Topics

- Gene inheritance
  - Genetic disorders
- Transcription
  - Mechanism
  - RNA processing
- Regulation of Gene Expression

The flow of information in a cell

The master set of instructions — DNA

Replication

Transcription

The messenger and intermediary — RNA

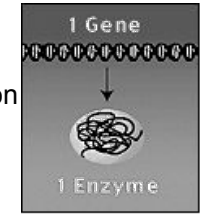
Translation

The product and executor — Protein

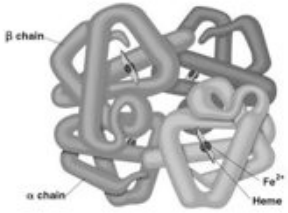
The Central Dogma

The flow of information in a cell

Oversimplification



One gene-one polypeptide?

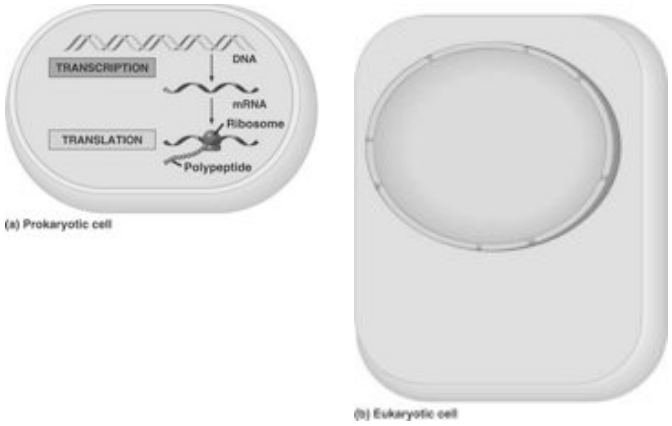


DNA is **Transcribed** into RNA

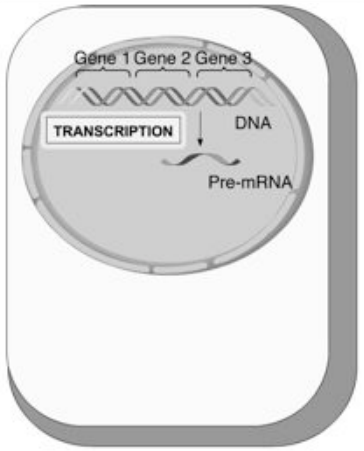
Q: Why not use DNA directly? Why bother with RNA?

- **Protection**. There is only **ONE** copy of each allele (gene) in the entire cell.
- **Amplification**. Transcription of one gene can make many RNAs
- **Efficiency.** Each RNA transcript can be repeatedly translated.

Basic Principles of Transcription and Translation



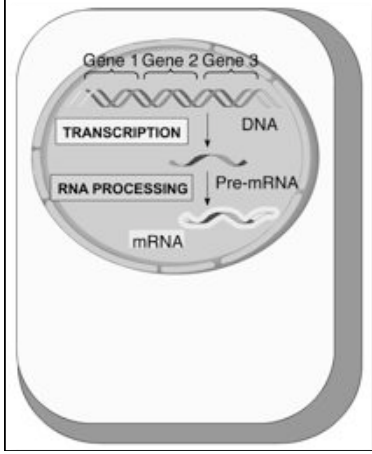
Gene Expression in Eukaryotes



Transcription to a "pre-mRNA" occurs in the nucleus. Also called a **primary transcript**.

