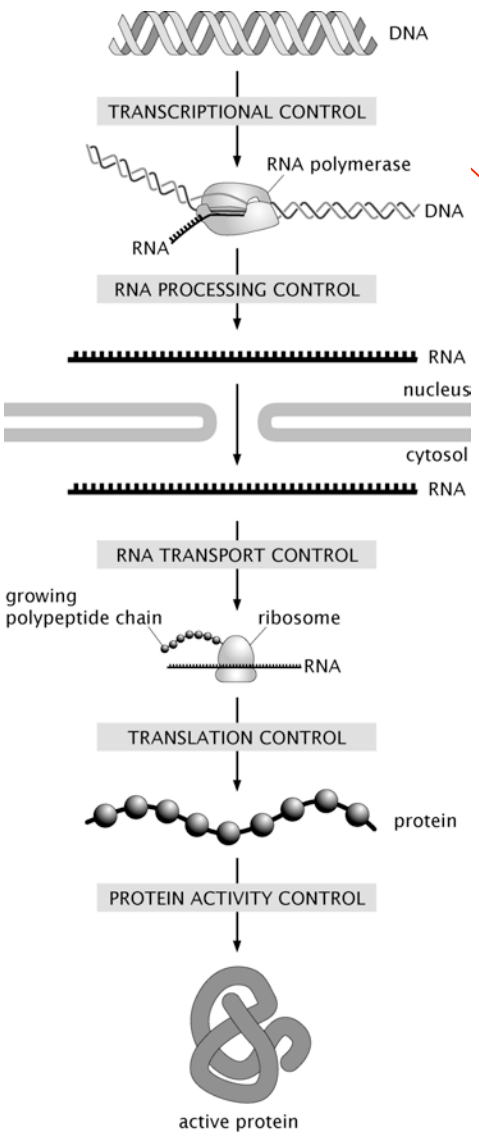
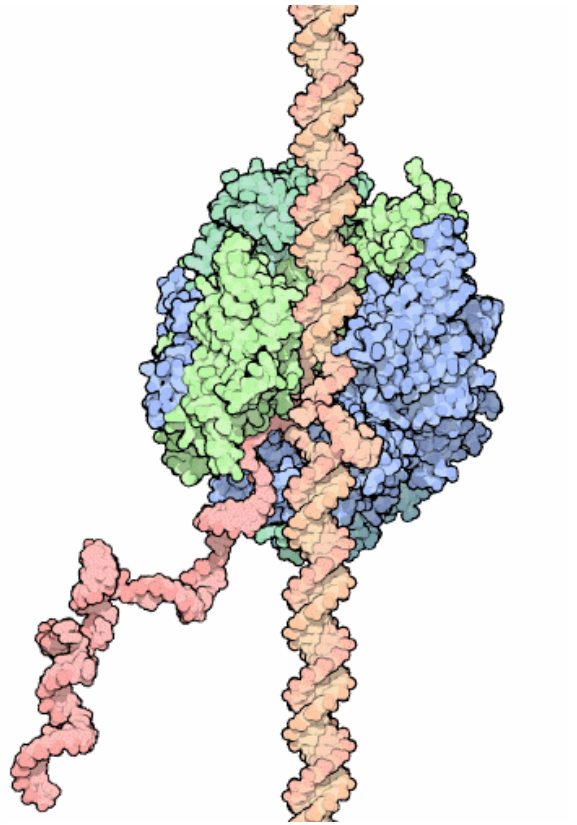
But, Genes Are Precisely Controlled: Transcriptional Regulation



- ***Regulation takes place very far upstrea***
  In particular, the "decision" is made whether or not to produce mRNA.
- ***Question: What are the molecules that mediate this control?***



Repressors: The Cartoon

- ***Repressor molecules inhibit action of RNA polymerase.***
- ***Repressors can be under the control of other molecules (i.e. inducers) that dictate when repressor is bound and not.***

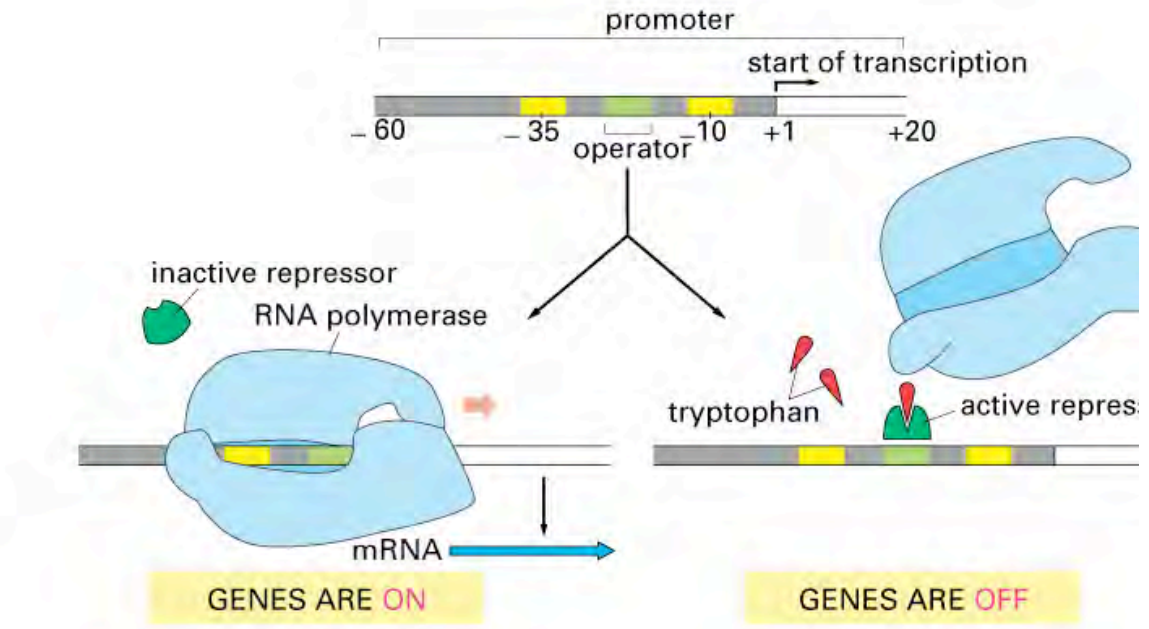
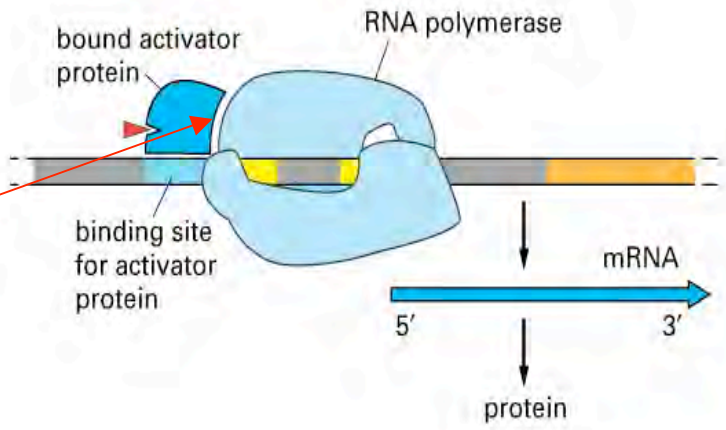


