

Report

Rewiring the Exit from Mitosis

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KEY WORDS

molecular network dynamics, feedback loops, MPF, Cdc20, cyclin degradation

ABBREVIATIONS

MPF	mitosis promoting factor
APC	anaphase promoting complex
SN	Saddle Node
SNIC	Saddle Node on an Invariant Circle

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ABSTRACT

The mitotic cell cycle can be described as an alternation between two states. During mitosis, MPF (mitosis promoting factor) is high and keeps inactive its numerous molecular antagonists. In interphase, MPF is inactivated, and the antagonists prevail. The transition between the two states is ensured by 'helper' molecules that favor one state over the other. It has long been assumed that active MPF (a dimer of cyclin B and cyclin-dependent kinase 1) induces exit from mitosis by activating APC:Cdc20, a ubiquitin ligase responsible for cyclin B degradation. The molecular details have not been fully worked out yet, but recent results show that MPF and the ubiquitin ligase are not involved in a simple negative feedback loop. While it is proven that MPF activates APC, new data suggest that MPF inhibits Cdc20, i.e., that MPF and Cdc20 are antagonists. We introduce this new idea into a published model for cell cycle regulation in *Xenopus laevis*, and study its dynamical behavior. We show that the new wiring permits oscillations with a simpler and smaller network than previously envisaged and that the antagonism between MPF and Cdc20 suggests a new interpretation of the spindle checkpoint.

INTRODUCTION

The molecular machinery that controls cell cycle progression is very conserved among eukaryotes.¹ Cell cycle events, most notably mitosis (M) and DNA synthesis (S), alternate during the cell cycle thanks to the timely regulation of cyclin-dependent kinases (CDKs).² Inactive if alone, CDKs are activated when bound to their regulatory subunits, known as cyclins. Availability of cyclins is the rate-limiting step for CDK:cyclin heterodimer formation because CDKs are present in excess throughout a normal cell cycle, and binding is fast and spontaneous. Not surprisingly, living cells modulate cyclin synthesis and degradation as a means to control CDK:cyclin activity. For example, during early embryonic division cycles of the frog (*Xenopus laevis*), the Cdk1:cyclin B complex responsible for mitosis (known as MPF, mitosis promoting factor) accumulates until the cell enters into mitosis and drops shortly thereafter. Accordingly, cyclin B is continuously synthesized during the cycle but is degraded only at the end of mitosis.^{3,4}

Central to cyclin B degradation is the ubiquitin ligase APC (anaphase promoting complex) (for a review, see Ref. 5). APC alone is unable to target cyclin B for degradation, its activation being dependent on the binding of either one of two cofactors, Cdc20 or Cdh1.⁶⁻⁸ Both complexes APC:Cdc20 and APC:Cdh1 are able to ubiquitinate cyclin B, but the two cofactors are thought to have different roles because they are thought to be differently regulated by MPF.

APC:Cdh1 degrades cyclin B during G₁ phase, whereas it is inactive in S, G₂ and M phases, when MPF is high.⁹ The reason is that MPF and Cdh1 are antagonists: APC:Cdh1 is an efficient ubiquitin ligase for cyclin B degradation, and MPF converts Cdh1 into a phosphorylated form that cannot bind to APC.⁸ Both experimentalists¹⁰ and theoreticians^{11,12} have stressed the importance of this antagonism which is said to lie at the heart of the cell cycle regulatory network. In this framework, the cell cycle has been explained as the alternation between two self-sustaining states: active MPF/inactive Cdh1 and vice versa.

A challenge for this model is to explain how the cell-cycle regulatory network can switch from one self-sustaining state to another. How does the active MPF/inactive Cdh1 state lose its stability? It is generally accepted that MPF activates APC:Cdc20, and therefore indirectly triggers its own inactivation.¹³⁻¹⁵ The effect of the negative feedback on the antagonism between MPF and APC:Cdh1 is dramatic. As cyclins start to be degraded by

APC:Cdc20, MPF loses the upper hand, APC: Cdh1 prevails and keeps degrading cyclin B throughout the whole next G₁ phase.

According to this model, it is crucial that MPF has opposite effects on the two cofactors: it inhibits Cdh1, making sure that there are two alternative states, but activates Cdc20, which guarantees that the mitotic state is transitory. However, recent evidence regarding Cdc20 regulation suggests that this is an oversimplified view. MPF exerts a double control on APC:Cdc20 formation. It is well known that MPF phosphorylates and activates APC, giving rise to a negative feedback signal (MPF → APC —| MPF).^{8,16,17} Less understood is the control that MPF exerts on Cdc20. Some studies report that MPF has no effect on Cdc20,⁸ but more conclusive and recent evidence suggests that MPF inhibits Cdc20,¹⁸ hinting that MPF and Cdc20 are antagonists involved in a positive feedback loop (MPF —| Cdc20 —| MPF).¹⁹⁻²¹ Summarizing, recent data show that MPF is involved in both positive and negative feedback effects with APC and Cdc20.

