MOLECULAR CELL PHYSIOLOGY - Post-Transcriptional Control & Protein Sorting

I. Transcription termination in prokaryotes (less is known about eukaryotic mechanisms)
- **A. Rho factor (a transcription-termination factor)**
  1. **The Rho protein is an ATPase that dislodges the 3' end of a growing RNA chain from the active site of RNA polymerase**
     - **a) Rho-dependent terminations are present in some 8-phage and *E. coli* genes**
  2. **Most operons have Rho-independent termination sites with two features**
     - **a) A series of U residues in transcribed RNA**
     - **b) A GC-rich region with several intervening nucleotides**
- **B. Attenuation**
  1. **An attenuator site is a DNA sequence where a choice is made by RNA polymerase between continued transcription and termination**
     - **a) Rapid translation of the leader sequence in an mRNA favors an RNA secondary structure that terminates transcription prematurely by a Rho-independent mechanism**
     - **b) Slow translation favors an alternative RNA secondary structure that does not cause termination**
- **C. In eukaryotes, RNA polymerases employ different termination mechanisms**
  1. **Polymerase-specific termination factor stops transcription of pre-rRNA genes by RNA polymerase I**
  2. **Cleavage and polyadenylation of the carboxyl-terminal domain (CTD) is coupled with termination of RNA polymerase II**
  3. **A series of poly-U residues leads to termination of RNA polymerase III**

II. RNA processing, regulation of processing, and signal-mediated transport through nuclear pores
- **A. Main steps of RNA processing**
  1. **5' capping: 7-methylguanosine is added to the 5' end of nascent mRNA that associates with the phosphorylated CTD of RNA polymerase II**
  2. **3' cleavage and polyadenylation: a conserved polyadenylation signal (AAUAAA) lies 10-30 nucleotides upstream from a poly(A) site**
     - **a) A GU- or U-rich site sequence downstream contributes to efficiency of cleavage**
  3. **RNA splicing to remove introns: carried out by a large ribonucleotide protein complex (spliceosome) composed of 5 small nuclear RNAs (snRNAs)**
- **B. Regulation of RNA processing**
  1. **Expression of some proteins is regulated by the processing of the primary transcript from the gene encoding them**
  2. **Alternative splicing of primary transcripts is regulated (sometimes by RNA-binding proteins) - splicing activators**
- **C. Signal-mediated transport through nuclear pores**
  1. **Macromolecules larger than 60 kDa must be actively transported through nuclear pores (entails ATP hydrolysis and conformational changes)**
  2. **For export and import, proteins must AA sequences that function as nuclear-export signal (NES) and/or nuclear-localization signal (NLS)**
  3. **Nascent RNA transcripts associate with various proteins, forming heterogeneous ribonucleoproteins (hnRNPs) that contain fully processed mRNAs (mRNPs)**

III. Protein sorting
- **A. Protein pathways**
  1. **Secretory pathway**
     - **a) Proteins are directed to rough endoplasmic reticulum by ER signal sequence**
     - **b) After translation, proteins move via transport vesicles to Gogi apparatus**
     - **c) Packaged (disulfide bonds, addition of carbohydrates, proteolytic cleavages, assembly into multimeric units) protein directed to cell surface (secretion), lysosome, or plasma membrane**

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2. Proteins released into cytosol

a) Processed to contain specific uptake-targeting sequences (these are later removed by proteases)

b) Imported into mitochondrion, chloroplast, peroxisome, nucleus

1) Mitochondria and chloroplasts contain organelle DNA, which encodes organelle rRNAs and tRNA, but few organelle proteins

2) Most mitochondrial and chloroplast proteins are encoded by nuclear genes, which are translated by cytosolic ribosomes and imported into organelles

c) May be sorted to other organellar compartments (second signal sequence)