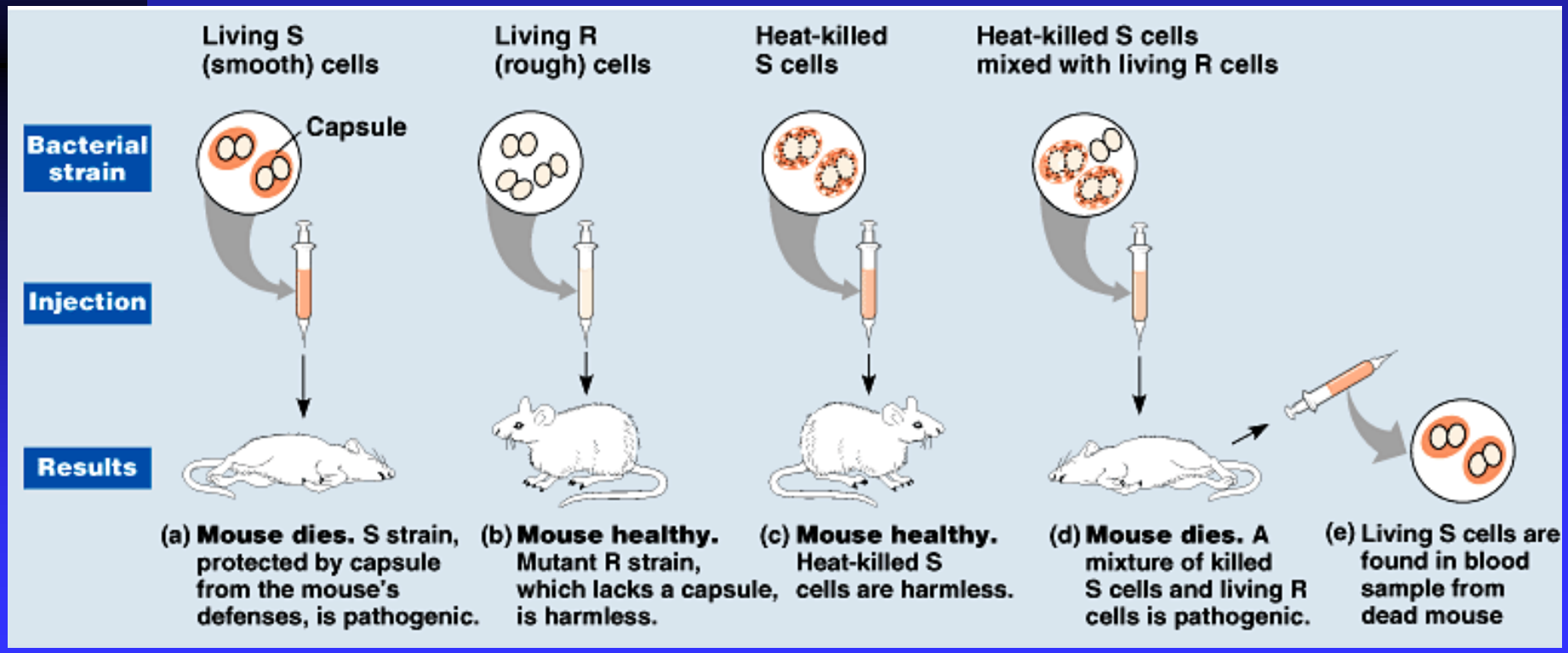
Introduction to Molecular Genetics

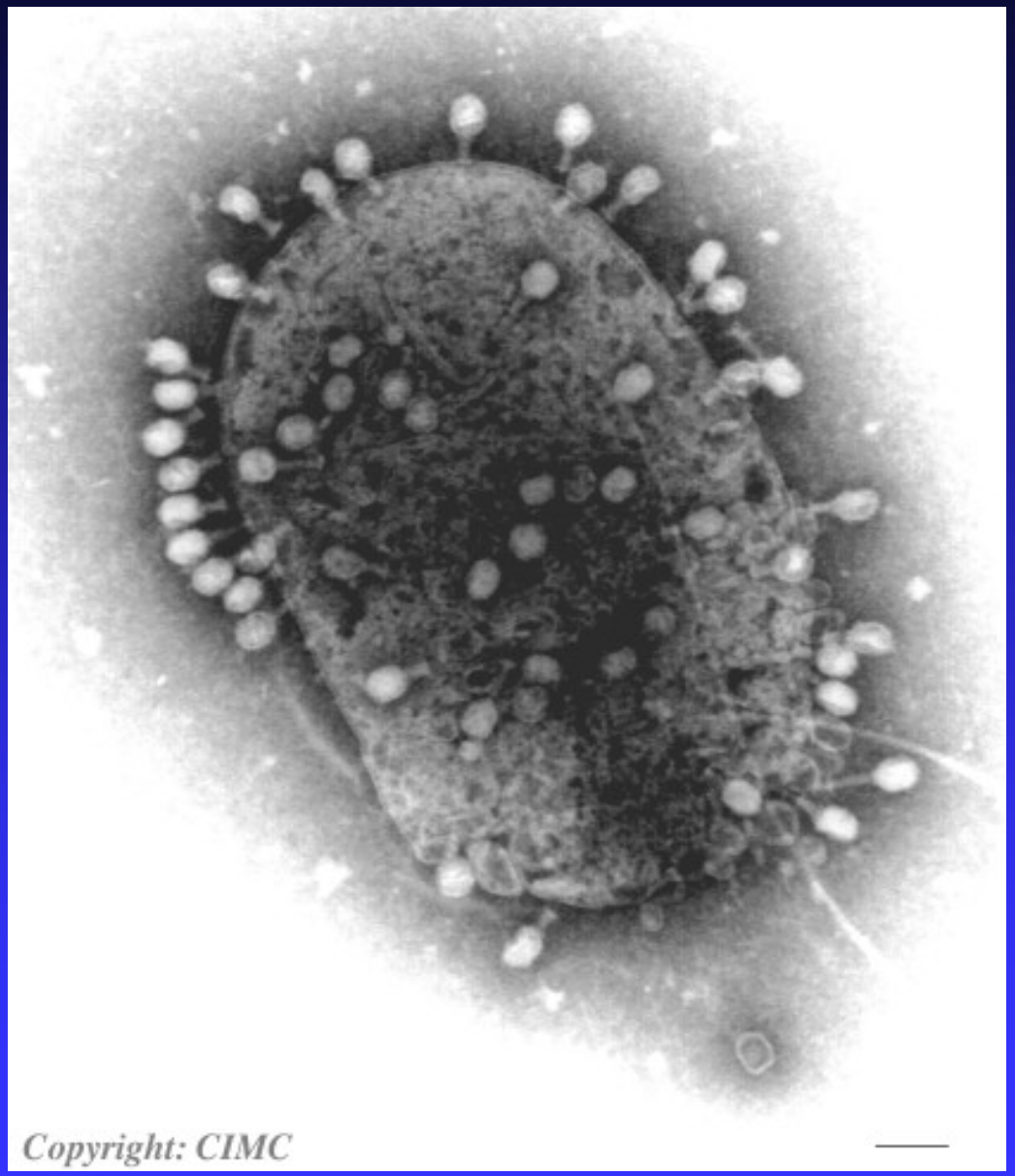
- 3 Views of the Genetic Material
- The Chemical Nature of the Genetic Material: Is it made of protein of nucleic acids?
  - Fred Griffith Experiment (1928)
  - Hershey and Chase Experiment (1952)
- 4 Requirements of DNA as the Director of Metabolism
- The Structure of the Genetic Material: Encoding Information
  - Rosalind Franklin (1952)
  - Watson and Crick (1952)
- DNA Self-Replication: Three Proposed Models
  - Messelson and Stahl (late 1950's)
  - DNA replication in prokaryotes and eukaryotes

The Search for the Genetic Material

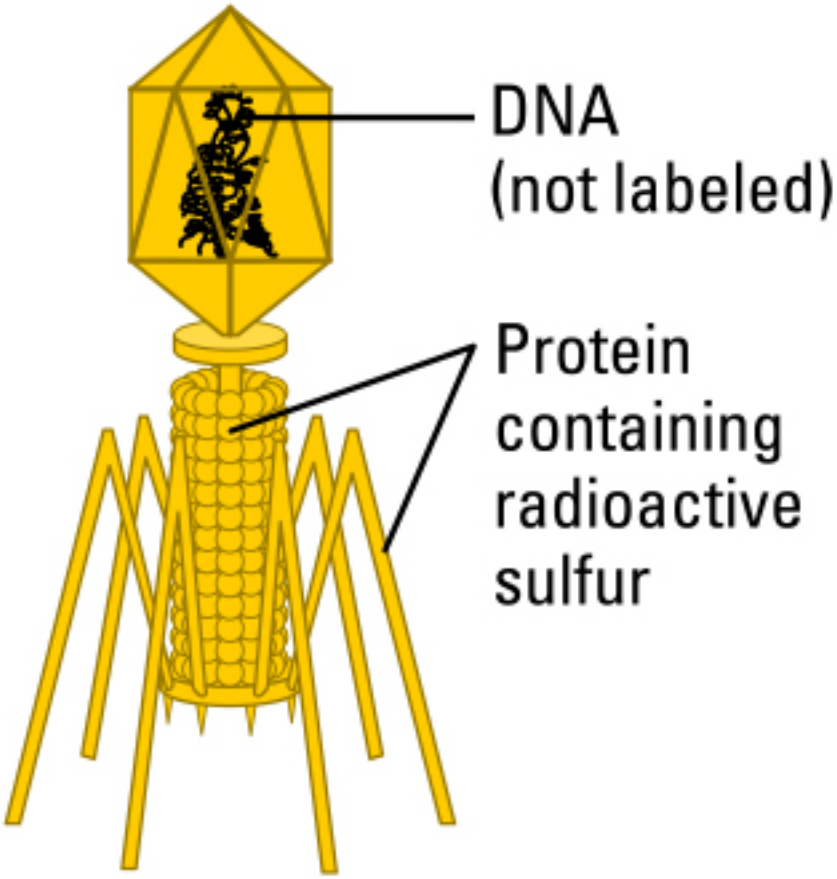
- Unlikely candidates: Lipids and CHO's
- Proteins vs. nucleic acids
  - Frederick Griffith, 1928

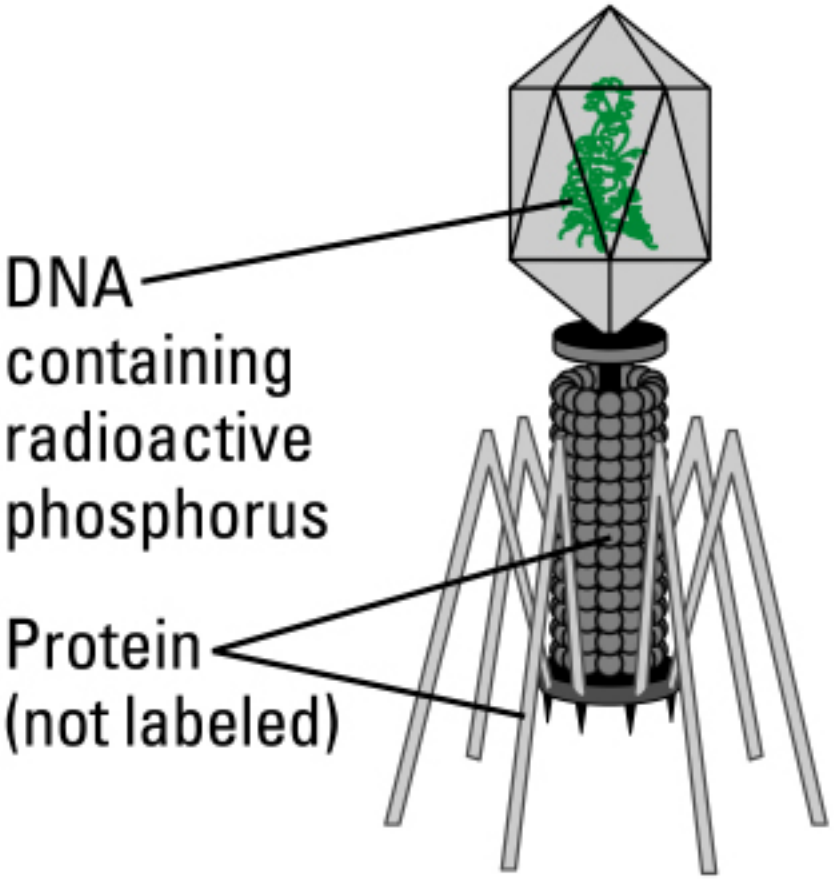


Bacteria phages infecting an *E. coli* bacterium

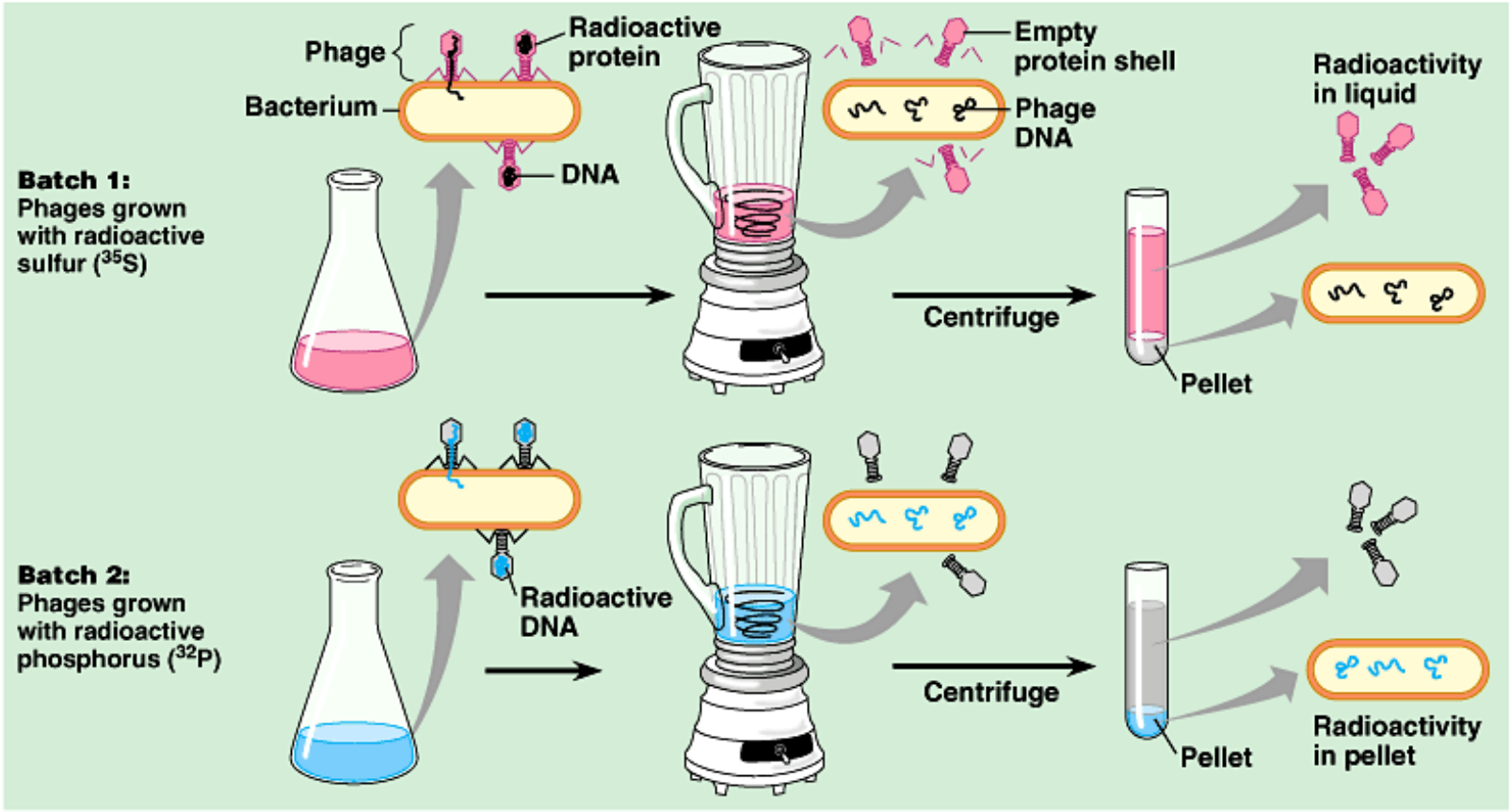


Bacteriophage Virus: Protein and DNA





The Hershey and Chase Experiment (1952)



(b) The experiment showed that T2 proteins remain outside the host cell during infection, while T2 DNA enters the cell.

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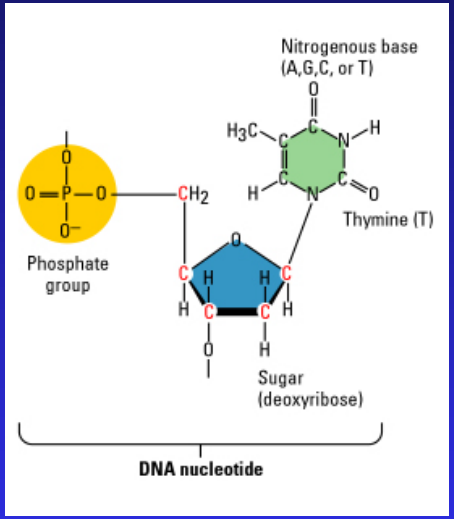
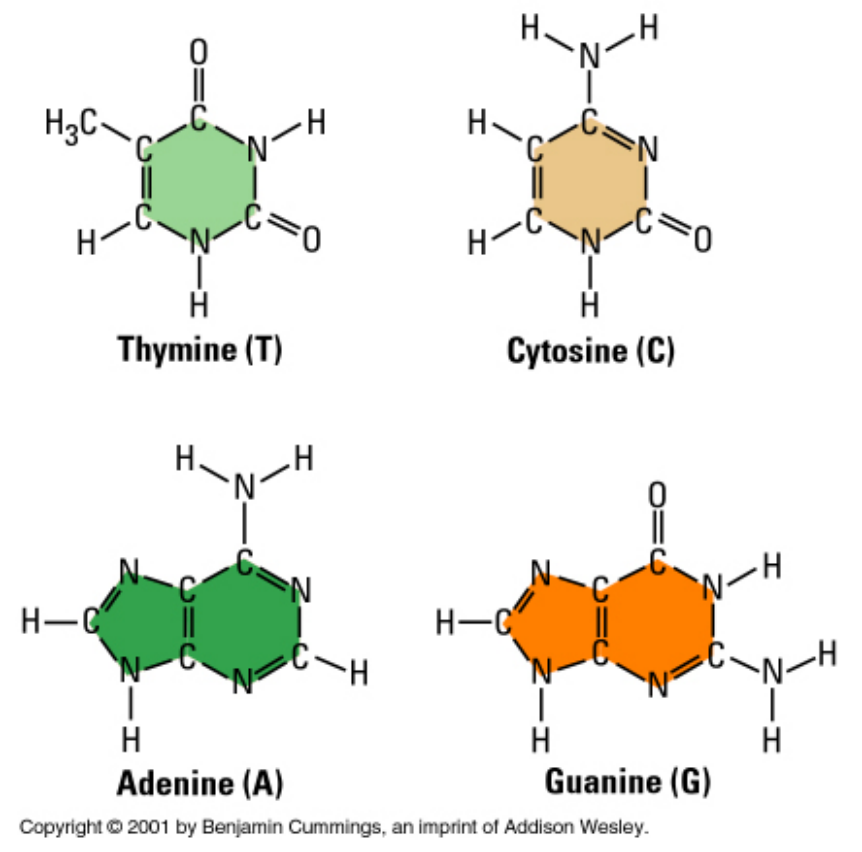
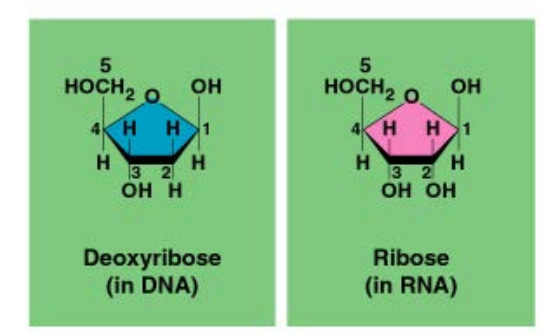
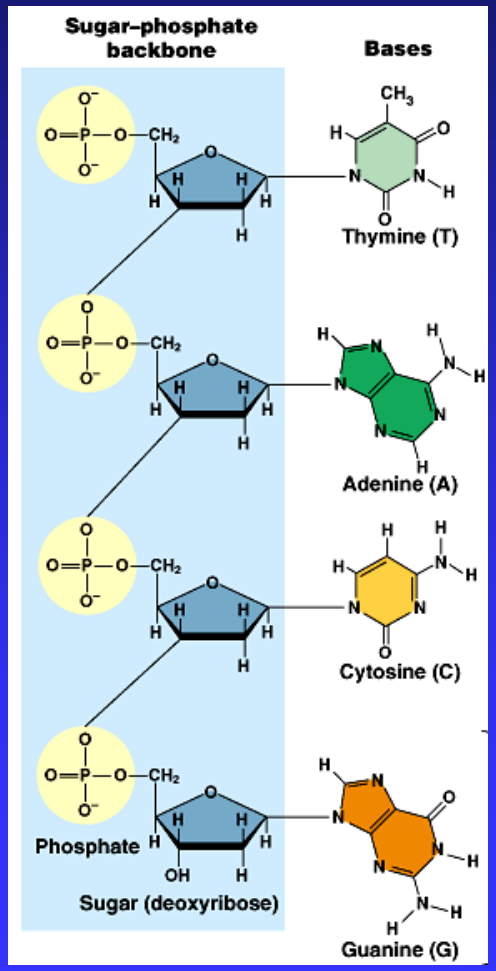
The Nucleotide and the 4 nitrogen bases: The Building Blocks of DNA

