Part 2
Simple network, complex function

Biological Oscillators

based on B. Novak and J.J. Tyson
Nature Review Molecular Cell Biology 2008, Vol 9,981-991

Week 3-4, 3/13-22/2018

Outline

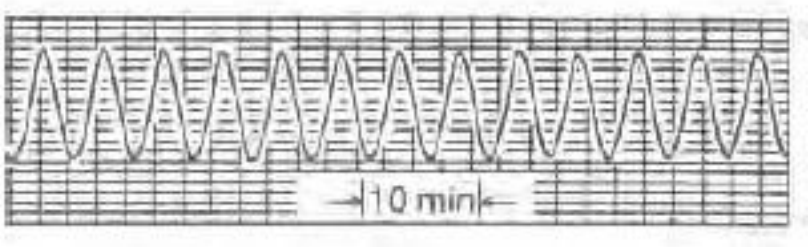
A. Importance of biological oscillators
B. Stability analysis of oscillation
C. Essential requirements for biochemical oscillators
D. Classification of oscillatory motifs

A. Importance of biological oscillators

Oscillations widely occur in Biology at all time scales

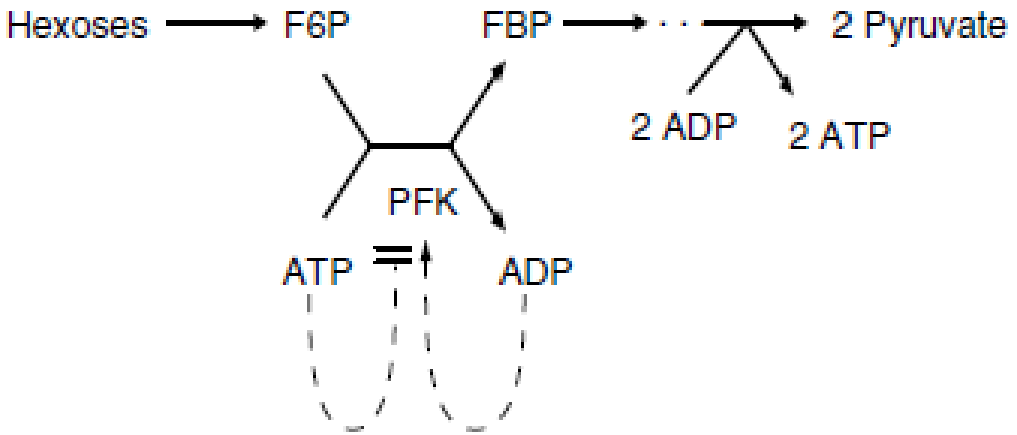
- Neuronal Oscillations (ms – s): membrane potential
- Cardiac Rhythms (s): pacemaker neuronal network
- Calcium Oscillations (s-min): calcium-regulated calcium release
- Biochemical Oscillations (min): metabolites oscillate
- Hormonal Oscillations (10 m - 24 h): Insulin, GnRH
- Cell Cycle (30 m-24 h): enzymatic and gene regulation
- Circadian Rhythms (24 h): enzymatic and gene regulation
- Ovarian Cycle (weeks-months): hormonal and gene regulation
- Predator and Prey Population Cycle (years): wolves and sheep

Example I: Glycolytic oscillation



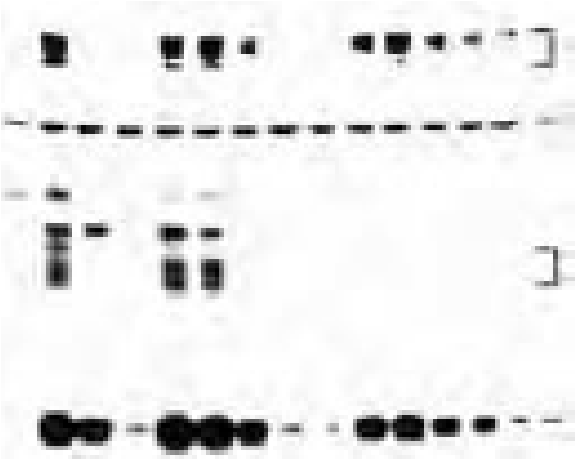
Sustained oscillation in NADH fluorescence in budding yeast.
Pye (1971)

Metabolic pathway responsive for the oscillation



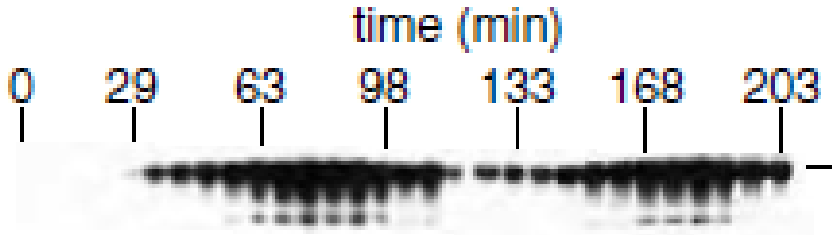
Simplified mechanism

Example II: Cyclin oscillation during cell cycle in synchronous yeast cell



Tyers et al (1993)

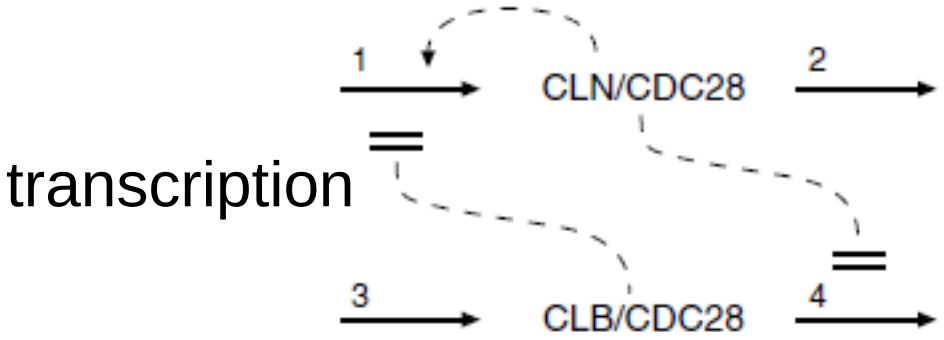
Cln2/CDC28 activity



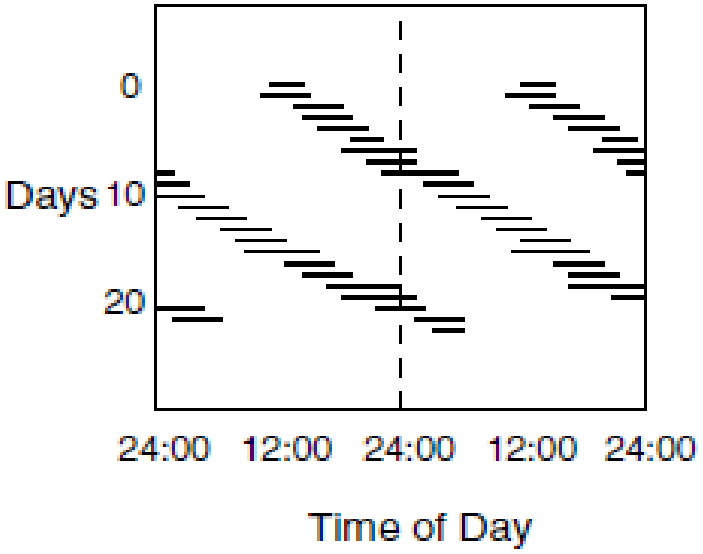
Surana et al (1991)

H1 CLB2/CDC28 activity

Molecular mechanism

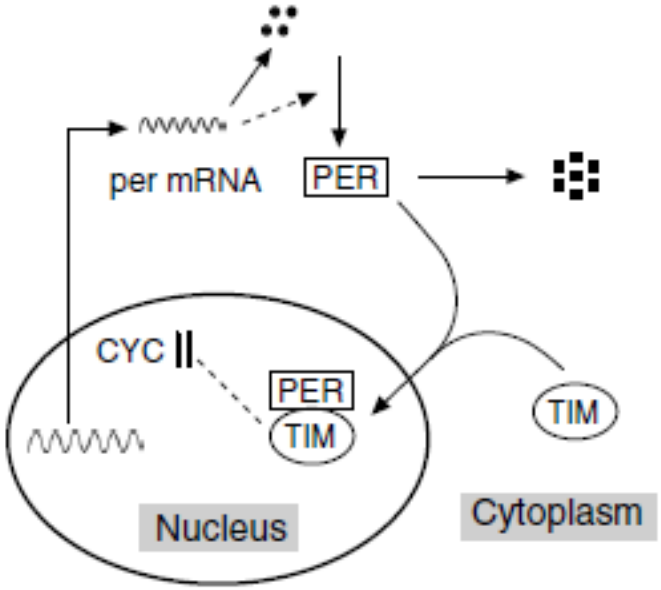


Example III: Endogenous circadian rhythm

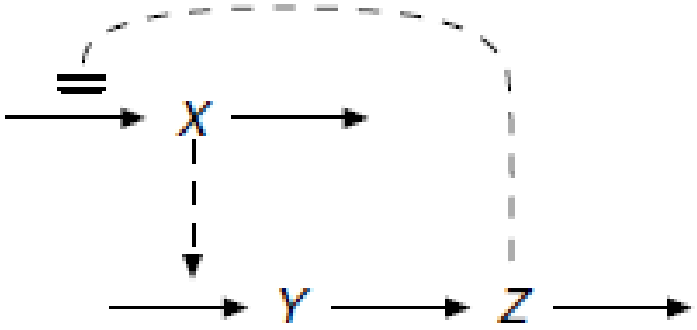


Sleep episodes of a human being isolated from all external temporal cues. *Siffre (1975)*

Molecular mechanism for Drosophila



Simplified model



B. Stability analysis of oscillation

Mathematical basis of stability analysis

$$\dot{x} = f(x, y)$$
$$\dot{y} = g(x, y)$$

system of two coupled differential equations

step 1 ↓

find nullclines and fixed point(s)

$$\dot{x} = 0 \rightarrow f(x_o, y_o) = 0$$
$$\dot{y} = 0 \rightarrow g(x_o, y_o) = 0$$

step 2 ↓

consider small deviation from fixed point

$$\tilde{x} \equiv x - x_o$$
$$\tilde{y} \equiv y - y_o$$

$$\tilde{x} \equiv x - x_o$$

$$\tilde{y} \equiv y - y_o$$

consider small deviation from fixed point

step 3 ↓

linearize around fixed point(s)

$$\dot{x} \approx \tilde{x} \left.\frac{\partial f}{\partial x}\right|_{(x_o, y_o)} + \tilde{y} \left.\frac{\partial f}{\partial y}\right|_{(x_o, y_o)} \equiv a\tilde{x} + b\tilde{y}$$

$$\dot{y} \approx \tilde{x} \left.\frac{\partial g}{\partial x}\right|_{(x_o, y_o)} + \tilde{y} \left.\frac{\partial g}{\partial y}\right|_{(x_o, y_o)} \equiv c\tilde{x} + d\tilde{y}$$

step 4 ↓

determine matrix A

$$A = \begin{bmatrix} a & b \\ c & d \end{bmatrix}$$

$$A = \begin{bmatrix} a & b \\ c & d \end{bmatrix}$$ determine matrix A

step 5 ↓ determine trace and determinant of A:

