

your own scent requires a much less sophisticated information processing system than trying to remember previous mates – a female always has her own scent available as a reference, so she can simply avoid males that smell too familiar.

The finding that females have evolved to mark their mates has two major implications. It suggests that the risk of encountering the same male twice must be large and so must the benefits of mating with more than one male. The crickets used in this study are not particularly unusual insects, they live in large populations at high densities [11] and are highly mobile. If a species like this has evolved a system for avoiding mating with the same male twice it suggests that similar abilities may be widespread. It also supports the argument that females can gain substantial benefits from mating with more than one male [12,13] and highlights the importance of what happens after a female mates. Indeed the possibility that females may often avoid mating with previous mates has such major implications that a degree of scepticism is warranted – there may be a lot of studies trying to find this phenomenon, with the danger that statistically significant results may sometimes occur by chance.

Although the sizes of the effects found in reports of female mating preference for novel males [2–7] are reasonably large, it is noteworthy that across studies the strength of the effect reported apparently decreases as sample size increases (effect sizes were calculated by converting p-values for experiments showing a mating bias to z-scores and dividing the z-score by the square root of the sample size; a linear regression of effect size against sample size gave a negative association ( $r^2 = 0.727$ ;  $F_{1,5} = 10.64$ ;  $p = 0.031$ )). This suggests that smaller studies where females fail to show discrimination against novel males may be being done but not being published, making the literature less objective than it should be. Nevertheless, Ivy *et al.* [7] provide the first study to show

that females both discriminate against males they have already mated, and that they do it by marking them as used goods. Males have been known to mark their mates for some time [14], so this sort of behaviour from females brings a little sexual equality to the mating game.

#### References

1. Jennions, M.D., and Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biol. Rev. Camb. Philos. Soc.* 75, 21–64.
2. Eakley, A.L., and Houde, A.E. (2004). Possible role of female discrimination against 'redundant' males in the evolution of colour pattern polymorphism in guppies. *Proc. R. Soc. Lond. B. Biol. Sci.* 271 (Suppl), 299–301.
3. Zeh, J.A., Newcomer, S.D., and Zeh, D.W. (1998). Polyandrous females discriminate against previous mates. *Proc. Natl. Acad. Sci. USA* 95, 13732–13736.
4. Bateman, P.W. (1998). Mate preference for novel partners in the cricket *Gryllus bimaculatus*. *Ecol. Ent.* 23, 473–475.
5. Archer, M.S., and Elgar, M.A. (1999). Female preference for multiple partners: sperm competition in the hide beetle, *Dermestes maculatus* (DeGeer). *Anim. Behav.* 58, 669–675.
6. Hosken, D.J., Martin, O.Y., Born, J., and Huber, F. (2003). Sexual conflict in *Sepsis cynipsea*: female reluctance, fertility and mate choice. *J. Evol. Biol.* 16, 485–490.

7. Ivy, T.M., Weddle, C.B., and Sakaluk, S.K. (2005). Females use self-referent cues to avoid mating with previous mates. *Proc. R. Soc. Lond. B. Biol. Sci.*, in press.
8. Bretman, A., Wedell, N., and Tregenza, T. (2004). Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proc. R. Soc. Lond. B. Biol. Sci.* 271, 159–164.
9. Tregenza, T., and Wedell, N. (2002). Polyandrous females avoid costs of inbreeding. *Nature* 415, 71–73.
10. Tregenza, T., and Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Anim. Behav.* 54, 979–984.
11. Sakaluk, S.K., Schaus, J.M., Eggert, A.K., Snedden, W.A., and Brady, P.L. (2002). Polyandry and fitness of offspring reared under varying nutritional stress in decorated crickets. *Evolution Int. J. Org. Evolution* 56, 1999–2007.
12. Zeh, J.A., and Zeh, D.W. (1996). The evolution of polyandry I: Intra-genomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. B. Biol. Sci.* 263, 1711–1717.
13. Hosken, D.J., and Stockley, P. (2003). Benefits of polyandry: A life history perspective. *Evol. Biol.* 33, 173–194.
14. Scott, D. (1986). Sexual mimicry regulates the attractiveness of mated *Drosophila melanogaster* females. *Proc. Natl. Acad. Sci. USA* 83, 8429–8433.