Figure 8-7 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Activators: The Cartoon

- ***Activator molecules enhance the action of RNA polymerase.***
- ***Activators can be under the control of other molecules (i.e. inducers) that dictate when activator is bound and not.***
- ***Activators "RECRUIT" the polymerase.***



Adhesive interaction between RNAP and activator

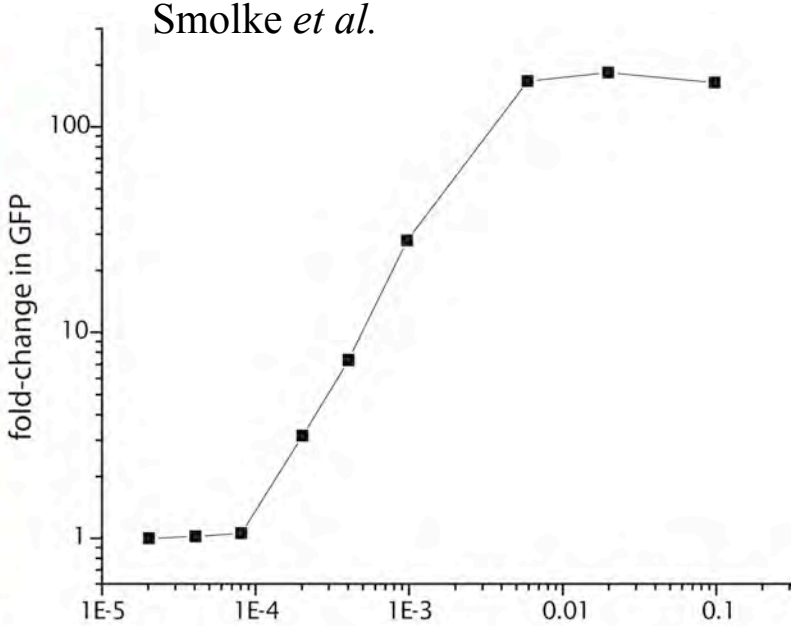
But quantitative data demands more than cartoons!

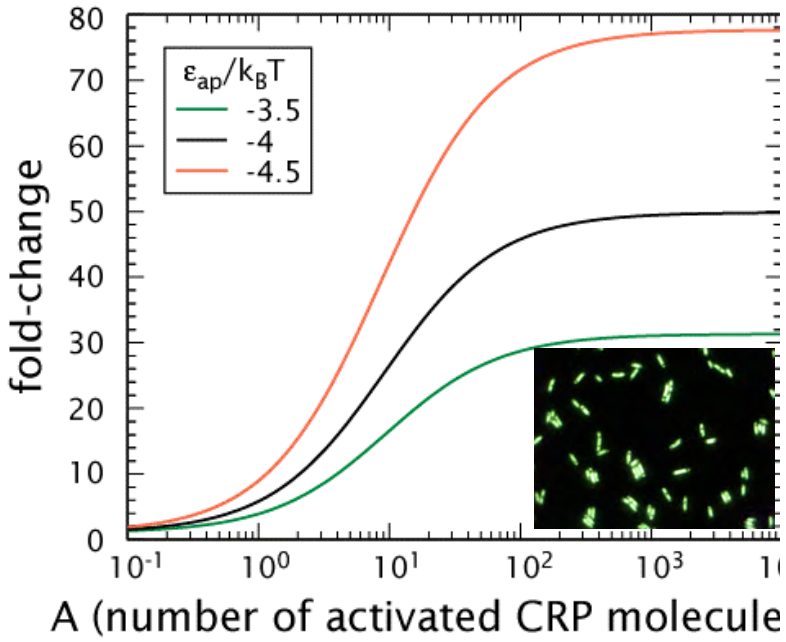
The Quantitative Measurement of Gene Expression: When, Where, How Much?

- ***The idea: by how many fold is the expression increased or decreased relative to some reference value.***
- ***To measure fold-change one can measure the expression level (for example using fluorescent reporter molecules) for the case of interest and for the reference state.***

$$\text{fold-change in promoter occupancy} = \frac{p_{bound}(A\neq}{p_{bound}(A=}$$

Smolke *et al.*

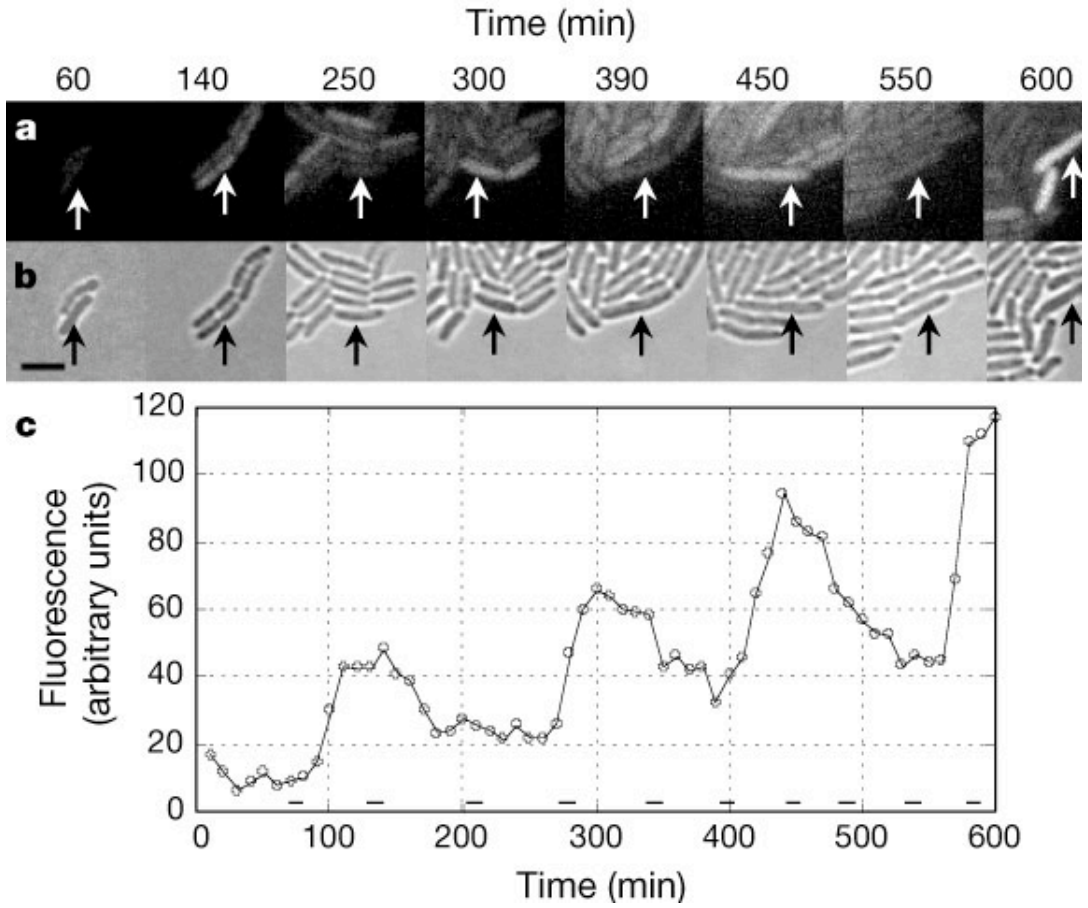




Quantitative Measurement of Gene Expression: When?

