

Bioinformatics 3

V18 – Kinetic Motifs

Fri, Jan 8, 2016

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^{*†}, Katherine C Chen^{*‡} and Bela Novak[§]

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

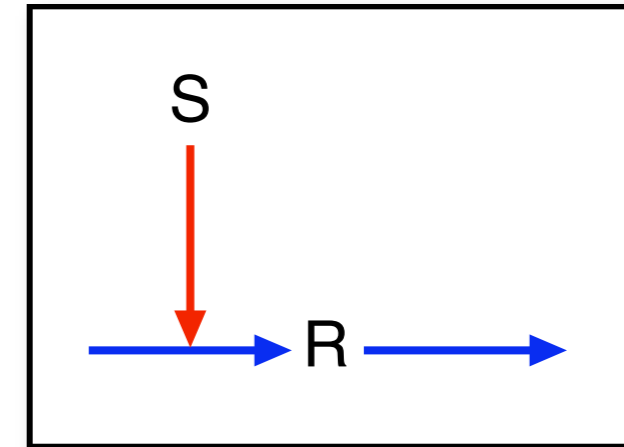
- 1) How do the magnitudes of signal **output** and signal duration depend on the **kinetic properties** of pathway components?
- (2) Can high signal **amplification** be coupled with **fast** signaling?
- (3) How are signaling pathways **designed** to ensure that they are **safely off** in the absence 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 V8)

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

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

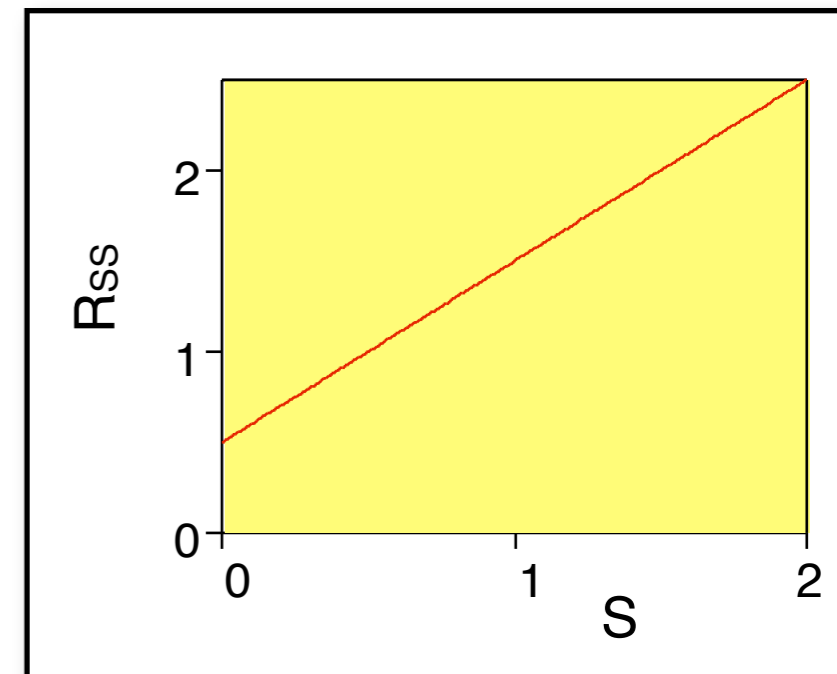


$$\frac{dR}{dt} = k_0 + k_1 S - k_2 R$$

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

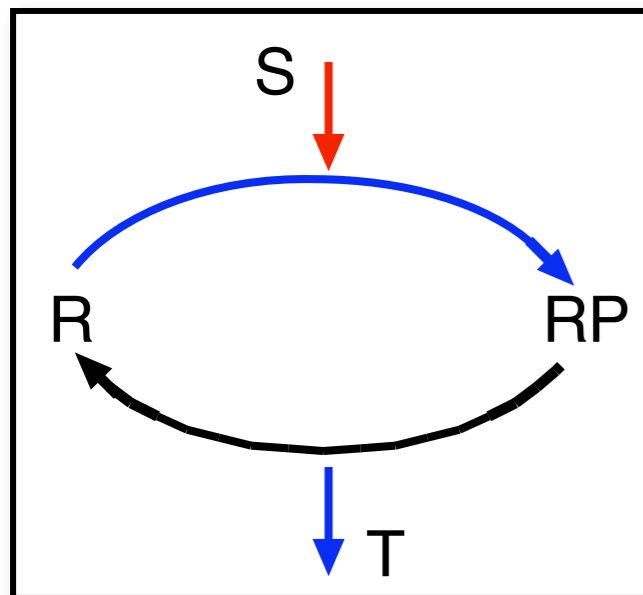
$$\left. \frac{dR}{dt} \right|_{R=R_{ss}} = 0 \Rightarrow R_{ss} = \frac{k_0 + k_1 S}{k_2} = \frac{k_0}{k_2} + \frac{k_1}{k_2} S$$

R_{ss} linearly dependent on S

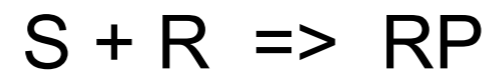


$$k_0 = 1, k_1 = k_2 = 2$$

phosphorylation/dephosphorylation



„forward“: R is converted to phosphorylated form RP
 „backward“: RP can be dephosphorylated again to R



with $R_{tot} = R + RP$

↑
phosphorylated form

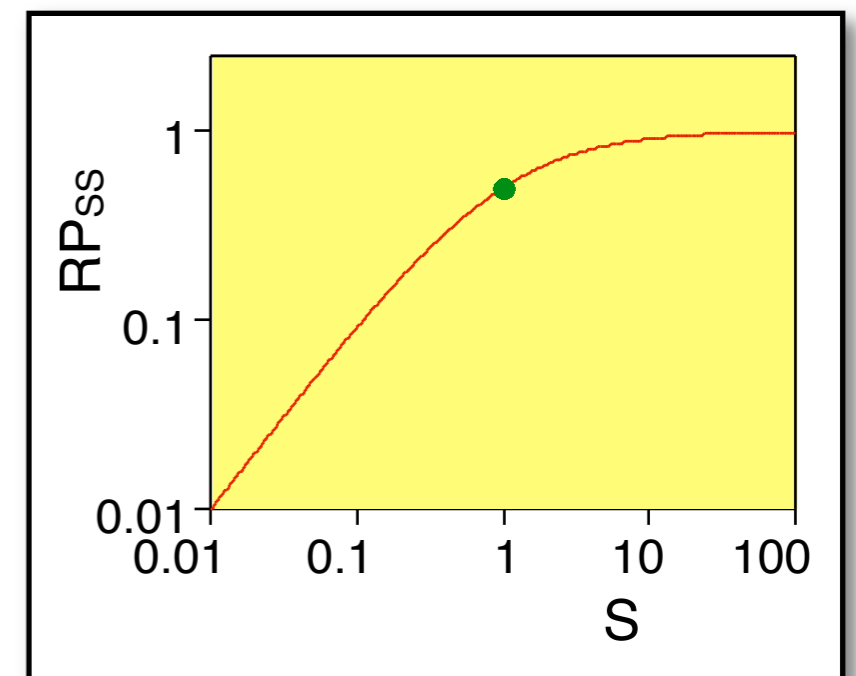
$$\frac{dRP}{dt} = k_1SR - k_2RP = k_1S(R_{tot} - RP) - k_2RP$$

Find steady state for RP: linear until saturation

$$RP_{ss} = \frac{k_1R_{tot}S}{k_1S + k_2} = \frac{R_{tot}S}{S + k_2/k_1} = \frac{R_{tot}S}{S + S_0}$$

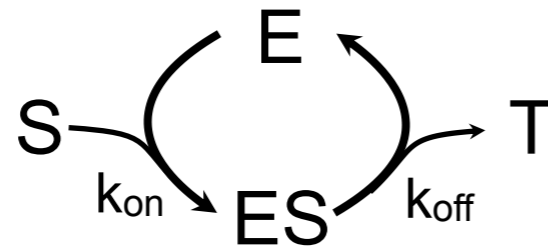
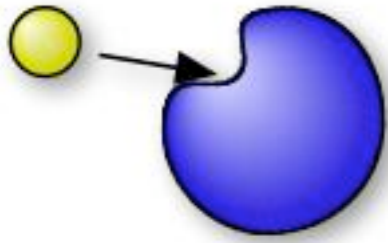
Output T proportional to RP level:

$$\frac{dT}{dt} = k_2RP$$



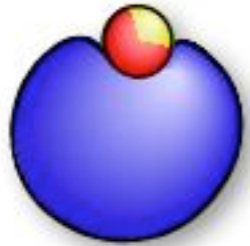
$R_{tot} = 1, S_0 = 1$

Enzyme: Michaelis-Menten-kinetics



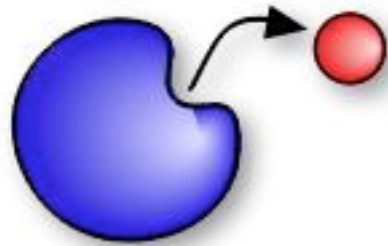
Reaction rate:

$$V = k_{off}ES$$



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

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



Total amount of enzyme is constant:

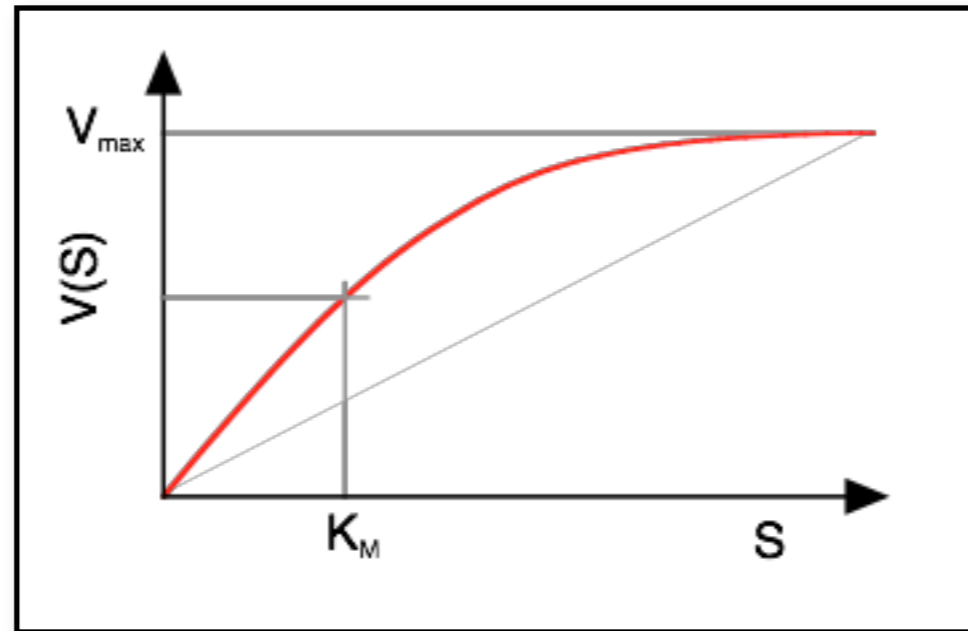
$$E_T = E + ES \quad \Rightarrow \quad ES = E_T \frac{S}{S + K_M}$$

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

The MM-equation

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

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



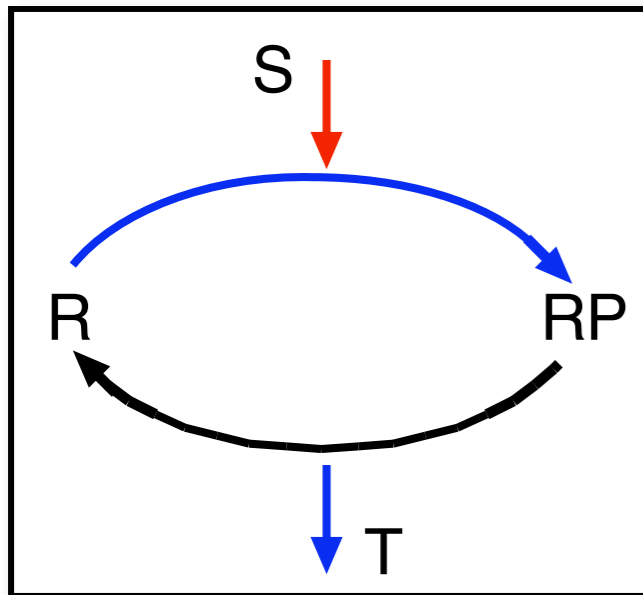
$$K_M = \frac{k_{off}}{k_{on}}$$

- 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.

