Clocks and Dominoes: Studies into Positive Feedback and the Continuum from Early Embryo to Somatic Cell

Joe Pomerening

Indiana University Department of Biology and The Biocomplexity Institute

Kavli Institute for Theoretical Physics - Biological Clocks

July 26, 2007

INDIANA UNIVERSITY
BLOOMINGTON

the biocomplexity institute

1. Digital Responses In Vivo: Mitotic Toggle Switches?

2. The Embryonic Mitotic Oscillator: Positive Feedback and the Emergent Properties of the CDK1/Cyclin B Module

3. Positive Feedback and Somatic Mitosis: To Enter, To Exit, To Not Oscillate?

Digital Biology Converted from Graded Stimuli

Division

Stimulus → CONVERSION → Response

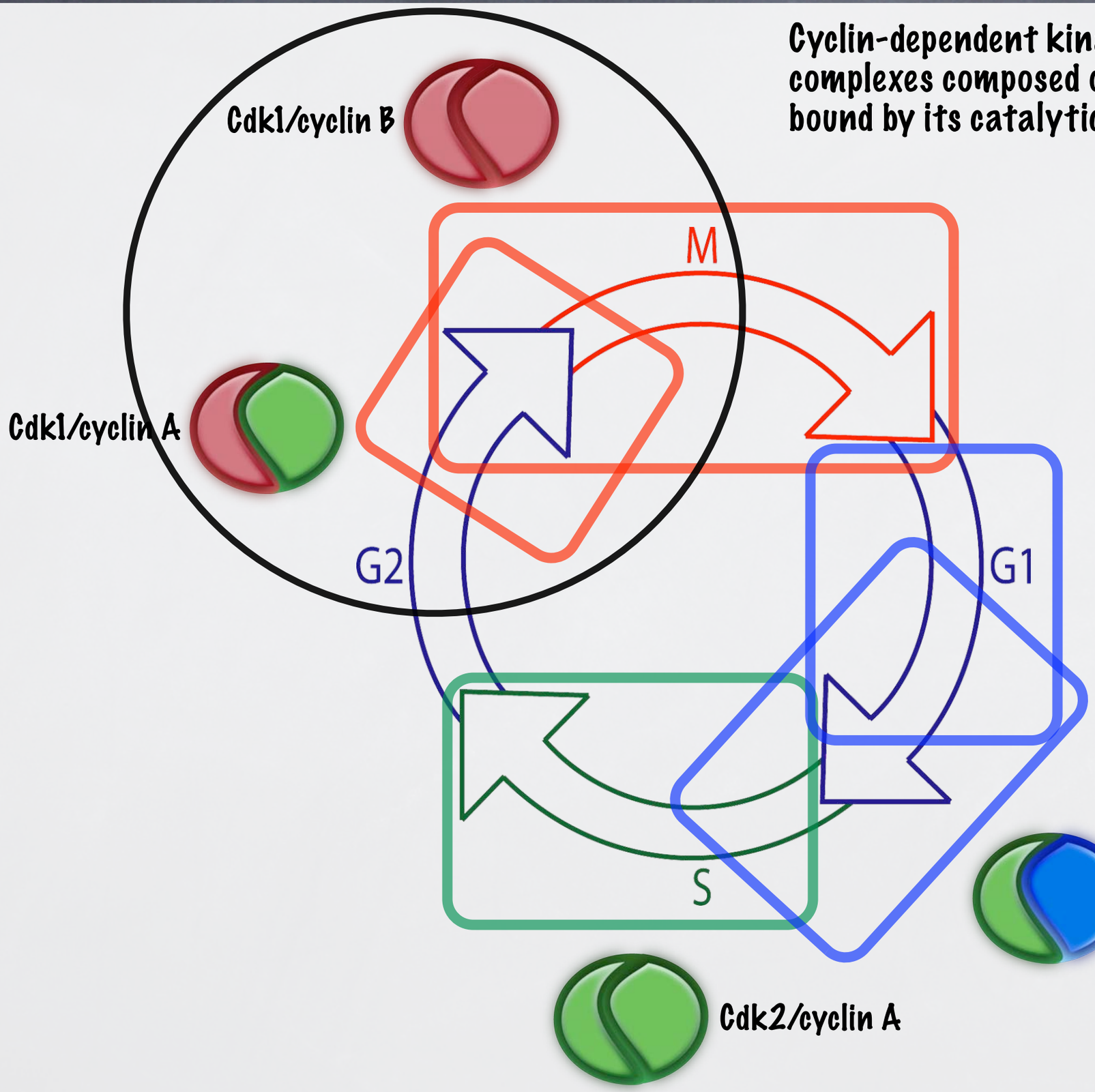
Cyclin

All-or-none character.

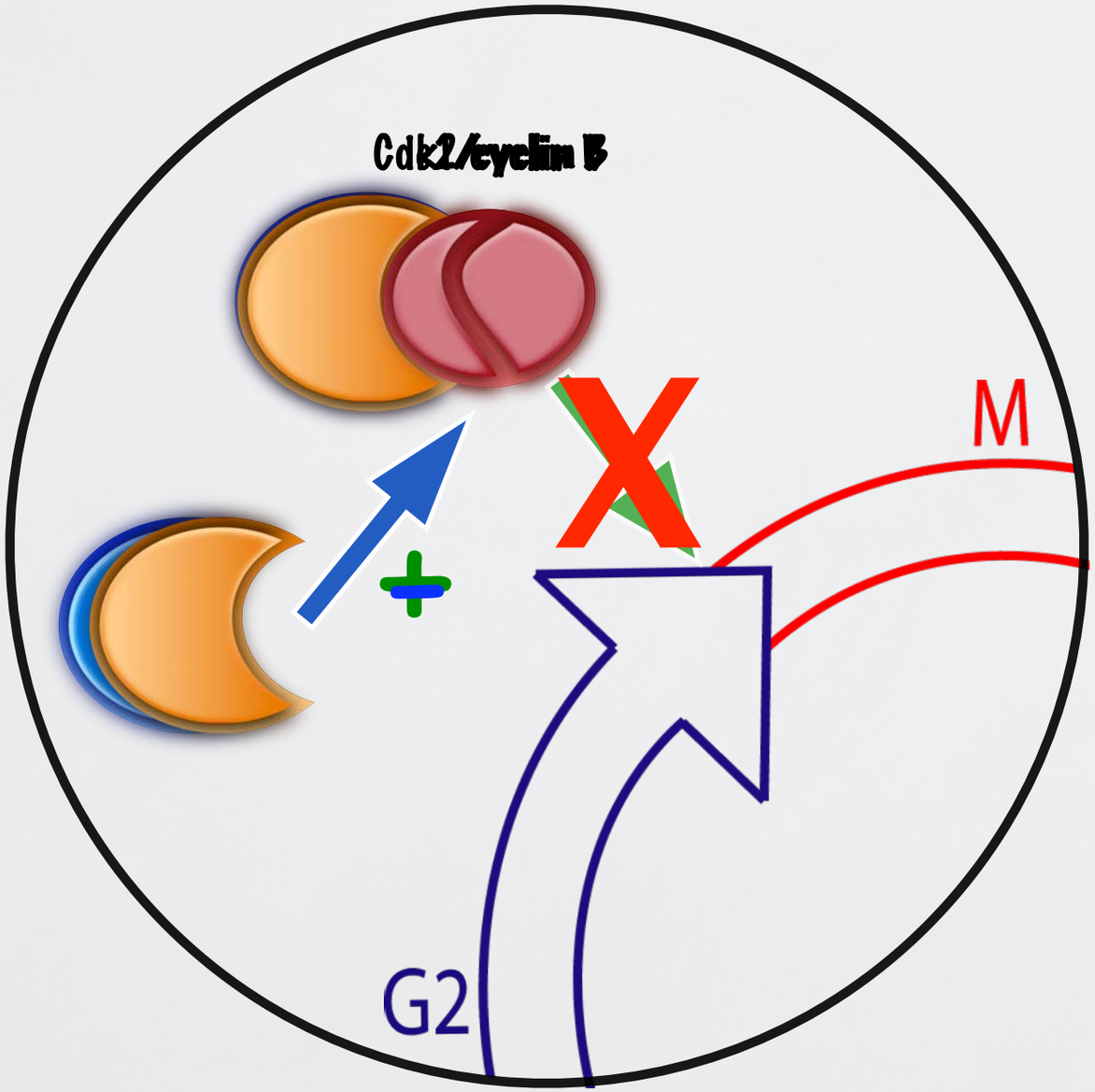
Cells must avoid "half-way" completion of mitosis; returning to a "G2 state".

What About Mitosis, its Inputs and Outputs?

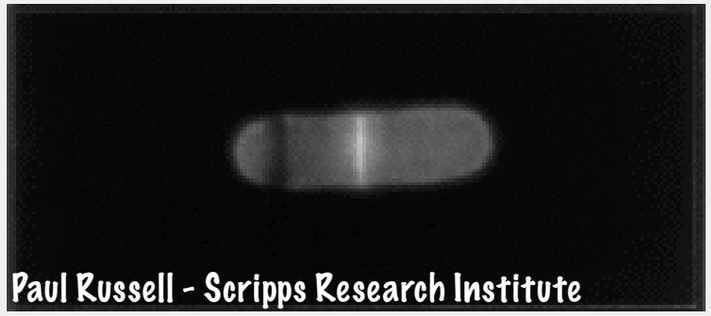
Cyclin-dependent kinases (CDKs) are heterodimeric complexes composed of a regulatory subunit (cyclin), bound by its catalytic counterpart.



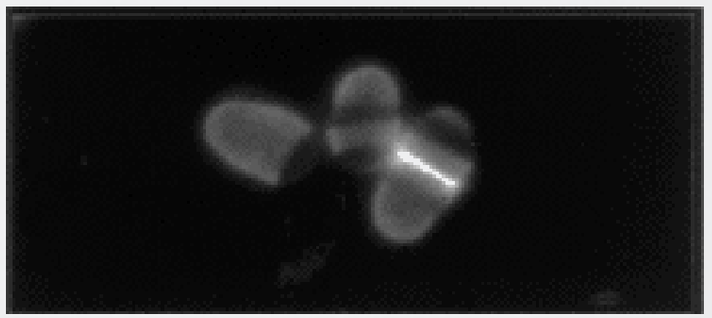
What About Mitosis, its Inputs and Outputs?



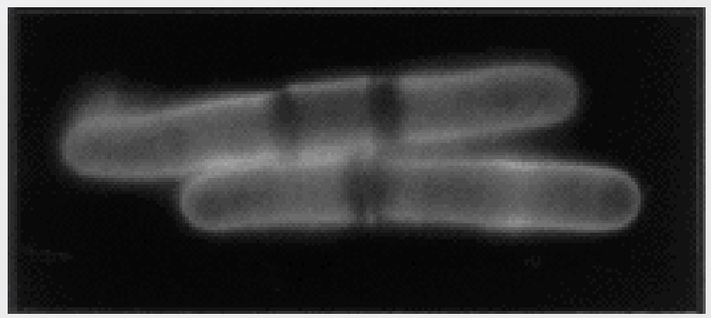
WT *S. pombe*



wee1-50



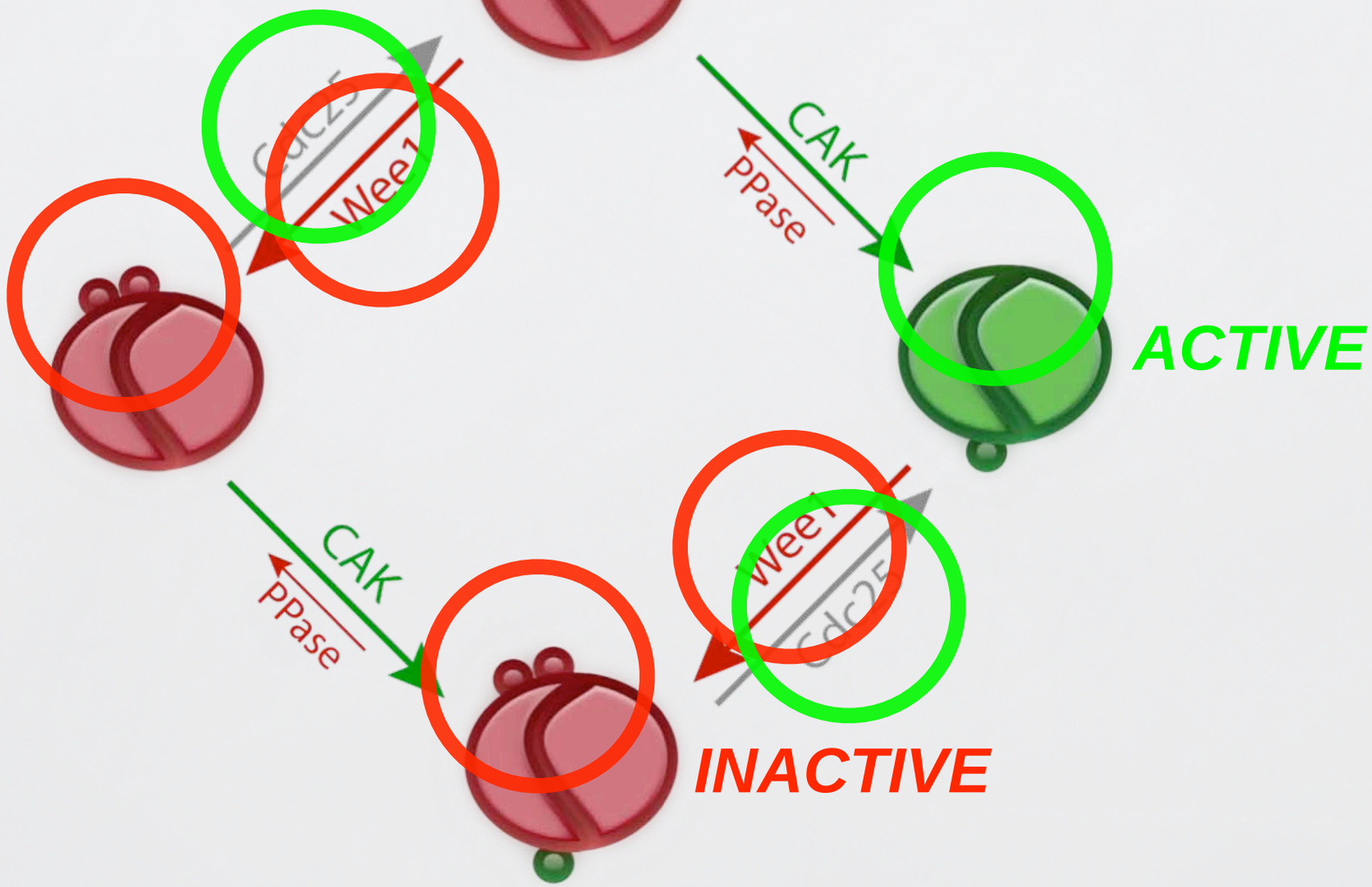
cdc25-22



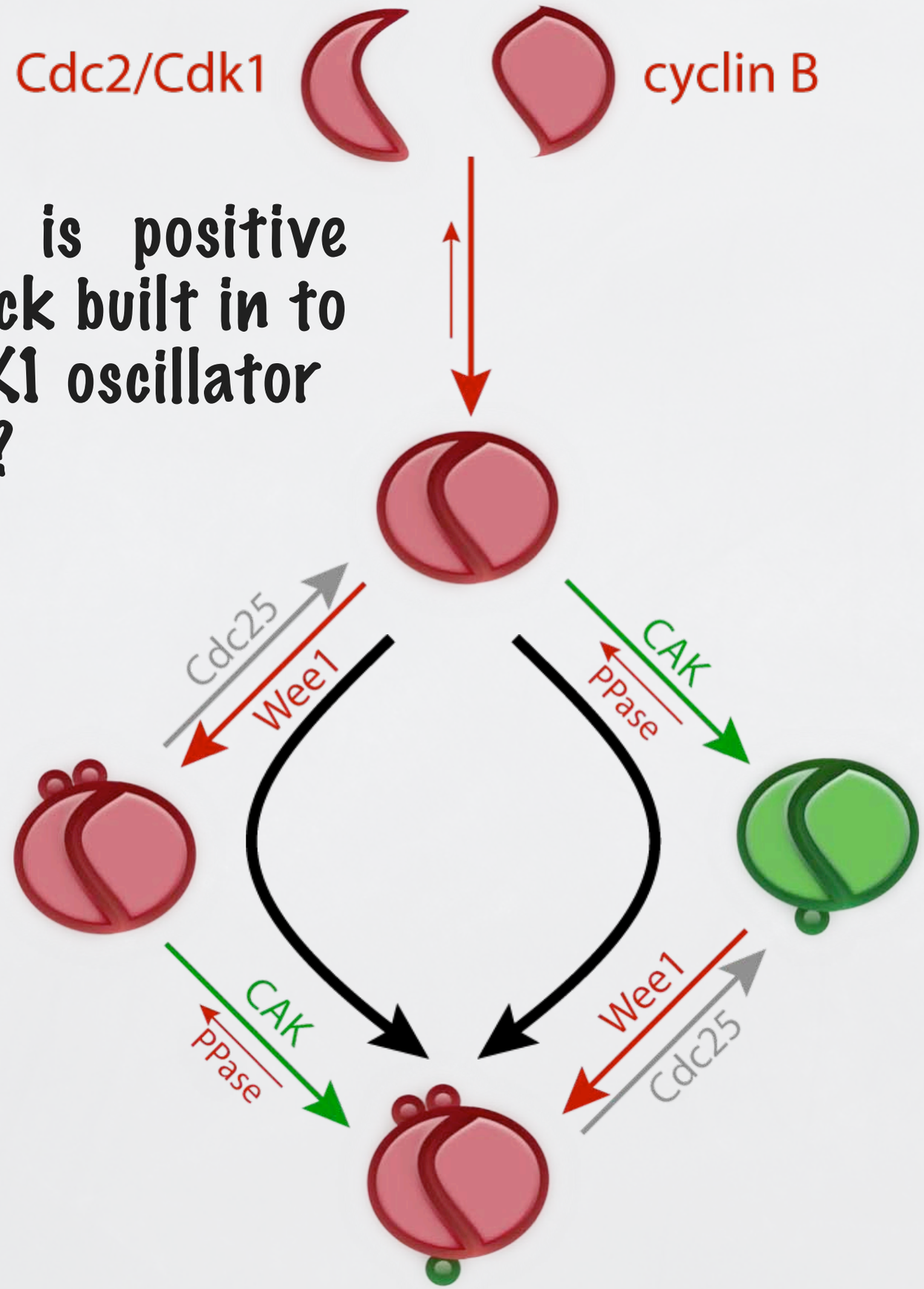
Cdc2/Cdk1

cyclin B

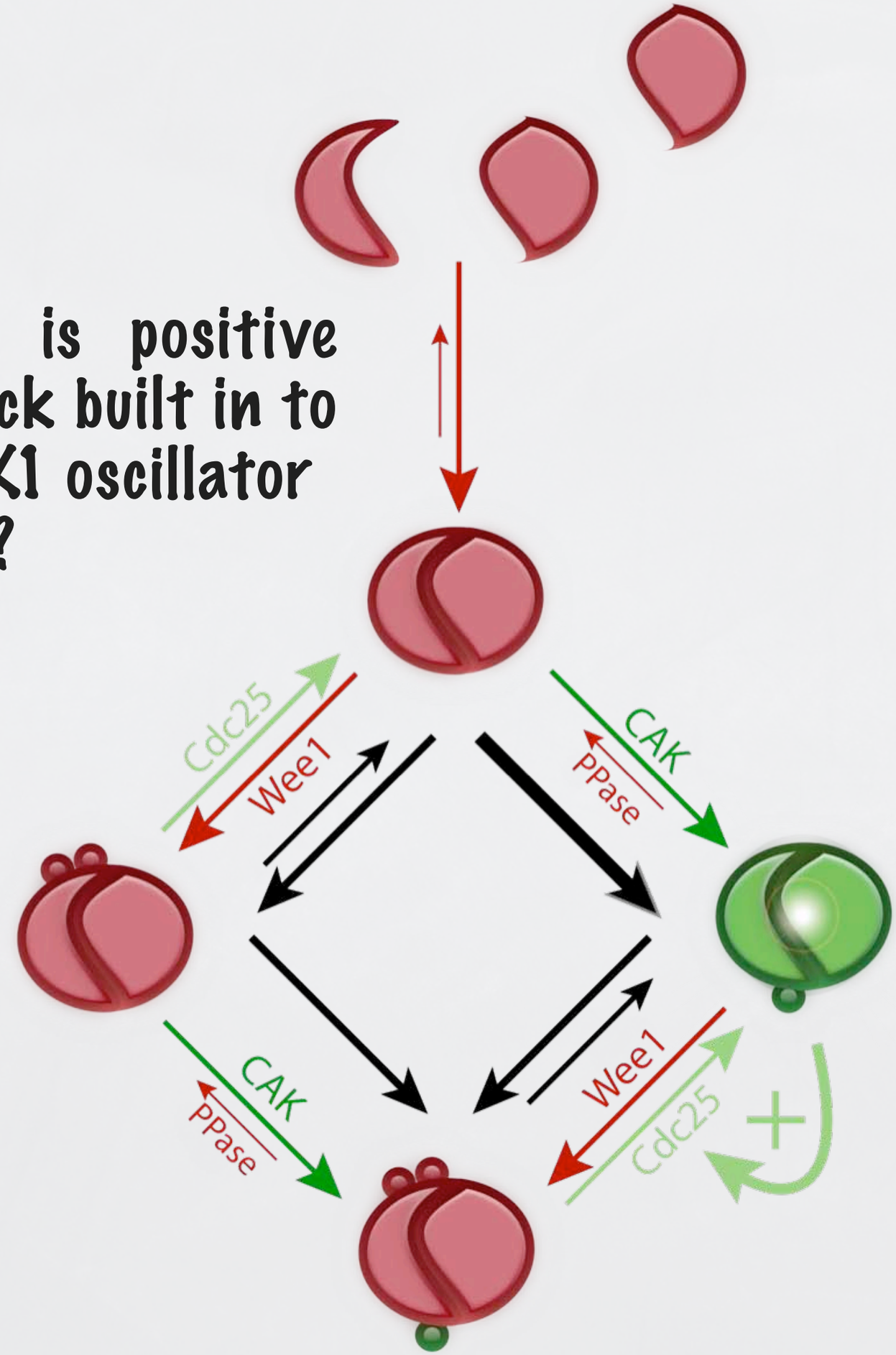
Cyclin binding is essential to CDK activation, but post-translational modifications truly engineer the "wiring" of the M-phase circuit.



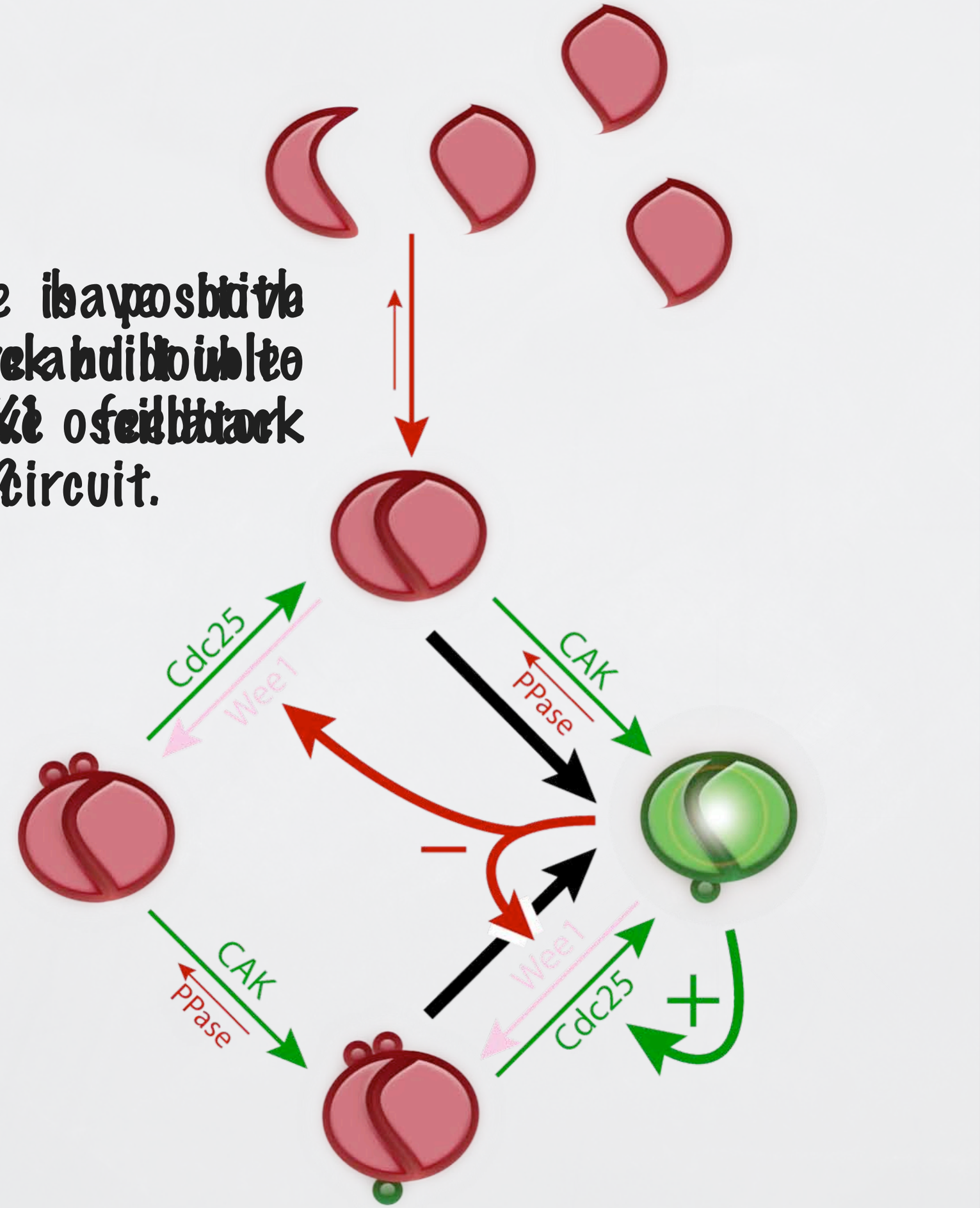
Where is positive feedback built in to the CDK1 oscillator circuit?



Where is positive feedback built in to the CDK1 oscillator circuit?

