Modeling from an experimentalist's perspective: How to mine the literature and design experiments to provide data that informs mathematical models of signaling networks.

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Virginia Tech

$$\frac{d\,CDK}{dt} = k_1 - (v_2' + v_2'' \cdot Cdh1) \cdot CDK$$

$$\frac{d\,Cdh1}{dt} = \frac{(k_3' + k_3'' \cdot Cdc20_A)(1 - Cdh1)}{J_3 + 1 - Cdh1} - \frac{(k_4' + k_4'' \cdot CDK \cdot M)\,Cdh1}{J_4 + Cdh1}$$

$$\frac{d\,IEP}{dt} = k_9 \cdot CDK \cdot M \cdot (1 - IEP) - k_{10} \cdot IEP$$

$$\frac{d\,Cdc20_T}{dt} = k_5' + k_5'' \frac{(CDK \cdot M)^4}{J_5^4 + (CDK \cdot M)^4} - k_6 \cdot Cdc20_T$$

$$\frac{d\,Cdc20_A}{dt} = \frac{k_7 \cdot IEP\,(Cdc20_T - Cdc20_A)}{J_7 + Cdc20_T - Cdc20_A} - \frac{k_8 \cdot MAD\,Cdc20_A}{J_8 + Cdc20_A} - k_6 \cdot Cdc20$$

Outline:

Modeling example: the cell cycle and checkpoints in frog egg extracts

Experimental test of a model

Mining the literature for data

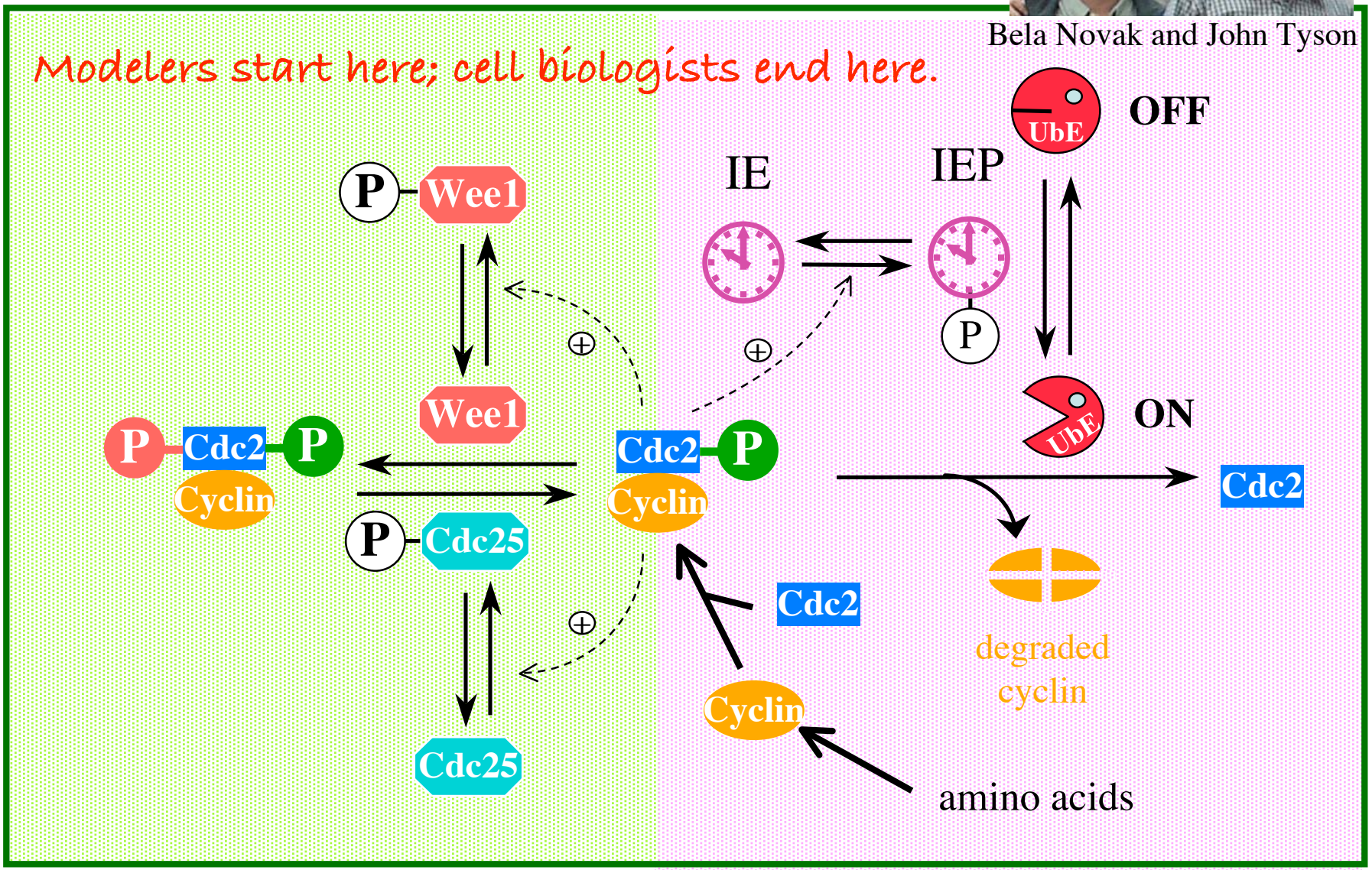
Designing experiments to inform a model

Building a model to guide further experimentation (cyclin E oscillator)

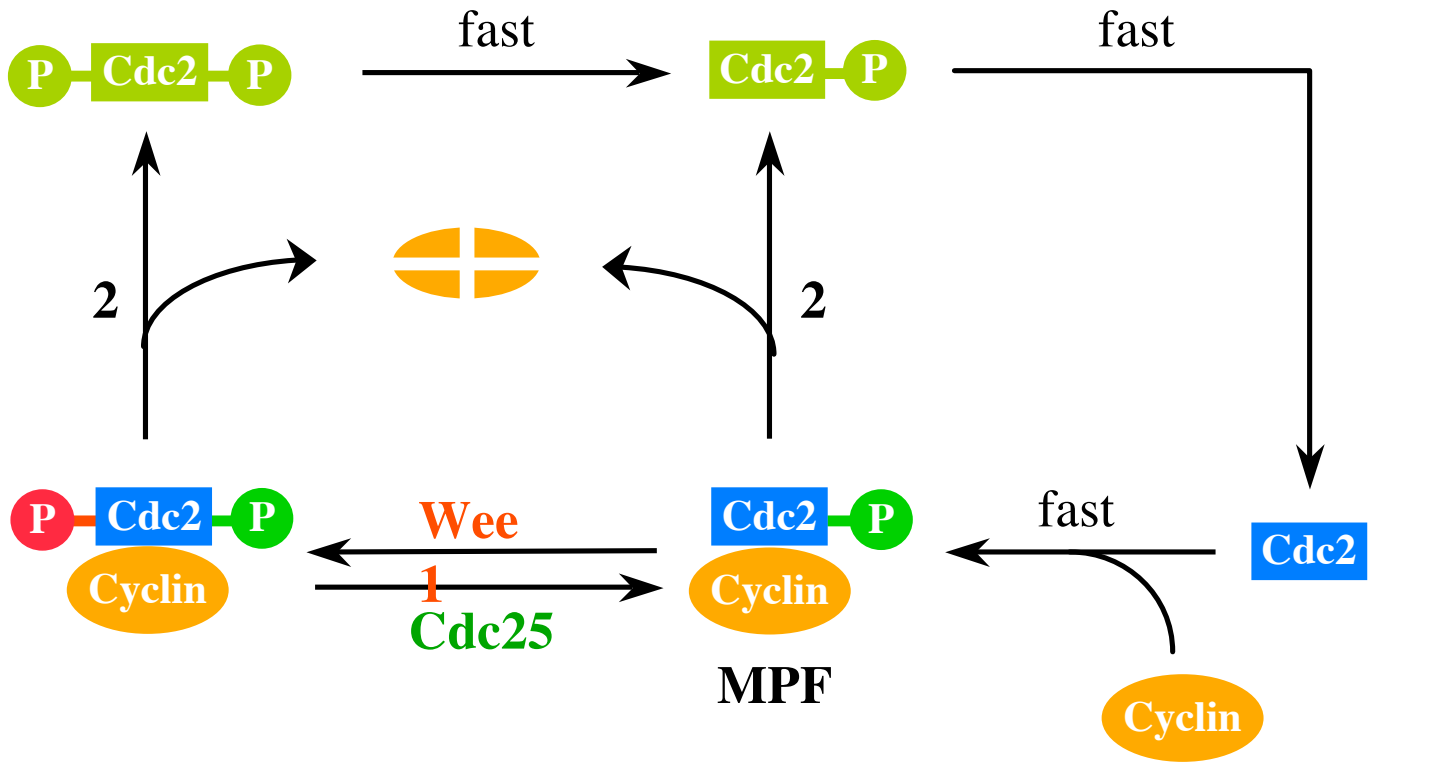
relationship advice for working with an experimental cell biologist

Mitosis is regulated by positive and negative feedback loops

Bela Novak and John Tyson



Molecular mechanism for M-phase control is translated into ordinary differential equations.

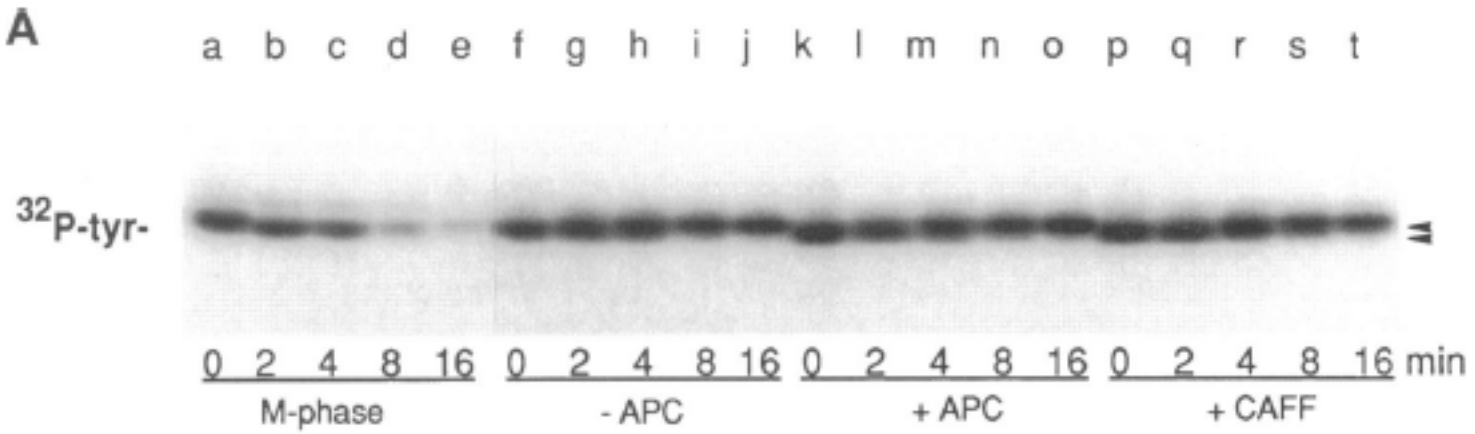


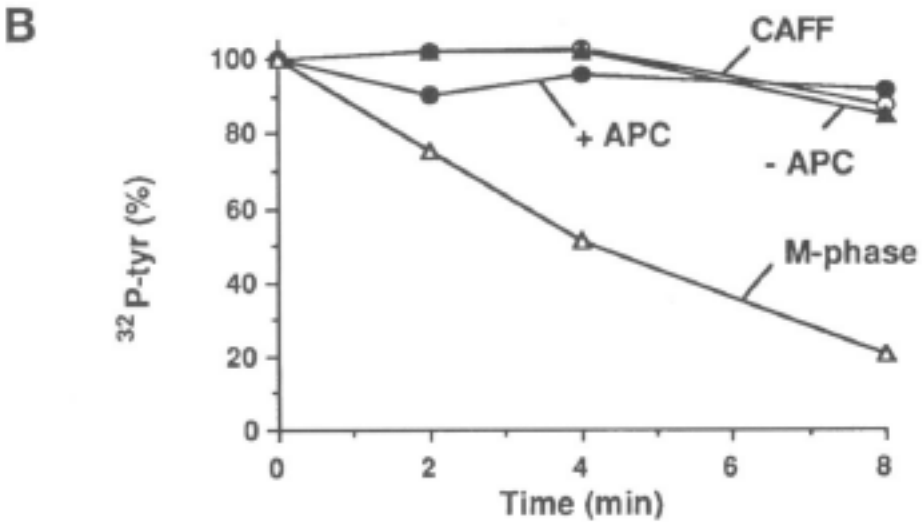
$$\frac{d\,\mathrm{MPF}}{dt} = k_1 - (k_{wee} + k_2) * \mathrm{MPF} + k_{25}\,(\mathrm{cyclin} - \mathrm{MPF})$$

$$\frac{d\,\mathrm{cyclin}}{dt} = k_1 - k_2 * \mathrm{cyclin}$$

Cell biologists are afraid of math!

Estimating rate constants from the literature:



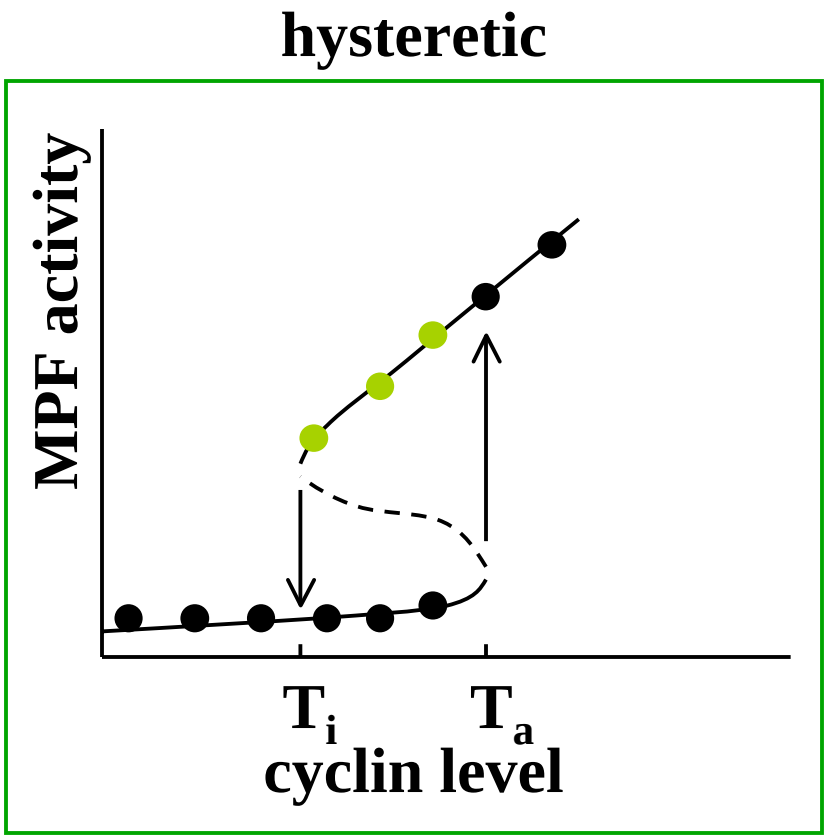


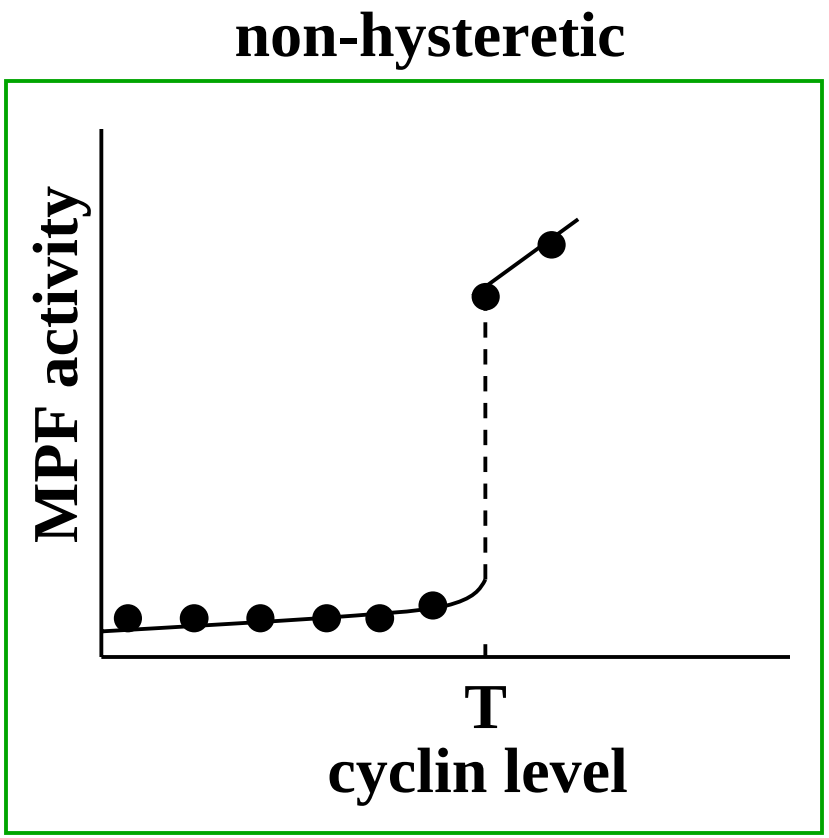
Cell biologists are binary thinkers.

Kumagai and Dunphy, 1995

Sha et al. (2003) - experimental test of a model

Prediction 1: The threshold concentration of cyclin B required to activate MPF is higher than the threshold concentration required to inactivate MPF.



