Analysis and Simulation of Biological Systems

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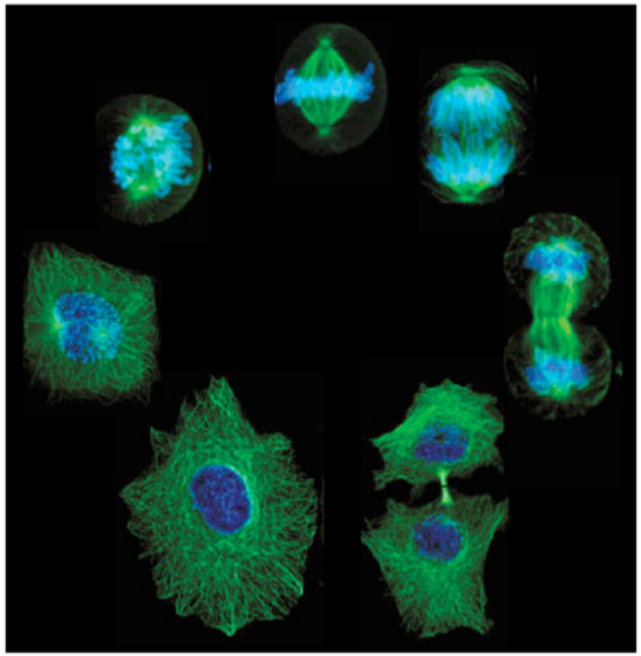
The Cell Cycle

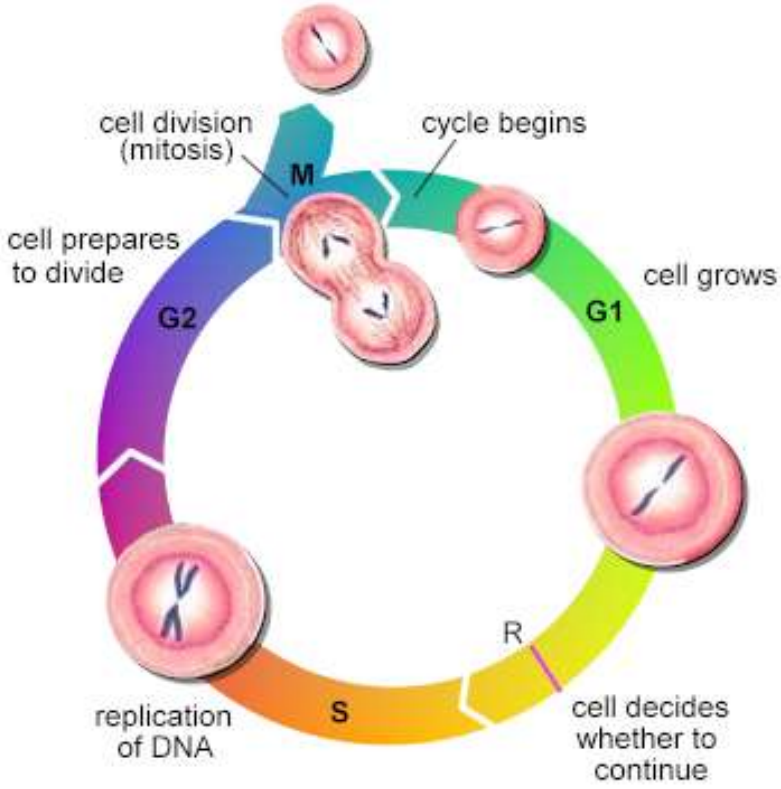
- Among the cellular processes the cell division cycle is one of the most fascinating due to
  - The fine regulation mechanisms used for the synchronization and proof checking of the process
  - The relevance for understanding the occurrence of many related diseases, like cancer



The Cell Cycle

- Each cell reproduces by performing an orderly sequence of events
- The classical model of cell cycle comprises four sequential phases

- S (synthesis) phase: the DNA is replicated to produce two identical copies
- G2 (gap) phase: new proteins are synthesized and cell approximately doubles in size
- M (mitosis) phase: matching chromosomes are pulled to opposite poles of the cell and cytokinesis pinches the cell in half
- G1 (gap) phase: each daughter cell replicates again after a gap period, or enters a quiescent phase (G0)

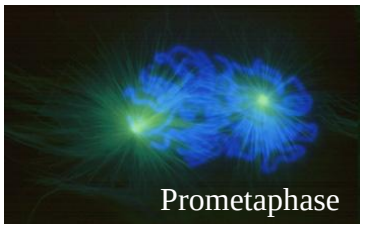


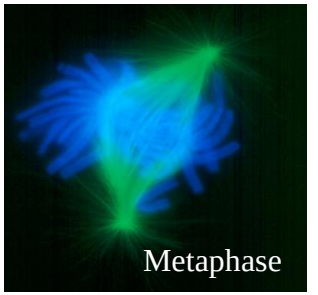
Proof Checking the Cell Cycle

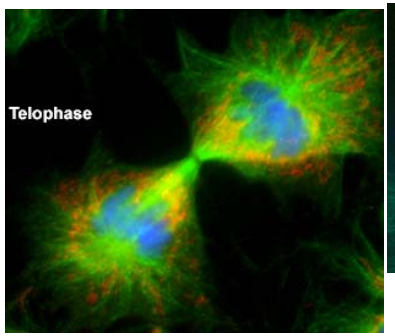
- The cycle progress through the four phases is regulated both by internal control mechanisms and by external signals
- For instance, a cell remains in the G1 phase (or enters G0) if the external condition are not favorable for cell replication (e.g. lack of nutrients)
- Once passed through the so-called restriction point, the cell commits itself to progress to the S phase and the process is not reversible
- Similarly, at the end of the G2 and M phases, there are other checkpoint mechanisms, which block the progress and completion of mitosis if the DNA synthesis or other processes have not been completed correctly

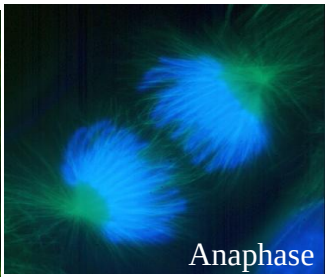
Mitosis

- Mitosis can be separated into five sub–phases
  - Prophase: the dispersed duplicated genome (chromatin) condenses into highly ordered structure (chromosome) and metabolic activity is reduced
  - Prometaphase: the nuclear envelope breaks into fragments and disappears and microtubules elongate from the centromeres
  - Metaphase: condensed chromosomes are aligned in the middle of the cell
  - Anaphase: the duplicated chromosomes are separated into two identical parts and move towards the opposite poles
  - Telophase: the chromosomes decondensate and become metabolic active, and the nuclei are reconstituted









Progression of the Cell Cycle

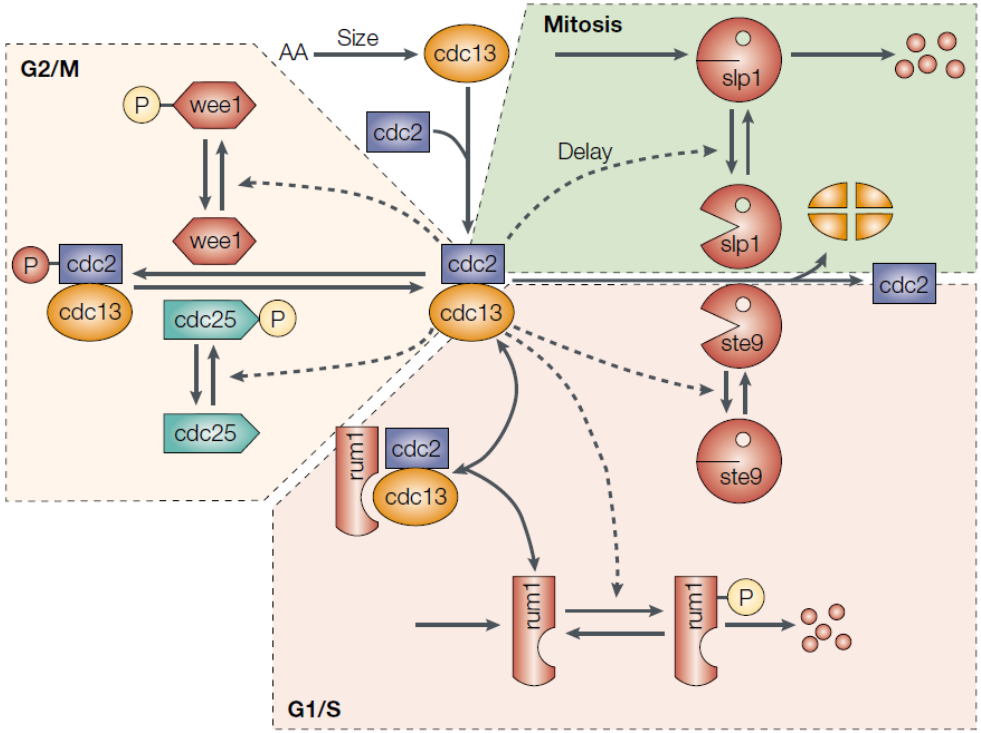
- The cell cycle is an ordered sequence of events, whose progression is strictly regulated by means of biochemical switches, responding to both internal and external conditions
- Several fundamental tasks have to be accomplished, ensuring the following features
  - the timeline of the cycle is respected
  - the events are activated in the correct order
  - each event is triggered only once per cycle (e.g. replicated DNA fragments are tagged, to distinguish from non-replicated ones)
  - the triggering events (biochemical switches) activate irreversible processes
  - the system should have the ability to perform these tasks in a certain range of environmental conditions
  - robustness against malfunctioning in the single steps has to be guaranteed
  - abnormal termination (apoptosis) procedure in case of fatal non repairable errors