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## Cell Cycle: Bistability Is Needed for Robust Cycling

***Xenopus* egg extracts have distinct Cdk-active and Cdk-inactive states at intermediate cyclin concentrations, a phenomenon known as bistability. A new study shows that this behavior is important for robust cell cycling.**

Nicholas Ingolia

Cyclins and cyclin-dependent kinases (Cdks) play a central role in the cell-cycle oscillator, but they do not function in isolation. A complex network of conserved interactions generates a number of feedback mechanisms that regulate Cdk activity. For instance, the active cyclin–Cdk complex activates the anaphase-promoting complex (APC), which in turn destroys cyclin, in order to produce an alternation between interphase and mitosis (reviewed in [1]). This APC-mediated negative feedback alone could drive the cell cycle [2], but there are other conserved Cdk regulators of the cyclin–Cdk

complex. In particular, there is positive feedback on Cdk activity mediated by Cdc25 and Wee1 (reviewed in [3]). This positive feedback results in bistability in Cdk activity – two possible, stable levels of Cdk activity for a fixed cyclin concentration [4,5]. A number of investigators previously suggested that this bistability may be required for the production of distinct interphase and mitotic states in the cell cycle [6]. Recent work by Pomerening *et al.* [7] has now tested this proposal experimentally by specifically ablating the positive feedback and verifying that this indeed results in defects in the cell cycle as predicted by modelling work.

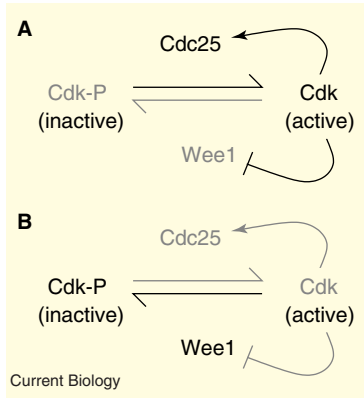


Figure 1. Positive feedback and bistability in Cdk activity.

Cdk is inhibited by Wee1-mediated phosphorylation, and this phosphorylation is removed by Cdc25. At constant, intermediate cyclin levels, there can be two stable states of Cdk activity. (A) Stable state with active Cdk maintaining active Cdc25 and inactive Wee1. (B) Stable state with inactive Cdk, permitting active Wee1 and no activation of Cdc25.

Active Cdk phosphorylates both the Wee1 kinase and the Cdc25 phosphatase, inhibiting the former and activating the latter. Wee1 and Cdc25 in turn feed back on Cdk activity by regulating an inhibitory phosphorylation site on the Cdk, with Wee1 promoting the phosphorylation and Cdc25 destroying it. As predicted by earlier modelling efforts ([6], reviewed in [8]), this positive feedback produces two stable

states of Cdk activity at intermediate levels of cyclin [4,5]. Active Cdk maintains inactive Wee1 and active Cdc25, suppressing inhibitory phosphorylation (Figure 1A). However, inhibited Cdk allows Wee1 to maintain the inhibitory phosphorylation and does not activate Cdc25 to remove it (Figure 1B). An analogous regulation involving the Cdk inhibitor Sic1 has been shown to produce bistability in budding yeast Cdk activity [9]. This regulation comprises widely conserved components that are not homologous to those in the Wee1/Cdc25 system. The similarity in regulatory mechanisms suggests that bistability in Cdk activity may be an important general feature of the cell-cycle oscillator.

It may seem that stable states of Cdk activity would be a problem for the cell cycle, which requires oscillating levels of Cdk activity. However, the bistability in Cdk activity is coupled to the APC-mediated negative feedback in a way that could drive switching between the Cdk-active and Cdk-inactive stable states. In this model, the bistable feedback loop would ensure abrupt and irreversible switching between a mitotic and an interphase state. Slow cyclin accumulation in interphase would trigger mitosis, a switch to the Cdk-active state,

while the activation of the APC would drive cyclin degradation and switch back to the Cdk-inactive interphase state.

