Molecular Biology

2025-2024

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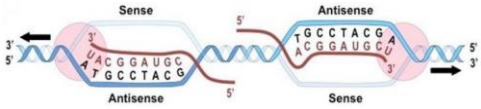
ARKAN ACADEMY

Transcription

- It is the process of making RNA from a DNA template
  - Transcription requires ***only one DNA template*** (one of the two strands) but DNA replication require 2 templates (both strands)
  - Each gene uses a specific strand, where the ***promoter*** present
  - RNA molecule is much ***shorter*** than DNA
  - RNA ***doesn't store genetic information*** in the cells

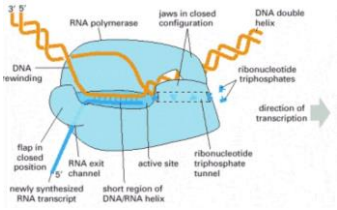
> ***Gene:*** The entire DNA sequence that is necessary for the synthesis of a ***functional RNA*** (mRNA, rRNA, tRNA, lncRNA, microRNA, etc.) or a ***polypeptide***, which may become a protein or a functional peptide(s)
>
> It contains *coding* region and non-coding *regulatory* elements (sequences)

- This process occurs from ***5' to 3'*** on the growing transcript molecule and appears from 3' to 5' on the DNA template
  - ***Transcript:*** It is the *RNA molecule* produced from transcription
  - ***Template strand:*** It is the DNA strand used in transcription and it is ***complementary*** to the transcript
    - ✓ Also called *non-coding* or *antisense* strands
  - ***Non-template strand:*** It is the other DNA strand, where it is ***identical*** to the RNA molecule but having T instead of U
    - ✓ Also called *coding* or *sense* strands

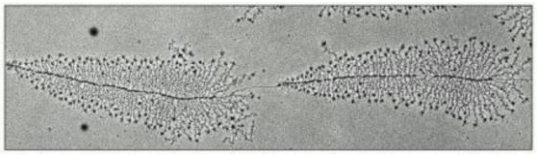


- Transcription involves the addition of monomers to the 3' end of the growing transcript (RNA)
  - Monomers are ribonucleotides (*ribonucleoside triphosphate NTP*) including ATP, CTP, GTP, UTP where they undergo <u>hydrolysis to provide energy</u>
  - ***RNA polymerase*** is the enzyme that catalyze the ***formation of phosphodiester bond***
    - ✓ It does ***not require primer***, where it can initiate synthesis de novo
    - ✓ Transcription is *less accurate* than replication because RNA polymerase is less accurate and having modest proofreading mechanism (making a mistake for every 10^4 nucleotide)
    - ✓ Transcription is a unilateral process

- Binding of RNA to DNA during transcription is temporary, where the newly synthesized portion of the RNA is bound to the DNA template but is ***released*** as RNA extends further

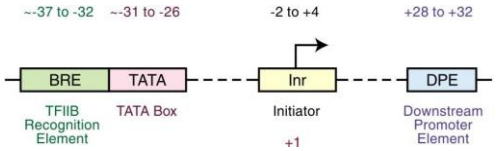


- ***Polysome:*** It is the synthesis of ***many RNA*** molecules from the ***same gene simultaneously***
  - Shorter chains are near to the starting point, and longer are near to the end



- ***Promoter:*** It is the sequence on the template where RNA polymerase ***initially binds***
  - Transcription starting (initiation) site (TSS) is the site where RNA synthesis starts (+1)
  - **<u>Prokaryotes</u>**
    - ✓ Promoter consist of ***consensus*** sequences located ***upstream*** to the transcription initiation site
    - ✓ It consists of 2 elements, (***-10***) and (***-35***) elements