Cell–Cycle Control System

- In the sequel we will follow the presentation given in

  Tyson, Chen, Novak, *Network Dynamics and Cell Physiology*, Nature Reviews Molecular Cell Biology 2:908–916, 2001

- The scheme represents a wiring diagram of the basic mechanisms involving in the regulation of the cell–cycle
- Each node represents a protein (or complex of proteins)
- Solid arrows represent the biochemical process that produce, degrade, activate or inactivate the attached proteins
- Dashed arrows represent the regulatory influence of one protein on a biochemical process

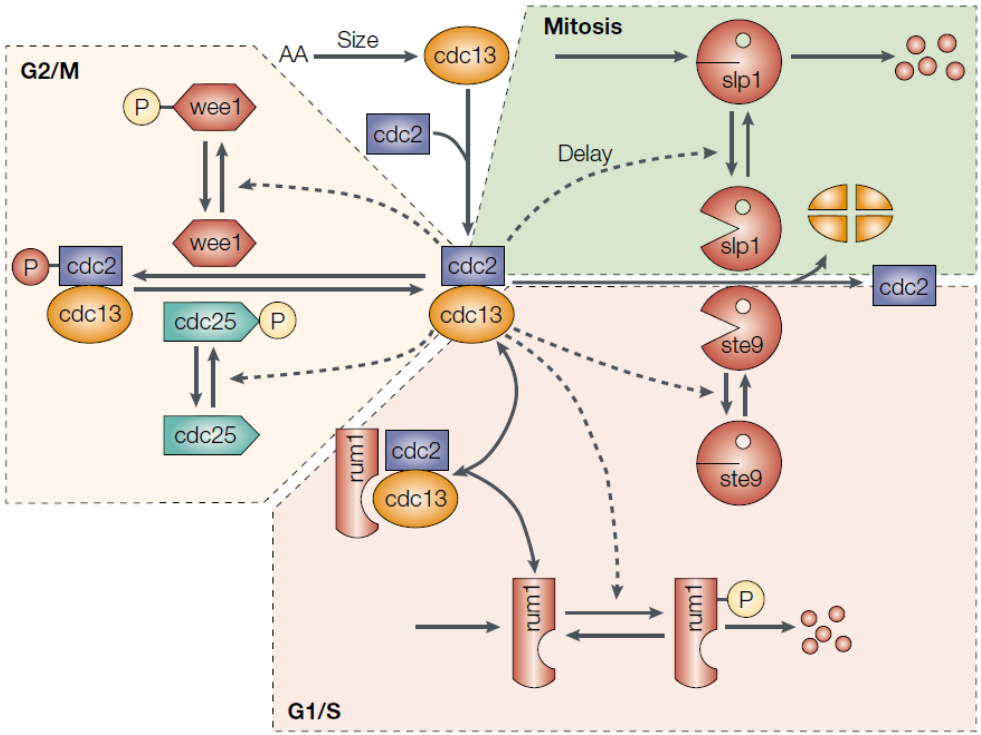


Cell – cycle control system in fission yeast

Tyson, Chen, Novak, Nature Reviews Molecular Cell Biology 2:908–916, 2001

Cell–Cycle Control System: G1 → S

- A central role is played by cyclin–dependent kinase (cdc2) in association with a B–type cyclin (cdc13)
- The control system can be divided into three modules
- The first module regulates the transition from G1 into S phase
- The amount of cdc2–cdc13 (Mitosis Promoting Factor, MPF) is low in G1 because cdc13 is degraded by ste9
- In addition, the dimeric complexes are bound to a stoichiometric inhibitor, rum1
- In turn, active MPF opposes its antagonist (ste9 and rum1) by phosphorylating them



Cell – cycle control system in fission yeast

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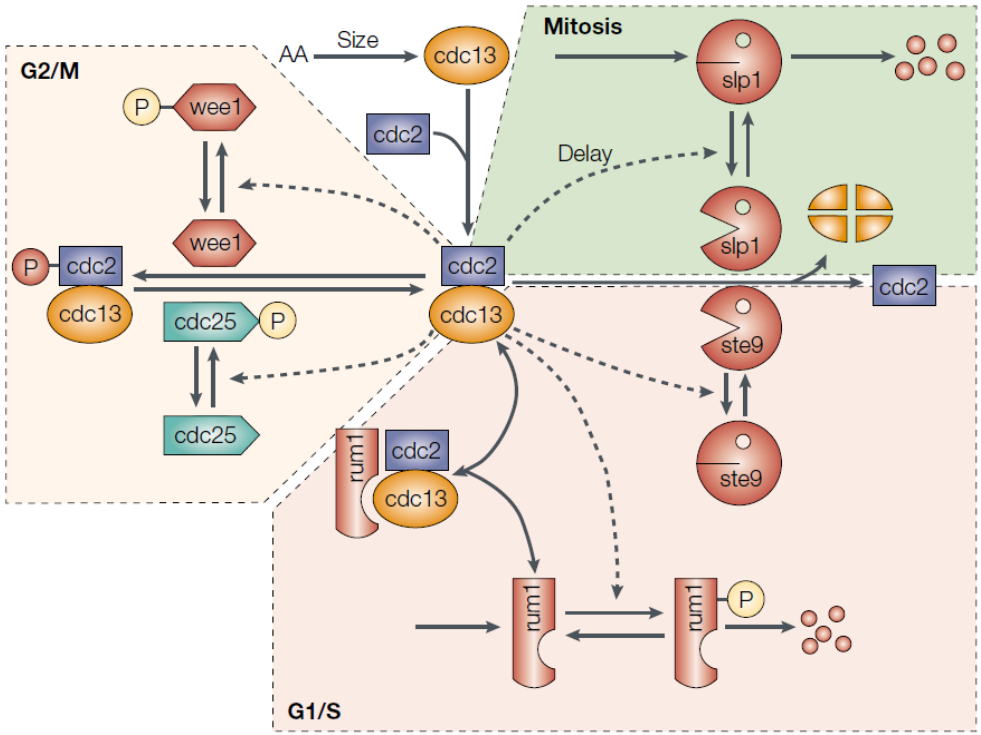
Cell–Cycle Control System: G2 → M

- At the G1–S transition, the balance of power shifts from ste9 and rum1 to MPF
- The amount of MPF is still kept under control by a second control module
- A tyrosine kinase, wee1, phosphorylates cdc2, thereby suppressing MPF activity
- On the other hand, MPF is supported by a tyrosine phosphatase, cdc25, which dephosphorylates it
- Both this molecule are regulated, in turn, by active MPF, establishing a negative and a positive feedback loop, respectively
- When the level of MPF becomes sufficiently high, the cell starts the M phase



