Supplementary Information for:

The Cdk1-APC/C cell cycle oscillator circuit functions as a time-delayed, ultrasensitive switch

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Computational Modeling

A Two-ODE Model of the Embryonic Cell Cycle. Here we construct a simple, experimentally-parameterized, two-ODE model of *Xenopus laevis* embryonic cell cycle oscillations. More complicated models have been proposed previously[1-4]. However, simpler models are more amenable to analytical analysis, highlight the essence of the Cdk1-APC/C^{Cdc20} system, include fewer adjustable parameters, and are easier to understand and compare to other activator-repressor circuits. Therefore we chose to begin with this simple model and added additional features as required.

This model is similar in spirit to models of the embryonic cell cycle originally proposed by Tyson and Novak[5], and in particular to the simplified two-ODE model they developed[3]. The main difference is that we used Hill functions rather than Goldbeter-Koshland functions to introduce nonlinearities into the model.

The first ODE of the two-ODE model describes the synthesis and destruction of the mitotic cyclins [Eq 1]:

$$\frac{d}{dt}Cyc = k_{synth} - k^{*}_{deg}Cyc \qquad \text{[Eq 1]}$$

We assumed that cyclin synthesis occurs at a constant rate k_{synth}, and that the apparent first order rate constant for cyclin degradation, k^*_{deg}, varied with the activity of Cdk1 as a result of the activation of APC/C^{Cdc20} by Cdk1, as described by:

$$k^*_{deg} = a_{deg} + b_{deg}\frac{Cdk1^{17}}{EC50^{17}_{deg} + Cdk1^{17}} \qquad \text{[Eq 2]}$$

The Hill exponent of 17 in Eq 2 was taken from the securin-CFP degradation response data shown in Fig. 1f. Combining Eqs 1 and 2 yielded:

$$\frac{d}{dt}Cyc = k_{synth} - \left(a_{deg} + b_{deg}\frac{Cdk1^{n_{deg}}}{EC50^{n_{deg}}_{deg} + Cdk1^{n_{deg}}}\right)Cyc \qquad \text{[Eq 3]}$$

Initially we assumed that there was no time lag between the activation of Cdk1 and the regulation of cyclin degradation; later we will relax this assumption.

Next we considered the activity of Cdk1, which depends upon cyclin synthesis, cyclin degradation, and the positive and double-negative feedback loops. The concentration of Cdk1 is known to be high compared to the peak concentration of the B-type cyclins[6, 7], and the affinity of these cyclins for Cdk1 is known to be high[8], so we assumed that the newly synthesized mitotic cyclin is quantitatively converted to cyclin-Cdk1 complexes. For simplicity we also assumed that these complexes are rapidly and quantitatively phosphorylated by the Cdk-activating kinase CAK. Under these assumptions, the synthesis of cyclin initially produces active Cdk1. These complexes can then be inactivated by Wee1 and re-activated by Cdc25, via the double-negative and positive feedback loops. Active Cdk1 complexes can also be eliminated through cyclin degradation. Overall the ODE for the net production of active Cdk1 is:

$$\frac{d}{dt}Cdk1 = k_{synth} + k_{Cdc25}Cdc25^*\left(Cyc - Cdk1\right) - k_{Wee1}Wee1^*Cdk1 - k^*_{deg}Cdk1 \qquad \text{[Eq 4]}$$

The quantity $Cdc25^*$ represents the concentration of active Cdc25C, which is a function of the active Cdk1 concentration. Because Cdc25C is one of the first proteins to be phosphorylated by

Cdk1 when *Xenopus* extracts enter mitosis[9], we assumed that the activity of Cdc25 is always in equilibrium with the activity of Cdk1. Experimental work has shown this steady-state response to be well-approximated by:

$$k_{Cdc25}Cdc25^{*} = a_{Cdc25} + b_{Cdc25}\frac{Cdk1^{11}}{EC50_{cdc25}^{11} + Cdk1^{11}} \qquad \text{[Eq 5]}$$

