

Bioinformatics 3

# V20 – Kinetic Motifs

Mon, Jan 13, 2014

# Modelling of Signalling Pathways

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OPINION  
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221

## Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell

John J Tyson<sup>\*†</sup>, Katherine C Chen<sup>\*‡</sup> and Bela Novak<sup>§</sup>

*Curr. Op. Cell Biol.* **15** (2003) 221

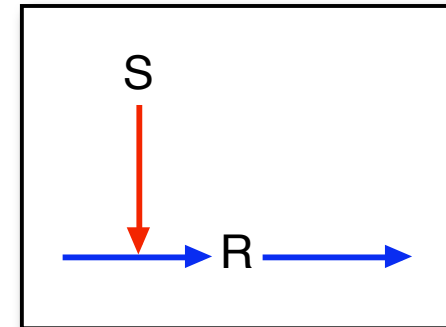
- 1) How do the magnitudes of signal **output** and signal duration depend on the **kinetic properties** of pathway components?
- (2) Can high signal **amplification** be coupled with **fast** signaling?
- (3) How are signaling pathways **designed** to ensure that they are **safely off** in the absence of stimulation, yet display high signal amplification following receptor activation?
- (4) How can **different agonists** stimulate the **same pathway** in distinct ways to elicit a sustained or a transient response, which can have dramatically different consequences?

# Linear Response

E.g., protein synthesis and degradation (see lecture VI0)

S = signal (e.g., concentration of mRNA)

R = response (e.g., concentration of a protein)

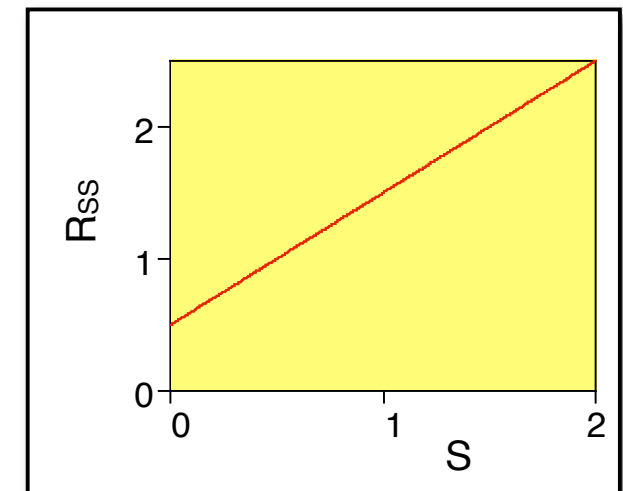


$$\frac{dR}{dt} = k_0 + k_1 S - k_2 R$$

At steady state (which implies  $S = \text{const}$ ):

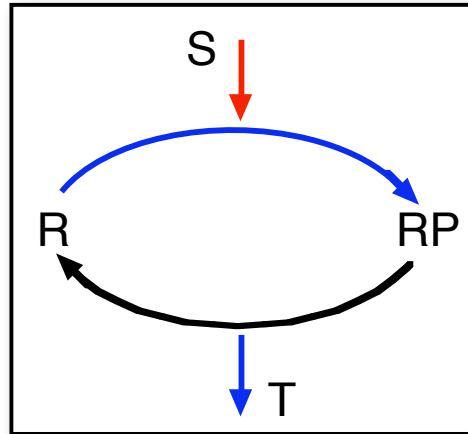
$$\left. \frac{dR}{dt} \right|_{R=R_{ss}} = 0 \Rightarrow R_{ss} = \frac{k_0 + k_1 S}{k_2} = \frac{k_0}{k_2} + \frac{k_1}{k_2} S$$

$R_{ss}$  linearly dependent on  $S$



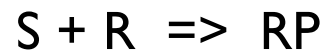
$$k_0 = 1, k_1 = k_2 = 2$$

# phosphorylation/dephosphorylation



„forward“: R is converted to phosphorylated form RP

„backward“: RP can be dephosphorylated again to R



with  $R_{\text{tot}} = R + \underset{\substack{\uparrow \\ \text{phosphorylated form}}}{RP}$

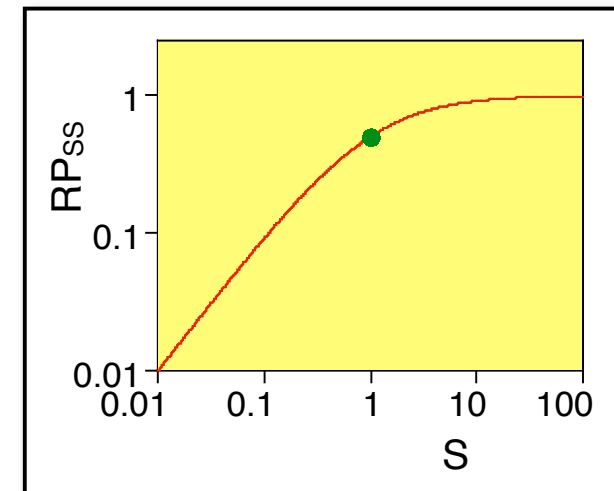
$$\frac{dRP}{dt} = k_1 SR - k_2 RP = k_1 S (R_{\text{tot}} - RP) - k_2 RP$$

Find steady state for RP: linear until saturation

$$RP_{ss} = \frac{k_1 R_{\text{tot}} S}{k_1 S + k_2} = \frac{R_{\text{tot}} S}{S + k_2/k_1} = \frac{R_{\text{tot}} S}{S + S_0}$$

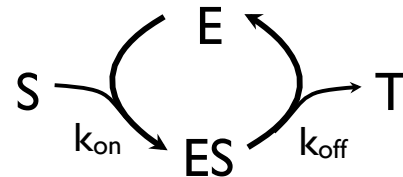
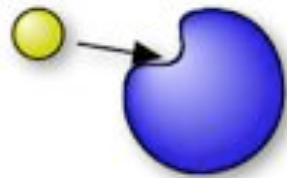
Output T proportional to RP level:

$$\frac{dT}{dt} = k_2 RP$$



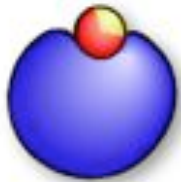
$$R_{\text{tot}} = 1, S_0 = 1$$

# Enzyme: Michaelis-Menten-kinetics



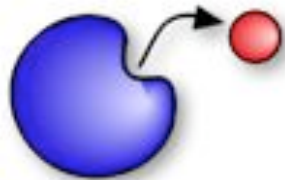
Reaction rate:

$$V = k_{\text{off}} ES$$



Steady state:  $k_{\text{on}} E \cdot S = k_{\text{off}} ES$

$$ES = \frac{k_{\text{on}} E \cdot S}{k_{\text{off}}} = \frac{E \cdot S}{K_M}$$



Total amount of enzyme is constant:

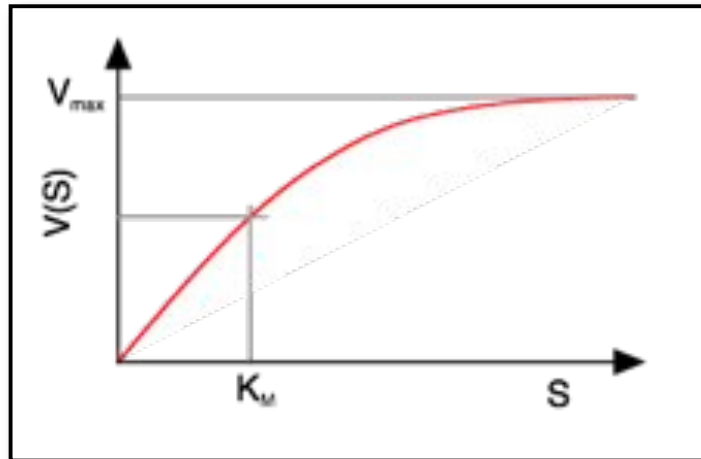
$$E_T = E + ES \quad \Rightarrow \quad ES = E_T \frac{S}{S + K_M}$$

turnover:  $V = V_{\text{max}} \frac{S}{S + K_M}$

# The MM-equation

Effective turnover according to MM:  $V = V_{max} \frac{S}{S + K_M}$

$$V_{max} = k_{off} E_T$$



$$K_M = \frac{k_{off}}{k_{on}}$$

Pro:

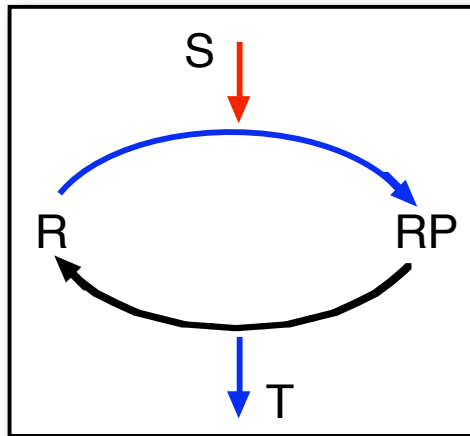
- analytical formula for turnover
- curve can be easily interpreted:  $V_{max}$ ,  $K_M$
- enzyme concentration can be ignored

Cons:

less kinetic information

$$k_{on}, k_{off}, E_T \Rightarrow V_{max}, K_M$$

# Sigmoidal Characteristics with MM kinetics



Same topology as before with Michaelis-Menten kinetics for phosphorylation and dephosphorylation.

