V18 – Kinetic Motifs in Signaling Pathways

-Types of kinetic motifs in signaling pathways

- -Application to cell cycle
- -Circadian clocks



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):

$$\frac{dR}{dt}\Big|_{R=R_{ss}} = 0 \quad \Longrightarrow \quad R_{ss} = \frac{k_0 + k_1 S}{k_2} = \frac{k_0}{k_2} + \frac{k_1}{k_2} S$$

Rss linearly dependent on S







phosphorylation/dephosphorylation



"forward": R is converted to phosphorylated form RP "backward": RP can be dephosphorylated again to R $S + R \implies RP$ $RP \implies R + T$ $\frac{dRP}{dt} = k_1 SR - k_2 RP = k_1 S(R_{tot} - RP) - k_2 RP$

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:

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



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

Enzyme: Michaelis-Menten-kinetics



 $S \leftarrow K_{on} \xrightarrow{E} K_{off} \xrightarrow{FS} K_{off}$ Reaction rate: $V = k_{off} ES$

Steady state: k

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



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

Total amount of enzyme is constant:

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

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

The MM-equation



Sigmoidal Characteristics with MM kinetics



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

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

$$V = V_{max} \; rac{S}{S+K_M} \; \; {
m this means that S} \; = {
m R_t} - {
m RP} \ {
m K_M} = {
m R_0}$$

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:

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

Parameters: $kI = I \pmod{s}^{-1}$, $k2 = I s^{-1}$, $R_0 = RP_0 = I \mod I$ Initial conditions: $R = I0 \mod, RP = 0$

Time courses for S = I, I.5, and 2, RP(0) = 0:

equilibrium is reached faster for stronger signal



Adaption - "sniffer"



Linear response modulated by a second species X

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

Steady state: R_{ss} independent of S

$$X_{ss} = \frac{k_3}{k_4}S \qquad \qquad R_{ss} = \frac{k_1k_4}{k_2k_3}$$

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



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

dEP	_	$k_3R ~ E$		$k_5 EP$
dt	_	$\overline{EP_0 + EP}$	_	$\overline{E_0 + E}$



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



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

$$\frac{dEP}{dt} = \frac{k_3RE}{EP_0 + EP} - \frac{k_5EP}{E_0 + E}$$



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):





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

Simplified Version of Cell Cycle Control System



G1 => S — Toggle Switch



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

Mutual Inhibition



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



Rate Equations: Toggle Switch



Stoichiometric matrix

"(C)" = catalyst

	RI	R2	R3	R4
Α	-1			
S	(C)			
R	Ι	– I	(C)	
Е		(C)	– I	I
EP			I	– I
Х		I		

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

$$\frac{dR}{dt} = \frac{dR1}{dt} - \frac{dR2}{dt} = k_1 A S - k_2 R E$$
$$\frac{dE}{dt} = \frac{dR4}{dt} - \frac{dR3}{dt}$$

Rate Equations: G1/S Module



	RI	R2	R3	R4	R5	R6
СусВ	-					
CdkI	-					
CycB:Cdk1	Ι	-1	(C)			Ι
СКІ		-1	-1	I		I
CKI:P ₃			I	-1		
CKI:P₃					-1	
CycB:Cdk1:CKI						-

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

Comparison: Matrices





	RI	R2	R3	R4
Α	– I			
S	(C)			
R	I	– I	(C)	
Е		(C)	– I	Ι
EP			I	-1
X				

	RI	R2	R3	R4	R5	R6
СусВ	-1					
CdkI	-1					
CycB:Cdk1	Ι	-1	(C)			Ι
CKI		-1	-1	I		I
CKI:P₃			Ι	-1		
CKI:P₃					-1	
CycB:Cdk1:CKI		I				-1

Difference: catalysts vs. substrates

Comparison: Equations



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

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

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

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

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

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



Rename species => same rate equations => same behavior

Predicted Behavior: G1 => S

Signal: cell growth = concentration of CycB, CdkI

Response: activity (concentration) of CycB:CdkI



Toggle switch:

=> above critical cell size, CycB:CdkI 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**

M => G1

Negative feedback loop oscillator

- i) CycB:CdkI 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

Overall Behavior



GI/S toggle => bistability

M/GI 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 posttranslational 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.

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

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- 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)



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)?



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



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

Peaks often at dawn and dusk.

Cluster tissues by similarity white fat skeletal muscle of peak phases cerebellum Tree in panel B shows that brainstem developmentally related organs hypothalamus tend to share circadian genes .

Three Examples



Pik3r1

18 24 30 36 42 48 54 60

time (CT)

Bioinformatics 3 – WS 16/17

IGF1

IGF1R →(

PIK3

V 18 -

time throughout body.

organs.

However PIK3r1 (regulatory

subunit for PIK3) peaks at

different times in different



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	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	SIc6a4	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	lgf1r	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!

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