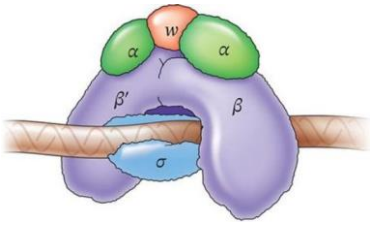
- ***Eukaryotes***
  - ✓ Eukaryotic promoter is more complex and involve ***upstream and downstream*** elements
  - ✓ It consists of many elements, where ***not all*** of them must be present in all genes
  - ✓ It involves core promoter region and other regulatory sequences
    - *Core promoter region* is where the RNA polymerase and transcription factors initially bind
  - ✓ These elements include:
    - *TFIIB recognition element (**BRE**):* Upstream the TSS and bind *TFIIB*
    - ***TATA box:** AT-rich* sequence, upstream the TSS, the most important to bind *RNA Pol II*
    - ***Initiator (Int):** Around* the TSS (includes the +1)
    - *Downstream promoter element (**DPE**):* **Downstream** to the TSS and the coding region



- Upstream = Before = toward the 5' end of the RNA molecule or the sense strand
- Downstream = After = toward the 3' end of the RNA molecule or the sense strand

RNA Polymerases

- **Prokaryotes:** They have ***one*** RNA polymerase
  - ➢ It consists of 2 major parts:
    - ✓ *Core polymerase* which includes ***two*** α, one β, one β', and one ω subunits and they have the full ability for **polymerization** of NTPs into RNA
    - ✓ σ *subunit* which is important for ***initiating*** transcription by binding the promoter but it is not required for the basic catalytic activity of the enzyme
  - ➢ It does **not require** any help from any proteins or factors

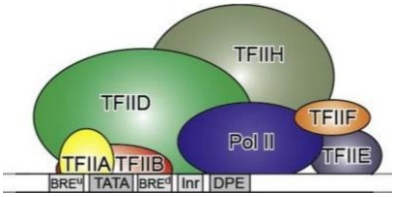


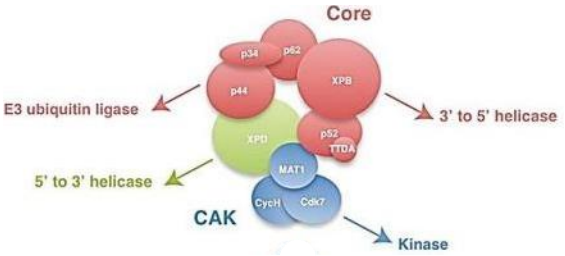
- **Eukaryotic** nuclei have ***three*** RNA polymerases (I, II and III)
  - ➢ *RNA polymerase I:* transcribes *rRNA* genes
  - ➢ *RNA polymerase II:* transcribes protein-encoding genes (*mRNA*), long noncoding RNA (*lncRNA*), and microRNA (*miRNA*)
  - ➢ *RNA polymerase III:* transcribes *tRNA* genes and **one** *rRNA* gene
  - ➢ They require the help of **transcription factors** especially general transcription factors
    - ✓ General = assemble and act on all promoters of all genes
    - ✓ They are important for positioning the polymerase on the promoter, separating the 2 strands of the DNA and pushing RNA polymerase forward (activating it)

- ***Initiation of transcription*** is a process involves the binding of RNA polymerase to the template and starting the synthesis of RNA

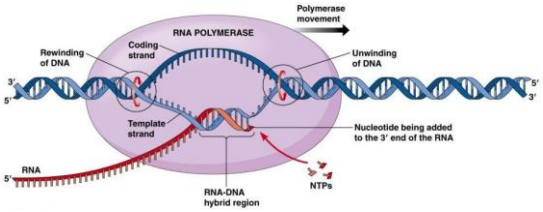
- **Prokaryotes**
  - *σ subunit identifies* the promoter and *guides* the core polymerase to the (-35) and (-10) elements
  - Without σ subunit the core polymerase can bind DNA *non-specifically and with low affinity*
  - The RNA polymerase is responsible for all the activities for transcription including identifying promoter, separating strands and adding nucleotides
  - After the addition of about 10 nucleotides, σ subunit is released

