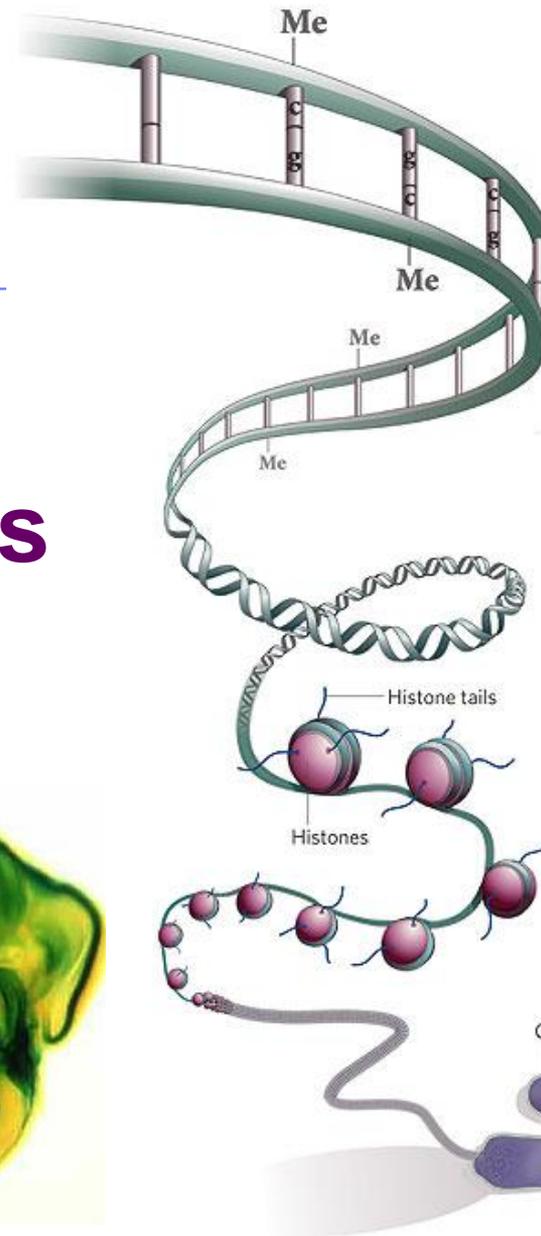


Control of Eukaryotic Genes



The two main components of the epigenetic code

DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

Histone modification

A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

Chromosome

The BIG Questions...

- How are genes turned on & off in eukaryotes?
- How do cells with the same genes differentiate to perform completely different, specialized functions?



(a) 5 weeks.



(b) 14 weeks.



(c) 20 weeks.

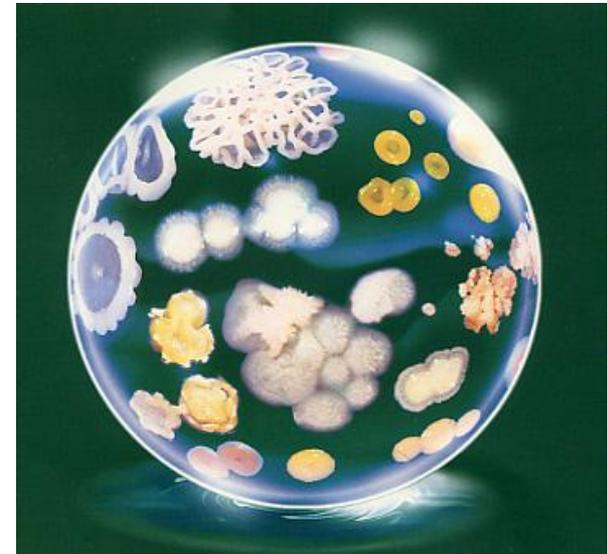
Evolution of gene regulation

■ Prokaryotes

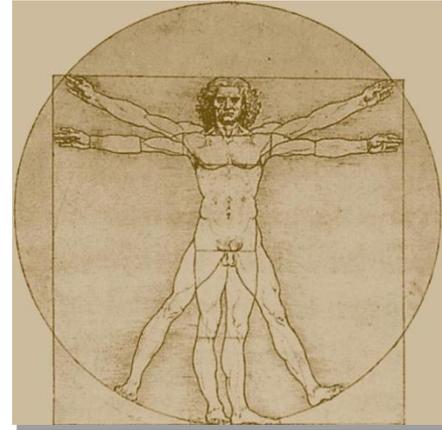
- ◆ single-celled
- ◆ evolved to grow & divide rapidly
- ◆ must respond quickly to changes in external environment
 - exploit transient resources

■ Gene regulation

- ◆ turn genes on & off rapidly
 - flexibility & reversibility
- ◆ adjust levels of enzymes for synthesis & digestion



Evolution of gene regulation



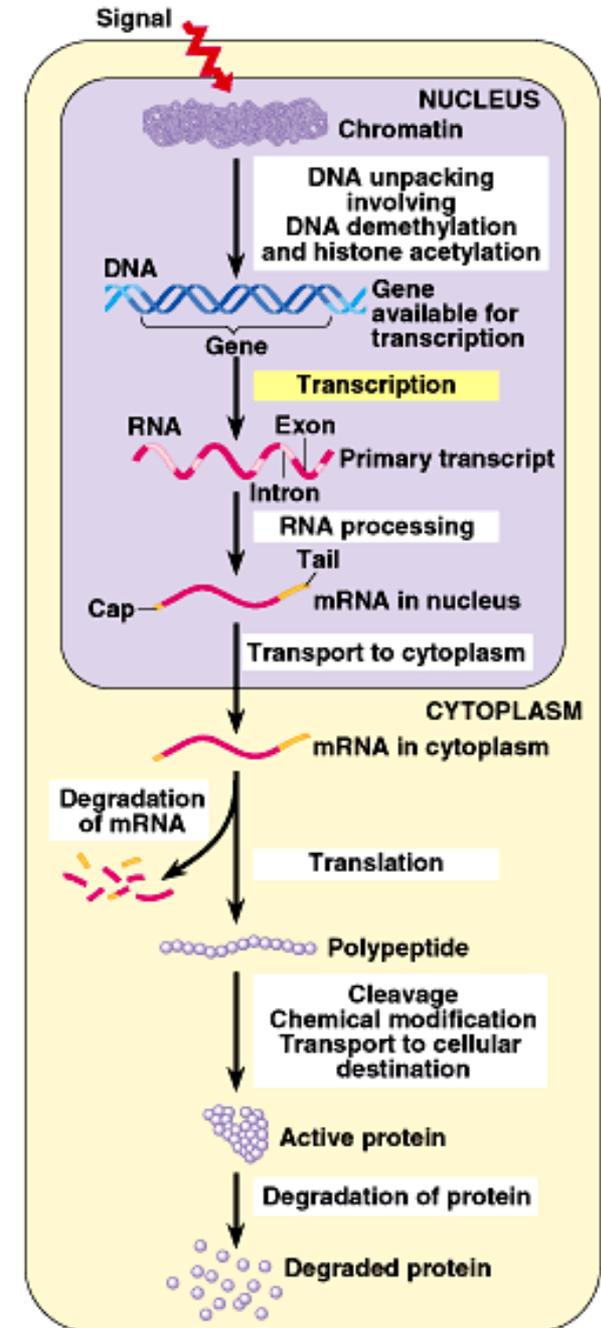
■ Eukaryotes

- ◆ multicellular
- ◆ evolved to maintain constant internal conditions while facing changing external conditions
 - homeostasis
- ◆ regulate body as a whole
 - growth & development
 - ◆ long term processes
 - specialization
 - ◆ turn on & off large number of genes
 - must coordinate the body as a whole rather than serve the needs of individual cells

Points of control

■ The control of gene expression can occur at any step in the pathway from gene to functional protein

1. packing/unpacking DNA
2. transcription
3. mRNA processing
4. mRNA transport
5. translation
6. protein processing
7. protein degradation

