Outline

Cell growth as the driver for cell cycle (in microbes):
coordination of growth and division

A basic principle organizing cell cycle control: why cyclin-dependent kinase activity must oscillate

Very messy regulatory machinery that provides the oscillations:
two potentially independent oscillatory mechanisms working in tandem

Chemical-kinetic modeling (in collaboration with John Tyson)

Coordination of growth and division:

Hartwell and Unger 1977:

The minimum time for the cell division cycle is significantly less than the mass doubling time.

Note: mass increase (ribosomes, etc) is approximately exponential with increasing cell size. Thus the 'mass doubling time'.

Growth and division are entrained because small cells are significantly less likely to initiate the cell division cycle than larger cells.

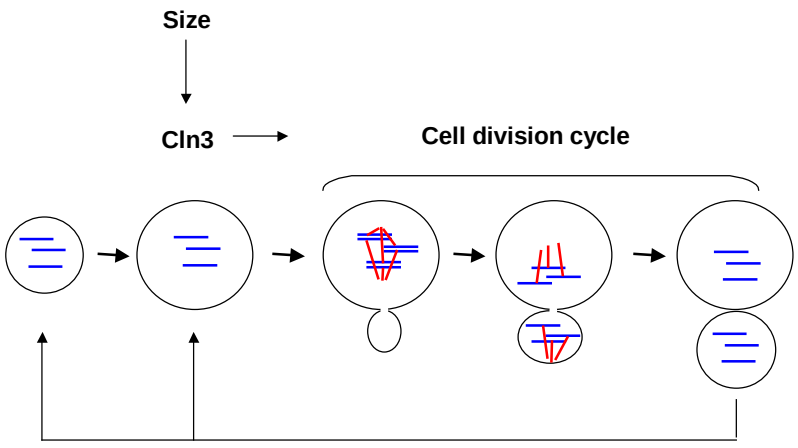
Coordination of growth and division:

Smaller cells are less likely to initiate the cell division cycle; the setpoint for the minimum size can be modulated by Cln3 (initiator cyclin) levels.

The mechanism remains unsolved.

Do cells measure nuclear/cytoplasmic ratios?

Is nuclear Cln3 a part of this measurement ratio?

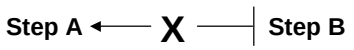


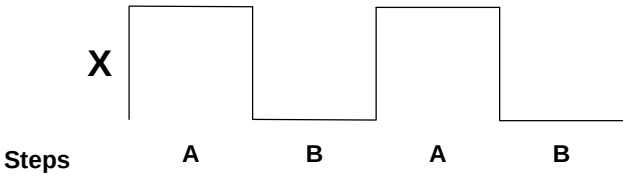
A good idea:

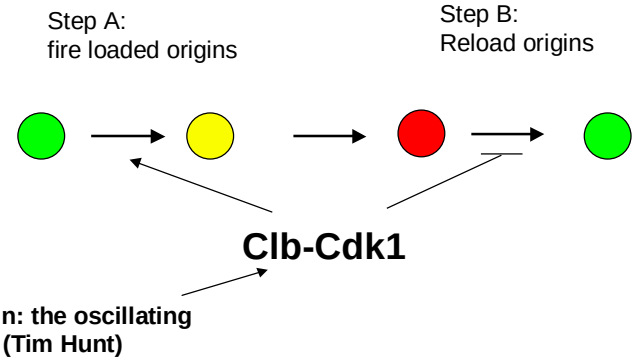
Make a biochemical oscillator, so that some enzyme activity **X** goes up and down.

Set up each bit of cell cycle machinery as two-step processes.

Then, connect up the cell division cycle machinery in the following way:







: Origins of DNA replication loaded

: Origins of DNA replication in use

: Origins of DNA replication unloaded

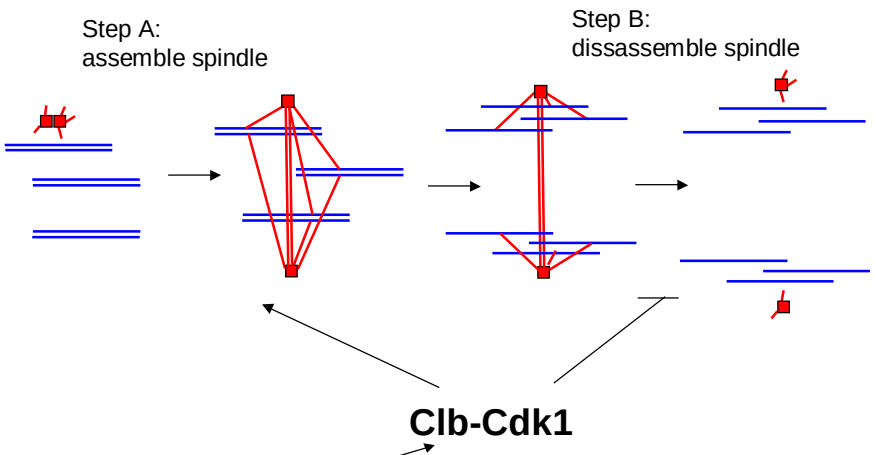
One part of regulation of origin loading
by Cdk activity: removing Mcm proteins from the nucleus

The Mcm complex is loaded at origins before firing.
It is essential for origin function. Cdk phosphorylation of the Mcm complex results in its exclusion from the nucleus.

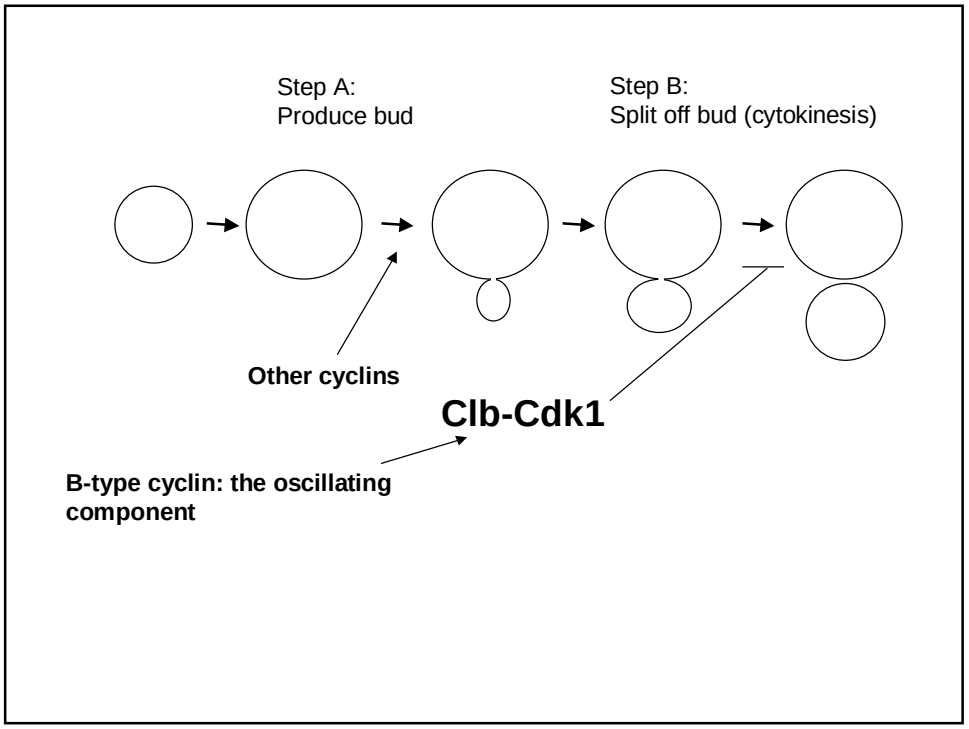
See the movie:

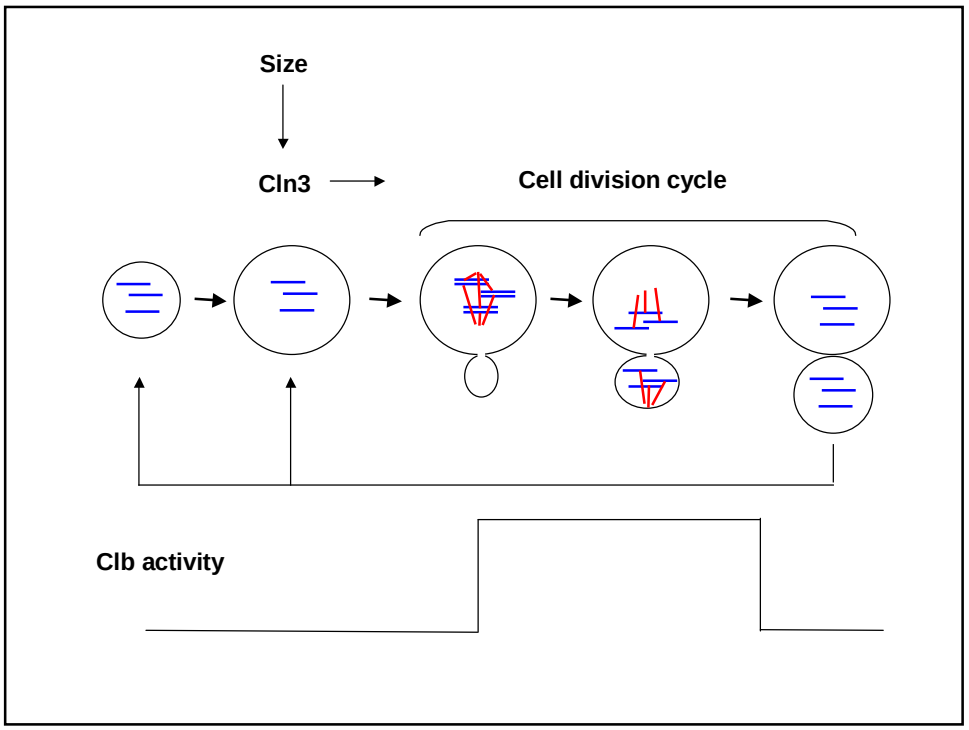
Note nuclear fluorescence only right after cell division.

(Subtle point: fluorescence lasts a bit longer in daughter nuclei than in mother nuclei)

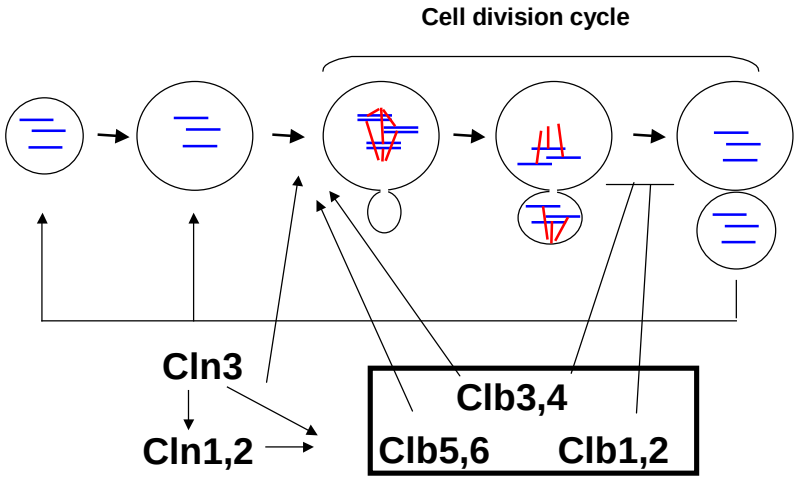


Watch another movie!

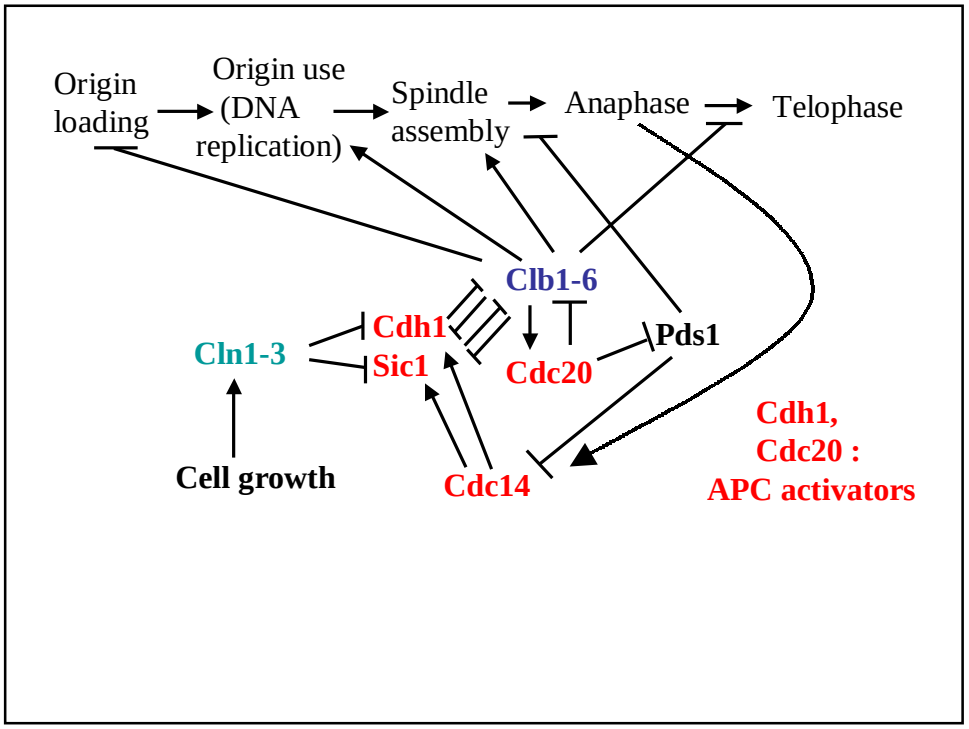

Step A:
Produce bud
Step B:
Split off bud (cytokinesis)
Other cyclins
Clb-Cdk1
B-type cyclin: the oscillating component


Size
Cln3
Cell division cycle
Clb activity

A complication: 9 cyclins!

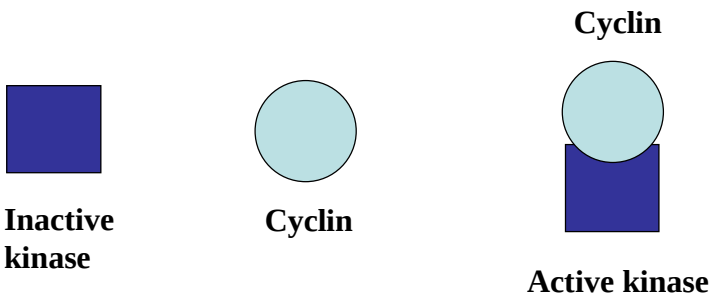


'The purpose of a Rube Goldberg Machine is to build the most complicated machine possible to perform a simple everyday task.'

