Computational Systems Biology

. . . Biology X – Lecture 8. . .

Bud Mishra

Professor of Computer Science, Mathematics, & Cell Biology

Modeling

Modeling Biomolecular Networks

- Agents and Modes:
  - **Species and Processes:** There are two kinds of agents:
    - **S-agents** (representing species such as proteins, cells and DNA): S-agents are described by concentration (i.e., their numbers) and its variation due to accumulation or degradation. S-agent's description involves differential equations or update equations.
    - **P-agents** (representing processes such as transcription, translation, protein binding, protein-protein interactions, and cell growth.) Inputs of P-agents are concentrations (or numbers) of species and outputs are rates.

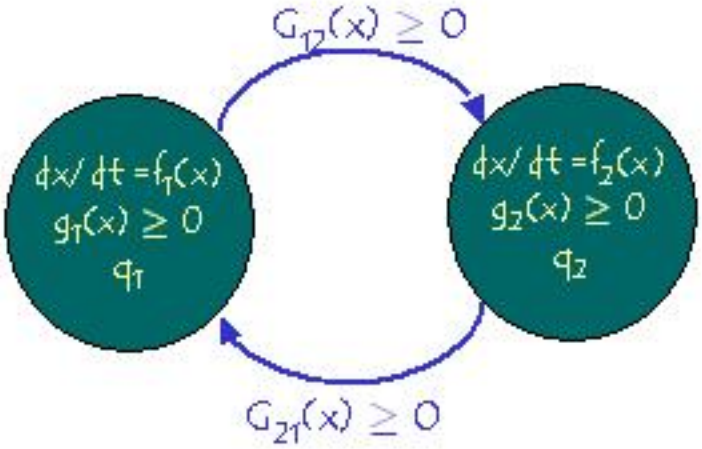
Agents & Modes

- Each agent is characterized by a state $x \in \mathbb{R}^n$ and
- A collection of discrete modes denoted by Q
- Each mode is characterized by a set of differential equations ($q_i \in Q$ & $z \in \mathbb{R}^p$ is control)

$$dx/dt = f_{qi}(x,z),$$

  - and a set of invariants that describe the conditions under which the above ODE is valid…
  - these invariants describe algebraic constraints on the continuous state…

Example of a Hybrid System



- q_1 and q_2 = two discrete modes
- x = continuous variable evolving as
  - $dx/dt = f_1(x)$ in mode q_1
  - $dx/dt = f_2(x)$ in mode q_2
- Invariants: Associated with locations q_1 and q_2 are
  - $g_1(x) \geq 0$ and $g_2(x) \geq 0$, resp.
- The hybrid system evolves continuously in disc. mode q_1 according to $dx/dt = f_1(x)$ as long as $g_1(x) \geq 0$ holds.
- If ever x enters the "guard set" $G_{12}(x) \geq 0$, then mode transition from q_1 to q_2 occurs.

Generic Equation

- Generic formula for any molecular species (mRNA, protein, protein complex, or small molecule):

$$dX/dt = \text{synthesis} - \text{decay} \pm \text{transformation} \pm \text{transport}$$

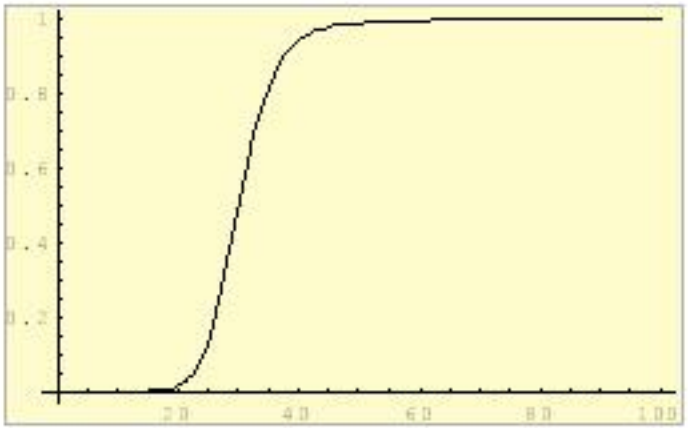
- Synthesis:
  - replication for DNA,
  - transcription of mRNA,
  - translation for protein
- Decay: A first order degradation process
- Transformation:
  - cleavage reaction
  - ligand binding reaction
- Transport: Diffusion through a membrane..

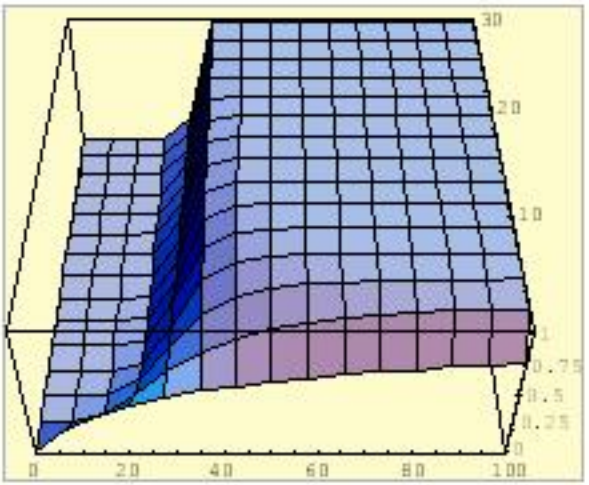
Model of transcription

- ν_{Xm} = Cooperativity coefficient
- κ_{Xm} = Concentration of X at which transcription of m is "half-maximally" activated.

- $\Phi(X, \kappa_{Xm}, \nu_{Xm}) = X^{\nu}/[\kappa^{\nu} + X^{\nu}]$
- $\Psi(X, \kappa_{Xm}, \nu_{Xm}) = \kappa^{\nu}/[\kappa^{\nu} + X^{\nu}] = 1 - \Phi(X, \kappa_{Xm}, \nu_{Xm})$
- A graph of function Φ = Sigmoid Function

Transcription Activation Function

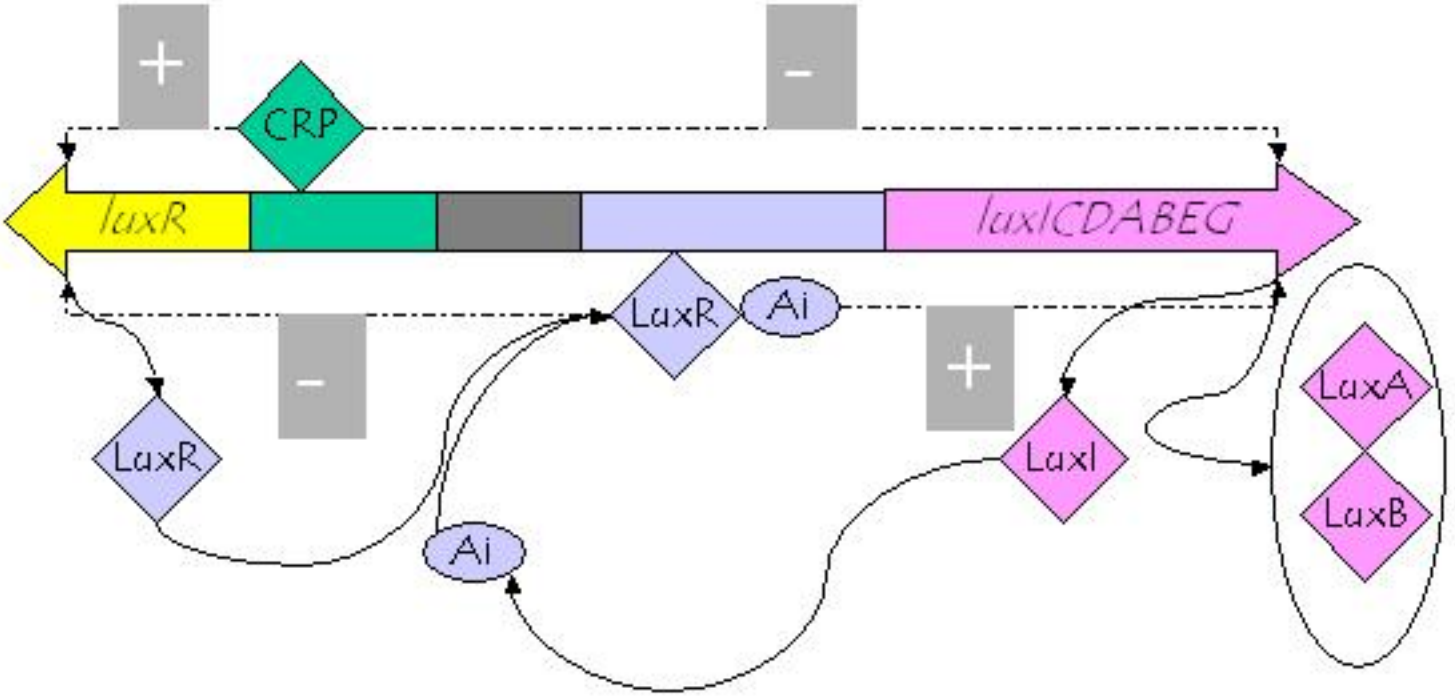




Quorum Sensing in V. fischeri

- Cell-density dependent gene expression in prokaryotes
  - Quorum = A minimum population unit
- A single cell of V. fischeri can sense when a quorum of bacteria is achieved—leading to bioluminescence…
- Vibrio fiscehri is a marine bacterium found as
  - a free-living organism, and
  - a symbiont of some marine fish and squid.
    - As a free-living organism, it exists in low density is non-luminescent..
    - As a symbiont, it lives in high density and is luminescent..
    - The transcription of the lux genes in this organism controls this luminescence

lux gene



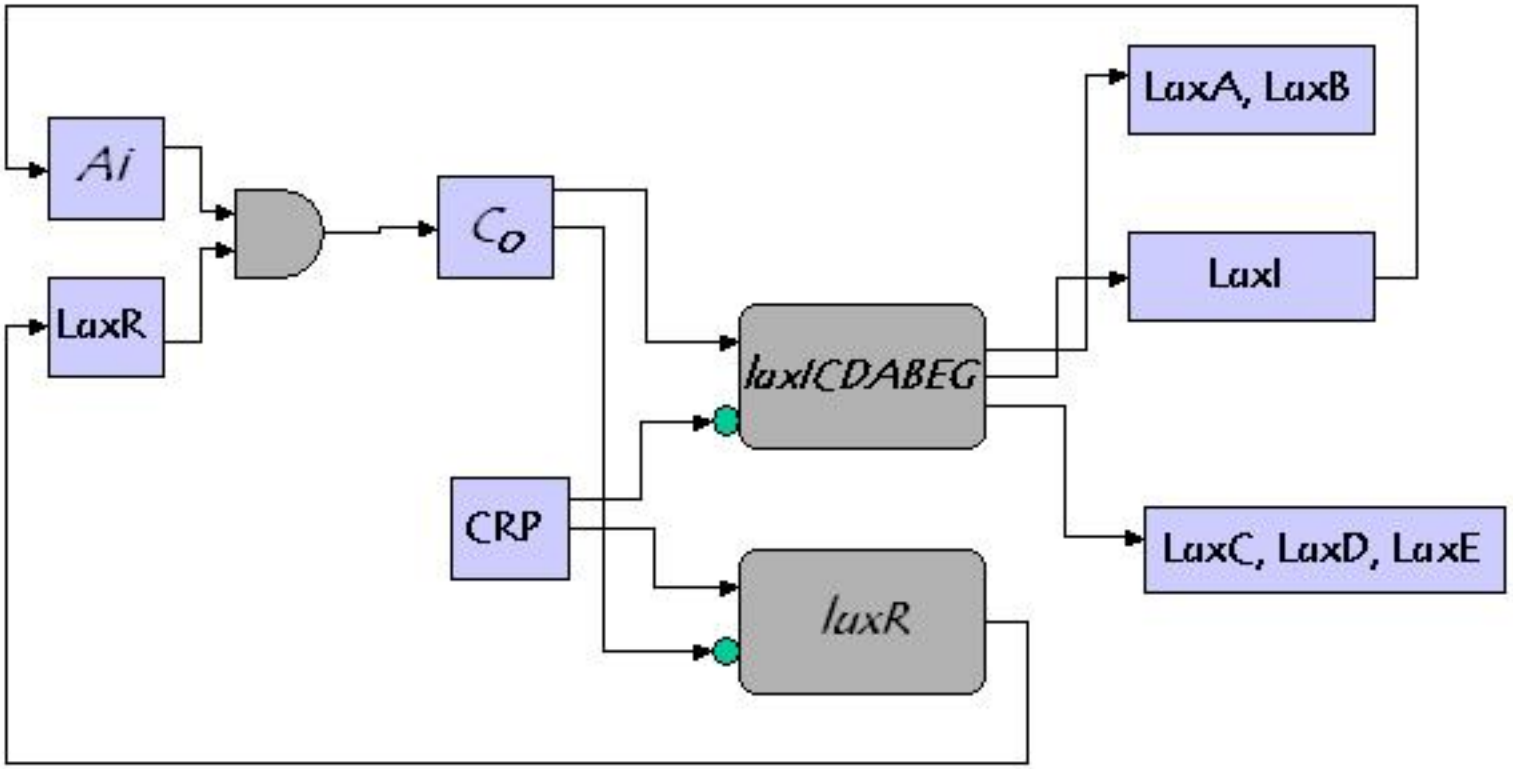
Quorum Sensing

- The *lux* region is organized in two transcriptional units:
  - O_L: containing *luxR* gene (encodes protein LuxR = a transcriptional regulator)
  - O_R: containing 7 genes *luxICDABEG*.
    - Transcription of *luxI* produces the protein LuxI, required for endogenous production of the autoinducer *AI* (a small membrane permeable signal molecule (acyl-homoserine lactone).
    - The genes *luxA* & *luxB* code for the luciferase subunits
    - The genes *luxC*, *luxD* & *luxE* code for proteins of the fatty acid reductase, needed for aldehyde substrate for luciferase.
    - The gene *luxG* encodes a flavin reductase.
    - Along with LuxR and LuxI, cAMP receptor protein (CRP) controls luminescence.

Biochemical Network

- The autoimmune inducer *Ai* binds to protein LuxR to form a complex C_0 which binds to the *lux box*.
- The *lux box* region (between the transcriptional units) contains a binding site for CRP.
- The transcription from the *luxR* promoter is activated by the binding of CRP.
- The transcription from the *luxICDABEG* is activated by the binding of C_0 complex to the *lux box*.
- Growth in the levels of C_0 and cAMP/CRP inhibit *luxR* and *luxICDABEG* transcription,

Biochemical Network



Notation

- x_0 = scaled population
- x_1 = mRNA transcribed from O_L
- x_2 = mRNA transcribed from O_R
- x_3 = protein LuxR
- x_4 = protein LuxI
- x_5 = protein LuxA/B
- x_6 = protein LuxC/D/E
- x_7 = autoinducer *Ai*
- x_8 = complex C_0

Evolution Equations...