$$\tau = trace(A) = a + d$$
$$\Delta = \det(A) = ad - bc$$

$$\Delta = \lambda_1 \lambda_2$$
$$\tau = \lambda_1 + \lambda_2$$

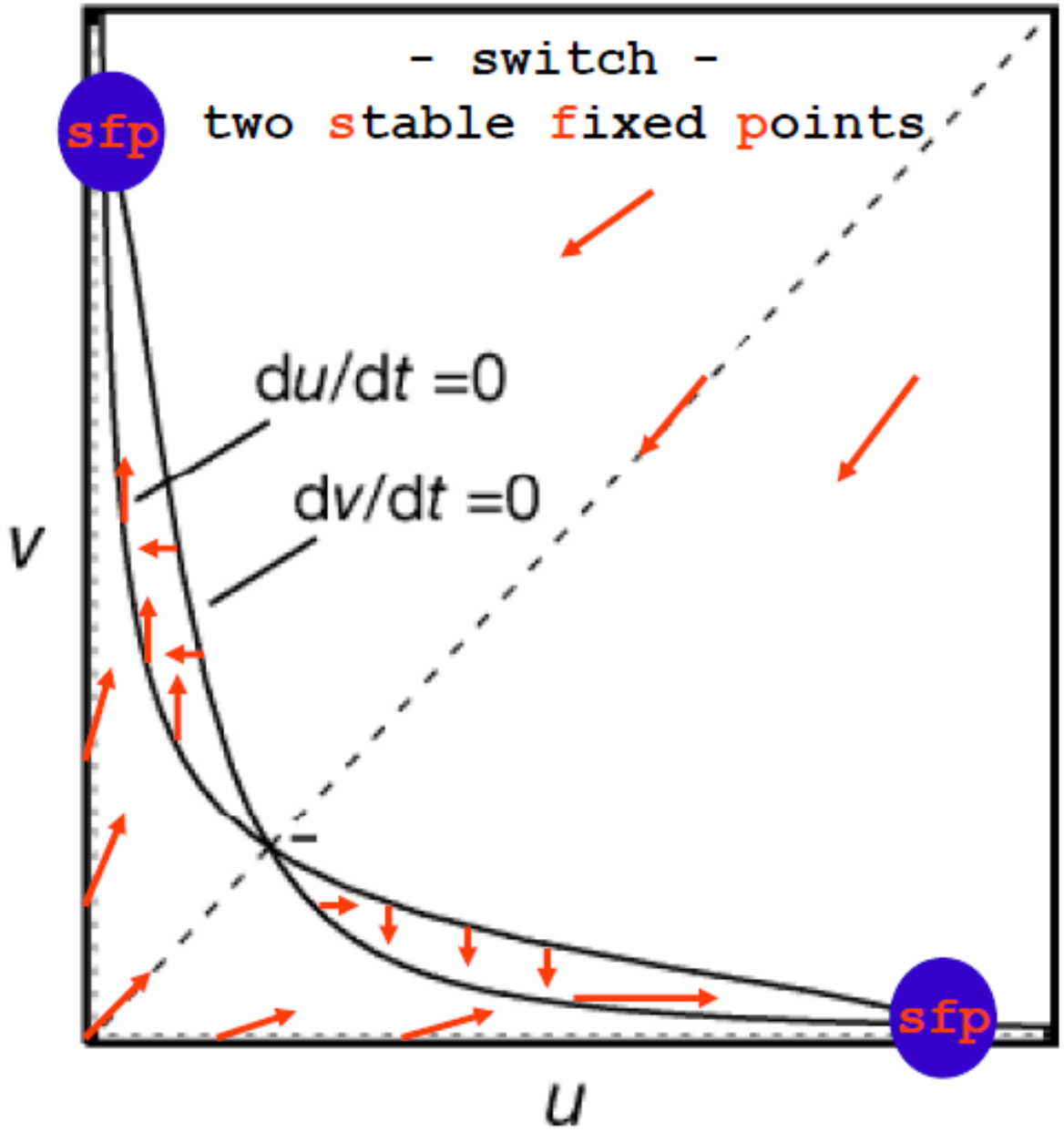
λ_1,λ_2, eigenvalue

step 6 ↓ determine stability of fixed point

only if $\tau < 0$ and $\Delta > 0$, (x_o, y_o) is a stable fixed point

!!! be careful: only valid for 2 dimensional systems !!!

Two stable fixed points and one unstable fixed point



nullclines:

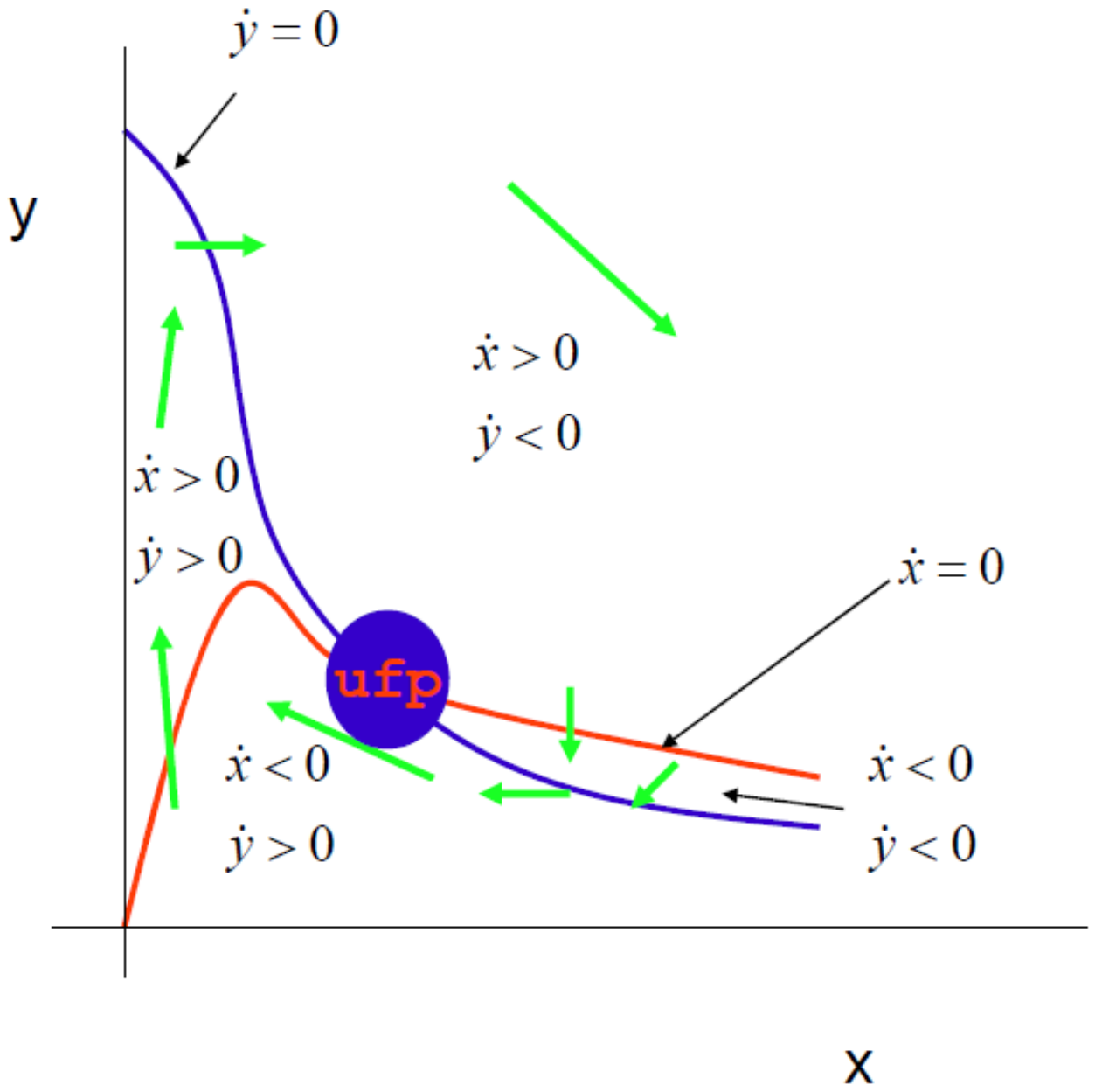
$$u = \frac{\alpha_1}{1+v^{\beta}}$$

$$v = \frac{\alpha_2}{1+u^{\gamma}}$$

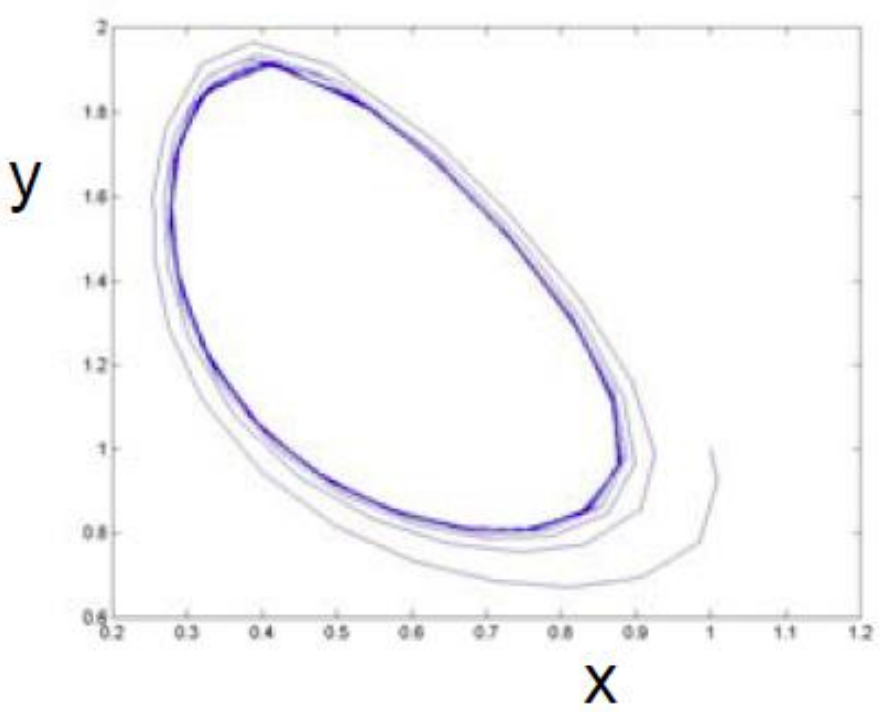
$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u$$

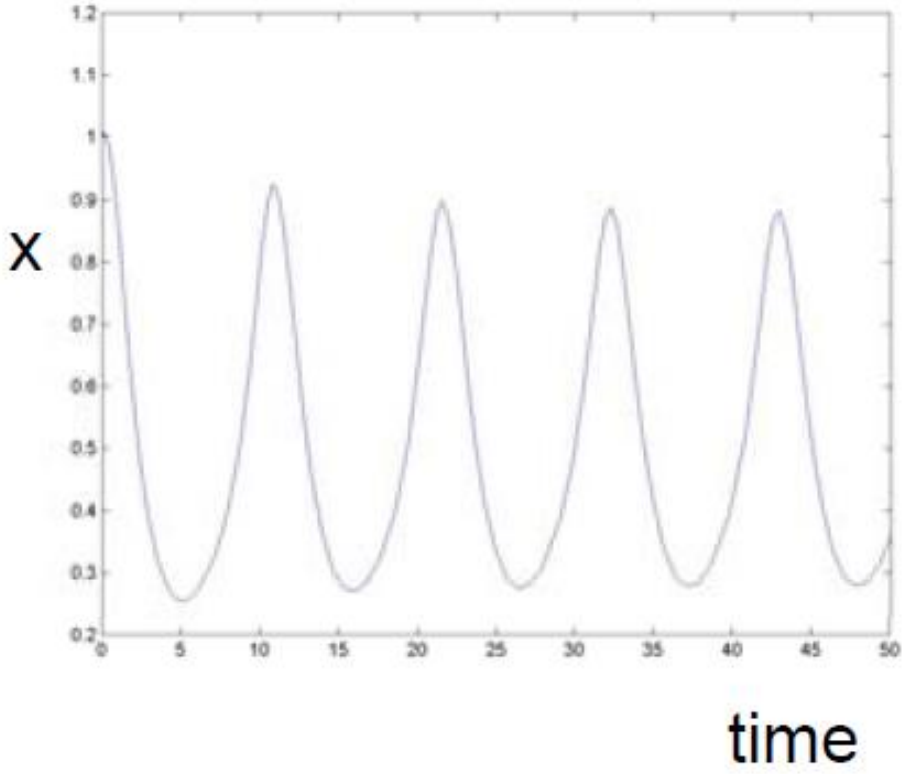
$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v$$

One unstable fixed point? ---- Oscillator



Oscillator (limit cycle)





A “toy” example

$$\dot{x} = -x + ay + x^2 y$$
$$\dot{y} = b - ay - x^2 y$$

model for glycolysis

nullclines:

$$y = \frac{x}{a + x^2}$$
$$y = \frac{b}{a + x^2}$$

fixed point:

$$x^* = b$$
$$y^* = \frac{b}{a + b^2}$$

stable or unstable ?

