From Gene to Protein

- Beadle and Tatum
  - Analyzed Fungi *Neurospora crassa* mutants
  - Mutants were UNABLE to grow without Arginine (an amino acid)
  - Other biochemical experiments indicated:
    - Precursor → Ornithine → Citrulline → Arginine
    - Each biochemical reaction requiring an enzyme

Hypothesis:
ONE gene ONE enzyme

- Beadle and Tatum

- Mutants could be classified into one of three groups
  - Grew with ornithine supplements
  - Grew with citrulline supplements
  - Grew only with arginine supplements

- Indicates that a gene is required for each step
  - Precursor → Ornithine → Citrulline → Arginine

Modifications to Beadle and Tatum's Hypothesis

- Reasons for modifications:
  - Enzymes can contain multiple protein and/or RNA subunits
  - Not all proteins are enzymes

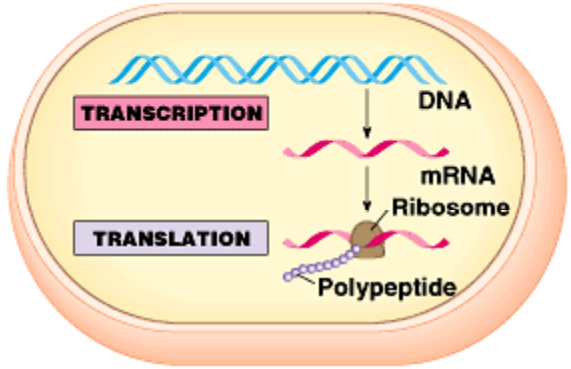
- ONE gene ONE polypeptide hypothesis
  - Still not entirely accurate as we will learn
    - Genes can encode for RNAs that are NOT used as a code for protein (ie NOT mRNA)
    - A single gene can be used to generate multiple different proteins

DNA → RNA → Protein

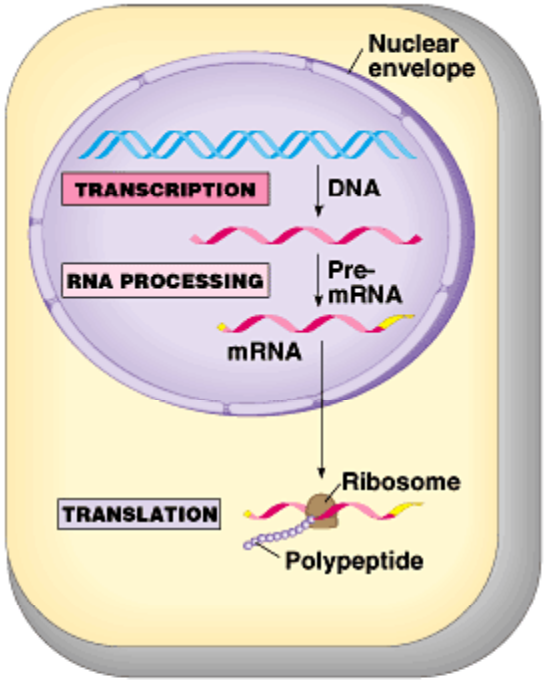
- **DNA → RNA**
  - Transcription
    - The production of ribonucleic acid using DNA as a template

- **RNA → Protein**
  - Translation
    - The production of a polypeptide using an RNA as a template

Transcription and Translation occur in BOTH procaryotic and eucaryotic cells

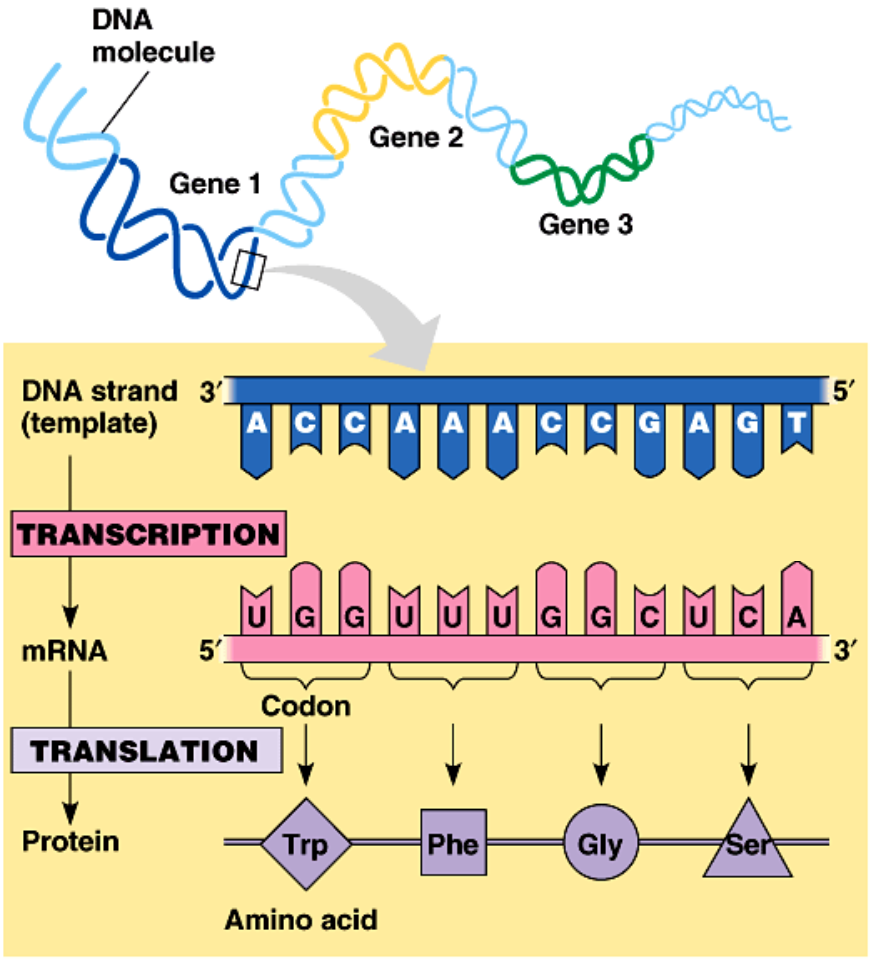


(a) Prokaryotic cell



(b) Eukaryotic cell

- Procaryotic Cells
  - No Nucleus
    - Transcription and Translation are coupled
- Eucaryotic Cells
  - Nucleus
    - Transcription and Translation occur in different cellular locations