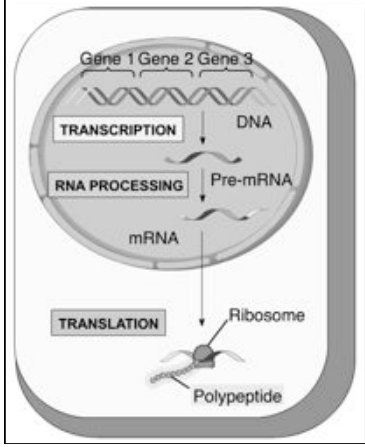
This is an exact replica of the gene sequence using **ribonucleotides** instead of deoxyribonucleotides

Gene Expression in Eukaryotes



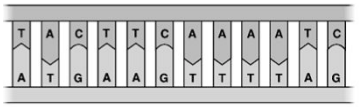
The pre-mRNA is "processed" into a **messenger RNA (mRNA)** *before leaving the nucleus*

Gene Expression in Eukaryotes



The mRNA sequence of nucleotides is **translated** into a sequence of amino acids in the cytoplasm with the help of **ribosomes**

Transcription/Translation

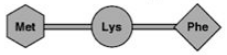


DNA

• One DNA strand is *transcribed* into RNA

RNA

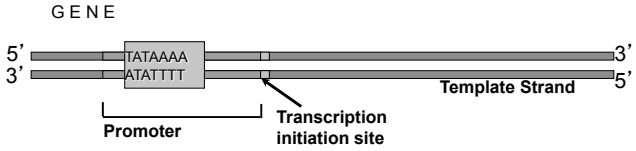
• The RNA is *translated* into protein

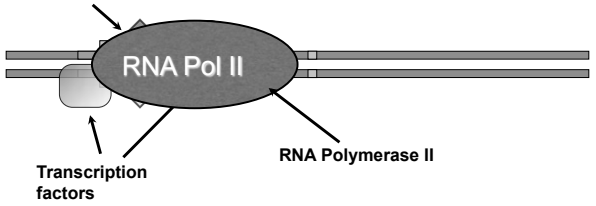


amino acids

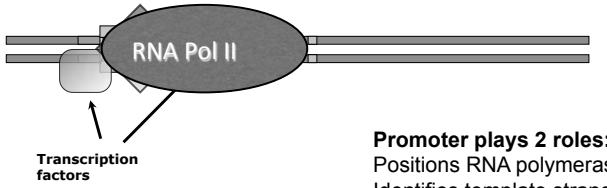
Protein

Transcription

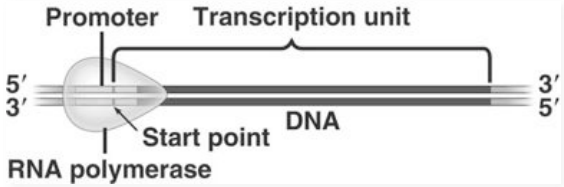




Transcription

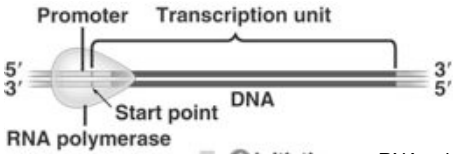


Promoter plays 2 roles:
Positions RNA polymerase
Identifies template strand



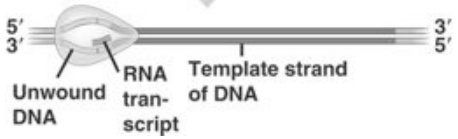
Transcription- 3 steps

1. Initiation



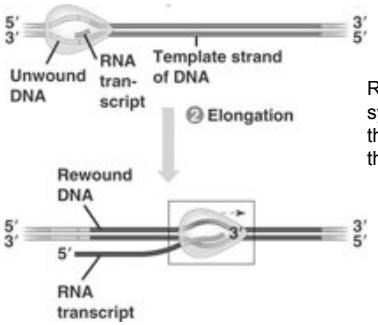
❶ Initiation

RNA polymerase and transcription factors bind the promoter, unwind the DNA strands and begins synthesizing at the start point.