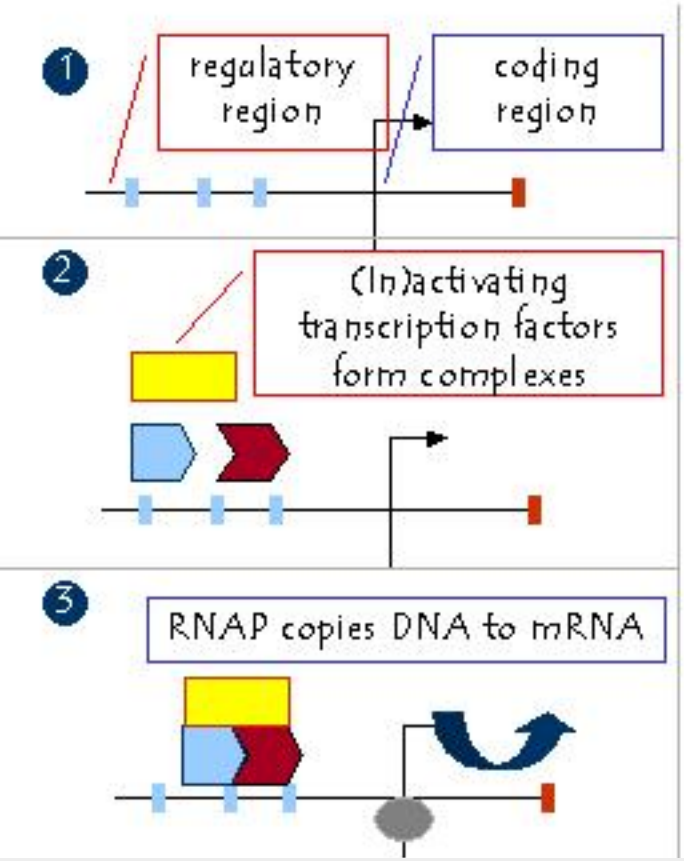
- $dx_0/dt = k_G x_0$
- $dx_1/dt = T_c[\Psi(x_8, \kappa_{C0}, \nu_{C0})\ \Phi(c_{CRP}, \kappa_{CRP}, \nu_{CRP})+b] - x_1/H_{RNA} - k_G x_1$
- $dx_2/dt = T_c[\Phi(x_8, \kappa_{C0}, \nu_{C0})\ \Psi(c_{CRP}, \kappa_{CRP}, \nu_{CRP})+b] - x_2/H_{RNA} - k_G x_2$
- $dx_3/dt = T_l x_1 - x_3/H_{sp} - r_{AiR} x_7 x_3 - r_{C0} x_8 - k_G x_3$
- $dx_4/dt = T_l x_2 - x_4/H_{sp} - k_G x_4$
- $dx_5/dt = T_l x_2 - x_5/H_{sp} - k_G x_5$
- $dx_6/dt = T_l x_2 - x_6/H_{sp} - k_G x_6$
- $dx_7/dt = x_0(r_{All} x_4 - r_{AiR} x_7 x_3 + r_{C0} x_8) - x_7/H_{Ai}$
- $dx_8/dt = r_{AiR} x_7 x_3 - x_8/H_{sp} - r_{C0} x_8 - k_G x_8$

Parameters

T_c	Max. transcription rate	ν_{CRP}	Cooperativity coef for CRP
T_l	Max. translation rate	κ_{CRP}	Half-max conc for CRP
H_{RNA}	RNA half-life	ν_{C0}	Cooperativity coef for C_o
H_{sp}	Stable protein half-life	κ_{C0}	Half-max conc for C_o
H_{up}	Unstable protein half-life	b	Basal transcription rate
H_{Ai}	$A/$ half-life	v_b	Volume of a bacterium
r_{AII}	Rate constant: LuxI $\rightarrow A/$	V	Volume of solution
r_{AiR}	Rate constant: $A/$ binds to LuxI	k_g	Growth rate
r_{C0}	Rate constant: C_o dissociates	x_{0max}	Maximum Population

Regulatory Networks

Transcription Initiation

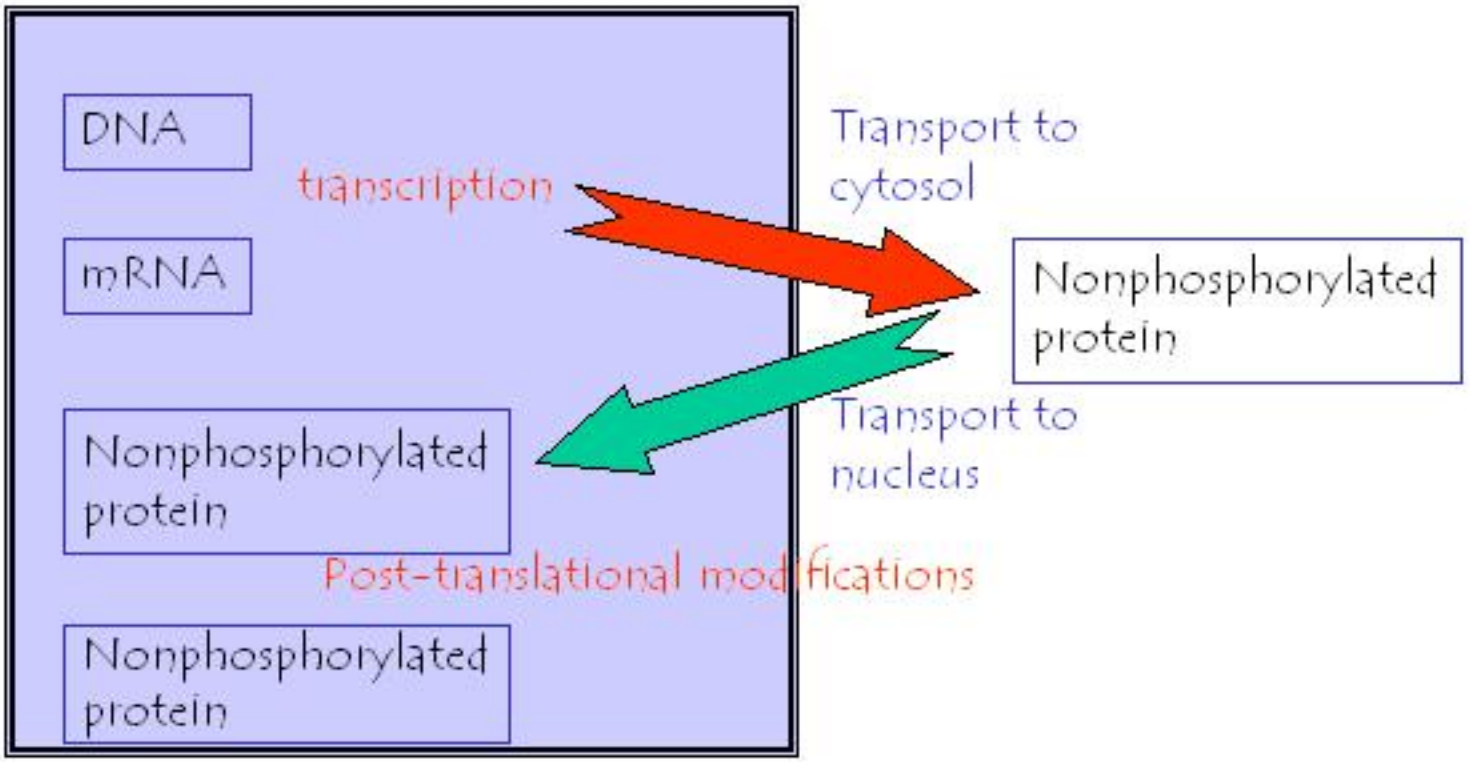


- Typically, TFs do not bind singly, but in complexes:
- Once bound to the DNA, TF complex allows RNA polymerase (RNAP) to bind to the DNA upstream of the coding region.
- RNAP forms a transcriptional complex that separates the two strands of DNA, thus forming an open complex, then moves along one strand of the DNA, step by step and transcribes the coding region into mRNA.

Regulatory Networks

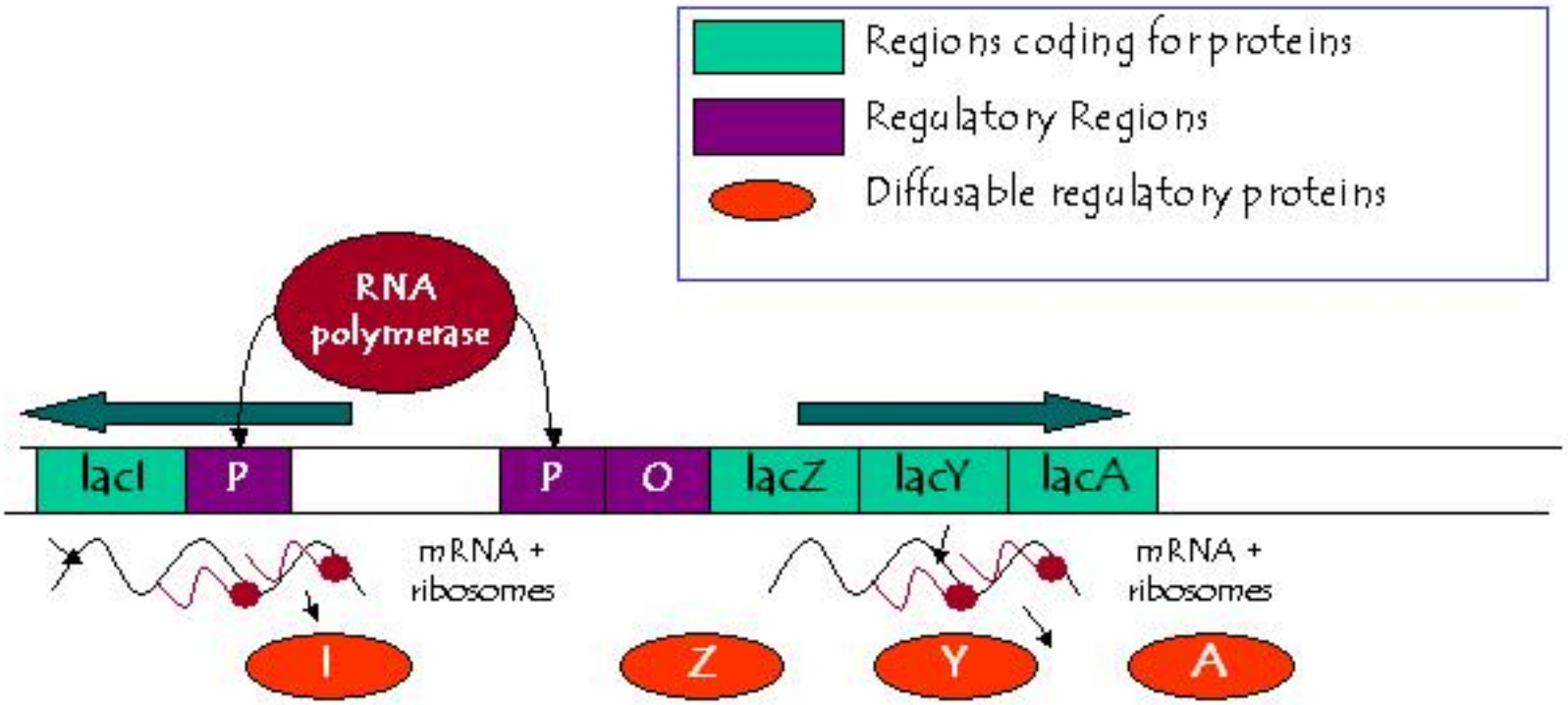
- All cells in an organism have the same genomic data, but the proteins synthesized in each vary according to cell type, time and environmental factors
- There are network of interactions among various biochemical entities in a cell (DNA RNA, protein, small molecules)

Gene Regulation



Transcriptional Regulation:

Example: The lac Operon



The *lac* Operon

- Regulates utilization of lactose by the bacterium *E. coli*.
- Lactose is not generally available to *E. coli* as a food substrate, so the bacterium does not usually synthesize the enzymes necessary for its metabolic use.
- There is an operon, called the *lac* operaon, normally turned off, that codes for three enzymes:
  - β-galactoside permease, β-galactosidase and β-thiogalactoside acetyl transferase.

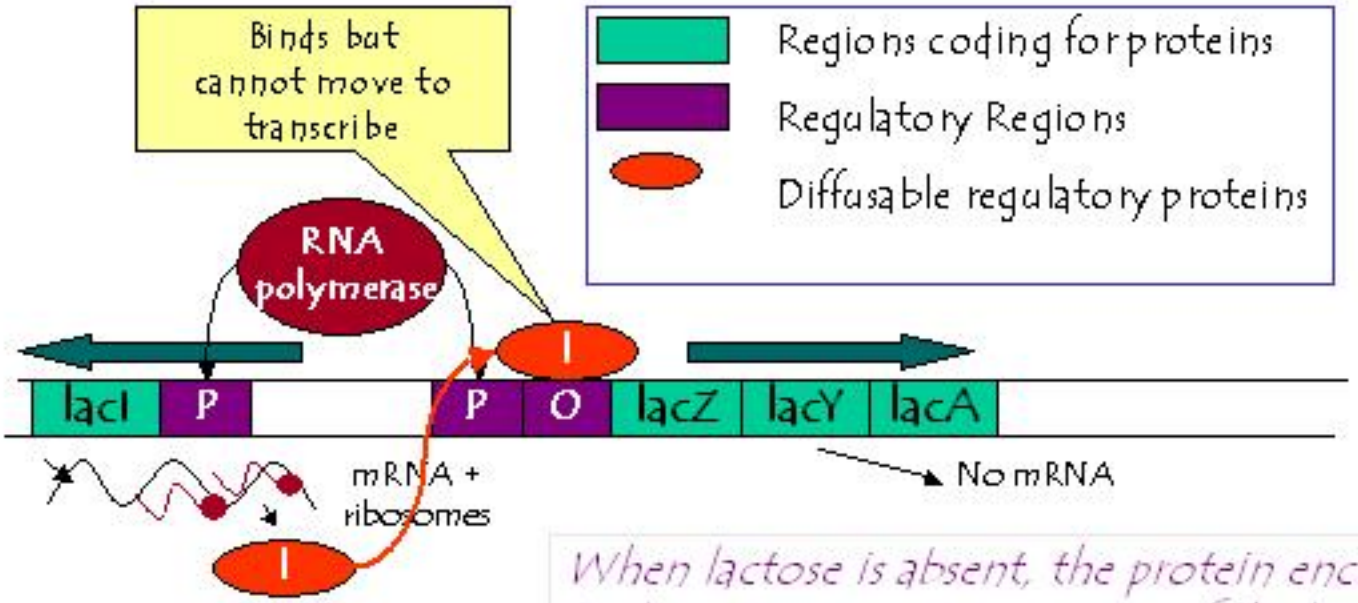
Activation of the *lac* operon

- If the bacterium is exposed to lactose, these enzymes work together to
  - transport lactose into the cell and
  - isomerizes lactose into allolactose (an allosteric isomer of lactose).
- The allolactose binds with a repressor molecule to keep it from repressing the production of mRNA.
- Production of allolactose turns on the production of mRNA, which then leads to production of more enzyme, enabling production of more lactose to allolactose...

