# Regulation of Gene Expression

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## I. Overview

- Gene expression: Multistep process producing functional RNA or protein.
- Primary regulation: Transcription (DNA → RNA) in both prokaryotes & eukaryotes.
- Eukaryotes: Additional regulation via posttranscriptional, posttranslational processes, and DNA accessibility.
- Types of genes:
    - Constitutive/housekeeping genes: Constant expression; needed for basic cellular functions.
    - Regulated genes: Expressed under specific conditions; may be cell-type specific (e.g., fibrinogen alpha chain in hepatocytes).
- Importance: Allows cellular differentiation, morphogenesis, and adaptability.

- Prokaryotes vs eukaryotes: Control mechanisms best understood in prokaryotes; many principles repeat in eukaryotes.

## II. Regulatory Sequences and Molecules

- Cis-acting elements: DNA sequences on the same chromosome influencing gene expression (e.g., promoters, operators).

- Trans-acting factors: Proteins or molecules that can diffuse and regulate genes on any chromosome (e.g., transcription factors).

- Mechanism: Regulatory proteins bind DNA via structural motifs:

    - Zinc finger
    - Leucine zipper
    - Helix-turn-helix

## III. Regulation of Prokaryotic Gene Expression

- Primary control: At transcription level.

- Efficiency: Regulating the first step prevents waste of

energy.

- Methods: Initiation control or premature transcription termination.

### A. mRNA Transcription from Operons

- Operon: Cluster of structural genes + regulatory sequences controlling proteins in a metabolic pathway.

- Polycistronic mRNA: Single mRNA encodes multiple proteins; allows coordinated regulation.

### B. Operators in Operons

- Operator: DNA segment where a repressor protein binds to block transcription.

    - Repressor bound: RNA polymerase cannot transcribe → no mRNA/protein produced.
    - Inducer present: Binds repressor → repressor releases operator → RNA polymerase initiates transcription.

- Example: Lactose (lac) operon – demonstrates both negative and positive regulation.

## C. Lac Operon

- Structural genes:
    - lacZ: β-galactosidase → lactose → glucose + galactose.
    - lacY: Permease → lactose transport into cell.
    - lacA: Thiogalactoside transacetylase → acetylates lactose (function unclear).
- Regulation: Maximally expressed only when lactose is present & glucose absent.
- Regulatory elements:
    - Promoter: RNA polymerase binds.
    - Operator (O): Repressor binds here.
    - CAP site: Activated by cAMP-CAP complex → enhances transcription.
- Repressor: LacI gene (trans-acting factor) binds operator with high affinity; LacI has its own promoter and is separate from the operon.

✅ Exam Points:

- Prokaryotic operons = polycistronic mRNA + cis-regulatory sequences + trans-acting factors.

- Lac operon: classic negative (repressor) + positive (CAP-cAMP) regulation example.

- Glucose preference → low cAMP → CAP inactive → lac operon not expressed.

### C. Lac Operon Regulation

#### 1. Only glucose available

- Lac operon repressed (turned off).

- Mechanism:

    - Repressor protein binds operator (O site) via helix-turn-helix motif.
    - RNA polymerase cannot bind promoter → transcription inhibited.

- Type of regulation: Negative regulation.

#### 2. Only lactose available

- Lac operon induced (maximally expressed).

- Mechanism:
    - Lactose → small amount converted to allolactose (inducer).
    - Allolactose binds repressor → repressor releases operator → RNA pol can transcribe.
    - cAMP–CAP complex binds CAP site (active adenylyl cyclase since glucose absent) → transcription initiation enhanced.
- Type of regulation: Positive regulation.
- Outcome: Single polycistronic mRNA → lacZ, lacY, lacA proteins synthesized.
- Note: lacI gene (repressor) is constitutive, always expressed.

3. Both glucose and lactose available

- Lac operon uninduced, transcription negligible.
- Mechanism:
    - Glucose inhibits adenylyl cyclase → no cAMP →

CAP site empty.
    - RNA pol cannot initiate efficiently even though operator is free.

- Term: Catabolite repression.

- Expression level: Basal (very low).

D. Tryptophan (trp) Operon

- Contains 5 structural genes → enzymes for Trp synthesis.

- Regulation: Negative control by Trp as corepressor + attenuation.

Mechanisms

1. Negative control:

    - Trp binds repressor → repressor binds operator → transcription blocked.

2. Attenuation:

    - Transcription starts but terminates prematurely if

Trp abundant.
    - Attenuator = hairpin (stem-loop) structure in mRNA → stops transcription.
    - If Trp scarce → ribosomes stall at Trp codons → hairpin does not form → transcription continues.

