Developmental Cell, Vol. 9, September, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.devcel.2005.08.006

Shake It, Don't Break It: Positive Feedback and the Evolution of Oscillator Design

In cell cycle control, a negative feedback oscillator design is shown to be reinforced with a positive feedback loop, giving a robust oscillatory architecture that is surprisingly common in biology.

The cell cycle was first shown to be controlled by an autonomous biochemical oscillator in *Xenopus* embryos and cell extracts. Cyclin synthesis was required for mitotic entry; remarkably, no other proteins needed to be newly synthesized (Murray and Kirschner, 1989). The ultimate mitotic inducer consists molecularly of a dimer of cyclin and the Cdc2 cyclin-dependent kinase catalytic subunit, activated by cyclin binding.

Entry into mitosis is associated with activation of cyclin proteolysis. This yields a minimal cell cycle oscillatory mechanism: a negative feedback oscillator. Start in a low-Cdk activity state permissive for cyclin accumulation; cyclin accumulation leads to high Cdk activity; high Cdk activity then activates cyclin proteolysis (negative feedback), resetting the system.

To drive the biology, high Cdk activity must phosphorylate target proteins, leading to entry into mitosis. Among these targets is the anaphase-promoting complex/cyclosome (APC/C), whose activation drives sister chromatid separation. The APC/C also ubiquinates cyclin, leading to its degradation (the negative feedback mechanism). Loss of Cdk activity is required for mitotic exit, presumably because Cdk phosphorylation of some target(s) keeps cells in mitosis; when the APC/C drives down cyclin levels, loss of these phosphorylations allows mitotic exit.

Thus, the out-of-phase rise and fall of cyclin versus APC/C results in two daughter cells ready for another cycle. Cdk and cyclin are essential for entry into mitosis, the APC/C is essential for completion of mitosis, and cyclin-Cdk inactivation is essential for exit from mitosis.

However, cyclin accumulation is not sufficient for production of active cyclin-Cdk complexes. The enzymatic activity of cyclin bound Cdc2 is inhibited by phosphorylation of Cdc2 Tyr15 (and Thr14 in animals) by kinases including Wee1. Tyr15 and Thr14 phosphorylations are reversed by the Cdc25 phosphatase. Wee1 and Cdc25 are themselves regulated, with Cdc25 (directly or indirectly) activated by cyclin-Cdc2 and Wee1 inhibited. *wee1* mutation in fission yeast causes advance of mitosis, and *cdc25* mutation causes inability to activate Cdc2 kinase and a lethal block to entry into mitosis (e.g., Fantes, 1981), consistent with Wee1 inhibiting and Cdc25 subsequently activating Cdc2 in every cell cycle.

Thus, the initial picture of a negative feedback oscillator was complicated by positive feedback: a small amount of Cdk activity could activate Cdc25 and/or inhibit Wee1, leading to amplification of Cdk activity. Positive feedback can lead to bistability: both the high-Wee1, low-Cdc25, low-Cdk state and the low-Wee1, high-Cdc25, high-Cdk state can be indefinitely stable at the same intermediate cyclin level provided cyclin proteolysis is blocked. This theoretical prediction was verified in *Xenopus* extracts (Pomerening et al., 2003; Sha et al., 2003).

In principle, positive feedback could tighten up the negative feedback oscillator: during the slow phase of cyclin accumulation, the system is trapped in the low Cdk activity branch of the bistable system, followed by explosive Cdk activation and a jump to the high Cdk activity branch. Cyclin proteolysis then activates, but the system can stay on the high branch until cyclin has been reduced to a very low level, whereupon the system jumps back to the low branch. These dynamics could cause a more reliable oscillator. Indeed, it is known from theoretical work (e.g., Pomerening et al., 2003) that a negative feedback oscillator is impossible without a time delay or some other mechanism giving irreversibility, since otherwise the system will rapidly find a stable intermediate state—good for many biological systems but bad for a cell cycle! Thus, positive feedback-induced bistability could provide a mechanism to make negative feedback operate robustly.