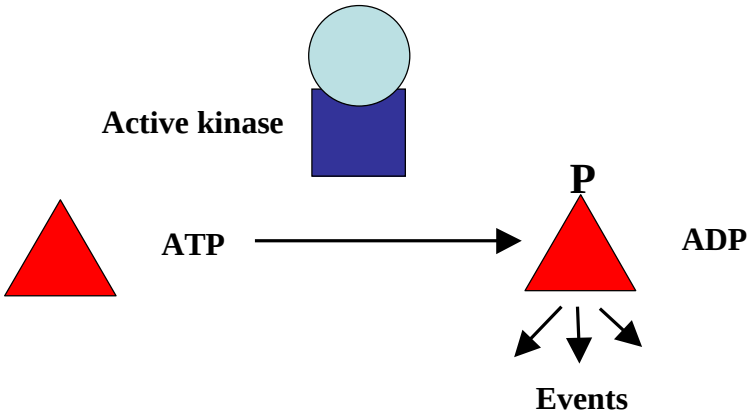


Oscillator components: cyclins

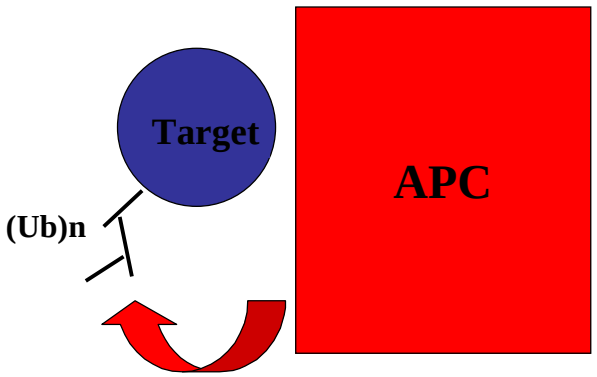
Cyclins work by activating a kinase.



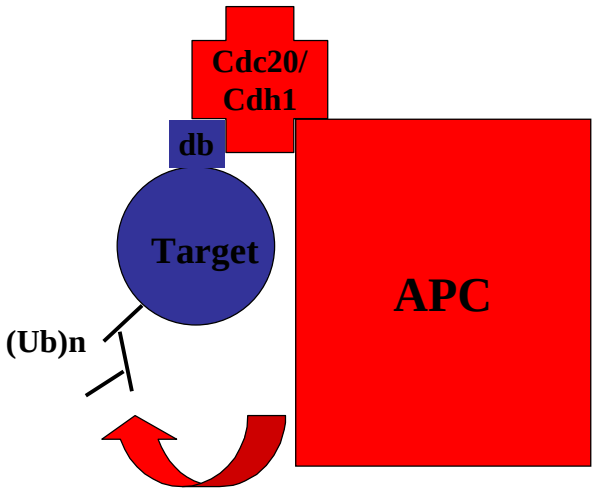
The kinase phosphorylates target proteins, and thereby regulates target activity.



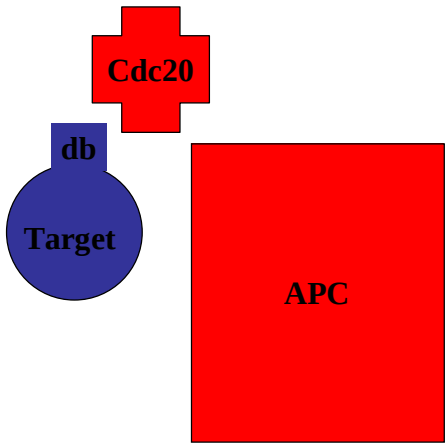
Oscillator components: APC



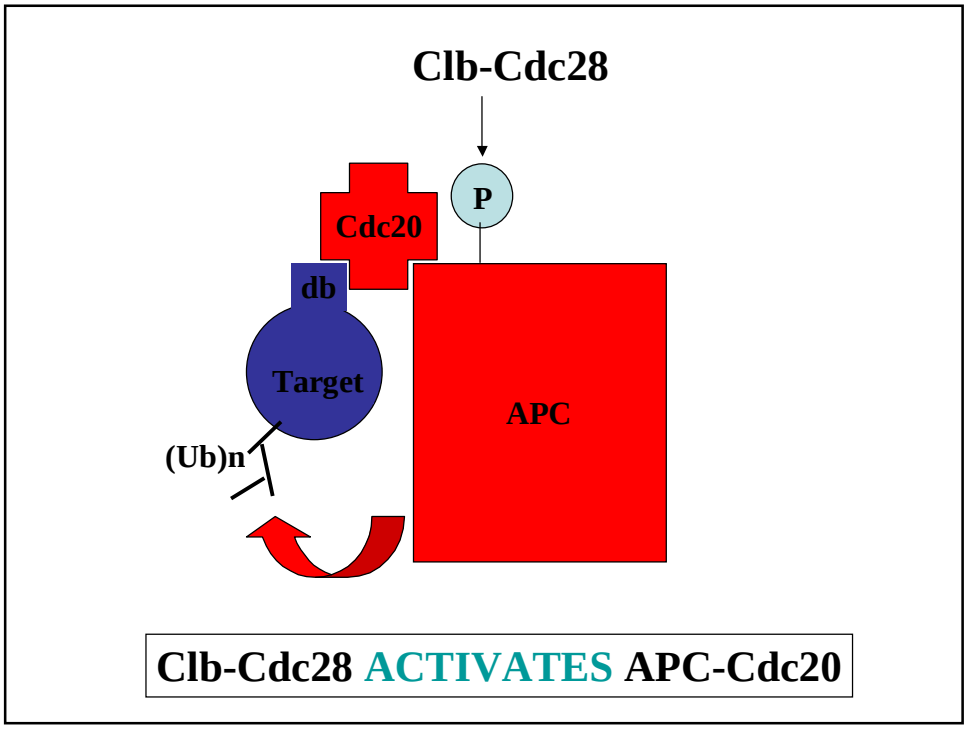
The anaphase-promoting complex (APC) ubiquitin ligase complex