Is this wiring consistent with the accepted role of APC:Cdc20 during exit from mitosis? We modify a published model for *Xenopus laevis*²² keeping track of these new data, and explore their effects on the dynamical behavior of the system. The rapid alternation of S and M phases that compose the early cycles in *Xenopus* are well suited to analyze the interaction between MPF and APC:Cdc20 because Cdh1 is missing, and cyclin B degradation is due to APC:Cdc20 only. At the end of the paper we will generalize our arguments.

THE MODEL

Biological basis. During the early stages of *Xenopus* development, MPF is controlled through two mechanisms, cyclin B degradation and Cdk1 phosphorylation, as summarized in Figure 1.

Cyclin B degradation. Only cyclin B synthesis is required to drive MPF oscillations in *Xenopus* extracts, all other mRNAs being dispensable.⁴ For this reason, in our model we keep track of cyclin synthesis and degradation only, while all other components are constant.

During *Xenopus* early development, cyclin B degradation is catalyzed by the complex APC:Cdc20, which is in turn subject to dual control by MPF. MPF phosphorylates and activates APC (call the phosphorylated form 'APCP'), a reaction required for the binding of APC to Cdc20.⁸ MPF also phosphorylates Cdc20 with an opposite effect, since phosphorylated Cdc20 (call it 'Cdc20P') has a reduced affinity for APC.¹⁸

We assume for the sake of simplicity that only the phosphorylated form of APC and the dephosphorylated form of Cdc20 can bind to form APCP:Cdc20. We also take

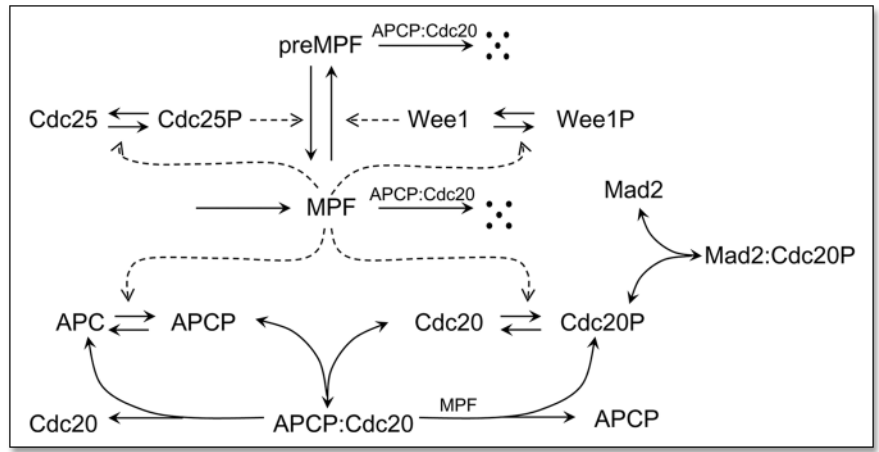


Figure 1. Wiring diagram describing the molecular network underlying cell cycle oscillations during *Xenopus* early development. Three modules can be recognized, one responsible for MPF phosphorylation and dephosphorylation (including MPF, Cdc25 and Wee1), the second which controls cyclin B degradation and therefore MPF inactivation (including MPF, Cdc20, APC), and the third involved in the spindle checkpoint (including Cdc20P and Mad2). See text for details.

into account the possibility that APCP:Cdc20 is destabilized either by APCP dephosphorylation or by MPF-catalyzed phosphorylation of Cdc20.

MPF phosphorylation. By phosphorylating and inactivating MPF, the protein kinase Wee1 complements APCP:Cdc20 in its role of antagonizing MPF activity.²³ MPF and Wee1 are mutual antagonists because they phosphorylate and inhibit each other (call the phosphorylated form of MPF 'preMPF'), forming a double-negative