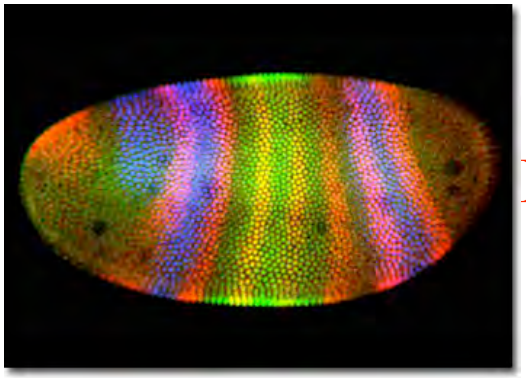
(Elowitz and Leibler)

- *Measurement of when genes are expressed.*
- *An example: the repressilator, a transcriptional regulatory network which leads to a time varying concentration of various gene products.*
- *The idea: stick an engineered set of genes into the cell and then turn them on.*

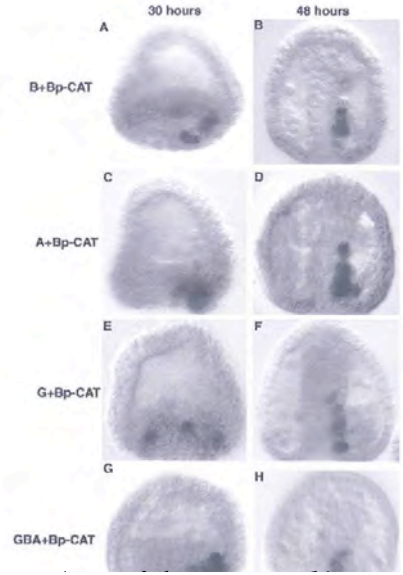


Quantitative Measurement of Gene Expression: Where?

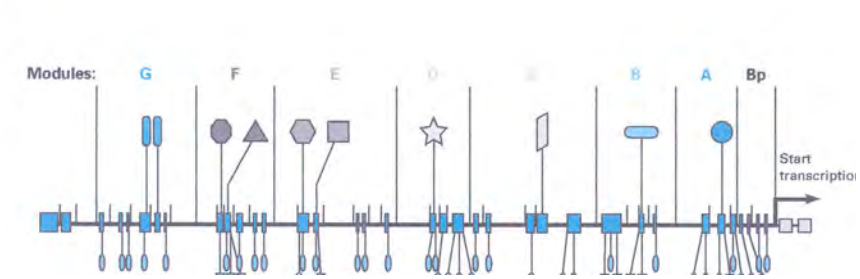
- ***Developmental biology is one of the most compelling arenas for thinking about spacetime gene expression.***

