

Master on Biophysics

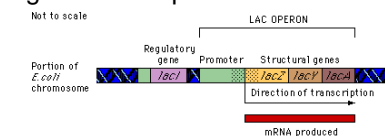
- Some ideas about regulation and the flow of information...
- Miquel Pons
- Institute for Research in Biomedicine and Department of Organic Chemistry. Universitat de Barcelona
- December 2009

Gene Circuits

- The levels of macromolecules in the cell are dynamically regulated
 - e.g. Enzymes allowing processing of Lactose by *E.coli* are only produced when
 - a) Lactose is available
 - b) An “easier” alternative, such as Glucose, is not present

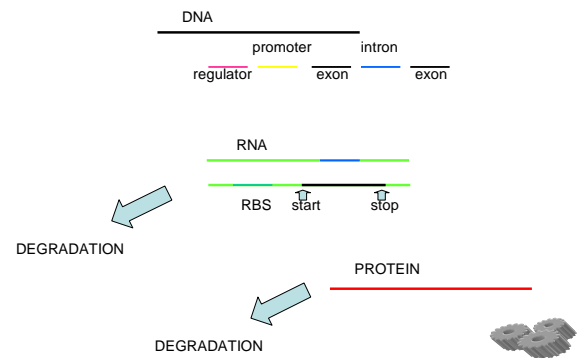
Operons

- Enzymes involved in a collective task are often physically clustered in the genome and controlled in a concerted way.
- E.g. The Lac operon



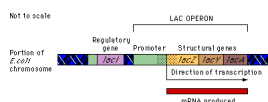
Lac Z : beta-galactosidase
Lac Y : permease
Lac A : transacetylase

From genes to proteins



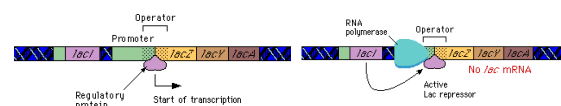
Transcription Regulation

- Transcription is the generation of RNA from DNA.
- RNA polymerases have to bind to specific sites in the DNA to start transcription. These sites are called **promoters**.
- Promoters can be repressed or activated



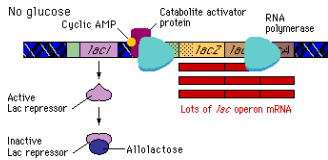
Transcription Regulation

- Promoters can be **repressed** or activated:
 - Repressors bind to the promoter and prevent RNA polymerases to start transcription



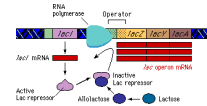
Transcription Regulation

- Promoters can be repressed or **activated**:
 - Activators bind to the promoter or neighbour regions and facilitate transcription



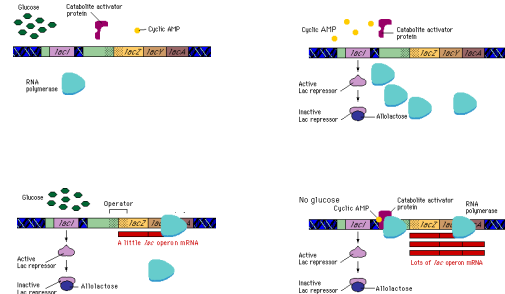
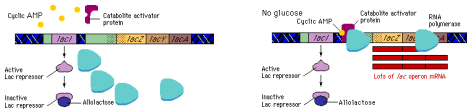
Transcription Regulation

- Repressors** and activators are produced or **modified** in response to regulatory events
 - E.g. the presence of the product to be acted upon by the gene products may inactivate the repressor, thus allowing the genes to be transcribed.



