

System-level feedbacks make the anaphase switch irreversible

Enuo He^a, Orsolya Kapuy^a, Raquel A. Oliveira^b, Frank Uhlmann^c, John J. Tyson^d, and Béla Novák^{a,1}

^aOxford Centre for Integrative Systems Biology, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom; ^bDepartment of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom; ^cChromosome Segregation Laboratory, Cancer Research UK London Research Institute, London WC2A 3PX, United Kingdom; and ^dDepartment of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

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The mitotic checkpoint prevents a eukaryotic cell from commencing to separate its replicated genome into two daughter cells (anaphase) until all of its chromosomes are properly aligned on the metaphase plate, with the two copies of each chromosome attached to opposite poles of the mitotic spindle. The mitotic checkpoint is exquisitely sensitive in that a single unaligned chromosome, 1 of a total of ~50, is sufficient to delay progression into anaphase; however, when the last chromosome comes into alignment on the metaphase plate, the mitotic checkpoint is quickly satisfied, and the replicated chromosomes are rapidly partitioned to opposite poles of the dividing cell. The mitotic checkpoint is also curious in the sense that, before metaphase alignment, chromosomes that are not being pulled in opposite directions by the mitotic spindle activate the checkpoint, but during anaphase, these same tensionless chromosomes can no longer activate the checkpoint. These and other puzzles associated with the mitotic checkpoint are addressed by a proposed molecular mechanism, which involves two positive feedback loops that create a bistable response of the checkpoint to chromosomal tension.

bistability | cell cycle | irreversible transition | mitotic checkpoint | spindle assembly checkpoint

The cell cycle is an ordered sequence of events by which cells replicate their chromosomes (S phase) and partition the identical sister chromatids to opposite poles of the mitotic spindle (M phase). In growing cells, temporal gaps separate S phase from M phase (G1-S-G2-M-G1- etc.). Progression through the cell cycle is characterized by irreversible transitions at the boundaries of these four phases: G1/S, G2/M, and M/G1. The M/G1 transition takes place in two steps: metaphase/anaphase (M/A; partitioning of sister chromatids) and telophase/G1 (T/G1; mitotic exit and return to G1 and cytokinesis). Specific, transient biochemical signals trigger these transitions, which are irreversible in the sense that, after the triggering signal disappears, the cell does not revert to the previous cell-cycle phase but is continually ratcheted forward through the G1-S-G2-M sequence.

The three major irreversible transitions are guarded by checkpoint mechanisms that delay or block the transitions until conditions are favorable to progress to the next phase of the cell cycle (1). At the restriction point, cells check that they have the proper growth factor signals and that their DNA is undamaged before they leave G1 and enter S phase. At the G2/M checkpoint, they check that DNA replication is completed before entering mitosis. Cells may pass the mitotic checkpoint only if the mitotic spindle is fully assembled and all chromosomes are properly aligned on the metaphase plate with sister chromatids attached to opposite poles of the spindle.

We have argued that the irreversibility of these transitions is based on system-level feedbacks in the molecular regulatory mechanisms of the checkpoints (2, 3). In particular, positive (or double-negative) feedback circuits in these regulatory networks create one-way toggle switches with two alternative stable steady states: the pre- and posttransition states. The checkpoints hold cells in the pretransition steady state until a triggering signal is generated to induce a switch to the posttransition steady state.

The switch is irreversible, because the cell is locked in the post-transition state even after the inducing signal disappears. This systems view of irreversibility is based on computational modeling (4–6) and is supported by experimental data (7–10) for some of these transitions (G1/S, G2/M, and T/G1).

