vii. Mechanism of Gene Transcription and Translation

vii. Mechanism of Gene Transcription and Translation, Genetic code, Gene structure, expression and regulation in eukaryotes, RNA polymerases, post-transcriptional modifications and Operon concept

Mechanism of Gene Transcription

Transcription is the process by which RNA is synthesized from a DNA template. In **eukaryotes**, it primarily occurs in the nucleus, whereas in **prokaryotes**, it occurs in the cytoplasm, often coupled with translation.

Eukaryotic Transcription

1. **Initiation**
   - **Chromatin Remodeling**: Transcription factors and chromatin-remodeling complexes (e.g., SWI/SNF) must relax local chromatin structure, allowing promoter access.
   - **Promoter Elements**: Common motifs include the **TATA box** (~25–30 bp upstream of start site), **BRE** (TFIIB recognition element), **INR** (initiator element), and **DPE** (downstream promoter element).
   - **Pre-Initiation Complex (PIC)**: General transcription factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH) sequentially assemble with **RNA polymerase II** at the core promoter, forming a stable platform for transcription initiation. TFIIH also has **helicase** and **kinase** activities, unwinding DNA and phosphorylating the C-terminal domain (CTD) of RNA Pol II.
2. **Elongation**
   - RNA Pol II leaves the promoter region once the CTD is sufficiently phosphorylated (particularly on Ser5 and Ser2 of the heptad repeats).
   - DNA is unwound ahead, forming an 8–9 bp RNA–DNA hybrid within the active site.
   - **Elongation Factors** (e.g., DSIF, NELF, P-TEFb) modulate Pol II processivity, relieving promoter-proximal pausing.
3. **Termination**
   - Eukaryotic termination is tied to **polyadenylation signals** in the pre-mRNA. Once RNA Pol II transcribes the poly(A) signal (AAUAAA), cleavage factors cut the nascent transcript, and an exonuclease (e.g., Xrn2) helps dislodge Pol II from the template.

Prokaryotic Transcription (Brief Contrast)

- In **bacteria**, a single RNA polymerase holoenzyme (α_2ββ′ω + σ) carries out transcription.
- **Sigma Factor (σ\sigmaσ)** confers promoter specificity (−35 and −10 regions).
- Transcription is terminated either via **rho-dependent** or **rho-independent** (hairpin loop) mechanisms.
- The **operon concept** (discussed in Section VII) encapsulates how multiple related genes can be transcribed from one promoter.

Mechanism of Translation

The Genetic Code

- **Definition**: A set of triplet mRNA codons specifying amino acids or stop signals.
- **Degeneracy**: Most amino acids are encoded by multiple codons.
- **Wobble Base Pairing**: The 3′ position of the mRNA codon can form non-canonical hydrogen bonds with the 5′ position of the tRNA anticodon, allowing one tRNA to read multiple synonymous codons.

Stages of Translation

1. **Initiation**
   - **Prokaryotes**: The small ribosomal subunit (30S) binds the **Shine-Dalgarno sequence** (AGGAGG) on mRNA, positioning the start codon (AUG) in the P-site. **Initiator tRNA (fMet-tRNAf^{Met})** pairs with AUG.
   - **Eukaryotes**: The small ribosomal subunit (40S) and associated factors (eIFs) recognize the 5′ cap and scan until reaching the **start codon** (usually AUG) within a **Kozak consensus**. Initiator tRNA (Met-tRNAi_ii) binds

in the P-site.

2. **Elongation**
   - Repeated cycles of **codon recognition (by aminoacyl-tRNA)**, **peptide bond formation** (catalyzed by the ribosomal RNA in the large subunit: 50S in prokaryotes, 60S in eukaryotes), and **translocation** of the ribosome along the mRNA.
   - **EF-Tu** (in bacteria) or **eEF1A** (in eukaryotes) delivers aminoacyl-tRNA to the A-site, ensuring fidelity. **GTP hydrolysis** provides energy for conformational changes.
3. **Termination**
   - Encountering a **stop codon** (UAA, UAG, UGA) in the A-site recruits **release factors** (RFs), triggering hydrolysis of the polypeptide from the tRNA in the P-site.
   - The ribosomal subunits, mRNA, and release factors then dissociate.

Gene Structure in Eukaryotes

1. **Exons and Introns**
   - **Exons**: Coding or expressed regions that remain in the mature mRNA.
   - **Introns**: Noncoding intervening sequences removed by **splicing**.
   - The presence of introns enables alternative splicing, increasing proteome complexity.
2. **Regulatory Elements**
   - **Promoters**: Regions immediately upstream of the transcription start site where general transcription factors and RNA Pol II assemble.
   - **Enhancers and Silencers**: DNA elements that can be located distally; they recruit activator or repressor proteins to modulate transcription.
   - **Insulators**: Boundary elements preventing enhancers from activating the wrong promoter.
3. **Untranslated Regions (5′ UTR and 3′ UTR)**
   - These regions flank the coding sequence and can regulate mRNA stability, localization, and translational efficiency (e.g., binding sites for RNA-binding proteins or miRNAs).