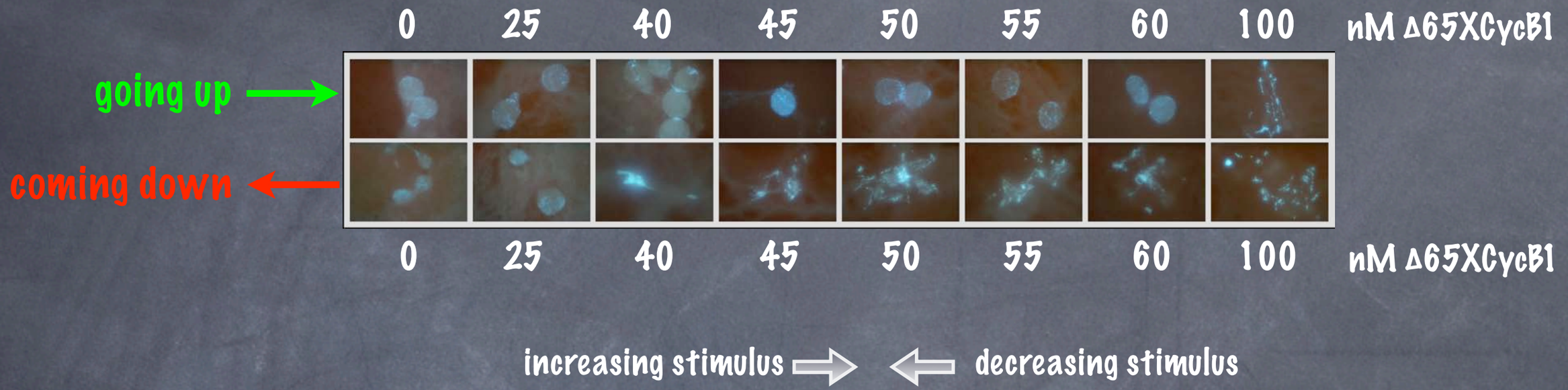


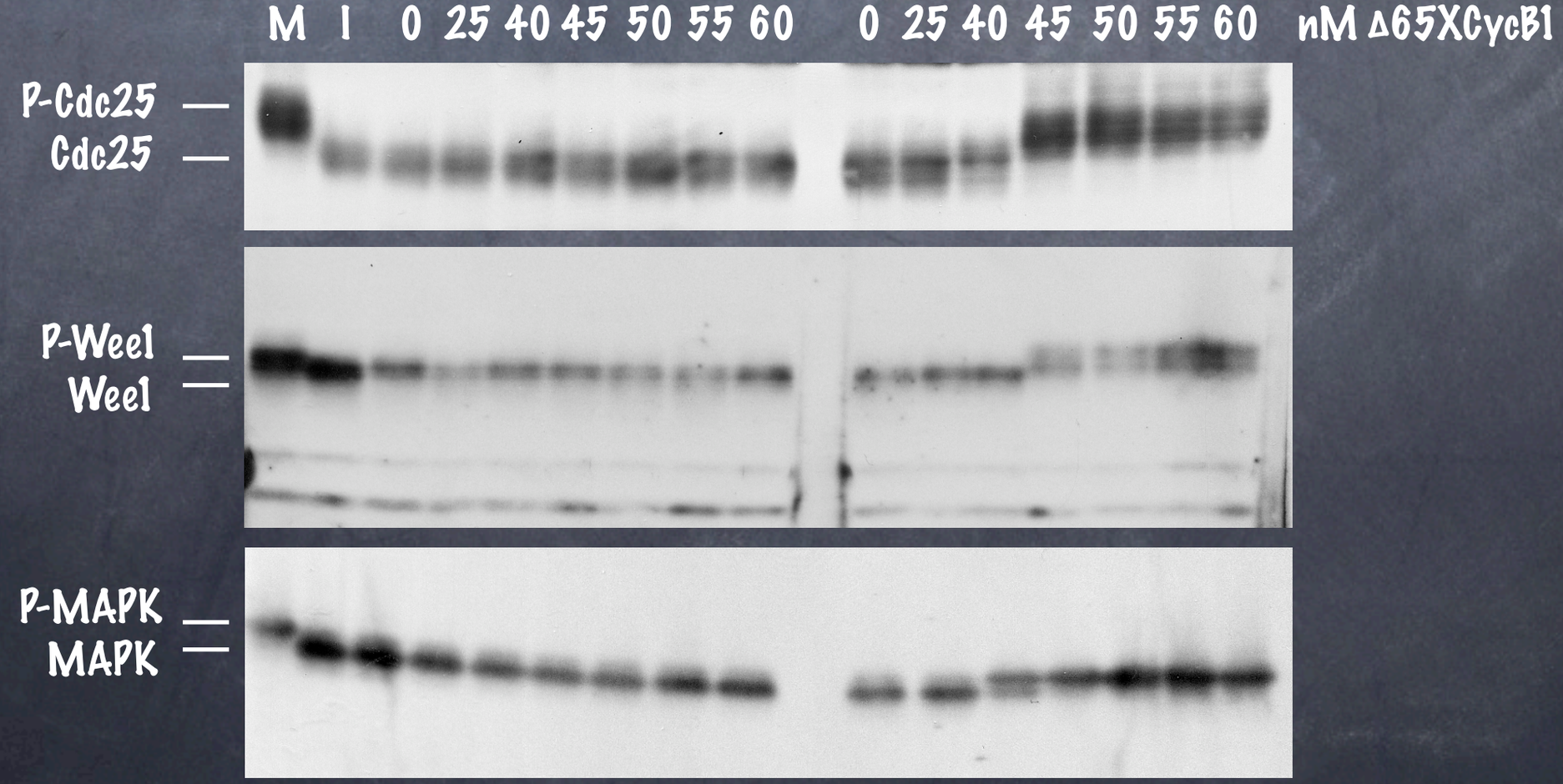
Where is a possible positive feedback loop? Where is a possible negative feedback loop? Where is a bistable circuit?



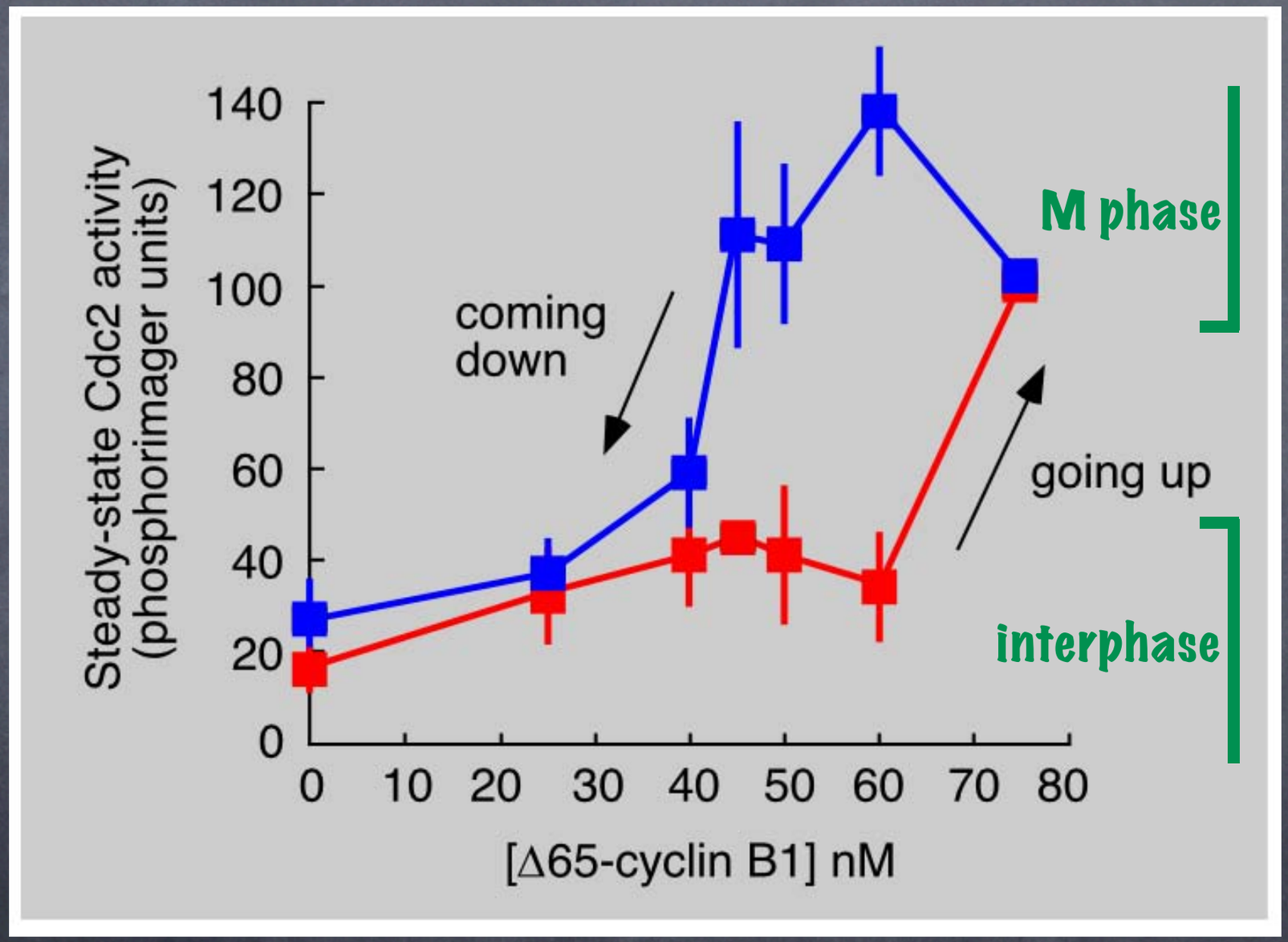
What is the steady-state response of CDK1 to cyclin B?

Cell Biological and Molecular Markers Exhibit Hysteresis





Hysteresis in CDK1 Activation



positive feedback

hysteresis

bistability

Testing a Mathematical Model of the Yeast Cell Cycle

Frederick R. Cross,* Vincent Archambault, Mary Miller, and Martha Klovstad

The Rockefeller University, New York, New York 10021

Submitted May 25, 2001; Revised September 25, 2001; Accepted October 10, 2001
Monitoring Editor: Mark J. Solomon

We derived novel, testable predictions from a mathematical model of the budding yeast cell cycle. A key qualitative prediction of bistability was confirmed in a strain simultaneously lacking *cdc14* and G1 cyclin

Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts

Wei Sha*, Jonathan Moore†, Katherine Chen*, Antonio D. Lassaletta*, Chung-Seon Yi*, John J. Tyson*, and Jill C. Sible*‡

*Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0406; and †Cancer Research UK London Research Institute, Clare Hall Labs, South Mimms, Herts, EN6 3LD, United Kingdom

Edited by Thomas D. Pollard, Yale University, New Haven, CT, and approved November 21, 2002 (received for review September 3, 2002)

Cells progressing through the cell cycle must commit irreversibly to mitosis without slipping back to interphase before properly segregating their chromosomes. A mathematical model of cell-cycle progression in cell-free egg extracts from frog predicts that irre-

activated by removal of the inhibitory phosphate groups. Because Cdc2 activates cyclin proteolysis, the rate of cyclin degradation in M phase exceeds its rate of synthesis, and cyclin concentration falls. However, according to the model, the extract

Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2

Joseph R. Pomerening*, Eduardo D. Sontag† and James E. Ferrell Jr*‡

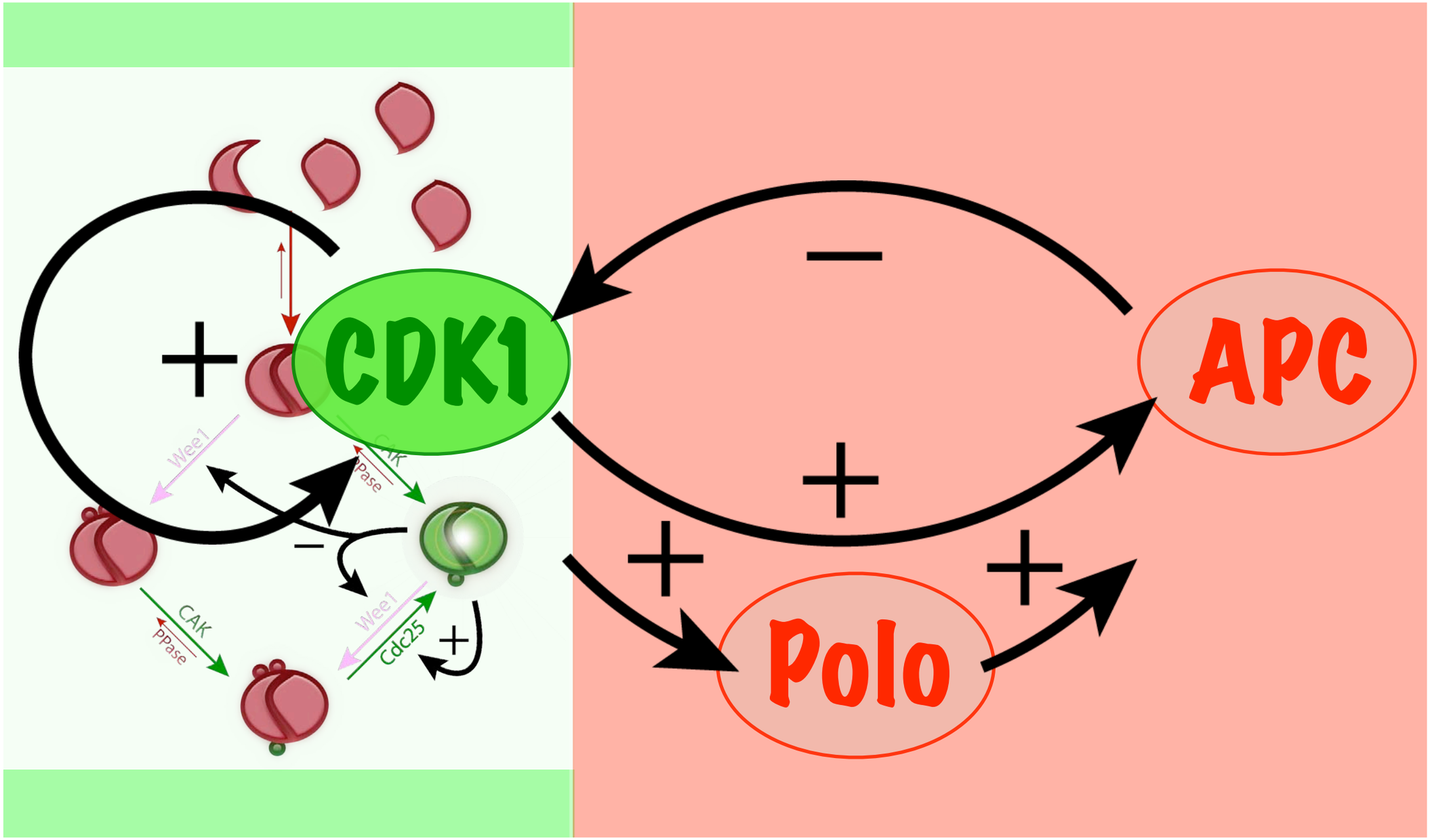
**Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5174, USA*
†Department of Mathematics, Rutgers University, New Brunswick, NJ 08903, USA
‡e-mail: james.ferrell@stanford.edu

Published online: 10 March 2003; DOI: 10.1038/ncb954

In the early embryonic cell cycle, Cdc2–cyclin B functions like an autonomous oscillator, whose robust biochemical

feedback[23], nor is it the only useful systems-level property that can arise from positive feedback loops (for example, sensitivity amplification[24] is a more robust property of

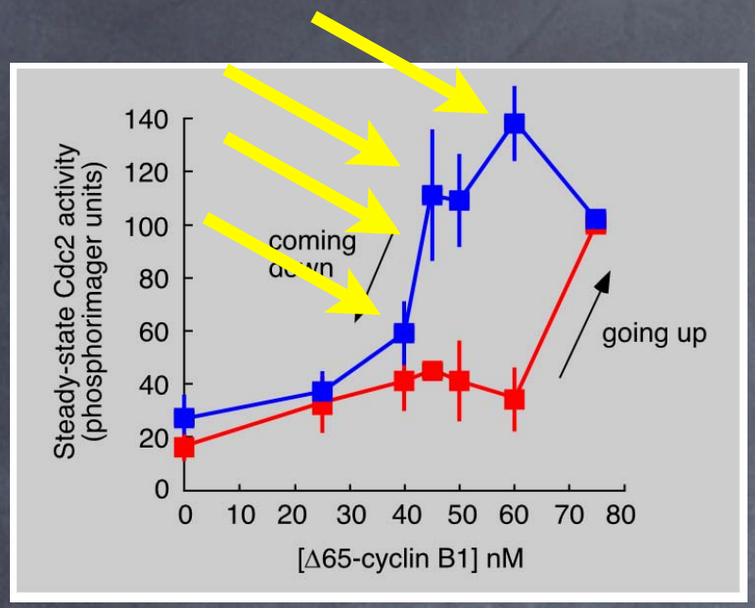
To oscillate, CDK1 must be inactivated



positive feedback

negative feedback

A Cell Biologist's Motivation Through Modeling

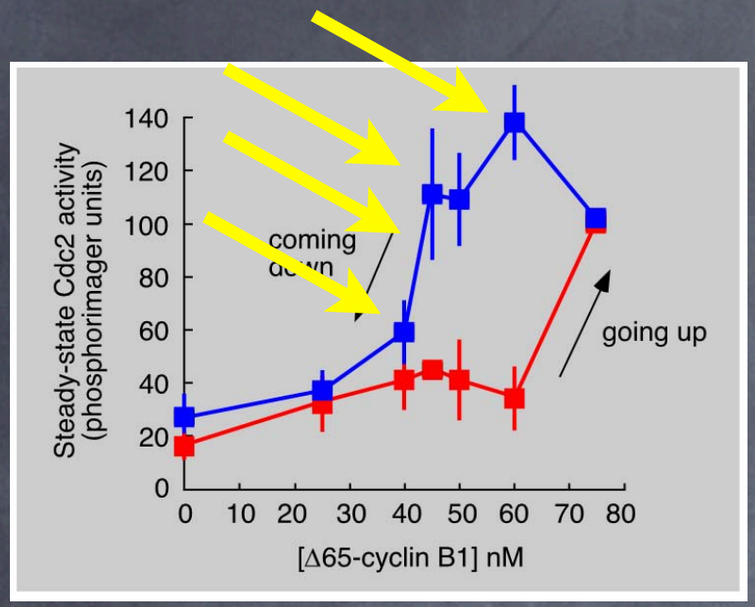


CDK1 activity is sustained and cell extracts remain in M phase at steady state, even as cyclin B levels are reduced.

What about the anaphase-promoting complex-mediated negative-feedback loop?

If CDK1 activity is reduced, how is APC activity maintained and remaining cyclin degraded?

A Cell Biologist's Motivation Through Modeling



CDK1 activity is sustained and cell extracts remain in M phase at steady state, even as cyclin B levels are reduced.

Some simplified possibilities:

1) The CDK1 -> APC activation loop is long enough for CDK1 inactivation to not cause immediate APC inactivation. Does not force the APC system to sustain activity.

2) The degradation of an inhibitor brings about APC activation, so CDK1 activity is not required to maintain APC activity. What inhibitor, and what is degrading it?

3) There is positive feedback intrinsic to APC activation, separate from the CDK1 module.

[Cell Cycle 4:8, 1107-1112; August 2005]; ©2005 Landes Bioscience

Report

Rewiring the Exit from Mitosis

Andrea Ciliberto[1,3]
Anna Lukács[1]
Attila Tóth[1]
John J. Tyson[2]
Béla Novák[1,*]

[1]Molecular Network Dynamics Research Group of Hungarian Academy of Sciences and Budapest University of Technology and Economics; Budapest, Hungary

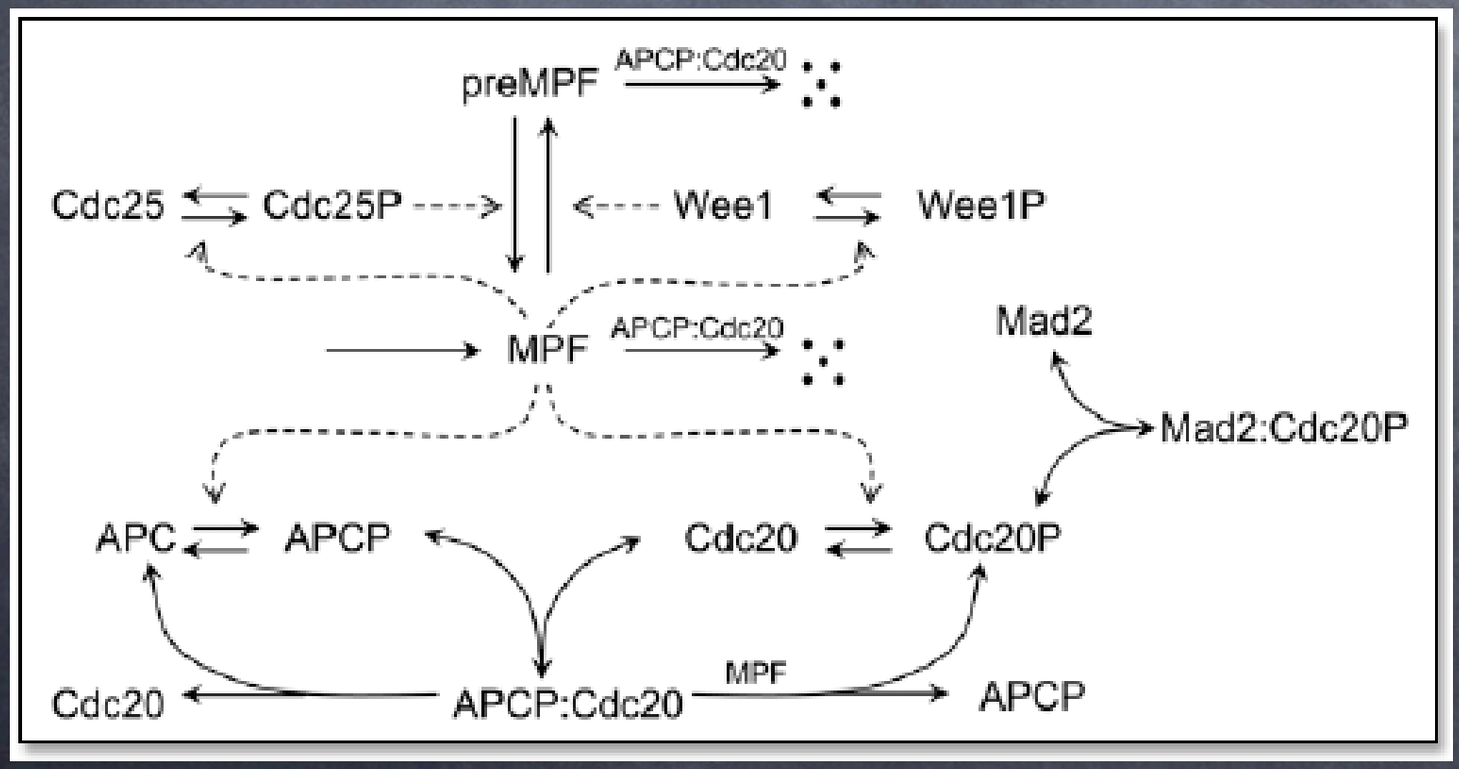
[2]Department of Biology; Virginia Polytechnic Institute and State University; Blacksburg, Virginia USA

[3]Current address: FIRC Insitute of Molecular Oncology; Milan, Italy

*Correspondence to: [illegible] Molecular Network Dynamics Research Group

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 [illegible] and that the antagonism between MPF and Cdc20 [illegible] checkpoint

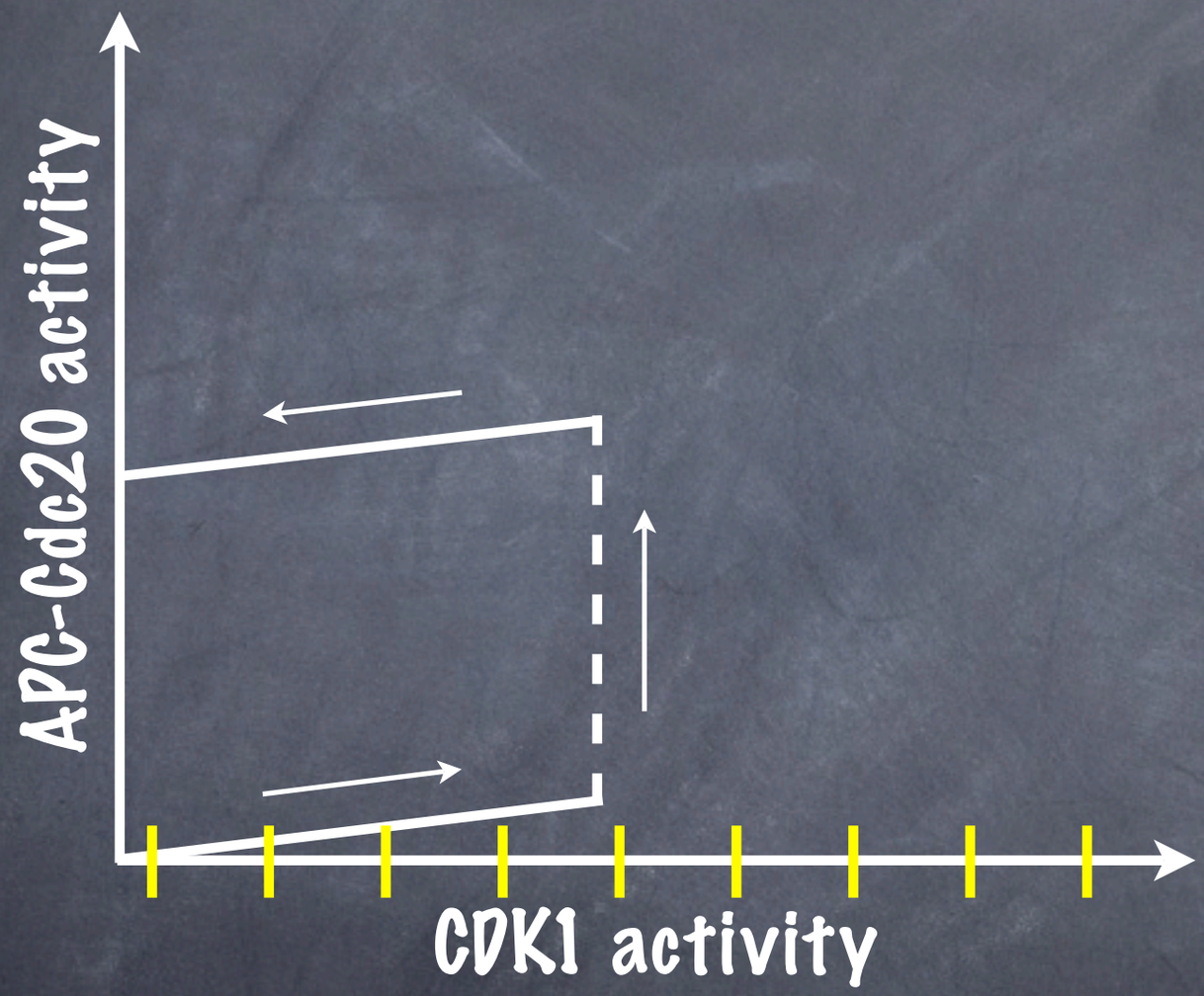


MPF

Cdc20P

Could this double-negative (positive) feedback make APC activation hysteretic?

Hysteresis in APC-Cdc20 Activation??

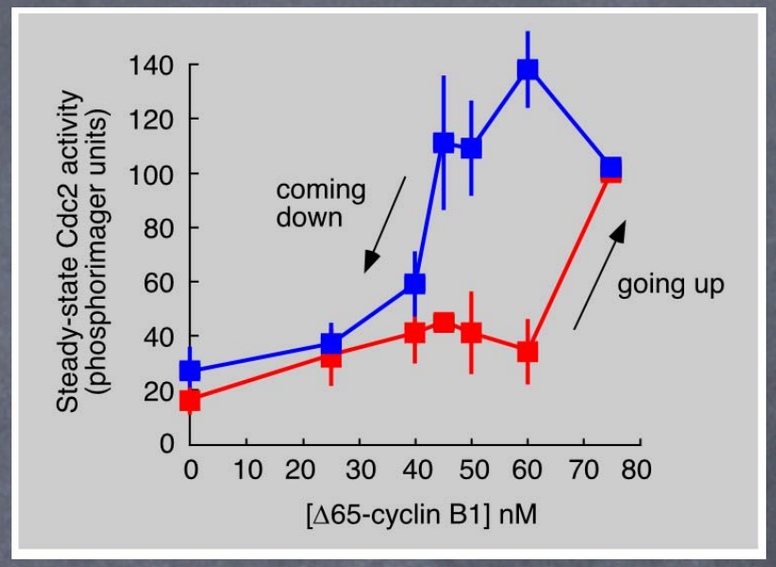


Attaining incremental CDK1 activites will be the experimental challenge here.

Summary-Part I

The CDK1/Wee1/Cdc25 System IS Bistable.

There is hysteresis in CDK1 activation: for some concentrations of cyclin, there are two discrete states; the thresholds for the ON- and OFF-states are different.



Wiring the switch this way ensures either interphase or M phase; there is no "settling" to an intermediate state.

