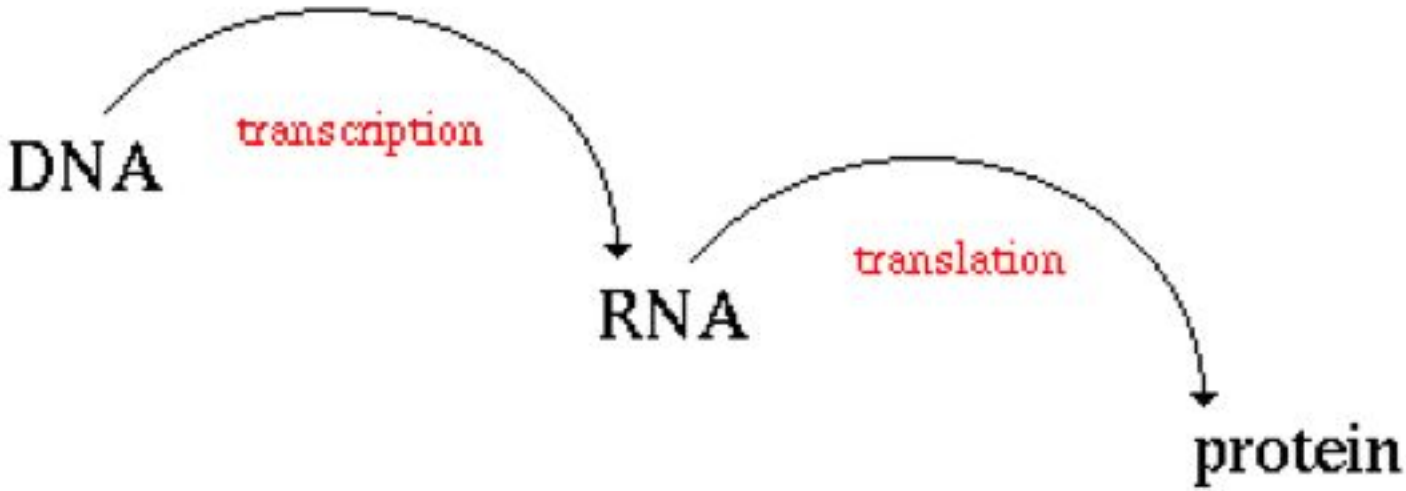
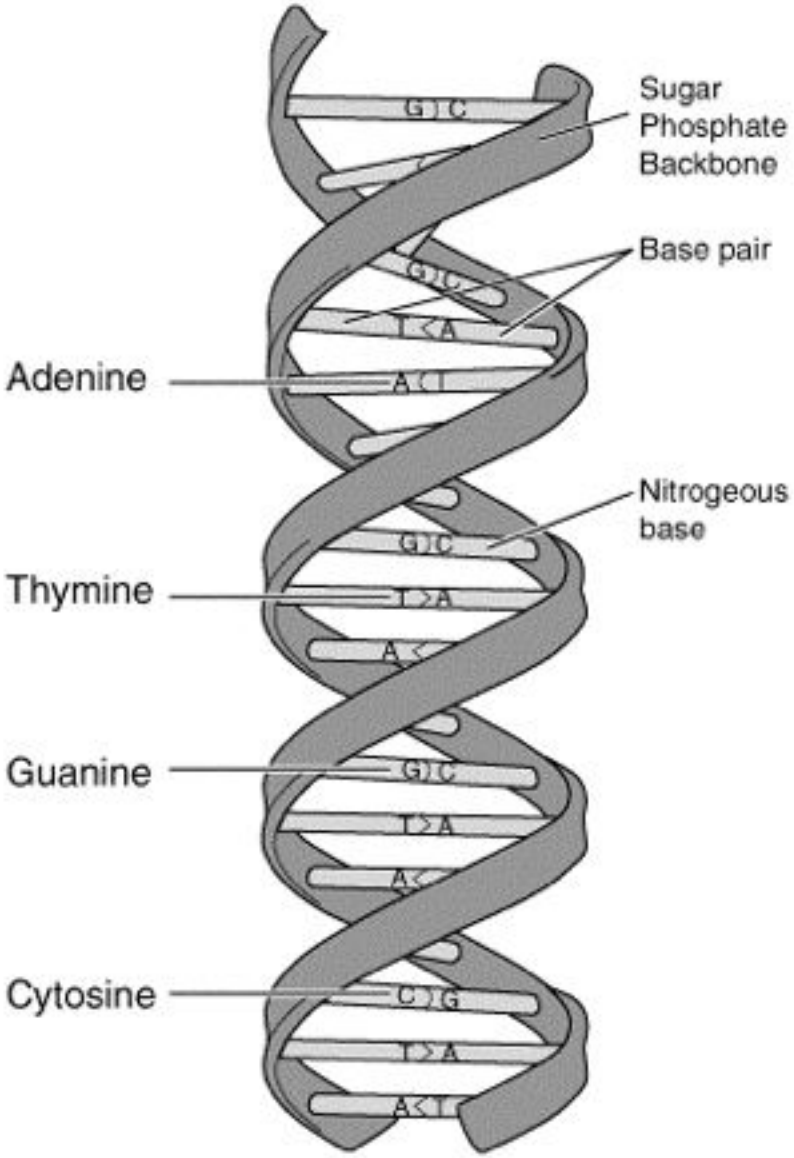
Eukaryotic Transcription

Central Dogma



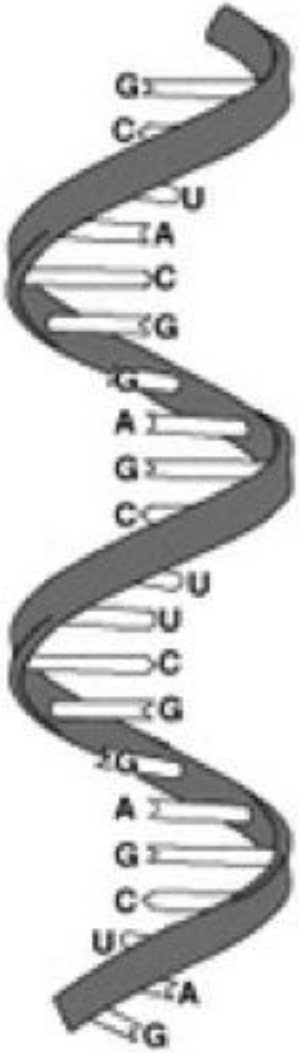
The Players…

- DNA is the genetic material
  - Building blocks are nucleotides
  - Capable of self-replication and synthesis of RNA



The Players…

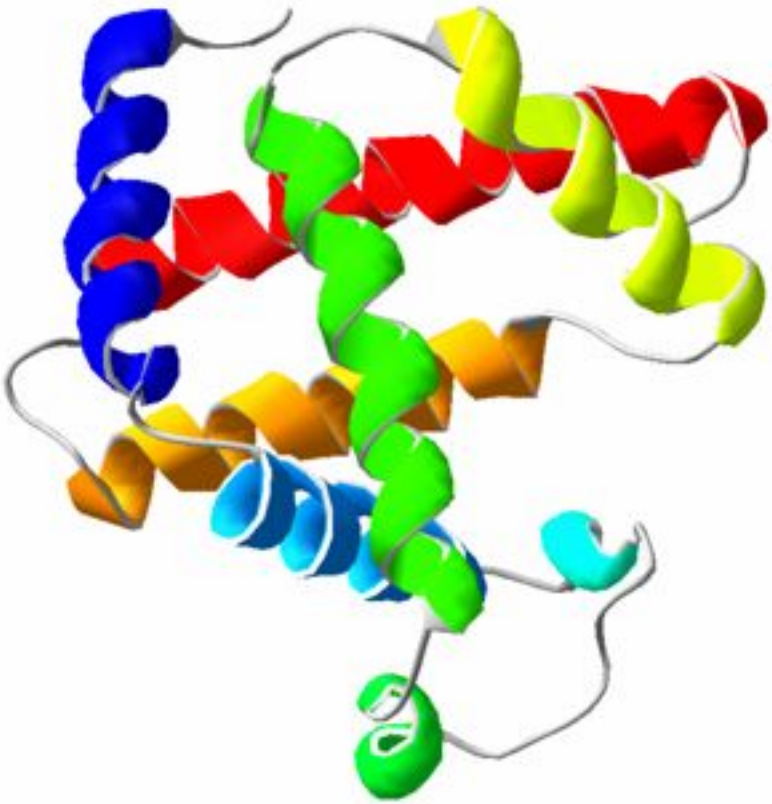
- DNA is the genetic material
  - Building blocks are nucleotides
  - Capable of self-replication and synthesis of RNA
- RNA is the middle man
  - mRNA, tRNA
  - Carries the genetic information into the cytoplasm and serves as template for protein synthesis