An autocatalytic reaction..

Transcriptional Regulation:

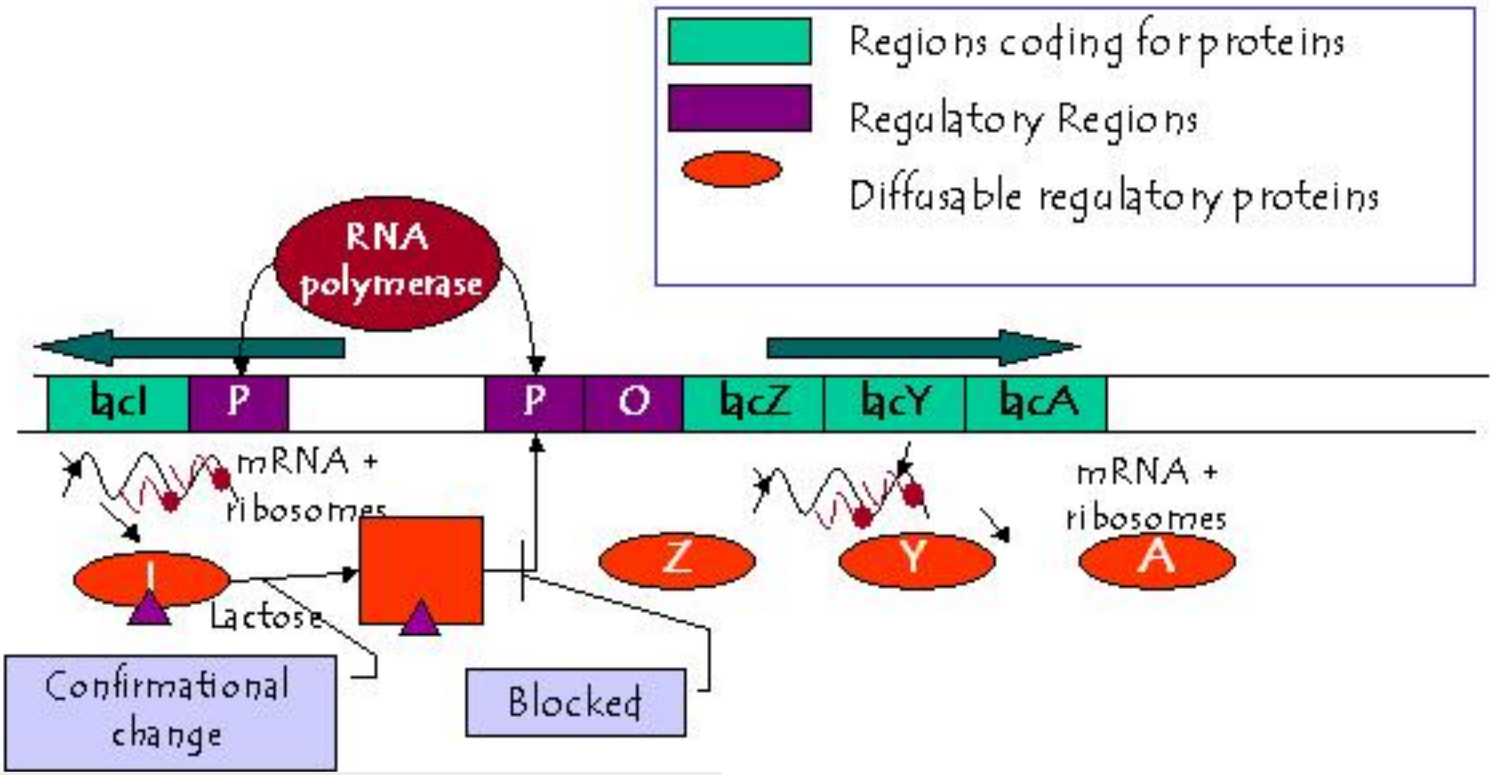
Example: The lac Operon



When lactose is absent, the protein encoded by lacI represses transcription of the lac operon

Transcriptional Regulation:

Example: The lac Operon



Mathematical Model

$$G + mP \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} X$$

- Production of enzyme is turned on by m molecules of the product allolactose P…
- G=Inactive state of the gene
- X=Active state of the gene
- In a large population of genes, the percentage of active genes is given by the chemical equilibrium:

$$p = [P]^m / (k_{eq}^m + [P]^m)$$

Production of mRNA

- The differential equation governing the (average) production of mRNA

$$dM/dt = M_0 + k_1 [P]^m/(k_{eq}^m + [P]^m) - k_2 M,$$

- where M is the concentration of mRNA that codes for the enzyme.
- Production of the enzymes (responsible for tarnsforming into allolactose substrate):

$$dE_1/dt = c_1 M - d_1 E_1;$$

$$dE_2/dt = c_2 M - d_2 E_2.$$

Lactose states

- S_0 = Concentration of the lactose that is exterior to the cell.
- S = Concentration of the lactose that is interior to the cell.
- [P] = Concentration of allolactose.

$$dS_0/dt = -\sigma_0 E_1 S_0/(k_0 + S_0)$$

$$dS/dt = \sigma_0 E_1 S_0/(k_0 + S_0) - \sigma_1 E_2 S/(k_s + S)$$

$$d[P]/dt = \sigma_1 E_2 S/(k_s + S) - \sigma_2 E_2 [P]/(k_p + [P])$$

Simplification

- Assume: mRNA is in quasi-steady state:

$$M = (k_1/k_2)\,[P]^m/(k_{eq}^m + [P]^m) + M_0/k_2;$$

- Assume: $d_1 = d_2$. Degradation is slow compared to cell growth. Also, $E_1 = E_2$.

$$d E_1/dt = c_1 M_0/k_2 + (c_1 k_1/k_2)\,[P]^m/(k_{eq}^m + [P]^m) - d_1 E_1;$$

- Assume: No delay in conversion of the lactose into allolactose:

$$d[P]/dt = \sigma_0 E_1 S_0/(k_0 + S_0) - \sigma_2 E_1 [P]/(k_p + [P]).$$

Dimensionless Form

- Dimensionless variables: $S_0 = k_0\, s$, $[P] = k_p\, p$, $E_1 = e_0\, e$, and $t = t_0\, \tau$...

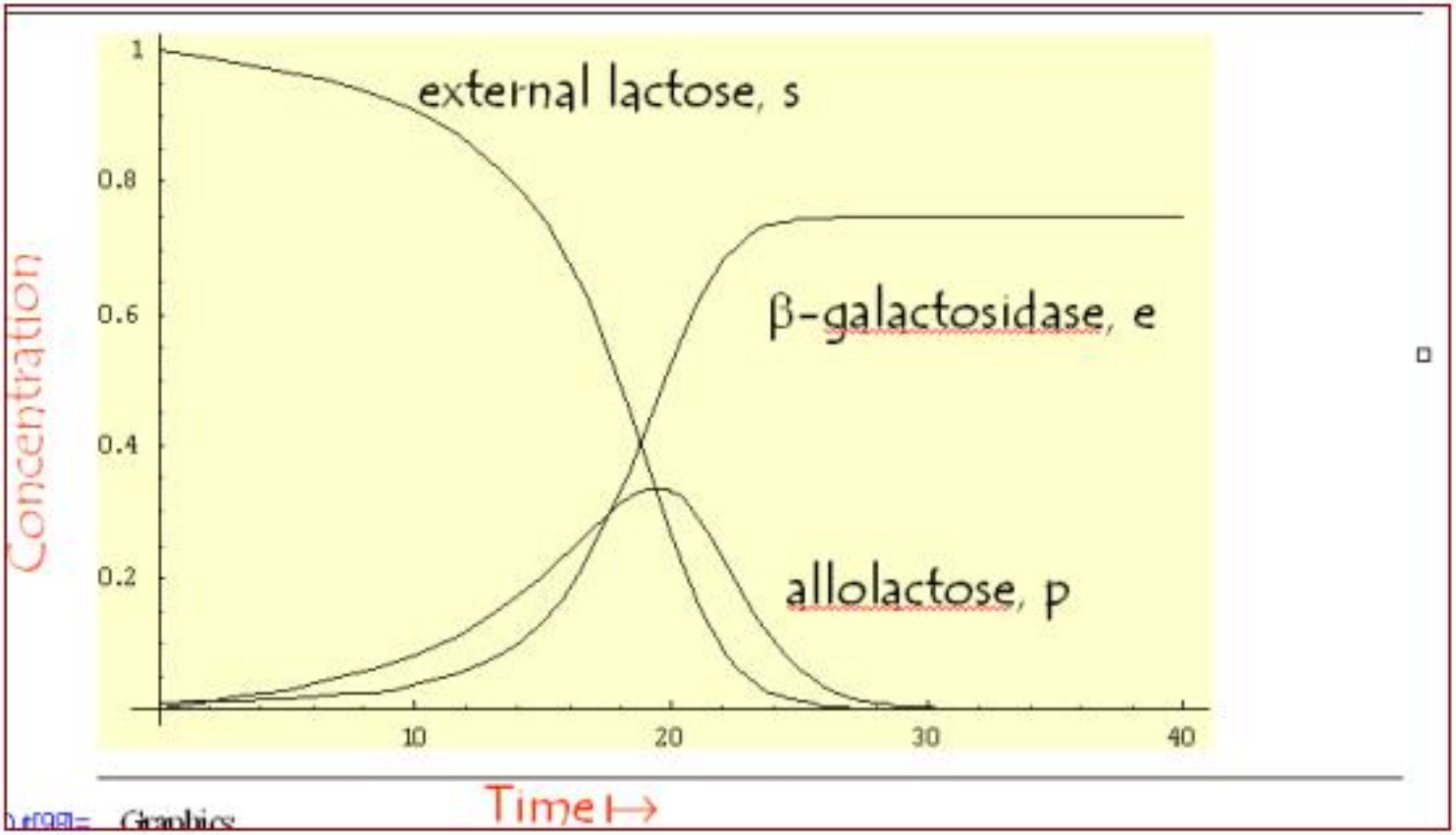
$$de/d\tau = m_0 + p^m/(\kappa^m + p^m) - \varepsilon\, e,$$

$$dp/d\tau = \mu\, e[s/(s+1) - \lambda\, p/(p+1)],$$

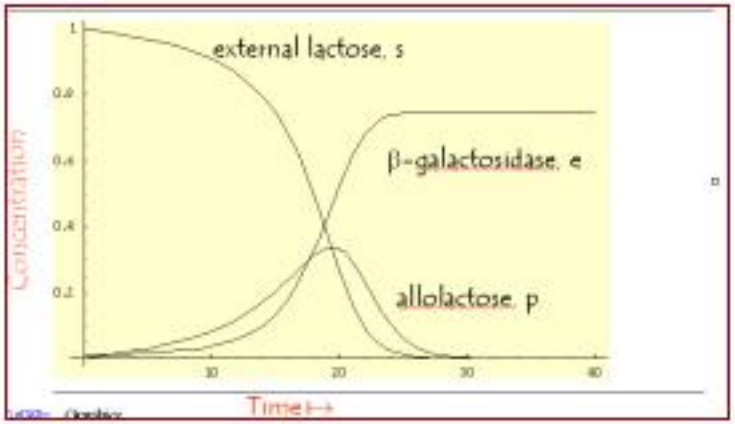
$$ds/d\tau = -e\, s/(s+1),$$

- where $e_0^2 = c_1 k_0 k_1/(\sigma_0 k_2)$, $t_0 = k+0/(e_0 \sigma_0)$, $\lambda = \sigma_2/\sigma_0$, $\mu = k_0/k_p$, $\kappa = k/k_p$, $m_0 = M_0/k_1$, and $\varepsilon = t_0\, d_1$...

Time Evolution



The *lac* operon

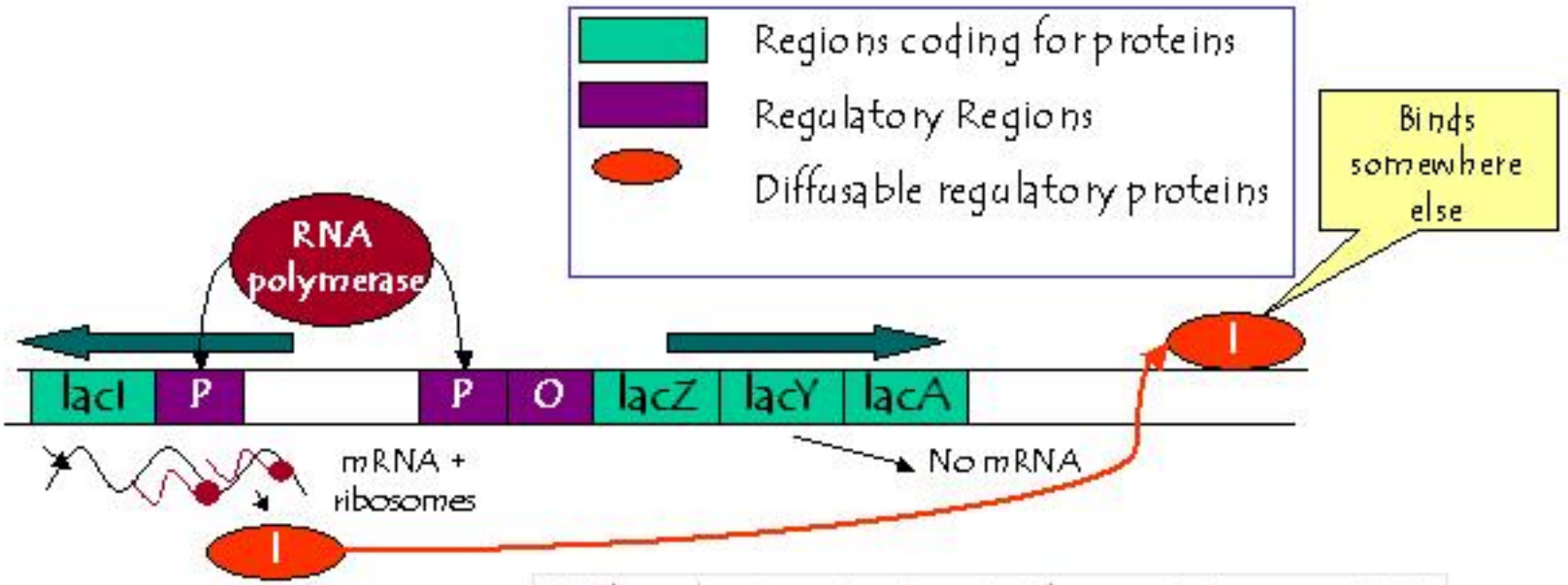


- If the amount of lactose is too small, then the lactose is gradually depleted, although there is no increase in enzyme concentration.
- However, if the lactose dose is sufficiently large, then there is an autocatalytic response, as the lac operon is turned on and enzyme is produced.
- The production of enzyme shuts down when the lactose stimuls is consumed, and the enzyme concentration gradually declines…