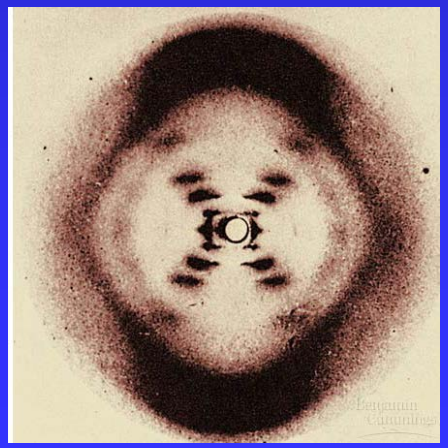


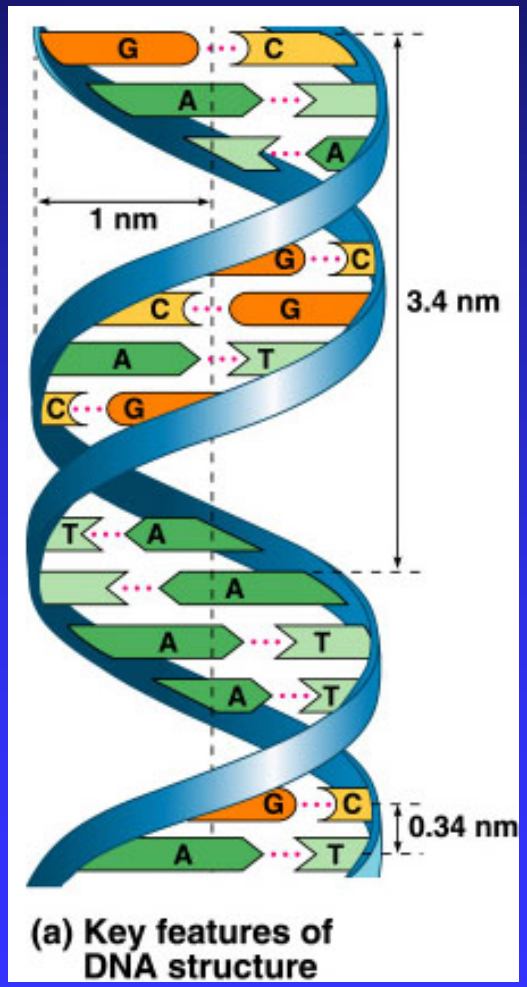




Rosalind Franklin: X-ray crystallography and the keys to the kingdom

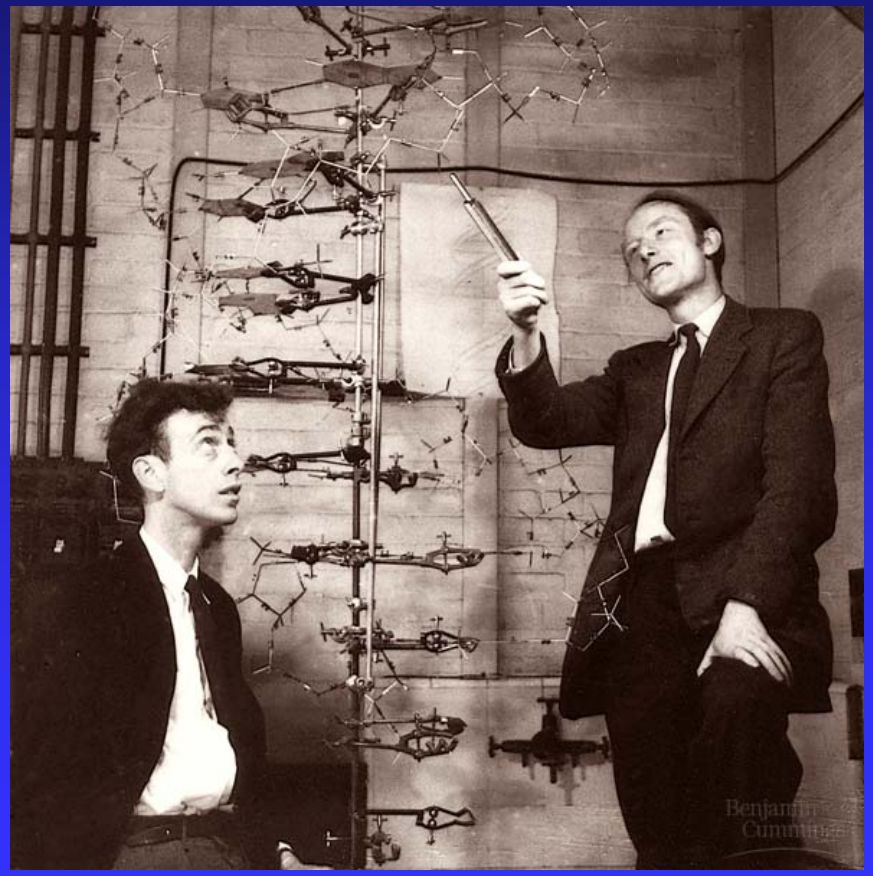




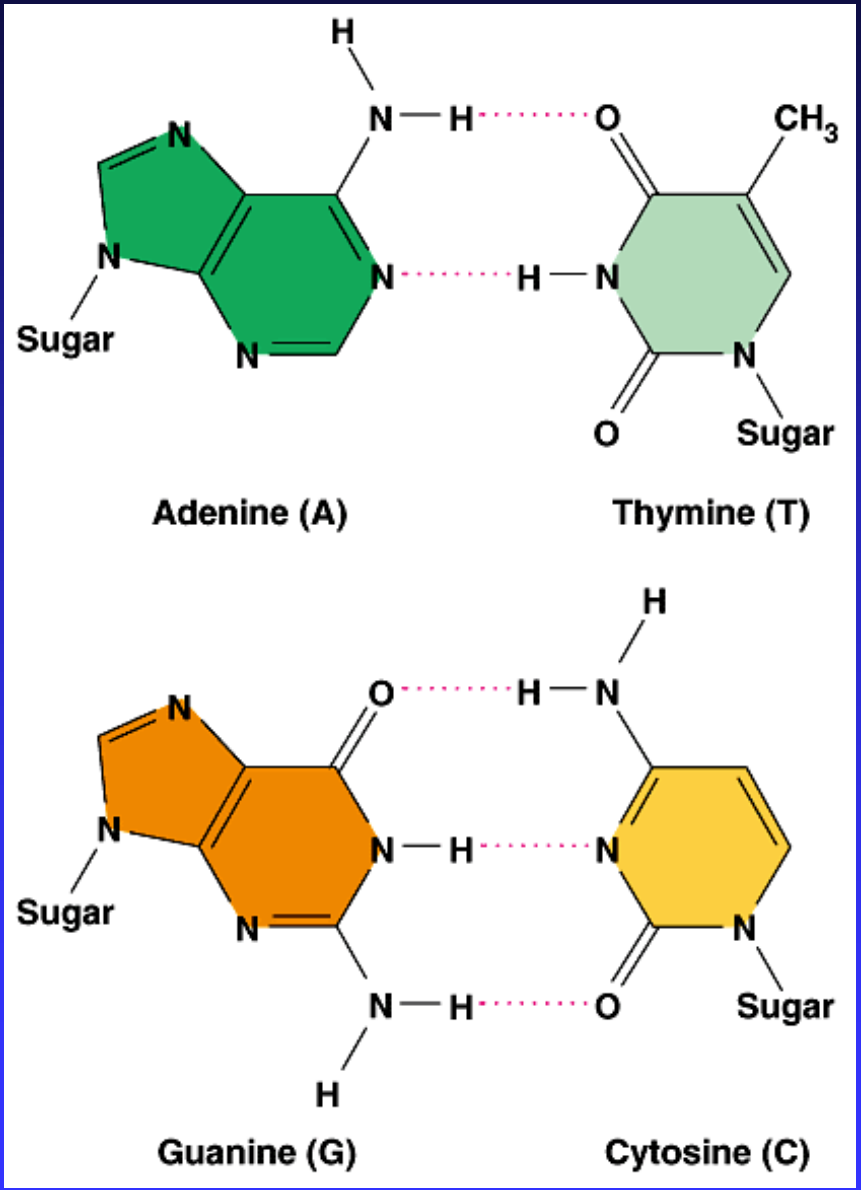


Watson & Crick and the Double Helix

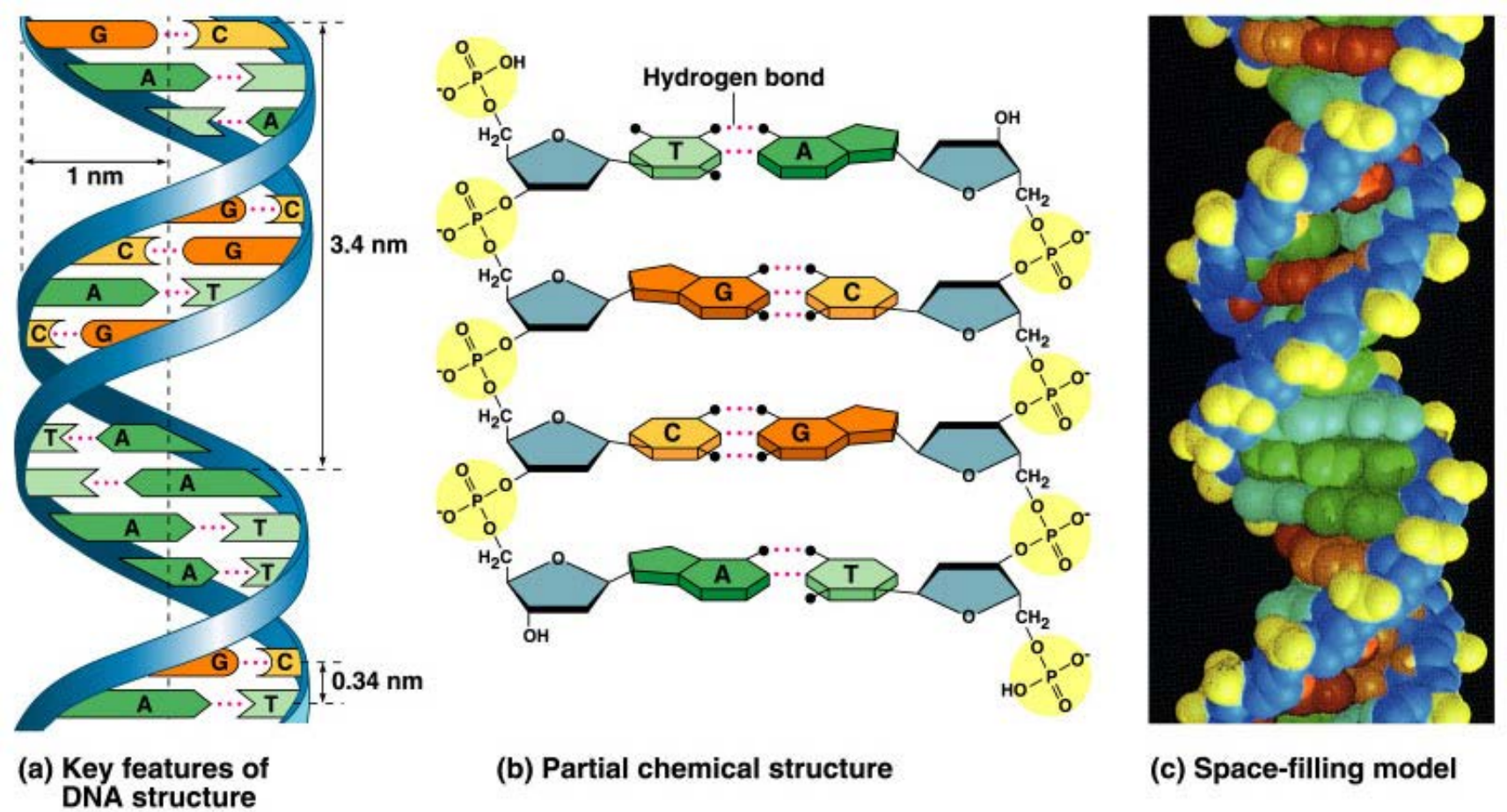
- Franklin:X-ray crystalography
- Helical dimensions of the molecule
- Chargaff's Rules on base pairing
- Nitrogen base chemistry



The chemical basis for the specificity of base pairing:



Putting together the greatest discovery of the 20th century



- Antiparallel arrangement
- Specificity of base pairing
- Unrestricted sequence

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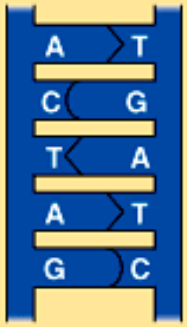
Why a double helix? Why not a single helix? W&C: a template for self-replication *Nature*: April 1953



(a) The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

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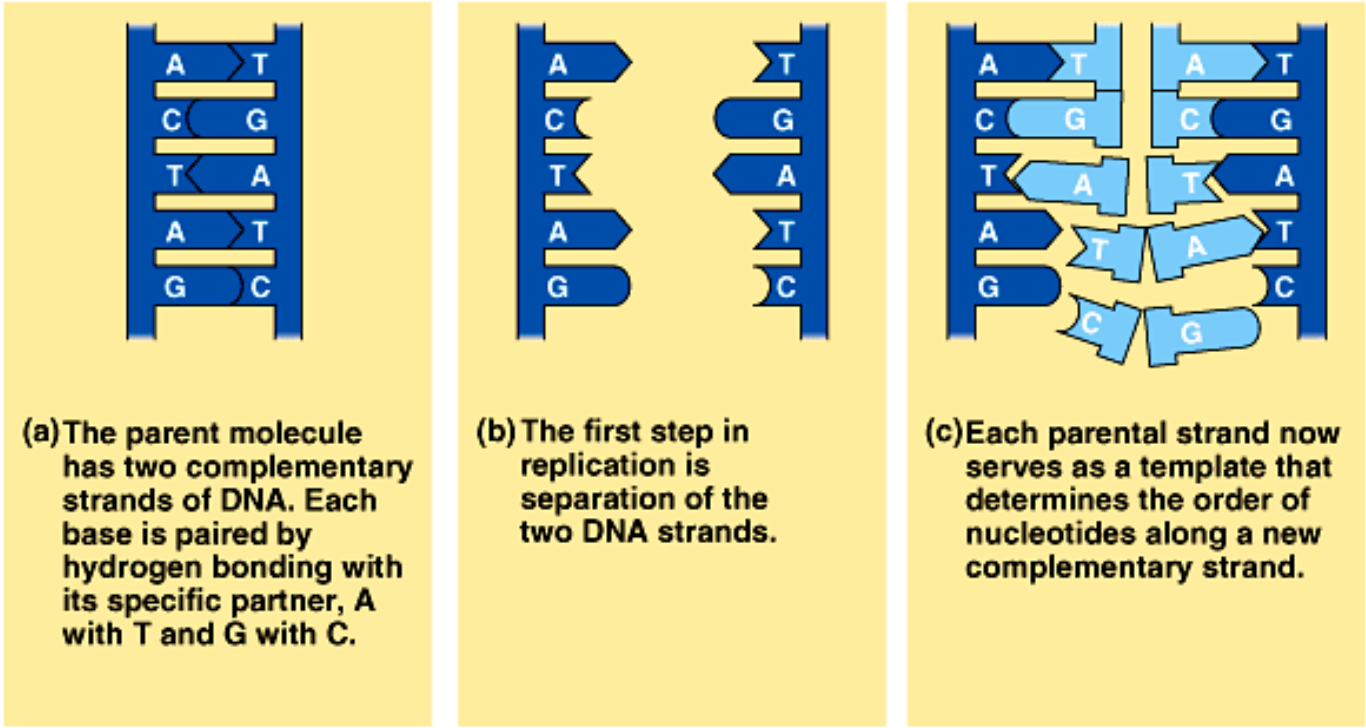

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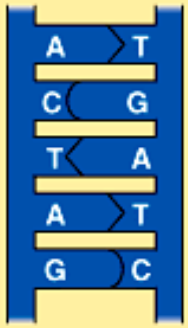

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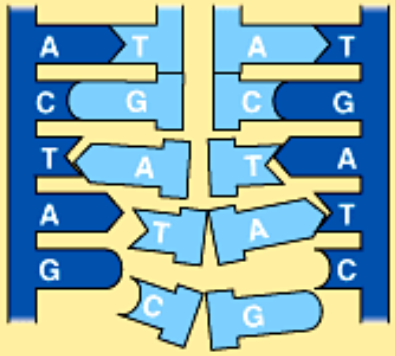

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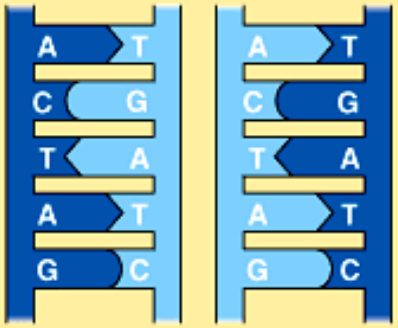
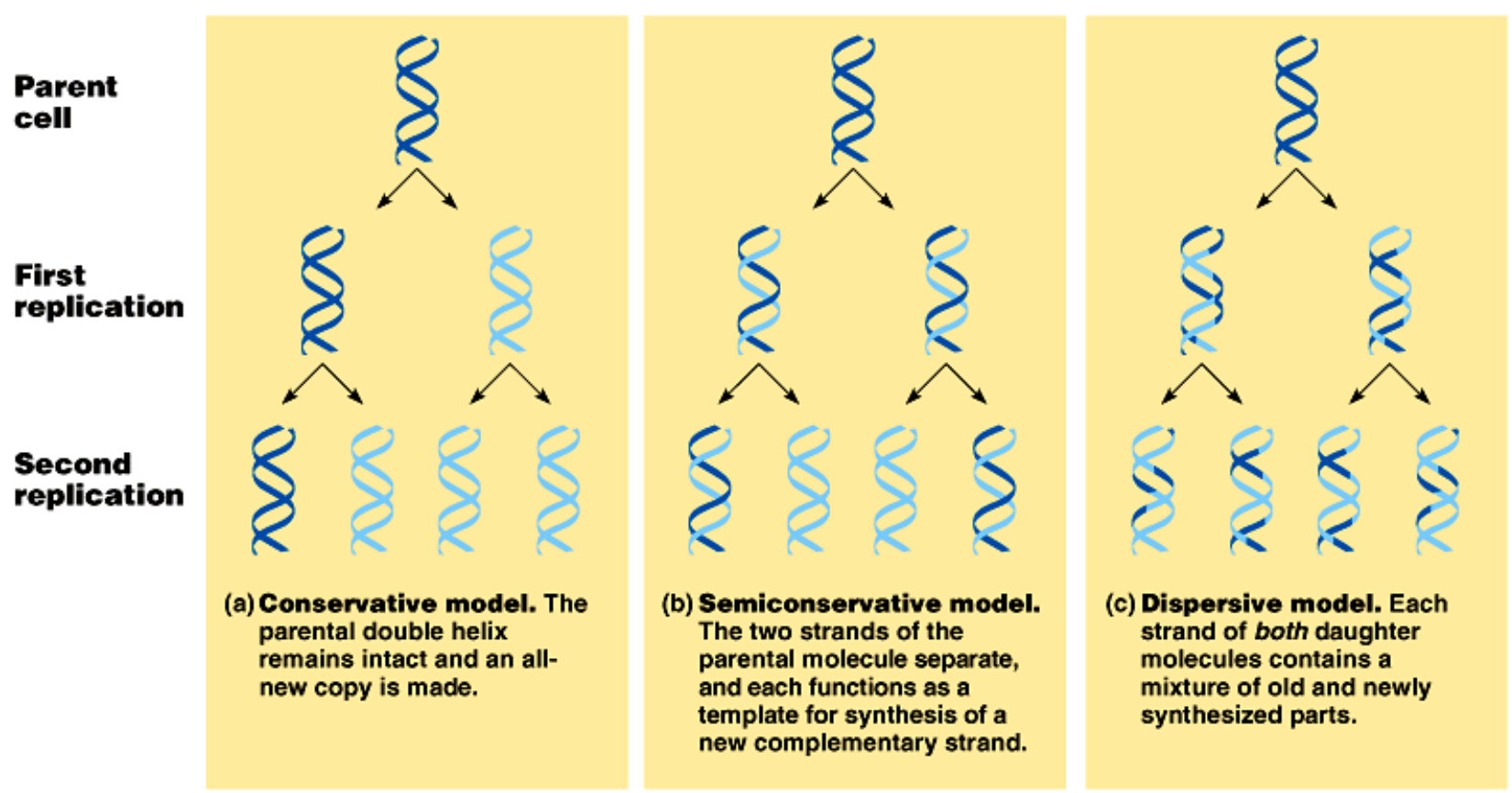
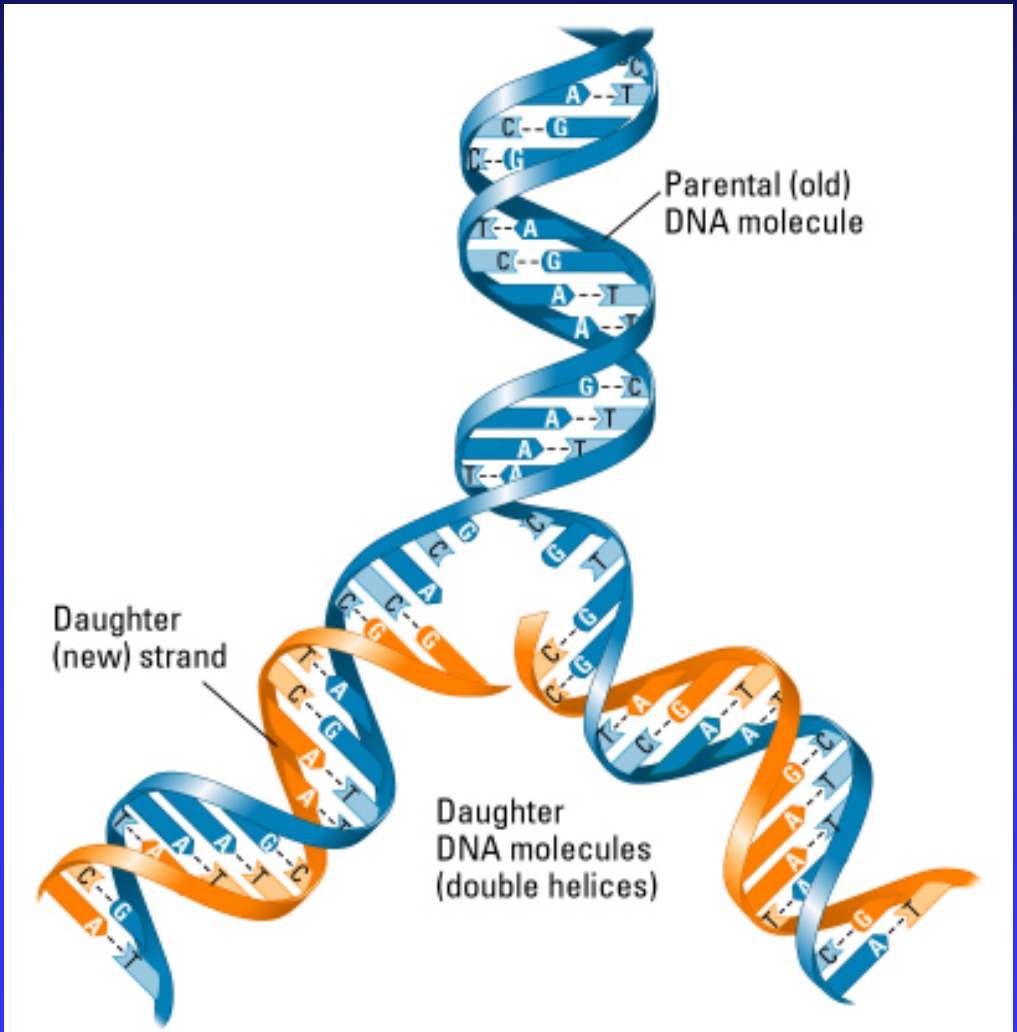