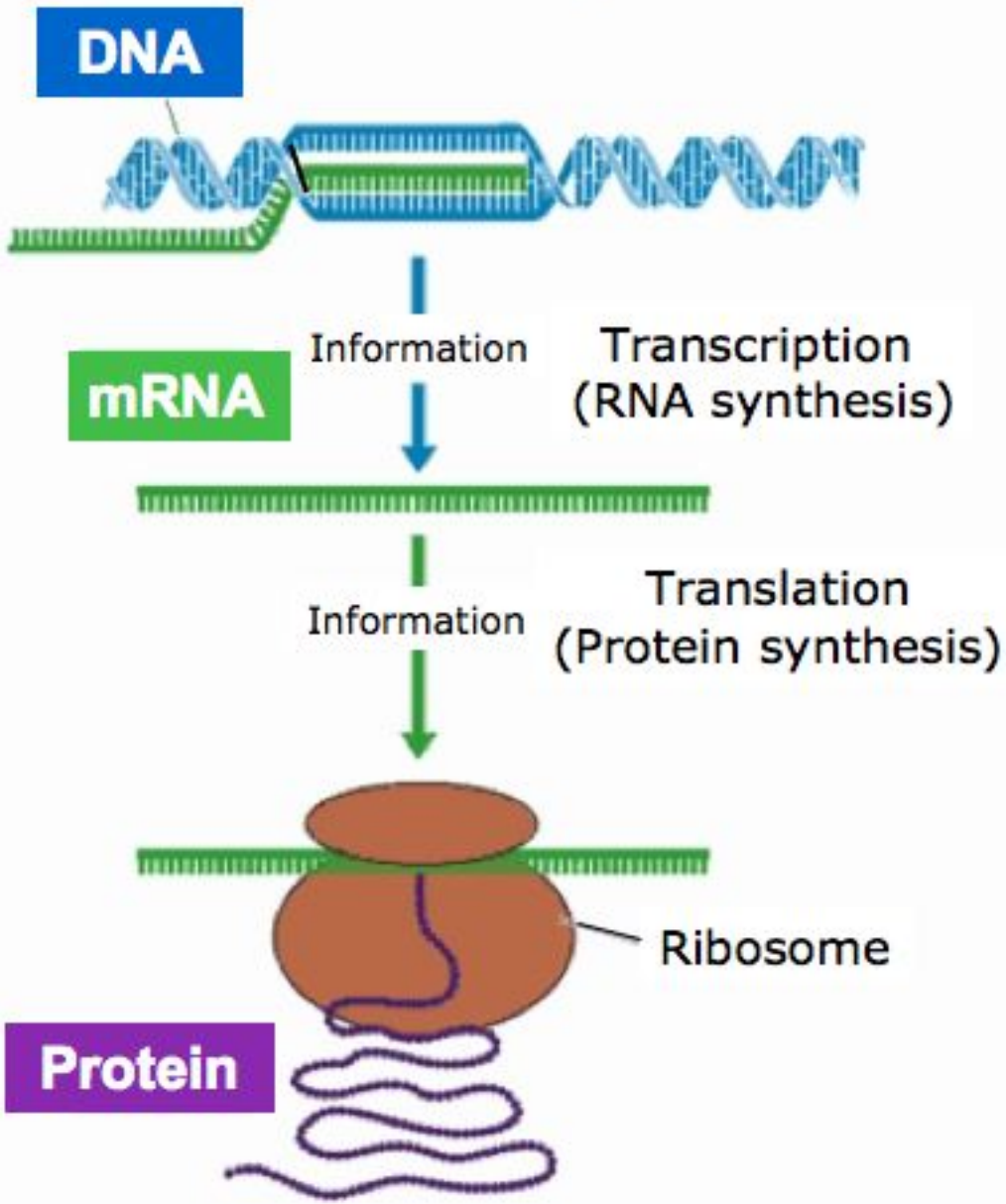
RNA

The Players…

- DNA is the genetic material
  - Building blocks are nucleotides
  - Capable of self-replication and synthesis of RNA
- RNA is the middle man
  - mRNA, tRNA
  - Structure and base sequence are determinants of protein synthesis and the transmission of genetic material
- Proteins are crucial for everything!
  - Building blocks are amino acids
  - Essential constituents of all living things
  - Examples: enzymes, hormones, antibodies



Central Dogma



Bacterial DNA

- 4.6×10^6 Nucleotides
- >500 – 4300 genes
- Single chromosome
- Usually circular
- Compact genome - closely packed genes

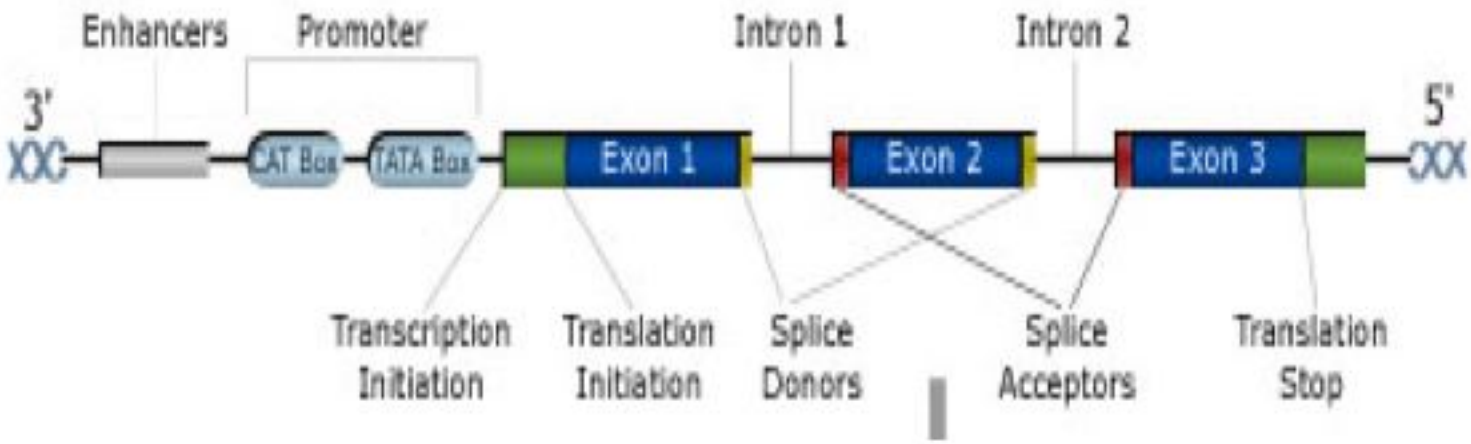
Eukaryotic DNA

- 3.2×10^9 nucleotides
- 2 meters of DNA if stretched from end to end
- Humans –
  - 30,000 genes
    - Exons, introns, regulatory DNA
  - Organized into 23 chromosomes
  - Linear
  - Lots of 'junk DNA'

Genes

- A gene is a region of DNA that encodes all the information needed to make a protein
- Often named for the function of the protein for which it encodes
- Genes are often considered the basic units of heredity

Typical Gene structure



- Promoter – non-coding sequence, directs RNA polymerase to the start of gene
  - CAT Box, TATA Box
- Enhancer/Silencer – regions to which activators/repressors bind to control transcription; located upstream of promoter
- Exon – coding sequence (sequences that encode the protein)
- Intron – non-coding sequence

Human cells contain ~30,000 genes

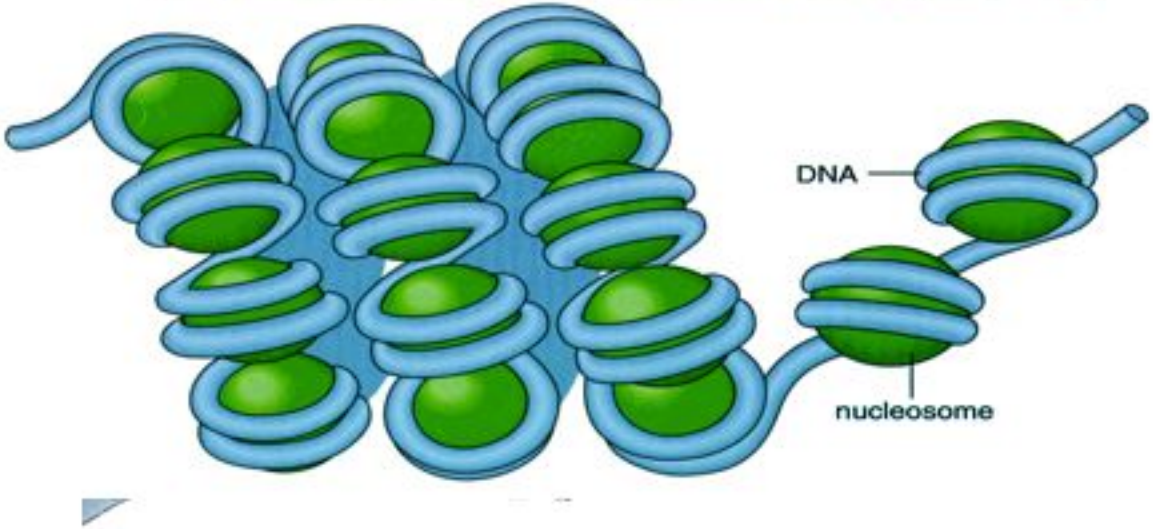
- Some are expressed in all cells all the time (housekeeping genes)
- Some are expressed as a cell enters a particular pathway of differentiation
- Some are expressed all the time in only those cells that have differentiated in a particular way
- Some are expressed only as conditions around and in the cell change

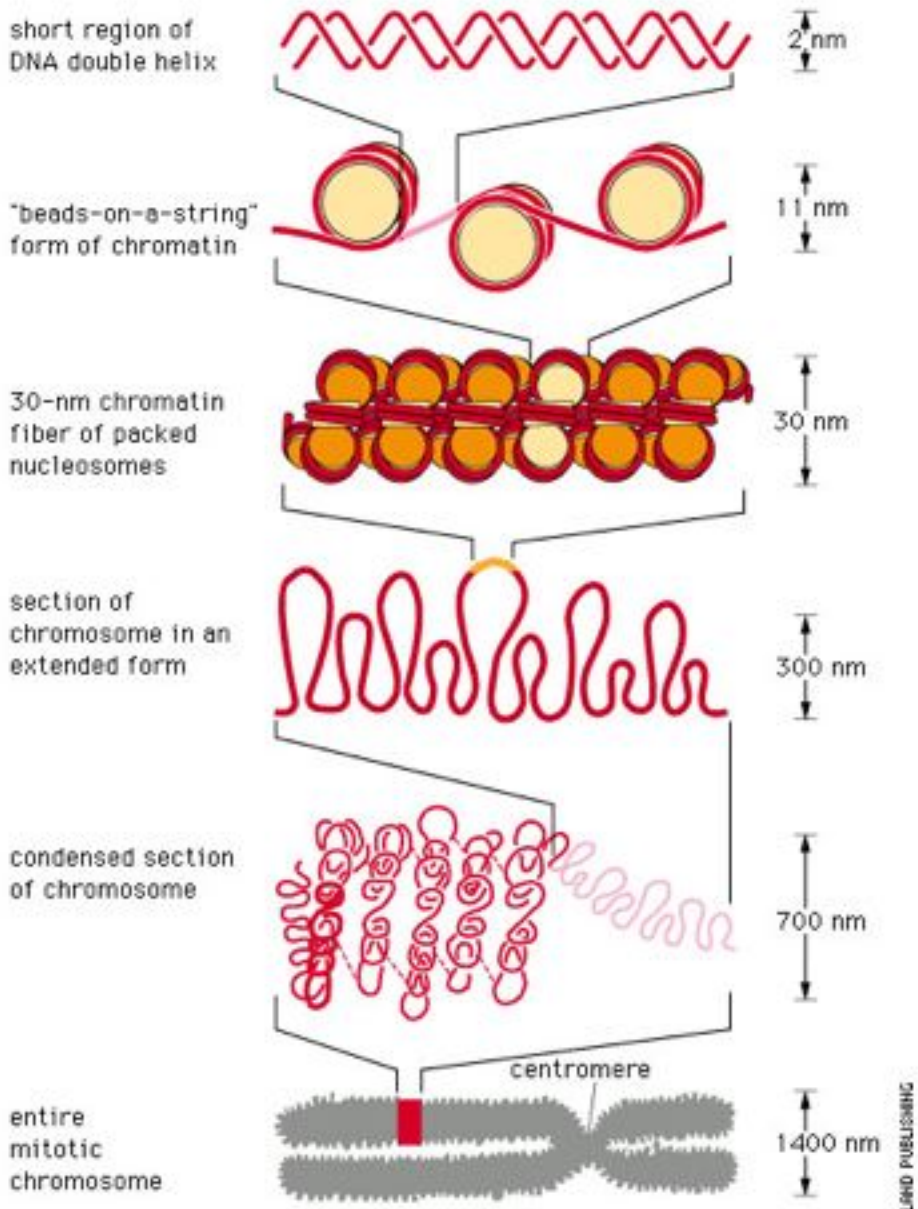
Eukaryotic Chromosomes

- Homologous chromosomes
- Contain genes and ‘junk DNA’
- Exist in different states throughout the life of the cell
- Typical structure:
  - Centromere
  - Two telomeres
  - Replication origin

