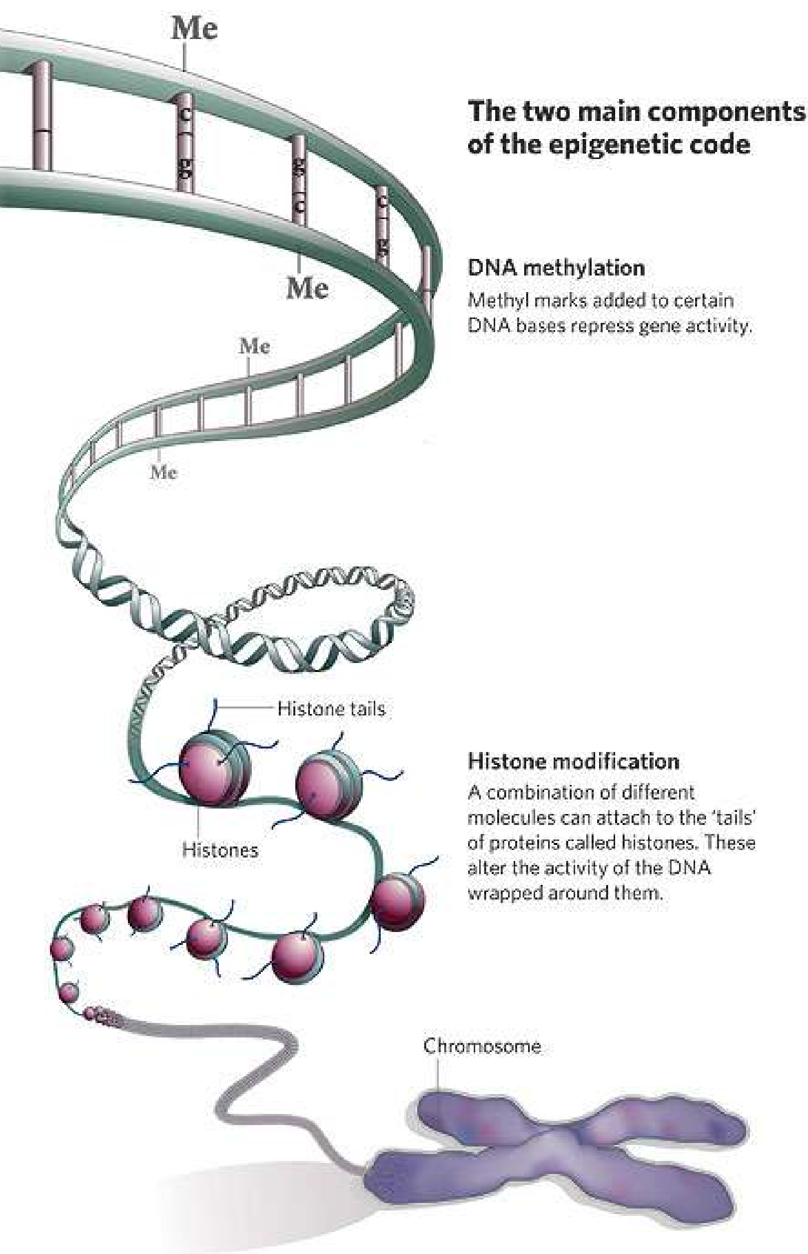
Control of Eukaryotic Genes

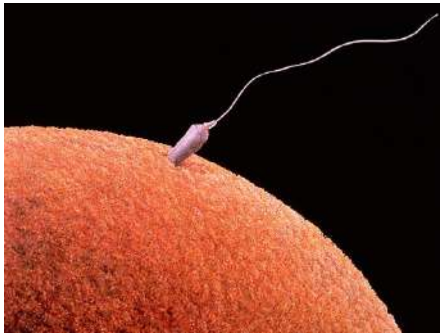


The two main components of the epigenetic code

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.