The matrix A is

$$A = \begin{bmatrix} -1 + 2x^* y^* & a + (x^*)^2 \\ -2x^* y^* & -(a + (x^*)^2) \end{bmatrix}$$

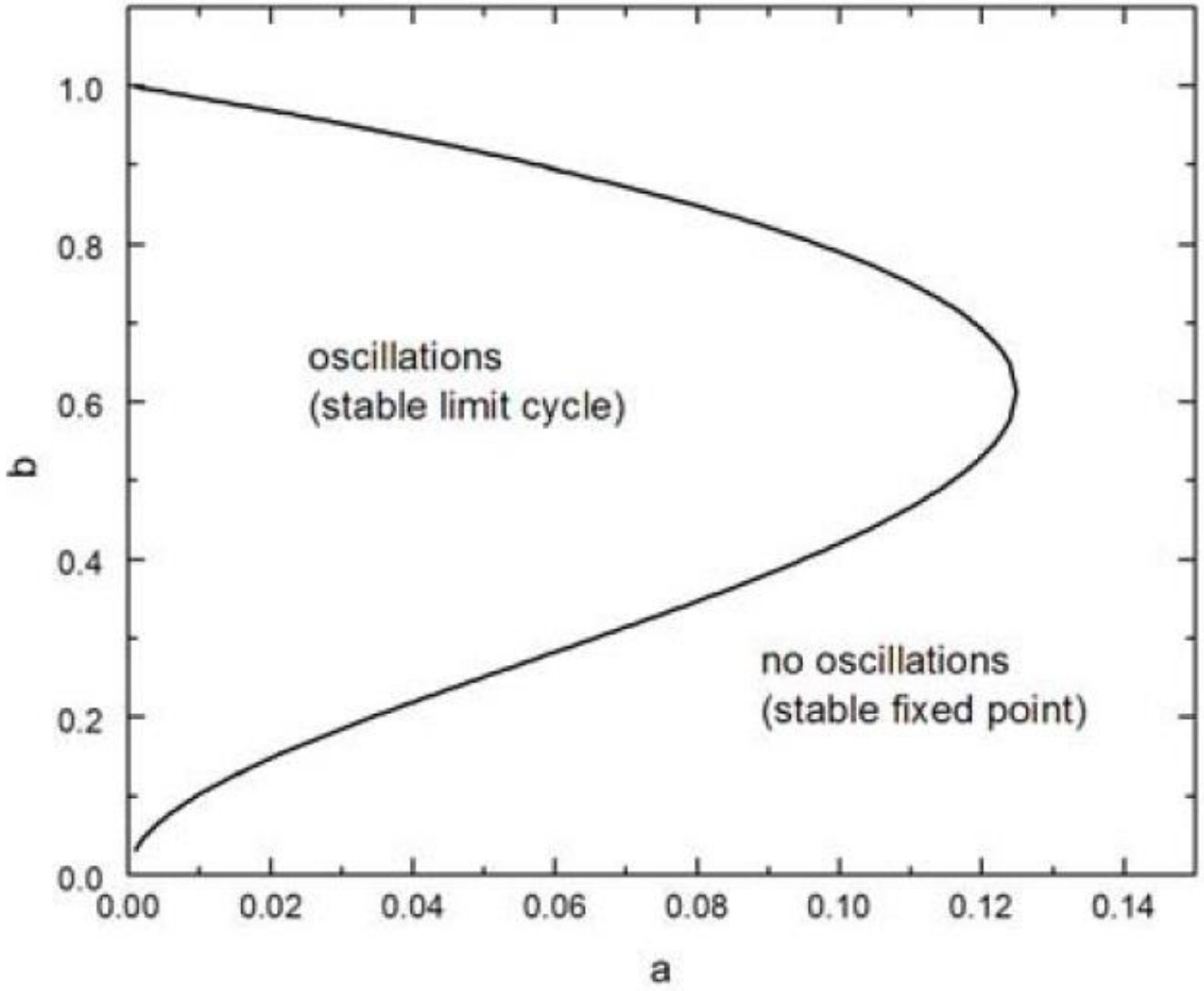
The determinant and trace are:

$$\Delta = a + b^2 > 0$$

$$\tau = -\frac{b^4 + (2a - 1)b^2 + (a + a^2)}{a + b^2}$$

When $\tau<0$, the fixed point is stable. When $\tau>0$, the fixed point is unstable

a-*b* parameter space indicating for which values of *a* and *b*, the systems exhibits stable oscillation



C. Essential requirements for biochemical oscillators

1) Negative feedback is necessary to carry a reaction network back to the 'starting point' of its oscillation.

2) The negative-feedback signal must be sufficiently delayed in time so that the chemical reactions do not settle on a stable steady state.

3) The kinetic rate laws of the reaction mechanism must be sufficiently 'nonlinear' to destabilize the steady state.

4) The reactions that produce and consume the interacting chemical species must occur on appropriate timescales that permit the network to generate oscillations.

The origins of time-delay

- **A physical constraint** *(e.g. the minimal time necessary to carry out transcription and translation, or the time needed to transport chemical species between cellular compartments).*

- **A long chain of reaction intermediates** *(as in a metabolic pathway).*

- **Dynamical hysteresis** *(overshoot and undershoot, as consequences of positive feedback in the reaction mechanism).*

Negative feedback with time delay

We consider a protein that represses the transcription of its own gene.

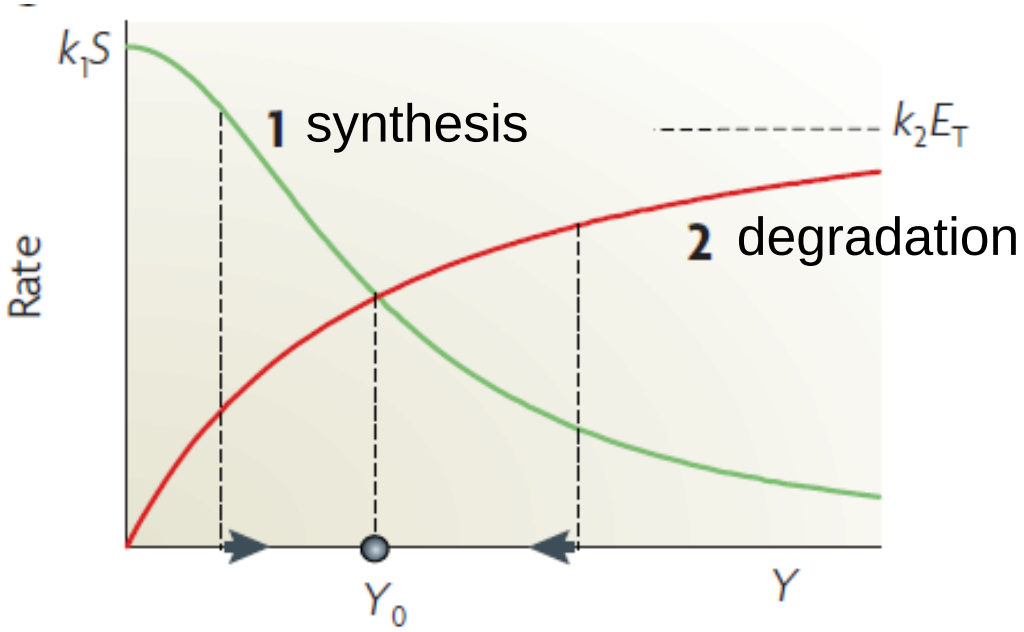
The change of the protein concentration (dY/dt) is given by:

$$\frac{dY}{dt} = \underbrace{k_1 S \frac{K_d^p}{K_d^p + Y^p}}_{\text{Production}} - \underbrace{k_2 E_T \frac{Y}{K_m + Y}}_{\text{Degradation}}$$

Production
K_d: binding affinity
p: hill coefficient
(oligomer of p)
$k_1 S$: basal rate of synthesis

Degradation
E_T: protease concentration
k_2: turn over rate
Km: Michaelis constant

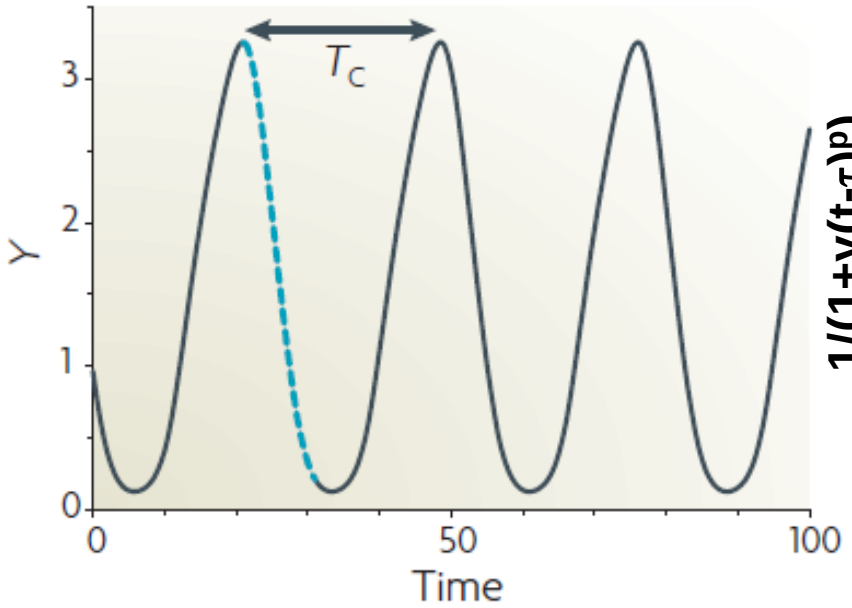
Without time delay, it is stable

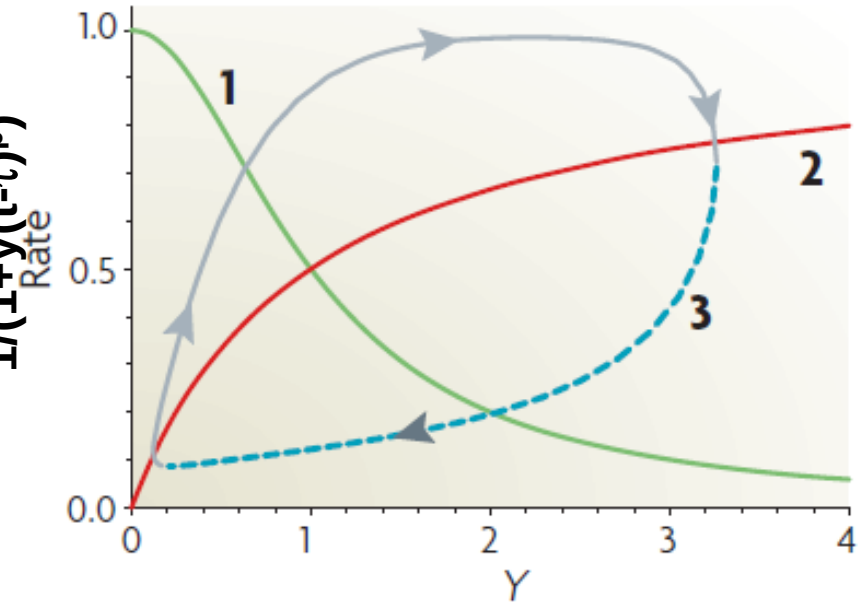


Time delay source I: explicit time delay

$$\frac{dY(t)}{dt} = k_1 S \frac{K_d^p}{K_d^p + Y(t-\tau)^p} - k_2 E_T \frac{Y(t)}{K_m + Y(t)}$$

- Suppose τ is the time required for transcription, translation and translocation to nucleus, the current synthesis (t) is depended on the Y protein synthesized at t-τ.
- With proper rate constants and time delay, it exhibits oscillation.