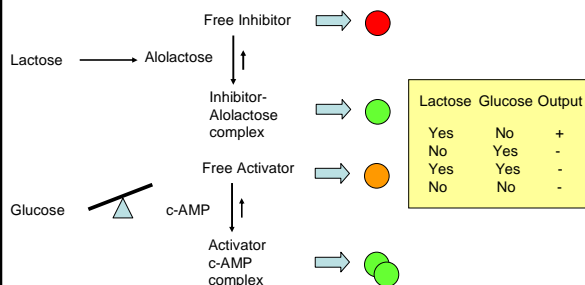
Transcription Regulation

- Promoters can be repressed or activated:
- Repressors** and **activators** are produced or **modified** in response to regulatory events
 - E.g. c-AMP is needed for the activator of the Lac operon to be operative and enhance the transcription of the Lac genes



http://www.phschool.com/science/biology_place/biocoach/lacoperon/intro.html

Regulatory circuits



Synthetic Circuits 1 (A bistable switch)

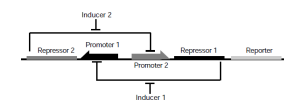
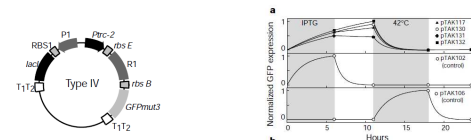


Figure 1 Toggle switch design. Repressor 1 inhibits transcription from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is induced by Inducer 2.



Gardner, Cantor, Collins Nature, 403, 339 (2000)

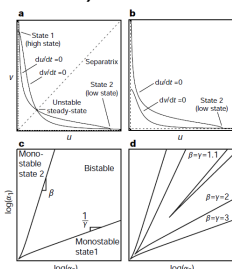
Synthetic Circuits 1 (A bistable switch)

The behaviour of the toggle switch and the conditions for bistability can be understood using the following dimensionless model for the network:

$$\frac{du}{dt} = \frac{\alpha_1}{1+u^2} - \nu \quad (1a)$$

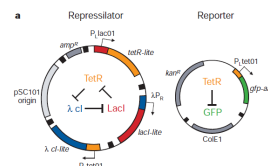
$$\frac{dv}{dt} = \frac{\alpha_2}{1+v^2} - \nu \quad (1b)$$

where u is the concentration of repressor 1, v is the concentration of repressor 2, α_1 is the effective rate of synthesis of repressor 1, α_2 is the effective rate of synthesis of repressor 2, β is the cooperativity of repression of promoter 2 and γ is the cooperativity of repression of promoter 1. The above model is derived from a biochemical rate equation formulation of gene expression^{24,25}. The final form of the toggle equations preserves the two most fundamental aspects of the network: cooperative repression of constitutively transcribed promoters (the first term in each equation), and degradation/dilution of the repressors (the second term in each equation).



Gardner, Cantor, Collins Nature, 403, 339 (2000)

Synthetic Circuits 2 (Oscillating output)



Elowitz, Leibler Nature, 403, 335 (2000)

Synthetic Circuits 2 (Oscillating output)

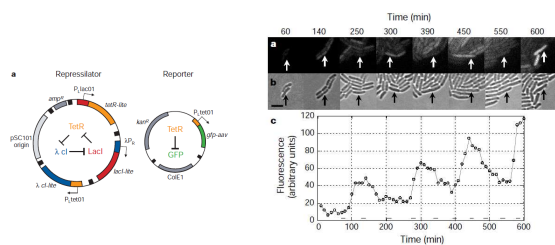
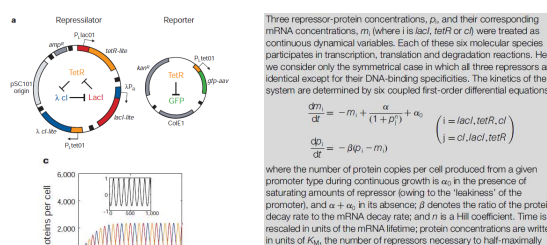


Figure 2 Repression in living bacteria. **a**, **b**. The growth and timecourse of GFP expression for a single cell of *E. coli* host strain MC4100 containing the repressilator plasmids (Fig. 1a). Snapshots of a growing microcolony were taken periodically both in fluorescence (**a**) and bright-field (**b**). **c**. The pictures in **a** and **b** correspond to peaks and troughs in the timecourse of GFP fluorescence density of the selected cell. Scale bar, 4 μ m. Bars at the bottom of **c** indicate the timing of septation events, as estimated from bright-field images.