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

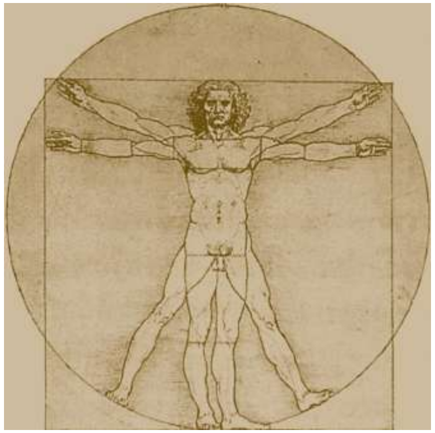
Prokaryotes

- 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



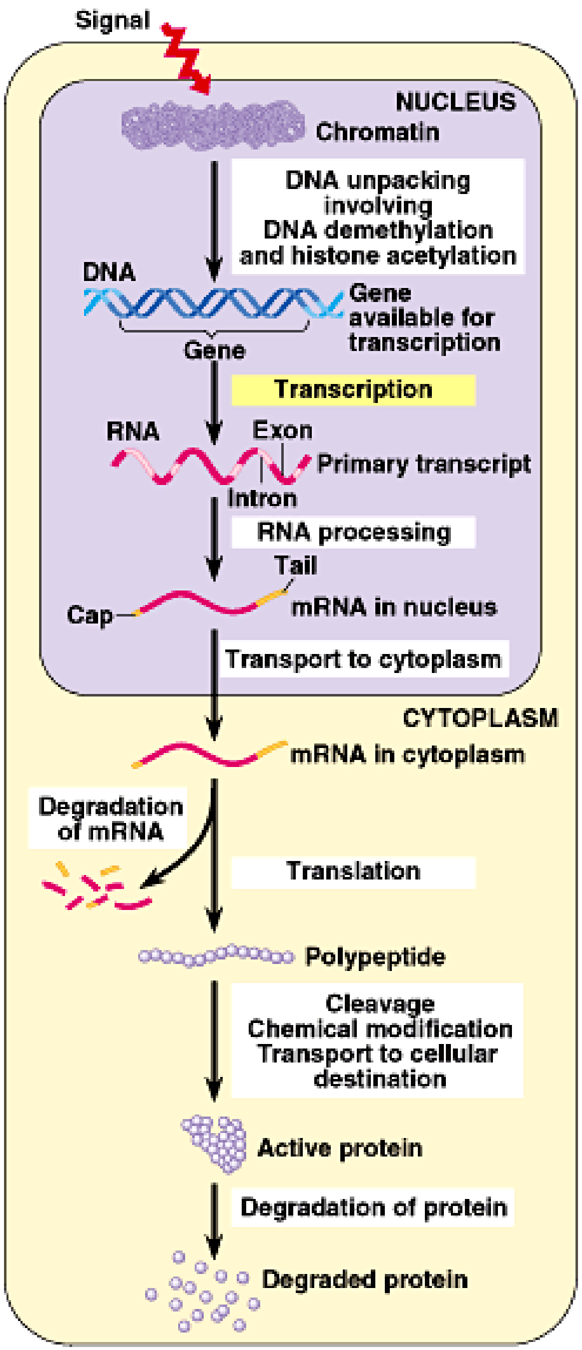
Eukaryotes

- 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

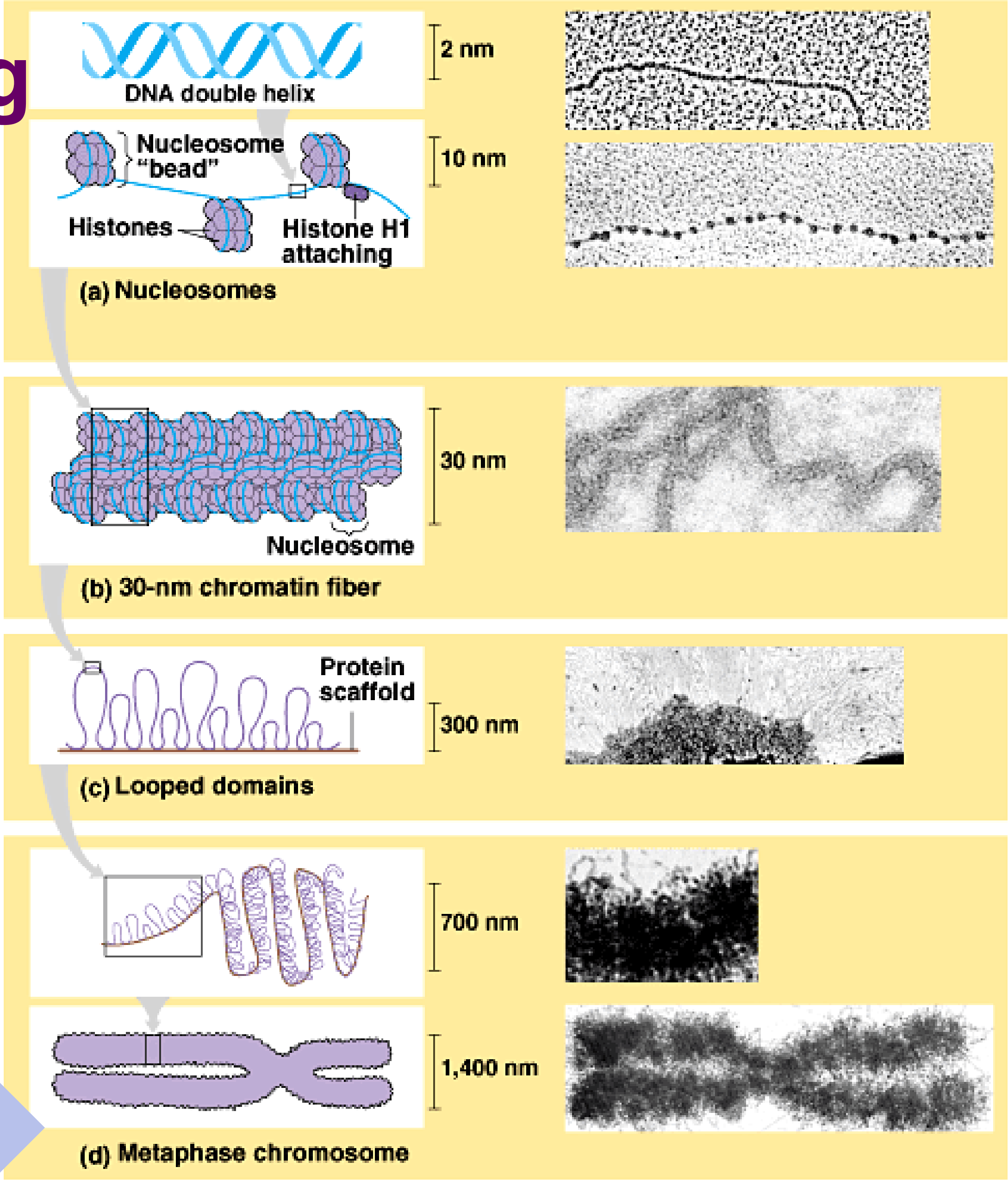
1. packing/unpacking DNA
2. transcription
3. mRNA processing
4. mRNA transport
5. translation
6. protein processing
7. protein degradation



1. DNA packing

How do you fit all that DNA into nucleus?

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

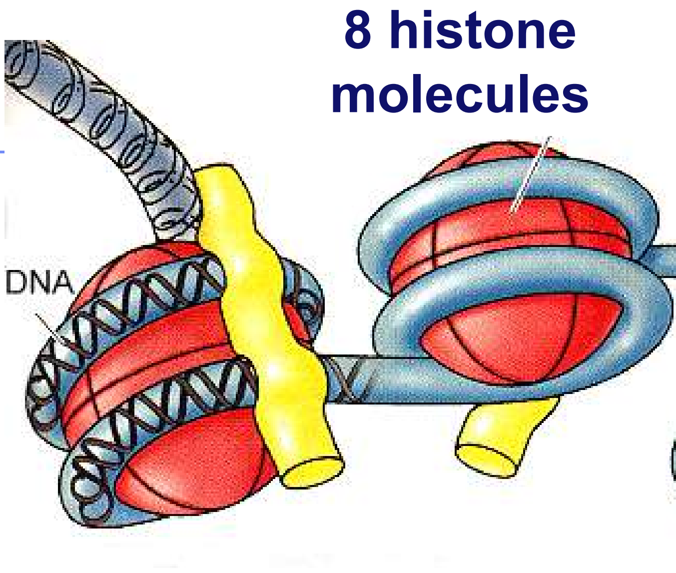


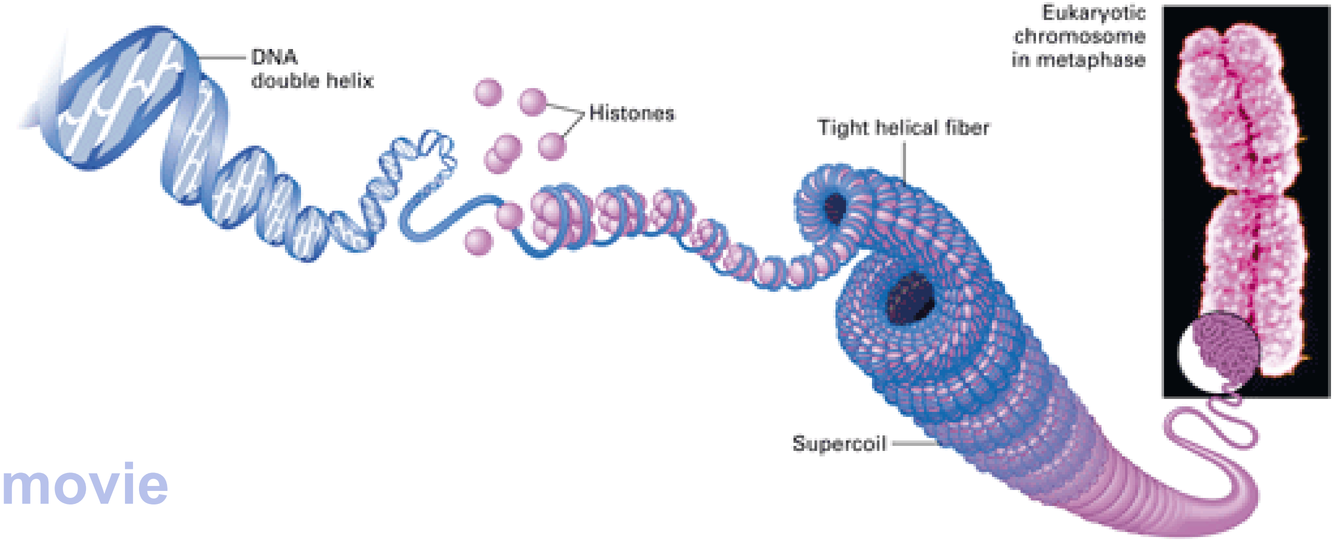
from DNA double helix to condensed chromosome

