# Cell Cycle Vignettes

## Bistability and Oscillations

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A molecular regulatory system can maintain itself indefinitely at a steady state where, for every time-varying species, the total rate of formation of the chemical is exactly balanced by its total rate of removal. In mathematical terms, we can write:

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = f_i\left(x_1, x_2, ..., x_n\right) - r_i\left(x_1, x_2, ..., x_n\right), \quad i = 1, ..., n \qquad [1]$$

where $x_i$ is the concentration of species $i$, and $f_i(x_1,x_2,\ldots,x_n)$ and $r_i(x_1,x_2,\ldots,x_n)$ are its rates of formation and removal. At a steady state, $\mathrm{d}x_i/\mathrm{d}t = 0$ for all $i$,

$$f_i\left(x_1, x_2, ..., x_n\right) = r_i\left(x_1, x_2, ..., x_n\right), \quad i = 1, ..., n\,. \qquad [2]$$

For any well-specified chemical reaction network, there always exists at least one steady state solution; call it $\mathbf{x}^0 = \left(x_1^0, x_2^0, ..., x_n^0\right)$. Such a steady state can be classified as either *stable* or *unstable* with respect to small perturbations away from the steady state. If all small perturbations (in any direction in the $n$-dimensional state space of the reaction network) eventually return to $\mathbf{x}^0$, then this steady state is said to be (locally asymptotically) stable. If there exist small perturbations (in some particular directions in state space) that depart from $\mathbf{x}^0$ and never return, then the steady state is said to be unstable.

Since the rates of formation and removal of any chemical species in a reaction network are algebraic functions of species concentrations, Eq. [2] is a system of $n$ nonlinear algebraic equations, which in general may have multiple solutions in the positive orthant: $x_i \geq 0$ for all $i$. In this case, we can denote the different steady states as $\mathbf{x}^0{}_j$, where $j = 1,\ldots,m$. A typical situation is the case of three steady states ($m = 3$), where two of the steady states are stable and one is unstable. This case is called *bistability*. In general, there may be more than two stable steady states, in which case we refer to

*tristability* or *multi-stability*. We may describe a system with $m > 1$ as having *multiple steady states* when we are not sure of the total number of stable steady states.

Bistability is a typical feature of reaction networks with positive feedback, although one must be aware that positive feedback is not always readily apparent in reaction networks as they are conventionally drawn. Double-negative feedback is a particularly common motif for bistability: $X_1$ inhibits $X_2$, and $X_2$ inhibits $X_1$. In this case, the two stable steady states are ($X_1$ active, $X_2$ inactive) and ($X_1$ inactive, $X_2$ active). The intermediate steady state ($X_1$ semi-active, $X_2$ semi-active) is unstable.

Multi-stability plays an important role in the theory of cell cycle progression. It is proposed that the characteristic states of cell cycle arrest (G1-arrest, G2-arrest, metaphase-arrest) correspond to alternative stable steady states of the underlying chemical reaction network controlling the activities of cyclin-dependent kinases (CDKs). In this view, cells progress through the cell cycle (G1 → S/G2 → M → G1 → …) by irreversible transitions from the G1 steady state to the S/G2 steady state (the G1-S transition, also called 'Start' or the 'Restriction Point'), from the S/G2 steady state to the M steady state (the G2-M transition), and from the M steady state to the G1 steady state (the metaphase-anaphase transition, also called 'exit from mitosis'). In this view, cell cycle checkpoints work by stabilizing one of these three steady states and preventing the transition to the next phase of the cell cycle.

Bistability is intimately connected to the existence of spontaneous limit cycle oscillations in regulatory networks with both positive feedback (to create alternative stable steady states) and negative feedback (to induce spontaneous switching between the two stable steady states). This combination of positive and negative feedbacks is precisely the case in the regulatory system governing the eukaryotic cell cycle (Tyson and Novak, 2008). In somatic cells, checkpoint signals prevent spontaneous cycling, but in some circumstances these checkpoints are removed, and the cell cycle proceeds as a spontaneous, unfettered, limit cycle oscillation. For example, during early embryogenesis, the fertilized egg undergoes a series of rapid cell divisions without growth, until it reaches the 'mid-blastula transition', when checkpoint proteins are expressed and the cell cycle regains the characteristic G1-S, G2-M and M-G1 transitions of somatic cells. It is also possible to create mutant yeast cells that lack checkpoint controls; these cells divide faster than they grow, getting smaller and smaller each cycle until they die. These observations suggest that, under most circumstances, periodic cell divisions are governed not by spontaneous limit cycle oscillations but by multi-stability and irreversible transitions (Tyson and Novak, 2008).