The separation of sister chromatids at anaphase is an apparent exception to our feedback view of irreversible cell-cycle transitions. In this case, it seems that the M/A transition is irreversible for thermodynamic reasons rather than network dynamic considerations. Sister chromatids are held together at metaphase by cohesin rings that oppose the pulling forces of the mitotic spindle on the bioriented chromosomes (11). At the M/A transition, a protease (called separase) is activated, which cleaves the cohesin rings, thereby allowing the sister chromatids to be pulled apart by spindle forces (12). Cohesin cleavage by proteolysis is a thermodynamically spontaneous reaction, and the metaphase alignment of chromosomes cannot be recreated simply by resynthesizing cohesin proteins. Thermodynamically spontaneous forces have pulled the sister chromatids apart, and they will not come back together again spontaneously, even if the inducing signal is removed and the cohesin rings are resealed. We consider this to be the first puzzle about the M/A transition.

(Puzzle 1) Is the M/A transition irreversible for thermodynamic reasons, unlike the other three cell-cycle transitions, which are irreversible because of regulatory feedback controls?

The M/A transition is puzzling in other respects as well. Its guard, the mitotic checkpoint, is activated in prometaphase by kinetochores that are not under tension, because the cohesin-bound chromatids have not yet achieved biorientation on the mitotic spindle (13, 14). In the pretransition state, chromosomes that are not under tension send a strong signal to the mitotic checkpoint to block cell-cycle progression. As soon as all of the chromosomes are properly aligned on the spindle, the mitotic checkpoint is lifted, separase is activated, cohesin rings are cleaved, and sister chromatids are pulled apart.

(Puzzle 2) Why is it that, before the M/A transition, zero tension prevents progression into anaphase, but after the transition, when tension is decreasing because the cohesin rings are broken, the control network is locked in a posttransition state and does not revert to the pretransition state (15, 16)?

Puzzle 2 has been bolstered by recent experimental manipulations (17–19) that make the M/A transition reversible by interfering with the tension-sensing mechanism.

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¹To whom correspondence should be addressed. E-mail: bela.novak@bioch.ox.ac.uk.

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A third feature of the mitotic checkpoint has been acknowledged as a puzzle for many years.

(Puzzle 3) How is it that a single unattached kinetochore is enough to block the M/A transition?

For a human cell, having 45 of 46 chromosomes properly aligned on the metaphase plate is not enough to satisfy the mitotic checkpoint (20). All 46 chromosomes must be bioriented and under tension. The mitotic checkpoint can reliably distinguish between the numbers $45/46 = 0.98$ and $46/46 = 1.00$. Biochemical control systems are not usually associated with such precision. Of course, one might turn these numbers around and claim that the mitotic checkpoint is telling the difference between $(46 - 45)/46 = 0.02$ and $(46 - 46)/46 = 0$ and that there is a big difference between nonzero and zero. However, this argument comes with its own puzzle. From this perspective, during prometaphase, the signal from the mitotic checkpoint drops from 1.00 to 0.02, but even the weakest signal is able to hold the checkpoint in the state of no additional progression. Hence, the kinetic processes that are trying to disengage the mitotic checkpoint must be very weak themselves. In that case, another puzzle is apparent.

(Puzzle 4) How is it that even a weak signal can keep the mitotic checkpoint engaged, but when the signal drops to zero, the checkpoint is rapidly disengaged?

In this paper, we address all four puzzles based on a proposal that the mitotic checkpoint network is indeed a one-way toggle switch. Our model requires, in addition, a positive feedback signal that accelerates disassembly of the mitotic checkpoint complex during anaphase. We speculate on the molecular basis of this signal and challenge experimentalists to confirm or refute our prediction.

Results

Mechanistic Details of the Mitotic Checkpoint. Cells enter M phase with replicated chromosomes, each one containing a pair of sister chromatids held together by cohesin proteins (11). To ensure precise partitioning of the sister chromatids at anaphase, every chromosome must biorient on the mitotic spindle, with sister chromatids attached to opposite poles (21). Proper segregation of sister chromatids is ensured by a surveillance mechanism, an error-correction mechanism, and a checkpoint mechanism. The surveillance mechanism senses the tension generated at kinetochores by the spindle-pulling force working against the resistance exerted by the cohesin rings (22, 23). In the absence of tension, the surveillance mechanism notifies the error-correction mechanism to destabilize the attachment of spindle microtubules to a kinetochore if that attachment is not under tension. Aurora-B kinase, a component of the chromosomal passenger complex, plays a central role in this error-correction process (24).

