Cell-Cycle Oscillator and Mitotic Trigger Wave -driven Spatiotemporal Coordination

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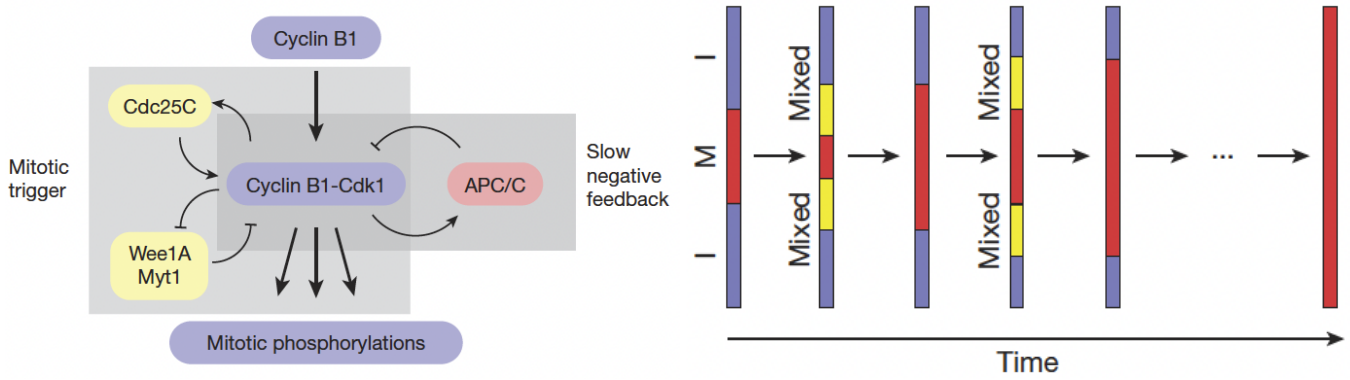
1 Introduction

A fertilized egg, despite its size, rapidly proceeds through mitosis with spatial coordination. There is abrupt reorganization in a cell during mitosis abrupt reorganization, and these processes are known to be driven by the activation of the protein kinase *Cdk1* [1, 2]. This activation likely occurs at the centrosome. While near-synchronous onset of mitosis can occur in typical somatic cells simply through random walk diffusion, the timescales become very large and unrealistic for diffusing to to all parts for larger, eukaryotic cells that can be as much as 60-100 times larger in radius.



(a) Cdk1-APC/C Signaling Circuit (b) Schematic Propagation of Cdk1 along 1D Tube

Figure 1: Trigger waves drive spatiotemporal coordinaton of mitosis. *Figures adopted from [3].*

Figure 1a shows the Cdk1-APC/C signaling circuit that is responsible for cell mitosis. The bistability of this system supports the possibility that tirgger waves might explain the near-synchronous spatiotemporal coordination of mitosis even in large cells. Figure 1b depicts a conceptual trigger wave-driven mitosis in a long thin tube containing cytoplasm with a uniform concentration of cyclin-B1 and Cdk1. Assuming that in some region of the tube the cytoplasm is in the mitotic, high Cdk1-activity state (shown in red) while the rest of the cytoplasm is in the interphase, low Cdk1-activity state (shown in blue), within some small distance of the interface, the cytoplasm will rapidly mix by diffusion. This results in an intermediate level

of Cdk1 activity in these regions (shown in yellow). If this activity is above the unstable steady state of the hysteretic Cdk1-Cyclin dynamical system, this slice of cytoplasm will flip to the mitotic state (changes yellow to red, in figure). The process of mixing and conversion repeats, and the mitotic state propagates down the tube at a constant velocity.

Chang et al. [3] showed, using an extract system that performs cell cycles in vitro, that mitosis does in fact spread through Xenopus cytoplasm via trigger waves, propagating at a linear speed of approximately $60\mu m$ min^{-1}. Further, they perturbed the feedback loops that give rise to the bistability of Cdk1 and showed that it alters the trigger wave dynamics. Then, by time-lapse imaging of intact egg, they show that trigger waves of Cdk1 activation are responsible for surface contraction waves [4]. Overall, they present evidence that Cdk1 trigger waves play a crucial role in ensuring spatiotemporal coordination of mitosis (onset) in large eggs. And in more general, the findings also suggest that trigger waves may be a more general mechanism responsible for coordinating biochemical events over large distances.