- **Eukaryotes**
  - Initiation must deal with the *packaging* of the DNA (nucleosomes and chromatin)
  - It involves formation of the *preinitiation complex* (PIC) which involves many transcription factors
    - ✓ The first transcription factor to assemble is ***TFIID*** then the others bind
    - ✓ Then ***RNA polymerase II*** binds
    - ✓ The last one to bind is ***TFIIH*** which is a multi-subunit factor having many activities:
      - **Helicase:** *unwinds* the DNA around the TSS and it is catalyzed by 2 subunits (***XPB, XPD***)
      - **Kinase:** Phosphorylates the **CTD** which *activates* the polymerase to start transcription
        - CTD is C-terminal domain in the RNA polymerase II
      - XPB and XPD have a role in the nucleotide excision **repair** also



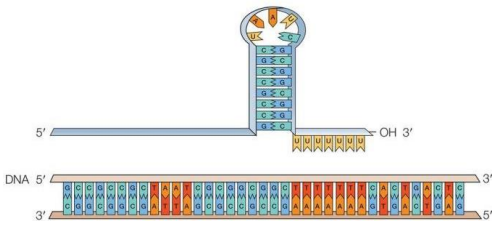


- ***Elongation*** involves ***unwinding*** of the DNA ahead of the polymerase, ***elongating*** RNA (adding nucleotide) and ***rewinding*** DNA behind the polymerase

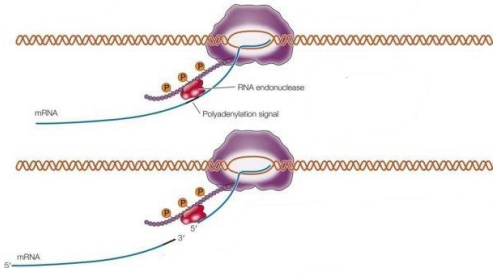


- ***Termination of transcription:*** Elongation continues until reaching a termination signal where the polymerase dissociated from the DNA and the RNA is released

- **Prokaryotes**
  - The most common termination signal (in E.coli) is a ***stable stem-loop structure*** consisting of *CG rich sequence* followed by a *U rich* sequence in the 3' end of the DNA

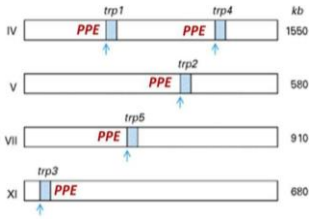


- **Eukaryotes**
  - Protein coding genes transcribes a ***polyadenylation signal*** in the 3' end of the RNA molecule
  - This signal is recognized by endonuclease and cleaved releasing the nascent transcript



- Protein involved in the ***same function or metabolic process*** are usually transcribed together
  - **Prokaryotes:** They have ***operons*** which are *polycistronic* genes that encode for different polypeptides having the same purpose
    - ✓ *Cistron:* it is alternative to the gene, where it is the region that encodes 1 polypeptide
    - ✓ *Polycistronic:* Many cistrons in the same transcriptional unit producing a single mRNA molecule that is translated into *many* polypeptides
    - ✓ *Monocistronic:* A gene that produces a single mRNA, translated into a *single* polypeptide
    - ✓ Prokaryotic genes can be either polycistronic (operons) or monocistronic, but eukaryotic genes are always monocistronic
    - ✓ Examples for operon: *Lac operon* (lactose metabolism), *Trp operon* (Tryptophan synthesis)

  - **Eukaryotes:** Genes that encode proteins with similar functions share ***PPE***
    - ✓ PPE (Proximal-Promoter Element) also called *response element*
    - ✓ They are *gene-specific* sequences *upstream* to the core promoter and important for strong expression
    - ✓ PPE can be **positive** (activation) or **negative** (inhibition)