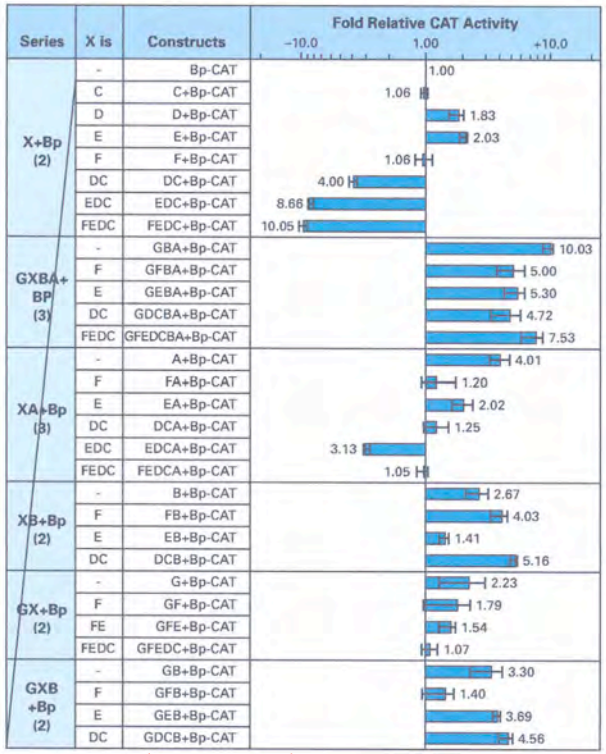


Fruit fly embryo



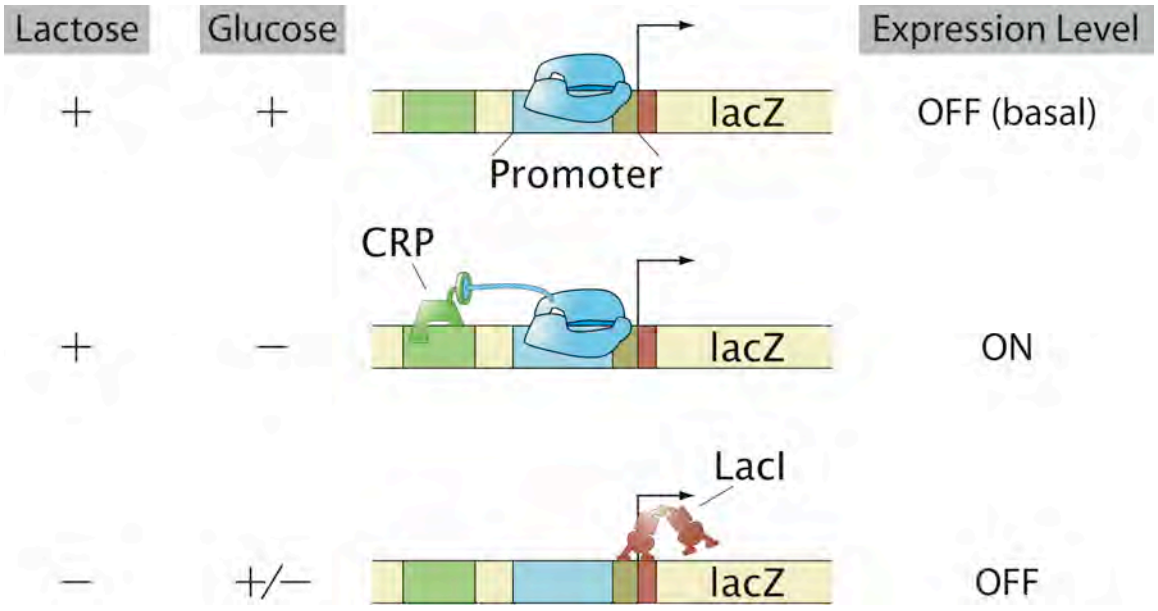
Sea urchin embryo





Battle cry: quantitative measurements demand quantitative models

The Lac Operon: The Hydrogen Atom of Gene Regulation

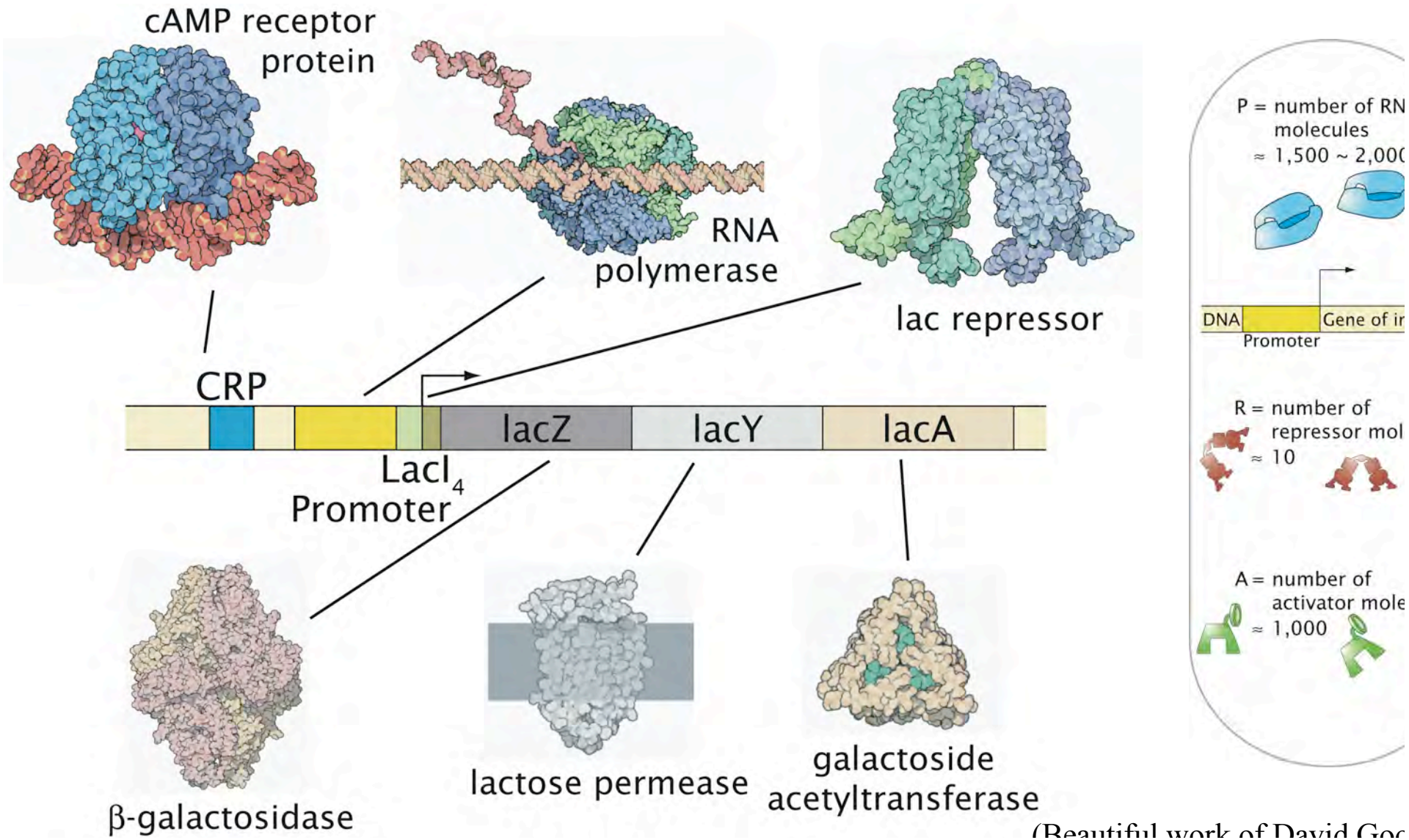


Monod

Saying a particular system is the "hydrogen atom" of a given subject is saying something very specific!