Table 1 Differential and algebraic equations*

$$\frac{d[\text{MPF}]}{dt} = k_{\text{cyc.syn}} \cdot k_{\text{wee}} \cdot [\text{MPF}] + k_{25}([\text{MPF}_{\text{total}}] - [\text{MPF}]) - (k_{\text{cyc.deg}} + k_{\text{cyc.deg}} \frac{[\text{APCP:Cdc20}]}{J_{\text{cyc.deg}} + [\text{MPF}_{\text{total}}]})[\text{MPF}]$$

$$\frac{d[\text{MPF}_{\text{total}}]}{dt} = k_{\text{cyc.syn}} - (k_{\text{cyc.deg}} + k_{\text{cyc.deg}} \frac{[\text{APCP:Cdc20}]}{J_{\text{cyc.deg}} + [\text{MPF}_{\text{total}}]})[\text{MPF}_{\text{total}}]$$

$$\frac{d[\text{Cdc20}]}{dt} = k_{20,\text{deph}} \cdot [\text{Cdc20P}] - (k_{20,\text{ph}} [\text{MPF}]) [\text{Cdc20}] + (k_{\text{apc.deph}} + k_{\text{diss}}) [\text{APCP:Cdc20}] - k_{\text{ass}} \cdot [\text{Cdc20}] \cdot [\text{APCP}]$$

$$\frac{d[\text{Mad2:Cdc20P}]}{dt} = k_{\text{ass}2} [\text{Mad2}] \cdot [\text{Cdc20P}] - k_{\text{diss}2} [\text{Mad2:Cdc20P}]$$

$$\frac{d[\text{APCP:Cdc20}]}{dt} = k_{\text{ass}} [\text{APCP}] \cdot [\text{Cdc20}] - (k_{\text{diss}} + k_{\text{apc.deph}} + k_{20,\text{ph}} [\text{MPF}]) [\text{APCP:Cdc20}]$$

$$\frac{d[\text{APCP}]}{dt} = k_{\text{apc.ph}} [\text{MPF}] \cdot [\text{APC}] - k_{\text{apc.deph}} [\text{APCP}] - k_{\text{ass}} [\text{APCP}] \cdot [\text{Cdc20}] + (k_{\text{diss}} + k_{20,\text{ph}} [\text{MPF}]) [\text{APCP:Cdc20}]$$

$$\frac{d[\text{Wee}]}{dt} = V_{\text{awee}} \frac{[\text{Wee}_{\text{total}}] - [\text{Wee}]}{J_{\text{wee}} + [\text{Wee}_{\text{total}}] - [\text{Wee}]} - V_{\text{iwee}} \cdot [\text{MPF}] \cdot [\text{Wee}]$$

$$\frac{d[\text{Cdc25P}]}{dt} = (V_{\text{a25}} \cdot [\text{MPF}] \cdot ([\text{Cdc25}_{\text{total}}] - [\text{Cdc25P}])) - \frac{V_{25} [\text{Cdc25P}]}{J_{25} + [\text{Cdc25P}]}$$

$$k_{\text{wee}} = [\text{Wee}_{\text{total}}] k'_{\text{wee}} + (k''_{\text{wee}} - k'_{\text{wee}}) [\text{Wee}]$$

$$k_{25} = [\text{Cdc25}_{\text{total}}] k'_{25} + (k''_{25} - k'_{25}) [\text{Cdc25P}]$$

$$[\text{APC}] = [\text{APC}_{\text{total}}] - [\text{APCP}] - [\text{APCP:Cdc20}]$$

$$[\text{Cdc20P}] = [\text{Cdc20}_{\text{total}}] - [\text{Cdc20}] - [\text{APCP:Cdc20}] - [\text{Mad2:Cdc20P}]$$

$$[\text{Mad2}] = [\text{Mad2}_{\text{active}}] - [\text{Mad2:Cdc20P}]$$

*The equations have been derived from the wiring diagram in Figure 1.

Table 2 Parameters and initial conditions

Rate constants (min⁻¹)

$k_{cyc, syn} = 0.4$	$k_{cyc, deg} = 2$	$k_{cyc, deg}^I = 0.08$	
$k_{apc, ph} = 0.04$	$k_{apc, deph} = 0.04$	$k_{20, deph} = 8$	$k_{20, ph} = 10$
$k_{ass} = 80$	$k_{diss} = 20$	$k_{ass2} = 10$	$k_{diss2} = 0.1$
$k_{25}^I = 0.2$	$k_{wee}^{II} = 10$	$V_{i25} = 0.2$	$V_{a25} = 1$
$k_{wee}^I = 0.1$	$k_{wee}^{II} = 4$	$V_{awe} = 0.2$	$V_{iwee} = 1$

Other constants (dimensionless)

$Mad2_{active} = 0.01$	$APC_{total} = 1$	$Cdc20_{total} = 1$	$Cdc25_{total} = 1$
$Wee1_{total} = 1$	$J_{wee} = 0.01$	$J_{cyc, deg} = 0.01$	$J_{25} = 0.01$