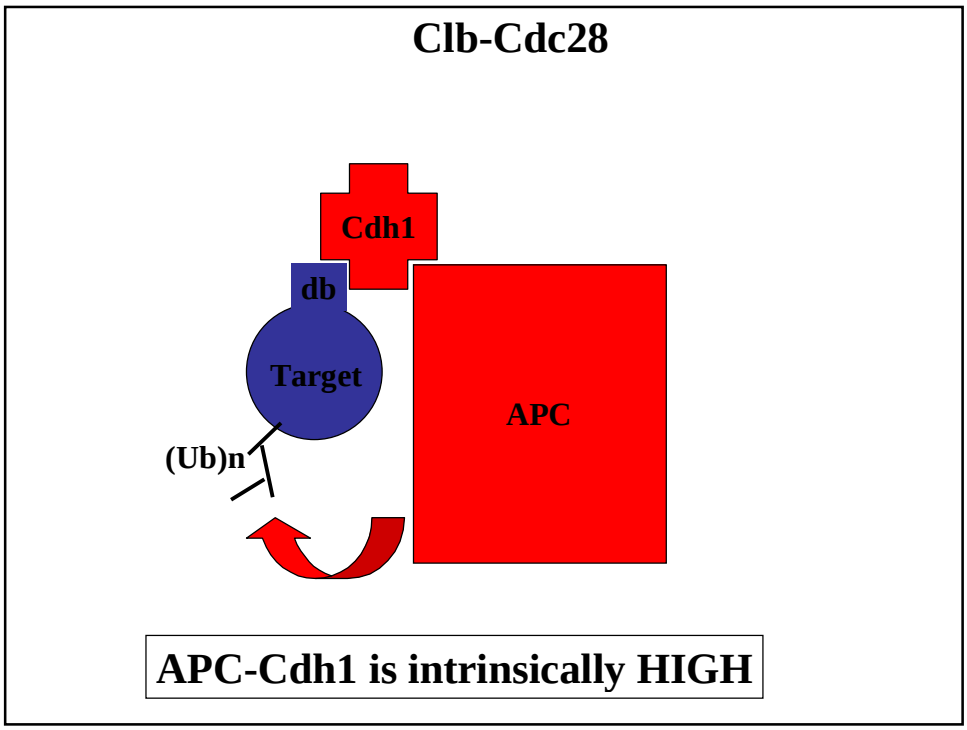


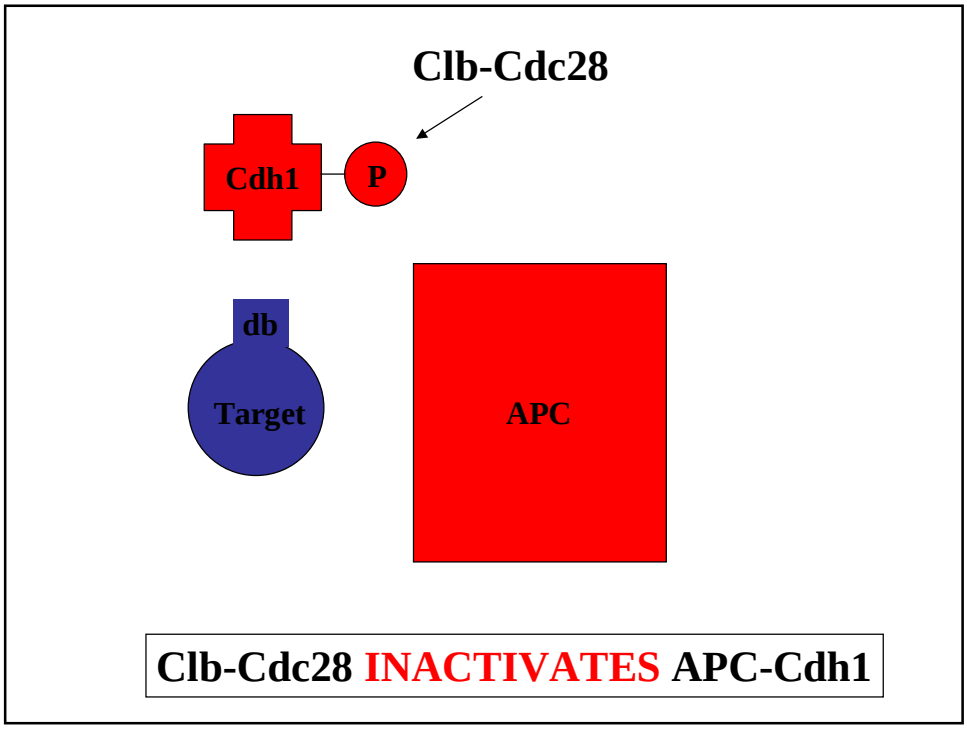
Cdc20 and Cdh1 are adapters that bring destruction-box-containing target proteins to the APC ubiquitin ligase complex

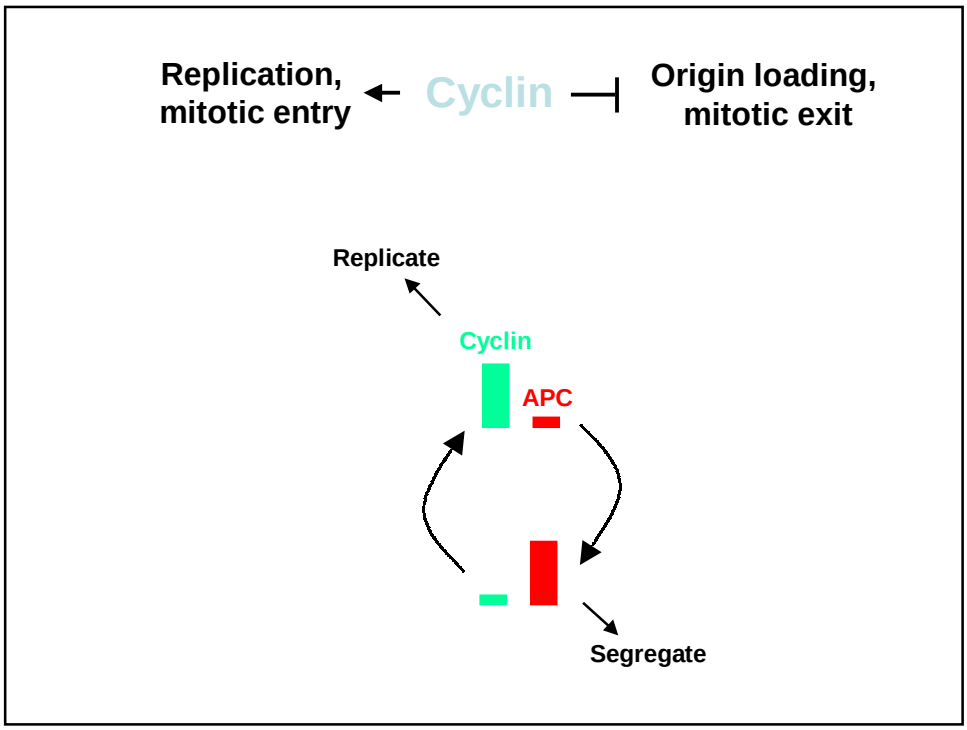


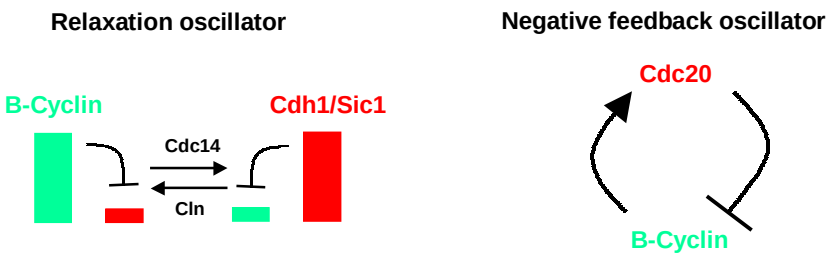
APC-Cdc20 activity is intrinsically LOW


Clb-Cdc28
P
Cdc20
db
Target
(Ub)n
APC
Clb-Cdc28 ACTIVATES APC-Cdc20

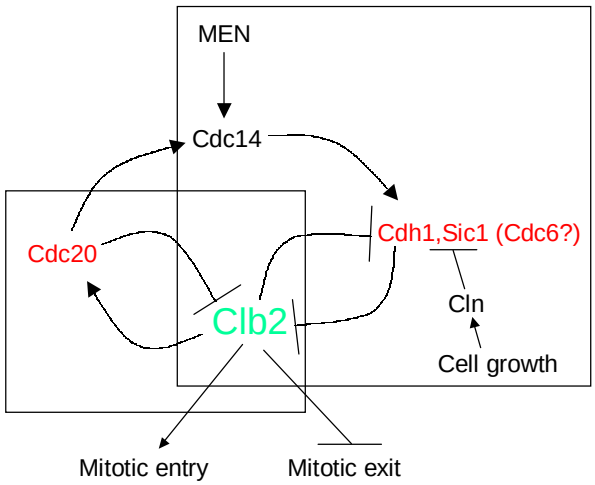

Clb-Cdc28
Cdh1
db
Target
(Ub)n
APC
APC-Cdh1 is intrinsically HIGH


Clb-Cdc28
Cdh1
P
db
Target
APC
Clb-Cdc28 INACTIVATES APC-Cdh1


Replication, mitotic entry
Cyclin
Origin loading, mitotic exit
Replicate
Cyclin
APC
Segregate

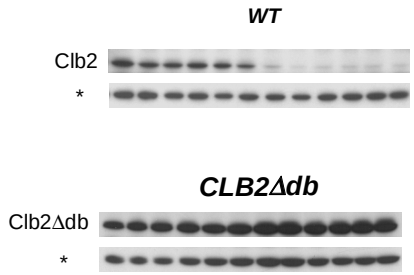


Circuitry for two different types of oscillators coexists in the wild-type budding yeast system.