Because error correction requires some time, the surveillance mechanism also signals the mitotic checkpoint to stop further progression through the cell cycle (i.e., cohesin cleavage at anaphase). Whether the checkpoint is activated directly by the surveillance mechanism (lack of tension) or indirectly by the error-correction mechanism (unattached kinetochores) is still an open question (25). In either case, the mitotic checkpoint keeps cohesin rings intact by blocking the activation of separase. Separase is kept inactive by a stoichiometric inhibitor, securin (12). At anaphase, the proteolysis of securin is initiated by an E3 ubiquitin ligase, the anaphase-promoting complex/cyclosome (APC/C), combined with a targeting subunit, Cdc20 (26, 27). The mitotic checkpoint blocks the onset of anaphase by activating Mad2, which binds to and inactivates Cdc20. The Mad2:Cdc20 complex, along with some auxiliary proteins, is called the mitotic checkpoint complex (MCC). After all chromosomes are bioriented and under tension, Mad2 is removed from Cdc20, active APC^{Cdc20} degrades securin,

active separase cleaves cohesins, and the mitotic spindle pulls sister chromatids to the opposing poles.

Because APC^{Cdc20} activation at anaphase results in cohesin cleavage and loss of tension at kinetochores, the error-correction mechanism and the mitotic checkpoint are in danger to be reactivated during anaphase (15, 16). However, microtubule-kinetochore attachments are stable, and APC^{Cdc20} stays active during normal anaphase, suggesting the existence of an inactivating mechanism that suppresses reactivation of the mitotic checkpoint during anaphase.

A crucial change taking place at the M/A transition is a drop in activity of cyclin B-dependent kinase (CDK^{CycB}) and an increase in activity of its counteracting protein phosphatase (CAPP). Four papers (17–19, 28) conclude that this abrupt drop in CDK^{CycB}/CAPP ratio during anaphase blocks reactivation of the error-correction mechanism and the mitotic checkpoint. Cohesins engineered with cleavage sites for tobacco etch virus (TEV) protease were expressed in *Drosophila* embryos (18) and budding yeast cells (17) arrested in metaphase by APC^{Cdc20} inactivation. By inducing TEV protease in these metaphase-arrested cells, cohesins are cleaved, and a pseudoanaphase is initiated. At first, sister chromatids move to opposite poles, but later, they start to oscillate between the two poles (18). TEV-induced cohesin cleavage is accompanied by reaccumulation of checkpoint proteins to kinetochores in both flies and yeast (17, 18).

Why is the checkpoint mechanism reactivated under these conditions? Because APC^{Cdc20} is inactive in these experiments, neither securin nor cyclin B is degraded; hence, separase activity stays low, and CDK^{CycB} activity stays high. Because separase activity stays low, the early anaphase activation of Cdc14, a CDK-counteracting phosphatase in budding yeast, is blocked. Because TEV-induced pseudoanaphase happens at high activity of CDK^{CycB} and low activity of Cdc14, CDK-dependent phosphorylation is a likely suspect for reactivation of the checkpoint machinery. An earlier paper came to the same conclusion for *Drosophila* embryos, because expression of a nondegradable cyclin B caused reactivation of the checkpoint mechanism during anaphase (28). The fact that similar effects are observed in yeast cells, fly embryos, and mammalian cells (19) suggests that this silencing mechanism may be universal among eukaryotes.

Furthermore, reduction of CDK-dependent phosphorylation during TEV-induced anaphase by a CDK inhibitor (p27) in fly embryos (18) or Cdc14 phosphatase in yeast cells (17) blocked reactivation of the checkpoint mechanisms during sister chromatid separation. These experiments confirmed the role of CDK^{CycB} in blocking the translocation of the chromosomal passenger complex from kinetochores to the spindle midzone during TEV-induced anaphase (17). Persistence of the chromosomal passenger complex at kinetochores during TEV-induced anaphase could account for reactivation of the checkpoint (19).