Transcription- 3 steps

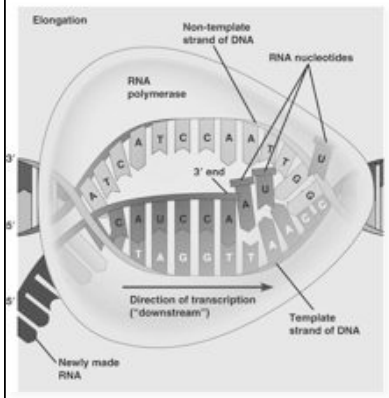
2. Elongation



RNA polymerase continues synthesizing an RNA complement in the 5’ to 3’ direction and rewinds the DNA behind it.

Transcription- 3 steps

2. Elongation

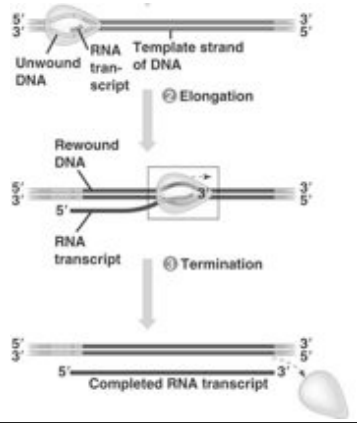


RNA polymerase continues synthesizing an RNA complement and rewinds the DNA behind it.

Do you remember how RNA and DNA nucleotides differ?

Transcription- 3 steps

3. Termination



The RNA polymerase “falls off” or is “pulled off” the template strand at places that make the RNA polymerase/ DNA interaction unstable.

Bacteria: termination signal

Humans: polyadenylation signal (AAUAAA)

RNA Processing

The RNA is not yet ready for translation and must be **processed** first

I. Alteration of RNA ends

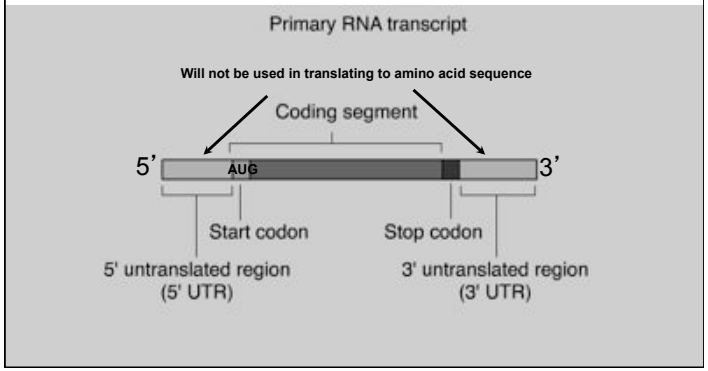
II. RNA splicing

Does 1 gene = >1 protein?