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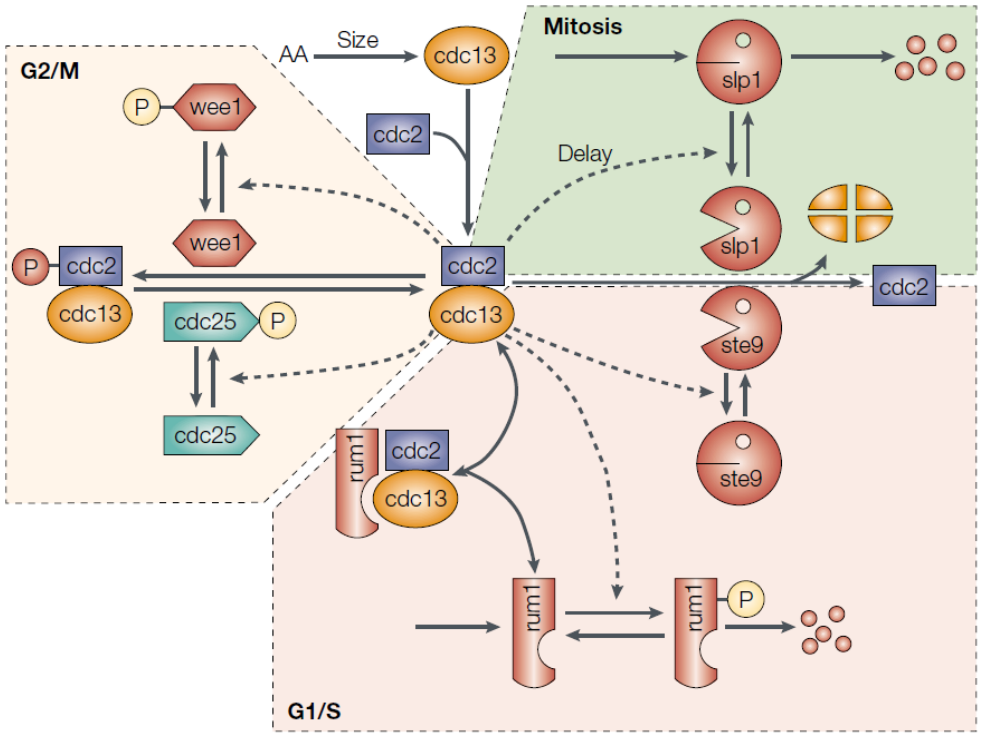
Cell–Cycle Control System: Mitosis

- The exit from mitosis is regulated by the third control module
- At completion of metaphase (chromosomes aligning), slp1 is activated
- slp1 promotes sister–chromatid separation (anaphase) and degradation of cdc13, enabling cell division
- The reduced level of MPF re-triggers the activity of ste9 and rum1, whereas slp1 becomes inactive
- The daughter cells are in the G1 phase and ready to repeat the process



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Cell–Cycle is a Widely Conserved Process

- The mechanisms underpinning cell–cycle regulation have been widely conserved among different species during evolution
- The main differences consist in the number of molecular complexes of each kind that regulate the various phases

Table 1 | **Proteins that regulate the eukaryotic cell cycle**

Fission yeast	Budding yeast	Frog egg	Mammal	Generic role
cdc2	Cdc28	Cdk1,2	Cdk1,2	Cyclin-dependent kinase
cdc13	Clb1–6	Cyclin A,B,E	Cyclin A,B,E	Cyclins
rum1	Sic1	Xic1	$p27^{Kip1}$	Stoichiometric inhibitor
ste9	Cdh1	Fizzy-related	Cdh1	APC auxiliary
slp1	Cdc20	Fizzy	$p55^{cdc}$	APC auxiliary
wee1	Swe1	Wee1	Wee1	Tyrosine kinase
cdc25	Mih1	Cdc25C	Cdc25C	Tyrosine phosphatase

Tyson, Chen, Novak, Nature Reviews Molecular Cell Biology 2:908–916, 2001

Cell Cycle in Mammalian Cells

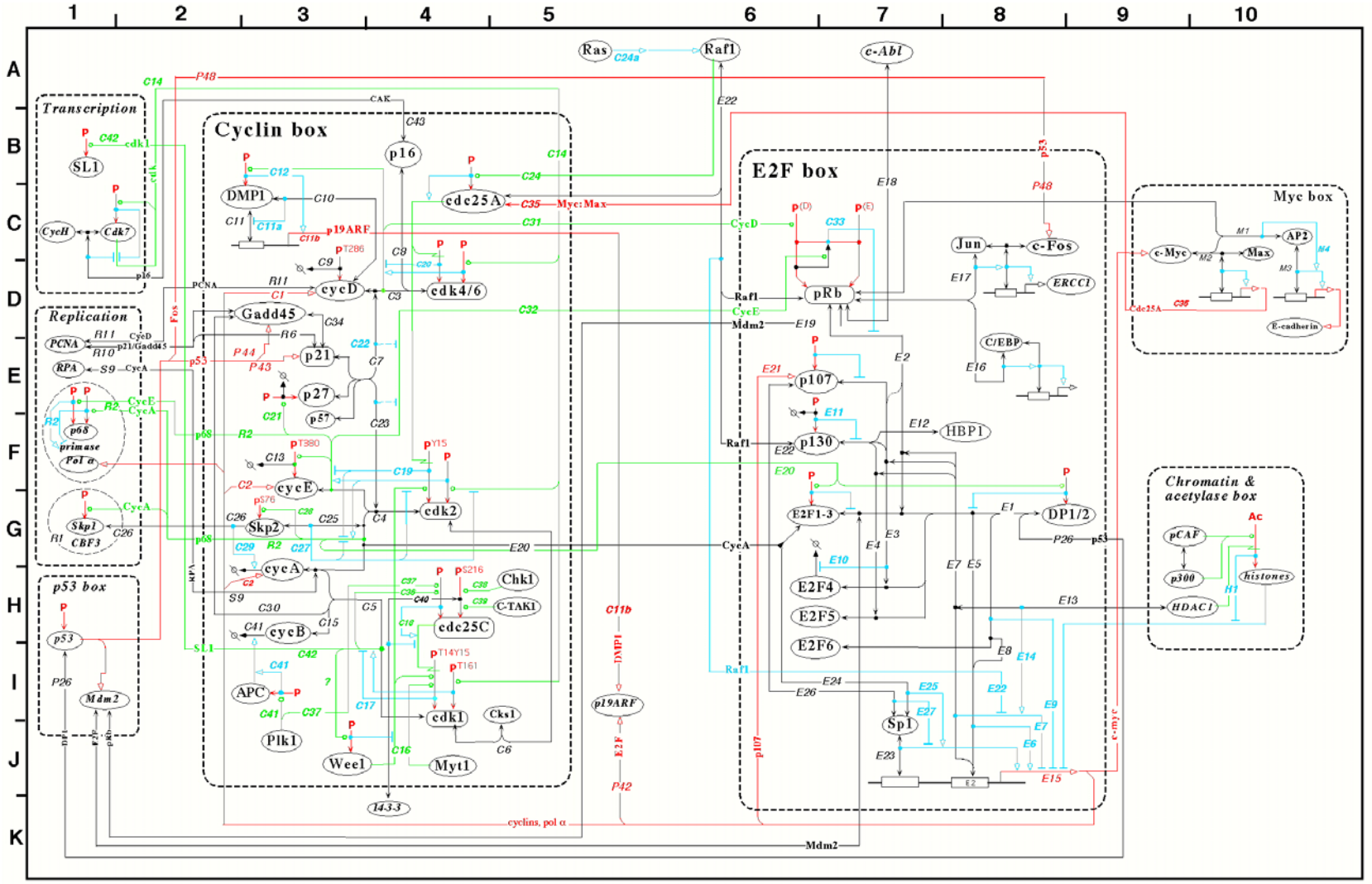


Figure 6A: The Cyclin - E2F cell cycle control system (version 3a - June 8, 1999)

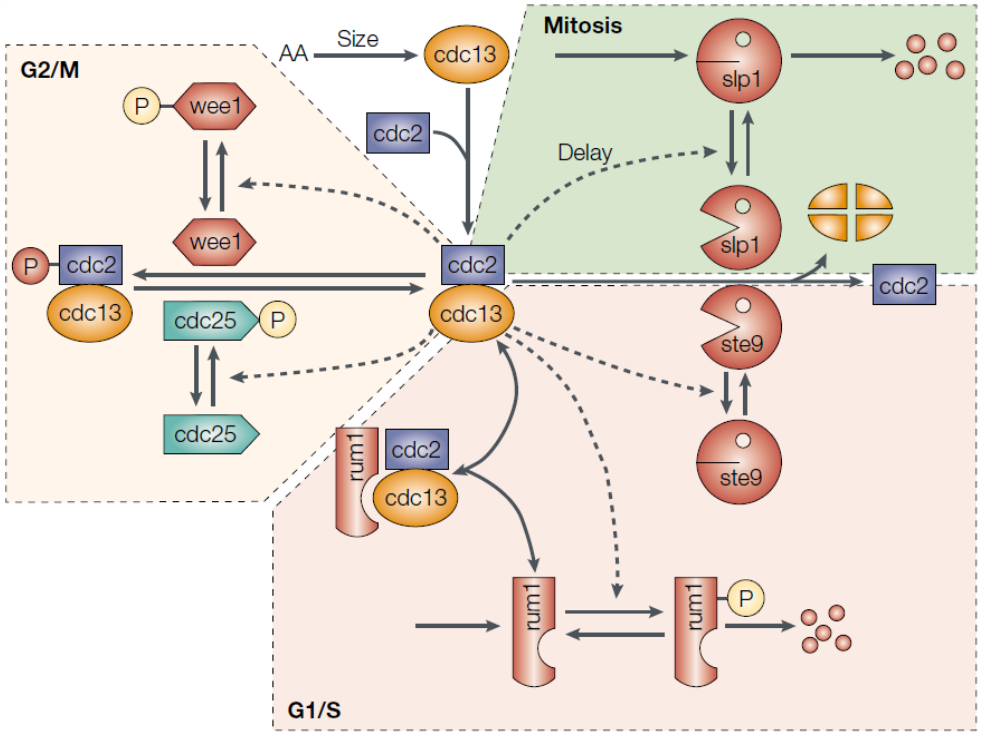
Kohn, Nature Reviews Molec. Biol Cell. 10(8):2703–2734, 1999