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



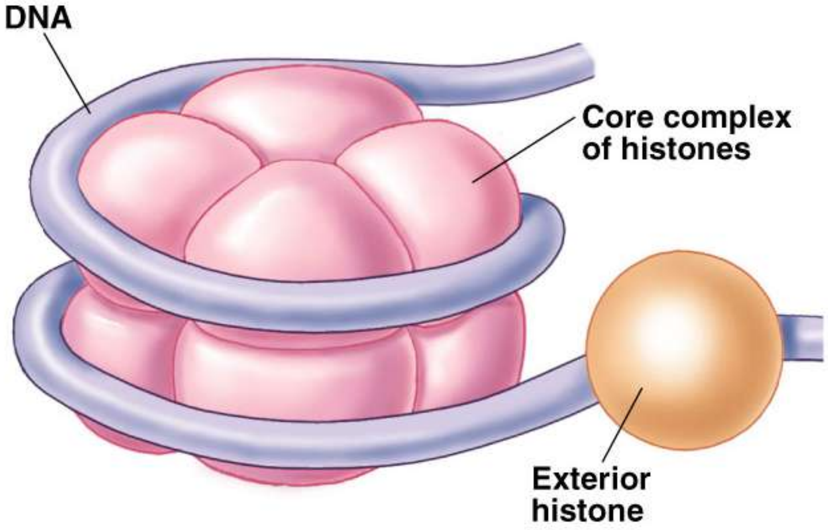


DNA packing movie

DNA packing as gene control

Degree of packing of DNA regulates transcription

- ◆ tightly wrapped around histones
  - no transcription
  - genes turned off

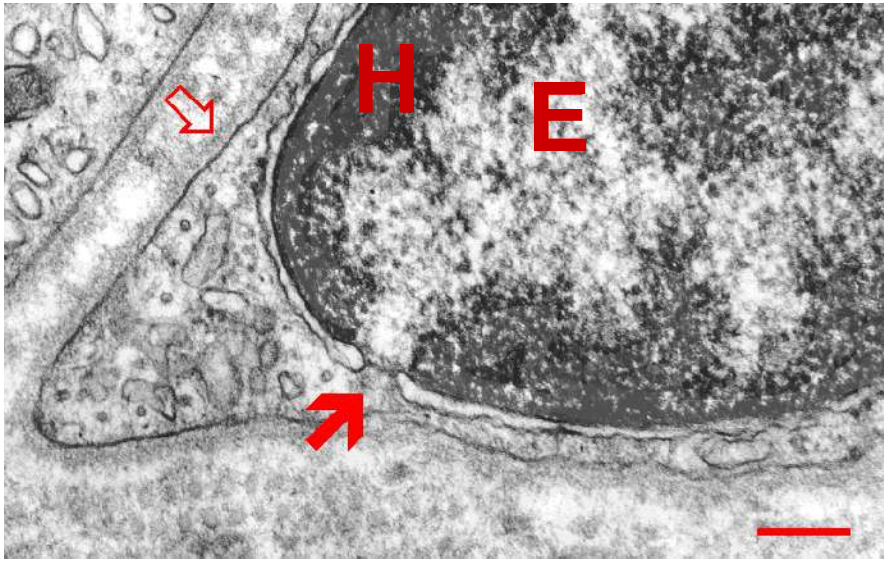


- heterochromatin

darker DNA (H) = tightly packed

- euchromatin

lighter DNA (E) = loosely packed



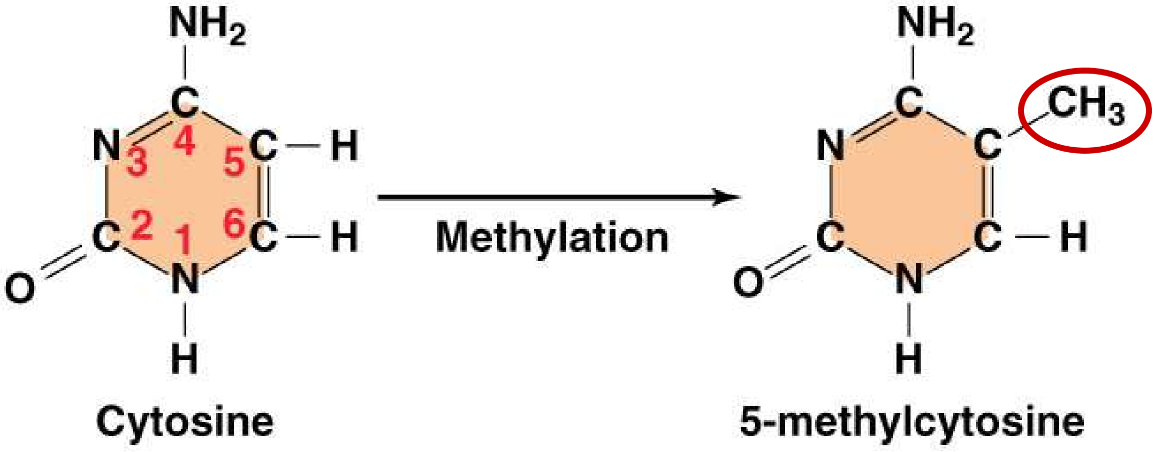
DNA methylation

Methylation of DNA **blocks transcription factors**

- no transcription

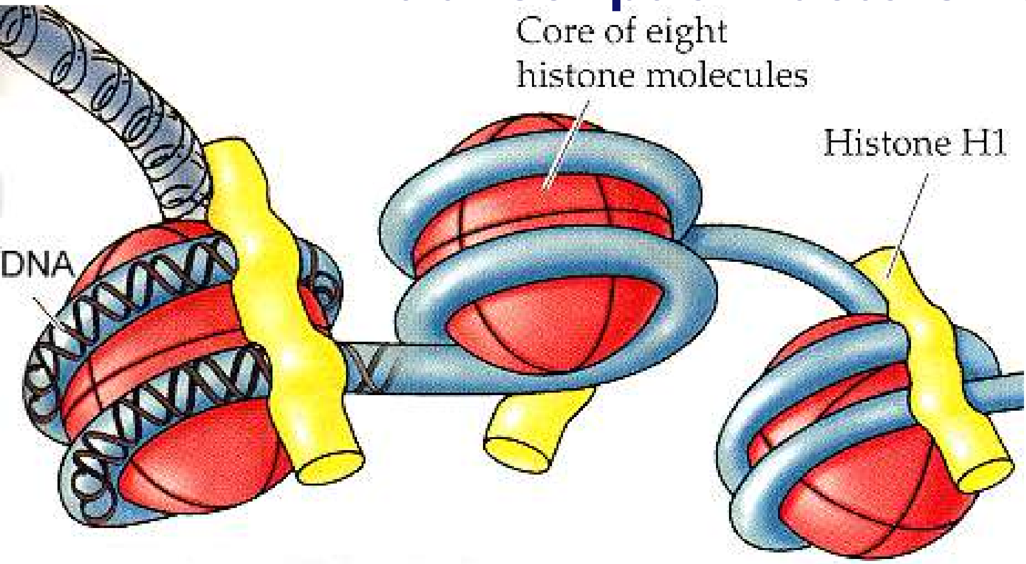
→ **genes turned off**

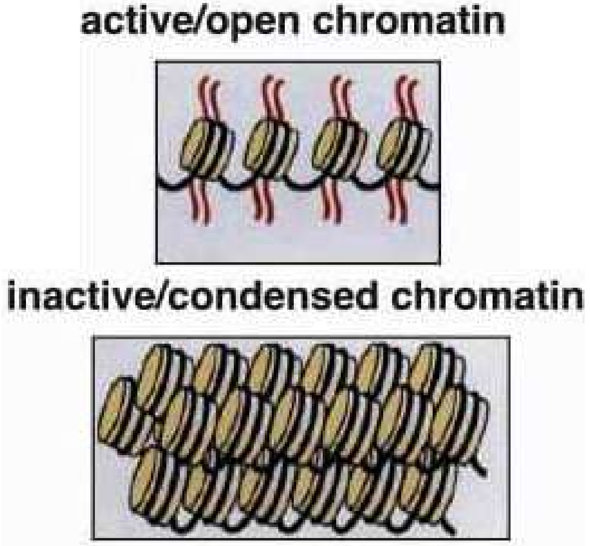
- **attachment of methyl groups ($-CH_3$) to cytosine**
  - **C = cytosine**
- **nearly permanent inactivation of genes**
  - **ex. inactivated mammalian X chromosome = Barr body**



Histone acetylation

- Acetylation of histones unwinds DNA
  - ◆ loosely wrapped around histones
    - enables transcription
    - genes turned on
  - ◆ attachment of acetyl groups ($-COCH_3$) to histones
    - conformational change in histone proteins
    - transcription factors have easier access to genes