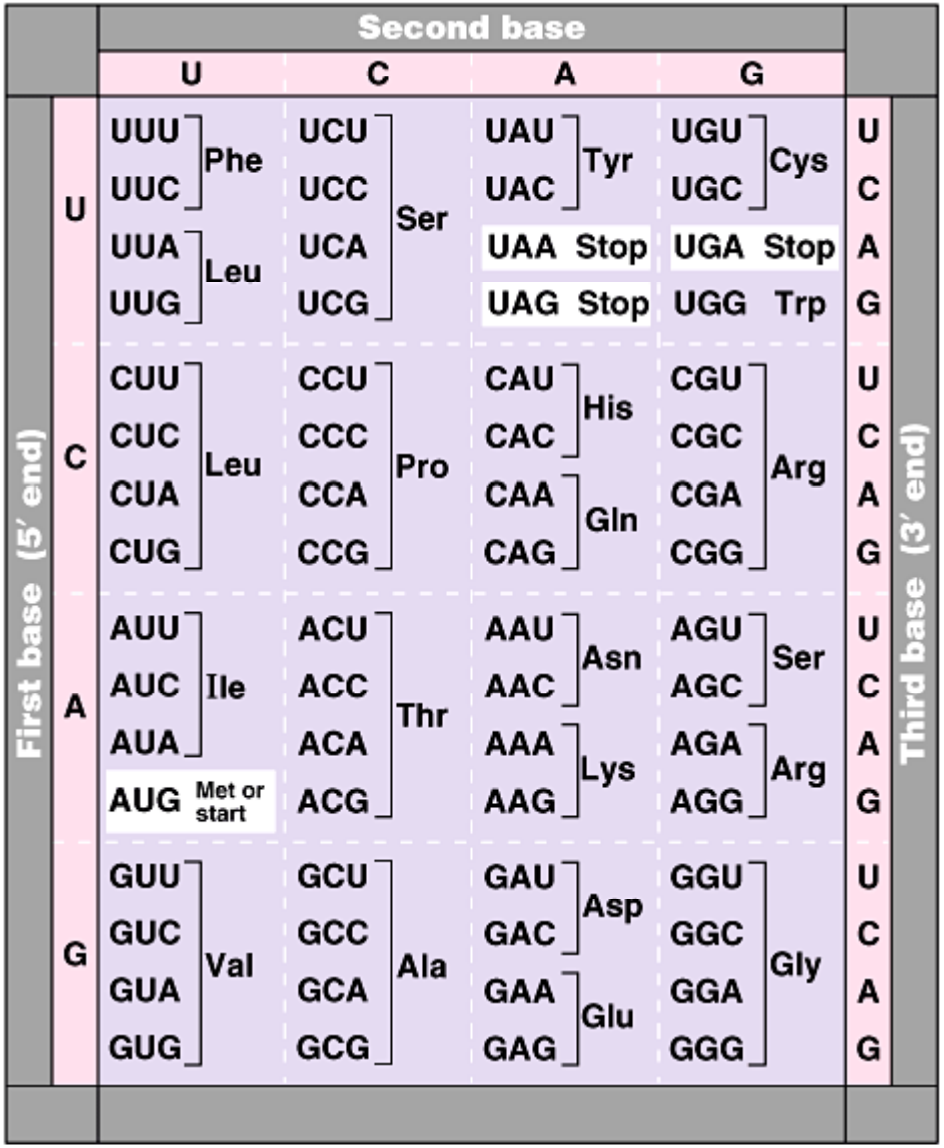
- One strand of DNA is used as a Template to produce a single strand of RNA
- RNA is produced in the 5’ to 3’ direction
  - The template DNA strand is read in the 3’ to 5’ direction
- In protein production, the template RNA (termed a messenger RNA) is read in the 5’ to 3’ direction
- 3 nucleotides, or a codon, code for a single amino acid

Genetic Code

- 4 RNA nucleotides
- 20 amino acids

- Theoretical
  - 2 letter code
    - 4 x 4 = 16 possibilities NOT ENOUGH
  - 3 letter code
    - 4 X 4 X 4 = 64 possibilities ENOUGH

- Experimental
  - Nirenberg produced an artificial poly U RNA and performed translation in a test tube
  - Produced a polypeptide with just phenylalanine



Genetic Code

- **Start**
  - AUG
- **Stop**
  - UAA, UAG, UGA

- Non overlapping
  - 9 nucleotides contains only 3 codons!
  - UGC AGU CCA

- Redundant
  - Some amino acids are coded for by multiple codons
    - Proline
      - CCU, CCC, CCA, CCG

- The genetic code is essentially the same in all 3 domains: bacteria, archaea, eucaryotes

Transcription

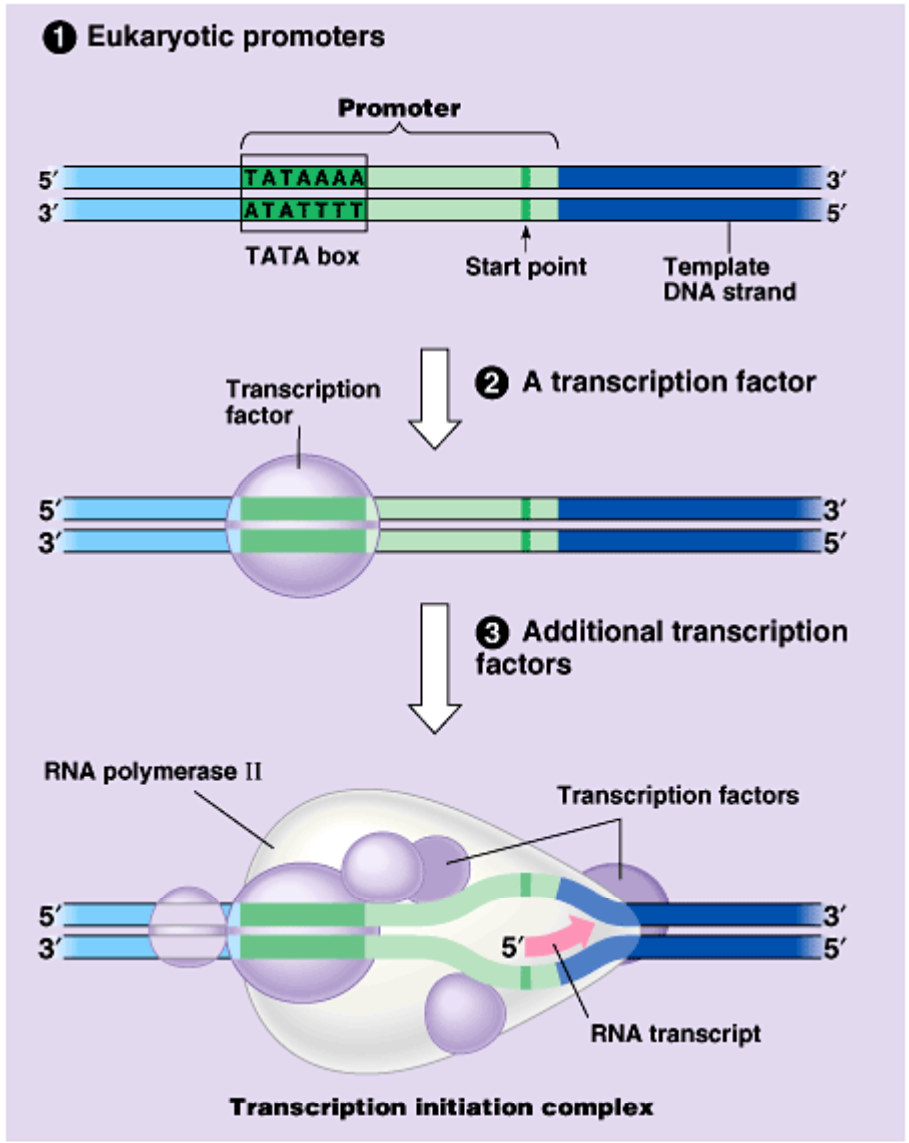
- The process by which a DNA template is used to build a strand of RNA
- RNA Polymerase
  - The enzyme responsible for the condensation/ dehydration reactions that build an RNA
- Occurs in the 5’ to 3’ direction
- Occurs in three stages
  - Initiation
  - Elongation
  - Termination