Key difference vs eukaryotes:

- Attenuation possible in prokaryotes because transcription & translation occur simultaneously.

- Eukaryotes have spatial separation → no attenuation.

### E. Coordination of Transcription and Translation

#### 1. Stringent Response

- Triggered by amino acid starvation in E. coli.

- Mechanism:

    - Uncharged tRNA binds ribosome → activates RelA enzyme → synthesizes ppGpp (alarmone).
    - ppGpp binds RNA pol → inhibits rRNA & tRNA synthesis.

    - mRNA for ribosomal proteins also partially inhibited.
    - mRNA for amino acid biosynthesis enzymes not inhibited.

- Purpose: Prevents wasteful ribosome production; prioritizes amino acid synthesis.

2. Regulatory r-proteins

- Excess ribosomal proteins (r-proteins) inhibit their own operons.

- Mechanism:

    - Specific r-protein binds Shine-Dalgarno (SD) sequence → blocks small ribosomal subunit → translation inhibited.
    - r-protein also binds rRNA (higher affinity).
    - Balances rRNA and r-protein synthesis → ensures proper ribosome assembly.

## ✅ Exam Points

- Lac operon: Negative (repressor) + Positive (CAP-cAMP).

- Catabolite repression: Glucose inhibits lac operon via low cAMP.
- Trp operon: Repressible, negative control + attenuation.
- Stringent response: ppGpp → inhibits rRNA synthesis during amino acid scarcity.
- r-protein feedback: Prevents overproduction; coordinates with rRNA.

## IV. Regulation of Eukaryotic Gene Expression

Eukaryotes have complex genomes and a nuclear membrane, requiring multiple regulatory mechanisms.

- Transcription is still the primary site of regulation.
- No operons in eukaryotes → use alternate strategies for coordinated regulation.
- Regulation occurs at multiple levels:
    - Transcriptional → transcription factors + cis-elements
    - Posttranscriptional → mRNA processing & stability

- Translational / Posttranslational → protein stability, processing, targeting

A. Coordinate Regulation

- Needed for simultaneous expression of functionally related genes, often on different chromosomes.

- Mechanism:

  - Specific transcription factor (STF) = trans-acting protein
  - Binds cis-acting regulatory sequences on each gene
  - STF has:
    - DNA-binding domain (DBD) → binds DNA
    - Transcription-activation domain (TAD) → recruits coactivators (e.g., histone acetyltransferases) + general transcription factors → RNA pol binds promoter → transcription

- Combinatorial control: Effect depends on protein composition of the complex

Examples of Coordinate Regulation

1. Galactose circuit (yeast)

    - Genes for galactose metabolism are on different chromosomes.
    - Gal4 (STF) binds UASGal (upstream activating sequence).
    - Gal80 inhibits Gal4 in absence of galactose.
    - Gal3 binds Gal80 when galactose present → Gal4 activates transcription.
    - Glucose inhibits Gal4 expression → prevents galactose use.

2. Hormone response system

    - Hormone response elements (HREs) = DNA sequences that bind trans-acting proteins
    - Hormones: bind intracellular receptors (steroids) or cell-surface receptors (peptides)

    a. Intracellular (nuclear) receptors

    - Examples: steroid hormones, vitamin D, thyroid hormone, retinoic acid
    - Domains: DNA-binding, transcriptional activation, ligand-binding
    - Mechanism: hormone binds receptor →

conformational change → dimerization → binds HRE (e.g., GRE) → recruits coactivators → transcription
- Can activate or repress target genes; allows coordinate expression across chromosomes

b. Cell-surface receptors

- Examples: insulin, glucagon, epinephrine
- Mechanism: hormone binds G-protein-coupled receptor → cAMP → protein kinase A → phosphorylates CREB (STF) → binds CRE (cis-element) → transcription of target genes (e.g., gluconeogenesis enzymes like PEPCK, glucose-6-phosphatase)

B. Messenger RNA Processing and Use

Eukaryotic mRNA undergoes processing before translation:

1. 5′ capping → stability & ribosome recognition
2. 3′ polyadenylation → stability, translation regulation
3. Splicing → remove introns

1. Alternative Splicing

- Generates tissue-specific protein isoforms from same pre-mRNA
- Mechanisms: exon skipping, intron retention, alternative splice donor/acceptor sites
- Example: Tropomyosin (TM) → tissue-specific isoforms
- Fact: >90% of human genes undergo alternative splicing

2. Alternative Polyadenylation (APA)

- Pre-mRNAs may have multiple cleavage/polyadenylation sites
- APA → different 3′ UTRs or coding sequences → affects translation & localization
- Example: IgM → secreted vs membrane-bound forms

Note: Alternative splicing + APA + alternative transcription start sites → ~20,000–25,000 genes → >100,000 proteins

