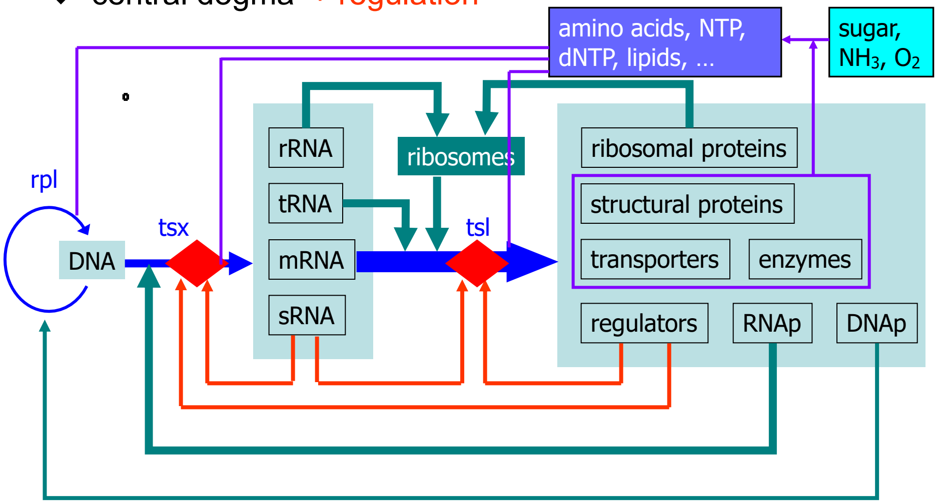
❖ central dogma + regulation



amino acids, NTP, dNTP, lipids, …

sugar, NH$_3$, O$_2$

rpl

DNA

tsx

rRNA

tRNA

mRNA

sRNA

ribosomes

tsl

ribosomal proteins

structural proteins

transporters

enzymes

regulators

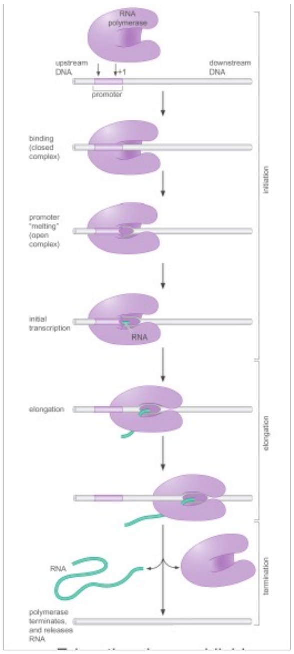
RNAp

DNAp

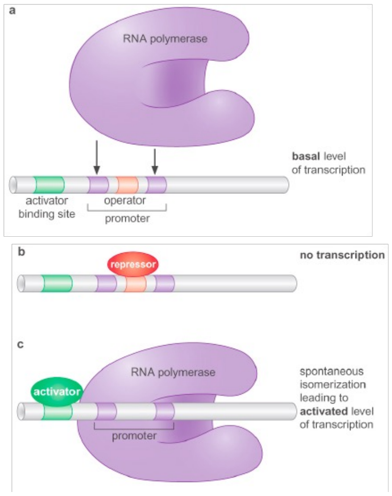
- tsx initiation control by transcription factors (TF)
- tsl initiation control by sRNA and RNA-binding proteins
- tsx termination control by sRNA and anti-terminators
- control of mRNA and protein degradation

coupled to environmental signals

transcriptional initiation and termination



tsx init control by activators, repressors

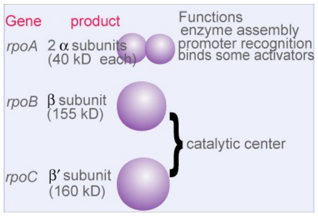


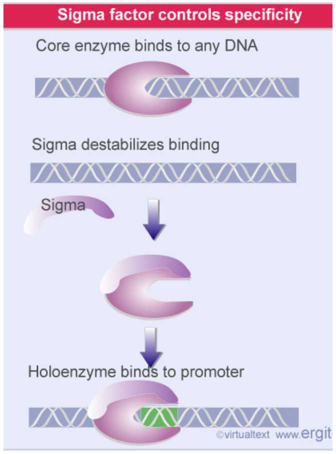
Topic 2: Transcription Initiation Control

A. Mechanisms of tsx initiation in bacteria

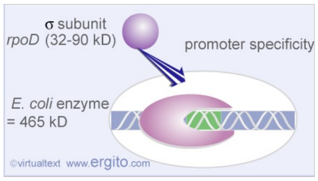
1. Components:

- core enzymes of RNA polymerase:



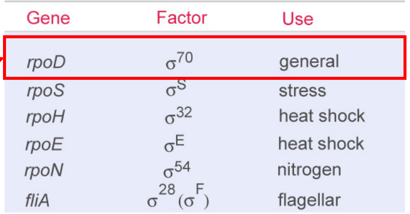


- sigma factor:



- *E. coli* has 6 different σ-factors

70-80% of genes; further regulated by TFs

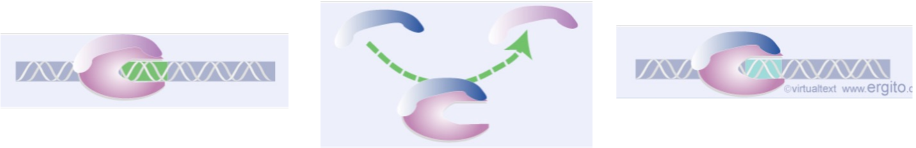


Gene	Factor	Use
rpoD	σ^{70}	general
rpoS	σ^{S}	stress
rpoH	σ^{32}	heat shock
rpoE	σ^{E}	heat shock
rpoN	σ^{54}	nitrogen
fliA	σ^{28} (σ^{F})	flagellar

- core promoter recognition sequences

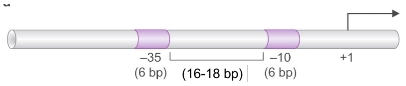
Factor	-35 Sequence	Separation	-10 Sequence
σ^{70}	TTGACA	16-18 bp	TATAAT
σ^{32}	CCCTTGAA	13-15 bp	CCCGATNT
σ^{54}	CTGGNA	6 bp	TTGCA
σ^{28} (σ^{F})	CTAAA	15 bp	GCCGATAA

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

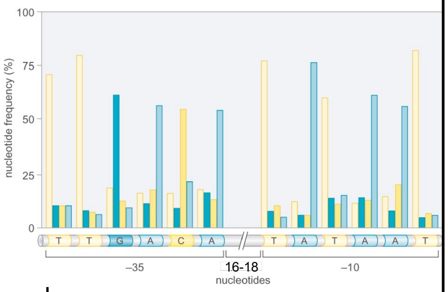


- *B. subtilis* has ~20 σ-factors (include sporulation, competence, …)
- generally, more complex the life style of organism, more sigma factors

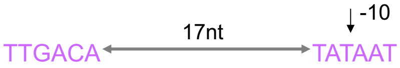
- core promoter recognized by σ^{70}-factor:



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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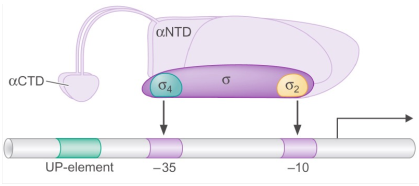
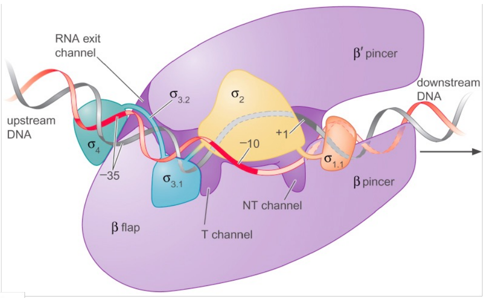
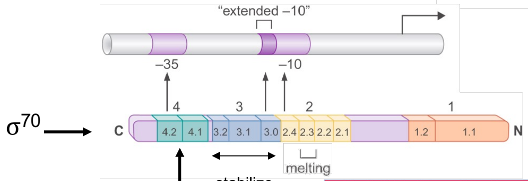
2. RNAp-promoter interaction

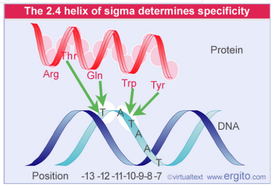


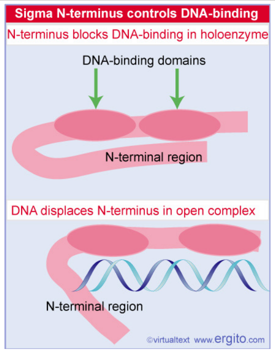




stabilize
open
complex

bends DNA at -35;
facilitates interaction
with upstream activators


The 2.4 helix of sigma determines specificity


Sigma N-terminus controls DNA-binding

N-terminus blocks DNA-binding in holoenzyme

DNA displaces N-terminus in open complex

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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)

$$\mathrm{R} + \mathrm{P} \underset{}{\overset{K_1}{\rightleftharpoons}} \mathrm{I}_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{I}_2 \overset{K_3}{\rightleftharpoons} \mathrm{RP_O}$$

(rapid equilibria)

(slow)

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

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

– opportunity for regulation, e.g., boost promoter binding probability

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

– fast end need not be faster
[cf: search kinetics]

– another opportunity for regulation