Elowitz, Leibler Nature, 403, 335 (2000)

Synthetic Circuits 2 (Oscillating output)



Three repressor-protein concentrations, p_i , and their corresponding mRNA concentrations, m_i (where i is $lacI$, $tetR$ or cI) were treated as continuous dynamical variables. Each of these six molecular species participates in transcription, translation and degradation reactions. Here we consider only the symmetrical case in which all three repressors are identical except for their DNA-binding specificities. The kinetics of the system are determined by six coupled first-order differential equations:

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1+p_i^n)^2} + \alpha_0 \quad (i = lacI, tetR, cI)$$

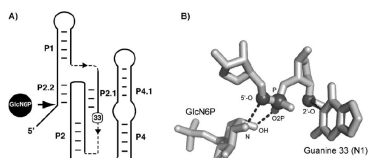
$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

where the number of protein copies per cell produced from a given promoter type during continuous growth is α_0 in the presence of saturating amounts of repressor (owing to the 'leakiness' of the promoter), and $\alpha + \alpha_0$ in its absence; β denotes the ratio of the protein decay rate to the mRNA decay rate; and n is a Hill coefficient. Time is rescaled in units of the mRNA lifetime; protein concentrations are written in units of $K_{0.5}$, the number of repressors necessary to half-maximally repress a promoter; and mRNA concentrations are rescaled by their translation efficiency, the average number of proteins produced per mRNA molecule. The numerical solution of the model shown in Fig. 1c used the following parameter values: promoter strength, 5×10^{-4} (repressed) to 0.5 (fully induced); transcripts per s ; average translation efficiency, 20 proteins per transcript; Hill coefficient, $n = 2$; protein half-life, 10 min; mRNA half-life, 2 min; $K_{0.5}$, 40 monomers per cell.

Elowitz, Leibler Nature, 403, 335 (2000)

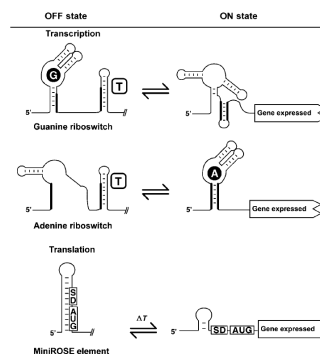
RNA-based Regulation

- RNA can combine its information transfer capacity with ligand recognition (aptamers), enzymatic action (ribozymes), and regulation (riboswitches).

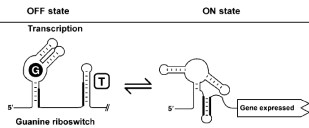


S. Blount, J. M. Jensen, J. C. Penedo, and D. A. Lafontaine ChemBioChem 2009, 10, 400 - 416

RNA-based Regulation



RNA-based Regulation



The transcription is aborted if nascent RNA adopts a "terminator" structure. Transcription riboswitches control the formation of terminator or alternative structures in response to ligand binding.

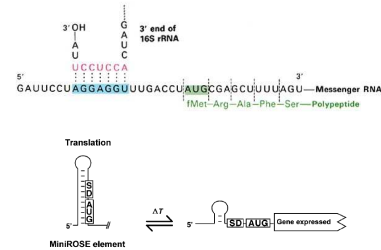
The control can be thermodynamic (controlled by the relative stability of the free and bound states) or kinetic (ligand binding has to take place before the transcription has continued into the expression region, otherwise may be ineffective).

Kinetic control adds an additional level of regulation as the response will depend on the rate of the RNA polymerase reaction, the availability of nucleotides, etc.

RNA-based Regulation

Translation initiation can be regulated by changes in the RNA conformation.

The Shine-Dalarno (ribosome binding site) and the initiation codon may be hidden or exposed in response to ligand binding.



RNA-based Regulation

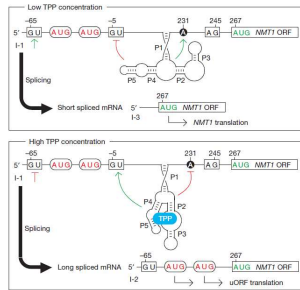


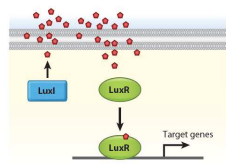
Figure 4 | Mechanism of TPP riboswitch-mediated alternative splicing of mRNA in *N. crassa*.