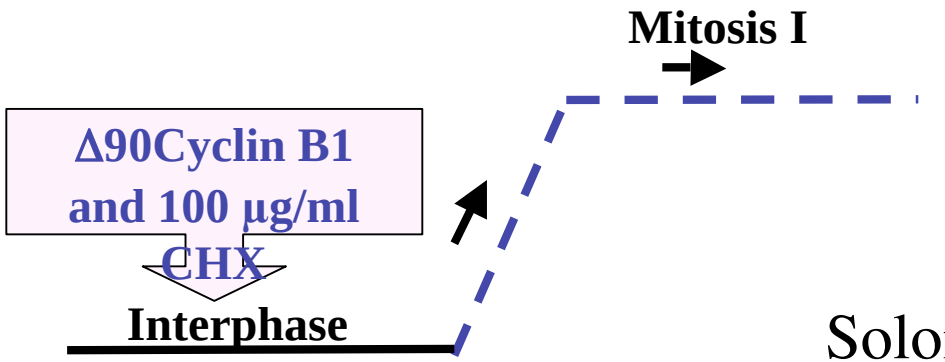


Testing Thresholds in Cycling Extracts

Wei Sha

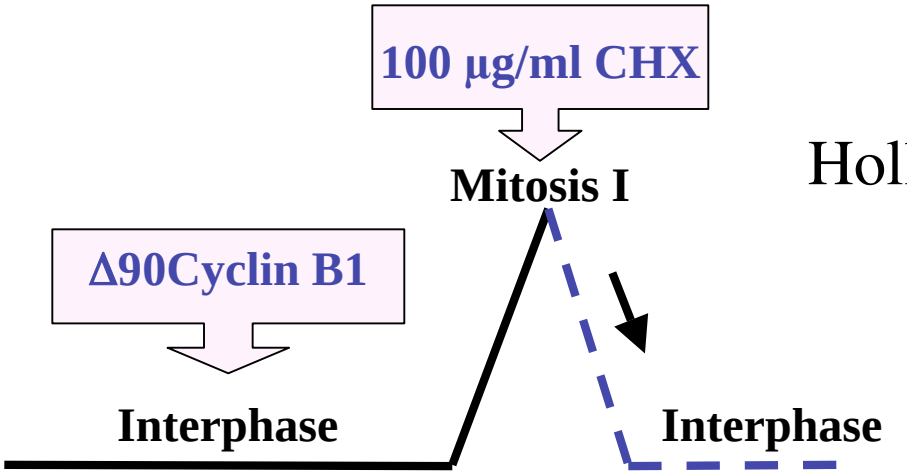
Tony Lassaletta

Testing activation threshold for Mitosis I



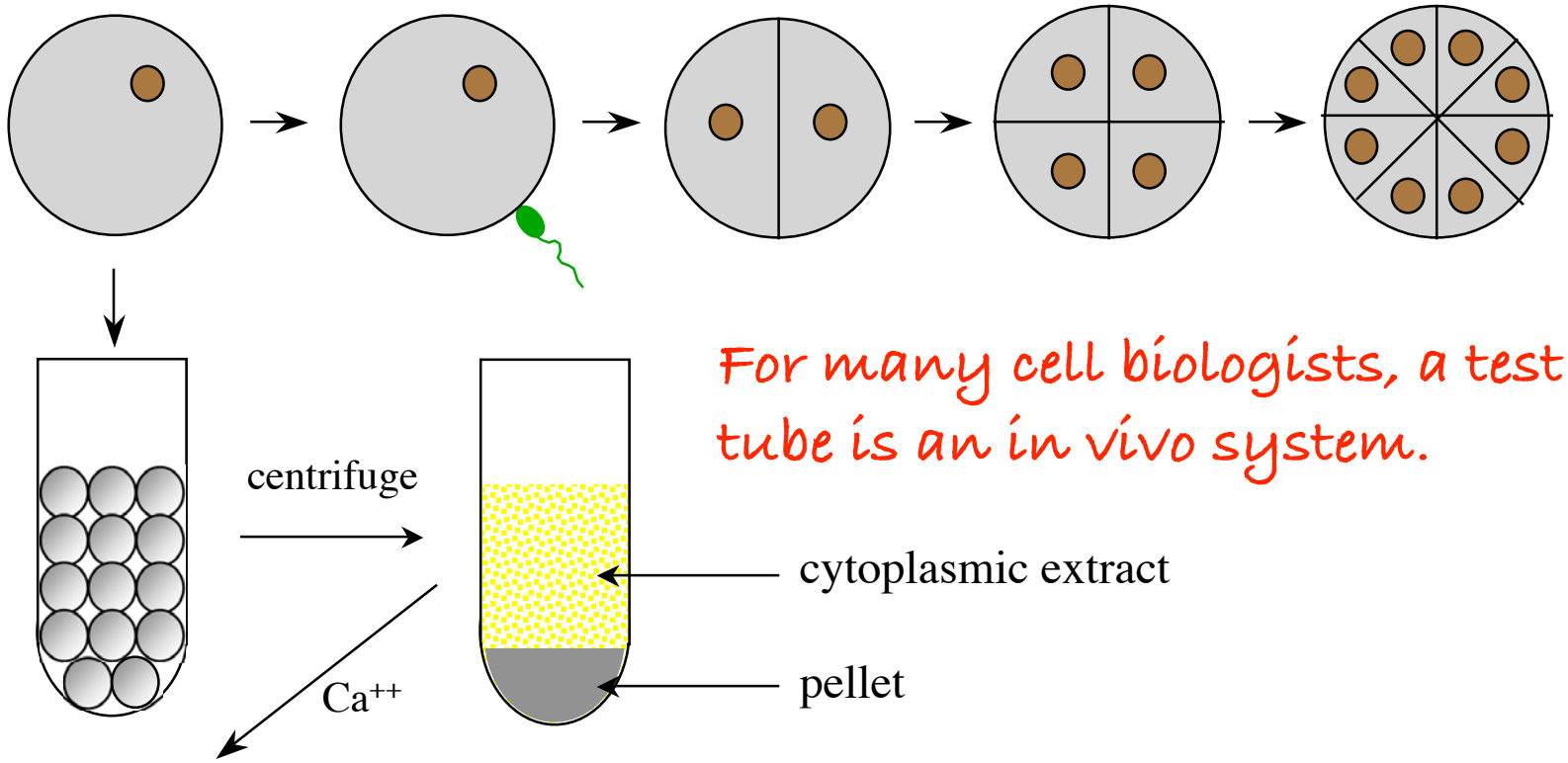
Solomon et al. 1990

Testing inactivation threshold for Mitosis I

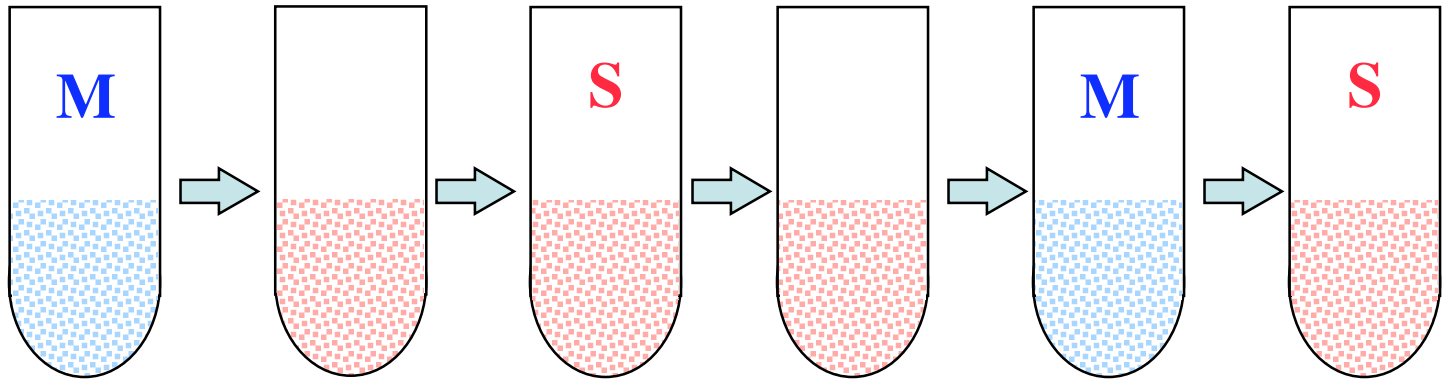


Holloway et al. 1993

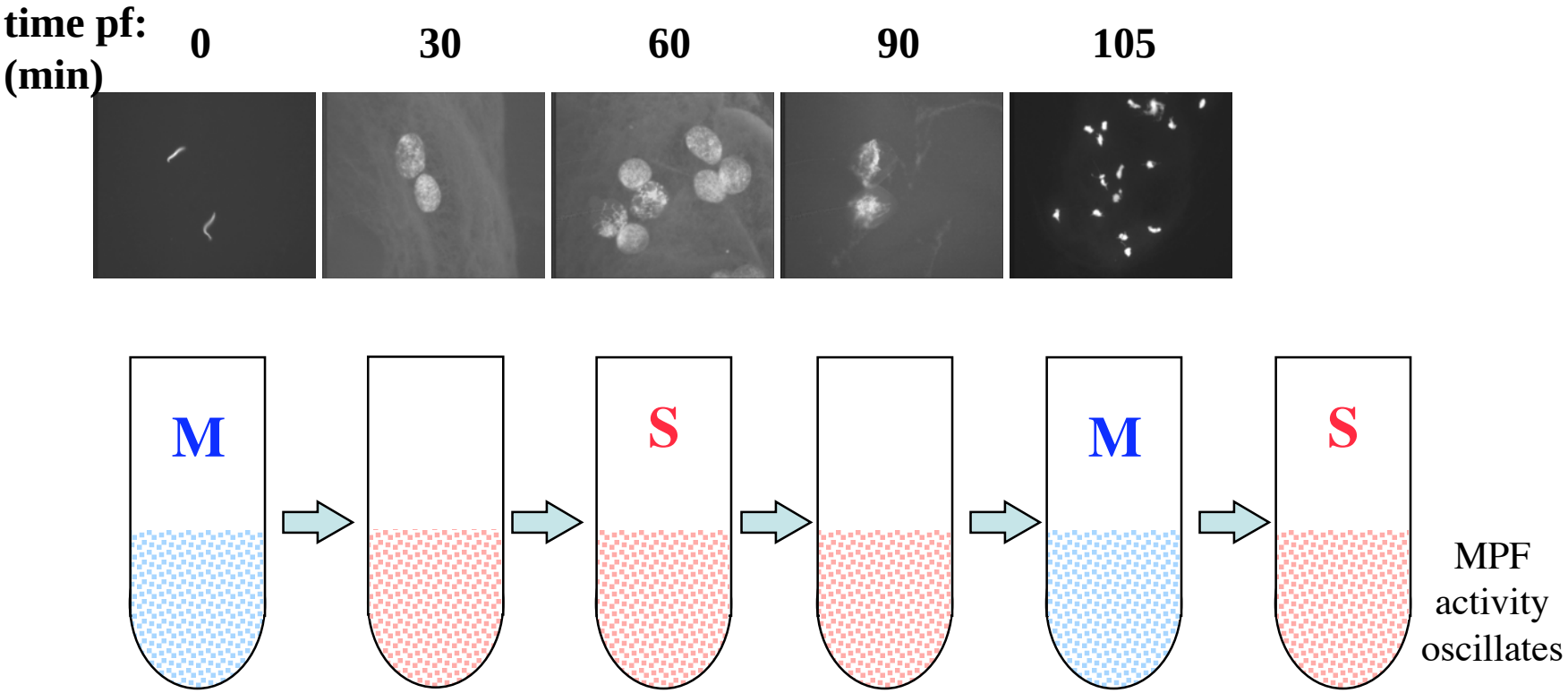
MPF activity oscillates in cell-free egg extracts.



For many cell biologists, a test tube is an in vivo system.

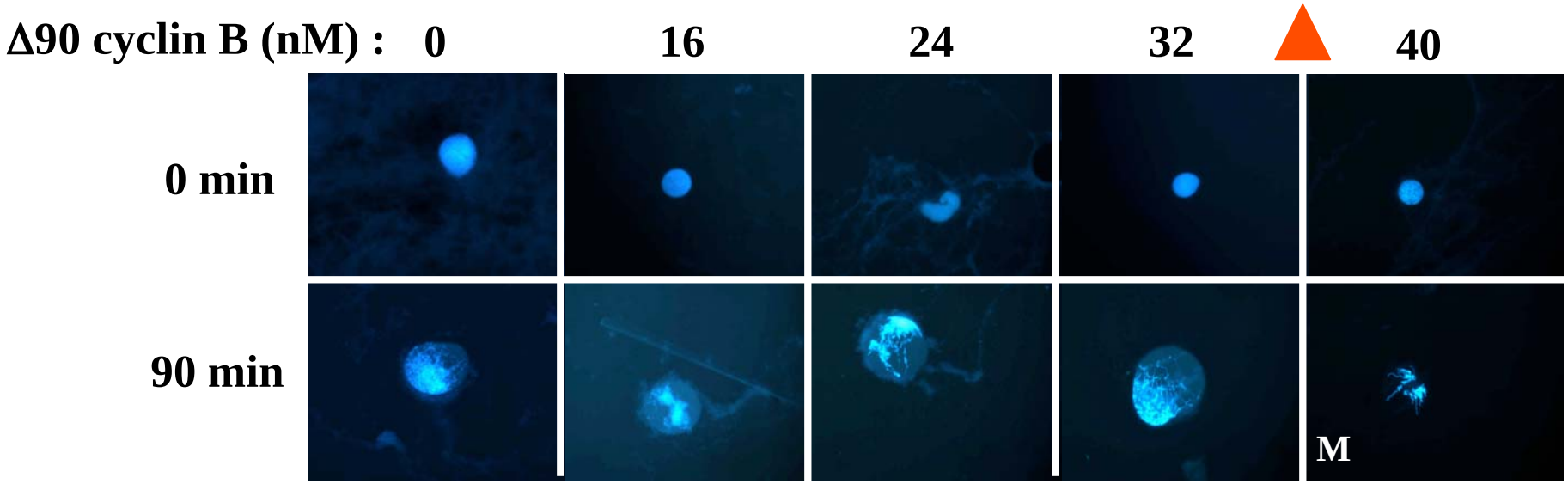


MPF oscillations correlate with changes in sperm morphology.

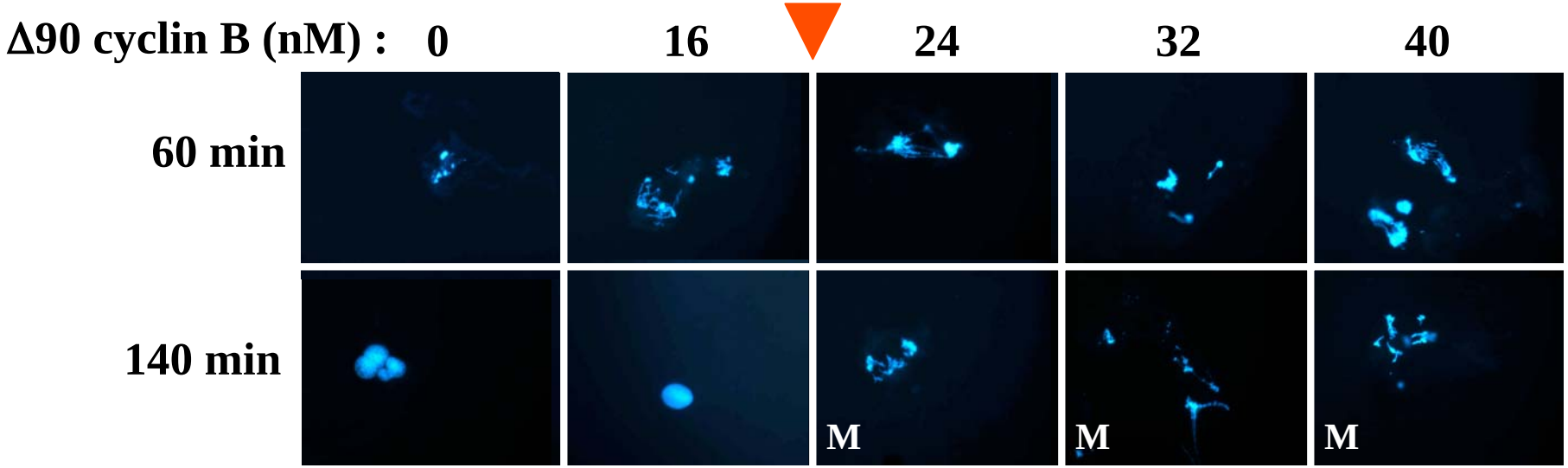


Cell biologists love microscopes!

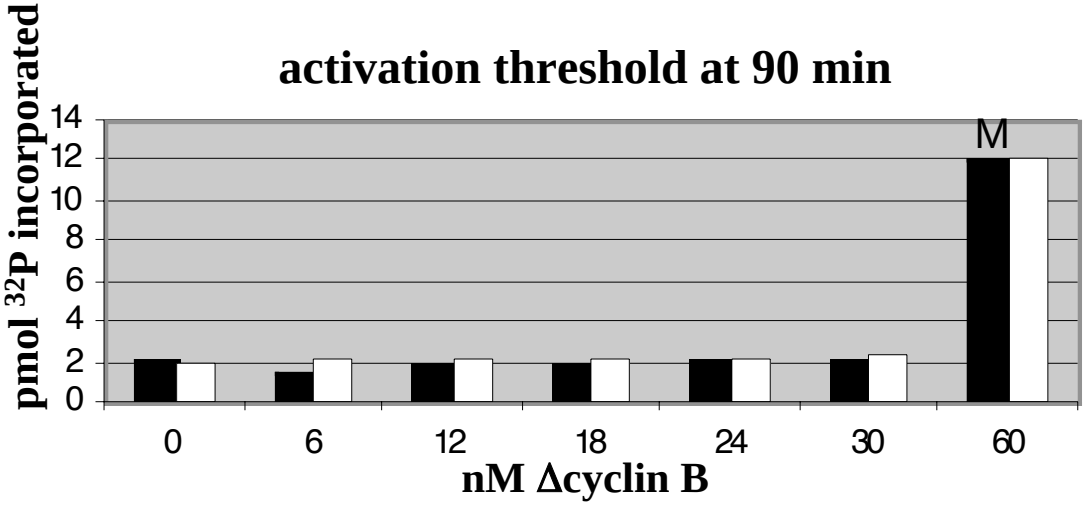
Testing the activation threshold for Mitosis I

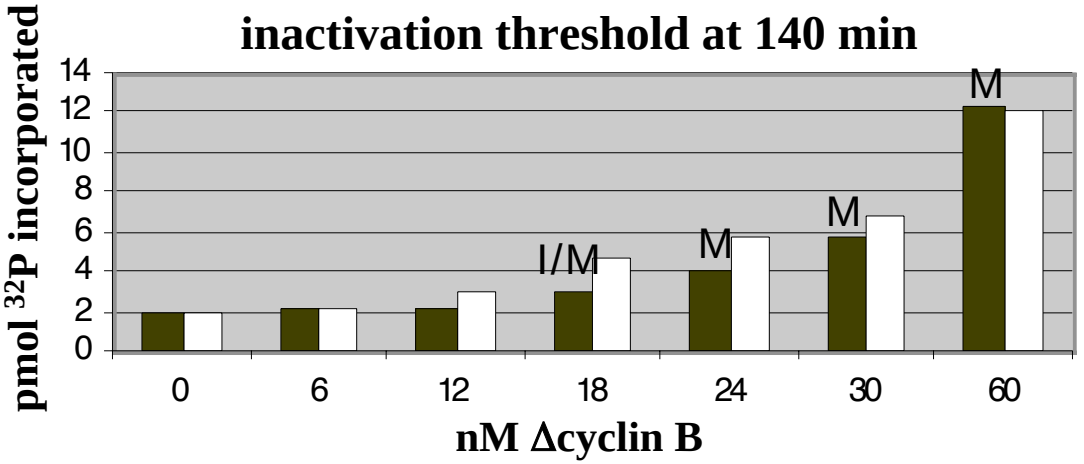


Testing the inactivation threshold for Mitosis I

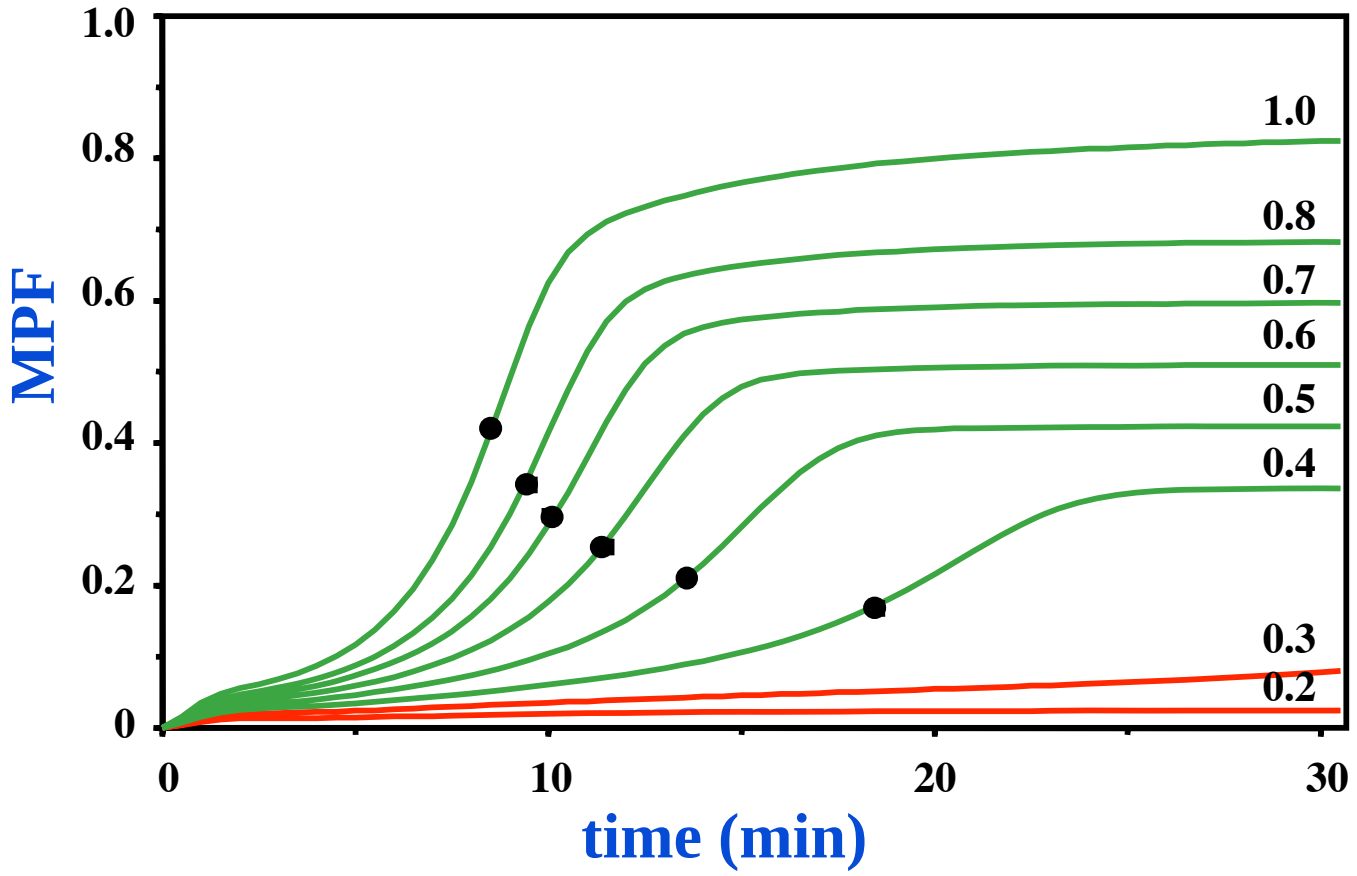


Prediction 1: The threshold concentration of cyclin B required to activate MPF is higher than the threshold concentration required to inactivate MPF.