Packaging the DNA – Chromosomal Organization

- DNA + protein = chromatin
- DNA + histone = nucleosome
- Chromatin Fiber
- Chromosome



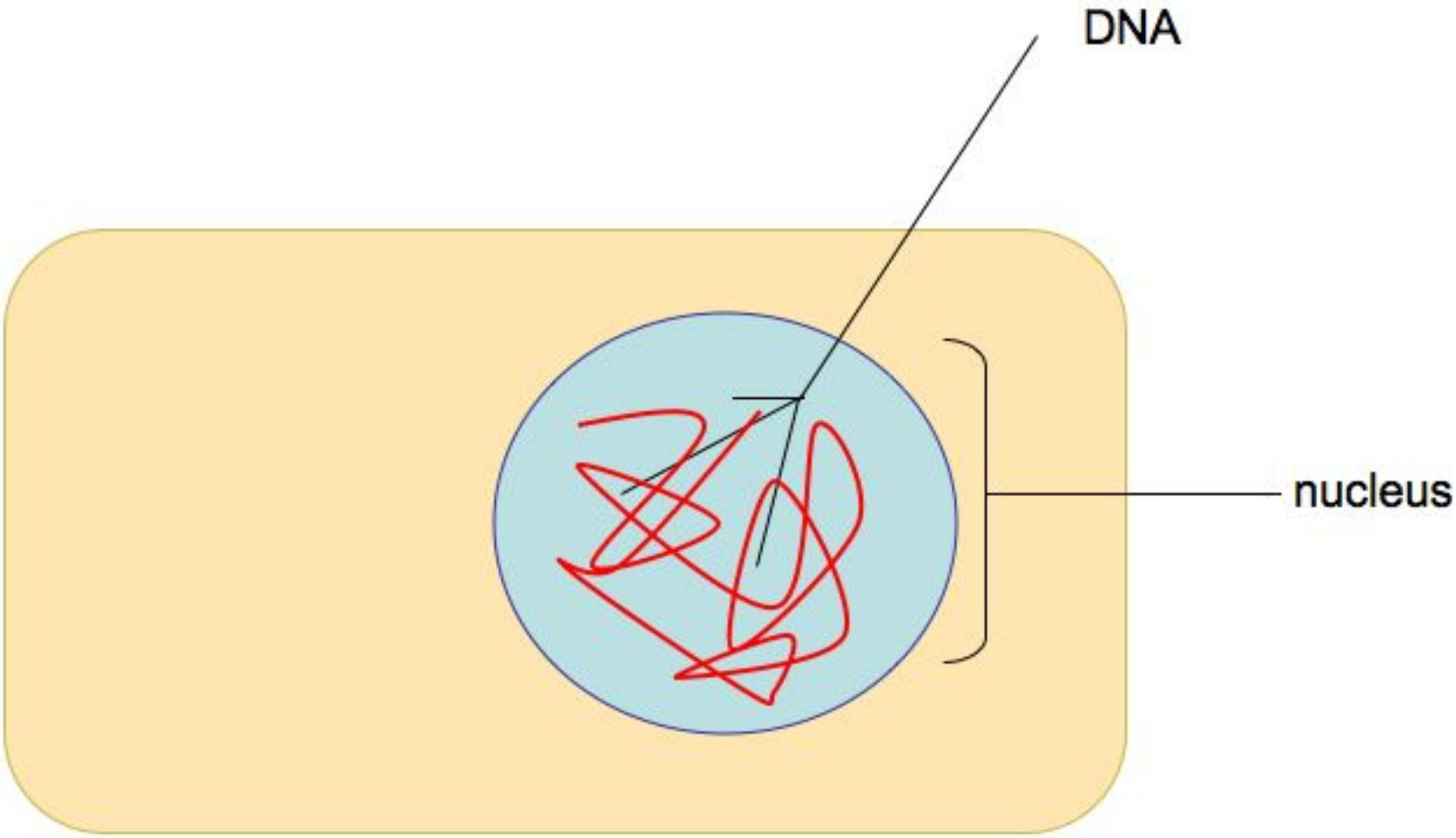


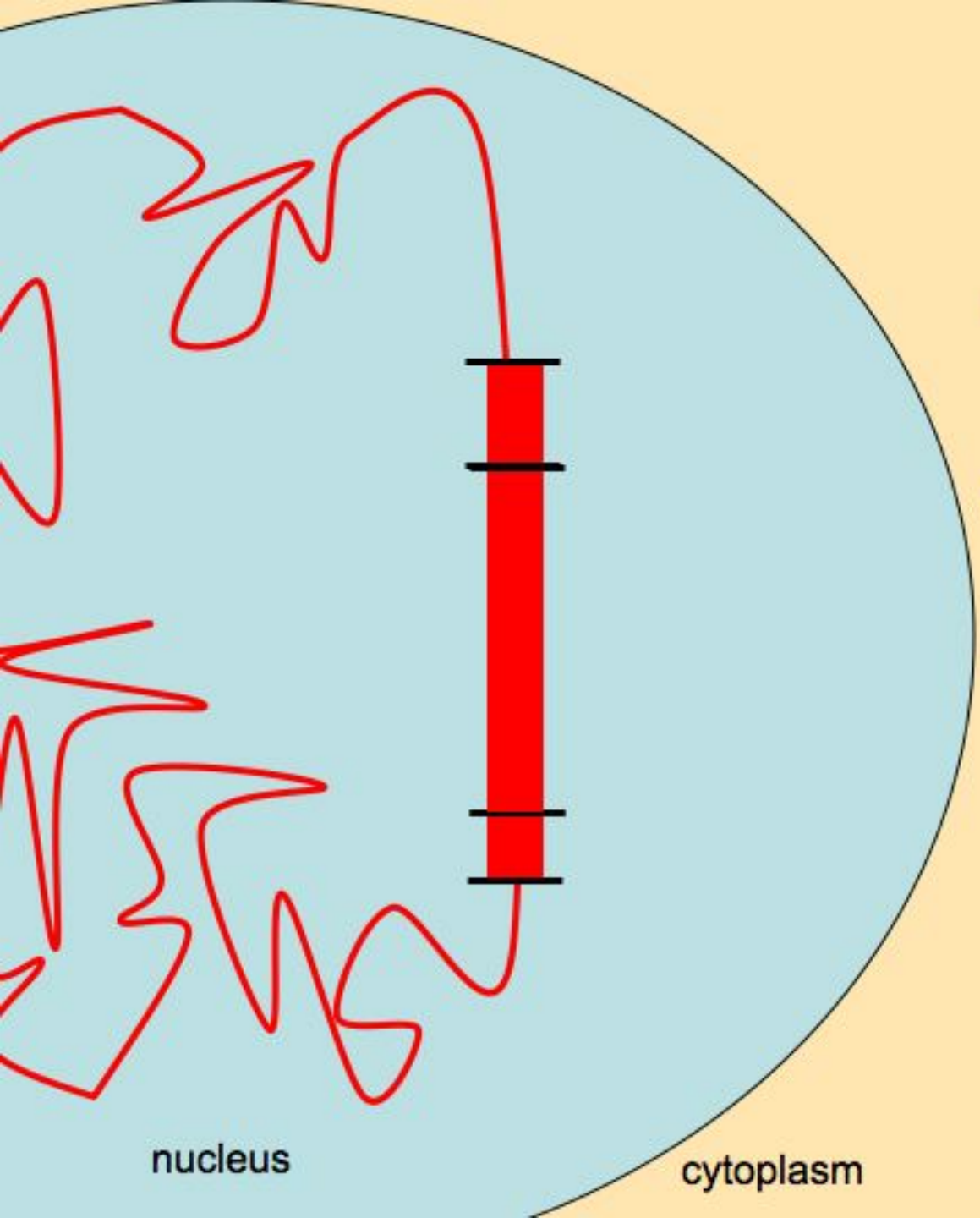
NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

TRANSCRIPTION

- Copying of one strand of DNA into a complementary RNA sequence by the enzyme RNA polymerase
- Necessary components for Transcription
  - DNA template
  - RNA polymerase
  - Transcription factors
  - Nucleoside triphosphates
- Three phases of transcription
  - Initiation
  - Elongation
  - Termination