M.Cheah, A.Wachter1, N.Sudarsan & R.BreakerNature,447, 24 May 2007| doi:10.1038/nature05769

Collective Regulation in Bacteria (Quorum Sensing Networks)

- Production and Perception of Signaling molecules is used to sense the population of cells of a given type and regulate target genes.
- Often related to pathogenicity, changes in life-style (planktonic-biofilms)...
- May integrate additional environmental inputs (temperature, pH, availability of nutrients...)

Collective Regulation in Bacteria (Quorum Sensing Networks)



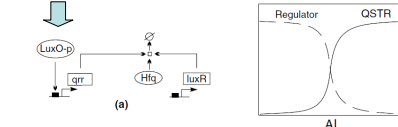
Often LuxI is under the control of LuxR leading to a positive feedback loop:

Presence of the Autoinducer increases its production causing a switch of all the population

W-L. Ngand B. L. Bassler Annu. Rev. Genet. 2009. 43:197-222

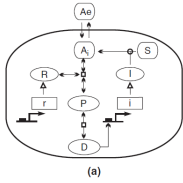
Collective Regulation in Bacteria (Quorum Sensing Networks)

Low cell densities (absence of AI)
LuxR RNA forms a complex and is degraded



High cell densities (AI increases)
LuxO non phosphorylated
Qrr not produced
LuxR RNA translated

Collective Regulation in Bacteria (Quorum Sensing Networks)



$$\frac{dr}{dt} = k_1 - k_2 r \quad \text{Synthesis and degradation of } r$$

$$\frac{di}{dt} = \frac{k_3 D}{K_4 + D} - k_5 i \quad \text{Production of } i \text{ is driven by } D$$

$$\frac{dR}{dt} = k_6 r - k_7 R - k_8 R \cdot A_i + k_{-8} P$$

Protein R is translated from its RNA (r), degraded, or converted into a complex with A but it can be released by dissociation of Complex P

$$\frac{dA_i}{dt} = k_{11} I - k_8 R \cdot A_i + k_{-8} P + k_{14}(A_e - A_i)$$

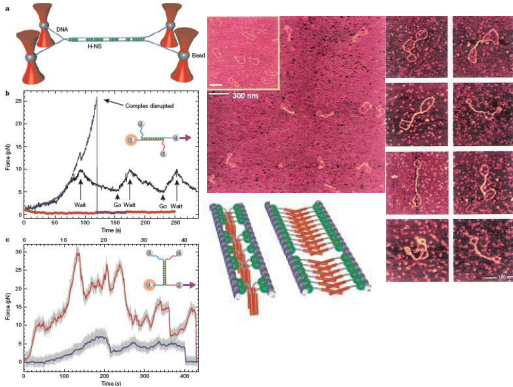
A_i is produced by I, sequestered in complex P and transported across the membrane following a concentration gradient

A. Goryachev, WIREs Systems Biology and Medicine, 1, 45-60, 2009
DOI: 10.1002/wsbm.027

Nucleoid associated proteins and bacterial adaption

5% of *E. coli* genes are regulated by H-NS (137 residues) in response to environmental signals like changes in osmolarity or temperature associated to colonization of a host in pathogenic strains

H-NS oligomers can bridge distant double helical DNA regions

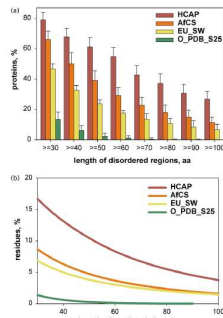


Native unfolded proteins

Review: A.L.Fink Current Opinion in Structural Biology, 2005, 15:35-41

- 30% of eucariotic proteins are completely or partially unfolded
- The proportion of genome encoding unstructured proteins increases with the complexity of the organism
 - 2.0% archaea, 4.2% eubacteria, 33% eucaryotes
- Disordered proteins are subjected to a strong evolutionary activity
 - 39% show short internal repeats vs, 14% in all proteins in Swiss-Prot
- It has been argued that native unfolded proteins should be considered a separate class on their own

A vast majority of the Human Cancer Associated Proteins (HCAP) contains substantial intrinsically unfolded regions