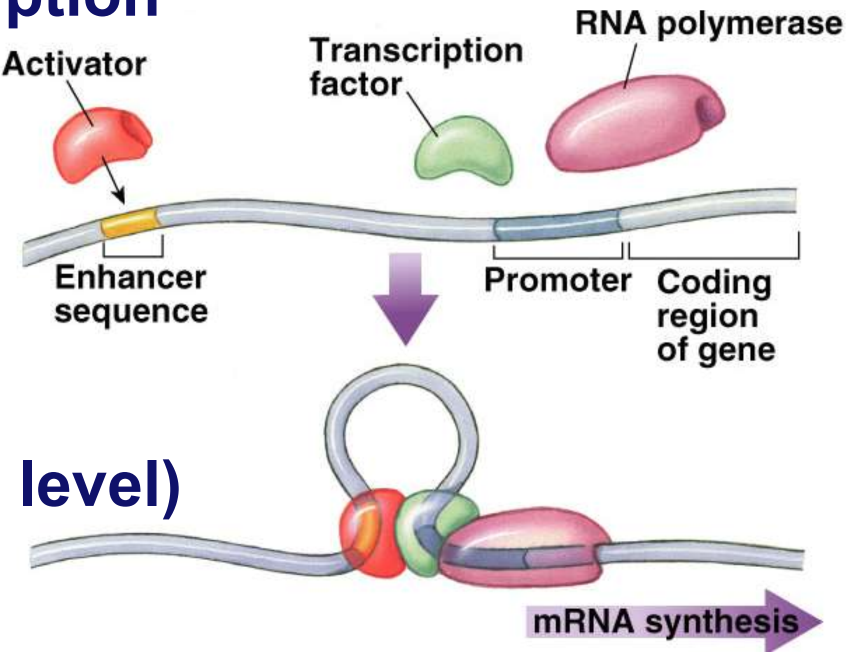




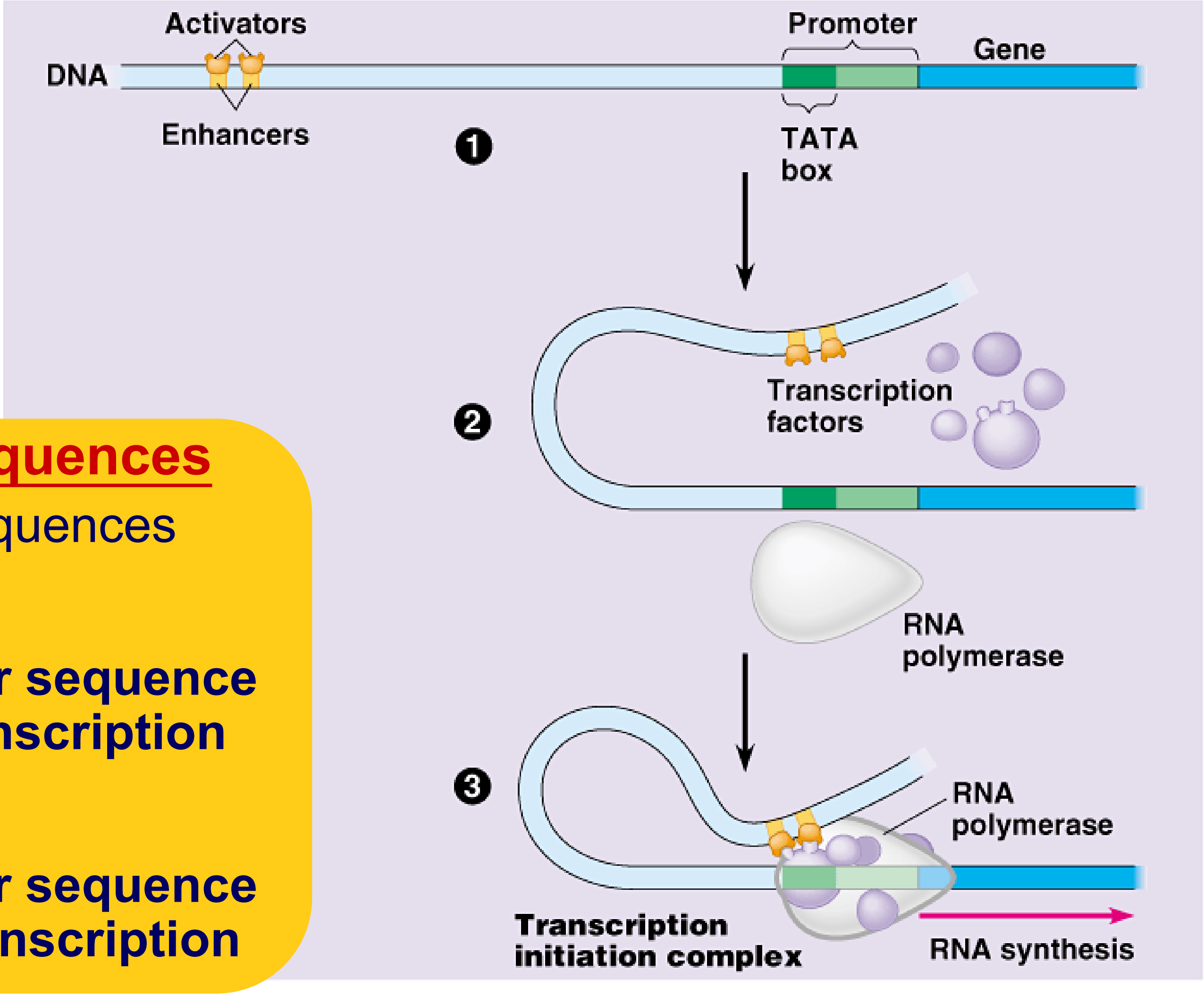
2. Transcription initiation

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

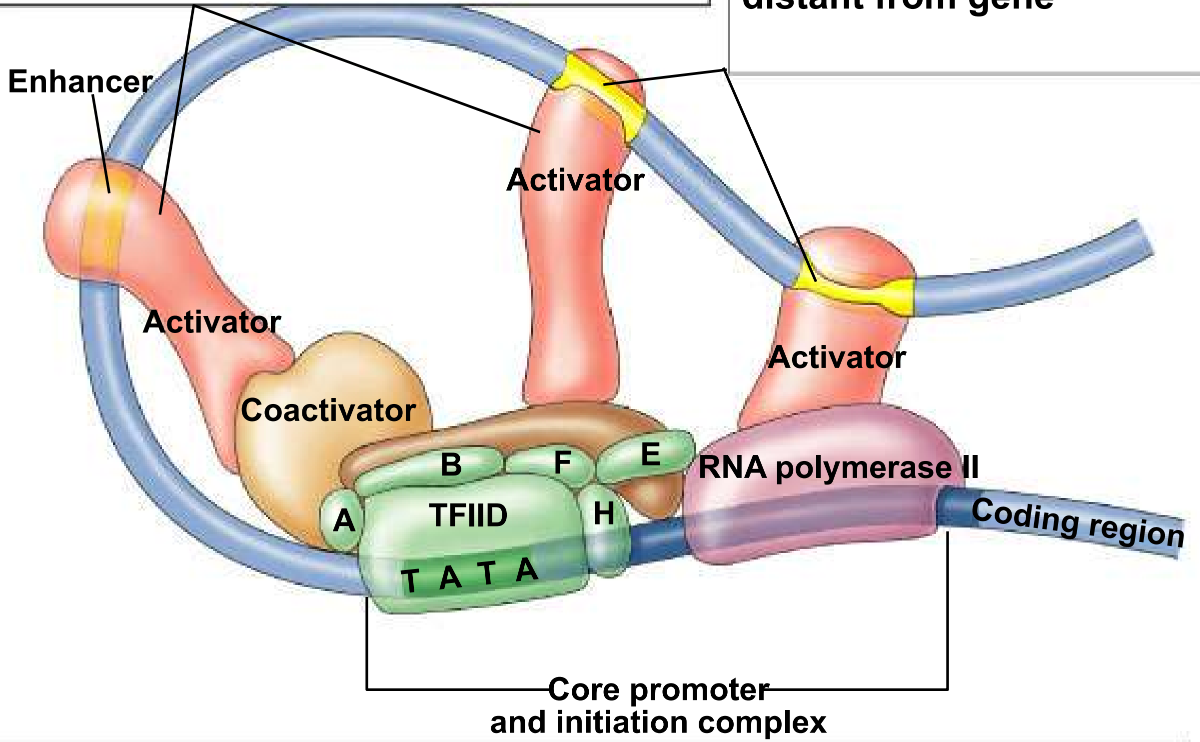
- **bind to enhancer sequence & block gene transcription**