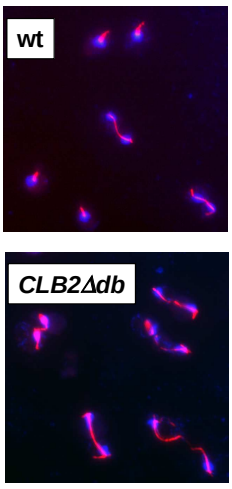
Clb2 is the main mitotic cyclin.

GAL-SIC1 CLB2 or *CLB2Δdb*: turn off *GAL-SIC1*, elutriate mid-cell cycle, release into α-factor

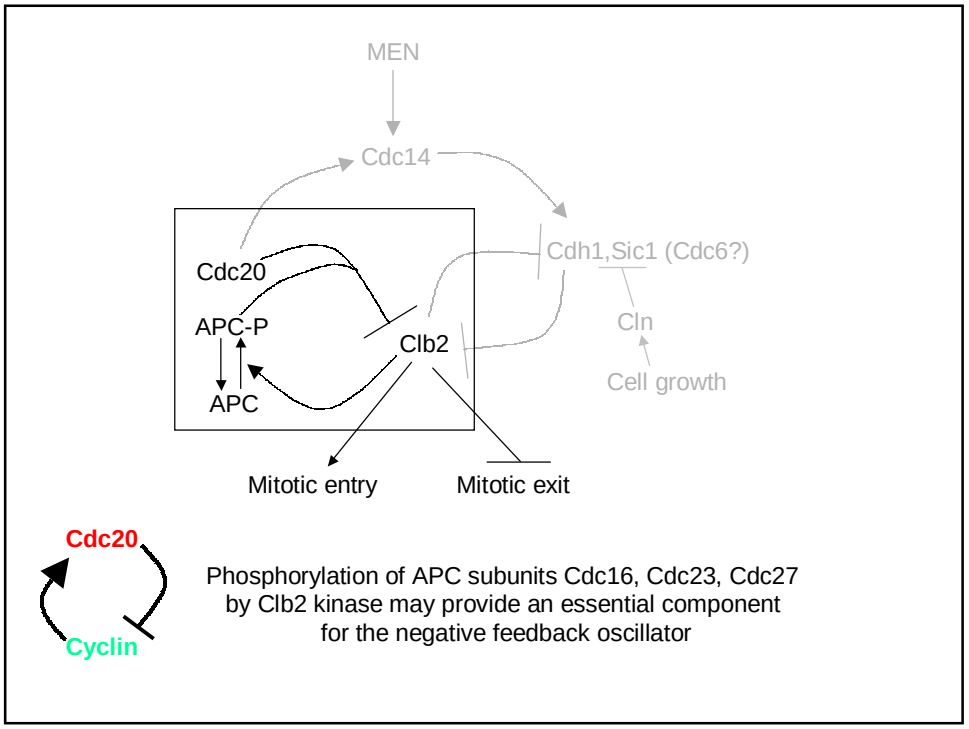


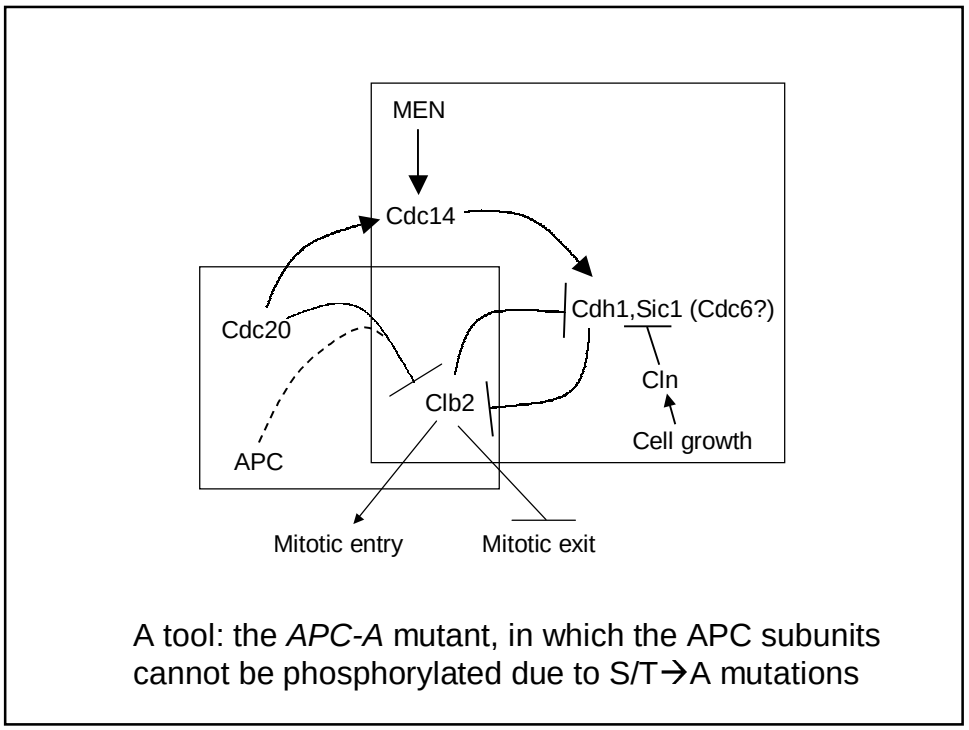
Removing the Clb2 destruction box prevents Clb2 degradation

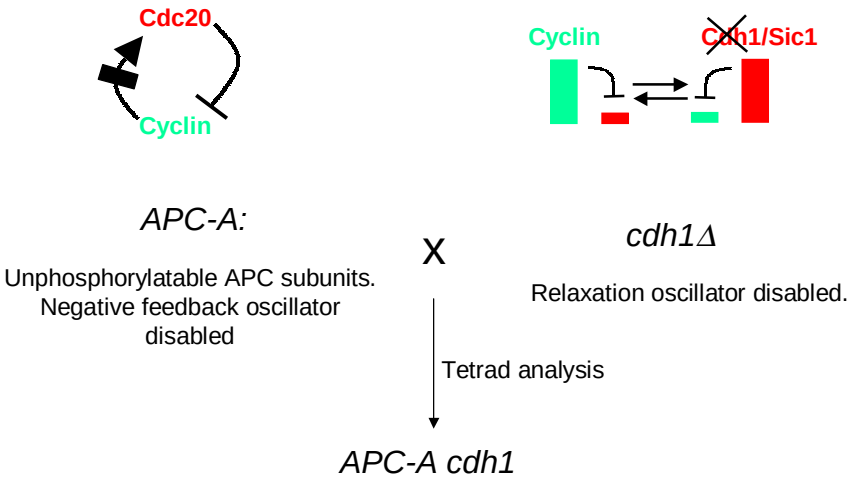
CLB2 GAL-SIC1 or *CLB2Δdb GAL-SIC1* : turn off *GAL-SIC1*