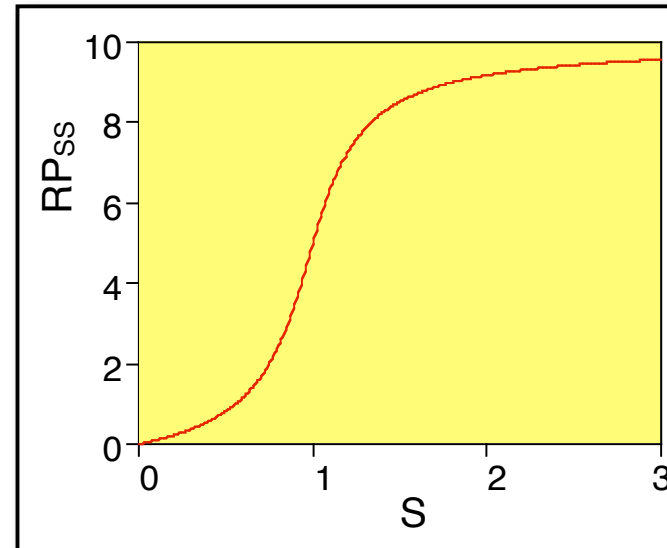
$$\frac{dRP}{dt} = \frac{k_1 S (R_t - RP)}{R_0 + (R_t - RP)} - \frac{k_2 RP}{RP_0 + RP} \stackrel{!}{=} 0$$

$$V = V_{max} \frac{S}{S + K_M} \quad \text{this means that } \begin{matrix} S = R_t - RP \\ K_M = R_0 \end{matrix}$$

Quadratic equation for RP

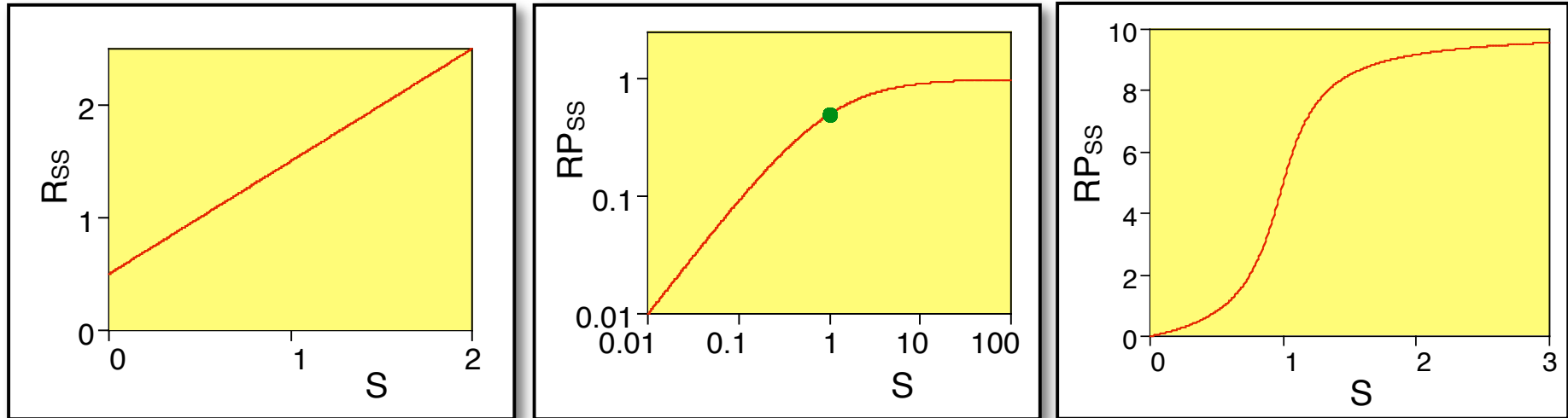
$$k_2 RP (R_0 + (R_t - RP)) = k_1 S (R_t - RP) (RP_0 + RP)$$

=> sigmoidal characteristics  
(threshold behavior)  
often found in signalling cascades



$$R_t = 10, R_0 = RP_0 = 1, k_1 = k_2 = 1$$

# Graded Response

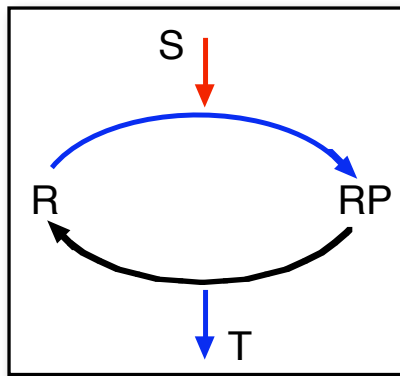


Linear, hyperbolic, and sigmoidal characteristic give the same steady state response independent of the previous history  
=> no hysteresis

BUT: In fast time-dependent scenarios,  
delay may lead to a modified response



# Time-dependent Sigmoidal Response



Direct implementation:

$$v_1 = \frac{Sk_1R}{R_0 + R}$$

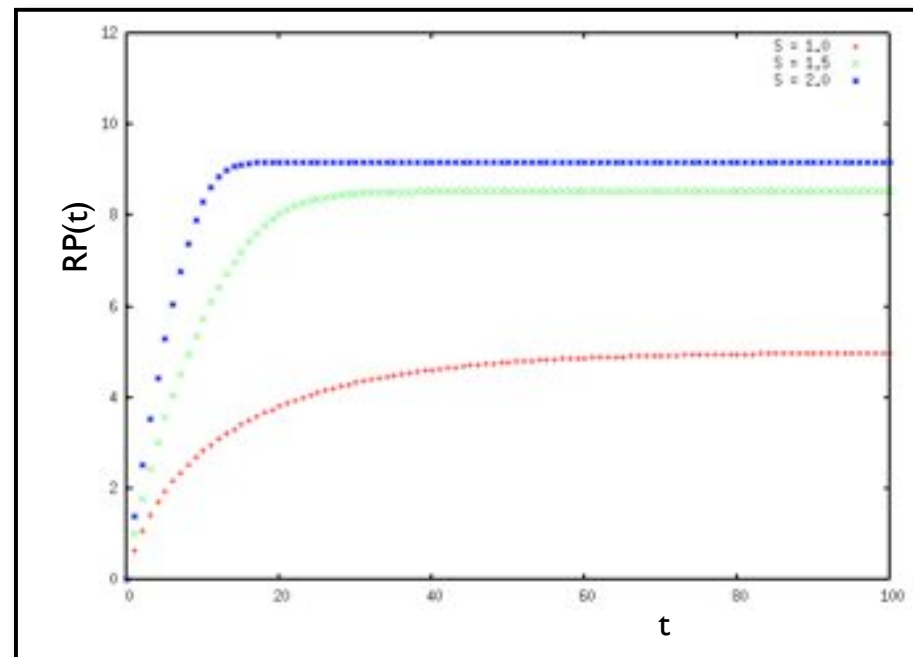
$$v_2 = \frac{k_2RP}{RP_0 + RP}$$

Parameters:  $k_1 = 1 \text{ (mol s)}^{-1}$ ,  $k_2 = 1 \text{ s}^{-1}$ ,  $R_0 = RP_0 = 1 \text{ mol}$

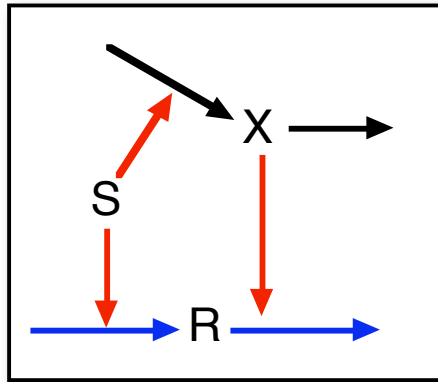
Initial conditions:  $R = 10 \text{ mol}$ ,  $RP = 0$

Time courses for  
 $S = 1, 1.5$ , and  $2$ ,  
 $RP(0) = 0$ :

equilibrium is reached  
 faster for  
 stronger signal



# Adaption - „sniffer“



Linear response modulated by a second species X

$$\frac{dX}{dt} = k_3 S - k_4 X$$

$$\frac{dR}{dt} = k_1 S - k_2 X R$$

Steady state:  $R_{ss}$  independent of S

$$X_{ss} = \frac{k_3}{k_4} S$$

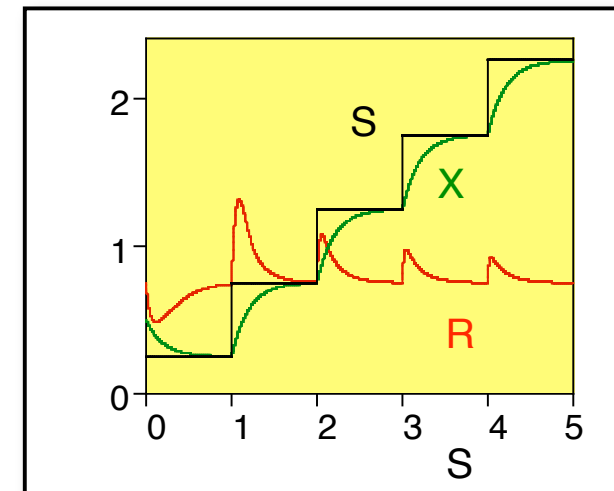
$$R_{ss} = \frac{k_1 k_4}{k_2 k_3}$$

R changes transiently when S changes, then goes back to its basal level.

found in smell, vision, chemotaxis, ...

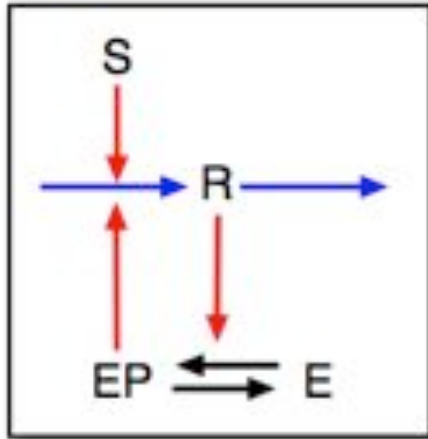
Note: response strength  $\Delta R$  depends on rate of change of S.