**History**

In an influential review of cell cycle regulation, Murray and Kirschner (1989) asked whether progression through the cell cycle is more like 'dominoes' (a dependent sequence of events: one falling domino pushing over the next) or a 'clock' (an autonomous oscillation, ticking along independent of the events being timed). There is good experimental evidence for both views. Early genetic analysis of the budding yeast cell cycle provided evidence for two parallel dependent sequences (the budding

sequence and the DNA replication sequence) that diverged in G1 phase (at the Start transition) and reconnected at the end of the cycle (exit from mitosis). On the other hand, biochemical studies of frog egg extracts suggested an autonomous oscillation of mitosis-promoting factor (MPF) that drives periodic DNA replication and mitosis, but generates periodic bursts of MPF activity quite independently of chromosomes and nuclei. In the first view, cell cycle progression is orchestrated by a gene regulatory network flipping genes on and off in a strict sequence intimately connected to cell growth. In the second view, cell cycle progression is governed by a protein interaction network that drives MPF activity up and down in waves of synthesis and degradation of cyclin proteins.

**Systems biology**

These two views were brought together by Novak and Tyson (1993) in an early example of 'molecular systems biology'. They used the wiring diagram in Fig. 1 to derive a mathematical model of spontaneous MPF oscillations in frog egg extracts and (in later papers) of cell cycle mutants in fission yeast. According to their theory, the regulation of CDK-cyclin activity by tyrosine phosphorylation and dephosphorylation of the CDK subunit, by Wee1 (the kinase) and Cdc25 (the phosphatase), creates a bistable switch that governs the transition from G2 phase into mitosis (a state of high CDK activity); see Fig. 2A. This switch has all the properties of a classic cell cycle checkpoint. To pass the checkpoint, a cell must fully replicate its DNA and grow to a sufficient size. Once past the checkpoint the transition is irreversible; the cell does not slip back into G2 phase and try to enter mitosis a second time. Rather, the cell must complete the stages of mitosis and activate cyclin degradation at the metaphase-anaphase transition. Cyclin degradation allows the bistable switch to be reset to the state of low CDK activity. Repeated cycles of DNA synthesis and mitosis correspond to repeated flipping of the switch from a stable steady state of low CDK activity (interphase) to a stable steady state of high CDK activity (M phase) and back again. (Physicists and engineers call this behavior a 'hysteresis loop'.)

Novak and Tyson showed, furthermore, that under the special conditions in an early frog embryo or in a frog egg extract, the CDK-cyclin control system can generate spontaneous oscillations of MPF activity, unconstrained by requirements of cell growth, DNA synthesis, DNA damage, or mitotic spindle functions. The spontaneous oscillations are driven by periodic bursts of cyclin degradation, which are in turn generated by the periodic activation of MPF by dephosphorylation of the CDK subunit. These spontaneous oscillations result from an interplay between the bistable switch and a negative feedback loop (MPF activates the cyclin degradation machinery which destroys cyclin subunits, causing a loss of MPF activity); see Fig. 2B. (Physicists and engineers call this behavior a 'relaxation oscillator'.)