Requirements for oscillation

"Proper choice of rate constants':

parameter constraints

S (signal strength),

p (nonlinearity of feedback)

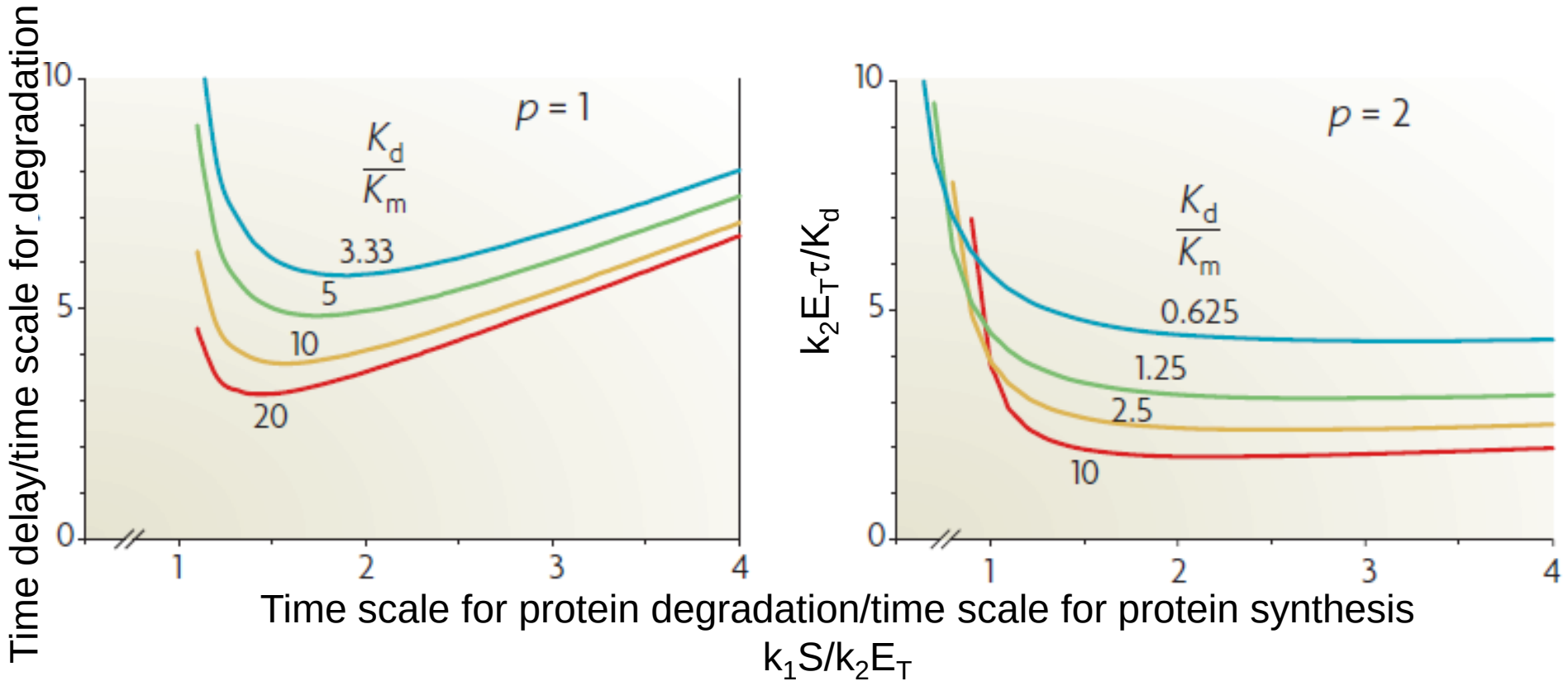
Km (nonlinearity of the removal step)

τ (duration of time delay)

Specifically:

- τ must be long enough
- p or Kd/Km increase, nonlinear enough
- Rate of opposing process must be appropriately balanced

Constraint curves for oscillation

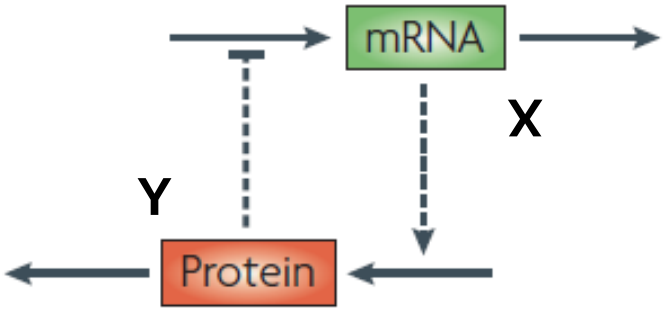


Nonlinearility *p* increase the possibility for oscillation

It is clear that these four elements (negative feedback, nonlinearity, time delay and timescale constraints) are generally needed for all biochemical oscillators, provided the notion of time delay is suitably generalized.

Time delay source II: a series of intermediates

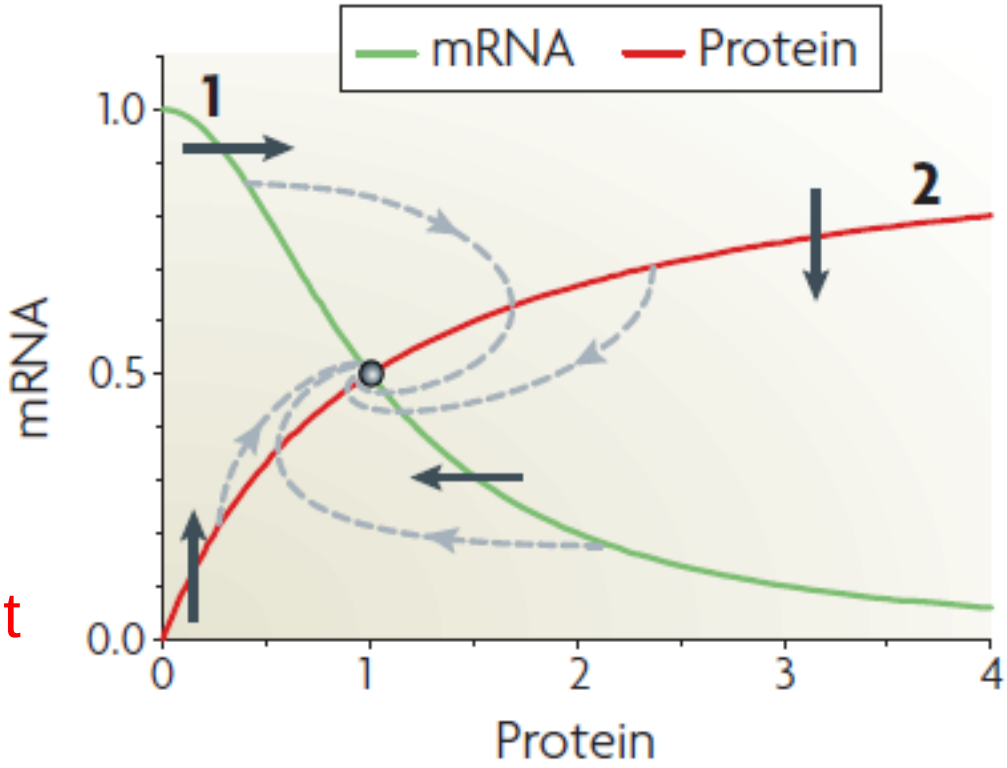
Scenario I



$$\frac{dX}{dt} = k_1 S \frac{K_d^p}{K_d^p + Y^p} - k_{dx} X$$

$$\frac{dY}{dt} = k_{sy} X - k_2 E_T \frac{Y}{K_m + Y}$$

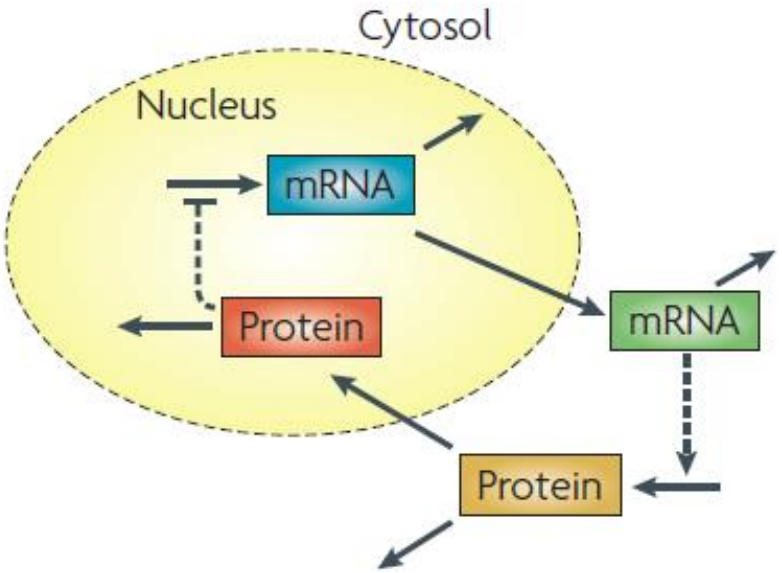
Result:

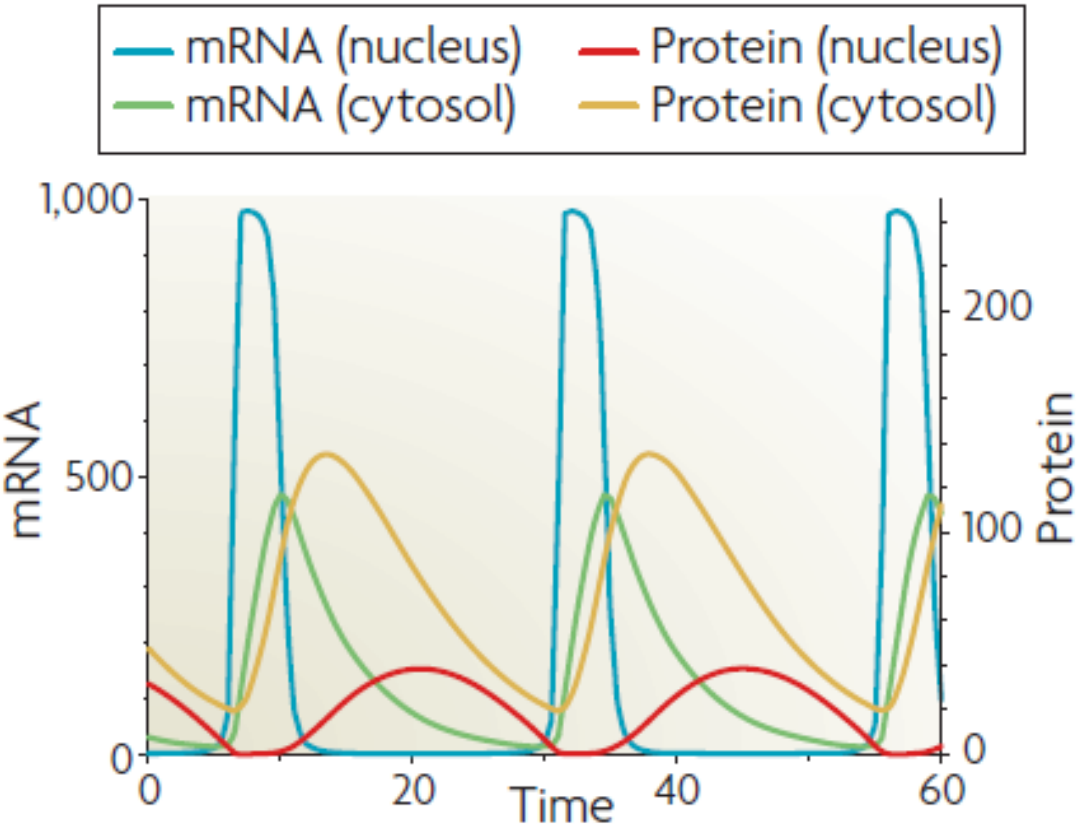


Two-component negative feedback loop is not sufficient to generate oscillation!