2 Results

This section expands on the implementation of the partial differential equation (PDE) model of Cdk1 activation and trigger wave propagation, and the model to extrapolate the point of mitotic wave origin from observed surface contraction waves.

2.1 PDE Model of Cdk1 Activation and Propagation

To computationally model the spatiotemporal coordination hypothesis, I re-implemented the proposed PDE model of *Cdk1* activation and propagation. In the model, I assumed that *cyclin-B1* was synthesized at a uniform, constant rate everywhere in the cytoplasm, except in a $0.5\mu m$ section of the tube, where the concentration of the mitotic activator *Cdc25C* was 50% higher in concentration. This inhomogeneity is meant to represent the centrosome where the onset of mitosis occurs first.

I was able to reproduce the results presented in Figure 1d of the paper. As expected, the activation of *Cdk1* occurred first in the high *Cdc25C* region, and then to spread linearly up and down the tube. This caused a V-shaped wavefront of *Cdk1* activation. The propagation rate, which is the slope of the diagonal wave fronts, was approximately $121\mu m$ min^{-1}, compatible with the estimates from Luther's formula. In regions farther from the centrosome, *cyclin* synthesis reached the threshold for *Cdk1* activation before the trigger wave arrived, resulting in a vertical front of *Cdk1* activity. However, with successive cycles, the trigger waves came to occupy more and more of the tube.

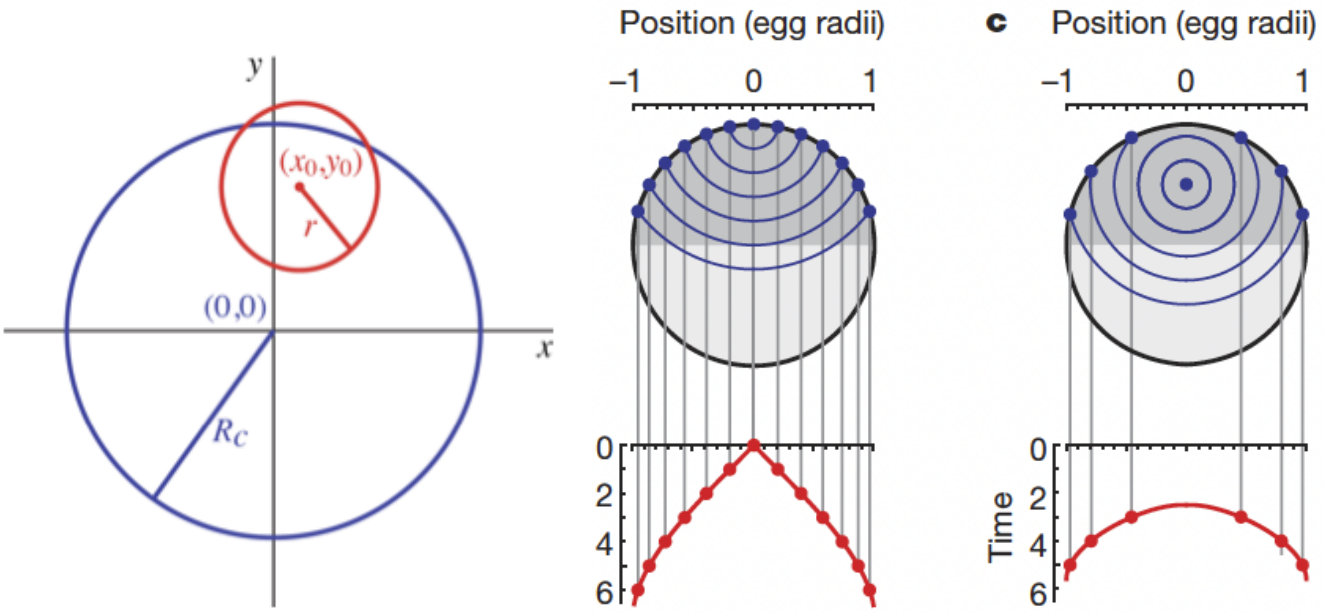
Implementation Challenges. *First*, with the parameters reported in the paper, I was not able to reproduce characteristic V-shape of the trigger wave propagation; they appeared as straight lines extending all the way to either end despite increasing the timescale of simulation or the total width of the tube. This suggested that diffusion effect was dominating, so I increased the concentration of the higher *Cdc25C* concentration region from 50% to 75%. These gave rise to similar patterns as can be seen in Figure 1d of the paper, except the trigger waves extend a little longer than shown there. And the propagation speed I obtained was about twice that reported in the paper ($121\mu m$ min^{-1} instead of $60\mu m$ min^{-1}). *Second*, writing and solving PDEs on Matlab was a maddening and time-consuming yet fun activity on its own!