Example of Competition

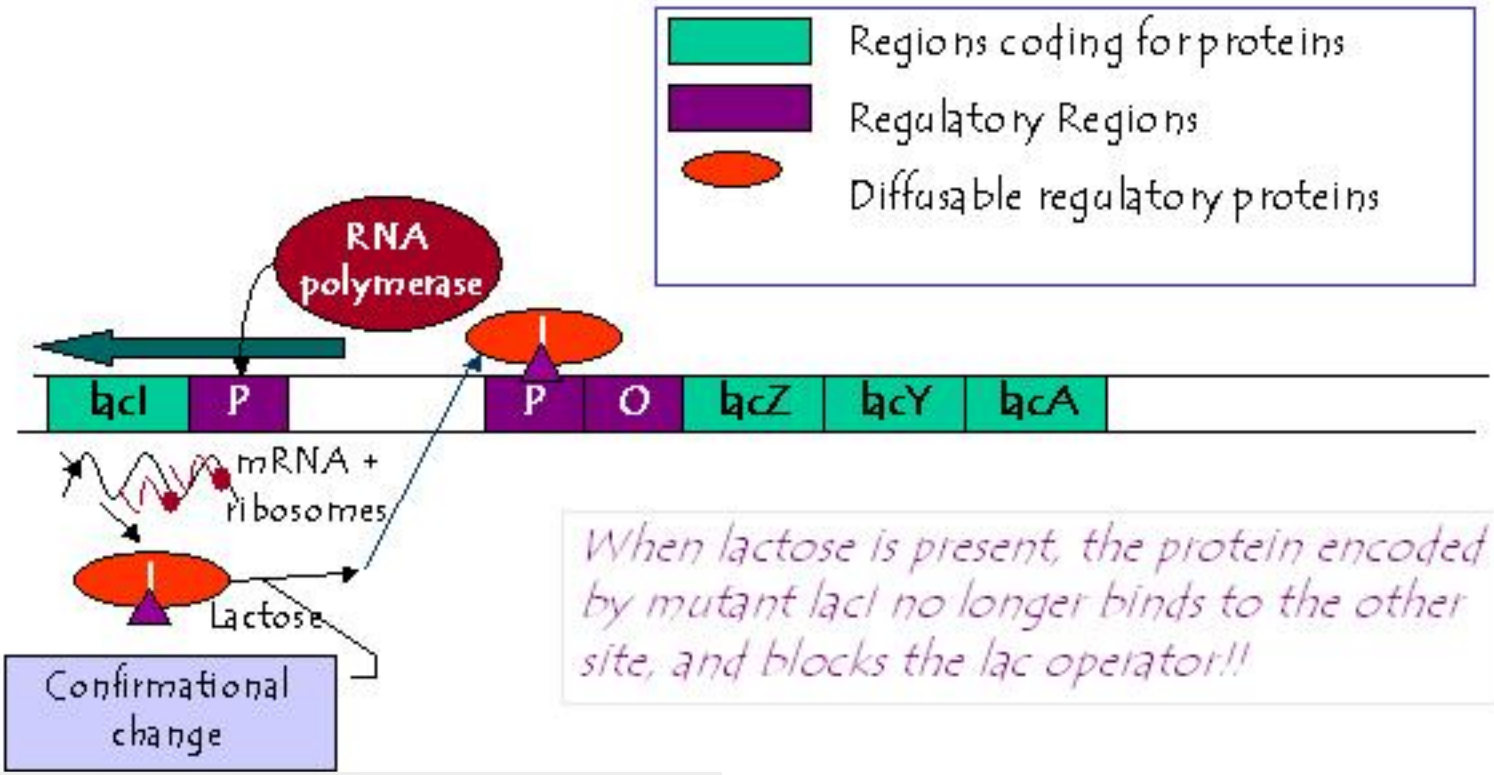
- The mutant Lac repressor X186:
  - This mutant represses transcription of the *lac* genes in the presence of lactose...
  - The mutant binds DNA so tightly that, in the absence of inducer (allolactose), it is sequestered on non-operator DNA sites.
  - The inducer weakens the binding of the mutant repressor; thus, allowing it bind to the *lac* operon.

Lac repressor X 186



When lactose is absent, the protein encoded by mutant lacI binds to some other site.

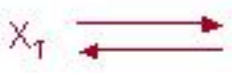
Lac repressor X 186



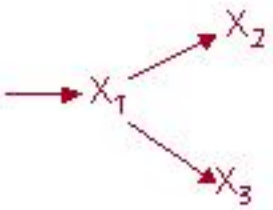
When lactose is present, the protein encoded by mutant lacI no longer binds to the other site, and blocks the lac operator!!

S-Systems

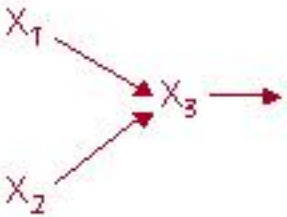
Graphical Representation



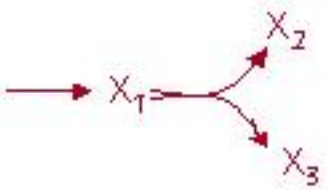
Reversible Reaction



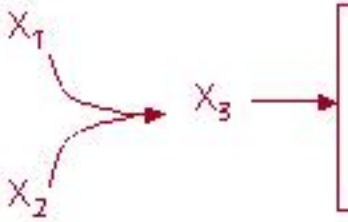
Divergence Branch Point: Degradation processes of X_1 into X_2 and X_3 are independent



Convergence Branch Point: Degradation processes of X_1 into X_2 and X_3 are independent

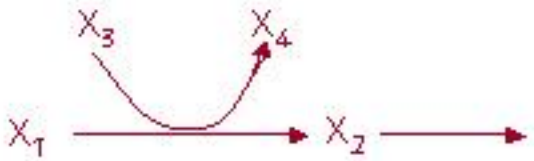


Single splitting reaction generating two products X_2 and X_3, in stoichiometric proportion.

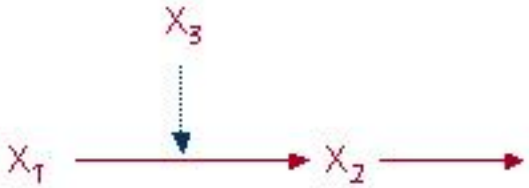


Single synthetic reaction involving two source components X_1 and X_2, in stoichiometric proportion.

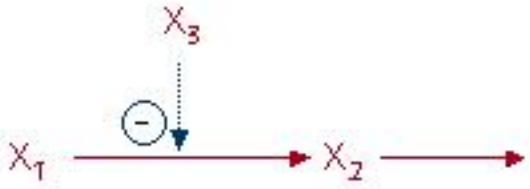
Graphical Representation



The reaction between X_1 and X_2 requires coenzyme X_3 which is converted to X_4

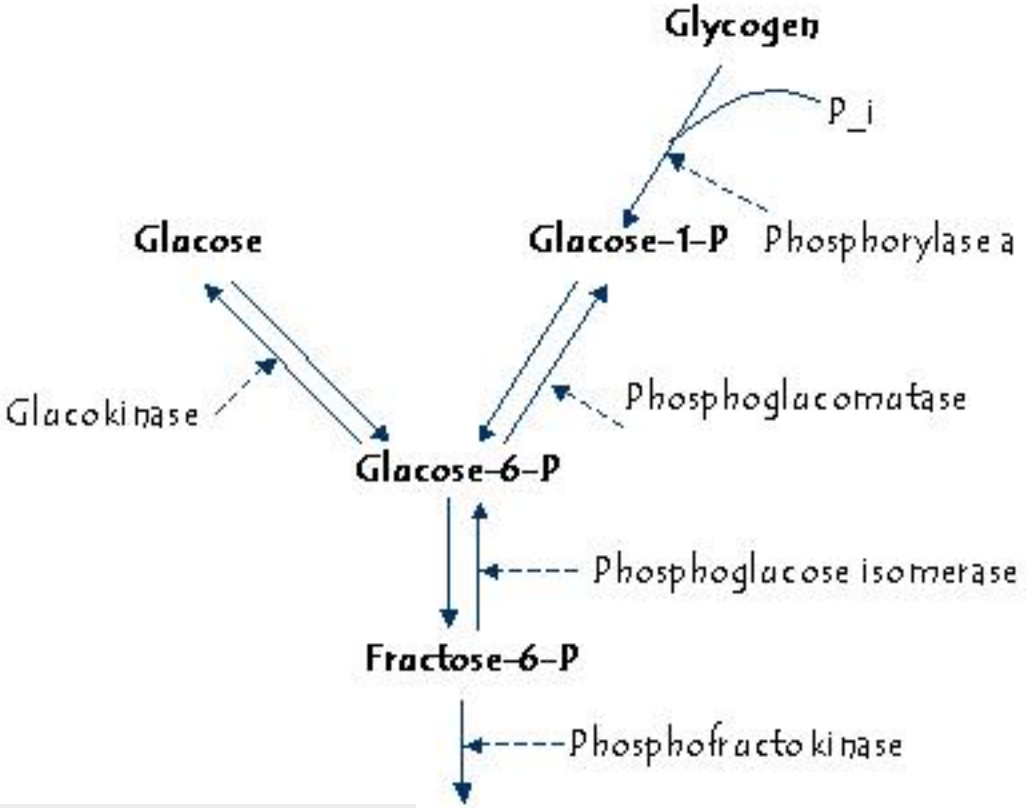


The conversion of X_1 into X_2 is modulated by X_3



The conversion of X_1 into X_2 is modulated by an inhibitor X_3

Glycolysis



S-Systems

- Dependent Variables: $X_i(t)$, $i=1,\ldots,n$, $0 \leq t$.
- System is described in terms of the temporal changes in dependent variables:
  - E.g., Instantaneous product formation in response to changes in the exogenous substrate, inhibitor or enzyme concentration…
  - Kinetic Laws: Relate a reaction rate to concentrations.
  - Reaction Rate = Instantaneous temporal rate of change in concentration of substrate or product.
- Is this information sufficient to deduce the dynamics of a biochemical system?

  Yes

Systems of Differential Equations

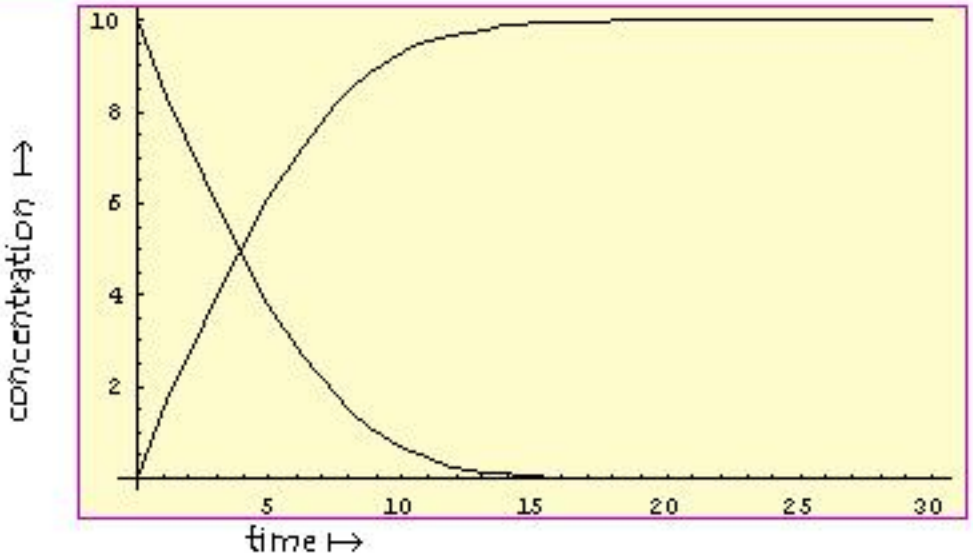
- dX_i/dt = (instantaneous) rate of change in X_i at time t = Function of substrate concentrations, enzymes, factors and products:

$$dX_i/dt = f(S_1, S_2, \ldots, E_1, E_2, \ldots, F_1, F_2, \ldots, P_1, P_2, \ldots)$$

- E.g. Michaelis-Menten for substrate S & product P:

1. $dS/dt = - V_{max}\, S/(K_M + S)$
2. $dP/dt = V_{max}\, S/(K_M + S)$

Michaelis-Menten



Temporal decrease in substrate concentration and increase in product concentration: with V_{max} =2 and K_M = 4. Substrate concentration at time 0 is 10, and product concentration at time 0 is 0.

General Form

- $dX_i/dt = V_i^+(X_1, X_2, \ldots, X_n) - V_i^-(X_1, X_2, \ldots, X_n)$:
  - Where $V_i^+(\cdot)$ term represents production (or accumulation) rate of a particular metabolite and $V_i^-(\cdot)$ represent s depletion rate of the same metabolite.
- Generalizing to n dependent variables and m independent variables, we have:

$$dX_i/dt =$$

$$V_i^+(X_1, X_2, \ldots, X_n, X_{n+1}, X_{n+2}, \ldots, X_{n+m})$$
$$- V_i^-(X_1, X_2, \ldots, X_n, X_{n+1}, X_{n+2}, \ldots, X_{n+m}):$$

- These n differential equations are called: the **systems equations**, or the **system description** or **Kirchhoff's node equation**

Canonical Forms

- S-systems result in Non-linear Time-Invariant DAE System.
- Note that: Given a system of equations with f and g being arbitrary rational functions, we can transform the system into a set of Differential Binomial Equation System with Linear Constraints:

$$dx_i/dt = \alpha\, x_1^{a1} \cdots x_n^{an} - \beta\, x_1^{b1} \cdots x_n^{bn}$$

$$\& \; \gamma_1 x_1 + \cdots \gamma_n x_n = 0$$

Transformation I

- Assume that an equation is given as
- $dx/dt = p(x(t), u(t))/q(x(t), u(t))$
  - A rational function. p & q are polynomials
  - $p(x(t), u(t)) = \alpha_1 m_1 + \cdots + \alpha_k m_k - \beta_1 p_1 - \cdots - \beta_l p_l$
  - where m's and p's are power-products with arbitrary power. α's and β's are positive-valued.

$$dx/dt = p(x(t), u(t))\, y(t)^{-1},$$
$$dc/dt = q(x(t), u(t)) - y(t),$$
$$c = 0.$$

Transformation II

- $dx/dt = \alpha_1 m_1 + \cdots + \alpha_k m_k - \beta_1 p_1 - \cdots - \beta_l p_l$

  $= (\alpha_1 m_1 - w(t)/k) + \cdots + (\alpha_k m_k - w(t)/k)$

  $- (\beta_1 p_1 - w(t)/l) - \cdots - (\beta_l p_l - w(t)/l)$

- Equivalent System

$$x(t) - \gamma_1(t) - \cdots - \gamma_k(t) + \gamma_{k+1}(t) + \cdots + \gamma_{k+l}(t) = 0$$

$$d\gamma_i/dt = \alpha_i m_i - w(t)/k, \quad 1 \le i \le k$$

$$d\gamma_j/dt = \beta_1 p_1 - w(t)/l, \quad k+1 \le j \le k+l$$

Canonical Forms

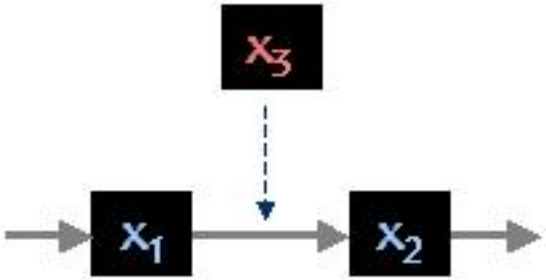
Theorem 1 *Every bio-chemical system arising from an S-system model can be expressed in a canonical form involving $r > n + m$ variables $Z_1, Z_2, \ldots, Z_r$:*

$$\begin{bmatrix} \dot{Z}_1 \\ \dot{Z}_2 \\ \vdots \\ \dot{Z}_r \end{bmatrix} = \begin{bmatrix} m_1^+(\mathbf{Z}) - m_1^-(\mathbf{Z}) \\ m_2^+(\mathbf{Z}) - m_2^-(\mathbf{Z}) \\ \vdots \\ m_r^+(\mathbf{Z}) - m_r^-(\mathbf{Z}) \end{bmatrix}, \tag{0.4}$$

$$\begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1r} \\ a_{21} & a_{22} & \cdots & a_{2r} \\ \vdots & \vdots & \ddots & \vdots \\ a_{s1} & a_{s2} & \cdots & a_{sr} \end{bmatrix} \begin{bmatrix} Z_1 \\ Z_2 \\ \vdots \\ Z_r \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ \vdots \\ 0 \end{bmatrix}, \tag{0.5}$$

where m_i^+'s and m_i^-'s are ratios of monomials and a_{ij}'s are constants in $\mathbb{R}[Z_1, \ldots, Z_r]$ with positive coefficients.