Model Derivation

- The ODE model can be derived by applying the techniques previously described, e.g. for the concentration of MPF

$$d/dt\,[\text{cdc2-cdc13}] = k_1 \cdot \text{size} - k_2[\text{cdc2-cdc13}] - k_3[\text{rum1}][\text{cdc2-cdc13}] + k_4[\text{rum1-cdc2-cdc13}] - k_5[\text{cdc2-cdc13}] + k_6[\text{cdc2P-cdc13}]$$

- The kinetic parameters are, in turn, determined by the activity of the other species
- For instance, the parameter k2 quantifies the degradation rate of cdc13, which depends on the activities of slp1 and ste9
- The model can be derived by applying the QSSA, assuming that the complexes formation is fast



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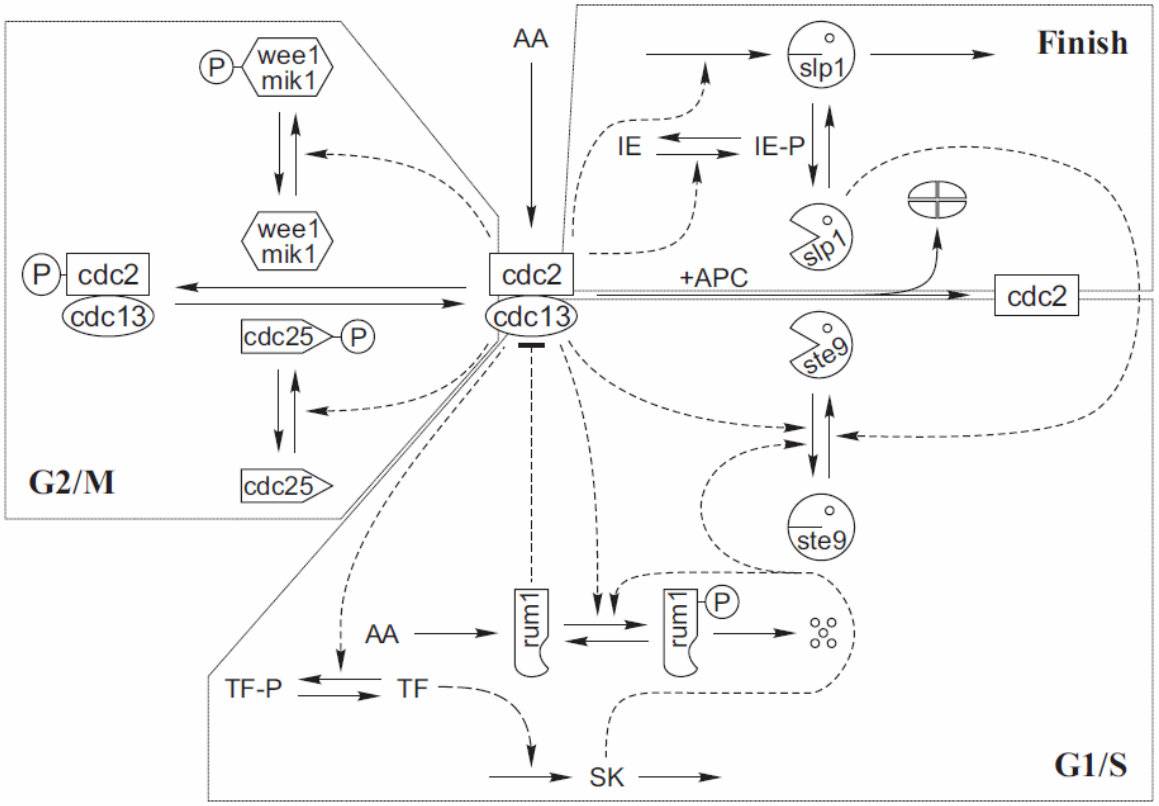
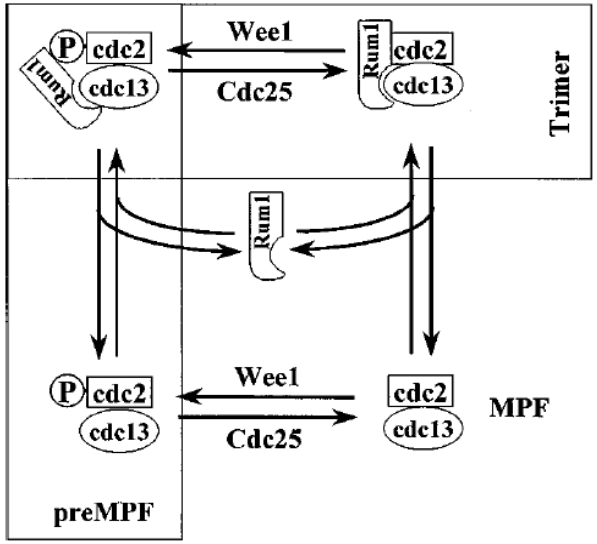
Further Regulatory Interactions

- In order to account for the effect of unknown mechanisms, the wiring diagram is extended with additional hypothesized regulatory molecules (Intermediary Enzyme Proteins, IEP, and Starter Kinases, SK)





Novak et al, Chaos 11(1):277–286, 2001

Model Equations

$$
\begin{aligned}
\frac{d\,[\mathrm{Cdc13_T}]}{dt} &= k_1\,M - \left(k_2' + k_2''\,[\mathrm{Ste9}] + k_2'''\,[\mathrm{Slp1}]\right)[\mathrm{Cdc13_T}]\,,\\
\frac{d\,[\mathrm{preMPF}]}{dt} &= k_{\mathrm{wee}}\left([\mathrm{Cdc13_T}] - [\mathrm{preMPF}]\right) - k_{25}\,[\mathrm{preMPF}]\\
&\quad - \left(k_2' + k_2''\,[\mathrm{Ste9}] + k_2'''\,[\mathrm{Slp1}]\right)[\mathrm{preMPF}]\,,\\
\frac{d\,[\mathrm{Ste9}]}{dt} &= \left(k_3' + k_3''\,[\mathrm{Slp1}]\right)\frac{1-[\mathrm{Ste9}]}{J_3+1-[\mathrm{Ste9}]}\\
&\quad - \left(k_4'\,[\mathrm{SK}] + k_4\,[\mathrm{MPF}]\right)\frac{[\mathrm{Ste9}]}{J_4+[\mathrm{Ste9}]},\\
\frac{d\,[\mathrm{Slp1}]}{dt} &= k_5' + k_5''\frac{[\mathrm{MPF}]^4}{J_5^4+[\mathrm{MPF}]^4} - k_6\,[\mathrm{Slp1_T}]\,,\\
\frac{d\,[\mathrm{Slp1^*}]}{dt} &= k_7\,[\mathrm{IEP}]\frac{[\mathrm{Slp1_T}]-[\mathrm{Slp1}]}{J_7+[\mathrm{Slp1_T}]-[\mathrm{Slp1}]} - k_8\frac{[\mathrm{Slp1}]}{J_8+[\mathrm{Slp1}]}\\
&\quad -k_6\,[\mathrm{Slp1}]\,,\\
\frac{d\,[\mathrm{IEP}]}{dt} &= k_9\,[\mathrm{MPF}]\frac{1-[\mathrm{IEP}]}{J_9+1-[\mathrm{IEP}]} - k_{10}\frac{[\mathrm{IEP}]}{J_{10}+[\mathrm{IEP}]},
\end{aligned}
$$

$$
\begin{aligned}
\frac{d\,[\mathrm{Rum1_T}]}{dt} &= k_{11} - \left(k_{12} + k_{12}'\,[\mathrm{SK}] + k_{12}''\,[\mathrm{MPF}]\right)[\mathrm{Rum1_T}]\,,\\
\frac{d\,[\mathrm{SK}]}{dt} &= k_{13}\,[\mathrm{TF}] - k_{14}\,[\mathrm{SK}]\,,\\
\frac{d\,[\mathrm{M}]}{dt} &= \mu\,M,\\
[\mathrm{Trimer}] &= \frac{2\,[\mathrm{Cdc13_T}]\,[\mathrm{Rum1_T}]}{\Sigma + \sqrt{\Sigma^2 - 4\,[\mathrm{Cdc13_T}]\,[\mathrm{Rum1_T}]}},\\
[\mathrm{MPF}] &= \frac{([\mathrm{Cdc13_T} - [\mathrm{preMPF}]])\,([\mathrm{Cdc13_T}] - [\mathrm{Trimer}])}{[\mathrm{Cdc13_T}]},\\
[\mathrm{TF}] &= G\left(k_{15}M, k_{16}' + k_{16}''\,[\mathrm{MPF}]\,, J_{15}, J_{16}\right)
\end{aligned}
$$