Pomerening *et al.* [7] began by employing mathematical models of the mitotic oscillator to test this prediction more quantitatively. They looked at two particular experimentally accessible variables in the Cdk system — the amount of cyclin and the level of Cdk activity. These variables are not perfectly correlated because some fraction of Cdk will be inactivated by phosphorylation. Pomerening *et al.* [7] studied oscillations as trajectories in a phase plane, a technique commonly used in mathematical models such as these. A possible state of the mitotic oscillator — the current levels of Cdk activity and of cyclin — is represented by a point in this phase plane. A cell cycle traces out a trajectory of cell states as cyclin levels and Cdk activity rise and then fall (Figure 2).

The simplest model of the cell cycle features only cyclin synthesis and APC-mediated cyclin degradation. An oscillation in this model involves increasing cyclin concentrations, with Cdk activity closely tracking cyclin levels. As Cdk activity rises, the APC is activated and triggers cyclin degradation. Because there is no other regulation of Cdk activity, the region of the trajectory where cyclin is accumulating is actually quite similar to the one where cyclin is being degraded. Furthermore, Cdk shows intermediate levels of activity during much of the cell cycle. For some plausible parameter values, the system cannot even sustain oscillations.

Positive feedback from Wee1 and Cdc25 substantially changes the trajectories of the oscillation in the model. Now, Wee1 suppresses Cdk activity, allowing cyclin accumulation with little increase in Cdk activity. At some point, this residual Cdk activity is high enough to activate Cdc25 and inactivate Wee1. This autocatalytic process results in rapid Cdk activation, which is soon followed by APC activation. Cyclin is then degraded but, because Cdk is not inhibited, Cdk activity remains

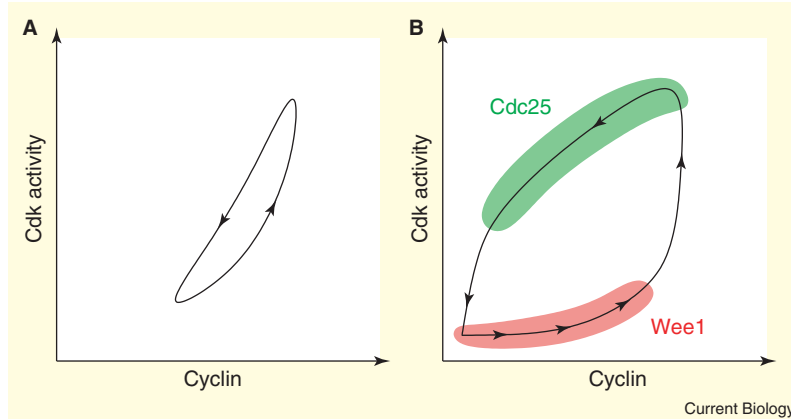


Figure 2. Trajectories of the cell-cycle oscillator.

The cell-cycle oscillations trace out loops in the phase plane of cyclin levels and Cdk activity. (A) Oscillations driven by Cdk/APC-mediated negative feedback alone. The time delay in formation of the cyclin–Cdk complex and activation of the APC is responsible for the slight asymmetry in the trajectory. (B) Oscillations in the presence of Wee1/Cdc25-mediated positive feedback. The portions of the trajectory characterized by active Cdc25 are shaded green and those by active Wee1 are shaded red. Note that the bottom of the trajectory, where cyclin is accumulating but Cdk activity is suppressed by Wee1, is slower than other parts of the trajectory.

high even as cyclin levels fall dramatically. This results in much greater cyclin clearance in mitosis, so the trajectory of Cdk activation and inactivation are quite distinct. Furthermore, the activation of Cdk is faster, with little time spent at an intermediate level of Cdk activity.

Pomerening *et al.* [7] are able to experimentally observe the oscillatory trajectory described above by measuring cyclin levels and Cdk activity in oocyte extracts with fine temporal resolution. They confirm that the mitotic oscillator follows a wide trajectory, with more than a 6-fold difference in cyclin levels between Cdk activation and inactivation. Furthermore, suppression of the positive feedback on Cdk activity results in a narrower and less robust trajectory of oscillation. This trajectory lingers in a phase of intermediate Cdk activity, compromising aspects of the cell cycle such as DNA synthesis. Without positive feedback, the oscillator is unable to produce distinct, alternating interphase and metaphase states, and in some cases entirely fails to oscillate.