Initial conditions (dimensionless)

$[MPF] = 0.015$	$[MPF_{total}] = 0.016$	$[Cdc20] = 0.652$	$[APCP] = 0.124$
$[APCP:Cdc20] = 0.320$	$[Wee1] = 0.718$	$[Cdc25P] = 0.281$	$[Mad2:Cdc20P] = 0.009$

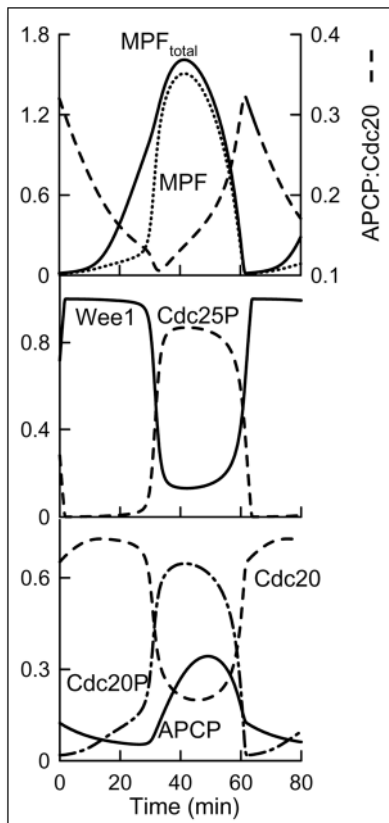


Figure 2. The molecular network generates limit cycle oscillations. In the upper panel are shown $[MPF]$ and $[MPF_{total}] = [MPF] + [preMPF]$. As the positive feedback formed by MPF and Cdc25P is engaged, preMPF is converted into MPF (the dotted and solid lines get close). In the middle panel are plotted the active forms of Cdc25 and Wee1, the phosphatase and the kinase that control MPF phosphorylation state. In the lower panel are plotted APCP, Cdc20 and Cdc20P. Notice that Cdc20 undergoes faster post-translational modifications than APC, so that Cdc20P reaches both maximum and minimum before APCP. Equations in Table 1, parameters and initial conditions in Table 2. Cycle time = 62 min.

feedback loop of the same type as the one formed by Cdc20 and MPF.²³ Activation of MPF (preMPF \rightarrow MPF) by the protein phosphatase Cdc25 is also regulated by positive feedback: MPF phosphorylates and activates Cdc25, and Cdc25 removes the

inhibitory phosphate group from MPF.^{24,25} In the rest of the paper we shall refer to this part of the network as the MPF phosphorylation module.

We model the MPF phosphorylation module following the approach used by Novak and Tyson,²² with a minor change. The differential equations for Wee1 and Cdc25 are not expressed as Goldbeter-Koshland functions,²⁶ rather we use saturation kinetics only for the reactions catalyzed by the phosphatases that act on Cdc25 and Wee1. All other reactions are

described using mass-action kinetics. We opted not to use Goldbeter-Koshland equations to describe the dynamics of molecular species that take part in multiple reactions (e.g., Wee1, which inhibits and is inhibited by MPF) because the assumption that substrate-enzyme complexes are negligible is not correct (Sabouri, Ciliberto, Novak, Tyson, unpublished).

The spindle checkpoint. We also aim to model the spindle checkpoint, a molecular device that blocks cells in M phase when chromosomes are not properly aligned on the metaphase plate. Although checkpoints are silent during early development, they can be induced by a strong signal. The spindle checkpoint can be stimulated in *Xenopus* extracts by adding nuclei and inhibitors of microtubule polymerization.²⁷ Experimental data on *Xenopus* extracts suggest that Mad2, an essential protein for the spindle checkpoint, sequesters the phosphorylated form of Cdc20 and thereby inhibits chromosome separation and stabilizes MPF.²⁰ We do not aim to model the detailed molecular circuitry of the spindle checkpoint, which is complicated and not clearly understood yet.²⁸ We simply introduce the complex Mad2:Cdc20P to account for the role of Mad2 in maintaining the checkpoint operational. In the model, the sum of the concentrations of Mad2 free and bound to Cdc20P is constant ($[Mad2_{active}]$). We do not keep track of Mad2 activation: we simulate an operational checkpoint by increasing the concentration of Mad2_{active}.

The wiring diagram in Figure 1, is translated into a system of ordinary differential equations (Table 1) using standard laws of chemical kinetics. For the chosen parameter set and initial conditions (Table 2) the system gives rise to oscillations, as shown in Figure 2.