Prediction 2: For cyclin levels marginally above the activation threshold, there is a dramatic “slowing-down” in the rate of MPF activation.



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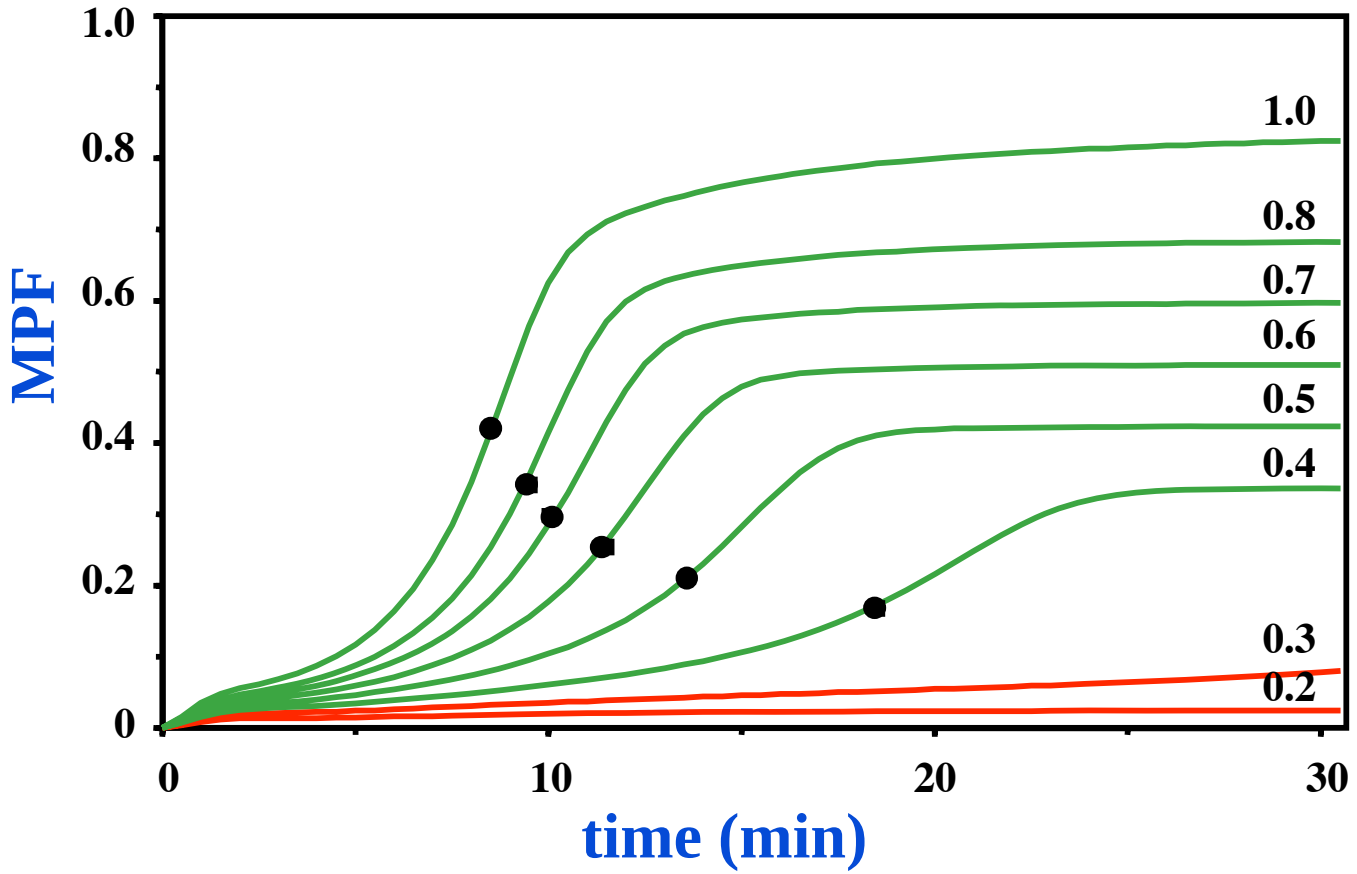
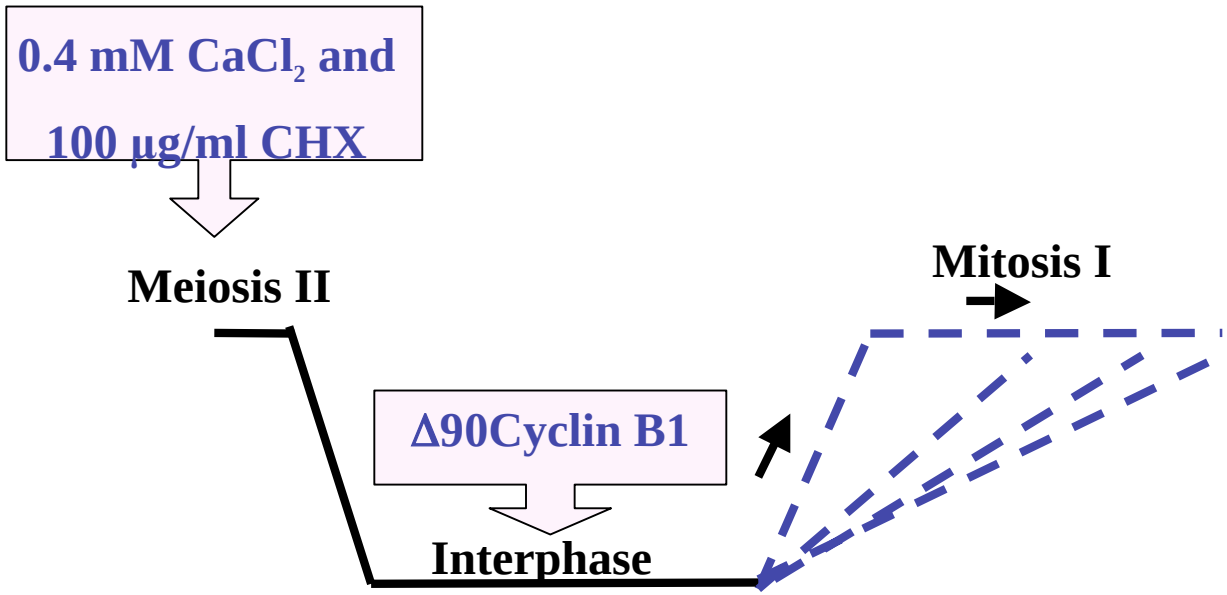


Marc Solomon found no evidence for this in 1990.

Cell biologists need a reason for doing a really difficult experiment.

Testing Lag Times in CSF-Released Extracts



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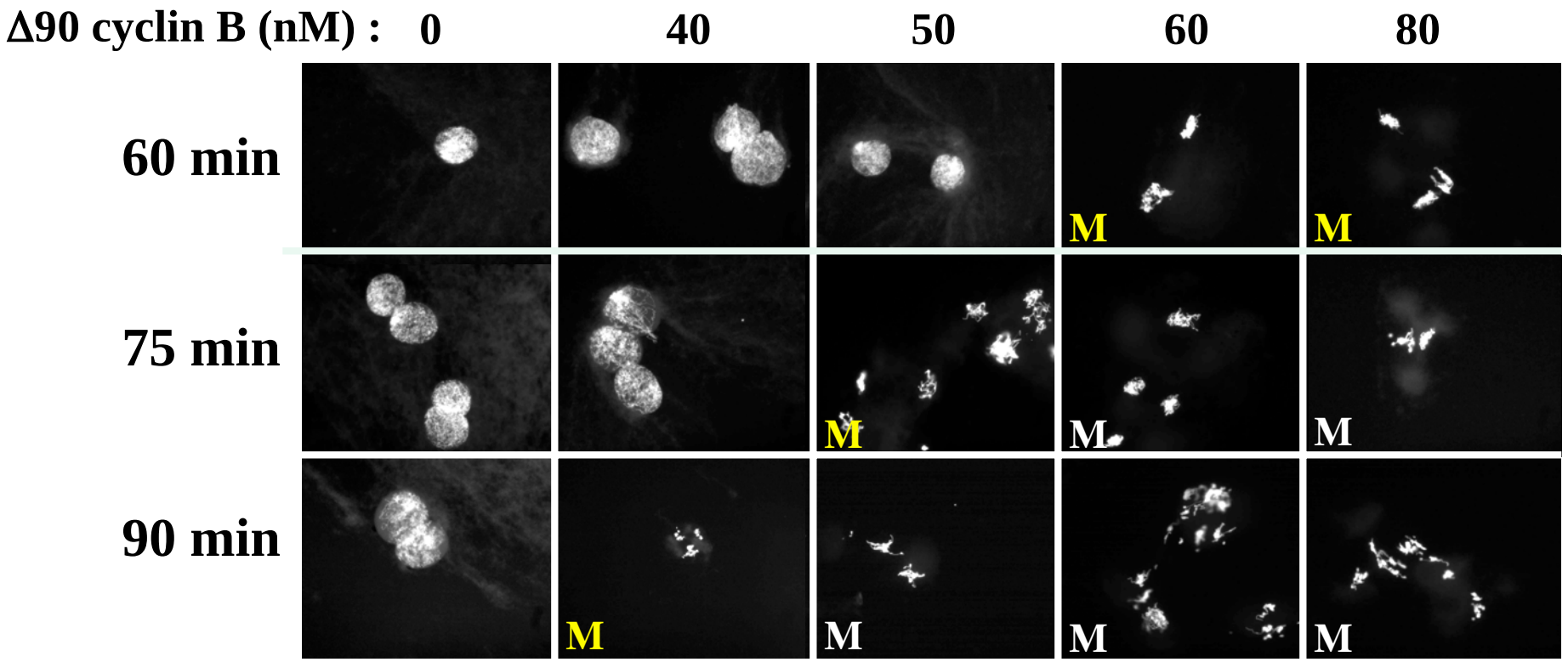
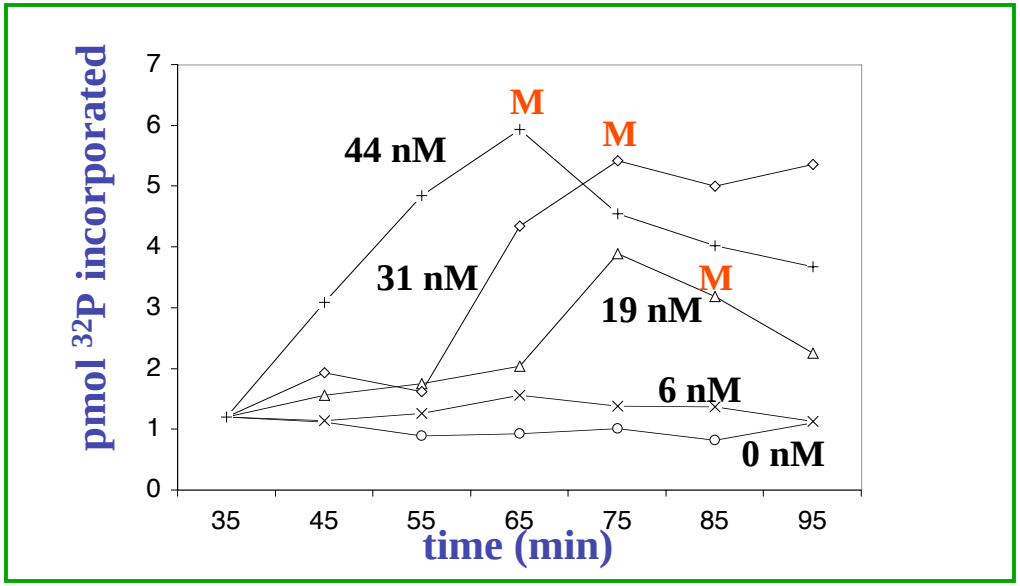
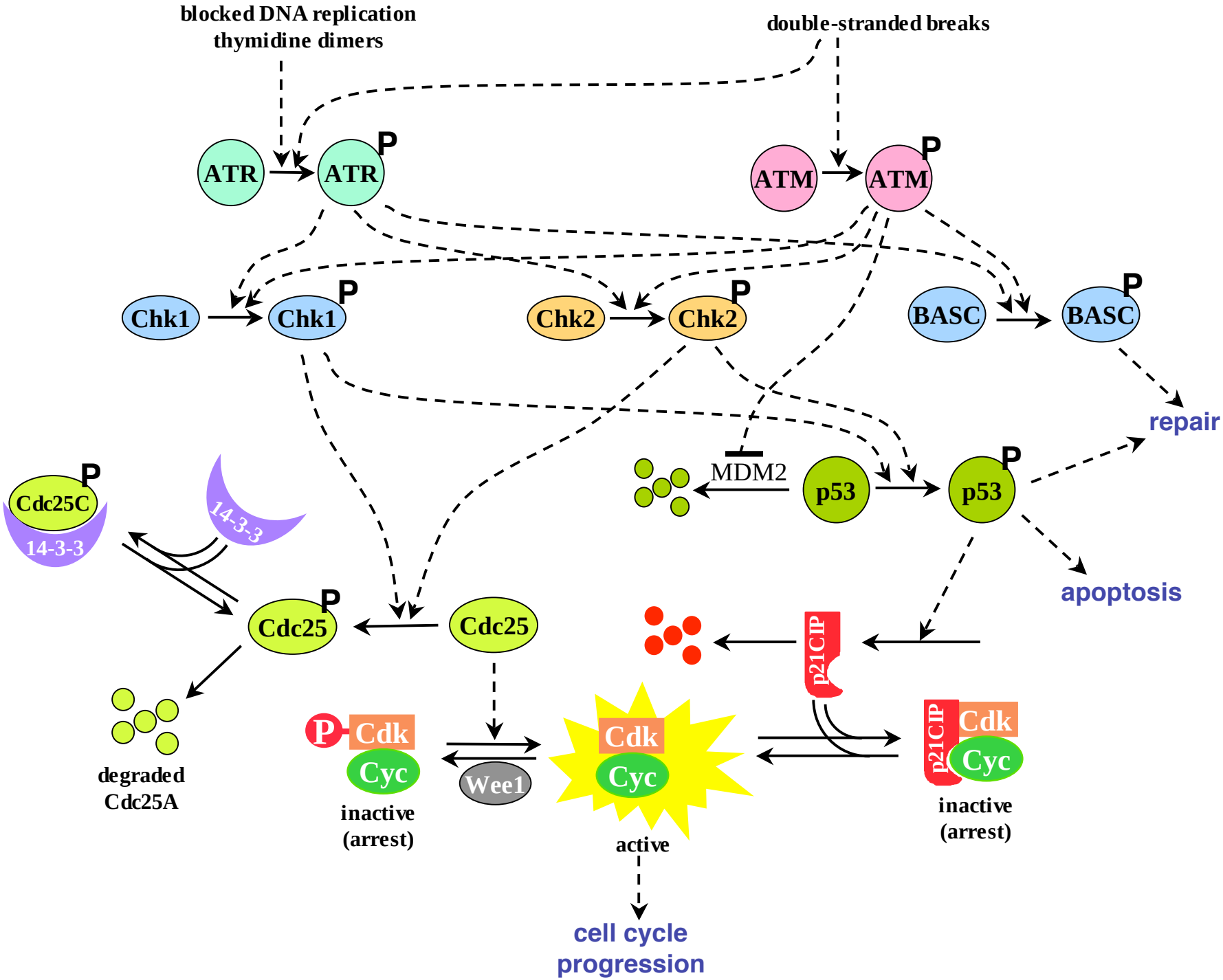


Did I mention, cell biologists love microscopes?