Testing this idea requires inactivating the positive feedback loop. What would happen in the simultaneous absence of Wee1 and Cdc25? 24 years ago (!), Fantes (1981) showed that while *cdc25* single mutants were dead, *wee1 cdc25* double mutants were viable, with advanced mitosis like *wee1* single mutants. The analysis implied that this was due to Wee1 inhibiting Cdc2, and Cdc25 relieving this inhibition. This was a remarkably prescient interpretation, obtained in the absence of any biochemical or molecular data whatsoever (i.e., precloning). Viability of the *wee1 cdc25* double mutant is odd, though, in comparison to the expectation of a highly defective cell cycle oscillator in the absence of the machinery for positive feedback! Subsequent work with the double mutant did indeed reveal some oddities, including apparently "quantized" cell cycle lengths, which could be attributed theoretically to loss of the positive feedback mechanism (Sveiczer et al., 2000). A simpler way to ablate the positive feedback system (that also gets around backup enzymes that can get at Cdc2-Y15 in the absence of Wee1 or Cdc25) was to remove the target site of inhibitory phosphorylation by mutating Cdc2-Tyr15 to phenylalanine. This mutant had marked mitotic abnormalities, apparently including difficulties exiting mitosis ("mitotic catastrophe" [Gould and Nurse, 1989]). Thus the Wee1/Cdc25/Cdc2 positive feedback system is quite important for the cell cycle oscillator to function, but the dynamics are hard to pursue with qualitative genetic results.

In a recent issue of *Cell*, new work by Pomerening et al. (2005) in the *Xenopus* extract system revisits these issues from an explicitly dynamical-systems perspective. First, an ordinary differential equations model was used to examine the theoretical consequences of reducing the positive feedback loops. Parameter choice in these kinds of models is critical; Pomerening et al. (2005) give some sense of how parameter-sensitive their conclusions are, but a computational method allowing a systematic exploration of this issue would likely be informative here to avoid anecdotality. In any case, modeling progressive weakening of positive feedback yields predictions of interesting dynamical behaviors such as blunting of peak-trough ratios, higher frequency cycling, and increased sensitivity of oscillations to parameter choices (a possible computational correlate of "noise").

These ideas are translated into the *Xenopus* extract experimental system by either doping the extract with added Cdc2-AF (unphosphorylatable mutant) or by running the system almost entirely with Cdc2-AF by using the expedient of constitutively inactivating endogenous Cdc2 with a mutant Wee1 and supplying exogenous Cdc2-AF (immune to Wee1). The first protocol represents a short-circuit of regulation of the endogenous system by positive feedback; the second should represent a near-complete abrogation of the positive feedback control. The results showed behavior consistent with the qualitative results of the modeling, in that oscillations were blunted with higher mitotic residence time, and the frequency of oscillations was increased: perhaps the frog extract version of fission yeast mitotic catastrophe (Gould and Nurse, 1989).

Intriguing quantitative measurements show the trajectory of the unperturbed or Cdc2-AF-subtituted system in the cyclin-Cdc2 activity plane. The wild-type system shows a wide sweep consistent with overshoot and hysteresis—indeed, even greater than predicted from positive-feedback alone (Pomerening et al., 2003), possibly due to intrinsic time delays in activation of cyclin proteolysis. In contrast, the trajectory is much tighter in the Cdc2-AF system, suggesting that this system not only lacks positive feedback, but also may approach a steady-state cyclin proteolysis activity level. The latter suggestion was confirmed by direct measurement.

Thus, even though the intrinsic negative feedback oscillator is clearly the primary driving mechanism, the positive feedback ratcheting mechanism is somewhere between important and essential for the functioning of this oscillator in the modern evolved *Xenopus* system. At the same time, lack of absolute essentiality of positive feedback has probably provided evolutionary flexibility. The Wee1/Cdc25 system has been exploited for the DNA damage response in many organisms, and in budding yeast it may be subsumed to response to bud growth/morphogenesis. In nonembryonic systems (fission yeast, budding yeast, animal somatic cells) there are additional positive feedback loops involving Cdh1-APC and Cdk stoichiometric inhibitors, which provide ratchets for the cyclin-APC/C negative feedback loop; these may serve the same systematic role as the Wee1/Cdc25 system. In fact, in budding yeast, these circuits may replace the Wee1/Cdc25 circuitry for the role of reinforcing bistability in general cell cycle regulation (Cross, 2003). Interestingly, though, the system architecture (a "relaxation oscillator") remains the same, and relaxation oscillators are found in biological systems unrelated phylogenetically to eukaryotic cell cycle control, such as circadian clocks (Leloup and Goldbeter, 2003) or the Caulobacter cell cycle (Holtzendorff et al., 2004). The reason for the frequent selection of this architecture is a subject for interesting speculation by Pomerening et al. (2005). In any case, this new work represents a promising methodological hybrid between theoretical computational studies and experimental work; these approaches may ultimately yield insight into evolutionary access to basic biological design principles.

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