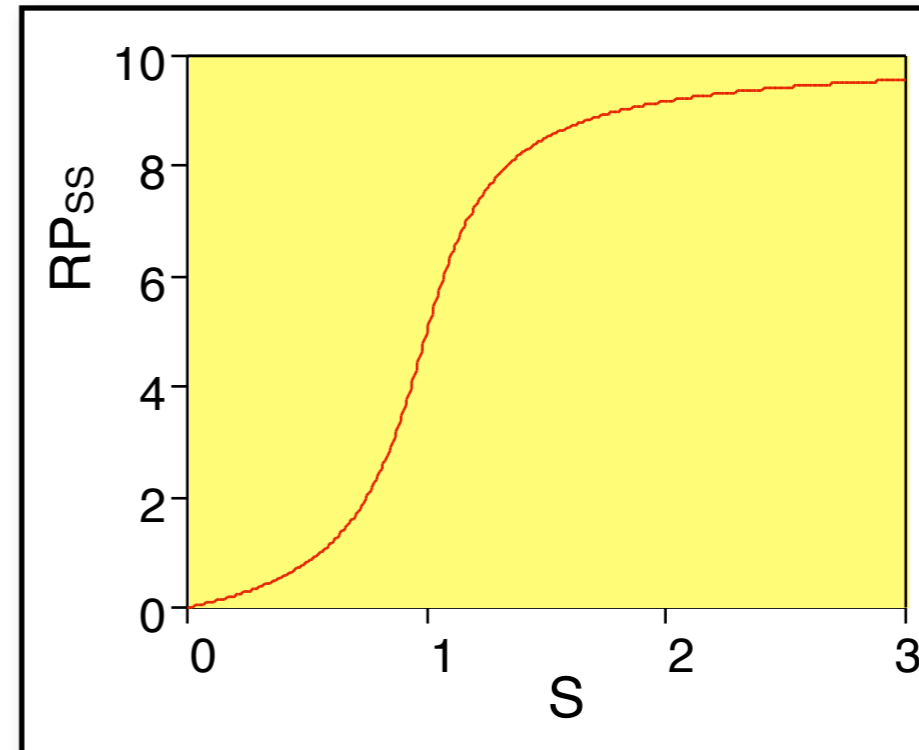
$$\frac{dRP}{dt} = \frac{k_1 S (R_t - RP)}{R_0 + (R_t - RP)} - \frac{k_2 RP}{RP_0 + RP} \stackrel{!}{=} 0$$

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

Quadratic equation for RP

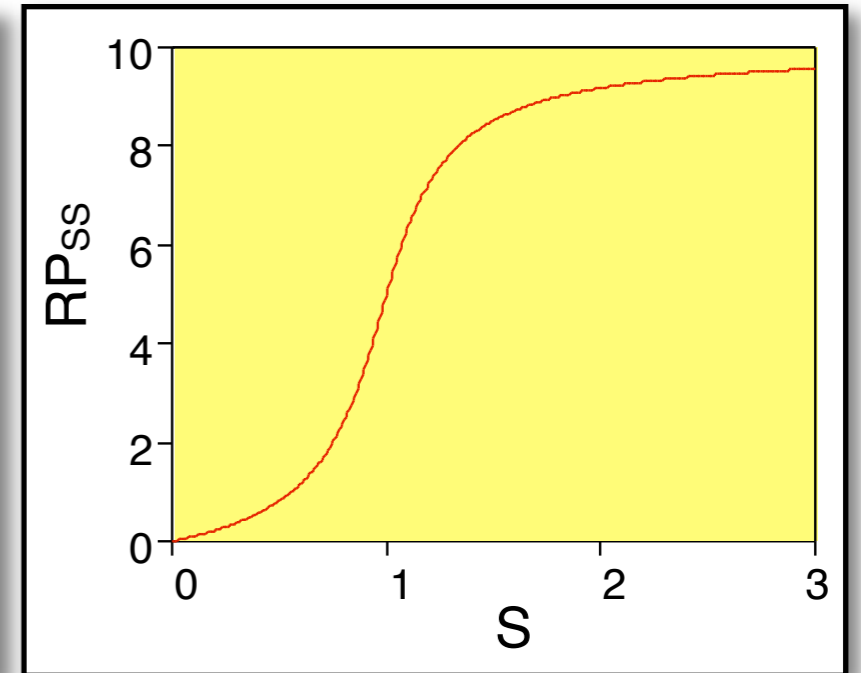
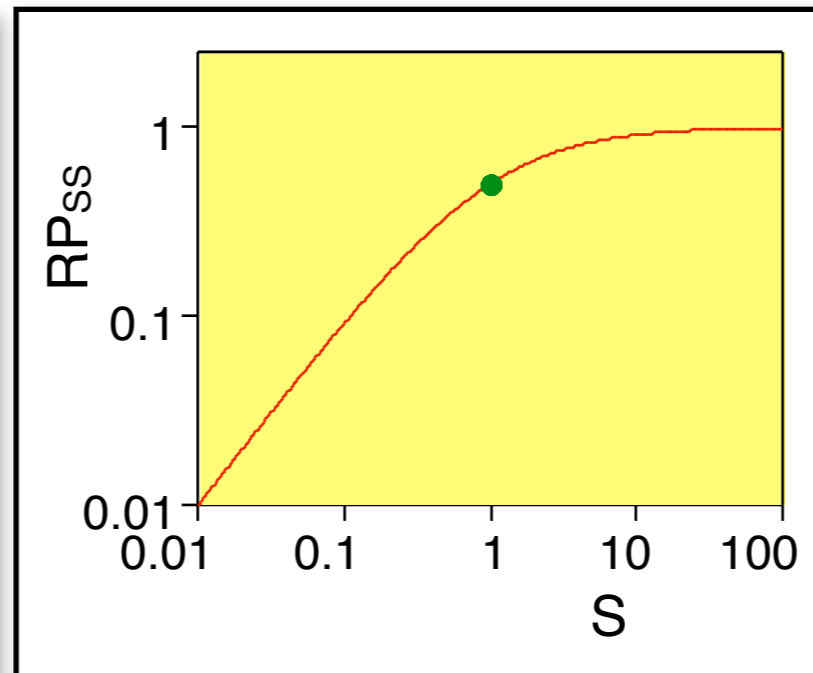
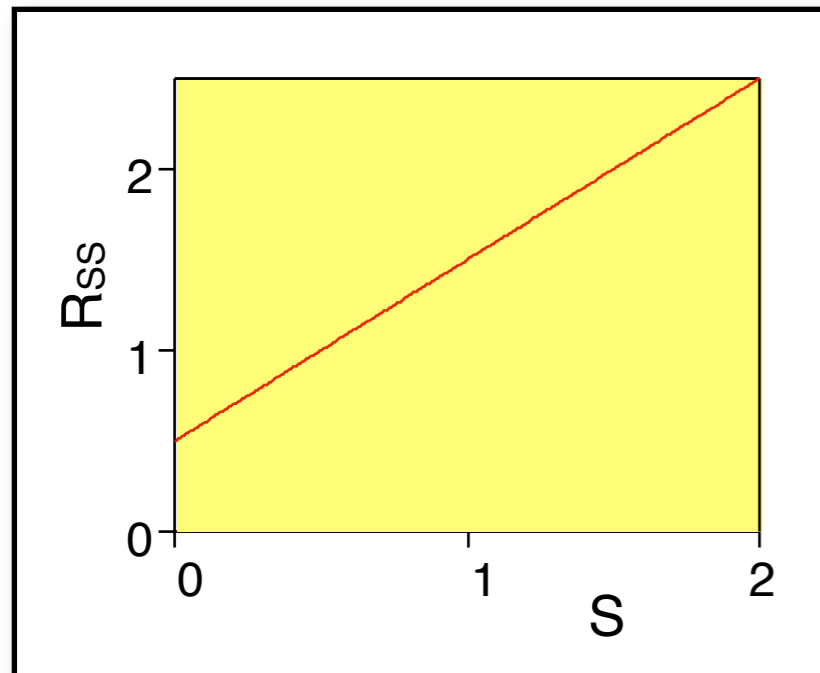
$$k_2 RP (R_0 + (R_t - RP)) = k_1 S (R_t - RP) (RP_0 + RP)$$

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



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

Graded Response

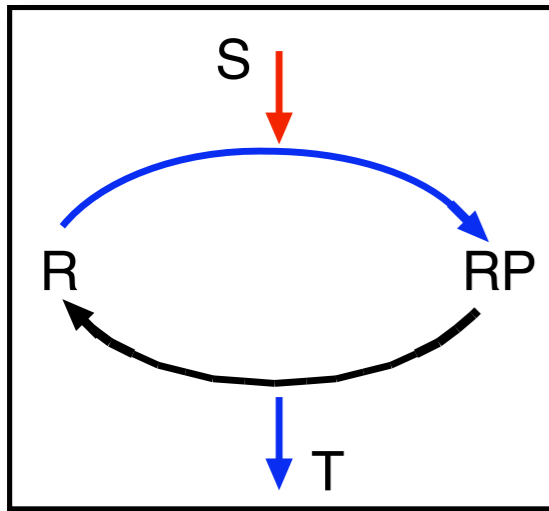


Linear, hyperbolic, and sigmoidal characteristic give the same steady state response independent of the previous history

=> no hysteresis

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

Time-dependent Sigmoidal Response



Direct implementation:

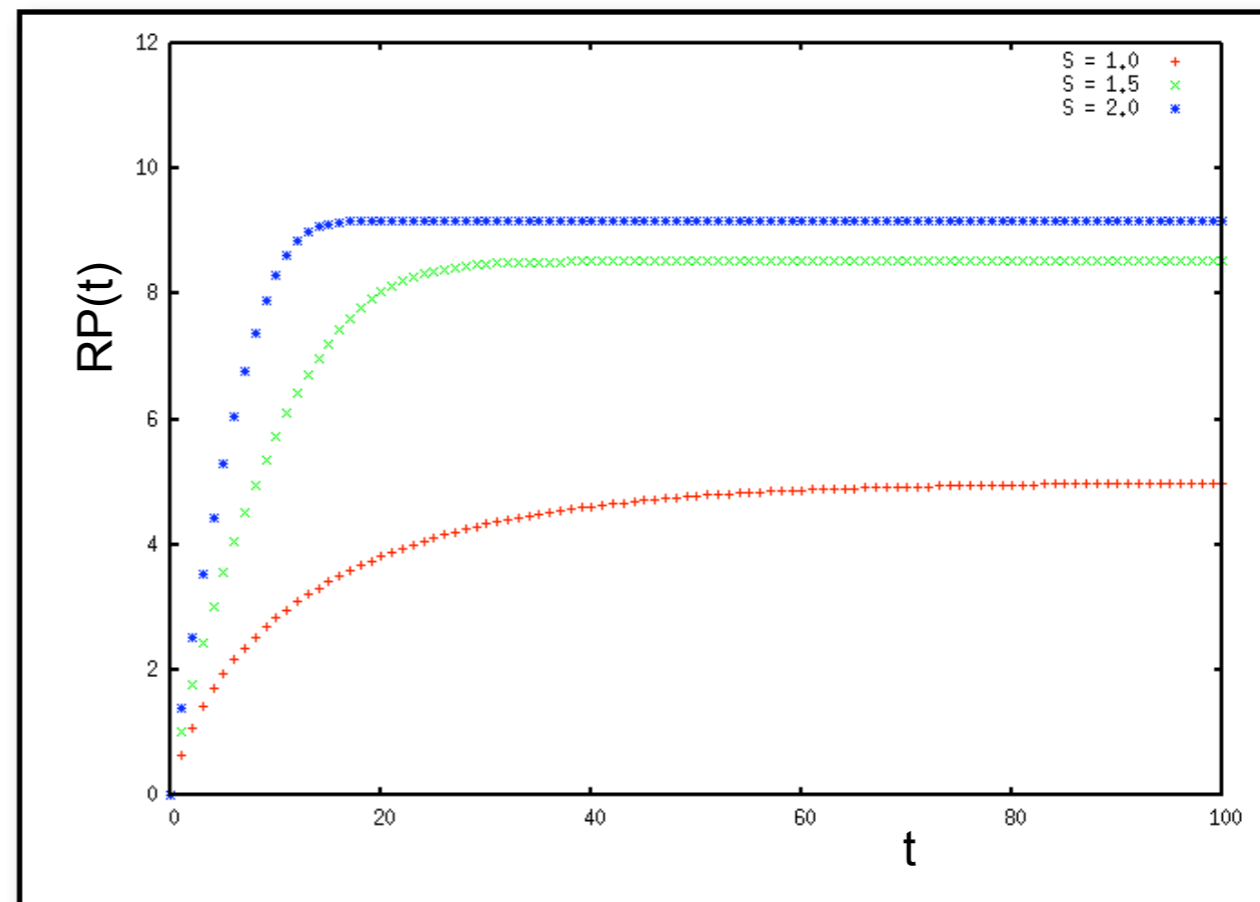
$$v_1 = \frac{Sk_1R}{R_0 + R} \quad v_2 = \frac{k_2RP}{RP_0 + RP}$$