Turning on Gene movie

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

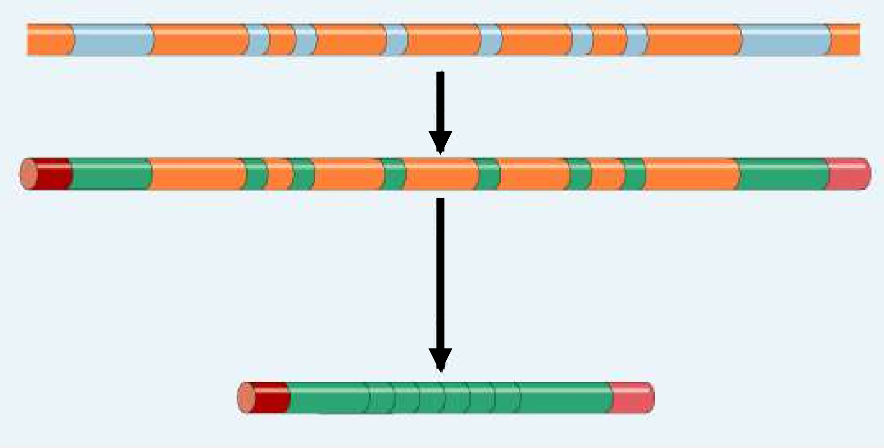


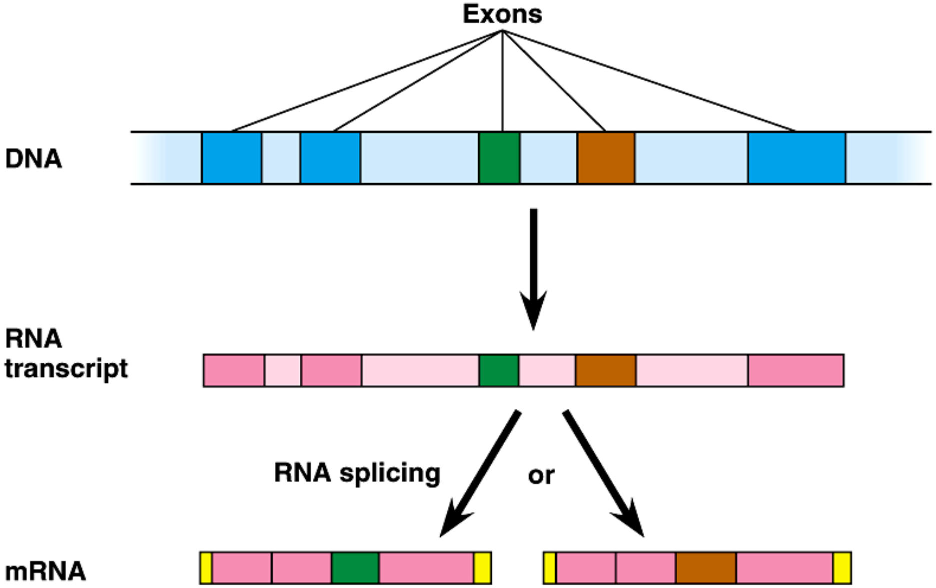
Initiation Complex at Promoter Site binding site of RNA polymerase

3. Post-transcriptional control

Alternative RNA splicing

- variable processing of exons creates a family of proteins

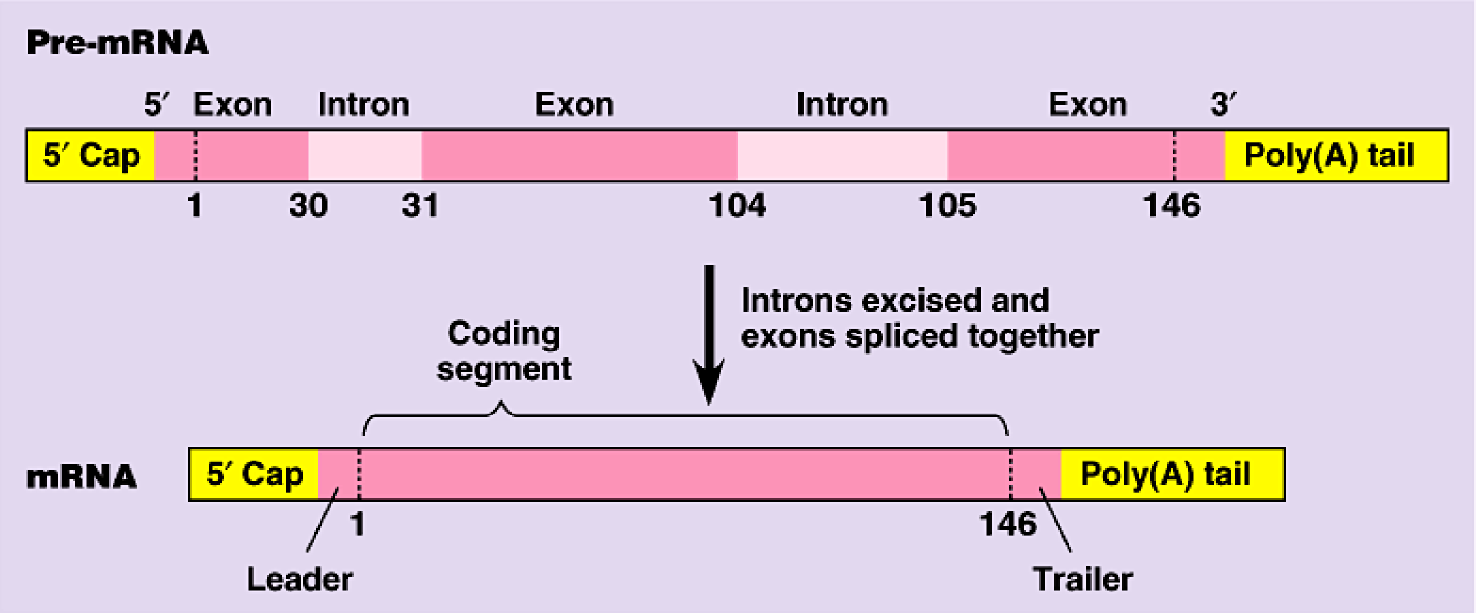




4. Regulation of mRNA degradation

Life span of mRNA determines amount of protein synthesis

- mRNA can last from hours to weeks



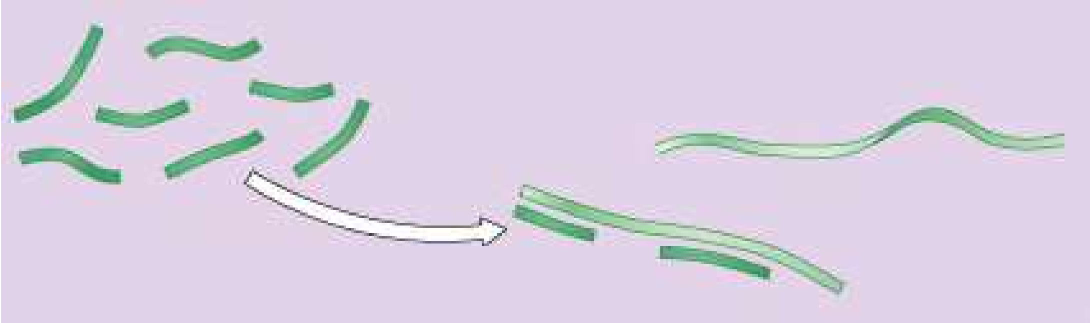
RNA processing movie

RNA interference

Small interfering RNAs (siRNA)