2.2 Estimating Cdk1 Wave Origin based on Time of Incidence at Surface

The paper finally presents an experiment that assess whether the trigger waves observed in extracts can also be seen in intact fertilized eggs. In this three-dimensional structure, the waves of *Cdk1* activation could originate at centrosome, spread outwards, and finally cause observable surface contraction waves (SCWs) at the cortical cytoskeleton. To connect the point of origin of these waves to the actually observable phenomena – time of their incidence at different points on the surface manifesting as SCWs, they devise a mathematical model using the great circle plane of the spherical egg cell. In Figure 2b, the blue circle represents the great plane of the egg while the red circle depicts that of the propagating spherical *Cdk1* activation wave, whose radius increases as a function of time and wave speed. Assuming that the centrosome lies on the y-axis and solving for time and x-position using the circle equations of the constant blue circle and time-dependent red circle, we find characteristic difference in shapes of the SCW incidence (see Figure 2b).

I was able to reproduce the theoretically predicted wavefronts in Figure 4b and 4c of the paper. I additionally obtained plots for a few more cases using the same relation to see the changing wavefronts; this is presented in Figure 3. These plots follow the same coordinate system as the one in Figure 2a and the negative sign on the wave origin y-coordinate denotes that it is located on the animal pole of the egg. Both x and y-coordinates are expressed as a fraction of the egg cell radius; hence, $y = 0.05$ lies close to the center of the egg, while $y = 0.95$ lies closer to the cytoskeleton. The gradual of change in shape of the wavefront from U-like to V-like can be seen as the position of origin shifts away from the center of the egg cell (increasing magnitude of y-coordinate).

Implementation Challenges. This implementation was fairly straightforward. The only portion that required some thought was in obtaining a good density of points to obtain a good estimate of the wavefront.



(a) Schematic of the incidence model. (b) Propagation wavefronts for different points of origin.

Figure 2: Surface contraction waves in intact fertilized Xenopus eggs. *Figures adopted from [3].*