=> non-monotonous relation for  $R(S)$



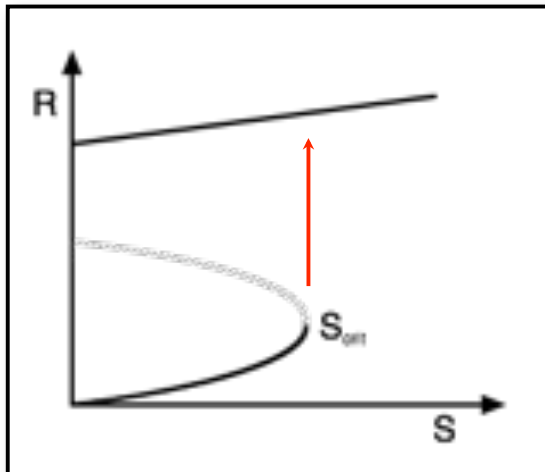
$$k_1 = 30, k_2 = 40, k_3 = k_4 = 5$$

# Positive Feedback



$$\frac{dR}{dt} = k_4 EP(R) + k_1 S - k_2 R$$

$$\frac{dEP}{dt} = \frac{k_3 R E}{EP_0 + EP} - \frac{k_5 EP}{E_0 + E}$$



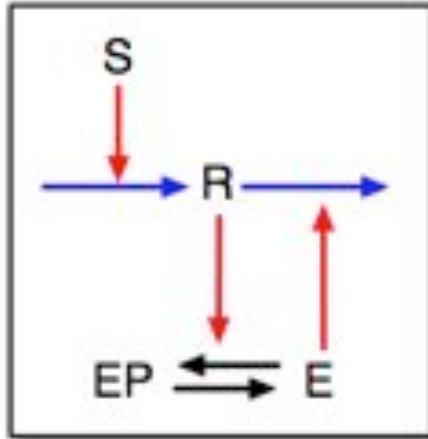
Feedback via R and EP

=> high levels of R will stay

**"one-way switch"** via bifurcation

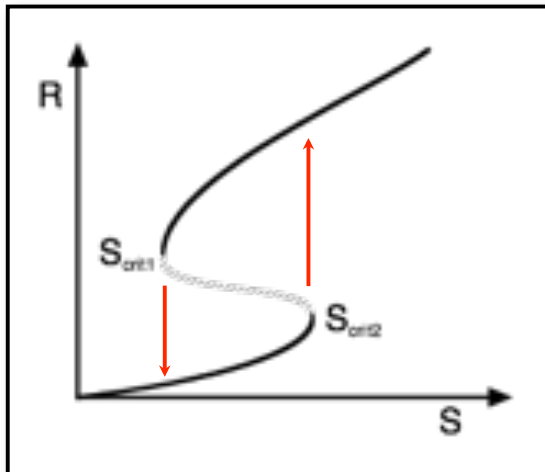
Found in processes that are "final":  
frog oocyte maturation, apoptosis, ...

# Mutual Inhibition - Toggle Switch



$$\frac{dR}{dt} = k_1 S - k_2 R - k_4 E(R)$$

$$\frac{dEP}{dt} = \frac{k_3 R E}{EP_0 + EP} - \frac{k_5 EP}{E_0 + E}$$

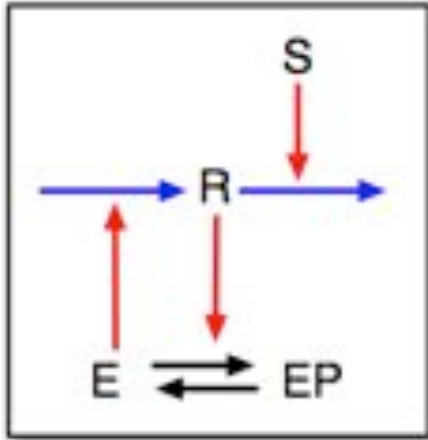


Sigmoidal "threshold" in  $E \rightleftharpoons EP$  leads to bistable response (hysteresis): **toggle switch**

Converts continuous external stimulus into two well defined stable states:

- lac operon in bacteria
- activation of M-phase promoting factor in frog eggs

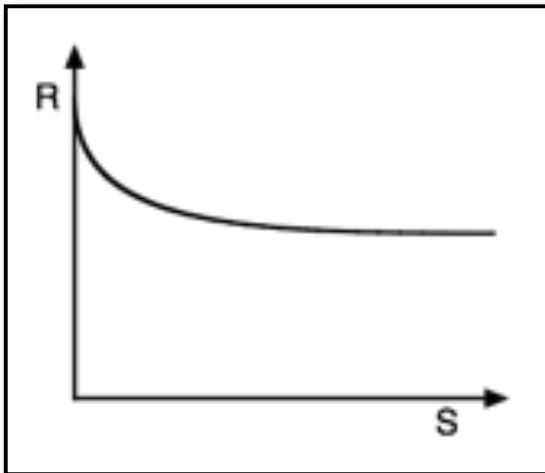
# Negative Feedback



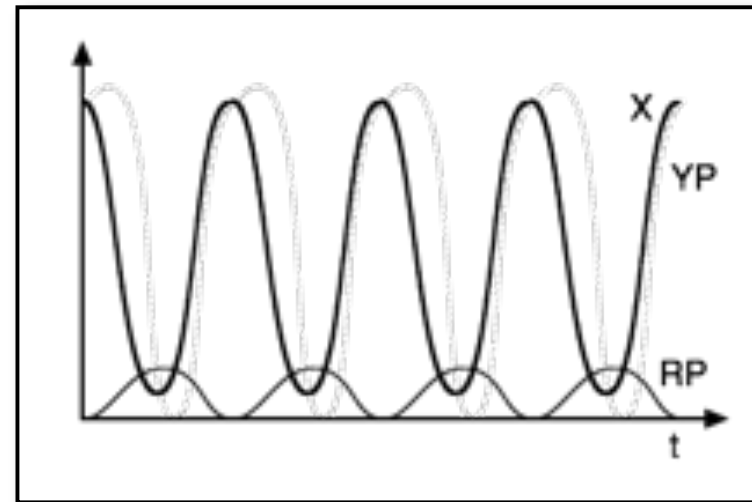
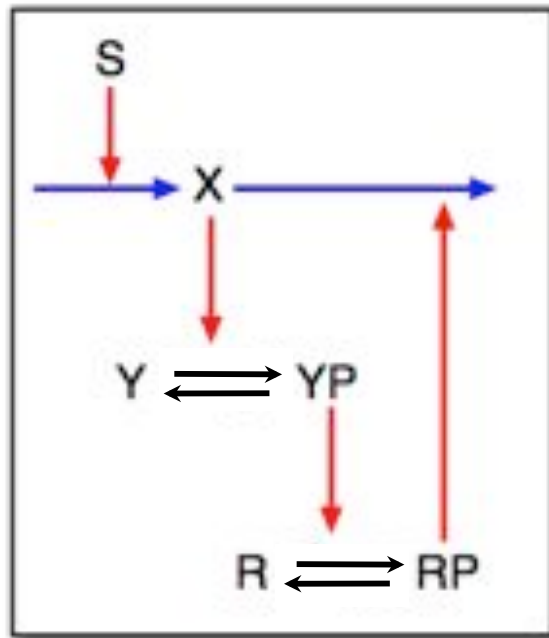
S controls the "demand" for R

=> **homeostasis**

found in biochemical pathways,  
no transient changes in R for steps in S  
(cf. "sniffer")



# Negative Feedback with Delay



Cyclic activation  $X \Rightarrow YP \Rightarrow RP \Rightarrow X$   
 $\Rightarrow$  **Oscillations** (in a range of S)