Time delay source II: a series of intermediates

Scenario II





Three(or more)-component feedback loop is possible to generate oscillation.

demonstrated with synthetic circuit by Elowitz and Leibler Nature 2000

Time delay source III: positive feedback

1) Time delay is a sort of memory: protein synthesis rate at the present time depends on protein concentration over some time in the past.

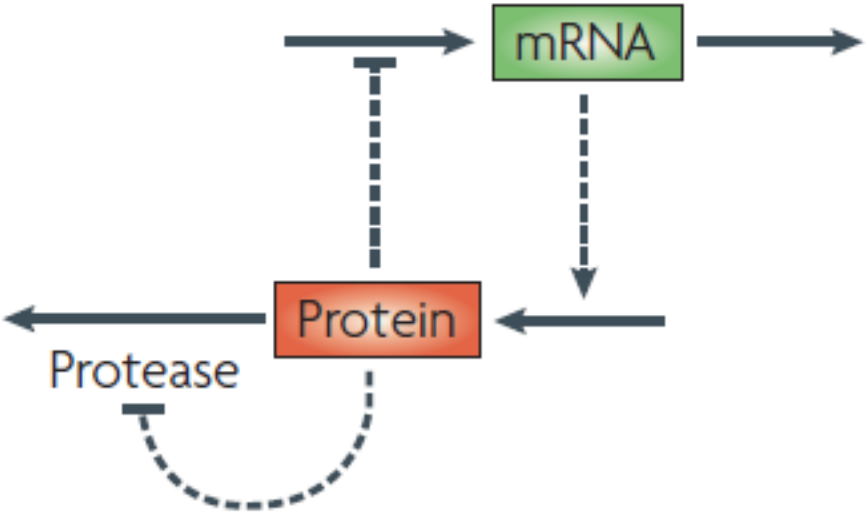
2) Memory is a property of biochemical systems with bistability: under identical chemical conditions, the system can be in either of two alternative stable steady states.

3) Which state a system occupies depends on its recent history (a phenomenon called hysteresis).

4) Hysteresis can prevent a system with negative feedback from finding its homeostatic steady state.

Time delay source III: positive feedback

Adding a feedback of Protein on its own stability by inhibiting the protease, it generate hysteresis.



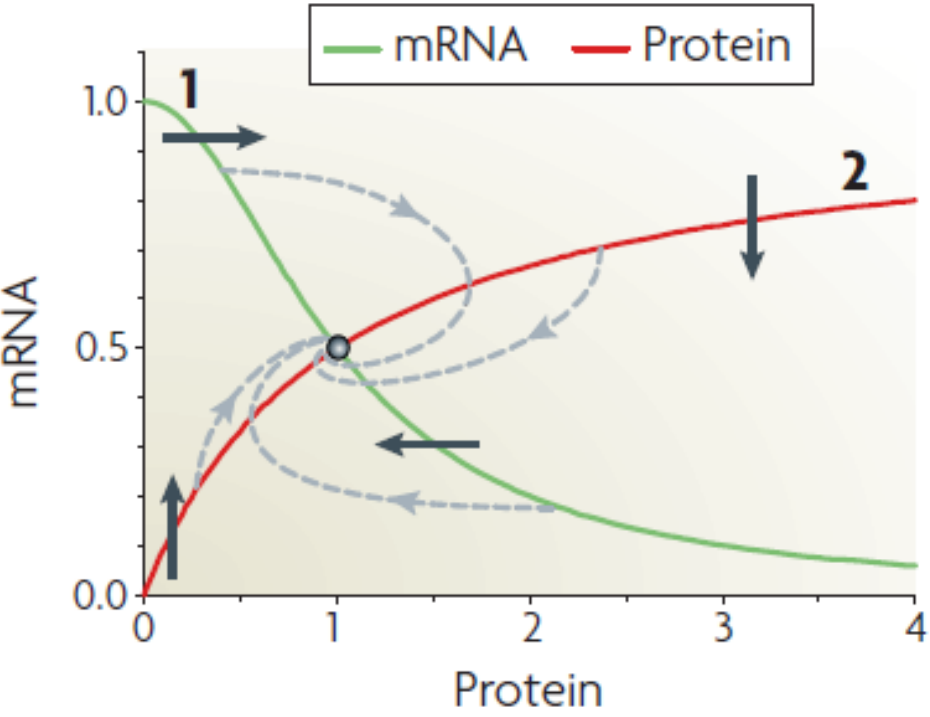
$$\frac{dX}{dt} = k_1 S \frac{K_d^p}{K_d^p + Y^p} - k_{dx} X$$

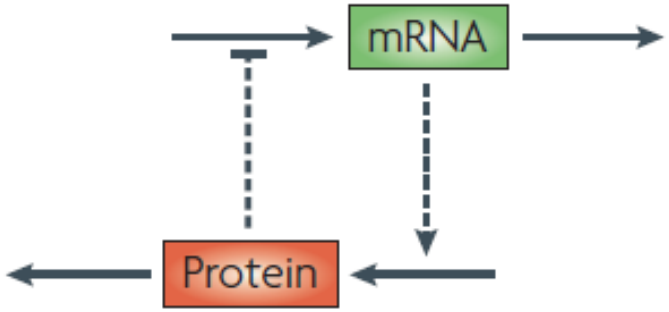
$$\frac{dY}{dt} = k_{sy} X - k_{dy} Y - k_2 E_T \frac{Y}{K_m + Y + K_I Y^2}$$

$k_{dy}Y$: alternative protein degradation pathway

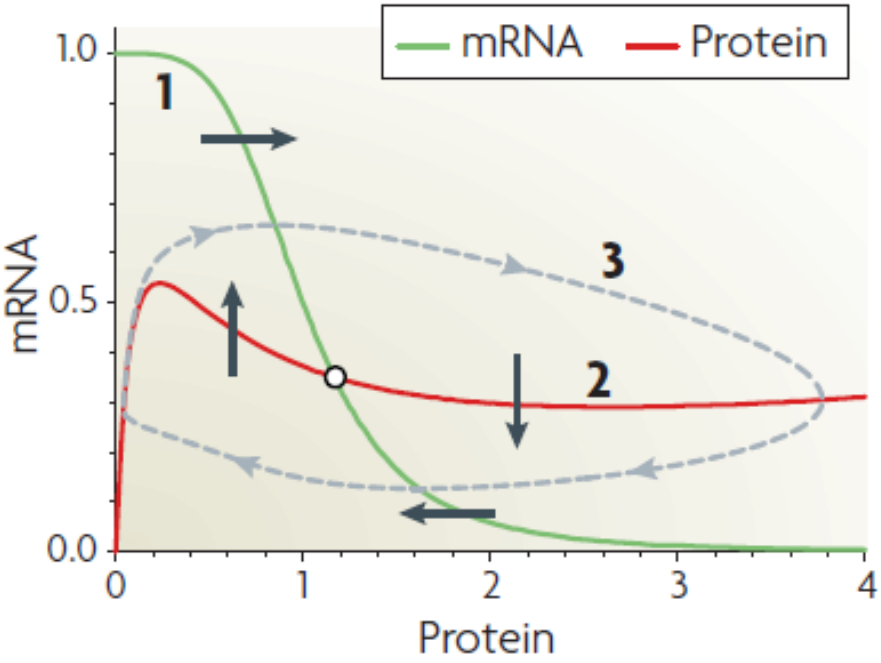
Time delay source III: positive feedback