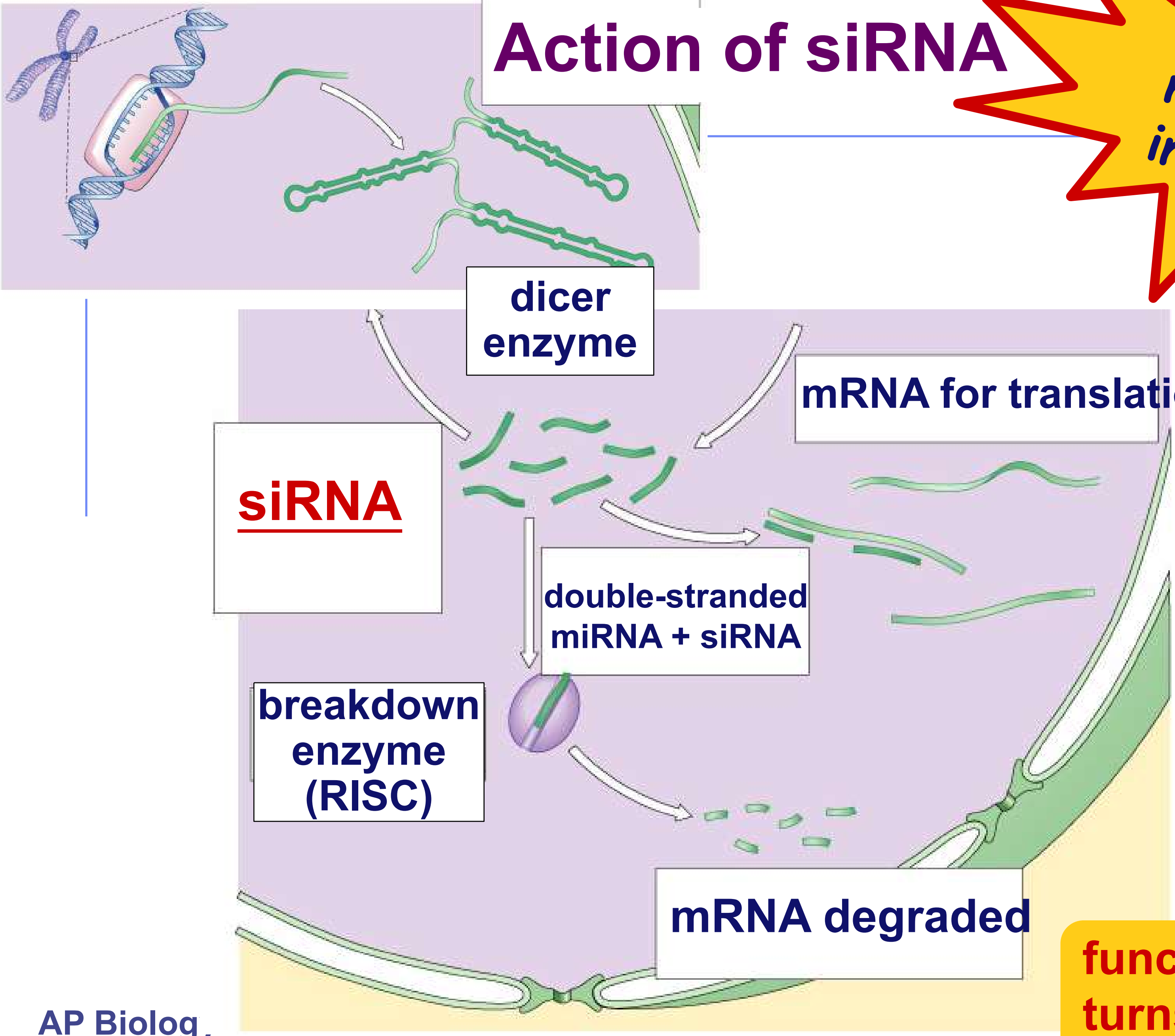
- short segments of RNA (21-28 bases)
  - bind to mRNA
  - create sections of double-stranded mRNA
  - "death" tag for mRNA
    - triggers degradation of mRNA
- cause gene "silencing"
  - post-transcriptional control
  - turns off gene = no protein produced

siRNA



Action of siRNA

Hot…Hot new topic in biology



dicer enzyme

mRNA for translation

siRNA

double-stranded miRNA + siRNA

breakdown enzyme (RISC)

mRNA degraded

functionally turns gene off

1990s | 2006

RNA interference

“for their discovery of RNA interference — gene silencing by double-stranded RNA”

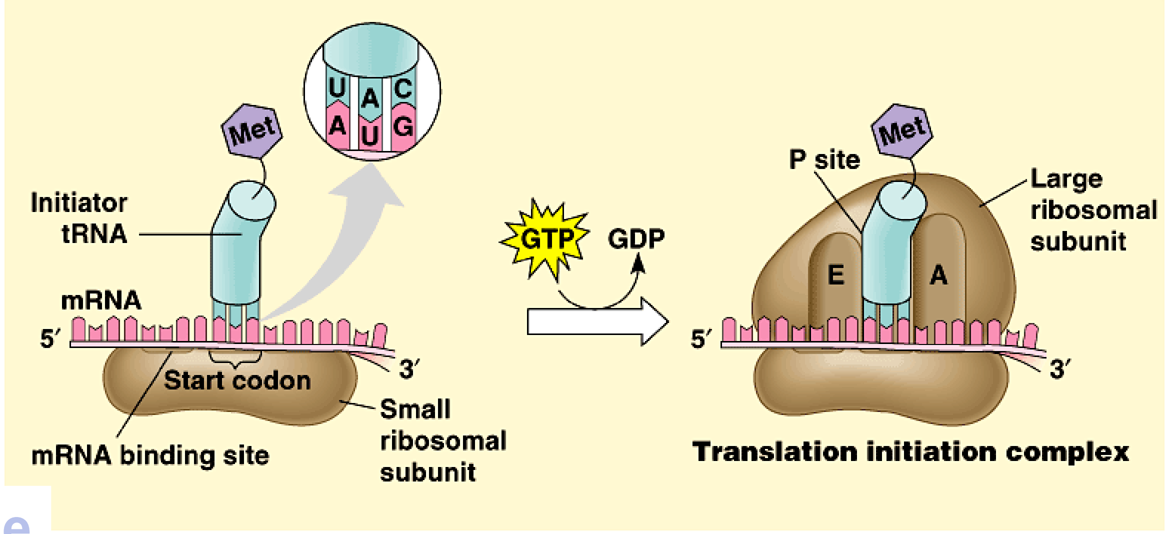
Andrew Fire
Stanford

Craig Mello
U Mass

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



Control of translation movie

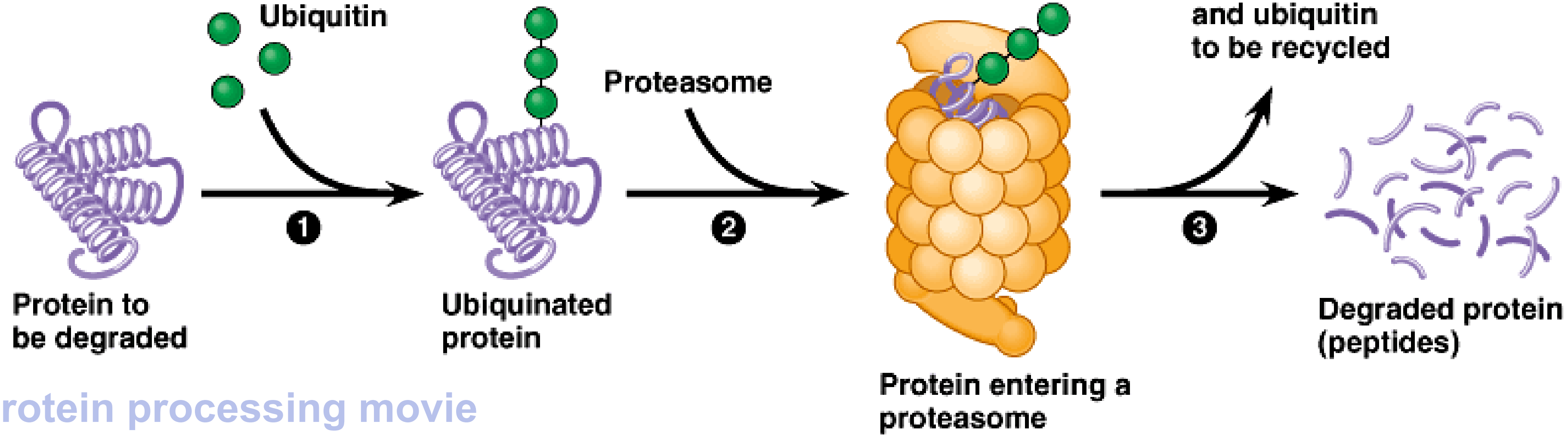
6-7. Protein processing & degradation

Protein processing

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

Protein degradation

- **ubiquitin** tagging
- **proteasome** degradation



Protein processing movie

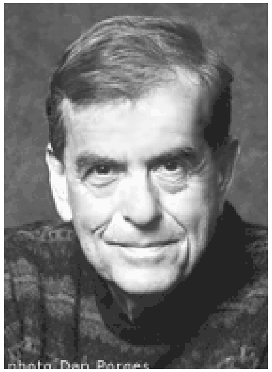
1980s | 2004

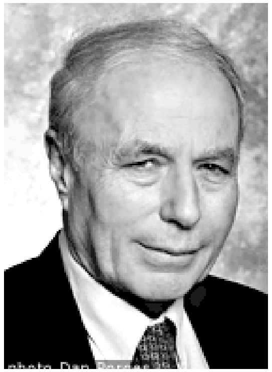
Ubiquitin

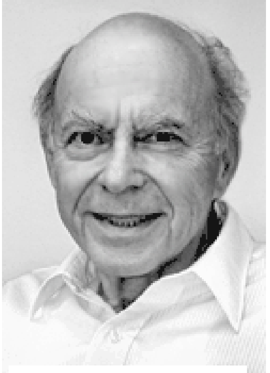
“Death tag”