Example:



$dX_1/dt = \alpha - V_1^-(X_1, X_3)$,

$dX_2/dt = V_1^-(X_1, X_3) - V_2^-(X_2)$.

- **Analysis of simple conversion of metabolite X_1 into X_2 that is catalyzed by X_3.**
- There is a constant flux into the system that replenishes the pool of X_1; V_1^+ is a constant = α.
- The degradation of X_1 depends on the concentration or pool size of X_1 and also on enzyme X_3; V_1^- depends on X_1 and X_3 but not on X_2.
- Production of X_2 constitutes the same process as degradation of X_1; $V_2^+ == V_1^-$.
- Degradation of X_2 depends only on its current concentration.

Power-law Presentation

- While the exact forms of V_1^- and V_2^- are not known, based on various models of binding, cooperativity, etc., it has been argued that they should be represented by "power-laws:"

$$V_1^-(X_1, X_3) \triangleq \beta X_1^a X_3^b$$

$$V_2^-(X_2) \triangleq \gamma X_2^c$$

- Final system:

$$dX_1/dt = \alpha - \beta X_1^a X_3^b$$

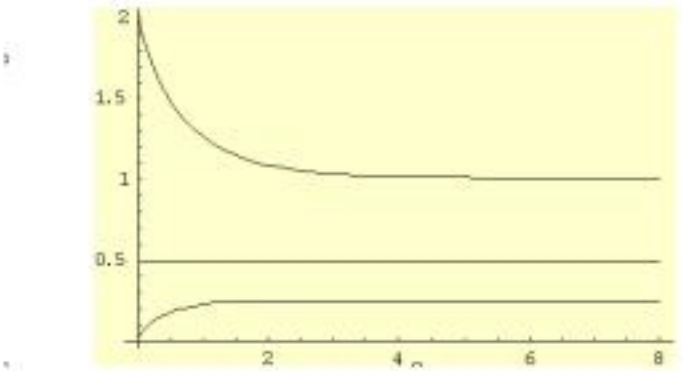
$$dX_2/dt = \beta X_1^a X_3^b - \gamma X_2^c$$

Simulation

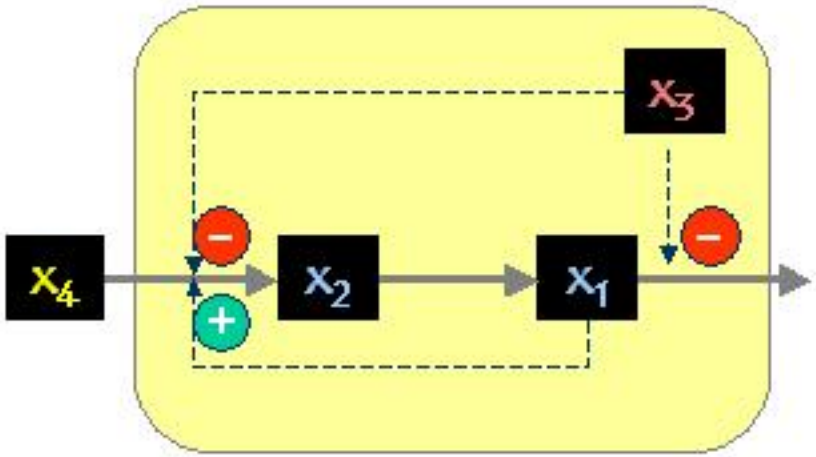
$$dX_1/dt = \alpha - \beta X_1^a X_3^b$$

$$dX_2/dt = \beta X_1^a X_3^b - \gamma X_2^c$$

- Parameters:
  - $\alpha = 1$,
  - $\beta = 1$
  - $\gamma = 1$,
  - $a = 0.5$,
  - $b = -1.0$, and
  - $c = 1.0$.



Example: Feedback



- X_2 is a dependent variable:
  - X_2 is the product of a reaction that uses X_4 as a substrate and
  - X_2 is activated by X_1 and inhibited by X_3.

$$dX_1/dt = 2X_2 - 1.2X_1^{0.5}X_3^{-1},$$

$$X_1(0) = 2,$$

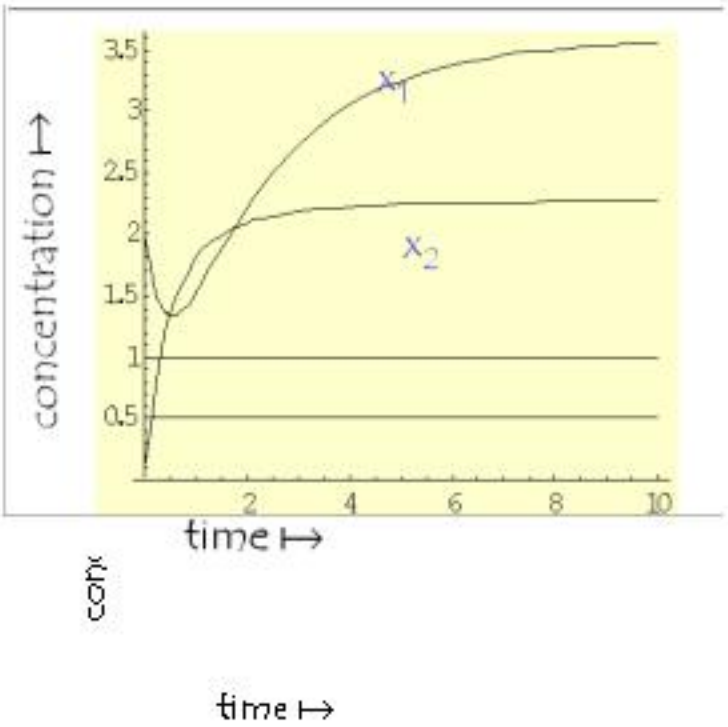
$$dX_2/dt = 2X_1^{0.1}X_3^{-1}X_4^{0.5} - 2X_2,$$

$$X_2(0) = 0.1,$$

$$X_3 = 0.5,$$

$$X_4 = 1.$$

Systems Equations



- Dynamics of X_1 and X_2 from the example are depicted:
- X_1 initially undershoots but ultimately reaches a level higher than at the beginning of the experiment.
- X_2 shows a simple monotonic increase.

Rate Constants

- In the following equation:

$$dX_i/dt = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}$$

  - α_i's and β_i's are rate constants in the production and the depletion terms respectively.
  - These terms are positive or zero, but cannot be negative.

- At any point, which term (production or depletion) dominates depends on the
  - rate constants: α_i and β_i
  - other parameters: g_{ij} and h_{ij}, and
  - the current concentration of all the metabolites that are involved in V_i^+ and V_i^-.

Steady State

- If all equations are balanced (i.e., production is balanced by depletion), then $dX_i/dt = 0, \forall i=1,\ldots,n$.
- Thus the steady-state is achieved at

$$0 = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}$$

  - or

$$\alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} = \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}$$

- A steady state is characterized by the condition that no metabolite is changing (i.e., that $dX_i/dt = 0$) and they remain constant..

Indices & Kinetic Order

- The roles of the kinetic order parameters: g_{ij} and h_{ij}:
  - i = the first index of the kinetic order and
  - j= second index of the kinetic order.
  - g_{ij} represents how the **production** of X_i is influenced by the variable X_j:
  - h_{ij} represents how the **degradation** of X_i is influenced by the variable X_j:
  - Positive kinetic orders indicate activating influences and negative kinetic orders express inhibition.
  - If the kinetic order is zero, then it indicates independence from the metabolite.

Interesting Properties of S-systems:

- S-systems can model "allometric relationship:"
  - dX_1/X_1 and dX_2/X_2 are linearly related..
  - Growth at "different scales:" The relative growth of two parts are very often linearly related. (Galileo, Thompson, Huxley, Needham & Adolph.)

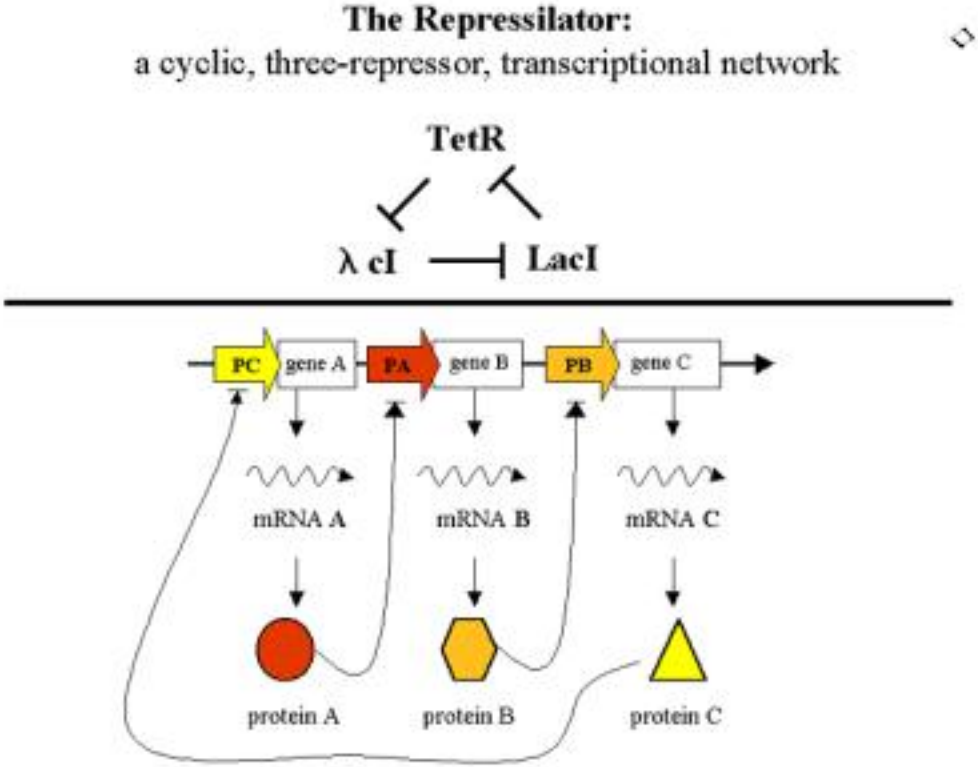
Interesting Properties of S-systems:

- S-systems can model "telescopic relationship:"
- Models at different levels.
  - First stage: Enzyme catalyzed relations constituting a chemical pathway.
  - Second stage: Interaction between organelles
  - Third Stage: Interactions between cells.
  - Final Stage: Dynamics of a system with different organs.

Purine Metabolism: Telescopy

- Example: Purine Metabolism:
  - **Lowest Level**: Inter-conversion of the various adenylates … using adenine, adenosine, adenyl succinate, AMP, ADP and ATP.
  - **Next Level**: Dynamic interactions between adenylates, guanylates, and oxypurines.
  - We could pool all adenine derivatives and consider this pool as one variable: "adenylates.".
  - **Final Level**: Synthesis and degradation of DNA and RNA and use a pool of all nucleosides and nucleotides.

An Artificial Clock



- Three proteins:
  - LacI, tetR & λ cI
  - Arranged in a cyclic manner (logically, not necessarily physically) so that the protein product of one gene is rpressor for the next gene.

LacI→ ¬ *tetR*; *tetR*→ TetR

TetR→ ¬ λ *cI*; λ *cI* → λ cI

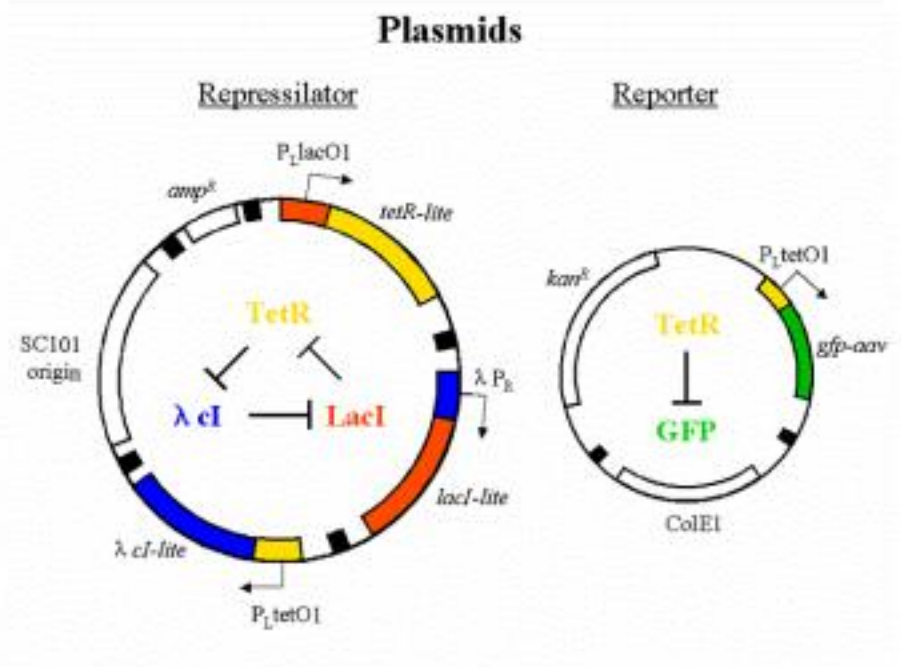
λ cI→ ¬ *lacI*; *lacI*→ LacI

uet et al., Antoniotti et al., Wigler & Mishra

Cycles of Repression

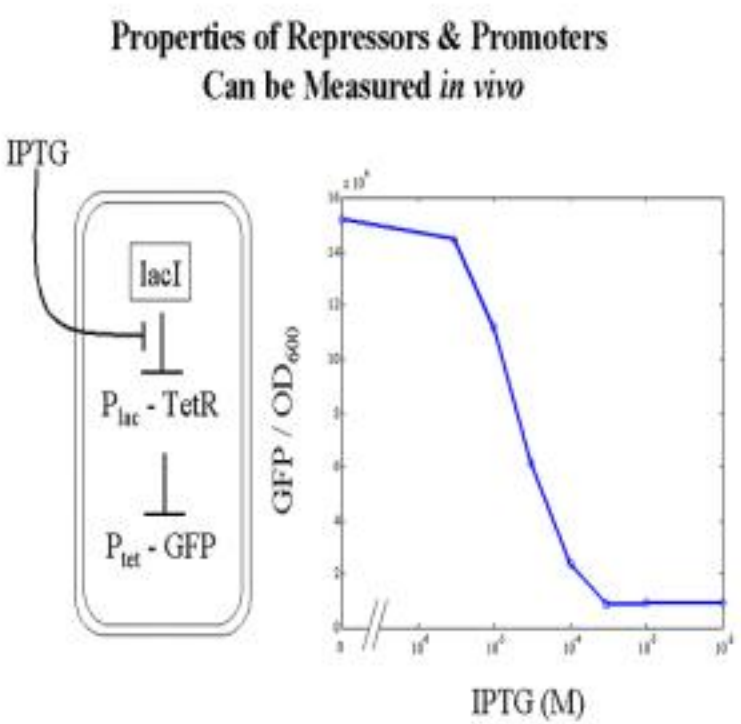
- The first repressor protein, LacI from E. coli inhibits the transcription of the second repressor gene, tetR from the tetracycline-resistance transposon Tn10, whose protein product in turn inhibits the expression of a third gene, cI from I phage.
- Finally, CI inhibits lacI expression,
- completing the cycle.