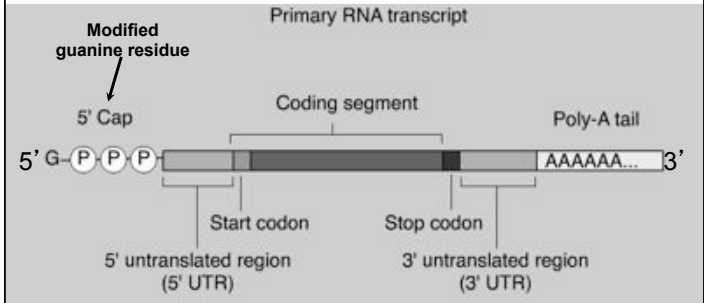
RNA Processing

The RNA is not yet ready for translation and must be **processed** first

I. Alteration of RNA ends

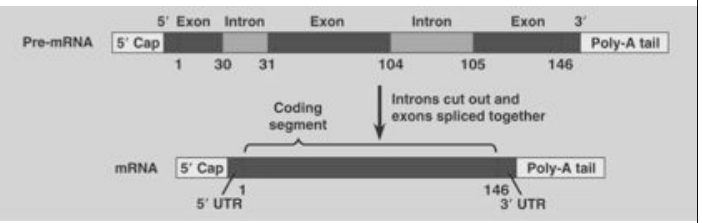


RNA Processing



The **5' cap** and **3' poly-A tail** help protect the RNA from being degraded

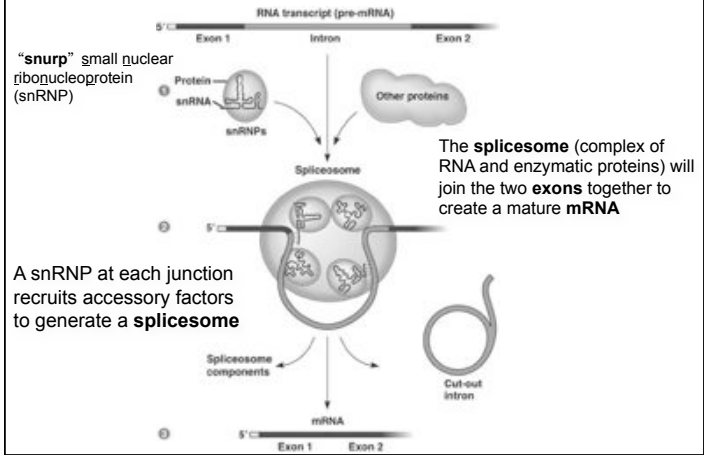
II. RNA splicing



DNA in eukaryotes contains both **exons** and **introns.**

Only **exon** sequences end up in the coding sequence of the protein.

How are introns removed?

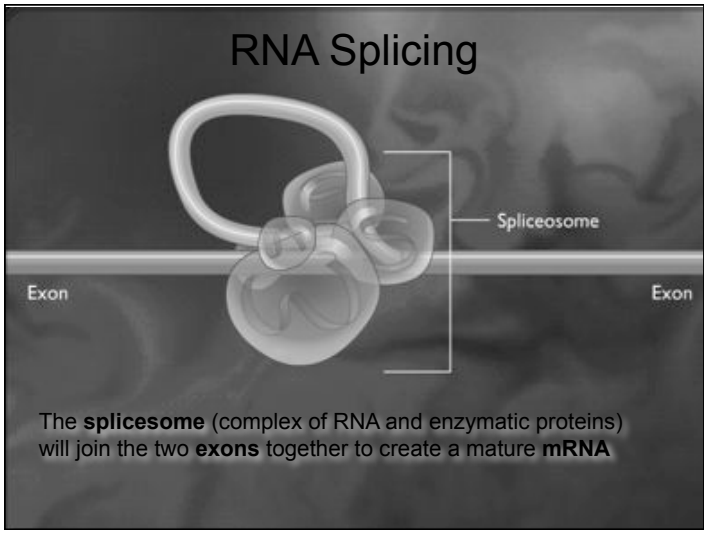


"**snurp**" small nuclear ribonucleoprotein (snRNP)

The **splicesome** (complex of RNA and enzymatic proteins) will join the two **exons** together to create a mature **mRNA**

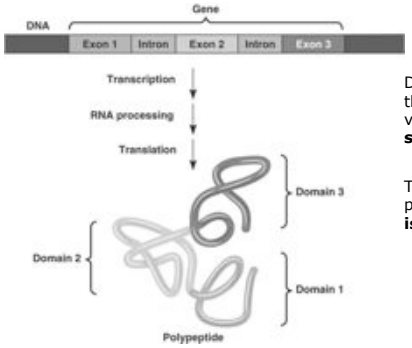
A snRNP at each junction recruits accessory factors to generate a **splicesome**

RNA Splicing



The **splicesome** (complex of RNA and enzymatic proteins) will join the two **exons** together to create a mature **mRNA**

What's the Deal with Introns?



Depending on how the cell treats them, introns can be exons, and vice-versa. Called **alternative splicing**.

This would create diversity in protein sequence. Different **isoforms** of a protein.