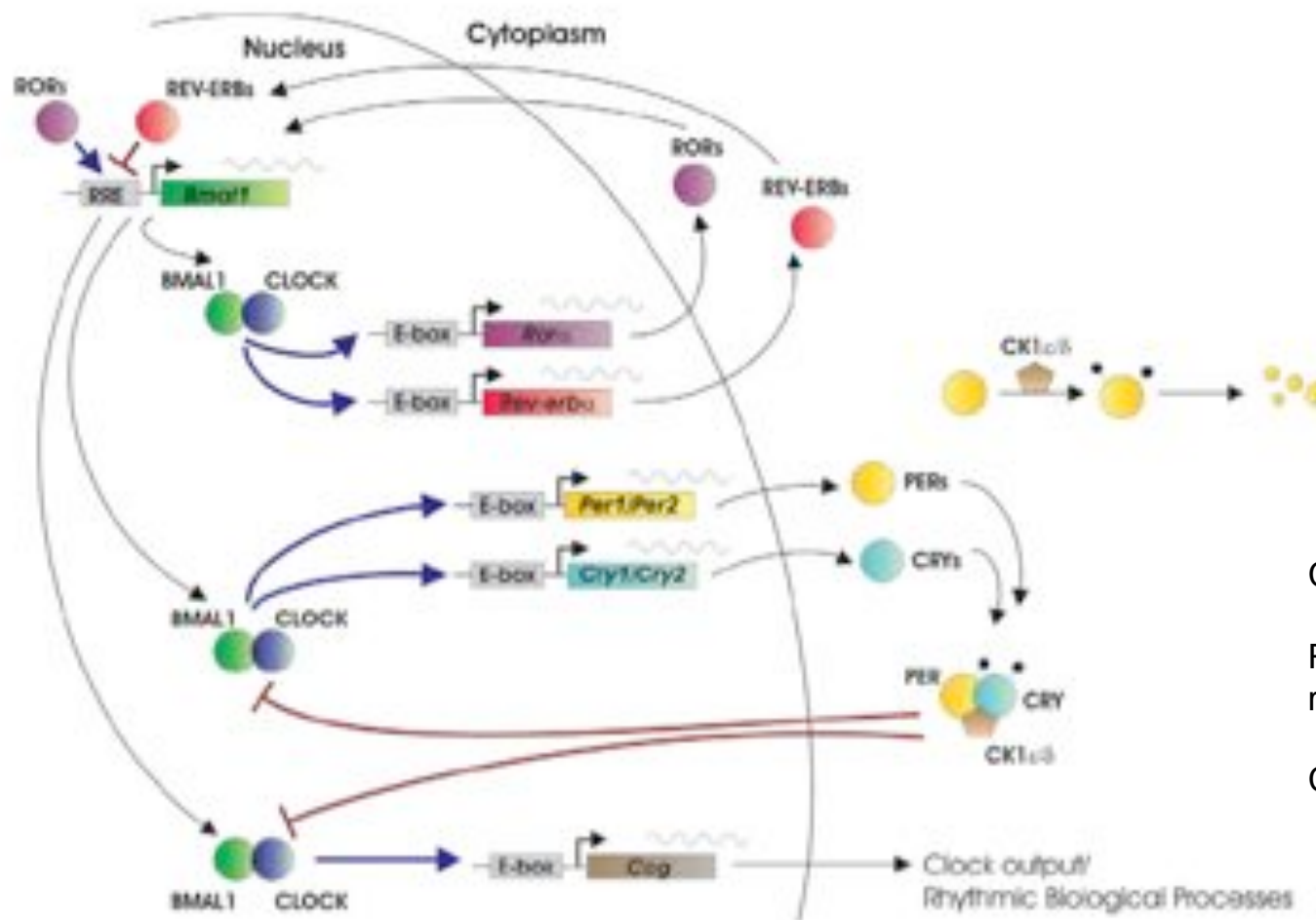
$$\frac{dX}{dt} = k_0 + k_1 S - k_2 X - k_7 RP X$$

$$\frac{dYP}{dt} = \frac{k_3 X Y}{Y_0 + Y} - \frac{k_4 YP}{YP_0 + YP}$$

$$\frac{dRP}{dt} = \frac{k_5 YP R}{R_0 + R} - \frac{k_6 RP}{RP_0 + RP}$$

Proposed mechanism  
for circadian clocks

# Circadian Clocks



PER: period

CRY: cryptochrome

CK1: casein kinase

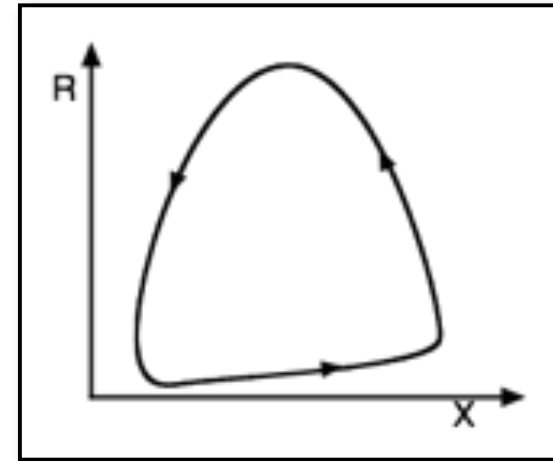
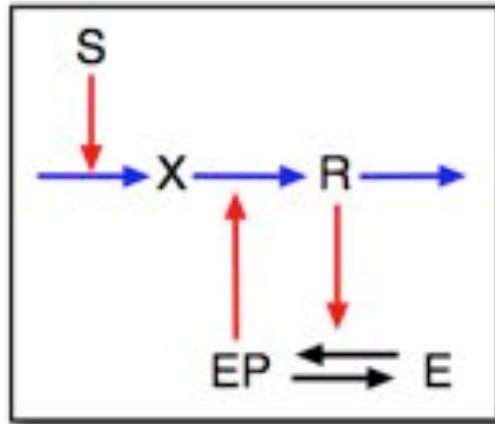
Rev-erb, ROR: retinoic acid-related orphan nuclear receptors

Cdg: clock-controlled gene(s)

Figure 1. A network of transcriptional-translational feedback loops constitutes the mammalian circadian clock.

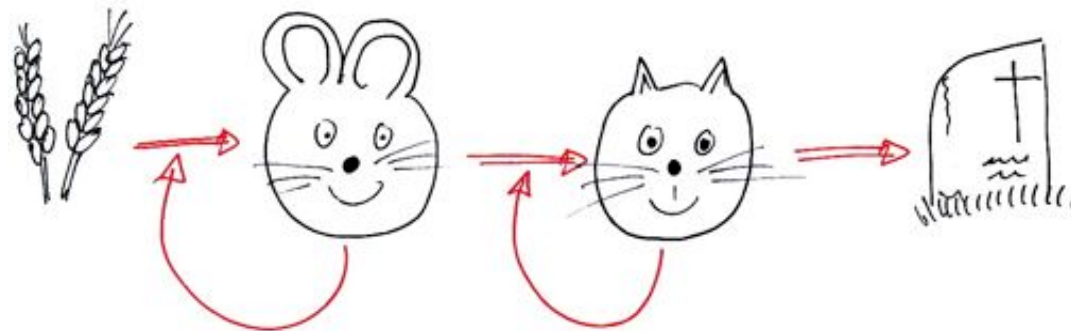
Ko & Takahashi Hum Mol Genet  
15, R271 (2006)

# Substrate-Depletion Oscillations



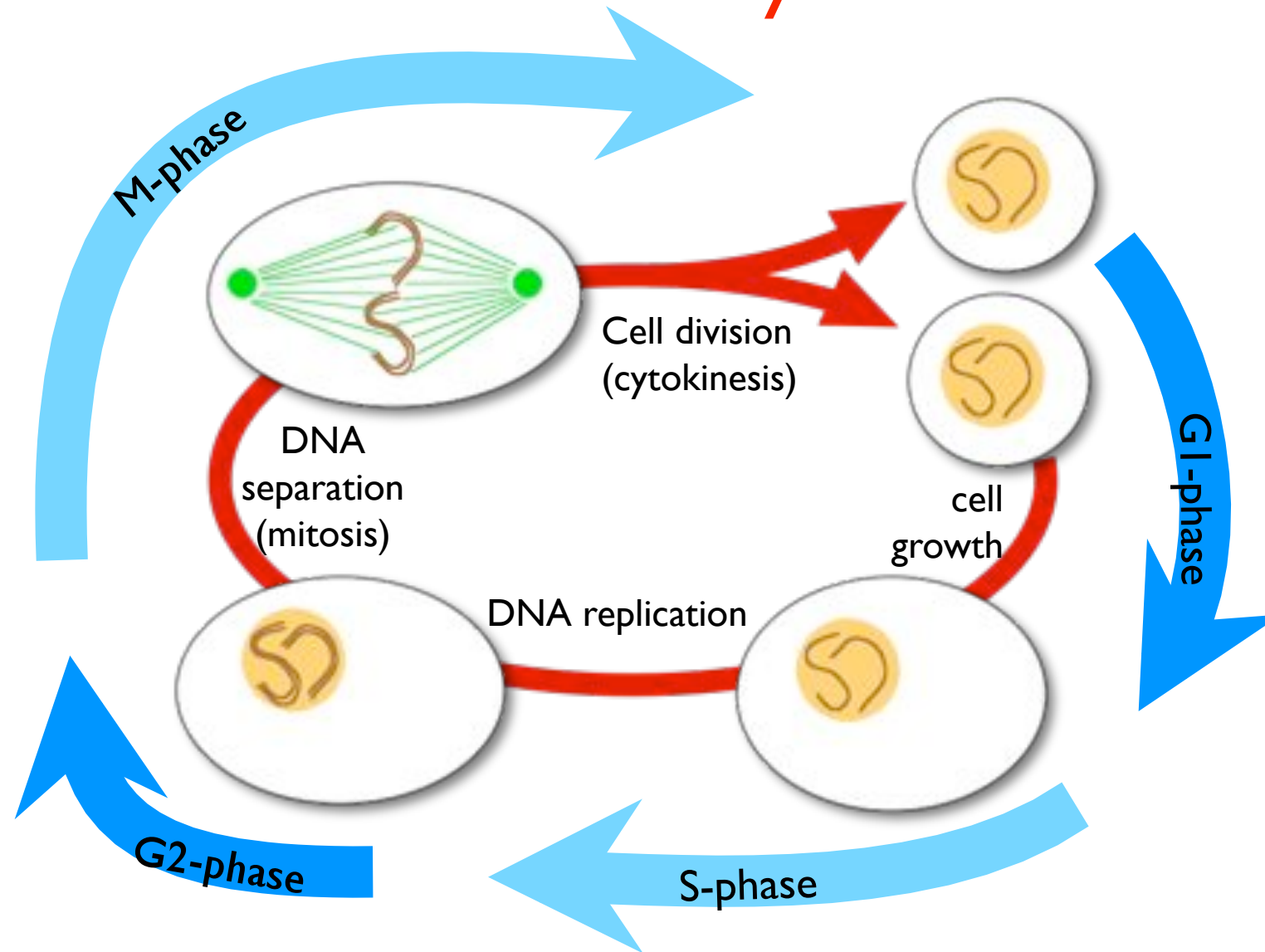
$R$  is produced in an **autocatalytic** reaction from  $X$ , finally **depleting**  $X$ ...

