Unit 8: Regulation of transcription in Pro/Eukaryotes

Topic: Eukaryotes-TFs/HSPs/ gene Silencing

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Transcriptional regulation in Eukaryotes
Transcription Factors

- Transcription factors are proteins excluding RNA polymerase involved in the process of converting, or transcribing, DNA into RNA.
- They have DNA-binding domains that give them the ability to bind to specific sequences of DNA called enhancer or promoter sequences.
- Some transcription factors called basal (general) transcription factors (e.g., TFIID, TFIIA, etc.) bind to a DNA promoter sequence near the transcription start site and help form the transcription initiation complex.
- Other transcription factors bind to regulatory sequences, such as enhancer or silencer sequences, and can either stimulate or repress transcription of the related gene. These regulatory sequences can be thousands of base pairs upstream or downstream from the gene being transcribed.
- Through these contacts and interactions, the transcriptional activation domain of the factor may then induce conformational changes in the assembled proteins, paving the way for the RNA polymerase to initiate transcription.

Refer to the Transcription factors table and their interaction with CAAT box and GC box; provided to you on your Whatsapp/E-mail group
Transcription factors possess characteristic structural motifs such as the zinc finger, the helix-turn-helix, the leucine zipper, and the helix-loop-helix that result from associations between amino acids within their polypeptide chains.

1- Zinc finger motifs:

A short peptide loop that forms when two cysteines in one part of the polypeptide and two histidines in another part nearby jointly bind a zinc ion; the peptide segment between the two pairs of amino acids then juts out from the main body of the protein as a kind of finger.

Mutational analysis has demonstrated that these fingers play important roles in DNA binding.

Refer to the Zinc finger motifs interaction figure; provided to you on your Whatsapp/E-mail group.
2- **helix-turn helix,**
- A stretch of three short helices of amino acids separated from each other by turns.
- Genetic and biochemical analyses have shown that the helical segment closest to the carboxy terminus is required for DNA binding.

3- **Leucine zipper,**
- A stretch of amino acids with a leucine at every seventh position.
- Polypeptides with this feature can form dimers by interactions between the leucines in each of their zipper regions.
- Usually, the zipper sequence is adjacent to a positively charged stretch of amino acids.
- When two zippers interact, these charged regions splay out in opposite directions, forming a surface that can bind to negatively charged DNA.

4- **helix-loop-helix,**
- A stretch of two helical regions of amino acids separated by a nonhelical loop.
- Upon dimerization, the positive charged amino acid loop easily binds to negative charged DNA.

Refer to the helix turn helix, leucine zipper, helix loop helix *figure*; provided to you on your Whatsapp/E-mail group
HEAT SHOCK PROTEINS

Heat shock proteins are a group of highly conserved proteins found in both eukaryotic and prokaryotic cells. They are involved in a wide range of cellular processes such as assisting protein folding and degradation of misfolded proteins, modulating signaling pathways and regulating immune responses.

The multi-functional nature of heat shock proteins enables them to play critical roles in the regulation of protein homeostasis and cell survival.

How do HSPs work?

One major function of chaperones is to prevent both newly synthesised polypeptide chains and assembled subunits from aggregating into non functional structures.

High temperatures and other stresses, such as altered pH and oxygen deprivation, make it more difficult for proteins to form their proper structures and cause some already structured proteins to unfold.

Heat Shock Proteins are induced rapidly at high levels to deal with this problem.
Heat Shock Proteins are classified by their molecular weight, size, structure, and function. They are divided into several families, namely -

1. **HSP100**
2. **HSP90**
3. **HSP70**
4. **HSP60** (chaperonin)
5. **Small Heat Shock Proteins**/ (alpha)-crystalline proteins
## Functions of HSP families

<table>
<thead>
<tr>
<th>Family</th>
<th>Major Functions</th>
</tr>
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<tbody>
<tr>
<td>Hsp 100</td>
<td>Stress tolerance, Protein disaggregation, thermo tolerance</td>
</tr>
<tr>
<td>Hsp 90</td>
<td>Regulatory interactions with signaling proteins, stabilization of misfolded proteins</td>
</tr>
<tr>
<td>Hsp 70</td>
<td>Protein folding, membrane transport of proteins, Auto regulation in heat shock response, anti apoptotic</td>
</tr>
<tr>
<td>Hsp 60</td>
<td>Protein folding (limited substrates in eukaryotic cytoplasm)</td>
</tr>
<tr>
<td>Hsp 40</td>
<td>Protein folding, co-chaperone for Hsp70</td>
</tr>
<tr>
<td>Small Hsp’s</td>
<td>Stabilization of misfolded proteins, thermotolerance, eye lens structural proteins</td>
</tr>
</tbody>
</table>
Why Don't Heat Shock Proteins Denature?

✓ Better Hydrogen Bonds
✓ Better Hydrophobic Internal Packing
✓ Enhanced Secondary Structure
✓ Helix Dipole Stabilization
When organisms are subjected to the stress of high temperature, they respond by synthesizing a group of proteins that help to stabilize the internal cellular environment.

These heat-shock proteins, found in both prokaryotes and eukaryotes, are among the most conserved polypeptides known.

Comparisons of the amino acid sequences of heat-shock proteins from organisms as diverse as E. coli and Drosophila show that they are 40 to 50 percent identical—a remarkable finding considering the length of evolutionary time separating these organisms.

Refer to the HSPs-HSE interaction figure; provided to you on your Whatsapp/E-mail group.
Post transcriptional gene silencing—RNAi

RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules. Historically, it was known by other names, including co-suppression, post-transcriptional gene silencing (PTGS), and quelling.

Andrew Fire and Craig Mello 1998

RNAi have Won Nobel

Craig C. Mello Andrew Fire

“trigger for gene silencing was not single-stranded RNA (ssRNA) but double-stranded RNA (dsRNA)”
RNA Interference (RNAi) is initiated when there is an introduction of double-stranded RNA in the cell, which is then recognized by the DICER proteins.

Dicer endoribonuclease being part of the RNase III family, cleaves double-stranded RNA (dsRNA) and pre-microRNA (pre-miRNA) into short double-stranded RNA fragments called small interfering RNA and microRNA, respectively.

These fragments are approximately 20-25 base pairs long with a two-base overhang on the 3' end.

Dicer facilitates the activation of the RNA-induced silencing complex (RISC), which is essential for RNA interference.

RISC has a catalytic component argonaute, which is an endonuclease capable of degrading messenger RNA (mRNA) and thus causing the gene silencing.

Refer to the RNAi mechanism figure; provided to you on your Whatsapp/E-mail group.
Biological role of gene silencing:

Refer to the summary figure explaining the role of gene silencing and the various dimensions it covers; provided to you on your Whatsapp/E-mail group.
Thank you for Understanding!!!
All the best for the exam.