Removing the Clb2 destruction box (exact endogenous gene replacement) blocks cells in late mitosis


MEN
Cdc14
Cdc20
APC-P
APC
Clb2
Cdh1,Sic1 (Cdc6?)
Cln
Cell growth
Mitotic entry
Mitotic exit
Cdc20
Cyclin
Phosphorylation of APC subunits Cdc16, Cdc23, Cdc27
by Clb2 kinase may provide an essential component
for the negative feedback oscillator


MEN
Cdc14
Cdc20
APC
Clb2
Cdh1,Sic1 (Cdc6?)
Cln
Cell growth
Mitotic entry
Mitotic exit
A tool: the *APC-A* mutant, in which the APC subunits
cannot be phosphorylated due to S/T→A mutations



APC-A:

Unphosphorylatable APC subunits. Negative feedback oscillator disabled

X

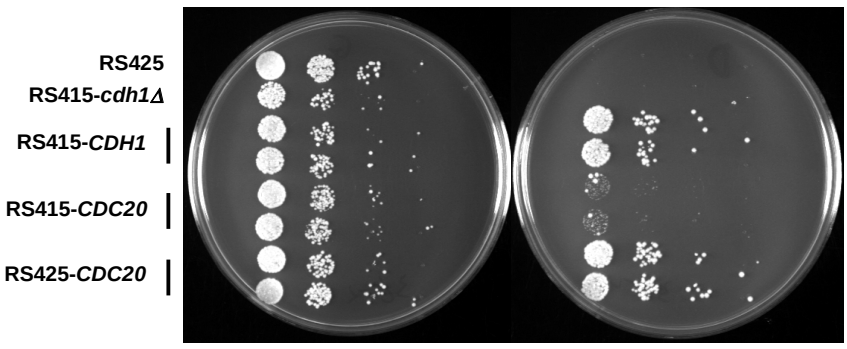
cdh1Δ

Relaxation oscillator disabled.

Tetrad analysis

APC-A cdh1

Cannot complete mitosis



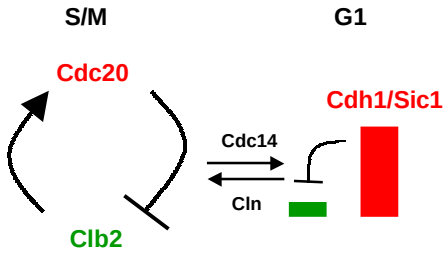
CDC16-A CDC23-A CDC27-A cdh1Δ

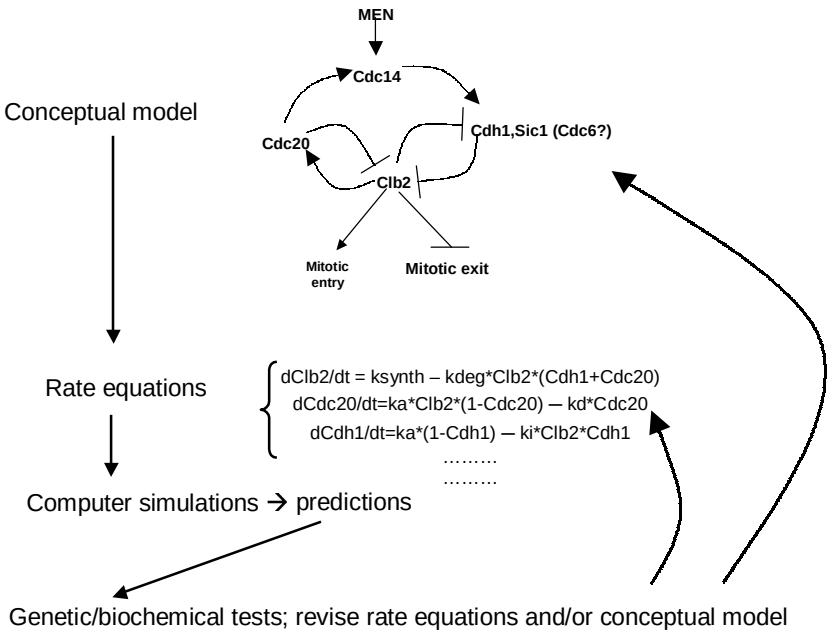
*ura3****::URA3::GAL-SIC1***

SIC1 or *CDC20* overexpression can restore *cdh1 APC-A* viability

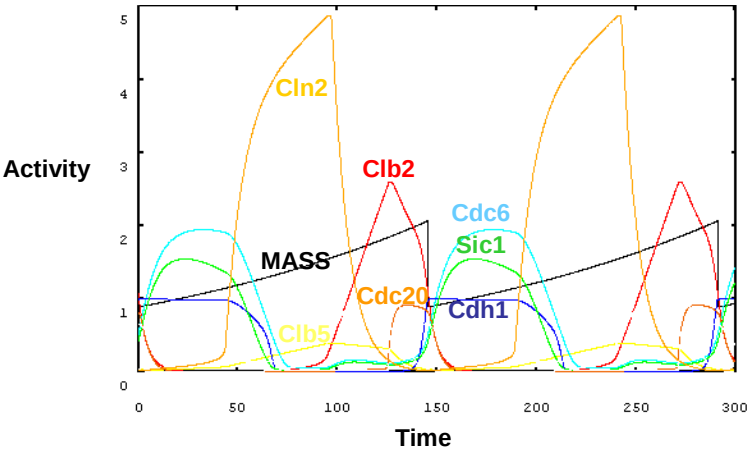
Disabling both oscillators blocks the cell cycle;

either one alone may be sufficient.



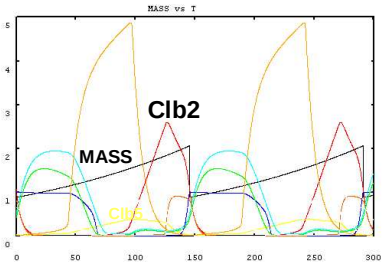


Realistic computational analysis* shows that a double-oscillator system can account for cell cycle regulation, in wild-type and in many mutant situations