Experiments with mammalian cells support these conclusions as well. In checkpoint-arrested metaphase cells, Cdk1 inhibition by flavopiridol causes delocalization of the chromosomal passenger complex from kinetochores and degradation of cyclin B (29). This cyclin B degradation is mediated by APC^{Cdc20} (30), suggesting that Cdk1 activity is required for maintenance of the mitotic checkpoint.

Model for the Anaphase Switch. The fact that the mitotic checkpoint does not reactivate during normal anaphase suggests that this checkpoint is indeed regulated by a one-way toggle switch, like the other three cell-cycle transitions. In the absence of tension at kinetochores, the mitotic checkpoint must have two alternative states: an active state in prometaphase when the error-correction and checkpoint mechanisms are turned on and an inactive state in anaphase when these mechanisms are turned off. An essential role of this bistable switch is to suppress the dangerous negative feedback loop, which could reactivate the checkpoint during anaphase.

planation for this feedback. An alternative mechanism for accelerated release of Cdc20 from the MCC during anaphase is suggested by the role of Cdc20 phosphorylation in MCC formation (36), which is explained in *Discussion*.

Dynamics of the Anaphase Switch. To illustrate the properties of the bistable anaphase switch, we plot one-parameter bifurcation diagrams (i.e., signal response curves) in Fig. 2. A one-parameter bifurcation diagram shows the steady-state level of a specific dynamical variable (e.g., X_{0A} , MCC, or CycB) as a function of the fraction of chromosomes under tension (X_{tens}). The signal (the bifurcation parameter) increases from zero to one as chromosomes align on the mitotic spindle during prometaphase, and the response (the dynamical variable) shows the state of the control system. The three plots in Fig. 2 look different, but they have the same qualitative features, namely two branches of stable steady states (mitotic checkpoint active and inactive; solid lines) separated by an intermediate branch of unstable steady states (dashed lines).

Mitotic progression starts with prometaphase cells on the upper branch of the curve, with X_{tens} close to zero. As individual chromosomes become bioriented, X_{tens} increases in small steps, and the state of the control system moves slowly to the right on the bifurcation diagram. This causes a decrease in the fraction of active, tensionless chromosomes (X_{0A}) according to Eq. 1, which is derived from the steady-state solution of Eq. S2 (*SI Text*):

$$X_{0A} = \frac{\theta}{\theta + ([\text{CAPP}]/[\text{CycB}])} (1 - X_{\text{tens}}), \quad [1]$$

where $\theta = k_{\text{an,cyc}}/k_{\text{in,capp}}$. In prometaphase, when the phosphatase to kinase ratio is low ($[\text{CAPP}]/[\text{CycB}] \ll \theta$), $X_{0A} \sim 1 - X_{\text{tens}}$. Although X_{0A} is decreasing, the checkpoint is kept active, Cdc20 is inactive, and cyclin B stays high. The system cannot overcome the checkpoint until the fraction of chromosomes under tension (X_{tens}) exceeds the bifurcation point, which is very close to one. When X_{tens} gets larger than the bifurcation point, the mitotic checkpoint is bypassed, Cdc20 is activated, and cyclin B is degraded. Although tension is lost on the kinetochores, the control system is now on the lower branch of stable steady states on the bifurcation diagram, with X_{0A} being small and X_{0I} being large.

The jump to the lower branch of stable steady states corresponds to dissociation of the MCC and inactivation of the mitotic checkpoint at metaphase. Because cyclin B and securin levels decrease simultaneously at the M/A transition, separase gets activated and starts to cleave cohesins. Loss of sister chromatid cohesion causes a decrease of X_{tens} but only after some time of $\text{APC}^{\text{Cdc20}}$ activation, because securin degradation and cohesin cleavage require some time. Therefore, first, the mitotic checkpoint is inactivated, and then the control system starts traveling to the left along the lower branch of stable steady states. By the time that all of the sister chromatids are segregated, X_{tens} is again close to zero, which it was at the beginning of mitosis. Along the lower branch of stable steady states, $[\text{CAPP}]/[\text{CycB}] \gg \theta$, and therefore, X_{0A} remains close to zero even as X_{tens} drops to zero. Therefore, Mad2 is not activated, and the checkpoint stays inactive.

