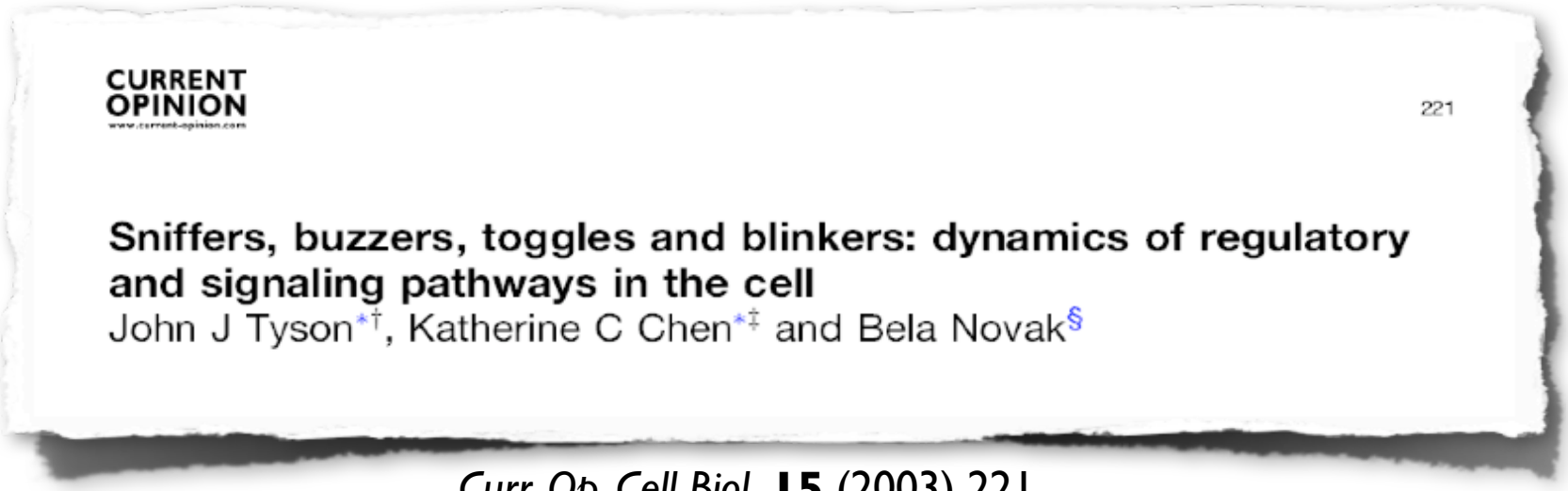
V24 – Kinetic Motifs in Signaling Pathways

- Types of kinetic motifs in signaling pathways
- Application to cell cycle
- Circadian clocks



CURRENT OPINION

221

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

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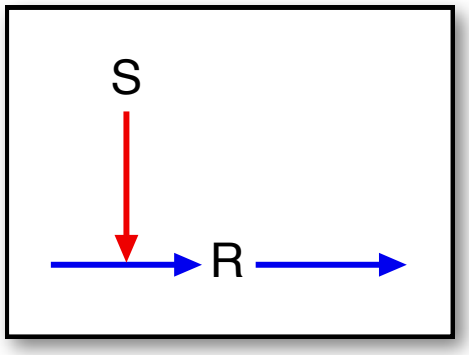
Curr. Op. Cell Biol. **15** (2003) 221

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)

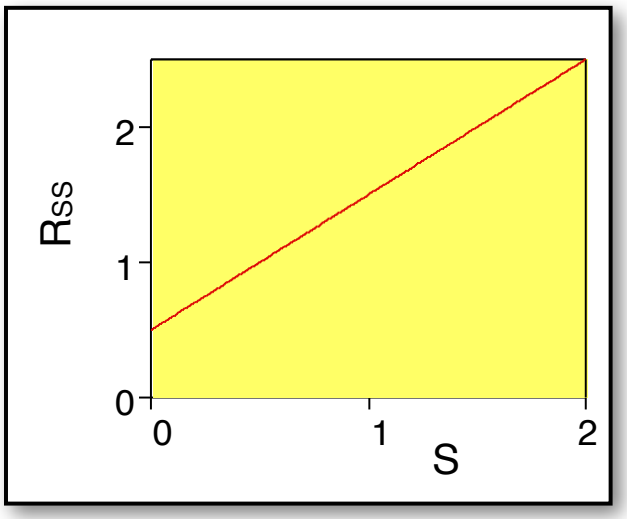


$$\frac{dR}{dt} = k_0 + k_1 S - k_2 R$$

At steady state (which implies S = const):

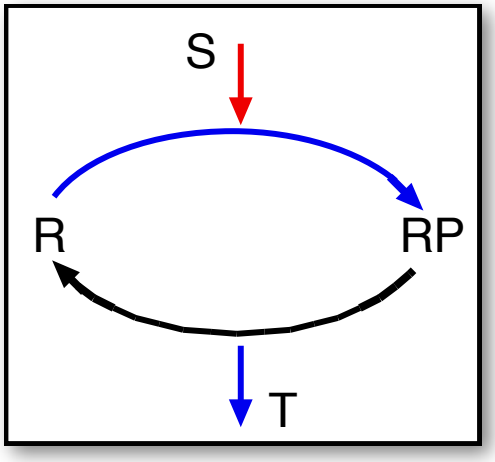
$$\left.\frac{dR}{dt}\right|_{R=R_{ss}} = 0 \quad \Rightarrow \quad R_{ss} = \frac{k_0 + k_1 S}{k_2} = \frac{k_0}{k_2} + \frac{k_1}{k_2} S$$

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

S + R => RP

RP => R + T

with $R_{tot} = R + RP$

↑ phosphorylated form

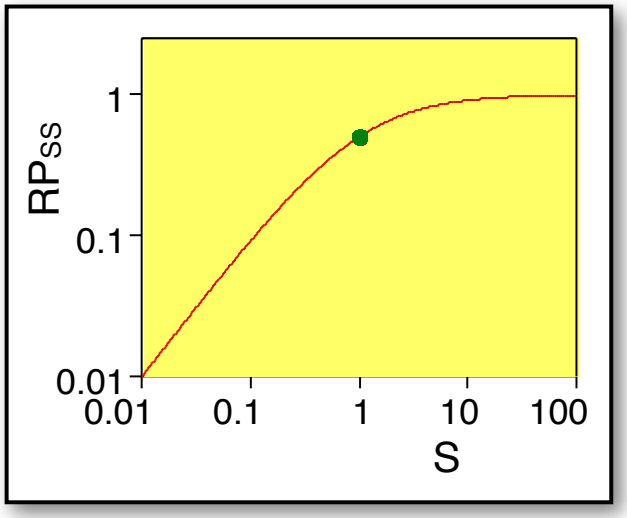
$$\frac{dRP}{dt} = k_1 SR - k_2 RP = k_1 S(R_{tot} - RP) - k_2 RP$$

Find steady state for RP: linear until saturation

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

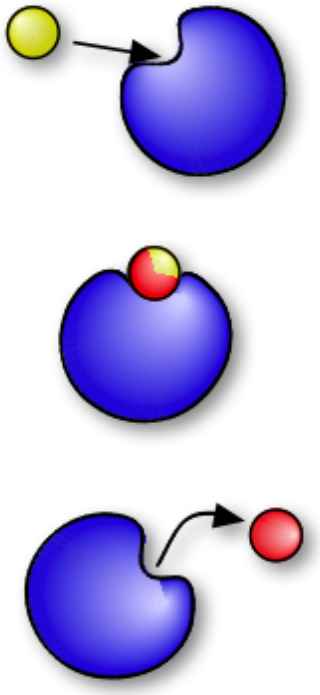
Output T proportional to RP level:

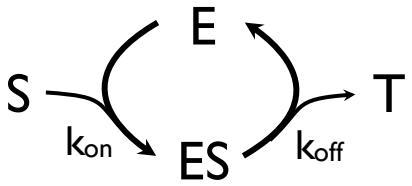
$$\frac{dT}{dt} = k_2 RP$$



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

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:

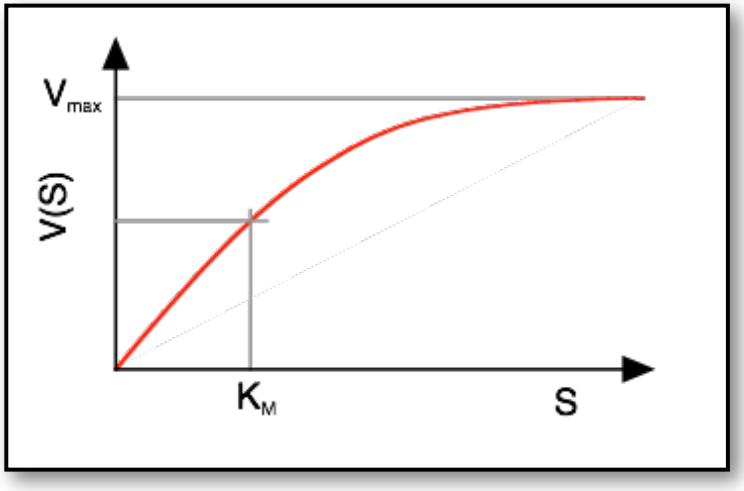
$$E_T = E + ES \quad \Rightarrow \quad ES = E_T \frac{S}{S + K_M}$$

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

The MM-equation

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

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



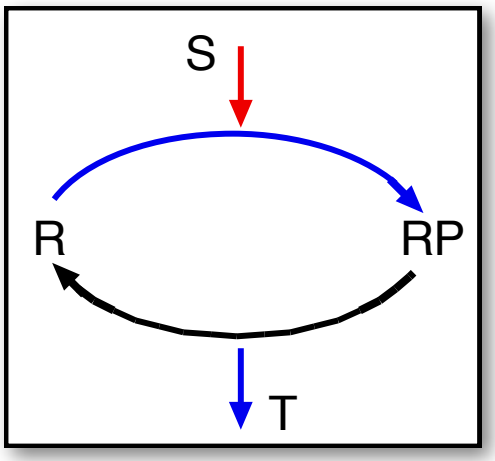
$$K_M = \frac{k_{off}}{k_{on}}$$

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 => V_{max}, K_M

Sigmoidal Characteristics with MM kinetics



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

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

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

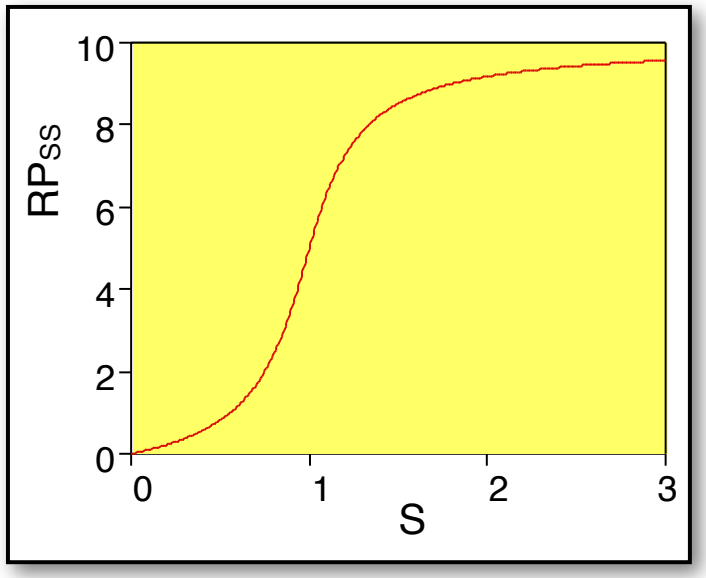
this means that $S = R_t - RP$

$K_M = R_0$

Quadratic equation for RP

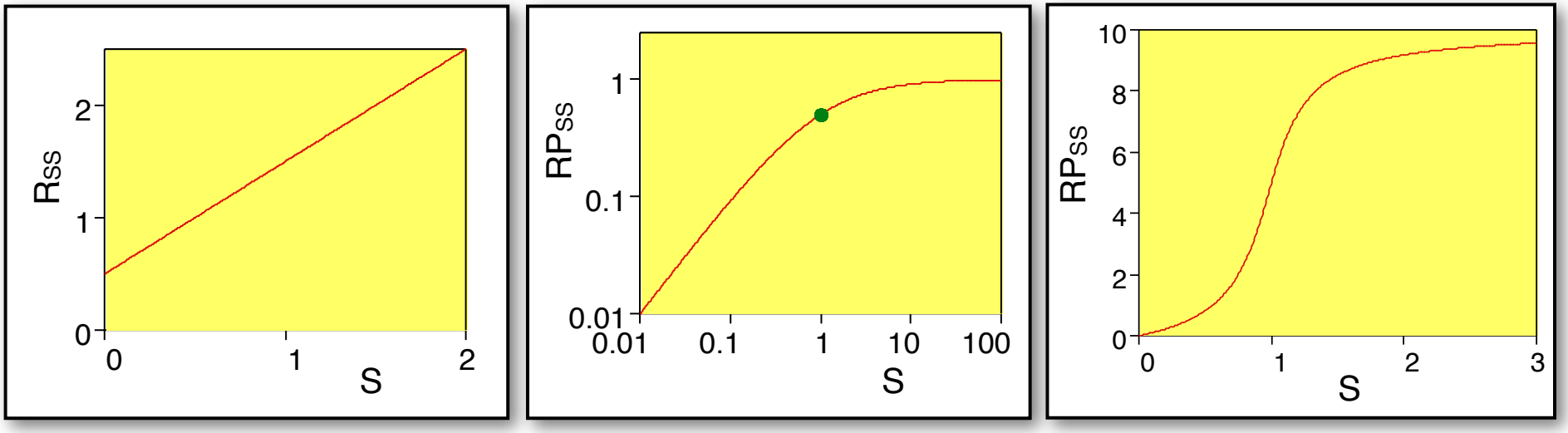
$$k_2\,RP(R_o + (R_t - R_p)) = k_1 S(R_t - RP)(RP_0 + RP)$$