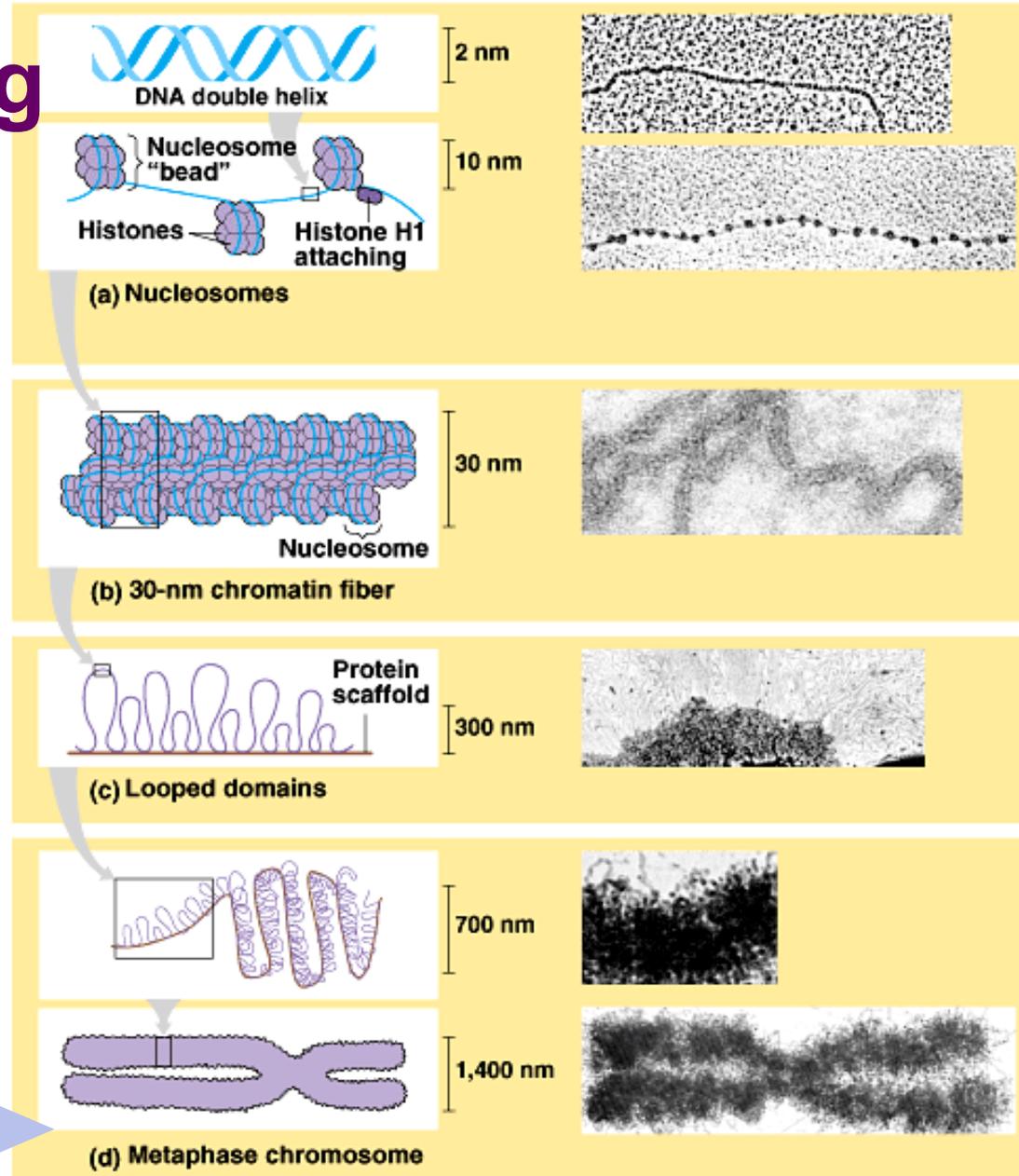


1. DNA packing

How do you fit all that DNA into nucleus?

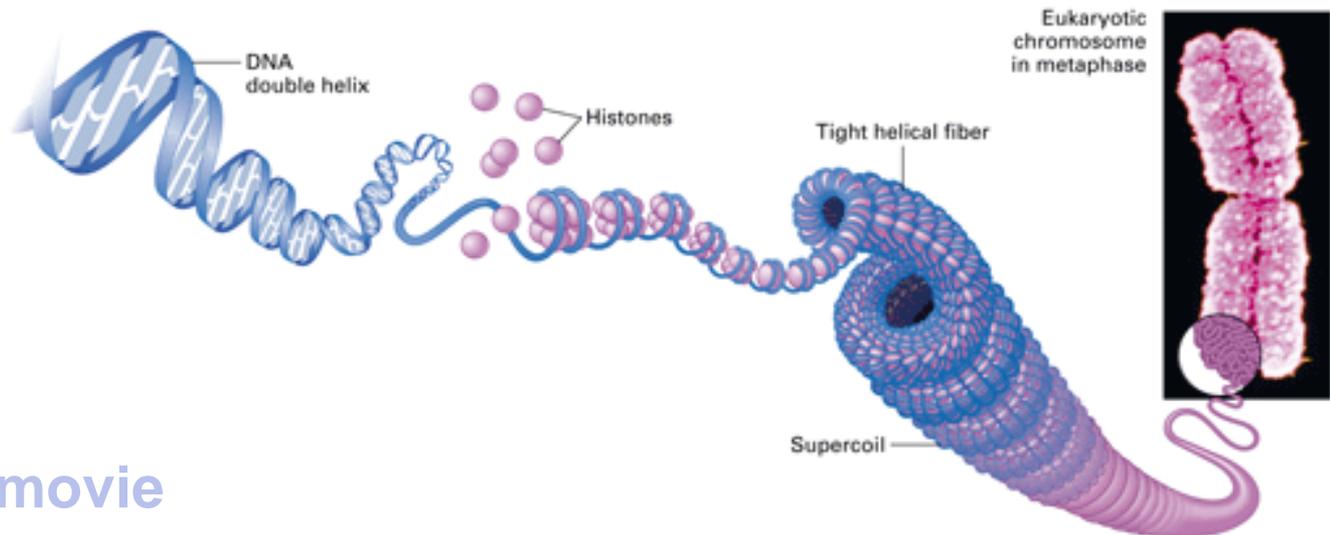
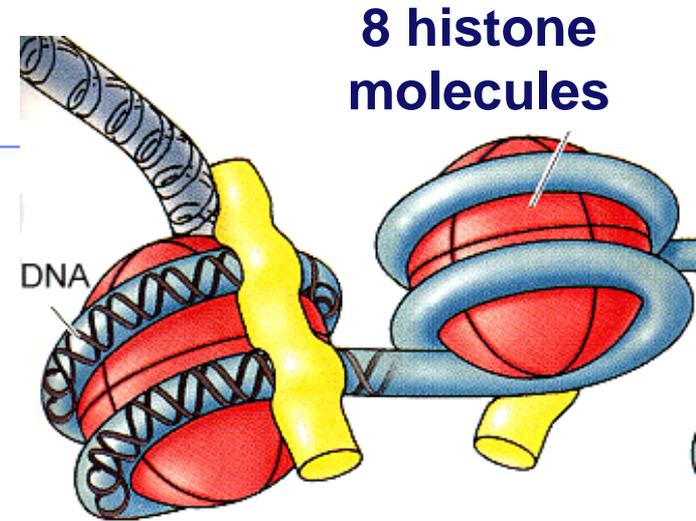
- ◆ DNA coiling & folding
 - double helix
 - nucleosomes
 - chromatin fiber
 - looped domains
 - chromosome

