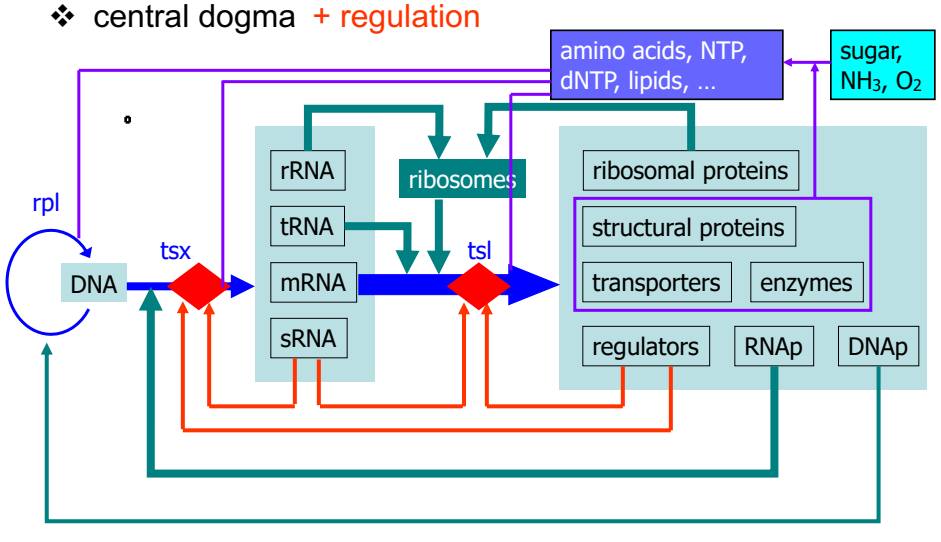
❖ central dogma + regulation

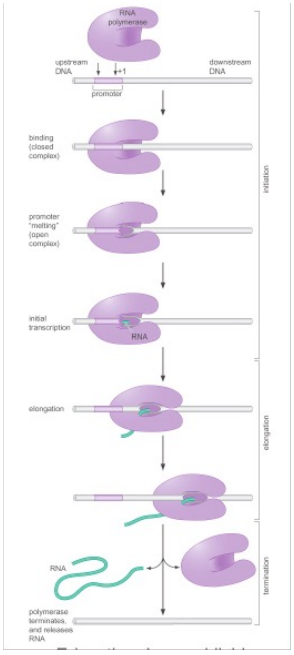


- tsx initiation control by transcription factors (TF)
- tsl initiation control by sRNA and RNA-binding proteins
- tsx termination control by sRNA and anti-terminators
- control of mRNA and protein degradation

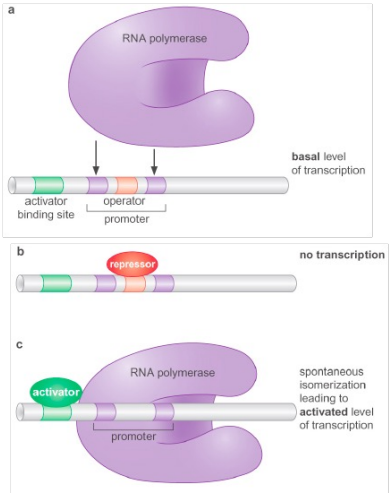
} coupled to environmental signals

1

transcriptional initiation and termination



tsx init control by activators, repressors



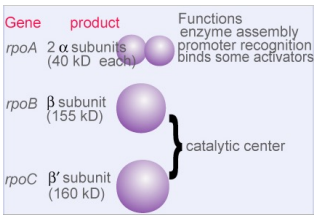
2

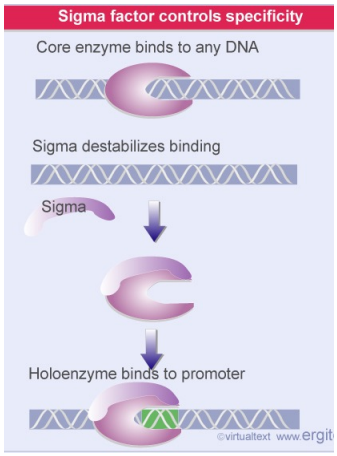
Topic 2: Transcription Initiation Control