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



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

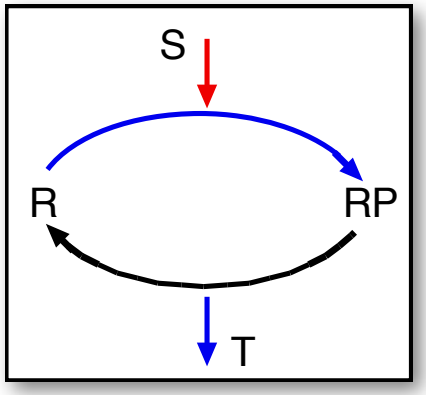
Graded Response



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



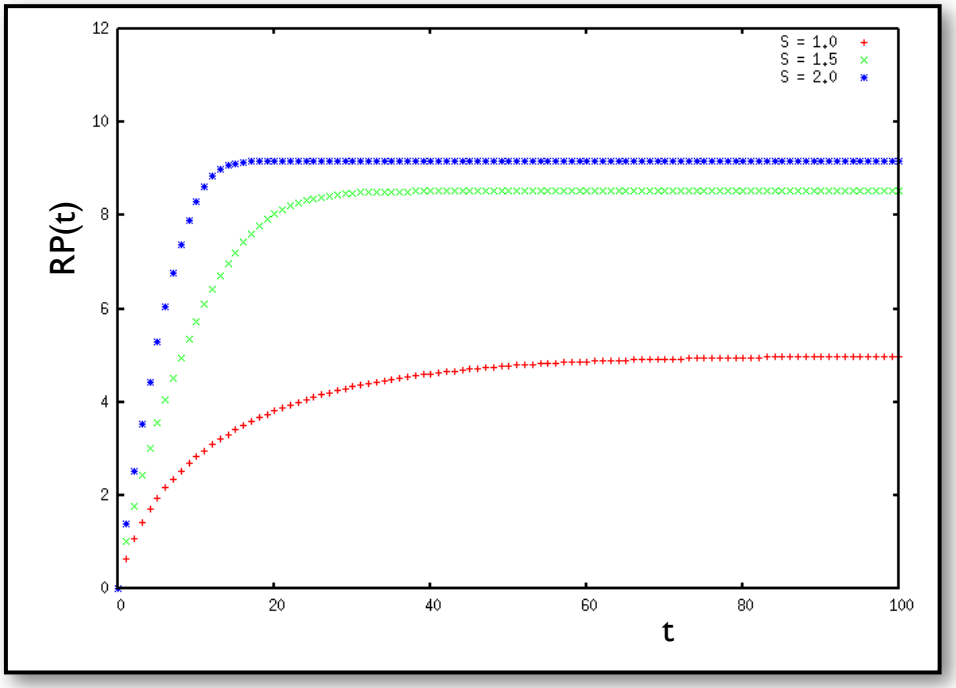
Direct implementation:

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

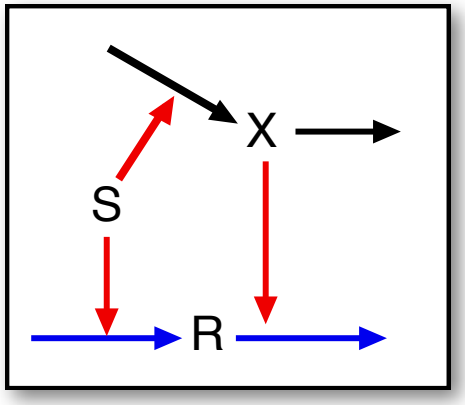
Parameters: k1 = 1 $(\text{mol s})^{-1}$, k2 = 1 s^{-1}, $R_0 = RP_0 = 1$ mol
Initial conditions: R = 10 mol, RP = 0

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

$$\frac{dX}{dt} = k_3 S - k_4 X \qquad \frac{dR}{dt} = k_1 S - k_2 X R$$

Steady state: R_{ss} independent of S

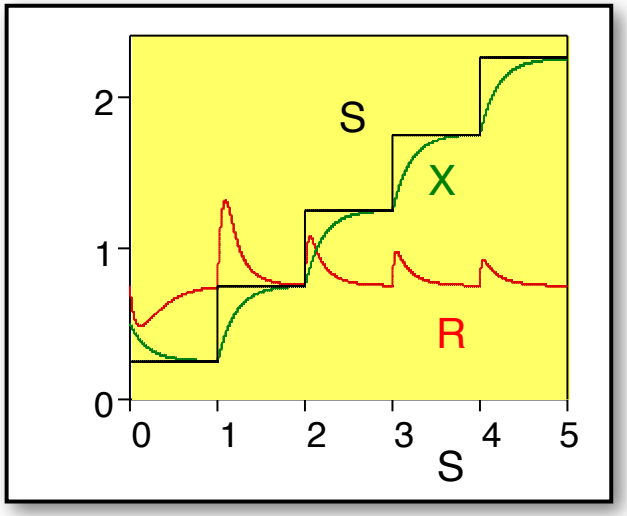
$$X_{ss} = \frac{k_3}{k_4} S \qquad R_{ss} = \frac{k_1 k_4}{k_2 k_3}$$

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

found in smell, vision, chemotaxis, …

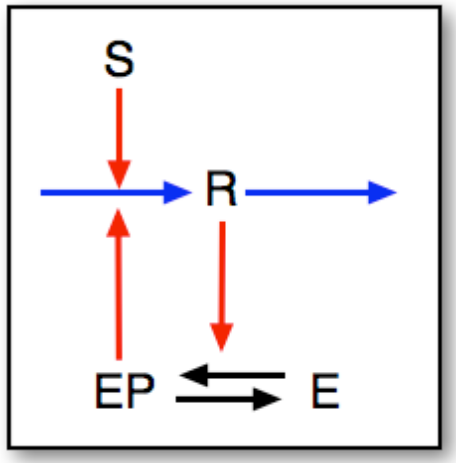
Note: response strength ΔR depends on rate of change of S.

=> non-monotonous relation for R(S)



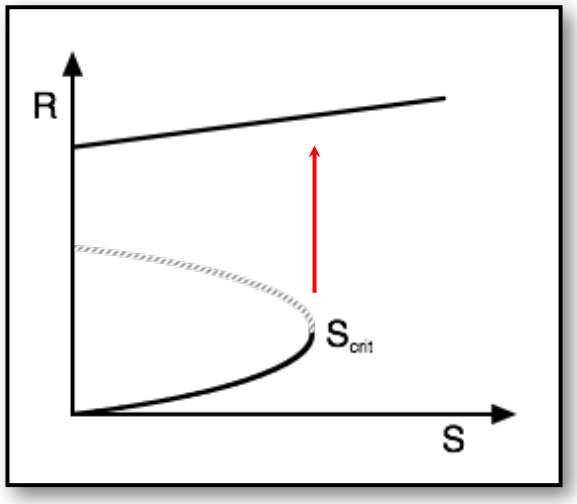
$k_1 = 30, k_2 = 40, k_3 = k_4 = 5$

Positive Feedback



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

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

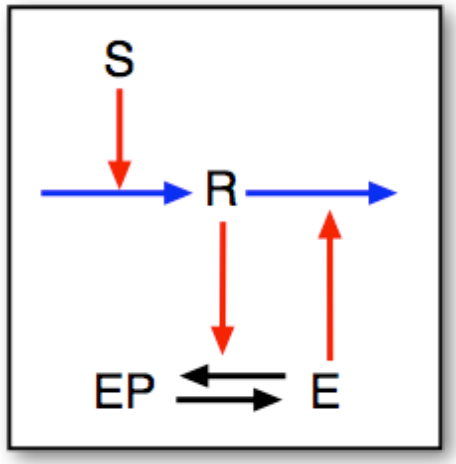


Feedback via R and EP
=> high levels of R will stay

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

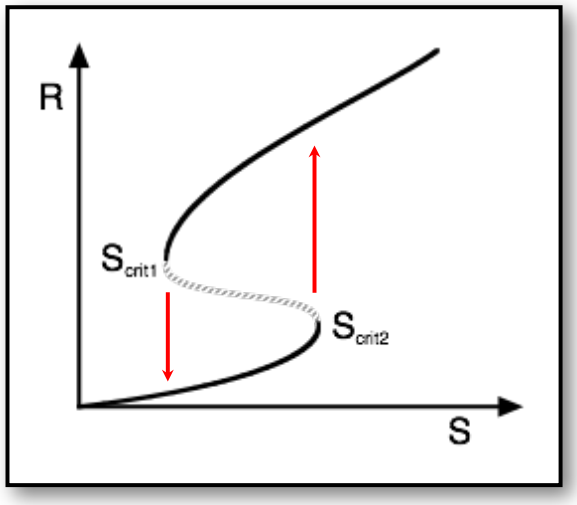
Found in processes that are "final":
frog oocyte maturation, apoptosis, …

Mutual Inhibition - Toggle Switch



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

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

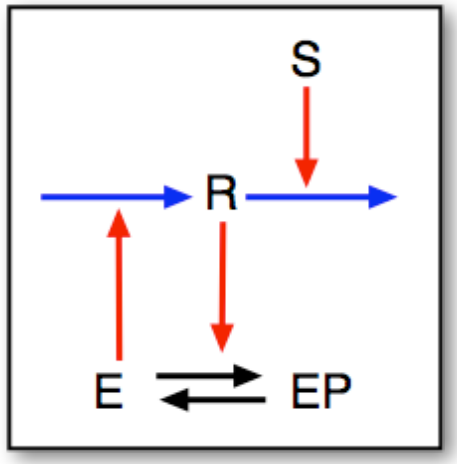


Sigmoidal "threshold" in E <=> EP leads to bistable response (hysteresis):
toggle switch (dt. *Kippschalter*)

Converts continuous external stimulus into two well defined stable states:

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

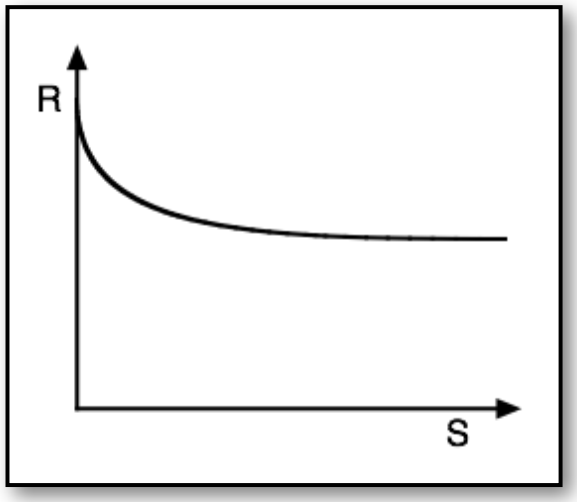
Negative Feedback



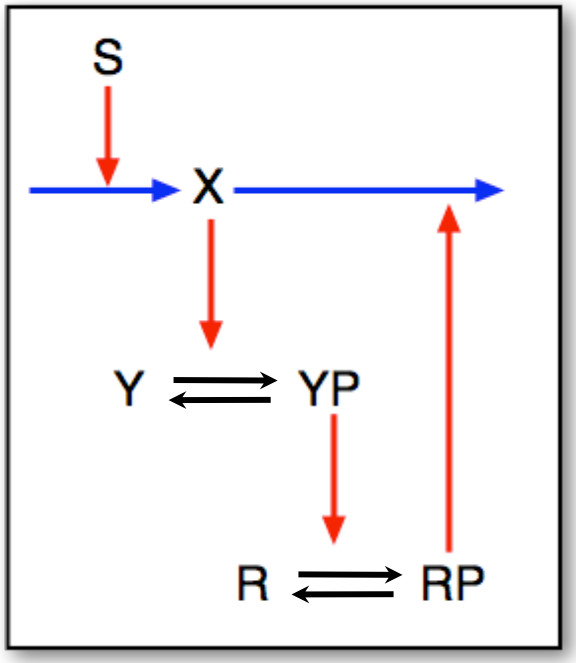
S controls the "demand" for R

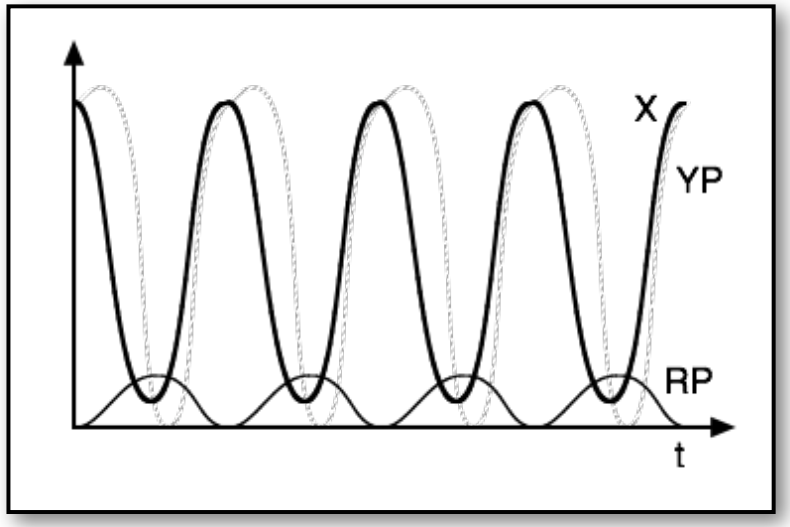
=> **homeostasis**

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



Negative Feedback with Delay





Cyclic activation X => YP => RP => X
=> **Oscillations** (in a range of S)

