Genetics
& Cell biology

◉ Sheet ○ Slides

Number

10

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Transcription in Eukaryotes

- Regulation in eukaryotes is more complex, in prokaryotes we have one RNA polymerase, in eukaryotes however we have 3 RNA polymerases
  - RNA Polymerase I transcribes rRNA genes
  - RNA polymerase III transcribes tRNA genes and one of the rRNA gene
    The process of transcription is almost the same with only a few variances between prokaryotes and eukaryotes
  - RNA Polymerase II is responsible for transcribing mRNA genes aka the protein coding regions, as well as microRNA genes
- We face an issue with eukaryotic cells, as they have nucleosomes (DNA coiled around a histone) which need to be unwound before transcription in order for the RNA polymerase to be able to read the sequence and transcribe mRNA

  ➔General Transcription Factor have several functions aiding in transcription:

  1. Bind DNA and remove histones
  2. Help RNA polymerase find the promoter sequence and position itself on it
  3. Function as helicases
  4. Activation of transcription by pushing the RNA polymerase forward

  ➔ called "General" as they work on multiple genes concerned with transcription (nonspecific)

  abbreviated as TF II ➔ meaning they work for RNA Polymerase II and are specified as TFIIA, TFIIB,….

  Were only concerned with the two following subtypes:

  A. **TFIID**: (the first one to bind) binds to the promoter and recruits all the other proteins
     Once it binds to the promoter, it bends it, signaling to the other proteins to move towards the target gene

  B. **TFIIH**: important in DNA repair system, as previously mentioned
     Also functions as a helicase: binds to promoter, pulling apart the two strands, unwinding the DNA exposing ssDNA to allow to the RNA Polymerase to initiate transcription

❖ Elongation

- Once the RNA Polymerase is attached to the promoter sequence, it needs a "push forward" or "fuel" to start transcription, which is provided by TFIIH acting as a kinase, by phosphorylating the tail of RNA polymerase II, this is the "switch" that allows the RNA Polymerase to separate from the promoter and begin transcribing the target gene
- The RNA Polymerase doesn't go on forever, the process is highly regulated

[minute 00:00-9:14]

❖ Termination

- Unlike prokaryotes, termination of transcription in eukaryotes uses the consensus sequence "AAUAAA" which is found on the mRNA
- When the tail is phosphorylated, and the RNA Polymerase starts to move, certain proteins bind to the phosphorylated site, one of which is the Polyadenylation factor
- The polyadenylation factors will move along with the mRNA as its being transcribed, until the termination sequence emerges, they will then detect it and jump over to it to, to cleave the mRNA separating it from the RNA Polymerase.
- polyadenylation factors ➔ after cleaving mRNA, they add several A bases (polyA tail) to the mRNA, making it "polyadenylated", and this is true for all mRNA molecules, and is used as distinctive feature to differentiate mRNA from other RNA molecules.
- The polyA tail is usually around 200 bases long
- The A bases being added aren't part of the DNA coding sequence, so the PolyA Polymerase doesn't need a template while adding them
- There is some distance between the termination signal and the cleaving site

❖ Capping and Splicing

- Proteins responsible for capping and splicing are associated with the tail, they move along the mRNA modifying it as they go.

  A. Capping:
    - The cap is a reversed guanine nucleotide added to the 5' end of the mRNA
    - The guanine base comes from a GTP molecule
    - Capping factors recognize the 5' end of the mRNA as soon as it emerges from the RNA Polymerase and add the guanine nucleotide in a reversed orientation

- Functions:
    1. Stabilizes mRNA preventing any kind of degradation from taking place at the 5' end by exonucleases
    2. Once mRNA is capped and polyadenylated, it is now ready to be transported outside the nucleus for translation, but without the cap, it will remain trapped inside the nucleus (this process is facilitated by cap-binding complex)
    3. Help in recognition of mRNA molecules for translation

B. Splicing
- Splicing Factors: RNA molecules functioning as enzymes by binding to proteins, and forming a complex known as snRNP (small nuclear ribonucleoprotien). As the mRNA emerges from the RNA Polymerase, the snRNP bind the introns at the borders, forming a splicosome, and once transcription is terminated, they perform their function and splice out the introns
- hnRNP (heterogeneous nuclear ribonuclearprotiens): they bind to introns marking them. They coat the introns, and once transcription is over, the coated segments are recognized

❖ Accuracy of Splicing
- Recognizes the difference in size between exons and introns:
    - Exons are very small in comparison to introns which are large in size
- Assembly of spliceosome occurs during transcription, but splicing only takes place once transcription is completed. The cell makes sure that all the introns are marked before splicing takes place
- Spliceosome components, called SR Protiens mark the 3' and 5' ends
- hnRNPs also insure that all introns are marked