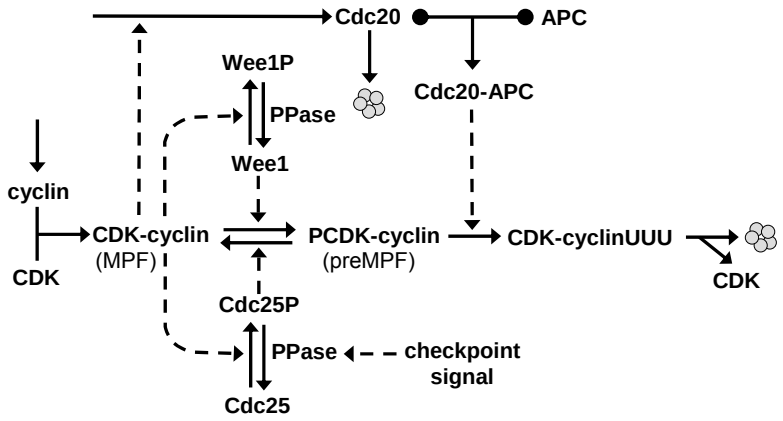


**Figure 1. CDK regulatory network for frog egg cell cycles.** In this network diagram, a solid arrow represents a biochemical reaction, and a dashed arrow represents a catalytic effect on a reaction. A T-shaped arrow represents the association of two proteins to form a complex. Black balls on the crossbar indicate a reversible binding reaction. The small gray balls represent proteolytic fragments of a protein. Newly synthesized cyclin B combines with CDK subunits (in excess) to form active CDK-cyclin dimers (called MPF). The CDK subunit is phosphorylated by Wee1 to form a less active form of MPF, called preMPF. The inhibitory phosphate group can be removed by Cdc25. Wee1 and Cdc25 activities are also regulated by phosphorylation, catalyzed by MPF: Wee1P is the less active form, and Cdc25P is the more active form. Cyclin B is labeled for proteolysis by an E3 ubiquitin ligase, the APC, which works in collaboration with a targeting subunit, Cdc20. The synthesis of Cdc20 is activated by MPF.

The mathematical model of Novak and Tyson not only gave a systematic account of a variety of experimental observations on fission yeast, budding yeast and frog eggs, but also made a series of striking predictions:

1. The 'cyclin threshold for MPF activation' that had been observed by Solomon et al. (1990) is only one-half of a hysteresis loop. There should be a separate, distinctly lower cyclin threshold for MPF inactivation (Fig. 2A).
2. For cyclin levels well above threshold, the time lag for MPF activation should be roughly constant (as observed by Solomon et al. (1990)), but as cyclin level approaches the activation threshold from above, the time lag for MPF activation should increase dramatically.
3. The cyclin level for MPF activation should be an increasing function of unreplicated DNA in an extract.
4. If the bistable switch is disabled by interfering with the inhibitory phosphorylation of CDK, then MPF oscillations may still be possible, but they will be faster, more smooth (not abrupt bursts of MPF activity) and probably damped (Fig. 2C).

Predictions 1 and 2 are generic properties of bistable systems with hysteresis. Prediction 3 was a novel proposal for the mode of action of a checkpoint signal that delays entry into mitosis. Prediction 4 relates to classic properties of oscillations driven by a 'simple negative feedback loop' in comparison to relaxation oscillations in a 'substrate-depletion relaxation oscillator'.

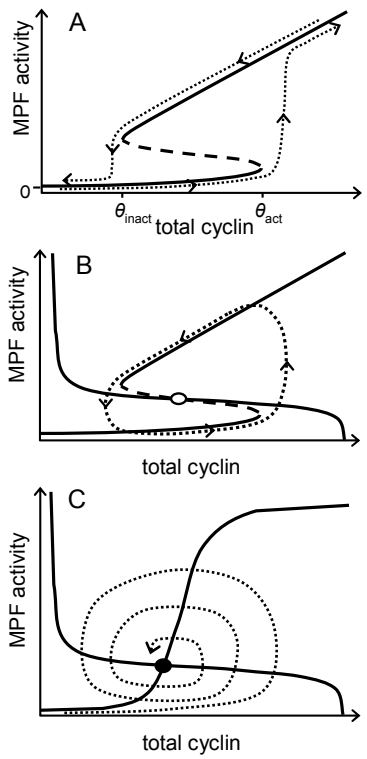


**Figure 2. Signal-response curves: MPF activity as a function of total cyclin.** (A) Bistable switch. If cyclin synthesis and degradation are blocked, then the total cyclin concentration ( [MPF] + [preMPF] ) in a frog egg extract can be manipulated experimentally. For [total cyclin] between the two thresholds, $\theta_{inact}$ and $\theta_{act}$, the control system has two stable steady states separated by an unstable steady state. The dotted line shows how MPF activity will respond to slowly increasing [total cyclin] and then slowly decreasing [total cyclin]. (B) Relaxation oscillations. If cyclin is steadily synthesized, then [total cyclin] will increase when MPF is in the low-activity state, because Cdc20 is unavailable, but once the system flips to the state of high MPF activity, then Cdc20 is produced and cyclin is degraded faster than it is synthesized. The bistable switch flips back to the low-activity state once [total cyclin] drops below the inactivation threshold. The white circle represents an unstable steady state. (C) Damped sinusoidal oscillations. If the CDK phosphorylation site (a tyrosine residue) is mutated to phenylalanine, then the positive feedback loops involving Wee1 and Cdc25 are disengaged and bistability is lost. The system now undergoes damped oscillations to a stable steady state (black circle). If there is enough time delay in the negative feedback loop through Cdc20-APC, then the control system might exhibit sustained oscillations, but they are quite distinct from the relaxation oscillations in panel B.