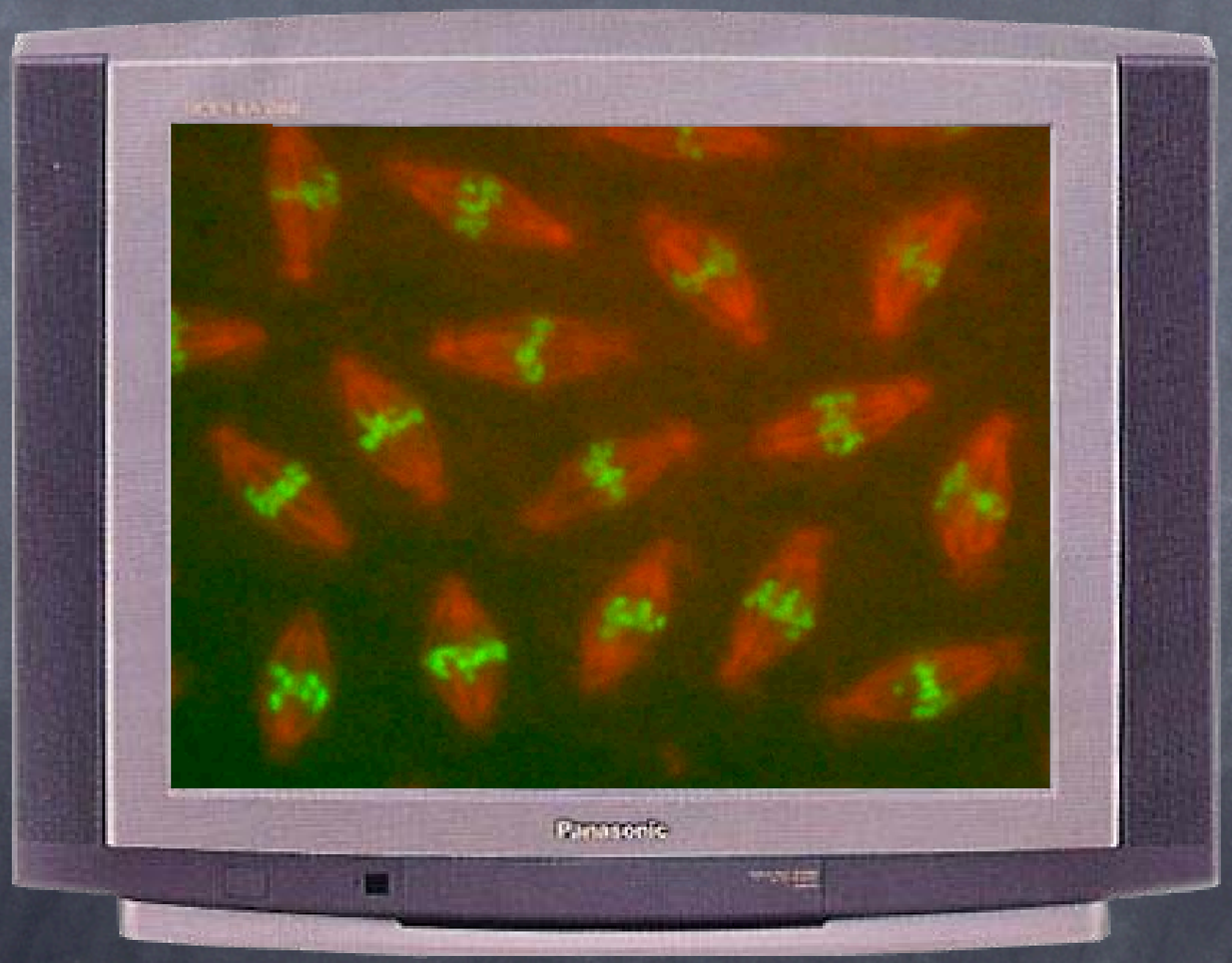
There may be sources of positive feedback in APC activation, and thus, its response may be bistable relative to CDK1 activity.

1. Digital Responses In Vivo: Mitotic Toggle Switches?

2. The Embryonic Mitotic Oscillator: Positive Feedback and the Emergent Properties of the Cdc2/Cyclin B Module

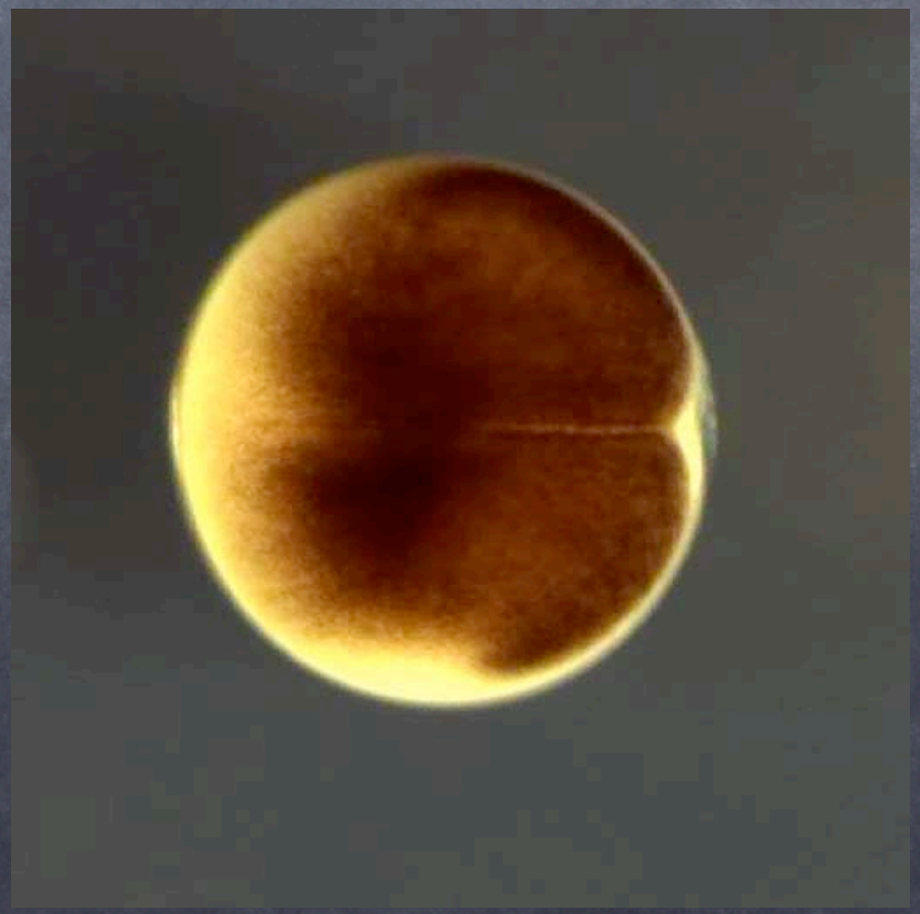
3. Positive Feedback and Somatic Mitosis: To Enter, To Exit, To Not Oscillate?

Positive Feedback: Simple Relationships Eliciting Complex and Decisive Responses in Many Biological Oscillatory Contexts

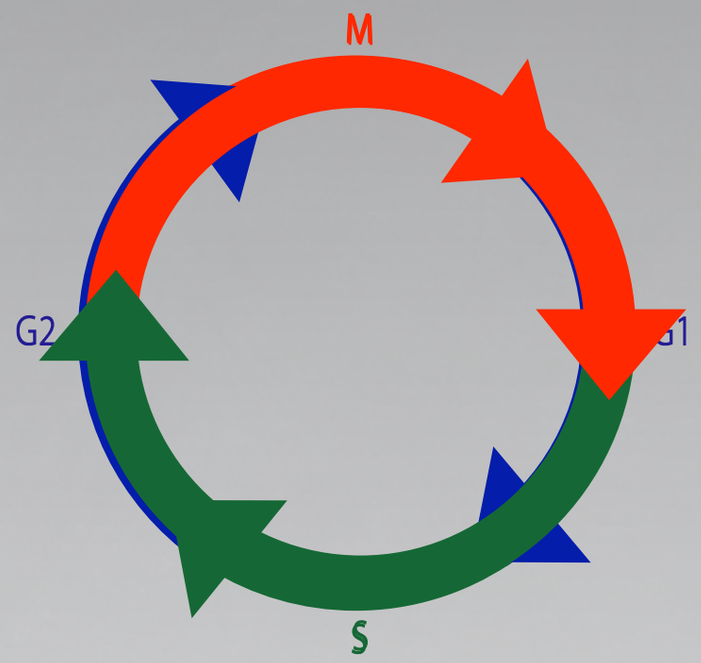


Biological Oscillators Are Diverse in Form and Function

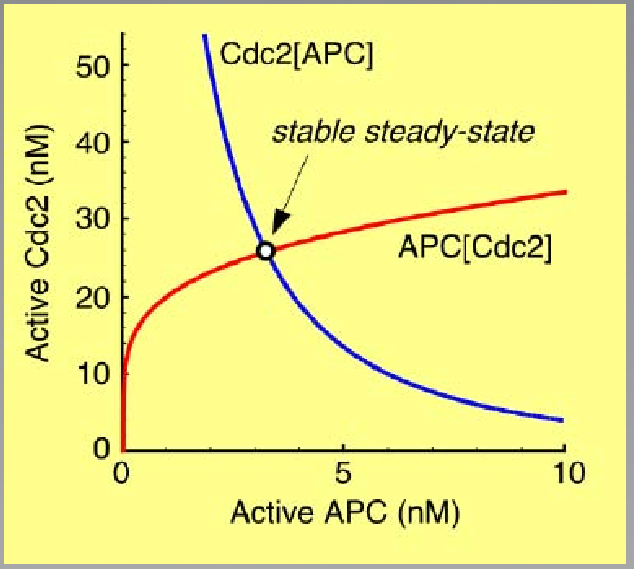
"Pulsatile" - oscillations of CDK1 activity in the embryo;
much like an action potential in a neuron;
no transcription necessary;
driven by rapid modifications of regulatory enzymes.

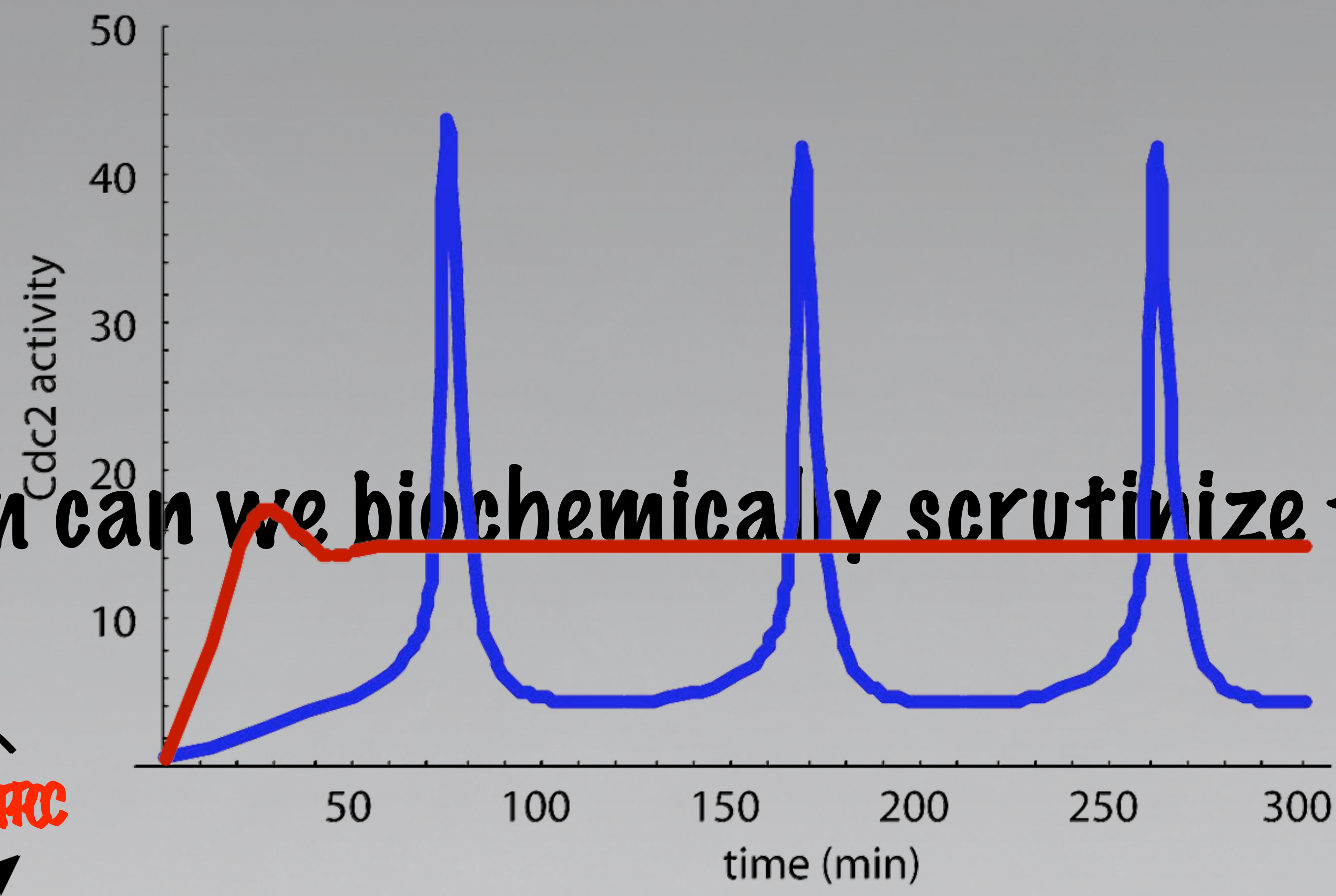


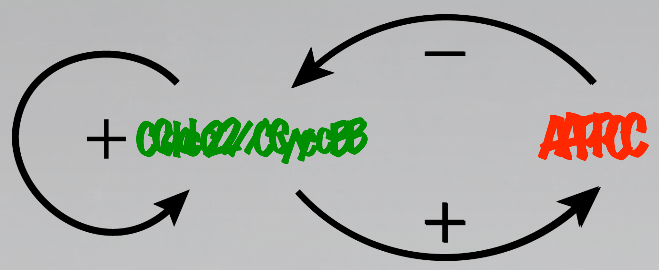
The somatic cell cycle circuitry has the same core components as an embryo, but the early embryonic cell cycle has no gap/growth phases, so oscillations are very rapid.



In what system can we biochemically scrutinize these oscillations?

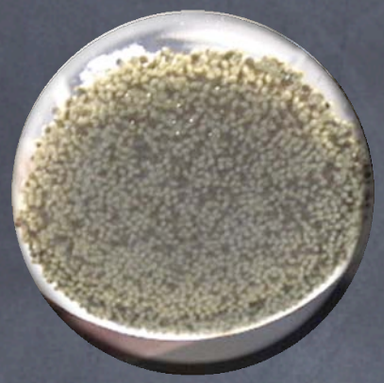






In a modeled two component system, a loss of positive feedback cannot facilitate oscillations.

Adding positive feedback, however, recovers sustained oscillations.



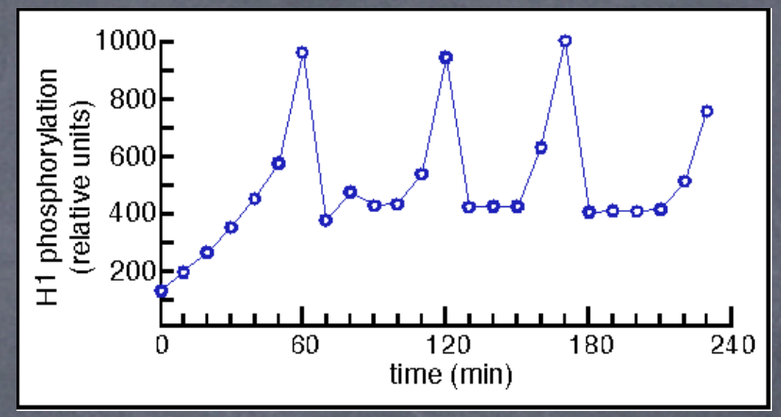
+

How to make a Xenopus egg extract

"cycling" egg extract

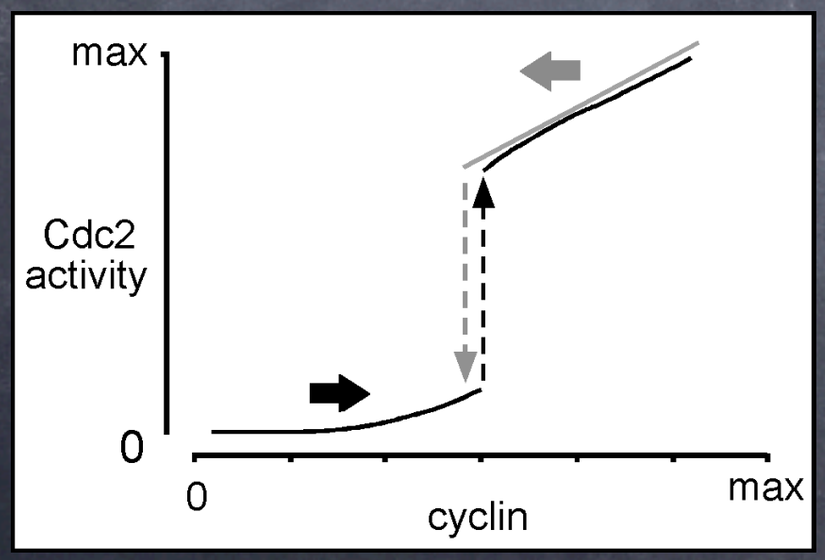
Oscillations of MPF Activity in Cycling Egg Extracts: What is the Systems-level Logic of This Oscillator?

phospho-histone H1

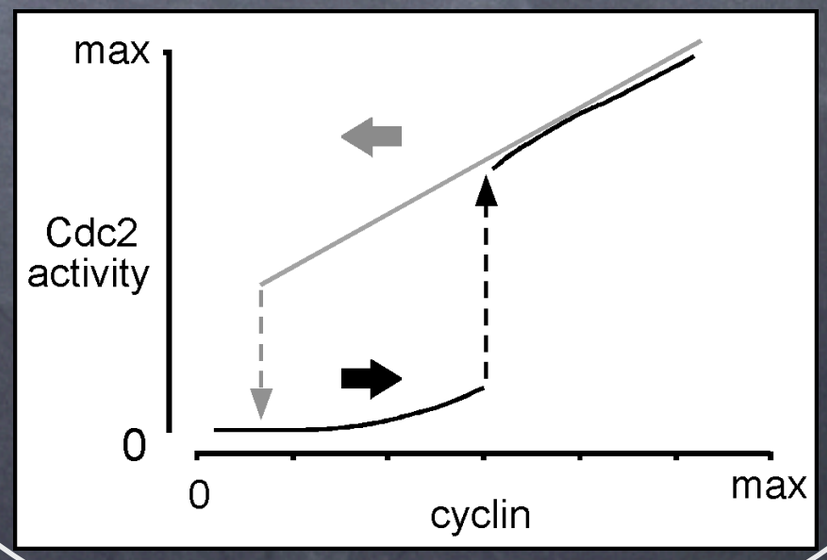


POSITIVE FEEDBACK

monostable system

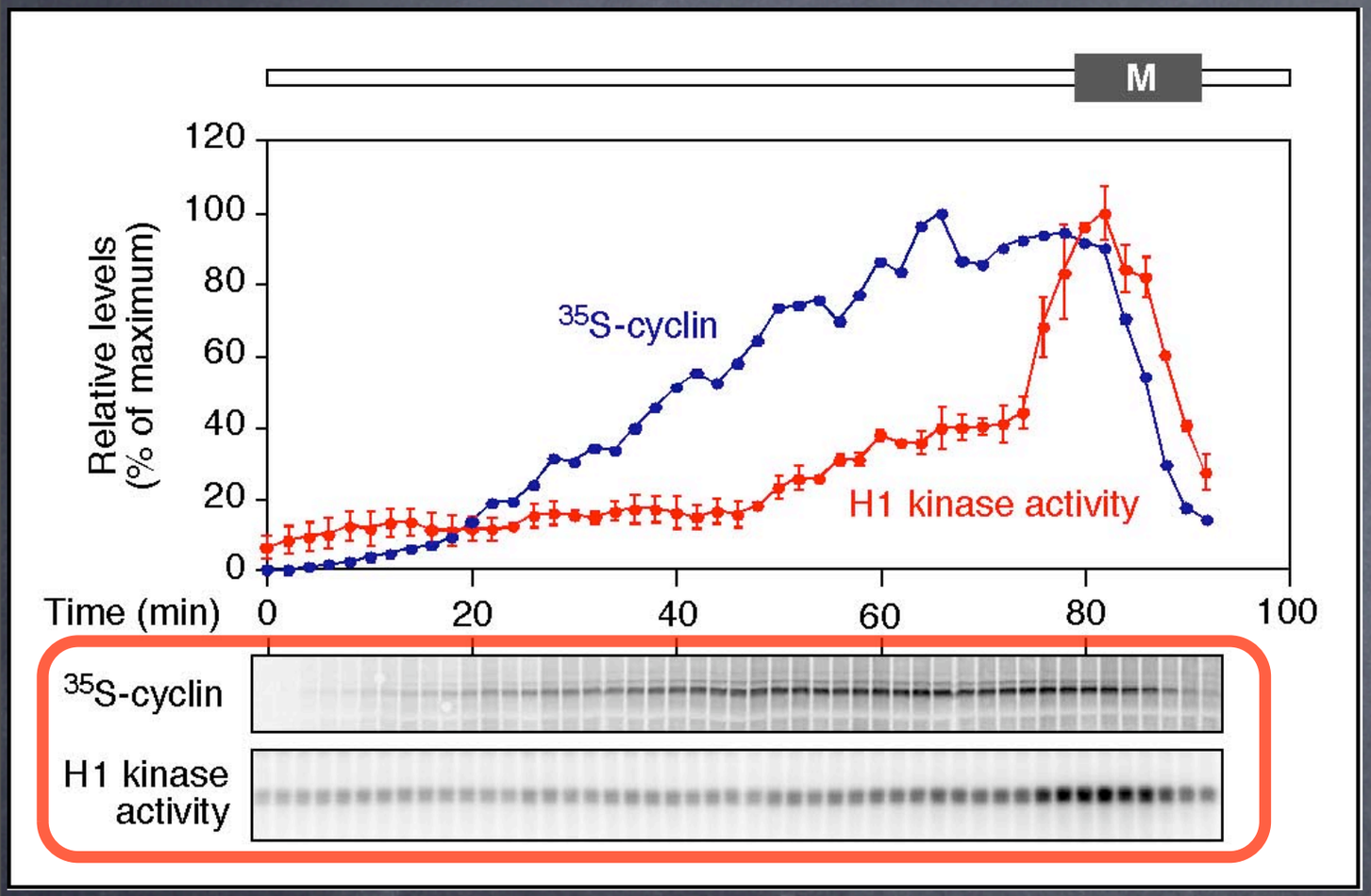


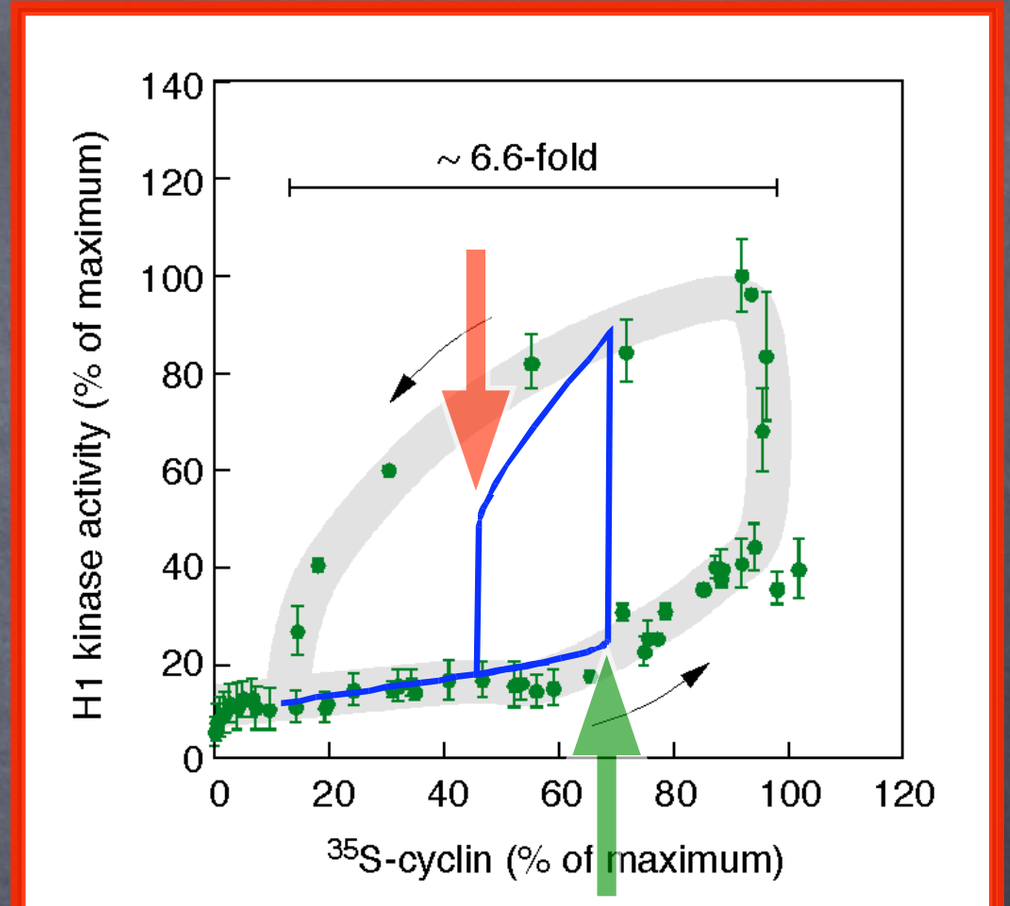
bistable system



negative feedback oscillator? ⟷ relaxation oscillator?

What is the Dynamical Relationship Between Cyclin B, CDK1, & APC?





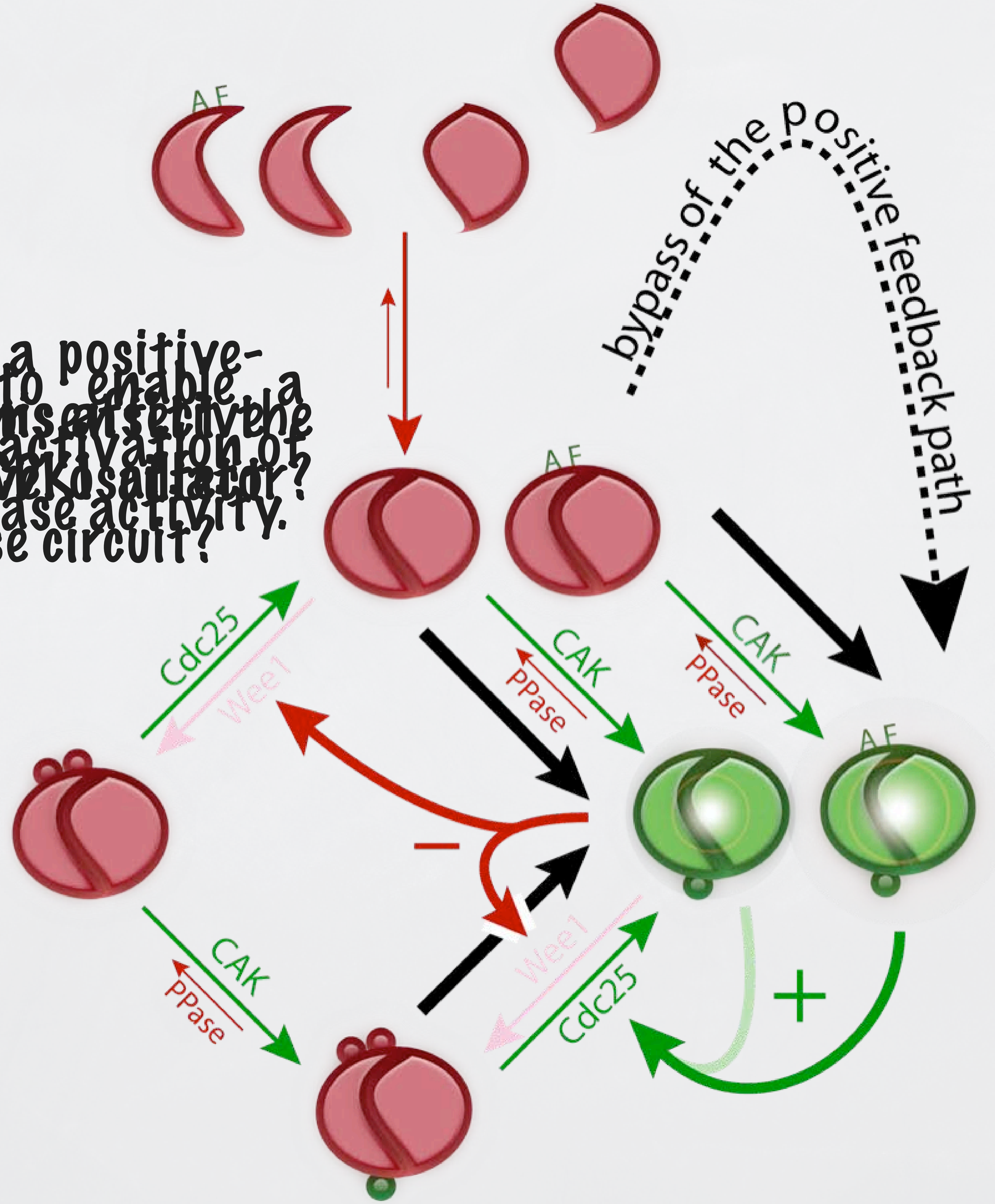
The stimulus-response of CDK1(Cyclin B) overshoots the steady-state hysteresis loop.