### 3. mRNA Editing

- Base changes after mRNA processing → alters protein sequence
- Example: Apolipoprotein B (apo B)
  - Liver → full-length apo B-100 → VLDL
  - Intestine → cytosine (C) → uracil (U) → stop codon → shorter apo B-48 → chylomicrons

### ✅ Exam Points

- Coordinate regulation: STFs + cis-elements → multiple genes on different chromosomes
- Galactose circuit: Gal4 (activator) + Gal80/Gal3 regulation
- Hormone responses: intracellular (steroids) vs cell-surface (peptides → cAMP → CREB)
- mRNA regulation: alternative splicing, APA, editing → protein diversity

### 4. Messenger RNA (mRNA) Stability

- The lifespan of an mRNA in the cytosol determines how much protein can be produced.

- Key examples: iron metabolism and RNA interference (RNAi).

a. Iron Metabolism

- Transferrin (Tf): plasma protein transporting iron.

- Tf binds transferrin receptors (TfR) → internalization → iron delivery.

- TfR mRNA: contains iron-responsive elements (IREs) in 3′-UTR → stem-loop structures.

- Iron regulatory proteins (IRPs) bind IREs:

    - Low iron: IRPs bind 3′-IRE → stabilize TfR mRNA → more TfR made.
    - High iron: IRPs dissociate → mRNA degraded → TfR decreased.

- Ferritin mRNA: has a 5′-IRE

    - Low iron: IRPs bind → block translation → less ferritin

- High iron: IRPs dissociate → ferritin synthesized to store excess iron

- Heme synthesis enzyme (ALA synthase 2) also contains 5′-IRE → regulated similarly.

b. RNA Interference (RNAi)

- Gene silencing by mRNA degradation or translation repression.

- Mediated by microRNAs (miRNAs) (~22 nt, noncoding).

- Processing steps:

  1. pri-miRNA → pre-miRNA by Drosha (nucleus)

  2. Export to cytoplasm → Dicer → short double-stranded miRNA

  3. Guide strand associates with RISC → hybridizes to 3′-UTR of target mRNA

  4. Outcome: translation repression or degradation (via Argonaute/Slicer)

- siRNA (short interfering RNA) can also trigger RNAi →

therapeutic potential

Example of RNAi therapy:

- Patisiran (2018) → treats hATTR amyloidosis
- siRNA inhibits abnormal TTR protein → prevents amyloid deposition

5. mRNA Translation Regulation

- Phosphorylation of eukaryotic initiation factor eIF-2 → inhibits translation initiation
- Prevents GDP→GTP exchange → blocks translation
- Kinases activated by:
    - Amino acid starvation
    - Heme deficiency in erythroblasts
    - Viral double-stranded RNA
    - Misfolded proteins in rough ER

C. Regulation through DNA Variation

Eukaryotic gene expression is influenced by DNA accessibility, gene copy number, and arrangement.

1. Access to DNA

- DNA + histone & nonhistone proteins → chromatin
- Euchromatin: transcriptionally active, decondensed
- Heterochromatin: transcriptionally inactive, condensed
- Histone modifications (acetylation, methylation, phosphorylation)
    - Reduce positive charge → loosen DNA → allow transcription factor access
- DNA methylation (CpG islands in promoters)
    - Hypomethylation → active genes
    - Hypermethylation → silenced genes
- These are epigenetic changes → heritable, no DNA sequence change

2. Gene Copy Number

- More gene copies → more protein product

- Example: Methotrexate resistance
    - DHFR gene amplification → more enzyme → survival despite drug

3. DNA Arrangement

- Immunoglobulin production in B cells:
    - Heavy/light chains = variable + constant regions
    - Variable region = somatic recombination of V, D, J segments → antibody diversity
- Pathologic rearrangements: chromosome translocations → disease

4. Mobile DNA Elements

- Transposons (Tn): mobile DNA segments
    - Move via transposase:
        - Direct transposition: cut & paste
        - Replicative transposition: copy inserted elsewhere
    - Retrotransposons: RNA intermediate → reverse transcription → new DNA copy

- Significance:
    - Contributes to genome variation
    - Can alter gene expression → cause disease (e.g., hemophilia A, Duchenne muscular dystrophy)
    - In bacteria: Tn on plasmids → antibiotic resistance

## ✅ Exam Points

- mRNA stability: iron metabolism (TfR/ferritin), RNAi (miRNA, siRNA)
- Translation regulation: eIF-2 phosphorylation → environmental stress response
- DNA-level regulation: chromatin accessibility, gene copy number, rearrangement, mobile DNA elements
- Epigenetics: histone modifications + DNA methylation → heritable gene expression control

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