Biological Model



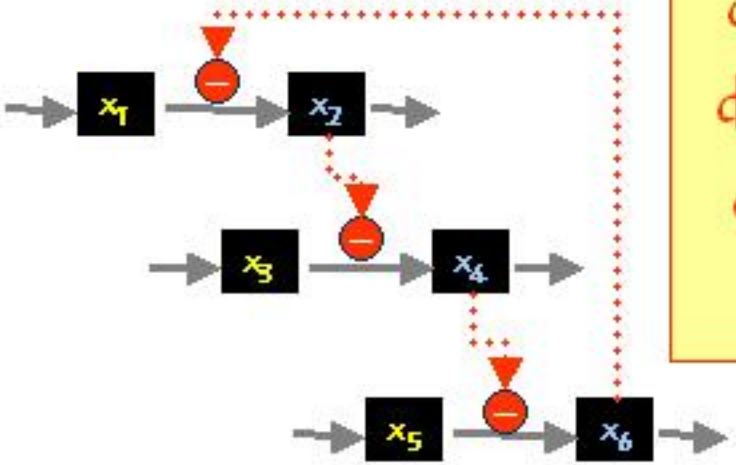
- Standard molecular biology: Construct
  - A low-copy plasmid encoding the repressilator and
  - A compatible higher-copy reporter plasmid containing the tet-repressible promoter PLtetO1 fused to an intermediate stability variant of gfp.

Reporter



- The inducer IPTG interferes with repression by LacI…A transient pulse of IPTG synchronizes a population of repressilator-containing cells.

Cascade Model: Repressilator?



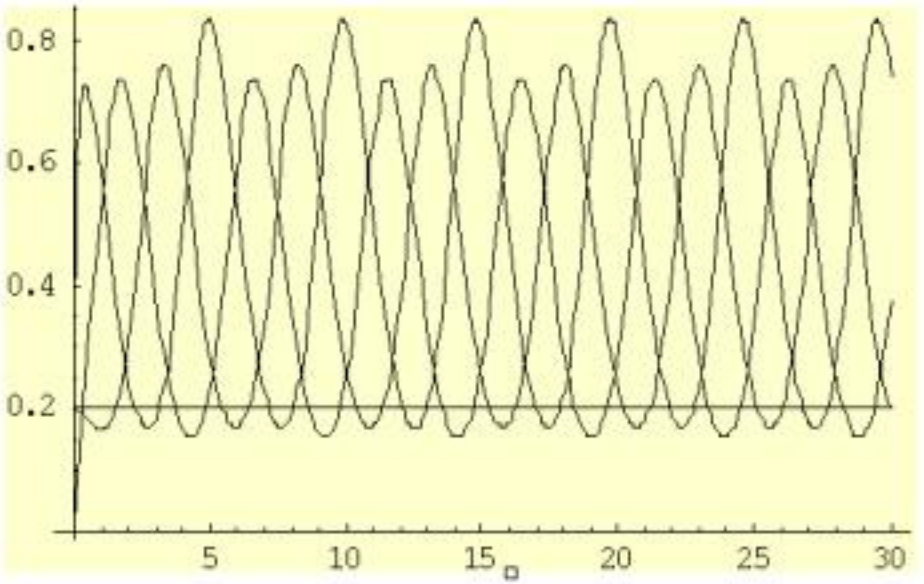
$$dx_2/dt = \alpha_2 X_6^{g26} X_1^{g21} - \beta_2 X_2^{h22}$$

$$dx_4/dt = \alpha_4 X_2^{g42} X_3^{g43} - \beta_4 X_4^{h44}$$

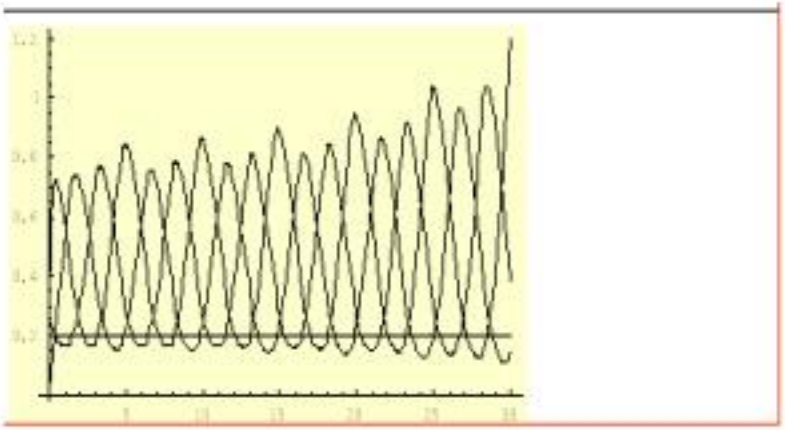
$$dx_6/dt = \alpha_6 X_4^{g64} X_5^{g65} - \beta_6 X_6^{h66}$$

$$X_1, X_3, X_5 = \text{const}$$

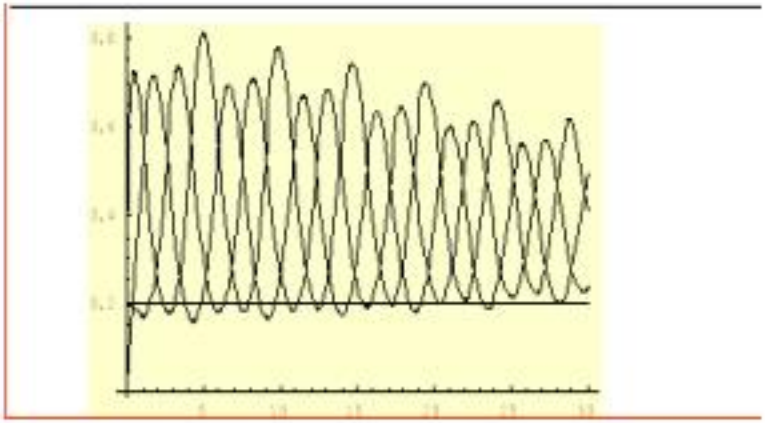
How Stable is This???



Robustness?



X_4 degrades slightly slowly



X_4 degrades slightly faster

Rescaled Symmetric System

- α = proteins/cell from *unrepressed* promoter
- $\alpha\,\rho$ = proteins/cell from *repressed* promoter
- β = protein : mRNA *decay* rate ratio
- n = Hill (*cooperativity*) coefficient

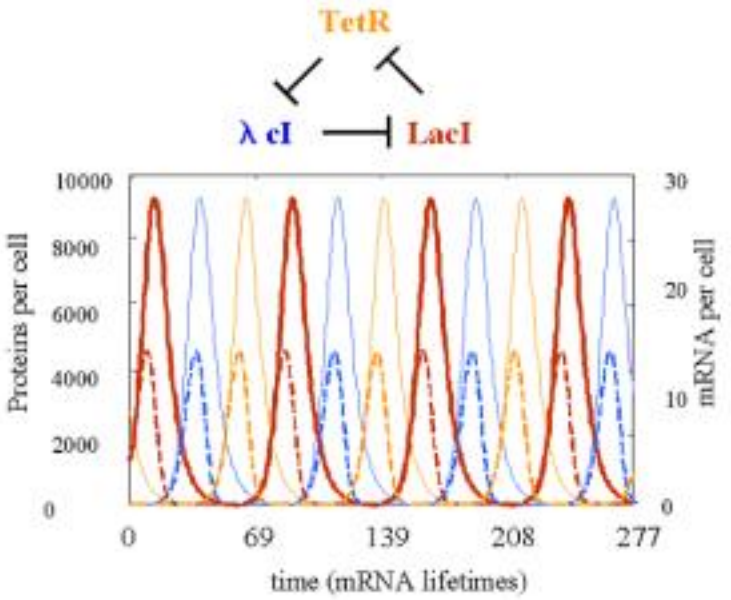
$$dm_i/dt = -\,m_i + \alpha/(1+p_j^{\,n}) + \alpha\,\rho$$

$$dp_i/dt = -\,\beta\,(p_i - m_i)$$

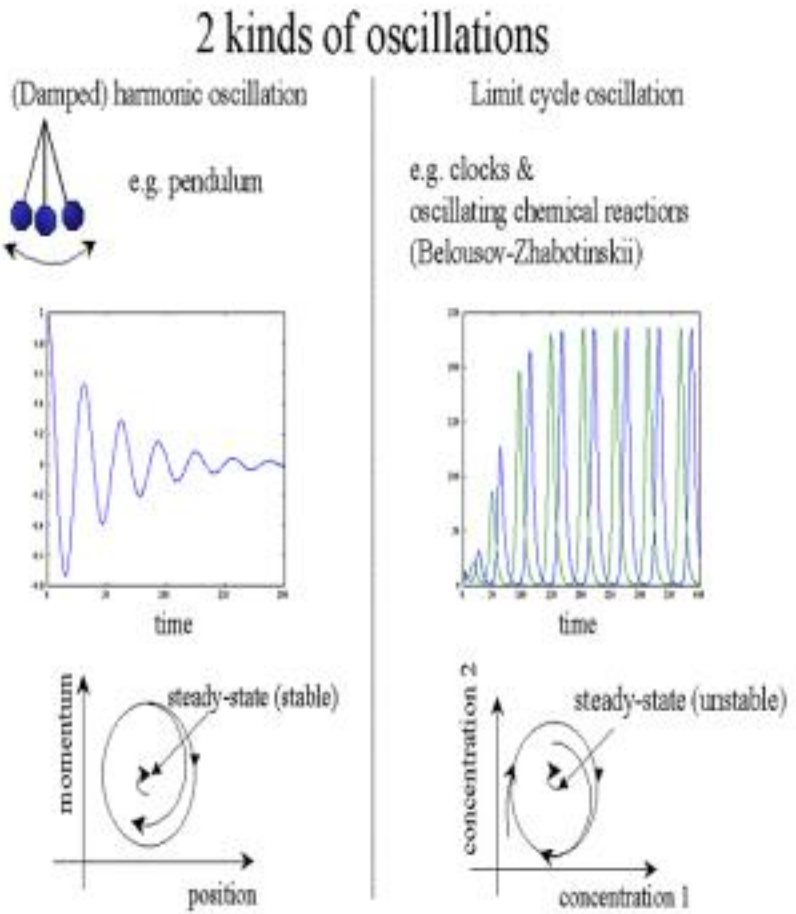
- where m_i = i^{th} [mRNA]; p_i = i^{th} [repressor protein]
  - i = lacI, terR, cI
  - j= cI, lacI, tetR
- Concentration units: K_M
- Time units: τ_{mRNA}

Oscillation

Numerical Integration of differential equations shows periodic behavior:

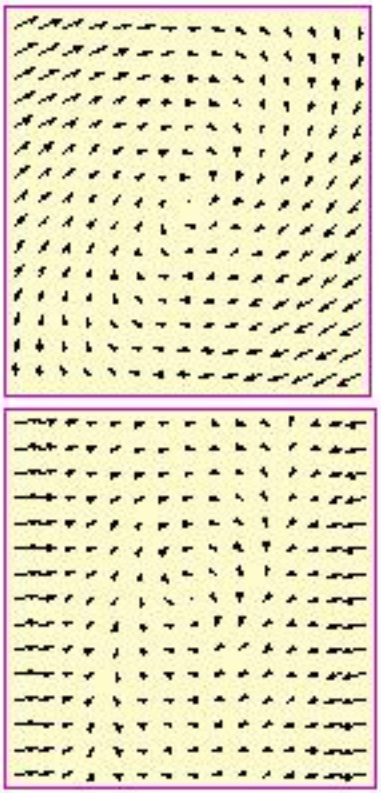


Stability Issue



- Unstable Oscillator:
  - Damped harmonic oscillator..
  - Asymptotically approaches a stable steady state value.
- Stable Oscillator:
  - Limit cycle oscillator.
  - The steady state in the interior of the cycle is repelling (unstable)

Phase Portrait

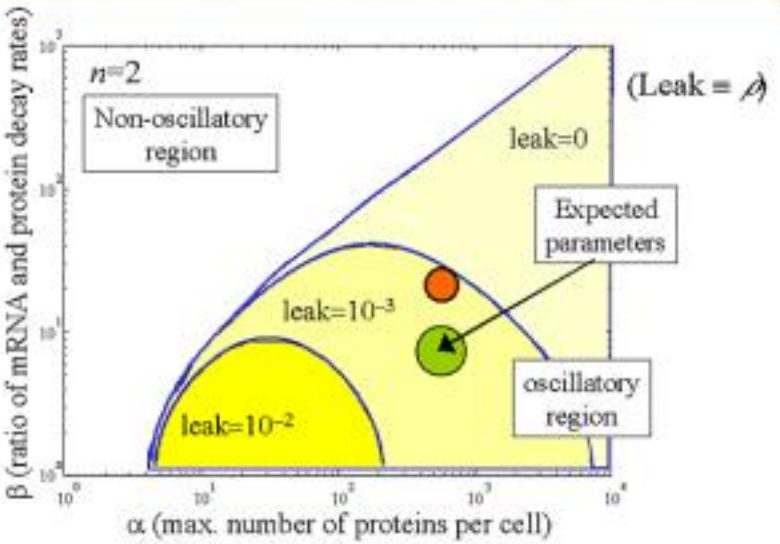


- Two phase portaits
  - a) Damped linear oscillator
    - $dx/dt = y - x$
    - $dy/dt = -x$
  - b) Limit cycle oscillator
    - $dx/dt = y - x^3 + x$
    - $dy/dt = -x$