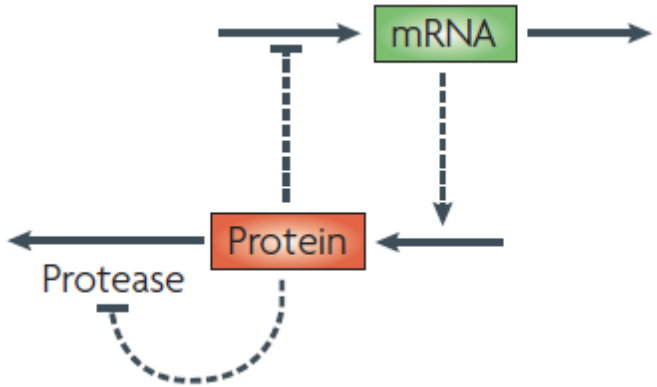
Without positive feedback



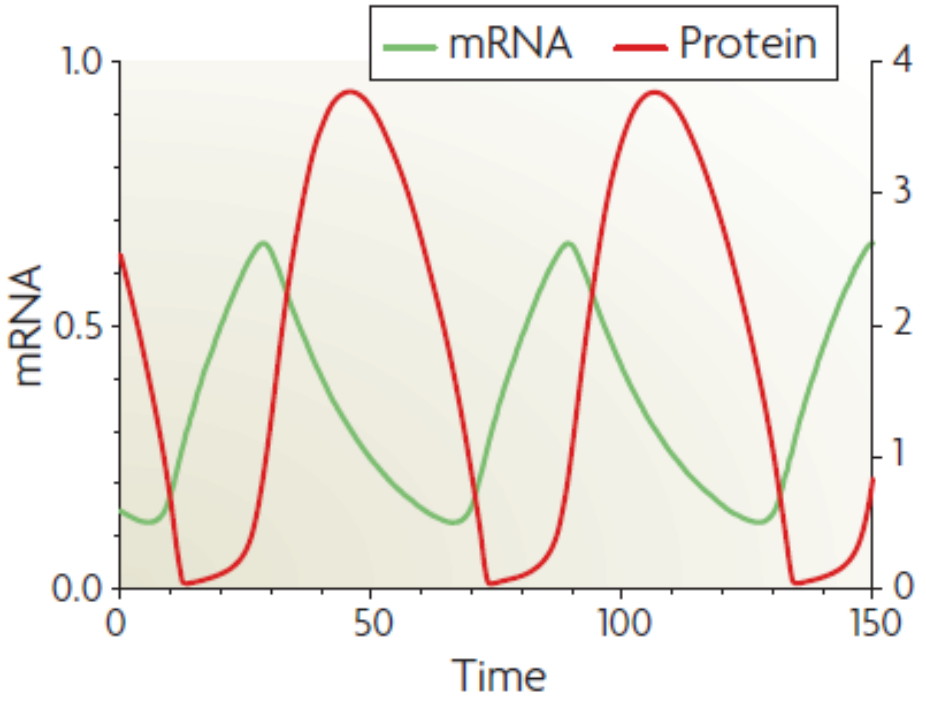


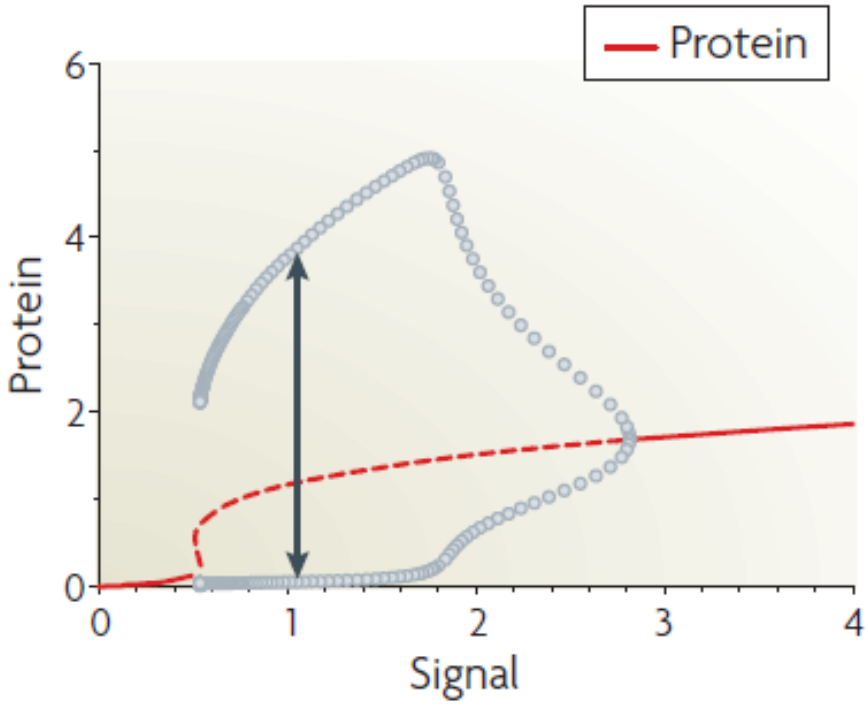
With positive feedback





Time delay source III: positive feedback

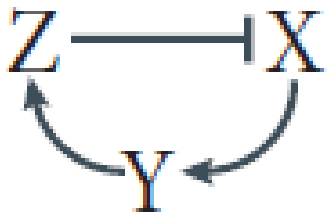


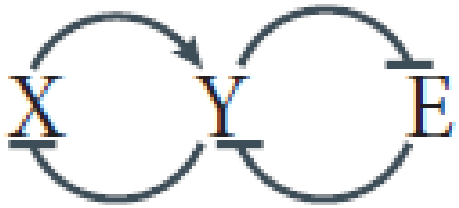


This mechanism has been proposed circadian rhythms in the reaction network that govers expression of the PER gene in drosophila by John Tyson (*J.J.Tyson et al. Biophysics J 1999)* Their idea is that PER dimer is less likely to be degraded by protease.

Biochemical interaction networks

In gene regulation network (GRN), protein interaction network (PIN) and metabolic control system (MICs), the basic network can be described as regulatory motifs such as:

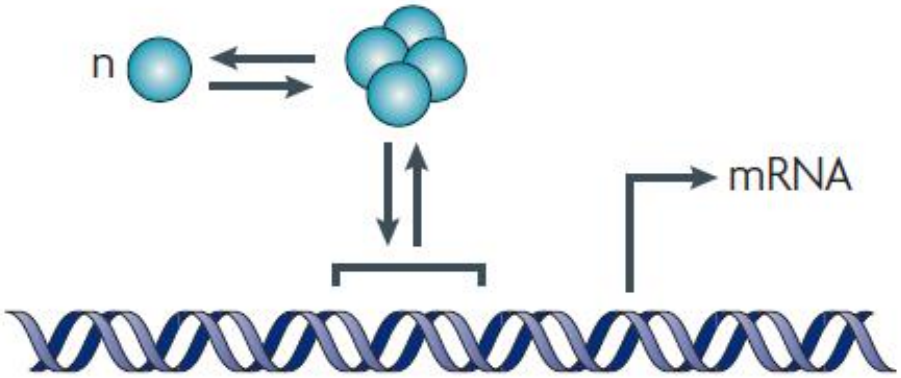


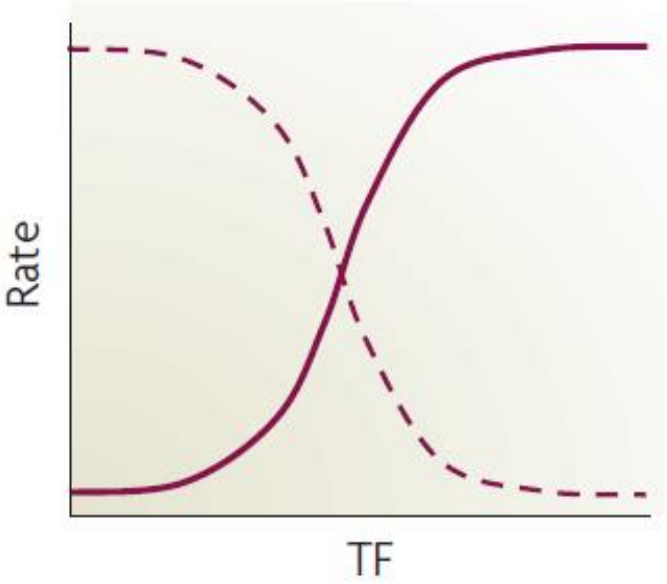


In these motifs, X ⟶ Y means 'X activates Y' and X ⟞ Y means 'X inhibits Y', X ⟜ Y means 'X either activates or inhibits Y'. Two white circles appear in the same motif have the same sign.

The source of nonlinearity

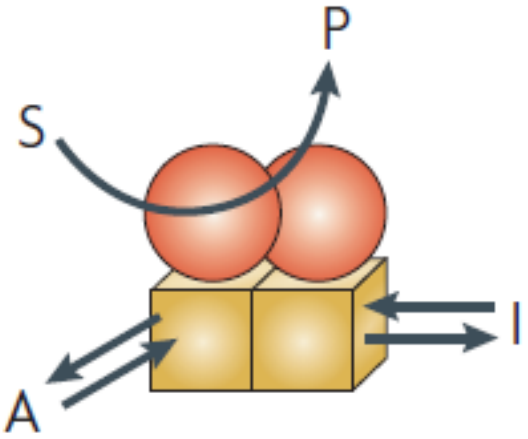
1. Oligomer binding: hill function $S^p / (K_d^p + S^p)$.

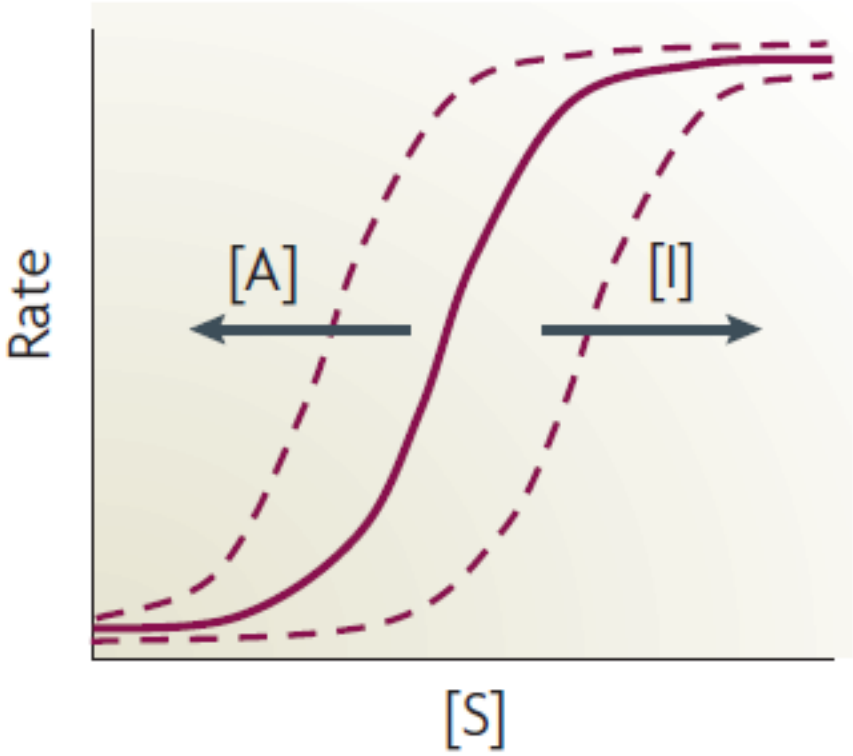




The source of nonlinearity

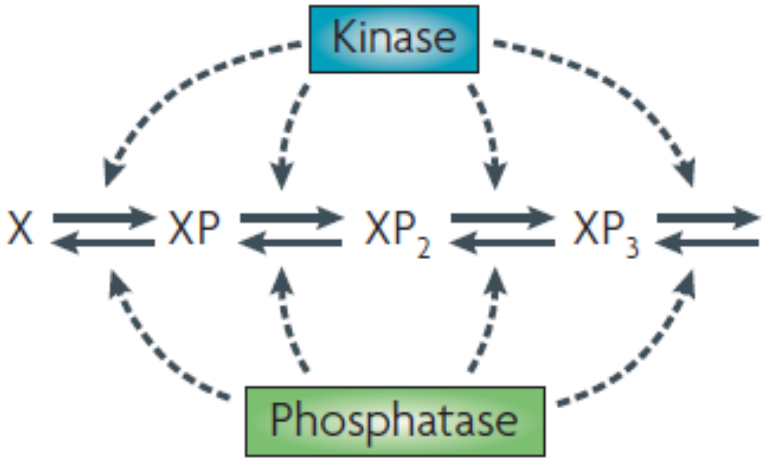
2. Cooperative and allostery: sigmoidal functions (hill)

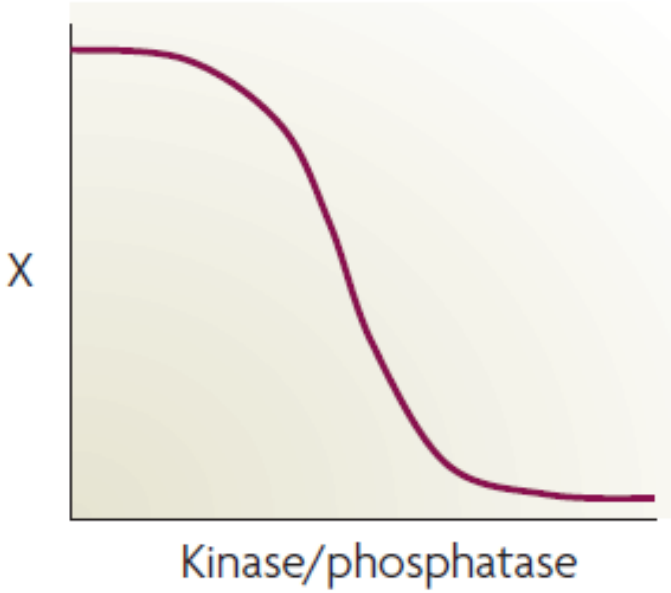




The source of nonlinearity

3. Multisite phosphorylation: reversible phosphorylation and dephosphorylation can create sigmoidal signal-response curve





D. Classification of oscillatory motifs

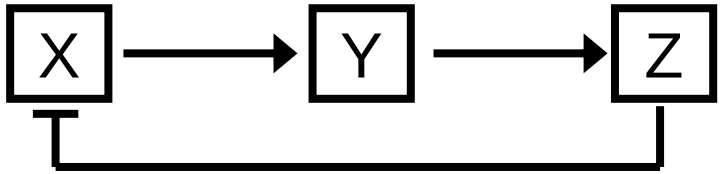
General scheme for classification:

1. Oscillator always involve a negative feedback loop.
    1. The authors knew of no examples of chemical oscillations without negative feedback.
    2. Negative feedback seems necessary to close a sequence of chemical states back onto itself
    3. In all the examples, use X to denote ‘activator’ and Y to denote ‘inhibitor’,Z to denote ‘intermediate’
2. Ignore the direct autocatalysis
3. Minimal three components and 3-4 interaction links