## Experimental verification

The first three predictions of the Novak-Tyson model were confirmed in frog egg extracts independently and simultaneously by Sha et al. (2003) and Pomerening et al. (2003); see Fig. 3. Slightly later, Pomerening et al. (2005) confirmed prediction 4 in a thorough and careful study of MPF oscillations in frog egg extracts; see Fig. 4.

In 1996, Kim Nasymth (1996) proposed that, at its heart, the budding yeast cell cycle is a repetitive alternation between two 'self-maintaining' states (i.e., stable steady states): a G1 state with low CDK activity, and an S-G2-M state with high CDK activity. This notion became the basis of a successful kinetic model of the budding yeast cell cycle by Chen et al. (2000). The model made many predictions that were tested in Fred Cross's laboratory. In particular, Cross et al. (2002) tested the idea that, under 'neutral' conditions, budding yeast cells could persist indefinitely in either the G1 state, with low CDK activity, or the S-G2-M state, with high CDK activity, depending on the immediately prior history of how the cells are brought into the neutral conditions. This experiment is explained in the vignette on "The Cell Cycle of Budding Yeast".

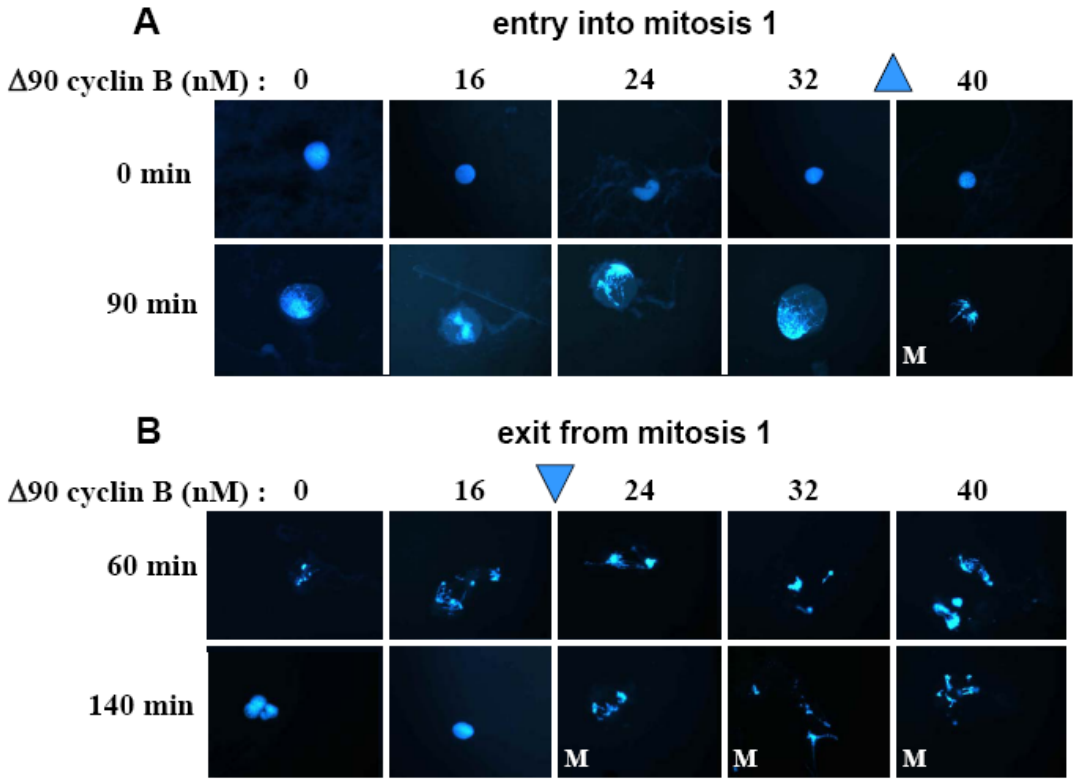


**Figure 3. Experimental confirmation of bistability in frog egg extracts.** From Sha et al. (2003), used by permission. (A) A frog egg extract, containing all the proteins in Fig. 1 except for cyclin, is prepared in the presence of sperm nuclei (indicators of MPF activity) and cycloheximide (a drug that prevents the synthesis of cyclin protein from endogenous mRNA). In the absence of cyclin subunits, the extract is blocked in interphase (t = 0), as indicated by the compact nucleus with intact nuclear membrane and dispersed chromatin (stained blue). At t = 0 the extract is supplemented with a measured amount of non-degradable (Δ90) cyclin, and 90 min later the extract is observed to see if the nuclei are in interphase (dispersed chromatin, intact membrane, low MPF activity) on in mitosis (condensed chromatin, breakdown of nuclear membrane, high MPF activity. Cyclin concentrations less than ~35 nM are insufficient to induce entry into mitosis, but [total cyclin] = 40 nM is above the