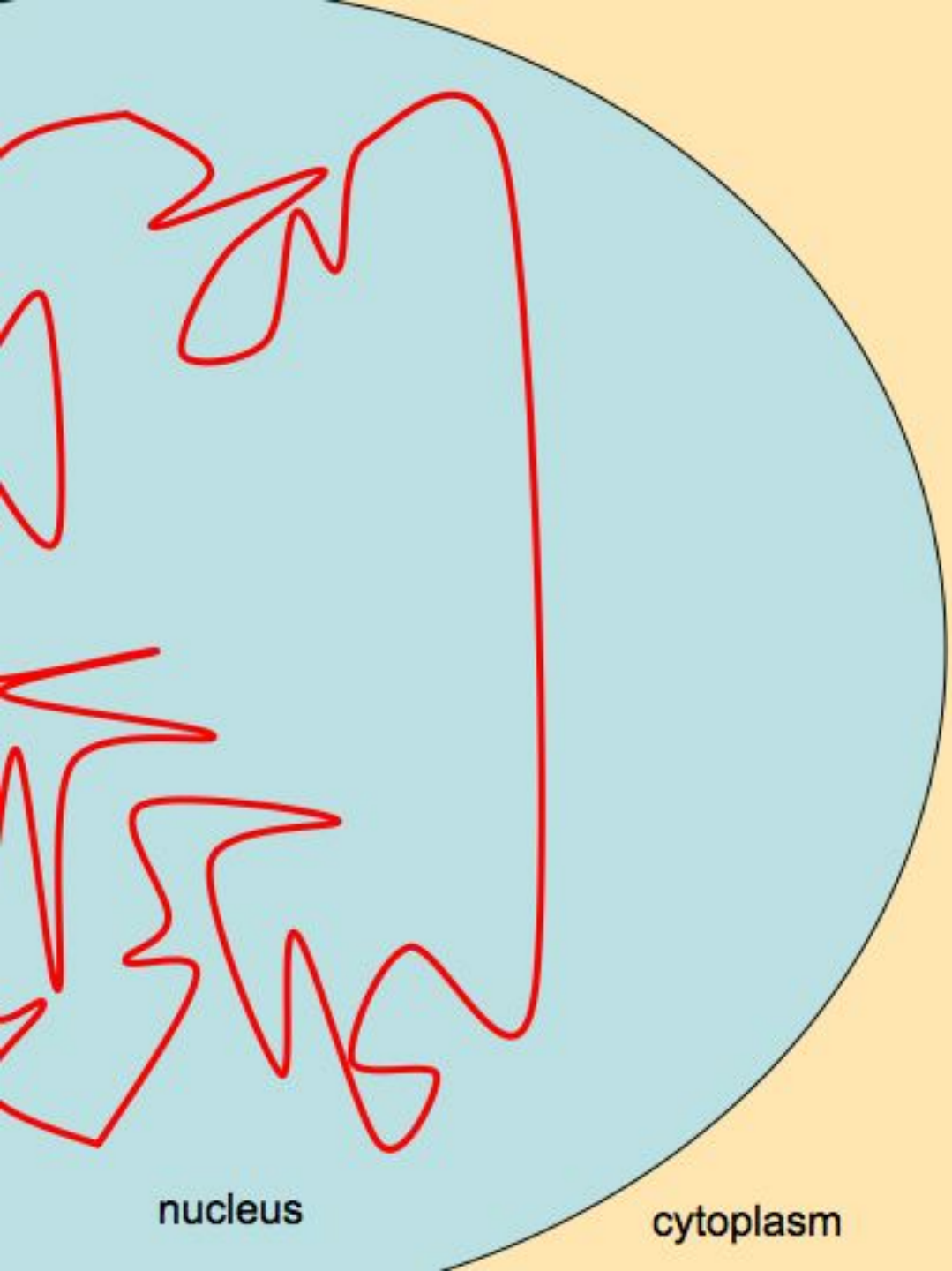
Genetic information is stored in the DNA within the nucleus of the cell





Every gene has three basic components

1. Promoter – non-coding sequence, directs RNA polymerase to the start of gene
2. Coding Region – encodes information directing which protein to produce
3. Termination Sequence – signals the end of a gene

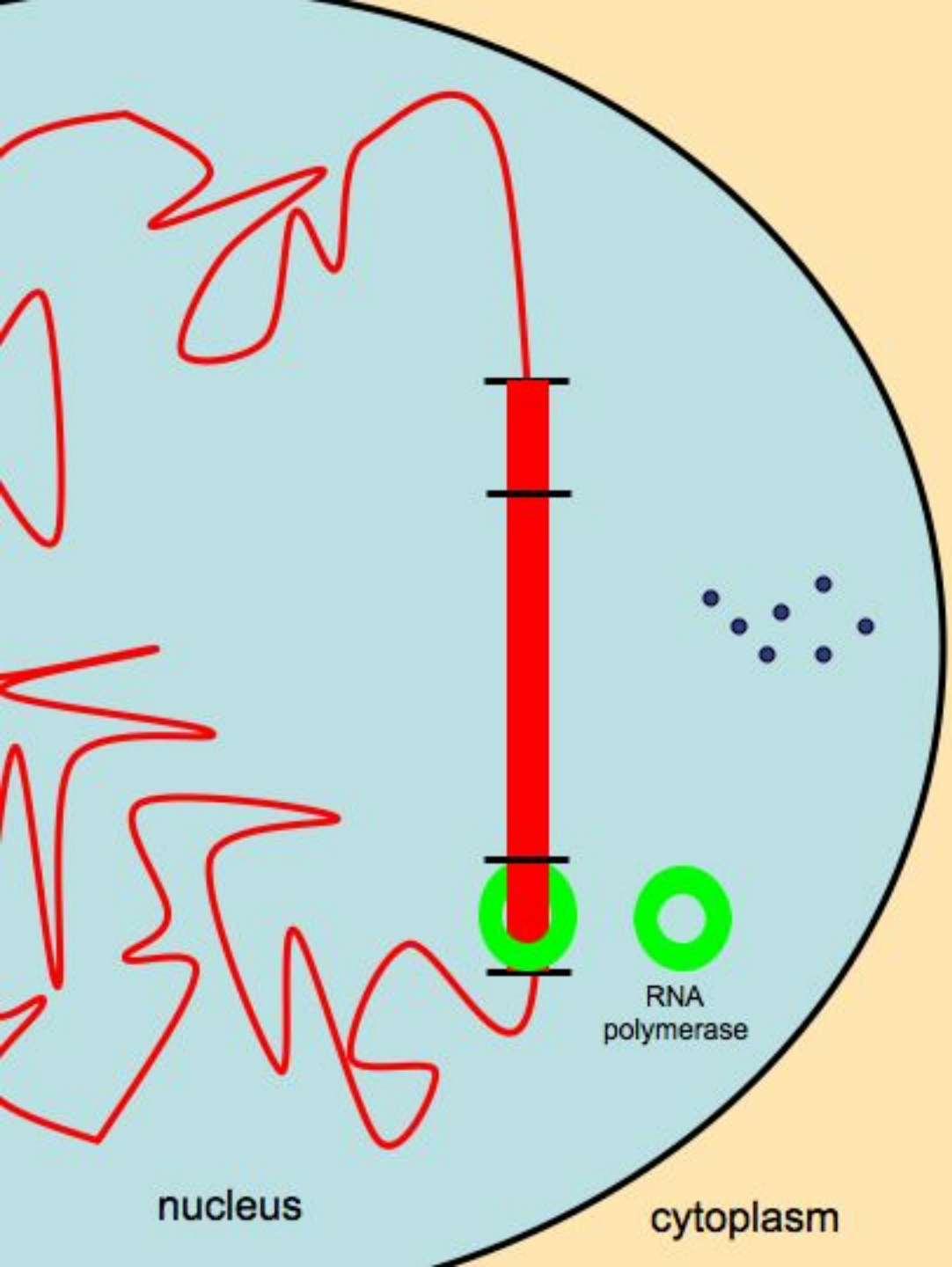


TRANSCRIPTION
is the first stage of protein synthesis

What is transcription?
Copying of a gene into a complementary RNA by the enzyme RNA polymerase

Transcription happens in three stages

1. Initiation
2. Elongation
3. Termination



TRANSCRIPTION
Initiation

Necessary components for Transcription:

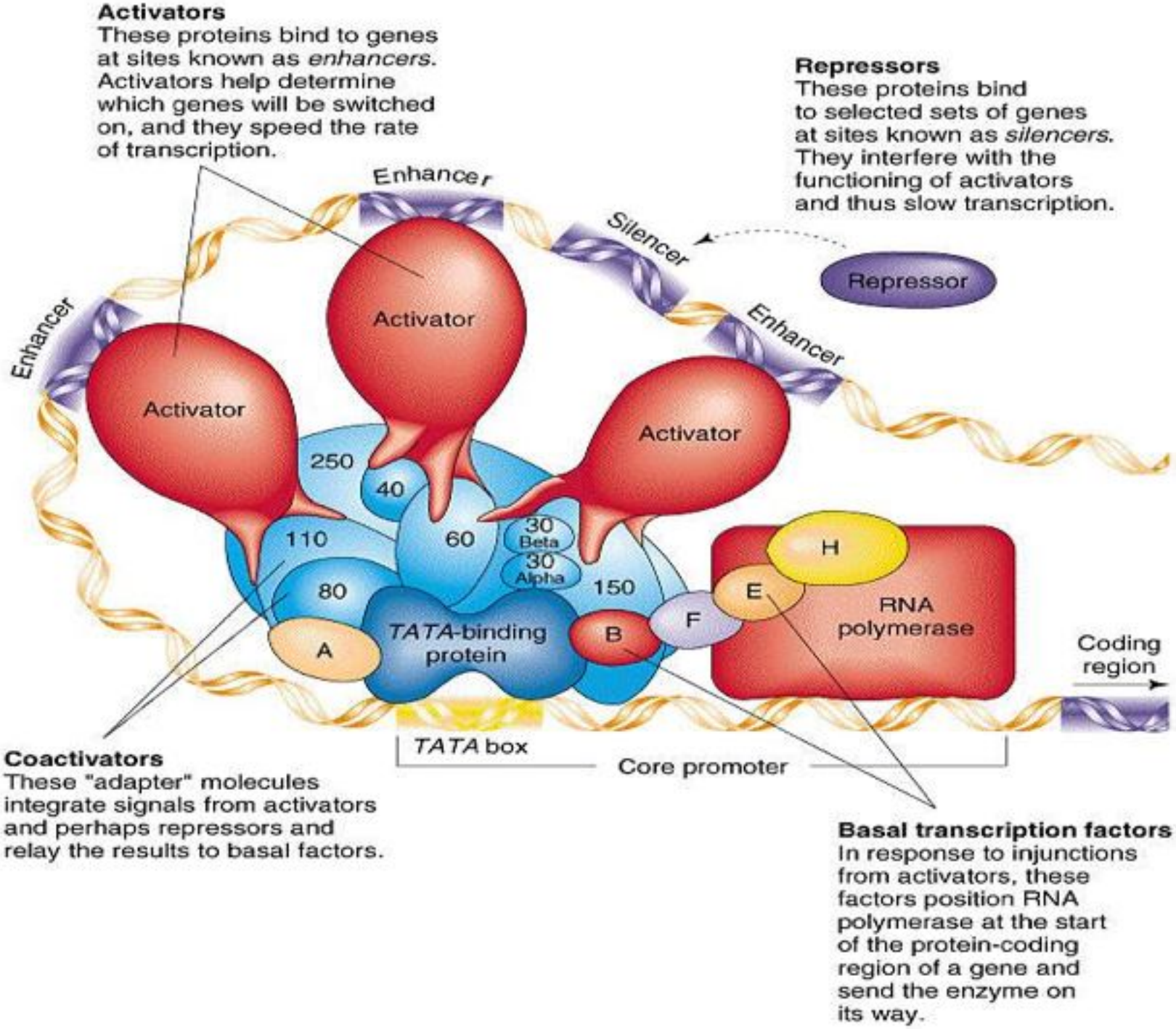
DNA template
RNA polymerase
Nucleoside triphosphates

RNA polymerase is responsible for reading the DNA code and building the RNA strand

RNA polymerase recognizes the promoter of the gene

Transcription Initiation in Eukaryotes

- ‘Unpacking’ of the DNA must occur
- Many Proteins must assemble at the promoter
  - RNA polymerase (I, II, or III)
  - General transcription factors
  - Activators
  - CoActivators
  - Repressors
  - Chromatin modifying proteins


Activators
These proteins bind to genes at sites known as *enhancers*. Activators help determine which genes will be switched on, and they speed the rate of transcription.
Repressors
These proteins bind to selected sets of genes at sites known as *silencers*. They interfere with the functioning of activators and thus slow transcription.
Enhancer
Silencer
Repressor
Enhancer
Enhancer
Activator
Activator
Activator
250
40
110
60
30 Beta
30 Alpha
80
150
A
TATA-binding protein
B
F
E
H
RNA polymerase
Coding region
TATA box
Core promoter
Coactivators
These "adapter" molecules integrate signals from activators and perhaps repressors and relay the results to basal factors.
Basal transcription factors
In response to injunctions from activators, these factors position RNA polymerase at the start of the protein-coding region of a gene and send the enzyme on its way.