Transcription

- **Initiation**
  - A region of the double stranded DNA serves to attract the RNA polymerase. This region of DNA is termed the **Promoter** Region
    - Promoter regions frequently contain a TATA box
  - Transcription Factors:
    - Proteins that interact with the nucleotides of the DNA
    - Recruit the RNA polymerase

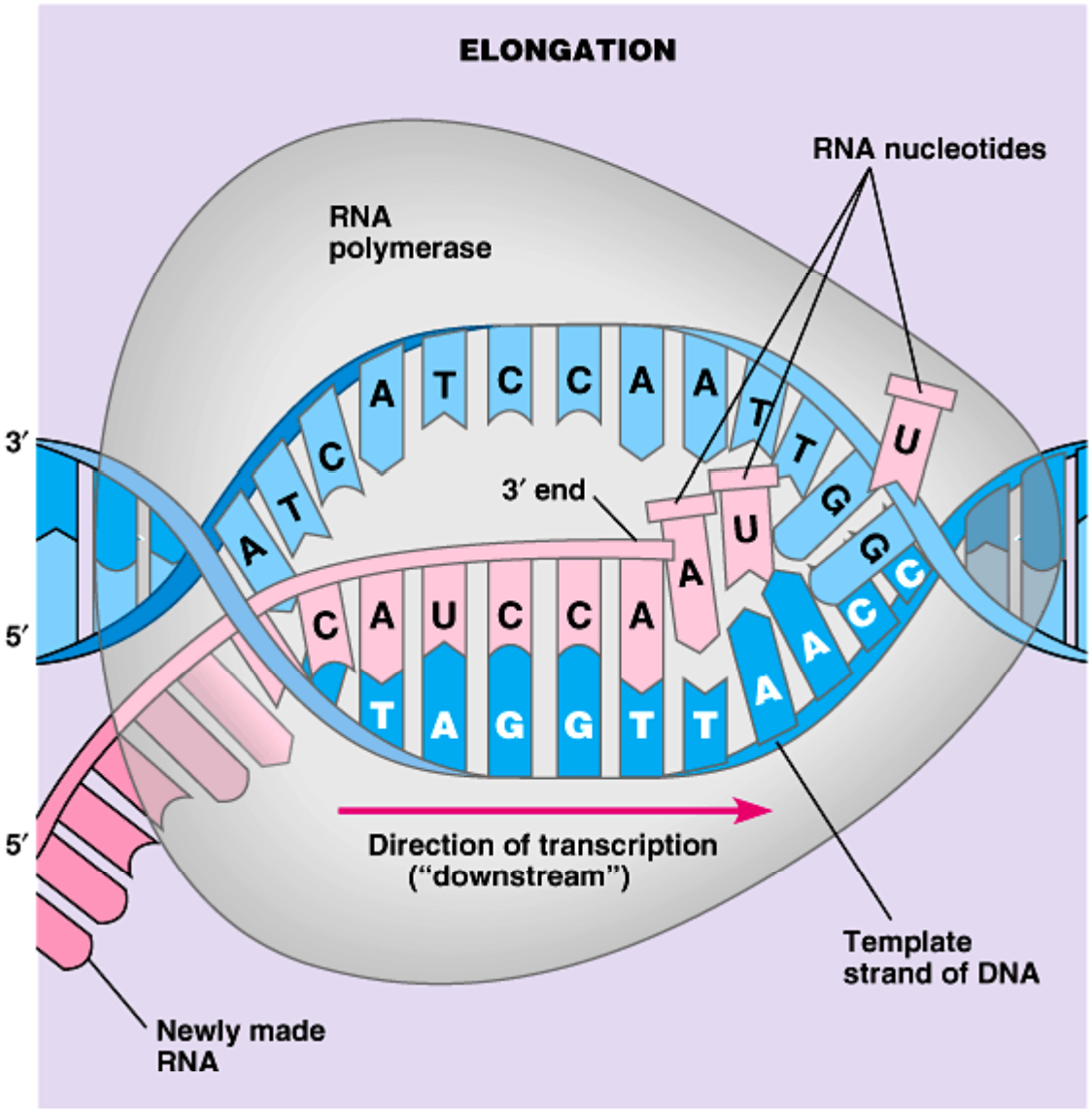
Transcription

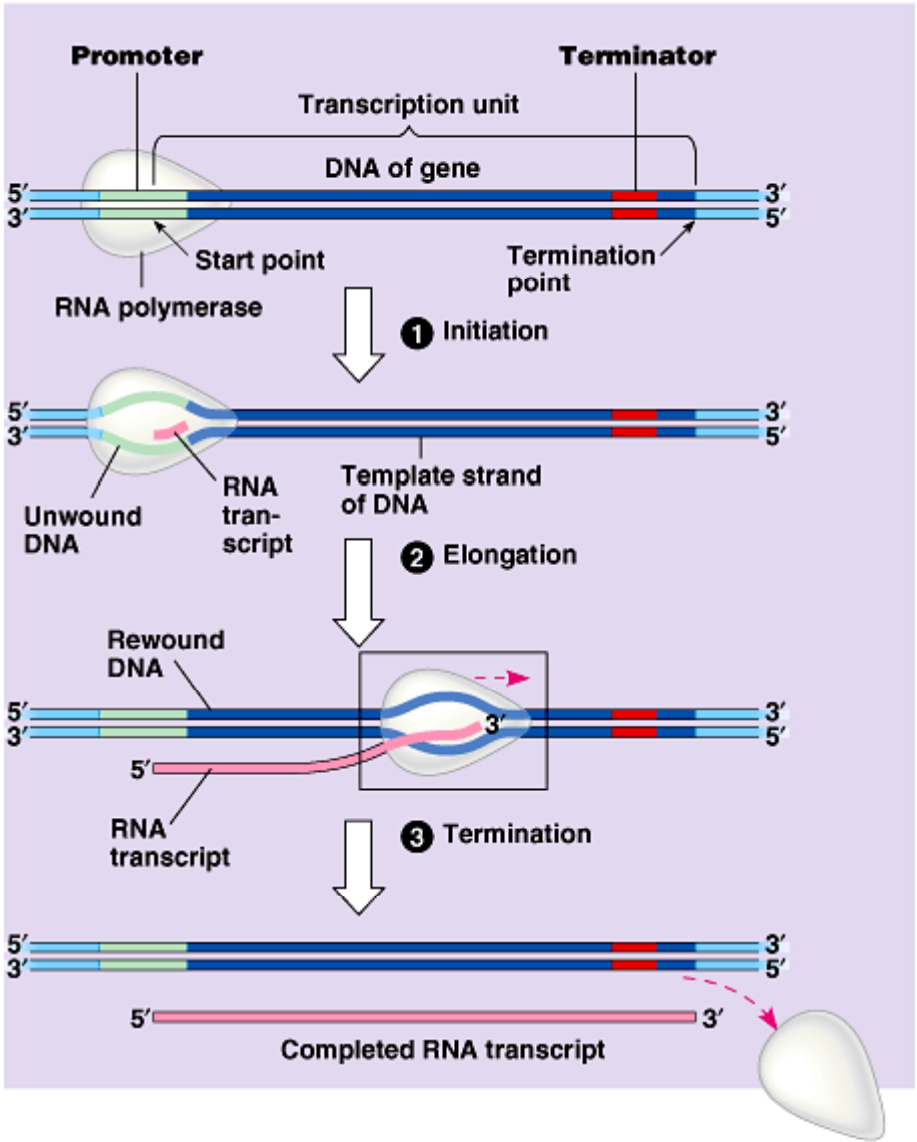
- **Initiation (cont'd)**
  - The transcription initiation complex results from the transcription factors and the RNA polymerase
  - The double helix is temporarily unwound as the RNA polymerase produces an RNA strand in the 5' to 3' direction
  - The DNA template is read by the RNA polymerase in the 3' to 5' direction



Transcription

- Elongation
  - Addition of nucleotides to the 3’ end of the growing RNA continues
- Termination
  - Procaryotes
    - Triggered by a sequence in the DNA called the terminator