A. Mechanisms of tsx initiation in bacteria

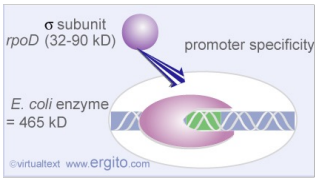
1. Components:

• core enzymes of RNA polymerase:





• sigma factor:



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• *E. coli* has 6 different σ-factors

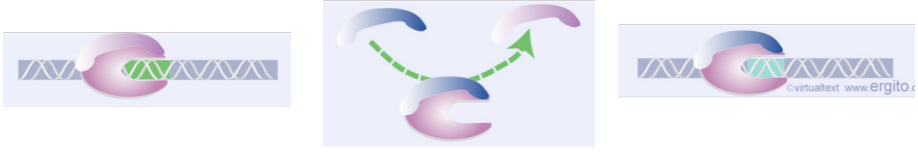
70-80% of genes; further regulated by TFs

Gene	Factor	Use
rpoD	σ^{70}	general
rpoS	σ^{S}	stress
rpoH	σ^{32}	heat shock
rpoE	σ^{E}	heat shock
rpoN	σ^{54}	nitrogen
fliA	σ^{28} (σ^{F})	flagellar

• core promoter recognition sequences

Factor	-35 Sequence	Separation	-10 Sequence
σ^{70}	TTGACA	16-18 bp	TATAAT
σ^{32}	CCCTTGAA	13-15 bp	CCCGATNT
σ^{54}	CTGGNA	6 bp	TTGCA
σ^{28} (σ^{F})	CTAAA	15 bp	GCCGATAA

• substitution of σ-factors ➔ recognize different set of promoters



• *B. subtilis* has ~20 σ-factors (include sporulation, competence, …)

• generally, more complex the life style of organism, more sigma factors

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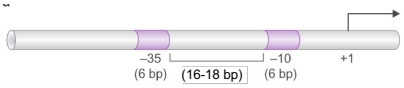
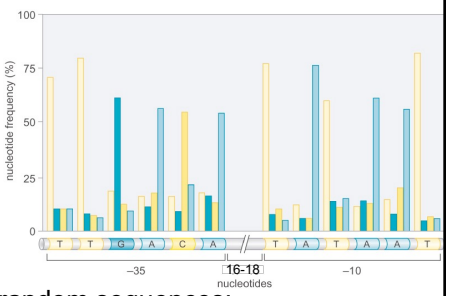
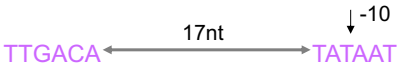
• core promoter recognized by σ^{70}-factor:

canonical promoter has fuzzy motif





• consensus sequence:



occurrence of fuzzy promoter motifs in random sequences:

• 3 out 6 matches in -35 region $\binom{6}{3} \times 0.25^3 \times 0.75^3 \approx 13\%$

• degeneracy in spacing (16-18bp) $\times 3$

• 4 out 6 matches in -10 region $\binom{6}{4} \times 0.25^2 \times 0.75^4 \approx 3\%$

➔ at given position in the genome, motif occurrence probability

$\approx 13\% \times 3 \times 3.3\% \approx 1.3\%$

(one occurrence every ~ 80bp, i.e., everywhere!)

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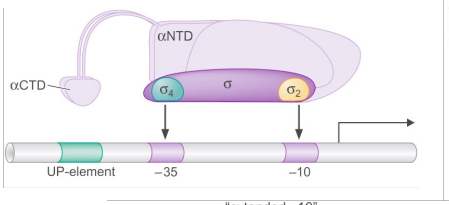
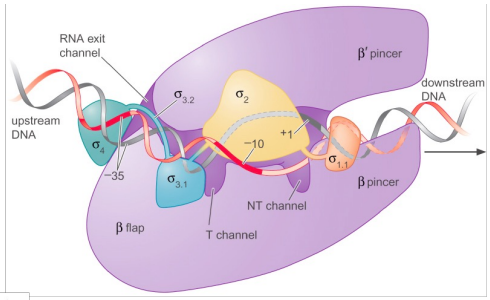
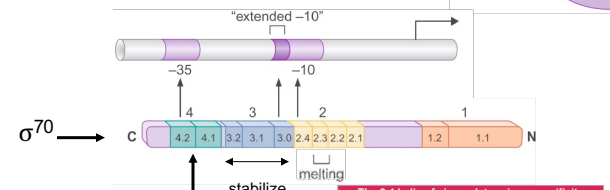
2. RNAp-promoter interaction