Dependence on Parameters

Analytical condition for oscillations:

$$\left(\sqrt{\beta}+\sqrt{\beta^{-1}}\right)^2 < \frac{3X_0^{\,2}}{4+2X_0} \qquad X_0 = \left.\frac{df}{dp}\right|_{\text{steady-state}}$$

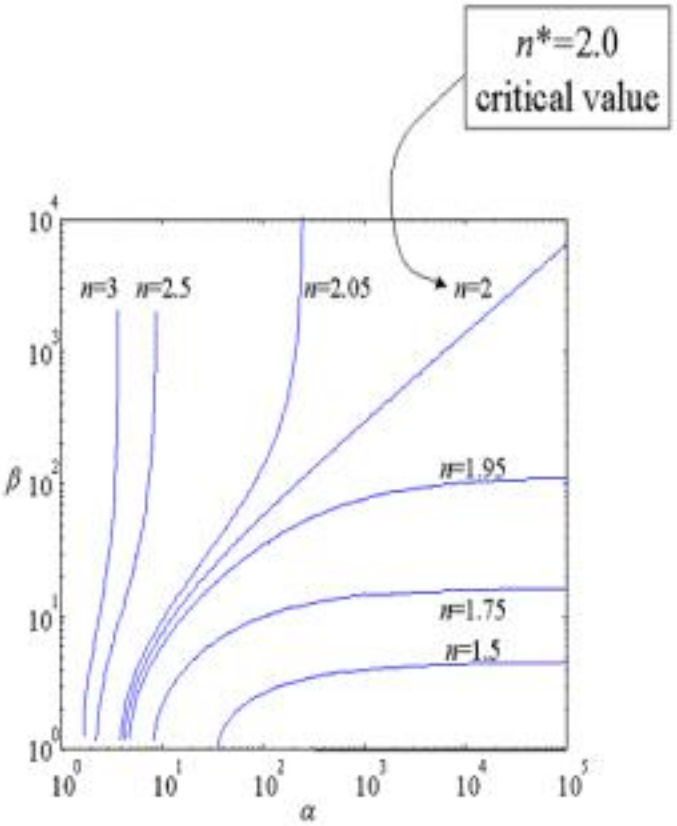


- $f = \alpha/(1+p^n)$
- $X_0 = df/dp|_{p=p0}$
  $= -\alpha n p_0^{n-1}/(1+p_0^n)^2$
- and p_0 is the solution to $p = \alpha/(1+p^n) + \alpha\rho$.
- The system of equations has a unique steady state which becomes unstable when

$$(\sqrt{\beta} + \sqrt{\beta^{-1}})^2 < 3X_0^2/2(2+X_0)$$

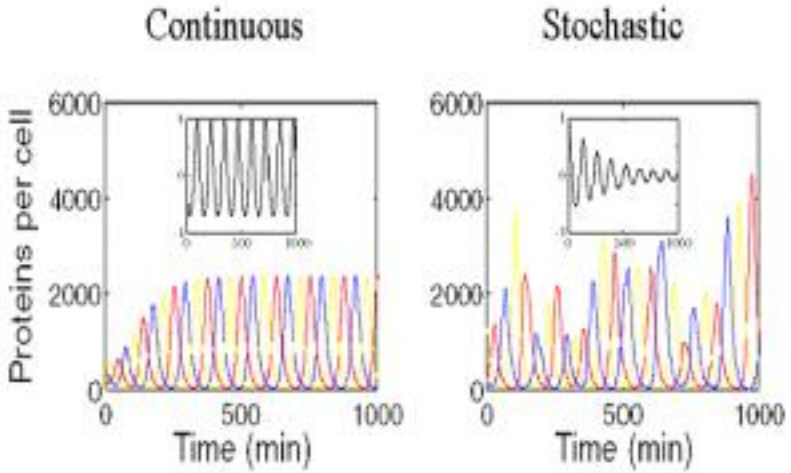
Cooperativity

The oscillatory domain depends on Hill coefficient



Stochasticity

Small numbers of molecules and discrete reaction events → significant variability



Circadian Clock

Circadian Oscillations

- "A model for circadian oscillations in the Drosophila period protein (PER)," Albert Goldbeter,
  - *Proc. R. Soc. Lond. B* (1995), **261**:319-324.
- A theoretical model:
  - Takes into account contemporary experimental observations
  - Model for circadian clock is based on
    1. multiple phosphorylation of PER protein
    2. the negative feedback exerted by PER on the transcription of the period (*per*) gene.

Model

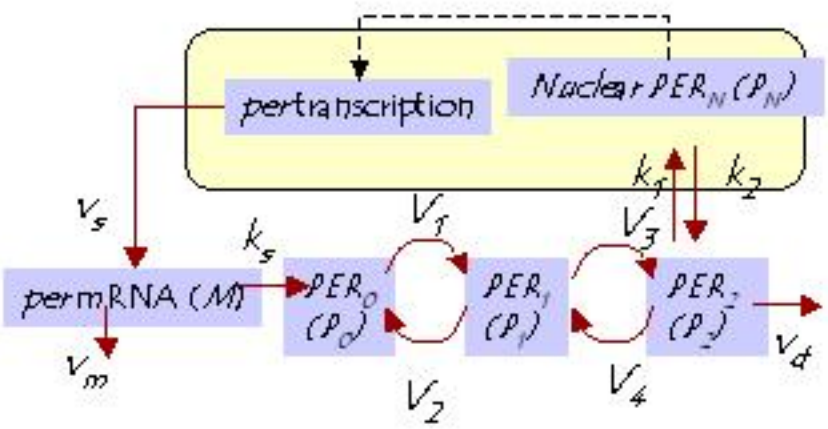
- This minimal biochemical model provides a molecular basis for circadian oscillation of the limit cycle type.
- During oscillations, the peak in per mRNA precedes by several hours the peak in total PER protein.
  - Accepted view: Multiple PER phosphorylation induces time delays which strengthen the capability of negative feedback to produce oscillation.
  - The rhythm occurs only when the maximum rate of PER degradation is in a range bounded by two critical values.
- Many unresolved issues:

n der Pol system,

Two Competing Biological Models

- Edery et al. (1994) Model:
  - Based upon multiple phosphorylation of PER and on repression of *per* transcription by a phosphorylated form of the PER protein.
- Abbott et al. (1995) Model:
  - Based upon the effect of a larger number of phosphorylated residues and their effect upon delaying the entry of the protein into nucleus and the resulting negative feedback effect on *per* transcription.

Circadian Oscillation of PER and *per* mRNA



- Assumptions:
  1. *per* mRNA is synthesized in the nucleus and transferred ti cytosol, where it is degraded.
     M = Cytosolic concentration of *per*
  2. Rate of synthesis of PER (by translation of *per* mRNA) is proportional to M.
  3. PER is multiply phosphorylated:
     $P_0 \mapsto P_1 \mapsto P_2$
  4. Phosphorylated PER is transported into the nucleus: P_N
  5. P_N acts directly as a repressor and reduces the *per* transcription rate.

Phosphorylation of PER

- PER is multiply phosphorylated:
  - To keep the model simple, only three states of the PER protein is considered:

P_0 = Unphosphorylated, P_1 = Monophosphorylated and P_2 = Biphosphorylated

  - The precise number of phosphorylated residues is still unknown. The role of PER phosphorylation is still unclear.
- Phosphorylation may control nuclear localization and/or degradation of PER.
  - Assume that the fully phosphorylated form P_2 is marked both for degradation and reversible transport into the nucleus.
- The effect of the nuclear form of PER (P_N) on the per transcription (M) is described by an equation of Hill ativity) coefficient of n = 4.

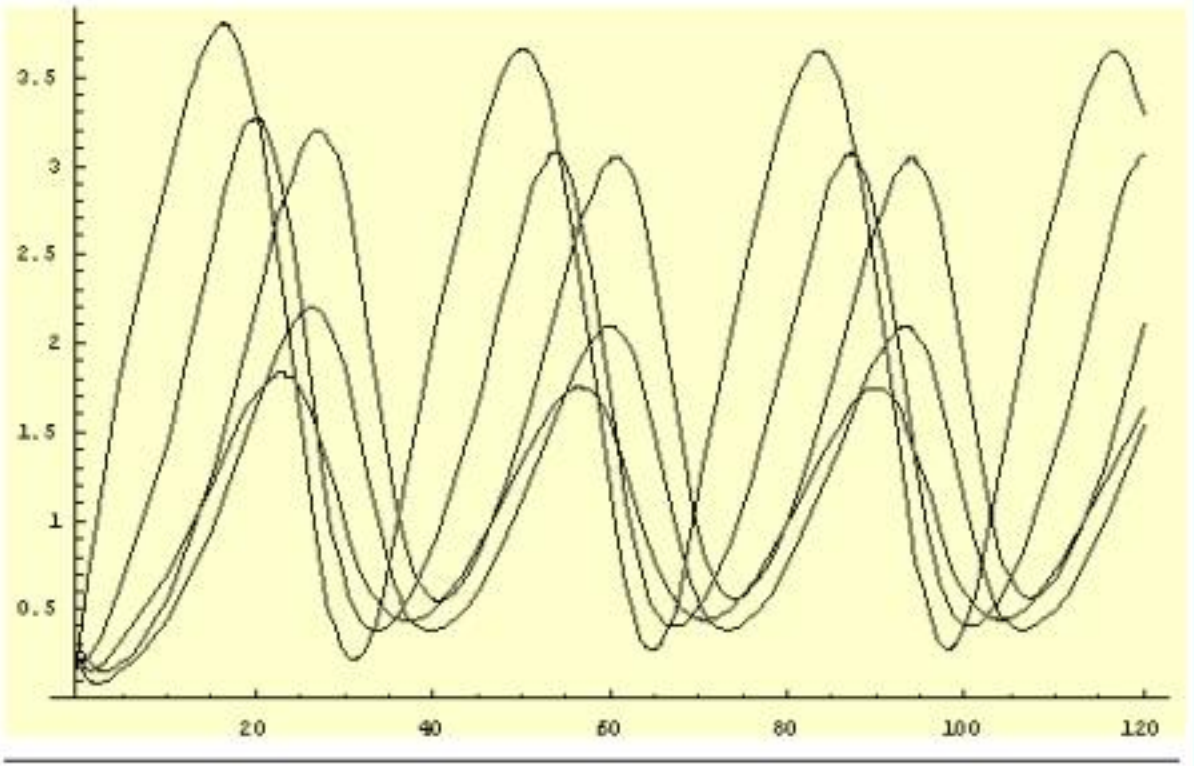
Differential Equations

- $dM/dt = v_s K_1^n/(K_1^n + P_N^n) - v_m M/(K_m+M)$
- $dP_O/dt = k_s M - V_1 P_O/(K_1+P_O) + V_2 P_1/(K_2 + P_1)$
- $dP_1/dt =$

  $V_1 P_O/(K_1+P_O) - V_2 P_1/(K_2+P_1) - V_3 P_1/(K_3+P_1) + V_4 P_2/(K_4+P_2)$
- $dP_2/dt =$

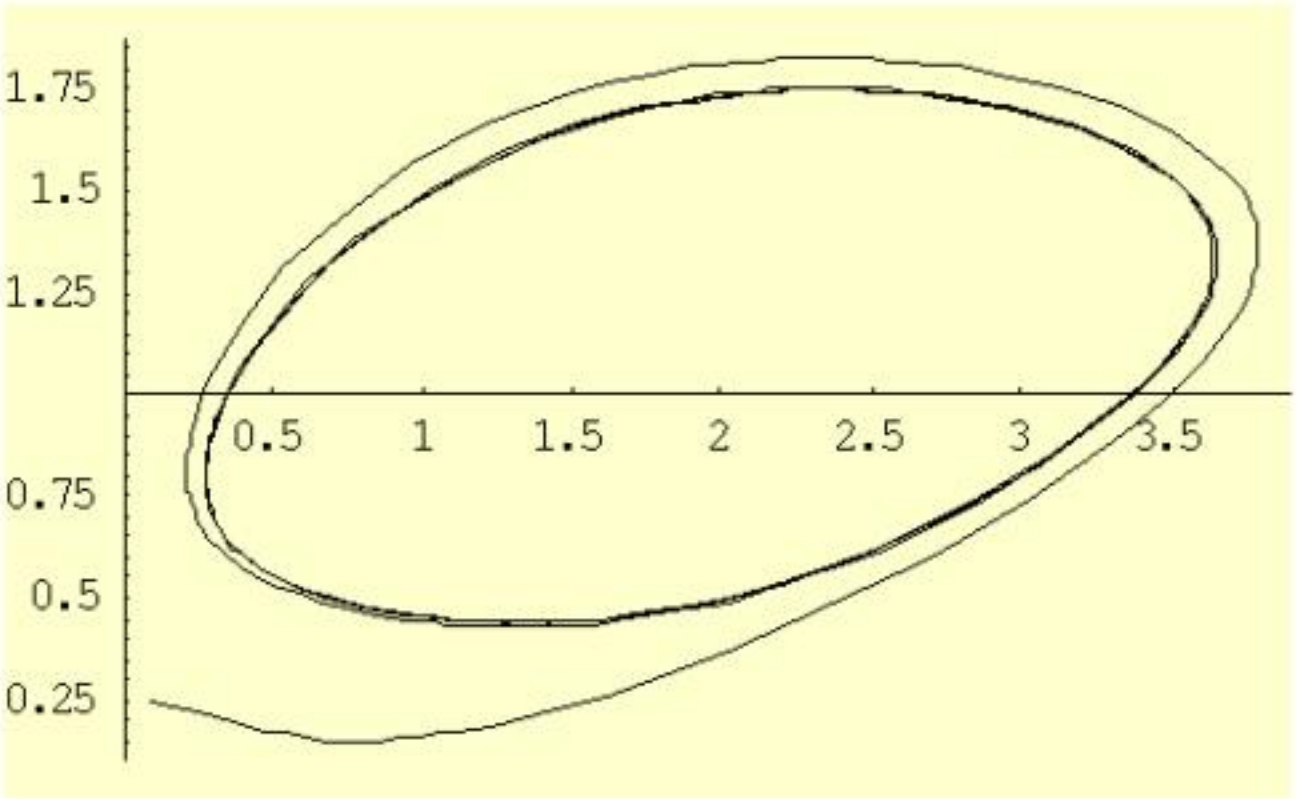
  $V_3 P_1/(K_3+P_1) - V_4 P_2/(K_4+P_2) - k_1 P_2 + K_2 P_N - v_4 P_2/(k_4+P_2)$
- $dP_N/dt = k_1 P_2 - k_2 P_N$
- $P_t = P_O + P_1 + P_2 + P_N$