The quantity $Wee1^{*}$ represents the concentration of active Wee1A, which is also a function of *Cdk1*. Like Cdc25C, Wee1A is an early substrate of Cdk1[9] and so it was assumed to be in equilibrium with the activity of Cdk1. Experimentally the Wee1A response has been determined to be well approximated by an inhibitory Hill function with a Hill exponent of 3.5[10]. Substituting the k_{deg}^{*}, $Cdc25^{*}$, and $Wee1^{*}$ response functions into Eq 4 yields:

$$\frac{d}{dt}Cdk1 = k_{synth} + \left(a_{Cdc25} + b_{Cdc25}\frac{Cdk1^{11}}{EC50_{Cdc25}^{11} + Cdk1^{11}}\right)(Cyc - Cdk1) - \left(a_{Wee1} + b_{Wee1}\frac{EC50_{Wee1}^{3.5}}{EC50_{Wee1}^{3.5} + Cdk1^{3.5}}\right)Cdk1 - \left(a_{deg} + b_{deg}\frac{Cdk1^{17}}{EC50_{deg}^{17} + Cdk1^{17}}\right)Cdk1 \qquad \text{[Eq 6]}$$

This completes the two ODE model of the Cdk1-APC/C^{Cdc20} system.

The dynamics of a two ODE system can be inferred by examining the fixed points or steady states of the system. These, in turn, can be mapped out by plotting the system's two nullclines[11]. In biochemical terms, the cyclin nullcline represents the steady-state level of cyclin as a function of the activity of Cdk1, and the Cdk1 nullcline represents the steady-state level of active Cdk1 as a function of cyclin. In mathematical terms, the nullclines are the curves that satisfy the equations $\frac{d}{dt}Cyc = 0$ and $\frac{d}{dt}Cdk1 = 0$.

An expression for the Cdk1 nullcline can be derived analytically, and the parameters for the equation have been obtained from *Xenopus* extract experiments[10, 12]. The result is an S-shaped curve that agrees well with the experimentally-observed hysteretic dependence of Cdk1 activity on cyclin concentration[12-14] (Fig. 3B).

An equation for the cyclin nullcline can also be obtained analytically, from Eq 3:

$$Cyc = \frac{k_{synth}}{k^*_{\deg}} = \frac{k_{synth}}{a_{deg} + b_{deg}\dfrac{Cdk1^{17}}{EC50^{17}_{deg} + Cdk1^{17}}} \qquad \text{[Eq 7]}$$

This equation can be plotted for various assumed values of k_{synth}, a_{deg}, b_{deg}, and $EC50_{deg}$. We can then compare the behaviors of the system to those that are possible with different assumed functions for the dependence of k^*_{deg} on Cdk1.

The parameters for the two-ODE model are listed in Table S1. Note that although all of the parameter choices are supported by experiments, some should still be taken with a grain of salt. For example, although the ratios of the *a* and *b* parameters are reasonably well constrained, their absolute values are less well constrained, in part because there is substantial extract-to-extract variability in the absolute timing of cell cycle events. Likewise for the *EC*50 values; their ratios are better constrained than their absolute values.

Note that the three steady state response functions in the model—for cyclin degradation, Wee1A inactivation, and Cdc25C activation—all are Hill functions with high Hill exponents. In the case of Wee1A and Cdc25C, we have been able obtain some ultrasensitivity in reconstitution experiments with purified recombinant proteins[10, 12] (active Cdk1, Wee1A or Cdc25C, and the catalytic subunit of PP2A), but the responses seen in extracts are generally more highly ultrasensitive[10, 12]. This suggests that some additional factor, like a Cdk1-regulated phosphatase[15] might contribute to the switch-like Wee1A and Cdc25C responses, and we suspect that the same may be true for APC/C activation.