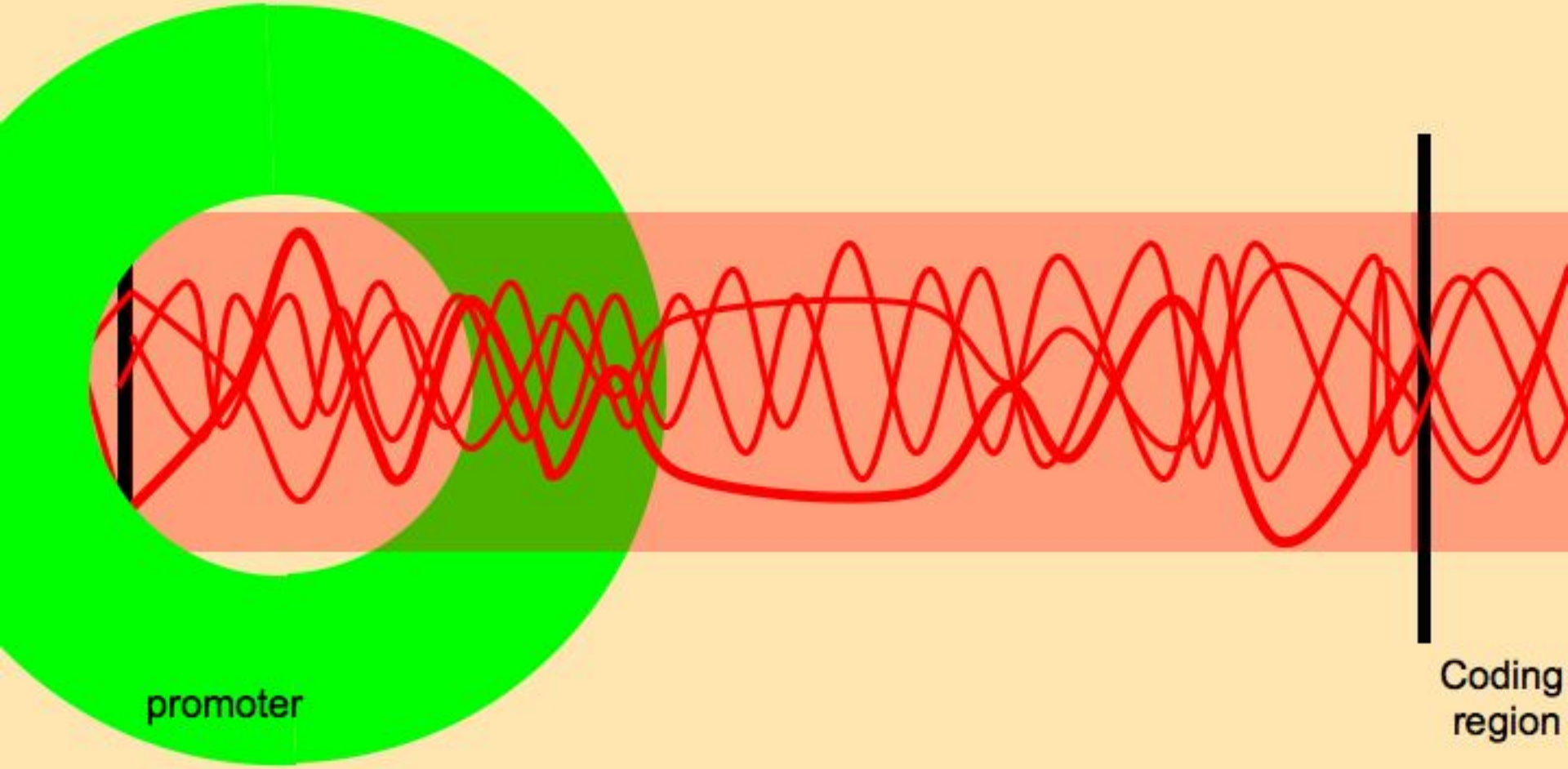
General Transcription Factors

- Must assemble at the promoter with the polymerase before txn can begin
- Help to position the RNA polymerase correctly at the promoter
- Aid in pulling apart the two strands of DNA
- Releases RNA polymerase from the promoter into elongation
- Aid RNApolII at all promoters

Activators, Repressors

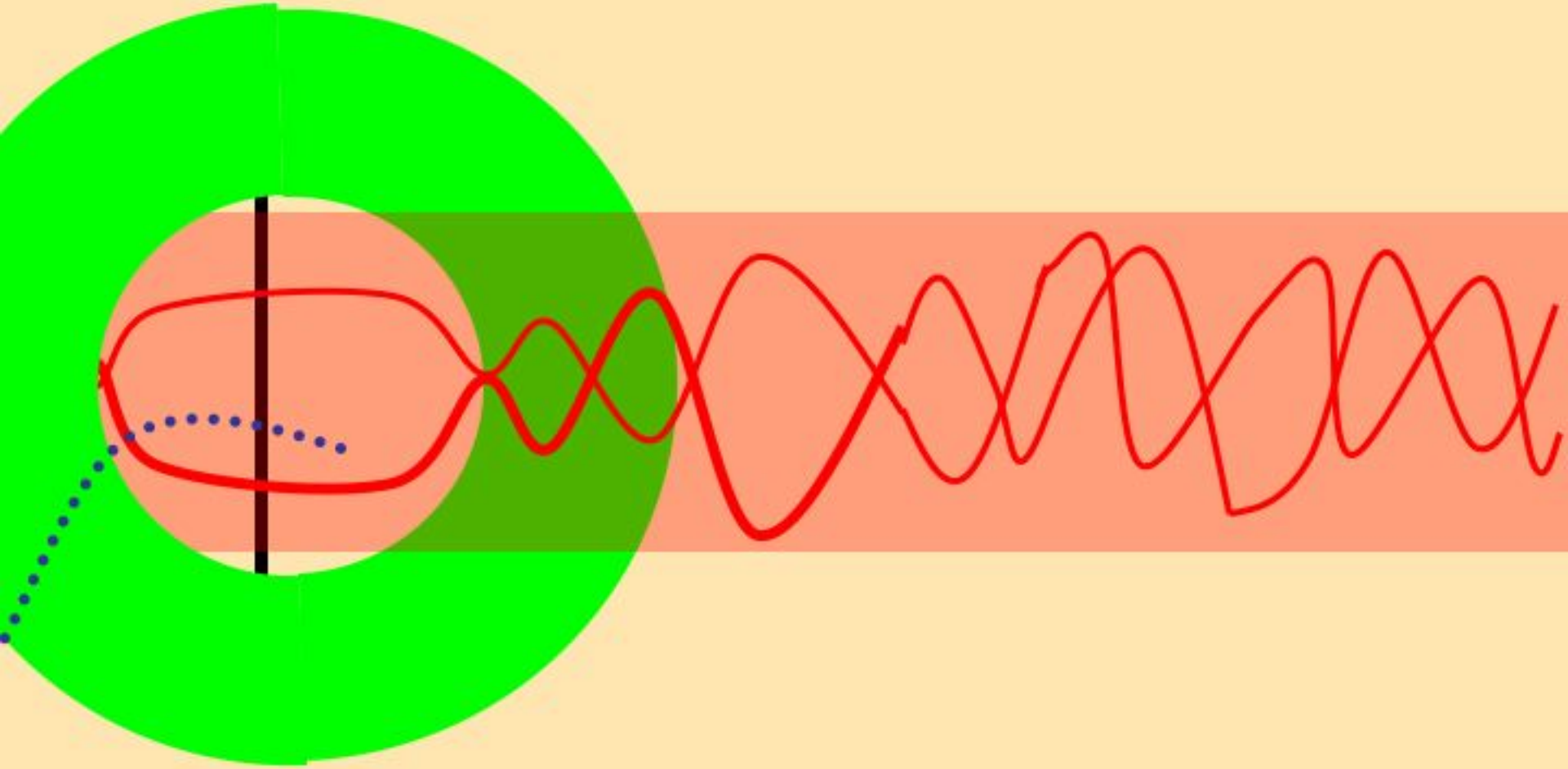
- Act in a more gene specific fashion
- Respond to the cell's need for a given protein and ensure the proper transcriptional control

The RNA polymerase binds to the promoter
and the DNA double helix begins to unwind