Is hysteresis merely a byproduct of the positive feedback, and the system really never needs to apply it, or does it infer the importance of positive feedback in the circuit?

How might a positive-feedback insensitive mutant of Cdk1 affect the M-phase circuit?

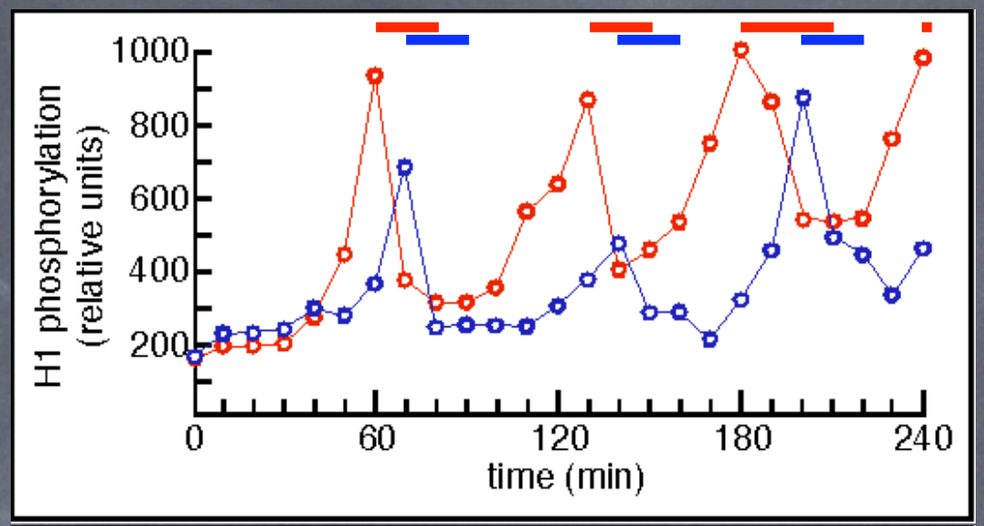
Predicted to enable a premature activation of CDK1AF kinase activity.

How would this affect the system as oscillator?



CDK1AF Accelerates Oscillations in a Cycling Egg Extract and Reduces Interphase Length

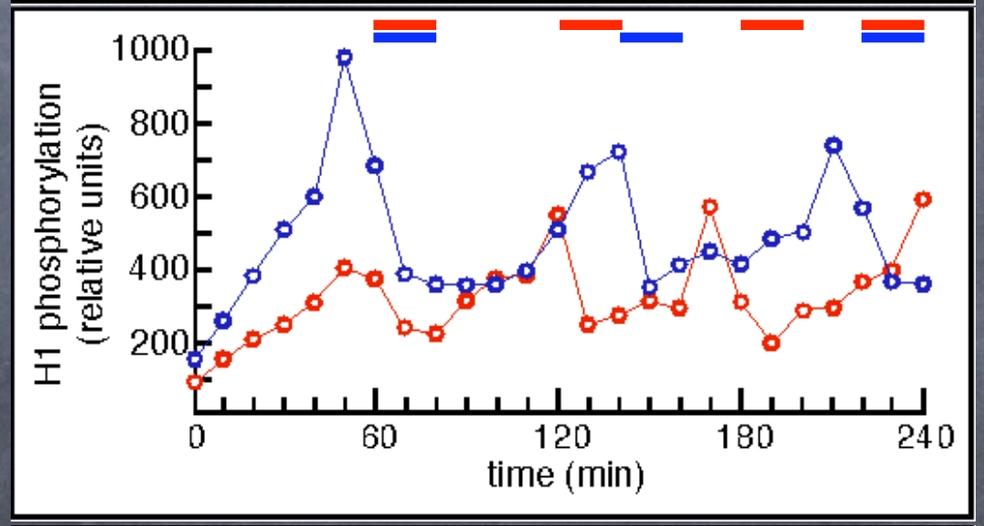
50 nM



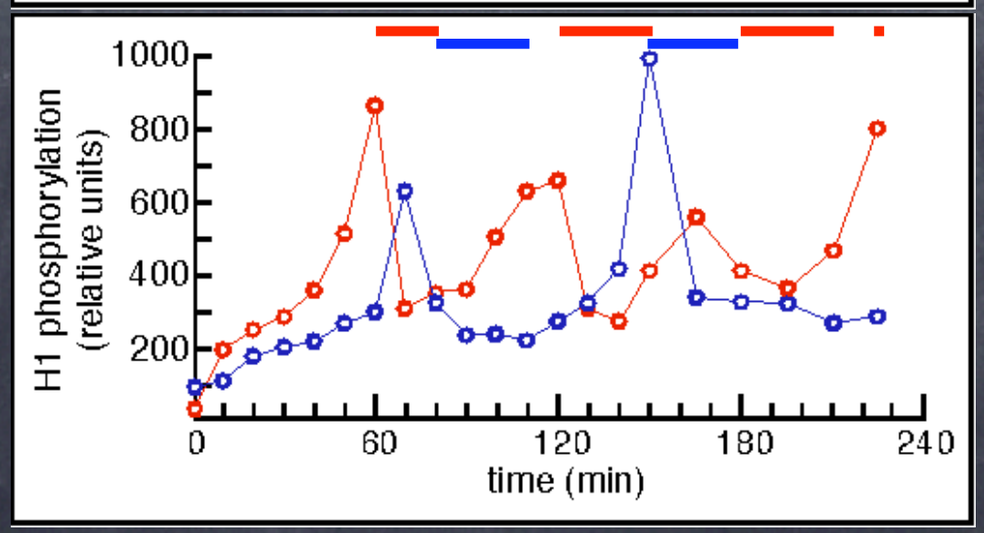
NEBD

visible nuclei

100 nM

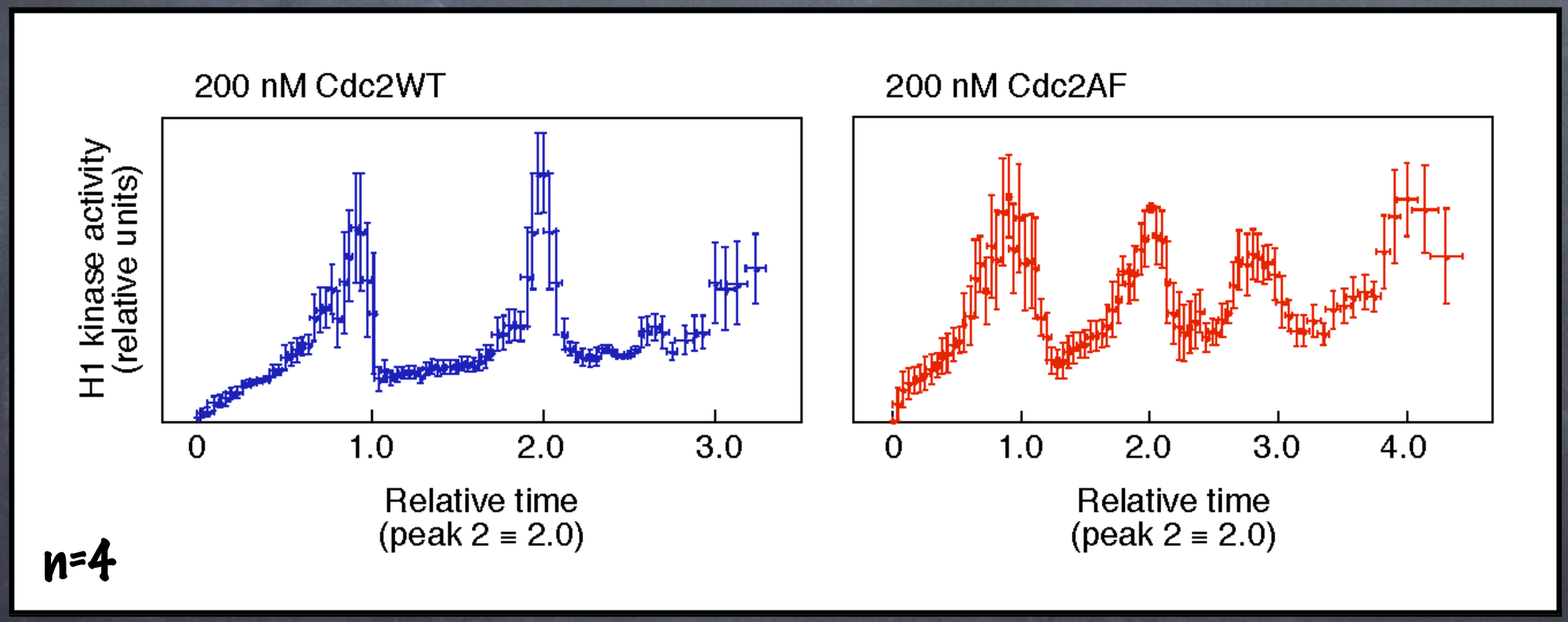


200 nM



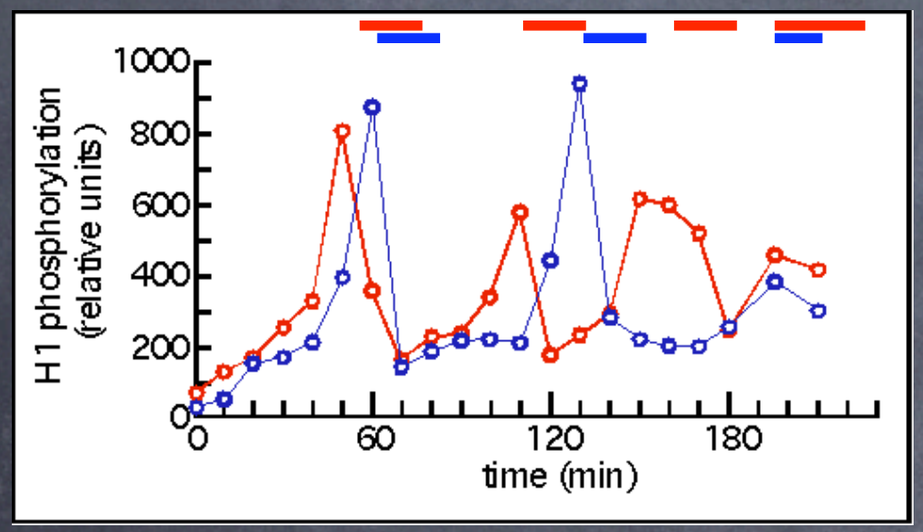
- XCDK1WT
- XCDK1AF

CDK1AF Accelerates Oscillations in a Cycling Egg Extract, Reduces Interphase Length, and Induces Damping



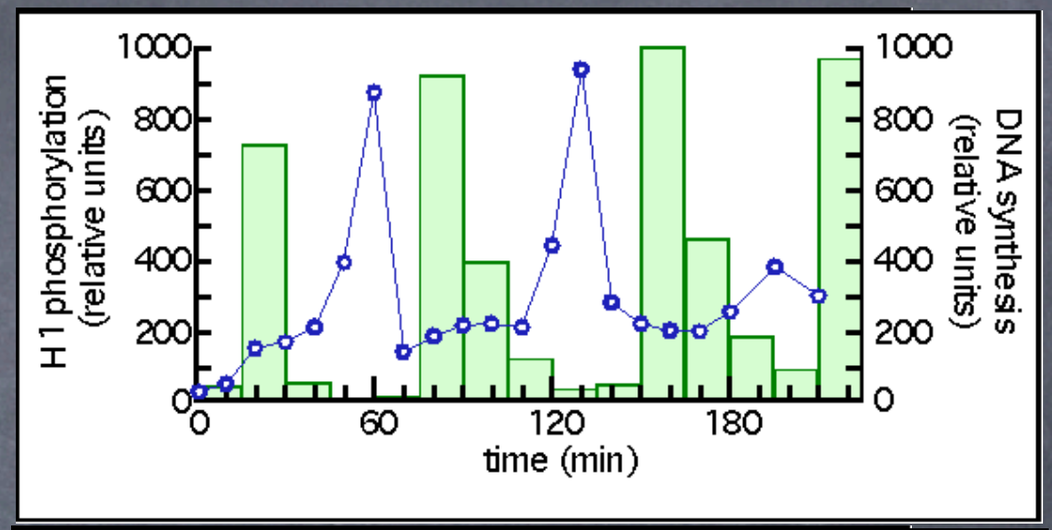
n=4

Does a CDK1AF-accelerated Extract Properly Relay Between M- and S-phases?

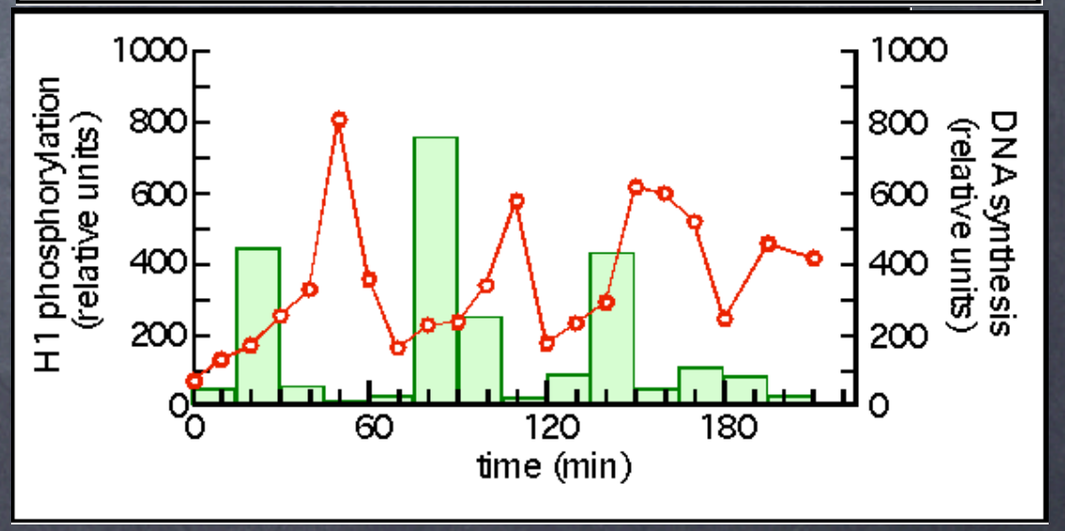


- XCDK1WT (200 nM)
- XCDK1AF (200 nM)

WT

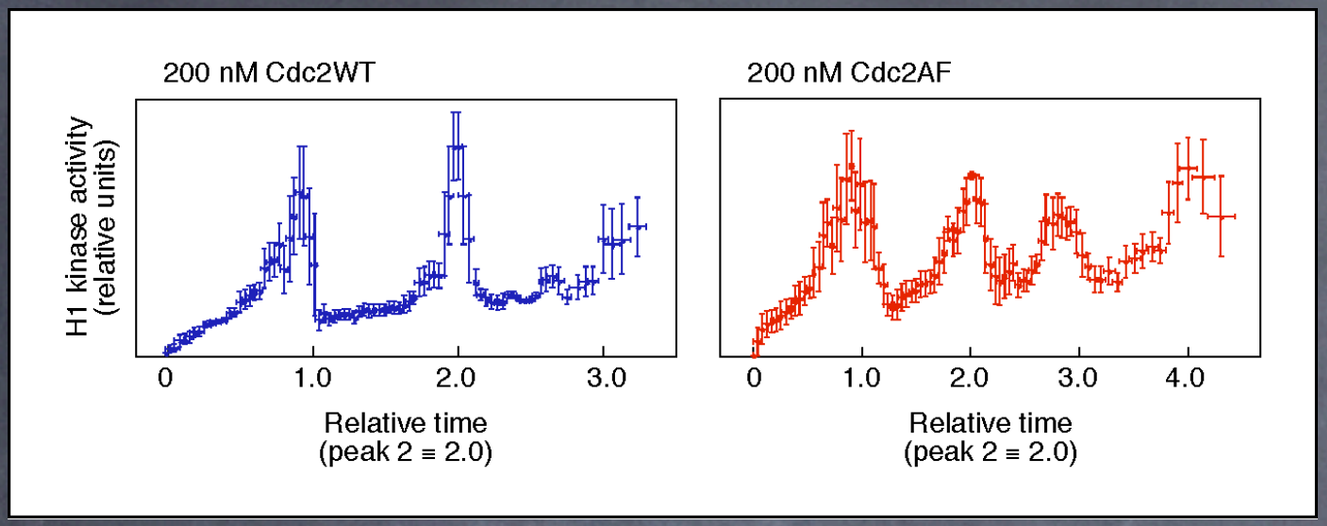


AF



DNA synthesis is eventually disrupted

Summary-Part II

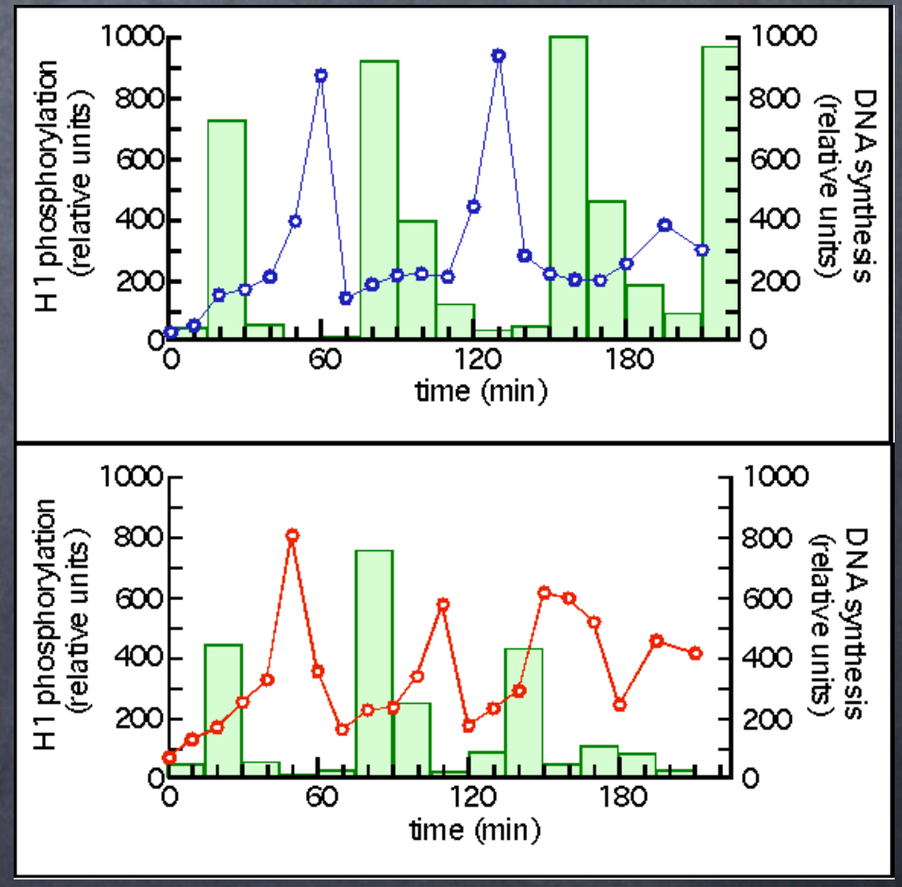


Bypassing positive feedback in CDK1 activation:

- accelerates oscillations in a cycling egg extract.
- reduces interphase length.
- induces damping of CDK1 activity.

As a consequence:

- DNA synthesis is inhibited.
- The discreteness between interphase and M-phase periods is compromised.

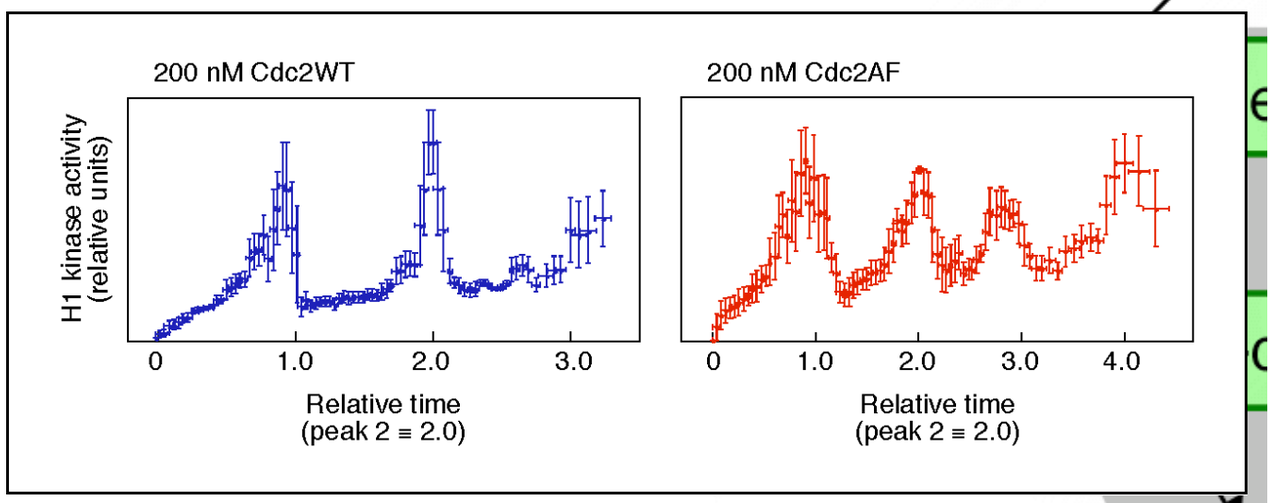


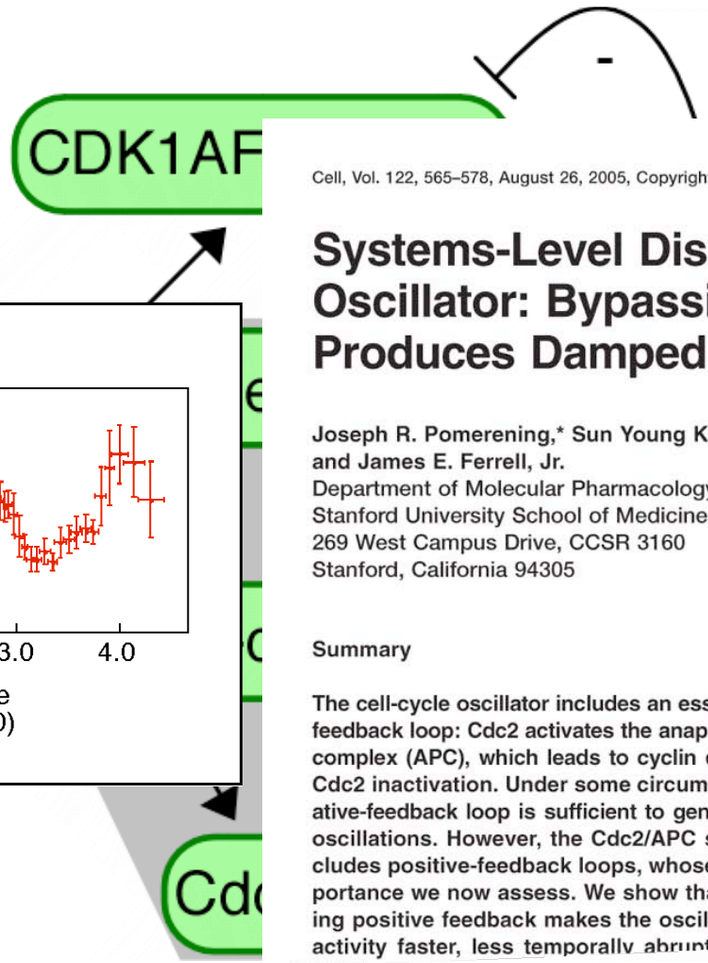
1. Digital Responses In Vivo: Mitotic Toggle Switches?

2. The Embryonic Mitotic Oscillator: Positive Feedback and the Emergent Properties of the Cdc2/Cyclin B Module

3. Positive Feedback and Somatic Mitosis: To Enter, To Exit, To Not Oscillate?

Dynamical Studies in Embryonic Extracts





Cell, Vol. 122, 565–578, August 26, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.06.016

Systems-Level Dissection of the Cell-Cycle Oscillator: Bypassing Positive Feedback Produces Damped Oscillations

Joseph R. Pomerening,* Sun Young Kim, and James E. Ferrell, Jr.
Department of Molecular Pharmacology
Stanford University School of Medicine
269 West Campus Drive, CCSR 3160
Stanford, California 94305

Summary

The cell-cycle oscillator includes an essential negative-feedback loop: Cdc2 activates the anaphase-promoting complex (APC), which leads to cyclin destruction and Cdc2 inactivation. Under some circumstances, a negative-feedback loop is sufficient to generate sustained oscillations. However, the Cdc2/APC system also includes positive-feedback loops, whose functional importance we now assess. We show that short-circuiting positive feedback makes the oscillations in Cdc2 activity faster, less temporally abrupt, and damped

cyclin B mRNA cycle faster than control embryos (Hartley et al., 1996). The accumulating cyclin B binds to the cyclin-dependent kinase Cdc2, and, under the proper circumstances, this complex becomes activated and phosphorylates mitotic substrates, driving the transition from interphase to mitosis. The transition from mitosis back to interphase is driven by a negative-feedback loop. Active cyclin B-Cdc2 brings about the activation of the APC, which results in the polyubiquitylation and proteolysis of cyclin (Hershko et al., 1994; King et al., 1995, 1996; Sudakin et al., 1995) (Figure 1A). Although the presence of a negative-feedback loop does not guarantee sustained oscillations of Cdc2 and APC activity, it is difficult to imagine an oscillator that does not contain a negative-feedback loop. Indeed, when APC-resistant cyclin mutants are added to interphase *Xenopus* egg extracts, the extracts approach a steady state of Cdc2 activity rather than oscillating (Murray et al., 1989). This implies that the negative-feedback loop

Embryos possess a positive-plus-negative feedback CDK1 oscillator.
How different is the systems-logic of the CDK1 module in somatic cells?
Is it more like a series of dependent, more highly-regulated switches?

Dominoes and Clocks: The Union of Two Views of the Cell Cycle

ANDREW W. MURRAY AND MARC W. KIRSCHNER

We review the recent advances in understanding transitions within the cell cycle. These have come from both genetic and biochemical approaches. We discuss the phylogenetic conservation of the mechanisms that induce mitosis and their implications for other transitions in the cell cycle.

THE CELL CYCLE IS THE SET OF EVENTS THAT IS RESPONSIBLE for the d[illegible] The recent advances in our

Hartwell and his colleagues (*1*) and later in the fission yeast by Nurse and his colleagues (*2*), the result was a description of the cell cycle as a set of dependent reactions. The basis of this dependency is discussed in the accompanying review by Hartwell and Weinert (*3*). The physiological and embryological approach was championed by researchers who favored marine and amphibian eggs. They argued that eggs and oocytes were the simplest systems for studying the basic processes of the cell cycle, because they were specialized for rapid cell division. The result of their investigations was a description of the cell cycle as a biochemical machine that oscillated between two states, mitosis and interphase, and whose oscillations were independent of the completion [illegible] any of the cell cycle events.

