DNA transcription and RNA processing in eukaryotic cells

I. How eukaryotic DNA is organized in the nucleus

1. The scale problem and the need for hierarchical packaging

Why do eukaryotic cells require the organization of DNA into layered structures within the nucleus, and what fundamental structural challenge does this arrangement address?

The nucleus of a human cell measures approximately **10 µm** in diameter but contains about **2 meters of DNA**. In eukaryotic organisms, this is accomplished by packaging DNA into chromatin.

- **Chromatin** is a nucleoprotein complex made up of DNA and histone and non-histone proteins. It organizes the eukaryotic genome and regulates its accessibility for essential processes like transcription, replication, and repair.

2. Levels of chromatin organization

a. Nucleosome formation

Nucleosome: The fundamental structural unit of chromatin, comprising approximately 147 base pairs of DNA coiled around a histone octamer, which consists of two copies each of the core histones H2A, H2B, H3, and H4.

b. Higher-order structures

Nucleosomes condense into 30-nm fibers, which are organized into looped domains attached to a protein scaffold. These loops create topologically associating domains (TADs), crucial for communication between enhancers and promoters.

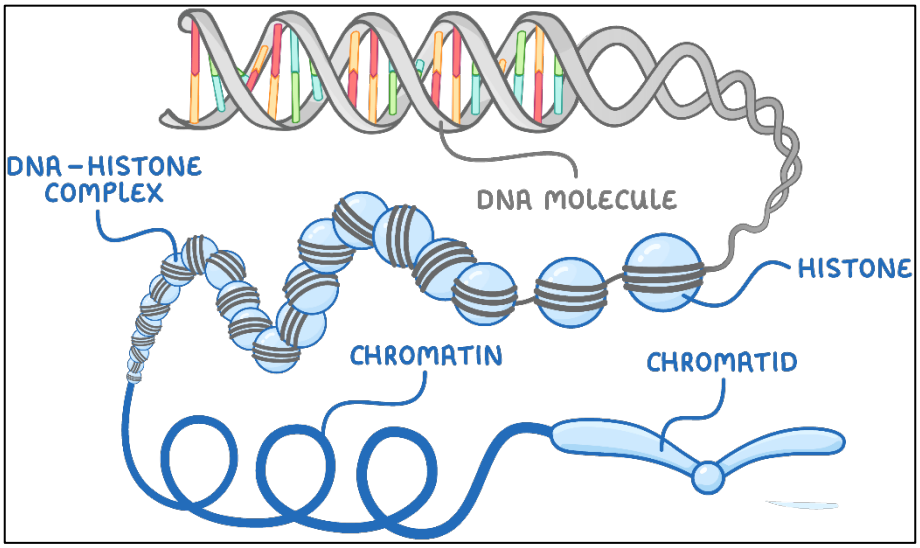


Figure 1: The diagram shows the hierarchical organization of eukaryotic DNA. DNA double helices associate with histone proteins to form nucleosomes. These nucleosomes fold into chromatin fibers, which then condense into chromatids at their most compact state. This structured packaging allows extensive DNA sequences to fit within the nucleus while preserving structural and functional integrity.

II. Overview of eukaryotic transcription

Transcription in eukaryotes occurs when the enzyme RNA polymerase synthesizes an RNA copy of a DNA strand in the nucleus. This process is the first step of gene expression, where genetic information is transcribed into RNA. The RNA then undergoes processing and translation, especially in the case of messenger RNA (mRNA), which contributes to cellular structure and function according to the genomic code.

The genetic code is a set of rules that specifies which amino acids or stop signals are represented by nucleotide triplets (codons) during protein synthesis, allowing genomic information to be translated into proteins.

1. Key enzyme: RNA polymerase II

RNA polymerase II (Pol II) synthesizes all messenger RNAs (mRNAs) and many noncoding RNAs. The initiation of transcription by Pol II depends on **g**eneral **t**ranscription **f**actor**s** (GTFs), as Pol II cannot directly recognize promoter sequences.

2. Promoter architecture

Typical promoters contain:

(1) **Core elements** (e.g., TATA box, initiator sequence)

(2) **Proximal regulatory elements**

(3) **Distal enhancers**, often tens or hundreds of kilobases away, brought into proximity via chromatin looping.

3. Steps of DNA transcription

(1) Transcription Initiation

Transcription initiation is a crucial phase of gene expression where RNA polymerase and transcription factors bind to a gene's promoter region, unwind the DNA, and start RNA synthesis at a specific nucleotide.

(2) Transcription elongation

1. RNA chain extension

RNA polymerase II synthesizes RNA in **the 5′ to 3′ direction** while maintaining an unwound region of about 15 base pairs of DNA. Accessory proteins enhance its efficiency and help it navigate DNA that is densely packaged around histones.

These accessory proteins, known as **transcription elongation factors,** assist RNA polymerase II in moving along the DNA. Notable examples include:

[1] **TFIIS**, which enables RNA polymerase II to recover from stalling and continue

a. transcription.

[2] **SPT5**, which stabilizes and enhances the processivity of RNA polymerase II during

a. elongation.