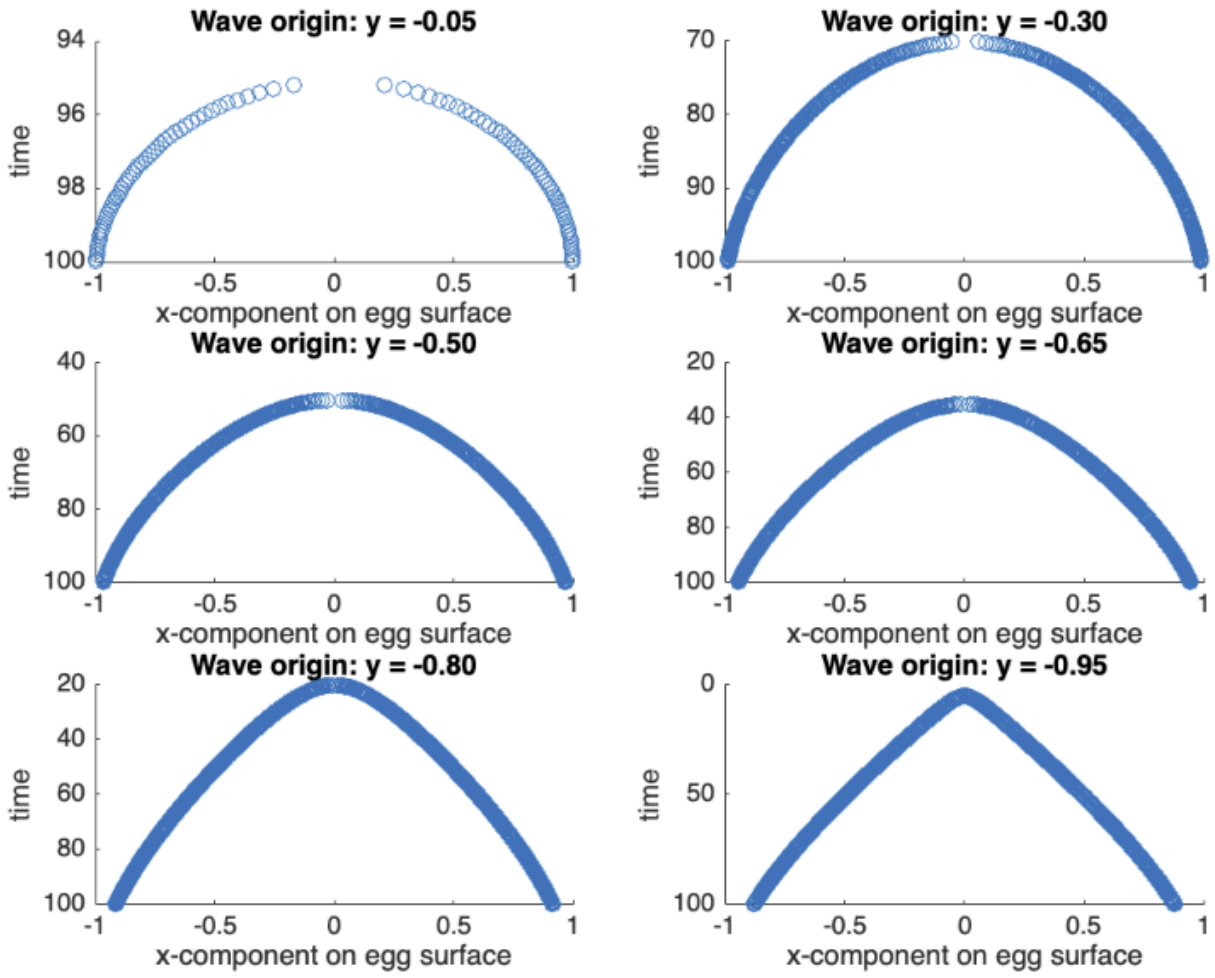


Figure 3: Wavefronts of *Cdk1* activation waves incident on the intact egg surface modeled for different points of origin.

3 Extensions

The mitotic trigger waves were modeled based on two ordinary differential equations (ODEs) that describe the synthesis/formation and degradation of *cyclin B1-Cdk1* complexes, and the interconversion of *cyclin B1-Cdk1* between active and inactive phosphorylation states (see Figure 1a). Spatial diffusion terms were added to these ODEs to arrive at the PDEs modeled above.

3.1 ODE Model of the Cdk1-APC/C Circuit

To better understand the dynamics of the *Cdk1-APC/C* signaling circuit, I modeled this signaling circuit using a two-ODE model [5]. In this model, *cyclin* synthesis is assumed to occur at a constant rate k_{synth}, while *cyclin* degradation occurs as a Hill function of the activity of *Cdk1* influenced by the activation of *APC/C*. This *cyclin* rate equation is presented in Equation 1.

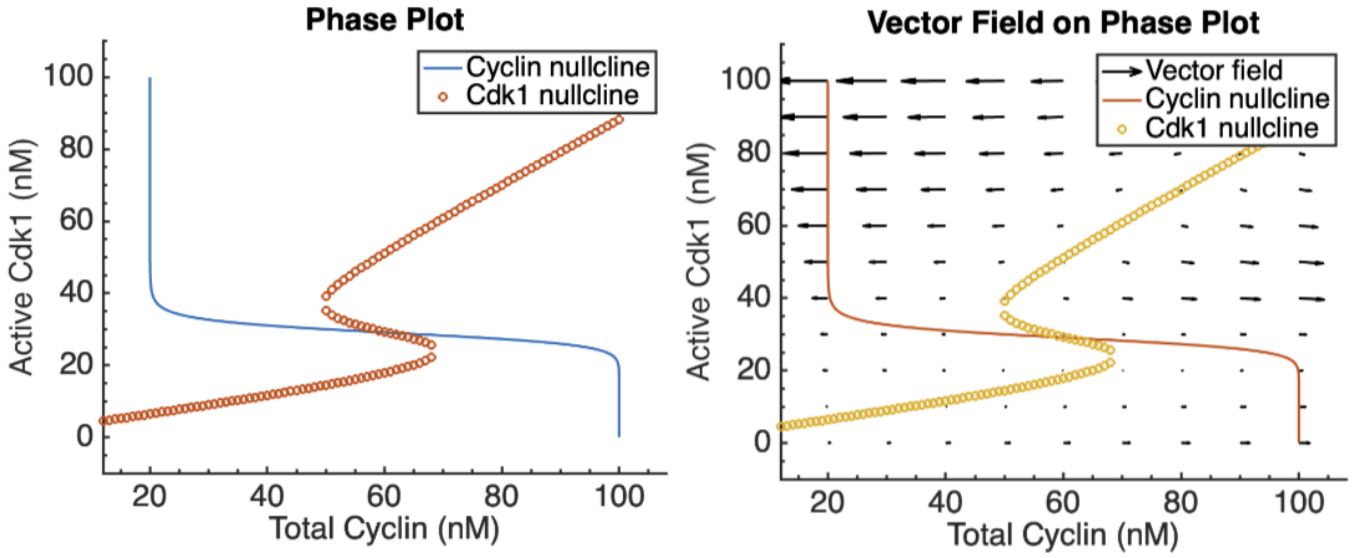
$$\frac{d}{dt}Cyc = k_{synth} - \left(a_{deg} + b_{deg}\frac{Cdk1^{n_{dtg}}}{EC50_{deg}^{n_{dkg}} + Cdk1^{n_{dxp}}}\right)Cyc \tag{1}$$