Cell cycle progression in the EARLY EMBRYO:

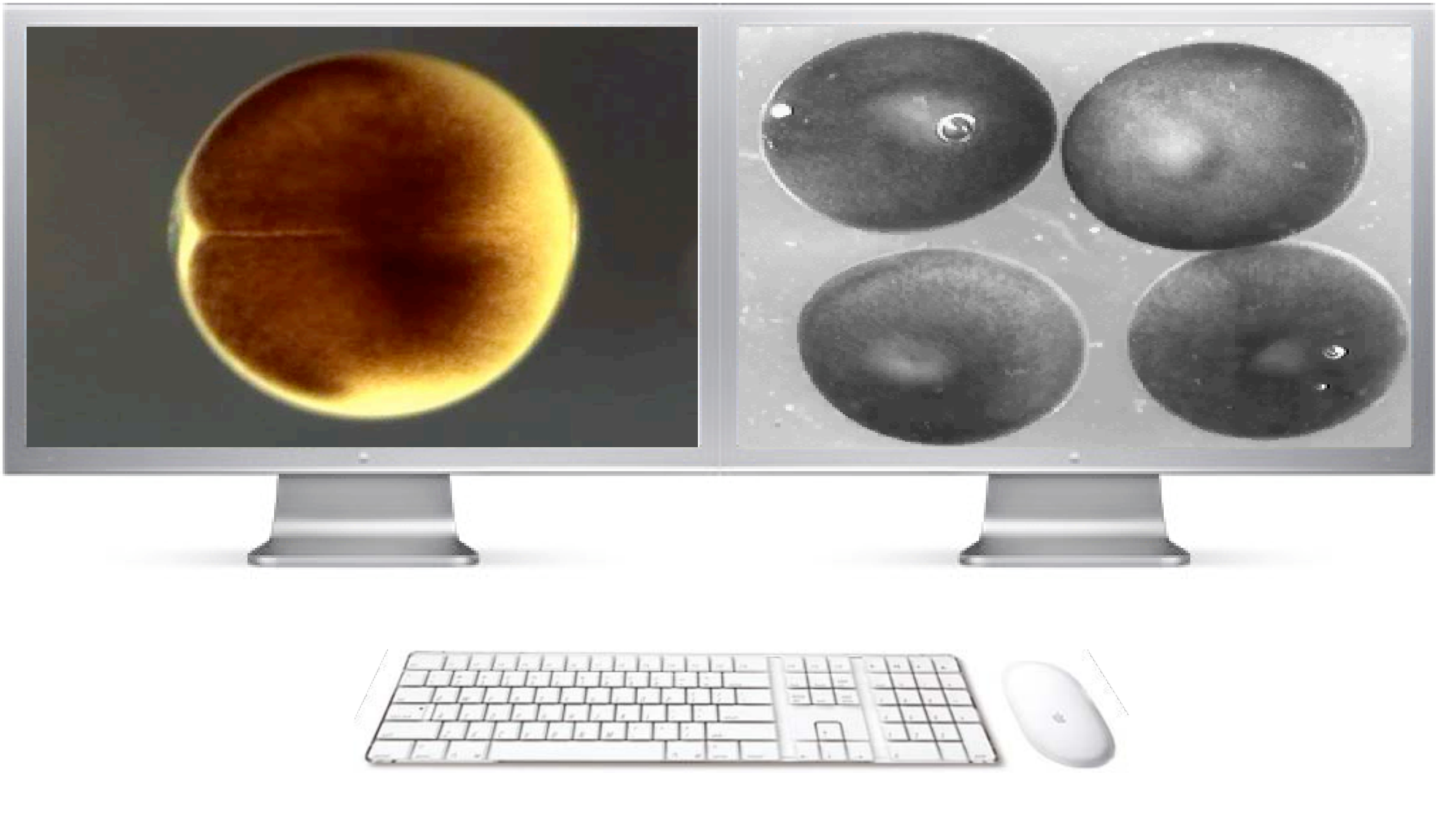
An autonomous oscillator, running just like a CLOCK - "Oscillations of M-phase-promoting activity independent of the completion of many of the cell cycle events."

Cell cycle progression in SOMATIC CELLS:

Sets of dependent reactions, just like DOMINOES - "Any fusion between two interphase cells at different stages of the cell cycle, the advanced nucleus (in G2) waits for the completion of events in the retarded nucleus (in G1) before progressing in the cell cycle."

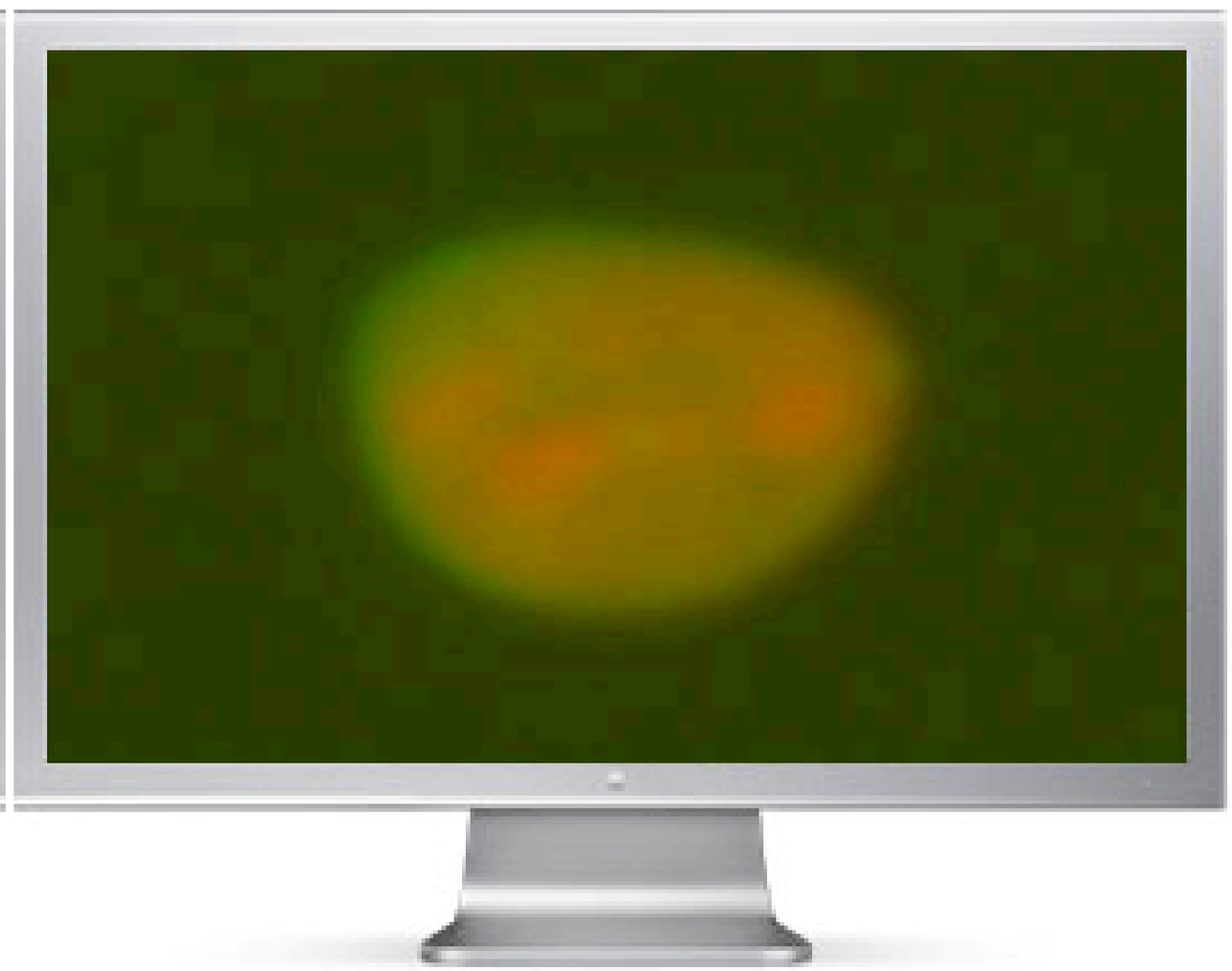
no growth or checkpoints until after division 12 (MBT), approximately 6 hpf

nocodazole-treated embryos

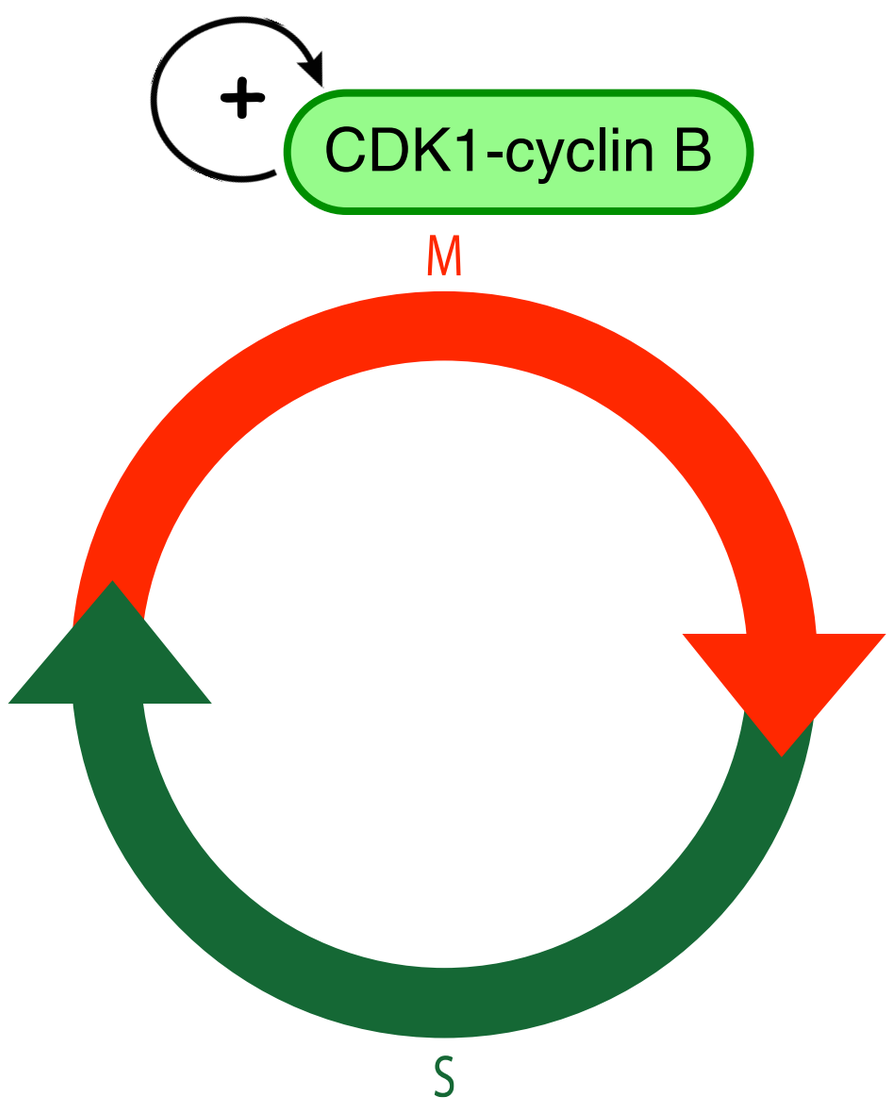


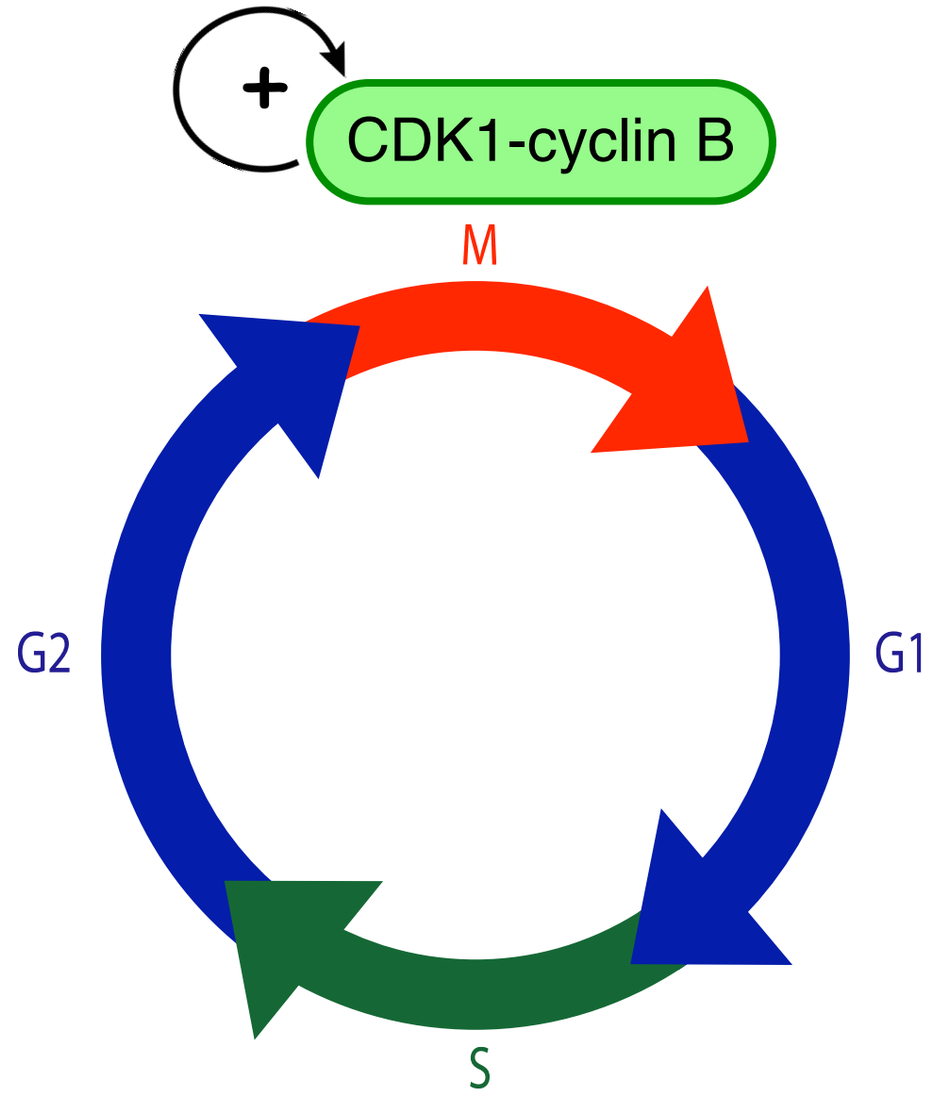
no growth or checkpoints until after division 12 (MBT), approximately 6 hpf

growth periods & checkpoints enabled; quiescence, differentiation, senescence



Do the design principles underlying the M-phase circuit differ between...





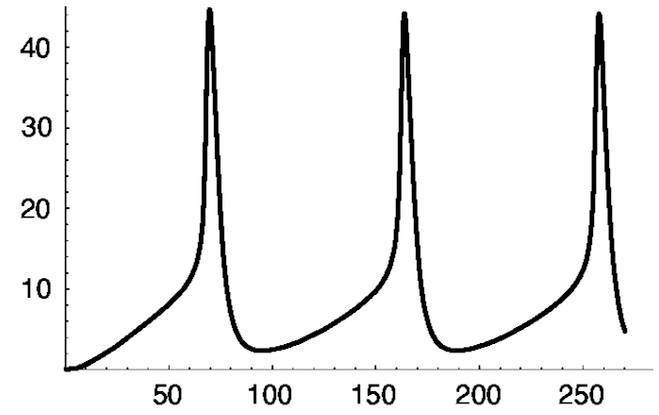
...and the basic biochemistry?

Somatic Controls = f(Transcriptional regulation? Checkpoints? Positive Feedback?)

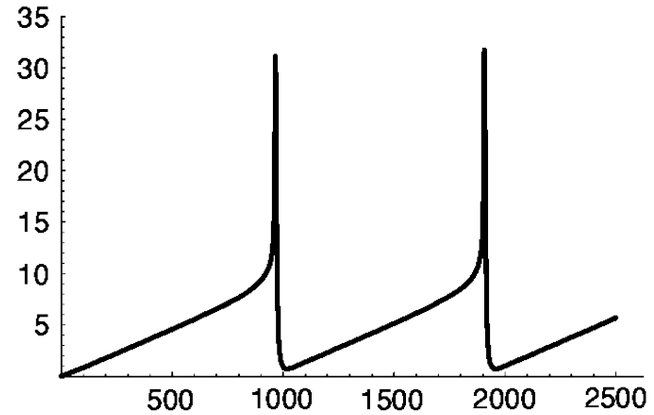
Is positive feedback a biochemical necessity for proper M-phase control, from embryo to adult?

If this IS the case then the somatic system may very well depend on positive feedback...

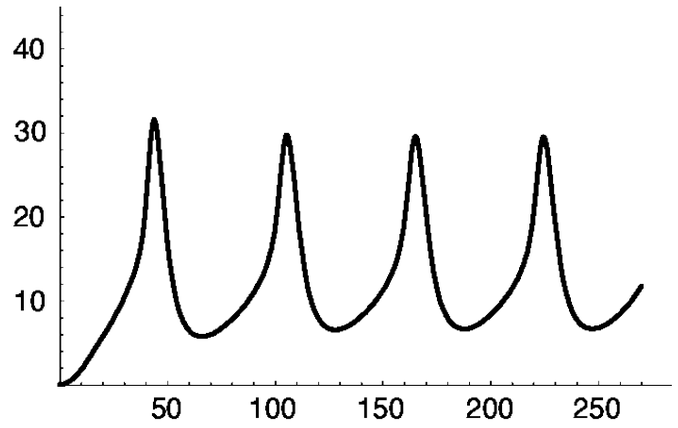
Simplest Case: Consider the Rate of Cyclin Synthesis & [CDK1AF]



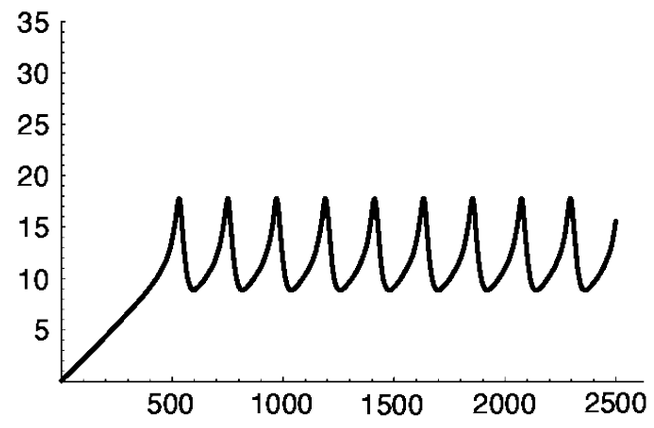
2 nM/min, 0 nM AF



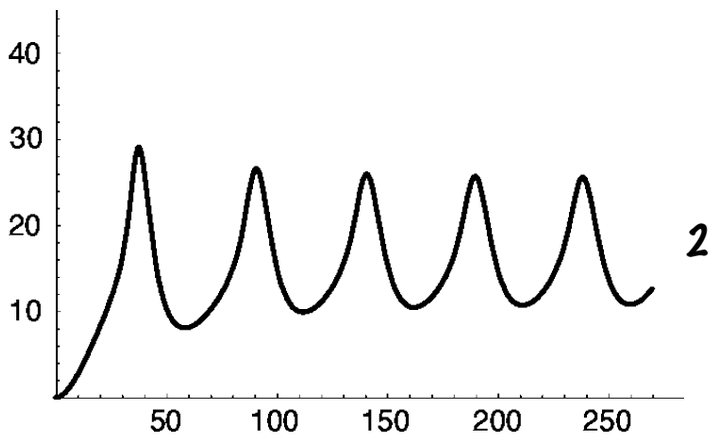
0.08 nM/min, 0 nM AF



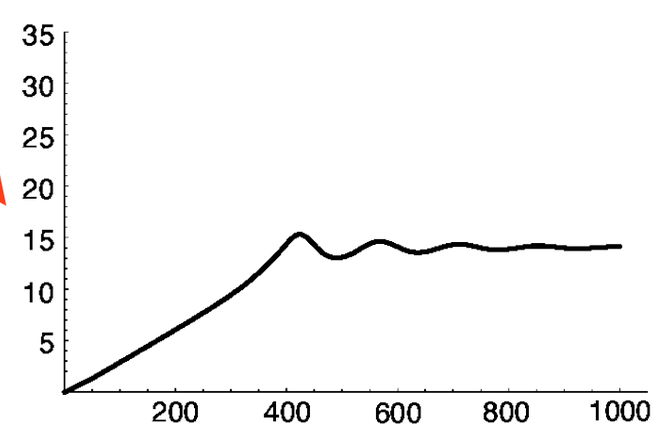
2 nM/min, 50 nM AF



0.08 nM/min, 50 nM AF



2 nM/min, 100 nM AF

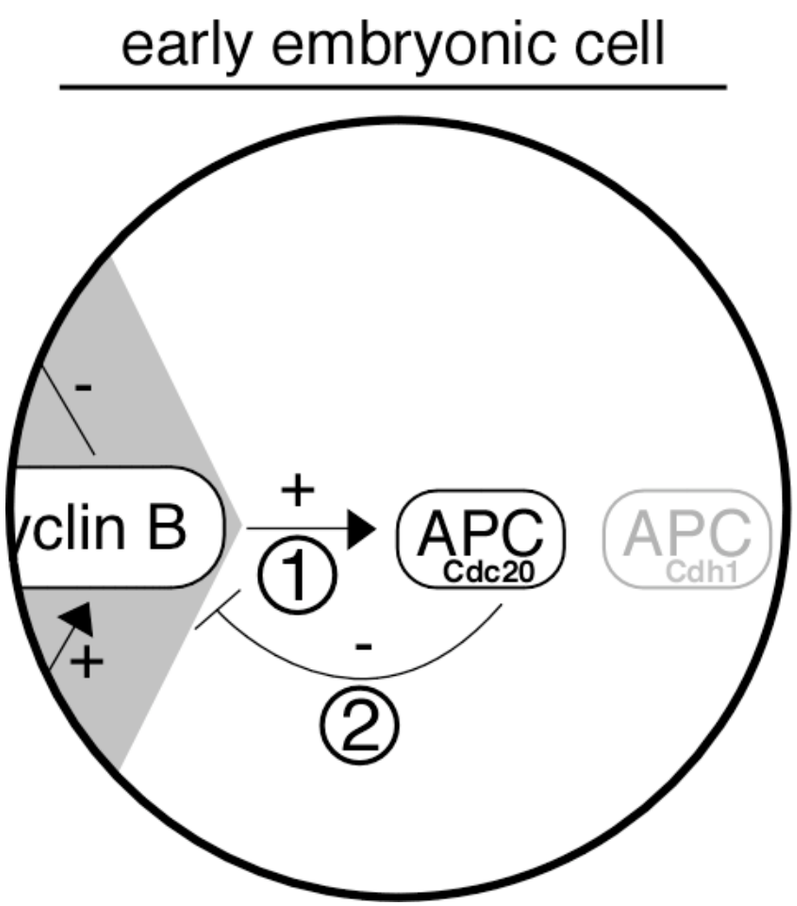


0.08 nM/min, 100 nM AF

EARLY EMBRYO example

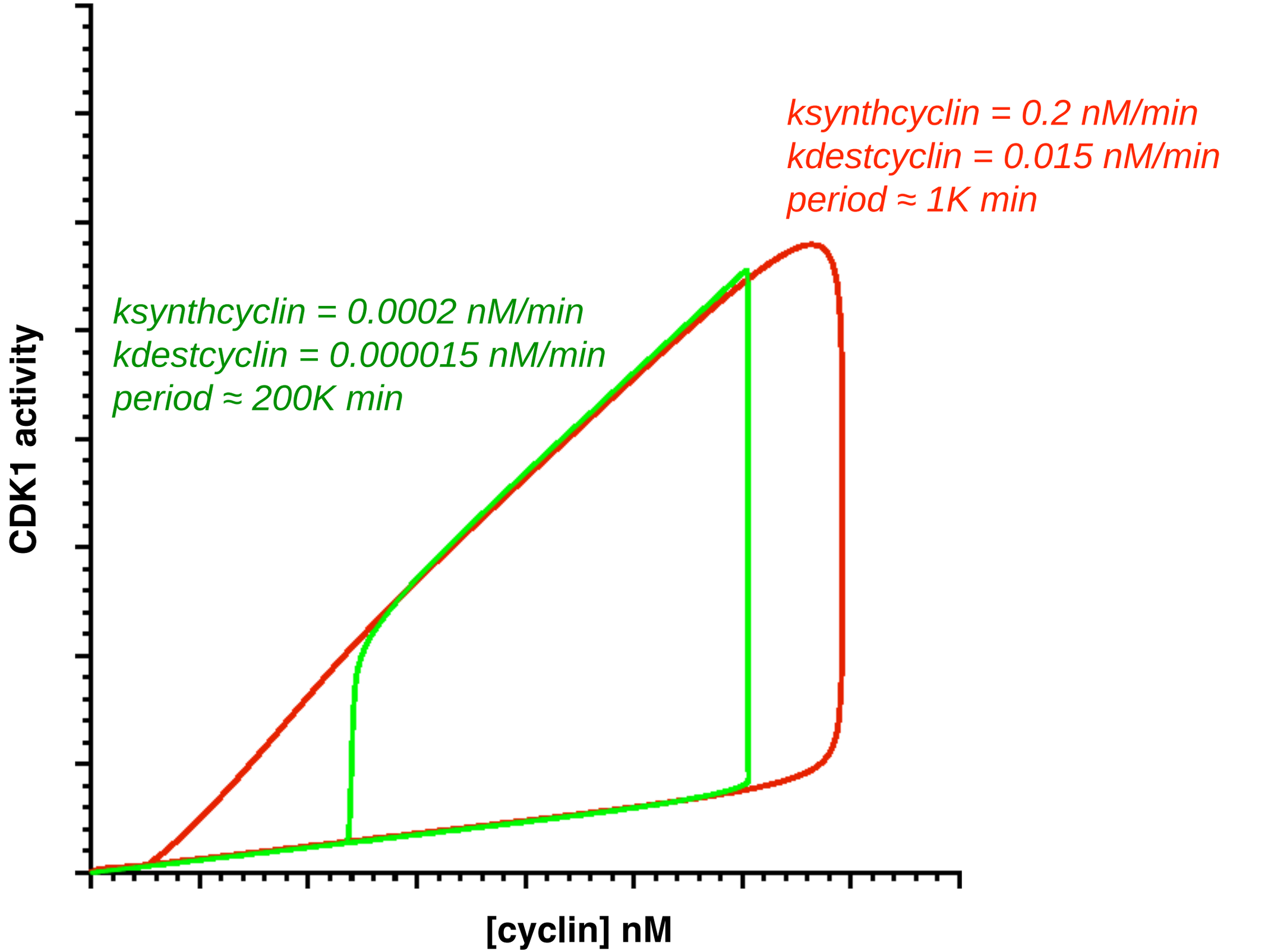
SOMATIC CELL example

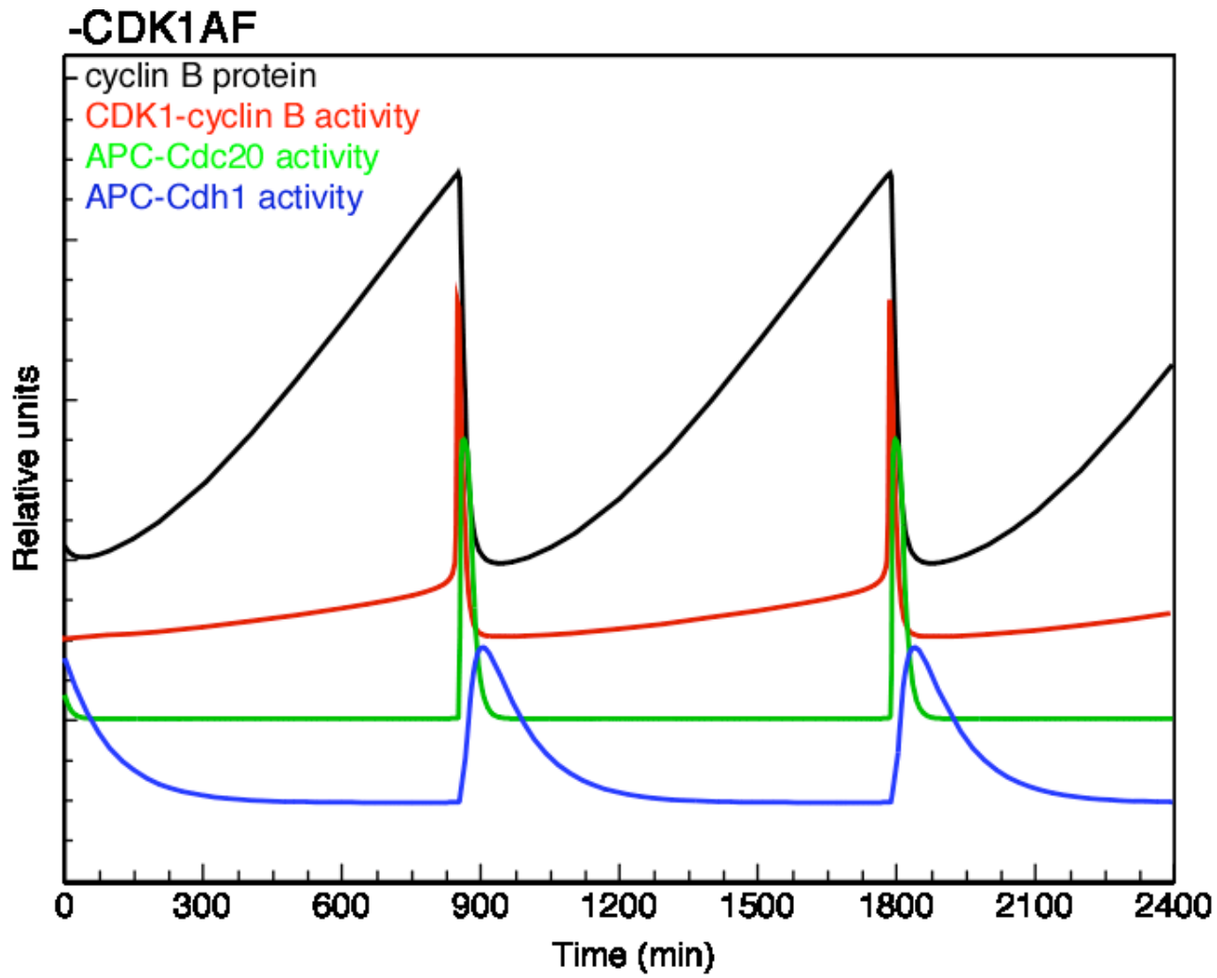
Negative-Feedback Loops and the Cell Cycle: From Early Embryo to Adult