(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

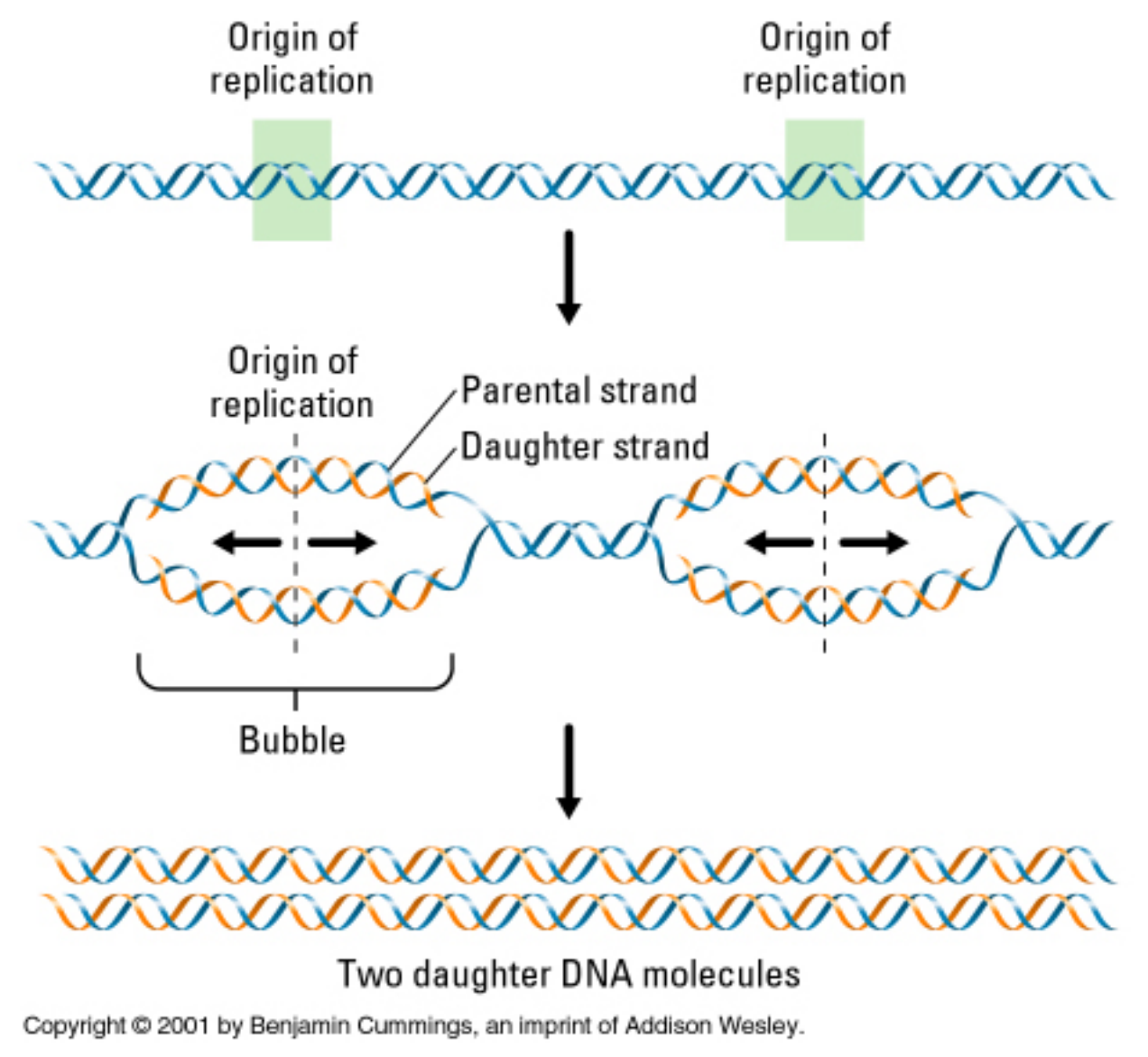
Testing the prediction of semiconservative replication: Three alternative models of DNA replication



DNA replication moving down the replication fork…



Replication with multiple origins



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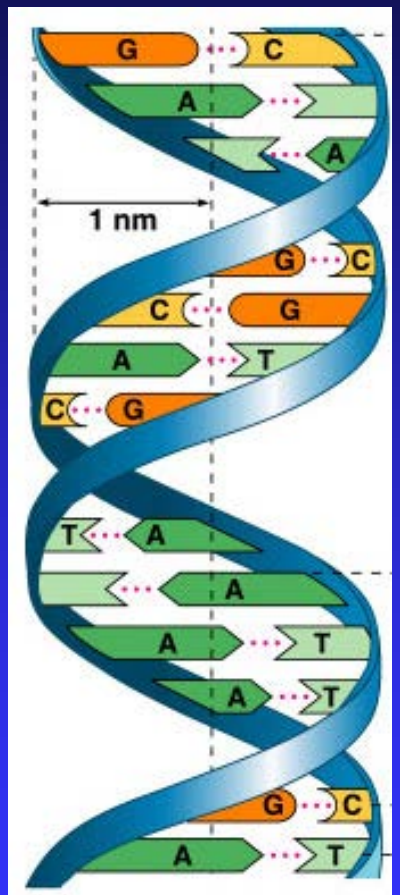
Expressing a Gene: Protein Synthesis

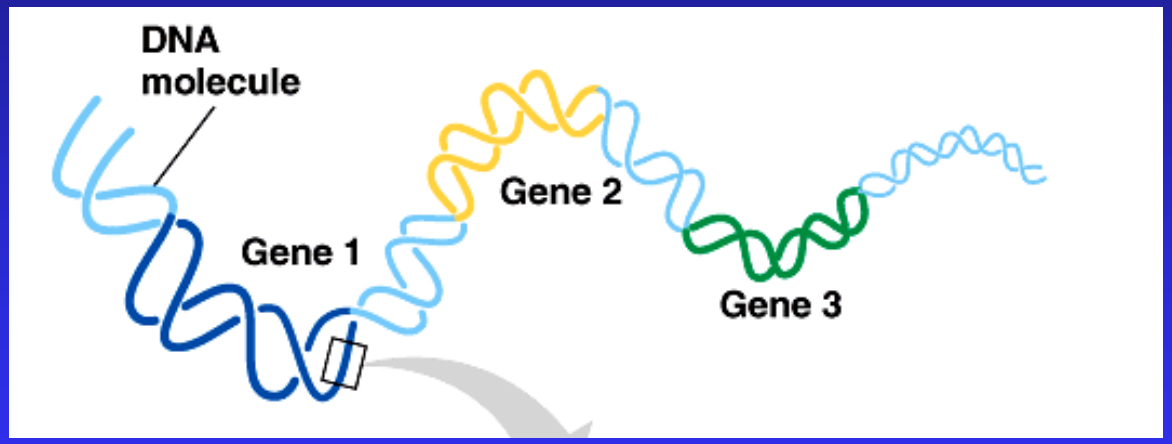
- DNA Self-Replication: The Semi-Conservative Model
  - DNA Polymerase & Mutations: a source of variation
- The Central Dogma: DNA → RNA → protein
- Transcription: A set of nuclear requirements
- Translation: The role of 3 RNA's in the cytoplasm
  - Messenger RNA (mRNA) : a working copy of the coded message
  - Transfer RNA (tRNA) : a set of cytoplasmic interpreters
  - Ribosomes (rRNA and protein): a cytoplasmic workbench
- Let's make a protein: initiation, elongation, termination
- Summary: 4 characteristics of the genetic code - triplet, redundant, unambiguous and universal

DNA and Genes



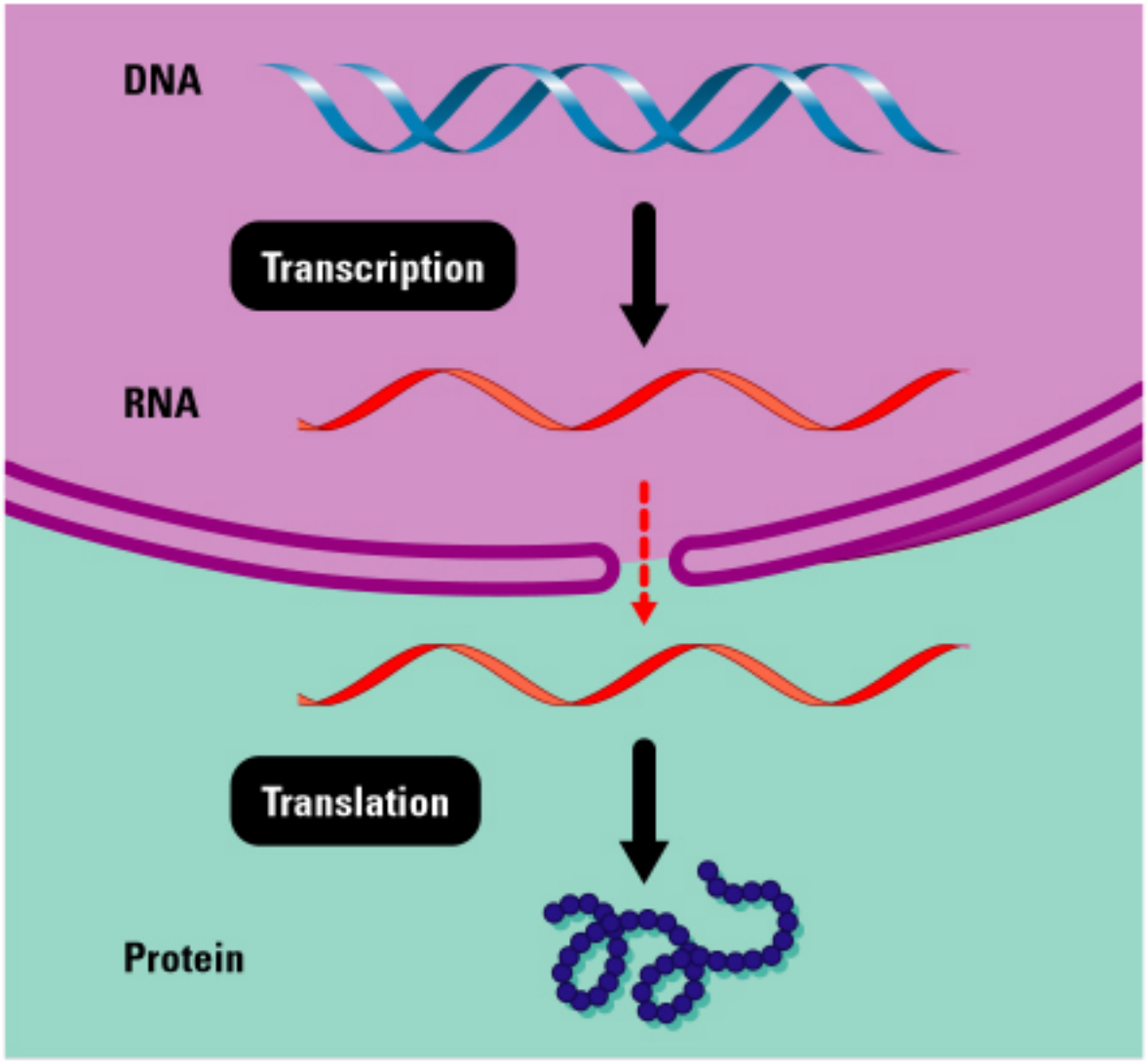
(a) Key features of DNA structure

- DNA: the genetic blueprint
  - The director of metabolism
  - A series of genes along the length of the DNA molecule

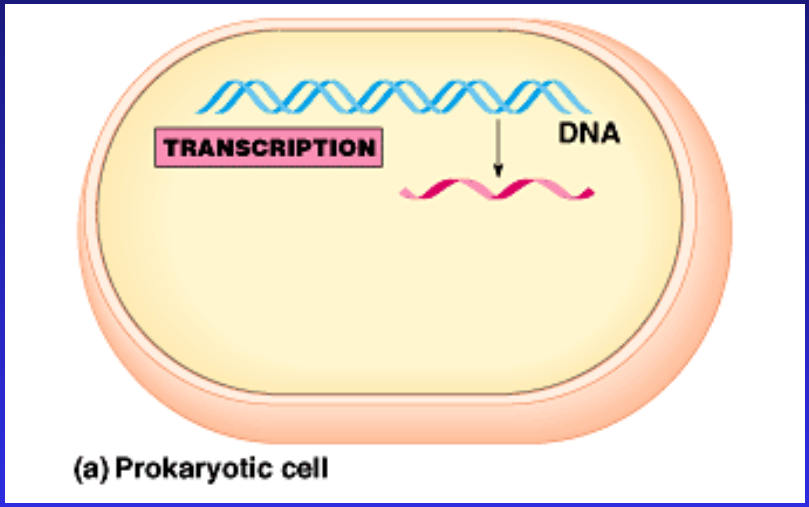


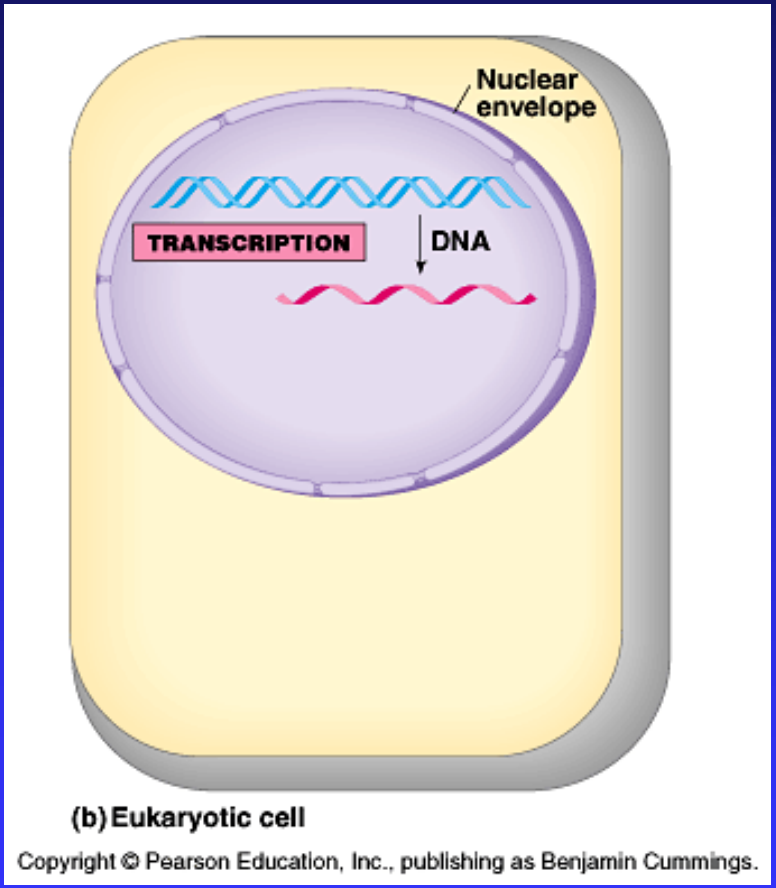
- Genes: a gene is specific linear sequence of nucleotides along the length of a DNA molecule that codes for the primary structure of a protein, I.e., its amino acid sequence.

Expressing genes: a two step process



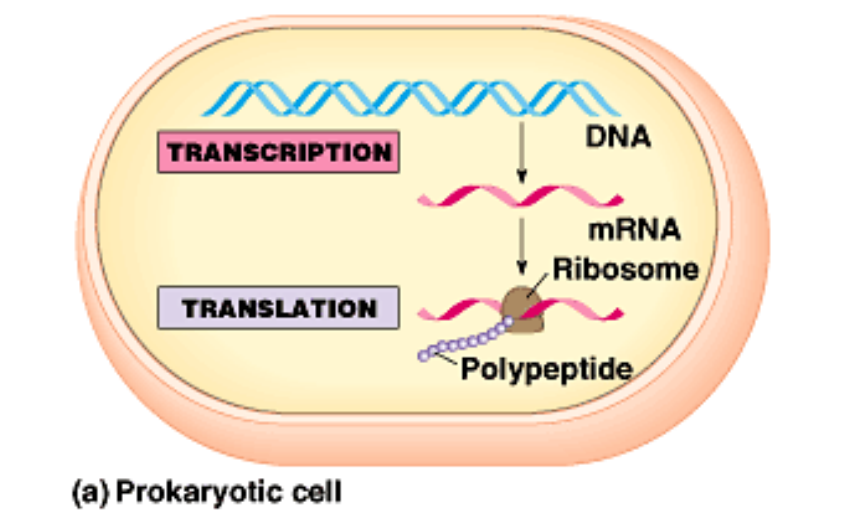
Overview: the roles of transcription and translation in the flow of genetic information

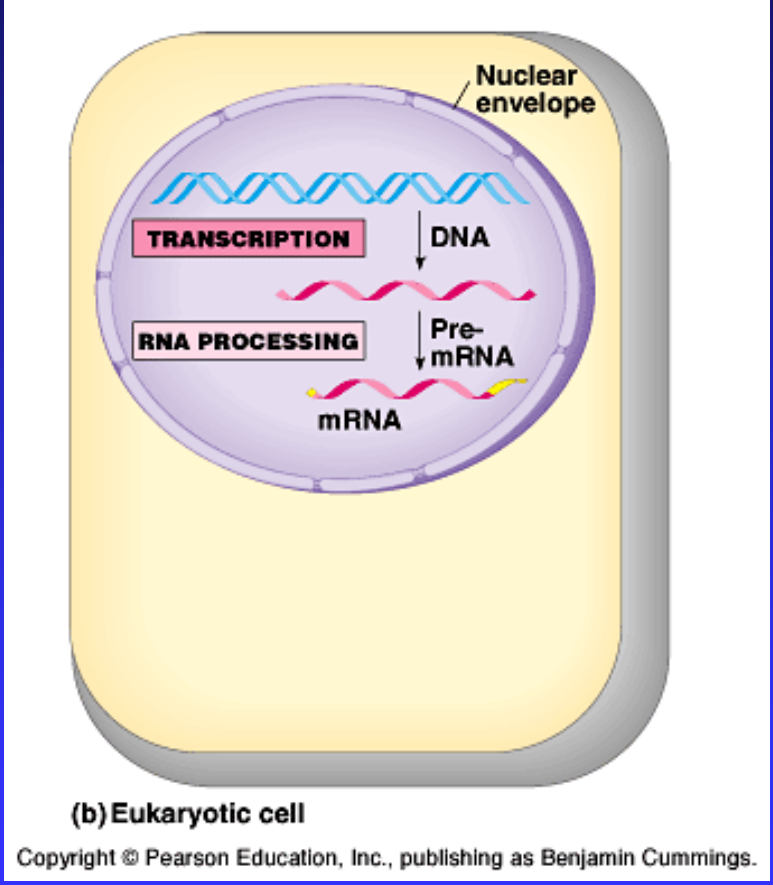




Transcription: The making of a working-copy of a gene.