Oscillatory motifs:
Class I, delayed feedback loop

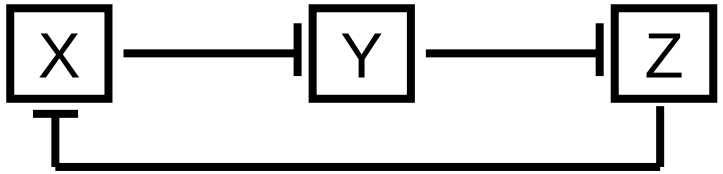
Since the total effects of the three component loop need to be negative feedback, there are two motifs:

I-a



Examples:
1. Circadian oscillation of PER in drosophila
2. Oscillation of p53 in response to ionizing radiation
   X=p53, Y=MDM2 mRNA, Z=MDM2 protein
3. Oscillation of NF-κB in response to TNF
   X= NF-κB, Y= IκB mRNA, Z= IκB protein

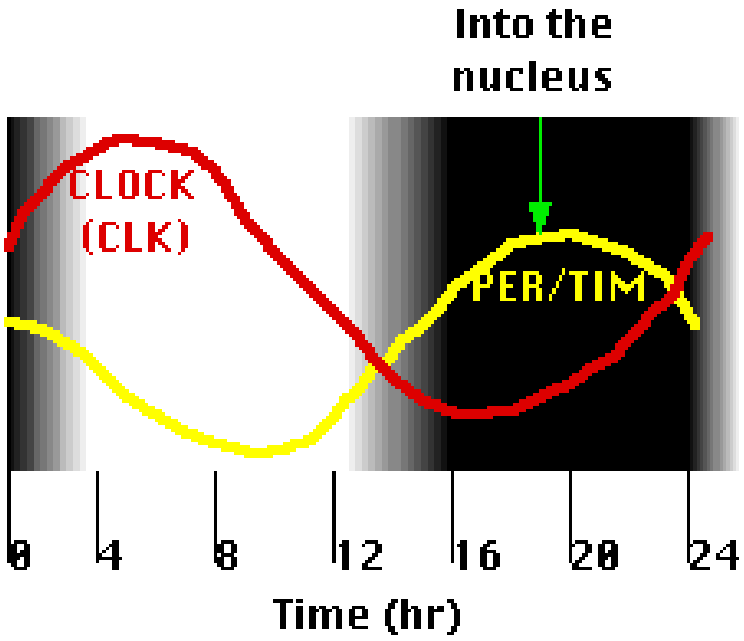
I-b



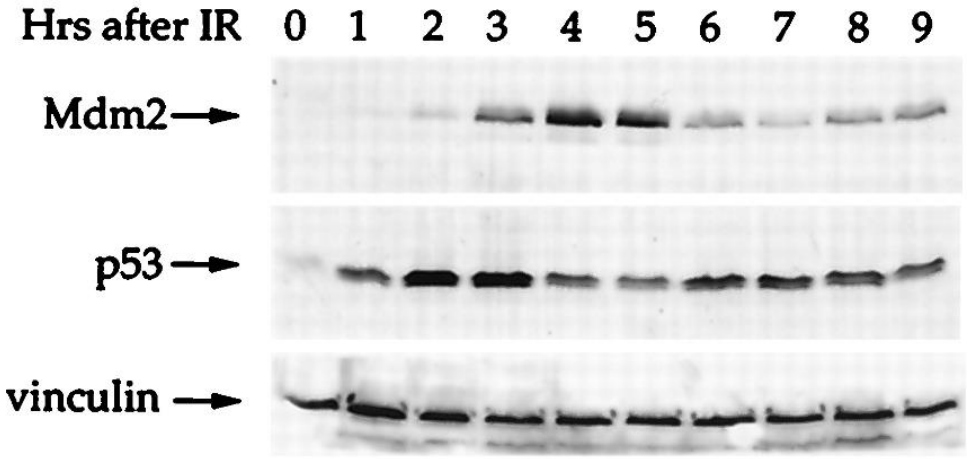
Examples:
1. Repressillator
   X=TetR, Y=cI, Z=LacI