- note: promoter doesn't need to be spliced off as it isn't transcribed into the mRNA in the first place (this was a student's question)

[9:14-20:30]

❖ Alternative Polyadenylation

- Many genes have a defined termination signal
- Certain genes contain 2 polyadenylation signals
  a. Proximal polyadenylation signal
  b. Distal polyadenylation signal
     ➔if the cell uses the distal polyadenylation signal, it results in the formation of a longer mRNA molecule
- Importance of Alternative Polyadenylation
  a. Formation of multiple proteins from the same primary transcript
     Calcitonin vs Calcitonin Gene Related Protein (CGRP)
     - 2 hormones produced from the same gene, the difference is:
     - in Calcitonin the polyadenylation signal occurs early on
     - in CGRP, the polyadenylation signal lies further downstream
     - the choice of polyA site for cleavage determines the protein to be transcribed following formation of mRNA from primary transcript
  b. formation of a longer mRNA with a binding site to microRNA
     - the binding of microRNA to mRNA destabilizes it and results in it's degradation,decreasing amount of mRNA available for translation, consequently decreasing protein concentration
       ➔ this is a mechanism to control how much protein is produced & influencing target functions

- in conclusion ➔ if we use PAS I, we generate a shorter mRNA transcript that is stable resulting in the production of more proteins. If we use PAS II, we include a site for microRNA binding resulting in a less stable mRNA molecule that gets rapidly degraded.
- This is important in regulating cell behavior; having small amount of proteins involved in cell differentiation,, or having large amounts of proteins involved in cell proliferation, dedifferentiation and cancer development.

- Student's question: "what causes the enzyme to recognize one PAS rather than the other?"

  ➔ there are regulatory proteins that bind and mask the first PAS, so the transcription of mRNA continues until the second PAS is reached, these proteins aren't well understood.

❖ Single nucleotide polymorphisms (SNP)

A. SNPs in PAS

- Single nucleotide variations between individuals
- Can be in the coding (transcribed) region
- The presence of SNPs within the polyadenylation signal can also alter the length of the mRNA
- Affects the half life of mRNA

B. SNPs in Promotor Sequence

- Affects binding of general transcription factors and RNA Polymerase binding
- Affects the binding efficiency of these proteins, which can have a regulatory role on the expression of affected gene

❖ Transport of mRNA

- If the mRNA is appropriately produced (capped, spliced, polyadenylated, of proper length), it gets transported outside the nucleus
- If the mRNA doesn't satisfy that criteria, or has any sort of defect, it will not be transported

❖ Degradation of mRNA

- mRNA molecules have a certain half life once in the cytoplasm
- The process is catalyzed by nucleases (endo and exo nucleases)
- In prokaryotes the half life of mRNA is around 3 minutes
- In eukaryotes, it is more stable, and its half life ranges from 30 minutes to 10 hours
- Half lives of genes:

a. Influential genes such as transcription factors, neurotransmitters, & growth factors

➔ have short half lives and are rapidly degraded (30 minutes)

b. Genes responsible for making rRNA&tRNA, which are used frequently and are essential for the cell

➔ their half life are relatively long (10 hours)

❖ Regulating Stability of mRNA

A. Iron Responsive Elements

- coding regions of mRNA that serve a regulatory function
- Found on mRNA responsible for the production of proteins involved in iron metabolism

- Ex: ferritin, transferrin receptor, ferroportin, divalent metal transporters
  - Once those genes are synthesized, their mRNA contains the IRE, and these IRE control the stability of the mRNA
  - In transferrin, IRE is found on the 3' end on the regulatory region
  - In ferritin, the IRE is found on the 5' end

B. Effect on Expression

➔ In the presence of excess levels of iron in the body "iron overload", iron needs to be stored, transferrin receptor isn't needed, but there is higher demand for ferritin.

When iron is abundant, it binds to IRE-BP, disabling the binding of IR-BP to ferritin mRNA

This prevents the degradation of the mRNA molecules allowing the production of more ferritin protein

Therefore, the iron itself causes the cell to produce more iron storage molecules

➔ In the case of iron deficiency, cells need iron, so production of transferrin receptor needs to be increased, while ferritin concentrations need to be reduced, especially in the liver.

at low iron levels, the IRE-BP will bind to the ferritin mRNA and, thus, the mRNA will be destabilized, making less ferritin protein

An opposite effect is seen on the stability of transferrin receptor mRNA