"Tout ce qui est vrai pour le Colibacille est vrai pour l'élép

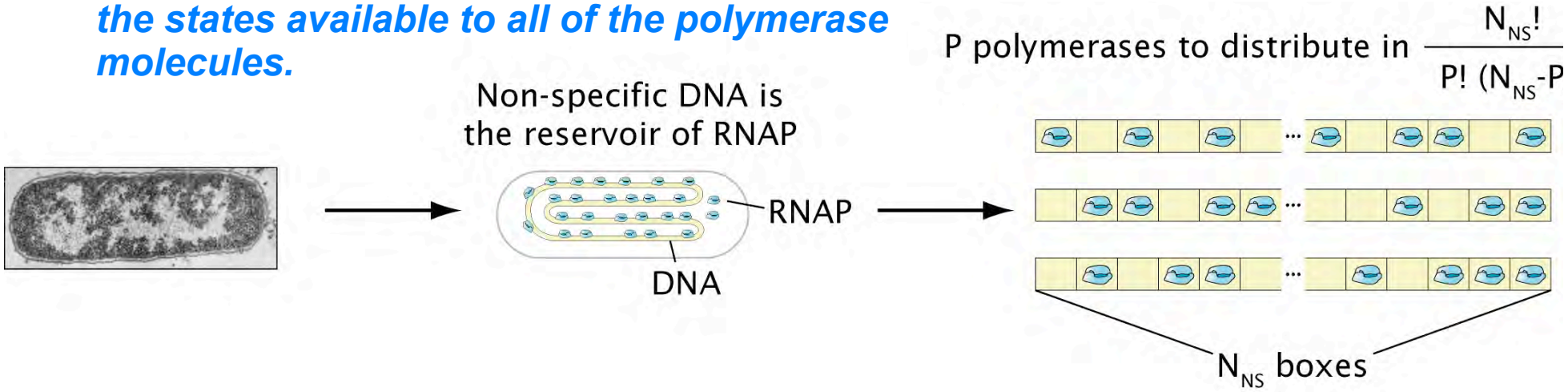
The Single Molecule Census



(Beautiful work of David Goo

Statistical Mechanics of Promoter Occupancy: Beyond the Cartoons

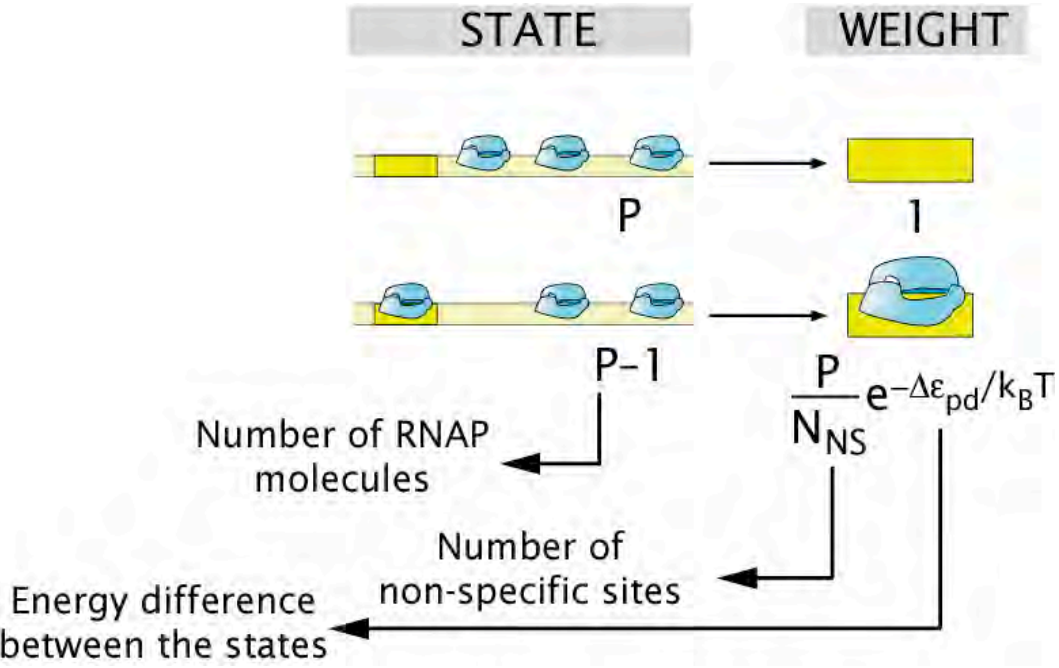
- ***The claim (hope): probability of promoter occupancy can tell us the extent to which gene is expressed.***
- ***The goal: compute the probability of promoter occupancy (like Ackers and Shea and others) as a ratio of promoter occupied states to all of the states available to all of the polymerase molecules.***



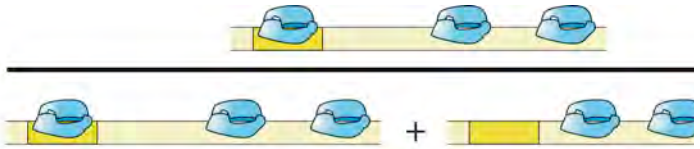
Why Bother? We are looking for knobs we can tune to change biological function and which permit us to find out whether the model is right or not.

$$\underbrace{Z(P;N_{NS})}_{\text{statistical weight - promoter unoccupied}} = \underbrace{\frac{N_{NS}!}{P!(N_{NS}-P)!}}_{} \times \underbrace{e^{-P\epsilon_{pd}^{NS}/k_BT}}_{\text{weight of each}}$$

Reckoning Promoter Occupancy



$p_{bound} =$

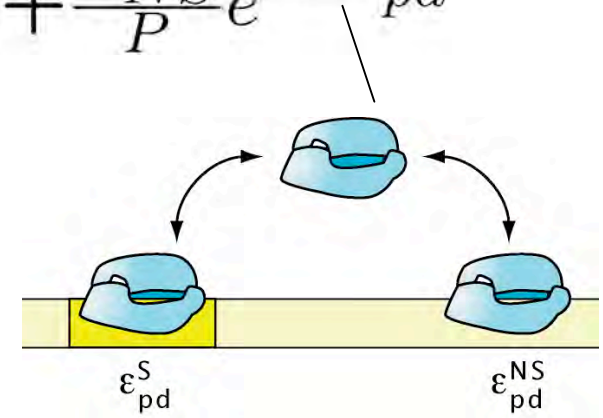


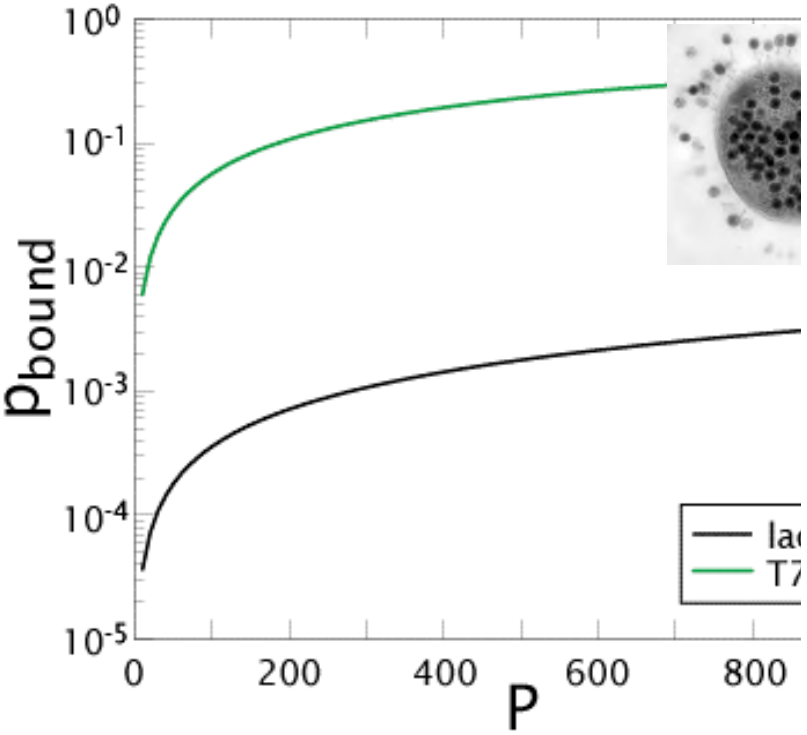
$$p_{bound} = \frac{1}{1+\frac{N_{NS}}{P} e^{\beta\Delta\epsilon}}$$

Essentially identical formula tells us open probability for ion channels!