- 9 ODEs
- 3 algebraic equations
- ~30 parameters

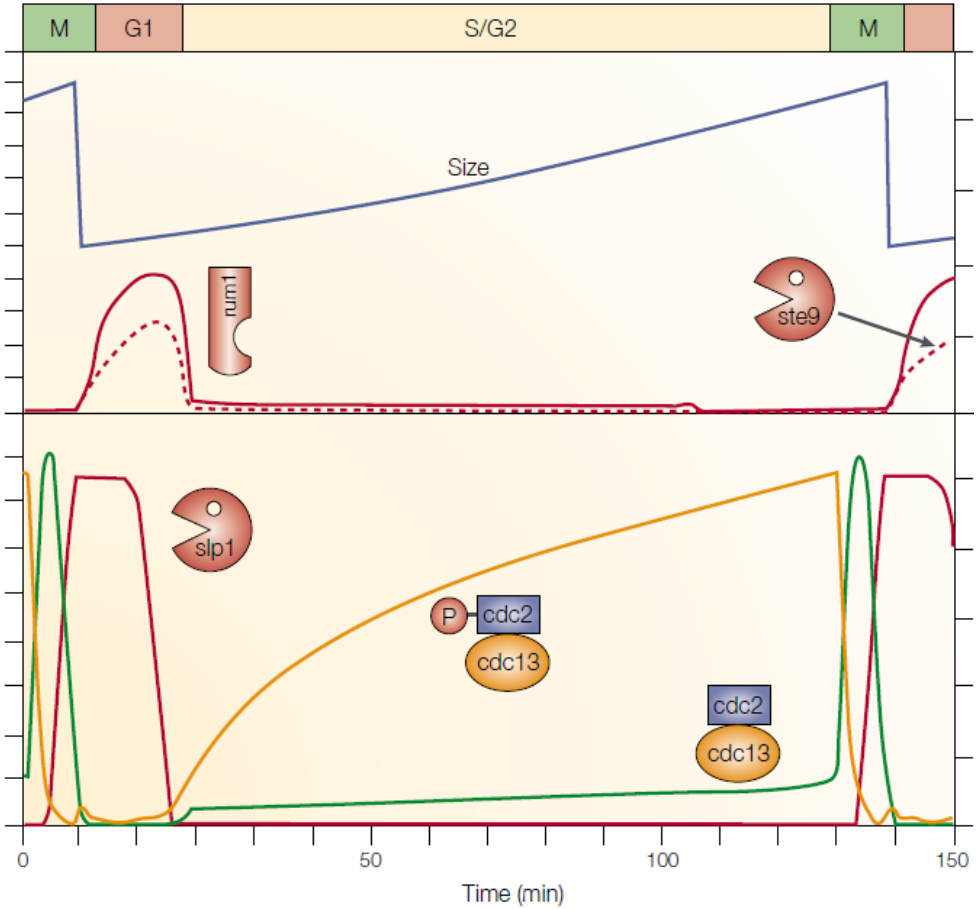
$$
\begin{aligned}
k_{\mathrm{wee}} &= k_{\mathrm{wee}}' + \left(k_{\mathrm{wee}}'' - k_{\mathrm{wee}}'\right)G\left(V_{\mathrm{awee}}, V_{\mathrm{iwee}}\,[\mathrm{MPF}]\,, J_{\mathrm{awee}}, J_{\mathrm{iwee}}\right),\\
k_{25} &= k_{25}' + \left(k_{25}'' - k_{25}'\right)G\left(V_{\mathrm{a25}}\,[\mathrm{MPF}]\,, V_{\mathrm{i25}}, J_{\mathrm{a25}}, J_{\mathrm{i25}}\right),\\
\Sigma &= [\mathrm{Cdc13_T}] + [\mathrm{Rum1_T}] + K_{\mathrm{diss}}.
\end{aligned}
$$

$$
G(a,b,c,d) = \frac{2ad}{b-a+bc+ad+\sqrt{(b-a+bc+ad)^2 - 4ad(b-a)}}
$$

Novak et al, Chaos 11(1):277–286, 2001

Time Courses of Wild–Type Cells

- The previous system can be solved by numerical integration to derive the time evolution of the single species for a wild–type cell



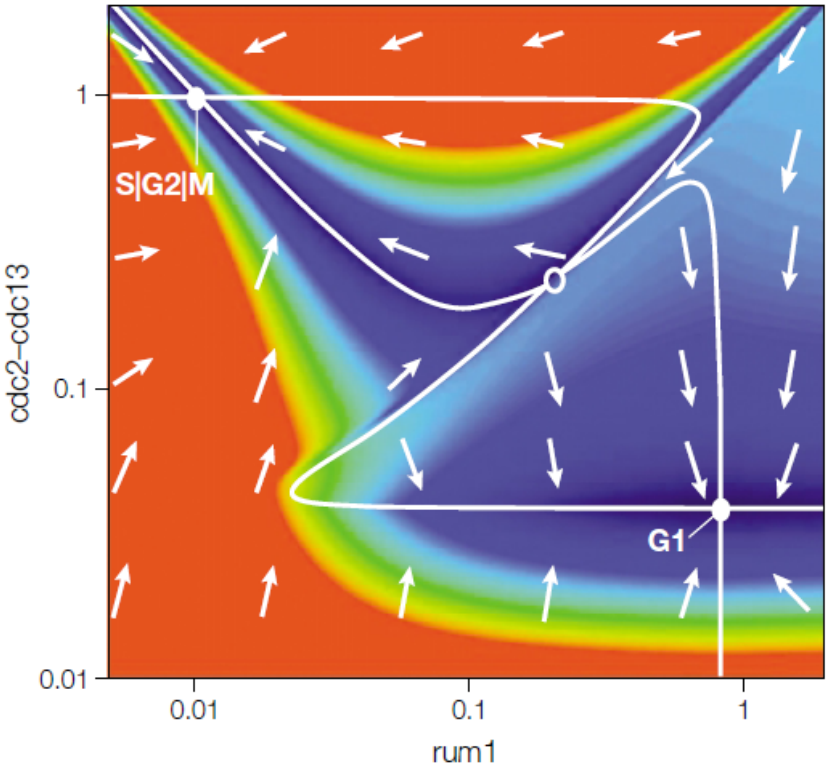
Tyson, Chen, Novak, Nature Reviews Molecular Cell Biology 2:908–916, 2001

Interpretation of the Model

- Numerical solution of biological models is a useful tool to predict the system's behavior under various conditions
- However, just like real biological experiments, they only provide data without any insight of the mechanisms underlying the particular behavior
- Understanding such mechanism by manual inspection of the equations is not a suitable approach for non–trivial systems
- We definitely need some tools to gain insight into why a system exhibit specific behavior, and how the latter depends on parameters values

Vector Field

- The behavior of a dynamical system can be suitably characterized by the corresponding vector field
- For instance, if we consider only changes of [MPF] and [rum1], the system behavior exhibits two stable equilibriums and an unstable one
- The system's trajectories follow the direction of the arrows
- They are attracted towards stable equilibriums and repelled by unstable ones
- Unfortunately it is not possible to suitably visualize vector fields of systems with more than two variables



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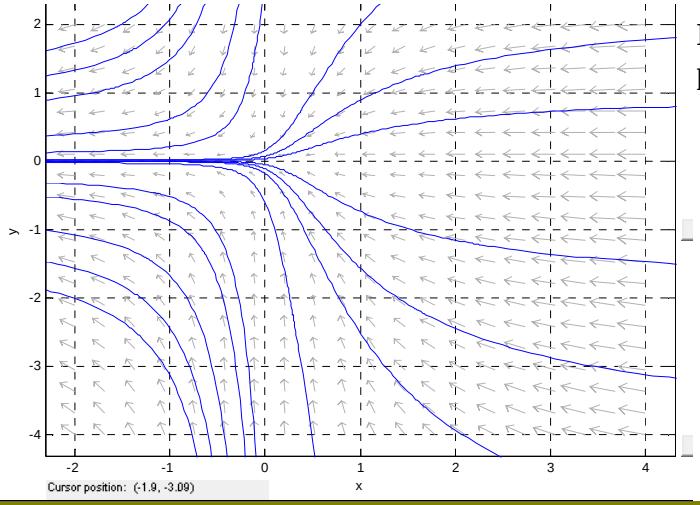
Bifurcation Theory