from DNA double helix to condensed chromosome



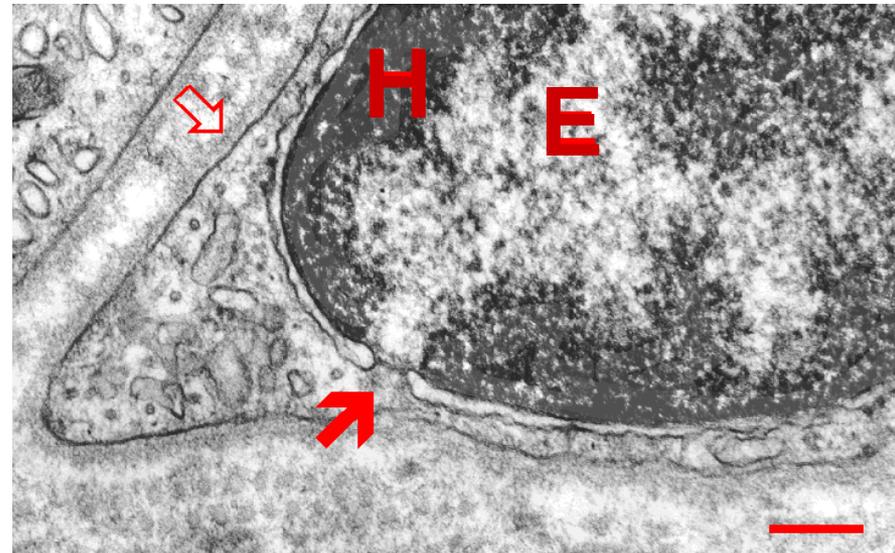
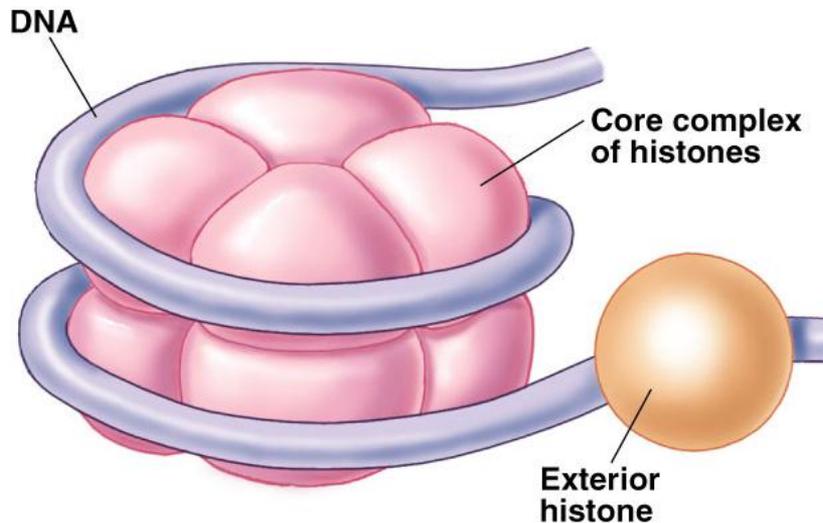
Nucleosomes

- “Beads on a string”
 - ◆ 1st level of DNA packing
 - ◆ histone proteins
 - 8 protein molecules
 - positively charged amino acids
 - bind tightly to negatively charged DNA



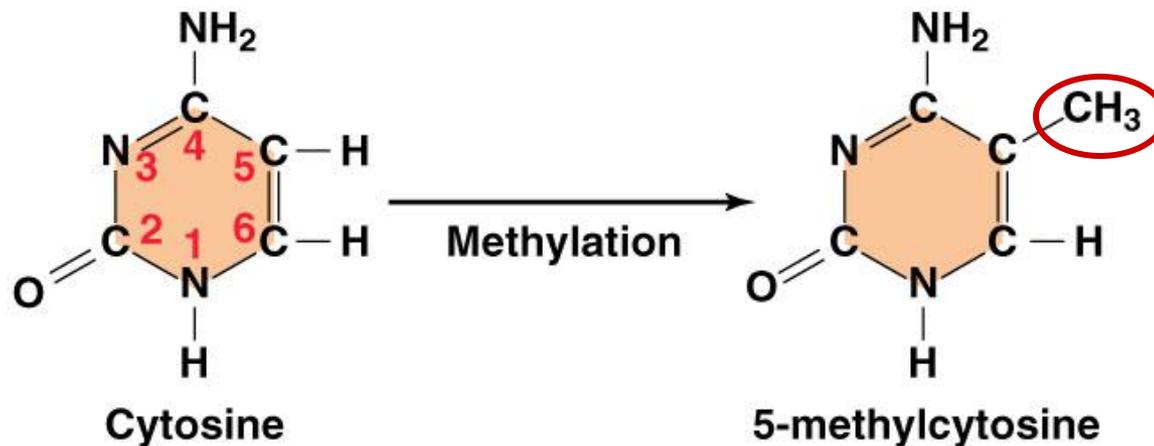
DNA packing as gene control

- Degree of packing of DNA regulates transcription
 - ◆ tightly wrapped around histones
 - no transcription
 - genes turned off
 - heterochromatin
darker DNA (H) = tightly packed
 - euchromatin
lighter DNA (E) = loosely packed



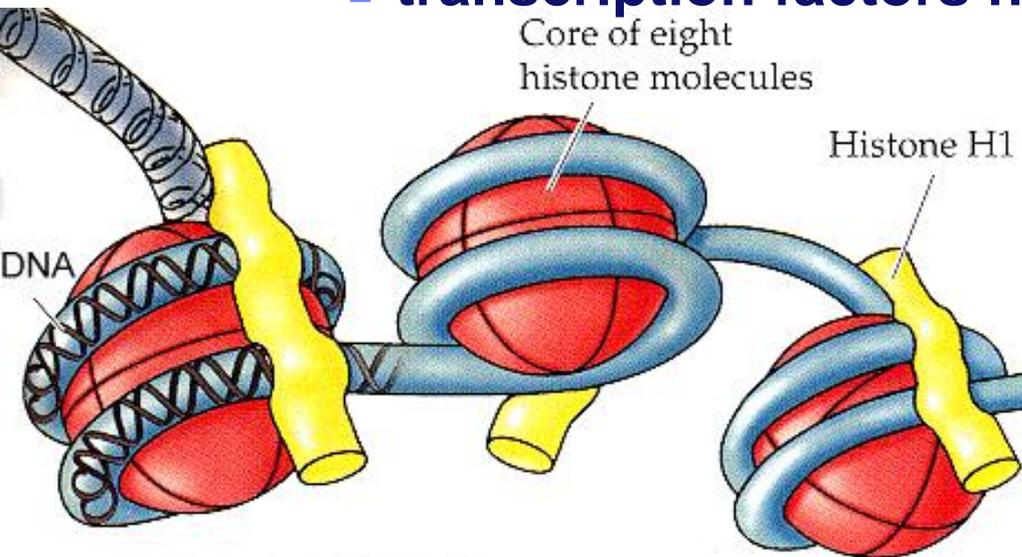
DNA methylation

- **Methylation of DNA** blocks transcription factors
 - ◆ no transcription
 - **genes turned off**
 - ◆ attachment of methyl groups ($-\text{CH}_3$) to cytosine
 - C = cytosine
 - ◆ nearly permanent inactivation of genes
 - ex. inactivated mammalian X chromosome = Barr body

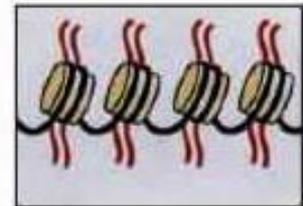


Histone acetylation

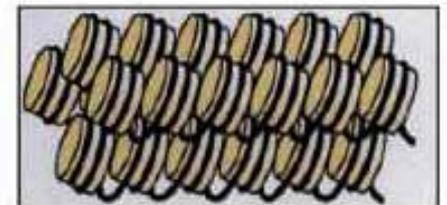
- Acetylation of histones unwinds DNA
 - ◆ loosely wrapped around histones
 - enables transcription
 - genes turned on
 - ◆ attachment of acetyl groups ($-\text{COCH}_3$) to histones
 - conformational change in histone proteins
 - transcription factors have easier access to genes