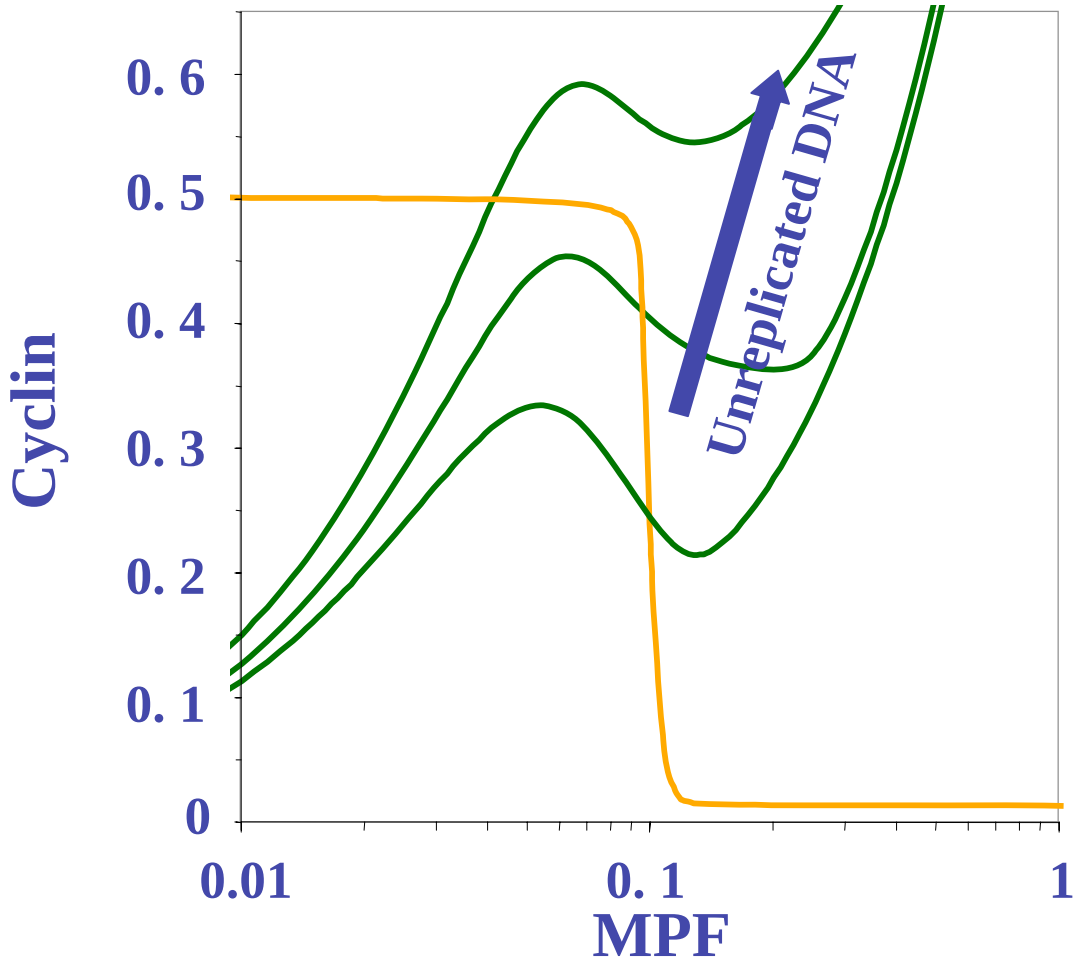
Testing Lag Times in CSF-Released Extracts:

H1 Kinase Assay

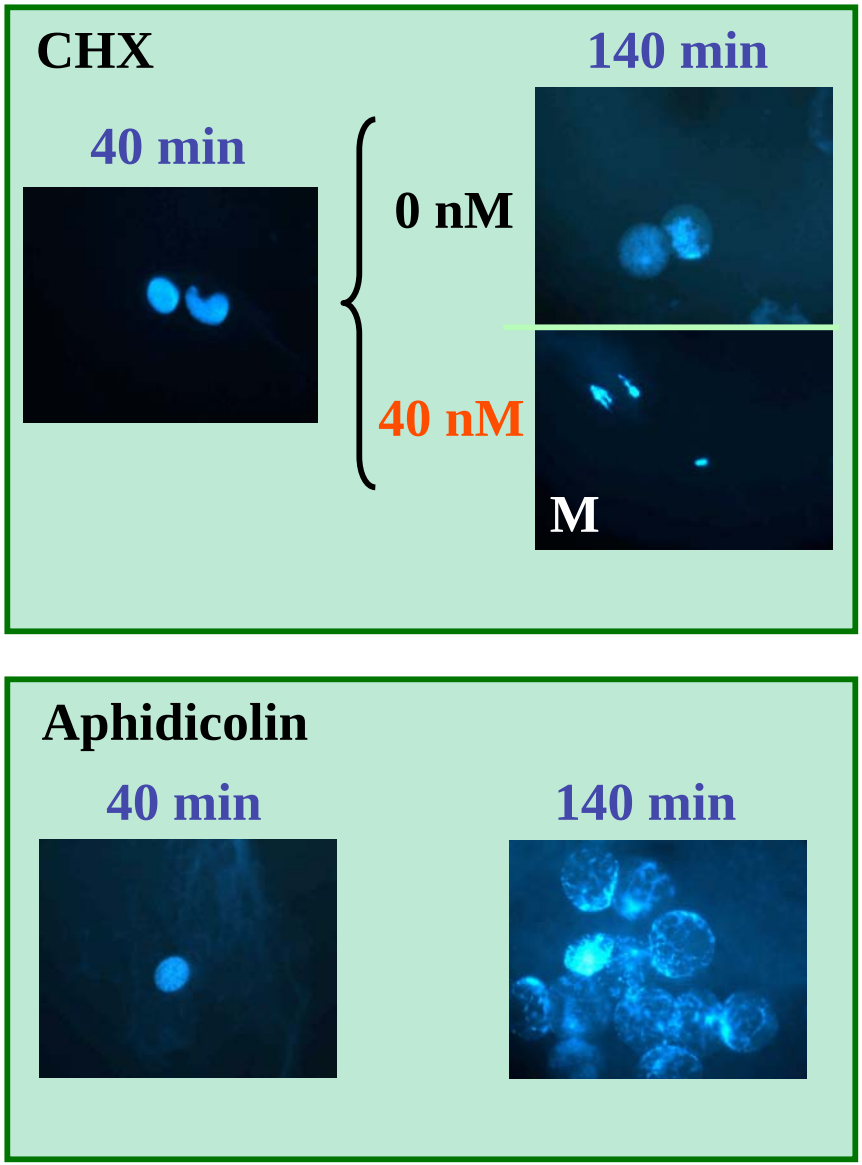



blocked DNA replication
thymidine dimers
double-stranded breaks
ATR
ATR
P
ATM
ATM
P
Chk1
Chk1
P
Chk2
Chk2
P
BASC
BASC
P
repair
Cdc25C
P
14-3-3
14-3-3
MDM2
p53
p53
P
apoptosis
Cdc25
P
Cdc25
degraded
Cdc25A
P
Cdk
Cyc
inactive
(arrest)
Wee1
Cdk
Cyc
active
p21CIP
p21CIP
Cdk
Cyc
inactive
(arrest)
cell cycle
progression

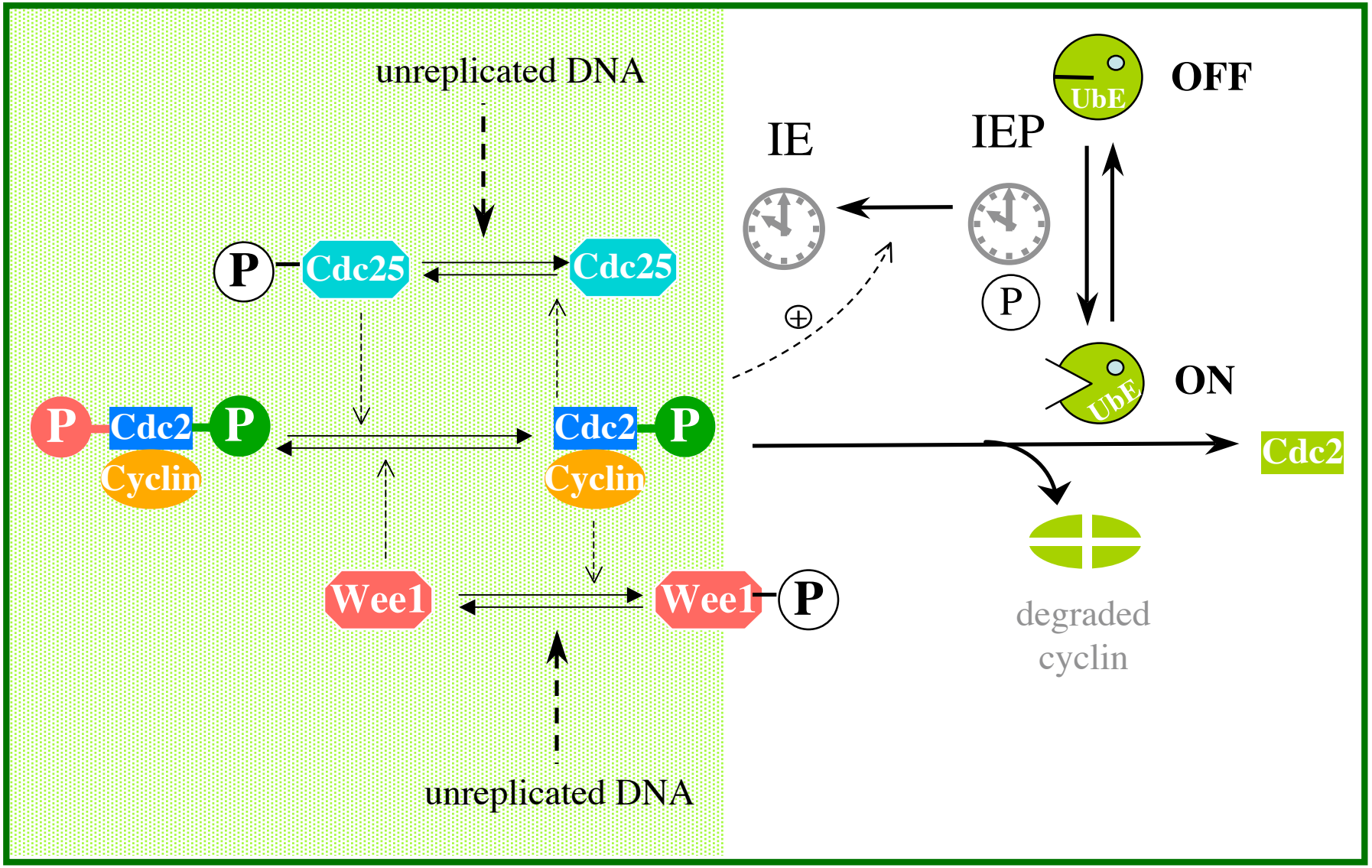
Prediction 3: The activation threshold of cyclin B is higher when a DNA replication checkpoint is engaged.



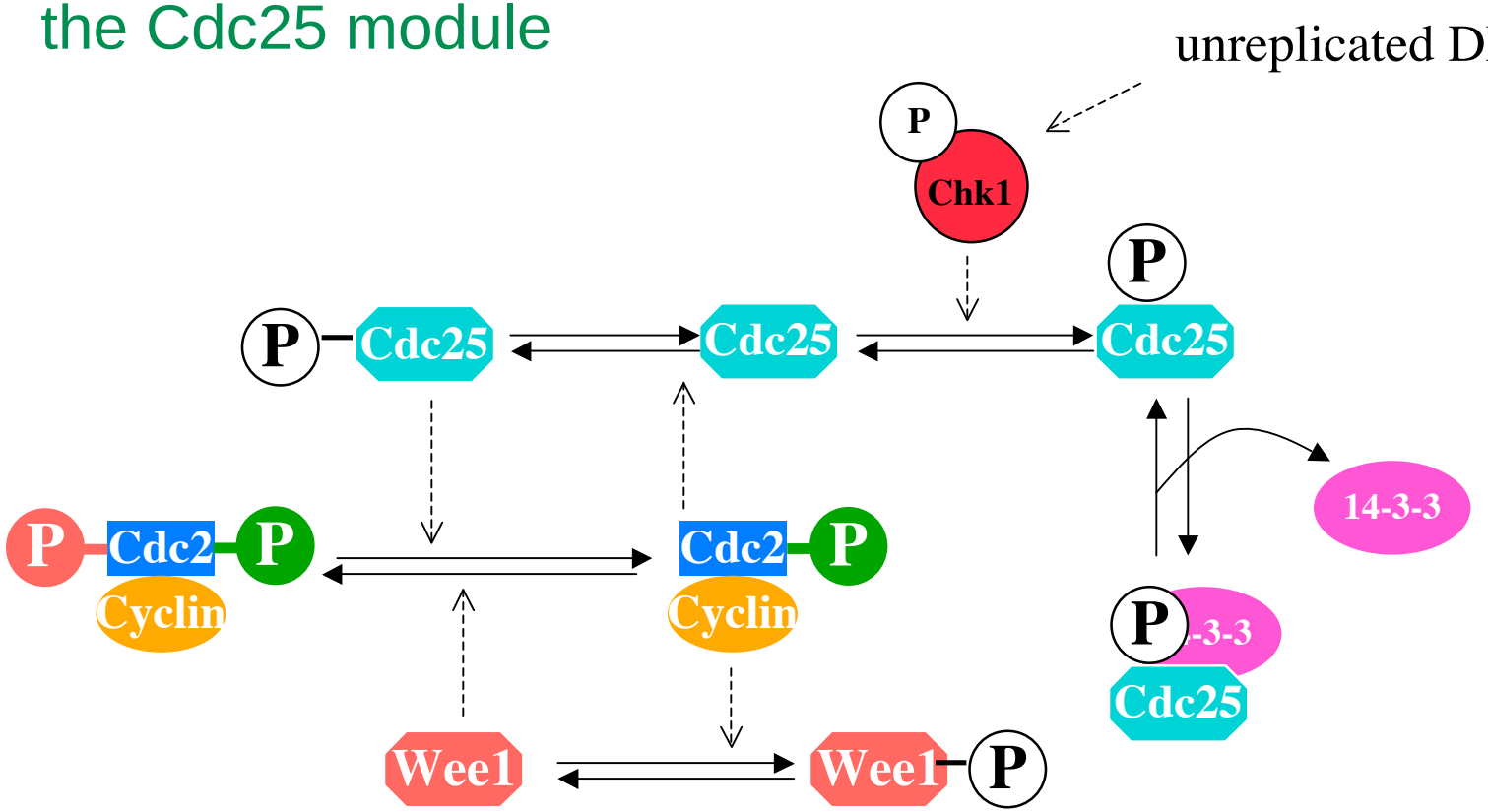
Testing Thresholds in Aphidicolin-Treated Extracts



The DNA replication checkpoint was modeled to affect the positive feedback.



Building a new model of the DNA replication checkpoint… the Cdc25 module



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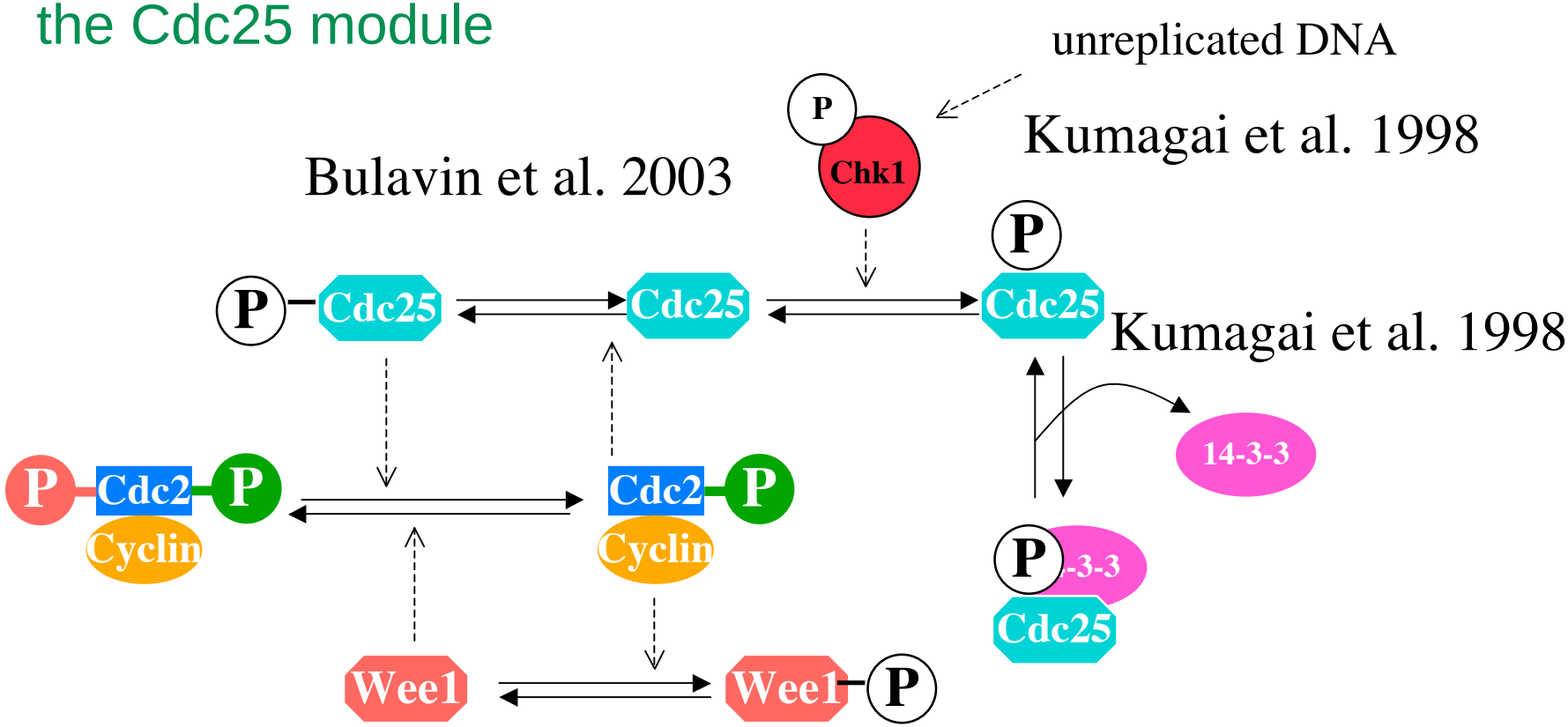

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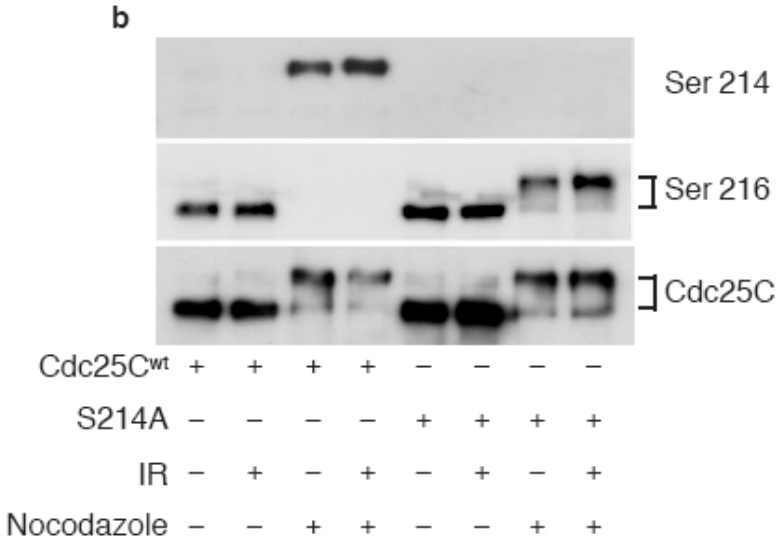
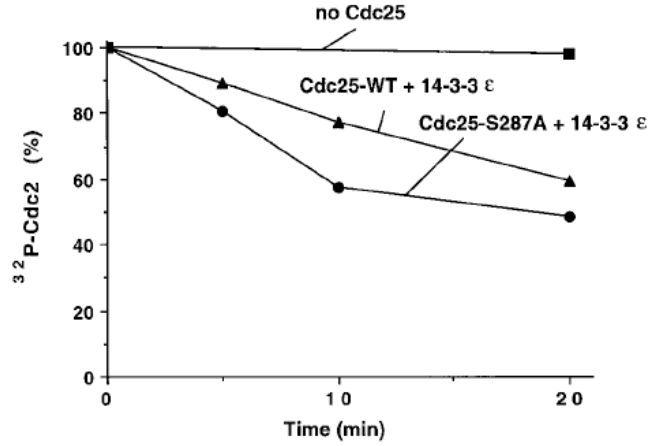
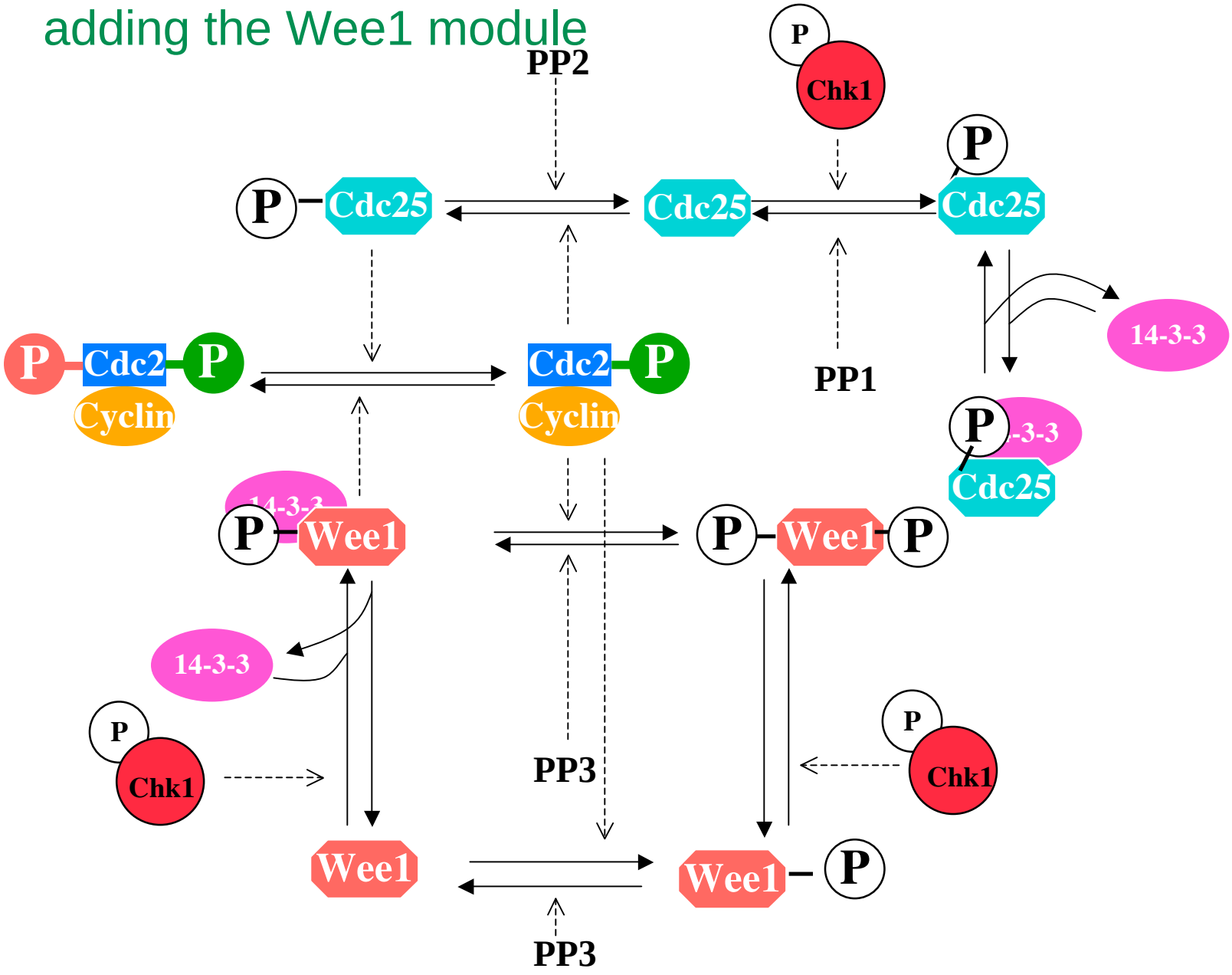