Basal Transcription at the Lac Promoter

$$p_{bound} = \frac{1}{1+\frac{N_{NS}}{P}e^{\beta\Delta\epsilon_{pd}}}$$





- ***Key insight: RNAP NOT bound in absence of helper molecules for ``normal'' promoters.***

The Action of Transcription Factors

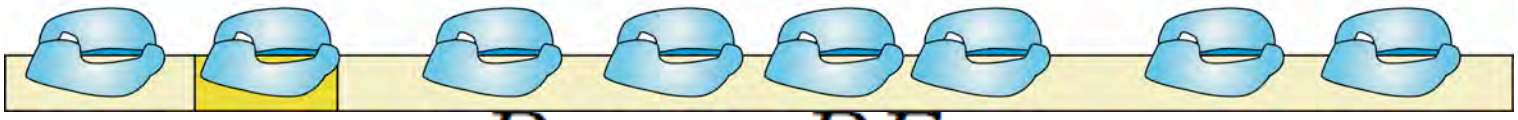
- ***The idea: The presence of transcription factors alters our previous result in a very simple way.***
- ***The interpretation: Activators make it seem like there are more polymerase molecules around, repressors make it seem like there are fewer.***

$$p_{bound} = \frac{1}{1 + \frac{N_{NS}}{PF_{reg}} e^{\beta \Delta \epsilon_{pd}}}$$

(A)

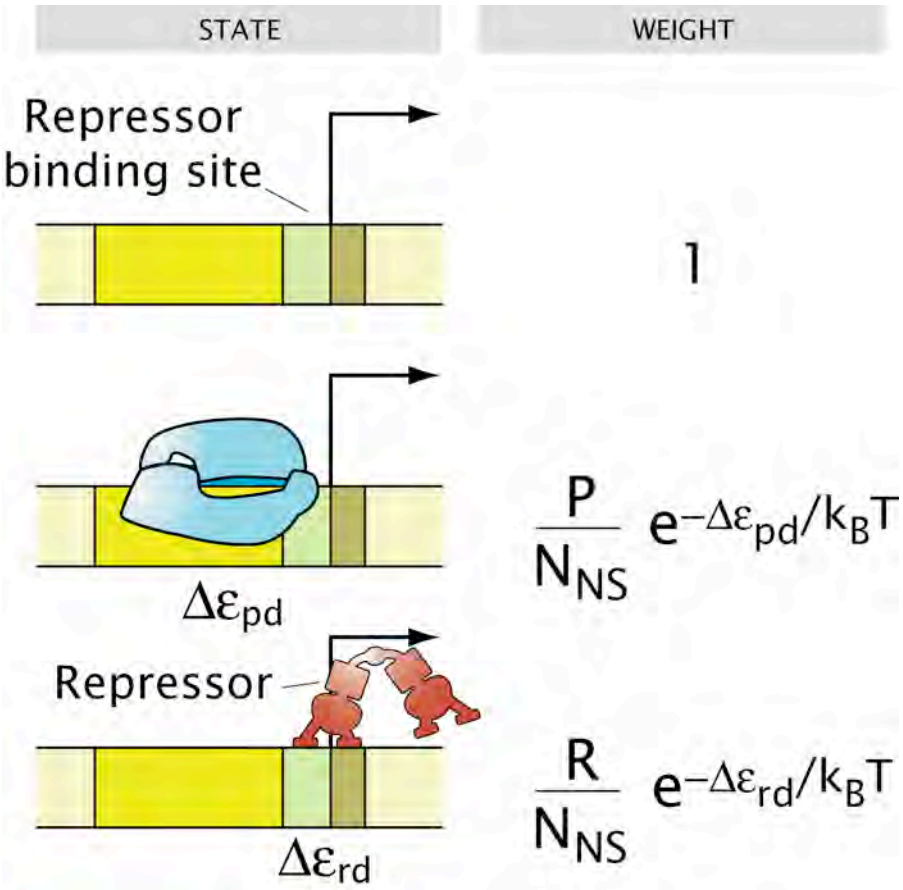
(B)

Freg = 2

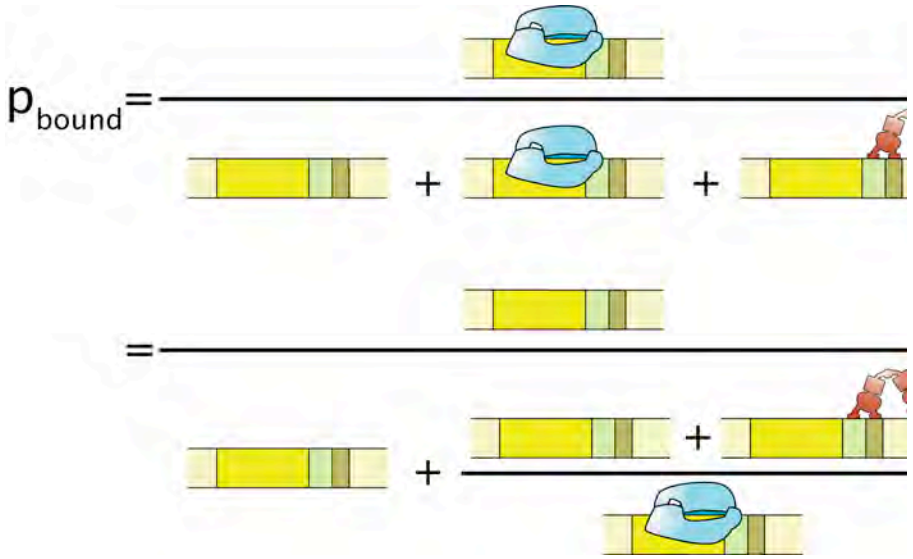


$$P_{eff} = PF_{reg}$$

Polymerase and Repressor Competing for the Same Real Estate



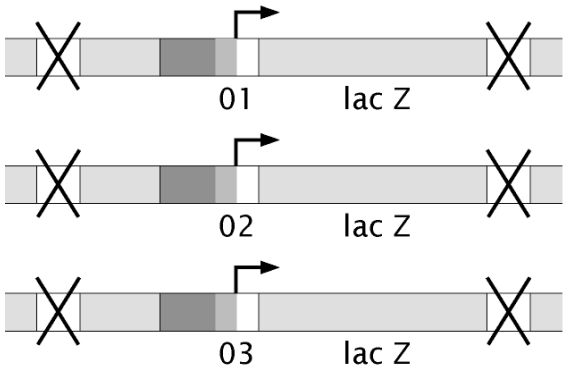
$$p_{bound} = \frac{1}{1 + \frac{N_{NS}}{PF_{reg}} e^{\beta \Delta \epsilon}}$$