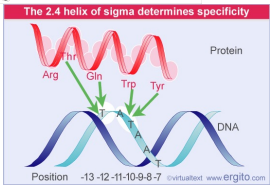


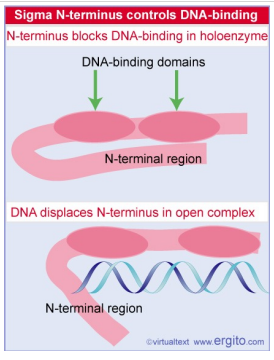




stabilize open complex

bends DNA at -35; facilitates interaction with upstream activators





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- avail. RNAp conc ~ 30 nM [McClure, 1983]
  ≈ 0.5 ~ 1 μM [Klumpp & Hwa, PNAS 2008]
  ≈ 1 μM (glc medium) [Balakrishnan et al, Science 2022]

- typical binding constants and rates

(rapid equilibria) (slow)

$$\mathrm{R + P} \underset{}{\overset{K_1}{\rightleftharpoons}} \mathrm{I_1} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{I_2} \overset{K_3}{\rightleftharpoons} \mathrm{RP_O}$$

(slow) (rapid equilibria)

$$K_1 / K_{ns} = 10^{-3} \sim 1 \quad \Rightarrow \quad \widetilde{K}_1 = \sum_{n \neq j}^{N} e^{(G_1 - G_{ns})/kT} = \frac{N \cdot K_1}{K_{ns}} = 10^4 \sim 10^7 \text{ nM}$$

  – promoter binding typically very weak, i.e., $[\mathrm{RNAp}] / \widetilde{K}_1 \ll 1$
  – opportunity for regulation, e.g., boost promoter binding probability

$$k_2 = 10^{-3} \sim 10^{-1} \text{ sec}^{-1}$$

  – fast end need not be faster
    [cf: search kinetics]
  – another opportunity for regulation

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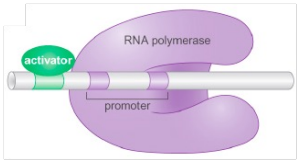
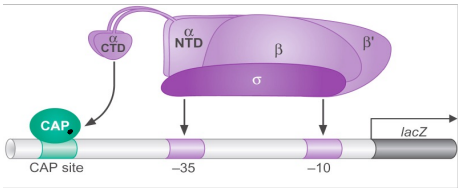
3. TF-RNAp interaction

- Recruitment:



e.g., CRP (activated by cAMP; aka CAP)



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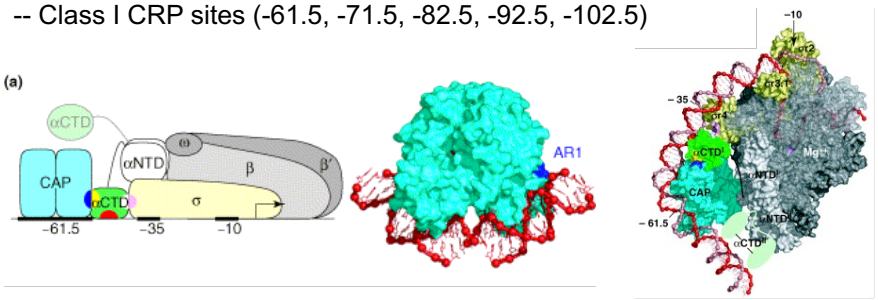
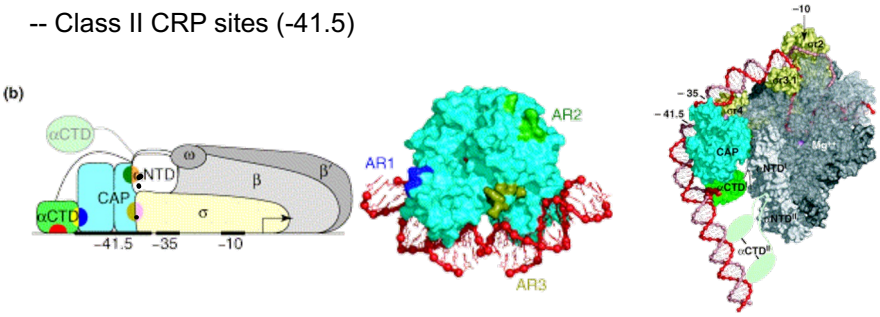
-- Class I CRP sites (-61.5, -71.5, -82.5, -92.5, -102.5)



