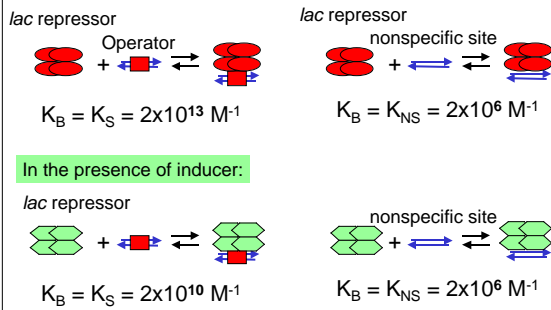


What do you learn from binding constants and rate constants?

Regulatory effects on RNA polymerase

Inducers
Repressors
Activators

Inducer lowers the K_B for repressor binding to operator



Specificity parameter (See also Appendix A)

Ratio of K_S to K_{NS} decreases 1000 fold in presence of inducer.

$$\text{Specificity} = \frac{K_S}{K_{NS}} = 10^7 \text{ in absence of inducer}$$

$$\frac{K_S}{K_{NS}} = 10^4 \text{ in presence of inducer}$$

Nonspecific sites are in vast excess over operator sites. Thus, in the presence of inducer, the repressor is redistributed so that the operator is not occupied.

Virtually all the repressor is associated with DNA

10 molecules of repressor/cell means $[P] = [R_4] = 1.7 \times 10^{-8} \text{ M}$

4.6×10^6 nonspecific sites on DNA/cell means
total $[D] = \text{total } [D_{NS}] = 7.64 \times 10^{-3} \text{ M}$

$$K_B = \frac{[DP]}{[D][P]} = K_{NS} = \frac{[R_4 D_{NS}]}{[R_4][D_{NS}]} = 2 \times 10^6 \text{ M}^{-1}$$

$$\frac{[R_4]}{[R_4 D_{NS}]} = \frac{1}{K_{NS} [D_{NS}]} = \frac{1}{(2 \times 10^6 \text{ M}^{-1})(7.64 \times 10^{-3} \text{ M})} = 6.5 \times 10^{-5}$$

Only about 1 in 15,000 repressor molecules is NOT bound to DNA.

Can calculate bound to free DNA while considering both specific and nonspecific binding

$$\text{Specificity} = \frac{K_S}{K_{NS}} = \frac{\frac{[R_4 D_S]}{[R_4][D_S]}}{\frac{[R_4 D_{NS}]}{[R_4][D_{NS}]}} = \frac{[R_4 D_S]}{[D_S]} \times \frac{[D_{NS}]}{[R_4 D_{NS}]}$$

$[R_4]_{\text{total}} - [D_S]_{\text{total}}$
since $[R_4]_{\text{free}}$ is negligible,
 D_S is close to saturated with R_4

$$\frac{K_S}{K_{NS}} = \frac{[R_4 D_S]}{[D_S]} \times \frac{[D_{NS}]}{[R_4]_{\text{total}} - [D_S]_{\text{total}}}$$

Measured Want to know Constants

Inducer shifts the distribution of repressor so that more is bound to nonspecific DNA

$$\frac{K_S}{K_{NS}} = \frac{[R_4 D_S]}{[D_S]} \times \frac{[D_{NS}]}{[R_4]_{total} - [D_S]_{total}}$$

$\frac{K_S}{K_{NS}} = 10^7$ in *absence* of inducer

$$\frac{[R_4 D_S]}{[D_S]} = 20 \quad \text{Most of the operators are bound by repressor.}$$

$\frac{K_S}{K_{NS}} = 10^4$ in *presence* of inducer

$$\frac{[R_4 D_S]}{[D_S]} = 0.02 \quad \text{Most of the operators are NOT bound by repressor.}$$

Converting ratios of bound/free to fractional occupancy

If $a/b = x$, then $a/(a+b) = x/(1+x)$.

If $\frac{a}{b} = x$, then $a = bx$

and

$$\frac{a}{a+b} = \frac{bx}{bx+b} = \frac{bx}{b(x+1)} = \frac{x}{x+1}$$

Fractional occupancy of operators

Note: $[R_4 D_S] + [D_S] = [D_S]_{tot}$

In the *absence* of inducer:

$$\frac{[R_4 D_S]}{[D_S]} = 20 \quad \text{then} \quad \frac{[R_4 D_S]}{[D_S]_{tot}} = \frac{20}{21} = 0.95$$

About 95% of operators are bound by repressor.

In the *presence* of inducer:

$$\frac{[R_4 D_S]}{[D_S]} = 0.02 \quad \text{then} \quad \frac{[R_4 D_S]}{[D_S]_{tot}} = \frac{0.02}{1.02} \approx 0.02$$

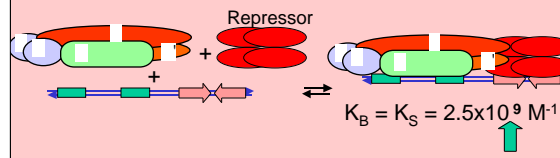
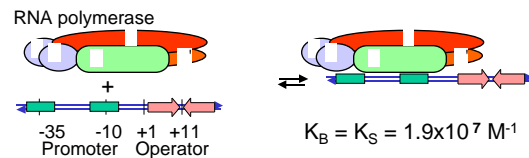
About 2% of operators are bound by repressor.

