

vi. Gene expression and regulation in prokaryotes

vi. Gene expression and regulation in prokaryotes, structure of prokaryotic gene, structure and functions of RNA polymerase and its subunits

Gene Expression and Regulation in Prokaryotes

1. General Principles of Prokaryotic Gene Regulation

- **Operon Concept:** In prokaryotes (e.g., *E. coli*), genes with related functions are often organized into operons—clusters of genes under the control of a single promoter and regulated by a common operator region.
- **Coupled Transcription and Translation:** Bacterial transcription and translation can occur simultaneously in the cytoplasm, as there is no nucleus. Regulation at the transcriptional level is therefore crucial for controlling protein levels.
- **Negative and Positive Control:** Regulatory proteins can **repress** or **activate** transcription:
 - **Repressors** bind to operator sequences and prevent RNA polymerase from initiating transcription.
 - **Activators** enhance the binding or activity of RNA polymerase, increasing gene expression.
- **Sigma Factor Specificity:** Bacteria utilize different sigma (σ) factors that recognize different promoter consensus sequences, enabling rapid reprogramming of the transcriptome under various environmental conditions (e.g., heat shock, nitrogen starvation).

2. Classic Examples of Prokaryotic Gene Regulation

1. lac Operon (Negative and Inducible Control)

- **Genes:** *lacZ*, *lacY*, *lacA* for lactose metabolism.
- **Regulation:**
 1. A **repressor** (LacI) binds the operator in the absence of lactose, blocking transcription.
 2. When lactose (or allolactose) is present, it inactivates the repressor, allowing expression of the operon.
- **CAP-cAMP** system exemplifies **positive regulation**: Low glucose → High cAMP → CAP-cAMP complex binds the promoter → Enhances RNA polymerase recruitment.

2. trp Operon (Negative and Repressible Control)

- **Genes:** *trpE*, *trpD*, *trpC*, *trpB*, *trpA* for tryptophan biosynthesis.
- **Regulation:** When tryptophan levels are high, tryptophan binds to the TrpR repressor, enabling it to bind the operator and shut off operon transcription.

3. Attenuation

- In some biosynthetic operons (e.g., *trp* operon), transcription can be terminated prematurely through a mechanism called **attenuation**, which depends on coupled transcription-translation and formation of secondary structures in the nascent RNA.

Structure of a Prokaryotic Gene

Although gene organization can vary, a typical **bacterial gene** (or operon) can be dissected as follows:

1. Promoter Region

- **Core Promoter Elements:**
 - – 35 Element: Usually **TTGACA** (consensus).
 - – 10 Element (Pribnow Box): Typically **TATAAT** (consensus).
- These motifs are recognized by the **sigma subunit** (σ^{70} in *E. coli* for “housekeeping” genes).

2. Operator (Regulatory) Sequences

- Often located near or overlapping the promoter.
- Binding sites for **repressor** or **activator** proteins that modulate RNA polymerase access to the DNA.

3. Coding Sequence (Structural Genes)

- Can be **monocistronic** (one gene per transcript) or **polycistronic** (multiple genes under one promoter, typical of bacterial operons).

4. Terminator

- **Rho-Independent Terminator:** An intrinsic hairpin-loop structure in the nascent RNA followed by a stretch of U residues leads to transcription termination.
- **Rho-Dependent Terminator:** Involves the rho protein (an RNA helicase) that terminates transcription when it catches up with the polymerase at a specific rut site on the mRNA.

Structure and Functions of RNA Polymerase and Its Subunits in Prokaryotes

Overview of Prokaryotic (Bacterial) RNA Polymerase

In *E. coli*, the **holoenzyme** that initiates transcription comprises multiple subunits. The “core enzyme” is responsible for the catalytic activity, but the presence of a **sigma factor** confers specificity to particular promoter sequences.

1. **Core Enzyme:** $\alpha 2 \beta \beta' (\omega)$
2. **Holoenzyme:** $\alpha 2 \beta \beta' \sigma (\omega)$

Here, ω (omega) is sometimes considered part of the core, though its role is less critical in basic catalysis (it aids in proper core assembly and stability). When the sigma subunit (σ) is bound to the core enzyme, the complete assembly is termed the **RNA polymerase holoenzyme**.

Subunits of Bacterial RNA Polymerase and Their Functions

1. **α (alpha) Subunits** (Two Copies)
 - **Gene:** *rpoA* in *E. coli*.
 - **Function:**
 - Involved in enzyme assembly (dimerization creates a platform for β and β' binding).
 - Important for interaction with transcriptional activators or upstream promoter elements (UP elements).
 - Play a role in the regulation of transcription by forming contacts with regulatory proteins.
2. **β (beta) Subunit**
 - **Gene:** *rpoB*.
 - **Function:**
 - Primary site for ribonucleoside triphosphate (rNTP) incorporation.
 - Contains a portion of the catalytic center responsible for polymerization activity.
3. **β' (beta prime) Subunit**
 - **Gene:** *rpoC*.
 - **Function:**
 - Provides essential residues for binding the DNA template.
 - Completes the catalytic center with the β subunit.
4. **ω (omega) Subunit**
 - **Gene:** *rpoZ*.
 - **Function:**
 - Facilitates the correct folding and assembly of the β subunit, stabilizing the RNA polymerase core.
 - Not strictly required for transcription in all conditions, but contributes to the integrity and robustness of the enzyme.
5. **σ (sigma) Factor**
 - **Gene:** *rpoD* for σ^{70} (the major housekeeping sigma factor in *E. coli*), though many alternative sigma factors exist (σ^{32} , σ^{54} , σ^{38} etc.) for specific stress or developmental conditions.
 - **Function:**
 - Recognizes promoter elements (– 35 and – 10 regions).
 - Positions the core enzyme at the transcription start site.
 - Dissociates after the initiation phase, leaving the core enzyme to elongate the RNA transcript.

Steps in Prokaryotic Transcription

1. Initiation

- Holoenzyme ($\alpha 2\beta\beta'\omega + \sigma$) binds to the promoter (– 35 and – 10 regions).
- DNA around the start site unwinds, forming the **open complex**.
- First few ribonucleotides are polymerized; once short transcripts of ~9 –10 nt are synthesized successfully, σ factor often dissociates.

2. Elongation

- Core enzyme ($\alpha 2\beta\beta'\omega$) continues RNA synthesis in the 5' → 3' direction.
- RNA polymerase unwinds DNA ahead of it and reanneals it behind, forming a transient “transcription bubble.”

3. Termination

- **Rho-independent:** Hairpin structure in the RNA + downstream U-tract leads to polymerase pausing and dissociation.
- **Rho-dependent:** Rho protein binds the rut site on RNA and travels toward polymerase, causing release when it catches the elongation complex.

Concluding Remarks

In **prokaryotes**, gene expression is intimately tied to the organization of the **operon**, the presence of specialized **regulatory sequences** (promoters, operators), and the dynamic function of **repressors/ activators**. The foundational enzyme driving transcription is the **RNA polymerase holoenzyme**, where the **core subunits ($\alpha 2\beta\beta'\omega$)** confer catalytic capabilities and the **sigma factor (σ)** provides promoter specificity. This mechanism allows bacteria to efficiently modulate gene expression in response to changing environmental conditions, employing strategies ranging from negative/positive regulatory loops (e.g., lac and trp operons) to the usage of alternative sigma factors for specialized genes.

This fundamental understanding of **prokaryotic transcription** sets the stage for exploring more complex eukaryotic regulatory networks, but even at the bacterial level, the versatility and adaptability of transcription regulation are central to survival, pathogenicity, and biotechnological applications.