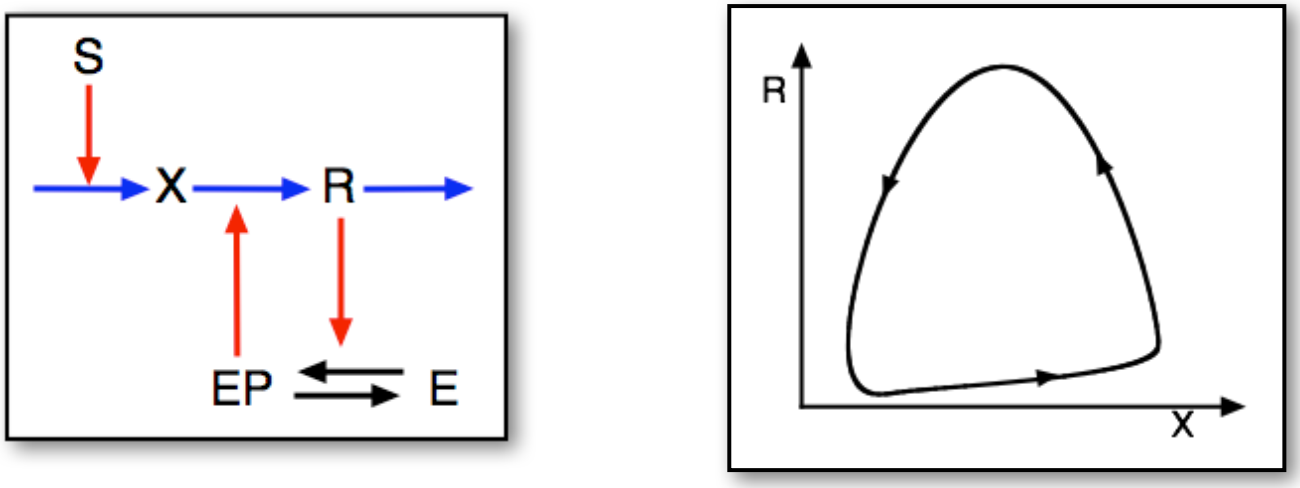
$$\frac{dX}{dt} = k_0 + k_1 S - k_2 X - k_7 RP\ X$$

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

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

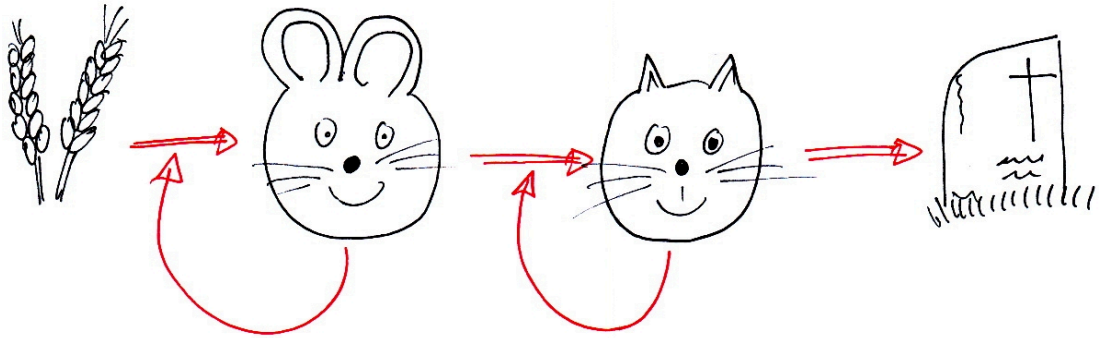
Proposed mechanism
for **circadian clocks**

Substrate-Depletion Oscillations

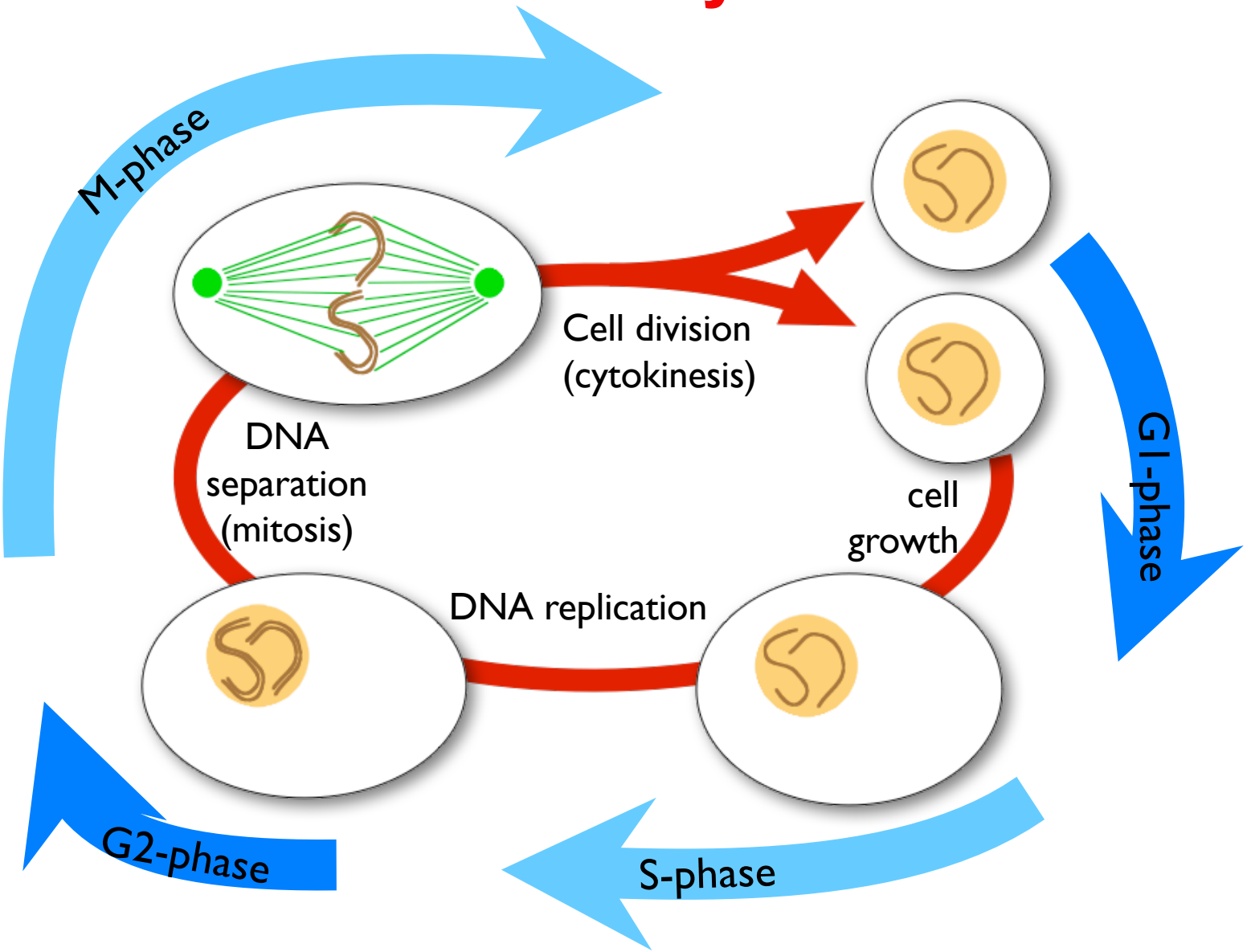


R is produced in an **autocatalytic** reaction from X, finally **depleting** X…

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



The Cell Cycle



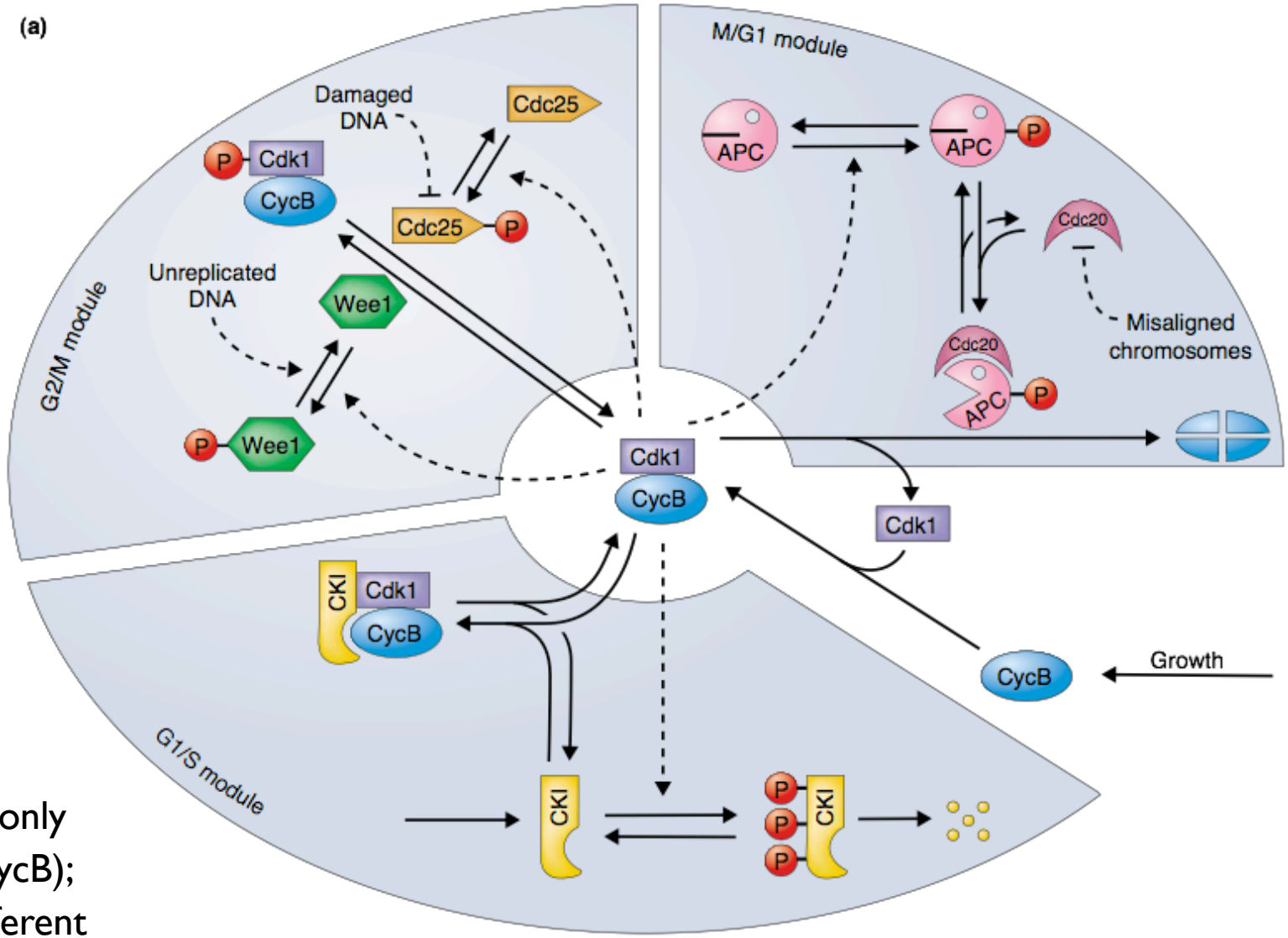
When to take the **next step**???