Bulavin et al. 2003

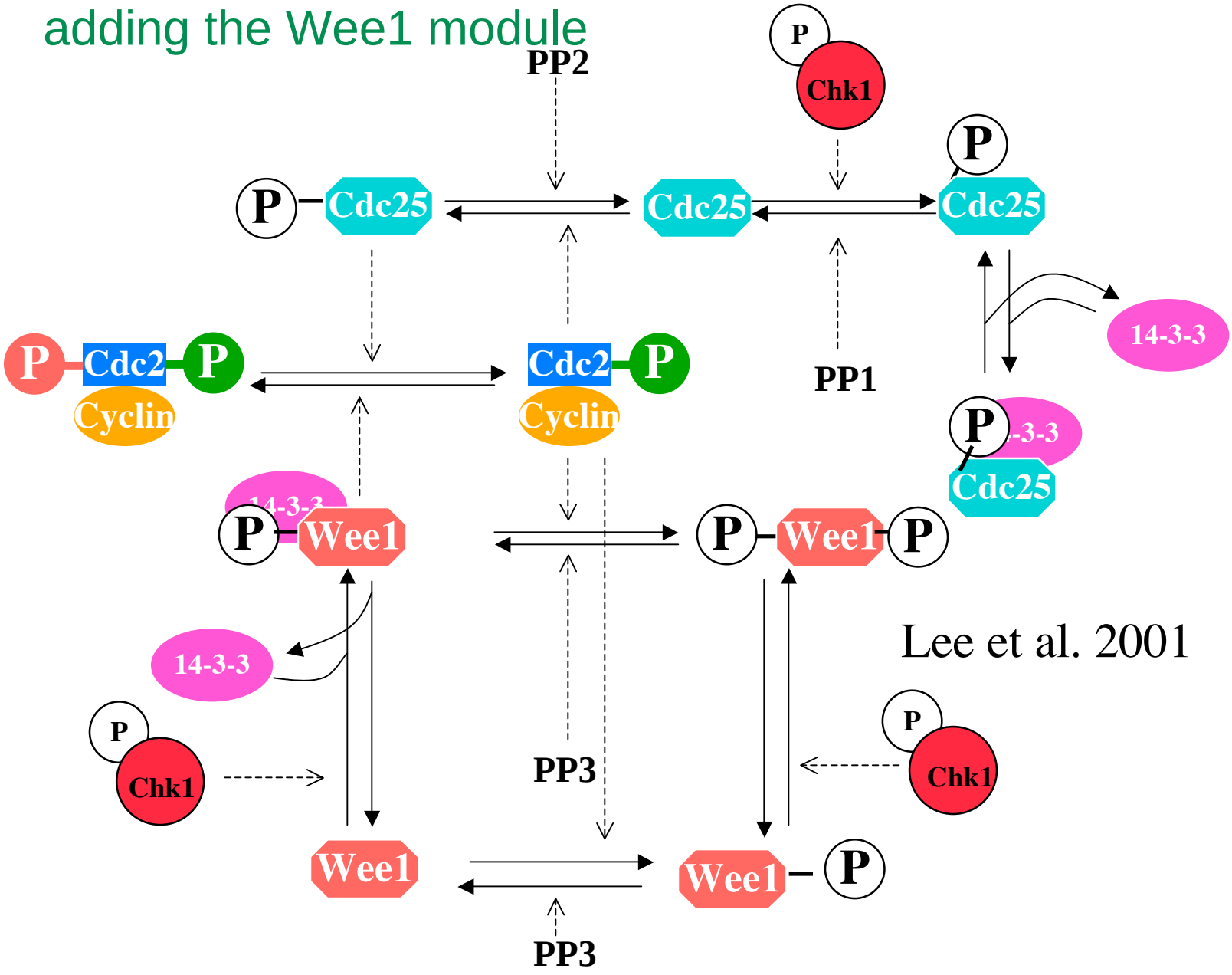


Kumagai et al. 1998

Building a new model of the DNA replication checkpoint… adding the Wee1 module

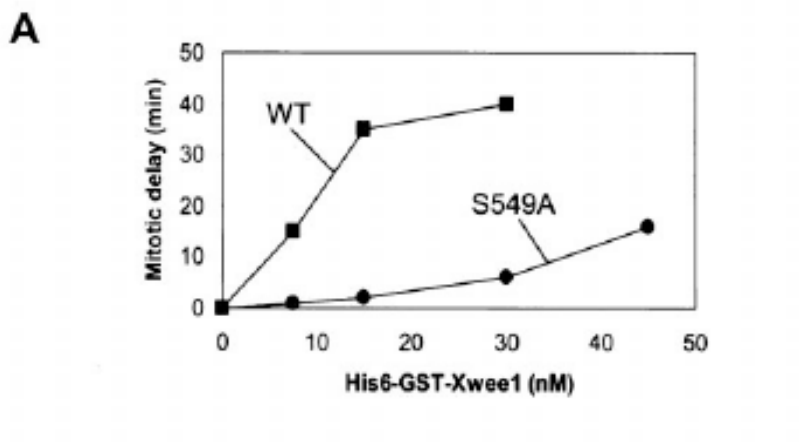


Building a new model of the DNA replication checkpoint… adding the Wee1 module



Lee et al. 2001

Building a new model of the DNA replication checkpoint… adding the Wee1 module

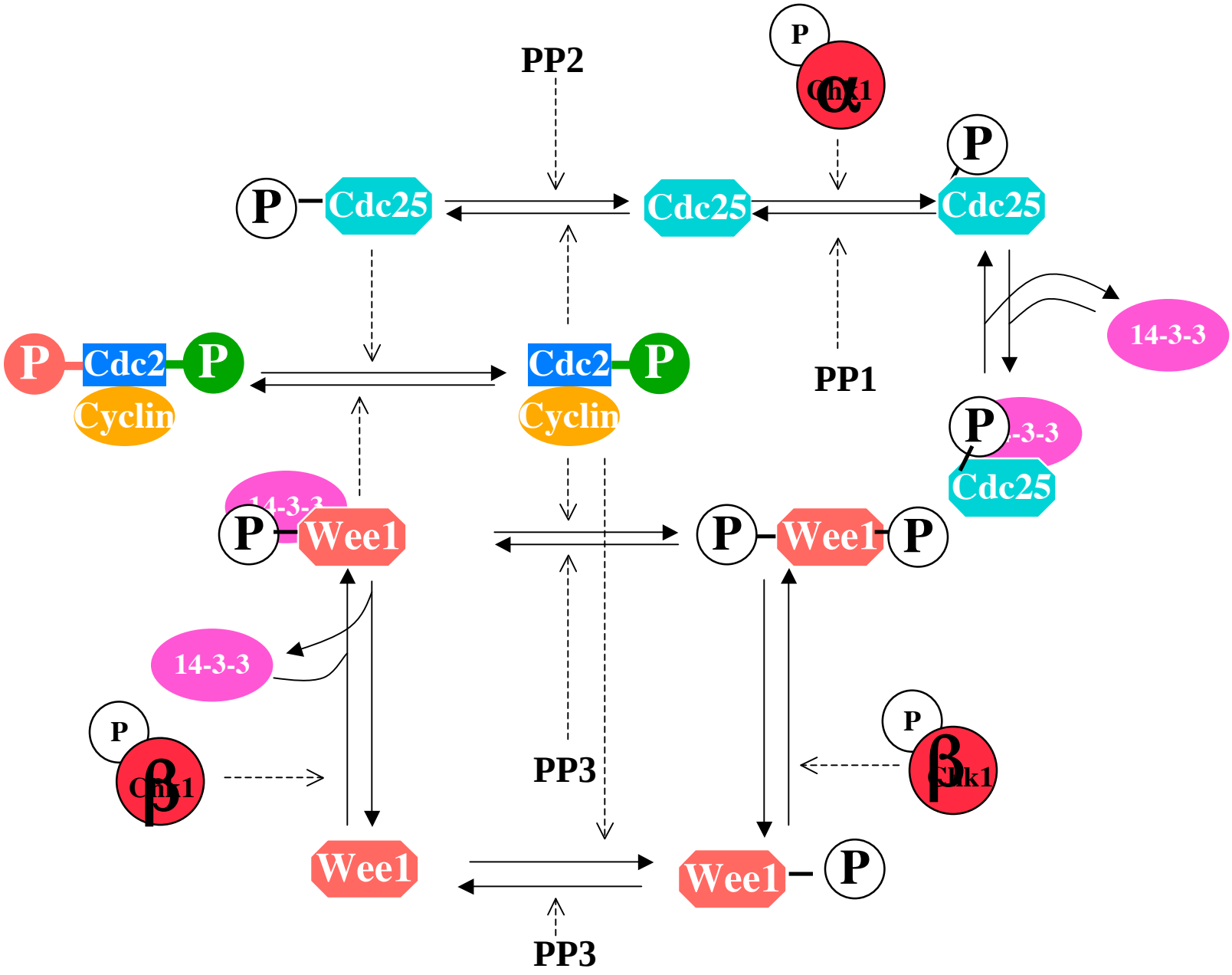


Lee et al. 2001

When mining the cell biology literature, read the fine print.

Although our data support a relationship between Xwee1, Xchk1, and 14-3-3 proteins in *Xenopus* egg extracts, a number of questions remain. Immunodepletion of Xchk1 from egg extracts does not abolish the binding of 14-3-3 proteins to Xwee1, suggesting the existence of at least one other kinase that phosphorylates Ser-549 of Xwee1 (our unpublished results). A comparable situation exists in the case of *Xenopus* Cdc25, which can be phosphorylated by Xchk1, Xcds1, and at least one other kinase (Kumagai *et al.*, 1998a; Guo and Dunphy, 2000). Thus far, we have not been able to detect an increase in kinase activity or binding of 14-3-3 proteins in the case of Xwee1 that has been immunoprecipitated from aphidicolin-treated extracts, which is consistent with previously published results (Kumagai and Dunphy, 1995; Mueller *et al.*, 1995a). However, only a small fraction of Xwee1 (~5%) is associated with 14-3-3 proteins in egg extracts, according to our immunoprecipitation studies, suggesting that a subpopulation of Xwee1 might be involved in this interaction. Approximately 10% or less of the Xwee1 becomes incorporated into the nuclei in cycling egg extracts, depending upon the experimental conditions. This portion of Xwee1 would presumably be the most accessible to checkpoint regulators. Moreover, as shown here, Xwee1 is differentially localized within the nucleus depending on whether it is associated with 14-3-3. These considerations suggest that there may be technical limitations in how Xwee1 can be assayed in checkpoint-activated extracts.

Building a new model of the DNA replication checkpoint…

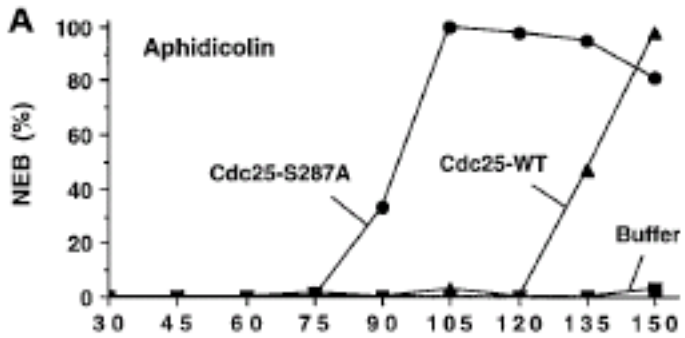


How do we compare data from the experimental literature to simulations of the model?

Literature: shows % nuclear envelope breakdown

Model: gives MPF activity

Experimental data from: Kumagai et al. 1998



How do we compare data from the experimental literature to simulations of the model?

Literature: shows % nuclear envelope breakdown

Model: gives MPF activity

a solution:

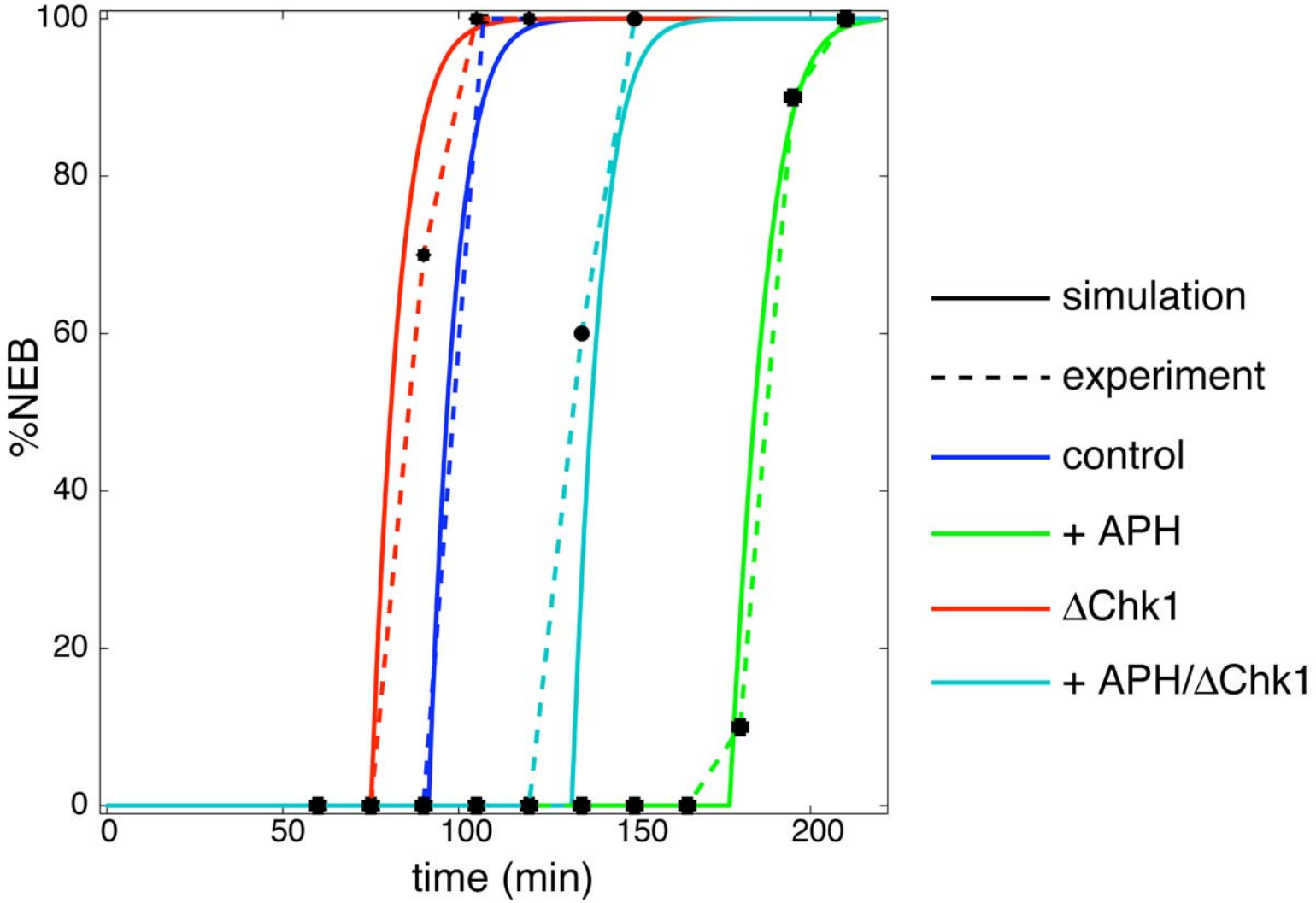
$$(15)\ d[\text{laminP}]/dt = k_{\text{phos lamin}}([\text{total lamin}] - [\text{laminP}])[\text{MPF}]$$

$$(16)\ \text{fNEB} = \frac{[\text{laminP}] - \theta}{[\text{total lamin}] - \theta} \bullet \text{Heaviside}\left(\frac{[\text{laminP}] - \theta}{[\text{total lamin}] - \theta}\right)$$

Effect of α & β on timing of mitosis (NEB) = nuclear envelope breakdown

Extract state	α	β	time for 50% NEB
control	0.2	1.5	100
+APH	0.6	4.5	175
ΔChk1	0.14	1.0	80
+APH ΔChk1	0.4	3.0	140

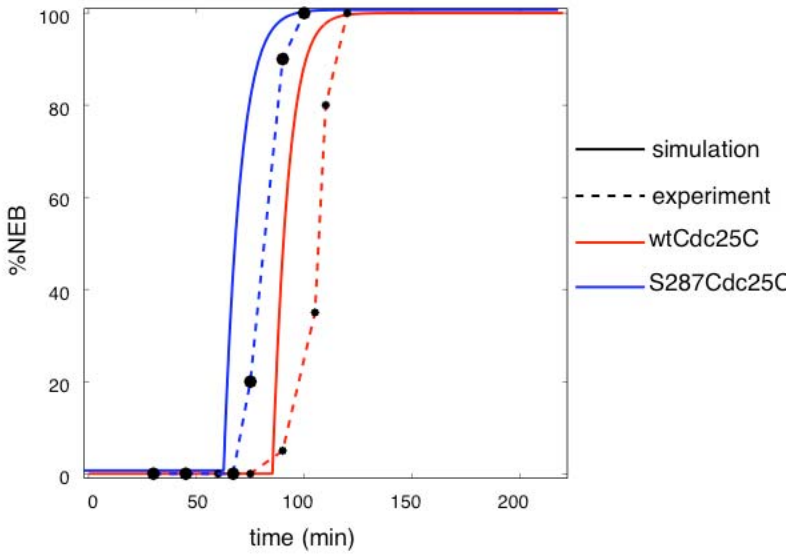
Comparing simulations with experimental data: testing the effect of unreplicated DNA



