

Gene expression in bacteria (Chapter 2)

Note: I am assuming that you remember the central dogma of molecular biology (DNA to RNA to protein), the basics of expression that is common to bacteria and eukaryotes (e.g., mRNA, rRNA, tRNA, etc.), genetic code, and basics of protein biochemistry.

I. Transcription

- A. RNA polymerase: $\alpha_2\beta\beta'\omega$ (core) + σ (holoenzyme)
 - 1. σ - binding of the core enzyme to the promoter (there are several σ s)
 - 2. ω - assembly of the polymerase
 - 3. no primer needed, has helicase activity
- B. DNA template strand – transcribed
- C. DNA coding strand – same as mRNA

- D. gene structure for σ^{70} -expressed genes: (Fig. 2.6)
-35 xx -10 xx (transcription start site) xx ATG start codon
TTGACA TATAAT A/G

- E. promoter strength – how efficiently RNA polymerase binds and initiates
 - 1. affected by consensus, presence or absence of upstream sequences

- F. steps of transcription (Fig. 2.8)
 - 1. core + σ = holoenzyme
 - 2. promoter recognition and binding (closed complex)
 - 3. isomerization (open complex)
 - 4. initiation (abort vs. σ escape)
 - 5. elongation
 - a. 17-bp transcription bubble
 - b. 8-9-bp RNA-DNA heteroduplex
 - c. rate = 30-100 bp/sec
 - d. pausing and backtracking
 - 6. termination

II. Termination

- A. **factor independent** (main) inverted repeat + polyA (Fig. 2.18)
 - 1. hairpin from inverted repeat
 - 2. weak duplex at A-U
- B. **factor-dependent (Rho)** (Fig. 2.19)
 - 1. untranslated mRNA (naked)
 - 2. exposes ill-defined terminator (C-rich)
 - 3. Rho complex binds
 - 4. ATP moves Rho toward polymerase
 - 5. requires pausing
 - 6. ancillary and not as strong as factor dependent

III. mRNA half-life

- A. usually 1-20 min (short compared with eukaryotes)
- B. regulated by RNases
- C. 3' end protected by terminator secondary structure

IV. Translation (page 89)

A. ribosomes

1. thousands of ribosomes/cell (varies with growth rate)
2. structure
 - a. large subunit (23S rRNA + 5S rRNA) and small subunit (16S rRNA)
 - b. numerous proteins
 - c. 23S rRNA is ribozyme that catalyzes peptidyl transfer
 - d. 16S rRNA initiation

B. initiation

1. Translation Initiation Region (TIR) = Ribosome Binding Site (RBS)/Shine-Delgarno Sequence (Fig. 2.30)
 - a. complementary to 16S rRNA
 - b. AGGAGGU 5-10 bases from ATG
 - c. some genes lack RBS – use ATG at 5' end of mRNA?
2. ATG start codon (others are rare [UUG, AUA])
3. fMet-tRNA^{Met} + mRNA + 30S ribosome +IF1-3 + 50S ribosome (Fig. 2.32)
 - a. formyl group usually cleaved off initial Met
 - b. initial Met usually leaved off peptide

C. elongation (Fig. 2.27)

1. amino acyl-tRNA + EF-Tu + EF-Ts + EF-G
2. 23S rRNA main catalytic player
3. codon usage issues based on GC content and redundancy of code

D. Termination

1. stop codons (UAA, UAG, UGA)
2. release factors

E. Polycistronic mRNAs

1. from same promoter (same operon)
2. internal RBS/TIR required
3. translational coupling
4. polarity
 - a. insertion of terminators
 - b. nonsense mutations + Rho

F. Protein folding

1. important for final function of protein and to help with localization
 - a. chaperones – help other proteins fold (general and specific)
 - b. chaperonins – macromolecular help refold improperly folded proteins (GroEL)
2. DnaK/Hsp70 - general
 - a. heat shock protein
 - b. cellular thermometer
 - c. also directs to degradation
 - d. highly conserved
3. others
 - a. trigger factor - prolyl isomerase
 - b. Clp proteases and chaperones