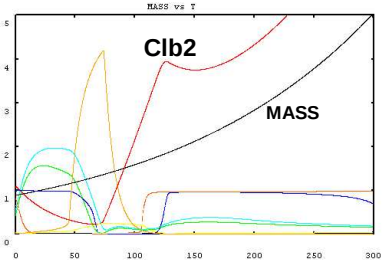
* K. Chen, J. Tyson

wt

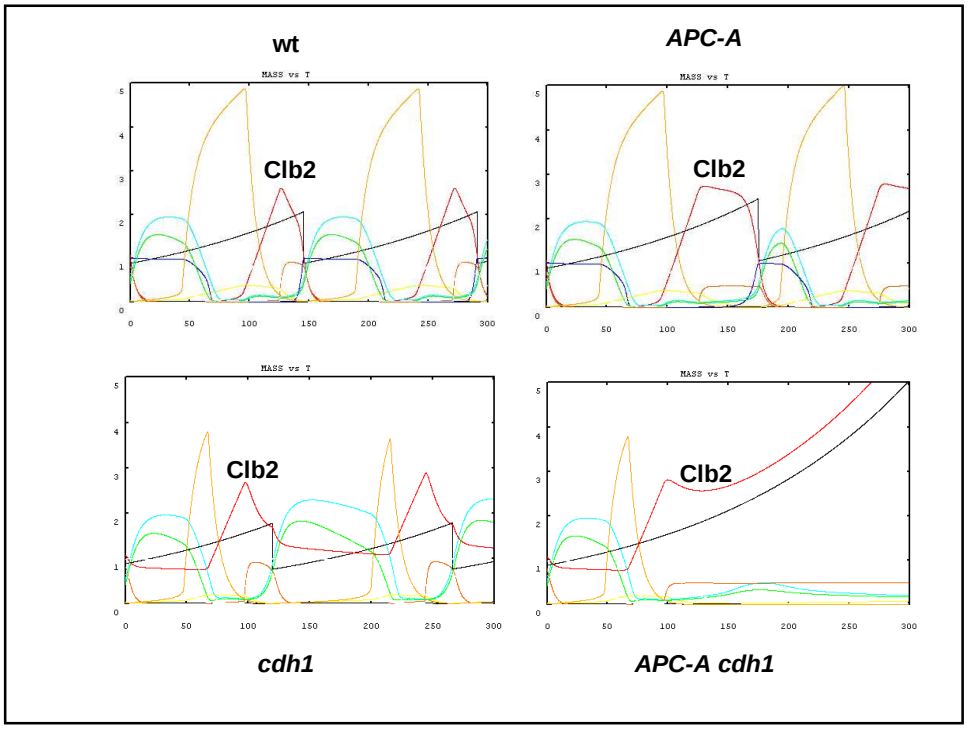


Cycles

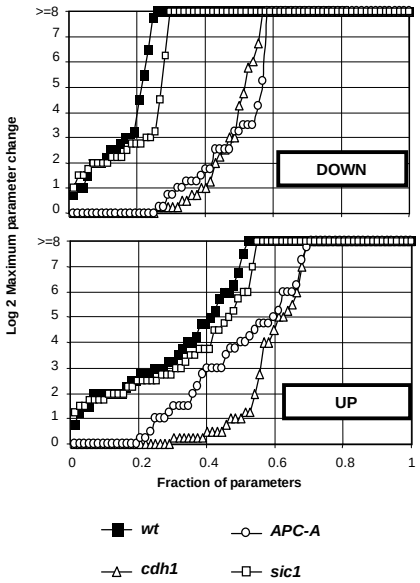
CLB2-db



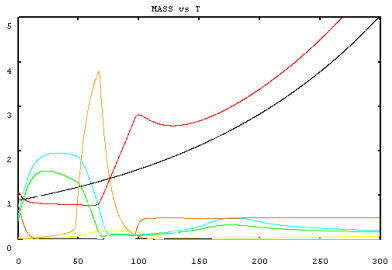
Mitotic arrest



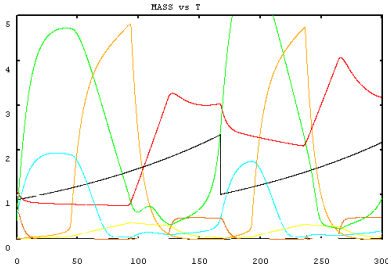
The wild-type model is reasonably robust; some 'viable' mutants are very fragile



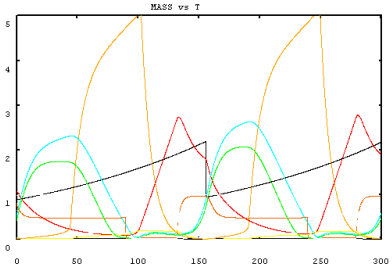
APC-A cdh1



APC-A cdh1* + multi-copy *SIC1



APC-A cdh1* + *GAL-CDC20



Parameter	Effect of change	Change
kdb2p	Increase **Cdc20**-dependent Clb2 degradation	Up 10X
ks20'	Increase constitutive ***CDC20*** transcription	Up 20X
ks20"	Increase Clb2-regulated ***CDC20*** transcription	Up 4X
kd20	Decrease **Cdc20** degradation	Down 8X
ka20'	Increase Phospho-APC-independent **Cdc20** activity	Up 7X
ksc1'	Increase constitutive ***SIC1*** transcription	Up 8X
ksc1"	Increase Swi5-regulated ***SIC1*** transcription	Up 90X
kaswi	Increase Swi5-regulated ***SIC1*** transcription	Up 10X
Jiswi	Increase Swi5-regulated ***SIC1*** transcription	Up 90X

cdh1 APC-A model: test systematic parameter variations for 'suppression' of 'inviability'