-- Class II CRP sites (-41.5)



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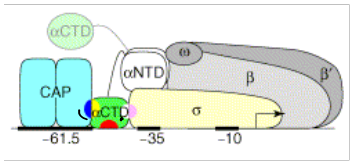
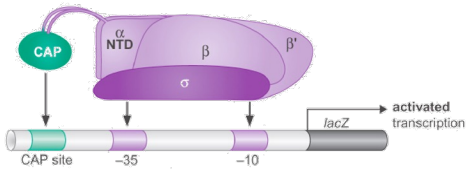
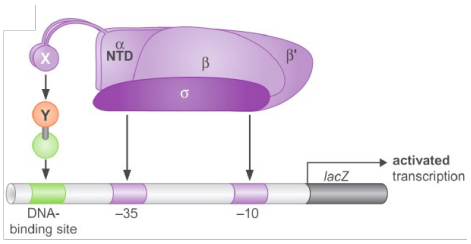
-- mechanism of activation? activator bypass experiments:

➔ glue-like attraction between CRP and α-CTD







(bacterial two-hybrid system)

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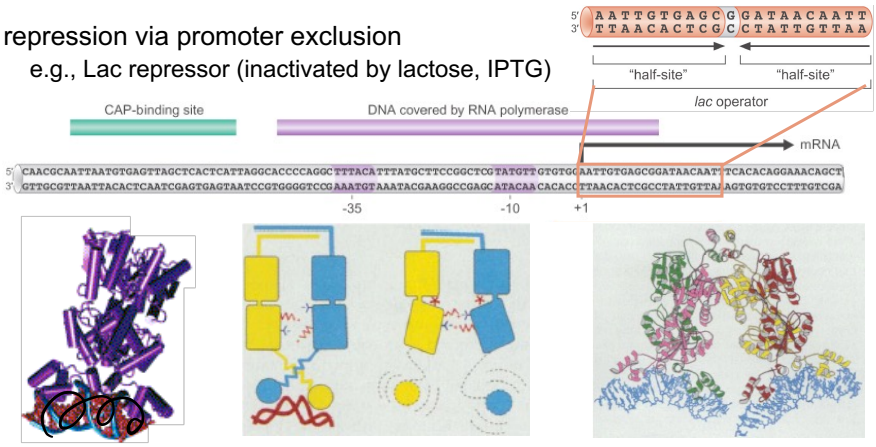
-- repression via promoter exclusion

e.g., Lac repressor (inactivated by lactose, IPTG)



dimer of Lac repressor

- minimal unit for DNA binding
- large portion of molecule for ligand binding and allosteric control of the DNA-binding domain
- wt Lac tetramerizes

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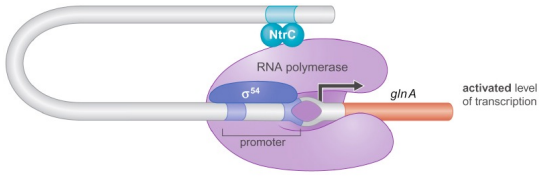
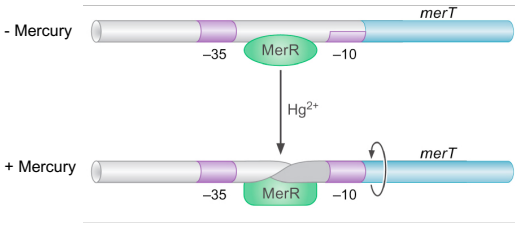
- Allosteric mechanisms of activation
  - NtrC (activated by phosphorylation under low nitrogen level): can activate σ^{54} from 1-2 kbp away; has ATPase activity