Simulation 1

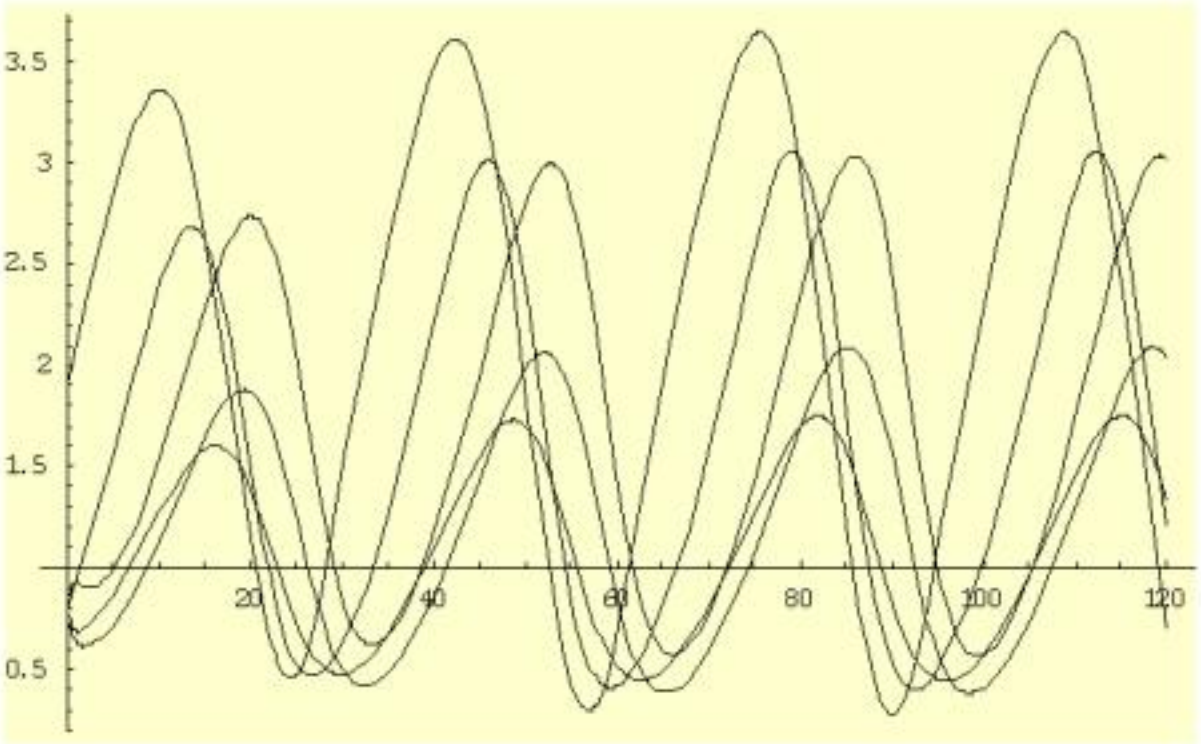


Out[158]= -Graphics-

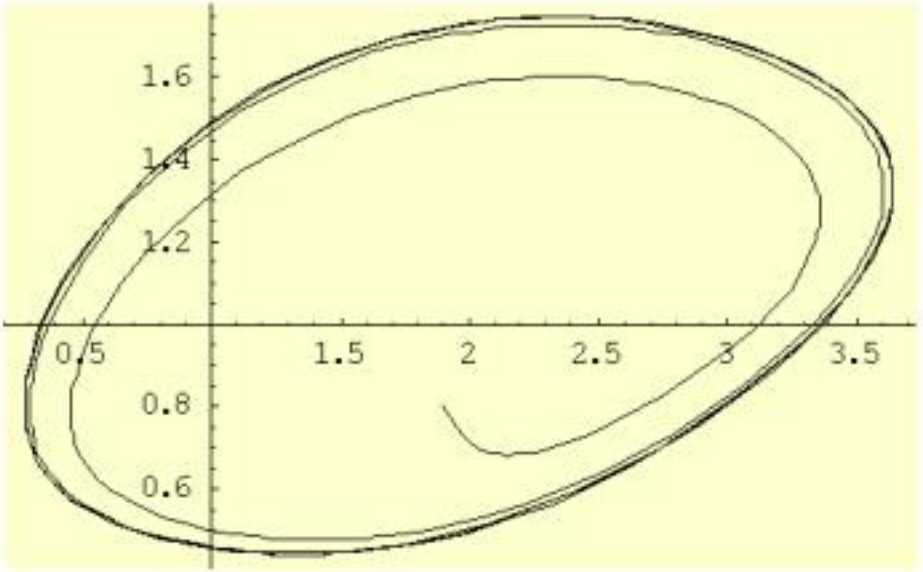
Phase Plane 1



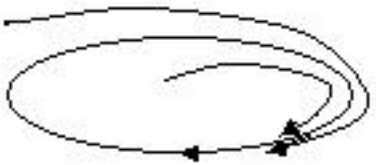
Simulation 2



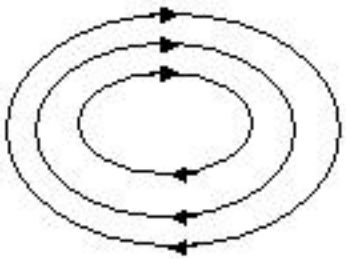
Phase Plane 2



Periodic Orbits and Limit Cycles



Stable Limit Cycle

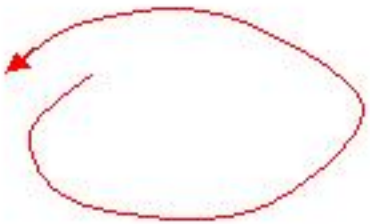


Not a Limit Cycle

- (Stable) Limit Cycle $\triangleq$ A periodic trajectory hich attracts other solutions to it.
- A member of a family of "parallel" periodic solutions (with linear centers) is not a limit cycle.
- Limit cycles are robust in two ways:

Robustness of Limit Cycles

- If perturbation moves state to different initial state away from the cycle, then the system will return to cycle…
  - e.g. Circadian rhythm: Phase adjusts after jet lag…
  - For a linear oscillator, this is not true; it will simply start oscillating along a different orbit and will never return to the original orbit.
  - If dynamics changes a little a limit cycle will still exist (can be proved using Poincare-Bendixon theorem.)



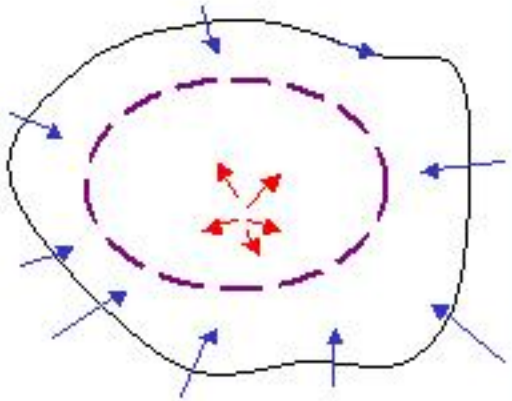
- Think of a linear oscilator:

$$dx/dt = y,\ dy/dt = -x + \varepsilon y$$

$$(\Rightarrow d^2x/dt^2 - \varepsilon\, dx/dt + x = 0)$$

  - Changes to a spiral orbit (whether stable or

Poincare-Bendixon Theorem



- For systems of **two** equations

  $dx/dt = F(x,y)$ & $dy/dt = G(x,y)$,

- The following criterion determines the existence of a limit cycle:
- **Suppose** a bounded region D in the plane is so that **no trajectory can exit D** (on boundary, the vector field (F,G) points inside or tangentially) and either there are **no steady states inside** or there is **a single steady state that is repelling** then there is a periodic orbit inside D.
- If the periodic orbit is unique then it is a limit cycle.

Bendixon's Criterion

- Given region D simply-connected (no holes)
- **if** the divergence of the vector field is **always positive or is always negative inside D**, then there **cannot be a periodic orbit inside D**:

$$F(x,y) = [f(x,y)\ g(x,y)]^T \ \&\ \operatorname{div} F = \partial f/\partial x + \partial f/\partial y$$

- By Gauss divergence theorem:

$$\int\int_D \operatorname{div} F\, dx\, dy = \int_C n \cdot F \neq 0.$$

- Thus F is not tangential to any closed path…No periodic orbit inside D!

Van der Pol Equation

- Consider a system involving two variables: e.g., an mRNA and a protein: x and y.
- For instance, consider the equations:

$$dx/dt = y - x^3 + x$$

$$dy/dt = -x$$

- In other words:

$$d^2x/dt^2 = dy/dt + (1-3x^2)\,dx/dt = (1-3x^2)\,dx/dt - x \text{ or}$$

$$d^2x/dt^2 + (3x^2-1)\,dx/dt + x = 0$$

- This system has a stable limit cycle!
- These equations were originally introduced to model a "self exciting" electric circuit.

Lienard Equations

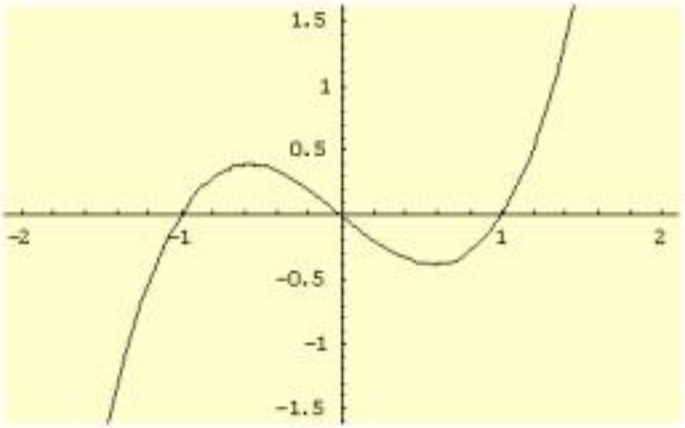
- Generalization of Van der Pol system:

$$d^2x/dt^2 + g(x)\, dx/dt + x = 0$$

  - If g(x) is zero, this is the linear oscillator.
  - The term involving dx/dt is a "frictional" term, where the friction depends on the position x.
  - For small x we are going to take g(x) negative so that it is an "anti-frictional" term
  - For large x we are going to take g(x) positive so that it is a "frictional" term

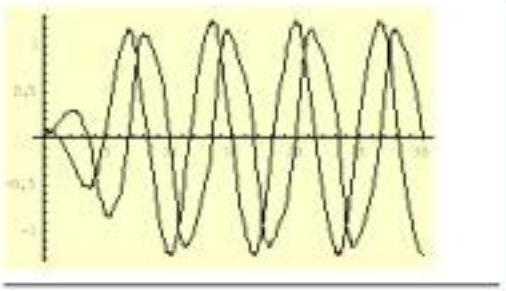
- This is sufficient to guarantee the existence of a robust limit cycle...

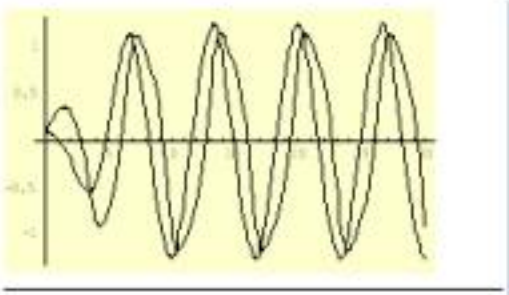
When $f(x) = -x + x^3$:

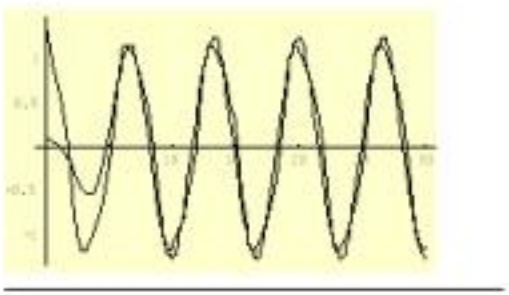


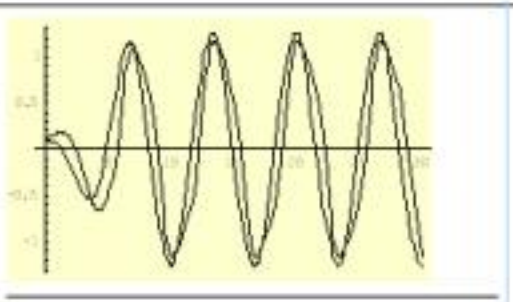
- The graph of $f(x) = -x + x^3$.
- Liénard's Equation: $dx/dt = y - f(x)$; $dy/dt = -x$.
  - f is an odd function of x
  - $f(x) < 0$ in (0,1) and $f(x) > 0$ in $(0, \infty)$
  - f is a strictly monotone increasing function of x (for $x > 1$)
  - f goes to infinity as x goes to ∞
- Sufficient to ensure a limit cycle.

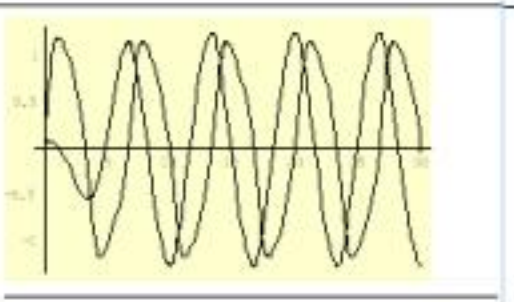
Van der Pol Equation

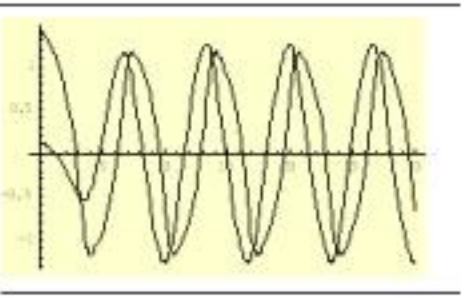




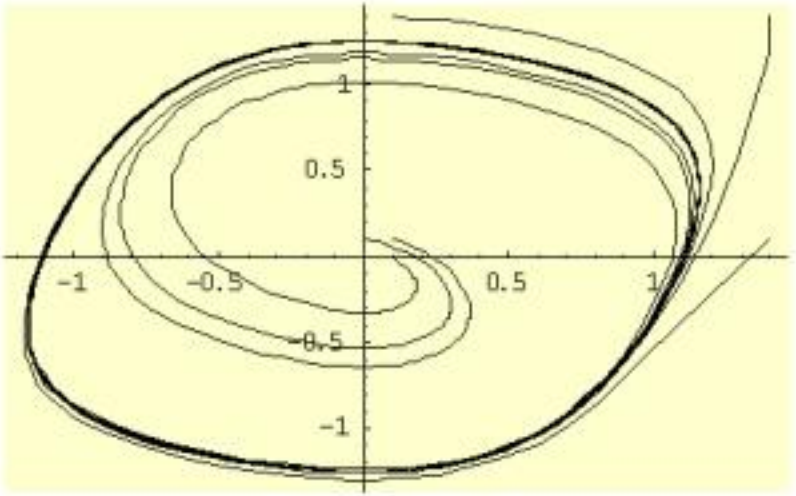








Van der Pol Equation



- Phase Portrait of the Lienard Equation for $f(x) = -x + x^3$ with $-1.4 \leq x \leq 1.4$ & $1.4 \leq y \leq 1.4$...
- Stability of the limit cycle follows from Poincare-Bendixon.

To be continued…

. . .