  - Eucaryotes
    - Not entirely understood, but the RNA is cleaved from the RNA polymerase following transcription of a poly adenylation signal AAUAAA





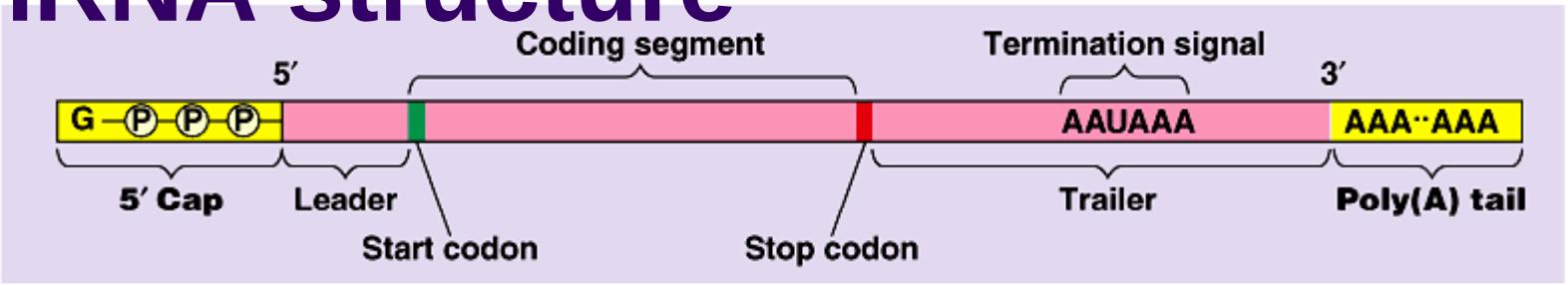
RNA processing

- Only occurs in eucaryotic cells
  - Alteration of the mRNA ends
  - RNA splicing
  - Pre-mRNA
- RNA Processing
  - mRNA

- **5’ end**
  - A modified guanine nucleotide is added

- **3’ end**
  - After the polyadenylation site AAUAAA a string of adenine nucleotides is added (between 50 – 250)

mRNA structure



- 5’ cap
- 5’ UTR
  - Untranslated region
    - Runs from the transcription start site (TSS) to the start codon (AUG)
- Coding region
- 3’ UTR
  - Stop codon until the poly (A) tail
- Poly(A) tail

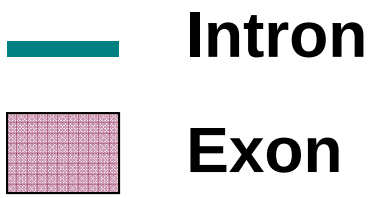
Alternative RNA splicing

- **Observation**: The actual size of many genomic regions used to produce an RNA transcript are MUCH larger than the actual mRNA used in the production of protein