In summary, mutual antagonism between the CDK^{CycB} and $\text{APC}^{\text{Cdc20}}$ creates a bistable switch that activates the mitotic checkpoint in prometaphase and blocks reactivation of the checkpoint in anaphase. Mutual antagonism in the model refers to the fact that active $\text{APC}^{\text{Cdc20}}$ promotes degradation of CycB as well as our assumption that CDK^{CycB} promotes activation of tensionless kinetochores, which are known to activate Mad2, an inhibitor of $\text{APC}^{\text{Cdc20}}$.

Point of No Return. The transition between two stable steady states of a bistable system is a cooperative (autocatalytic) process with a point of no return. If the transition is disturbed before the point of no return, the bistable switch will return to the initial state; if the transition is disturbed after the point of no return, it

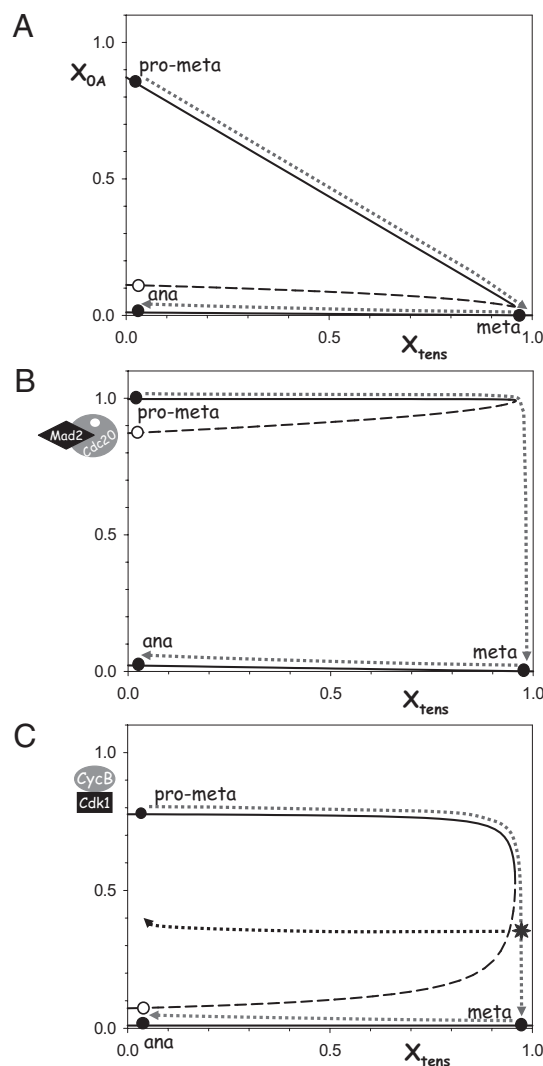


Fig. 2. Signal-response curve for the irreversible anaphase switch. (A–C) Three different dynamical variables—the fraction of active tensionless chromosomes (X_{0A}), Mad2:Cdc20 complex (MCC), and cyclin B-dependent kinase activity (CDK^{CycB})—are plotted as functions of the fraction of chromosomes under tension (X_{tens}). For $X_{\text{tens}} \sim 0$, the network is bistable with two alternative stable steady states (black circles)—active (MCC ~ 1) and inactive (MCC ~ 0)—separated by an unstable steady state (white circle). The solid lines show how the stable steady states depend on the value of X_{tens} , and the intermediate dashed line traces the unstable steady states that stand between the two loci of stable steady states. The gray dotted lines indicate progression from prometaphase to metaphase to anaphase. During prometaphase, the chromosomes are coming into alignment on the mitotic spindle (i.e., X_{tens} is steadily increasing to one). When all of the chromosomes are bioriented ($X_{\text{tens}} \sim 1$), the mitotic checkpoint switches from the active to the inactive state. During anaphase, X_{tens} is decreasing, because cohesin rings are cleaved; however, the mitotic checkpoint does not revert to its active state, because the lower steady state is stable for all values of X_{tens} between one and zero. The asterisk and the dotted trajectory back to the prometaphase state are related to the point of no return, which is explained in the text.