The activity of *Cdk1* depends on cyclin synthesis k_{synth}, cyclin degradation modeled above, and the positive and double-negative feedback loops. 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. The correspinding rate equation is shown in Equation 2, where $Cdk1$ represents the amount of active *Cdk1*.

$$\frac{d}{dt}Cdk1 = k_{synth} + \left(a_{Cdc25} + b_{\text{Cdc25}}\,\frac{Cdk1^{11}}{EC50_{Cdc25}^{11} + Cdk1^{11}}\right)(Cyc - Cdk1) - \left(a_{\text{Wee1}} + b_{\text{Wee1}}\,\frac{EC50_{\text{Wee1}}^{3.5}}{EC50_{\text{Weel}}^{3.5} + Cdk1^{35}}\right)Cdk1 - \left(a_{deg} + b_{\text{deg}}\,\frac{Cdk1^{17}}{EC50_{deg}^{17} + Cdk1^{17}}\right)Cdk1 \tag{2}$$

To identify the stable state of this two-ODE system, I solved the rate equations analytically to obtain their nullclines and produced a phase plot of *active Cdk1* and *total cyclin*. These results are presented in Figure 4. The steady state was obtained at around $[Cdk1_{act}] = 29nM$ and $[Cyclin_{tot}] = 61nM$. This value and the nullclines obtained are in good agreement with previous work [5]. Further, to visualize their oscillatory region, I plotted the vector fields on the phase plots along with the nullclines (see Figure 4b); it is easy to verify that a limit-cycle exists and the oscillatory regions agrees with previous work, too.

Implementation Challenges. *First*, the rate equation of *Cdk1* (Equation 2) was not easy to solve analytically, and neither Matlab nor Mathematica produced a



(a) (b)

Figure 4: Phase plots of the two-ODE representation of the Cdk1-APC/C circuit.

nullcline expression for *Cdk1* as a function of *total cyclin* analytically. Consequently, I used Matlab's numeric solver to obtain *Cdk1* levels at multiple amounts of *cyclin*. An interesting challenge here was that, in the hysteretic region, there are multiple solutions to *Ckd1* levels for the same amount of *cyclin*. So it involved a bit of a coding exercise to search different, mutually-exclusive intervals for additional solutions for *Cdk1* in the hysteretic region, while keeping the running time of the slow *vpasolve* function in check. *Second*, the vector field obtained from Matlab's *quiver* is hard to interpret in regions of small vector magnitude; I used WolframAlpha to look the oscillatory region instead (not presented here).

3.2 Oscillations in Total Cyclin and Active Cdk1 levels

I again used the same parameters as for the PDE models to simulate these ODEs to keep them consistent. The resulting simulated time-course of *Cdk1* and *cyclin* concentrations are shown in Figure 5. The system clearly exhibits oscillatory behavior. The periods of both *Ckd1* and *cyclin* were about 90 minutes, as interpreted from the plots (see codebase). These shapes and obtained values are consistent with previously published work [5, 6].

Implementation Challenges. No challenges in particular. I implemented the ODE equations as Matlab expressions and not surprisingly, this made the implementation less repetitive, easy to reuse, and easy to fix mistakes with equations. The ODE simulations were quite long in terms of computation time.