  - MerR: activate σ^{70}-mediated tsx by twisting promoter DNA



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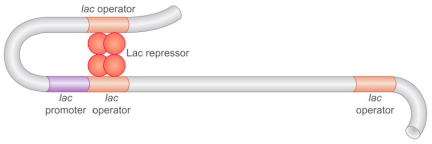
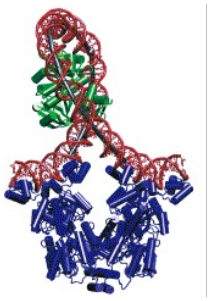
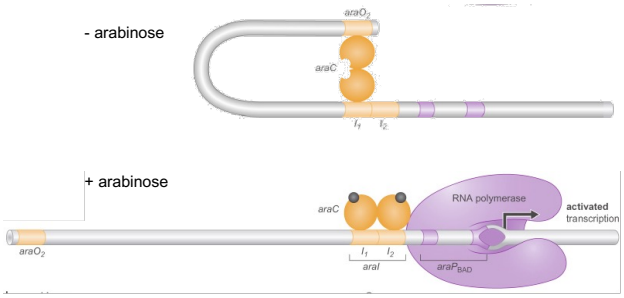
- DNA looping
  - tight repression by lac tetramers





  - strong regulation by AraC



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B. Basic Models of Transcriptional Control

1. tsx init by RNAp alone

$$\underbrace{\text{RNAp} + \text{promoter}}_{\text{“}P\text{”}} \underset{}{\overset{K_p}{\rightleftharpoons}} \text{RNAp} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAp} + \text{promoter} + \underbrace{mRNA}_{\text{“}m\text{”}}$$

- mRNA level: $\frac{d}{dt}[m] = \alpha \cdot \mathcal{P} - \beta \cdot [m]$

  α: probability of promoter occupation by RNAp

  β: mRNA degradation

- steady-state mRNA level (measurable): $[m^*] = \alpha \cdot \mathcal{P} / \beta$
- from protein-DNA interaction, expect $\mathcal{P} = 1 \Big/ \left(1 + \widetilde{K}_p / [P]_{av}\right)$

  where $[P]_{av}$ = avail RNAp conc ≈ 0.5 ~ 1 μM

  $\widetilde{K}_p = N \cdot K_p / K_{ns} = 10^4 \sim 10^7$ nM

➔ for RNAp by itself, $\mathcal{P} \approx [P]_{av} / \widetilde{K}_p \ll 1$

➔ TF can modulate $\mathcal{P}$ or α

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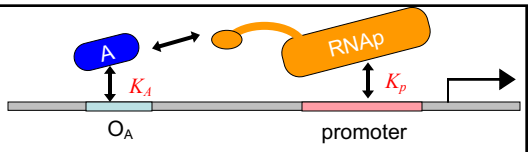
2. Activation by recruitment

How does gene expression depend on [A]?



Strategy: [Shea & Ackers, 1985]

-- assume $[m^*] = \alpha \cdot \mathcal{P}([A],[P])/\beta$

-- $\mathcal{P}$ computed according to thermodynamics (assumes thermal equilibrium)

Recall for operator site alone: $p_A = [A]_{tot} \Big/ \left([A]_{tot} + \widetilde{K}_A\right)$

[will drop tilde and subscript "tot" from here on]

Total probability of RNAp binding to promoter in the presence of A:

$$\mathcal{P}([A],[P]) = \frac{W(0,1)+W(1,1)}{W(0,0)+W(0,1)+W(1,0)+W(1,1)}$$

where $W(\sigma_A, \sigma_P)$ = weight of { operator A is occupied (σ_A =1) or unoccupied (σ_A =0); promoter is occupied (σ_P =1) or unoccupied (σ_P =0) }

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Dependence of the total probability of RNAp-promoter binding on A:

$$\mathcal{P}([A],[P]) = \frac{W(0,1)+W(1,1)}{W(0,0)+W(0,1)+W(1,0)+W(1,1)}$$

Form of $W(\sigma_A, \sigma_P)$: let $W(0,0)=1$ (since only ratio of weights matter)

$$W(0,1) = [P]/K_p, \quad W(1,0) = [A]/K_A$$
$$W(1,1) = \omega \cdot ([A]/K_A) \cdot ([P]/K_P)$$

derived from stat mech

$\omega = e^{-E_{int}/k_BT}$ ("cooperativity factor")

check: P by itself, i.e., [A]=0, $p_P = \dfrac{W(0,1)}{W(0,0)+W(0,1)} = \dfrac{[P]/K_P}{1+[P]/K_P}$