active/open chromatin



inactive/condensed chromatin



2. Transcription initiation

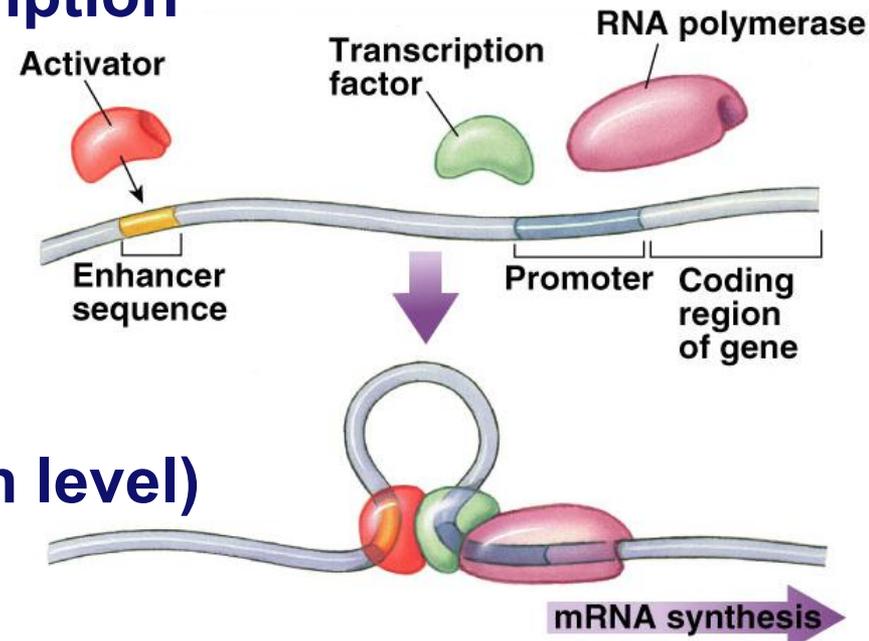
■ Control regions on DNA

◆ promoter

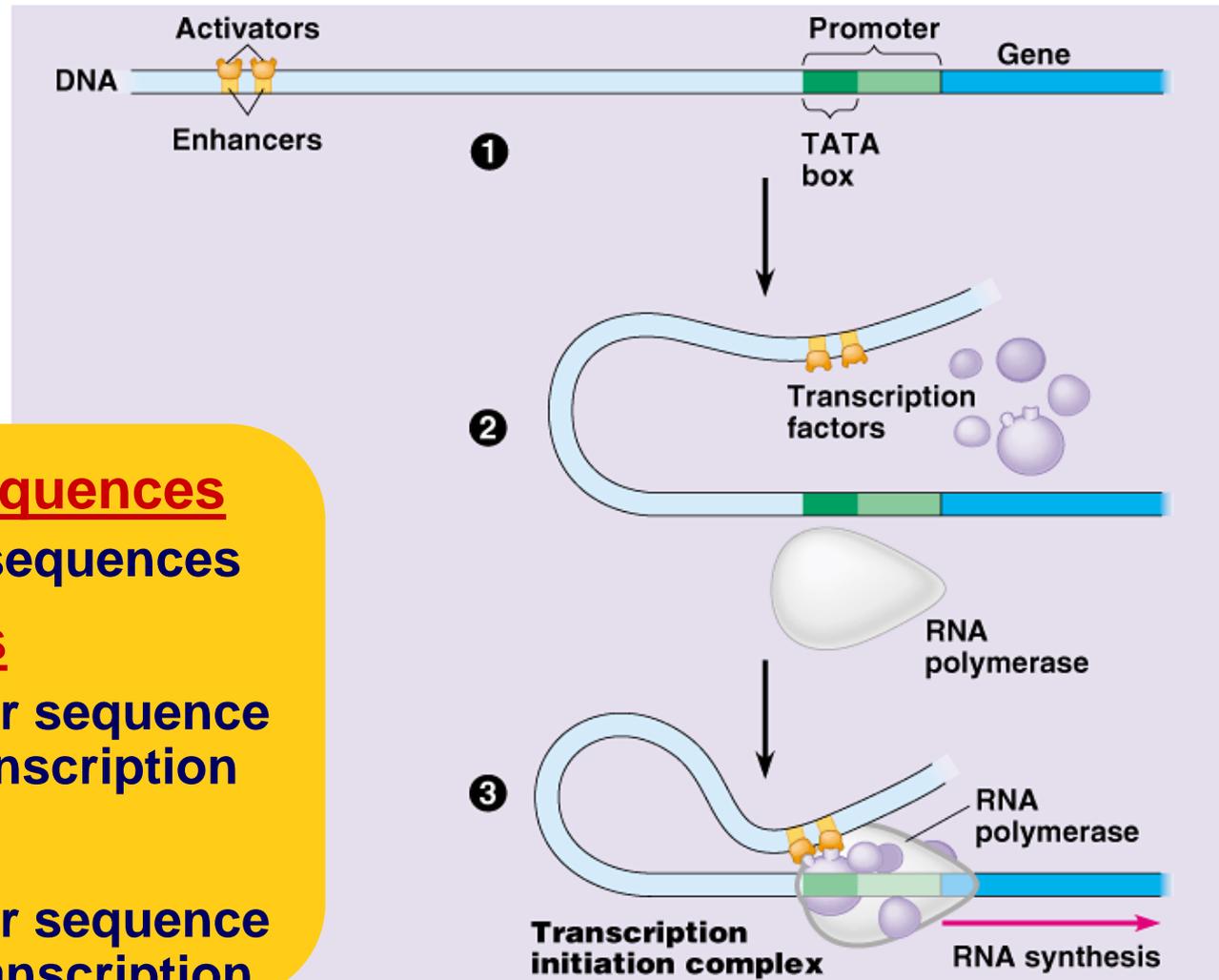
- nearby control sequence on DNA
- binding of RNA polymerase & transcription factors
- “base” rate of transcription

◆ enhancer

- distant control sequences on DNA
- binding of activator proteins
- “enhanced” rate (high level) of transcription



Model for Enhancer action



- **Enhancer DNA sequences**
 - ◆ distant control sequences
- **Activator proteins**
 - ◆ bind to enhancer sequence & stimulates transcription
- **Silencer proteins**
 - ◆ bind to enhancer sequence & block gene transcription

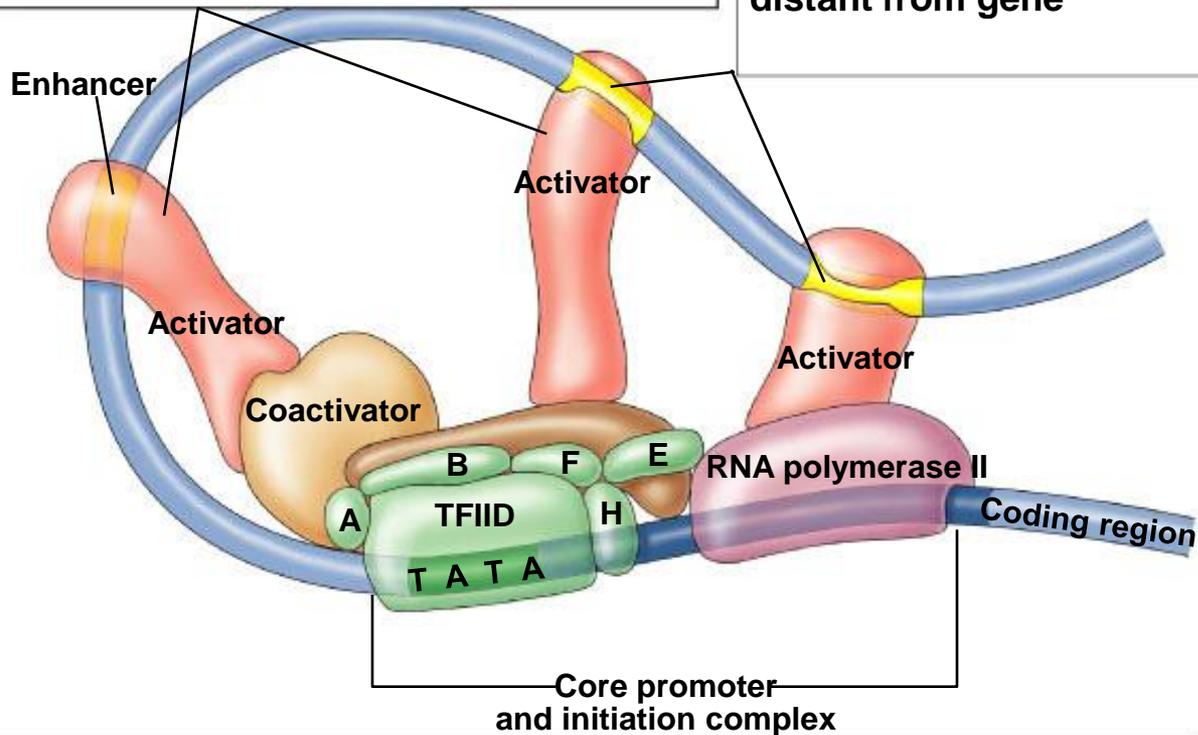
Transcription complex

Activator Proteins

- regulatory proteins bind to DNA at distant enhancer sites
- increase the rate of transcription