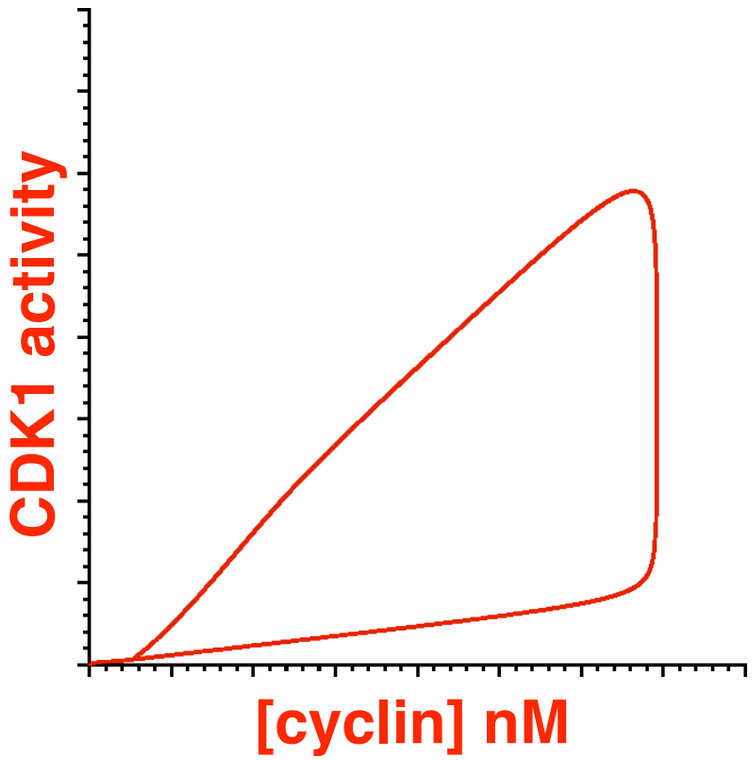


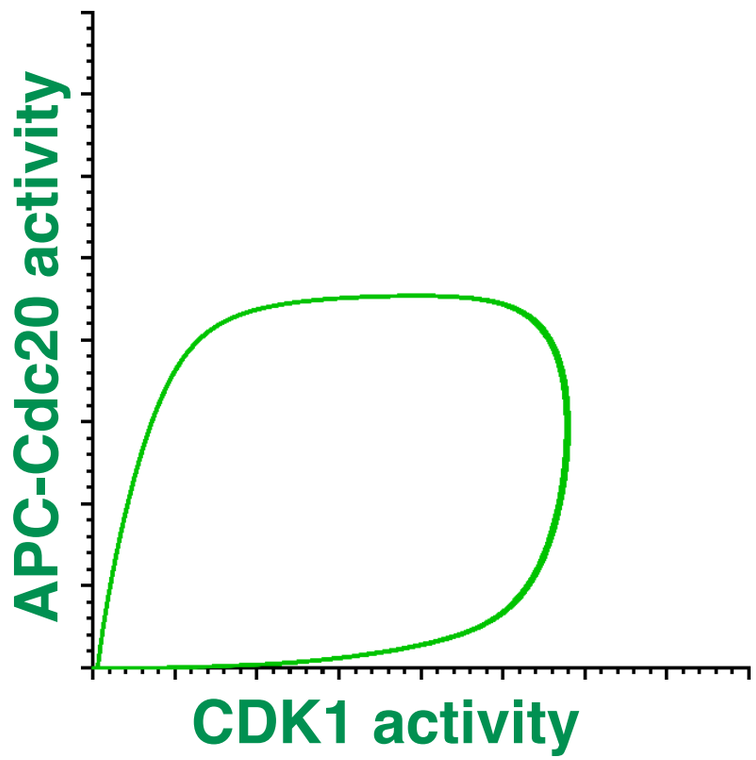
- **16 coupled ordinary differential equations**
  ksynth (cyclin) - "stimulus parameter"
  kdest (APC-Cdc20 & APC-Cdh1) - "destruction parameter"
- **44 parameters total**
- **scripted in *Mathematica***

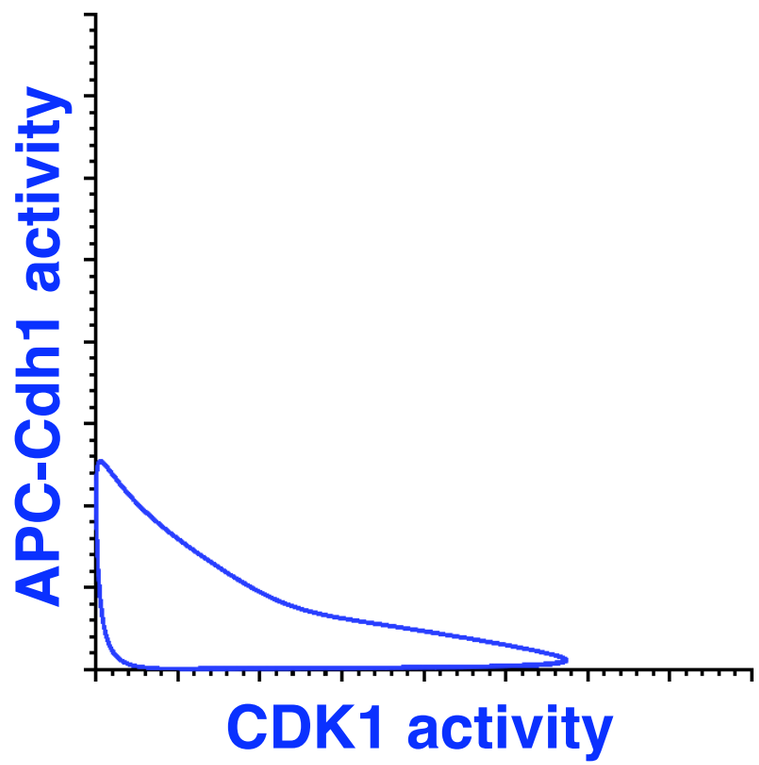
The "Somatic" Model & its Overshoot of the Hysteresis Loop




-CDK1AF
cyclin B protein
CDK1-cyclin B activity
APC-Cdc20 activity
APC-Cdh1 activity
Relative units
0
300
600
900
1200
1500
1800
2100
2400
Time (min)


CDK1 activity
[cyclin] nM


APC-Cdc20 activity
CDK1 activity


APC-Cdh1 activity
CDK1 activity

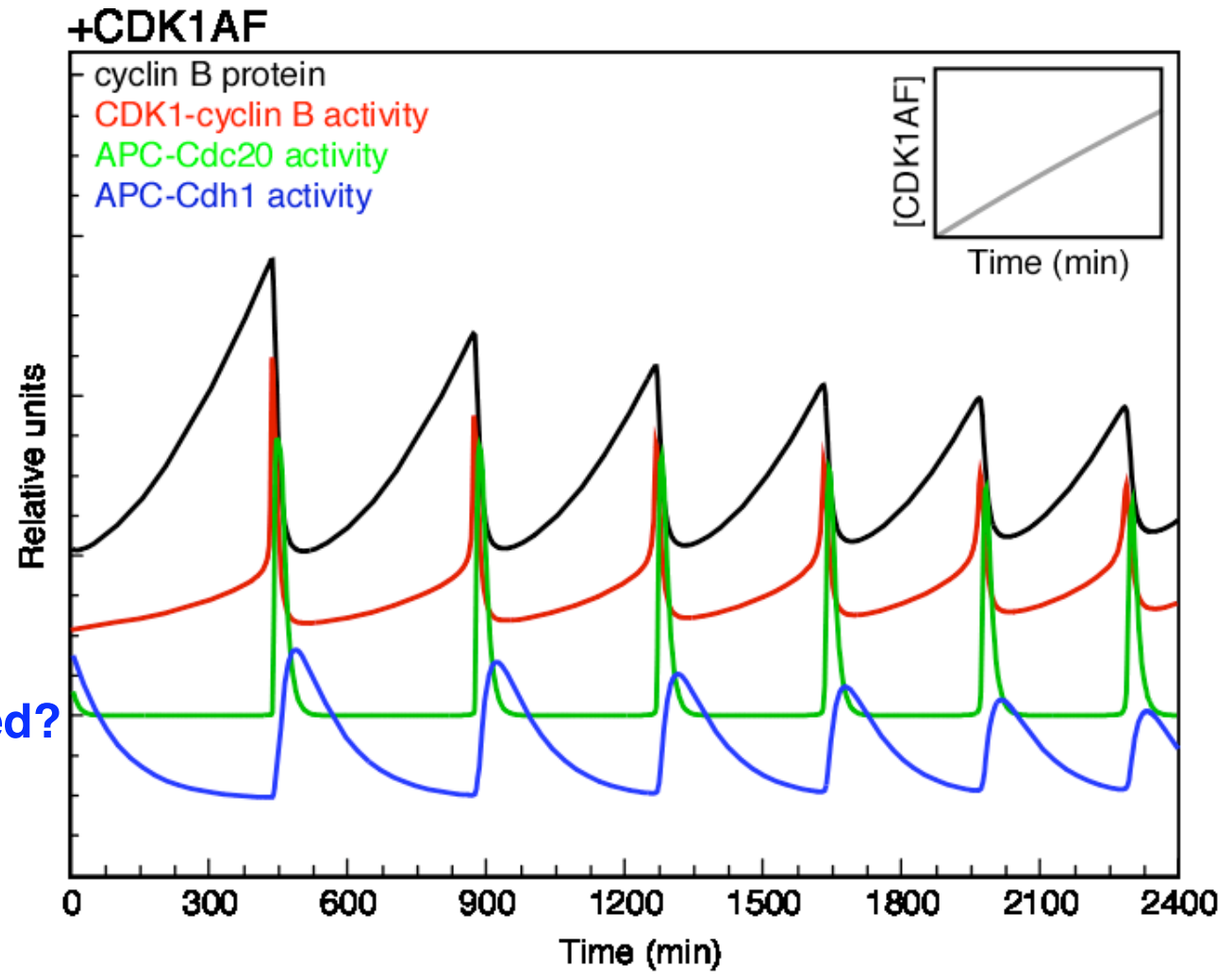
1) Early mitosis?

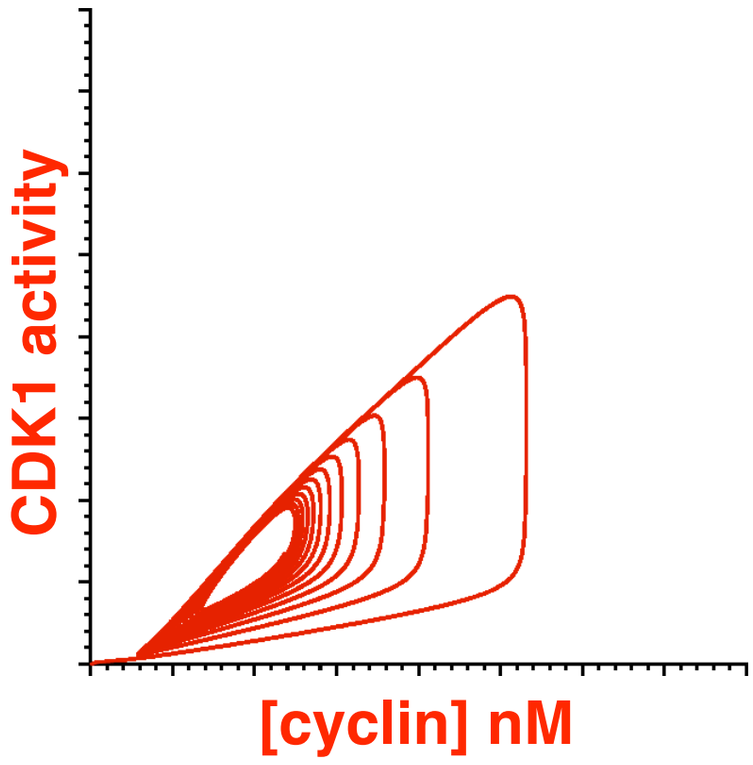
2) Increased frequency?

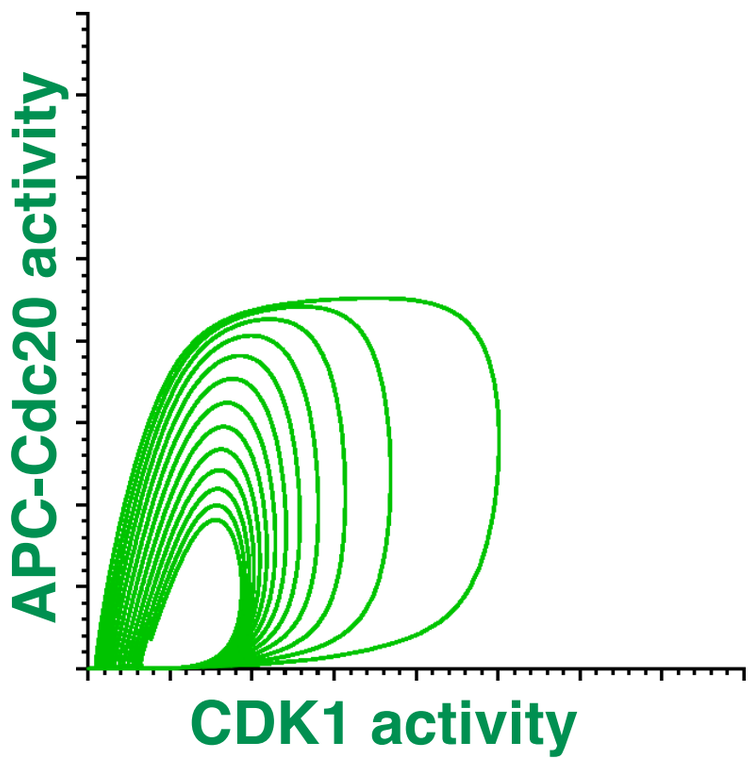
3) Some damping?

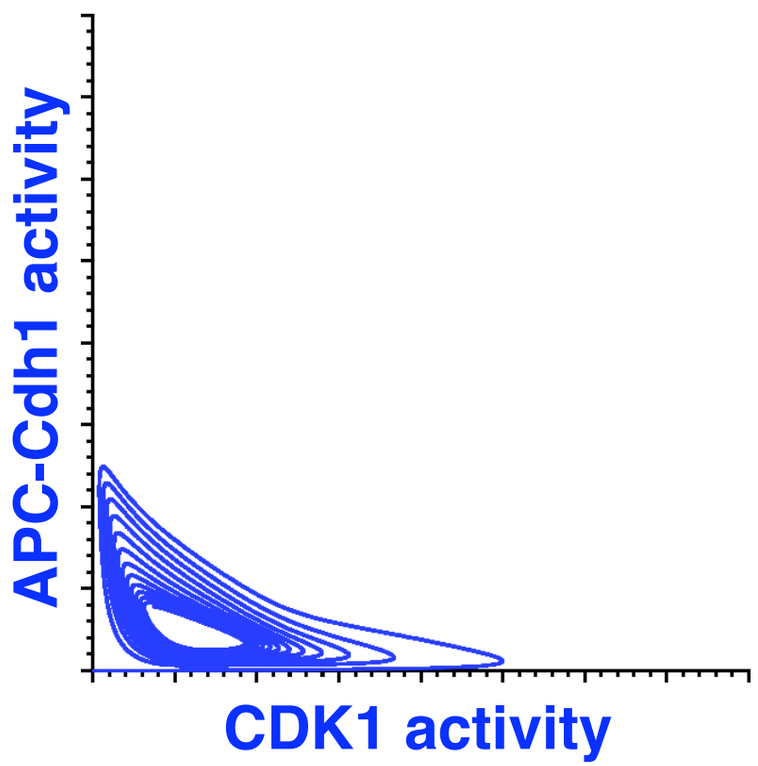
4) Cdc20 activity maintained?

5) Cdh1 activity period reduced?

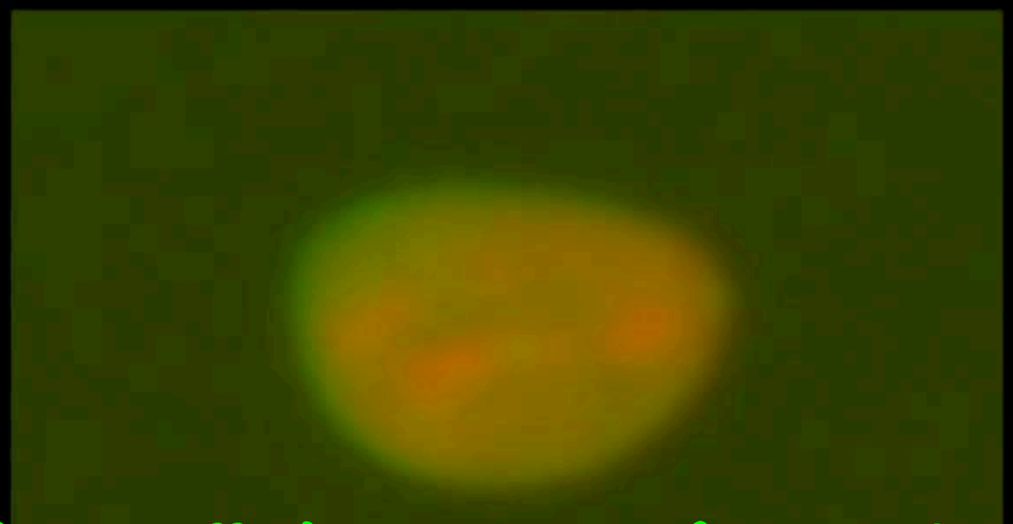








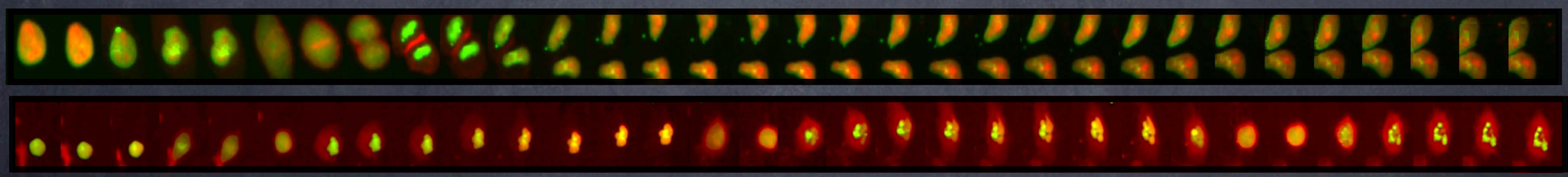
YFP-Lamin As A Nuclear Biosensor



How will the positive-feedback short-circuit affect NEB/division/NER?

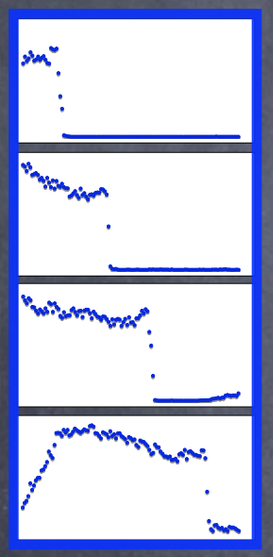
YFP-Lamin A1
MBS-Red
CFP-CDK1WT (not shown)

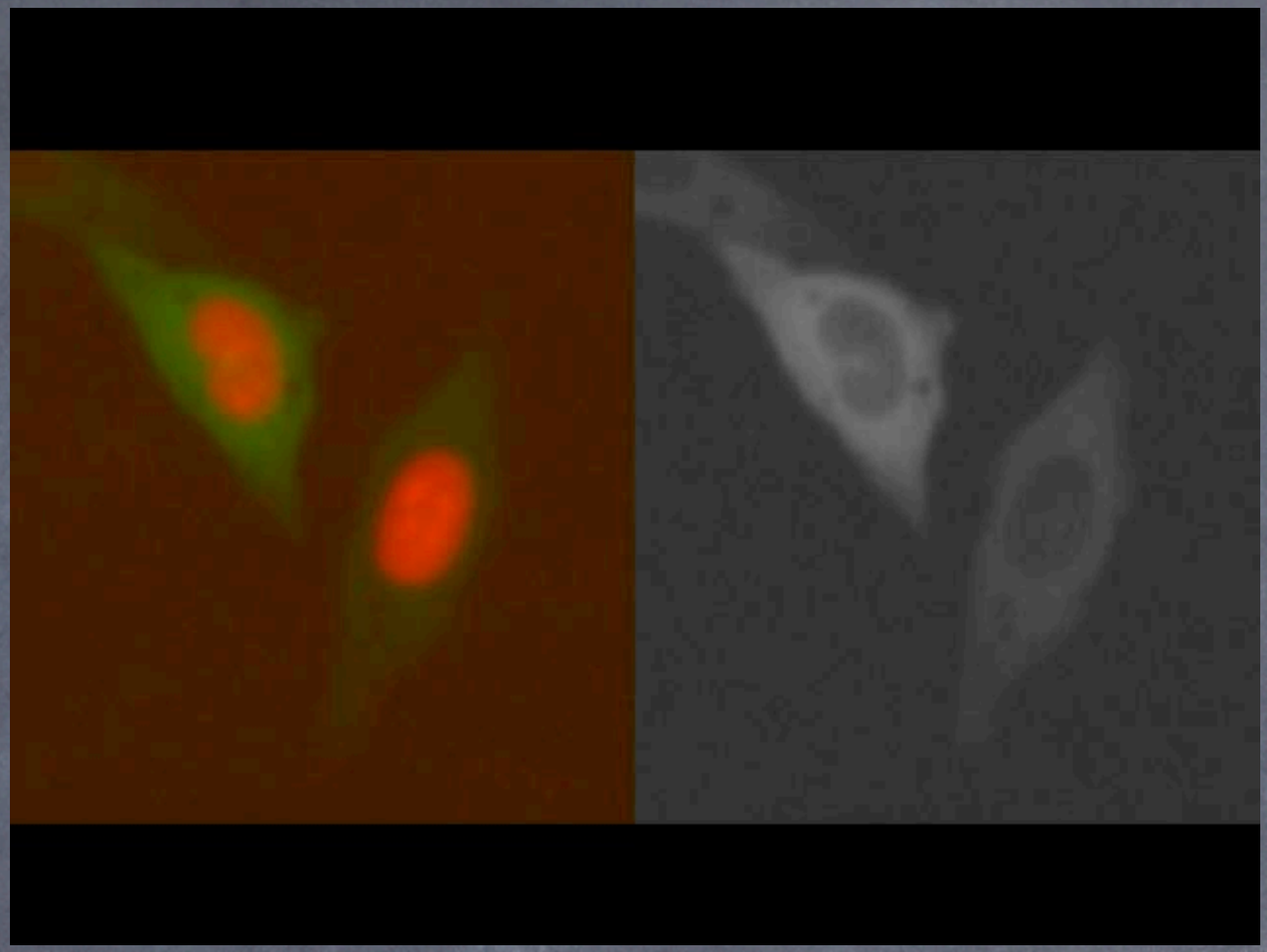
YFP-Lamin A1
MBS-Red
CFP-CDK1AF (not shown)



Cyclin B1-YFP As A Negative-Feedback & CDK1 Sensor

CDK1WT

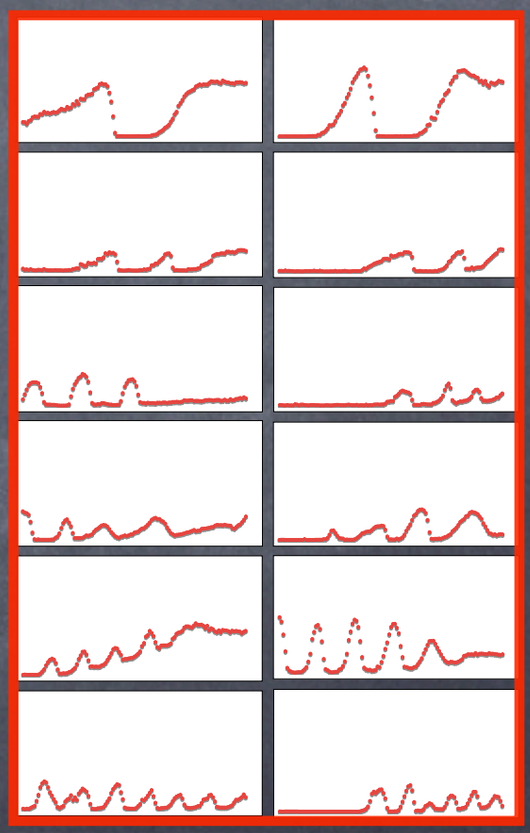


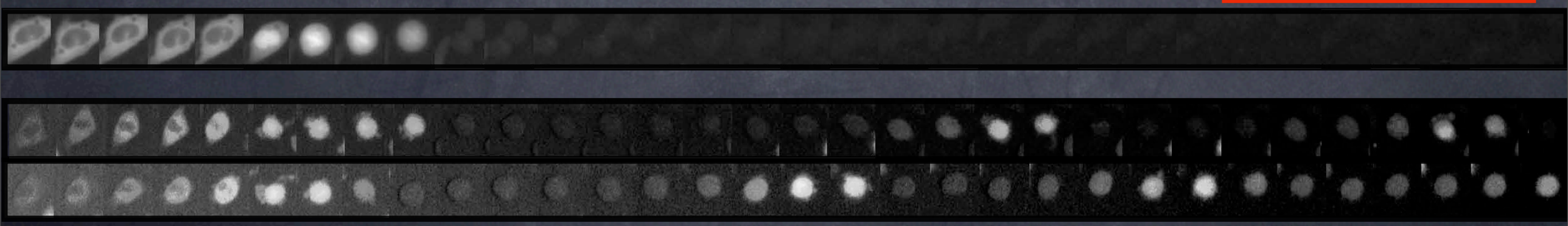


Cyclin B1-YFP
MBS-Red
CFHP-CDK1AF (knott shknown)