- Bifurcation theory is the study of topological changes of the vector field, i.e. changes in the qualitative behavior of the solutions of a system of differential equation
- A bifurcation occurs when a small smooth change of a parameter's value causes a sudden qualitative change in the dynamical behavior of the system
- Let us consider, for example, the following system

$$\dot{x} = \alpha - x^2$$

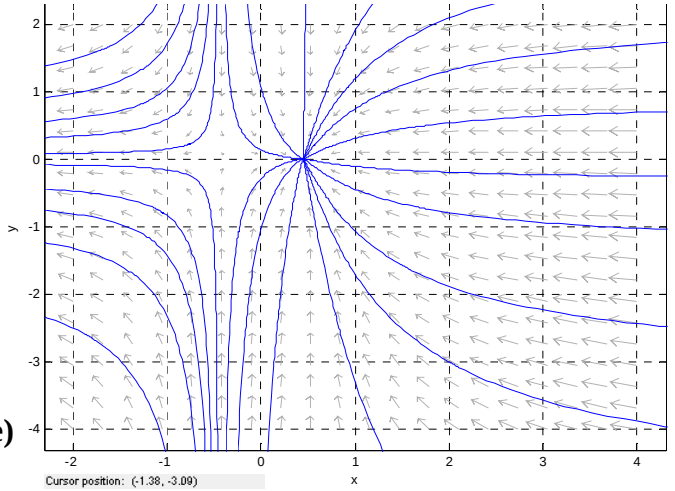
$$\dot{y} = -y$$

$\alpha < 0$



No equilibrium points

$\alpha > 0$

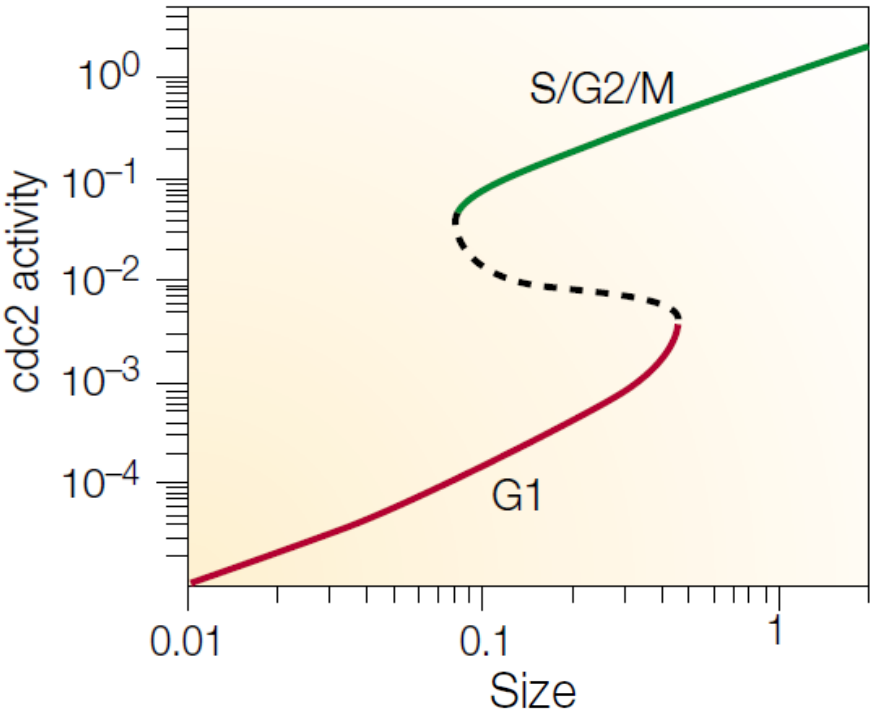


Two equilibrium points (one stable)

Bifurcation Analysis: G1–S

- It is possible to visualize the bifurcations in a multidimensional space pairwise, focusing on the variables of interest
- In the case of the cell cycle we can choose the cell size and cdc2 activity

- The G1–S control module is a bistable switch
- At intermediate cell size there are two stable steady–states (solid lines) and an unstable one (dashed line)
- Bistability is a result of the antagonism between MPF and ste9/rum1
- Note that the G1→S bifurcation is robust against small fluctuations in cell size



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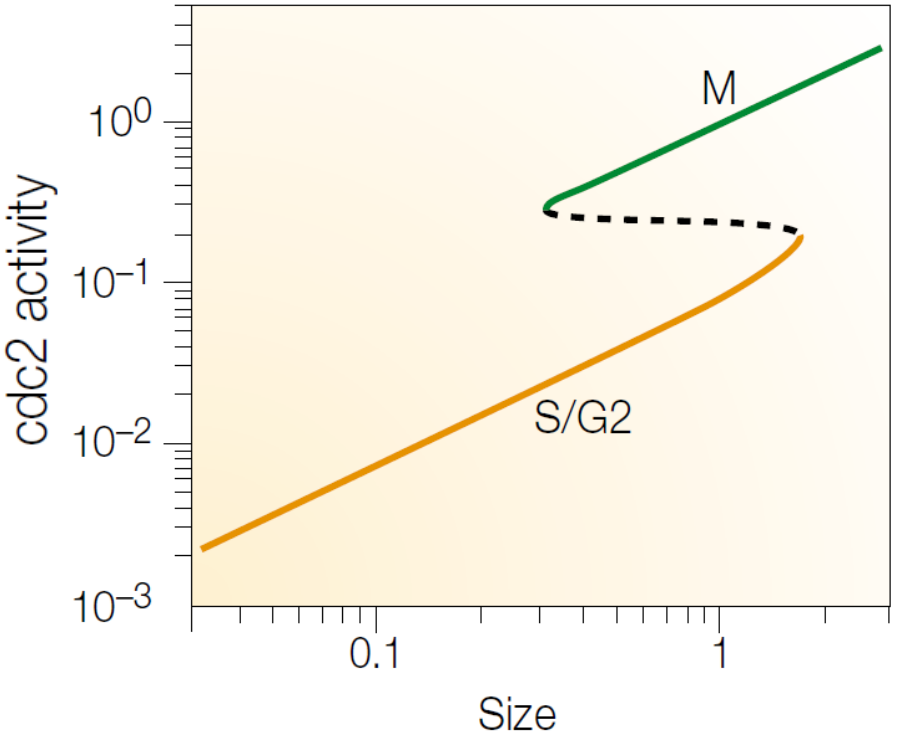
Bifurcation Analysis: G2–M

- Also the G2–M module is a bistable switch
- The transition from G2 phase into mitosis is driven by growth: when cell size reaches 1.8, the stable G2 steady–state is lost by a saddle point bifurcation and the transition to M takes place
- Bistability here results from MPF inactivating its enemy (wee1) and activating its friend (cdc25)



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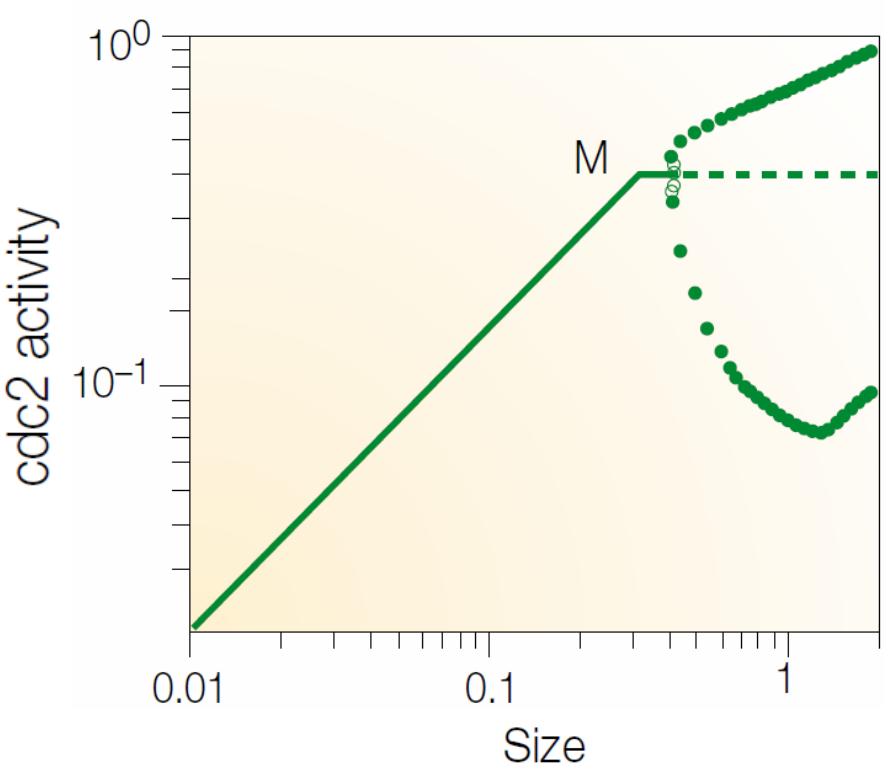
Bifurcation Analysis: M