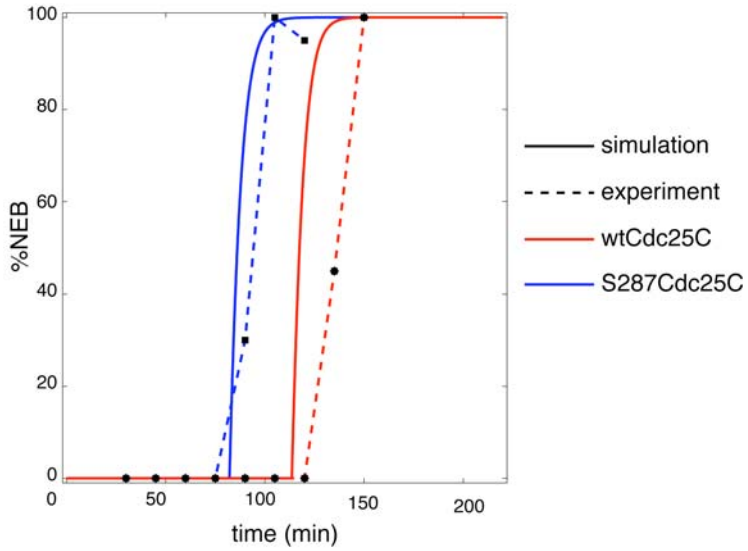
Experimental data from: Kumagai et al. 1998

Comparing simulations with experimental data: testing the Cdc25 module

control



+ aphidicolin



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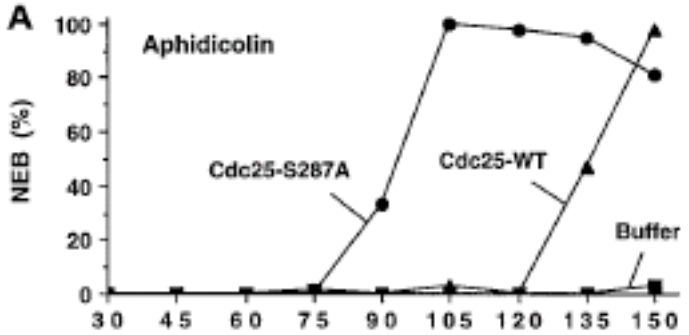
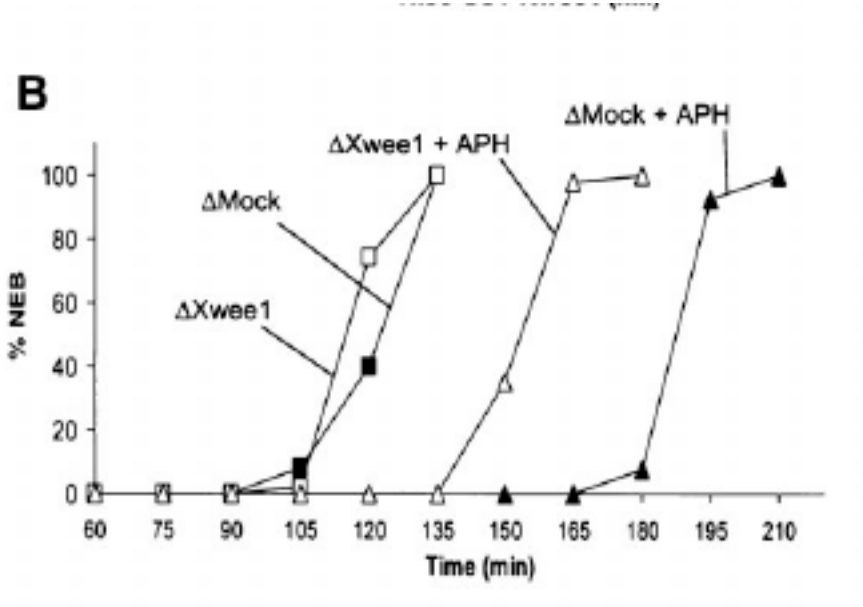
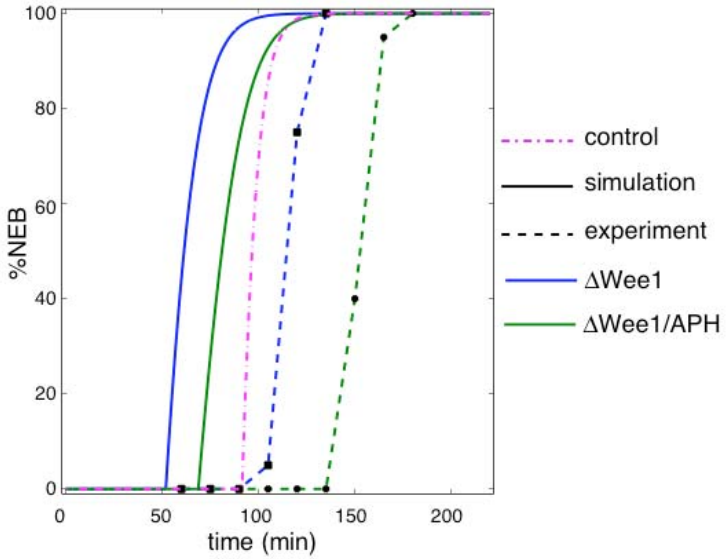


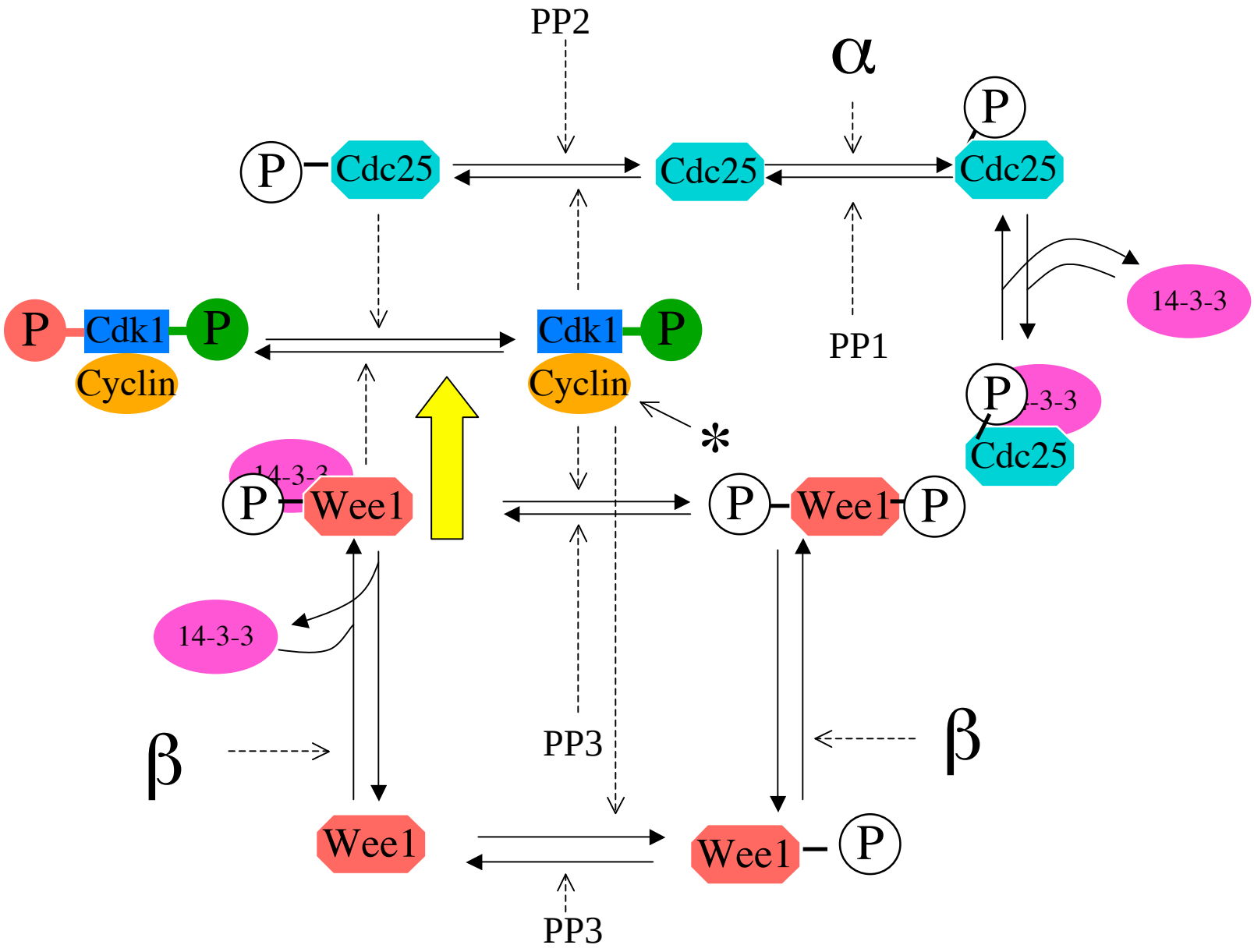
Comparing simulations with experimental data: testing the Wee1 module



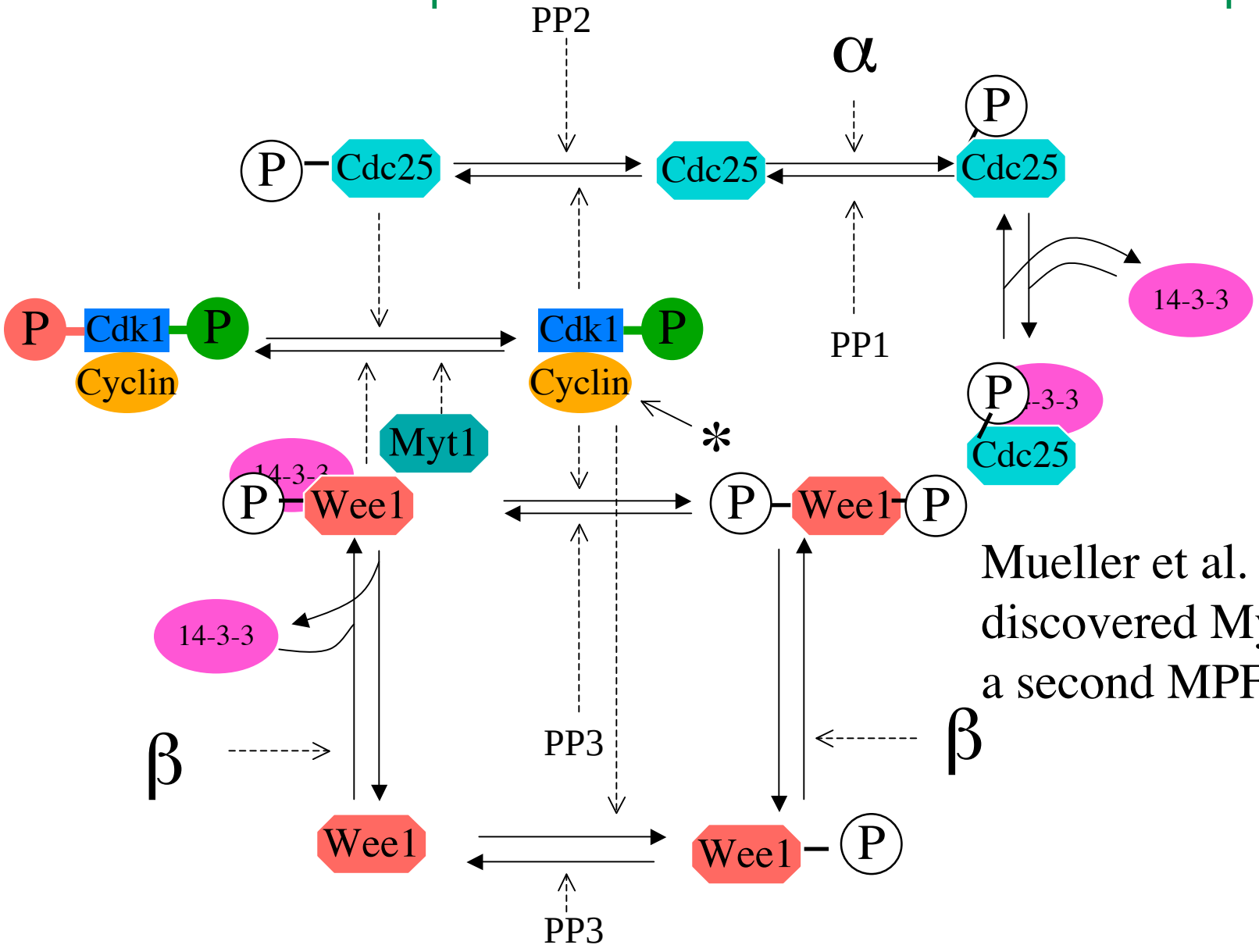


Experimental data from: Lee et al. 2001

Where is the problem with the model?



Where is the problem with the model? - what information can we obtain from the experimental literature to address this problem



Mueller et al. (1995) discovered Myt1, a second MPF kinase.

Where is the problem with the model? - what information can we obtain from the experimental literature to address this problem

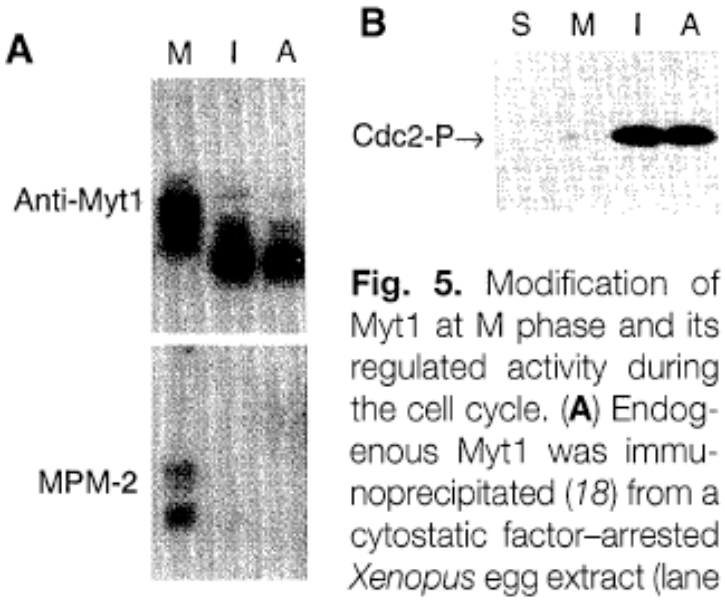
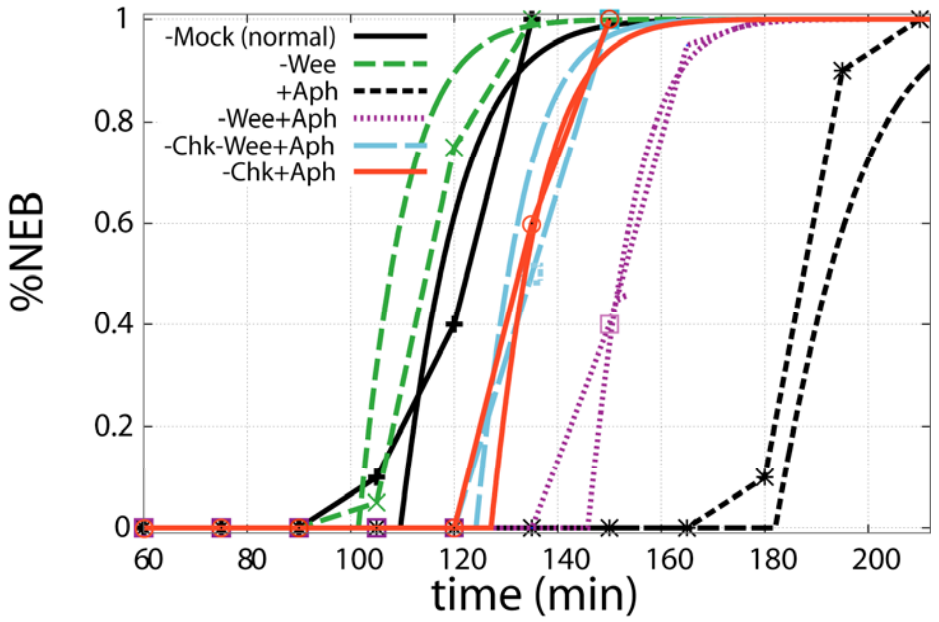


Fig. 5. Modification of Myt1 at M phase and its regulated activity during the cell cycle. (**A**) Endogenous Myt1 was immunoprecipitated (*18*) from a cytostatic factor–arrested *Xenopus* egg extract (lane M), an interphase extract (lane I), or an S phase–blocked extract that had been treated with aphidicolin (50 μg/ml) in the presence of sperm nuclei (1000 per microliter) (lane A). These samples were immunoblotted with anti-Myt1 (top panel) or monoclonal antibody MPM-2 (bottom panel). (**B**) The kinase activity of the various forms of immunoprecipitated Myt1 was measured as described in Fig. 2 with the N133A form of Cdc2 as the substrate (S). Lane S depicts a control assay without added Myt1 protein.



Take home message so far:

1. Mining the experimental literature is tedious but worthwhile.

2. “Qualitative” data can inform quantitative models and quantitative models can provide critical qualitative information.

3. Experiments to test and validate models require deliberate design.

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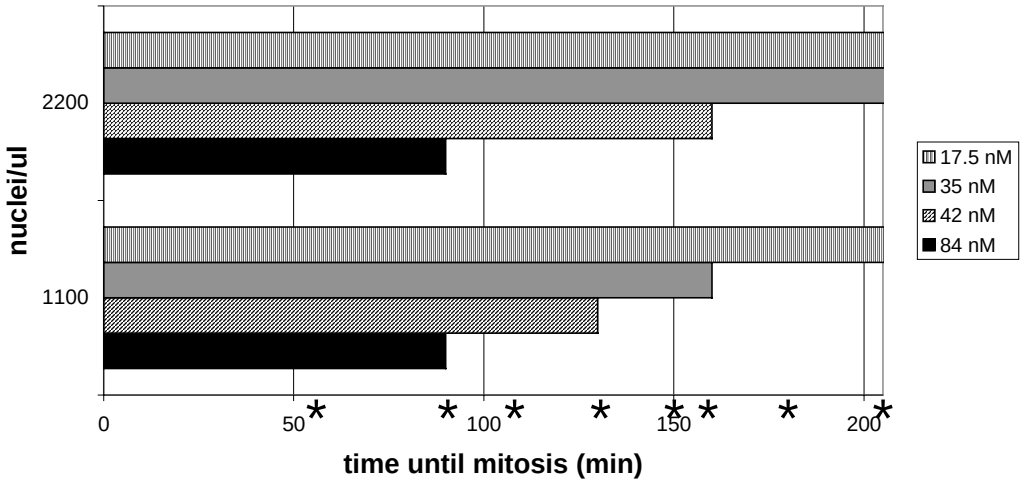