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

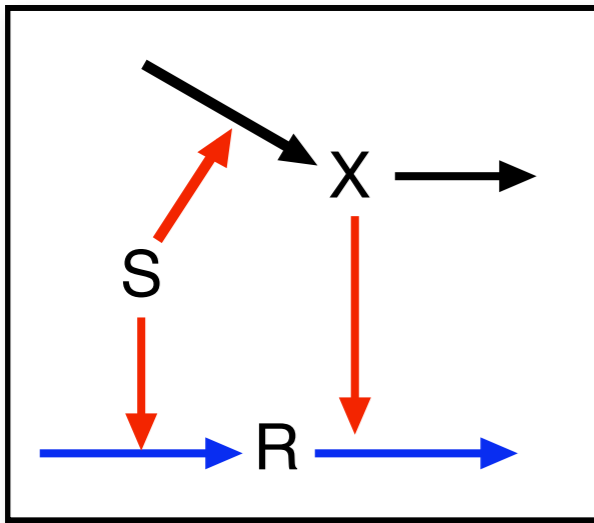
Initial conditions: $R = 10 \text{ mol}$, $RP = 0$

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

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

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

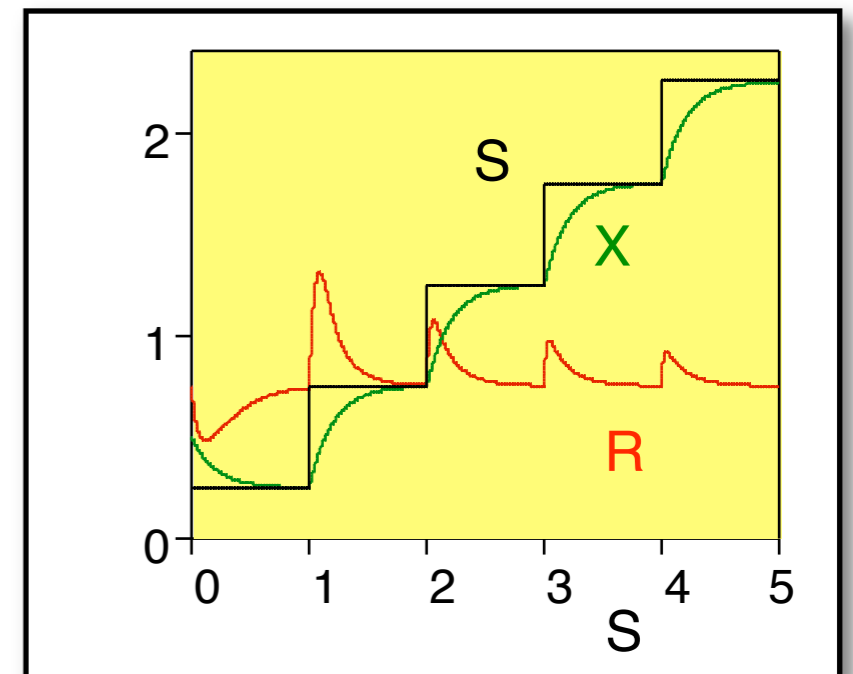
$$R_{ss} = \frac{k_1 k_4}{k_2 k_3}$$

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

found in smell, vision, chemotaxis, ...

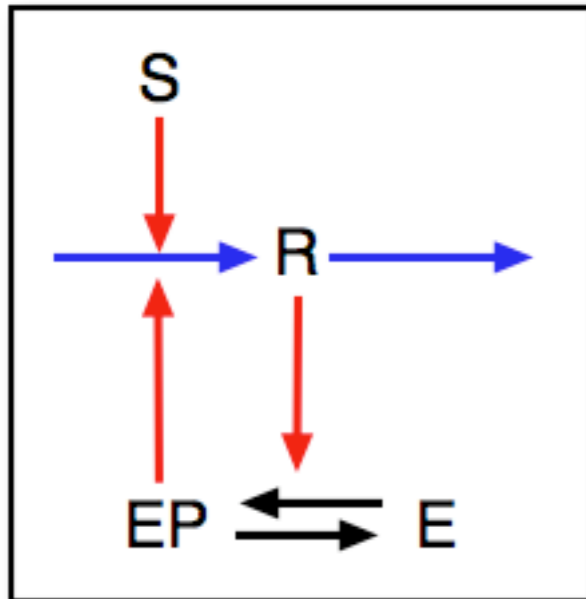
Note: response strength ΔR depends on rate of change of S.

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



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

Positive Feedback



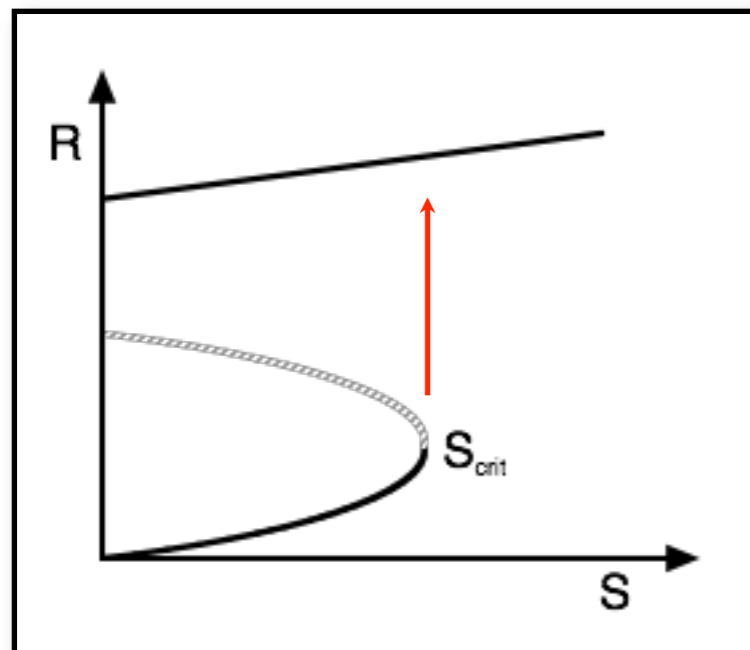
$$\frac{dR}{dt} = k_4 EP(R) + k_1 S - k_2 R$$

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

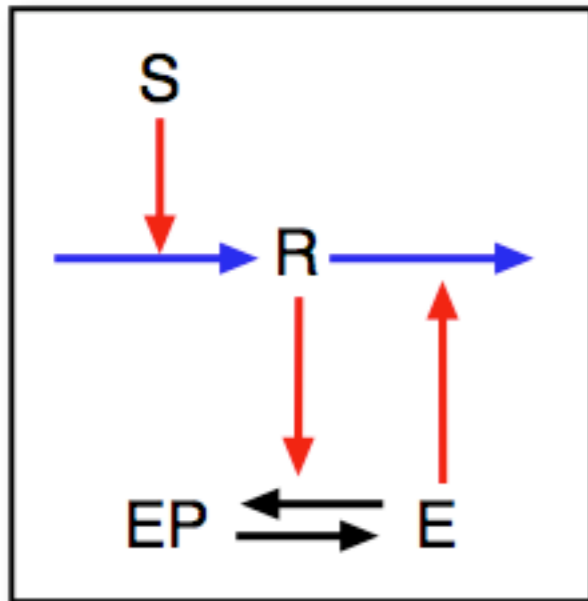
Feedback via R and EP
=> high levels of R will stay

"one-way switch" via bifurcation

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

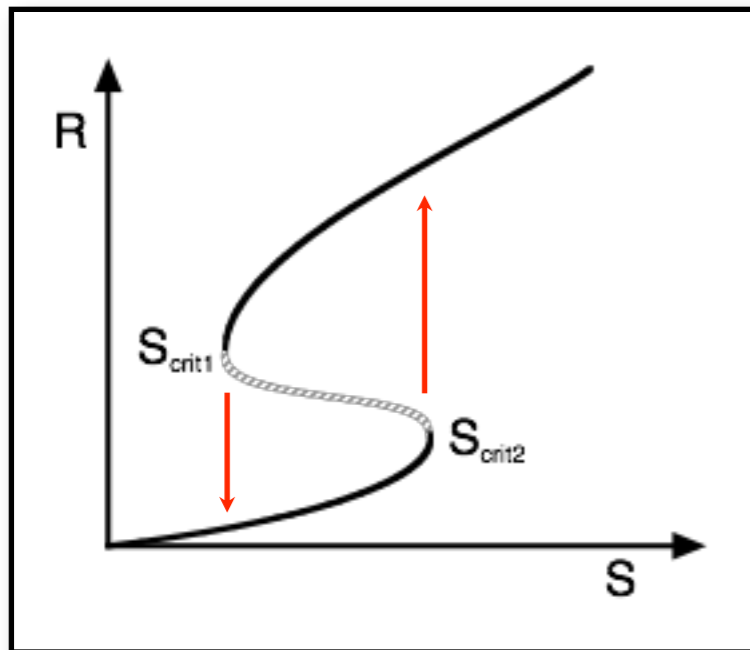


Mutual Inhibition - Toggle Switch



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

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

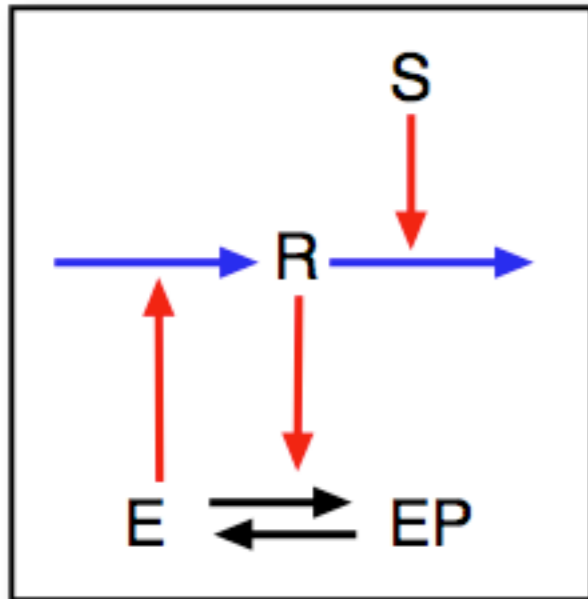


Sigmoidal "threshold" in $E \rightleftharpoons EP$ leads to bistable response (hysteresis): **toggle switch**

Converts continuous external stimulus into two well defined stable states:

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

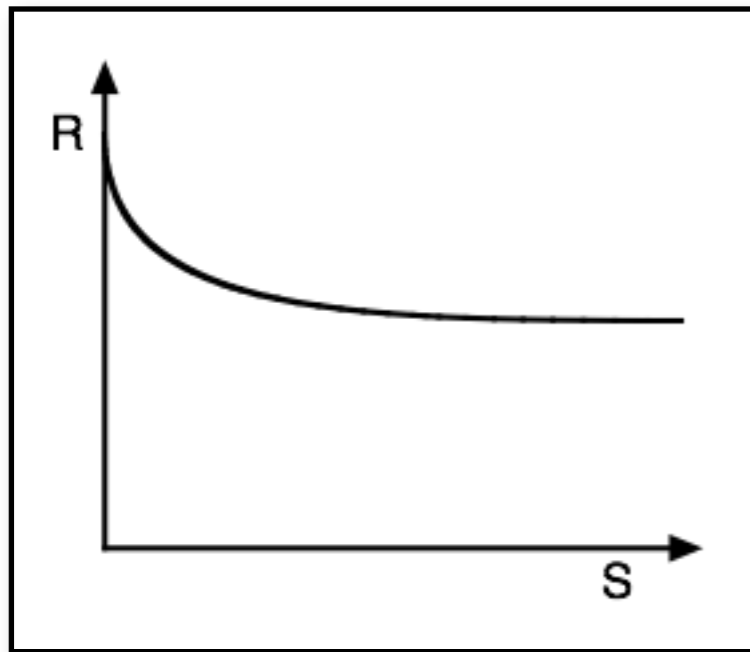
Negative Feedback



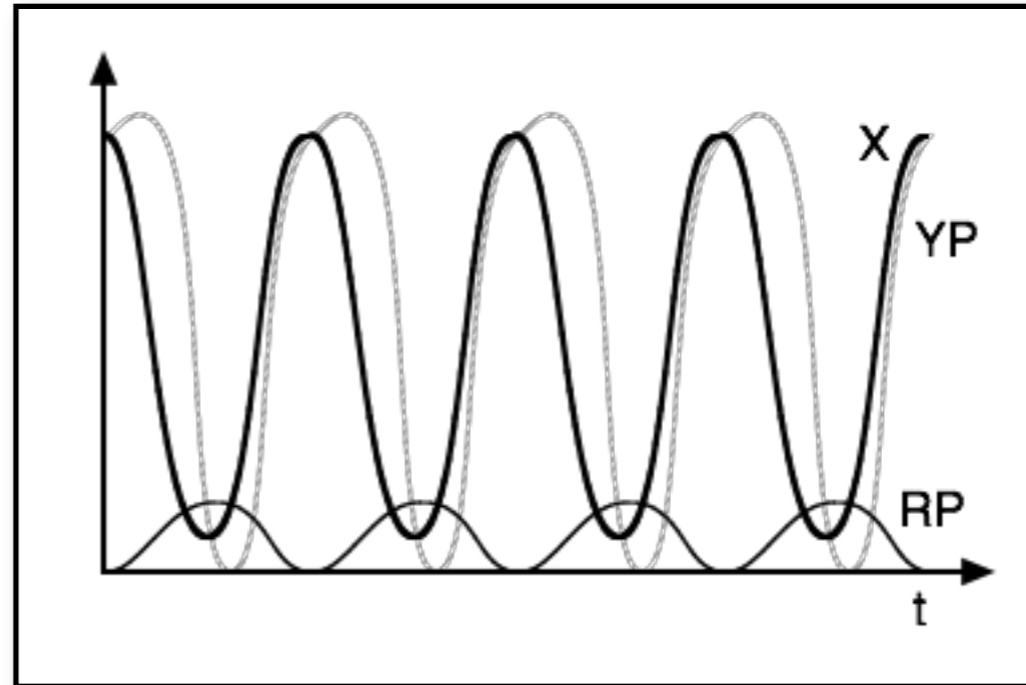
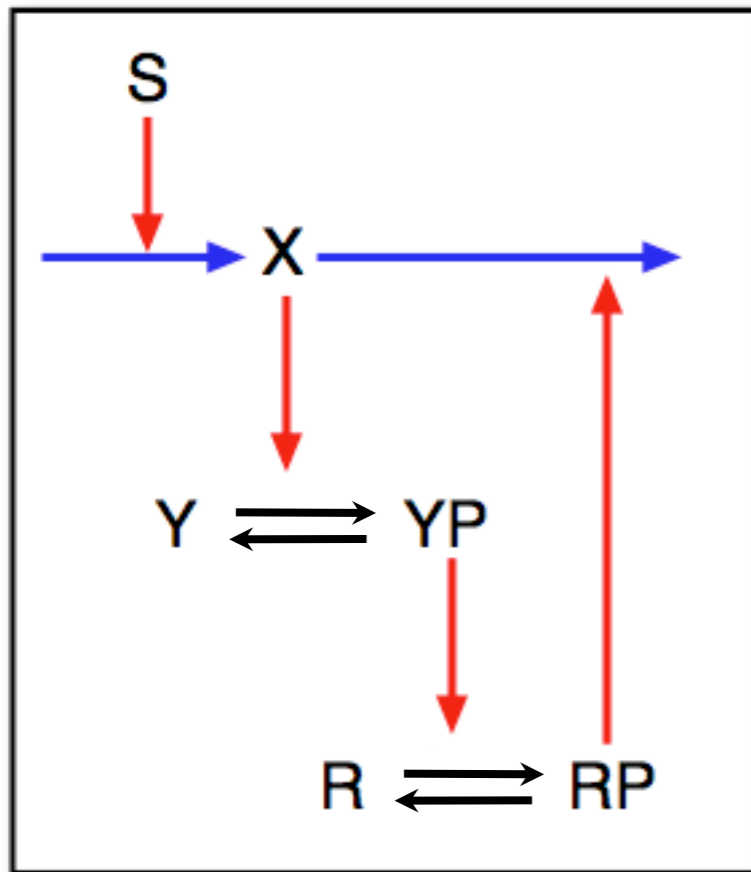
S controls the "demand" for R

=> **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)

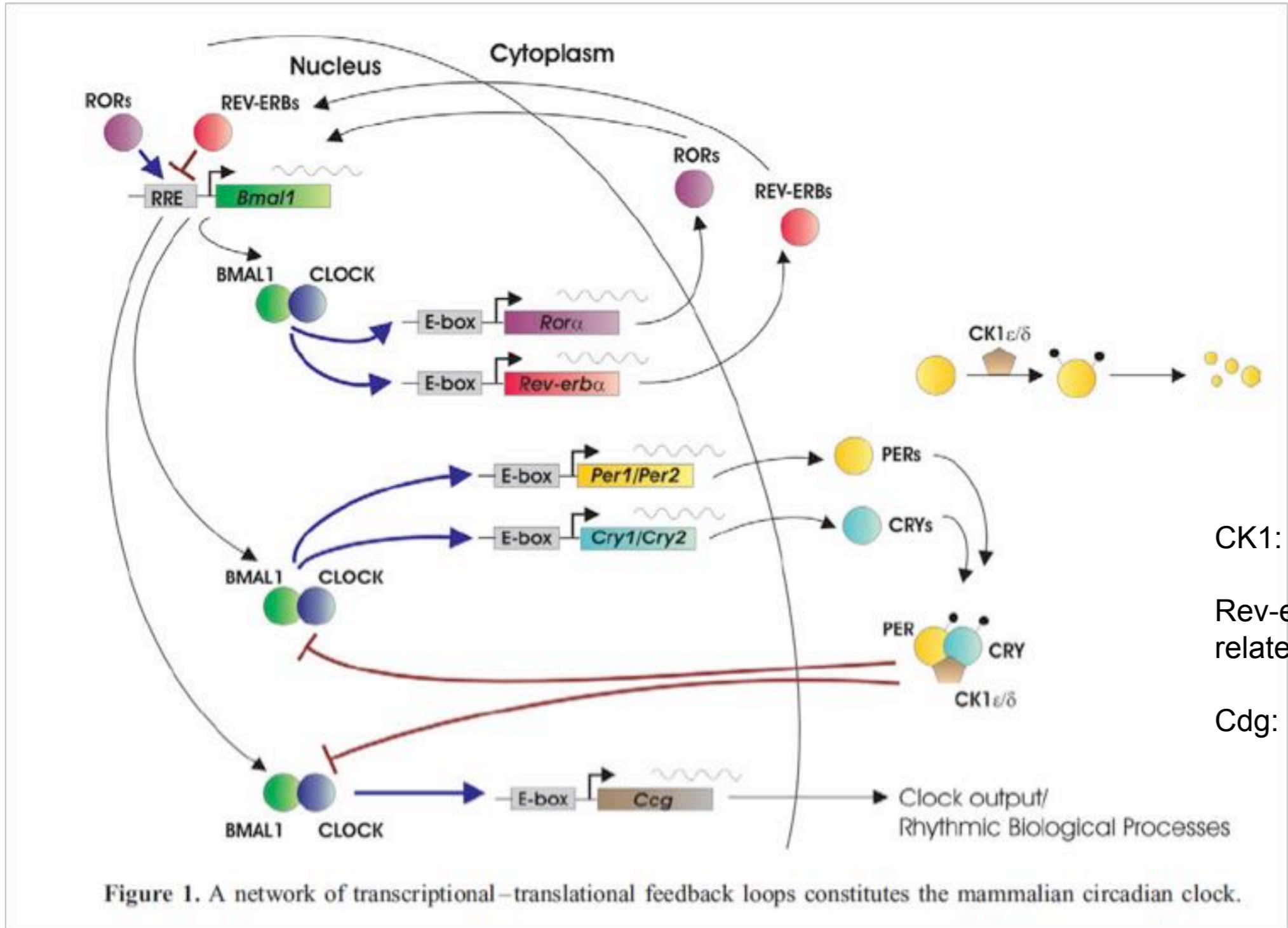
$$\frac{dX}{dt} = k_0 + k_1 S - k_2 X - k_7 RP X$$

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

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

Proposed mechanism
for circadian clocks

Circadian Clocks



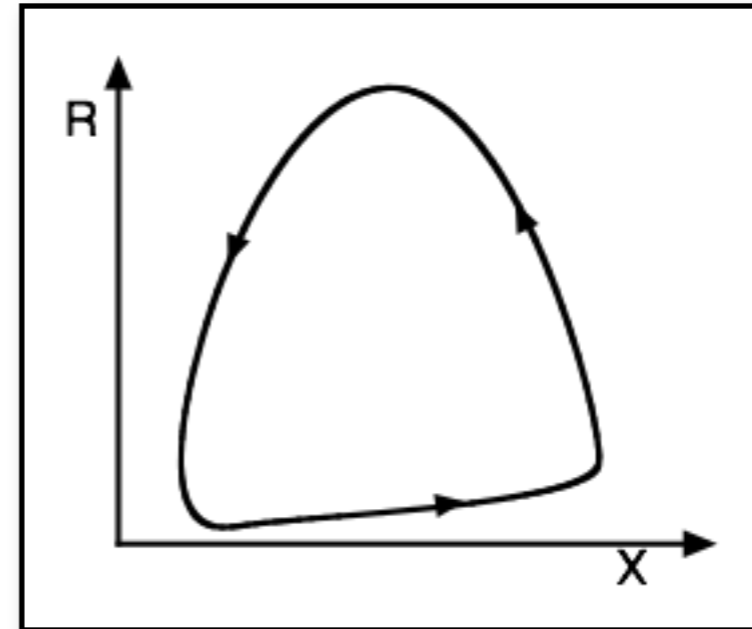
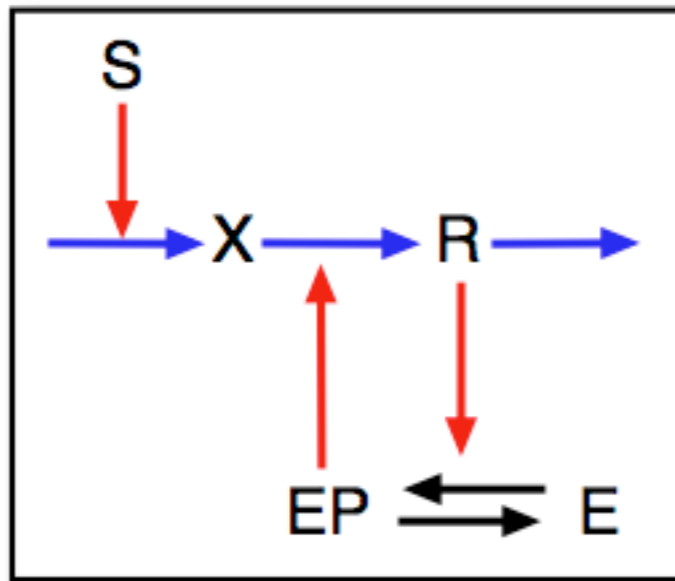
PER: period
CRY: cryptochrome

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.

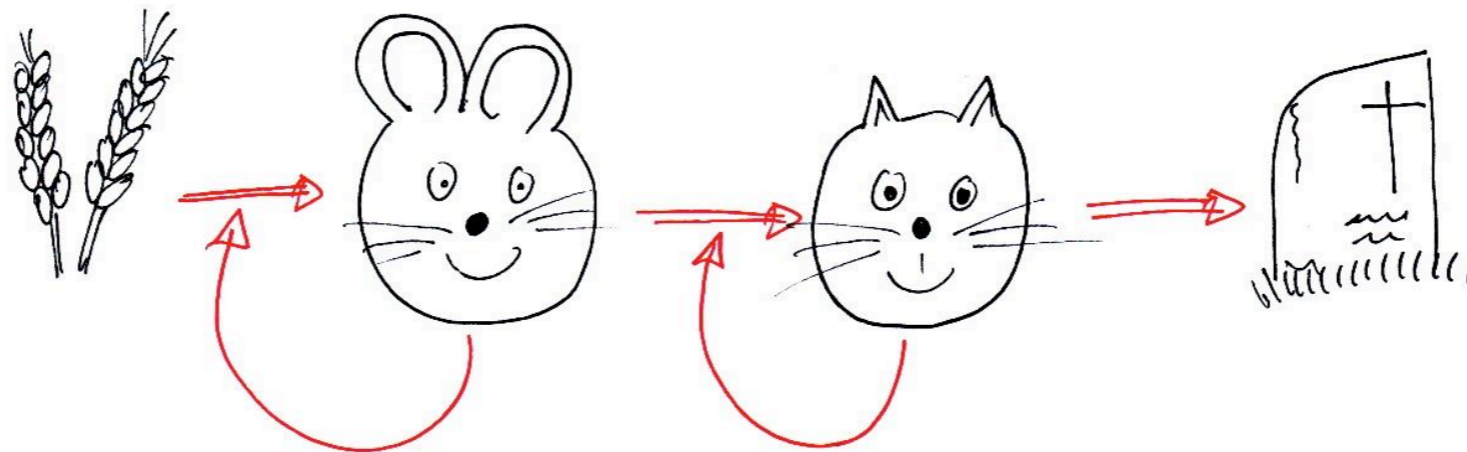
Ko & Takahashi Hum Mol Genet
15, R271 (2006)