YFP channel alone

CDK1AF



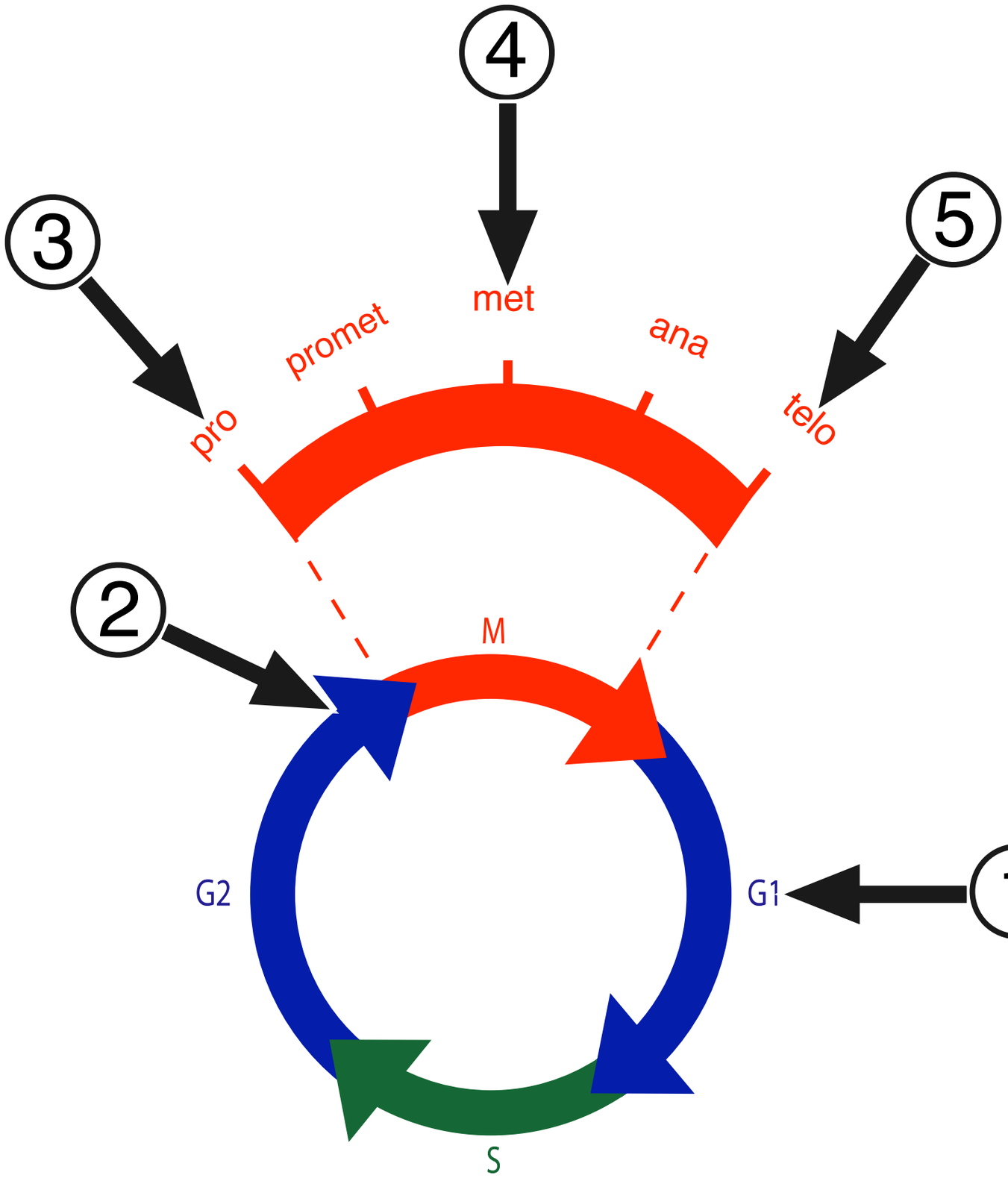


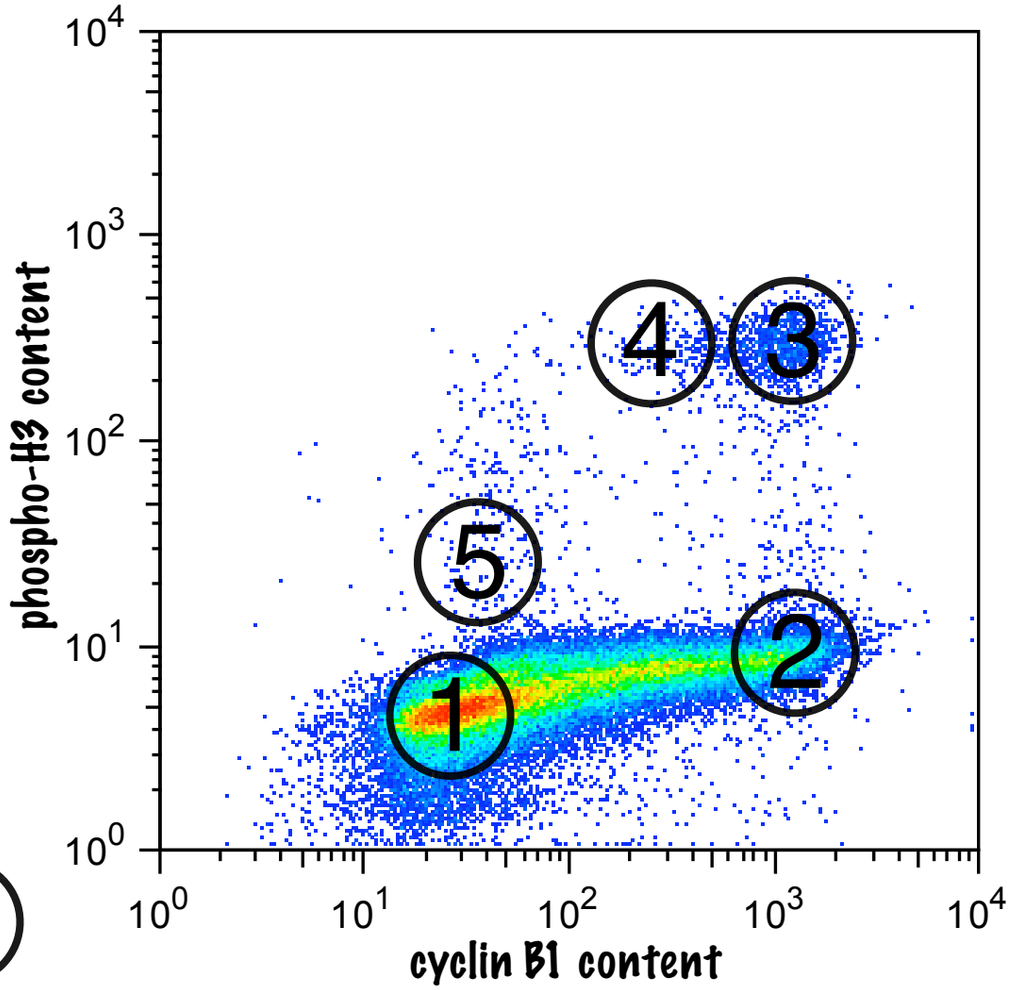
Which mitotic cyclins are involved in the aberrant M-phase-like/interphase-like oscillations that were observed in single cells during the live-cell imaging?

How can we dissect what is going on in large a population of cells WITHOUT extreme perturbation (even asynchronous), but WITH molecular sensitivity?

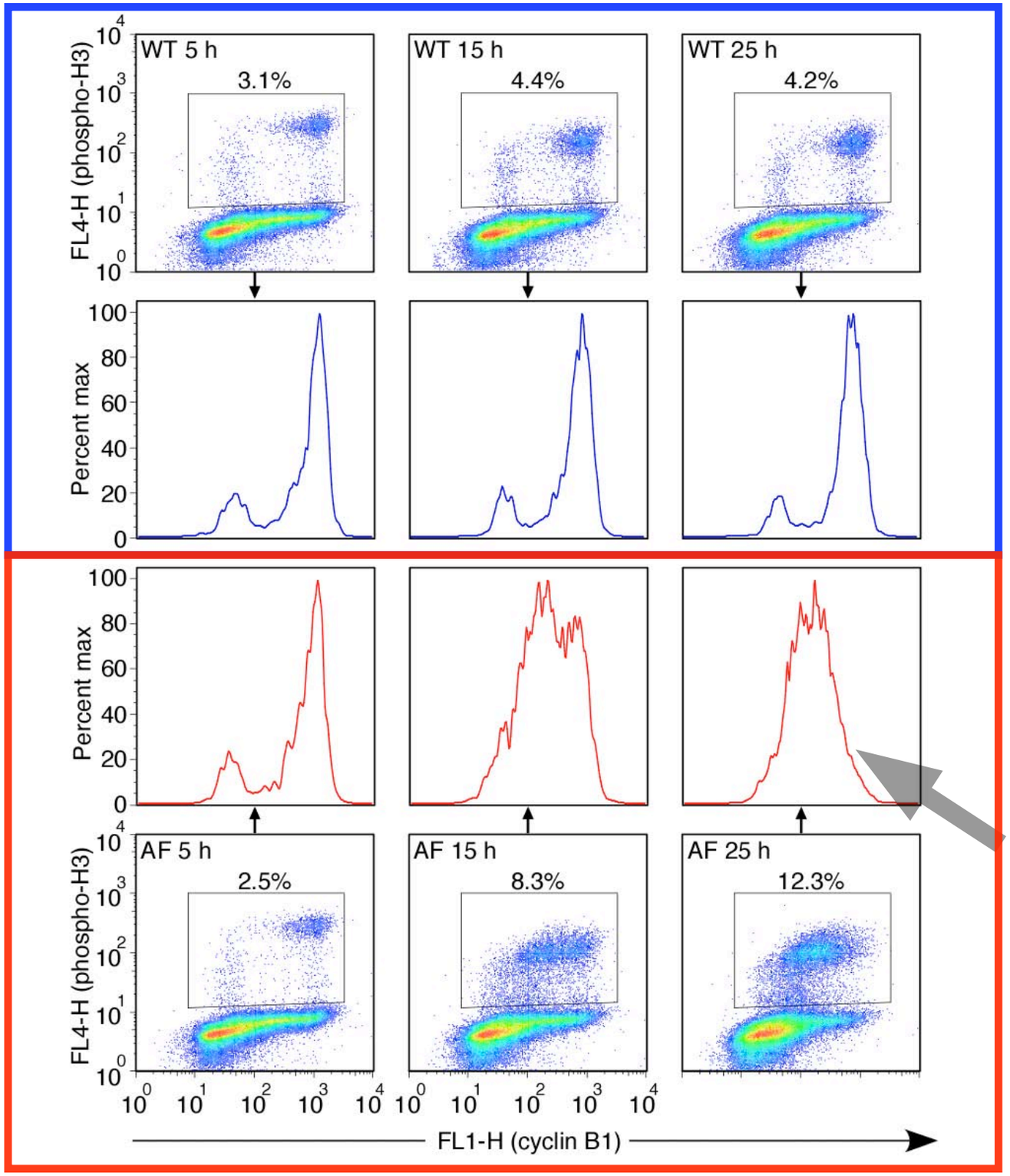
The BANE of CELL BIOLOGISTS: If not working in egg extracts, or "imaging" live individual cells with limited sets of sensors, we are working with cell populations.

Single-Cell Analysis of Cyclin B1 Content



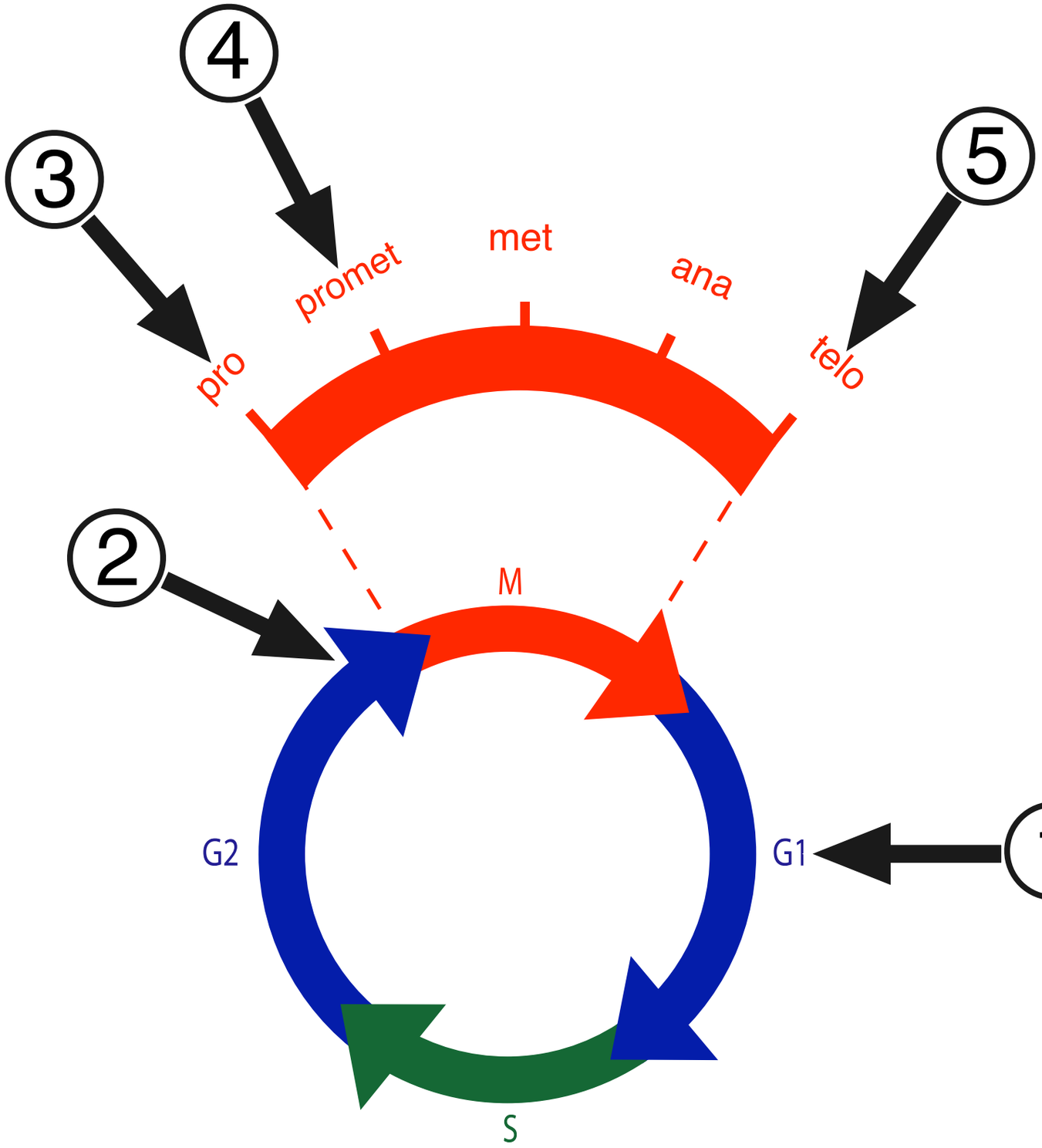


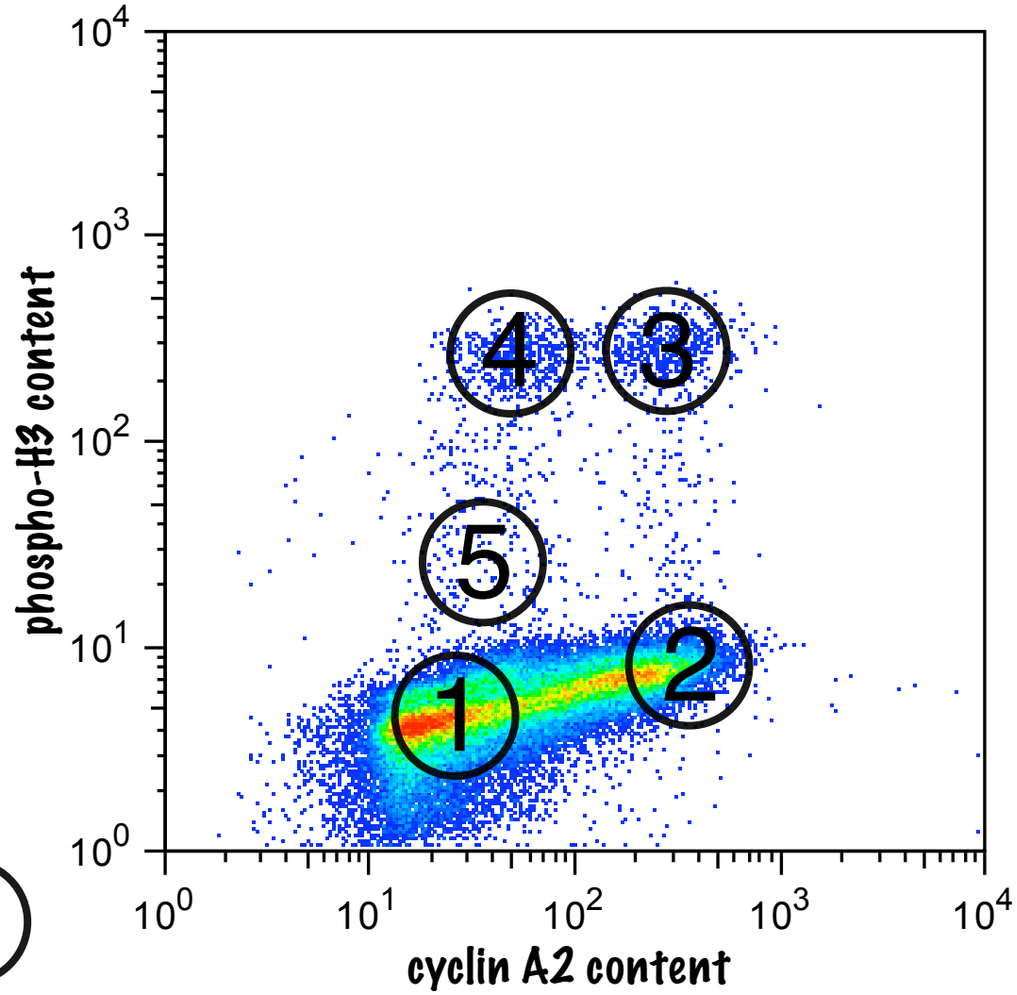
Single-Cell Analysis of Cyclin B1 Content



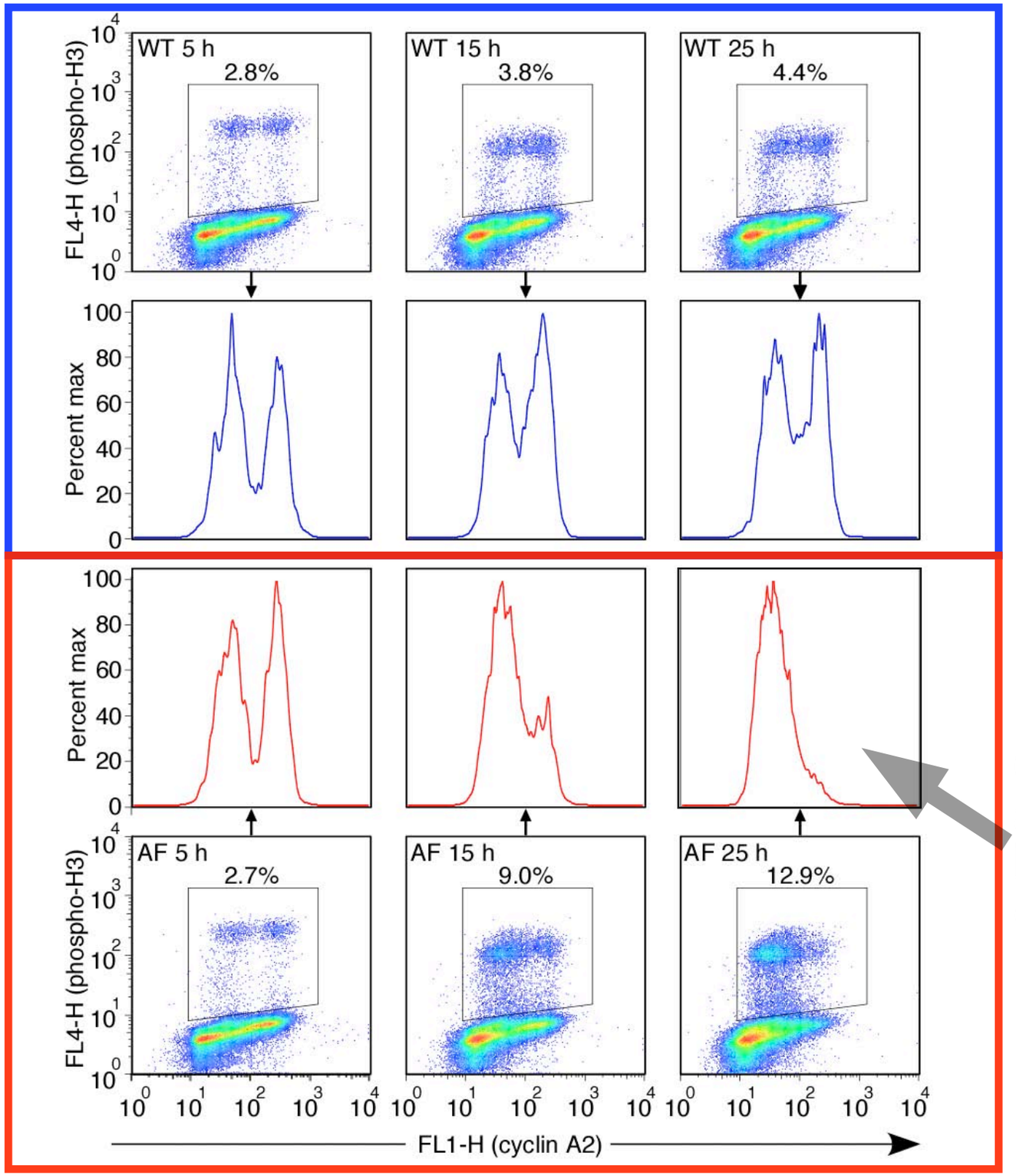
CDK1AF causes MORE cells to enter an M-phase-like state, though they are expressing INTERMEDIATE levels of cyclin B1

Single-Cell Analysis of Cyclin A2 Content





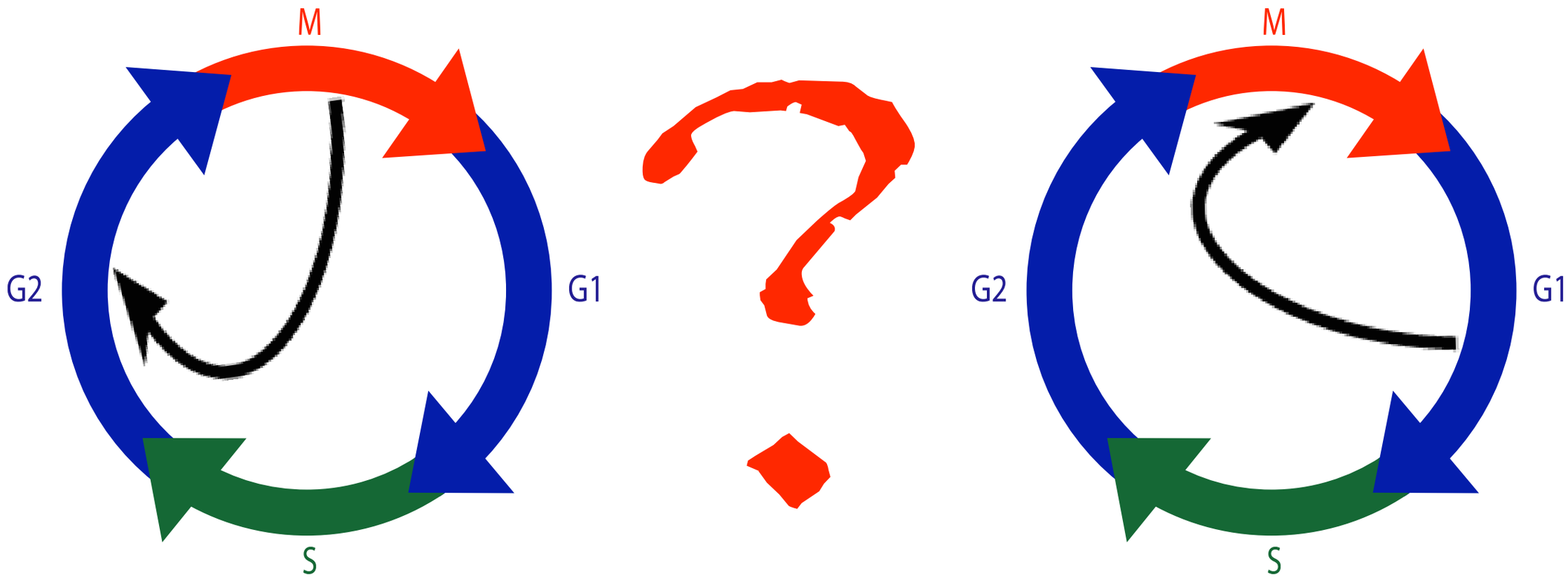
Single-Cell Analysis of Cyclin A2 Content



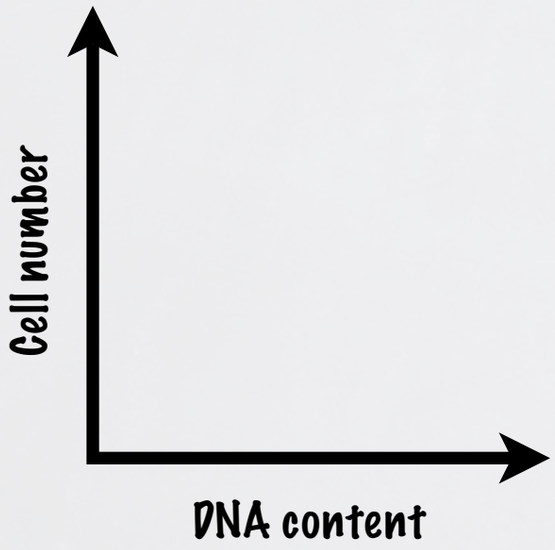
CDK1AF causes an accumulation of M-phase-like cells that are devoid of cyclin A2

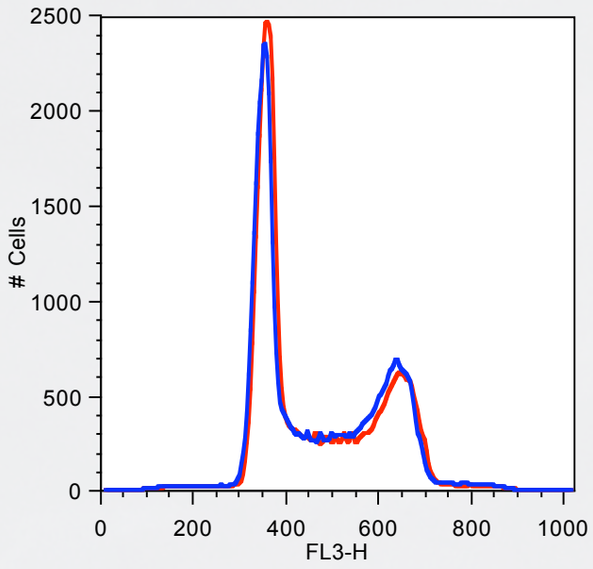
With cyclin A2 absent from the perturbed, M-phase-like populations, cyclin B1 is the sole mitotic cyclin driving the abnormal CDK1AF-induced oscillations.