Experimental data for Class I-a

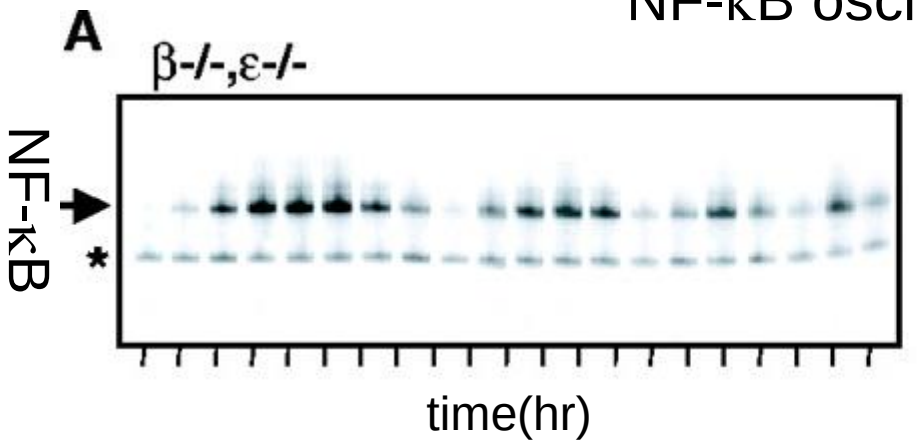
Drosophila circadian rhythm

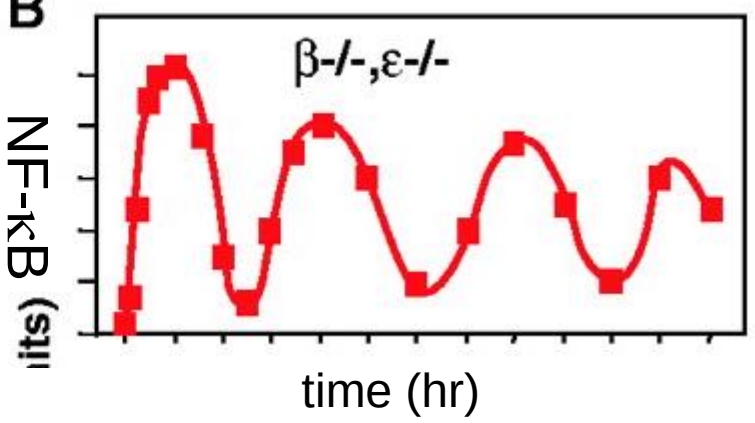


P53-MDM2 oscillation



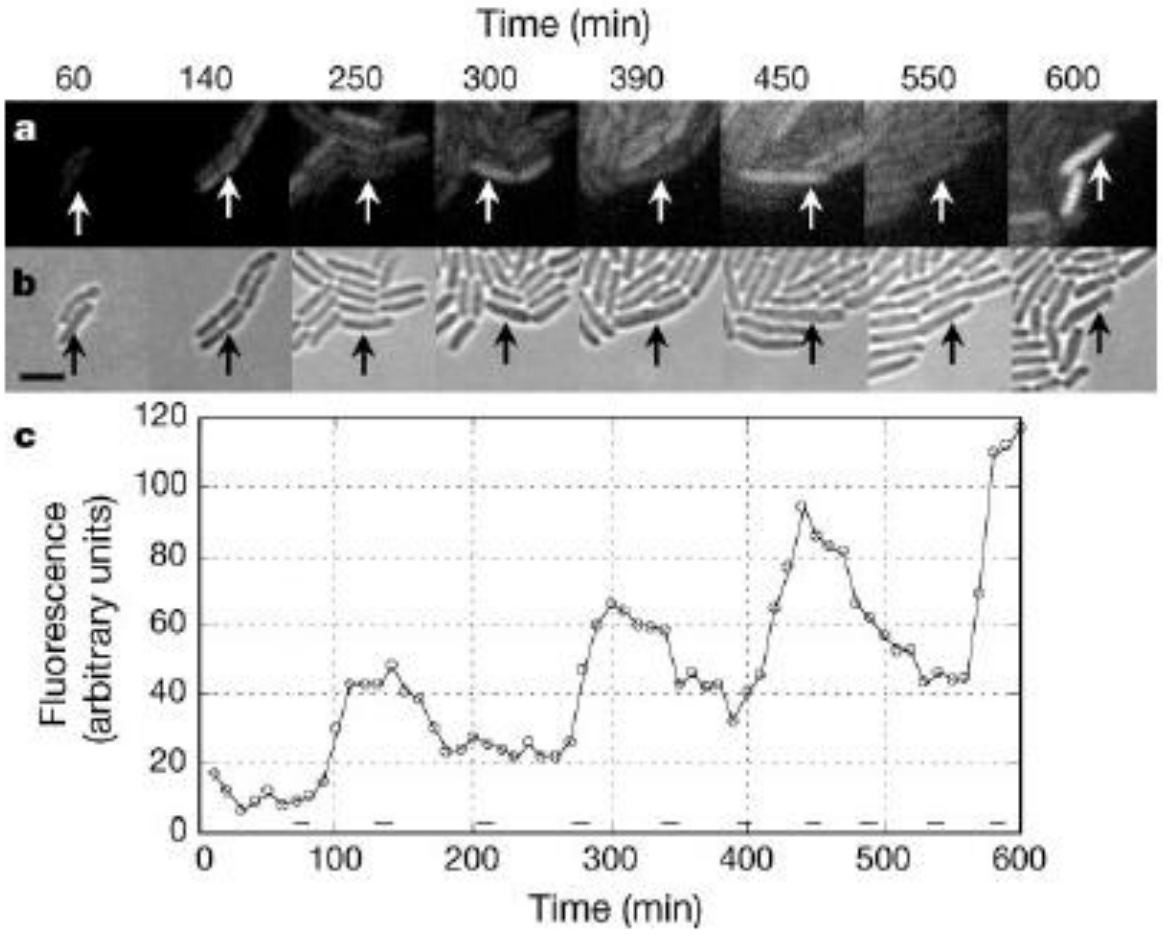
NF-κB oscillation





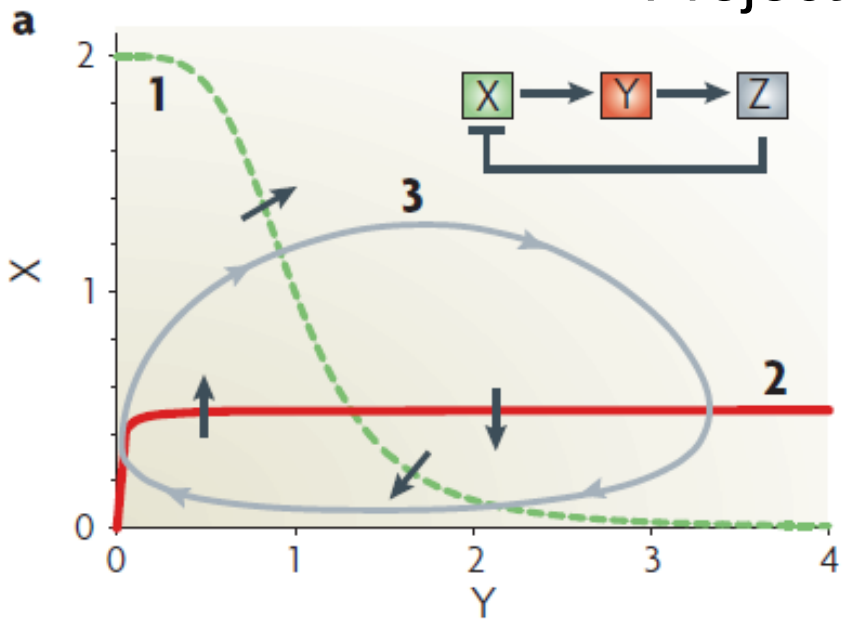
Experimental data for Class I-b

repressillator

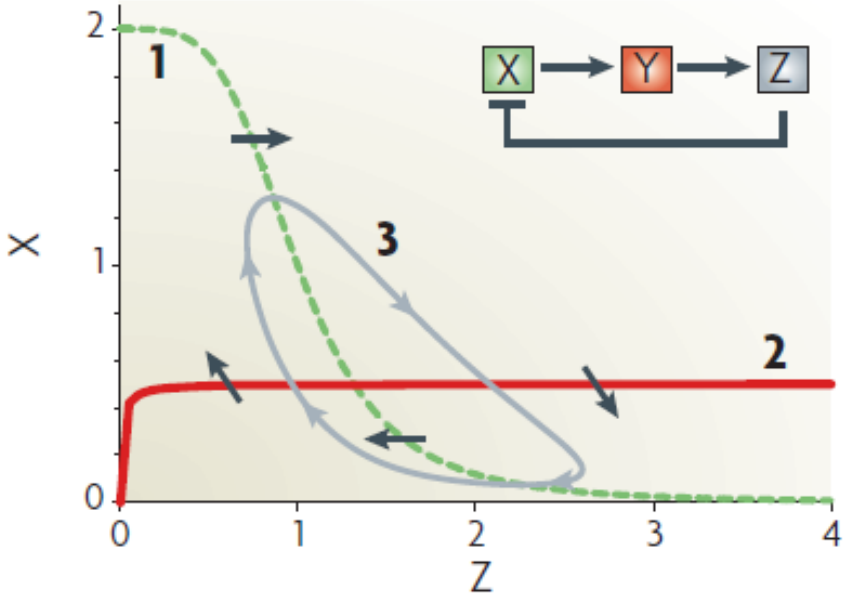


Stability analysis of I-a motif

Projection of 3D onto 2D



Limit cycle trajectory does not cross curve 1 in a horizontal direction because the rate of synthesis of X depends on the concentration of Y some time in the past.

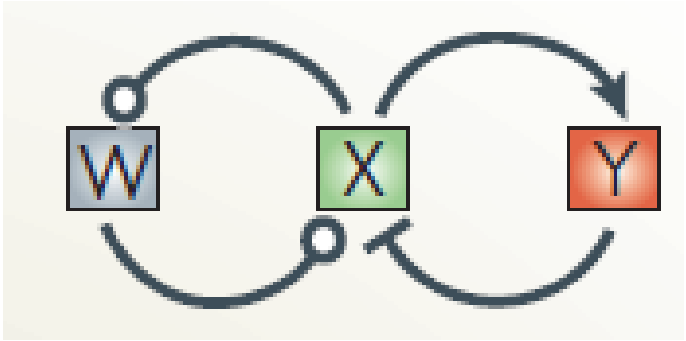


Limit cycle trajectory does not cross curve 2 in a vertical direction because the rate of synthesis of Z depends on the concentration of X some time in the past.

Oscillatory motifs: Class II, amplified negative-feedback loops

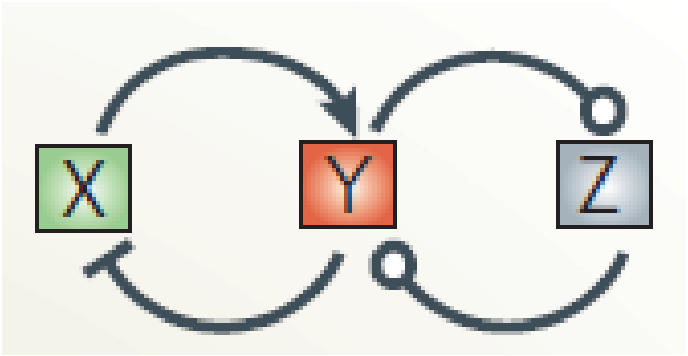
Since the W-facilitated positive feedback amplification can be on either activator or inhibitor, and the amplification could be either ++ or - - loop, there are four motifs in two groups:

II-a (2)



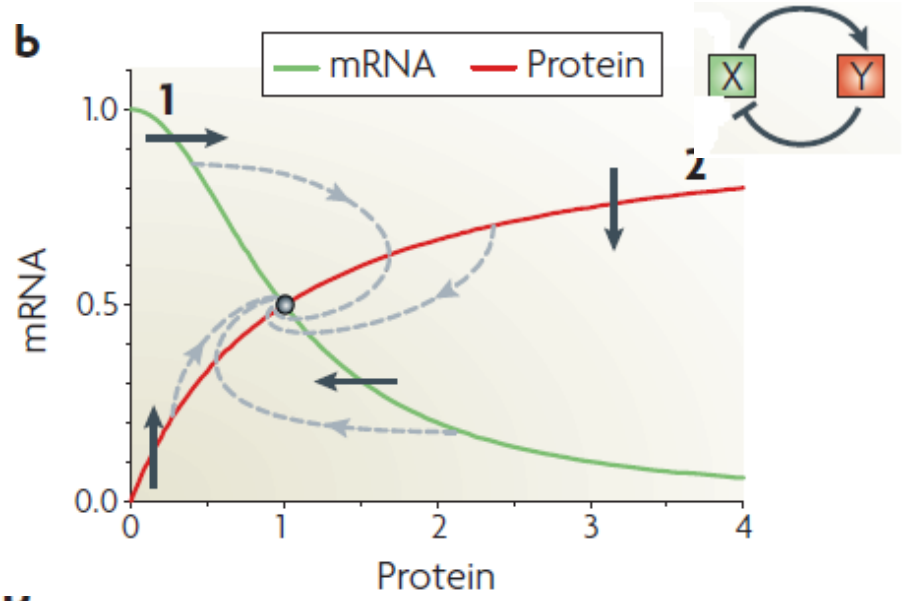
1. Mitosis-promoting factor (MPF) in frog egg extract. X=MPF, Y=Cdc20, W=Cdc25
2. J. Hasty's synchronized synthetic oscillator

II-b (2)

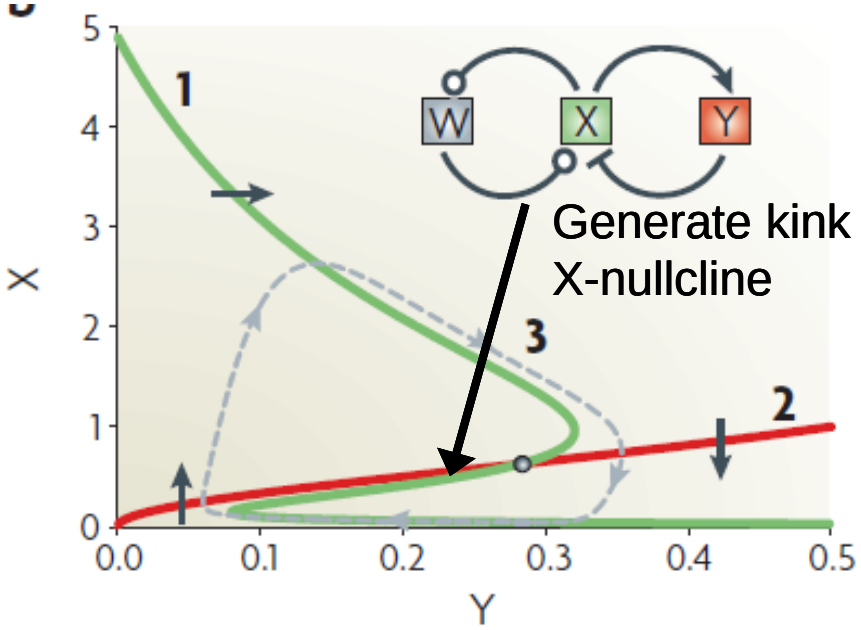


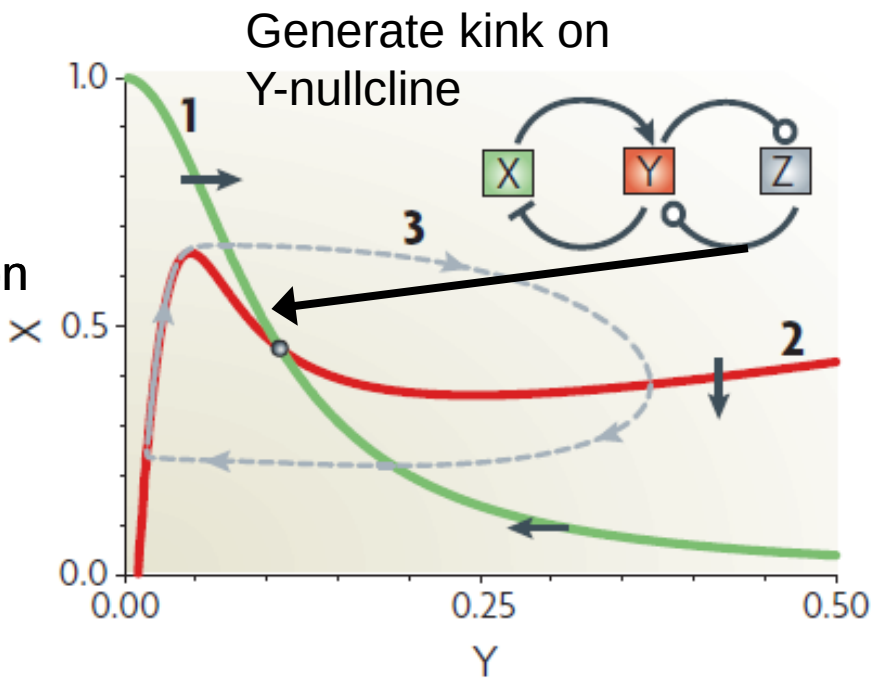
Periodic DNA replication in the absence of cell division
X=Cdc10, Y=Cig2, Z=Rum1

Stability analysis of Class II oscillator



The nullclines are kinked by amplification. The trajectories are forced to wheel around the unstable steady state

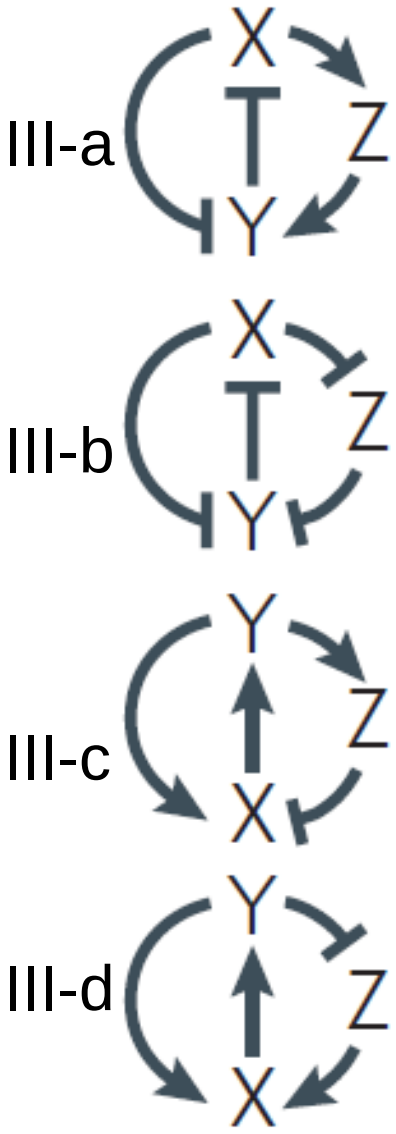




Oscillatory motifs:Class III, incoherently amplified negative-feedback loops

Four motifs:
Each consists of a (1) three-component negative-feedback loop (oscillatory) and a (2) two-component positive-feedback loop (amplification).
Each also contains an incoherent feed-forward loop.

Example: III-d: glycolytic oscillation (early model)
Y=phospho-fructokinase
X=fructose-6-phosphate+ADP
Z=fructose-1,6-phosphate+ATP
Etc.



Other possibilities or more complex topology

B. Novak and J.J. Tyson, Nature Review Molecular Cell Biology 2008, Vol 9,981-991