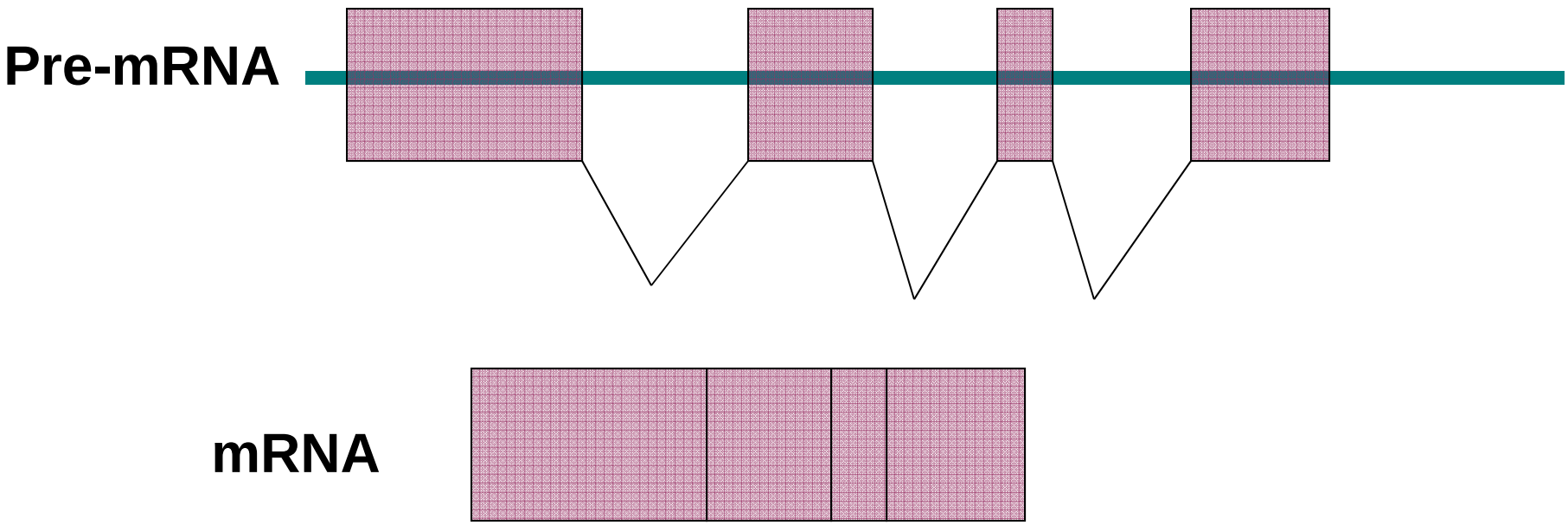
- **Introns**
  - Non-coding segments of an RNA that are removed prior to translation
  - “Intervening”

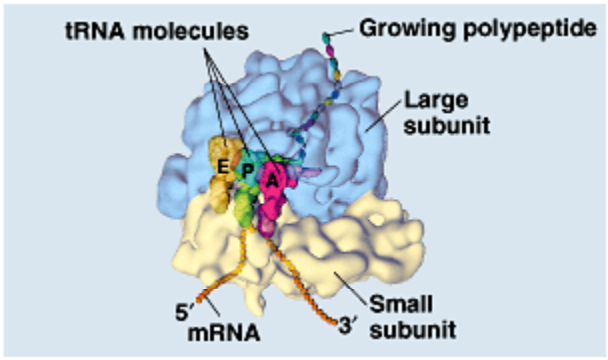
- **Exons**
  - The coding portion of an RNA that is used for translation

RNA splicing

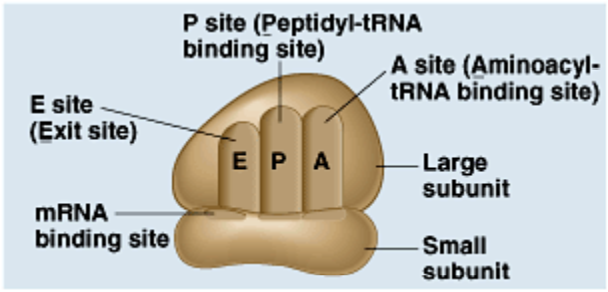


- The removal of introns and the splicing together of exons
- The reaction is catalyzed by a multi-protein RNA complex termed the Spliceosome

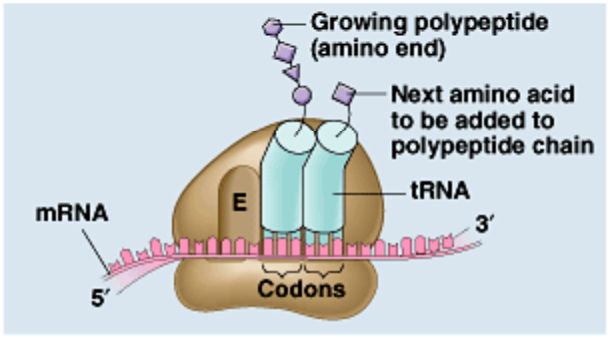




(a) Computer model of functioning ribosome



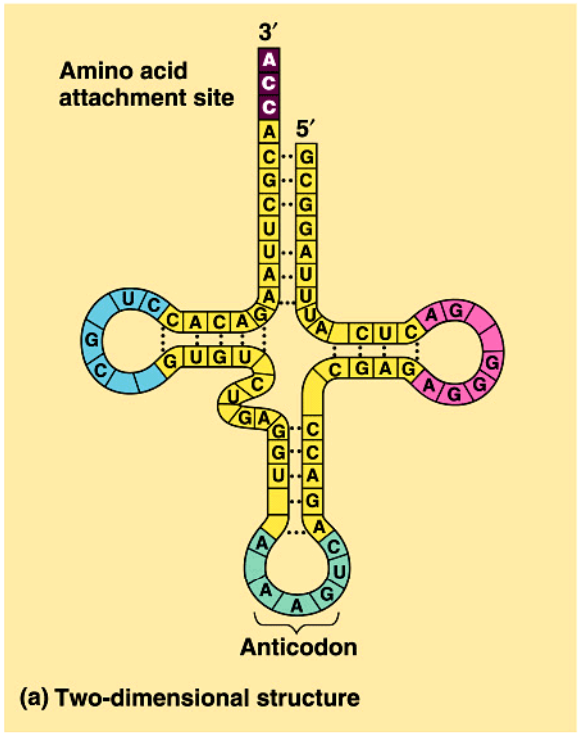
(b) Schematic model showing binding sites