    - ✓ Examples
      - *Serum Response Element (SRE):* Induced by *growth factors* which bind tyrosine kinase receptors and activate Ras-RAF-MEK pathway to activate ERK, promoting cell proliferation
      - *cAMP Response Element (CRE):* Induced by specific hormones that bind GPCR activating adenylyl cyclase producing cAMP that activates PKA that activates CRE and that regulates proliferation, survival and differentiation
      - *Hormone Response Element (HRE):* Induced by *thyroid* and *steroid* hormone
        - Testosterone binds the androgen receptor (AR), then dimerize and translocate to nucleus
    - ✓ The ***same gene*** can have ***many PPE***, such as:
      - *6-phosphofructo-2-kinase/fructose-2,6-bisphosophate 3* (***PFKFB3***) expression
        - It has response elements sensitive to progesterone, estrogen, hypoxia, cardiac rhythm

PFKFB3 regulates glycolysis and gluconeogenesis

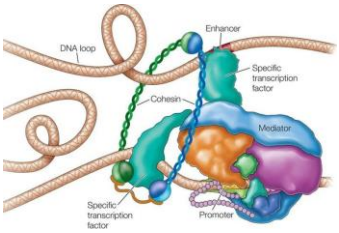
      - *Angiopoietin like protein 8* (***ANGPTL8***) expression
        - Inducers of anabolism (*feeding*, *glucose*, *insulin*) **stimulates** it by transcription factors called *SREBP-1c* and *ChREBP*
        - Inducers of catabolism (*fasting* and *glucocorticoids*) **suppress** its expression

ANGPTL8 regulates metabolism of lipoproteins and triglycerides, and inhibit lipoprotein lipase (LPL)

- *Tissue-specific transcription factors* are proteins regulate the expression of a gene in different tissues
  - Only basal transcription factors = ***Minimal*** expression
  - Activators = ***High*** expression
  - Repressors = ***No*** expression

Insulin is highly expressed in the pancreatic bets cell but not in the neuron

- ***Enhancers:*** Specialized regulatory sequences, that ***stimulate*** expression
- ***Silencers:*** Specialized regulatory sequences, that ***inhibit*** expression
  - There are 500,000 to over 1 million enhancers in the human genome, accounting for 10% or more of total genomic DNA
  - Both of them require the binding of gene-specific, cell-specific transcription factors
    - ✓ Enhancers are binding sites for *activators*
    - ✓ Silencers are binding sites for *repressors*
  - They can regulate expression ***regardless orientation and location***



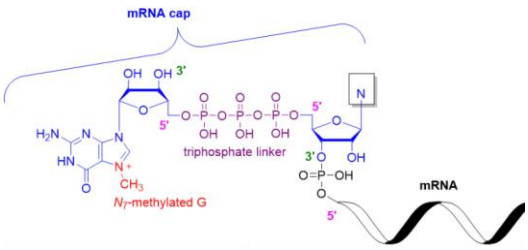
    - ✓ If the enhancer is far from the gene to be regulated, DNA is flexible to form *loops* allowing the activator or repressors to interact with the general transcription factors on the promoter via a ***mediator*** protein
    - ✓ Loops are *stabilized* via *cohesion* and *CTCF proteins*
    - ✓ Enhancers and silencers are restricted to interact with promoters in the ***same TAD***
      - ***TAD:*** Topologically associated *domains* divided by ***insulator*** sequences

- Transcription factor binding sites can be identified by ***chromatin immunoprecipitation***.
  - In the cell, the interaction between protein and DNA is non-covalent
  - During this technique, parts of the transcription factors are chemically *cross-linked* to the DNA then DNA is isolated and fragmented
  - The fragments are immunoprecipitated with an *antibody* against a specific transcription factor
  - The cross-links are reversed, and DNA fragments are analyzed by ***PCR*** to test for the presence of a specific DNA sequence or by next-generation DNA ***sequencing*** microarrays or microarrays to identify all the binding sites for the transcription factor within the genome

- The mRNA molecule initially produced from transcription in eukaryotic cells is ***nascent (pre-mRNA)*** and must be processed and modified in order to be transported into the cytosol and translated
- There are 3 Process done on the pre-mRNA in the nucleus to produce a ***mature mRNA***