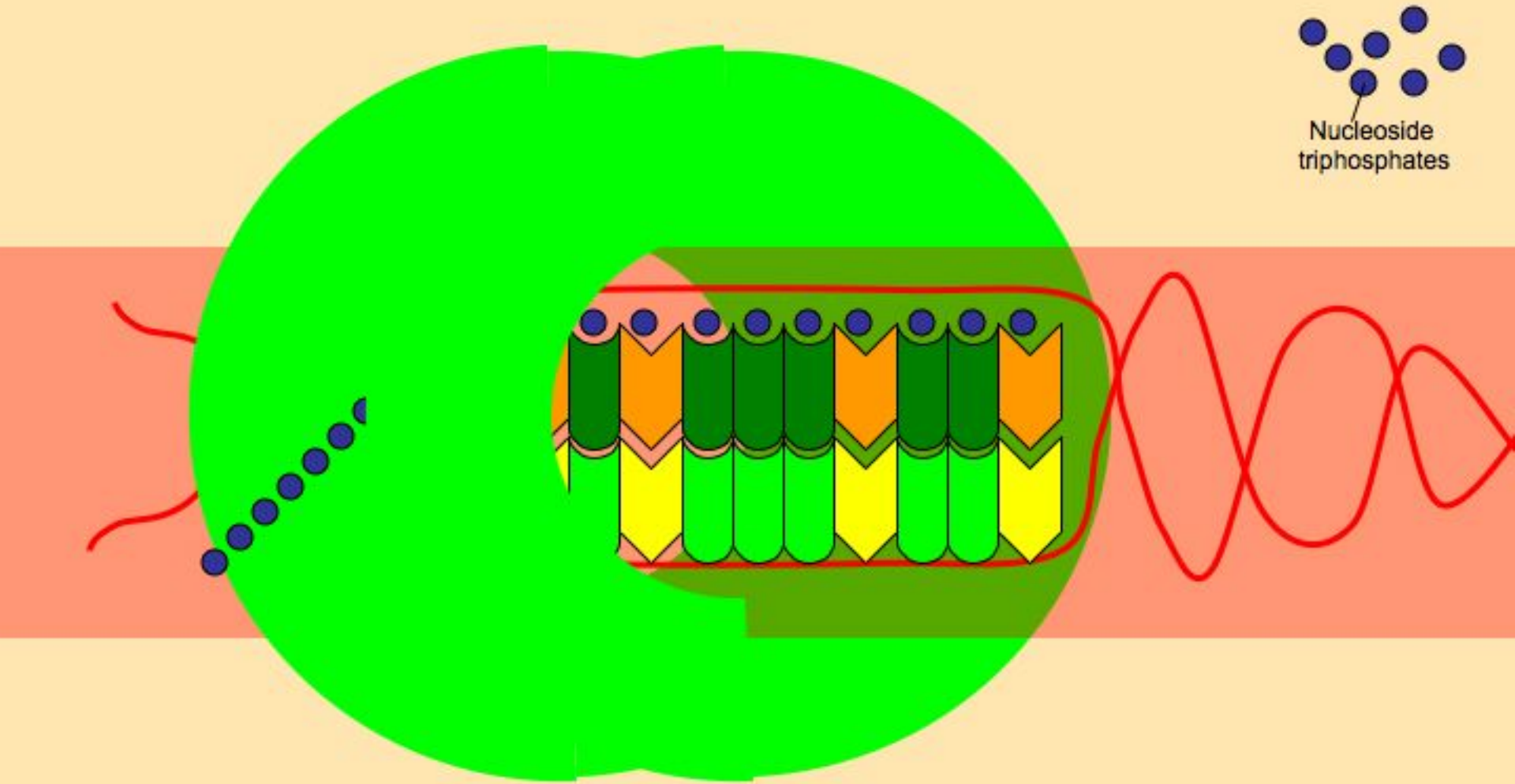
TRANSCRIPTION INITIATION

Once the RNA polymerase reaches the coding region
It reads one strand of the DNA and begins to build a new mRNA molecule



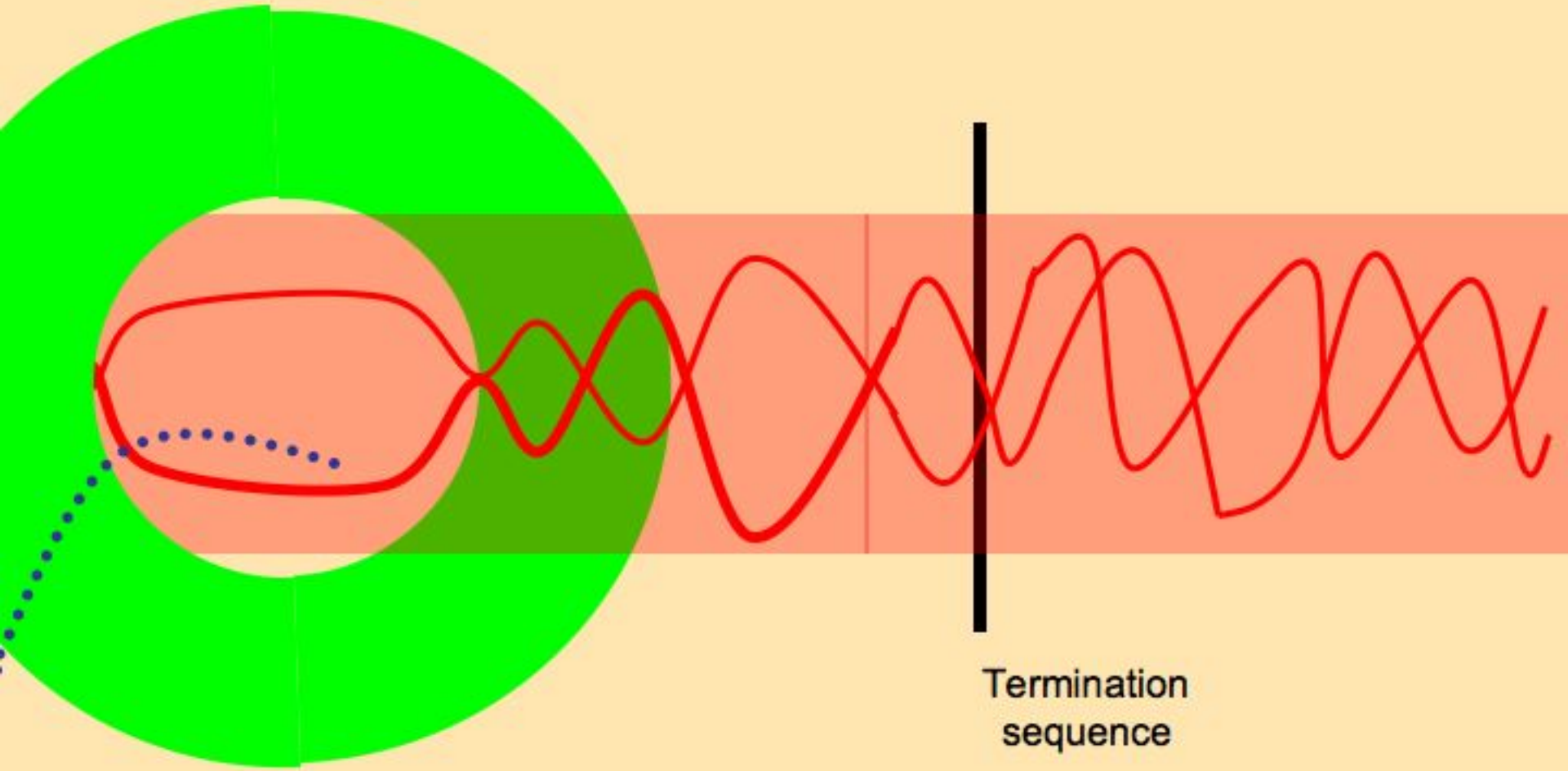
TRANSCRIPTION ELONGATION

As the RNA polymerase reads each nucleotide in the DNA, it brings in A complementary nucleotide of RNA forming a new mRNA molecule.

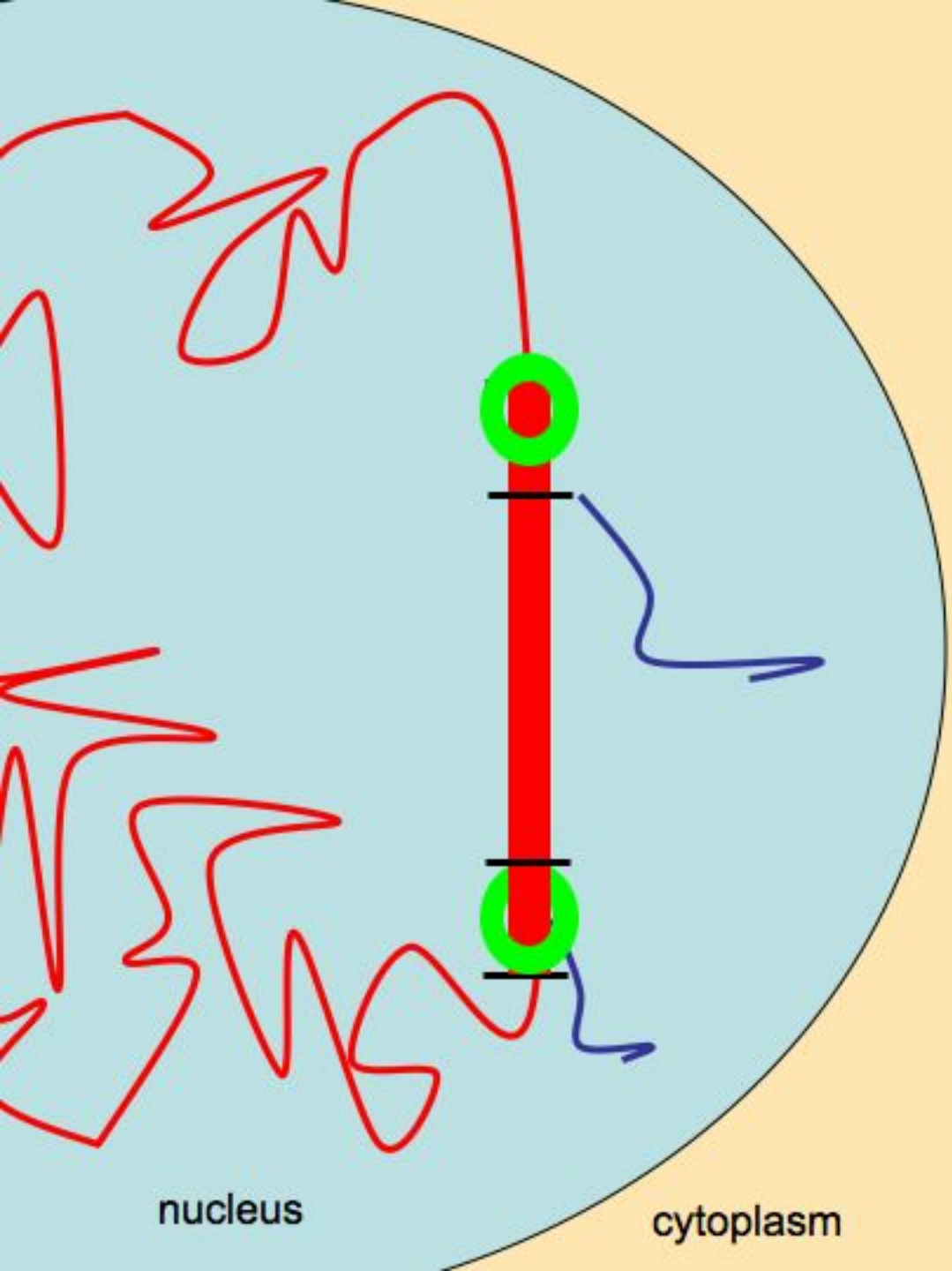


As the mRNA is made, it dissociates from DNA and the DNA rewinds

When the RNA polymerase reaches a termination sequence, RNA polymerase and the new mRNA fall off the DNA strand