Delay Differential Equations (DDEs). The ODE model presented above does not account for the experimentally observed time lag between the activation of Cdk1 and the activation of cyclin degradation. The first way we added this time lag was through a two-DDE model:

$$\frac{d}{dt}Cyc[t] = k_{synth} - \left(a_{deg} + b_{deg}\frac{Cdk1[t-\tau]^{17}}{EC50_{deg}^{17} + Cdk1[t-\tau]^{17}}\right)Cyc[t] \qquad \text{[Eq 8]}$$

$$\frac{d}{dt}Cdk1[t] = k_{synth} + \left(a_{Cdc25} + b_{Cdc25}\frac{Cdk1[t]^{11}}{EC50_{Cdc25}^{11} + Cdk1[t]^{11}}\right)\left(Cyc[t] - Cdk1[t]\right) - \left(a_{Wee1} + b_{Wee1}\frac{EC50_{Wee1}^{3.5}}{EC50_{Wee1}^{3.5} + Cdk1[t]^{3.5}}\right)Cdk1[t] - \left(a_{deg} + b_{deg}\frac{Cdk1[t-\tau]^{17}}{EC50_{deg}^{17} + Cdk1[t-\tau]^{17}}\right)Cdk1[t] \qquad \text{[Eq 9]}$$

The advantages of this model include its compactness and simplicity. In addition, it is easy to analyze the contributions of ultrasensitivity and time lags separately. One disadvantage is that the time lags in a DDE model are more absolute that the time lags in an ODE model are. In addition the DDE model implicitly assumes a time lag in both the activation of cyclin degradation and the inactivation of cyclin degradation, and the latter time lag has not been experimentally demonstrated. For this reason we also formulated a multistep ODE model, described below, as an alternative way of introducing a time lag in APC/C^{Cdc20} activation.

A Multistep Model of APC/C^{Cdc20} Activation. Although the details of APC/C^{Cdc20} activation are incompletely understood, we can examine in general terms how the high degree of ultrasensitivity and long time delay might arise. The activation of APC/C^{Cdc20} depends upon Cdk1 activity, and during an unperturbed mitosis human APC/C becomes phosphorylated on at least seven subunits and at more than 30 sites[16, 17]. Here we hypothesize that activation of APC/C^{Cdc20} depends upon *n* independent Cdk1-dependent phosphorylation events (Fig. S5). The net amount of ultrasensitivity for such a multisite phosphorylation system depends both on the number of steps and on how cooperative the process is; if the last step is infinitely cooperative, an *n*-site phosphorylation reaction will yield a Hill exponent of *n*[10, 12, 18, 19]. If we arbitrarily assume that the last step in the process is 100-times more favorable than the preceding steps, a 25 to 30-site phosphorylation reaction will yield a Hill exponent of 16 to 18 (Fig. S5), similar to the experimentally measured ultrasensitivity (Figs. 1, 2). The same multistep mechanism can also

account for the observed time delay. If we choose kinetic parameters so that APC activation is half-maximal 35 min after a step-like activation of Cdk1 (cf. Fig. 5c), the 30-site phosphorylation model yields a time delay of approximately 15 min (Fig. S5). Thus, the observed steady-state and dynamic behaviors the negative feedback loop are consistent with a multistep phosphorylation/activation mechanism, with the nominal number of steps being approximately 25-30.

We can use this same mechanism to formulate an ODE model (rather than a DDE model) that incorporates a realistic time lag into the negative feedback loop. The model is a variation on the 2-ODE model depicted in Eqs 1 and 6. We assume that there are 30 phosphorylated forms of APC, and only the 30th form is active:

$$\frac{d}{dt}Cyc = k_{synth} - \left(a_{deg} + b_{deg}APC_{30}\right)Cyc \qquad \text{[Eq 10]}$$

$$\frac{d}{dt}Cdk1 = k_{synth} + \left(a_{Cdc25} + b_{Cdc25}\frac{Cdk1^{11}}{EC50_{Cdc25}^{11} + Cdk1^{11}}\right)\left(Cyc - Cdk1\right) - \left(a_{Wee1} + b_{Wee1}\frac{EC50_{Wee1}^{3.5}}{EC50_{Wee1}^{3.5} + Cdk1^{3.5}}\right)Cdk1 - \left(a_{deg} + b_{deg}APC_{30}\right)Cdk1 \qquad \text{[Eq 11]}$$