Simplified Version of Cell Cycle Control System

cdc =
"cell division cycle"

Cdk1: cyclin dependent kinase 1

Simplification: assume only one type of cyclins (CycB); in reality there are different ones



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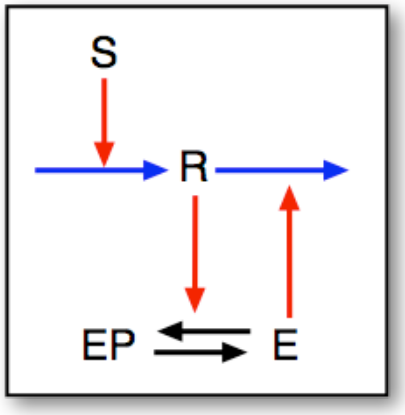
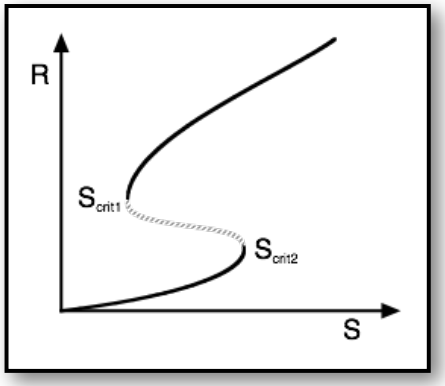
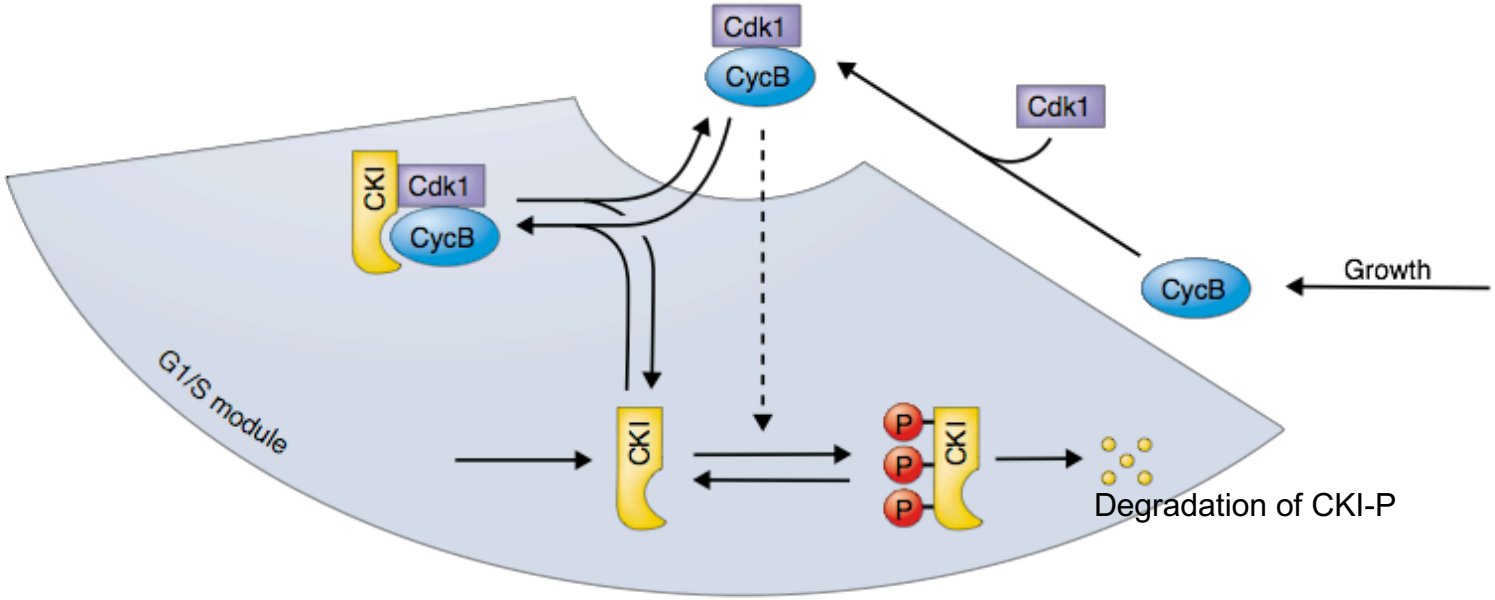
G1 => S — Toggle Switch



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





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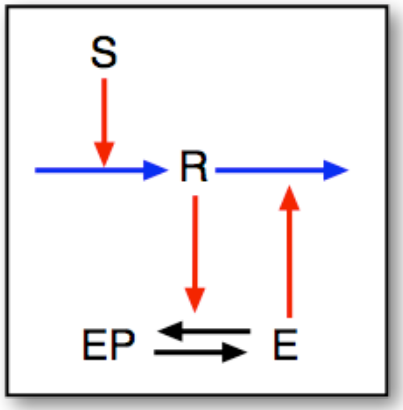
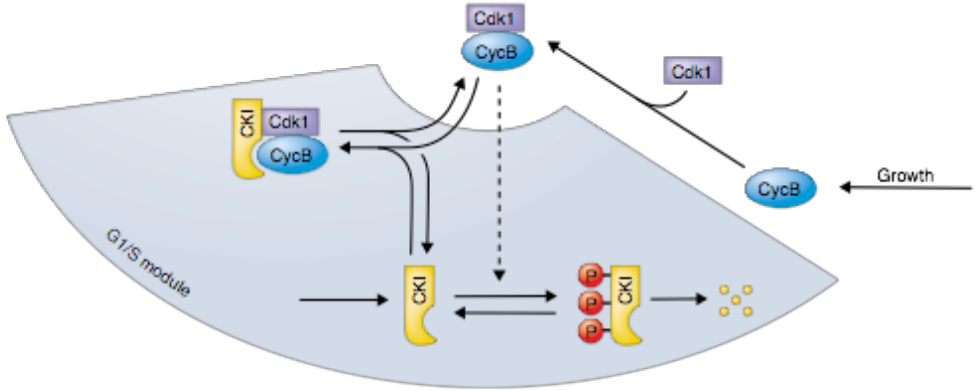
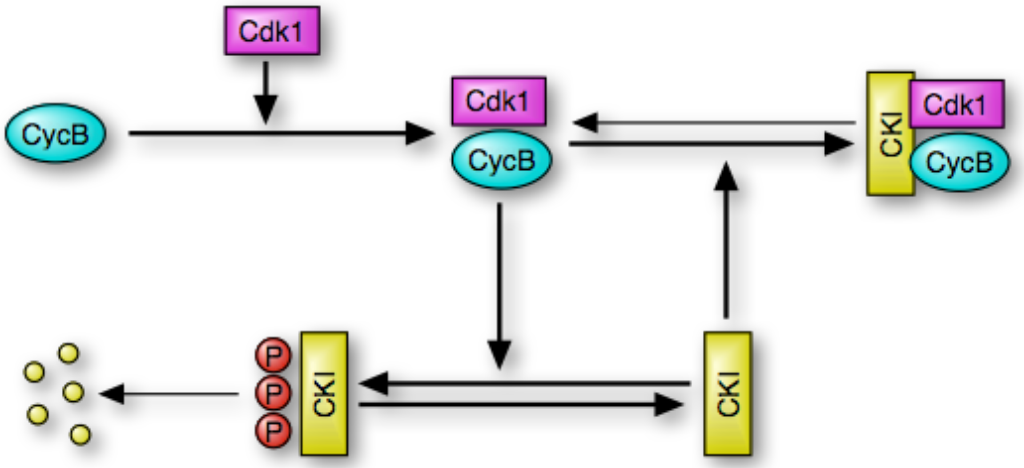
Mutual Inhibition



???

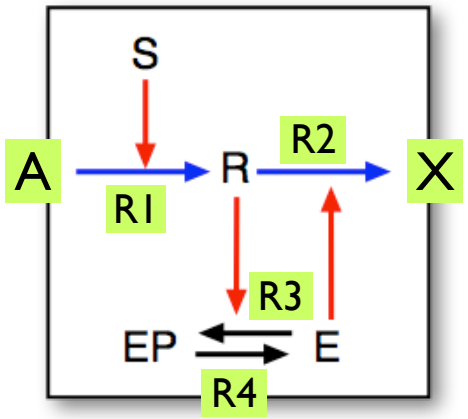


Assume: CycB:Cdk1:CKI is stable <=> dissociation is very slow



=> same **topology**
<=> 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		

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

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

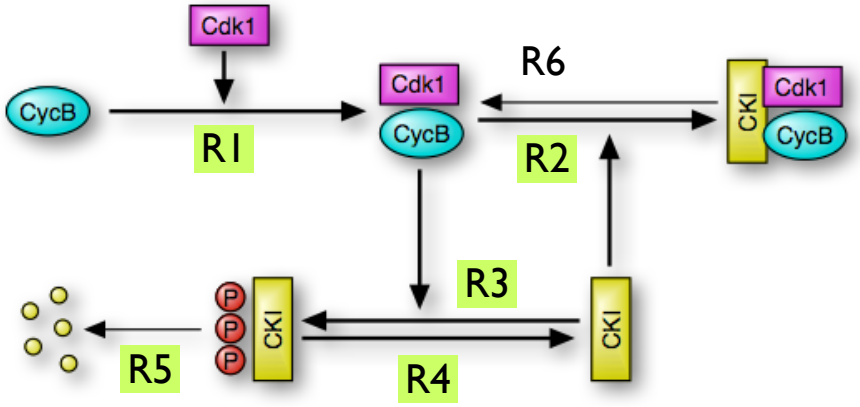
$$\frac{dR3}{dt} = \frac{k_3\ R\ E}{E_0 + E}$$

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

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

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

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

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

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

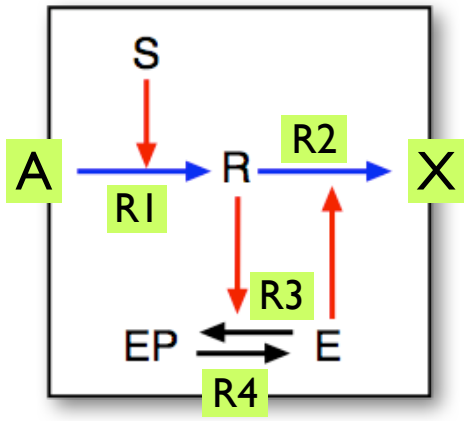
$$\frac{dR3}{dt} = \frac{k_3\,[\mathrm{CycB{:}Cdk1}]\,[\mathrm{CKI}]}{K_3 + [\mathrm{CKI}]}$$

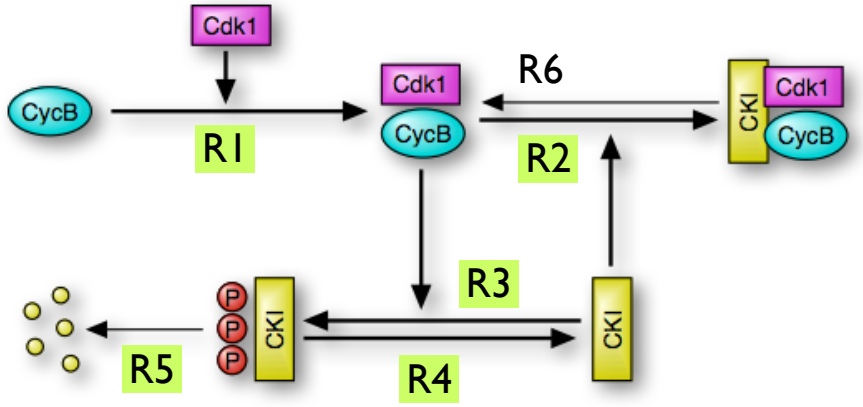
$$\frac{dR4}{dt} = \frac{V_4\,[\mathrm{CKI{:}P_3}]}{K_4 + [\mathrm{CKI{:}P_3}]}$$

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

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

Comparison: Matrices



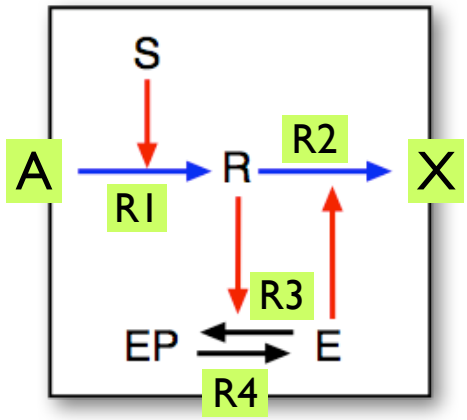


	R1	R2	R3	R4
A	–1			
S	(C)			
R	1	–1	(C)	
E		(C)	–1	1
EP			1	–1
X		1		

	R1	R2	R3	R4	R5	R6
CycB	–1					
Cdk1	–1					
CycB:Cdk1	1	–1	(C)			1
CKI		–1	–1	1		1
CKI:P_3			1	–1		
CKI:P_3					–1	
CycB:Cdk1:CKI		1				-1