RESULTS

At the beginning of an oscillation, MPF is inhibited by Wee1. Cyclin synthesis drives a slow increase of MPF until a critical threshold, when the positive feedbacks between Cdc25 and MPF and between Wee1 and MPF are engaged. After this point, Cdc25 is activated and Wee1 inhibited (Fig. 2, middle panel) while preMPF is dephosphorylated, giving rise to a steep increase in MPF (Fig. 2, upper panel) (see ref. 22 for a more detailed description of this process).

In our model, as MPF increases it both inhibits and activates the formation of the APCP:Cdc20 complex. In this context, oscillatory behavior may seem counterintuitive. Cyclin B degradation occurs when MPF reaches the peak of the oscillation and exerts the strongest inhibitory effect on Cdc20. How is it possible? The rate constants for Cdc20 and APC phosphorylation and dephosphorylation ($k_{20, ph}$, $k_{apc, ph}$ and $k_{20, deph}$, $k_{apc, deph}$ respectively) play key roles in the oscillatory behavior. The inhibitory effect of MPF

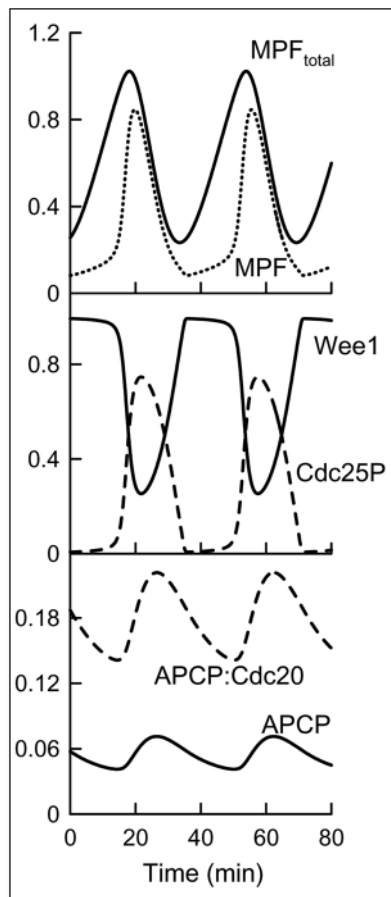


Figure 3. Limit cycle oscillations in a system where Cdc20 cannot be phosphorylated. Notice that in this system APCP:Cdc20 builds up when MPF starts to accumulate, without delay. Accordingly, the cell cycle is faster (cycle time = 35 min). Equations in Table 1, parameters in Table 2, except for $k_{20,ph} = 0$.

initially prevails because Cdc20 is phosphorylated promptly, while APCP builds up more slowly ($k_{20,ph} \gg k_{apc,ph}$), (Fig. 2, lower panel). As few APCP:Cdc20 complexes can form, MPF activity stays high for 10–20 min, during the time when Cdc25 is fully active. Eventually, the positive effect that MPF exerts on APCP:Cdc20 formation (i.e., production of APCP) overcomes the negative one (i.e., formation of Cdc20P), APCP avidly binds to the unphosphorylated fraction of Cdc20 and APCP:Cdc20 builds up. Cyclin B starts to be degraded, MPF level drops, the inhibition on Cdc20 is relieved, and a pool of unphosphorylated Cdc20 is rapidly created. As APCP dephosphorylation is slower than Cdc20P dephosphorylation ($k_{20,deph} \gg k_{apc,deph}$), APCP does not drop as quickly as Cdc20P, and APCP:Cdc20 keeps increasing while MPF decreases.

Eventually the cell exits from mitosis. MPF drops so low that it does not produce enough APCP to bind the large pool of available Cdc20, and cyclin B can accumulate again. The newly formed MPF is phosphorylated by Wee1, and a new oscillation starts.

Separating the two modules. In the model there is one negative feedback (MPF \rightarrow APC \dashv MPF) and three positive feedbacks, two involved in MPF phosphorylation (MPF \rightarrow Cdc25 \rightarrow MPF and MPF \dashv Wee1 \dashv MPF), and one in cyclin degradation (MPF \dashv Cdc20 \dashv MPF). Theory predicts that oscillations can arise in a two component system composed of one positive and one negative feedback.²⁹ We asked whether, given the parameter set in Table 2, our system would oscillate without either MPF phosphorylation or Cdc20 inhibition.