Operator mutants decrease the affinity of repressor for operator

- Appendix B

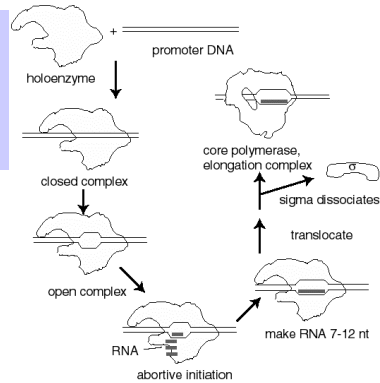
Effect of repressor on RNA polymerase at the promoter

Repressor **increases** affinity of polymerase for promoter



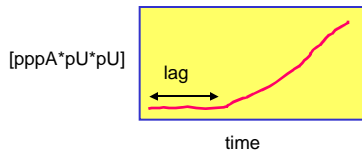
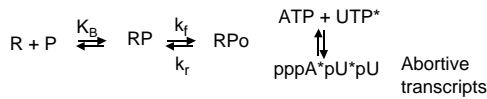
Repressor **decreases rate** of transition from closed to **open complex**

Events at initiation of transcription



Abortive initiation assay

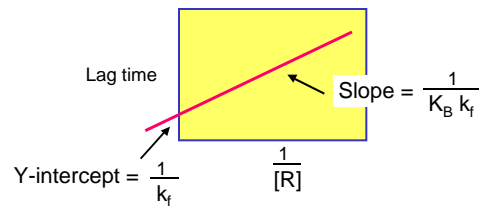
Let R = RNA polymerase, P = promoter (closed), and Po= promoter (open)



Measure k_f and K_B from lag time vs. $1/[R]$

Lag time in abortive initiation assay is inversely proportional to $[R]$.

$$\text{Lag time} = \frac{1}{K_B k_f} \times \frac{1}{[R]} + \frac{1}{k_f}$$



Effect of *lac* Repressor on RNA polymerase

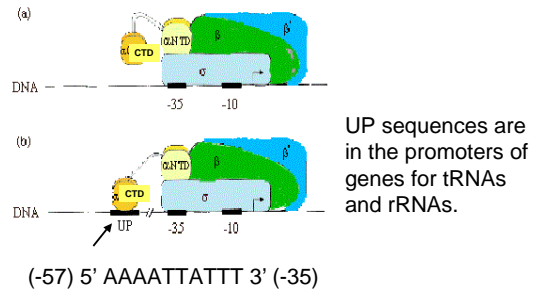
- RNA polymerase binds **more** tightly to the promoter when Repressor is bound to the operator.
 - Effect on K_b **favours** transcription.
- The k_f for the closed to open transition is **less** when repressor is bound to the operator.
 - This k_f effect accounts for the repression of the *lac* operon in the absence of inducer.

Activation of transcription by CAP

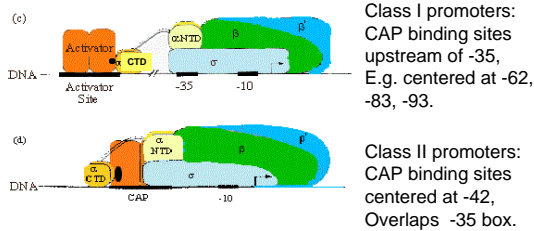
- cAMP-CAP interacts **directly** with RNA polymerase
- Binds to alpha subunit of RNA polymerase
- For *lac* operon, this increases K_B for binding of polymerase to promoter.
- At other operons, CAP binds to a different surface of the alpha subunit and increases k_f for isomerization from closed to open complex.

CAP illustrates the fact that a single protein can interact with RNA Pol via different contact surfaces

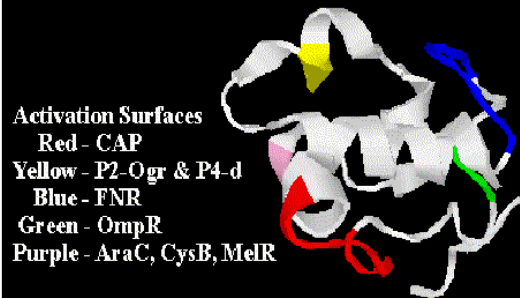
The CTD of the alpha subunit of RNA Pol can bind to UP sequences



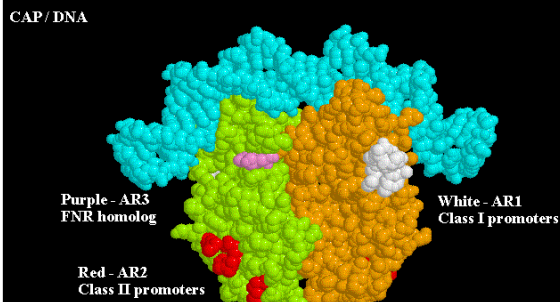
The CTD of the alpha subunit of RNA Pol can interact with activators



CTD of alpha subunit of RNA Pol has multiple contact sites for activators



CAP has 2 activation regions

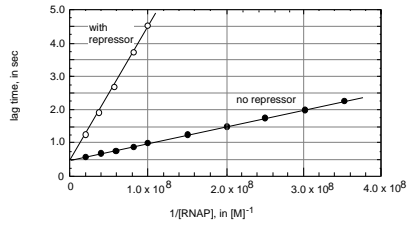


Interactions of CAP with alpha subunit of RNA polymerase

AR1 (residues 156-164) of CAP:
 At class I promoters, AR1 in the downstream subunit "sees" residues 258-265 of CTD of alpha; increases K_B .

AR2 (residues 19, 21, 96, 101)
 At class II promoters, AR2 in the downstream subunit "sees" alpha NTD residues 162-165, increasing k_i for isomerization from closed to open complexes.

Example Problem



k_f

What is the value of the forward rate constant (k_f) for closed to open complex formation under the two different conditions?