Overview: the roles of transcription and translation in the flow of genetic information





<u>Translation</u>: The uncoding of the working-copy into a sequence of amino acids.

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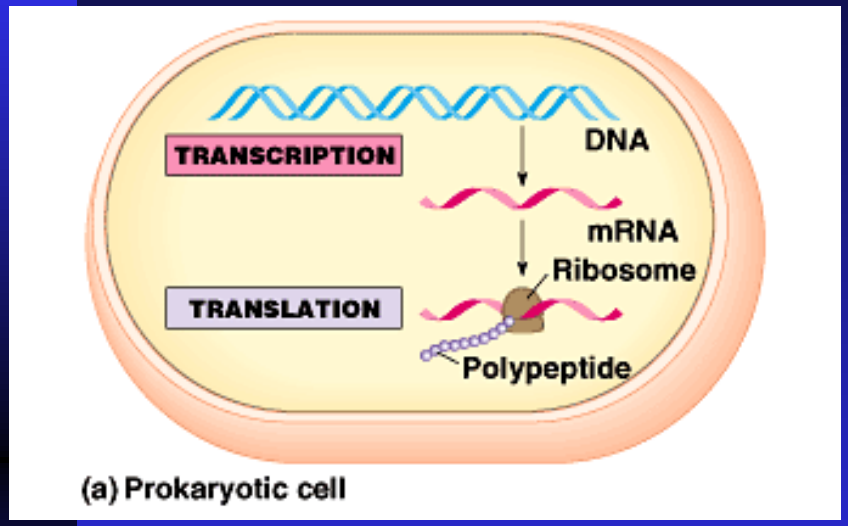
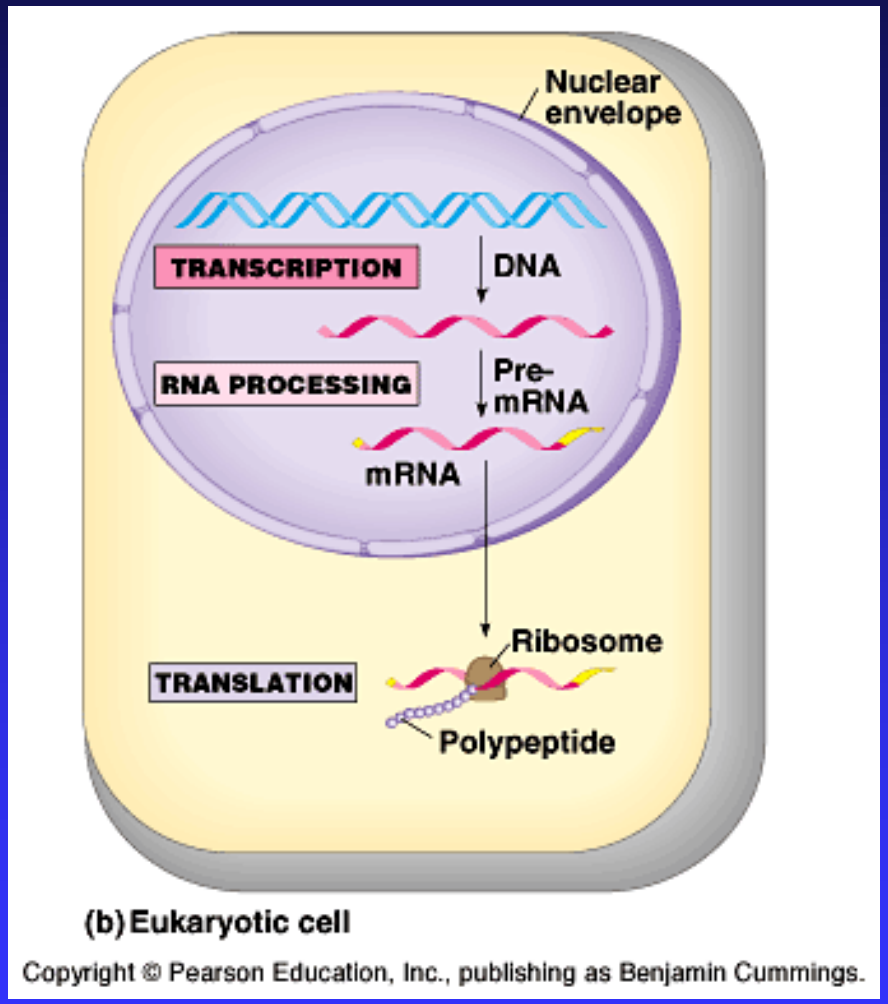


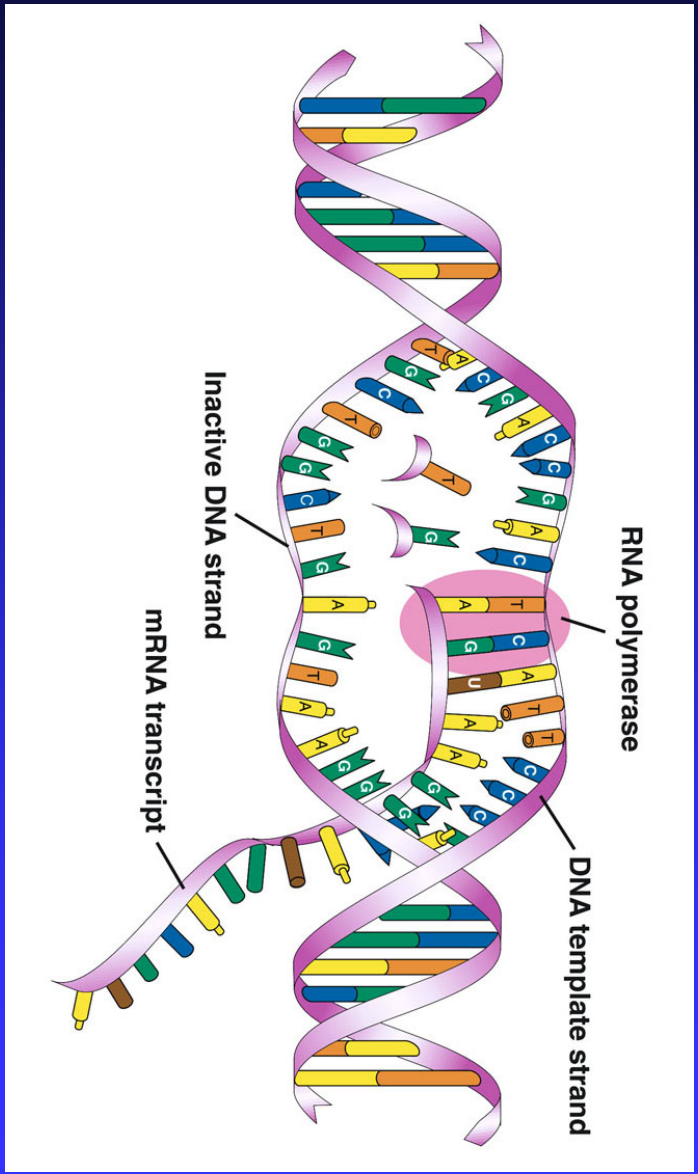


Transcription: The making of a working-copy of a gene.

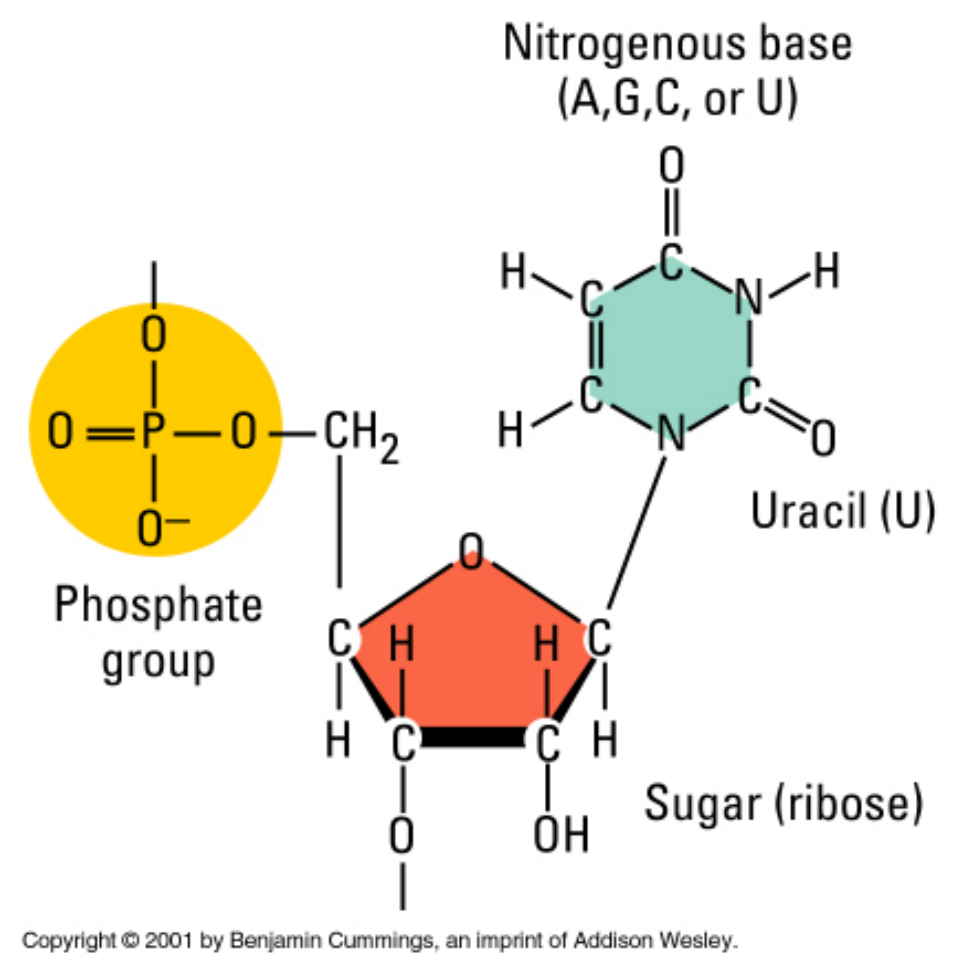
Translation: The uncoding of the working-copy into a sequence of amino acids.

The Details of Transcription: DNA ⟶ mRNA

- RNA polymerase
- Ribonucleotides: A, U (uracil), G, and C
- Specificity of base pairing
- The transcription bubble
- The *template* and *gene* strands
- The promotor sequence



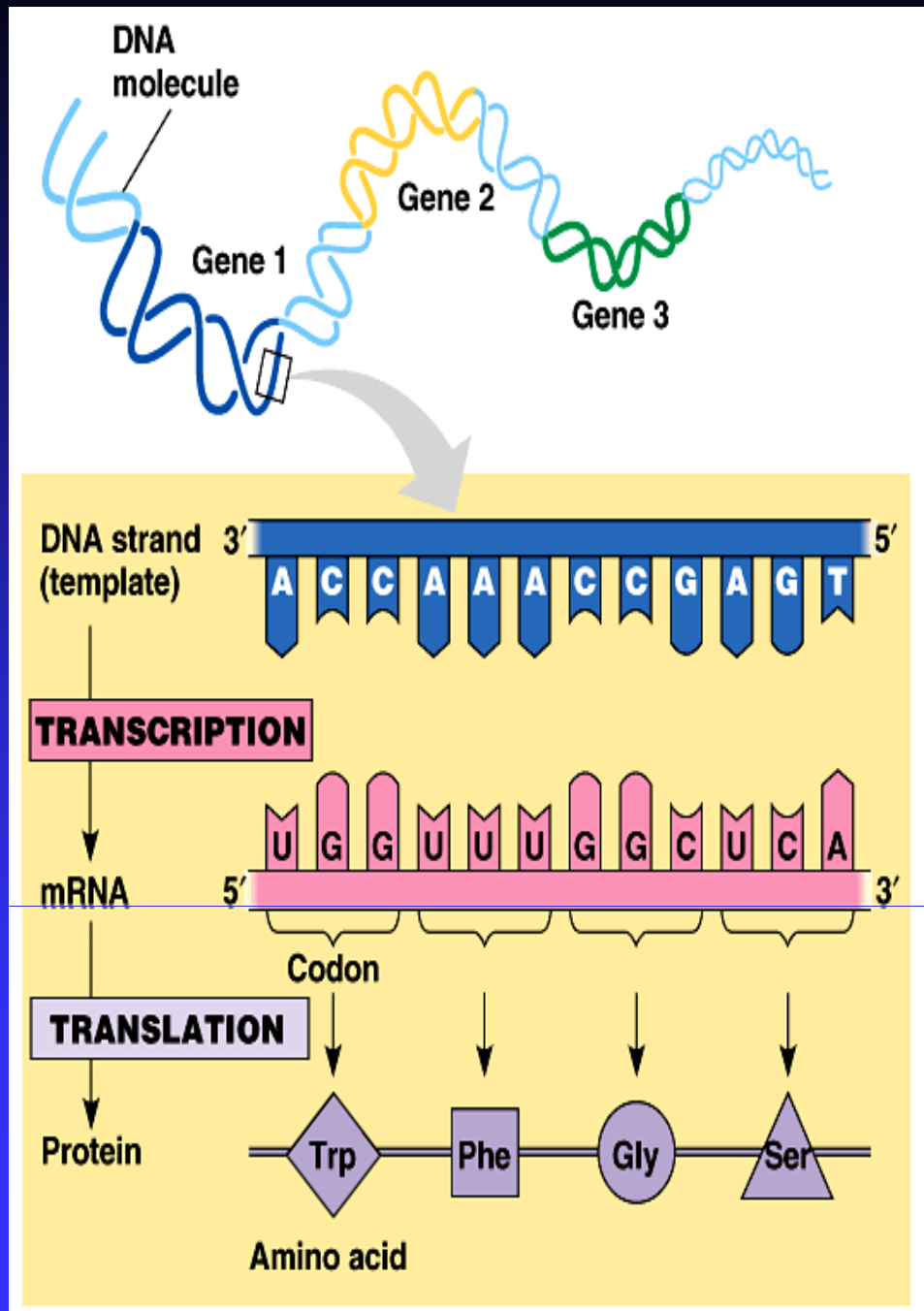
Nucleotides:The Building Block of Nucleic Acids



Translation:

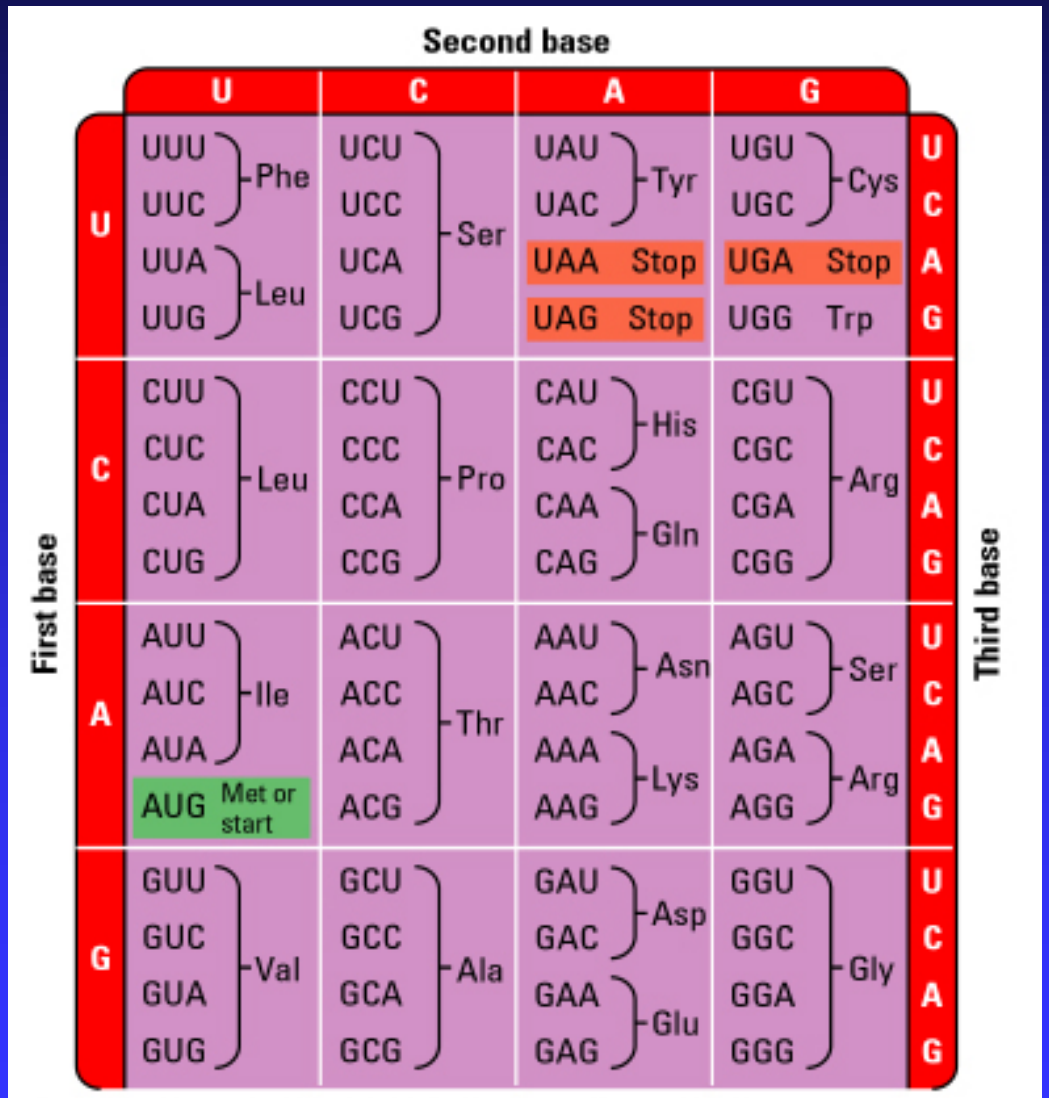
How can only four nucleotides code for 20 different amino acids?