- 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

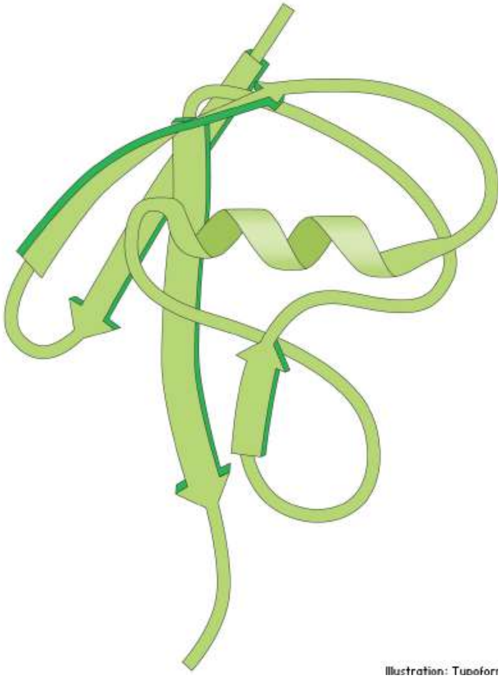
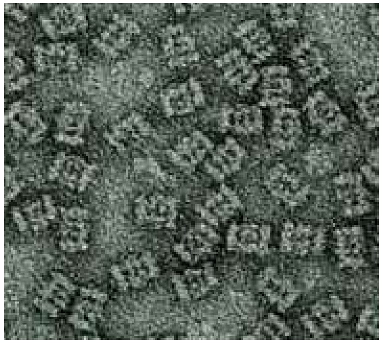


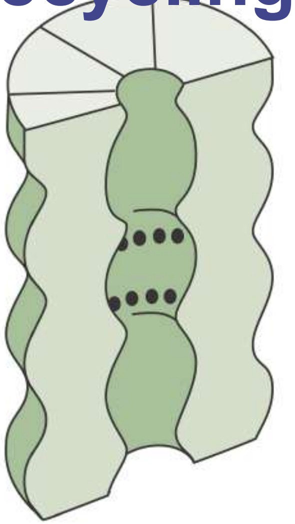
Illustration: Typofor

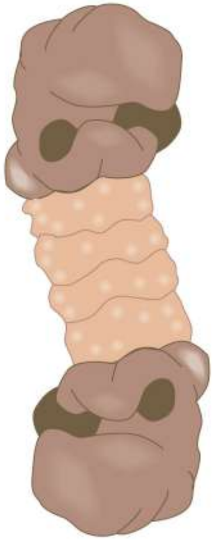
Proteasome

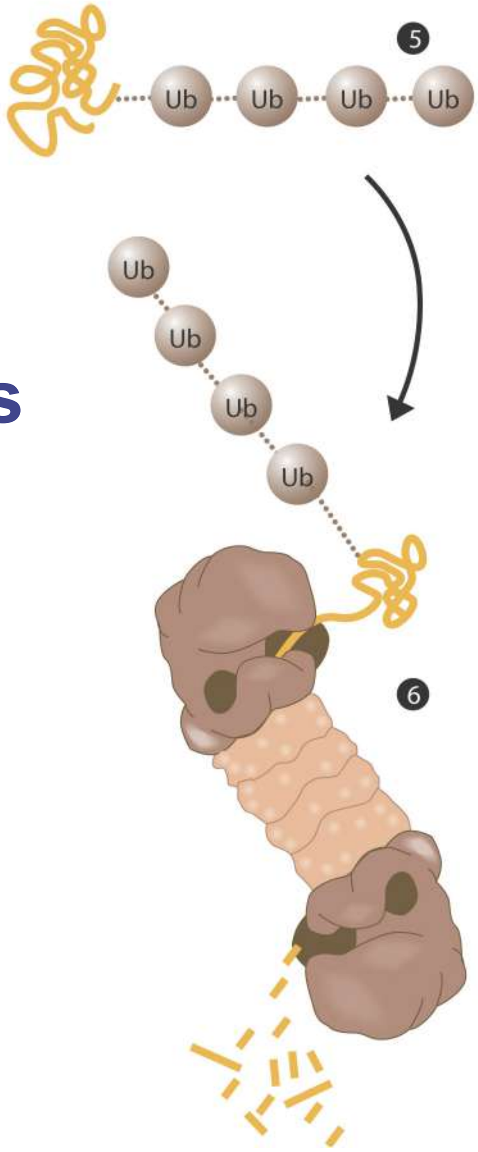
Protein-degrading “machine”

- cell’s waste disposer
- breaks down any proteins into 7-9 amino acid fragments
- cellular recycling