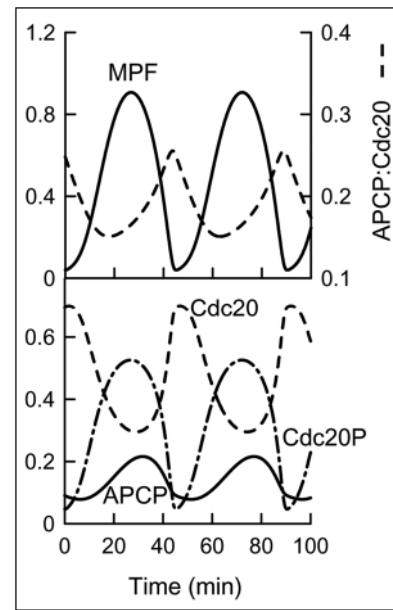


Figure 4. Limit cycle oscillations in a system where MPF cannot be phosphorylated. As soon as MPF drops, it rises again because it is not inhibited by Wee1. Therefore, APCP:Cdc20 starts to increase faster than in normal cells, and cycles are shorter (cycle time = 45 min). Equations in Table 1, parameters in Table 2, except for $k'_{wee} = 0$ and $k''_{wee} = 0$.

Nonphosphorylatable Cdc20. Limit cycle solutions still exist in a mutant where Cdc20 cannot be phosphorylated by MPF ($k_{20,ph} = 0$), (Fig. 3). When the antagonism between MPF and Cdc20 is lacking, APCP:Cdc20 accumulates as soon as MPF is able to effectively phosphorylate APC, roughly at the same time as the MPF/Cdc25 loop is engaged. As a result, oscillations are faster (cycle time = 35 min compared to wild type cycle time = 62 min) and have a smaller amplitude than in normal cells. Clearly, cells with normal Cdc20 have a longer cycle because the antagonism between MPF and Cdc20 delays APCP:Cdc20 formation.

The positive feedback loops of the MPF phosphorylation module give rise to bistability, as predicted theoretically²² and verified experimentally.^{30,31} In agreement with these observations, if we eliminate cyclin synthesis and degradation ($k_{cyc,syn} = 0$, $k_{cyc,deg} = 0$, $k'_{cyc,deg} = 0$), our model shows bistability (data not shown).

Nonphosphorylatable MPF. Early measurements could not detect any MPF-tyrosine phosphorylation during *Xenopus* early development,³² but more recent data showed that a certain extent of phosphorylation occurs.³³ Injection of the phosphatase Cdc25A in *Xenopus* embryos advances embryonic development;³³ nevertheless, cells still enter and exit from mitosis, implying that the cell cycle engine must be able to oscillate with very small or no contribution from MPF phosphorylation and dephosphorylation. In agreement with these observations, our model can oscillate with an excess of Cdc25, when preMPF is negligible (not shown). Furthermore, the model predicts that oscillations persist when preMPF is absent, as with a nonphosphorylatable form of MPF ($k'_{wee} = 0$ and $k''_{wee} = 0$), (Fig. 4). As expected, the period of oscillation is shorter and the amplitude of oscillation is smaller. Notice that, without inhibitory phosphorylation, MPF starts to accumulate immediately after the cell exits from mitosis.

Spindle checkpoint. Mad2, very low in cycling cells, blocks the extract in metaphase if added in excess, even in the absence of nocodazole.²⁰ To understand how the model explains the role of Mad2, we analyzed a simplified model lacking MPF phosphorylation, since this module does not contribute to the checkpoint. In addition, we assumed that APCP:Cdc20, Cdc20 and Mad2:Cdc20P have reached steady states so that only MPF and APCP are dynamic variables. In the phase plane of a cycling cell ($[Mad2_{active}] = 0.01$), (Fig. 5A), the MPF nullcline is a Z-shaped curve, due to the antagonism