Similar to Lotka-Volterra system (autocatalysis for  $X$ , too):





# The Cell Cycle



**When** to take the **next step**???

# Cell Cycle Control



Frederick  
Cross,  
Rockefeller University



Catherine  
Oikonomou

Oscillatory networks underlie the

- circadian clock,
- the beating of our hearts, and
- the cycle of cell division, which creates two cells from one, driving the reproduction and development of living systems.

Already simple genetic circuits can give rise to oscillations.

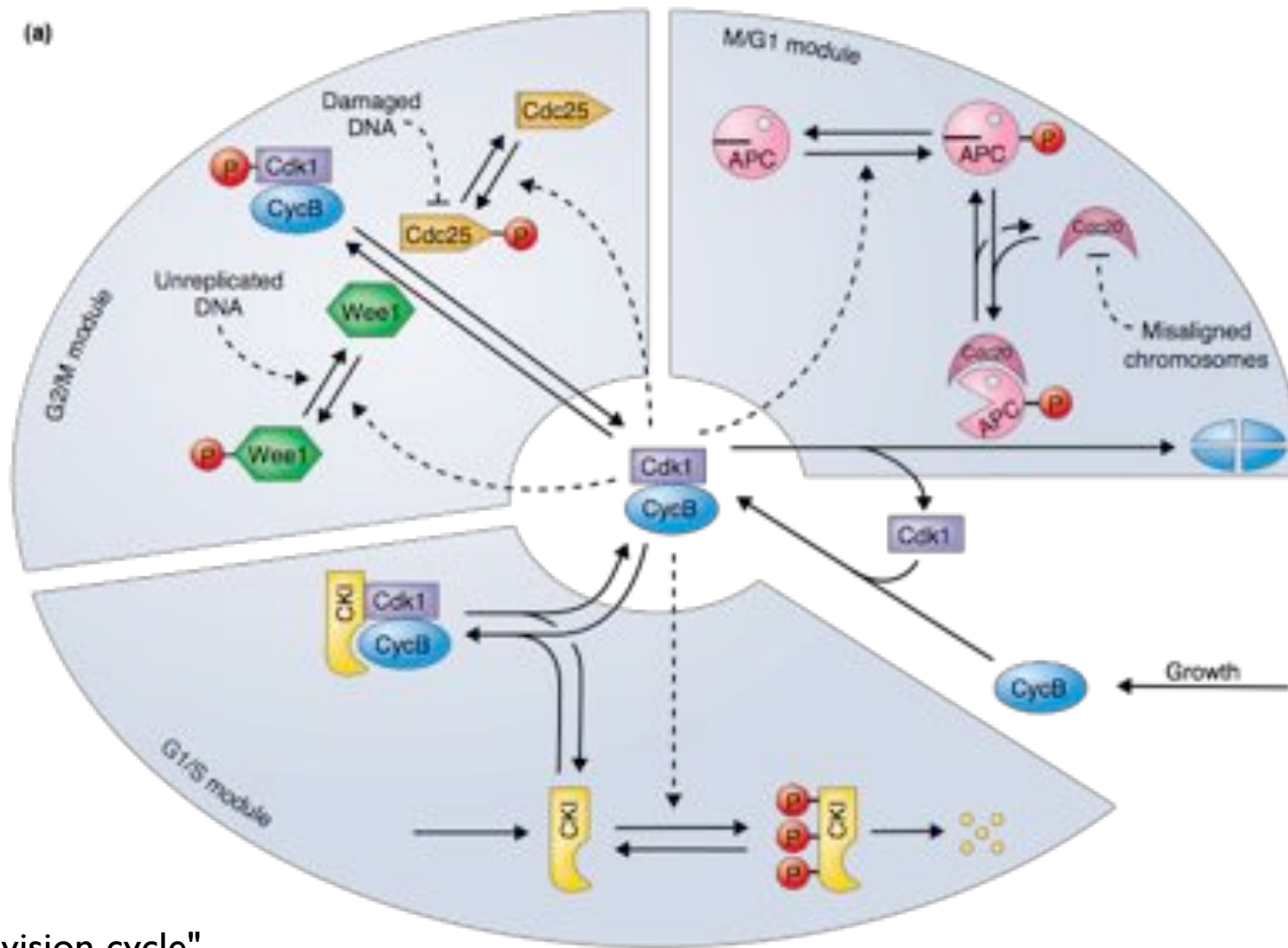
E.g., a negative feedback loop  
 $X \rightarrow R \dashv X$  can yield oscillations  
(X activates R, which inhibits X, so that R goes down, so that X goes back up. . .).

Such a circuit requires significant non-linearity or a time delay to keep from rapidly settling to a constant steady state.

An oscillator of this sort is thought to be the core of many eukaryotic cell cycles.

Oikonomou & Cross, Curr. Opin. Genet Devel. 20, 605 (2010)

# Cell Cycle Control System



cdc =  
"cell division cycle"

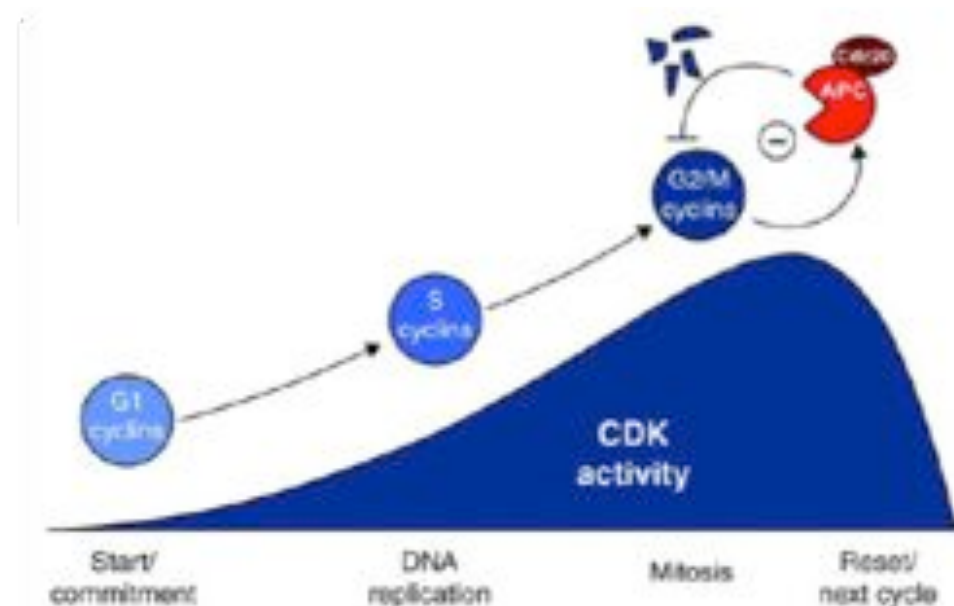
Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

# Feedback loops control cell cycle

A negative feedback loop can give rise to oscillations. Here, such an oscillator forms the core of eukaryotic cell cycles.

Cyclin-CDK acts as activator, and APC-Cdc20 acts as repressor.

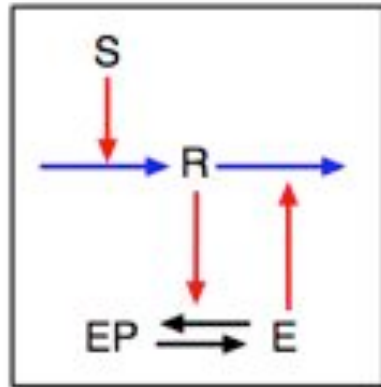
Non-linearity in APC-Cdc20 activation prevents the system from settling into a steady state.



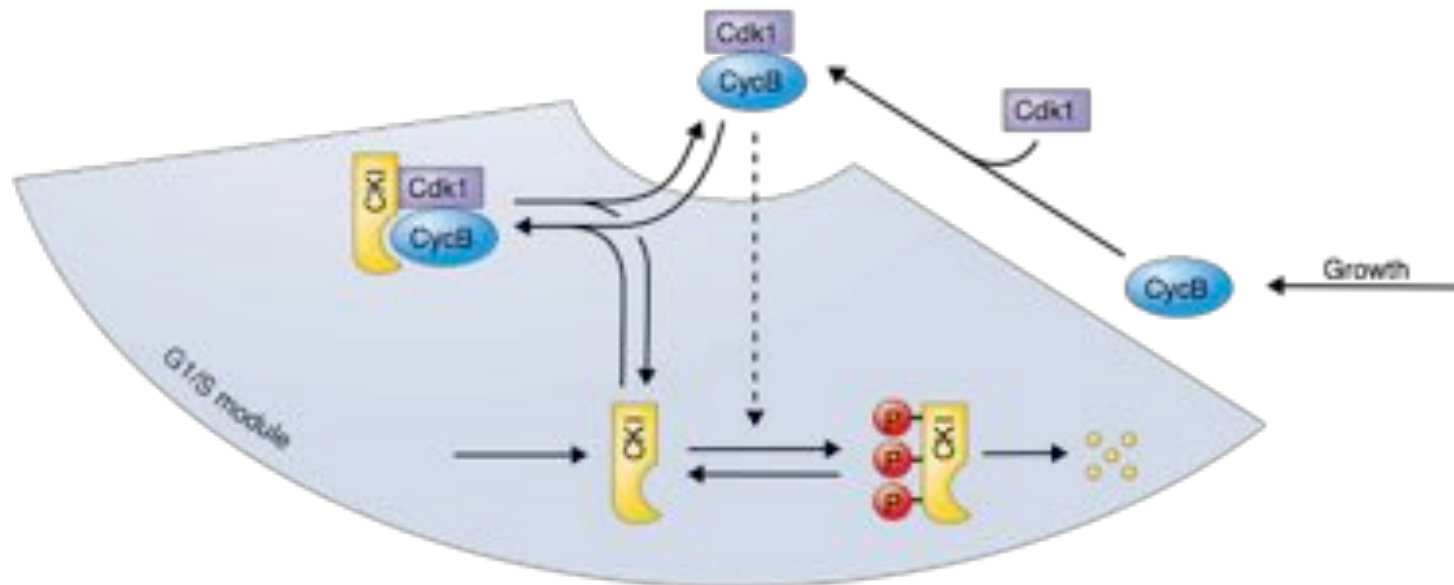
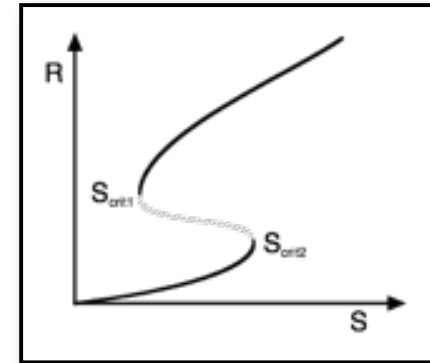
- CDKs require the binding of a cyclin subunit for activity. These cyclin partners can also determine the localization of the complex and its specificity for targets.
- At the beginning of the cell cycle, cyclin-CDK activity is low, and ramps up over most of the cycle. Early cyclins trigger production of later cyclins and these later cyclins then turn off the earlier cyclins, so that control is passed from one set of cyclin-CDKs to the next.
- The last set of cyclins to be activated, the G2/M-phase cyclins, initiate mitosis, and also initiate their own destruction by activating the APC-Cdc20 negative feedback loop. APC-Cdc20 targets the G2/M-phase cyclins for destruction, resetting the cell to a low-CDK activity state, ready for the next cycle.