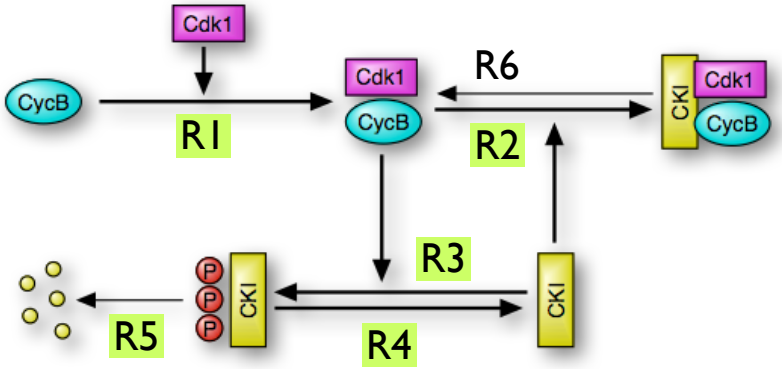
Difference: catalysts vs. substrates

Comparison: Equations



$$\frac{dR1}{dt} = k_1\ A\ S$$
$$\frac{dR2}{dt} = k_2\ R\ E$$
$$\frac{dR3}{dt} = \frac{k_3\ R\ E}{E_0 + E}$$
$$\frac{dR4}{dt} = \frac{V_4\ EP}{EP_0 + EP}$$

$$\frac{dR}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} = k_1\ A\ S - k_2\ R\ E$$
$$\frac{dE}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt} = \frac{k_3\ R\ E}{E_0 + E} - \frac{V_4\ EP}{EP_0 + EP}$$



$$\frac{dR1}{dt} = k_1\ [\text{CycB}]\ [\text{Cdk1}]$$
$$\frac{dR2}{dt} = k_2\ [\text{CycB:Cdk1}]\ [\text{CKI}]$$
$$\frac{dR3}{dt} = \frac{k_3\ [\text{CycB:Cdk1}]\ [\text{CKI}]}{K_3 + [\text{CKI}]}$$
$$\frac{dR4}{dt} = \frac{V_4\ [\text{CKI:P}_3]}{K_4 + [\text{CKI:P}_3]}$$

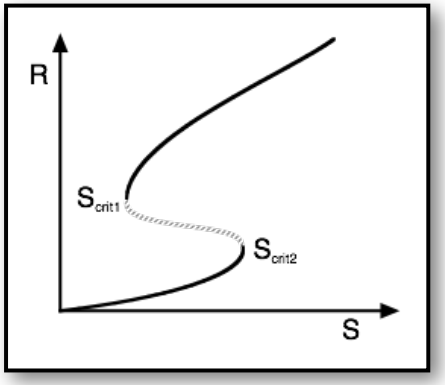
$$\frac{d[\text{CycB:Cdk1}]}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} + \frac{dR6}{dt}$$
$$\frac{d[\text{CKI}]}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt} - \frac{dR2}{dt} + \frac{dR6}{dt}$$

Rename species => same rate equations => same behavior

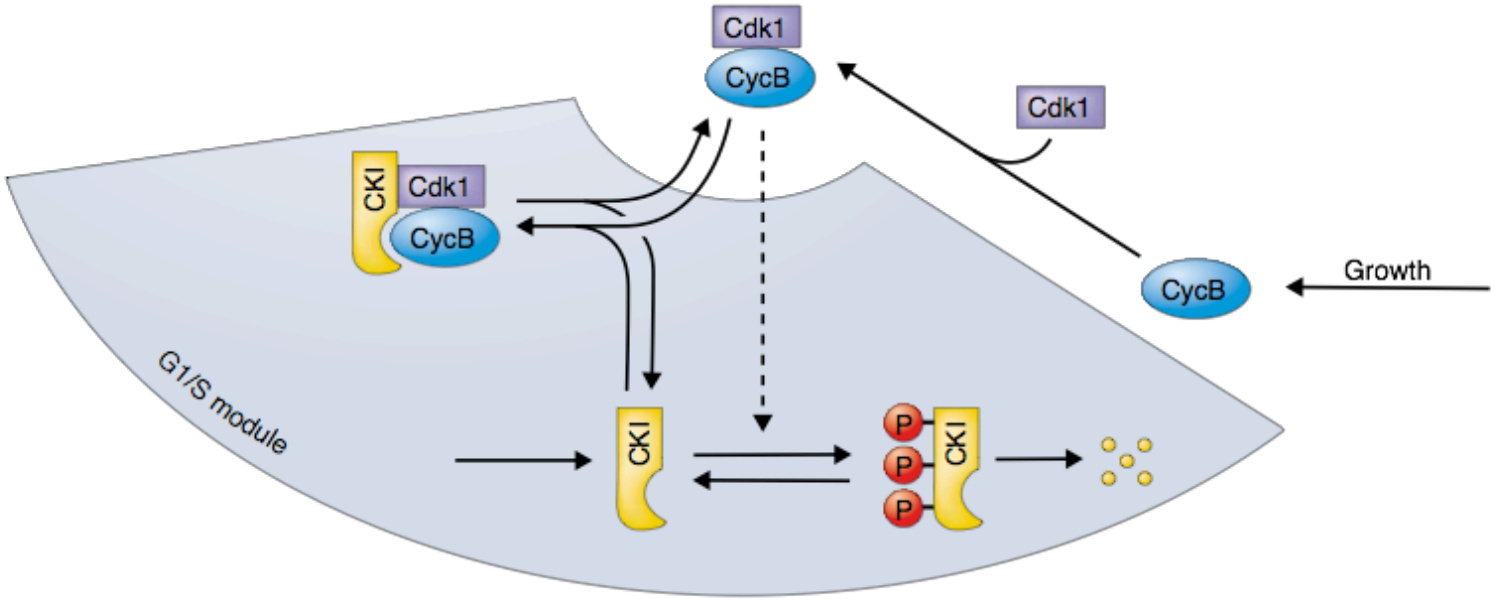
Predicted Behavior: G1 => S

Signal: cell growth = concentration of CycB, Cdk1

Response: activity (concentration) of CycB:Cdk1

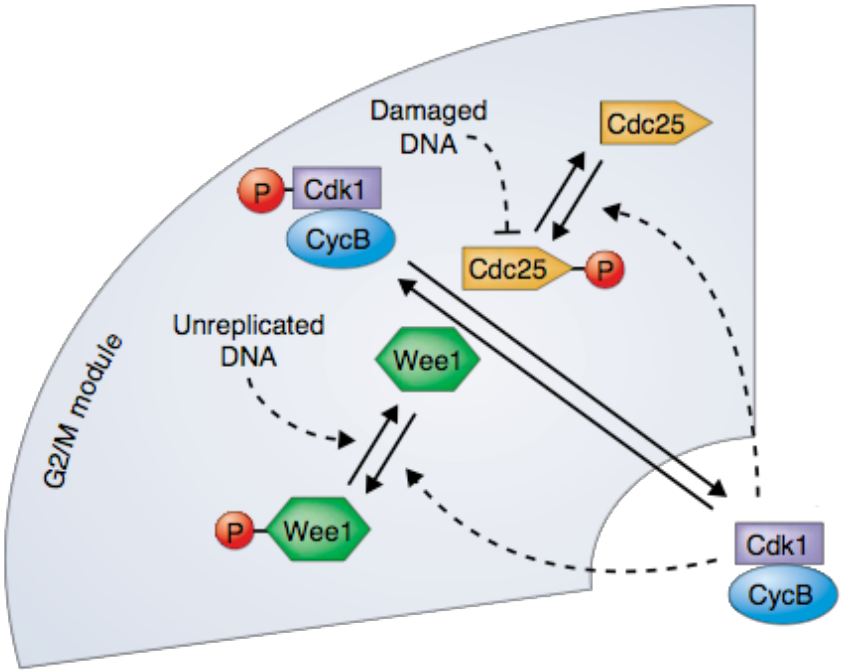


Toggle switch:
=> above critical cell size, CycB:Cdk1 activity will switch on



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

G2 => M



Dual 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)

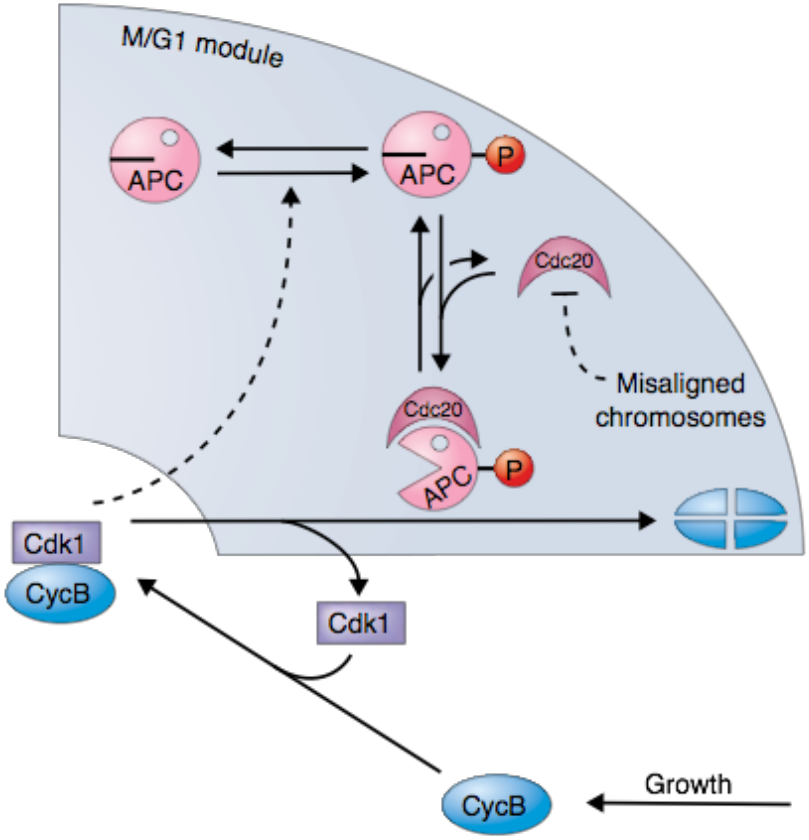
=> when the cell **grows** further during the second gap phase G2, the activity of CycB:Cdk1 will **increase** by a further **step**

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

M => G1

Negative feedback loop oscillator

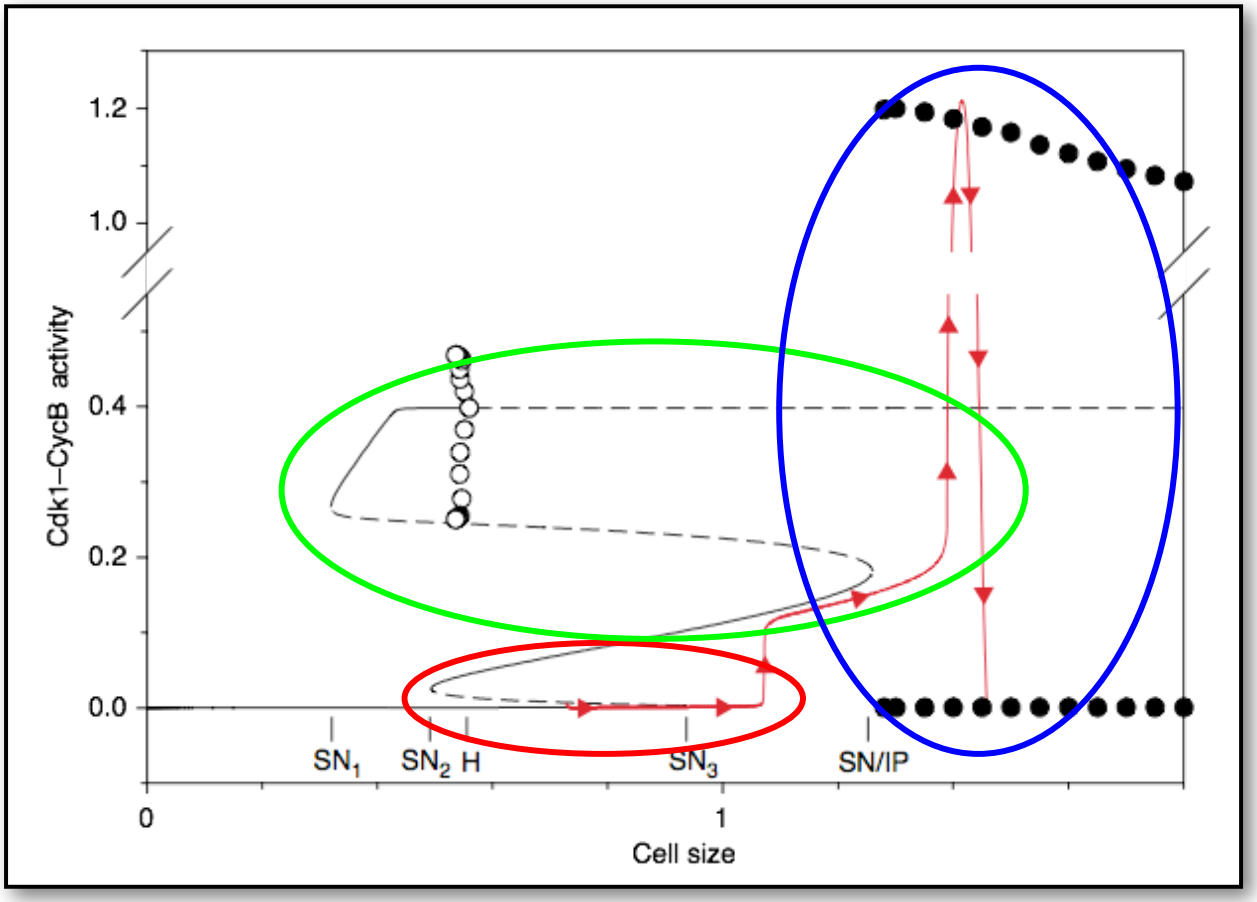
i) CycB:Cdk1 activates anaphase promoting complex (APC)
ii) APC-P activates Cdc20
iii) Cdc20:APC-P degrades CycB