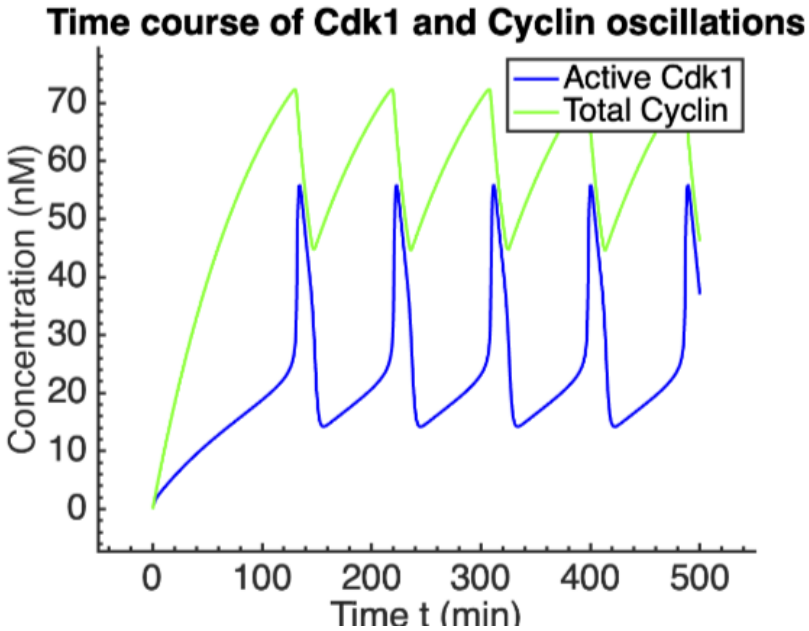


Figure 5: Time-course of active *Cdk1* and total *Cyclin* concentrations from the two-ODE model.

3.3 Suppressing Positive Feedback Damps Oscillations

To further dissect the dynamics of the oscillations produced in the Cdk1-APC/C circuit, I tried to assess the effect of short-circuiting the positive feedback loops in the circuit relating *active Cdk1* levels, namely those of *Cdc25* and *Wee1A*. In Equation 2, this is encapsulated by the second and third terms. These terms represent a Hill function-defined sensitivity *active Cdc25C* and *Wee1A* levels, respectively, both of which are functions of *Cdk1* and are early substrates in the phosphorylation downstream of *Ckd1*. Hence, they are assumed to be in equilibrium with *Cdk1* levels and the steady-state can be represented as shown in Equation 3 for *Cdc25* and similarly for *Wee1A*. Hence, positive feedback can be suppressed by decreasing the rate constants k_{Wee1A} (not shown here) and k_{Cdc25}. This in turn is the same as numerically dividing the second and third terms of Equation 2 by a positive whole number greater than one. To this end, I introduced an additional parameter into the two-ODE model called *pos_fb_strength*. This parameter is pre-multiplied with the *Wee1A* and *Cdc25* -related terms of *Cdk1*'s rate equation during ODE simulation. Lower the value of *pos_fb_strength*, lower the strength of positive feedback.

$$k_{Cdc25}Cdc25^{*} = a_{Cdc25} + b_{Cdc25}\frac{Cdk1^{11}}{EC50_{cdc25}^{11} + Cdk1^{11}} \tag{3}$$

The obtained results from this perturbation experiment are presented in Figure 6. It is clear that as positive feedback is suppressed, the oscillations are first sustained but with reduced amplitdue; after that, the oscillations are damped and they eventually disappear as the strength of positive feedback decreases further. In summary, in the limit of slow cyclin synthesis and destruction, bistability is strictly required for oscillations. This observation is consistent with previously published results [6].