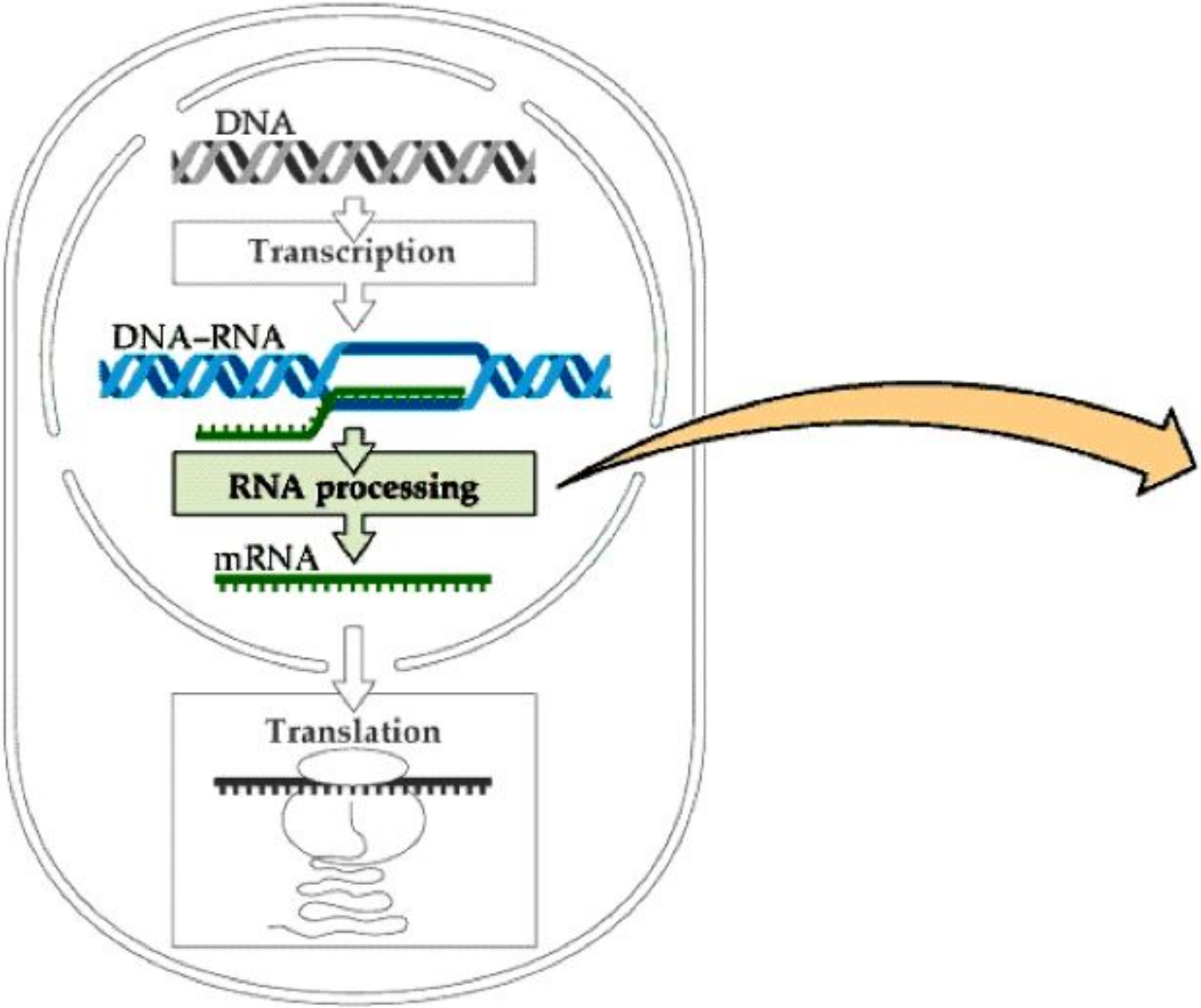


TRANSCRIPTION TERMINATION



RNA Processing

After the mRNA copies the genetic information from the DNA in the nucleus it travels into the cytoplasm, which is where translation takes place, but in order for the RNA to exit the nucleus it first has to be "processed" properly.

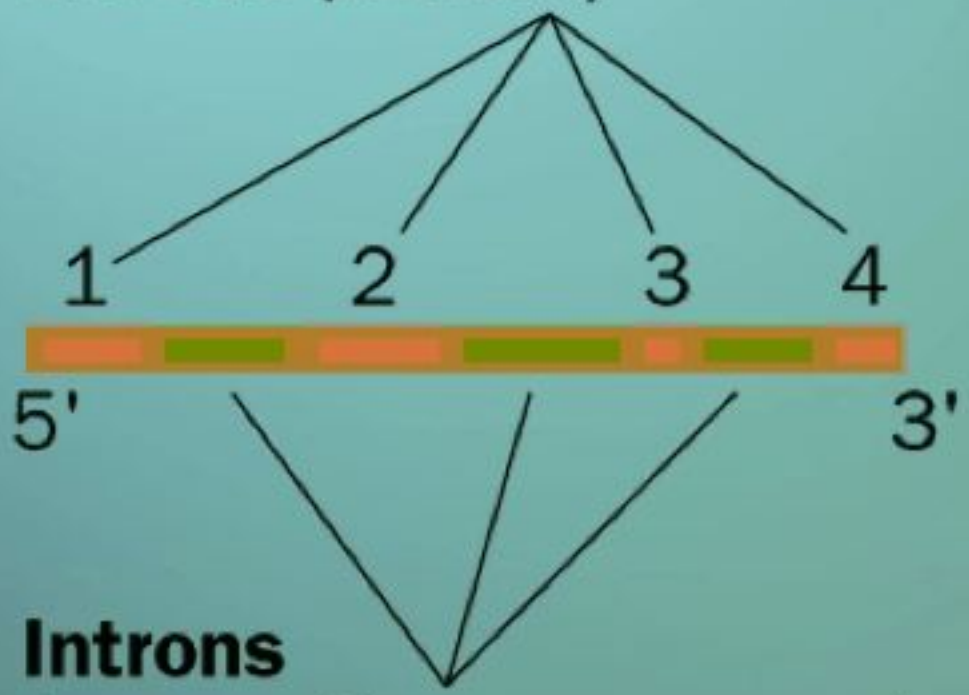

DNA
Transcription
DNA–RNA
RNA processing
mRNA
Translation

Transcription Elongation & RNA Processing

- Elongation in Eukaryotes is tightly coupled to RNA Processing
- RNA Processing
  - Capping
  - Slicing
  - Polyadenylation

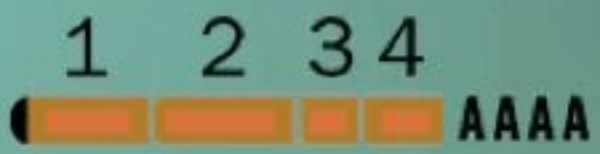
pre-mRNA

Exons (sequences that encode protein)



Introns (non-coding sequences)

Mature mRNA



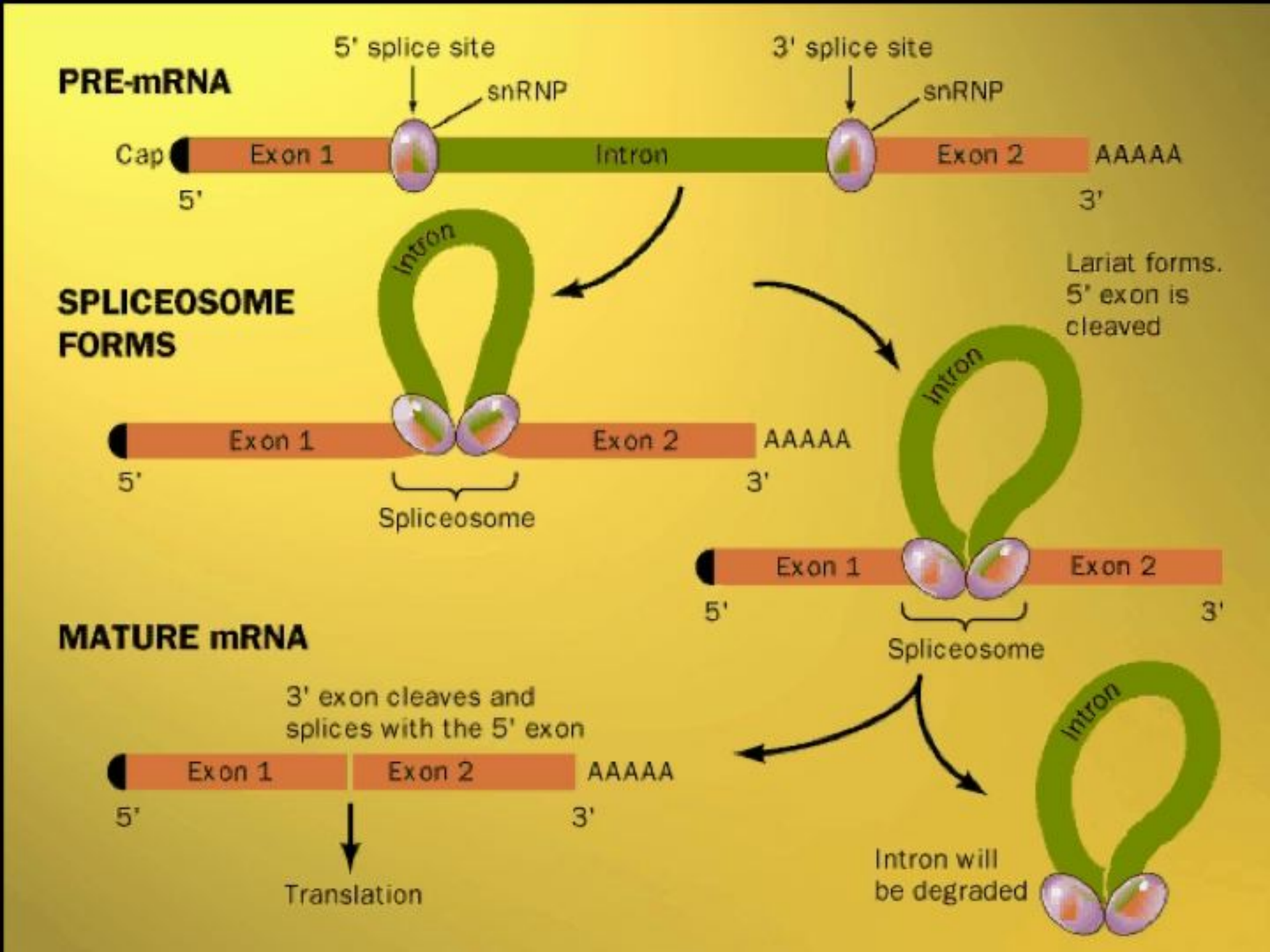
Exons 1–4 are now adjacent, ready for translation.