Oikonomou & Cross, Curr. Opin. Genet Devel. 20, 605 (2010)

# GI => S — Toggle Switch

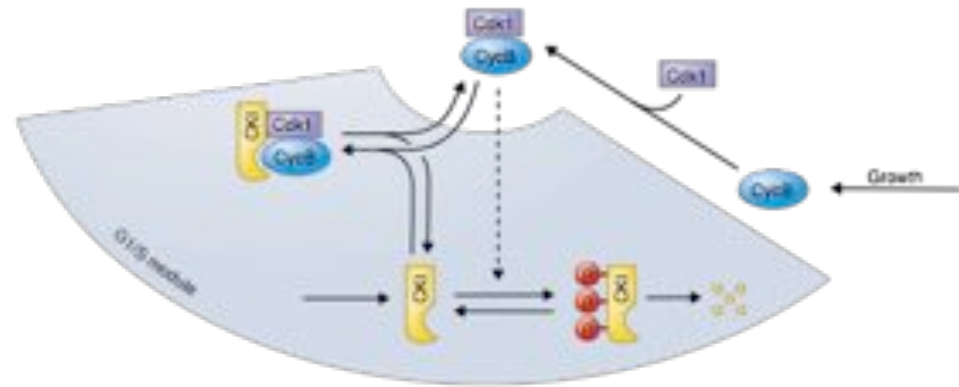
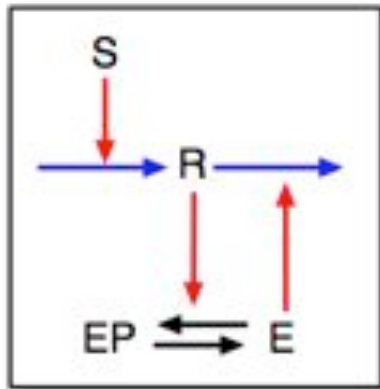


Mutual inhibition between  
CdkI-CycB and CKI  
(cyclin kinase inhibitor)

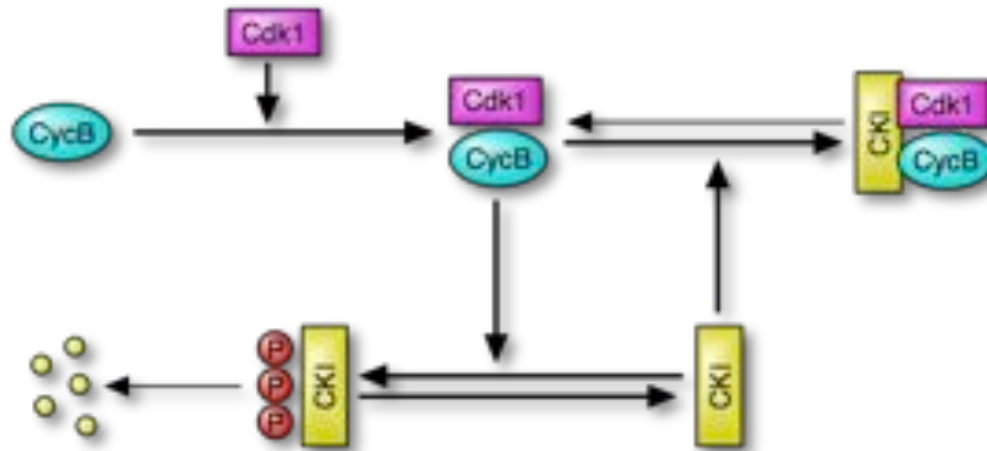


Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

# Mutual Inhibition



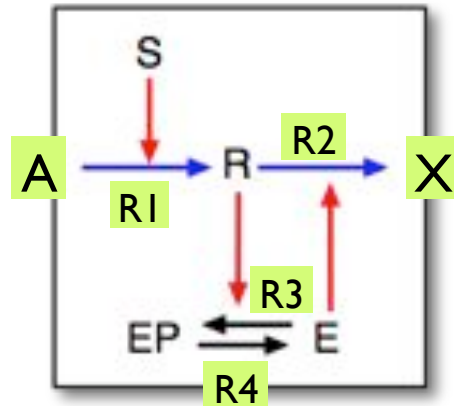
Assume: CycB:Cdk1:CKI is stable  $\Leftrightarrow$  dissociation is very slow



$\Rightarrow$  same **topology**  
 $\Leftrightarrow$  same bistable  
**behavior** (?)



# Rate Equations: Toggle Switch



Stoichiometric  
matrix  
"(C)" = catalyst

	R1	R2	R3	R4
A	-1			
S	(C)			
R	1	-1	(C)	
E		(C)	-1	1
EP			1	-1
X		1		

$$\frac{dR1}{dt} = k_1 A S$$

$$\frac{dR2}{dt} = k_2 R E$$

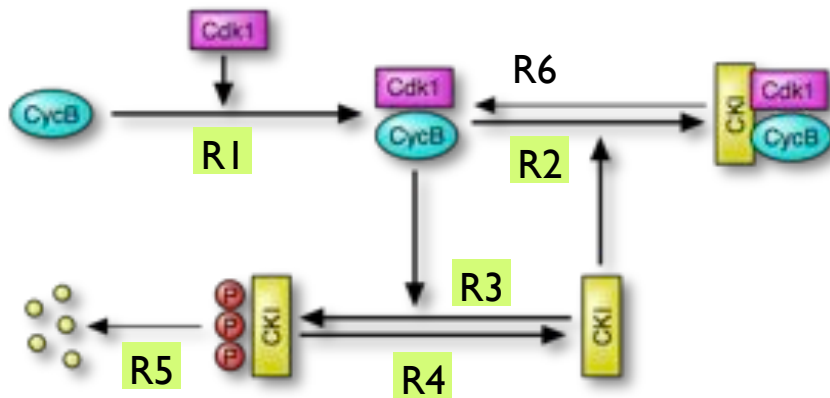
$$\frac{dR3}{dt} = \frac{k_3 R E}{E_0 + E}$$

$$\frac{dR4}{dt} = \frac{V_4 EP}{EP_0 + EP}$$

$$\frac{dR}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} = k_1 A S - k_2 R E$$

$$\frac{dE}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt}$$

# Rate Equations: G1/S Module



	R1	R2	R3	R4	R5	R6
CycB	-					
Cdk1	-					
CycB:Cdk1		-	(C)			
CKI		-	-			
CKI:P <sub>3</sub>				-		
CKI:P <sub>3</sub>					-	
CycB:Cdk1:CKI						-

$$\frac{dR1}{dt} = k_1 [\text{CycB}] [\text{Cdk1}]$$

$$\frac{dR2}{dt} = k_2 [\text{CycB:Cdk1}] [\text{CKI}]$$

$$\frac{dR3}{dt} = \frac{k_3 [\text{CycB:Cdk1}] [\text{CKI}]}{K_3 + [\text{CKI}]}$$

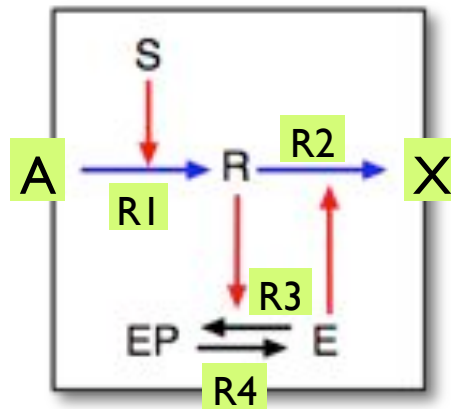
$$\frac{dR4}{dt} = \frac{V_4 [\text{CKI:P}_3]}{K_4 + [\text{CKI:P}_3]}$$

$$\frac{d[\text{CycB:Cdk1}]}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} + \frac{dR6}{dt}$$