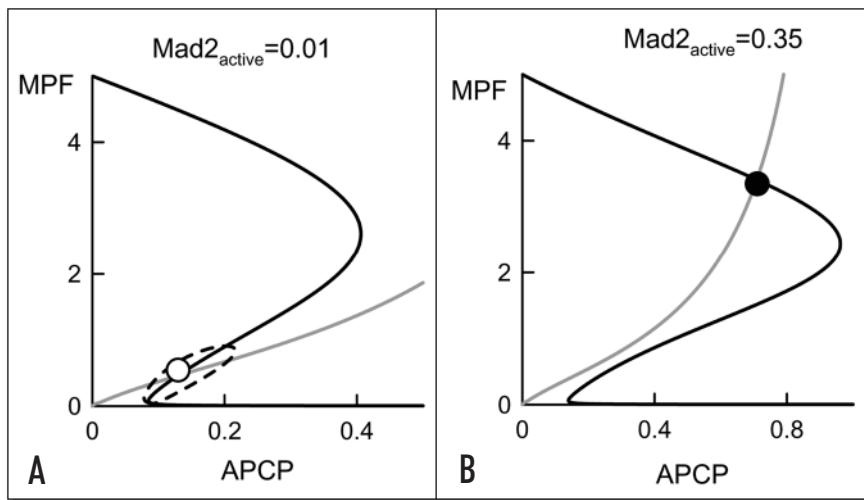


Figure 5. Phase plane analysis for the simplified model without MPF phosphorylation. Equations in Table 1 and parameters in Table 2, except for $k_{wee}' = 0$ and $k_{wee}'' = 0$. (A) Checkpoint not engaged ($[Mad2_{active}] = 0.01$). The MPF nullcline (black line) is Z-shaped, the APCP nullcline (grey line) is a hyperbola. The two nullclines meet in a steady state, which is unstable (white-filled dot). Around the unstable steady state there is a stable limit cycle (the simulation of Fig. 4, is superimposed on the phase plane, dashed line). (B) Checkpoint engaged ($[Mad2_{active}] = 0.35$). The steady state becomes stable, with high MPF activity.

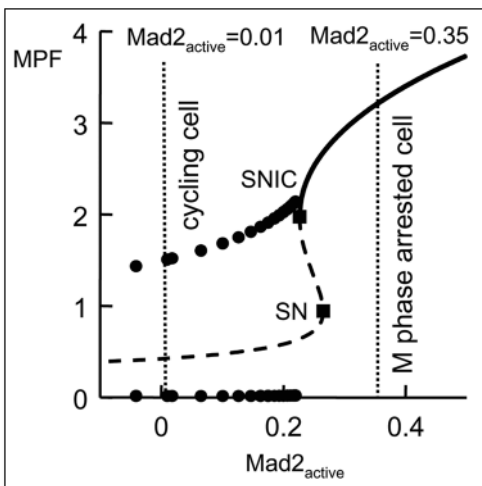


Figure 6. Bifurcation diagram for the model in Table 1, parameters in Table 2. Stable steady states (solid line) and unstable steady states (dashed line) of MPF are plotted as a function of the bifurcation parameter, $[Mad2_{active}]$. Oscillatory solutions are born for small values of $[Mad2_{active}]$ —filled dots mark the maxima and minima of the oscillations. As $[Mad2_{active}]$ increases, oscillatory solutions disappear at a SNIC (Saddle Node on an Invariant Circle) bifurcation at $[Mad2_{active}] = 0.22$. After this point, there are three steady states in the region $0.22 < [Mad2_{active}] < 0.26$, one stable and two unstable. Further increasing $[Mad2_{active}]$, the system undergoes a saddle node (SN) bifurcation at $[Mad2_{active}] = 0.26$, and only one stable steady state with high MPF activity is left. During a normal cycle, $[Mad2_{active}]$ is negligible and the system lies in the oscillatory region. When the spindle checkpoint is engaged, $[Mad2_{active}]$ is large and the cell is blocked in M phase.

between MPF and Cdc20. The APCP nullcline is a hyperbola, which increases as MPF increases. The two nullclines meet in an unstable steady state, surrounded by a stable limit cycle. When $[Mad2_{active}]$ is increased to 0.35 (i.e., the checkpoint is operational) (Fig. 5 B) the MPF nullcline moves to the right, and the unstable steady state becomes a stable steady state with

high MPF activity. In this setting, MPF phosphorylates Cdc20 which is sequestered by Mad2, and the formation of the complex APCP:Cdc20 is inhibited. Notice that according to the model, the spindle checkpoint relies on MPF activity to inhibit Cdc20 and block the cell in M.

DISCUSSION

Oscillations. Experimental data suggest that repetitive cell cycles in early embryos rely on cyclin B degradation.³⁴ According to the standard view, oscillations originate from the negative feedback loop between MPF and APC:Cdc20. Theory predicts that a negative feedback loop can oscillate only if formed by at least three species.²⁹ Hence, a loop where MPF activates APC:Cdc20 which in turn inactivates MPF cannot oscillate. To account for sustained oscillations and the delay between MPF activation and cyclin B degradation,¹³ an additional and as yet unidentified molecule (the intermediary enzyme ‘IE’) has been proposed between MPF and APC:Cdc20.²²

Recent experimental data suggest that this story is too simple. Although MPF favors APC:Cdc20 formation by activating APC, it also preserves cyclin B stability by inhibiting Cdc20. In other words, MPF acts at the same time on the brake and the accelerator. This double and opposing control does not abrogate the capability of the system to oscillate, actually it permits a simplification of the molecular network because it allows oscillations without the hypothetical intermediary enzyme, IE. As MPF activity rises at the onset of mitosis, the brake has a stronger effect at first (i.e., Cdc20 is inhibited), and cyclin B is not degraded. Slowly, however, the pressure on the accelerator increases (i.e., APCP builds up), until it overcomes the brake, APCP:Cdc20 is formed, cyclin B is degraded, and MPF is inactivated. Once MPF drops to low levels, APCP is dephosphorylated, and cyclin B starts to accumulate again. As in previous models, there is a delay between activation of MPF and formation of APCP:Cdc20, but in our case it is not due to a molecule like IE. It rather emerges from the dynamics of the system, from the antagonism $MPF \rightarrow Cdc20 \rightarrow MPF$ which delays firing of the negative feedback $MPF \rightarrow APC \rightarrow MPF$.