- A singlet code?
  - 1 ntd/aa
- A doublet code?
  - 2 ntds/aa
- A triplet code?
  - 3 ntds/aa



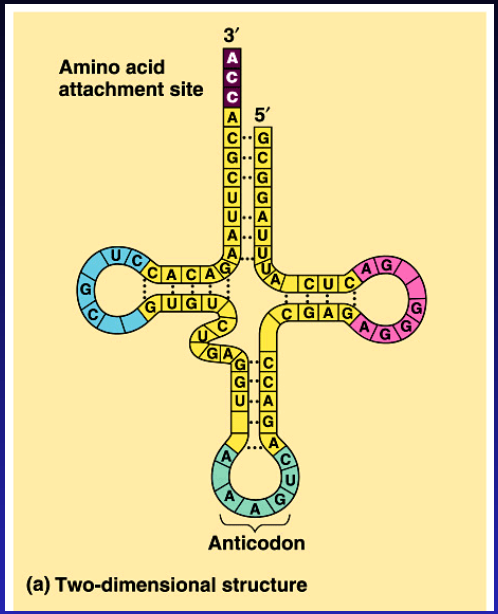
Reading the working blueprint: A codon table for mRNA translation

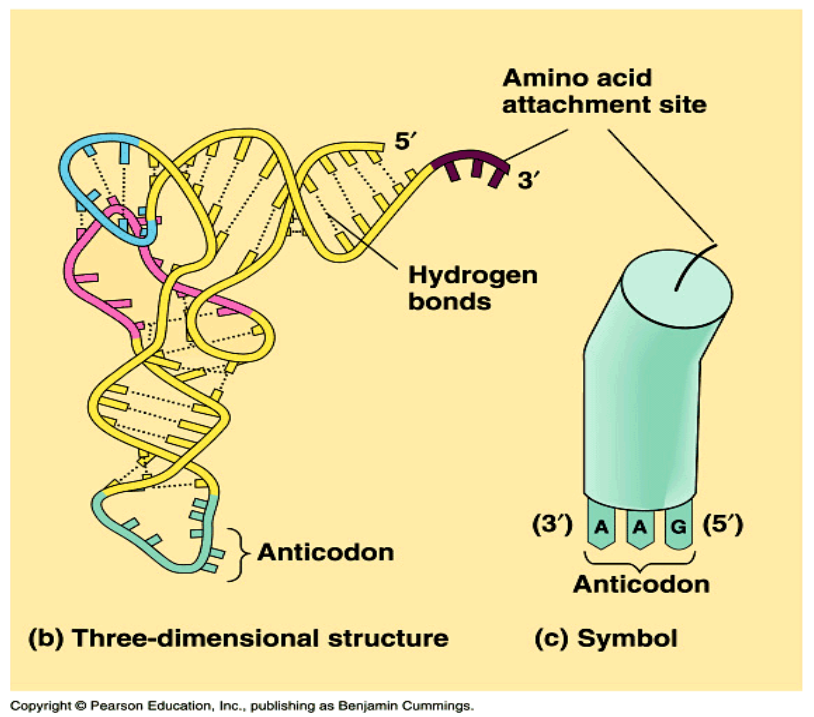
- The "Start codon"
- Redundancy of codons
- Unambiguity
- Chain terminators: stop codons
- Universal nature of the code



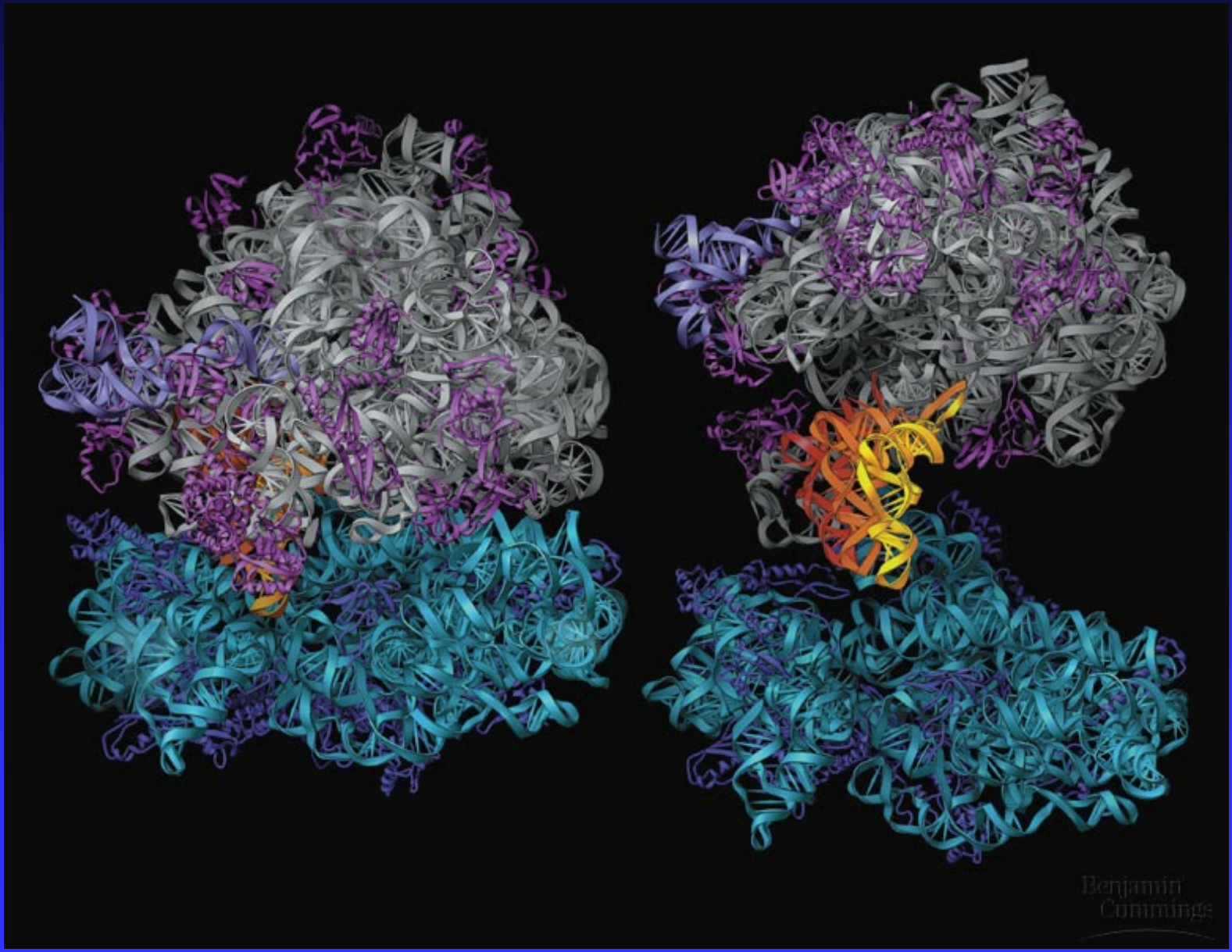
The structure and function of transfer RNA (tRNA)

- 75-80 nucleotides in length
- Transcribed from the DNA
- Functions as the code interpreter:
  - Amino acid attachment site
  - Anticodon

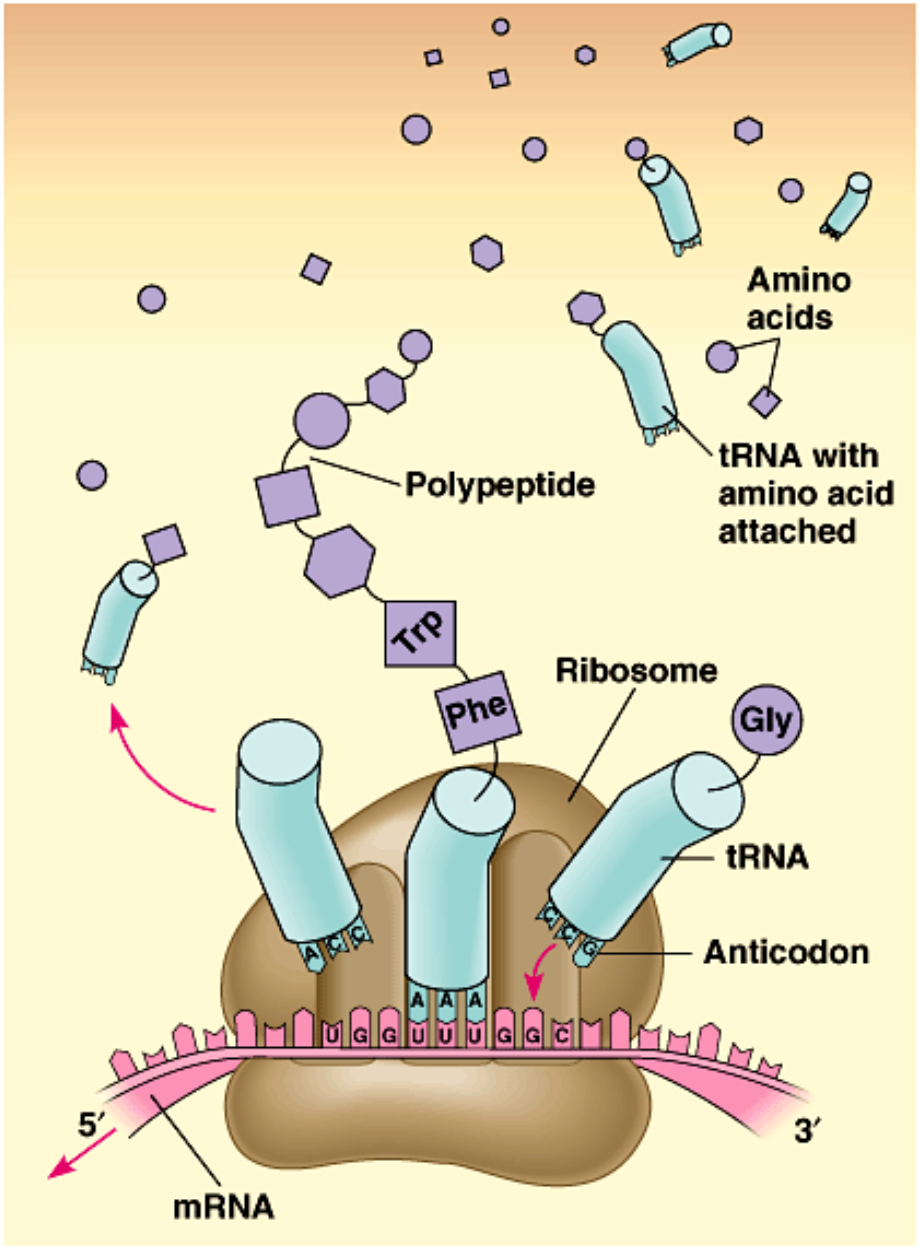




A Ribosome in 3D: protein + rRNA

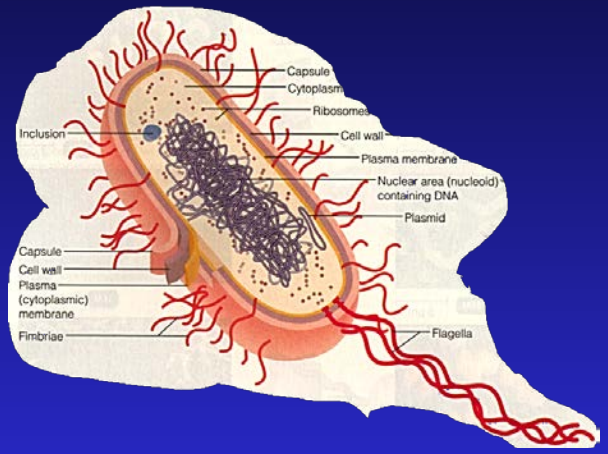


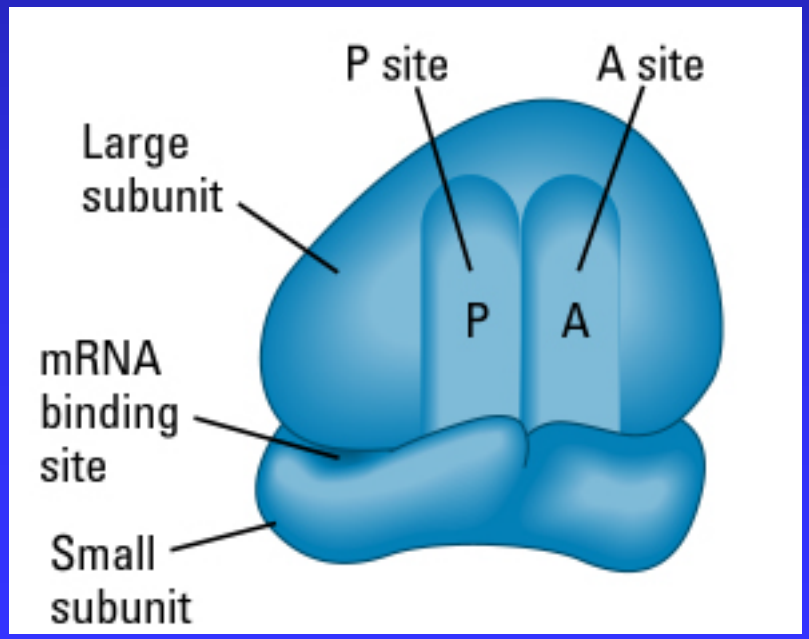
Translation: the basic concept



Ribosomes: Macromolecular particles composed of rRNA + a few proteins

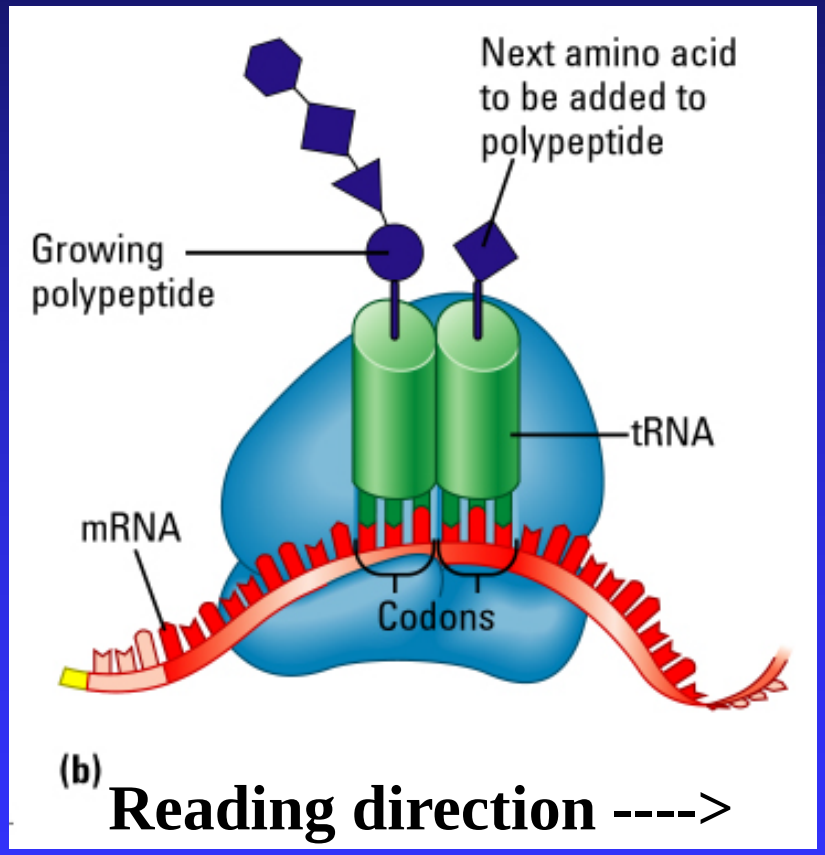
- A molecular workbench
- About 30,000/cell
- Small and large subunits
  - Coordinate codon-anticodon pairing
  - Catalyze peptide bond formation



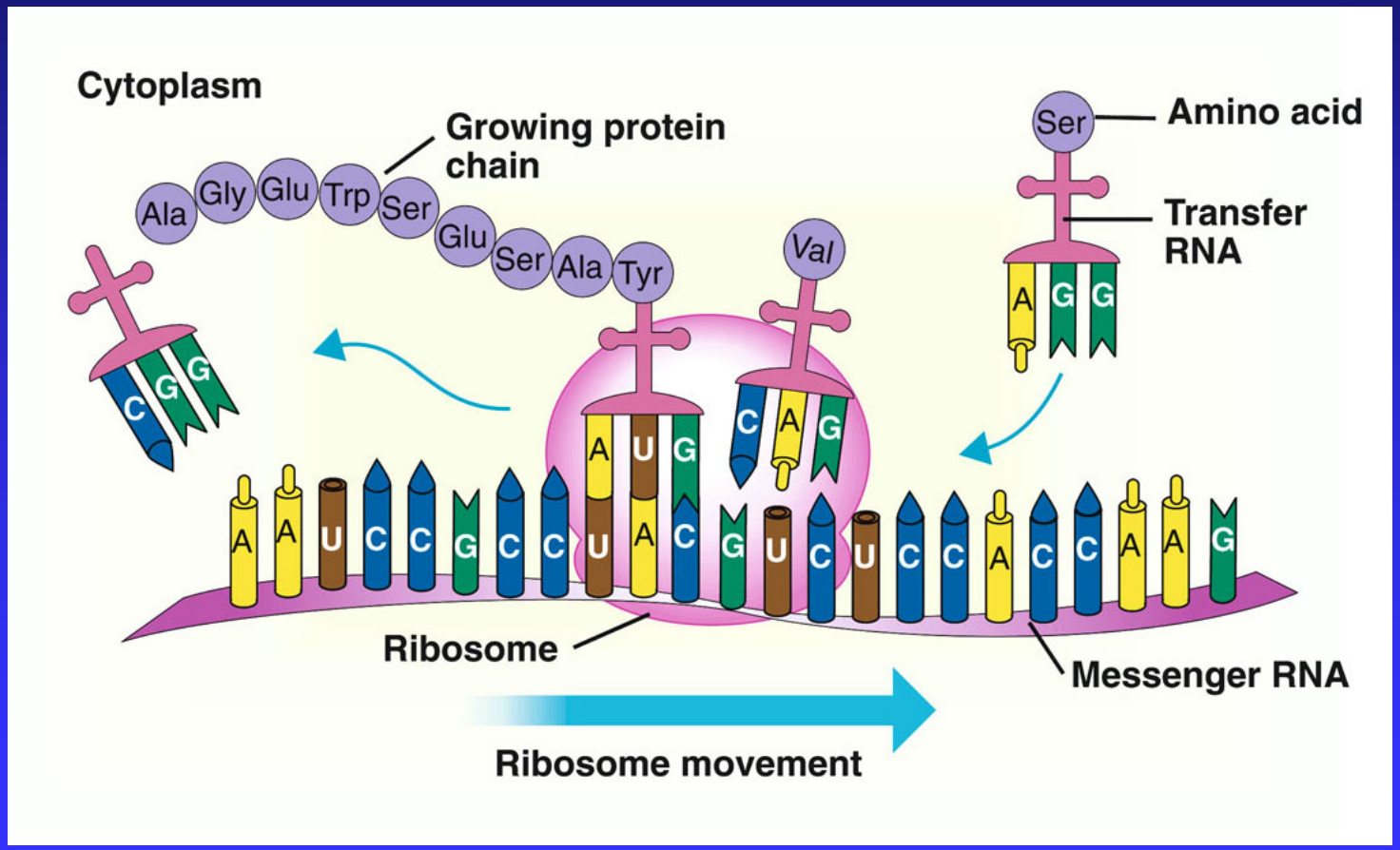


Translation: The interaction of all three types of RNA

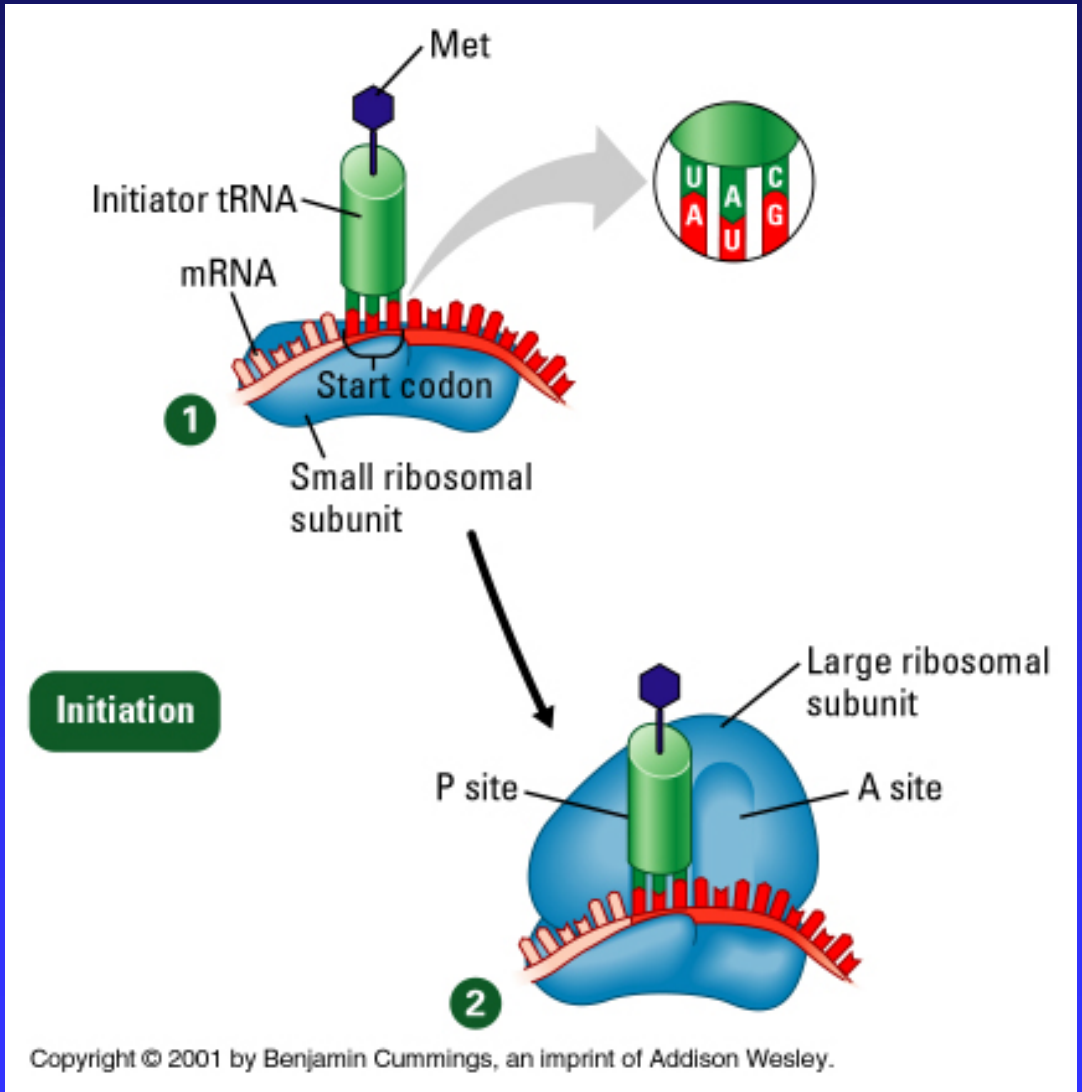
- mRNA, tRNA and a ribosome (rRNA)
- The ribosome has 2 binding sites
  - A site (A=amino acid)
  - P site (P=polypeptide)