1. Capping (Addition of a Cap)

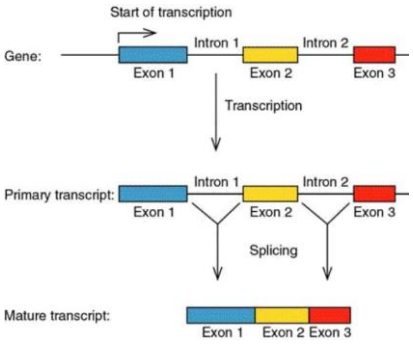
- The *first modification* occurs as RNA polymerase II has <u>produced a few nucleotides</u> of pre-mRNA
- Cap is added on the *5' end* of the pre-mRNA
  - The cap consists of ***7- methylguanosine*** molecule



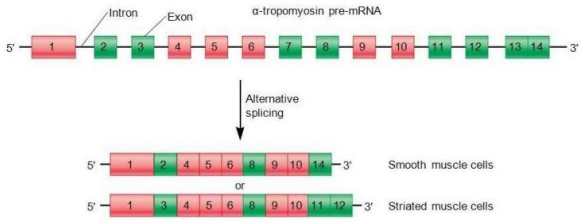
- Its importance is:
  - It *differentiates mRNA* from other RNA molecules (Other RNAs are not capped)
  - It *stabilizes* the mRNA and *protect* it from degradation by nucleases
  - It *signals the 5' end* of eukaryotic mRNAs, and helps in the *translation* of mRNAs to proteins
  - It recruits proteins necessary for *splicing and polyadenylation*
  - It helps in *exporting* RNA to the cytoplasm where nuclear exporters recognize the cap

2. Splicing

- ***Introns:*** Specific DNA sequences in the protein-coding genes that are transcribed but ***not translated***
- ***Exons:*** Protein-***coding*** regions that are transcribed and ***translated***
- The primary transcript (pre-mRNA) contains both introns and exons where the *introns are removed* by RNA splicing producing mature transcript or mature mRNA



- ***Alternative splicing:*** Transcripts are spliced in different ways to produce different mRNAs and *different proteins*
  - These proteins are known as ***protein isoforms***, which are highly related gene products that perform essentially the same biological function
  - It explains the huge gap between the proteins and protein coding transcripts that the number of genes
  - It involves the *removal of some exons* but ***not the rearrangement*** of them
  - ***Alternative exons:*** Exons can be removed via alternative splicing
  - ***Constitutive exons:*** Essential exons, can't be removed
- Eukaryotic transcription units produce mRNAs that encode only one protein, thus termed **monocistronic**

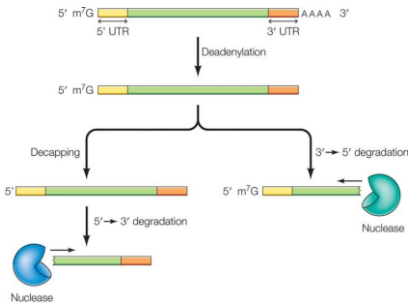


3. Polyadenylation

- The pre-mRNA is cleaved after a *certain sequence (AAUAAA)* in the 3' ends of mRNAs
- Poly-A polymerase then adds ~***200 'A' nucleotides*** from ATP
  - Poly-A polymerase does *not require a template* and the poly-A tail is *not encoded in the genome*
- It helps in *translation*, mRNA *transport* from the nucleus to the cytosol and it *stabilizes* mRNA and prevent its degradation

- Transport of mRNA from the nucleus to the cytoplasm, where it is translated into protein, is ***highly selective*** and is associated to correct RNA processing
- Defective mRNA molecules like interrupted RNA (not capped, poly A tailed, mRNA with inaccurate splicing, very long mRNA) are not transported outside the nucleus

- Degradation of mRNAs is initiated by:
  - Shortening of polyA tail and then ***3' to 5' exonucleases***
  - Decapping (removal of cap) and then ***5' to 3' exonucleases***



- The half-lives of bacterial mRNA are about 3 minutes
- The half-lives of eukaryotic mRNAs can be on average 30 minutes but can be longer
  - As the stability of mRNA increases, its half-life increases and stays more in cytosol producing higher amount of proteins

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