Enhancer Sites

regulatory sites on DNA distant from gene

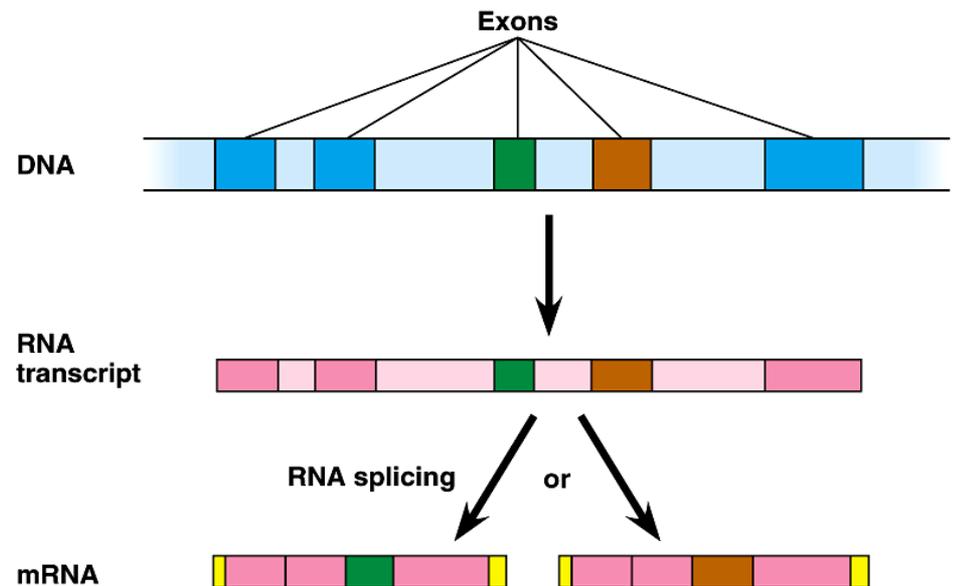
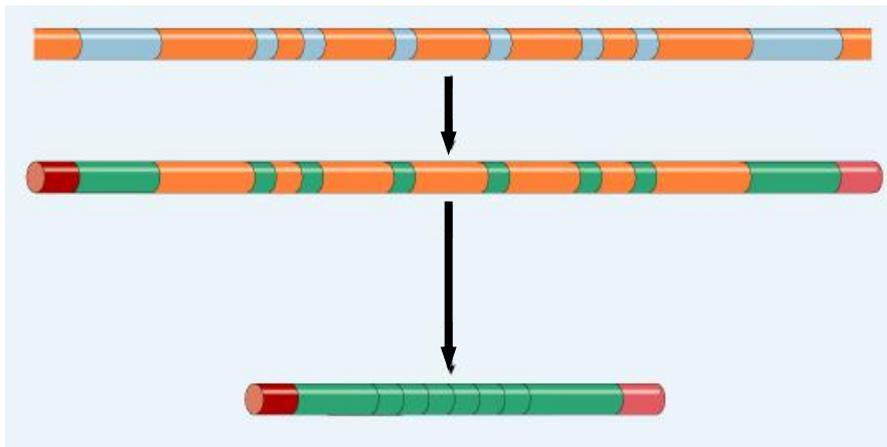


Initiation Complex at Promoter Site binding site of RNA polymerase

3. Post-transcriptional control

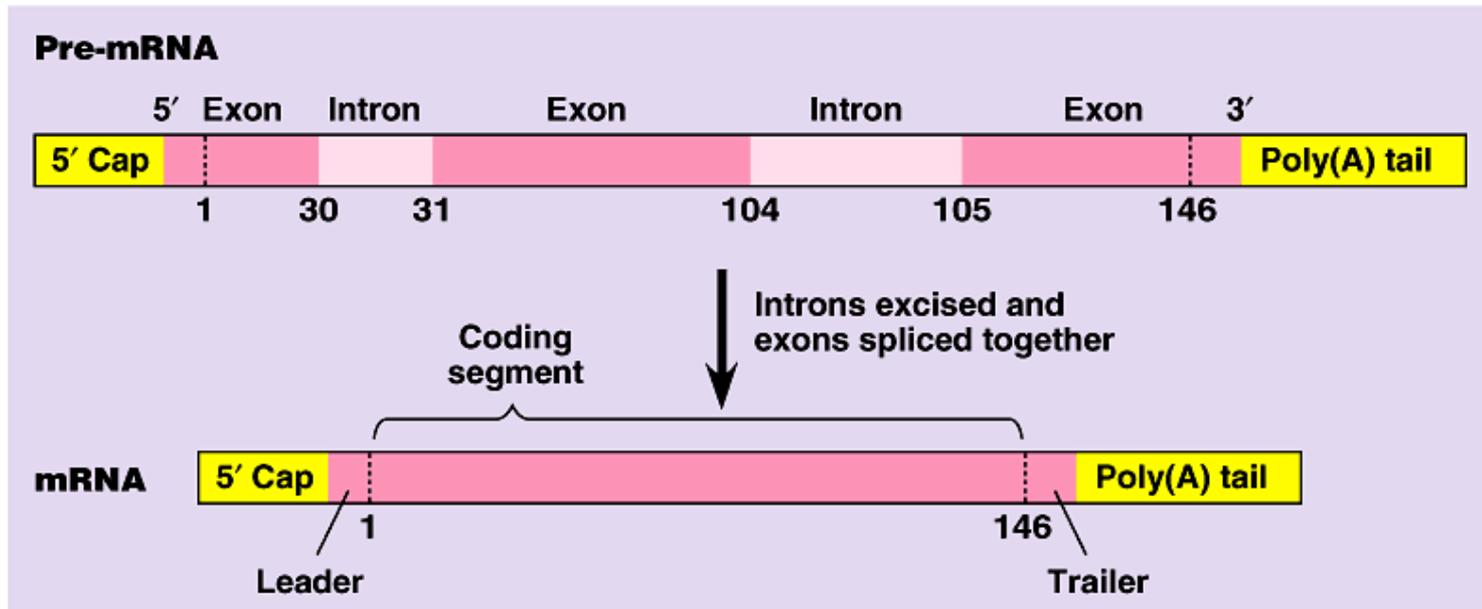
■ Alternative RNA splicing

- ◆ variable processing of exons creates a family of proteins



4. Regulation of mRNA degradation

- Life span of mRNA determines amount of protein synthesis
 - ◆ mRNA can last from hours to weeks

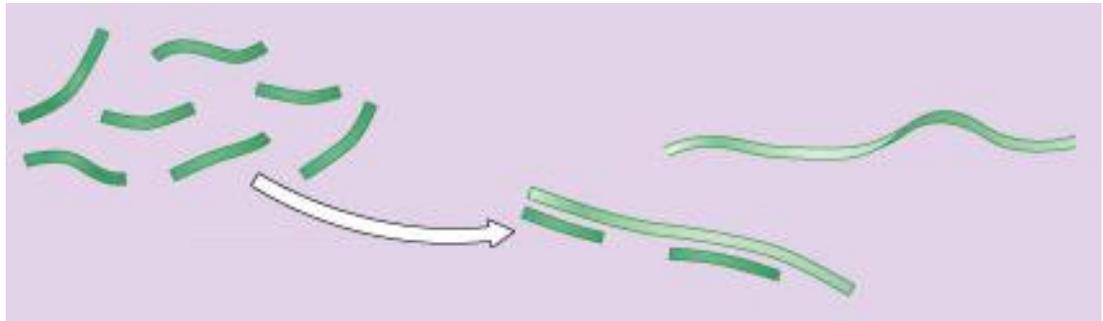


RNA interference

NEW!