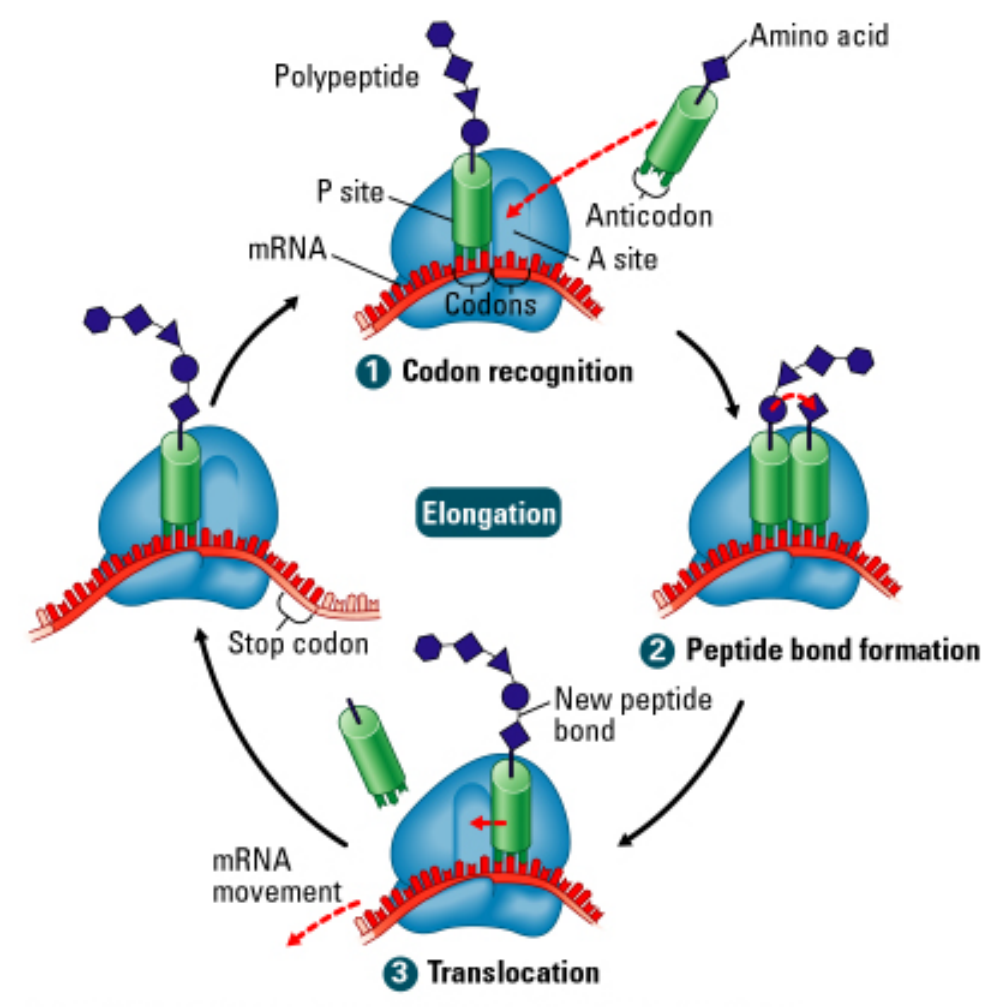
Translation: Reading the mRNA blueprint… a 3 step process



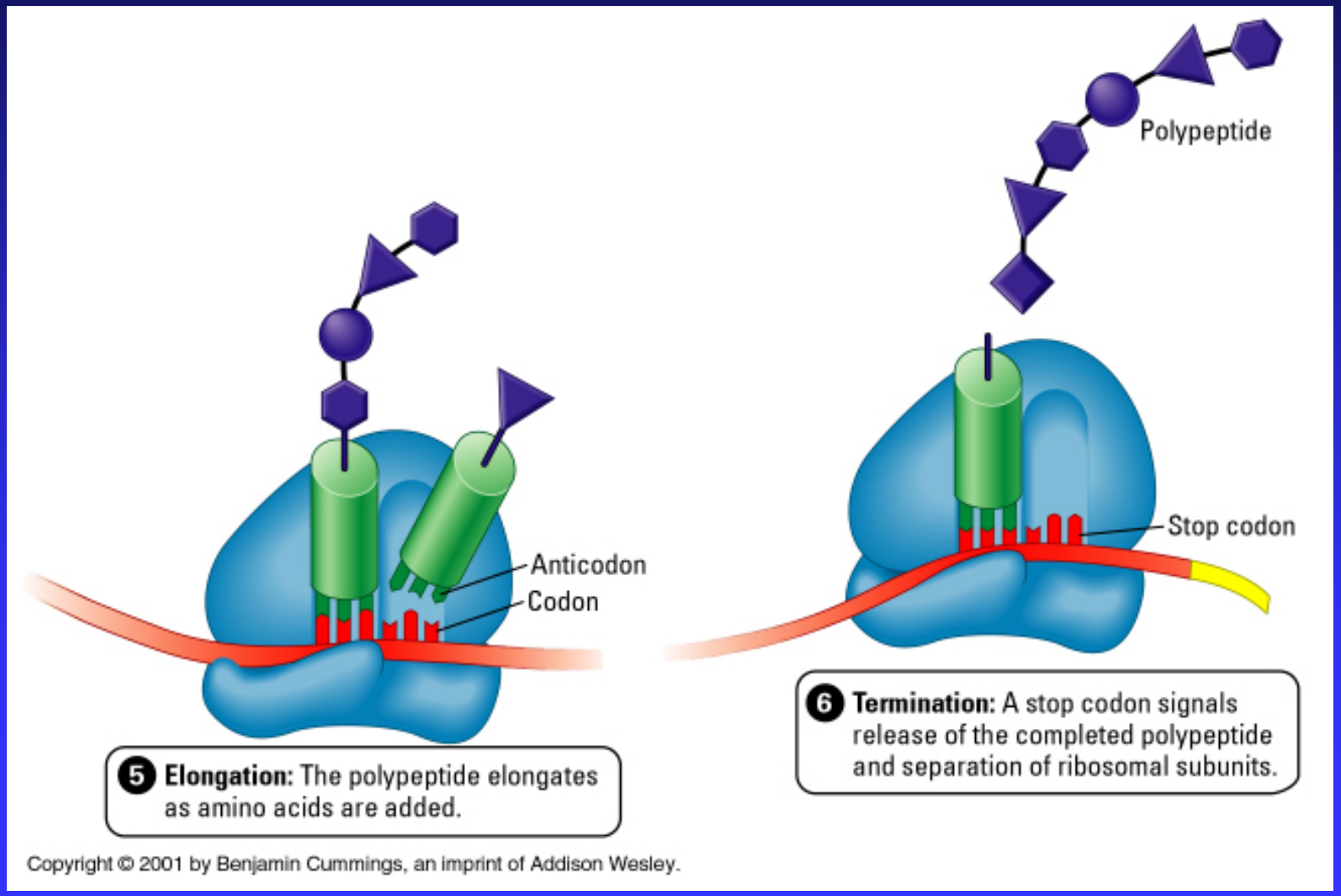
Step 1 - Initiation: When a ribosome begins to read the mRNA transcript at the start codon.



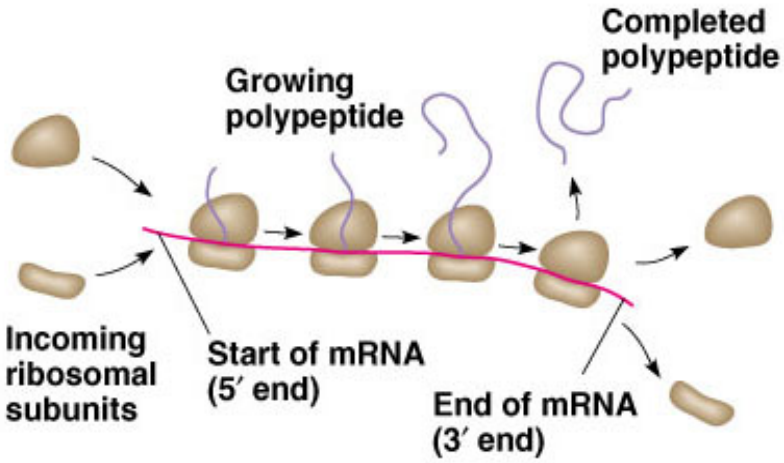
Step 2 - Elongation: Reading the chain of codons in a cycle, from one codon to the next



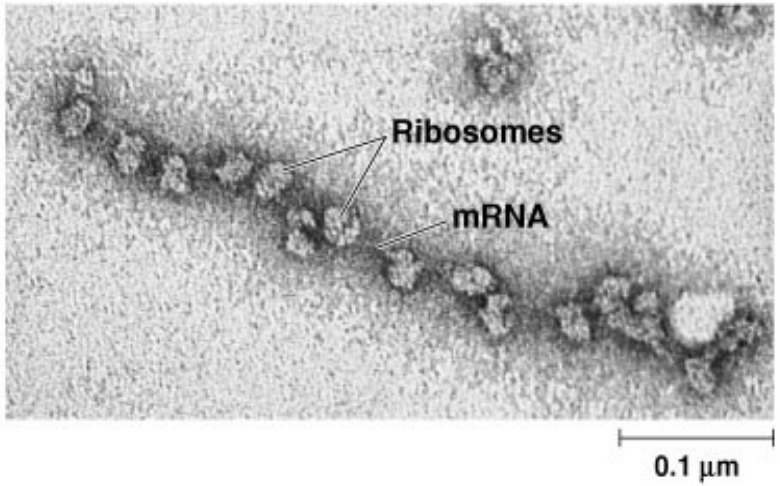
Step 3 - Termination: A stop codon signals the end of the mRNA transcript, and the completion of the translation process



Protein synthesis always involves MASS PRODUCTION by polyribosomes (polysomes):

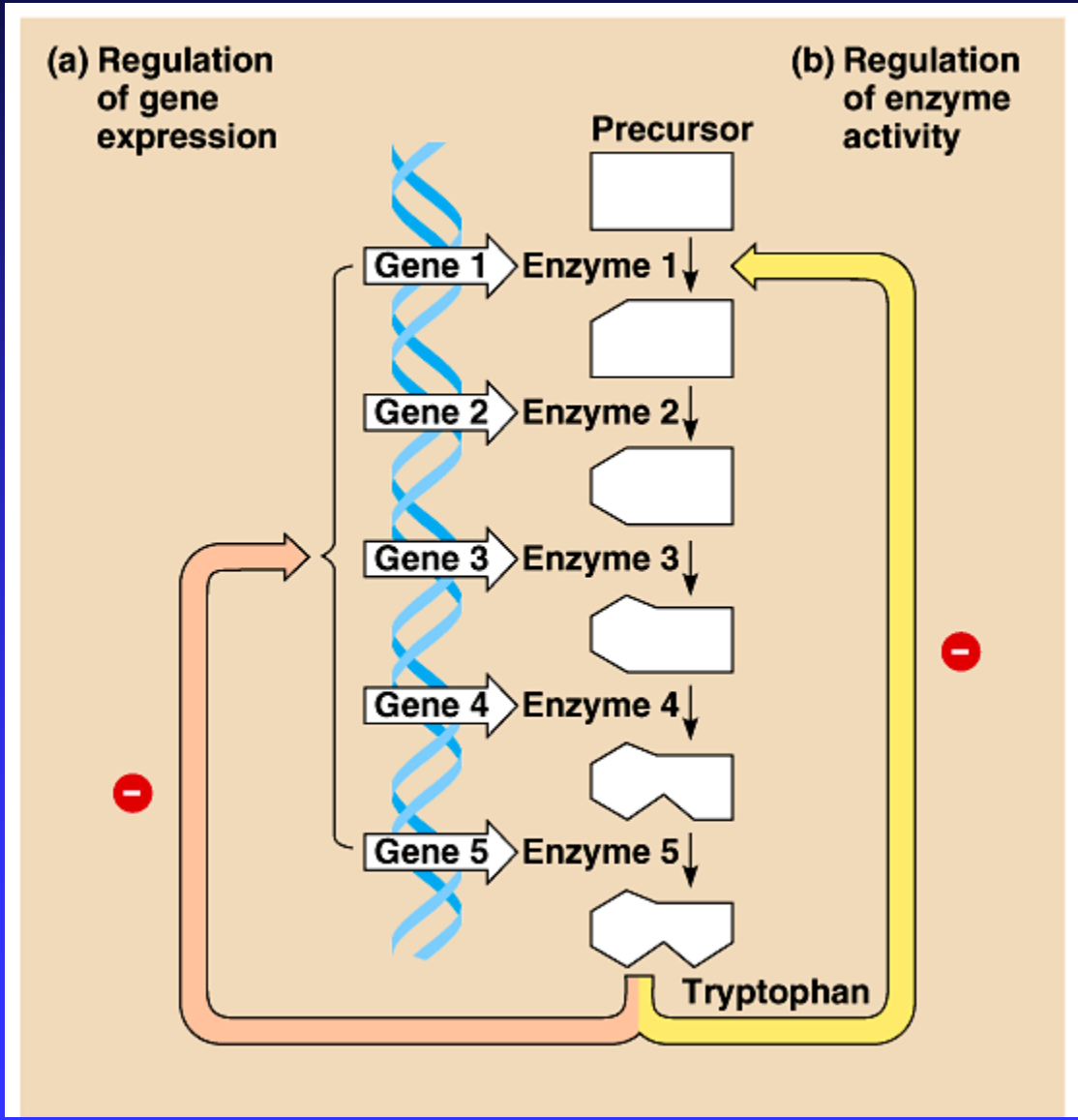


(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

Regulation of a metabolic pathway involves regulation at two levels: Gene expression and enzyme activity



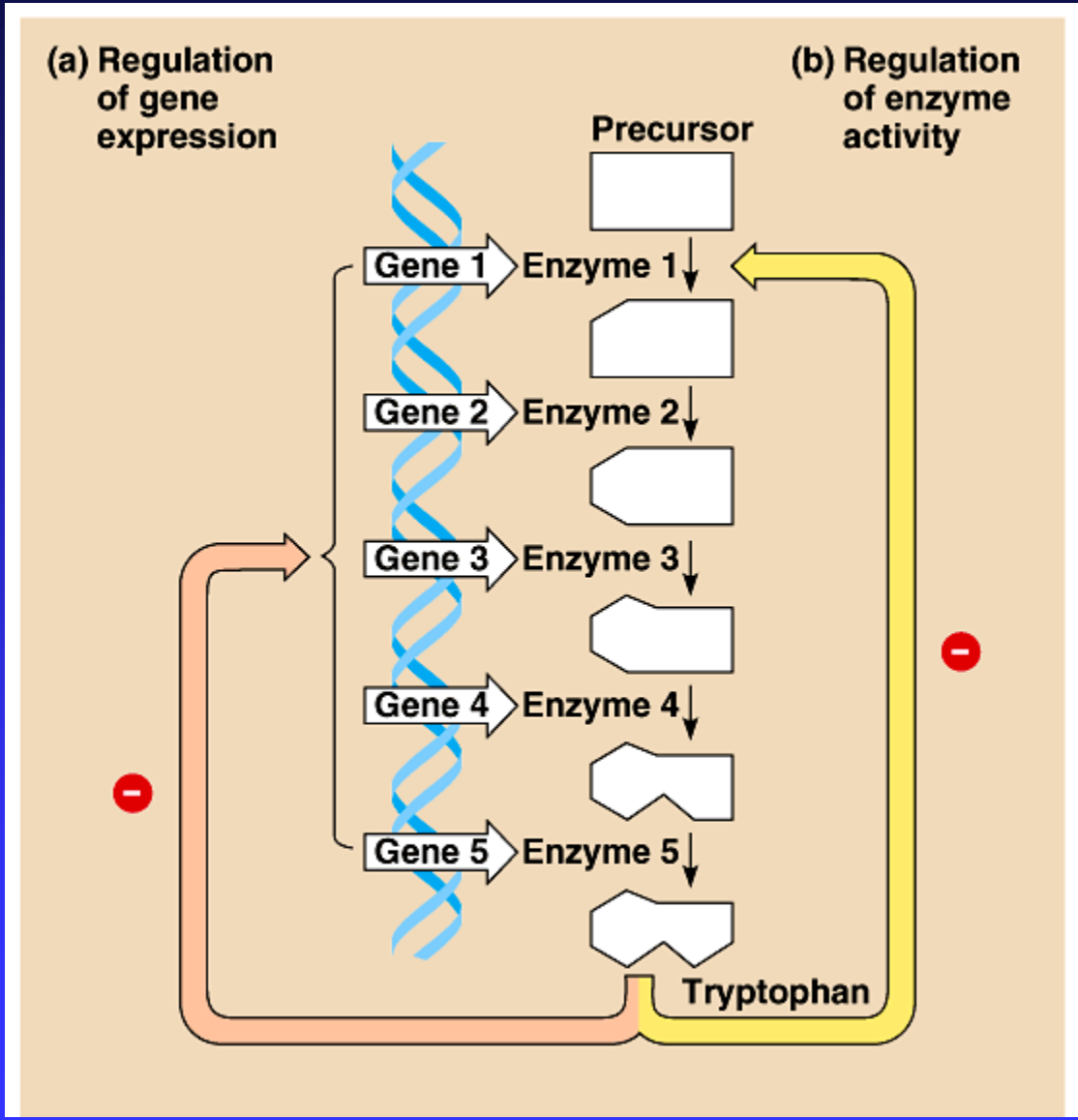
Four Structural Requirements of DNA as the Genetic Material

- 1. Account for diversity: The genome must store a vast amount of information - *Encode the specificity and variability of life.*
- 2. Capacity to self-replicate: As cells divide each must get a complete and "exact" copy of the genome - *Efficient DNA replication.*
- 3. Expressivity: To carry out metabolism the instructions to synthesize needed enzymes must me capable of expression - *Guide the synthesis of proteins.*
- 4. Capacity to control expression: Metabolism can be regulated at the genomic level - *Differential expression of the genome.*

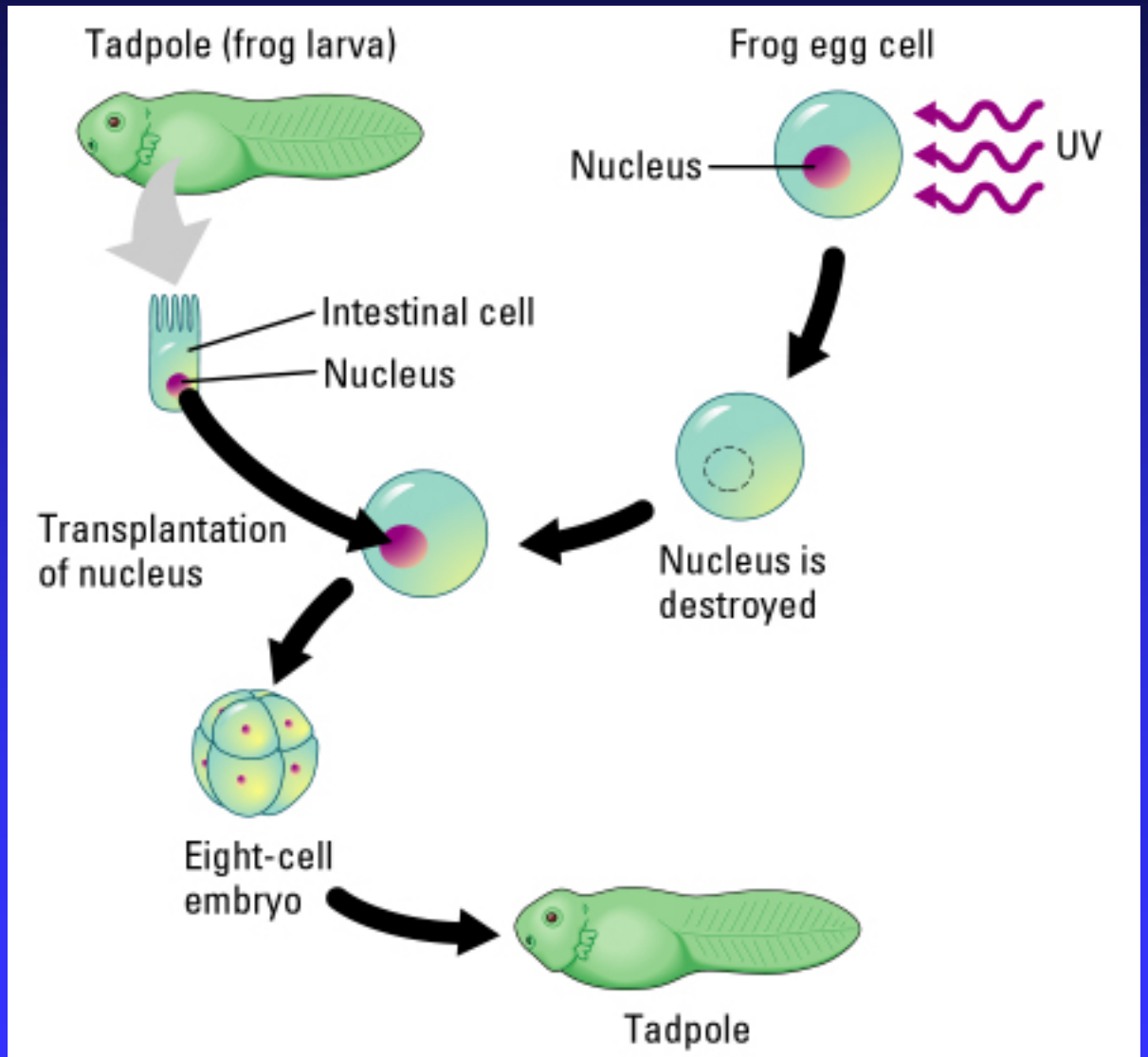
Controlling Gene Expression

- What makes cells different ?
  - A simple frog experiment
  - Liver, muscle and skin cells
- Controlling Gene Expression in Prokaryotic Cells
  - Transcriptional Control
    - Constitutive genes: strong and weak promoters
    - Inducible Genes: on/off with inducers
- Controlling Gene Expression in Eukaryotic Cells
  - 5 Levels of Control: Transcriptional Control, Transcript Processing Control, Transport Control, Translational Control, Post-Translational Control
- Sources of genetic variation