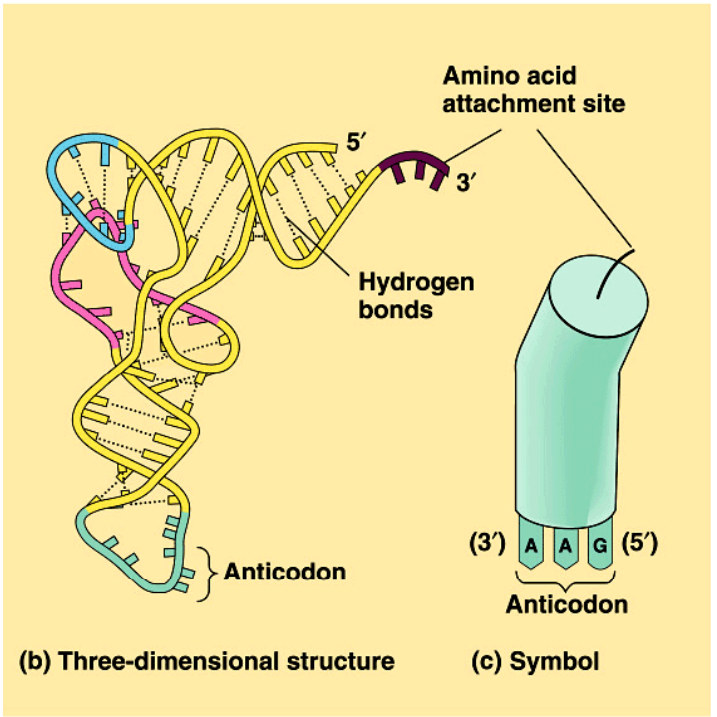


(c) Schematic model with mRNA and tRNA

Translation

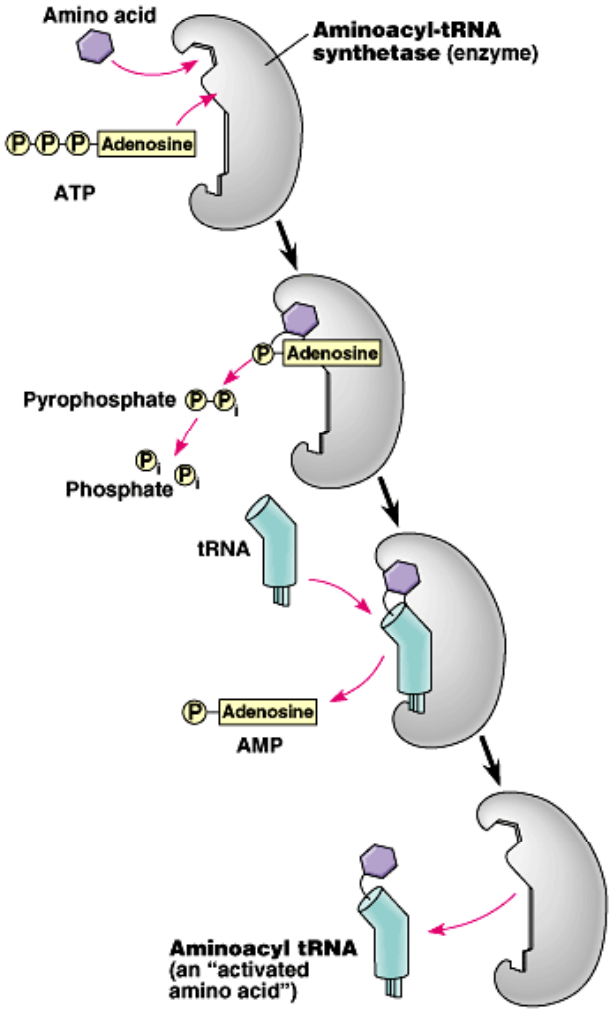
- mRNA
  - Transcribed and processed RNA from a gene
- Ribosome
  - Proteins
  - Ribosomal RNA
- tRNA
  - Transfer RNA
    - Contains an anticodon
    - Contains an amino acid





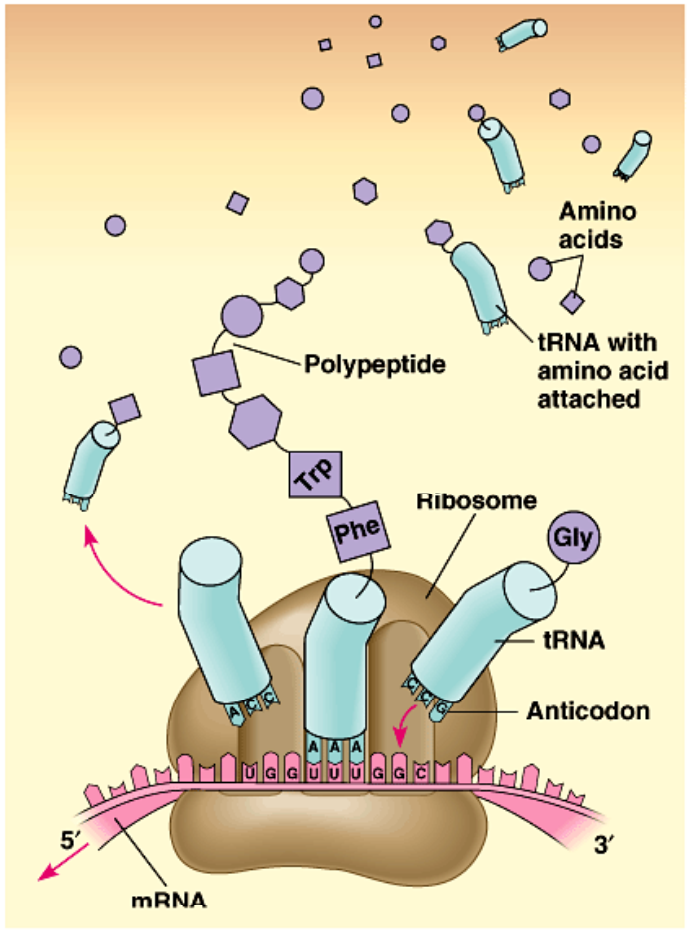
- The Single stranded RNA that is the transfer RNA forms hydrogen bonds amongst its nucleotides giving it a 3 dimensional shape
- Amino acid forms a covalent bond with the 3’ end of the tRNA
- The anticodon, 3’ to 5’ forms hydrogen bonds with the codon, 5’ to 3’ of the mRNA

Addition of the amino acid to the tRNA is catalyzed by an enzyme: *aminoacyl-tRNA synthetase*