will proceed on to the final state. Furthermore, by breaking the positive feedback loop (the mutual antagonism between CDK^{CycB} and $\text{APC}^{\text{Cdc20}}$), it should be possible to abolish bistability of the anaphase switch and observe reactivation of the error-correction/checkpoint mechanism during anaphase.

A point of no return was observed in experiments by Clute and Pines (37) and Hagting et al. (38). Using live-cell imaging, these studies (37, 38) showed that both securin and cyclin B are de-

graded during metaphase in mammalian cells. The addition of a microtubule-stabilizing drug (taxol) halfway through the degradation of these APC^{Cdc20} substrates blocked additional degradation of securin and cyclin B, indicating reactivation of the checkpoint and inactivation of APC^{Cdc20}. Our interpretation of this experiment is based on Fig. 2C. During a normal M/A transition (light gray dotted line), cyclin B is degraded all of the way down to the lower stable steady state, but in response to taxol treatment halfway through the process (dark gray dotted line), active kinetochores start signaling again (X_{tens} drops), APC^{Cdc20} is inactivated, cyclin B degradation is halted, and the control system reactivates the mitotic checkpoint (Fig. S14).

Reactivation of the checkpoint is observed when cohesin cleavage is carried out by TEV in the absence of APC^{Cdc20} activity (17, 18). In *cdc20Δ* cells, the mitotic checkpoint is not bistable (SI Text and Fig. S2), and therefore, the checkpoint reactivates during TEV-induced anaphase. In the absence of APC^{Cdc20}, the cyclin B level remains high throughout the process. As a consequence, [CAPP]/[CycB] $\ll \theta$ at all times, and $X_{0A} \sim 1 - X_{\text{tens}}$ as the chromosomes come into alignment (X_{tens} increases to one) and the sister chromatids start to separate (X_{tens} decreases to zero). Hence, during TEV-induced anaphase, tensionless kinetochores start signaling again, and the checkpoint is reactivated.

Sensitivity of the Mitotic Checkpoint for a Single Unattached Kinetochores. The model in Fig. 1 has two positive feedback loops: (i) mutual antagonism between the CDK^{CycB} and APC^{Cdc20}, which creates the bistable switch, and (ii) a self-activation loop, whereby APC^{Cdc20} promotes its own activation by disrupting the MCC, as shown in ref. 35. These two positive feedback loops, working synergistically, ensure that a single unaligned chromosome can keep the mitotic checkpoint active in prometaphase (20), and additionally, the checkpoint can be rapidly disengaged at the M/A transition.

To illustrate how these positive feedback loops work together, we plot (Fig. 3) the rates of activation and inactivation of Mad2 as a function of total active Mad2, $[\text{Mad2A}]_T = [\text{Mad2A}] + [\text{Mad2A:Cdc20}]$ (details of the calculation in SI Text). Because the activation rate of Mad2 depends on the signaling state of chromosomes, it is plotted for different values of X_{tens} . Without the feedback loops, both rate curves would be straight lines: the inactivation rate of Mad2A would increase directly proportional to $[\text{Mad2A}]_T$, whereas the activation rate would decrease with $[\text{Mad2A}]_T$, because it is proportional to the concentration of inactive Mad2, $[\text{Mad2I}] = [\text{Mad2}]_T - [\text{Mad2A}]_T$ (Fig. S3 shows the rate curves without Cdc20 auto-activation feedback, when the Mad2 inactivation rate is a straight line). Because of the feedback loops, both rate curves are nonmonotonic, with distinct maxima. Wherever the two rate curves intersect, $[\text{Mad2A}]_T$ is not changing, and therefore, the system has a steady state. For most values of X_{tens} , the two rate curves intersect at three points (two stable steady states separated by an unstable steady state). The left and right stable states represent checkpoint inactive and active states, respectively. As X_{tens} increases, the unstable steady state and active state come closer together and fuse when X_{tens} reaches the saddle-node bifurcation point ($X_{\text{tens}} = X_{\text{SNoff}} = 0.958$). For $X_{\text{tens}} > X_{\text{SNoff}}$, the only remaining steady state is the checkpoint inactive state, and the system moves in that direction. The fact that the bifurcation happens at a value of X_{tens} close to one is a consequence of the slow Mad2 inactivation rate (k_{inad}) at high Mad2 values (greater than one). However, at low Mad2 values (less than one), Mad2 is rapidly inactivated (Fig. 3) because of our assumption that APC^{Cdc20} catalyzes the inactivation of Mad2. Consequently, the control system switches rapidly from the active to the inactive state (Fig. 2 and Fig. S14). If Mad2 inactivation not catalyzed by APC^{Cdc20}, the silencing of the checkpoint would happen too slowly (Figs. S1B and S3).