Spindle checkpoint. The ideas presented in this paper are in strong agreement with the intuitive analysis presented by D’Angiolella et al.^{20,35} They noticed that “until completion of spindle assembly, cyclin B-Cdk1 helps restrain APC/C^{Cdc20} activation. This control may also ensure that APC/C^{Cdc20} inhibition is rapidly extinguished once the checkpoint is satisfied. At completion of spindle assembly, initial loss of Cdk1 activity by small amounts of active APC/C^{Cdc20} will be rapidly amplified by switching the balance towards dephosphorylation of Cdc20. Presumably this dephosphorylation is more rapid than dephosphorylation of core APC/C subunits so that a window of active APC/C^{Cdc20} can ensue”.³⁵

A quantitative translation of their arguments can be followed in the bifurcation diagram of Figure 6, where steady states and oscillations of MPF are plotted as functions of $[Mad2_{active}]$. During mitotic arrest, Mad2 keeps Cdc20 inactive and sequestered, with the help

of MPF. When the checkpoint signal vanishes, Mad2 is inactivated, the steady state with high MPF activity is lost through a SNIC bifurcation, and the system is attracted by stable limit cycles. During the transition (not shown) Cdc20 is dephosphorylated, APC:Cdc20 builds up, and MPF is inactivated.

From a modeler's perspective the spindle checkpoint can be viewed as a molecular mechanism that moves the network out of the oscillatory region into a stable steady state, just like many other known surveillance mechanisms (e.g., DNA replication checkpoint, size control checkpoint, and the morphogenesis checkpoint).^{11,36,37}

Suggested experiment. A cell blocked in M phase by the spindle checkpoint provides an ideal experimental setup to discriminate between the possibility that MPF activates or inhibits Cdc20. Exit from mitosis requires Cdc20 activity, both for cyclin B degradation and chromosome segregation. If MPF activates both APC and Cdc20, then inhibiting MPF (e.g., with specific drugs or adding CDK inhibitors—'CKI') surely would cause mitotic arrest. Instead, if MPF and Cdc20 are antagonists, the inhibition of MPF will indirectly activate Cdc20, driving the cell out of mitosis regardless of the checkpoint. D'Angiolella et al. performed this experiment in *Xenopus* extracts, and the results showed that indeed Cdc20 and MPF are antagonists.²⁰

In *Xenopus* extracts the interpretation is straightforward because Cdh1 is not present. The same experiment was previously done in budding yeast.³⁸ Cells were arrested in metaphase with nocodazole and MPF was inhibited by overexpression of the budding yeast CKI gene, *SIC1*. Exit from mitosis could be observed by following cyclin B degradation and chromosome separation. However, the interpretation of this observation is complicated by the presence of Cdh1 in budding yeast. Cdh1 is an antagonist of MPF and it likely replaces inactive Cdc20 (see for example refs. 16 and 39 for Cdh1's possible role in securin degradation). Whether Cdc20 is activated or inactivated by MPF, Cdh1 could lead the cell out of mitosis after MPF inhibition by Sic1. To avoid problems with the interpretation of the results, the experiment should be performed in a *cdh1-deletion* mutant. If MPF inhibition would induce cyclin B and securin degradation in this mutant as well, it would be an indication that Cdc20 is an antagonist of MPF in yeast as well as in frog egg.

If it will be confirmed that both Cdc20 and Cdh1 are antagonists of MPF, they will prove more similar than previously thought. Nevertheless, they play different roles in the cell cycle dynamics, due to their differential binding to APC. Cdc20 binds preferentially to the form of APC phosphorylated by MPF,⁸ so that the MPF/APC/Cdc20 network is a combination of positive and negative feedback and as such has a built-in capability to oscillate, as explained in this paper. By contrast, Cdh1 binds to APC regardless of the phosphorylation state of APC.⁸ Lacking the negative feedback, the MPF/APC/Cdh1 network gives rise to bistability but cannot oscillate.

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