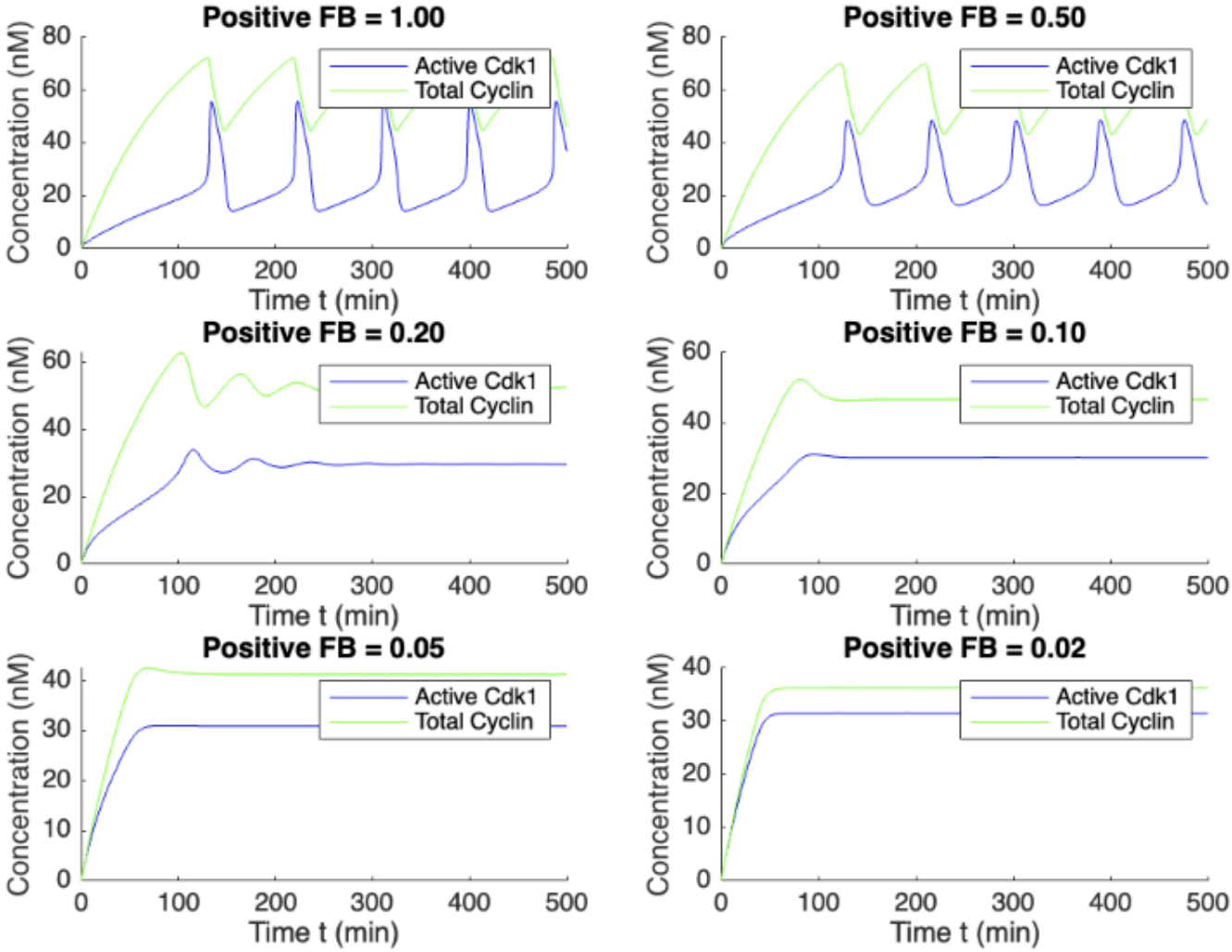


Figure 6: Effect of suppressing positive feedback on the oscillation dynamics of the Cdk1-APC/C circuit based on the two-ODE model.

4 Conclusion

It is evident from computational modeling experiments reproduced here and from the experimental results presented previously [3] that trigger waves are a crucial phenomena in ensuring spatiotemporal coordination of mitosis in embryonic egg cells. Furthermore, this along with previous work on trigger waves in diverse contexts [7] presents strong evidence that owing to slow speed of random walk diffusion, trigger waves might be a supporting mechanism for communicating over large cellular and/or tissue distances to coordinate and synchronize biochemical events.

Analyzing the *Cdk1-APC/C circuit* in particular was useful to understand the dynamics of the biochemical events that drive mitosis in cells. It helped develop a more comprehensive picture of mitotic trigger waves. But more broadly, the final analysis on perturbing the strength of positive feedback loops especially, provided insight into the roles of different subcircuits within a given dynamical system and how oscillations can emerge out of combinations of subcircuits whose individual behaviors are not oscillatory.

Challenges. Overall, there was some difficulty in finding parameters for published ODE models. While this was very directly supplied with base paper [3] and required only minor tweaking, for the other ODE models, trying out parameters based on the information in the paper was particularly challenging. Specific challenges during implementation are included in relevant sections of this report.

Code Availability

The code for all the experiments performed in the project can be accessed from: `https://github.com/karthik-d/dynamical-modeling-of-biological-systems/tree/main/project_mitotic-trigger-waves`. This project is primarily based on Chang et al. [3].

References

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