Transcription in Prokaryotes

Jörg Bungert, PhD
Phone: 352-273-8098
Email: jbungert@ufl.edu
Objectives

- Understand the basic mechanism of transcription.
- Know the function of promoter elements and associating proteins.
- Know the transcription cycle: initiation, elongation, termination.
- Know and understand the regulation of the lac-operon.
- Understand regulation by the PhoR/PhoB two-component system in *E.coli*.
- Know and understand the mechanism of antitermination.

Reading: Lodish 7th edition, chapter 7 (pp. 282-288).
Basic Principles of Transcription

(a)
Mechanisms of bacterial transcription initiation

A. RNA polymerase
   1. Synthesizes RNA from one strand of a double-stranded DNA template
   2. In E. coli, a single RNA pol synthesizes most, if not all mRNA, rRNA, tRNA
   3. RNA pol holoenzyme: 4 subunits: α, β, β’, σ
      a. Core enzyme (α, β, β’): synthesizes RNA randomly on ds DNA templates
      b. σ factor: confers promoter binding and specificity
   4. σ factor(s)
      a. Most E. coli promoters bound by σ^{70} – associated RNA pol
      b. Allows correct binding and transcription initiation at specific promoters
      c. Sigma released when nascent RNA is released upon transcription initiation; core enzyme continues transcription elongation
      d. Alternative sigma factors recognize different promoters
Overview: a transcription unit is a sequence of DNA transcribed into a single RNA, starting at the promoter and ending at the terminator.

During transcription, the bubble is maintained within bacterial RNA polymerase, which unwinds and rewinds DNA, maintains the conditions of the partner and template DNA strands, and synthesizes RNA.

- **Promoter**
- **proximal**
- **distal**
- **Terminator**
- **Upstream**
- **Downstream**
- **DNA coding strand**
- **Rewinding point**
- **Unwinding point**
- **DNA template strand**
- **RNA binding site**
- **RNA-DNA hybrid**
- Enzyme movement
The core RNA polymerase (alpha, beta) associate with the sigma factor (mostly sigma 70) to generate the RNA polymerase holoenzyme. The sigma factor is required for recruiting the RNA polymerase to the promoter. The active site contains a magnesium ion that is required for the catalytic activity.
Model of elongating RNA polymerase
B. Promoters and promoter complexes

1. Promoter – DNA sequence that binds RNA polymerase to initiate transcription
2. Transcription initiation – synthesis of first phosphodiester bond in nascent RNA
3. Position +1 – position of nucleotide in DNA template that encodes the first nucleotide of mRNA
4. Typical prokaryotic promoters recognized by E. coli $\sigma^{70}$ – RNA pol share important pol recognition sequences
   a. -10 region (Pribnow box): TATAAT consensus sequence
   b. -35 region: TTGACA consensus sequence
   c. different promoters have similar, but not identical -10 and -35 region sequences
   d. mutations within these regions alter promoter strength & function
   e. distance between -10 and -35 regions important
   f. strength of promoter mostly determined by affinity of RNA pol for promoter DNA sequences
   g. region unwound by pol appears to be between -9 and +3 (includes right end of -10 seq. and extending to just downstream of transcription initiation site)
5. Synthesis of RNA in 5’ -> 3’ direction; nucleotides added to 3’ end from ribonucleotide triphosphate precursors
Basal Promoter Elements

Promoter: The combination of DNA sequence elements required for the recruitment of RNA polymerase

-35 and -10 elements are recognized by sigma factor

UP element: AT rich element that interacts with C-terminal domain of the alpha subunit of RNA polymerase
RNA Polymerase Subunits and Promoter Recognition

The sigma subunit interacts with the -10 and -35 region, the alpha subunits contact the AT-rich UP element.
Not all RNA polymerase complexes transcribe until the end of the gene. Many transcription complexes dissociate from the template after adding a couple of rNTPs, a process called abortive transcription.
Elongation, Termination

A. Elongation
   1. Rate of elongation with E. coli RNA pol = ~40 nucleotides/sec.; T3 RNA pol = ~200 nucleotides/sec.
   2. E. coli RNA pol covers (footprints) ~28-35 bp of DNA during elongation
   3. Mechanism for overcoming “stalled” polymerase during elongation

B. Termination in prokaryotes
   1. Dependent on specific DNA sequences (terminator)
   2. Transcription complex dissociates and RNA pol and nascent RNA released
   3. Rho dependent termination –
      a. requires termination factor (protein) rho
      b. Mechanism not fully understood (see model)
   4. Rho independent termination - Involves formation of stem-loop secondary structure encoded by DNA template and formed by nascent RNA (see figure)
Pyrophosphorolytic Editing: The polymerase backtracks and removes an incorrectly inserted ribonucleotide by reincorporation of PPI.

Hydrolytic editing: The polymerase backtracks and cleaves the RNA, removing error-containing sequence. The process is stimulated by Gre factors, which also function as elongation stimulators.
**TERMINATION**

At transcription stop site, polymerase releases completed RNA and dissociates from DNA.

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**Rho-independent termination**: intrinsic terminators consist of a short inverted repeat (about 20 nucleotides) followed by a stretch of 8 A:T base pairs. The resulting RNA forms a stem-loop structure, which disrupts the elongation complex. A stretch of A:U base pairs in the DNA/RNA hybrid are weaker than other base pairs and are more easily disrupted as a consequence of stem loop formation.