- The mitosis module behaves rather differently from the previous ones
- At large size, the mitotic steady–state becomes unstable and stable limit cycles are born
- These oscillations are generated by a negative feedback loop
- Synthesis of cdc13 causes an increase of cdc2 activity
- After a certain time delay, slp1 is activated and destroys cdc13



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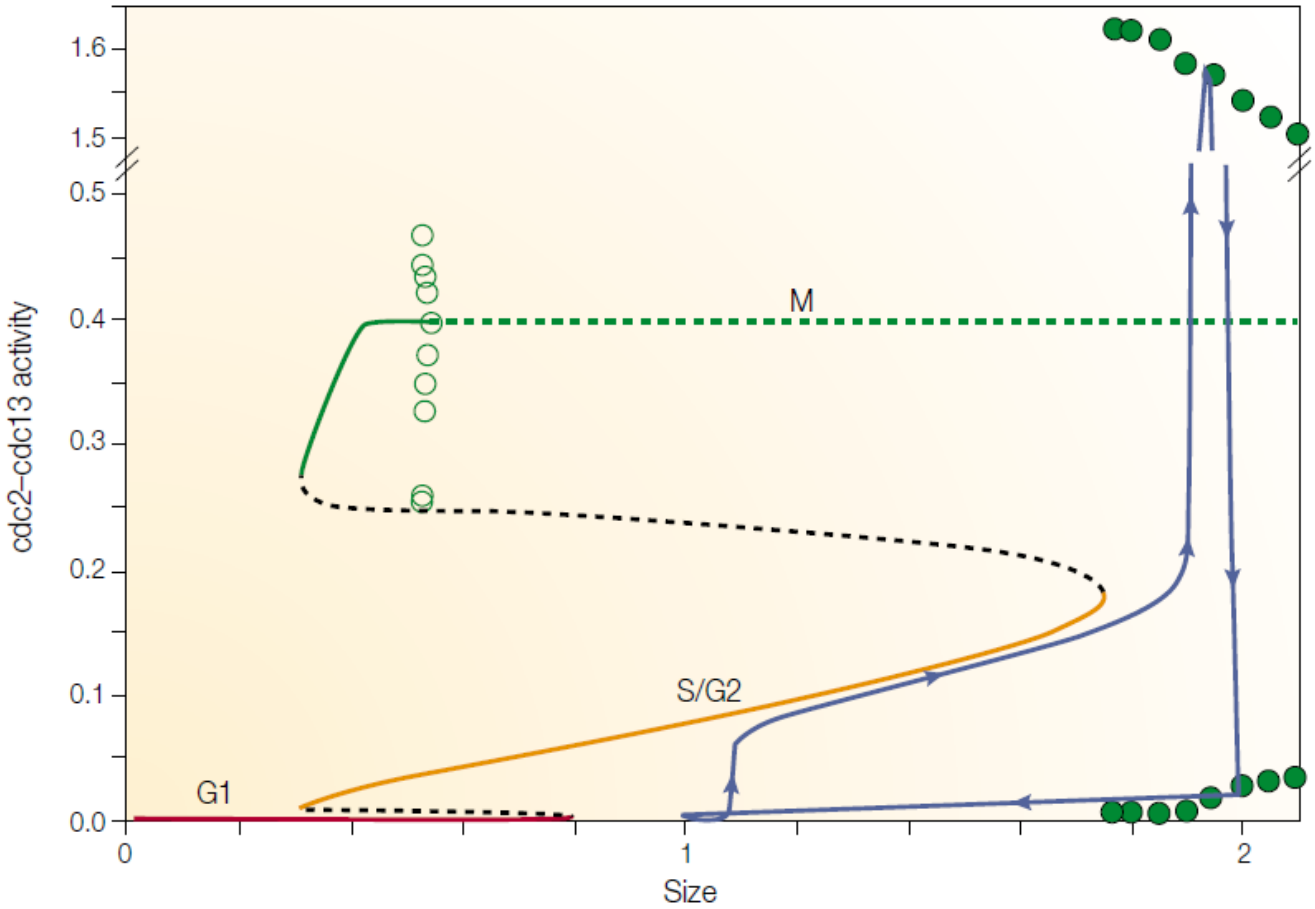
Wild–Type Cells Behavior

- Now we can put together the three modules to examine the cell–cycle in terms of bifurcations analysis



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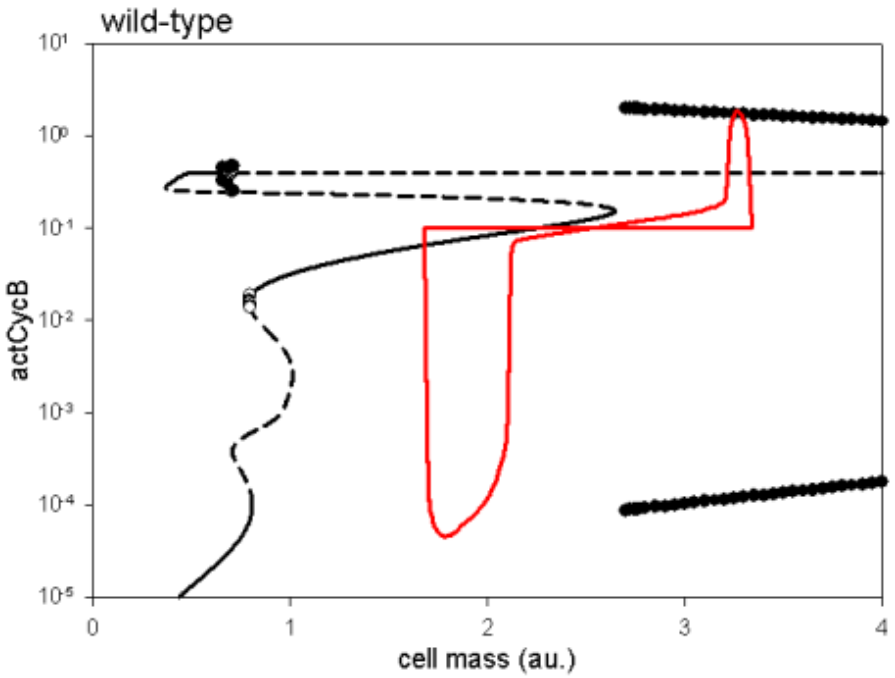
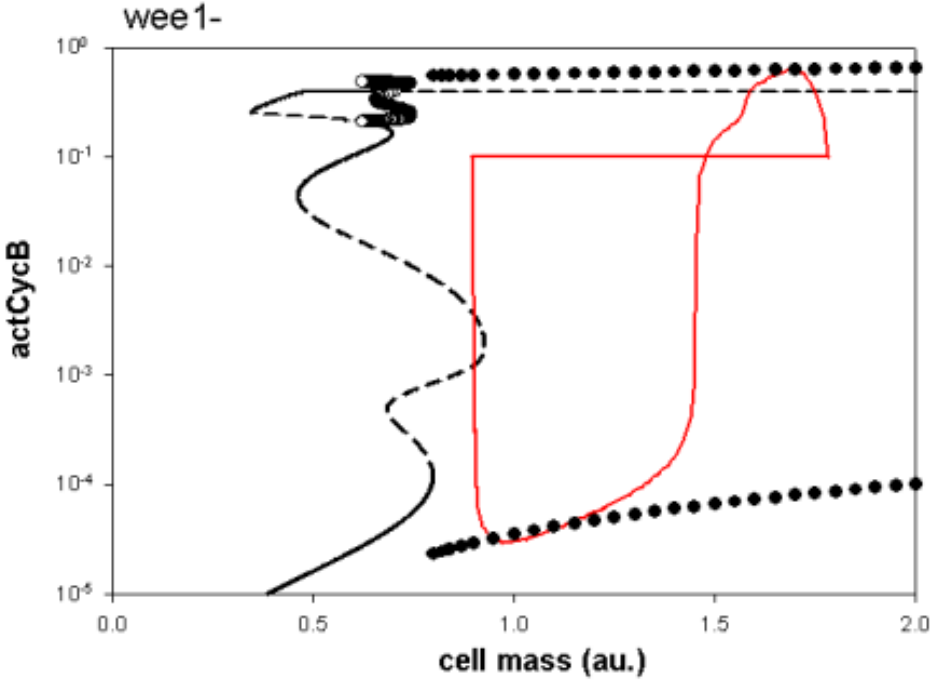
wee1$^-$ Mutant

- In wee1$^-$ mutants, cdc2 is not phosphorylated as normal and the G2 checkpoint malfunctions
- These mutants enter mitosis and divide at an abnormally small size, resulting in a much shorter S/G2 and an extended G1 phase