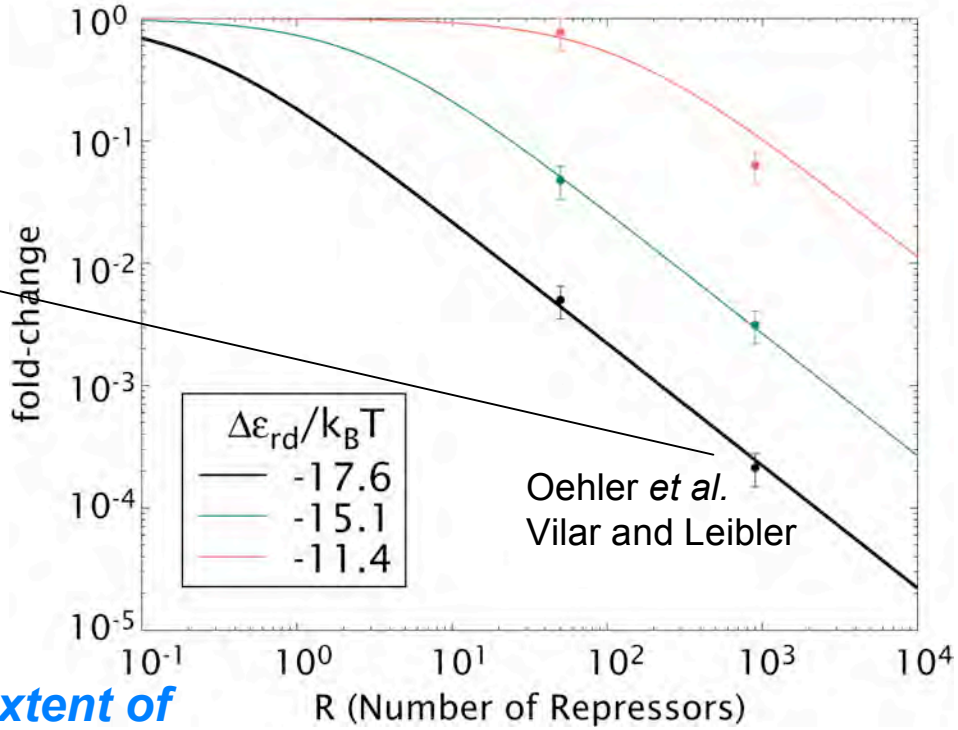
- ***Model predicts concentration dependence of repression for a single repressor binding site.***
- ***Extent of repression depends upon the strength of the binding site.***
- ***We need a better molecular census!***

Statistical Mechanics of a Single Repressor Binding Site

$$F_{reg} = (1 + \frac{R}{N_{NS}} e^{-\beta \Delta \epsilon_{rd}})$$



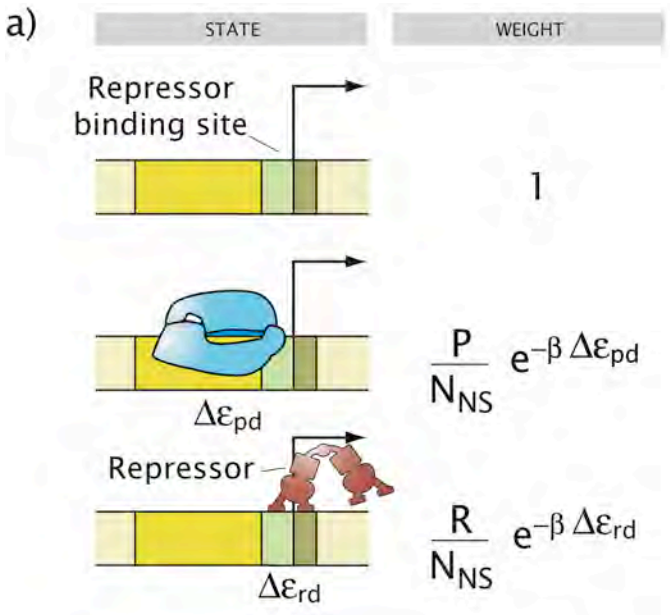
repression		
50	R	900
200		4700
21		320
1.3		16

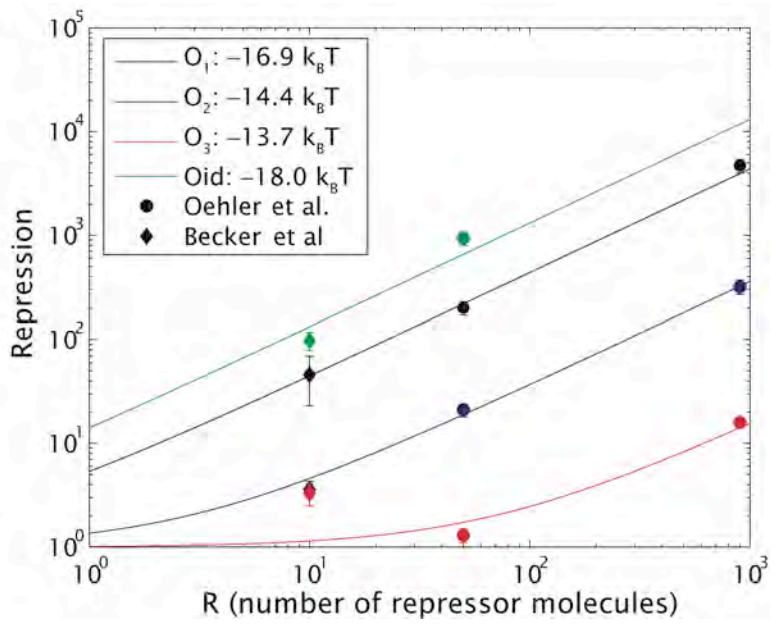


- ***Data from Oehler et al. examines the extent of repression for different binding strengths of the primary operator.***
- ***Model predicts how repression depends upon strength of binding site and number of repressors.***

Statistical Mechanics of a Single Repressor Binding Site

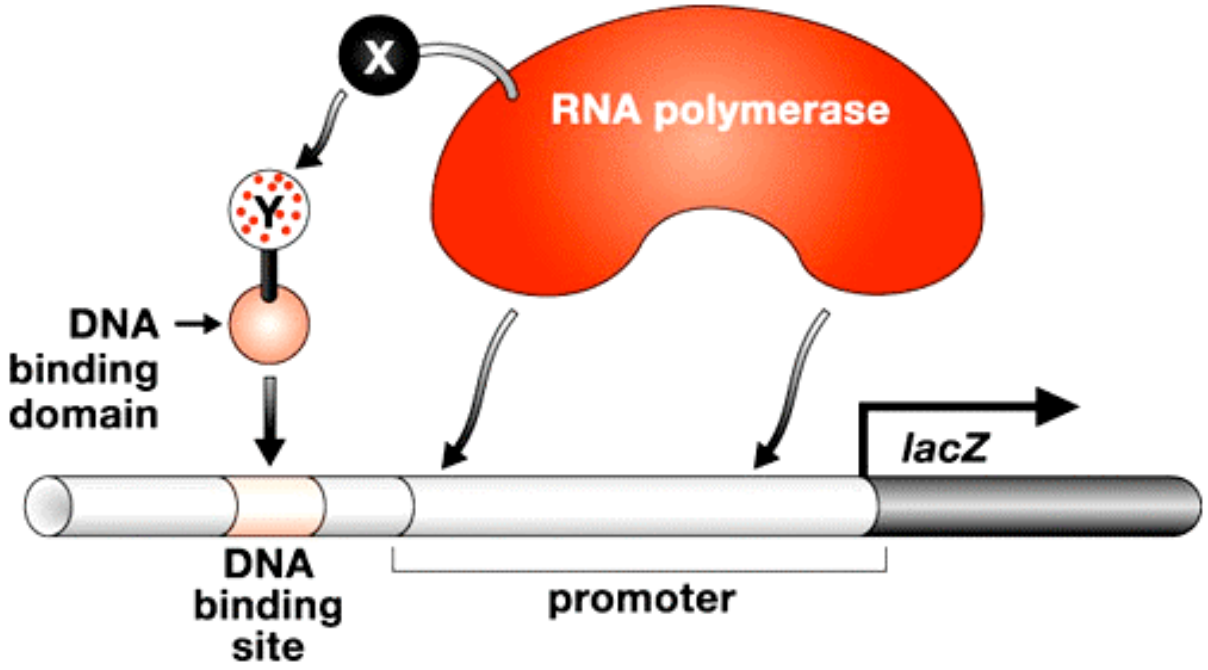
$$F_{reg} = (1 + \frac{R}{N_{NS}} e^{-\beta \Delta \epsilon_{rd}})$$





