation of exogenous cyclin. This system is probably the closest we can come to having the biochemist's ideal: a homogeneous bag of enzymes performing a complex task. The lack of checkpoints (unless one performs special tricks) and nonessential features one associates with more complex cell cycles in tissue culture (such as the need for nuclei, microtubules, subcompartments, DNA, etc.) allows the fundamental cell cycle oscillator to be studied in relative isolation. This system also exhibits conserved features of eukaryotic cell cycles: feedback loops. Thus, the rates of inhibitory phosphorylation of Cdc2 (mediated by Wee1 and Myt1) are decreased and the rates of dephosphorylation of these sites (mediated by Cdc25 proteins) are increased at the transition into mitosis (4). A second critical feedback is that cyclin synthesis, by inducing entry into mitosis and consequent activation of the anaphase-promoting complex, also leads to its own destruction. From these components and this basic wiring diagram one can hand wave one's way through the interphase-to-mitosis transition. But do we really understand this process? That's where mathematical modeling comes in.

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There are many reasons to model a complex process quantitatively. It can be reassuring to plug the complete set of enzyme and substrate concentrations and their respective kinetic properties into a set of equations and have one's favorite process pop out. But that doesn't happen until you already know everything. More useful is modeling when significant ignorance remains so that the act of modeling points to important gaps to be filled or suggests interesting behavioral aspects of the system that can be tested. That's the goal John Tyson and collaborators have tackled for over a decade. Most of this work was done in collaboration with Bela Novak (Budapest University of Technology and Economics) who brought his experimental experience studying the *Schizosaccharomyces pombe* cell cycle. Their first joint effort was a model of the basic cell cycle in *Xenopus* egg extracts (5). The underlying logical approach was remarkably straightforward: Compile a large set of conceptually simple differential equations describing the rate of change of given components (say cyclin concentration or Cdc2 with inhibitory phosphorylations) and let a computer sort out the resulting complex web of interrelationships. What resulted was a good description of the state of knowledge at the time. Many models could do that, however, because the degrees of freedom are unbounded. However, what also came out of the simulations were some nonintuitive predictions not made by other published models. The key one tested in the Sha *et al*. article (1) was that the system would display hysteresis, thereby explaining its bistability: two stable states, interphase and mitosis.

The approach to testing this prediction is at once elegant and technically tricky. What is needed are accurate measurements of how much cyclin it takes to push an egg extract into mitosis and how much it takes to hold it there. The first measurement is easy: Just determine how much of a nondegradable cyclin needs to be added to an extract in interphase lacking any endogenous cyclin to induce entry into mitosis. The second measurement is more complicated because the determination of how much cyclin is needed to maintain mitosis requires that some of the cyclin that was necessary to get you into mitosis in the first place be removed. The solution was, essentially, to use a mixture of de-

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*E-mail: Mark.Solomon@Yale.edu.

gradable and nondegradable cyclins to induce mitosis and to determine how much nondegradable cyclin is required to keep the extract in mitosis after the extract itself has removed (degraded) the degradable cyclin. Sha *et al.* (1) allowed the extract to synthesize its own cyclin as the source of the degradable cyclin. The answer is that between 32 and 40 nM of cyclin is necessary to induce mitosis but that only 16–24 nM of cyclin is required to keep an extract in mitosis, demonstrating hysteresis. The size of this hysteretic effect doesn't matter for the gross behavior of the system, just for the experimental difficulty of detecting it. Thus, hysteresis, and not other theoretical possibilities (1), underlies the all-or-none irreversible transitions between interphase and mitosis.

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Two other theoretical predictions were also verified by Sha *et al.* (1). The first is a "critical slowing down," meaning that it takes longer and longer to enter mitosis as the cyclin concentration is reduced close to the threshold concentration for mitotic entry. The second prediction was that induction of a DNA replication checkpoint (by adding many nuclei and inhibiting DNA replication) would increase the cyclin threshold for entry into mitosis. The critical slowing-down test emphasized how important a close collaboration between theoreticians and experimentalists is to successful studies such as this one. For years Tyson and Novak would berate experimentalists who had performed time-course experiments at varying cyclin concentrations (4), asking why they didn't observe a critical slowing down. The experiments, although detailed from a typical biochemical perspective, just didn't have the resolution (either temporally or in terms of cyclin concentration) to reveal this effect. The needs of the experimentalist and the theoretician were on different scales, and only a close collaboration can communicate and coordinate those requirements.

Similar evidence for hysteresis in the *Xenopus* system has been obtained by Joseph Pomerening and James Ferrell, Jr. at the Department of Molecular Pharmacology and Biochemistry, Stanford University, Stanford, CA (personal communication). They first considered whether the system exhibited bistability, reasoning that bistability would prevent reaching an intermediate state (partial mitosis); that it would lock the cell/extract into one state, preventing sliding back to the other state; and that it would allow the system to cycle indefinitely, without settling into an intermediate steady state. The last property was key to them as simple models invoking the feedbacks discussed earlier led to damping of the oscillations. They were led to examine whether the system exhibited hysteresis, as this property would be expected of a bistable system but not of a system governed by a system with highly cooperative transitions but lacking bistability. Their values for the cyclin thresholds for entering and exiting mitosis are very similar to those determined by Sha *et al.* (1).

The *Xenopus* system isn't the only one in which experimental tests of quantitative cell cycle models have been undertaken. The Tyson group has modeled various aspects of cell cycles in organisms ranging from *Xenopus* to budding yeast to fission yeast (5–9). The predictions for the yeast cell cycles are much more difficult to test than those in the *Xenopus* system. Nevertheless, Fred Cross and colleagues (10) recently tested some of the predictions of a model of the *Saccharomyces cerevisiae* cell cycle (9). They found that the model correctly predicted a bistability and the quantitative relationship between cell size and a G_1 cyclin, but incorrectly predicted certain genetic relationships. Although in this case John Tyson is not a collaborator, he and a coworker are thanked "for help in understanding and using the model," again demonstrating how important these interactions are in testing and advancing realistic models.

One of the most important "results" of the Sha *et al.* (1) study goes unmentioned in the article, although it has been alluded to above: the close collaboration between experimental and computational biologists. Even the best quantitative models have reduced impact if they are not tested experimentally. Theoreticians can know a field extremely well (in this case, better than most professional cell cyclists) but may lack an appreciation of technical limitations (or of how easy another experiment may be). Conversely, an experimentalist without close contact with a theoretician is unlikely to become sufficiently motivated to undertake the relevant experiments necessary to advance a model. Thus, it is obviously no coincidence that this work blossomed after Jill Sible moved to Virginia Polytechnic, where John Tyson had long been searching for a collaborator. An alternative "model" for such hybrid work is provided by the similar studies from Jim Ferrell's lab (see above), in which case the theoretician and the experimentalist were the same person. As time goes on and as interest in the quantitative study of complex biological systems advances (11), many more studies at the intersection of theory and experiment will help advance our understanding of the cell cycle and other processes, both at the quantitative level and at the level of "how does it really work?"

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