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

Circadian clocks in mammals and plants

Most organisms (animals, plants, fungi and cyanobacteria) enhance their fitness by coordinating their development with daily environmental changes through molecular timekeepers (circadian clocks)

Mammals display circadian rhythms in behavioural and physiological processes, such as

- sleep
- feeding
- blood pressure and
- metabolism

Roles in **plants** e.g.:

- opening of flowers in the morning and their closure at night

Circadian rhythms are guided by **external light–dark signals**
that are integrated through intrinsic central and peripheral molecular clocks

McClung Plant Cell 18, 792 (2006)

Circadian rhythms

(1) Circadian rhythms are the subset of biological rhythms with period of 24 h.
The term circadian combines the Latin words ‘‘circa’’ (about) and ‘‘dies’’ (day).

(2) Circadian rhythms are **endogenously generated** and **self-sustaining**.

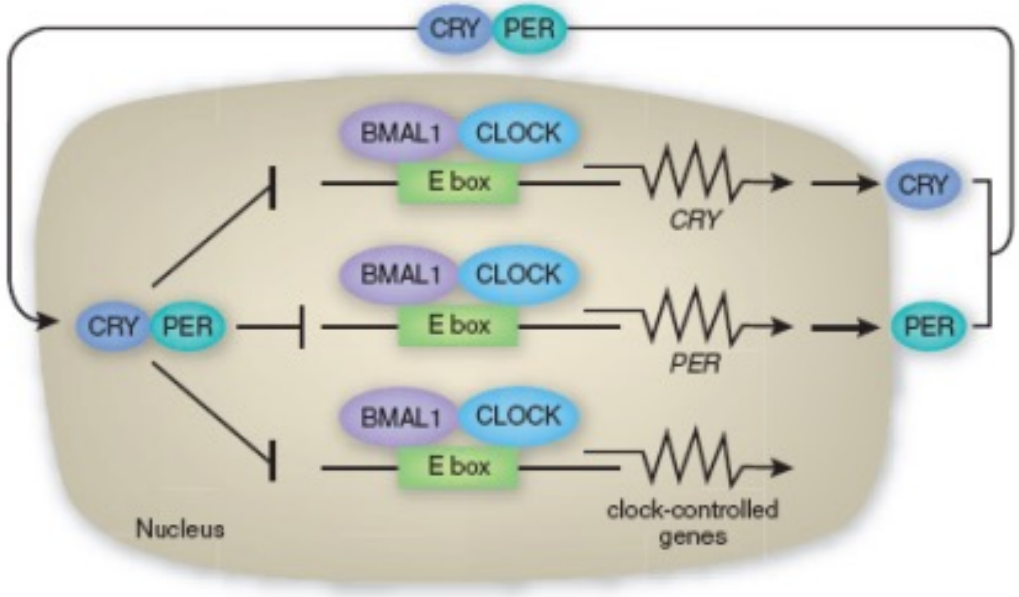
They persist under constant environmental conditions, typically constant light (or dark) and constant temperature.
Under these controlled conditions, the free-running period of **24 h** is observed.

(3) For all circadian rhythms the **period** remains relatively **constant** over a range of ambient temperatures.

This is thought to be one property of a general mechanism that buffers the clock against changes in cellular metabolism.

McClung Plant Cell 18, 792 (2006)

Basic molecular elements of mammalian clocks



This is the **minimal scheme** for the mammalian clock.

It requires several interconnecting transcriptional, translational and post-translational loops to achieve gene expression with circadian periodicity

(a) 2 TFs **CLOCK** and **BMAL1** **heterodimerize**.

(b) BMA1:CLOCK binds to the **E-boxes** in the promoters of
-the *PER* and *CRY* genes,
- and of clock-controlled genes,
and activate their transcription.

(c) The translated PER and CRY proteins dimerize in the cytosol, enter the nucleus and **inhibit** CLOCK-BMAL1–activated transcription.

Sancar,
Nat. Struct. Mol. Biol. 15, 23 (2008)

Circuit of circadian rhythms in mammals

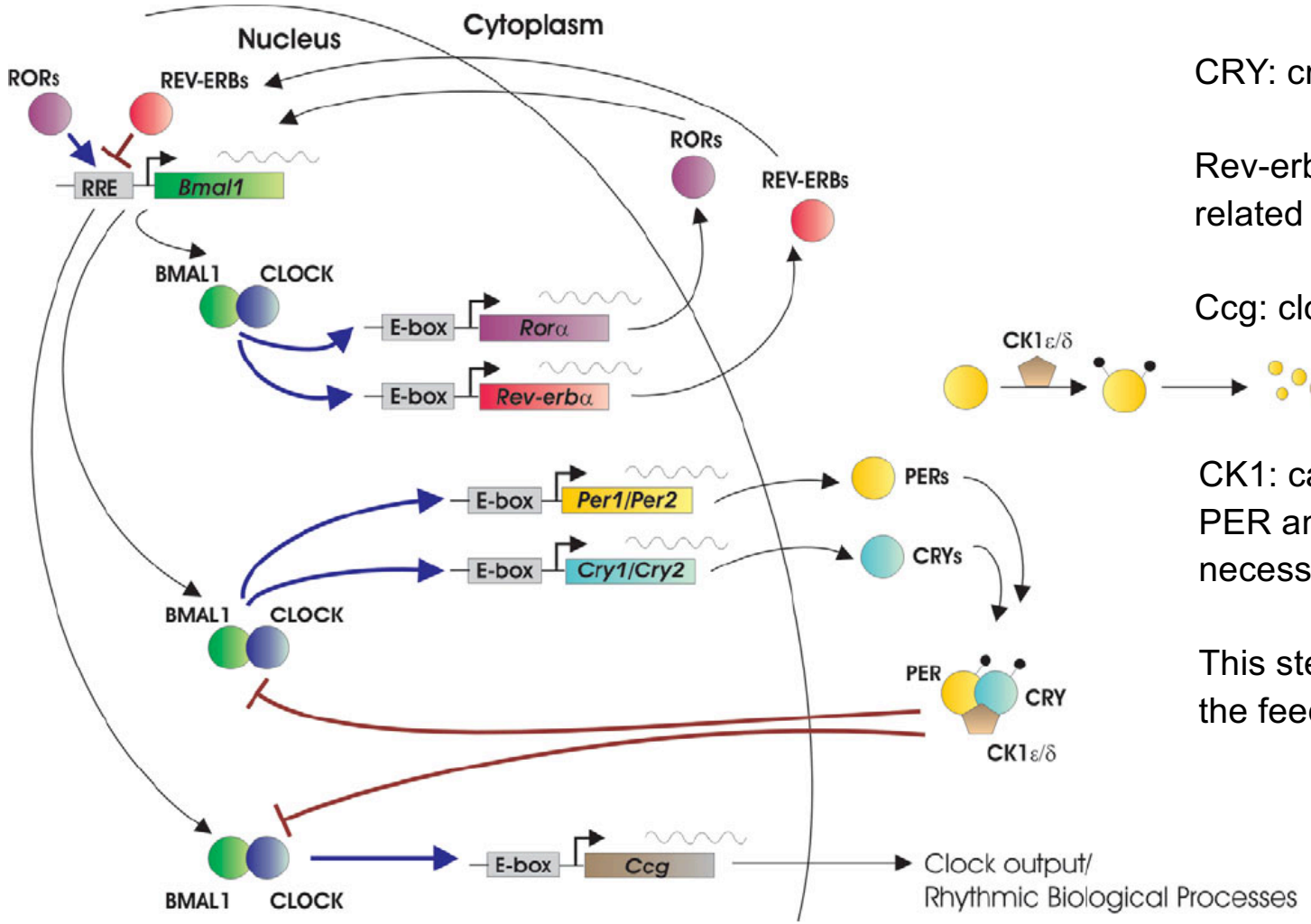


Figure 1. A network of transcriptional–translational feedback loops constitutes the mammalian circadian clock.

PER: period

CRY: cryptochrome

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

Ccg: clock-controlled genes

CK1: casein kinase; phosphorylates PER and CRY;
necessary for their dimerization

This step serves to slow down the feed-back cycle.

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

Are circadian rhythms relevant for bioinformatics?

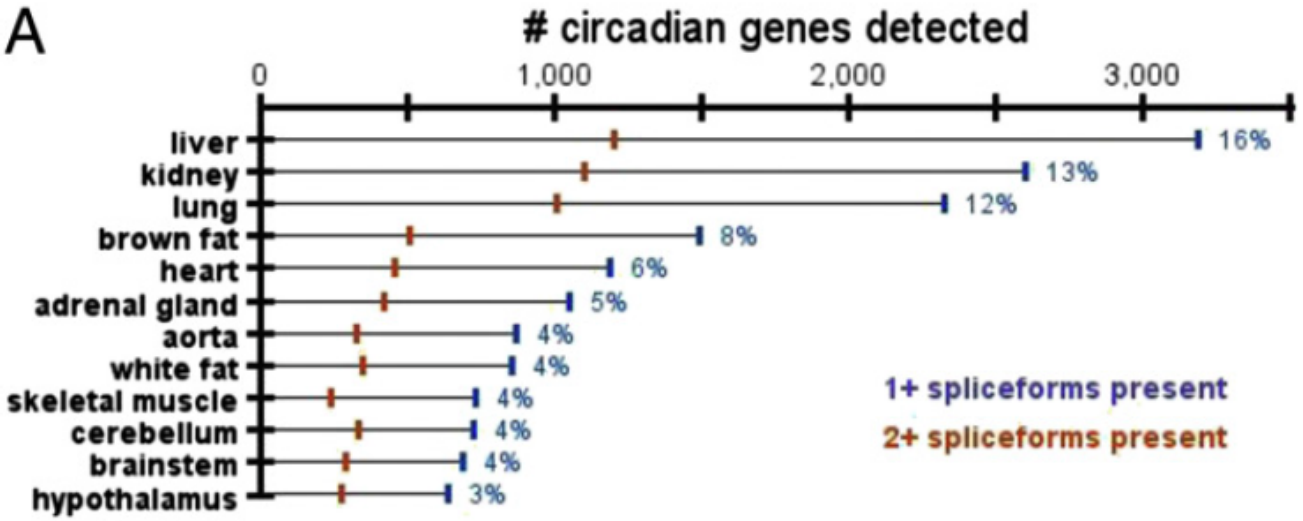
A circadian gene expression atlas in mammals: Implications for biology and medicine

Ray Zhang[a,1], Nicholas F. Lahens[a,1], Heather I. Ballance[a], Michael E. Hughes[b,2], and John B. Hogenesch[a,2]

[a]Department of Pharmacology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104; and [b]Department of Biology, University of Missouri, St. Louis, MO 63121

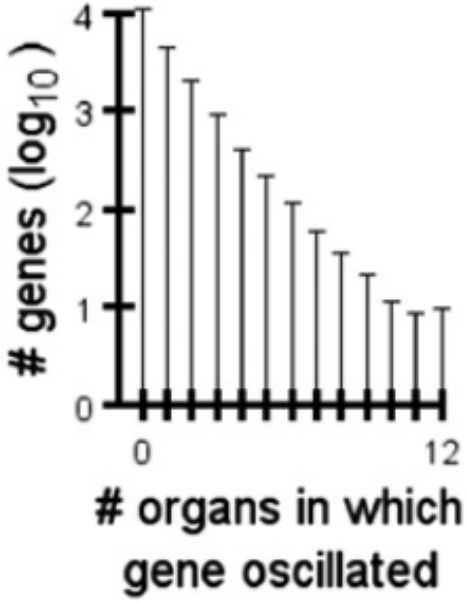
- RNA-seq and DNA arrays to quantify transcriptomes of 12 mouse organs at 2 hour/6 hour intervals