$$\frac{d}{dt}APC_0 = -k_{APCphos}Cdk1APC_0 + k_{APCdephos}APC_1 \qquad \text{[Eq 12]}$$

$$\frac{d}{dt}APC_1 = k_{APCphos}Cdk1APC_0 - k_{APCdephos}APC_1 - k_{APCphos}Cdk1APC_1 + k_{APCdephos}APC_2 \qquad \text{[Eq 13]}$$

$$\frac{d}{dt}APC_2 = k_{APCphos}Cdk1APC_1 - k_{APCdephos}APC_2 - k_{APCphos}Cdk1APC_2 + k_{APCdephos}APC_3 \qquad \text{[Eq 14]}$$

$$\vdots$$

$$\frac{d}{dt}APC_{29} = k_{APCphos}Cdk1APC_{28} - k_{APCdephos}APC_{29} - c\cdot k_{APCphos}Cdk1APC_{29} + \frac{1}{c}\cdot k_{APCdephos}APC_{30} \qquad \text{[Eq 15]}$$

$$\frac{d}{dt}APC_{30} = c \cdot k_{APCphos}Cdk1APC_{29} - \frac{1}{c} \cdot k_{APCdephos}APC_{30} \quad \text{[Eq 16]}$$

The constant *c* reflects the cooperativity of the phosphorylation and dephosphorylation reactions. In Fig. S5b, c we assume it to be 10, which allowed both the effective Hill coefficient of the response of APC_{30} to $Cdk1$ to be 18 (Fig. S5b) and the time lag after a step function increase of Cdk1 to 90 nM to be 15 min (Fig. S5c), comparable to the experimental results. Thus, it allowed us to directly compare the 32-ODE model together with the 2-ODE and DDE models. As shown in Fig. S4f, the three models produced similar oscillation profiles of Cyc and Cdk1 in their time courses even though their oscillation periods are somewhat different. The 32-ODE and 2-DDE models also share very similar trajectories of the limit cycles in phase plane (Fig. S5e).

Next, we asked whether the 32-ODE model (and more generally, any multi-step-ODE model) which incorporates a realistic time lag of 15 min into the negative feedback loop also promotes the robustness of oscillations to parameter variations, as we have seen with the DDE model with an explicit time delay of 15 min. To this end, we performed bifurcation analyses for seven parameters at given values of the Hill coefficient n_H ranging from 0 to 17, and compared the ranges of parameters that yielded limit cyclesfor the multi-step-ODE model and the 2-ODE models. Note that to allow for a direct comparison of the bifurcation diagrams of the multi-step-ODE and 2-ODE models, we had to tune the parameters for the multi-step-ODE model so that it always shared the exact same shape of the response function of active APC to active Cdk1 as the one in the 2-ODE model, that is, the two models should have comparable effective Hill coefficients and EC50s. To achieve this, we first calculated the number of steps of APC phosphorylation which gave an effective Hill coefficient comparable to the n_H for the APC to Cdk1 response in the 2-ODE model. For example, 5, 10, 30 steps generate the effective Hill coefficients of 4.4, 7.6, and 16.9. Next, for any given n_H, we solved the ratio of the

dephosphorylation to phosphorylation rates assuming the EC50 was to be kept at 32 nM. Finally, we tuned an APC speed factor (a multiplicative factor that tunes the dephosphorylation and phosphorylation rates simultaneously) to allow the resulting effective time lag of 15 min. Together, these allowed us to compare the multi-step-ODE models including a realistic time lag of 15 min into the negative feedback loop to the 2-ODE model, and for all seven parameters shown in Fig. S5g the multi-step-ODE model was more robust with respect to parameter variations than the 2-ODE model.

Numerical Simulations. The ODE and DDE models were solved numerically using MATLAB R2010b (MathWorks, Natick MA) or Mathematica 7 (Wolfram Research, Champaign IL). Bifurcation analysis was done either with the MATLAB package MatCont[20], for the ODE models, or with a custom-written MATLAB package for the DDE models. Model parameters are listed in Table S1. Stochastic simulations were carried out using the Gillespie algorithm[21].

Supplemental References

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