P given A, i.e., [A]=∞, $p_{P|A} = \dfrac{W(1,1)}{W(1,0)+W(1,1)} = \dfrac{\omega \cdot [P]/K_P}{1+\omega \cdot [P]/K_P}$

➔ promoter strength effectively increased (K_p ➔ K_p/ω)

Compact notation: $W(\sigma_A,\sigma_P) = ([A]/K_A)^{\sigma_A} \cdot ([P]/K_P)^{\sigma_P} \cdot \omega^{\sigma_A \sigma_P}$

then $\mathcal{P}([A],[P]) = \sum_{\sigma_A} W(\sigma_A,\sigma_P=1) \Big/ \sum_{\sigma_A,\sigma_P} W(\sigma_A,\sigma_P)$

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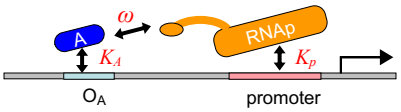
$$\mathcal{P}([A],[P]) = \frac{[P]/K_P + \omega\cdot([A]/K_A)\cdot([P]/K_P)}{1+[A]/K_A + [P]/K_P + \omega\cdot([A]/K_A)\cdot([P]/K_P)}$$

- function of $[A]$ and $[P]$, parameterized by K_A, K_B, ω
- typical parameter range:
  - promoters weak: $[P]/K_P \ll 1$
  - TF concentration: $[A] = 1 \sim 1000$ nM
  - operators tunable: $K_A = 1 \sim 1000$ nM
  - cooperativity weak: $\omega = 10 \sim 100$ (typically ~ 20)



➔want promoter activity as function of $[A]$

"basal level" = $\mathcal{P}_{lo}$

- expected behavior
  - low state: for $[A]=0$, $\mathcal{P} = \frac{[P]/K_P}{1+[P]/K_P} \approx [P]/K_P \ll 1$

$$\Rightarrow \quad \mathcal{P} \approx \mathcal{P}_{lo} \text{ as long as } \omega\cdot[A]/K_A \ll 1$$

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$$\mathcal{P}([A],[P]) = \frac{[P]/K_P + \omega\cdot([A]/K_A)\cdot([P]/K_P)}{1+[A]/K_A + [P]/K_P + \omega\cdot([A]/K_A)\cdot([P]/K_P)}$$

- function of $[A]$ and $[P]$, parameterized by K_A, K_B, ω
- typical parameter range:
  - promoters weak: $[P]/K_P \ll 1$
  - TF concentration: $[A] = 1 \sim 1000$ nM
  - operators tunable: $K_A = 1 \sim 1000$ nM
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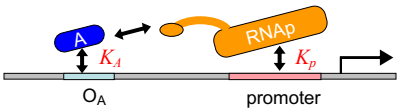

➔want promoter activity as function of $[A]$

"basal level" = $\mathcal{P}_{lo}$

- expected behavior
  - low state: for $[A]=0$, $\mathcal{P} = \frac{[P]/K_P}{1+[P]/K_P} \approx [P]/K_P \ll 1$

$$\Rightarrow \quad \mathcal{P} \approx \mathcal{P}_{lo} \text{ as long as } \omega\cdot[A]/K_A \ll 1$$

  - high state: for $[A] \gg K_A$, can consider A always bound to O_A

$$\Rightarrow \quad \mathcal{P}_{hi} \approx \frac{\omega\cdot[P]/K_P}{1+\omega\cdot[P]/K_P} \le 1$$

  - maximal fold-change ("capacity"): $\mathcal{P}_{hi}/\mathcal{P}_{lo} \approx \omega\cdot\frac{1+[P]/K_P}{1+\omega\cdot[P]/K_P} \le \omega$