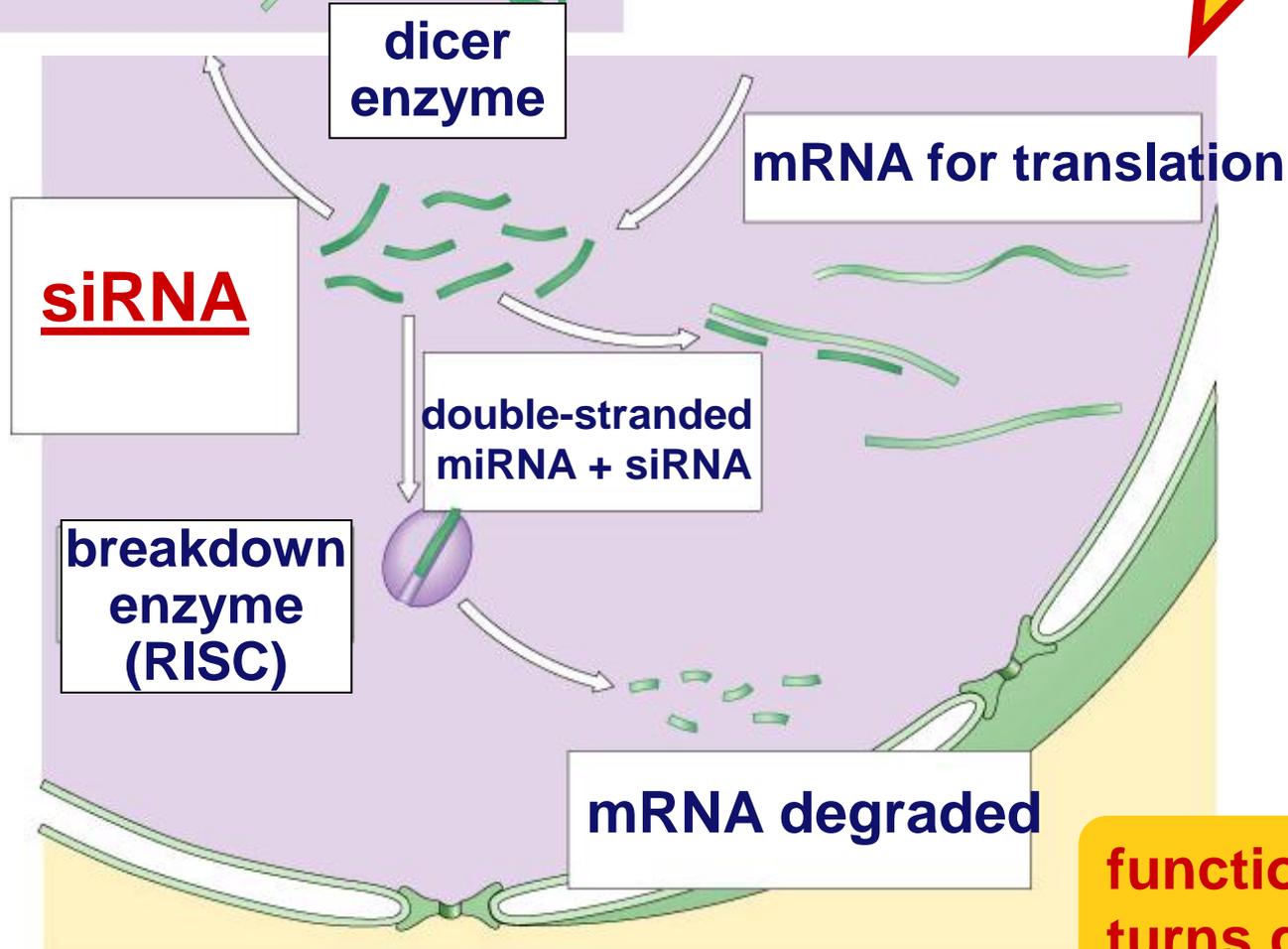
- Small interfering RNAs (siRNA)
 - ◆ short segments of RNA (21-28 bases)
 - bind to mRNA
 - create sections of double-stranded mRNA
 - “death” tag for mRNA
 - ◆ triggers degradation of mRNA
 - ◆ cause gene “silencing”
 - post-transcriptional control
 - turns off gene = no protein produced

siRNA



Action of siRNA

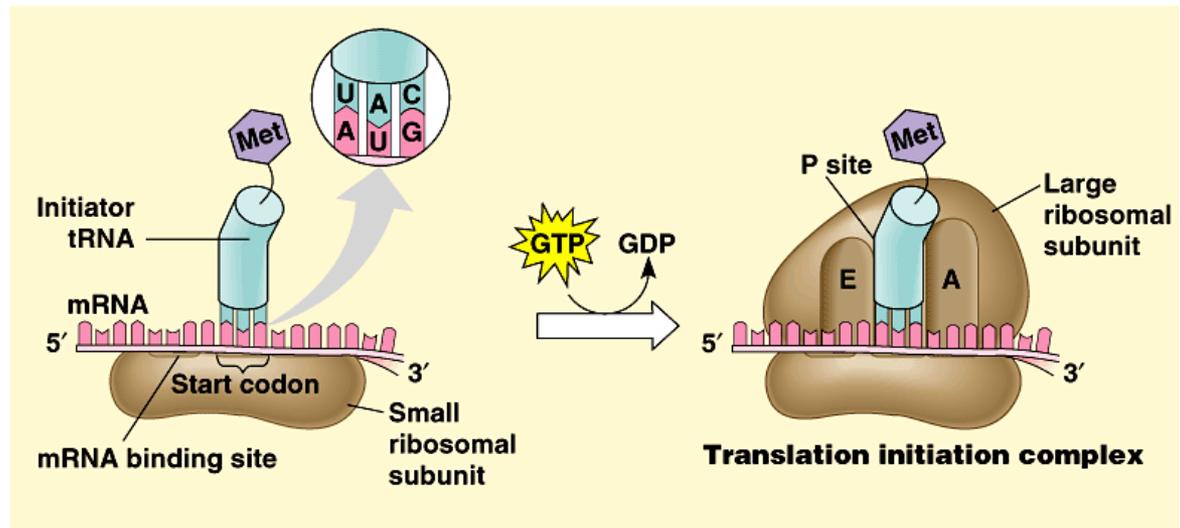
Hot...Hot
new topic
in biology



functionally
turns gene off

5. Control of translation

- **Block initiation of translation stage**
 - ◆ regulatory proteins attach to 5' end of mRNA
 - prevent attachment of ribosomal subunits & initiator tRNA
 - block translation of mRNA to protein



