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Programme: M.Sc., Biomedical Science

Course Code: BM35C5

Course Title: Molecular Biology

Unit-III

Transcription

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Unit III:

Transcription: Concept of transcription, RNA polymerases, transcriptional factors, regulatory elements, Mechanism of transcription in Prokaryotes and eukaryotes, Distinction between prokaryotic and eukaryotic transcription. Concept and mechanism of post transcriptional modification- 5‟ capping, polyadenylation, splicing of nuclear pre-mRNA, nuclear export of mRNA-mRNA stability

PRESENTATION: 3

mRNA Stability and Transport

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- Messenger RNA (mRNA) stability and transport are critical regulatory processes in gene expression.
- They determine the lifespan of mRNA molecules and their ability to be translated into proteins.
- These mechanisms ensure that the appropriate proteins are produced at the right time and place within a cell.

1. mRNA Stability

mRNA stability determines how long an mRNA molecule persists in the cell before being degraded. This stability is crucial for controlling the levels of proteins synthesized in response to cellular needs and environmental stimuli.

a. Mechanisms of mRNA Stability

1. 5' Cap and 3' Poly(A) Tail:

1. **5' Cap**: The 5' end of eukaryotic mRNA is modified with a 7-methylguanosine cap. This cap protects the mRNA from degradation by 5' exonucleases and facilitates ribosome binding during translation.
2. **3' Poly(A) Tail**: A string of adenine residues is added to the 3' end of the mRNA. The poly(A) tail stabilizes the mRNA and enhances translation. Poly(A)-binding proteins (PABPs) protect the tail from degradation.

2. Cis-Regulatory Elements:

- AU-Rich Elements (AREs): Found in the 3' UTR of many mRNAs, AREs are binding sites for proteins that can either stabilize or promote mRNA degradation.

- Stem-Loop Structures: Secondary structures in the mRNA can affect stability by influencing the binding of proteins or microRNAs.

3. Trans-Acting Factors:

RNA-Binding Proteins (RBPs) : These proteins bind to specific sequences in the mRNA and modulate its stability. For example, HuR stabilizes mRNAs, while TTP promotes decay.

microRNAs (miRNAs): These small RNAs bind to complementary sequences in the 3' UTR of target mRNAs, leading to translational repression or mRNA degradation through the RNA-induced silencing complex (RISC).

4. Codon Optimality:

The use of "optimal" codons, which match abundant tRNAs, can enhance translation efficiency and mRNA stability. Conversely, "non-optimal" codons can slow down translation and increase mRNA degradation.

b. Pathways of mRNA Decay

1. Deadenylation-Dependent Decay:

The first step in mRNA decay is the shortening of the poly(A) tail by deadenylases. Once the tail is sufficiently short, the mRNA becomes unstable and is degraded.

2. Decapping Pathway:

After deadenylation, the mRNA can be decapped by the decapping complex (Dcp1-Dcp2). This exposes the mRNA to 5' to 3' exonucleases, such as Xrn1, which rapidly degrade the mRNA.

3. Endonucleolytic Cleavage:

- Some mRNAs are cleaved by endonucleases within their coding or untranslated regions, leading to the rapid degradation of the resulting fragments.

4. Nonsense-Mediated Decay (NMD):

- NMD targets mRNAs containing premature stop codons (nonsense mutations) and prevents the production of truncated, potentially harmful proteins.

5. RNA Surveillance Pathways:

- Other pathways, such as No-Go Decay (NGD) and Non-Stop Decay (NSD), target mRNAs that cause stalled ribosomes or lack a stop codon, respectively.

2. mRNA Transport

Following transcription in the nucleus, mRNA molecules need to be transported to the cytoplasm for translation. The spatial and temporal regulation of mRNA transport is essential for localized protein synthesis, particularly in polarized cells like neurons.

a. Nuclear Export

1. Nuclear Pore Complex (NPC):

- mRNAs are exported through the NPC, a large proteinaceous channel that regulates the exchange of molecules between the nucleus and the cytoplasm.

2. mRNA Export Factors:

- Export factors, such as the TREX (Transcription-Export) complex and Nxf1/Tap (nuclear export factor 1), bind to mRNA and facilitate its transport through the NPC.

3. RNA Modifications:

- Modifications like N6-methyladenosine (m6A) enhance mRNA export by recruiting reader proteins (e.g., YTHDC1) that interact with the export machinery.

4. Quality Control Mechanisms:

- Only fully processed mRNAs, with a 5' cap, spliced exons, and a poly(A) tail, are exported. This prevents the export of defective or unprocessed transcripts.

b. Cytoplasmic Localization

1. Localization Signals:

- mRNAs contain localization signals within their sequences that direct them to specific cytoplasmic regions. These signals are recognized by motor proteins or localization machinery.

2. Motor Proteins and Cytoskeleton:

- Kinesin and dynein transport mRNAs along microtubules, while myosin moves them along actin filaments. This ensures that mRNAs reach specific subcellular destinations, such as the leading edge of migrating cells or synaptic terminals in neurons.

3. Localized Translation:

- Once localized, mRNAs are often translated in response to local signals. This allows for rapid, spatially restricted protein synthesis, critical for processes like synaptic plasticity in neurons and cell polarity in epithelial cells.

3. Biological Significance

1. Gene Expression Regulation:

- mRNA stability and transport control the timing and location of protein synthesis, allowing cells to respond dynamically to internal and external cues.

2. Developmental Processes:

- During development, mRNA localization and localized translation are crucial for processes like asymmetric cell division, axis formation, and tissue differentiation.

3. Cellular Responses:

- Cells use mRNA stability and transport to regulate responses to stress, hormones, and other stimuli, ensuring precise control over protein production.

4. Disease Implications:

- Defects in mRNA stability or transport can lead to diseases. For example, mutations affecting mRNA stability are linked to cancer, where oncogenes may be overexpressed due to increased mRNA stability. Similarly, neurodegenerative diseases like Fragile X syndrome involve disrupted mRNA transport and localization.

4. Techniques to Study mRNA Stability and Transport

1. RNA Sequencing (RNA-Seq):

- Measures mRNA abundance and provides insights into stability by comparing steady-state levels of mRNA across conditions.

2. RIP-Seq (RNA Immunoprecipitation Sequencing):

- Identifies mRNAs associated with specific RBPs, providing insights into the regulatory networks controlling mRNA stability and transport.

3. Live-Cell Imaging:

- Allows the visualization of mRNA dynamics in living cells, revealing details about mRNA transport, localization, and translation in real-time.

4. CLIP-Seq (Crosslinking and Immunoprecipitation Sequencing):

- Identifies binding sites of RBPs on mRNAs, shedding light on how these proteins regulate mRNA fate.

5. m6A-Seq:

- Maps m6A modifications on mRNAs, providing insights into how these marks influence mRNA stability and transport.

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