Experiment ⇄ Computation

CLB2-db →

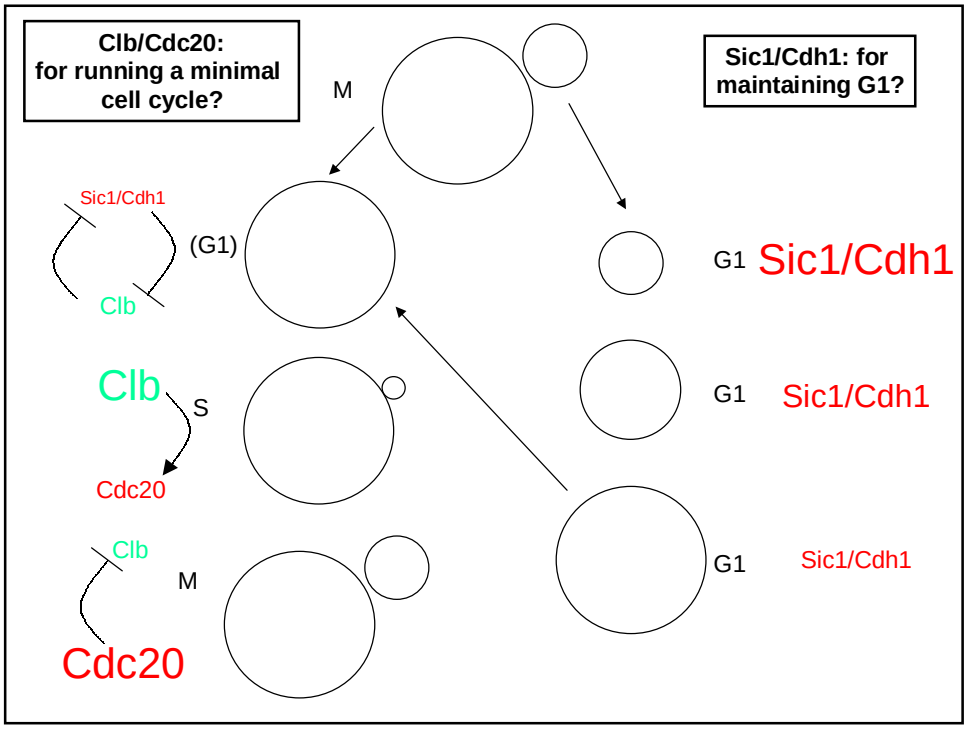
← ***cdh1 sic1 GAL-CDC20***

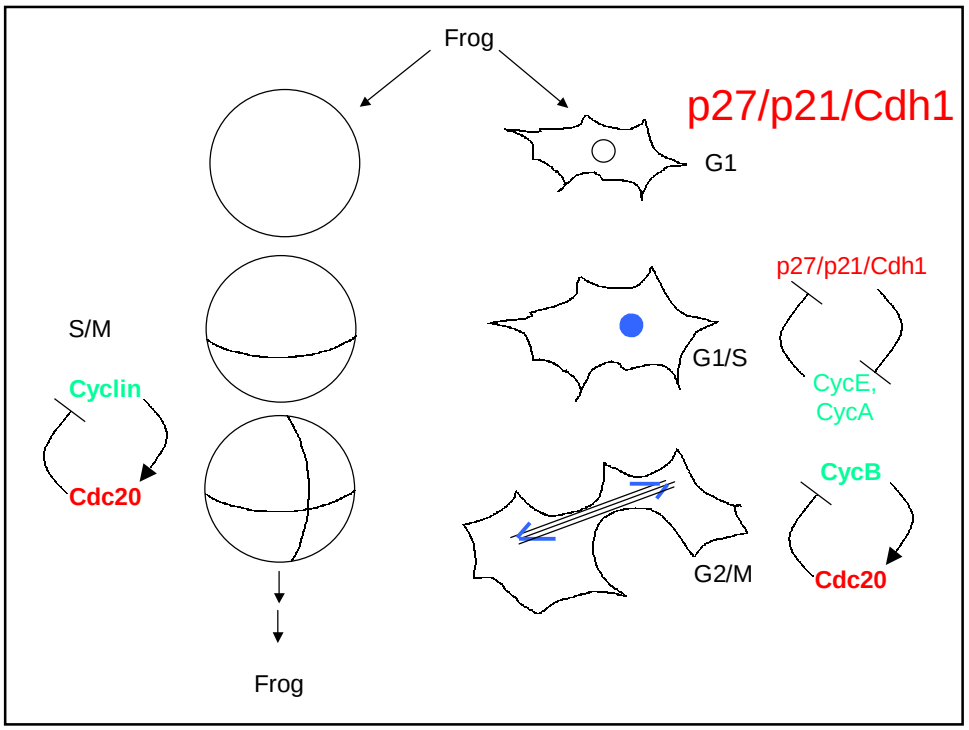
APC-A →

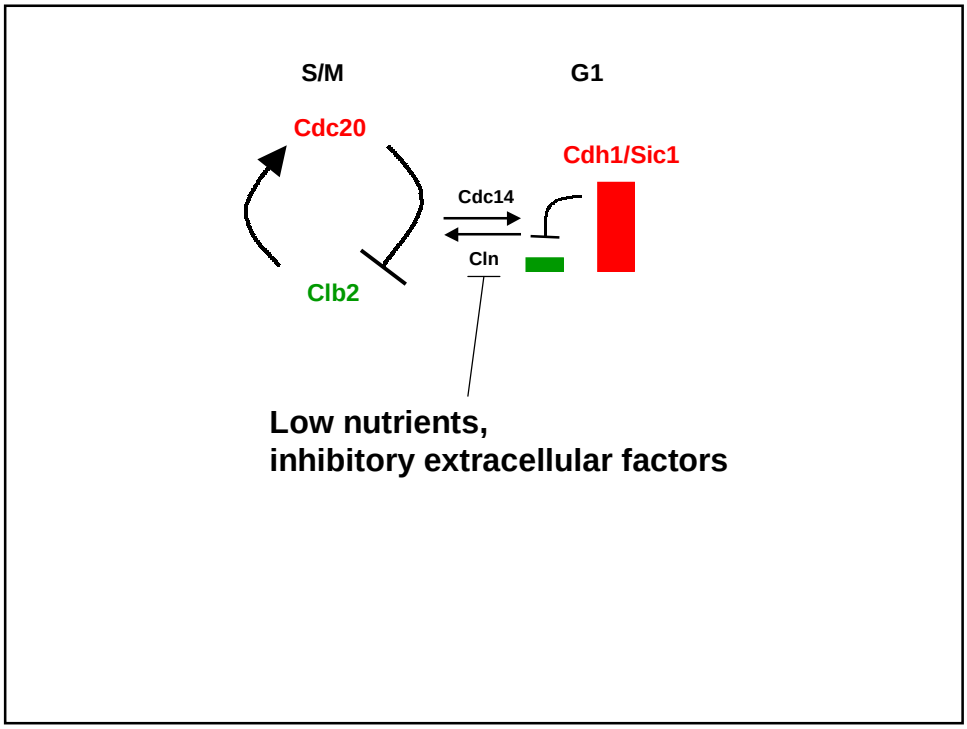
← ***APC-A cdh1***

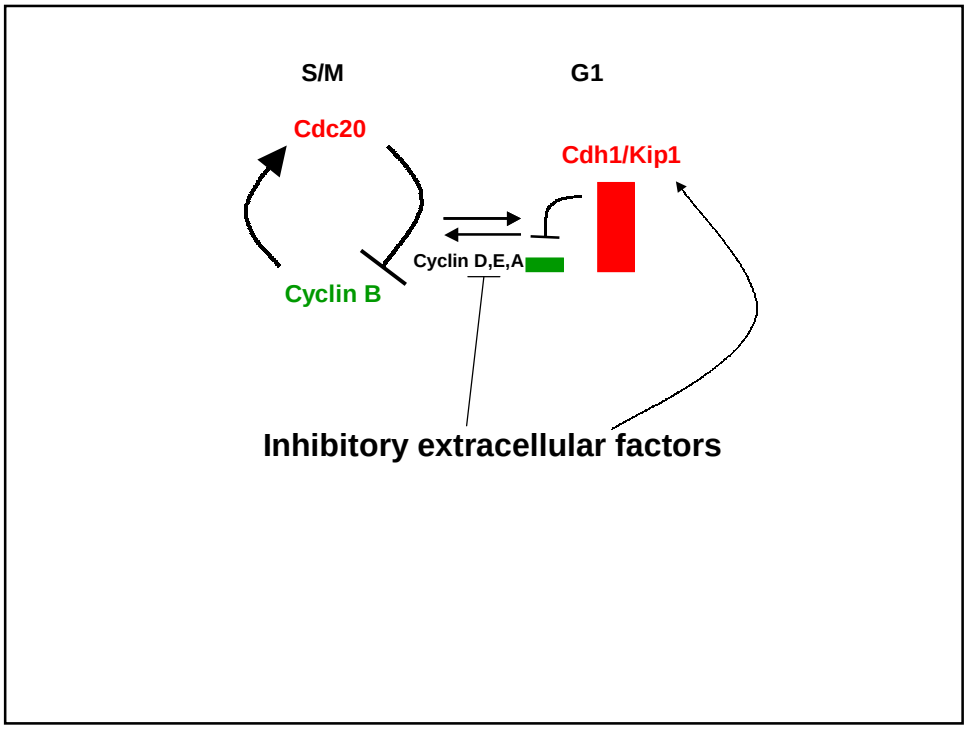
APC-A cdh1+ →
CDC20,SIC1 **overexpression**

Why would cells have two functional oscillators?
Maybe they function at different times…


Clb/Cdc20:
for running a minimal
cell cycle?
Sic1/Cdh1: for
maintaining G1?
M
Sic1/Cdh1
(G1)
Clb
G1
Sic1/Cdh1
Clb
S
Cdc20
G1
Sic1/Cdh1
Clb
M
Cdc20
G1
Sic1/Cdh1


Frog
p27/p21/Cdh1
G1
S/M
Cyclin
Cdc20
p27/p21/Cdh1
G1/S
CycE,
CycA
CycB
G2/M
Cdc20
Frog


S/M
G1
Cdc20
Cdh1/Sic1
Cdc14
Cln
Clb2
Low nutrients,
inhibitory extracellular factors


S/M
G1
Cdc20
Cdh1/Kip1
Cyclin D,E,A
Cyclin B
Inhibitory extracellular factors

Ralph Wäsch (CLB2-db)

Vincent Archambault (model testing)

Jamie Bean (movies)

John Tyson, Kathy Chen (Virginia Tech)