Differences between Prokaryotic and Eukaryotic Gene Expression

Characteristic	Prokaryotic	Eukaryotic
Transcription location	cytoplasm	nucleus
Translation location	Linked to transcription	cytoplasm
promoter	Shared by several genes	One gene = one promoter

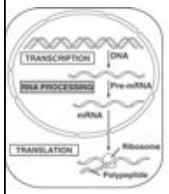
Differences between Prokaryotic and Eukaryotic Gene Expression

Characteristic	Prokaryotic	Eukaryotic
Transcription location	cytoplasm	nucleus
Translation location	Linked to transcription	cytoplasm
promoter	Shared by several genes	One gene = one promoter
Gene structure	Not interupted by introns	Transcribed regions often interrupted by non-coding introns
Post-transcriptional regulation	none	Introns removed 5' cap and 3' poly A tail added

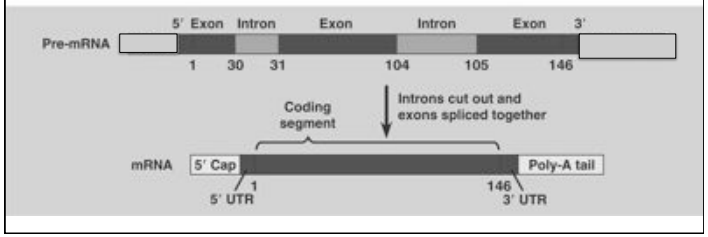
The Forms and Functions of RNA

Form	Function
Primary Transcript	The precursor to ALL RNAs. mRNAs begin as primary transcripts and are then processed to begin mature mRNAs.
messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes

RNA Processing



RNA processing. RNA processing begins during transcription and ends before the RNA leaves the nucleus as a **messenger RNA (mRNA)**



The Forms and Functions of RNA

Form	Function
Primary Transcript	The precursor to mRNA, rRNA or tRNA before being processed by splicing or cleavage.
messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes
snRNA	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA
Transfer RNA (tRNA)	Serves as adapter molecule in protein synthesis; translates mRNA codons into amino acids
Ribosomal RNA (rRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes

The Many Functions of RNA

What makes RNA so versatile?

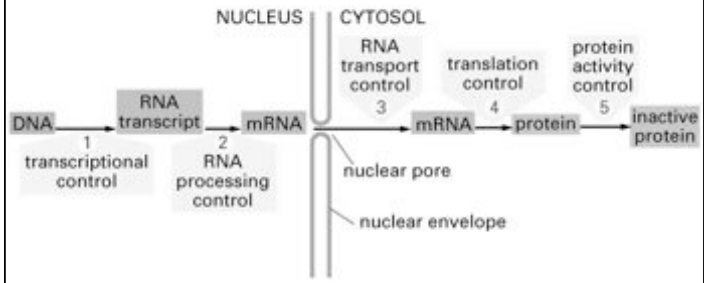
1. RNA is single-stranded. It can form hydrogen bonds with its bases to DNA, proteins or other RNAs.

2. RNA can develop base pairing with its own strand to create varied forms of 3-dimensional structure.

3. RNA's varied structure and functional groups can allow it to act as a type of enzyme... called **ribozymes**

Some RNAs are 'self-splicing'!

Regulation of Gene Expression



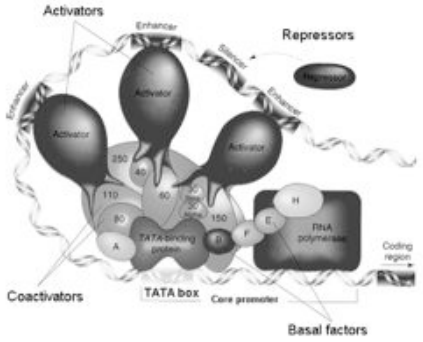
Gene expression is precisely regulated

Transcriptional Regulation in Eukaryotes

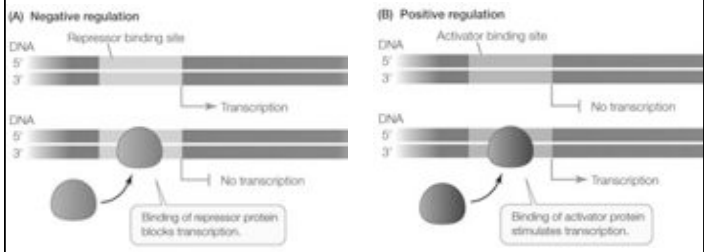
TATA-box: core promoter sequence bound by RNA pol, in 50-70% of promoters