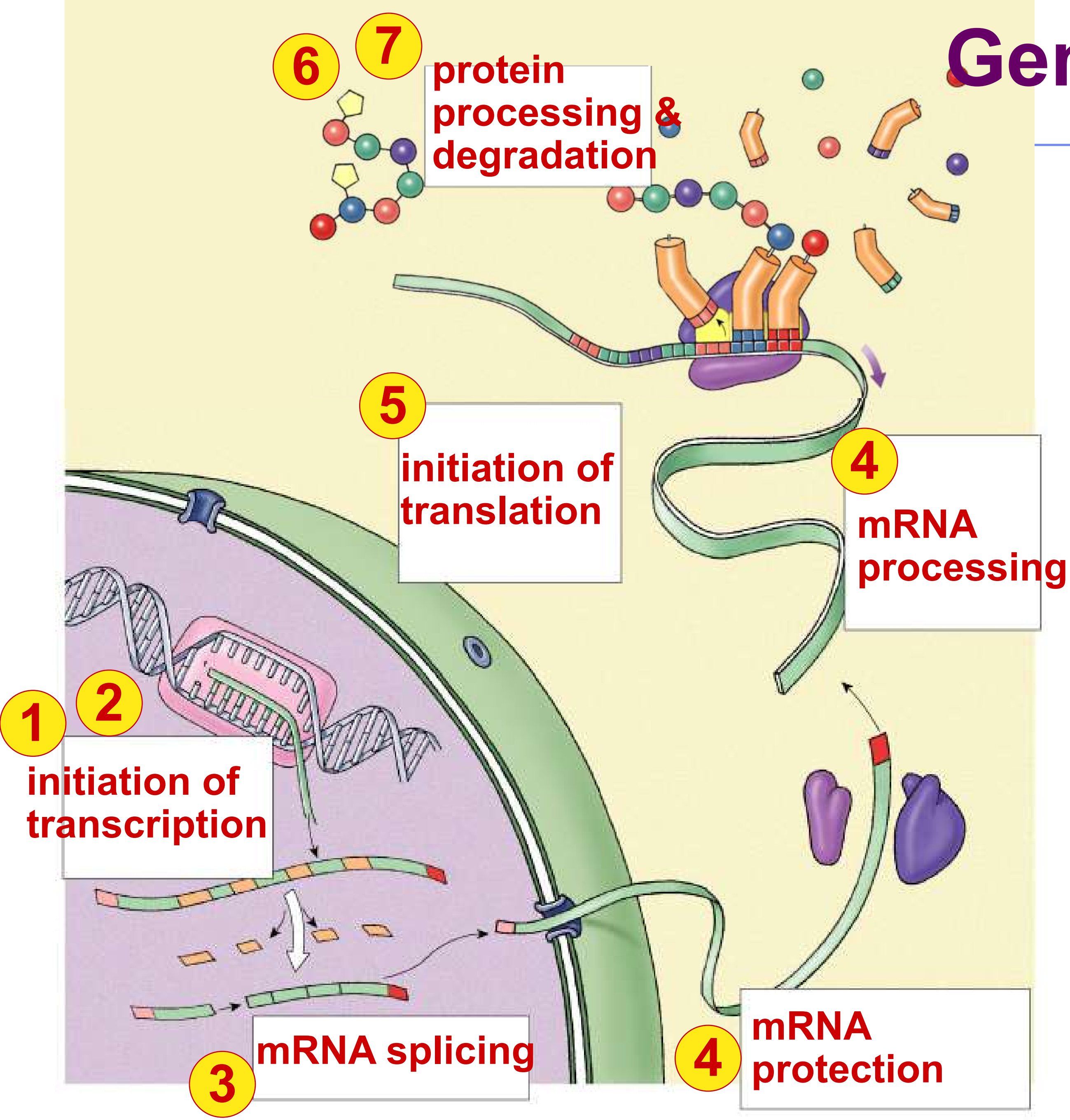






play Nobel animation

Gene Regulation



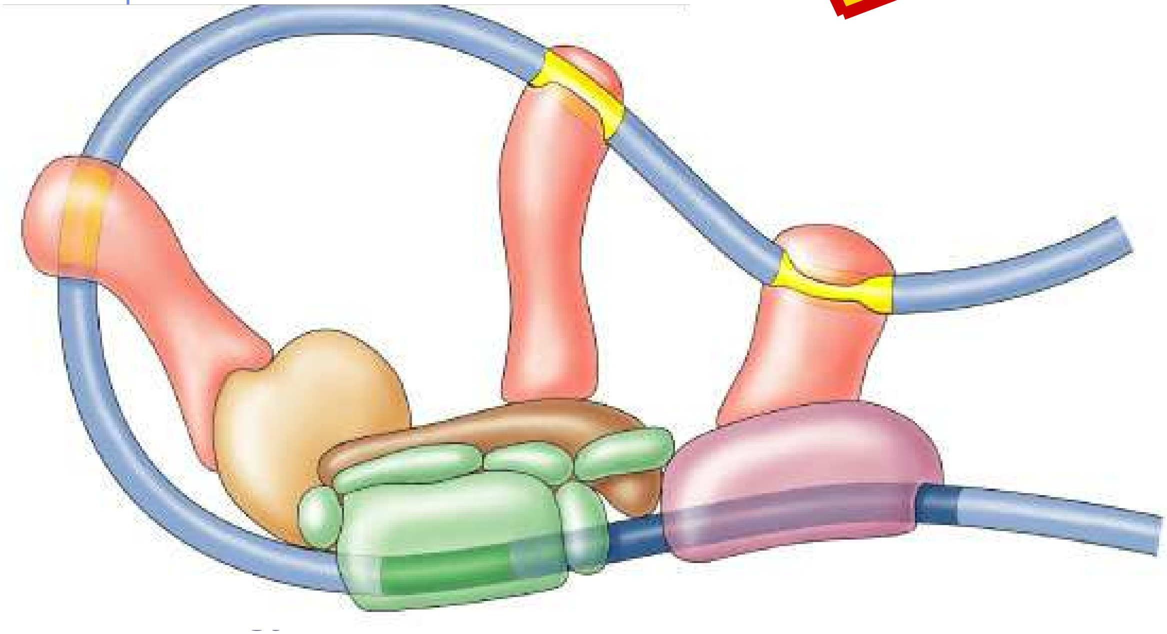
1 & 2. transcription
- DNA packing
- transcription factors

3 & 4. post-transcription
- mRNA processing
  - splicing
- 5’ cap & poly-A tail
- breakdown by siRNA

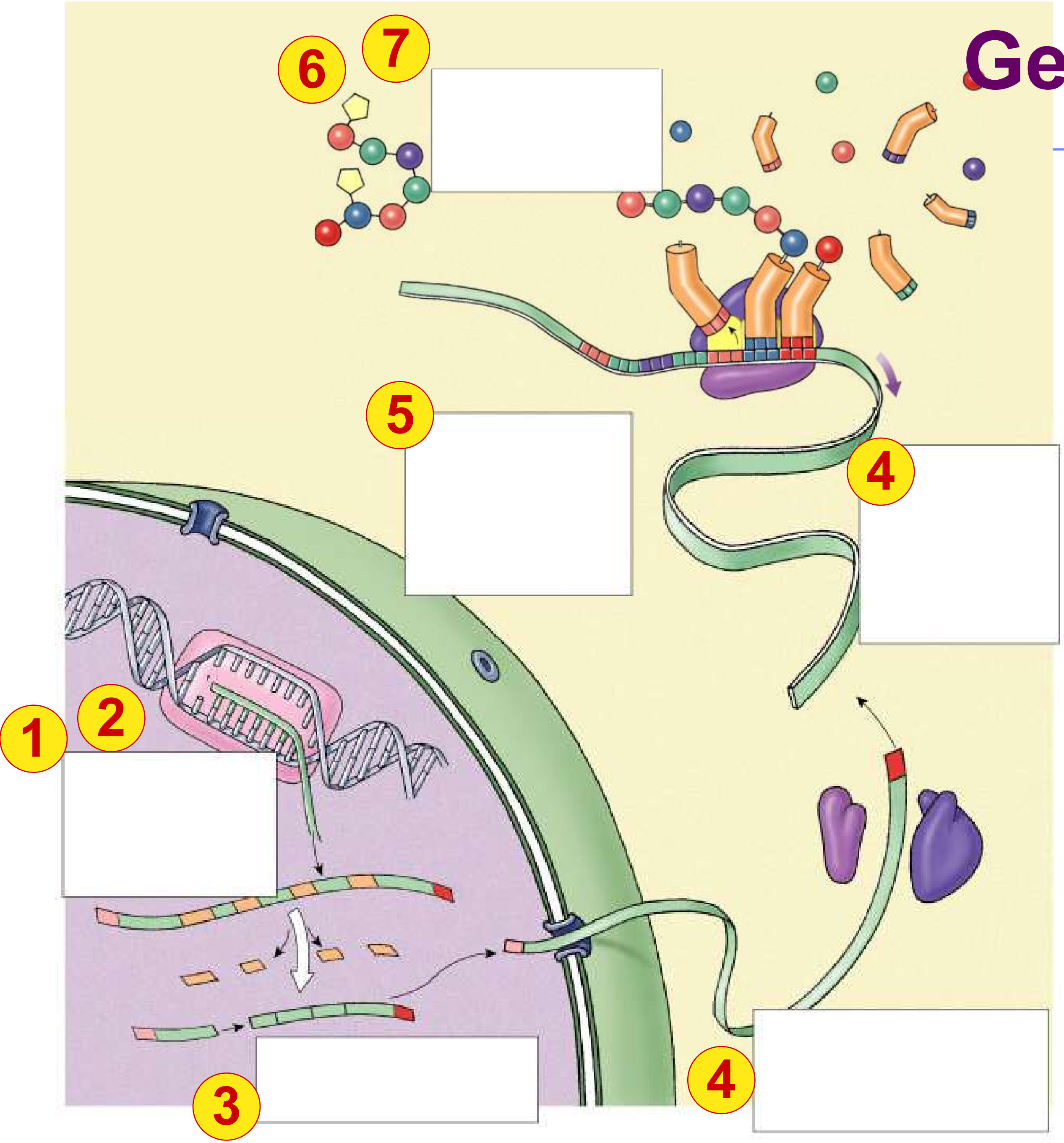
5. translation
- block start of translation

6 & 7. post-translation
- protein processing
- protein degradation

Turn your
Question Genes on!



Gene Regulation



1 & 2. ____________________

- ________________________
- ________________________

3 & 4. ____________________

- ________________________
- ________________________
- ________________________
- ________________________

5. ____________________

- ________________________

____

6 & 7. ____________________

- ________________________
- ________________________