These features of the mitotic checkpoint must be robust properties of the model (i.e., they must be exhibited not only for specific parameter values, which are illustrated in Figs. 2 and 3,

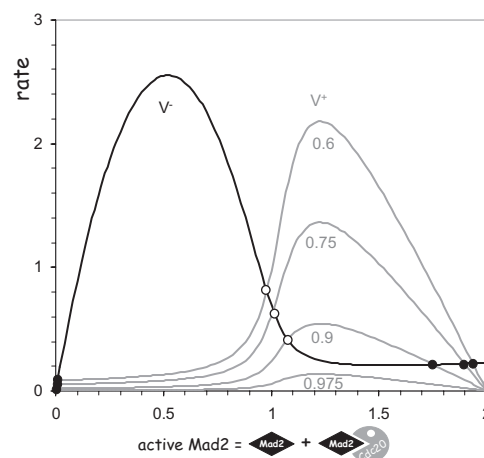


Fig. 3. Two feedback loops make the irreversible anaphase switch robust. Because they are functions of total active Mad2, we plot the rates of Mad2 activation (V^*) and inactivation (V^-). Both rate curves are sharply peaked because of the feedback loops in the reaction network (Fig. 1). The activation rate curve is drawn for increasing values of X_{tens} from 0.6 to 0.975. Points of intersection (circles) of the black and gray curves are steady states of Mad2 activity (black, stable; white, unstable). In prometaphase, Mad2 is active (black circles on the right), but as the last chromosome comes into alignment on the mitotic spindle (as X_{tens} increases above ~ 0.95), the stable active state disappears by coalescing with the unstable saddle point. The mitotic checkpoint must now transition to the Mad2-inactive steady state (black circles on the left), and it does so quickly, because the inactivation rate constant increases as Mad2 activity drops.

but over a broad range of reasonable parameter values). For a single unattached chromosome to keep the checkpoint active, the termination point of the upper branch of stable steady states must lie in the interval $(N_T - 1)/N_T < X_{\text{SNoff}} < 1$, where N_T is the total number of chromosomes in the cell. For the checkpoint not to reactivate during anaphase, the mitotic checkpoint must be bistable for $X_{\text{tens}} = 0$ (i.e., termination point of the lower branch of stable steady states, X_{SNon} must be a negative number). In SI Text and Fig. S4, we show that these two requirements are indeed robust properties of our model.

Discussion

Our model resolves the four puzzles in the introduction as follows:

- (Puzzle 1) The M/A transition is indeed governed by a bistable toggle switch similar to the other cell-cycle transitions. This prediction (2, 3) has been confirmed experimentally (17–19), and we now propose a reasonable mechanism for bistability based on multiple positive feedback loops in the mitotic checkpoint.
- (Puzzle 2) Zero tension on kinetochores is consistent with the mitotic checkpoint being either active (in prometaphase) or inactive (in anaphase), because the checkpoint has two stable steady states for all values of N_{tens} (the number of chromosomes under tension) between zero and $N_T - 1$. This is a robust property of the proposed mechanism.
- (Puzzle 3) A single unattached kinetochores can block the M/A transition, because the bifurcation point, where the mitotic checkpoint switches from active to inactive is a number between $N_T - 1$ and N_T . This is also a robust property of the model.
- (Puzzle 4) Rapid disengagement of the mitotic checkpoint at the M/A transition is explained by a presumptive self-activation loop, whereby active APC^{Cdc20} promotes dissociation of the MCC into inactive Mad2 and active Cdc20.