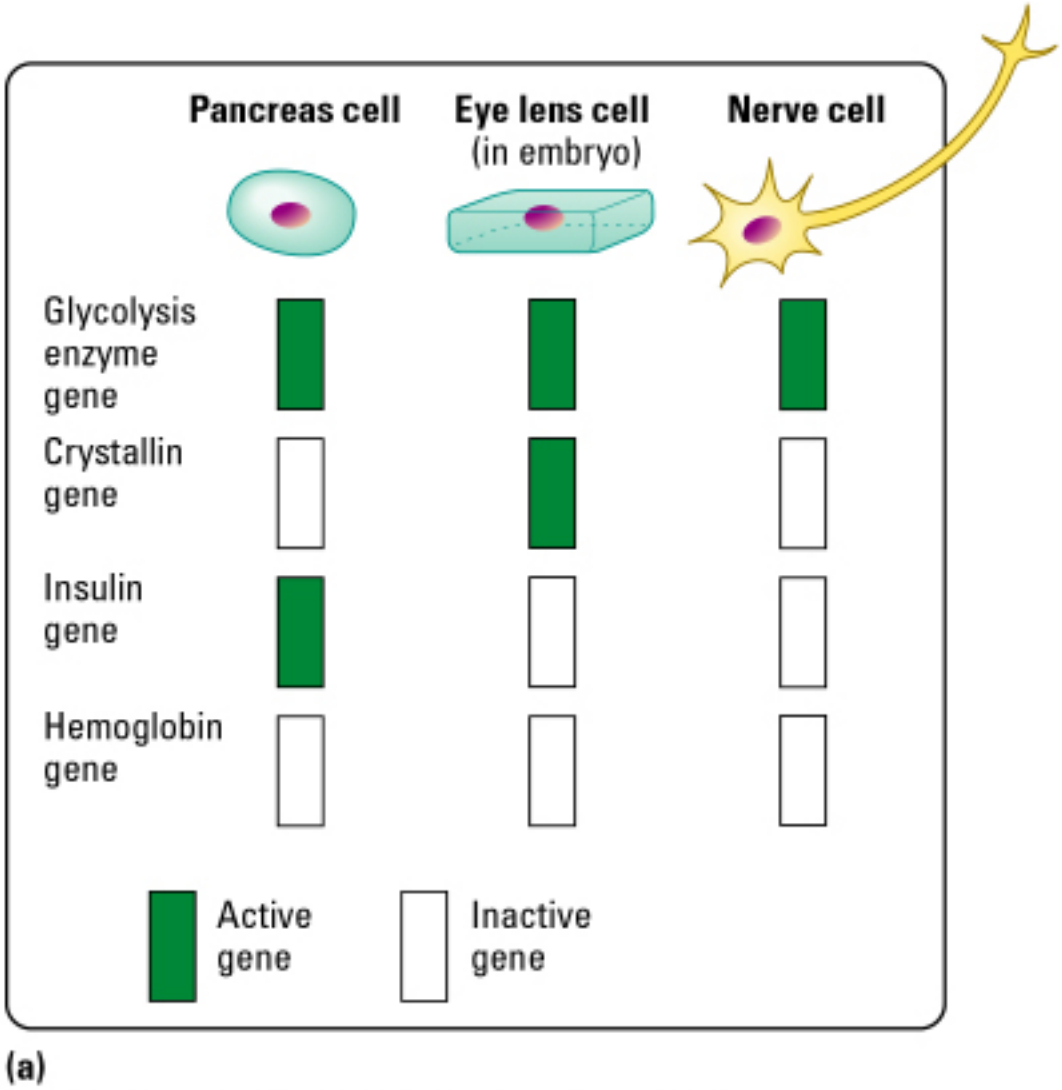
Regulation of a metabolic pathway involves regulation at two levels: Gene expression and enzyme activity



Do differentiated somatic cells have a complete or partial genome?



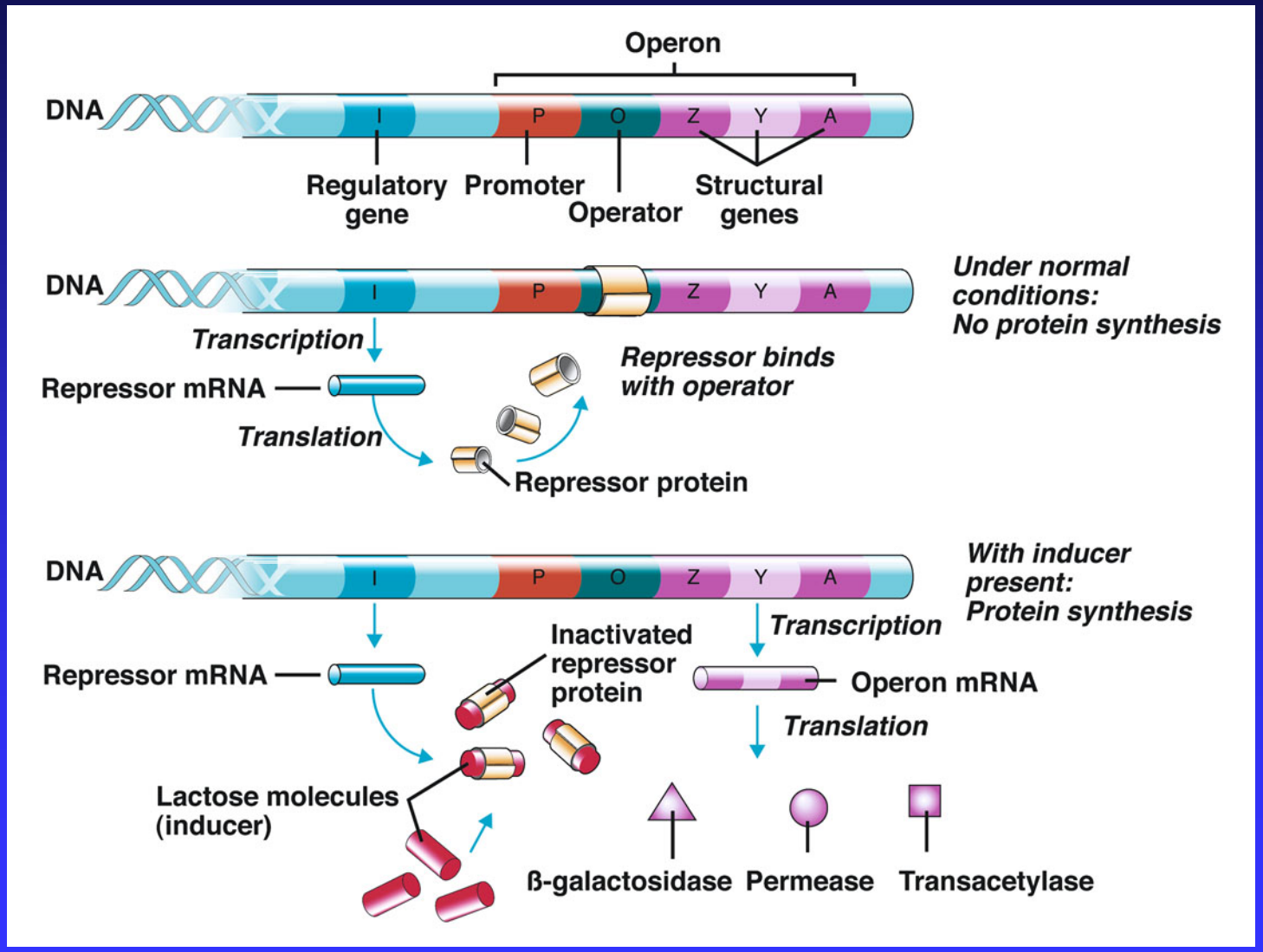
The basis of cellular specialization? Differential gene expression!



(a)

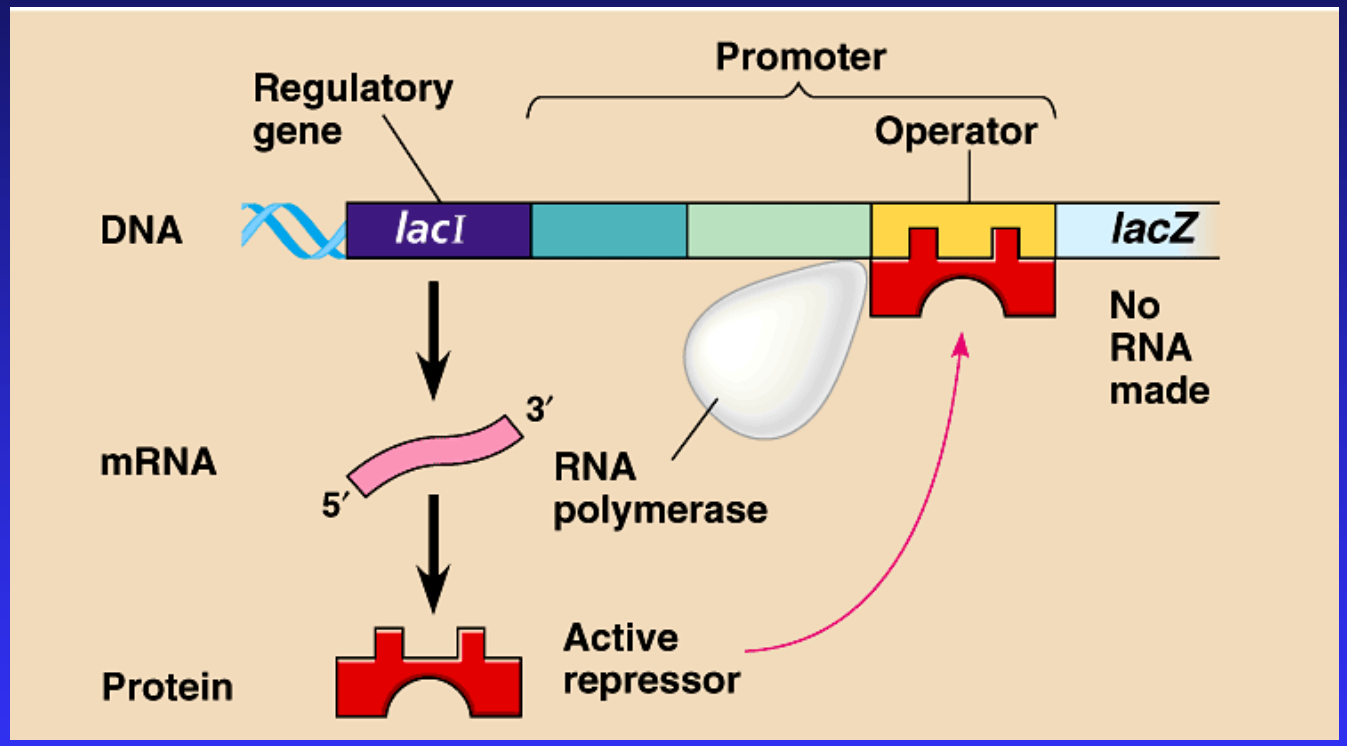
Among bacteria, turning a gene or a group of genes ON and OFF often involves OPERONS:

OPERON = Structural Genes and Regulatory Genes



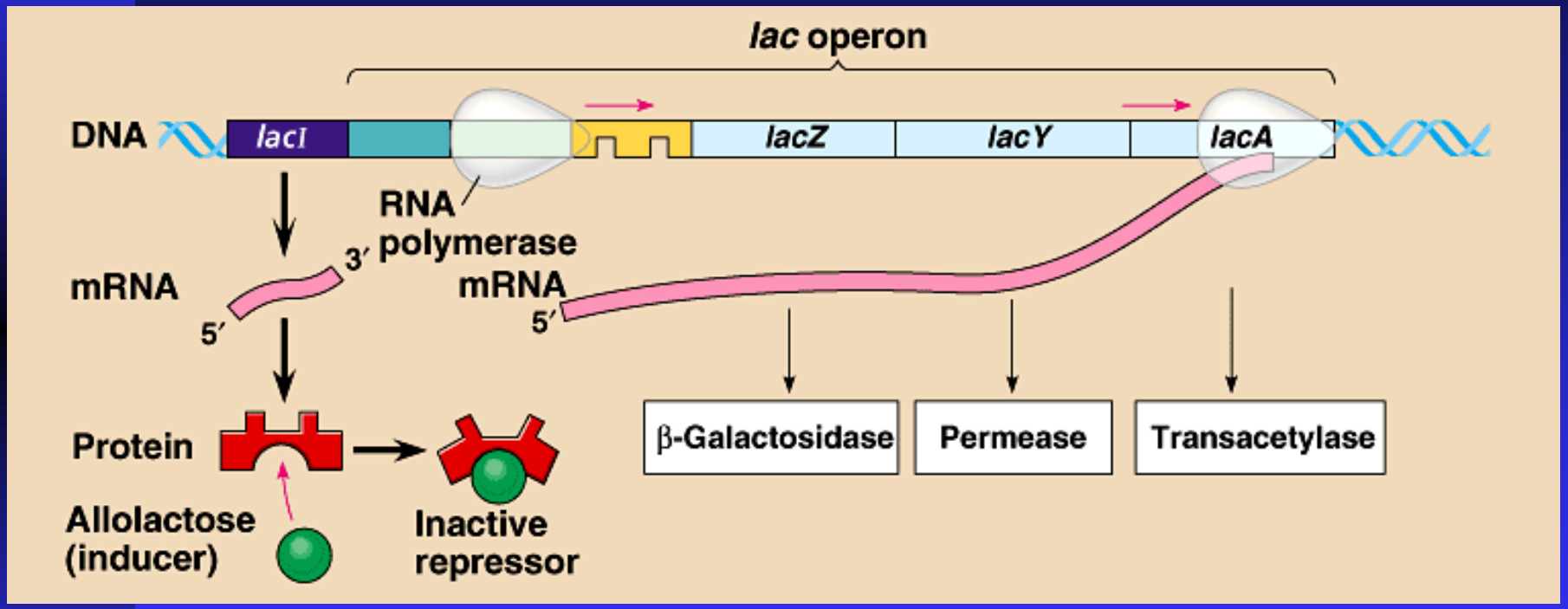
The lac operon: regulated synthesis of INDUCIBLE ENZYMES

A set of genes that are OFF except when they're ON.



When lactose is absent, the repressor protein is able to block transcription and the operon is turned OFF.

The lac operon: regulated synthesis of inducible enzymes

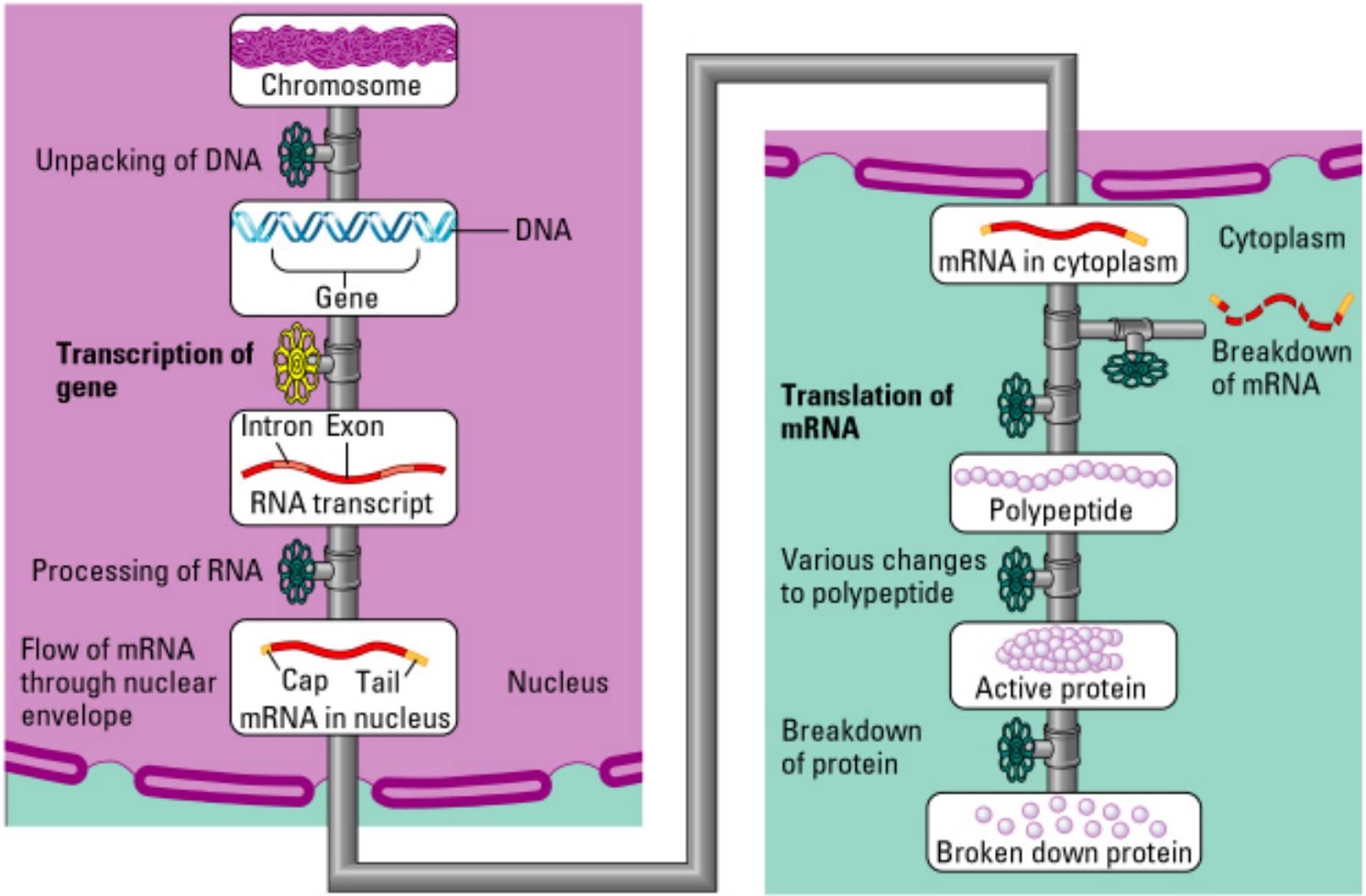


- When lactose is present, the repressor protein becomes inactive and the structural genes get transcribed and translated.