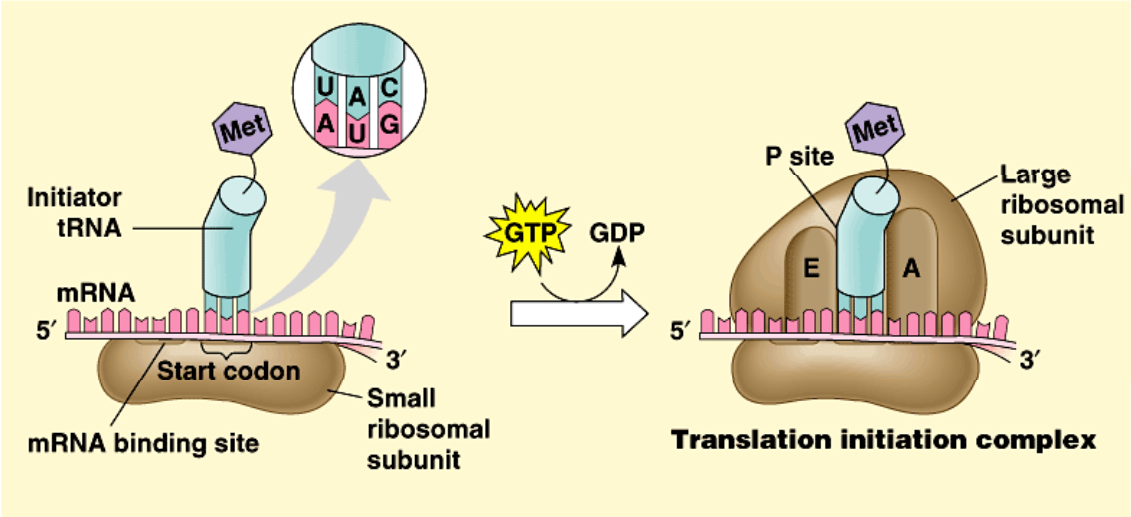
- 20 different aminoacyl-tRNA synthetases
  - One for each amino acid
- The addition of the amino acid to the tRNA uses ATP

Translation



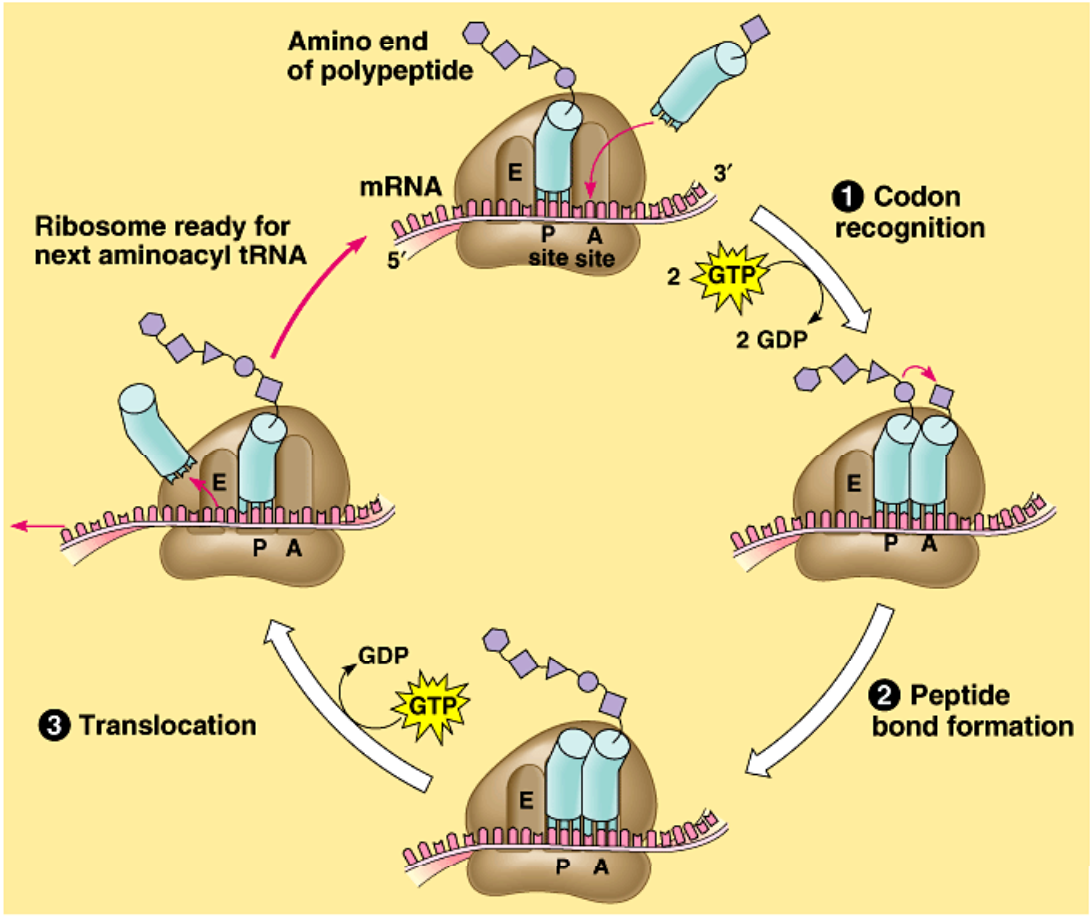
- **Initiation**
  - Ribosome, mRNA, tRNA association
- **Elongation**
  - Covalent peptide bond formations between successive amino acids
- **Termination**
  - Dissociation of ribosome, mRNA, and tRNA