We are not certain about the molecular mechanism of the self-activation loop. In the model, we assume that Cdc20 activity directly promotes dissociation of the MCC. Presumably, active

APC^{Cdc20} ubiquitinates some component of the MCC, which leads to its dissociation and the inactivation of Mad2. The target of APC/C-dependent ubiquitination could possibly be Cdc20 or Mad2 (35). Alternatively, APC^{Cdc20}-dependent ubiquitination and subsequent degradation of cyclin B might account for dissociation of the MCC if, as has been suggested (36), the phosphorylation of Cdc20 by CDK^{CycB} is required to stabilize the MCC (Fig. S5). This alternative mechanism is examined in *SI Text* and shown to have robust control properties similar to the model in Fig. 1 (compare Fig. S6 with Fig. 2, and Fig. S7 with Fig. 3).

We have attributed irreversibility of the M/A transition to a one-way toggle switch based on double-negative feedback between CDK^{CycB} and APC^{Cdc20}. Other double-negative feedback loops are in operation during entry into and exit from mitosis, and their potential interactions with the mitotic checkpoint should be kept in mind. The well-known mutual antagonism between CDK^{CycB} and APC^{Cdh1} underlies the mitotic exit toggle switch that ensures irreversibility of the transition from anaphase to G1 of the next cell cycle (2). The mitotic exit toggle switch is reinforced by mutual antagonism between CDK^{CycB} and its stoichiometric inhibitor (Sic1 in budding yeast, Rum 1 in fission yeast, and p27^{Kip1} in mammalian cells) (6, 10). In addition, entry into mitosis (the G2/M transition) is governed by a toggle switch based on mutual antagonism between CDK^{CycB} and Wee1 kinase, which is reinforced by mutual activation between CDK^{CycB} and Cdc25 phosphatase (4, 39, 40). These switches must also be flipped to the off state (Cdc25 inactive and Wee1 active) as the cell exits mitosis. At present, it is not clear how these semiautonomous switches influence one another as a cell progresses from metaphase to anaphase to telophase to G1 phase of the next cell cycle.

Our toggle switch model for the M/A transition accounts for inactivation of the mitotic checkpoint as cells exit M phase and become established in G1 phase. However, how does the mitotic checkpoint get reset to its active state at the beginning of the next mitosis? The explanation of this puzzle lies in the complex regulation of APC^{Cdc20}. The APC/C core must be phosphorylated by CDK^{CycB} at ~50 sites before it can be activated by Cdc20 (41). In the present model, we have ignored this level of regulation, because the phosphorylation and dephosphorylation of APC/C lags behind the activation and inactivation of CDK^{CycB}. However, the effect of incompletely activated APC/C on the bifurcation diagram can be interpreted in terms of the cyclin B degradation parameter $k_{\text{dyc},\text{c}20}$ in Eq. S1 and β in Eq. S1'. If APC/C is not fully phosphorylated and therefore, APC^{Cdc20} is not fully active, then the rate of APC^{Cdc20}-dependent degradation of cyclin B is compromised. At small values of β , the saddle-node bifurcation point X_{SNon} , which is normally a negative number, becomes positive (i.e., the mitotic checkpoint can switch to the active state when X_{tens} is small). Therefore, in early prometaphase when CDK^{CycB} is active but APC/C is not yet phosphorylated and only a few chromosomes are bioriented, the control system can jump to the upper steady state in Fig. 2 (i.e., the mitotic checkpoint can turn on).

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