Substrate-Depletion Oscillations

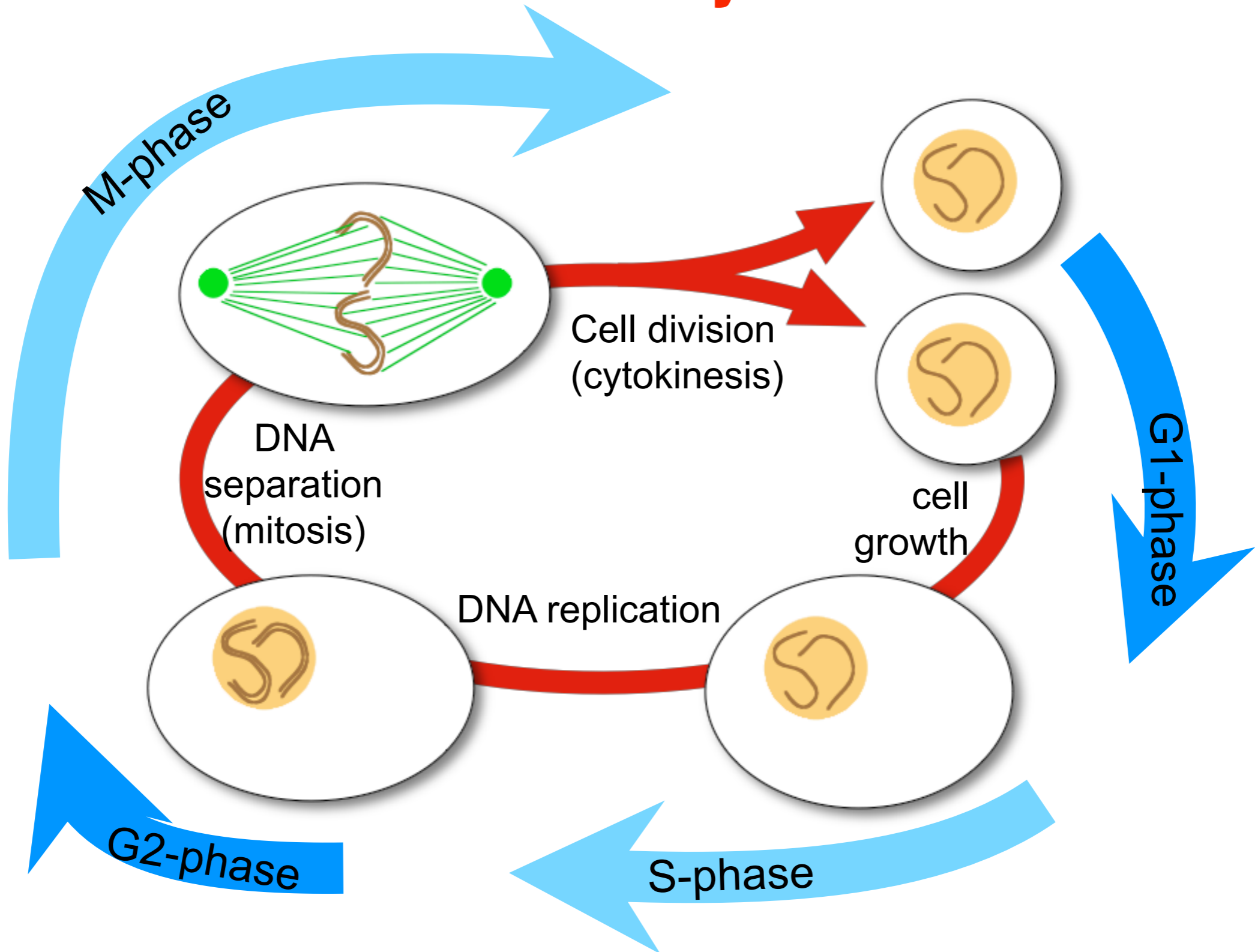


R is produced in an **autocatalytic** reaction from X , finally **depleting** X ...

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.

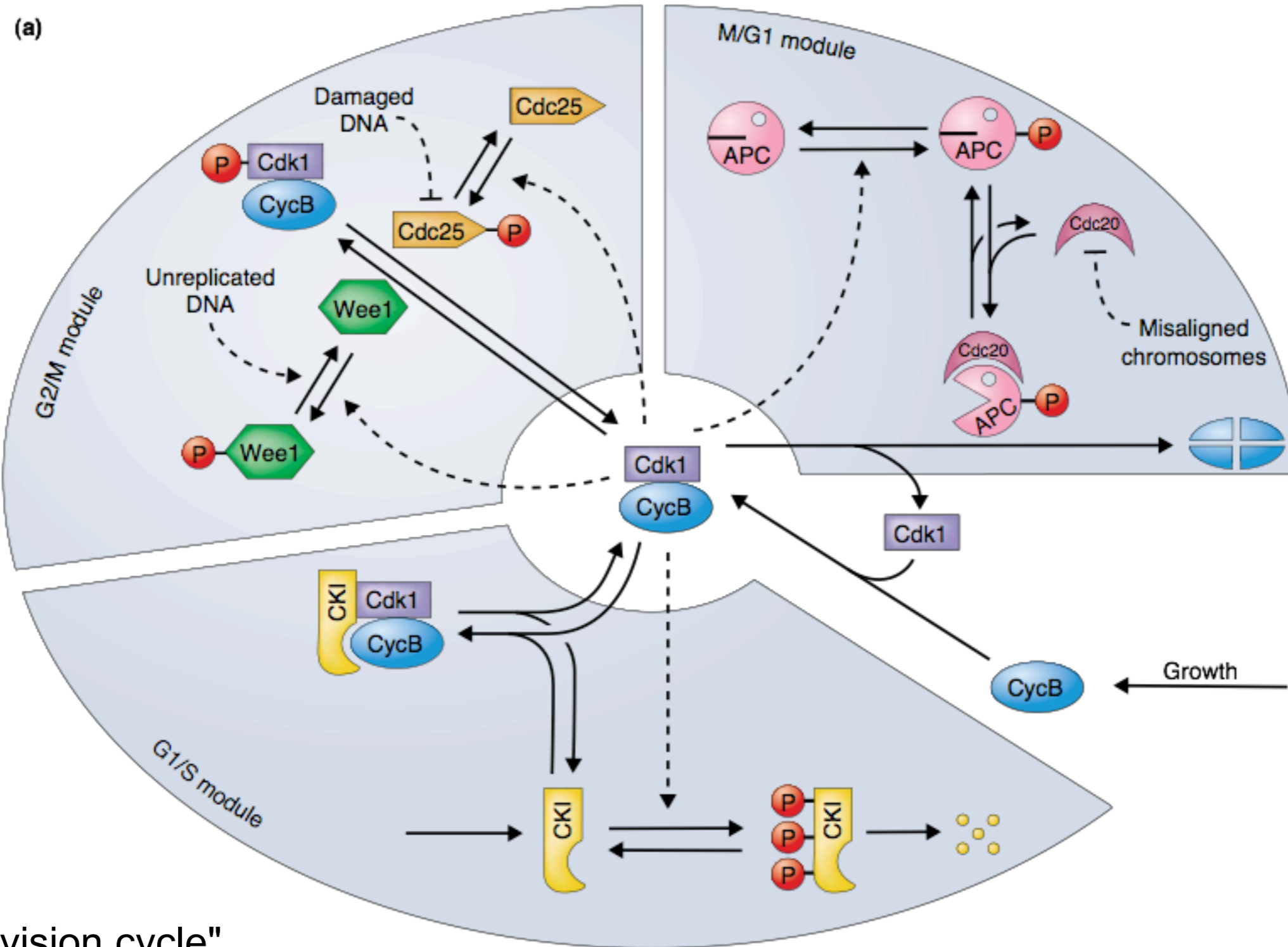
E.g., a negative feedback loop
 $X \rightarrow R \dashv X$ can yield oscillations
(X activates R, which inhibits X, so that R goes down, so that X goes back up. . .).

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"

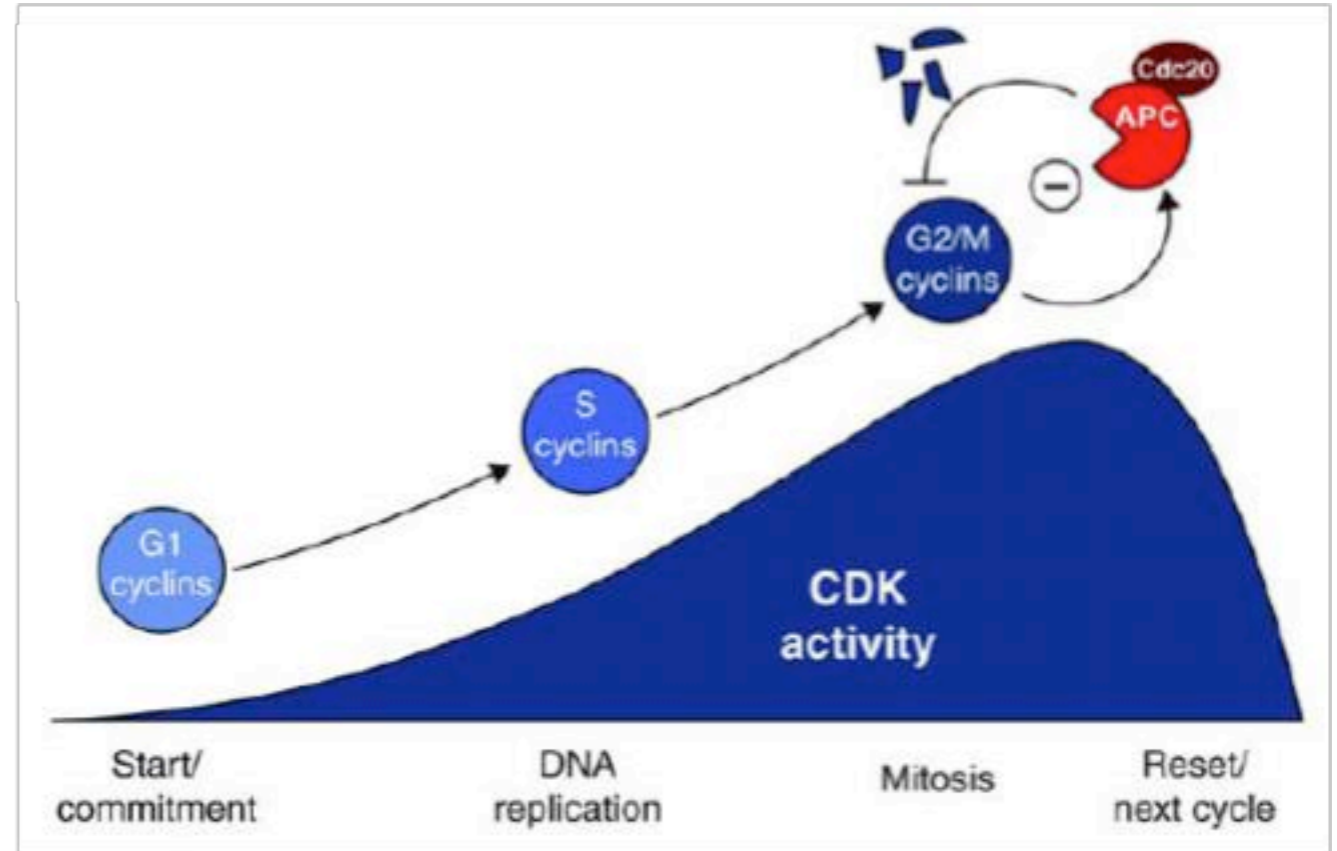
Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

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.

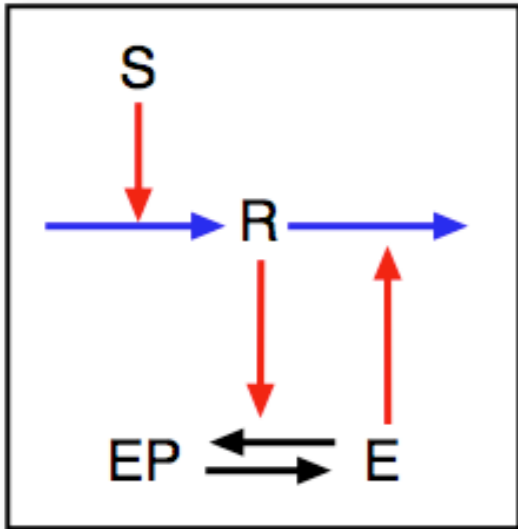
Non-linearity in APC–Cdc20 activation prevents the system from settling into a steady state.