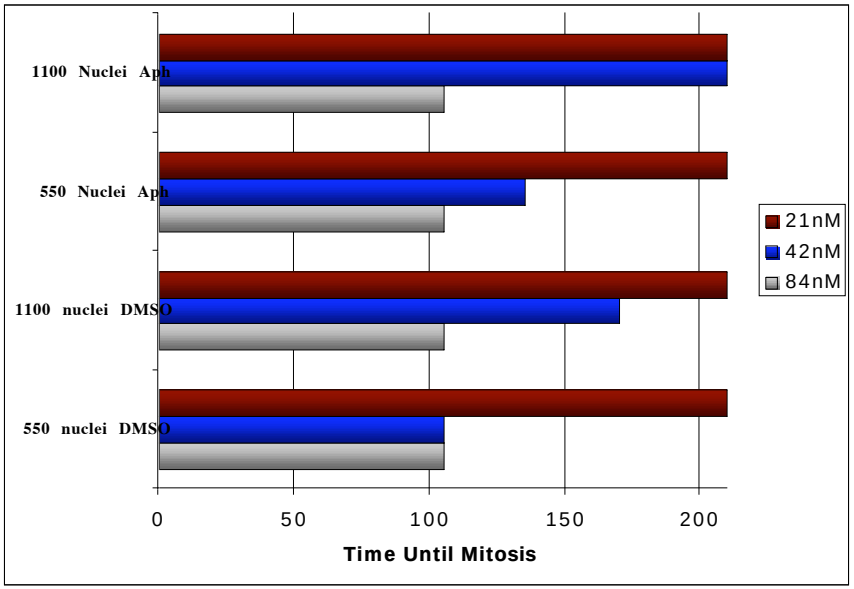

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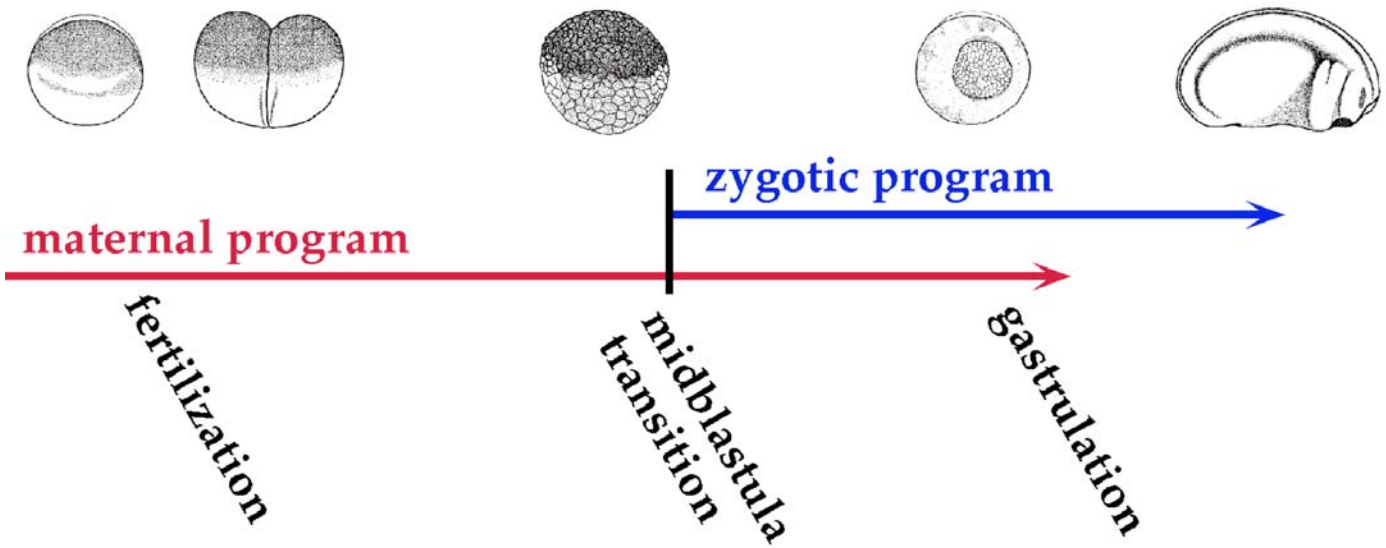
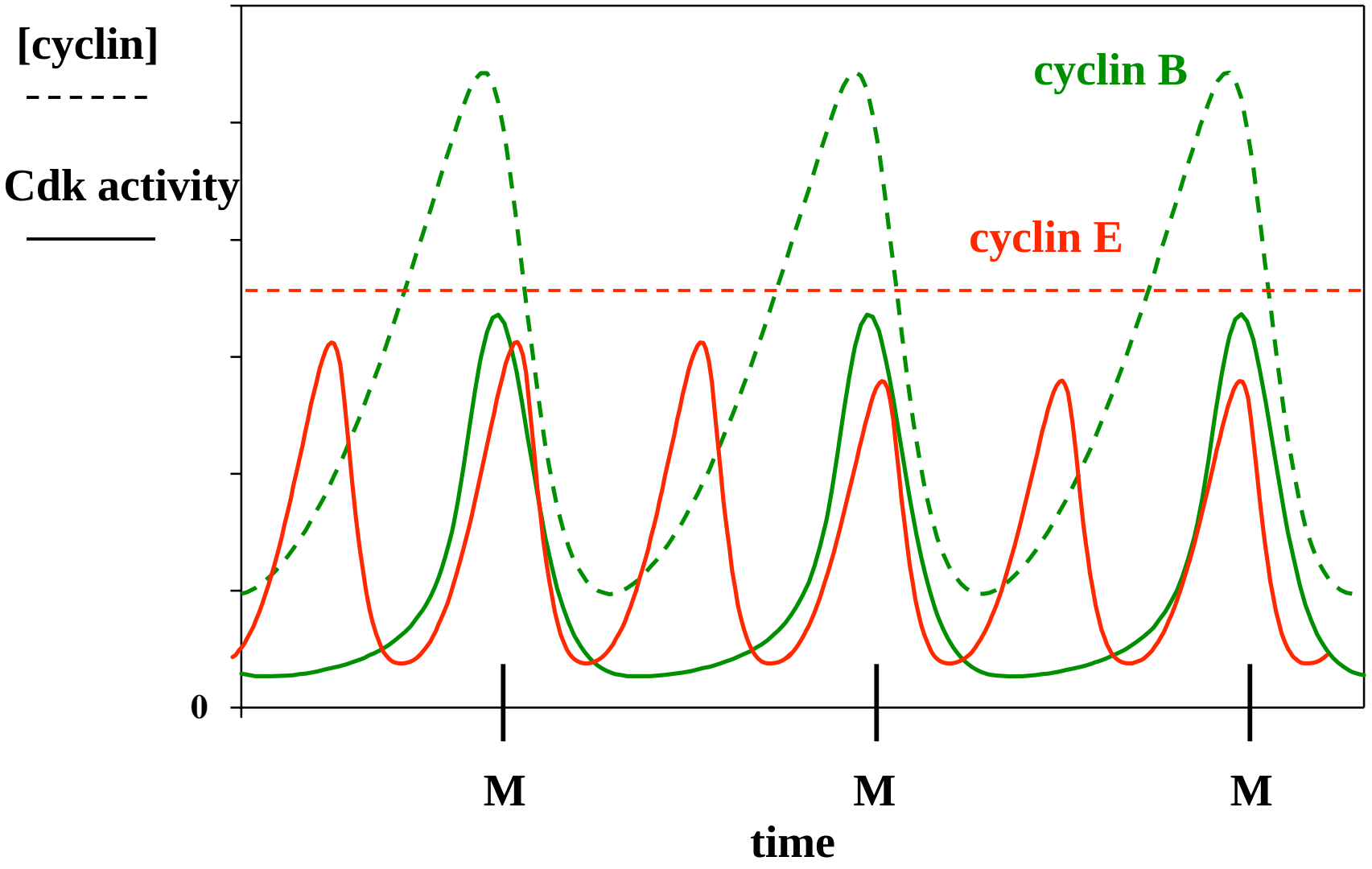
3. Experiments specifically designed to inform models require deliberate design.

Treatment of extract	Source of reagent	[Δcyclin B]	Predicted time for 50% NEB (-APH)	Predicted time for 50% NEB (+APH)
untreated	N/A	0.45	98 min	>500 min
		0.6	67 min	145 min
ΔWee1	antibody from Zymed®	0.45	90 min	>500 min
		0.6	61 min	100 min
ΔCdc25C	antibody given by Maller[83]	0.45	>500 min	>500 min
		0.6	>500 min	>500 min
2xWee1	cDNA given by Murakami[84]	0.45	90 min	>500 min
		0.6	61 min	100 min
2x Cdc25C	cDNA given by Maller[83]	0.45	48 min	67 min
		0.6	35 min	42 min

Understanding cell cycle control in a dynamic in vivo context.



Cyclin E/Cdk2 activity oscillates independently of cyclin E level.



Building the cyclin E oscillator

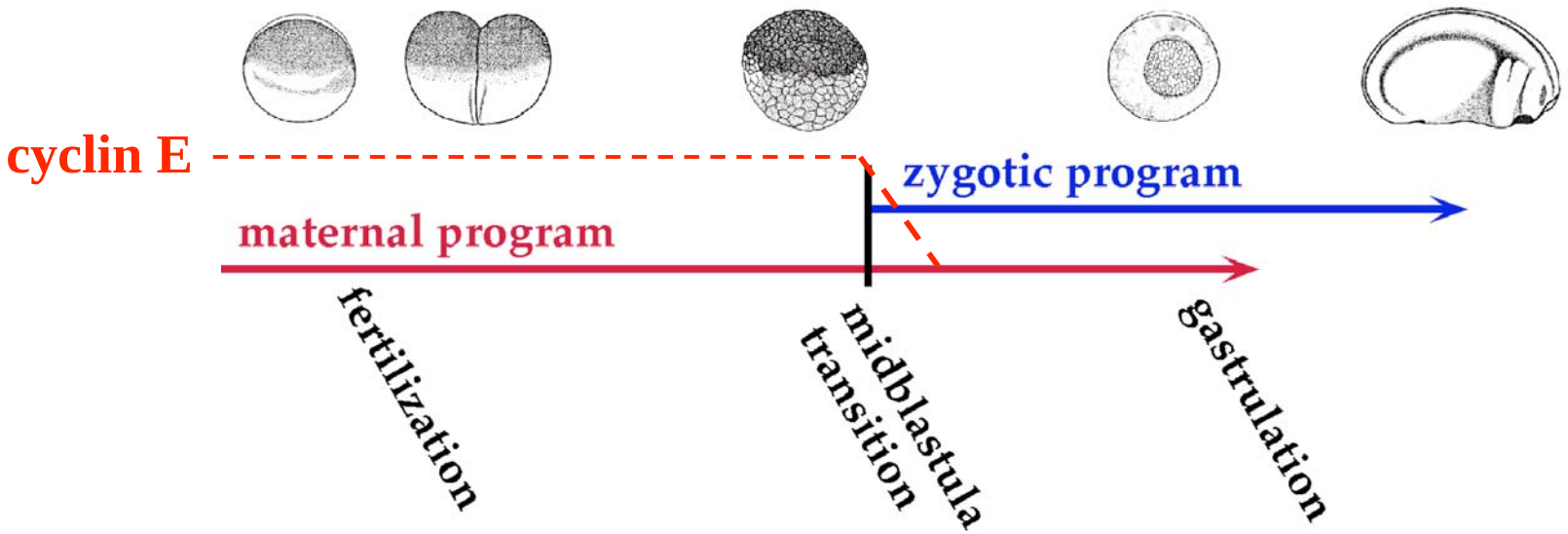
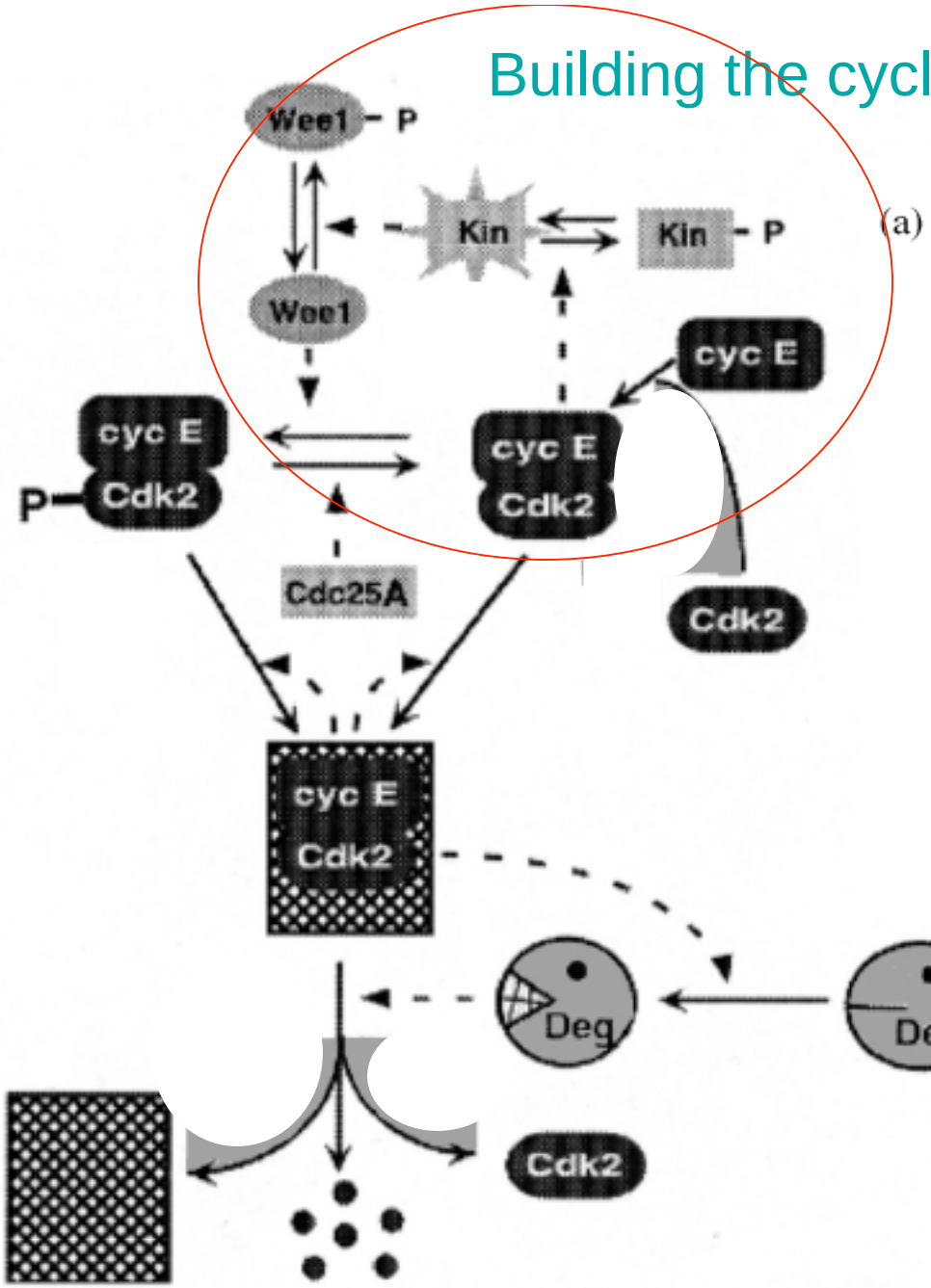
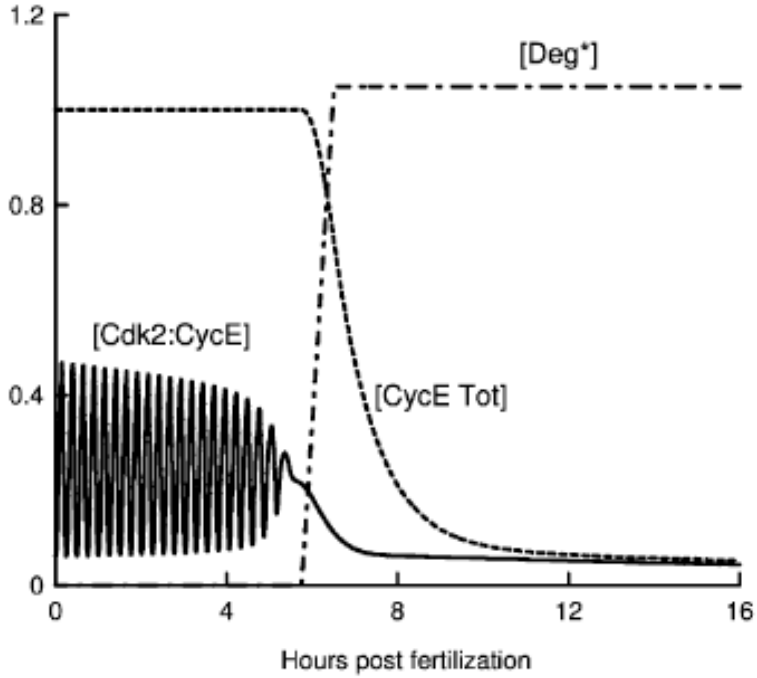


Table 1
Key assumptions of the cyclin E/Cdk2 kinetic model

1	Constant level of cyclin E	[25,26,31,32]
2	No abundant CKI inhibitor	[33,50]
3	Regulation of Cdk2 activity by phosphorylation	[37,40]
4	Negative feedback loop in Cdk2 oscillations	To be tested
5	Timing of cyclin E degradation linked to Cdk2 activity	[25], Fig. 1
6	No return of Cdk2 activity in Xic-injected embryos	Fig. 2
7	Degradation of cyclin E independent of transcription	[25], Fig. 3

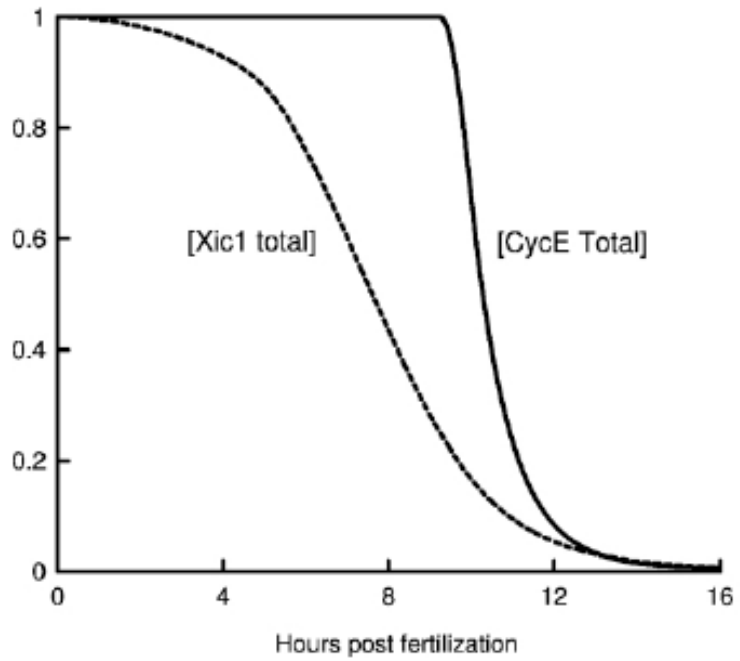
Building the cyclin E oscillator



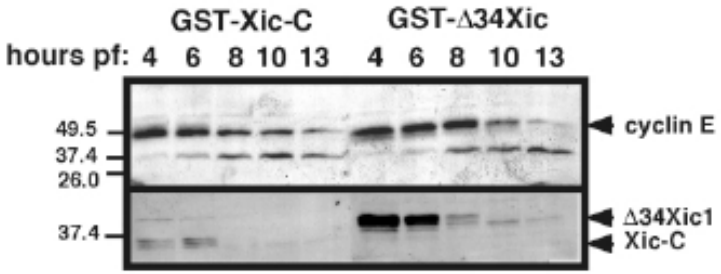


Testing predictions of the model

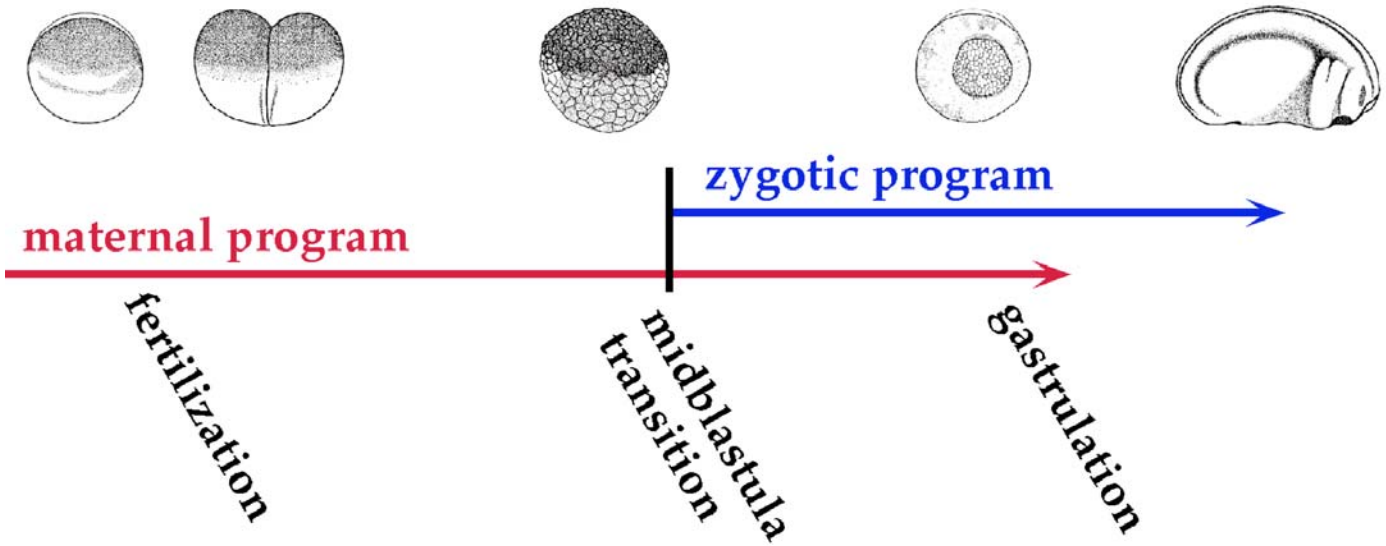
(c)



(d)



Threats to genomic integrity elicit different responses at different stages of development.