Are these cells experiencing a G2-M defect, or are they daughter cells with an M exit-G1 initiation defect.



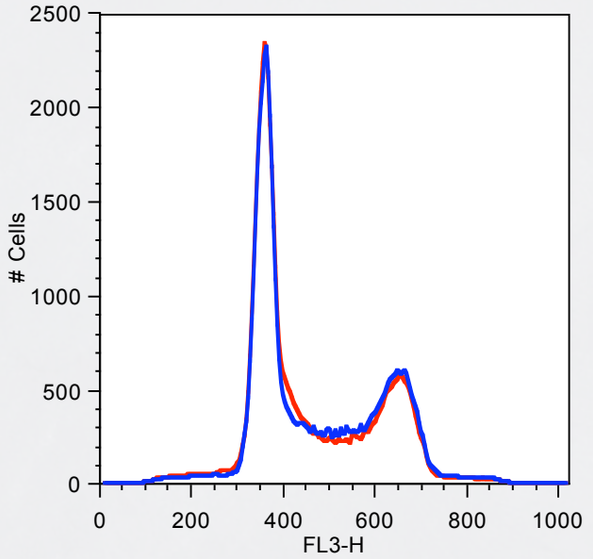
No, but they do begin to pile up between 2N and 4N DNA content cells. Cells seem to be rapidly moving from G1 to S phase.



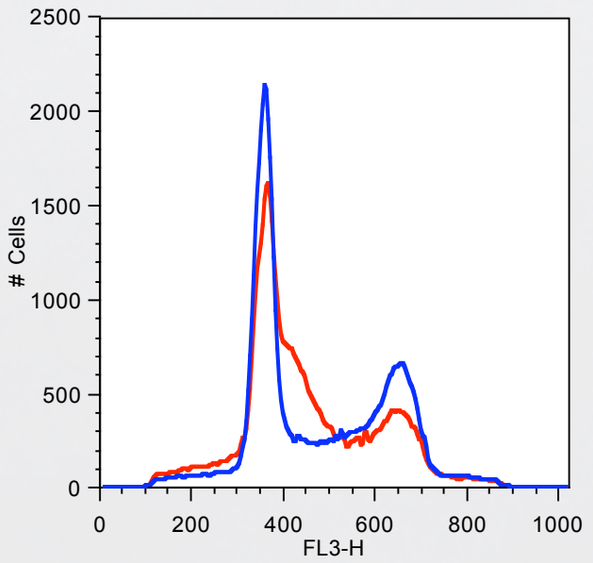


Post-transfection (h)

5



15



25

Live-Cell Analyses of G1-, S-, and M-phase Progression

CDK1WT-transfected w/MBS & PCNA-YFP

S end/G2 start (8:32:34 PM)

G2 duration ≈1.75 h

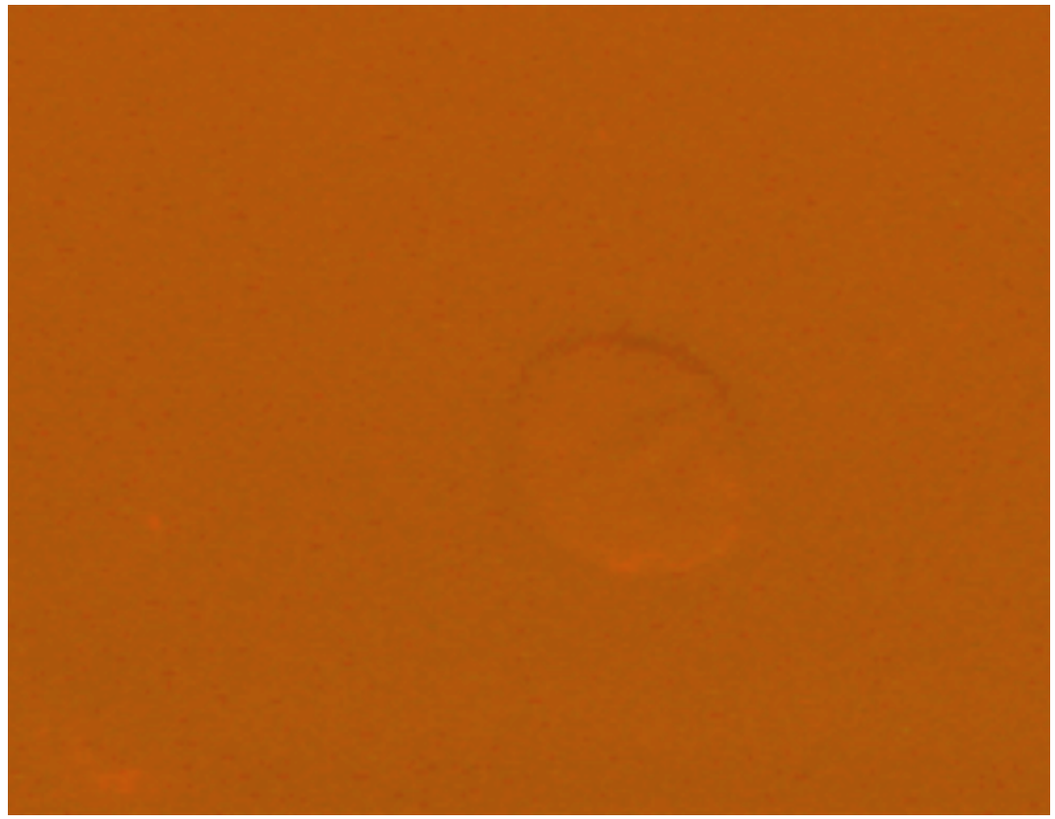
M (10:12:12 PM)

M duration ≈0.67 h

cytokinesis/G1 start (10:53:20 PM)

G1 duration ≈8.5 h

S start (7:25:51, 7:31:48 AM)



CDK1AF-transfected w/MBS & PCNA-YFP

cytokinesis/G1 start (11:02:07 PM)

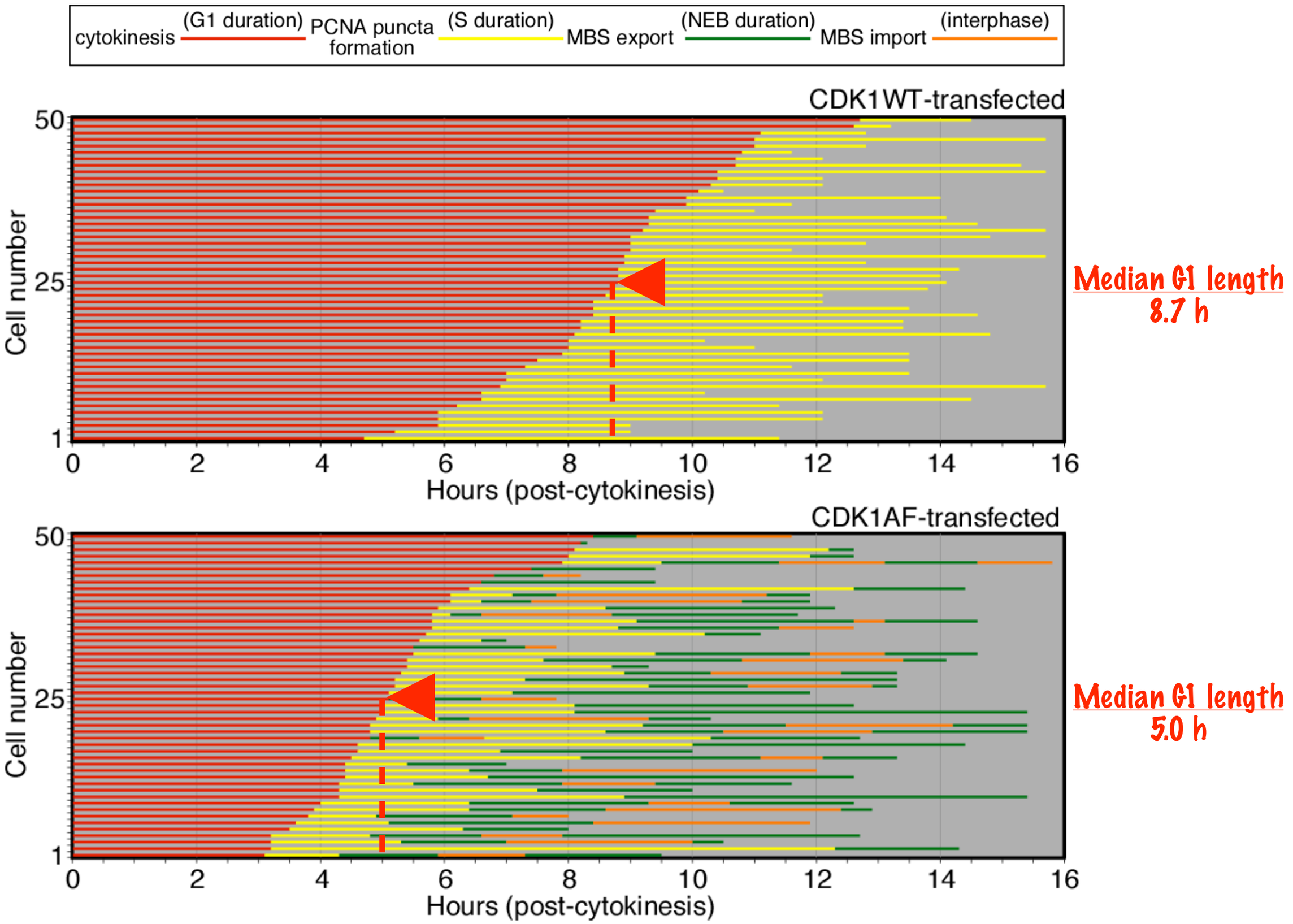
G1 duration ≈3.5 h

S start (2:30:31 AM)

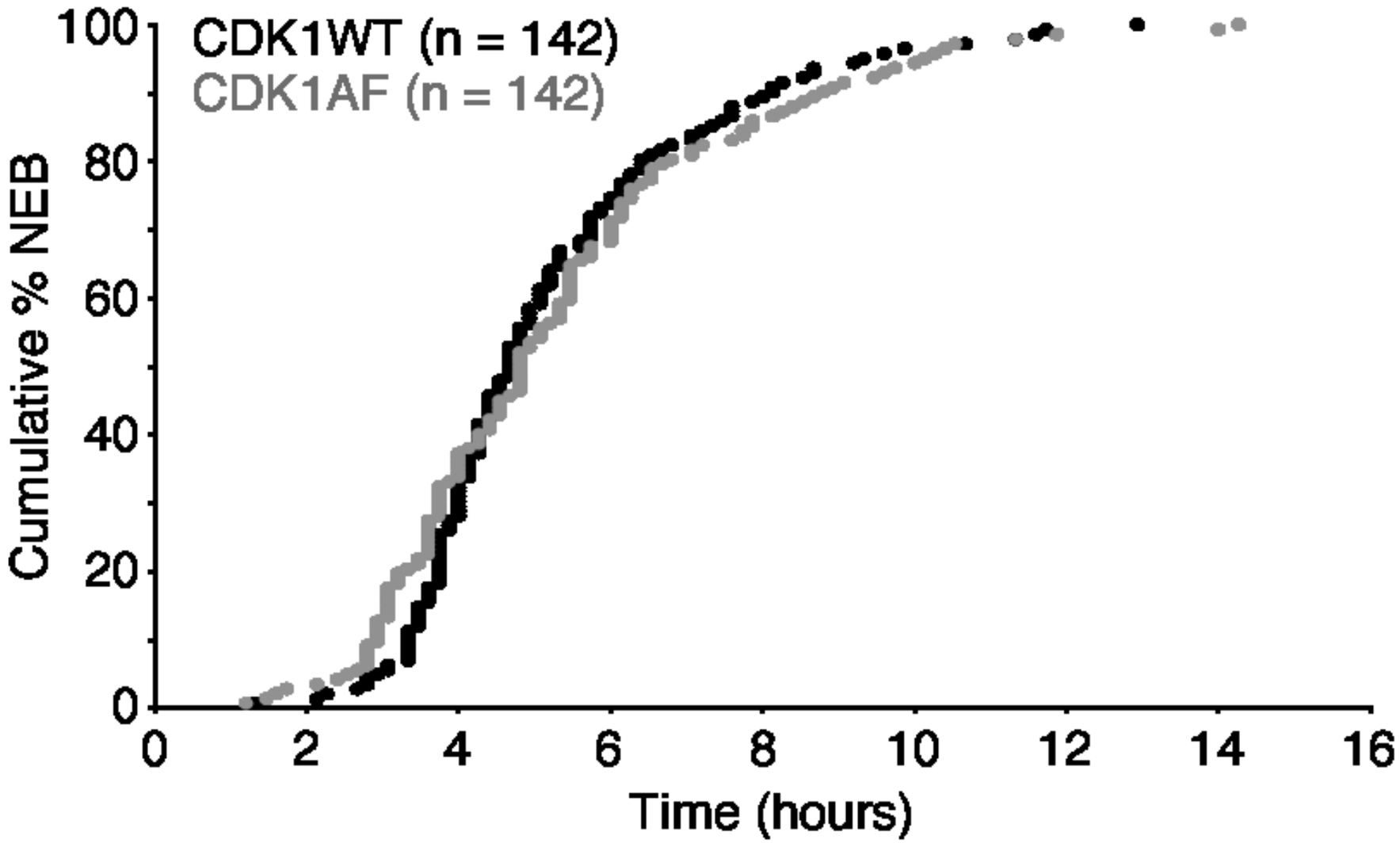
S duration ≈2.0 h

NEB (3:57:57, 4:27:42 PM)

Live-Cell Analyses of G1-, S-, and M-phase Progression



Does CDK1AF cause early mitosis in a cell population released from thymidine?



1) Early mitosis? ✗

2) Increased frequency? ✓

3) Some damping? ✓

4) Cdc20 activity maintained? ✓

5) Cdh1 activity period reduced? ✓

Current Biology 17, 85–91, January 9, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.11.066

Report

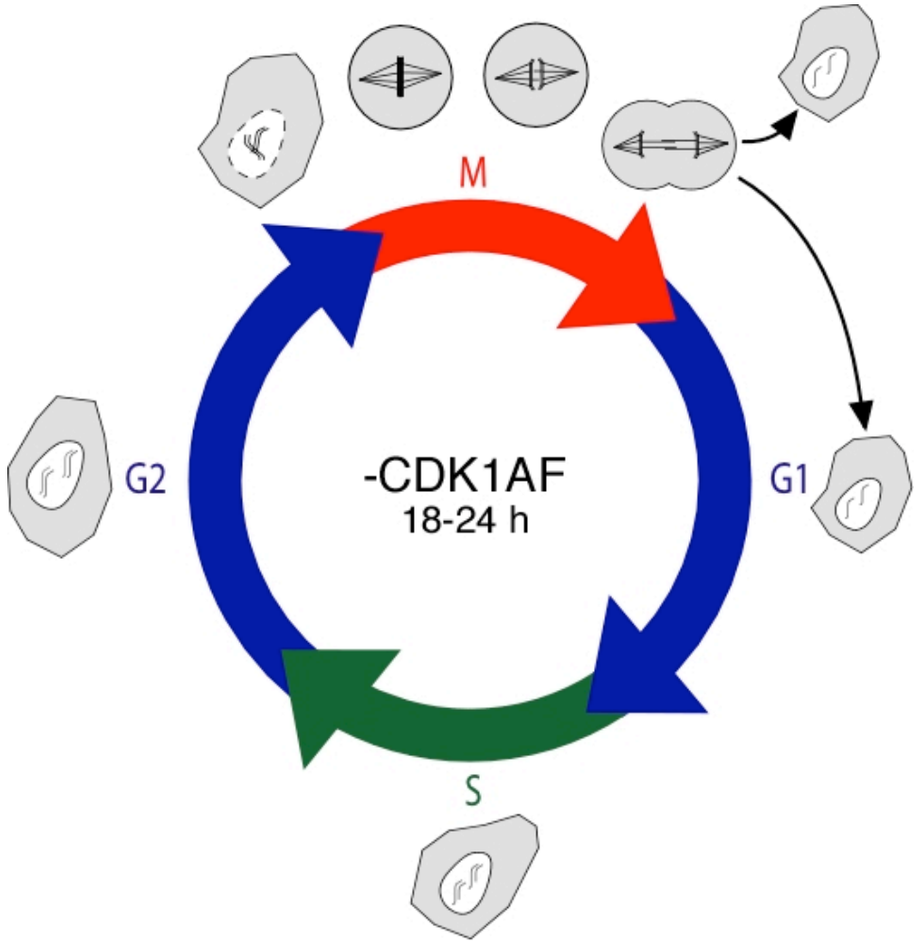
Cyclin A2 Regulates Nuclear-Envelope Breakdown and the Nuclear Accumulation of Cyclin B1

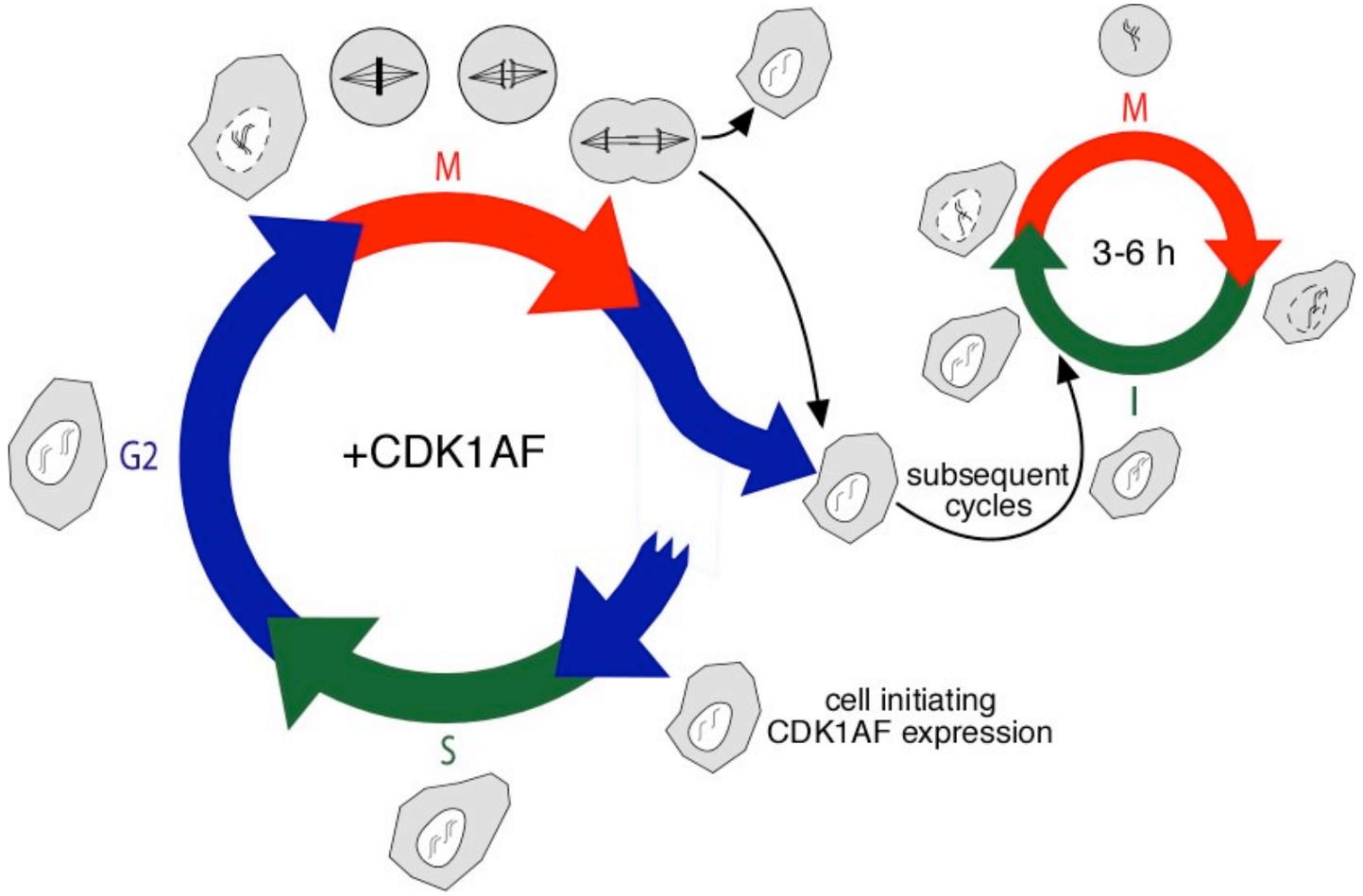
Delquin Gong,[1,5,*] Joseph R. Pomerening,[1] Jason W. Myers,[1] Christer Gustavsson,[2] Joshua T. Jones,[2] Angela T. Hahn,[1,3] Tobias Meyer,[2] and James E. Ferrell, Jr.[1,4,*]

[1] Department of Chemical and Systems Biology, CCSR, MC 5174

[2] Department of Chemical and Systems Biology,

pathways [8]. We synchronized cells by double thymidine block and transfected them with the cyclin d-siRNAs singly or in combination during the first thymidine washout. 24 hr after transfection, cells were released from the second thymidine block. We assessed cyclin levels by immunoblotting (Figure 1A) and followed mitotic [illegible] by automated epifluorescence microscopy, [illegible] fluorescent mitotic biosen-





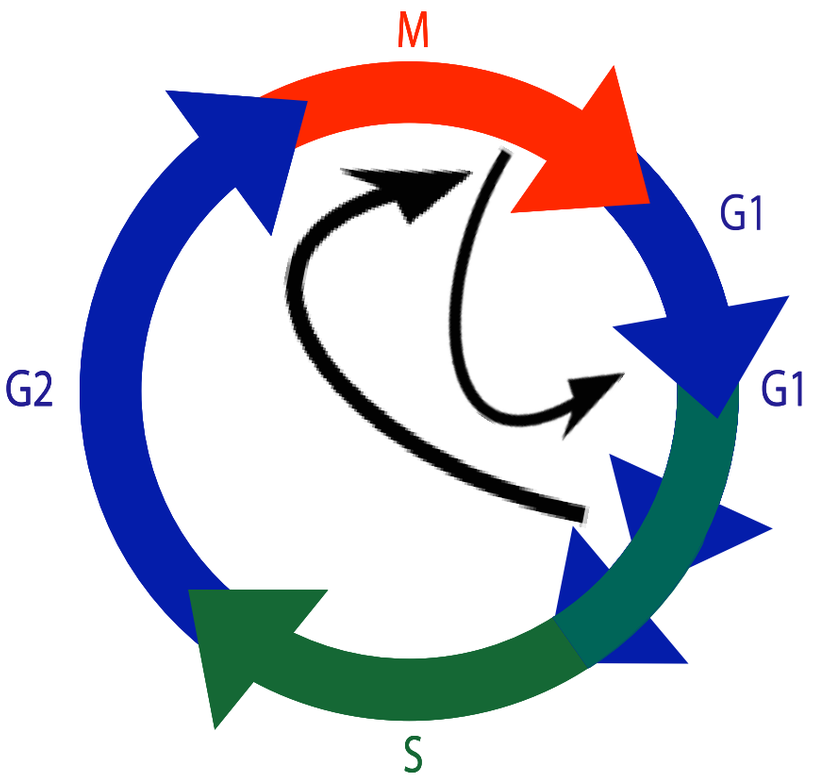
Summary-Part III

Bypassing the positive feedback loops responsible for CDK1 activation with CDK1AF causes multiple cellular defects within a SOMATIC CELL type, including:

exchanges of M-phase-like and interphase-like periods with intermediate M-phase phosphorylation states;

periods of cyclin B stability and instability, when there should only be ONE per cell cycle;

a rapid oscillatory behavior that emerges from cells that divide, and either enter an M-phase-like state from G1 (16% of the time), or cells shorten G1 (by approx. 4 h), induce a precocious S phase, then stimulate an M-phase-like state (84% of the time).



Positive feedback in CDK1 activation provides discrete M- and S-phases in the embryonic context (to oscillate properly).

Positive feedback in CDK1 activation ensures a sufficient G1 period, and thus, a properly delayed S phase onset in the somatic context (no oscillation).

Thank You.

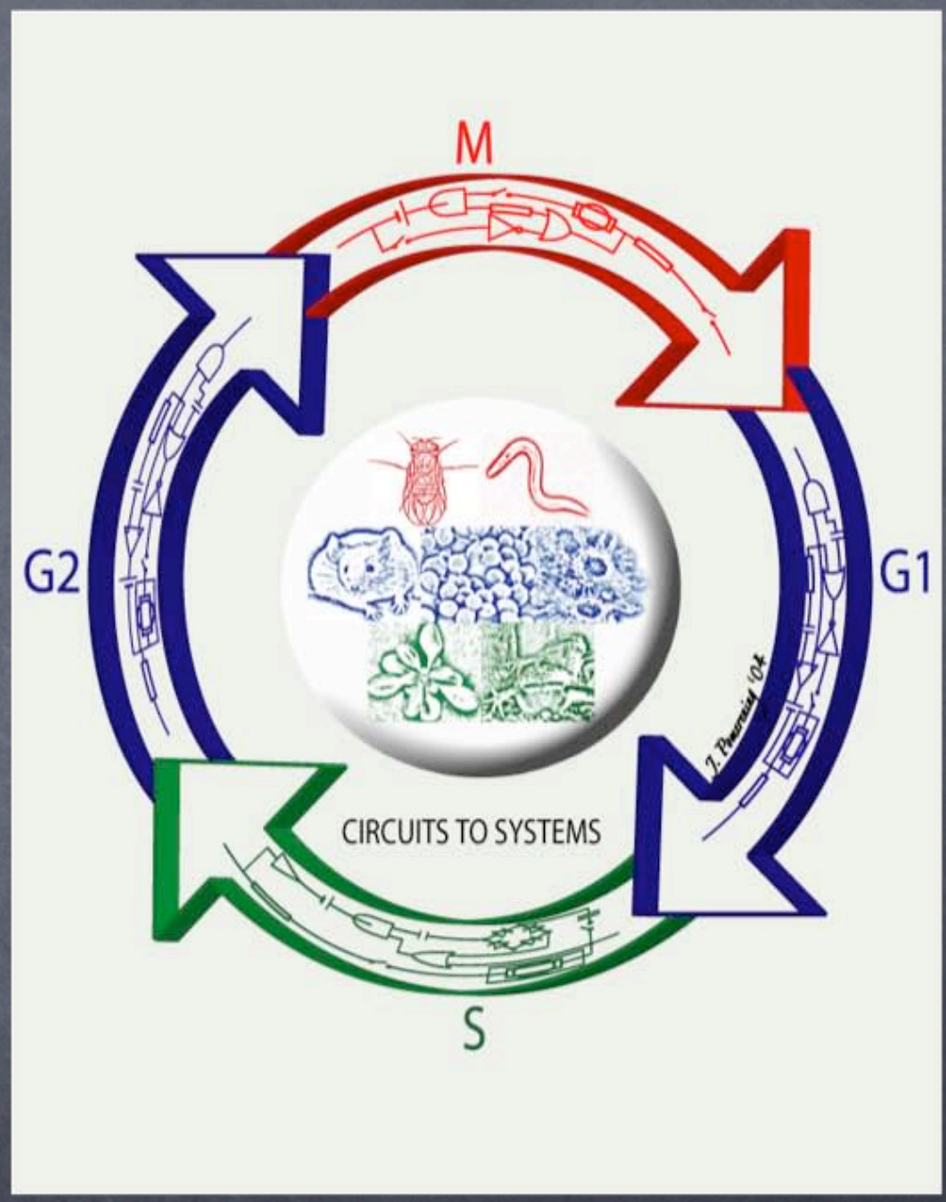
Ferrell Lab
-Prof. Jim Ferrell
-Sun Young Kim
-Jason Myers
-Delquin Gong

Meyer Lab
-Prof. Tobias Meyer
-Josh Jones
-Angie Hahn

Tom Wehrman
Mark Hammer

Stanford HTBC Facility

M. Cristina Cardoso
Max Delbrück, Berlin



Quantitative Chemical Biology Program

Quantitative Chemical Biology

Kavli Institute for Theoretical Physics - University of California-Santa Barbara