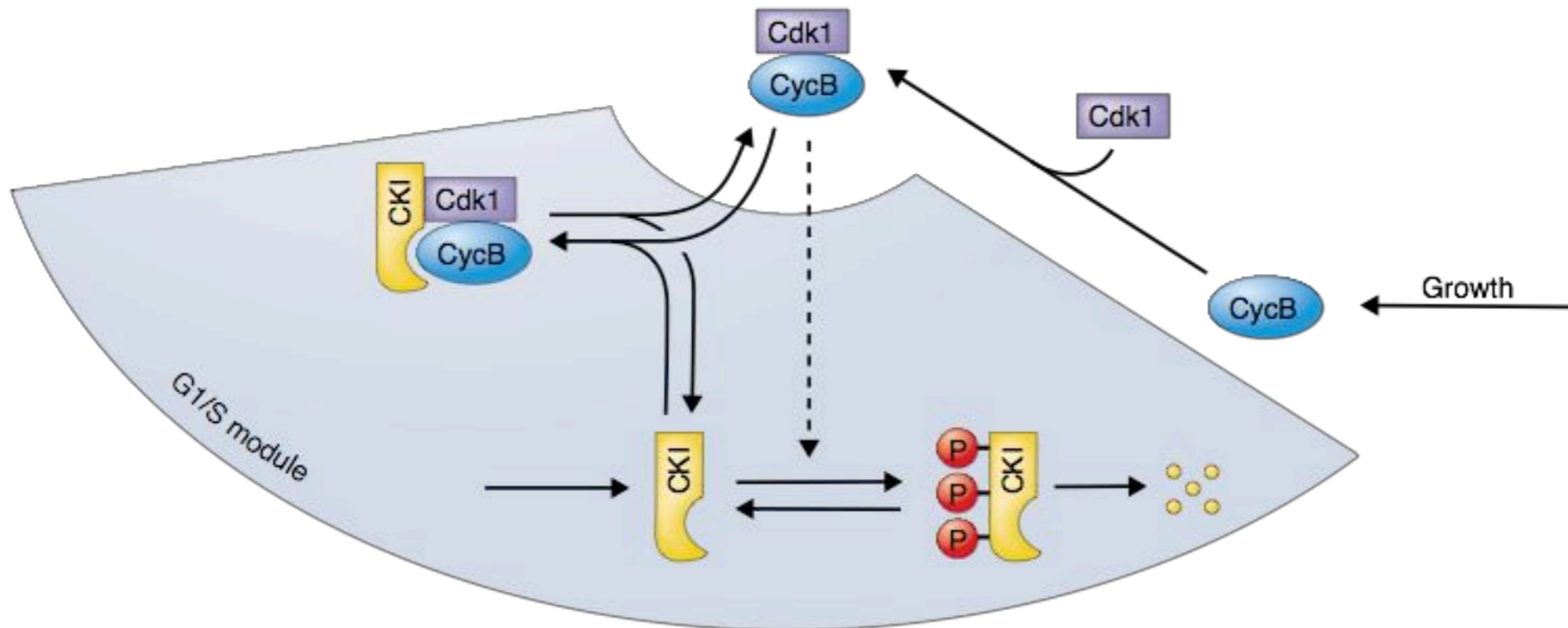
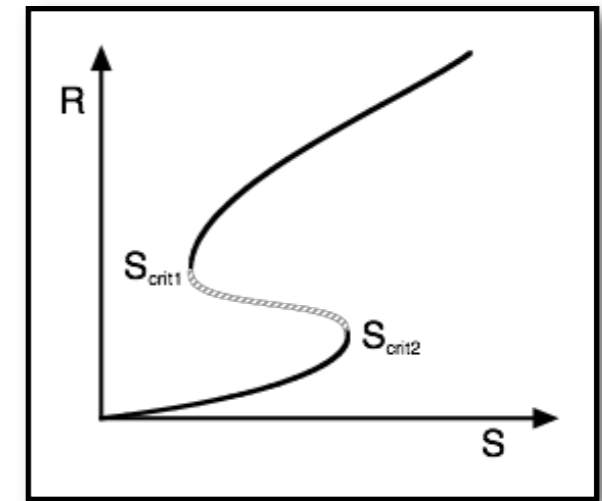
- CDKs require the binding of a cyclin subunit for activity. These cyclin partners can also determine the localization of the complex and its specificity for targets.
- At the beginning of the cell cycle, cyclin–CDK activity is low, and ramps up over most of the cycle. Early cyclins trigger production 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)

G1 => S — Toggle Switch

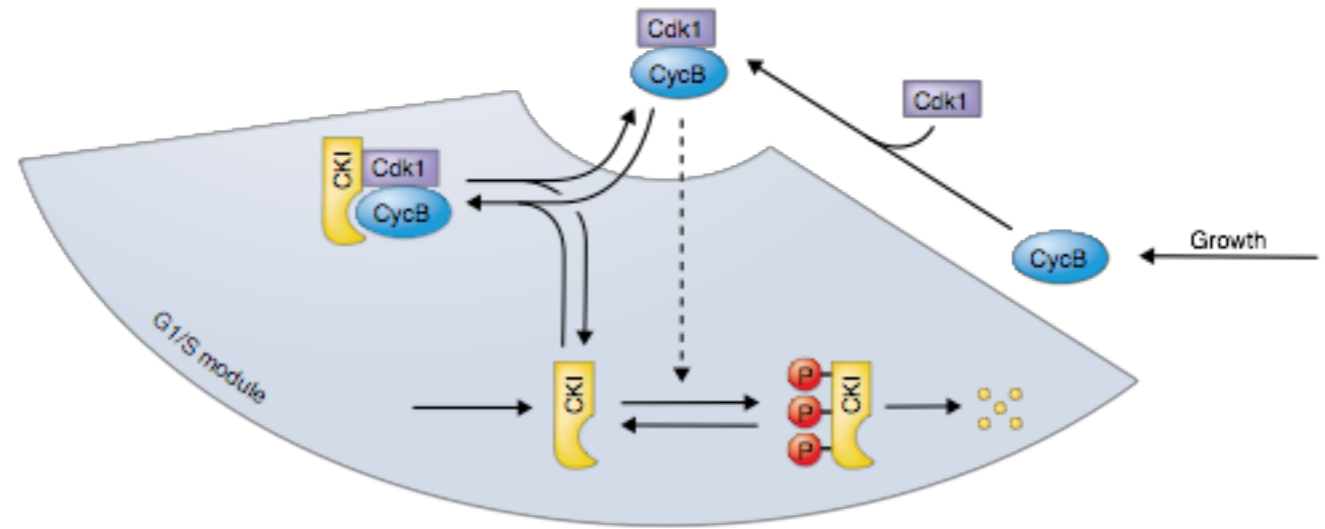
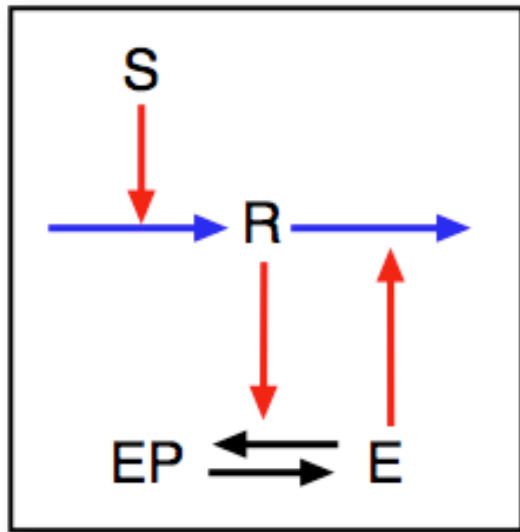


Mutual inhibition
between Cdk1-CycB
and CKI
(cyclin kinase inhibitor)

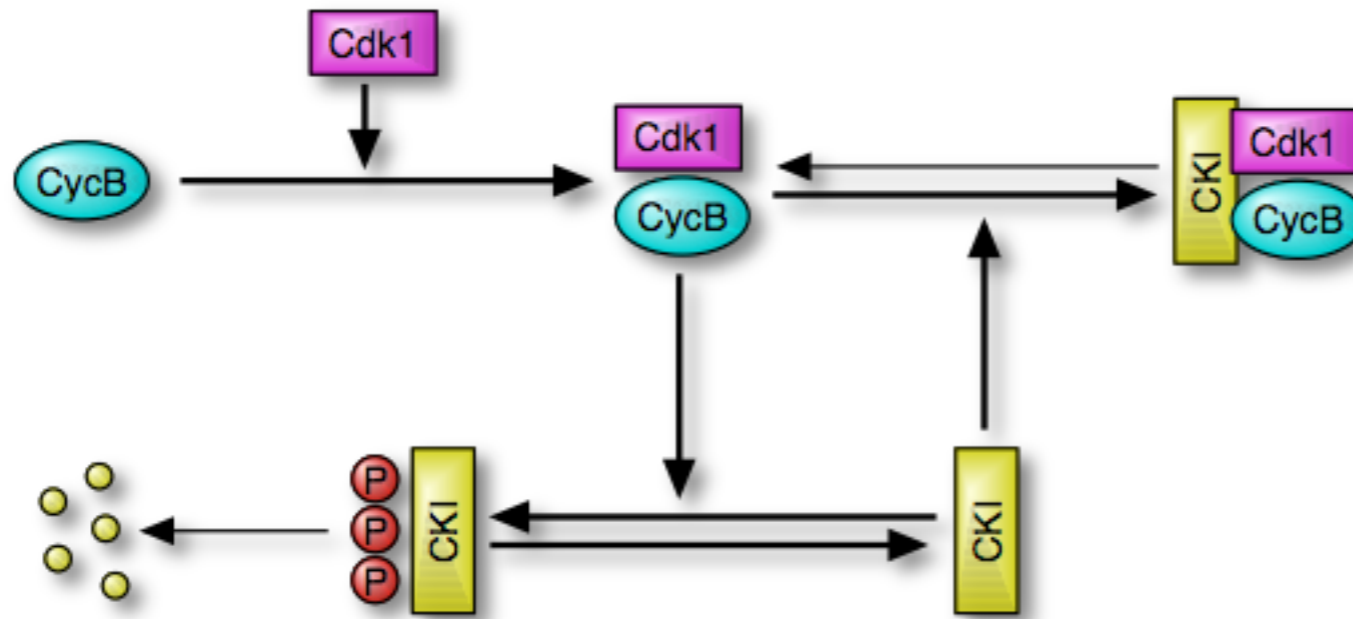


Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

Mutual Inhibition

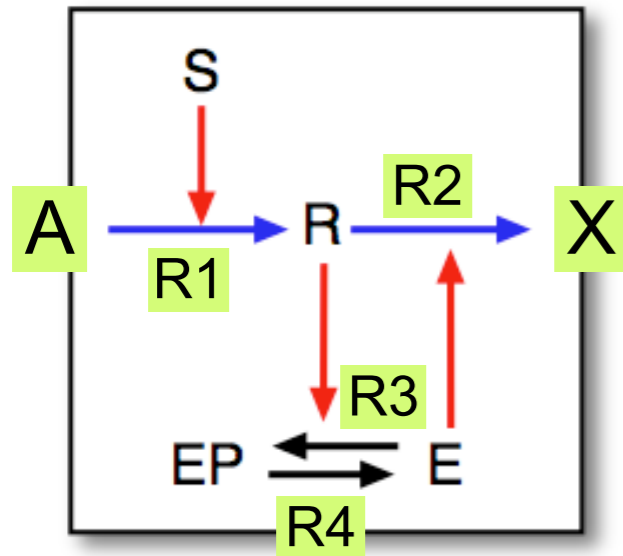


Assume: CycB:Cdk1:CKI is stable \Leftrightarrow dissociation is very slow



\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		

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

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

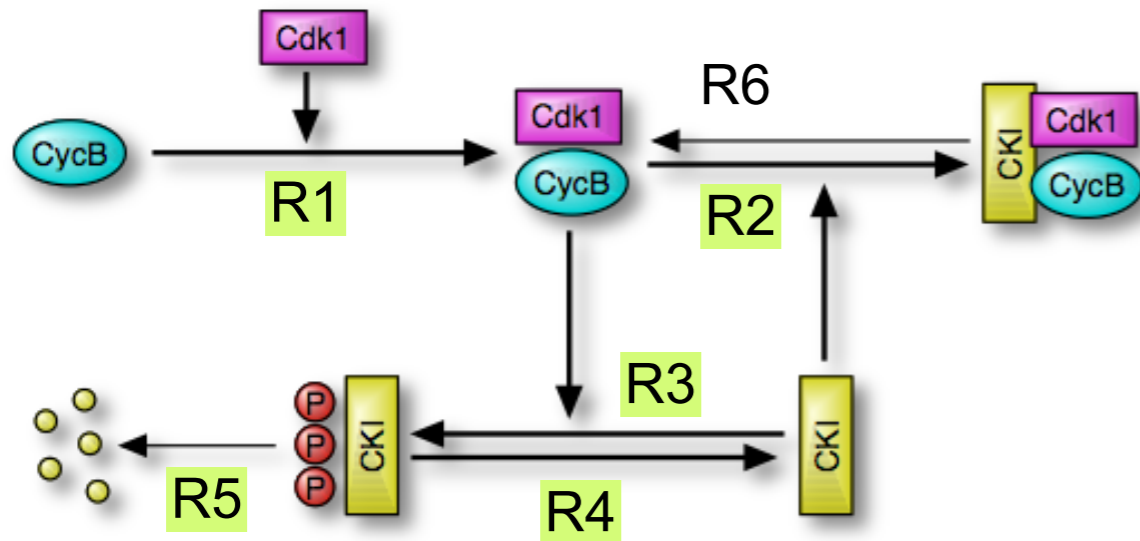
$$\frac{dR3}{dt} = \frac{k_3 R E}{E_0 + E}$$

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

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

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

Rate Equations: G1/S Module



	R1	R2	R3	R4	R5	R6
CycB	-1					
Cdk1	-1					
CycB:Cdk1	1	-1	(C)			1
CKI		-1	-1	1		1
CKI:P ₃			1	-1		
CKI:P ₃					-1	
CycB:Cdk1:CKI		1				-1