RNA capping

- Cap= modified guanine nucleotide
- 5 ‘ end of growing pre-mRNA
- Only pre-mRNAs are capped, this helps to distinguish them from other RNAs
- Cap plays an important role in translation

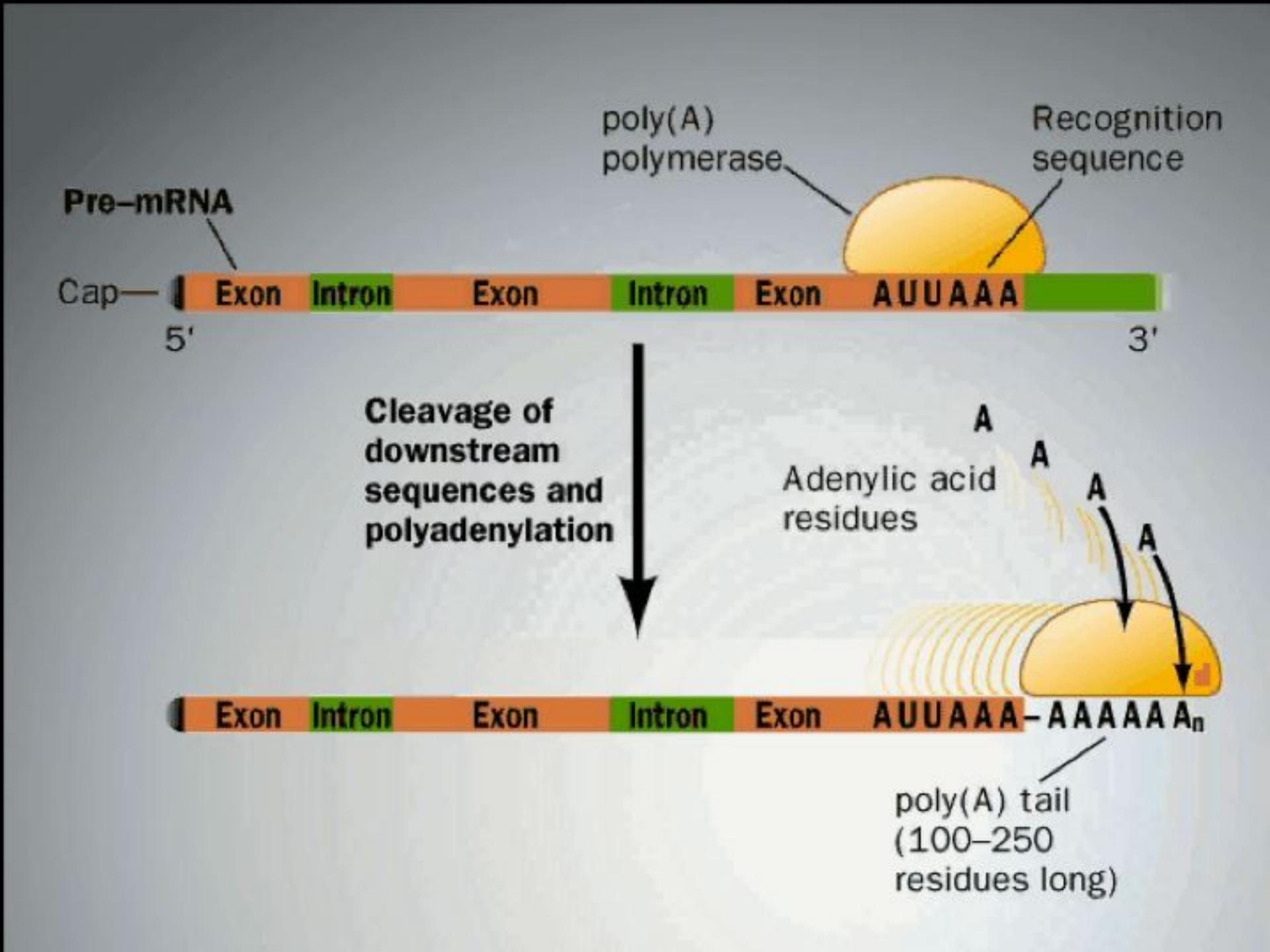
RNA splicing

- Removal of intron sequences
- Most splicing occurs on pre-mRNA
- Two phosphoryl-transfer reactions join the ends of the exons, removing the intron as a lariat
- Performed by the Spliceosome
  - RNA and Proteins


PRE-mRNA
5' splice site
snRNP
3' splice site
snRNP
Cap
Exon 1
Intron
Exon 2
AAAAA
5'
3'
SPLICEOSOME FORMS
Intron
Exon 1
Exon 2
AAAAA
5'
3'
Spliceosome
Lariat forms. 5' exon is cleaved
Intron
Exon 1
Exon 2
5'
3'
Spliceosome
MATURE mRNA
3' exon cleaves and splices with the 5' exon
Exon 1
Exon 2
AAAAA
5'
3'
Translation
Intron
Intron will be degraded

Poly-A Tail and Transcription Termination

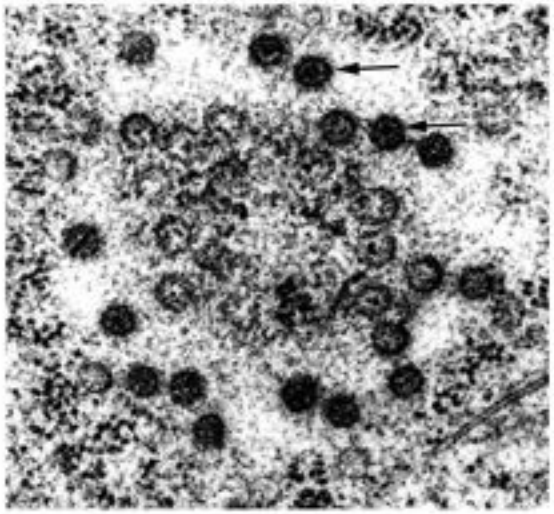
- Long sequence (often several hundred) of adenine nucleotides added to the tail (3' end) of the pre-mRNA
- Polyadenylate polymerase
- Added to growing transcripts that contain a specific sequence --- AAUAAA
- Aids in mRNA stability, protects it from exonucleases
- Important for transcription termination, export of the mRNA out of the nucleus, and translation


poly(A)
polymerase
Recognition
sequence
Pre–mRNA
Cap—
Exon
Intron
Exon
Intron
Exon
AUUAAA
5'
3'
Cleavage of
downstream
sequences and
polyadenylation
A
A
A
A
Adenylic acid
residues
Exon
Intron
Exon
Intron
Exon
AUUAAA–AAAAAA$_n$
poly(A) tail
(100–250
residues long)

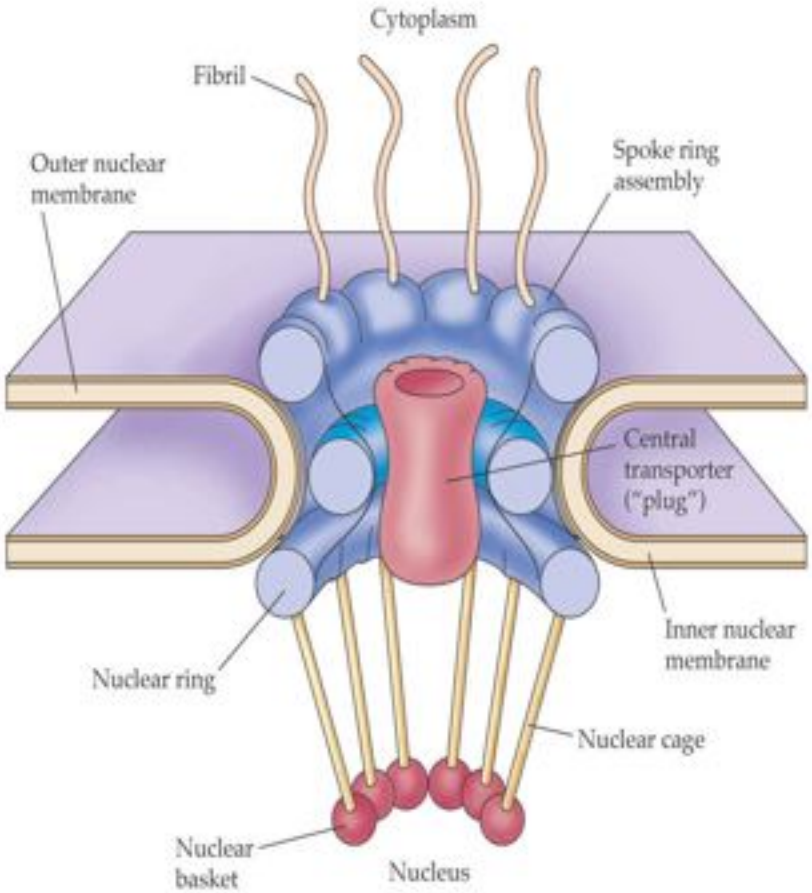
Fully processed mRNAs are exported for translation

- Through nuclear pore complex
  - Recognizes and transports ONLY completed mRNAs

(B)



(A)



Differences between prokaryotic and eukaryotic gene expression

1. In eukaryotes, one mRNA = one protein. (in bacteria, one mRNA can be polycistronic, or code for several proteins).
2. DNA in eukaryotes forms a stable, compacted complex with histones. In bacteria, the chromatin is not in a permanently condensed state.
3. Eukaryotic DNA contains large regions of repetitive DNA, whilst bacterial DNA rarely contains any "extra" DNA.
4. Much of eukaryotic DNA does not code for proteins (~98% is non-coding in humans); in bacteria often more than 95% of the genome codes for proteins.
5. Sometimes, eukaryotes can use controlled gene rearrangement for increasing the number of specific genes. This happens rarely in bacteria.
6. Eukaryotic genes are split into exons and introns; in bacteria, genes are almost never split.
7. In eukaryotes, mRNA is synthesized in the nucleus and then processed and exported to the cytoplasm; in bacteria, transcription and translation can take place simultaneously off the same piece of DNA.