	Disordered Region (%)	Length of longest disordered region
FRAT-1 proto-oncogene	86.7	85
EW5 oncogene	79.9	286
FUS oncogene	72.6	252
Cyclin-dep.kin. inhib. p57	71.5	156
AF4 proto-oncogene	71.3	430
c-jun proto-oncogene	64	117
L-myc-1 proto-oncogene	64	85
Homeobox protein Hox 11	61.5	111
c-fos proto-oncogene	61	107
N-myc proto-oncogene	57.1	85
C-ski oncogene	57	155
Mdm2 oncoprotein	56.8	81
c-myc proto-oncogene	56.3	94
Tumor protein p73	54.9	121
Tumor suppressor p53	47.6	66

Iakoucheva, et al. J.Mol.Biol., 323, 573 (2002)



Unfolded domains respond to interactions and use external momentum (binding energy) to direct interaction pathways

The importance of being unfolded

Folded structures are often preorganized favoring tight (constitutive) binding. Intrinsically unfolded binding sites may have similar energies in the free and bound form: regulation

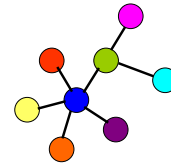


Unfolded structures may acquire different folds by binding different targets.



The importance of being unfolded (2)

Unfolded structures display EVOLVABILITY: neutral mutations may generate variability from which new interactions may be created.



Network dynamics in the 10^3 - 10^6 years time scale

Reinterpreting the STRUCTURE-FUNCTION paradigm

FOLDENESS ↔ ORDER ↔ FUNCTION

FOLDENESS INFORMATION FUNCTION

UNFOLDENESS INFORMATION FUNCTION

THE NEW PARADIGM HYPOTHESIS:
INFORMATION OF INTRINSICALLY UNFOLDED
PROTEINS IS DYNAMICALLY PROGRAMMED WITHIN A
SEQUENCE FRAMEWORK SELECTED TO AVOID
FORMATION OF AMYLOIDS

REVIEW ARTICLE

Bacteria as computers making computers

Antoine Danchin

Génomique des Génomés Bactériens, Institut Pasteur, Paris, France

FEMS Microbiol Rev 33 (2009) 3–26

Génomique des Génomés Bactériens, Institut Pasteur, 28 rue du Docteur Roux 75724 Paris Cedex 15, France. Tel.: +331 4568 8442; fax: +331 4568 8948; e-mail: antoine.danchin@pasteur.fr

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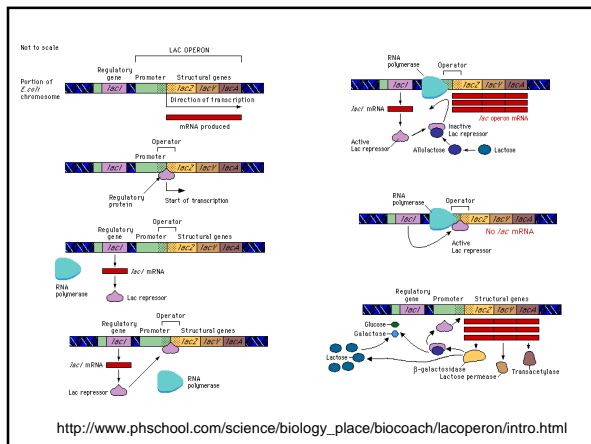
DOI:10.1111/j.1574-6976.2008.00137.x

Editor: Michael Gekker

Keywords: minimal genome, operating system, algorithmic complexity, junk DNA, APOBEC, ADAF.

Abstract

Various efforts to integrate biological knowledge into networks of interactions have produced a lively microbial systems biology. Putting molecular biology and computer sciences in perspective, we review another trend in systems biology, in which reactivity and information replace the usual concepts of differential equations, feedback and feedforward loops and the like. Noting that the processes of gene expression separate the genome from the cell machinery, we analyse the role of the separation between machine and program in computers. However, computers do not make computers. For cells to make cells requires a specific organization of the genetic program, which we investigate using available knowledge. Microbial genomes are organized into a palimpsest (the name emphasizes the role of the corresponding functions from the time of the origin of life, comprising a constructor and a replicator, and a genome (emphasizing community-relevant genes), made up of genes that permit life in a particular context. The cell duplication process supposes rejuvenation of the machine and replication of the program. The palimpsest also possesses genes that enable information to accumulate in a ratchet-like process down the generations. The systems biology must include the dynamics of information creation in its future developments.



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