**Rho dependent termination**: terminators are not characterized by specific RNA elements that fold in secondary structure. The Rho factor is recruited to rut sites (Rho utilization) on the single stranded RNA.
Rho Independent Termination

**Figure 9.27** Intrinsic terminators include palindromic regions that form hairpins varying in length from 7 to 20 bp. The stem-loop structure includes a G-C-rich region and is followed by a run of U residues.

**Figure 12-1** Rho-independent termination of transcription in *E. coli*. The sequence of mRNA synthesized near a termination site (t) contains a string of U residues preceded by a G/C-rich region with dyad symmetry (red boxes). The bases of the symmetrical region of the mRNA form a stem-loop by base pairing. This structure interacts with RNA polymerase and causes it to pause during elongation. This pausing coupled with the weak base pairing of rU-dA base pairs at the termination site displaces the mRNA chain and signals the polymerase to release from the template. [See T. Platt, 1981, *Cell* 24:10.]
Model for rho Dependent Termination

Rho binds as a hexameric protein complex to specific sequences called RUT (rho utilization) sites. The complex also binds ATP and moves along the RNA ultimately disrupting the interactions between the RNA polymerase and the RNA.

Banerjee et al., Journal of Microbiology, 2007
Prokaryotic Transcriptional Regulation

A. Lac operon

A. Trp operon

A. Gln regulation by NTRC – “enhancer” function
The Lac-Operon

$\text{inducer}$

$\text{repressor}$

$\text{lacZ}$

$\text{lacY}$

$\text{lacA}$

$\beta$-galactosidase

transacetylase

$\text{P2} \rightarrow \text{P1}$

$\text{O3} \rightarrow \text{A} \rightarrow \text{O1} \rightarrow \text{O2}$

$\text{CAP-eAMP}$

$\text{+1 (transcription start site)}$

Promoter

$\text{CAP site}$

Operator

$\text{lacZ}$

$E. \text{coli lac}$ transcription-control genes
The -35 region of the lac operon is not optimal for Pol binding. CAP helps polymerase bind to the promoter by interacting with the C-terminal domain of the alpha subunit. In the absence of lactose, a tetramer of lac repressor binds two operators.

CAP: Catabolite Activator Protein
also known as CRP (cAMP receptor protein)
After entering the cell lactose is converted to allolactose, mediated by β-galactosidase, which is also encoded by the lac-operon. Allolactose binds the lac repressor and causes an allosteric change resulting in loss of DNA binding activity. Glucose lowers the levels of cAMP, which is an allosteric effector of CAP. CAP does not interact with its binding site and the polymerase is recruited with low affinity leading to low levels of transcription.
What happens in the presence of glucose (with or w/out lactose)?

E. coli prefers to use glucose over other sugars for energy and as a carbon source.

In the presence of both glucose and lactose, glucose is preferentially metabolized and proteins encoded by the lac operon are synthesized at very low levels.

Therefore, another regulatory signal must be required to monitor glucose levels and activate the lac operon (in addition to inactivation of the lac repressor by allolactose).

*Catabolite repression:*

A breakdown product (catabolite) of glucose modulates intracellular levels of cAMP:
- High glucose levels lower cAMP levels
- Low glucose levels increase cAMP levels

CAMP binds to CAP (catabolite gene activator protein) and cAMP-CAP complex activates transcription of the lac operon by binding to the lac promoter region.

CAP alone (w/out bound cAMP) will not stimulate transcription.

CAP-cAMP binds to lac operon just upstream from RNA pol binding site in promoter.

CAP-cAMP binding to adjacent DNA seq. facilitates RNA pol binding to promoter, thereby stimulating rate of transcription initiation.
Regulation of the lac-operon

In the absence of glucose the cAMP concentration is high, cAMP binds to CAP, CAP binds to its recognition site and enhances the recruitment of RNA polymerase leading to high level transcription.
The Lac Operator and the Lac Repressor

Lac Repressor interacts with the DNA via a helix-turn-helix motif
cAMP induced binding of CAP to DNA

CAP binds to DNA via a helix-turn helix motif. It binds as a dimer. cAMP induces a conformation change that allows DNA binding.
The PhoR/PhoB two-component regulatory system
Regulation of Gene Expression in the trp Operon

Transcription attenuation model of the trp operon. When tryptophan is limiting (−tryptophan) TRAP is not activated. During transcription, antiterminator formation (A and B) prevents formation of the terminator (C and D), which results in transcription of the trp operon structural genes. When tryptophan is in excess (+tryptophan) TRAP is activated. Tryptophan-activated TRAP can bind to the (G/U)AG repeats and promote termination by preventing antiterminator formation. The overlap between the antiterminator and terminator structures is shown. Numbering is from the start of transcription.

Babitzke and Gollnick, J. Bacteriology, 2001
Regulation of Gene Expression by NtrC

NtrC control expression of genes involved in nitrogen metabolism. At low nitrogen levels NtrC binds to DNA and activates transcription. In case of the glnA gene NtrC regulates the transition from a closed to open transcription complex, an example of allostery. NtrC interacts with a specialized sigma factor (sigma 54) which directs the RNA polymerase to a specific set of genes containing variations in the consensus promoter sequence.