One heartening aspect of this work is that the role of positive feedback can be understood through modelling even without quantitative information about the

biochemistry of the system. Furthermore, these models can produce specific, semi-quantitative predictions that are experimentally testable. This approach is particularly valuable in cases such as the cell-cycle oscillator, where feedback plays a major role. In these networks, dynamical systems theory can predict the range of possible behaviors and identify the key factors determining the behavior of a particular system. Modelling also emphasizes how functions in regulatory networks should be attributed to patterns of interactions rather than to specific proteins. Both Wee1 and Cdc25 mediate positive feedback in Cdk activity. However, these proteins are regulated in other ways to control cell-cycle progression rather than to provide positive feedback on Cdk activity [10,11]. This positive feedback loop in the network, rather than any specific protein's biochemical activity, provides the bistability that is important for the function of the cell-cycle oscillator.

#### References

1. Murray, A.W. (2004). Recycling the cell cycle: cyclins revisited. *Cell* 116, 221–234.
2. Goldbeter, A. (1991). A minimal cascade model for the mitotic oscillator involving cyclin and Cdc2 kinase. *Proc. Natl.*

- Acad. Sci. USA* 88, 9107–9111.
3. Coleman, T.R., and Dunphy, W.G. (1994). Cdc2 regulatory factors. *Curr. Opin. Cell Biol.* 6, 877–882.
4. Pomerening, J.R., Sontag, E.D., and Ferrell, J.E., Jr. (2003). Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nat. Cell Biol.* 5, 346–351.
5. Sha, W., Moore, J., Chen, K., Lassaletta, A.D., Yi, C., Tyson, J.J., and Sible, J.C. (2003). Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proc. Natl. Acad. Sci. USA* 100, 975–980.
6. Novak, B., and Tyson, J.J. (1993). Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos. *J. Cell Sci.* 106, 1153–1168.
7. Pomerening, J.R., Kim, S.Y., and Ferrell, J.E., Jr. (2005). Systems-level dissection of the cell-cycle oscillator: bypassing positive feedback produces damped oscillations. *Cell* 122, 565–578.
8. Ingolia, N.T., and Murray, A.W. (2004). The ups and downs of modeling the cell cycle. *Curr. Biol.* 21, R771–R777.
9. Cross, F.R., Archambault, V., Miller, M., and Klovstad, M. (2002). Testing a mathematical model of the yeast cell cycle. *Mol. Biol. Cell* 13, 52–70.
10. Kumagai, A., and Dunphy, W.G. (1996). Purification and molecular cloning of Plx1, a Cdc25-regulatory kinase from *Xenopus* egg extracts. *Science* 273, 1377–1380.
11. Michael, W.M., and Newport, J. (1998). Coupling of mitosis to the completion of S phase through Cdc34-mediated degradation of Wee1. *Science* 282, 1886–1889.

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## Quality Control: Another Player Joins the ERAD Cast

The quality control system known as ERAD removes misfolded proteins from the ER to the cytosol for degradation. The AAA ATPase Cdc48p and ubiquitin ligases play crucial roles; their relationship has been unclear, but recent work has shown that the membrane protein Ubx2p links their functions in yeast.

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Endoplasmic reticulum-associated degradation (ERAD) is a quality control system that recognises and disposes of misfolded or unassembled polypeptides in the ER. Disposal involves the retrotranslocation of aberrant proteins across the ER membrane to the cytosol, where

they are ubiquitinated prior to degradation by the proteasome. The delivery of ubiquitinated substrates to the proteasome depends on a member of the AAA ATPase family, namely Cdc48p in yeast, or its homologue p97 (also known as valosin-containing protein, VCP) in mammalian cells [1]. Cdc48p/p97 is involved in a number of other cellular processes, including cell-cycle

regulation, transcriptional regulation and homotypic membrane fusion [2]. The recruitment of Cdc48p/p97 into these diverse pathways requires specific adaptor proteins; for example, Cdc48p/p97 complexed with the adaptors Npl4p and Ufd1p functions in proteolysis, whereas the adaptor p47 recruits Cdc48p/p97 into a membrane fusion role.

Npl4p and Ufd1p engage Cdc48p in proteolysis by virtue of their ability to bind specifically to polyubiquitin chains on proteins tagged for proteasomal degradation [2]. These polyubiquitin chains are initially built up on suitable lysyl residues in target proteins by the action of E3 ubiquitin ligases. Two E3 ubiquitin ligases have been implicated in ERAD in yeast,