- *Data from Oehler et al. examines the extent of repression for different binding strengths of the primary operator.*
- *Model predicts how repression depends upon strength of binding site and number of repressors.*

Activator Bypass Experiments



Genes and Signals, © 2002 by Cold Spring Harbor Laboratory Press, Chapter 1, Figure 8

Exploring Regulatory Diversity

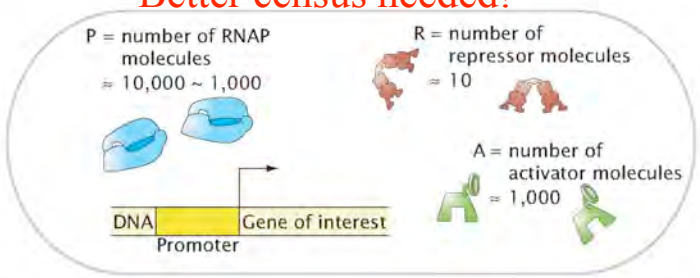
Table 1

Regulation factors for several different regulatory motifs.

Case	Regulation factor (F_{reg})	
1. Simple repressor	$(1+r)^{-1}$	$\left(1+\frac{[R]}{K_R}\right)^{-1}$
2. Simple activator	$\frac{1+ae^{-\frac{\varepsilon_{ap}}{k_BT}}}{1+a}$	$\frac{1+\frac{[A]}{K_A}f}{1+\frac{[A]}{K_A}}$
3. Activator recruited by a helper (*H*)	$\frac{1+a\frac{1+he^{-\frac{\varepsilon_{hd}}{k_BT}}}{1+h}e^{-\frac{\varepsilon_{ap}}{k_BT}}}{1+a\frac{1+he^{-\frac{\varepsilon_{hp}}{k_BT}}}{1+h}}$	$\frac{1+\frac{[H]}{K_H}+\frac{[A]}{K_A}f+\frac{[A][H]}{K_AK_H}f_{\omega}}{1+\frac{[H]}{K_H}+\frac{[A]}{K_A}+\frac{[A][H]}{K_AK_H}\omega}$
4. Repressor recruited by a helper (*H*)	$\left(1+\frac{1+he^{-\frac{\varepsilon_{hr}}{k_BT}}}{1+h}r\right)^{-1}$	$\frac{1+\frac{[H]}{K_H}}{1+\frac{[H]}{K_H}+\frac{[R]}{K_R}+\frac{[R][H]}{K_RK_H}\omega}$
5. Dual repressors	$(1+r_1)^{-1}(1+r_2)^{-1}$	$\left(1+\frac{[R_1]}{K_{R_1}}\right)^{-1}\left(1+\frac{[R_2]}{K_{R_2}}\right)^{-1}$
6. Dual repressors interacting	$\left(1+r_1+r_2+r_1r_2e^{-\frac{\varepsilon_{r_1r_2}}{k_BT}}\right)^{-1}$	$\left(1+\frac{[R_1]}{K_{R_1}}+\frac{[R_2]}{K_{R_2}}+\frac{[R_1][R_2]}{K_{R_1}K_{R_2}}\omega\right)^{-1}$
7. Dual activators interacting	$\frac{1+a_1e^{-\frac{\varepsilon_{a_1p}}{k_BT}}+a_2e^{-\frac{\varepsilon_{a_2p}}{k_BT}}+a_1a_2e^{-\frac{\varepsilon_{a_1p}+\varepsilon_{a_2p}+\varepsilon_{a_1a_2}}{k_BT}}}{1+a_1+a_2+a_1a_2e^{-\frac{\varepsilon_{a_1p}+\varepsilon_{a_2p}}{k_BT}}}$	$\frac{1+\frac{[A_1]}{K_{A_1}}f_1+\frac{[A_2]}{K_{A_2}}f_2+\frac{[A_1][A_2]}{K_{A_1}K_{A_2}}f_1f_2\omega}{1+\frac{[A_1]}{K_{A_1}}+\frac{[A_2]}{K_{A_2}}+\frac{[A_1][A_2]}{K_{A_1}K_{A_2}}\omega}$
8. Dual activators cooperating via looping	$\frac{1+a_1e^{-\frac{\varepsilon_{a_1p}}{k_BT}}+a_2e^{-\frac{\varepsilon_{a_2p}}{k_BT}}+a_1a_2e^{-\frac{\varepsilon_{a_1p}+\varepsilon_{a_2p}+F_{loop}}{k_BT}}}{(1+a_1)(1+a_2)}$	$\frac{1+\frac{[A_1]}{K_{A_1}}f_1+\frac{[A_2]}{K_{A_2}}f_2+\frac{[A_1]}{K_{A_1}}\frac{[A_2]}{K_{A_2}}f_1f_2\omega}{\left(1+\frac{[A_2]}{K_{A_2}}\right)\left(1+\frac{[A_1]}{K_{A_1}}\right)}$
9. Repre		
10. *N* no		

Regulati… symbol … in terms … regulatio… TF we in… the conc… the DNA…

Better census needed!



Key point: We can work ou… the regulation factor for ma… other scenarios including other looping scenarios.

STATE	WEIGHT
	1
	$\frac{P}{N_{ns}}\,e^{-\Delta\varepsilon_{pd}}$
	$\frac{A}{N_{ns}}\,e^{-\Delta\varepsilon_{a_1d}}$
	$\frac{A}{N_{ns}}\,e^{-\Delta\varepsilon_{a_2d}}$
	$\frac{A}{N_{ns}}\frac{A}{N_{ns}}\,e^{-(\Delta\varepsilon_{a_1d}+\Delta\varepsilon_{a_2d})}$
	$\frac{P}{N_{ns}}\frac{A}{N_{ns}}\,e^{-(\Delta\varepsilon_{pd}+\Delta\varepsilon_{a_1d}+\varepsilon_{a_1p})}$
	$\frac{P}{N_{ns}}\frac{A}{N_{ns}}\,e^{-(\Delta\varepsilon_{pd}+\Delta\varepsilon_{a_2d}+\Delta\varepsilon_{a_2p})}$
	$\frac{P}{N_{ns}}\frac{A}{N_{ns}}\frac{A}{N_{ns}}\,e^{-(\Delta\varepsilon_{pd}+\Delta\varepsilon_{a_1d}+\Delta\varepsilon_{a_2d}+\varepsilon_{a_1p}+\varepsilon_{a_2p}+F_{loop})}$

Synergistic Activation