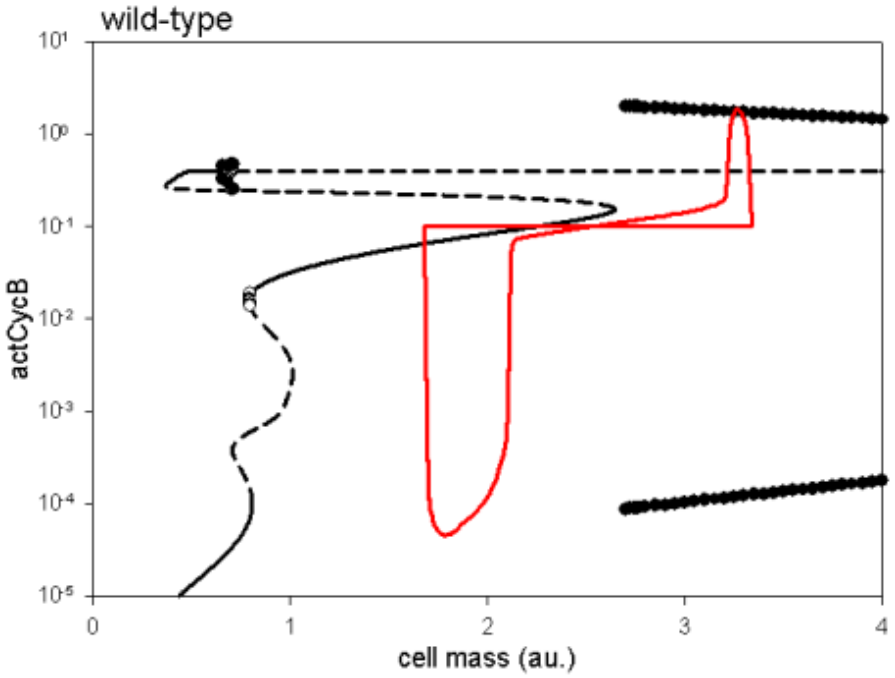


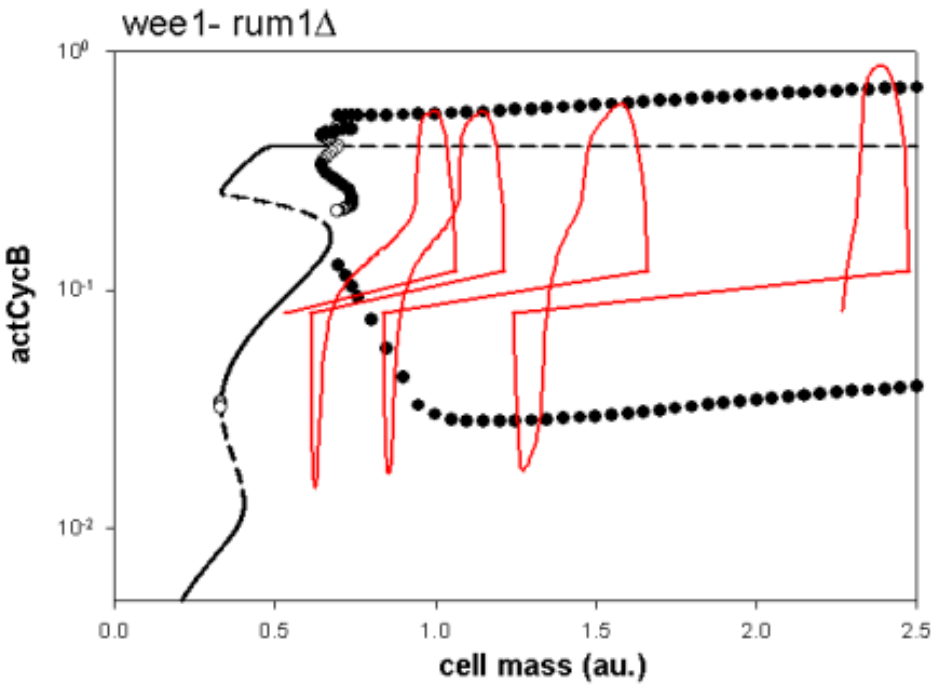


http://mpf.biol.vt.edu (Tyson's group web site)

wee1^{-} rum1Δ Mutant

- wee1 – rum1Δ result in a loss of viability
- The cells divide faster than they grow, becoming progressively smaller until they die





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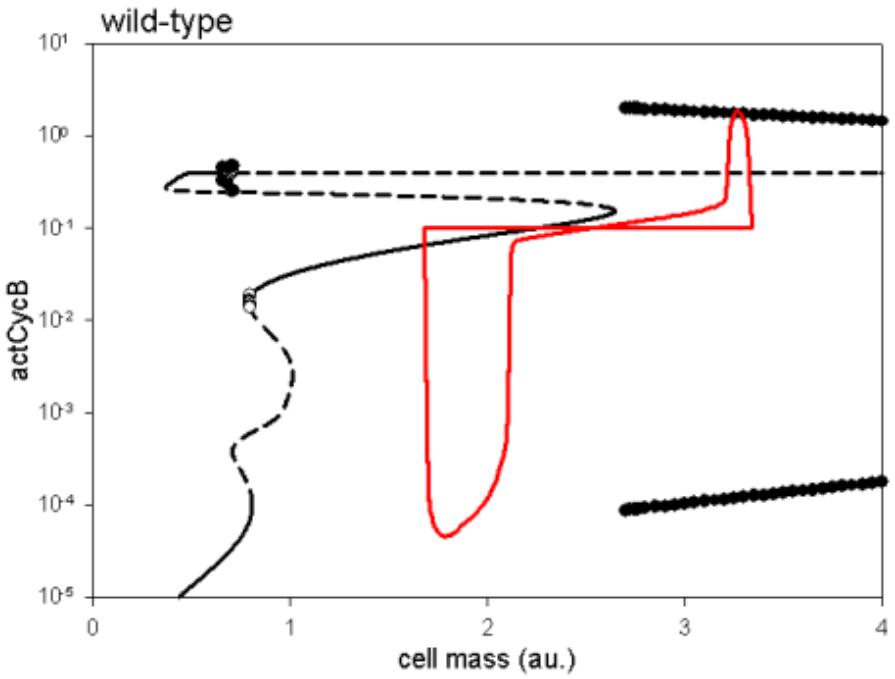
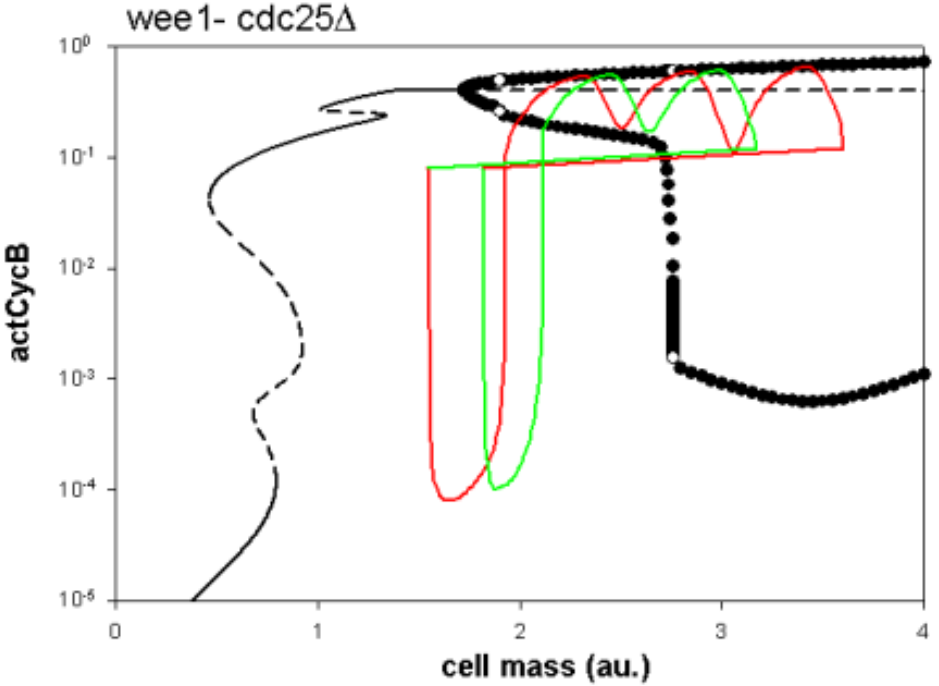
wee1- cdc25Δ Mutant

- If wee1 is missing then cells should not need cdc25 as well; indeed such mutation is viable, but causes an abnormal cycle
- Experiments show that three subpopulations are formed, with distinctly different cycle times (90, 160 and 230 min)





References

- Tyson, Chen, Novak, *Network Dynamics and Cell Physiology*, Nature Reviews Molecular Cell Biology 2:908–916, 2001
- Novak et al, *Mathematical model of the cell division cycle of fission yeast*, Chaos 11(1):277–286, 2001