[3] **FACT** complex, which helps RNA polymerase II traverse nucleosomes by temporarily disrupting histone-DNA interactions.

Together, these factors work to promote efficient transcription through chromatin in eukaryotic cells.

2. Chromatin remodeling during elongation

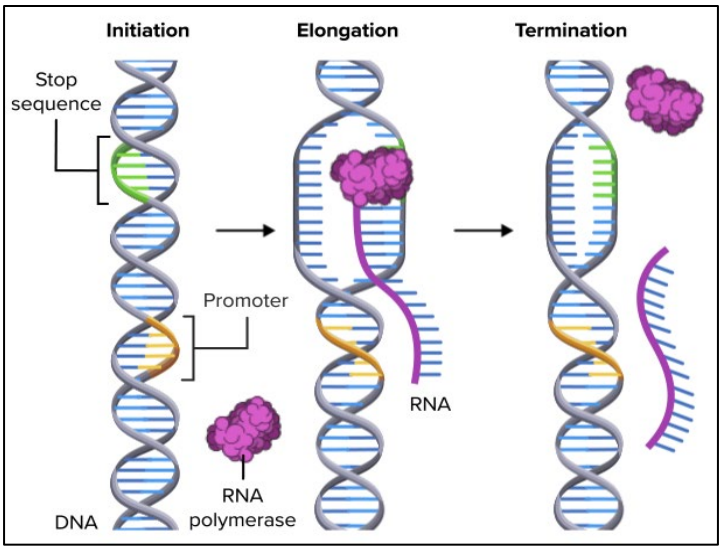
As RNA polymerase II moves along the DNA to synthesize RNA, it displaces the DNA from histone proteins. This is aided by the FACT complex, which temporarily changes the DNA-histone structure, allowing transcription to continue. Other proteins later help reposition the histones correctly, preserving the cell's gene regulatory information.

(3) Transcription termination

Transcription termination is a crucial process where RNA polymerase stops RNA synthesis, releases the new transcript, and detaches from the DNA template at the end of a gene.

This process involves the cleavage of the new transcript after the **polyadenylation signal** (AAUAAA) and includes several key mechanisms:

Figure 2: The figure on the right illustrates the three main stages of transcription: initiation, elongation, and termination. It shows RNA polymerase binding to a promoter, moving along the DNA template strand in the 3'-to-5' direction, and synthesizing mRNA in the 5'-to-3' direction. Finally, RNA polymerase detaches from the double helix.



4. RNA processing in eukaryotic cells

Eukaryotic RNA modifications is the processes that transform a newly synthesized RNA transcript into a **mature, functional RNA** molecule suitable for cellular use, by removing non-coding sequences from the primary transcript.

What are the steps of RNA processing?

1- **5′ Capping:** An RNA molecule receives a 5′ cap at its terminal end to protect against degradation and ensure proper use.

2- **Splicing**: Non-coding regions, called introns, are removed, while the coding sequences, known as exons, are joined together.

3- **3′ Polyadenylation:** A polyadenylate tail is added to the 3′ end of the RNA molecule to improve stability and assist in its transport from the nucleus.

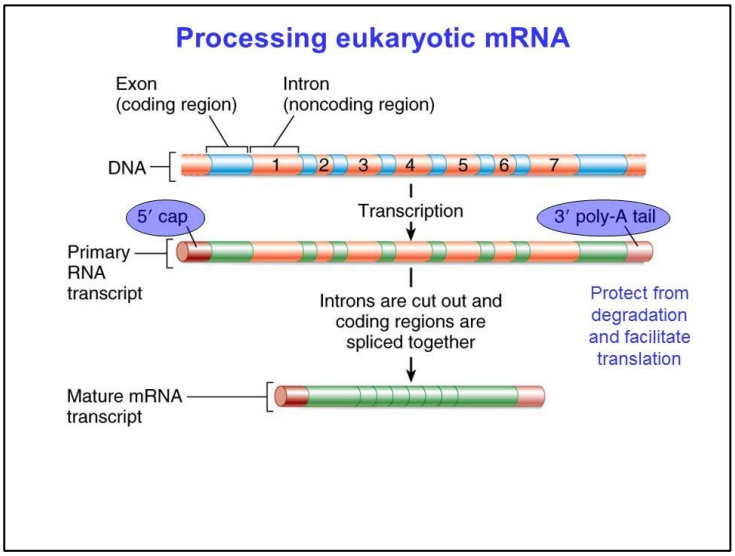


Figure 3: The figure shows that exons (coding sequences) and introns (noncoding sequences) is transcribed into a pre-mature RNA then removed by splicing . During transcription, a 5′ cap is added to the 5′ end, and polyadenylation occurs at the 3′ end to enhance RNA stability and aid translation.

What is next?

The RNA is now ready for its next act. After capping, editing, and the addition of a stabilizing tail, the mature RNA is ready to leave the nucleus. It moves into the cytoplasm, prepared to interact with ribosomes and initiate translation, where our DNA is translated into a working protein. We'll explore a different aspect of this story next lecture!