General transcription factors: interact with most promoters, help position the RNA polymerase

Activators/Repressors: highly gene specific, allows varied gene expression



Positive vs. Negative Gene Regulation



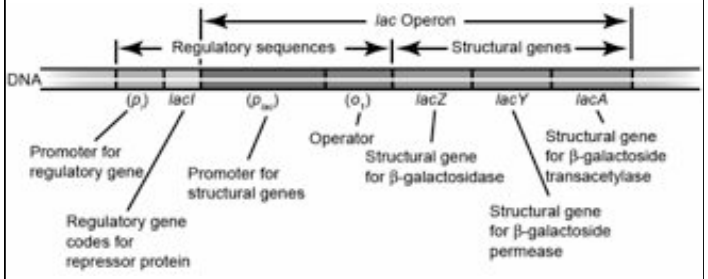
Inducible gene expression: expressed only when needed by the cell, involves repressors and activators

Constitutive gene expression: actively expressed at all times

Transcriptional Regulation in Prokaryotes

Operon: cluster of genes regulated by a single promoter

Operator: repressor binding site that helps regulate expression

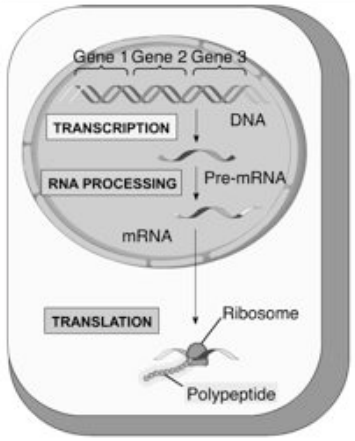


Why regulate gene expression?

Conserve energy
- regulating at level of transcription is most efficient
- protein synthesis is highly endergonic

To be able to respond to the environment
- Allows greater variety of proteins made as needed

Gene Expression in Eukaryotes



The mRNA sequence of nucleotides is **translated** into a sequence of amino acids in the cytoplasm with the help of **ribosomes**

The Code has a Reading Frame

Every three bases codes for an amino acid

Each triplet is called a **codon**

with three letter words representing codons

Example in frame:
"The red dog ate the cat"

Frameshifted by one:

"T her edd oga tet hec at"

The Nucleotide Sequence is a Code

AUGACCAAACCGAGUUGA

Cracking the Code

RNA

UUUUUUUUUUUUUUUUUUUUU...

↓

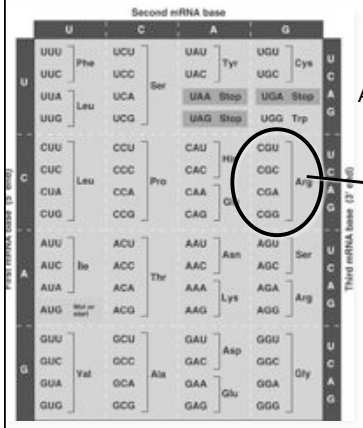
Protein

Phe-Phe-Phe-Phe-Phe...

AUG ACC AAA CCG AGU UGA

First position (5′ end)	Second position U	C	A	G	Third position (3′ end)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

The Code is Redundant



AUG (Met): always the starting codon

There are multiple **codons** for most amino acids

3rd base "wobble"

There is **NO** ambiguity...

Overview of amino acids