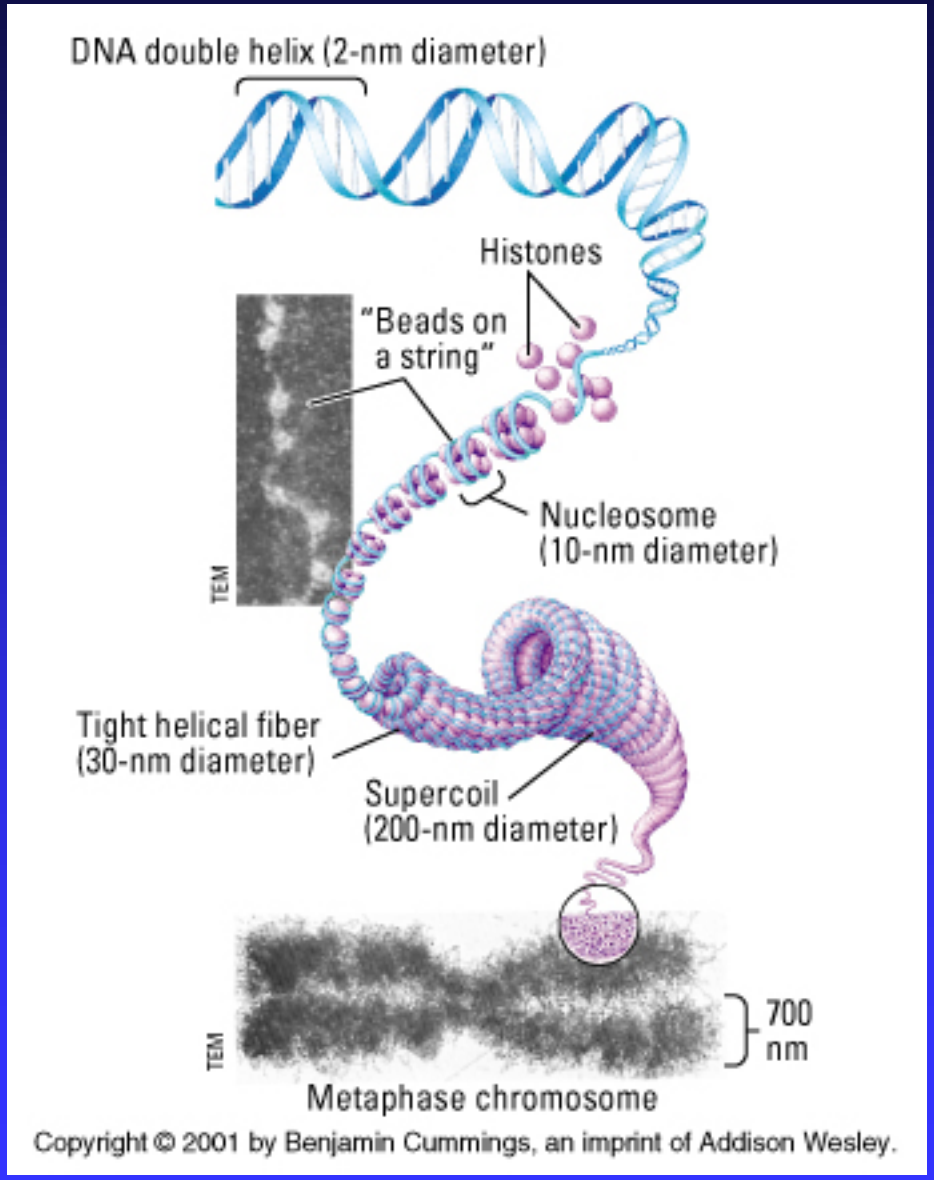
Eukaryotic Control of Genes and Sources of Genetic Variation

- Gene Expression in Eukaryotic Cells
  - 5 Levels of Control:
    - Transcriptional Control
    - Transcript Processing Control
    - Transport Control
    - Translational Control
    - Post-Translational Control
- Sources of Genetic Variation
  - Recombination through meiosis
  - Mutations
    - Types of mutations
    - Causes of mutations

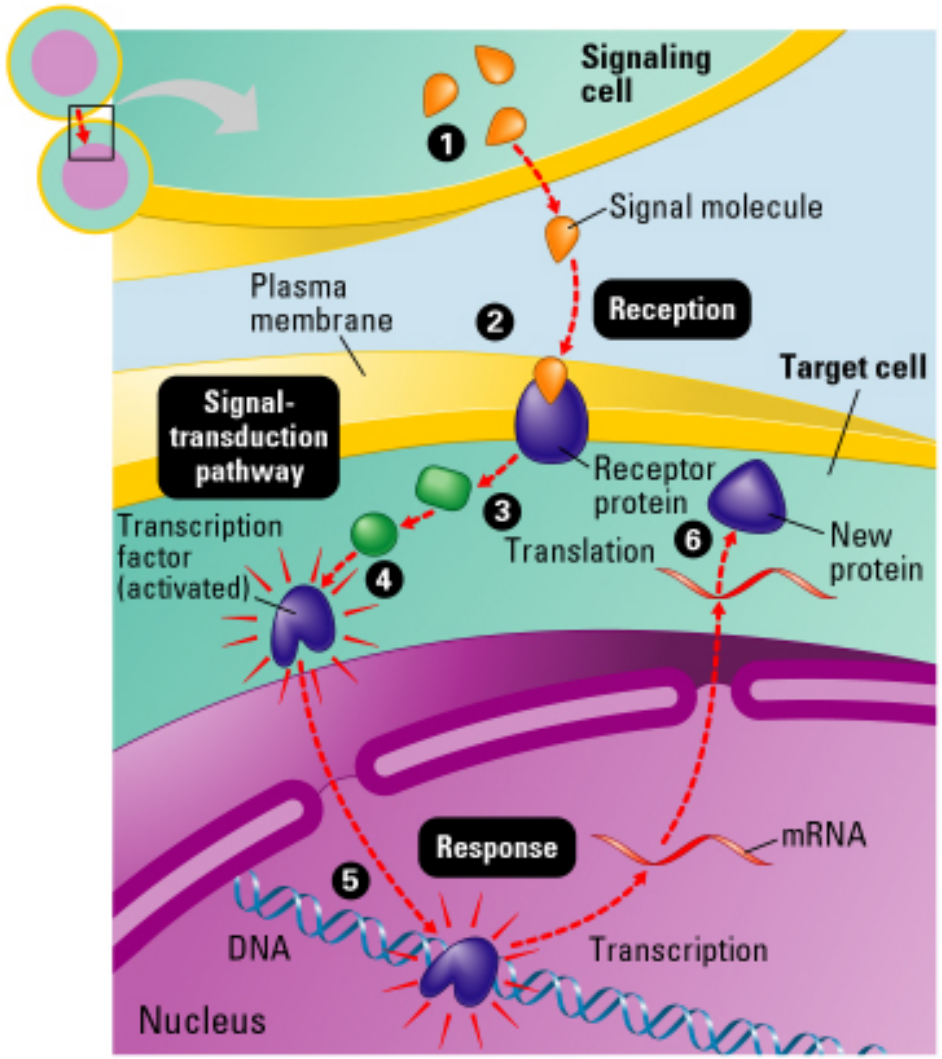
Controlling gene expression in eukaryotic cells:



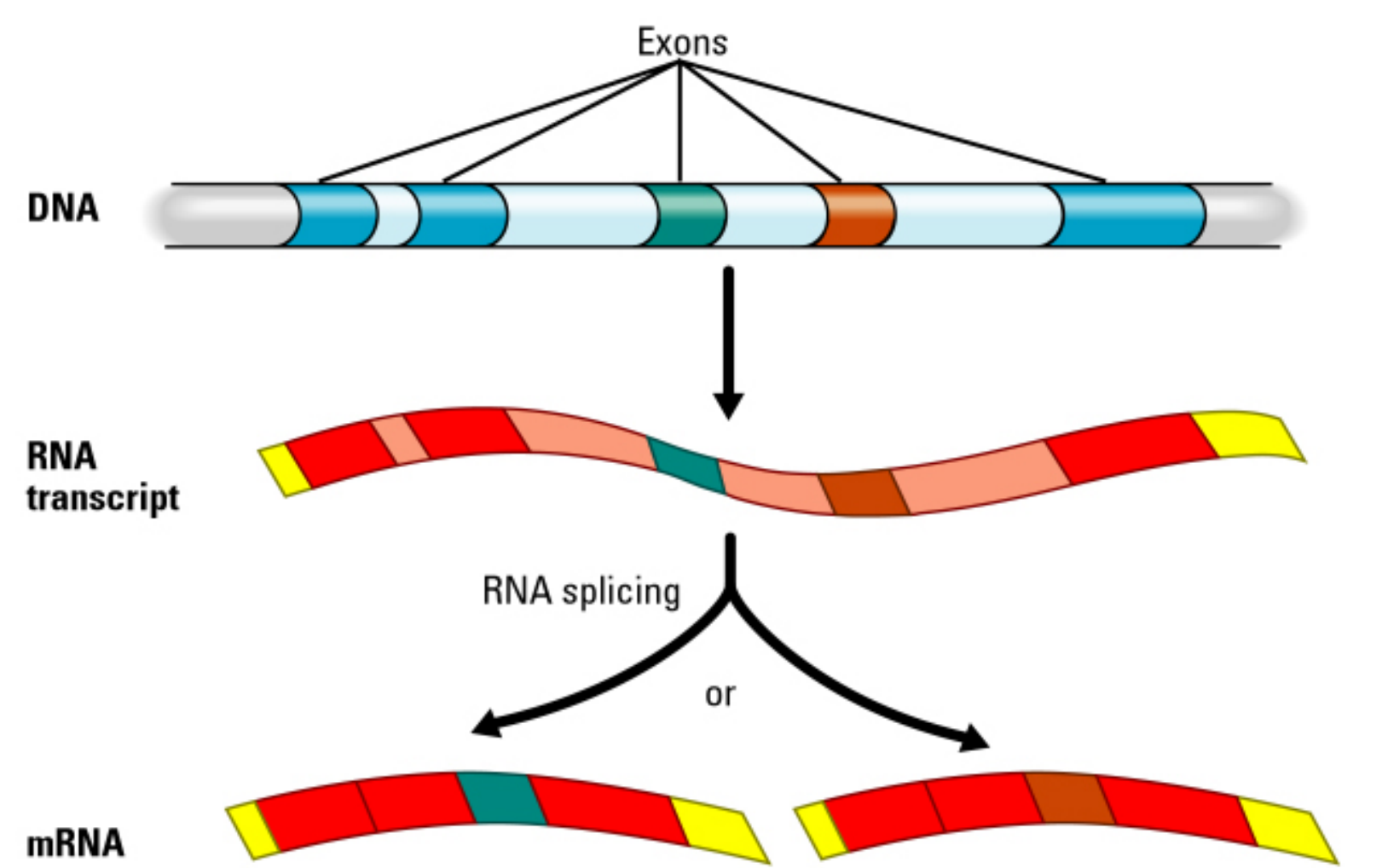
The genome must be accessible:



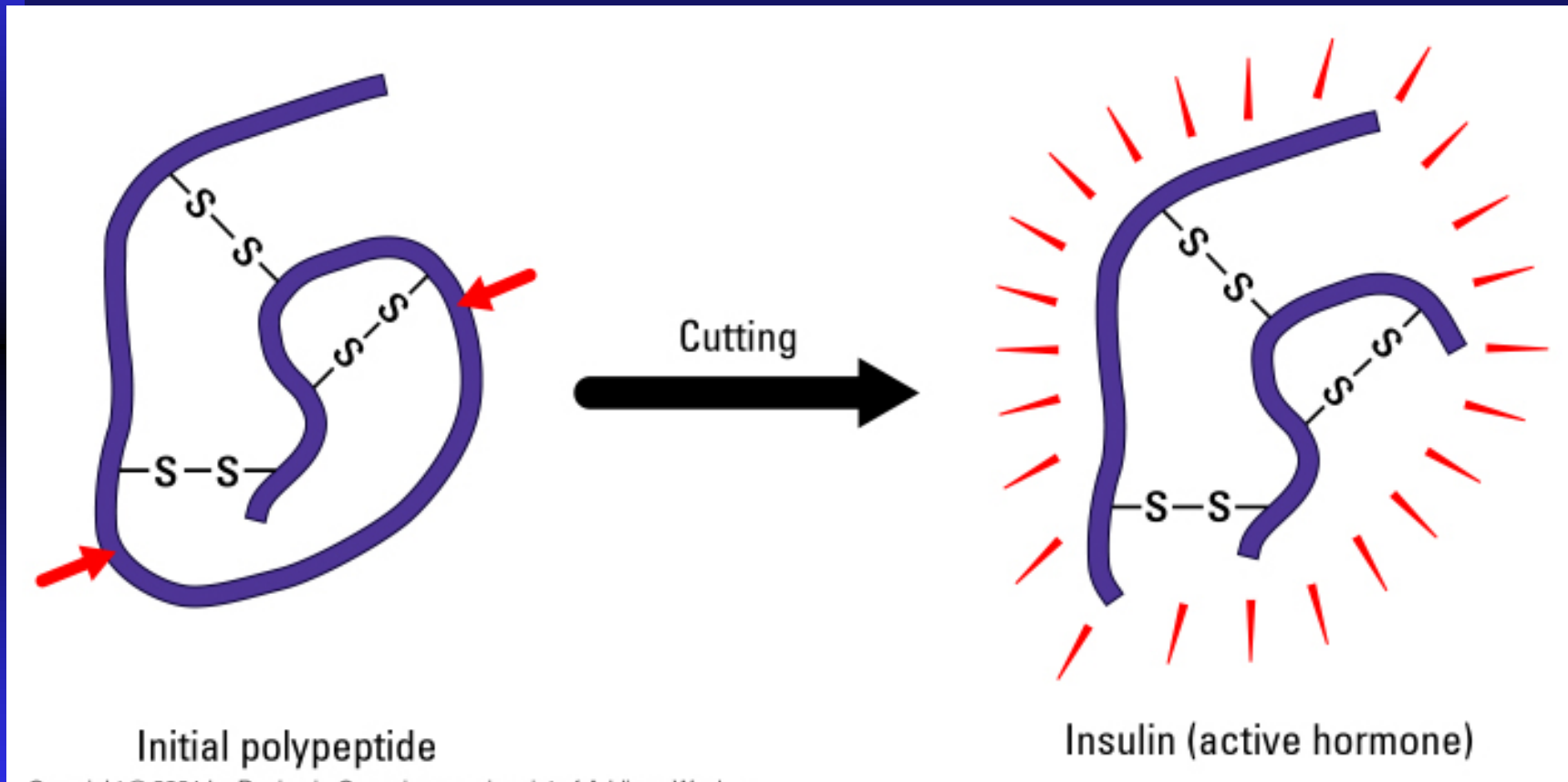
Extracellular effectors on transcriptional control:



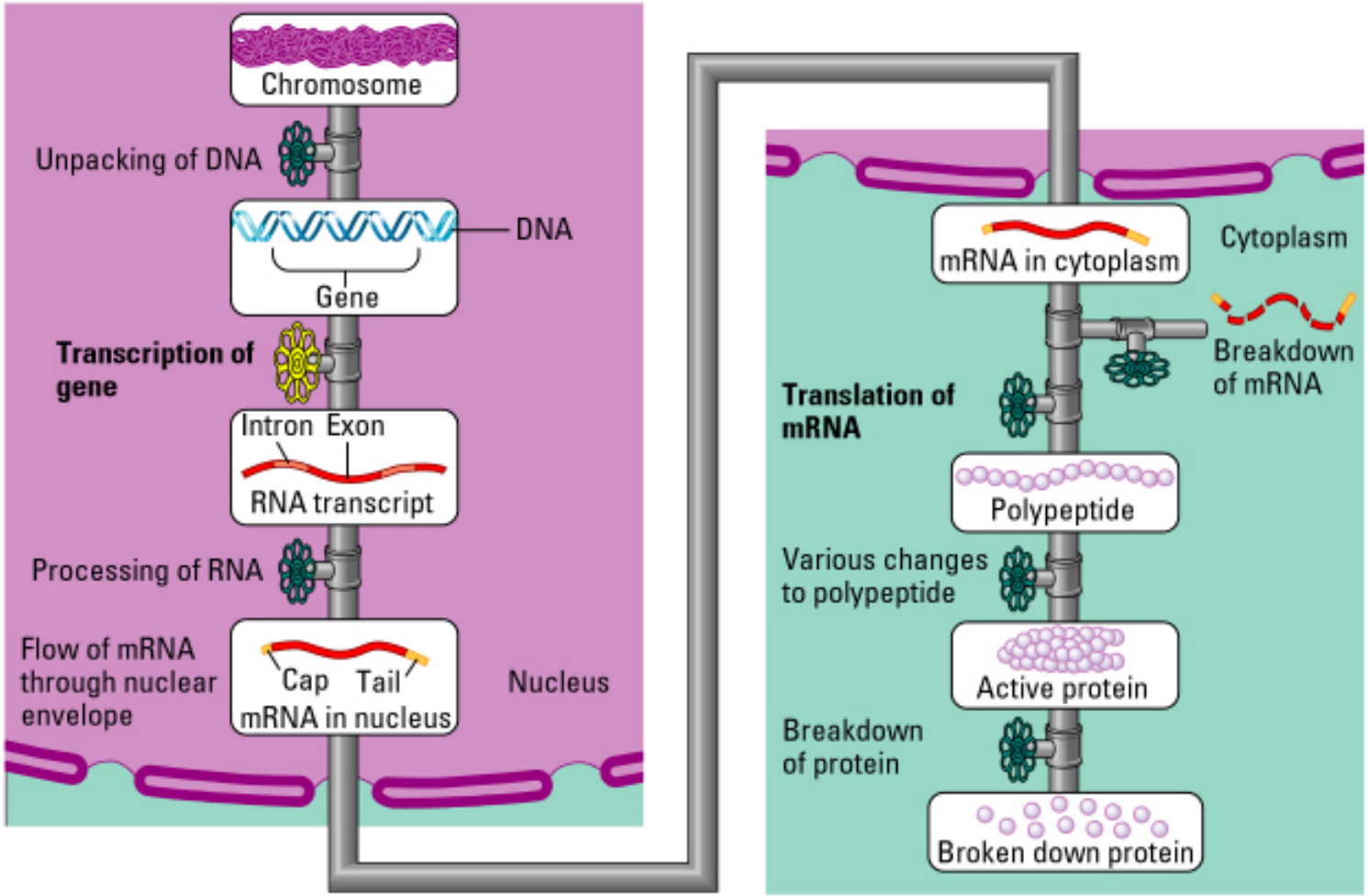
Post-transcriptional Processing:



Post-translational processing:



Controlling gene expression in eukaryotic cells:



Genetic Mutations: The Ultimate Source of New Alleles and Genetic Variation

- What are the sources of Genetic Variation?
- When are mutations significant?
  - Somatic Mutaions
  - Gametic Mutations
- Types of mutations
  - Base Substitutions
  - Frameshift Mutations
- Sources of mutations
  - Spontaneous Errors
  - Induced Mutations
    - Physical and Chemical Mutagens

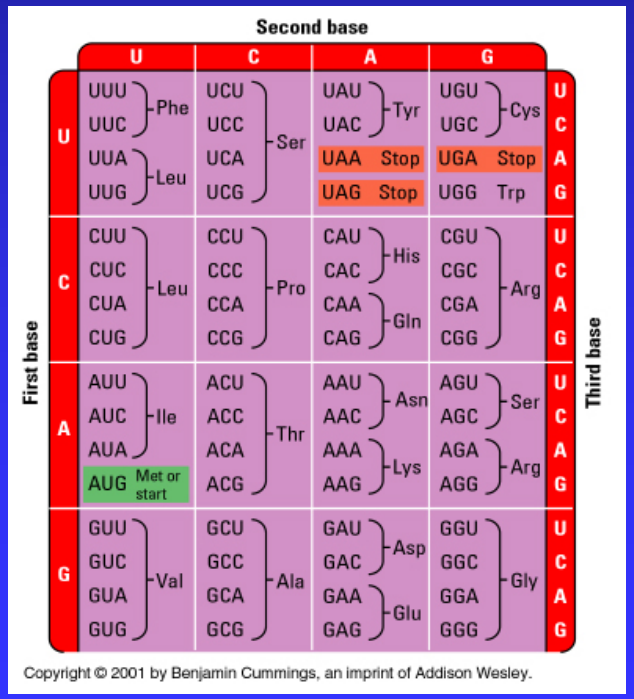
Somatic and Gametic Mutations

- Somatic Mutations: non-hertiable
  - Autosomal recessives are masked
  - Dominant alleles will be expressed
  - Most evident when they occur early in development
  - Cancer is the exception
- Gametic Mutations: heritable
  - Dominant alleles will first be expressed in F1
  - X-linked will be expressed in F1 males
  - Autosomal Recessives will remain masked until prevalence in population increases.

Two Basic Types of Mutations:

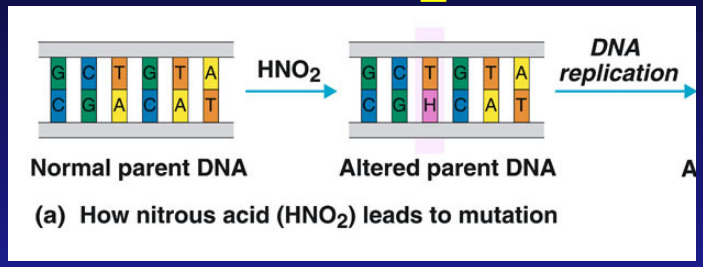
Mutations can alter a gene product or change the way its expression is regulated.

- 1. Base Substitutions or Point Mutations
  - Neutral mutation
  - Missense mutation
  - Nonsense mutation
- 2. Frameshift Mutation
  - Insertions or deletions

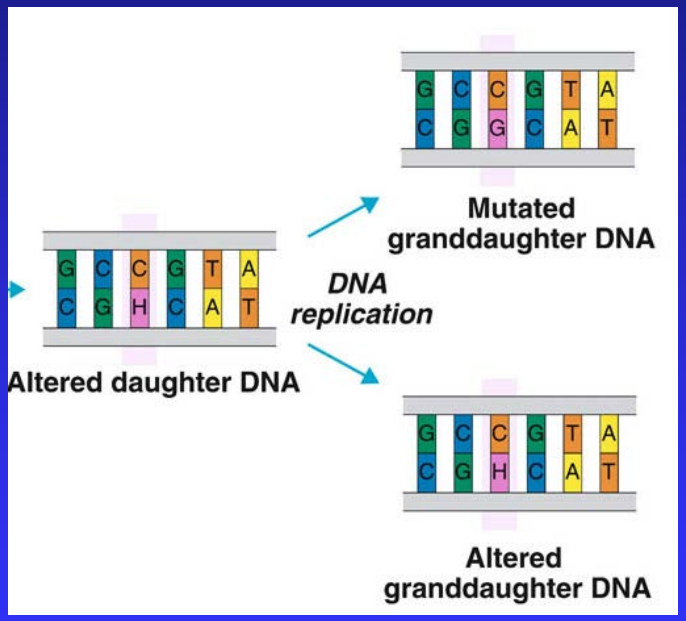


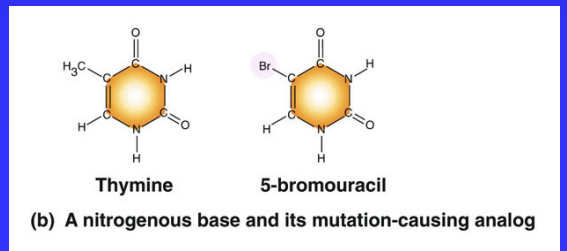
Mutations: Causes and Repair

- Spontaneous Mutations
- Induced Mutations
  - Chemical Mutagens
    - Conversion
    - Substitution
    - Frameshift
  - Physical Mutagens
- Proof reading and Repair
  - Mismatch Repair
  - Excision Repair



(a) How nitrous acid (HNO_2) leads to mutation





(b) A nitrogenous base and its mutation-causing analog