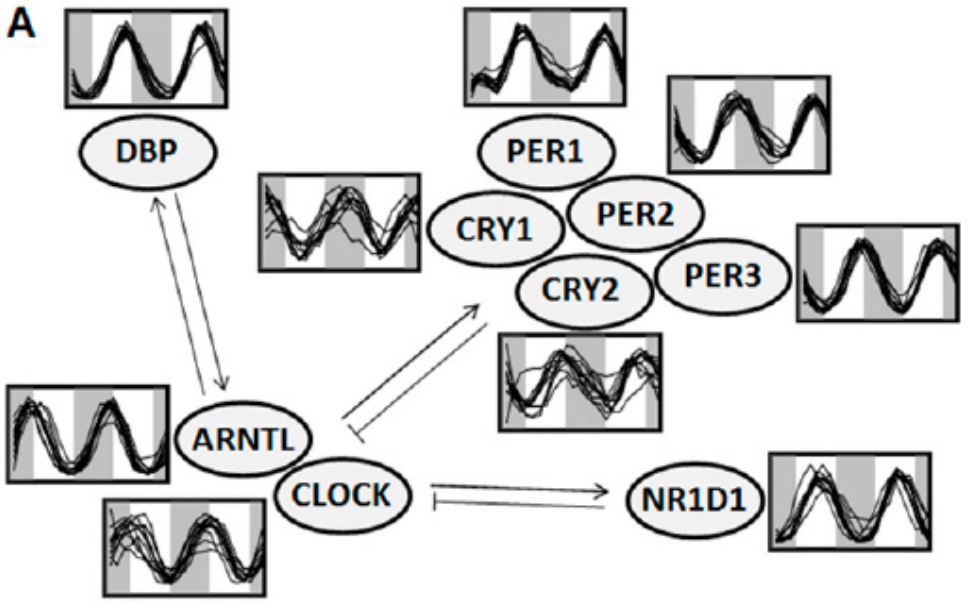
- **Circadian genes**: defined as genes that oscillate with 24 hour-period (project on sine/cosine functions)



Liver contained most circadian genes (-> metabolism), Brain tissue the fewest („the brain never sleeps“)

Globally oscillating genes in mouse tissue





Only 10 genes oscillated in all organs:

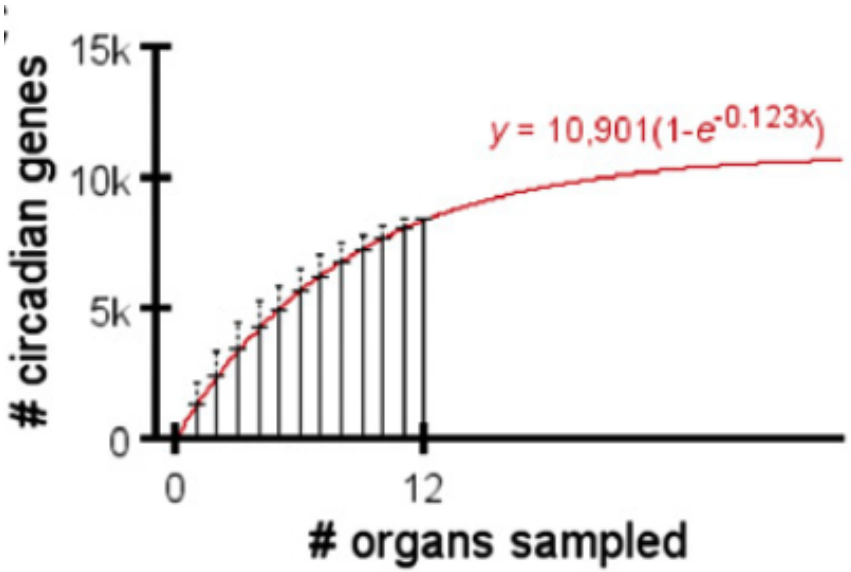
Arntl, Dbp, Nr1d1, Nr1d2, Per1, Per2, and *Per3* (core clock factors – **as expected**), and *Usp2*, *Tsc22d3*, and *Tspan4*.

Usp2 - Ubiquitin carboxyl-terminal hydrolase 2

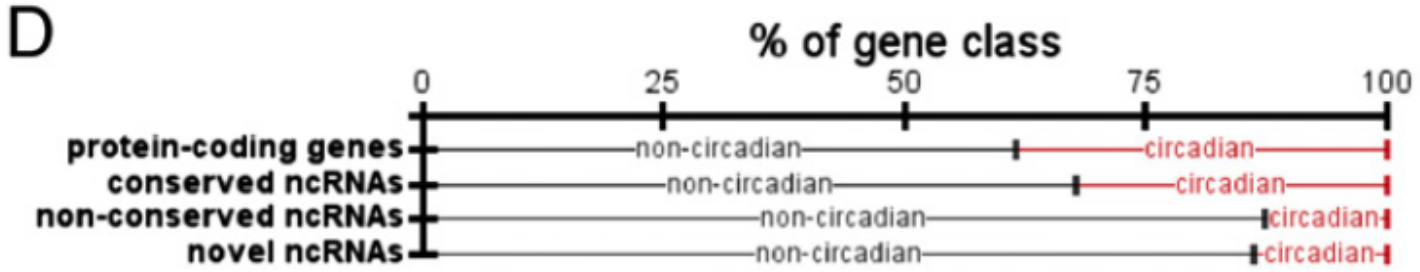
Tsc22d3 - TSC22 domain family protein 3

Tspan4 - The protein encoded by this gene is a member of the transmembrane 4 superfamily, also known as the tetraspanin family.

Overlap of genes/organs (B), how many expected (C)?

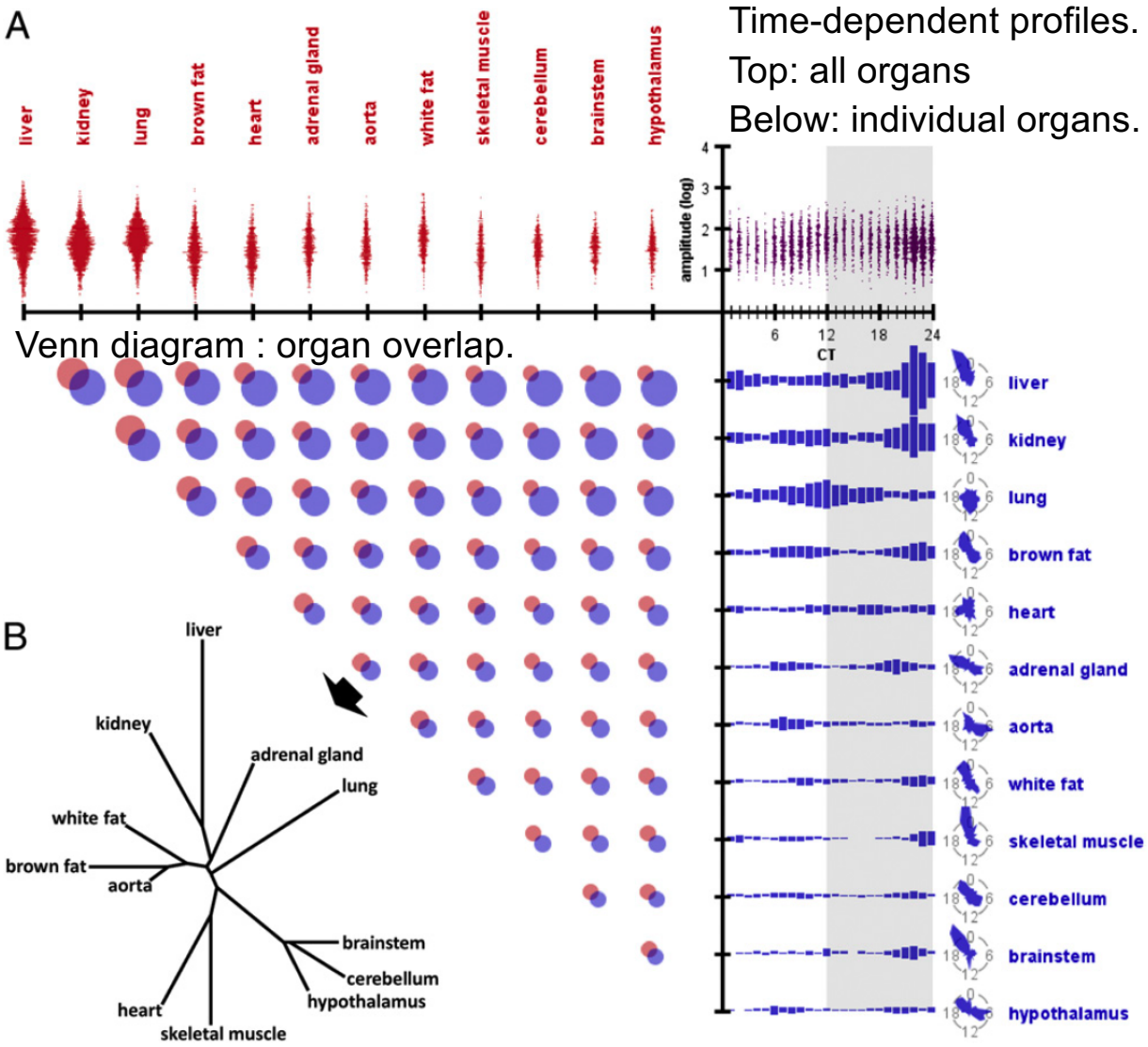


Extrapolation shows that 55% of all genes are expected to show circadian expression in some organ.



Also non-coding RNAs show circadian expression (at lower frequencies).
No individual ncRNA oscillated in more than five organs.
(ncRNA expression is known to be organ-specific).
Conserved ncRNAs means that they are conserved between human and mouse.

(A) Phases + overlap, (B) similarity



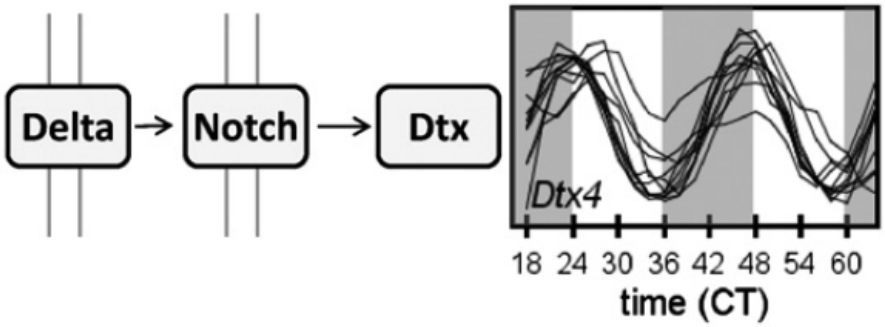
Time-dependent profiles.
Top: all organs
Below: individual organs.

Most circadian genes show organ-specific expression (small overlap).

Peaks often at **dawn** and **dusk**.

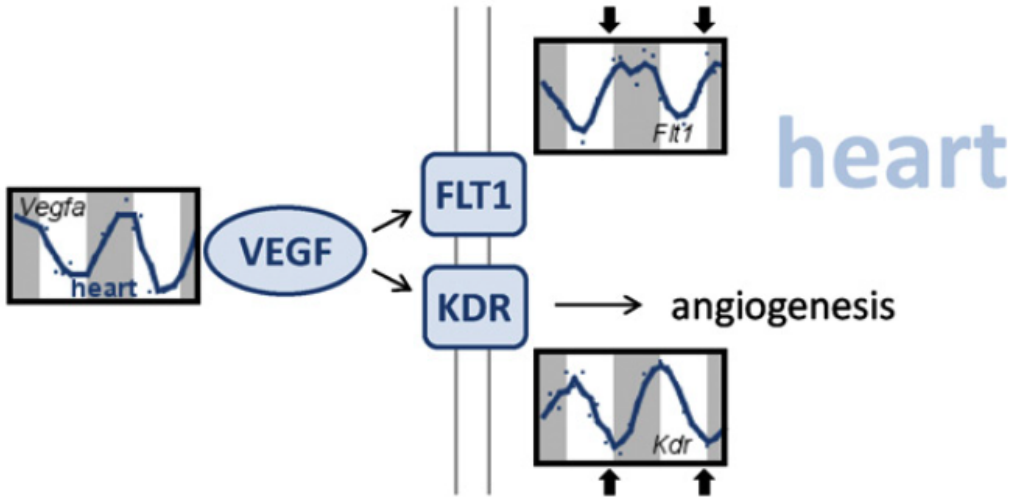
Cluster tissues by similarity of peak phases
Tree in panel B shows that developmentally related organs tend to share circadian genes .