threshold (blue up-triangle) for mitotic entry. (B) In a second experiment, the extract is supplemented with variable amounts of non-degradable cyclin at t = 0, but cycloheximide is not added until t = 60 min. By t = 60 min, the nuclei in each sample have been driven into mitosis 1 by a combination of the non-degradable cyclin added at t = 0 and the degradable cyclin subunits synthesized from the extract's endogenous mRNA. As the extracts try to exit from mitosis 1, the endogenous cyclin subunits are degraded by Cdc20-APC, but the exogenous Δ90 cyclin subunits resist degradation. Cycloheximide prevents any further cyclin synthesis in the extracts. At t = 140 min the extracts are assayed for the cell-cycle phase of the nuclei. Cyclin concentrations greater than ~20 nM are sufficient to maintain the nuclei in a mitotic state. [Total cyclin] = 16 nM is below the threshold (blue down-triangle) for inactivation of MPF and exit from mitosis. Cyclin concentrations of 24 and 32 nM are clearly in the bistable region: the nuclei can persist stably in interphase or in mitosis, depending on whether they are prepared initially in interphase or mitosis.

## Irreversibility

The concept of bistability provides an immediately obvious and intuitively satisfying explanation of the irreversibility of progression through the cell cycle. If cell cycle transitions are a result of passing from one stable steady state to another, then it is clear that the reverse transition cannot follow the same path. Once the transition is

made, then a completely different set of circumstances must be brought into play to accomplish the reverse transition. For example, in Fig. 2A the activation of MPF is accomplished by increasing total cyclin concentration above the threshold, $\theta_{act}$, where the unstable steady state merges with and annihilates the steady state of low MPF activity and forces the system to switch to the steady state of high MPF activity. If subsequently the cyclin level is caused to decrease, the control system does not jump back to the lower steady state at $\theta_{act}$, which would be the case for a 'reversible' transition. Rather, the cyclin level must decrease below a much smaller threshold, $\theta_{inact}$, before the down-jump occurs.