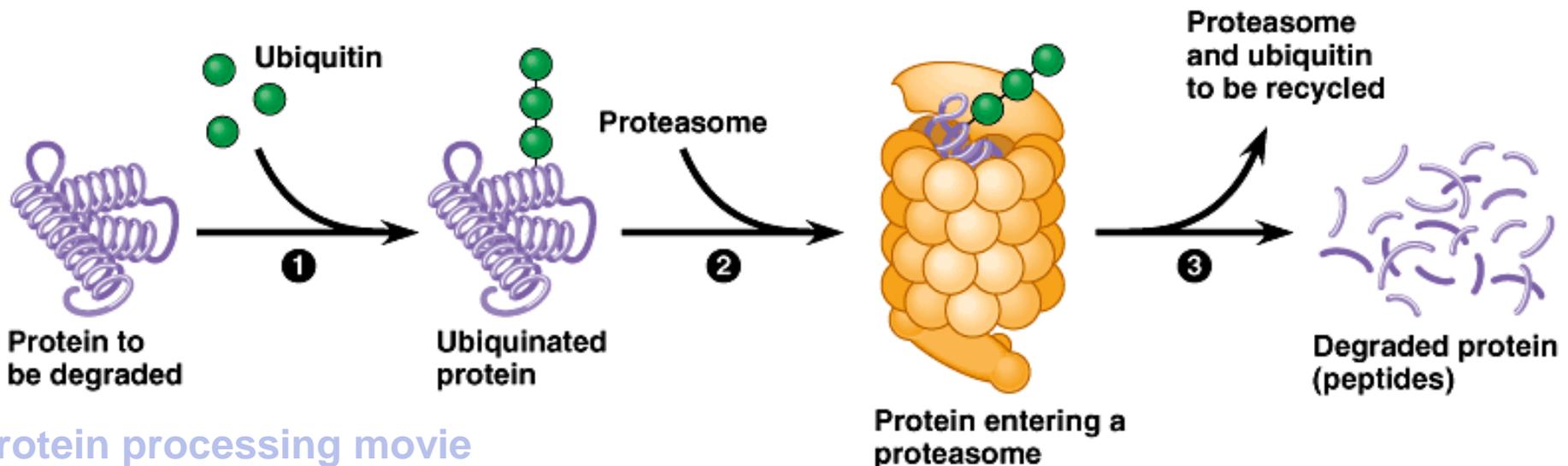
6-7. Protein processing & degradation

■ Protein processing

- ◆ folding, cleaving, adding sugar groups, targeting for transport

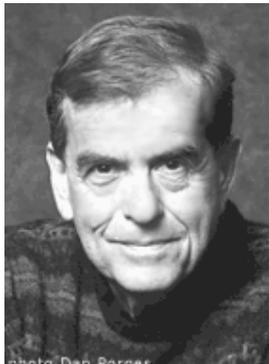
■ Protein degradation

- ◆ ubiquitin tagging
- ◆ proteasome degradation



Ubiquitin

- “Death tag”
 - ◆ mark unwanted proteins with a label
 - ◆ 76 amino acid polypeptide, ubiquitin
 - ◆ labeled proteins are broken down rapidly in “waste disposers”
 - proteasomes



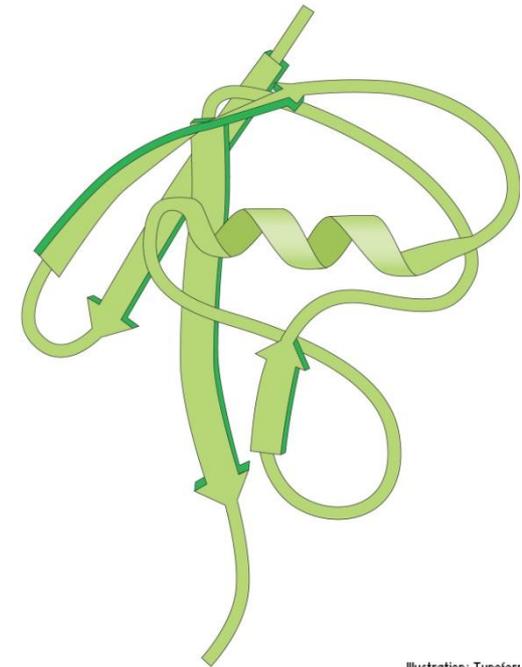
Aaron Ciechanover
Israel



Avram Hershko
Israel

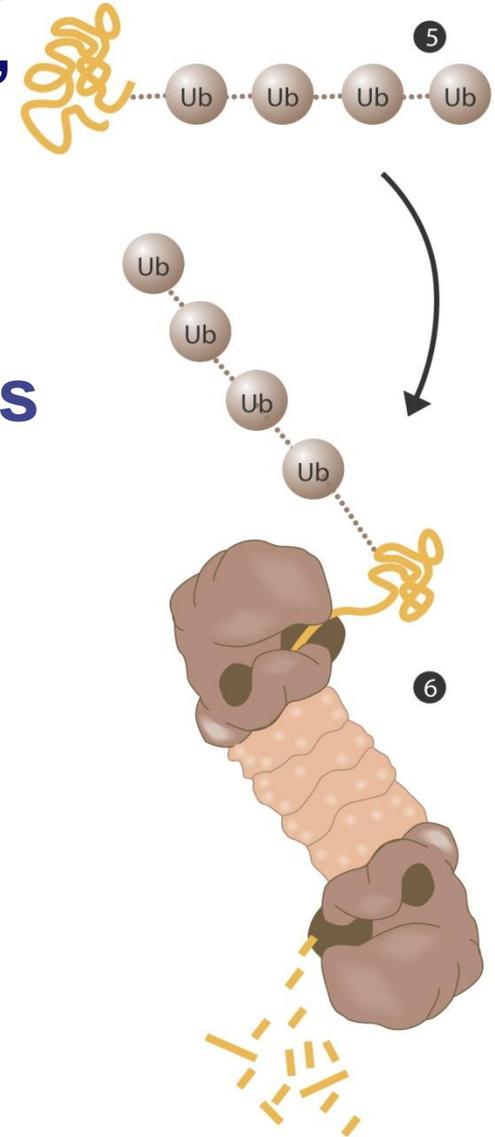
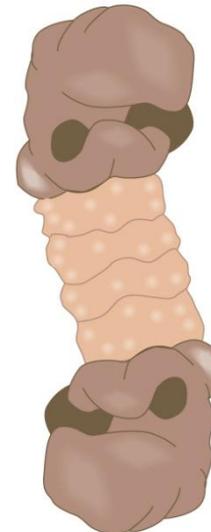
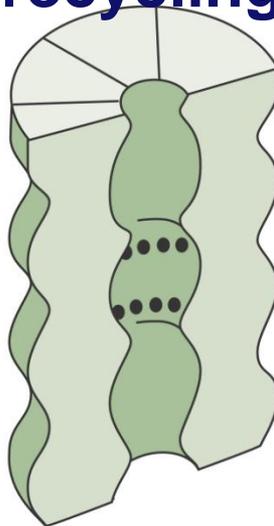
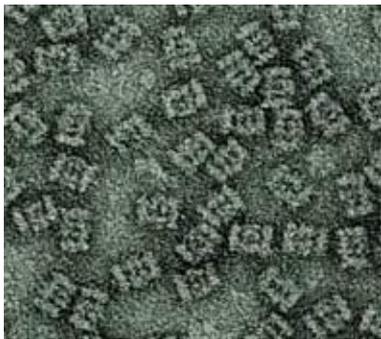