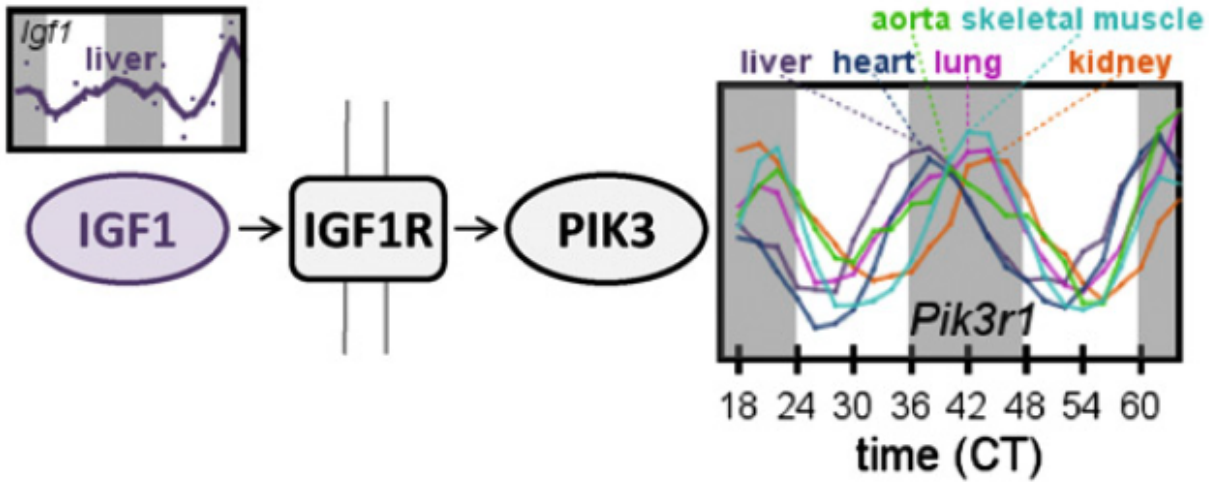
Three Examples



(1) *Dtx4*, a Notch pathway E3 ubiquitin ligase, oscillated in phase with *Arntl* in all organs

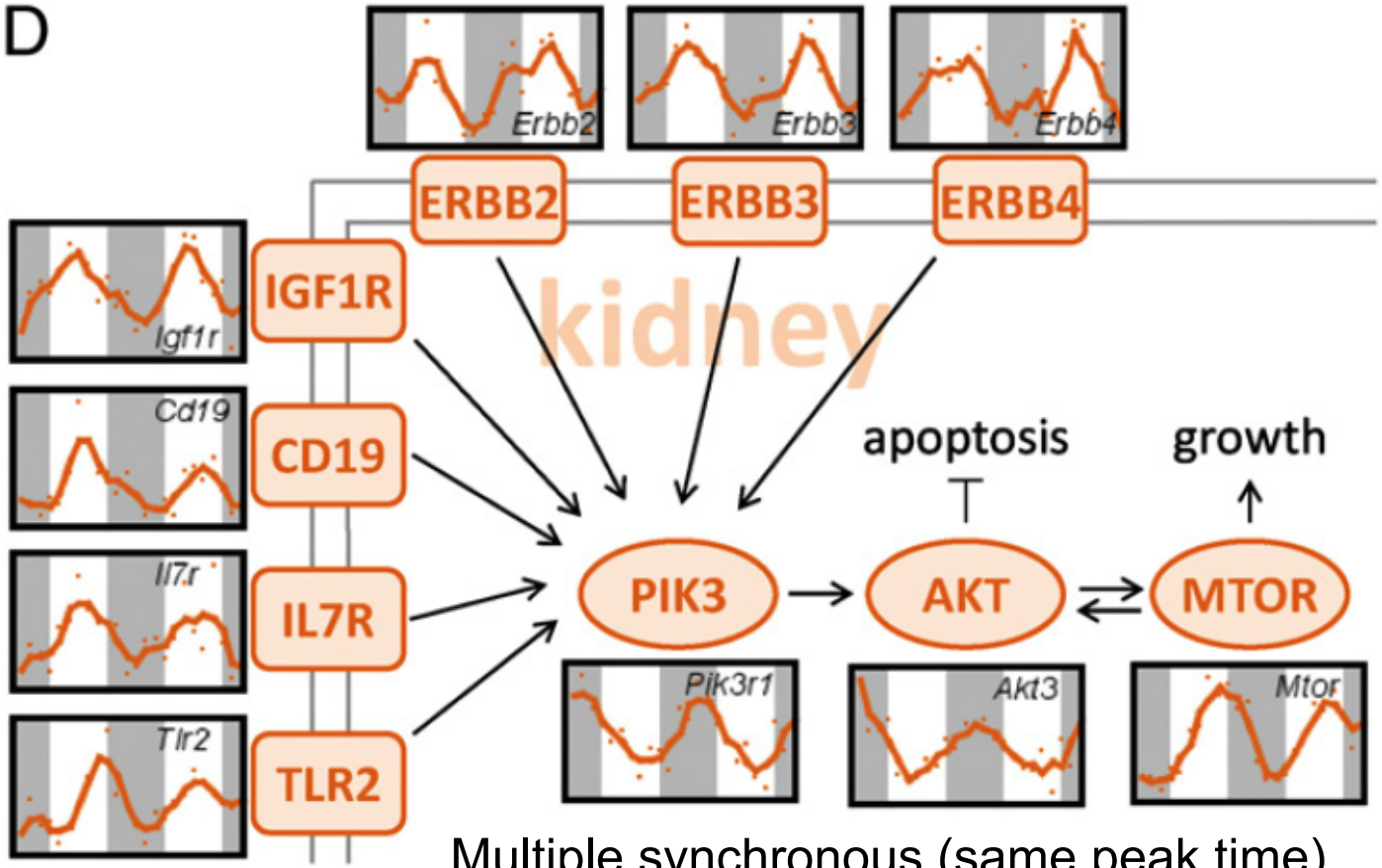
(2) Two VEGF-receptors FLT1 and KDR are expressed alternatively. Arrows: times of anti-phasing.





(3) IGF1 is most produced in liver -> peaks at the same time throughout body. However PIK3r1 (regulatory subunit for PIK3) peaks at different times in different organs.

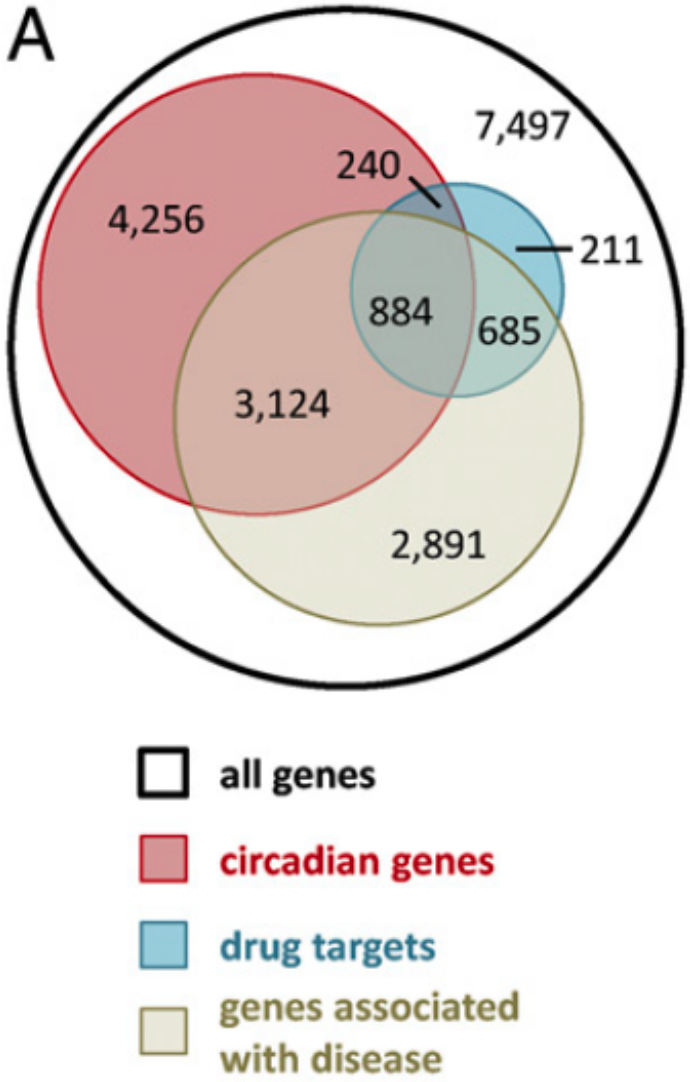
Multiple coordinated pathways control PIK3-AKT-MTOR



Multiple synchronous (same peak time) receptors feed into PIK3-AKT-MTOR pathway that controls growth and apoptosis.

All of them oscillate only in **kidney**!

Many drug-targets show circadian expression



Relevance: drug response will differ significantly depending on day/night time of application

Unclear whether these effects are taken into account during clinical studies

Relevance: mouse -> humans, drugs

Table 1. Drugs of the top-100 best-seller list that target circadian genes and have half-life < 6h

Rank	Sales, $	Trade name	Indications	Circadian-gene targets	Organs in which targets oscillate
2	1.46 b	Nexium	Gastritis, GERD, Esophagitis	*Atp4a*	L
5	1.28 b	Advair Diskus	Asthma, Chronic obstructive pulmonary di...	*Serpina6, Pgr, Nr3c2, Adrb2, Pla2g4a*	Lu, H, L, K, S, A
11	794 m	Rituxan	Rheumatoid arthritis, Non-Hodgkin's lymp...	*Fcgr2b, Ms4a1, Fcgr3*	L, K, S
20	538 m	Diovan	Hypertension, Heart failure	*Slc22a6, Agtr1a, Slco1b2, Car4, Kcnma...*	H, AG, L, K, S
27	431 m	Vyvanse	Attention deficit hyperactivity disorder	*Adra1b*	L
32	392 m	Tamiflu	Influenza	*Neu2, Neu1, Ces1g, Slc22a8, Slc15a1, ...*	Lu, L, BF, K, C
33	383 m	Ritalin	Attention deficit hyperactivity disorder	*Slc6a4*	AG, K
37	348 m	AndroGel	Hypogonadism	*Slc22a4, Slc22a3, Ar, Cyp1a1, Cyp2b10...*	Lu, H, BS, WF, AG...
38	346 m	Lidoderm	Pain	*Slc22a5, Cyp2b10, Egfr, Abcb1a*	Lu, H, AG, BF, L,...
44	304 m	Seroquel XR	Bipolar disorder, Major depressive disor...	*Htr2c, Htr1b, Htr2a, Chrm2, Drd4, Adr...*	Lu, H, BS, WF, AG...
45	289 m	Viagra	Erectile dysfunction	*Cyp1a1, Pde6g, Abcc5, Abcc10, Pde5a, ...*	Lu, H, BS, WF, AG...
47	281 m	Niaspan	Hyperlipidemia	*Slco2b1, Slc22a5, Qprt, Slc16a1*	Lu, H, BS, AG, WF...
48	279 m	Humalog	Diabetes mellitus T2	*Igf1r*	K
49	274 m	Alimta	Mesothelioma, Nonsmall cell lung cancer	*Tyms, Atic, Gart, Slc29a1*	Lu, H, BS, BF, L,...
54	267 m	Combivent	Asthma, Chronic obstructive pulmonary di...	*Slc22a5, Slc22a4, Chrm2, Adrb1, Adrb2*	Lu, H, BS, BF, K,...
56	262 m	ProAir HFA	Asthma, Chronic obstructive pulmonary di...	*Adrb1, Adrb2*	Lu, K, S
62	240 m	Janumet	Diabetes mellitus T2	*Slc47a1, Slc22a2, Prkab1, Abcb1a, Dpp4*	H, BS, AG, Hy, L,...
66	236 m	Toprol XL	Hypertension, Heart failure	*Slc22a2, Adrb1, Adrb2, Abcb1a*	Lu, H, AG, BF, L,...
71	220 m	Vytorin	Hyperlipidemia	*Hmgcr, Cyp2b10, Soat1, Abcc2, Anpep, ...*	Lu, H, BS, AG, BF...
78	209 m	Aciphex	Gastritis, GERD, Esophagitis	*Cyp1a1, Atp4a, Abcg2*	Lu, H, BS, WF, L,...
90	189 m	Lunesta	Insomnia	*Ptgs1, Tspo, Gabra3*	Lu, H, AG, K
98	173 m	Prilosec	Gastritis, GERD, Esophagitis	*Cyp1a1, Atp4a, Abcg2, Cyp1b1, Abcb1a*	Lu, H, BS, WF, AG...
99	171 m	Focalin XR	Attention deficit hyperactivity disorder	*Slc6a4*	AG, K

Rank and sales are based on USA 2013 Q1 data from Drugs.com. A, aorta; AG, adrenal gland; BF, brown fat; BS, brainstem; C, cerebellum; H, heart; Hy, hypothalamus; K, kidney; L, liver; Lu, lung; S, skeletal muscle; WF, white fat.

About half of top-100 drugs have half lives < 6 hours!