➔ for maximal control, want weak promoter such that $\omega\cdot[P]/K_P \ll 1$

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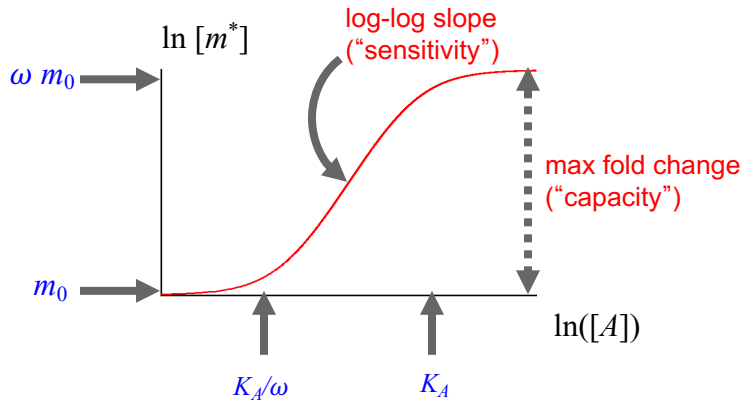
take $\omega[P]/K_P \ll 1$ from here on, then $\mathcal{P} \approx \frac{[P]}{K_P} \cdot \frac{1+\omega\cdot[A]/K_A}{1+[A]/K_A}$

$\Rightarrow \quad [m^*] = \alpha\cdot\mathcal{P}/\beta \approx m_0 \cdot \frac{1+\omega\cdot[A]/K_A}{1+[A]/K_A}, \qquad m_0 \equiv \frac{\alpha\,[P]}{\beta\,K_P}$



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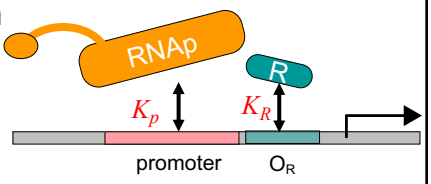
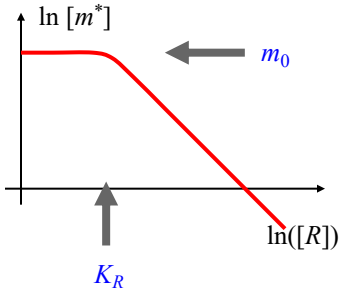
3. Repression by promoter occlusion

$W(\sigma_R = 1, \sigma_P = 0) = [R]/K_R,$
$W(\sigma_R = 0, \sigma_P = 1) = [P]/K_P,$
$W(\sigma_R = 1, \sigma_P = 1) = 0$



[promoter and O_R cannot be simultaneously occupied]

$$\mathcal{P} = \frac{W(0,1)+W(1,1)}{W(0,0)+W(0,1)+W(1,0)+W(1,1)} = \frac{[P]/K_P}{1+[P]/K_P+[R]/K_R} \propto \frac{1}{1+[R]/K_R}$$



-- large $[R]$ can provide arbitrarily strong repression according to model
-- "promoter leakage" provides the lower limit on $[m^*]$
-- high TF conc often generate toxic side effects

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4. Activation by catalysis (rather than recruitment)

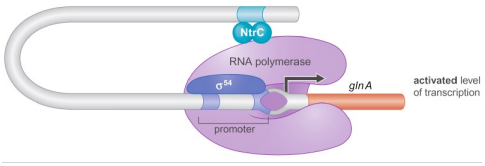
$$\underbrace{\text{RNAp} + \text{promoter}}_{\text{“}\mathcal{P}\text{”}} \underset{}{\overset{K_P}{\rightleftharpoons}} \text{RNAp} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAp} + \text{promoter} + \underbrace{mRNA}_{\text{“}m\text{”}}$$

- mRNA level: $\frac{d}{dt}[m] = \alpha \cdot \mathcal{P} - \beta \cdot [m]$

  tsx init rate ↑ ↑ probability of promoter occupation by RNAp; ↓ mRNA degradation

- steady-state mRNA level (measurable): $[m^*] = \alpha \cdot \mathcal{P} / \beta$

for σ54 promoters, the rate of promoter opening catalyzed by activator



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4. Activation by catalysis (rather than recruitment)

$$\underbrace{\text{RNAp} + \text{promoter}}_{\text{“}\mathcal{P}\text{”}} \underset{}{\overset{K_P}{\rightleftharpoons}} \text{RNAp} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAp} + \text{promoter} + \underbrace{mRNA}_{\text{“}m\text{”}}$$