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

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

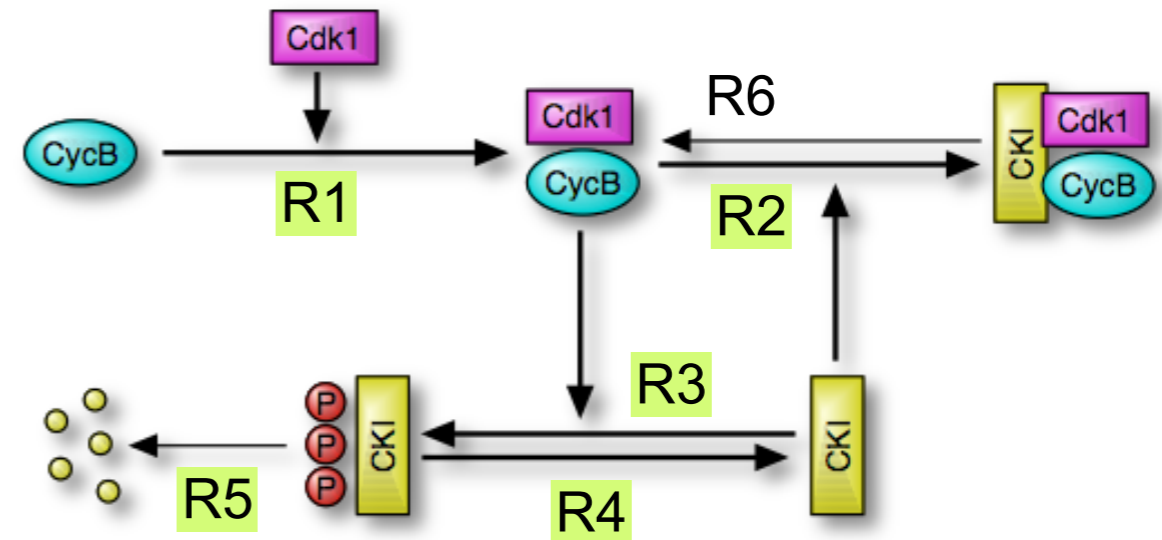
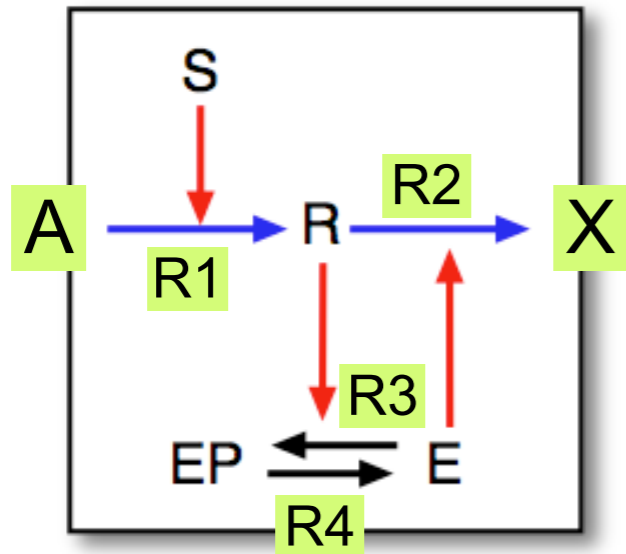
$$\frac{dR3}{dt} = \frac{k_3 [\text{CycB:Cdk1}] [\text{CKI}]}{K_3 + [\text{CKI}]}$$

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

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

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

Comparison: Matrices

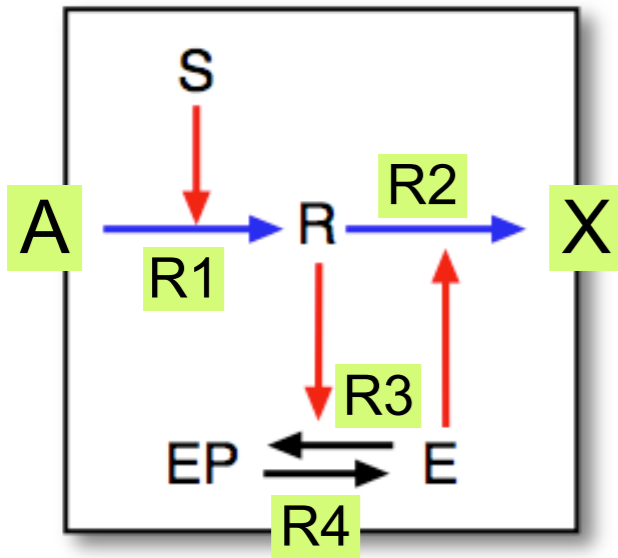


	R1	R2	R3	R4
A	-1			
S	(C)			
R	1	-1	(C)	
E		(C)	-1	1
EP			1	-1
X		1		

	R1	R2	R3	R4	R5	R6
CycB	-1					
Cdk1	-1					
CycB:Cdk1	1	-1	(C)			1
CKI		-1	-1	1		1
CKI:Pi ₃			1	-1		
CKI:Pi ₃					-1	
CycB:Cdk1:CKI		1				-1

Difference: catalysts vs. substrates

Comparison: Equations



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

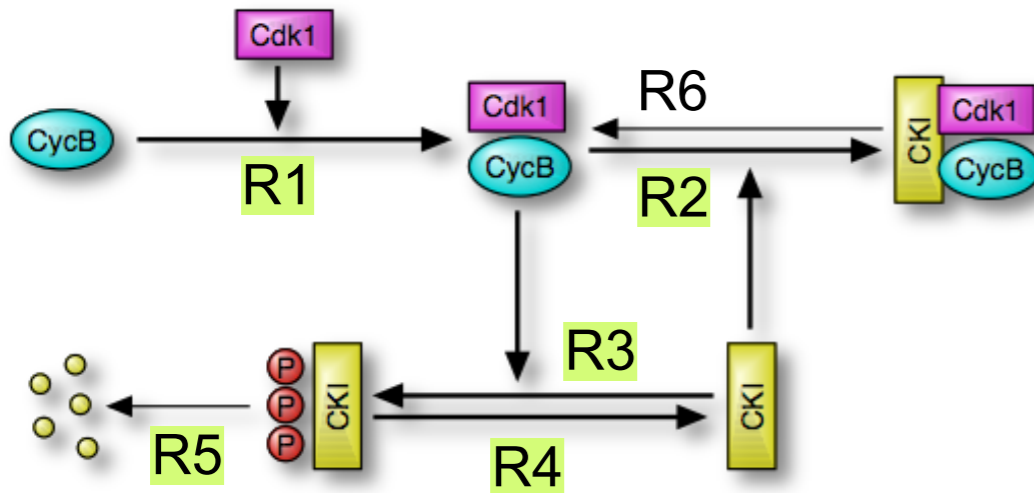
$$\frac{dR2}{dt} = k_2 R E$$

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

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

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

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



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

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

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

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

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

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

Rename species => same rate equations => same behavior

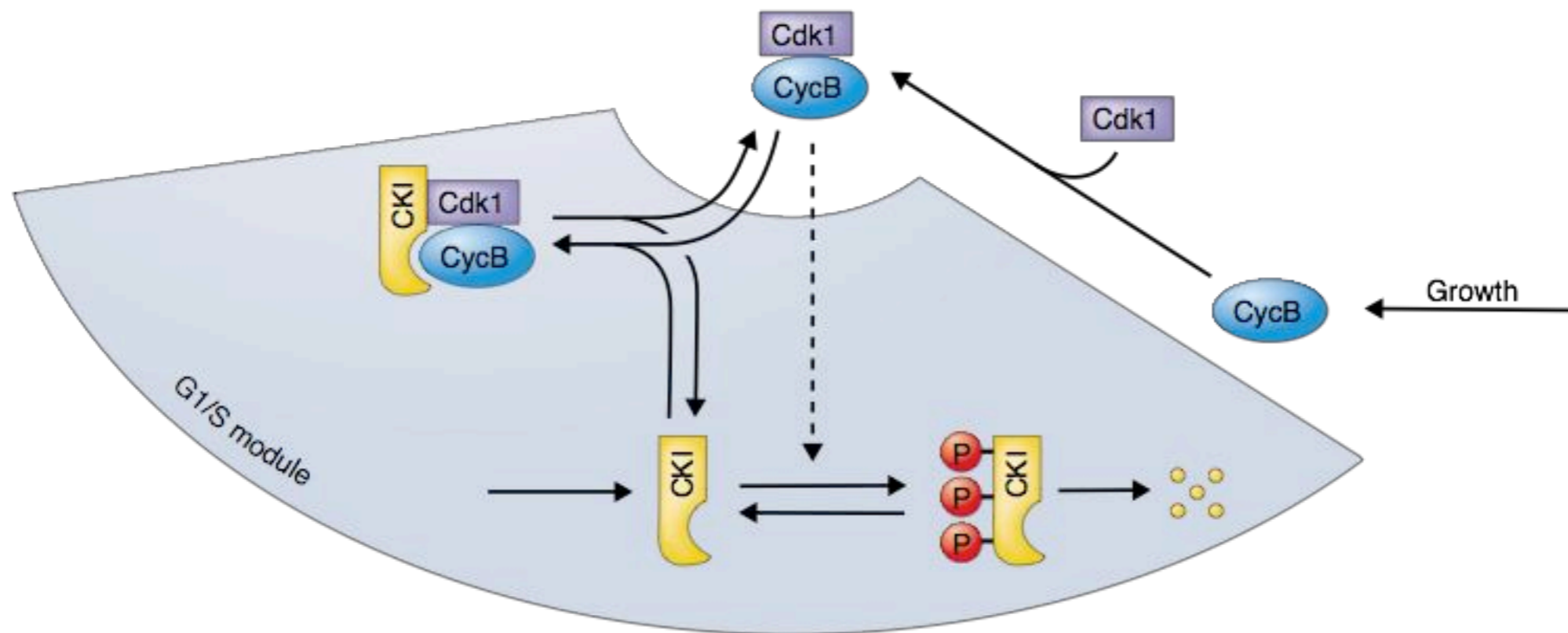
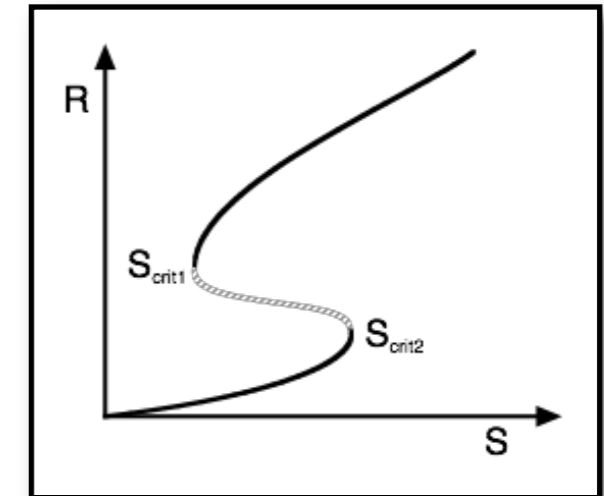
Predicted Behavior: G1 => S

Signal: cell growth = concentration of CycB, Cdk1

Response: activity (concentration) of CycB:Cdk1

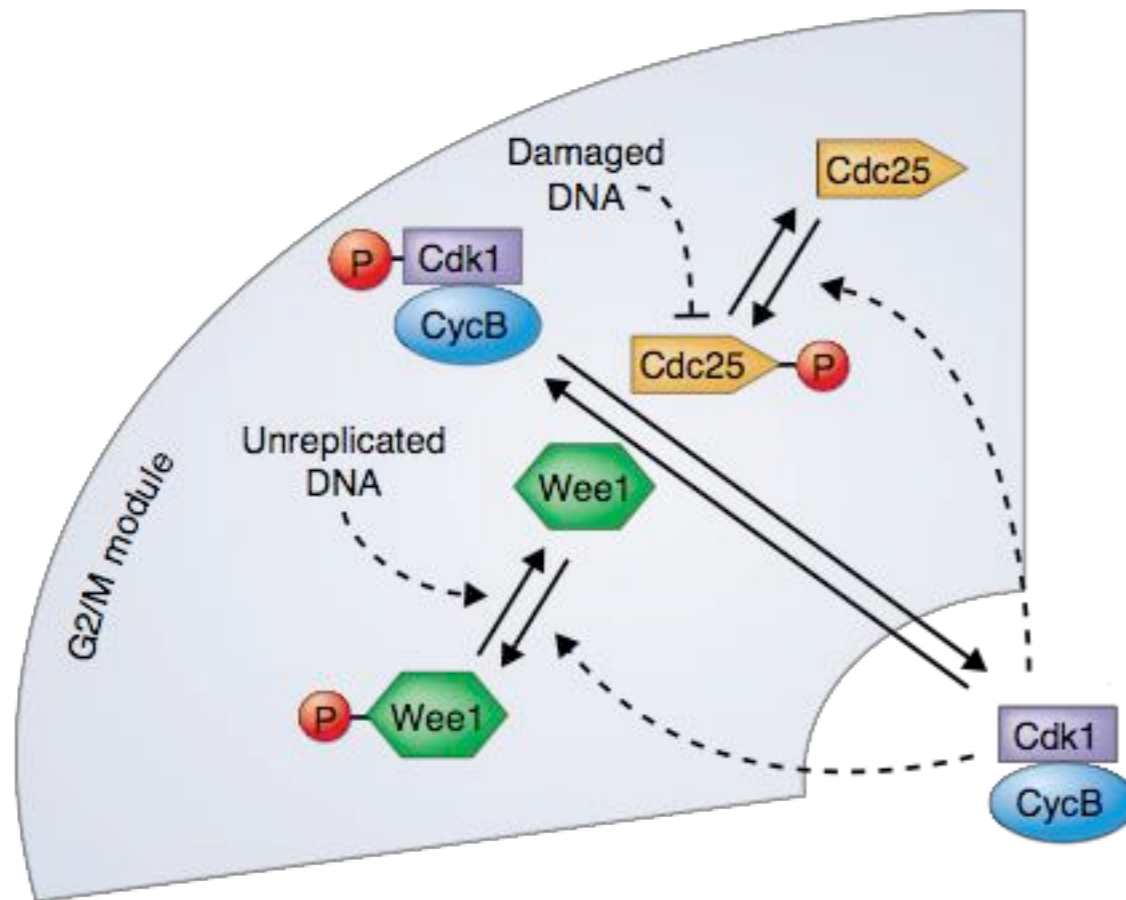
Toggle switch:

=> above critical cell size CycB:Cdk1 activity will switch on



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

G2 => M



Toggle switch:

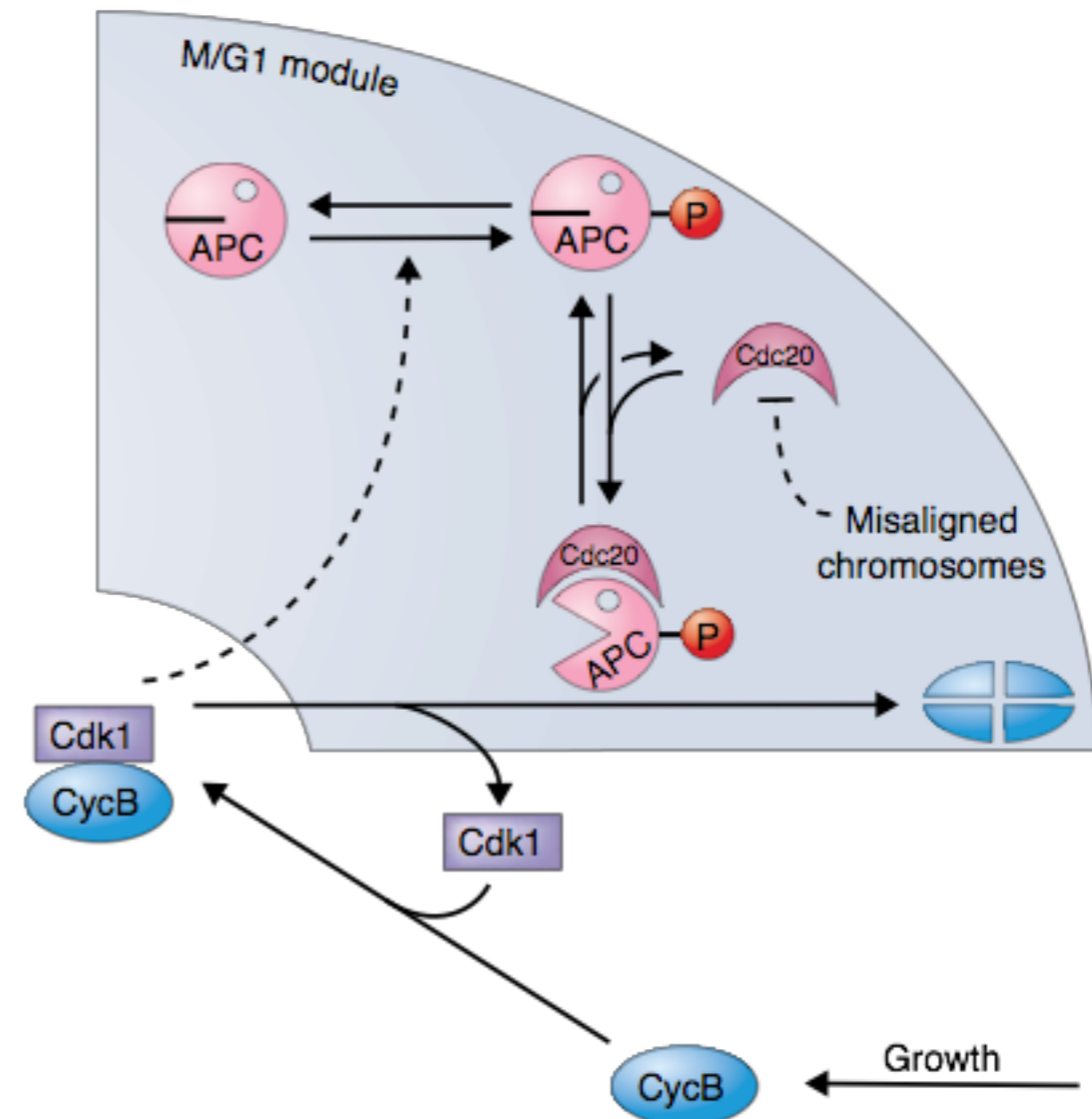
- **mutual activation** between CycB:Cdk1 and Cdc25 (phosphatase that activates the dimer)
- **mutual inhibition** between CycB:Cdk1 and Wee1 (kinase that inactivates the dimer)

=> when the cell **grows** further during the second gap phase G2, the activity of CycB:Cdk1 will **increase** by a further **step**

M => G1

Negative feedback loop oscillator

- i) CycB:Cdk1 activates anaphase promoting complex (APC)
- ii) APC activates Cdc20
- iii) Cdc20 degrades CycB



Behavior:

at a critical cell size

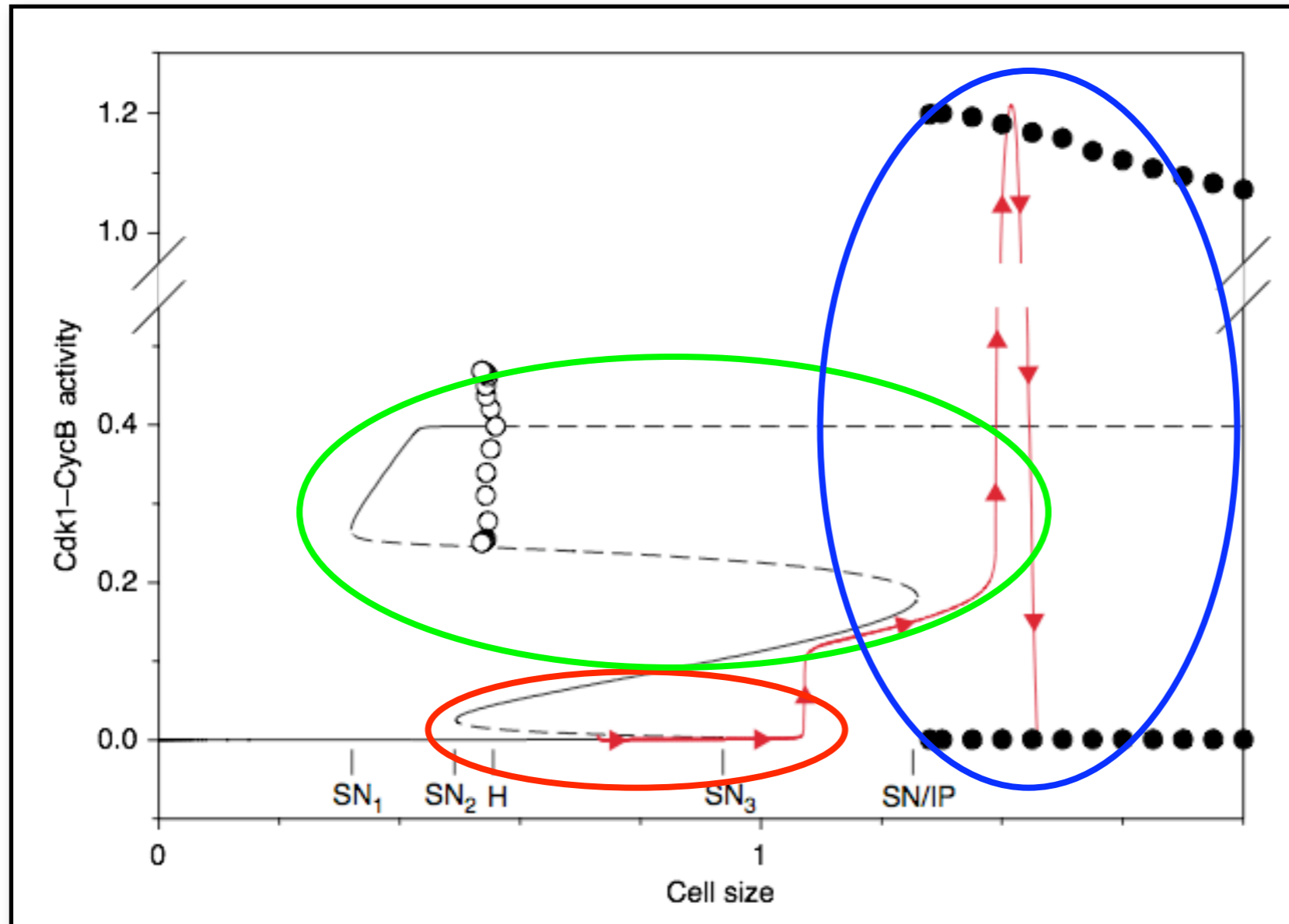
CycB:Cdk1 activity increases and **decreases** again

=> at low CycB:Cdk1 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

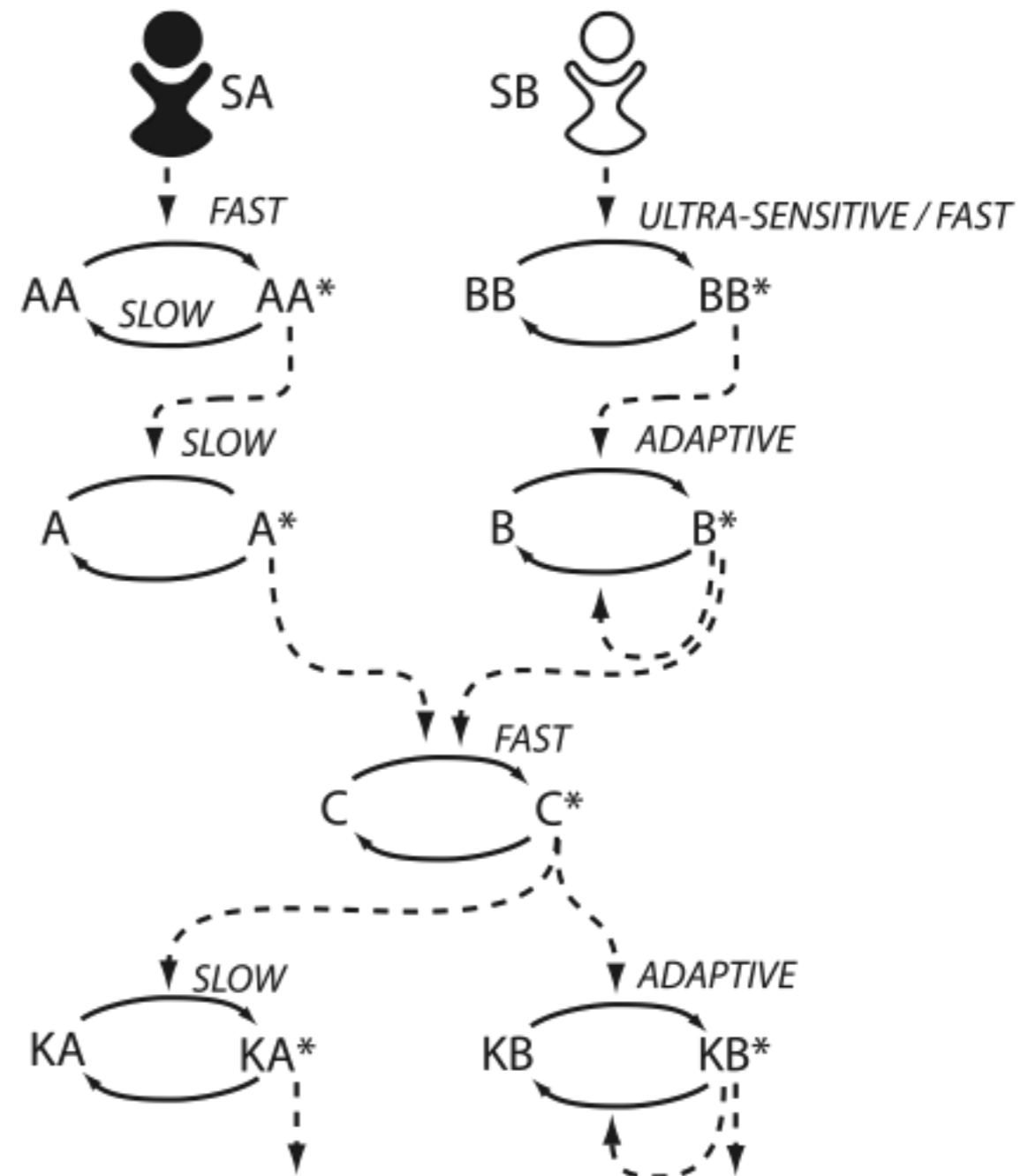
Preventing Cross-Talk

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