Gene Expression and Regulation in Eukaryotes

1. **Chromatin Modifications**
   - **Histone Acetylation (HATs/HDACs)**: Acetylated histones reduce nucleosome compaction, promoting transcription; deacetylation reverses this.
   - **DNA Methylation (CpG Islands)**: Generally associated with transcriptional repression.
2. **Transcription Factors**
   - **General (Basal) TFs**: Required for Pol II binding (TFIID, etc.).
   - **Specific TFs**: Bind enhancers/silencers, recruit coactivators or corepressors, and modulate transcription rate.
   - **Mediator Complex**: Bridges sequence-specific TFs and the Pol II machinery, influencing transcription initiation and elongation.
3. **Regulatory RNAs (miRNAs, lncRNAs)**
   - **microRNAs (miRNAs)**: ~22 nt RNAs that bind target mRNAs, causing translational repression or mRNA degradation.
   - **Long Noncoding RNAs (lncRNAs)**: Serve as scaffolds for chromatin modifiers or splicing factors, influencing gene expression at multiple levels.

RNA Polymerases in Eukaryotes

1. **RNA Polymerase I**
   - Synthesizes **pre-rRNA** (28S, 18S, 5.8S rRNAs).
   - Located in the nucleolus; specialized for high-level ribosome component production.
2. **RNA Polymerase II**
   - Transcribes **mRNAs**, most **snRNAs**, and some **lncRNAs**.
   - Has a **C-terminal domain (CTD)** with heptapeptide repeats (YSPTSPS) crucial for promoter clearance, elongation, and processing factor recruitment.

3. **RNA Polymerase III**
    - Transcribes **tRNAs**, **5S rRNA**, and other small noncoding RNAs.
    - Recognizes internal or upstream promoter elements (e.g., Box A, Box B).

(Additional polymerases exist in plants, e.g., Pol IV and Pol V for siRNA-related pathways.)

Post-Transcriptional Modifications (Eukaryotic mRNA)

1. **5′ Capping**
    - Addition of an inverted **7-methylguanosine (m^7G)** cap to the 5′ end.
    - Protects mRNA from exonucleases, aids nuclear export, and helps ribosomes recognize the mRNA.
2. **3′ Polyadenylation**
    - **Poly(A) polymerase** adds a poly(A) tail (~50–250 adenines) to the 3′ end.
    - Enhances mRNA stability and facilitates export and translation initiation.
3. **Splicing**
    - Removal of **introns** by the **spliceosome** (snRNPs U1, U2, U4, U5, U6).
    - **Alternative Splicing** can generate multiple protein isoforms from a single gene, contributing to proteomic diversity.
4. **RNA Editing**
    - Some transcripts undergo **base modifications** (e.g., C-to-U, A-to-I) that change codons and potentially protein function.

Operon Concept

1. **Prokaryotic Paradigm**
    - An **operon** is a functional unit of gene regulation and expression, comprising structural genes, a promoter, an operator, and regulatory proteins.
    - **lac Operon**: Inducible system (repressor inactivated by inducer, allolactose) for lactose metabolism.
    - **trp Operon**: Repressible system (operon switched off by high levels of the end product, tryptophan).
2. **Eukaryotic Considerations**
    - Eukaryotes usually have **monocistronic mRNAs** (one gene per transcript). True “operons” are rare but do exist in some eukaryotes (e.g., *Caenorhabditis elegans*).
    - Eukaryotic gene regulation is often more complex, involving enhancers, insulators, chromatin states, and combinatorial control by multiple transcription factors.

Concluding Remarks

Gene transcription in eukaryotes is an intricate process regulated at multiple levels—from **chromatin dynamics** and **RNA polymerase specificity** to **post-transcriptional mRNA modifications** and **RNA-based regulatory mechanisms**. The **genetic code** unifies transcriptional output (mRNA) with **translation**, ensuring the correct amino acid sequence is built into functional proteins. Although eukaryotes lack the classic operon architecture that typifies **prokaryotic** systems, understanding the **operon concept** offers a comparative framework for gene regulation, illuminating how organisms manage coordinated expression of functionally related genes.

Ultimately, these processes—gene transcription, post-transcriptional refinement, and translation—are the molecular cornerstones of **gene expression**, driving cellular identity, development, and adaptation across the tree of life.