- mRNA level: $\frac{d}{dt}[m] = \alpha \cdot \mathcal{P} - \beta \cdot [m]$

  tsx init rate ↑ ↑ probability of promoter occupation by RNAp; ↓ mRNA degradation

- steady-state mRNA level (measurable): $[m^*] = \alpha \cdot \mathcal{P} / \beta$

for σ54 promoters, the rate of promoter opening catalyzed by activator

model: $\alpha \Rightarrow \alpha_{\sigma_A}$

$$\alpha \cdot \mathcal{P} \Rightarrow \sum_{\sigma_A} \alpha_{\sigma_A} \cdot W(\sigma_A, \sigma_P = 1) \Big/ \sum_{\sigma_A, \sigma_P} W(\sigma_A, \sigma_P)$$

$$\Rightarrow \quad [m^*] \approx m_0 \cdot \frac{1 + \frac{\alpha_1}{\alpha_0} \cdot \omega \cdot [A]/K_A}{1 + [A]/K_A}, \qquad m_0 \equiv \frac{\alpha_0}{\beta} \frac{[P]}{K_P}$$

➔ same form as recruitment, but capacity increased by α_1/α_0
➔ large fold change, but dedicated components

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- "Advantages of the σ^{54} system:
  - very low basal rate for small α_0
    (activators need to consume ATP to catalyze open complex)
  - large capacity w/o need for large ω
    (recall also that very large ω can reduce capacity)
  - can activate from a long distance away (via DNA looping -- later)
- but in most bacteria species, there is at most one σ^{54} factor
  (compared to many families of σ^{70} factors)
- possible disadvantages?
  long distance activation can create unintentional cross talk unless
  different promoters are kept far apart (require long chromosomes)
  or separated by "insulating elements" (not available for prokaryotes)

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5. Induction of TF

$$X + I \underset{k_-}{\overset{k_+}{\rightleftharpoons}} XI$$

dissociation constant $K_I \equiv \frac{[X]\cdot[I]}{[XI]} = \frac{k_-}{k_+}$

$[X]_{tot} = [X] + [XI]$

$$[XI] = [X]_{tot}\frac{[I]}{[I]+K_I} \approx [X]_{tot}\frac{[I]_{tot}}{[I]_{tot}+K_I}$$

usually $[I]_{tot} \gg [X]_{tot}$, so $[I] \approx [I]_{tot}$

will drop the subscript "tot" from here on

"activated TF" X^* = form of TF able to bind specifically to DNA
or able to activate RNAp

if $X^* = XI$, then $[X^*] = [X]_{tot}\frac{[I]}{[I]+K_I}$

if $X^* = X$, then $[X^*] = [X]_{tot}\frac{K_I}{[I]+K_I}$

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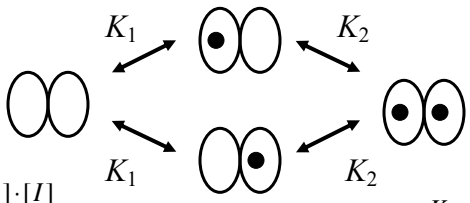
often TF are dimers (X_2)



$$K_1 \equiv \frac{[X_2]\cdot[I]}{[X_2 I]} \qquad K_2 \equiv \frac{[X_2 I]\cdot[I]}{[X_2 I_2]}$$

$$[X_2]_{tot} = [X_2]\cdot\left(1 + 2\frac{[I]}{K_1} + \frac{[I]^2}{K_1 K_2}\right)$$

- non-cooperative ($K_1 = K_2$): $[X_2] = [X_2]_{tot} \Big/ \left(1 + \frac{[I]}{K_1}\right)^2$
- strongly cooperative ($K_2 \ll K_1$): $[X_2] \approx [X_2]_{tot} \Big/ \left(1 + \frac{[I]^2}{K_1 K_2}\right)$
  (e.g., binding of 2nd molecule much easier after 1st is bound)

Hill function

➔ active TF could be X_2, $X_2 I$, or $X_2 I_2$

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