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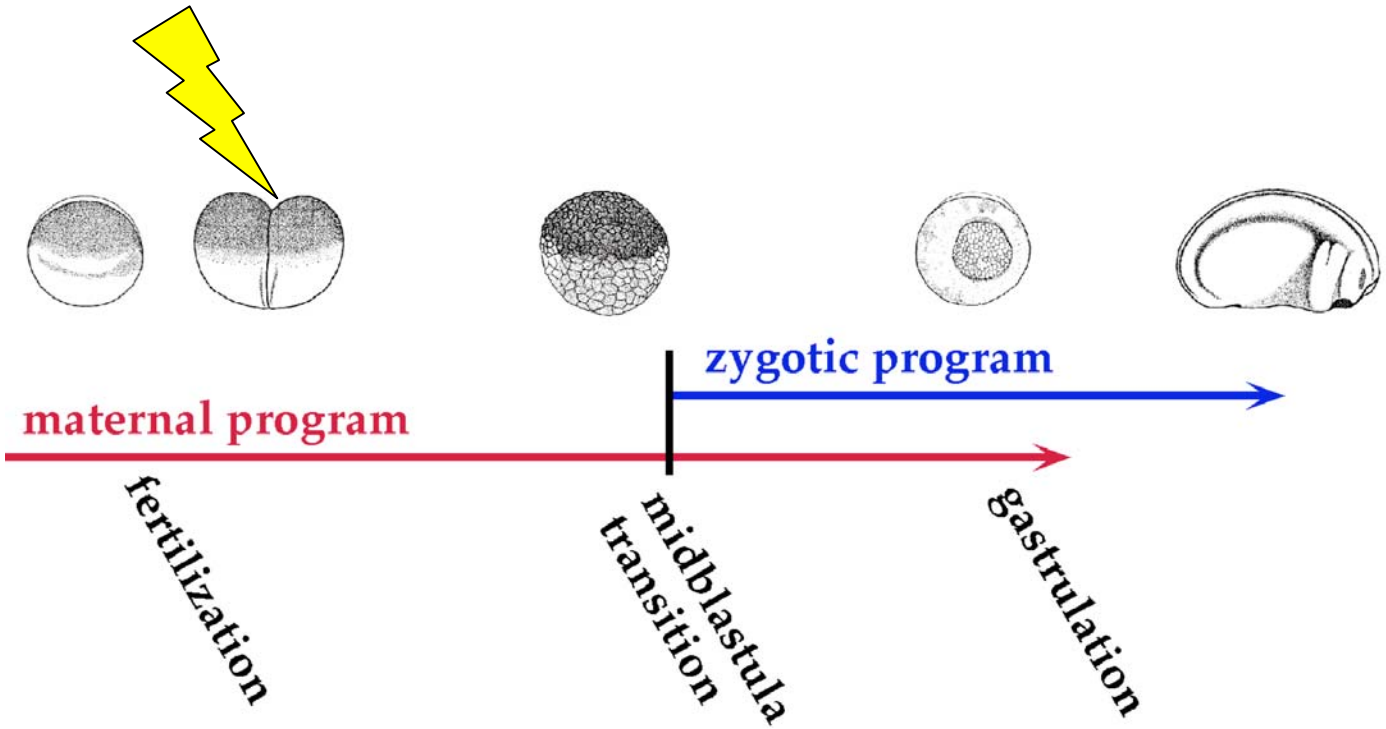

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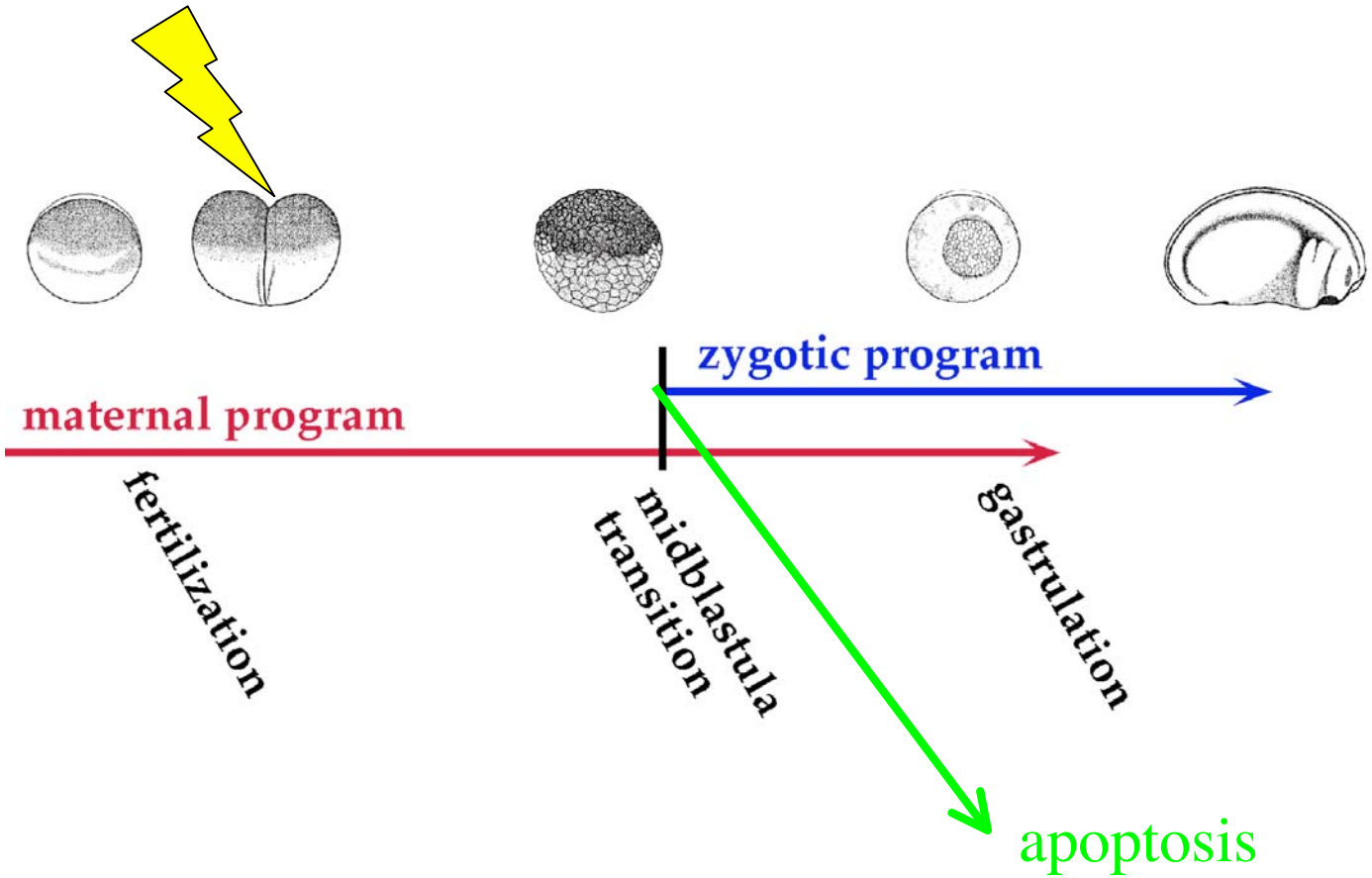

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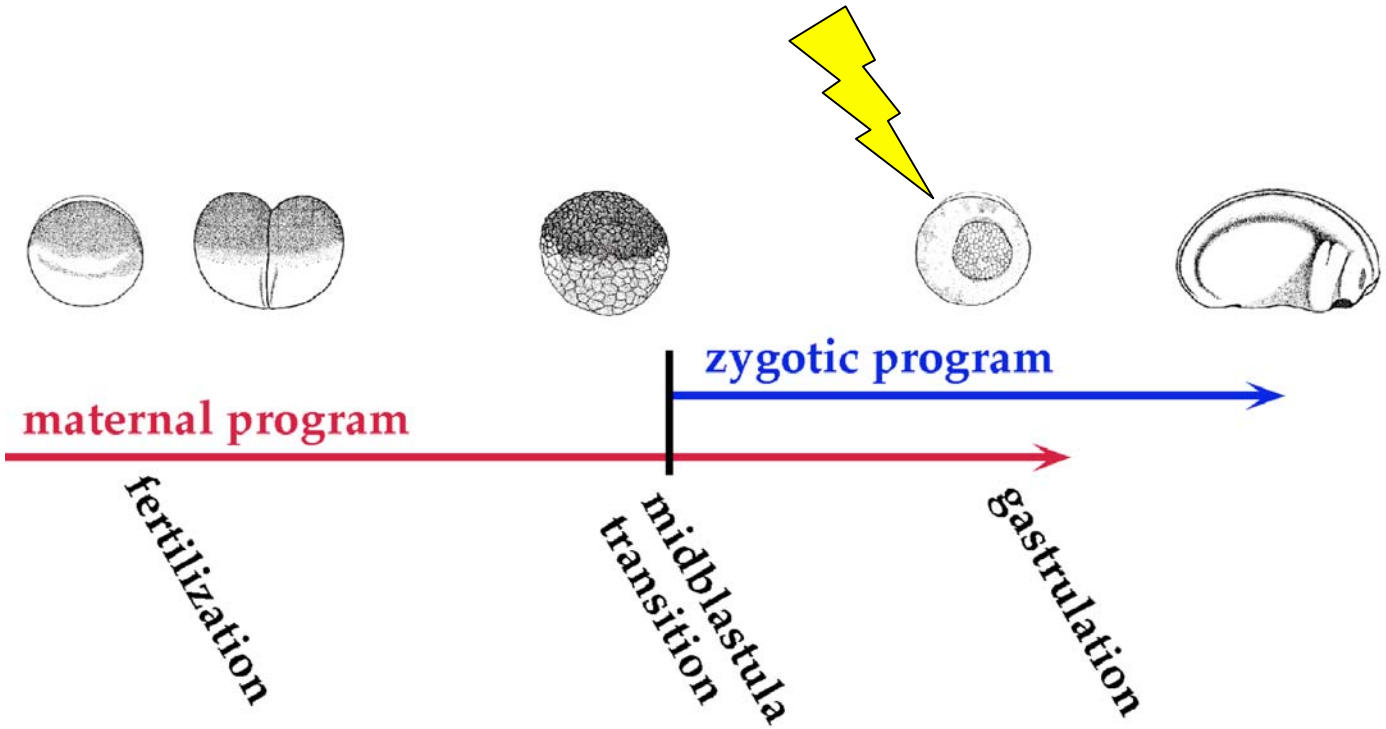

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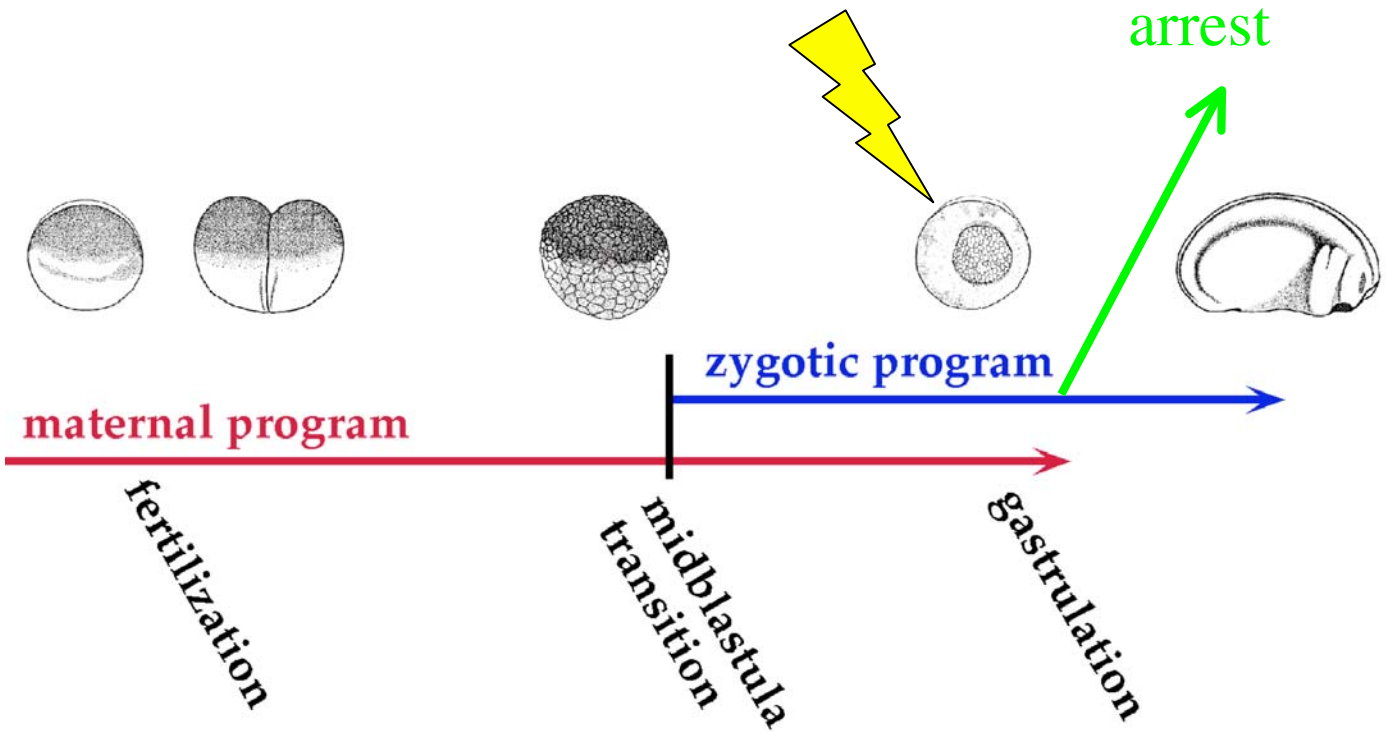
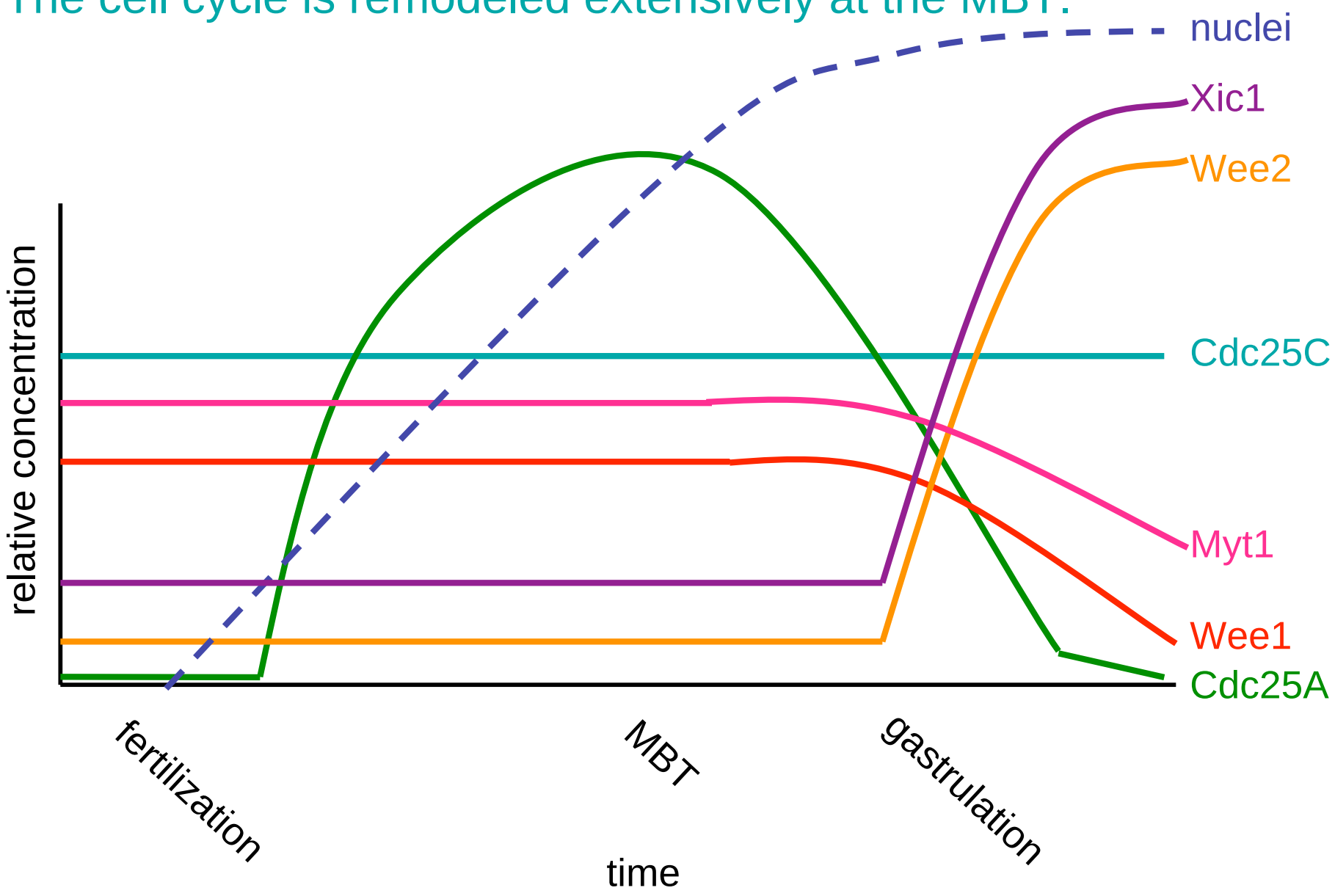


The cell cycle is remodeled extensively at the MBT.



The cell cycle is remodeled extensively at the MBT.

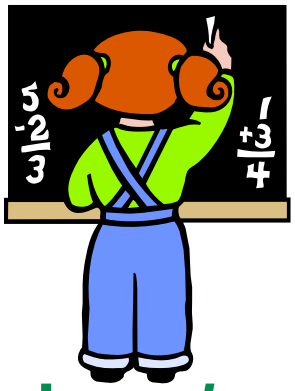
variable	pre-MBT	MBT through gastrulation	post-gastrulation
[nuclei] δ	1-4096/μl	4096-~10,000/μl	will plateau due to cell growth
[Cdc25A]	high, cycles 2-12	decreases	low
[Wee1] less active	high	decreases	very low
[Wee2] more active	absent	increases at gastrulation	relatively high
[Myt1]	basal	basal	decreased (based on RNA)[87]
Chk1 activity	low	transiently active*	decreased

Can we use the simple cell cycles of *X.laevis* embryos to develop a fundamental understanding of the regulation of the cell cycle and the engagement of cell cycle checkpoints?

Can we use the remodeling cell cycles of the *X. laevis* embryo as a model to define quantitative and qualitative elements that determine a cell's response to DNA damage?

Can we discover plasticity in the DNA damage response at the level of ecosystems? Does this give insight into amphibian decline?

THE BIG PICTURE



experimentalists

Models that are:

Predictive

Testable

Understandable

theoreticians / modelers

Data that are:

Quantitative

Kinetic

Interpreted