Irwin Rose
UC Riverside

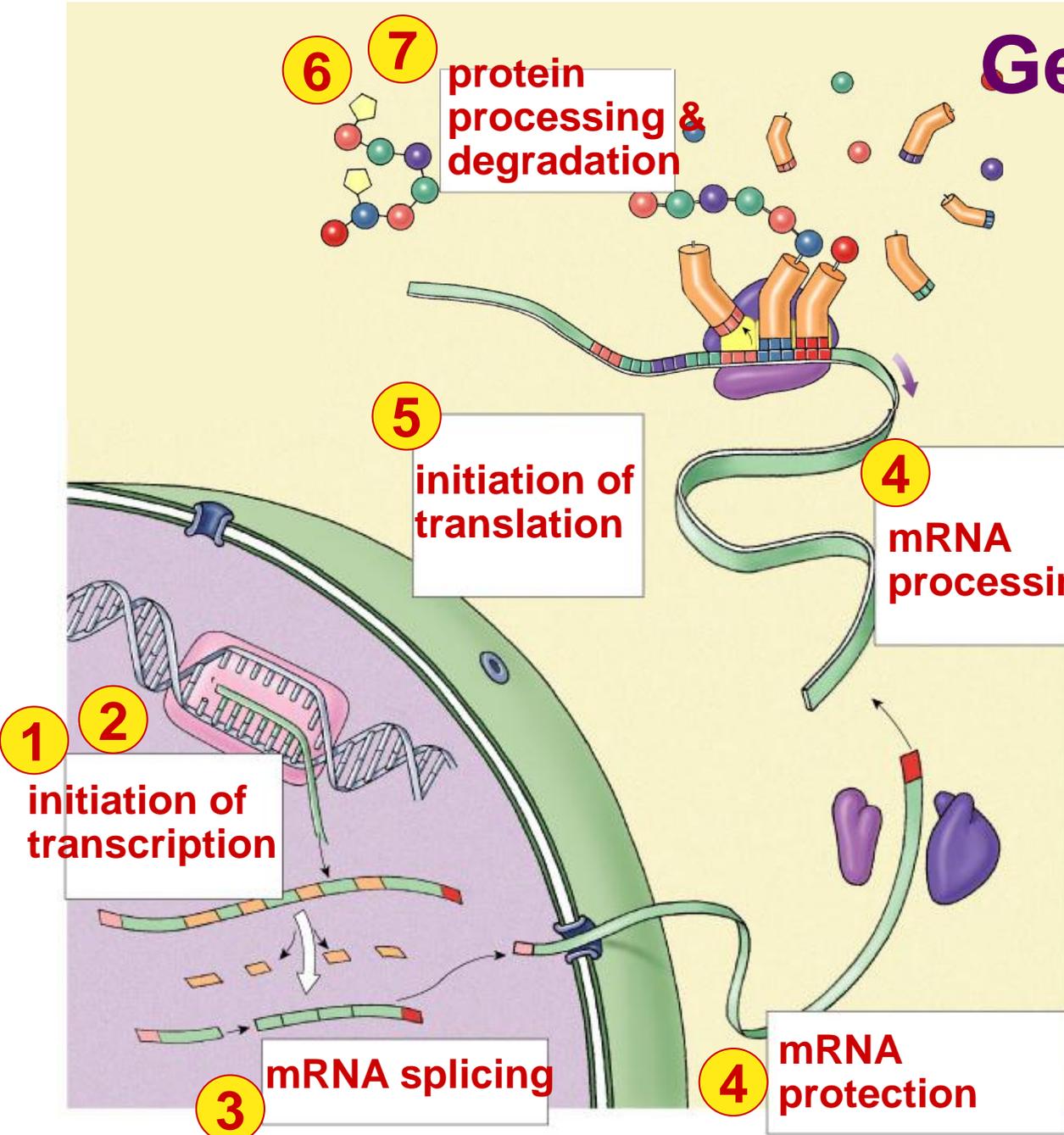


Proteasome

- Protein-degrading “machine”
 - ◆ cell’s waste disposer
 - ◆ breaks down any proteins into 7-9 amino acid fragments
 - cellular recycling



Gene Regulation



1 & 2. transcription

- DNA packing
- transcription factors

3 & 4. post-transcription

- mRNA processing
- splicing
- 5' cap & poly-A tail
- breakdown by siRNA

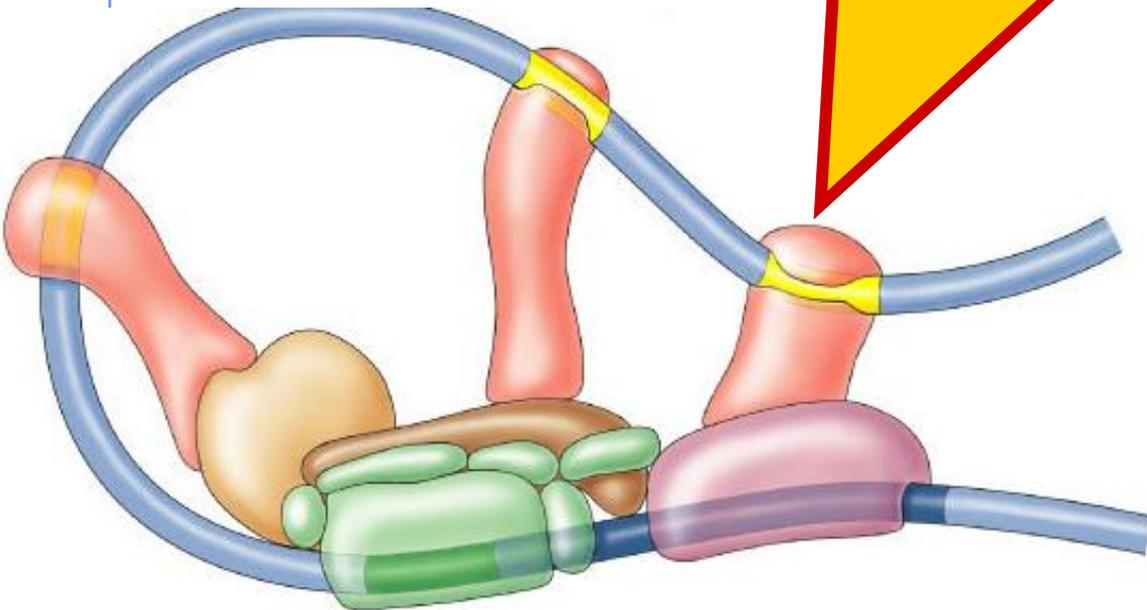
5. translation

- block start of translation

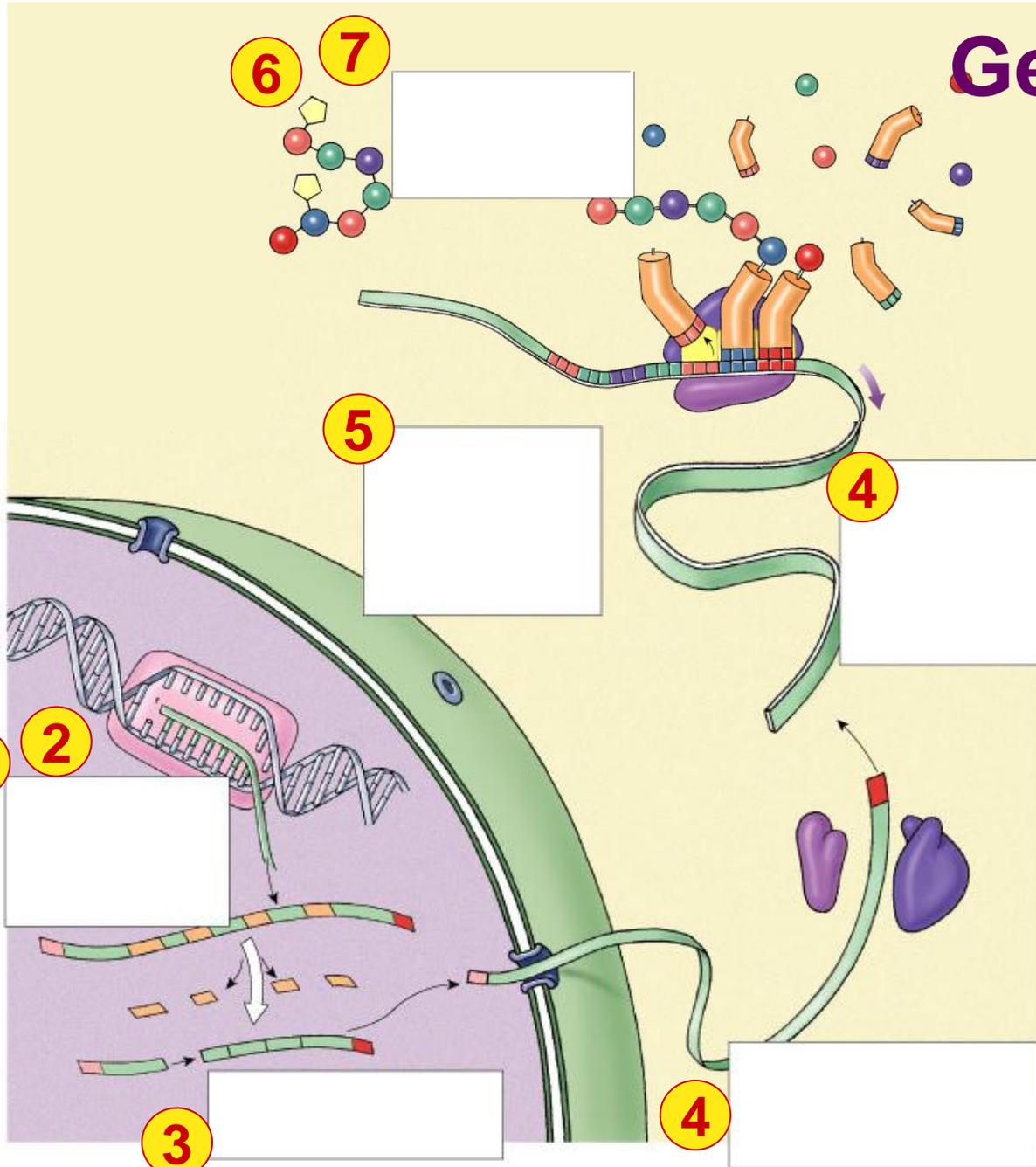
6 & 7. post-translation

- protein processing
- protein degradation

**Turn your
Question Genes on!**



Gene Regulation



1 & 2. _____

- _____
- _____

3 & 4. _____

- _____
- _____
- _____
- _____

5. _____

- _____
- _____

6 & 7. _____

- _____
- _____