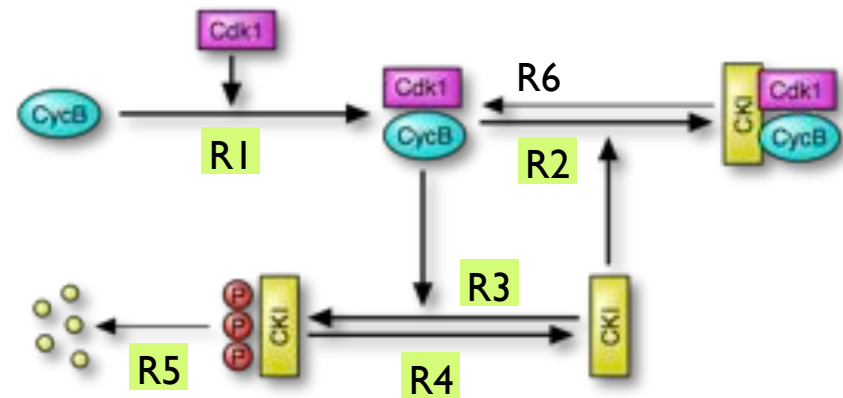
$$\frac{d[\text{CKI}]}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt} - \frac{dR2}{dt} + \frac{dR6}{dt}$$



# Comparison: Matrices



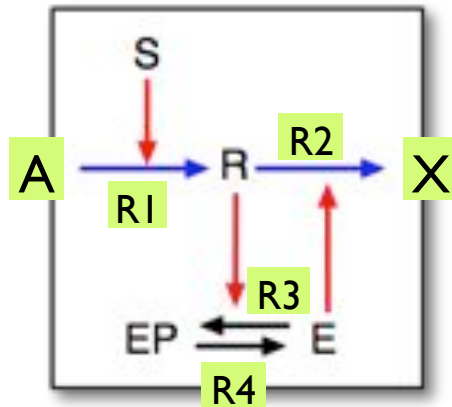
	R1	R2	R3	R4
A	-I			
S	(C)			
R	I	-I	(C)	
E		(C)	-I	I
EP			I	-I
X		I		



	R1	R2	R3	R4	R5	R6
CycB	-I					
Cdk1	-I					
CycB:Cdk1	I	-I	(C)			I
CKI		-I	-I	I		I
CKI:P <sub>3</sub>			I	-I		
CKI:P <sub>3</sub>					-I	
CycB:Cdk1:CKI		I				-I

Difference: catalysts vs. substrates

# Comparison: Equations



$$\frac{dR1}{dt} = k_1 A S$$

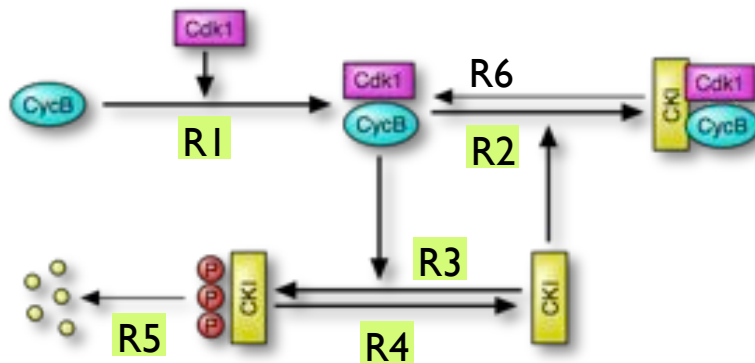
$$\frac{dR2}{dt} = k_2 R E$$

$$\frac{dR3}{dt} = \frac{k_3 R E}{E_0 + E}$$

$$\frac{dR4}{dt} = \frac{V_4 EP}{EP_0 + EP}$$

$$\frac{dR}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} = k_1 A S - k_2 R E$$

$$\frac{dE}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt} = \frac{k_3 R E}{E_0 + E} - \frac{V_4 EP}{EP_0 + EP}$$



$$\frac{dR1}{dt} = k_1 [\text{CycB}] [\text{Cdk1}]$$

$$\frac{dR2}{dt} = k_2 [\text{CycB:Cdk1}] [\text{CKI}]$$

$$\frac{dR3}{dt} = \frac{k_3 [\text{CycB:Cdk1}] [\text{CKI}]}{K_3 + [\text{CKI}]}$$

$$\frac{dR4}{dt} = \frac{V_4 [\text{CKI:P}_3]}{K_4 + [\text{CKI:P}_3]}$$

$$\frac{d[\text{CycB:Cdk1}]}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} + \frac{dR6}{dt}$$

$$\frac{d[\text{CKI}]}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt} - \frac{dR2}{dt} + \frac{dR6}{dt}$$

Rename species => same rate equations => same behavior

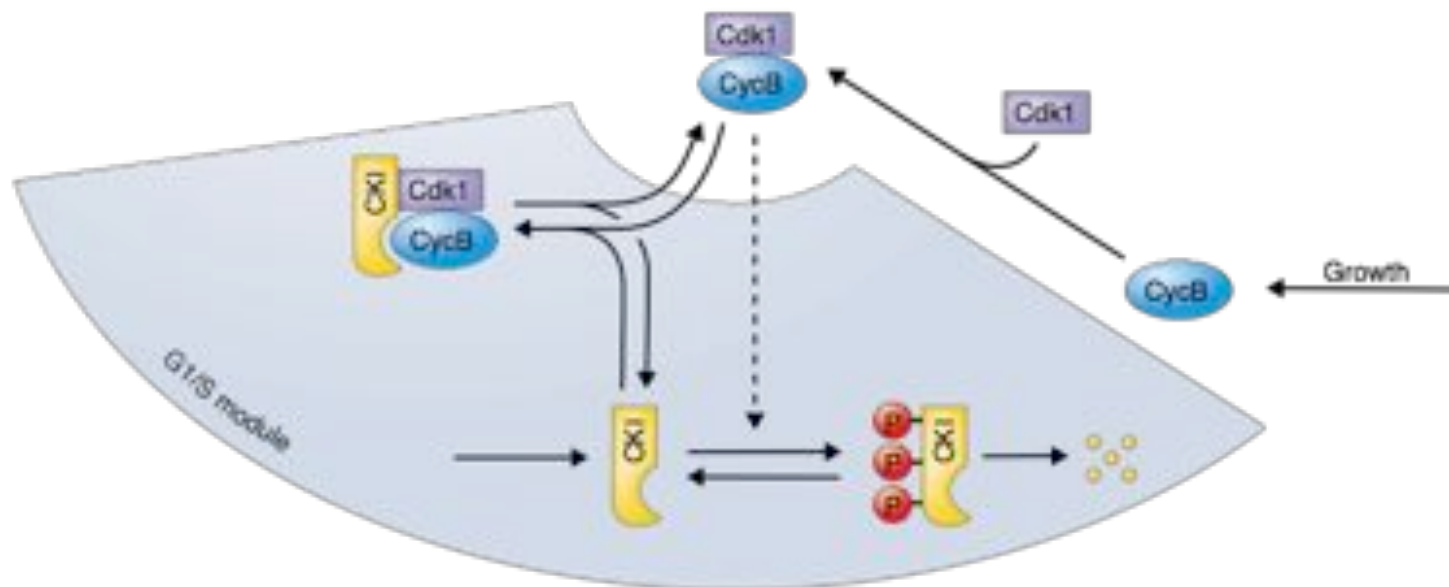
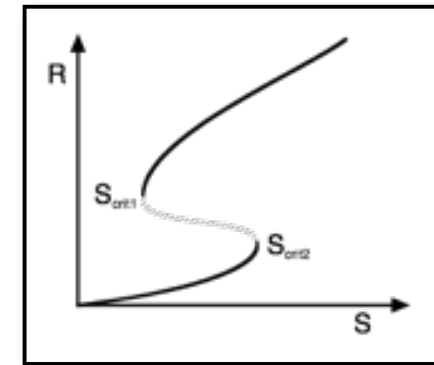
# Predicted Behavior: $G1 \Rightarrow S$

Signal: cell growth = concentration of CycB, CdkI

Response: activity (concentration) of CycB:CdkI

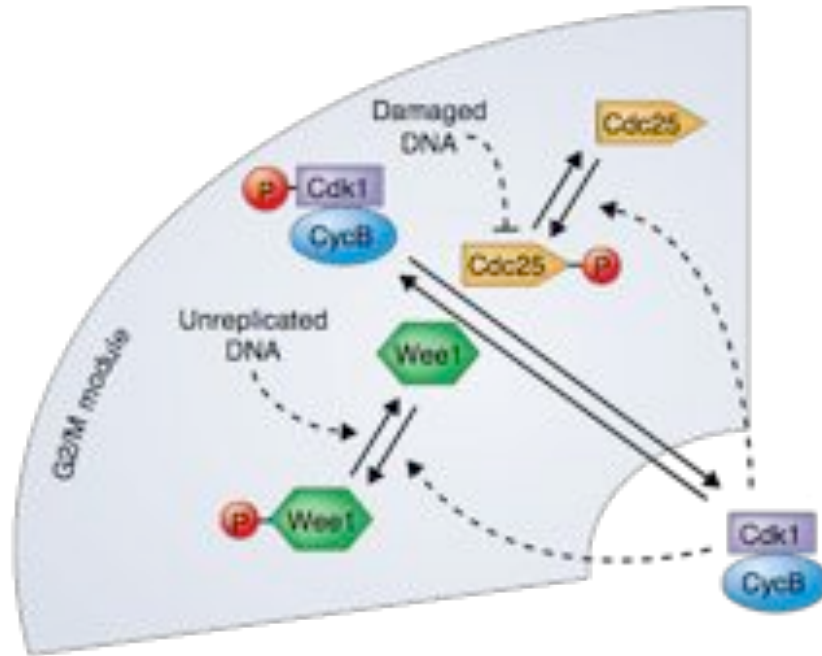
Toggle switch:

$\Rightarrow$  above critical cell size CycB:CdkI activity will switch on



Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

# G2 => M



**Toggle** switch:

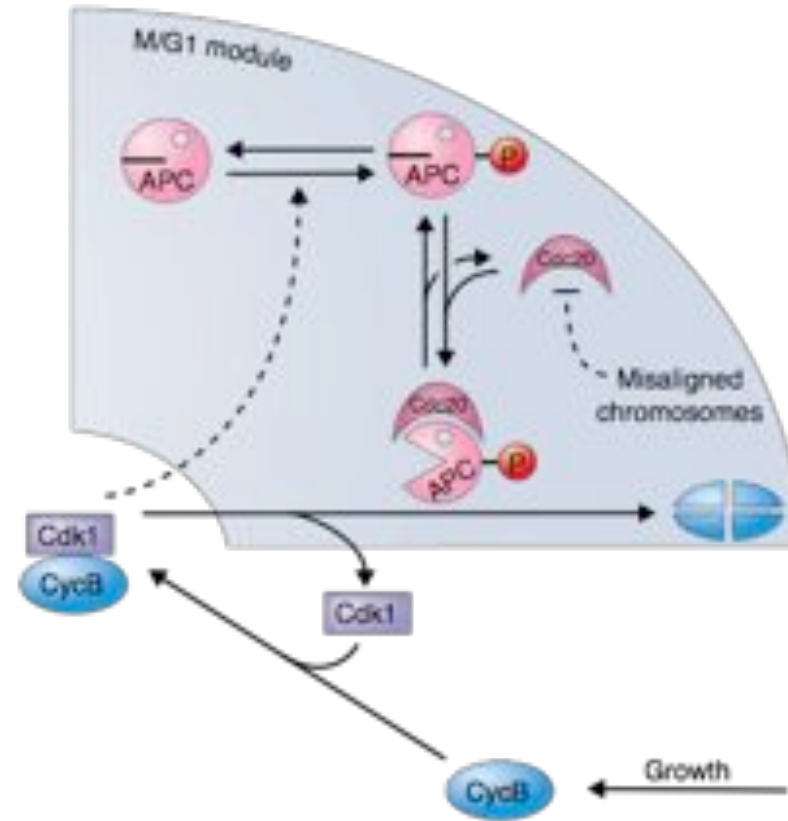
- **mutual activation** between CycB:CdkI and Cdc25 (phosphatase that activates the dimer)
- **mutual inhibition** between CycB:CdkI and WeeI (kinase that inactivates the dimer)

=> when the cell **grows** further during the second gap phase G2, the activity of CycB:CdkI will **increase** by a further **step**

M => GI

## Negative feedback loop oscillator

- i) CycB:CdkI activates anaphase promoting complex (APC)
- ii) APC activates Cdc20
- iii) Cdc20 degrades CycB



## Behavior:

at a critical cell size

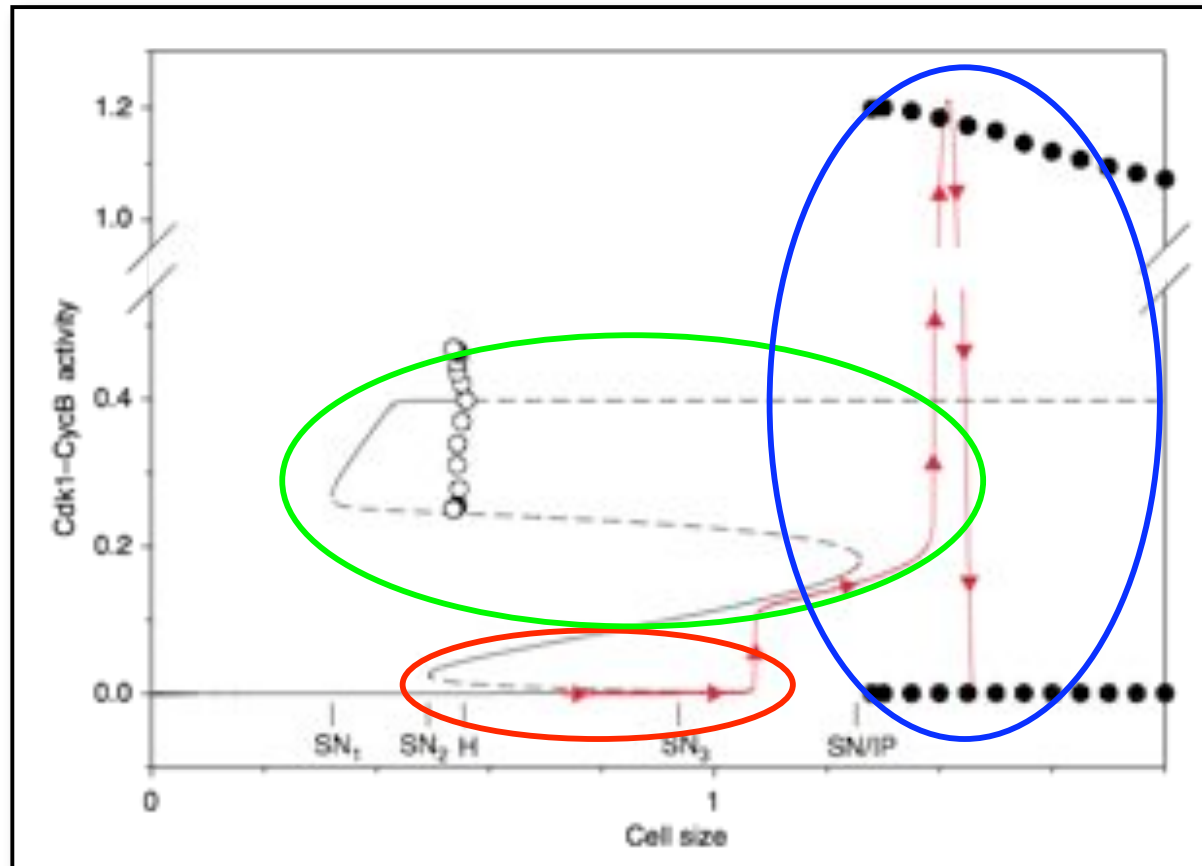
CycB:CdkI activity increases and **decreases** again

=> at low CycB:CdkI level, the G1/S toggle switches off again,

=> cell cycle completed

Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

# Overall Behavior



Cell divides at size 1.46

=> daughters start  
growing from  
size 0.73

=> switches to  
replication at  
size 1.25

G1/S toggle => bistability

M/G1 oscillator

G2/M toggle => bistability

Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

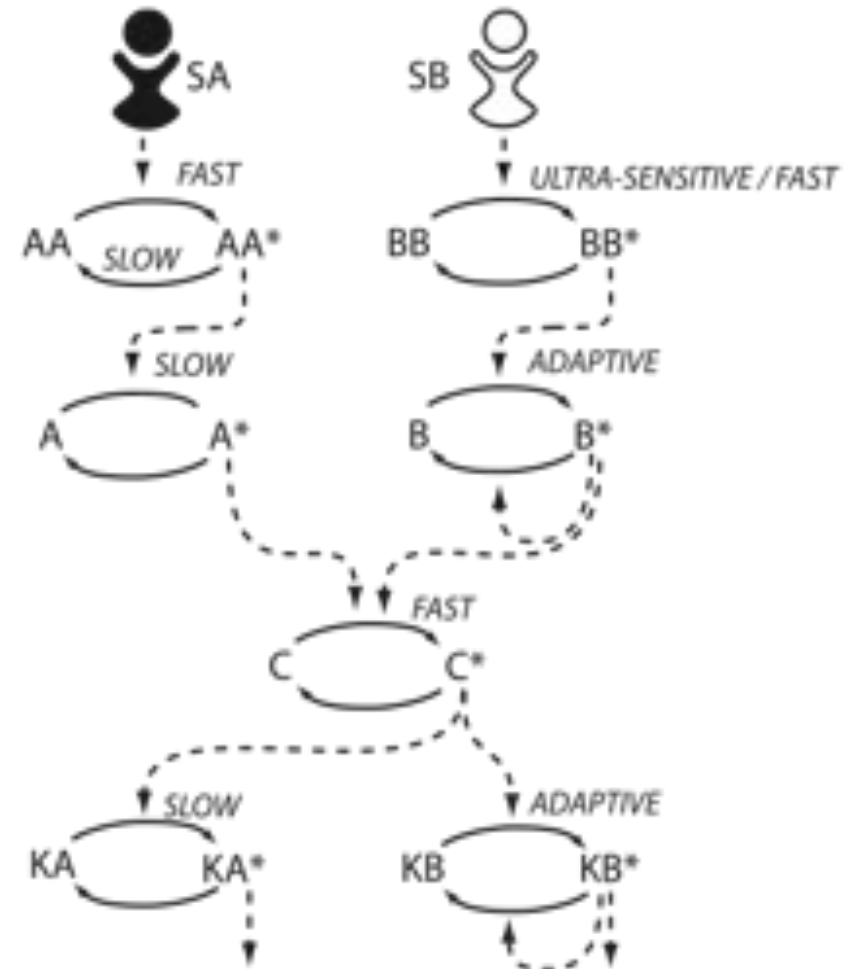
# Preventing Cross-Talk

Many enzymes are used  
in multiple pathways

=> how can different signals cross  
the same kinase?

=> different temporal signature  
(slow vs. transient)

=> Dynamic modelling!



# Summary

## **Today:**

Behavior of cell cycle control circuitry from its modules:

two toggle switches + one oscillator

=> map biological system onto motif via

- stoichiometric matrices
- rate equations