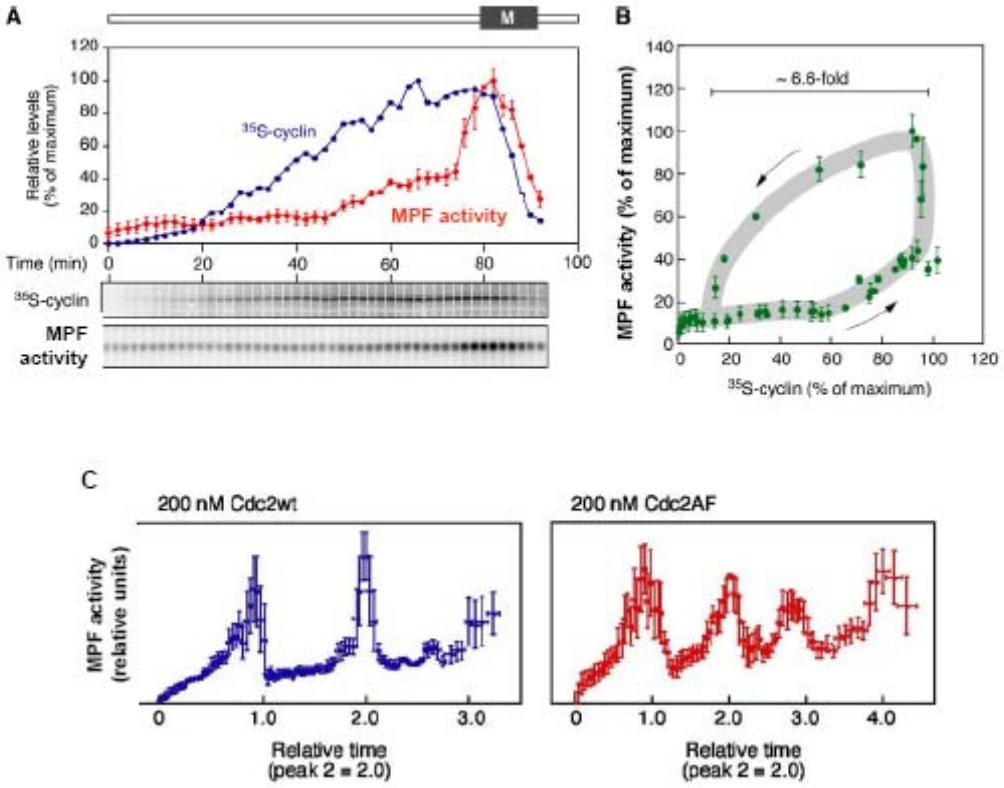


**Figure 4. Experimental confirmation of relaxation oscillations in frog egg extracts.** From Pomerening et al. (2005), used by permission. (A) In a 'cycling' extract, cyclin subunits are continuously synthesized (blue curve), but MPF activity (red curve) is low until total cyclin exceeds the threshold for MPF activation. The abrupt activation of MPF drives nuclei into mitosis (M). As the extract exits from mitosis, cyclin is degraded by Cdc20-APC, and MPF activity falls as a result. (B) The data in panel A is projected onto state space (MPF activity versus total cyclin level), as in panel A of Fig. 2B. (C) Confirmation of prediction 4. To a cycling extract, containing ~200 nM endogenous Cdc25wt (wild-type) protein, is added 200nM of either Cdc25wt protein or Cdc2AF protein, which cannot be phosphorylated and inhibited by Wee1. Hence, in the extract on the right there are roughly equal amounts of Cdc2wt and Cdc2AF subunits, compared to the 'control' extract on the left which contains ~400 nM Cdc2wt subunits. The mutant kinase subunits compromise the positive feedback loops in the model (Fig. 1) and change the properties of MPF oscillations. Compared to the blue curve, the MPF oscillations in the red curve are faster, more sinusoidal and noticeably damped, exactly as predicted by the mathematical model.

In the budding yeast case, the switch from the low-CDK state to the high-CDK state is driven by Cln-dependent kinase activity, but the switch back is driven by a completely different mechanism, dependent on the activities of Cdc20-APC (degradation of B-type cyclins) and Cdc14 (a CDK-counteracting phosphatase). In this case, the up-jump is a one-way switch: it is induced by Cln-dependent kinase, but after the switch is made, the Cln-kinase activity can drop to 0 and the CDK activity will remain high. To down-jump occurs by means of a different one-way switch. In the 'neutral' condition (Cln = Cdc20 = Cdc14 = 0), the control system can persist indefinitely in either the low-CDK or the high-CDK state. For more details, see the vignettes on "The Cell Cycle of Budding Yeast" and "Irreversible Transitions in the Cell Cycle".

**Checkpoints**

The concept of bistability also provides a natural framework for understanding the mechanisms of checkpoint controls. The function of a checkpoint is to block or delay progression of a damaged cell into the next phase of the cell cycle. For example, DNA damage should block cells from entering S phase, incomplete DNA ligation should block cells from entering M phase, misaligned chromosomes should block cells from exiting mitosis. If these transitions are governed by saddle-node bifurcations of a bistable system (as in Fig. 2A), then the transition can be delayed or blocked completely by raising the threshold for the transition ($\theta_{act}$). From the mathematical model of the transition it is immediately obvious which components control the location of the threshold. For example, in Fig. 1 it is the activity of the CDK-counteracting phosphatase (PPase) that is the vulnerable point.

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