Translation Initiation

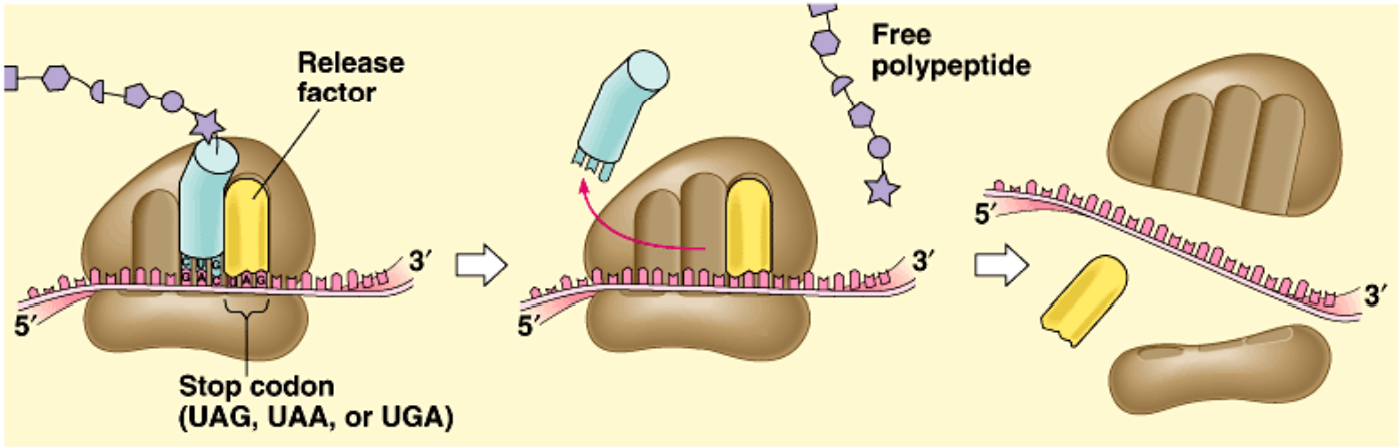


- Small subunit binds to the mRNA
- The start codon is recognized by the small subunit
- The initiator tRNA containing the amino acid Met is recruited
- The large subunit binds in a process that utilizes GTP, forming the translation initiation complex

Translation Elongation

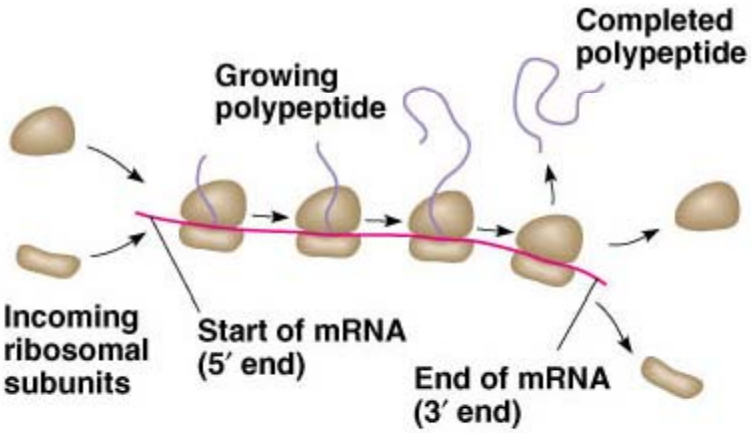


Translation Termination

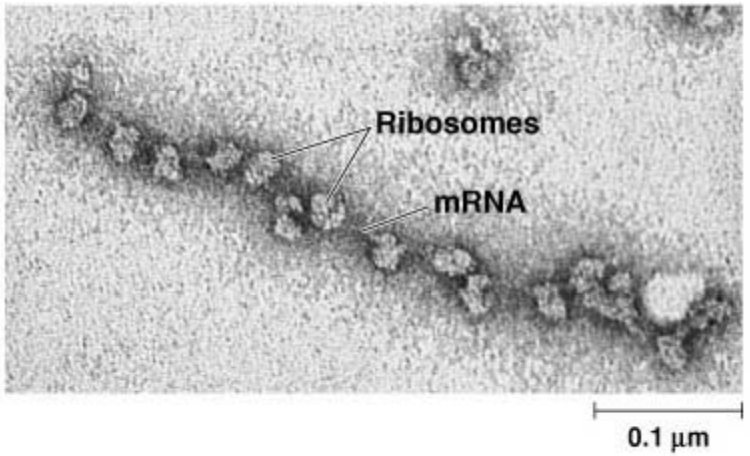


- A stop codon, UAA, UAG, UGA recruits a protein release factor
- The bond between the tRNA and the polypeptide is hydrolyzed by the release factor
- Dissociation of the mRNA, ribosome, and release factor

Polyribosomes or Polysomes



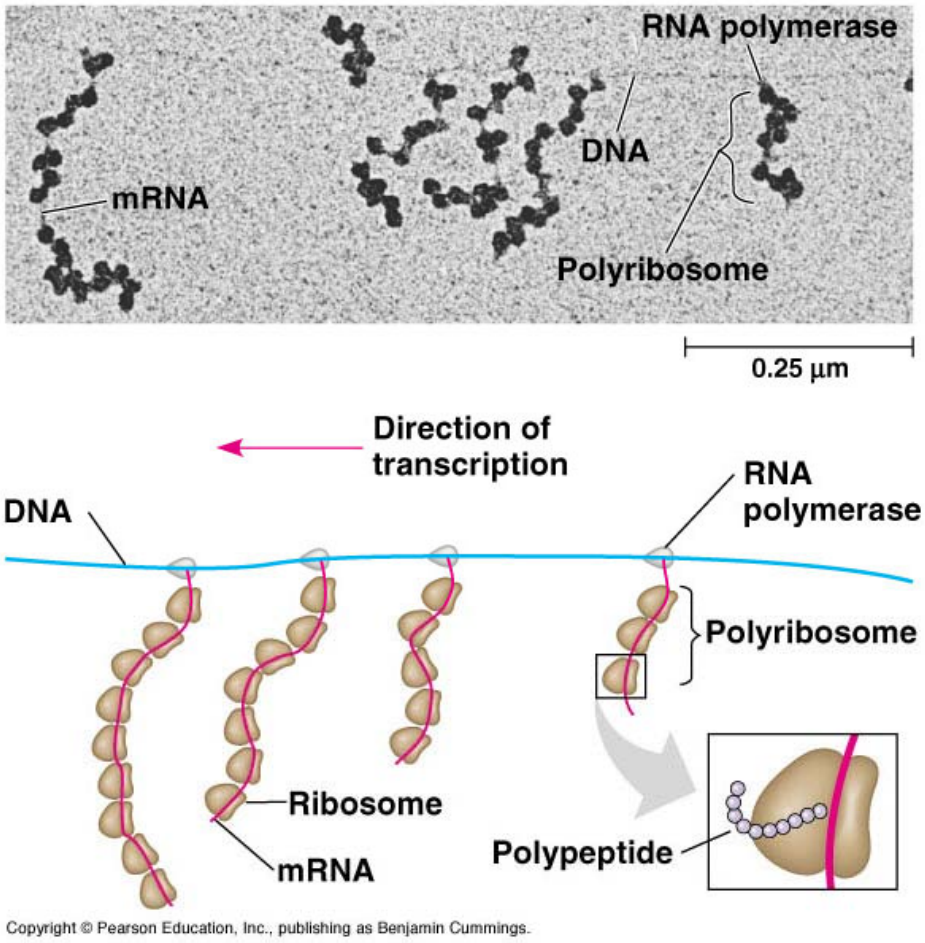
(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



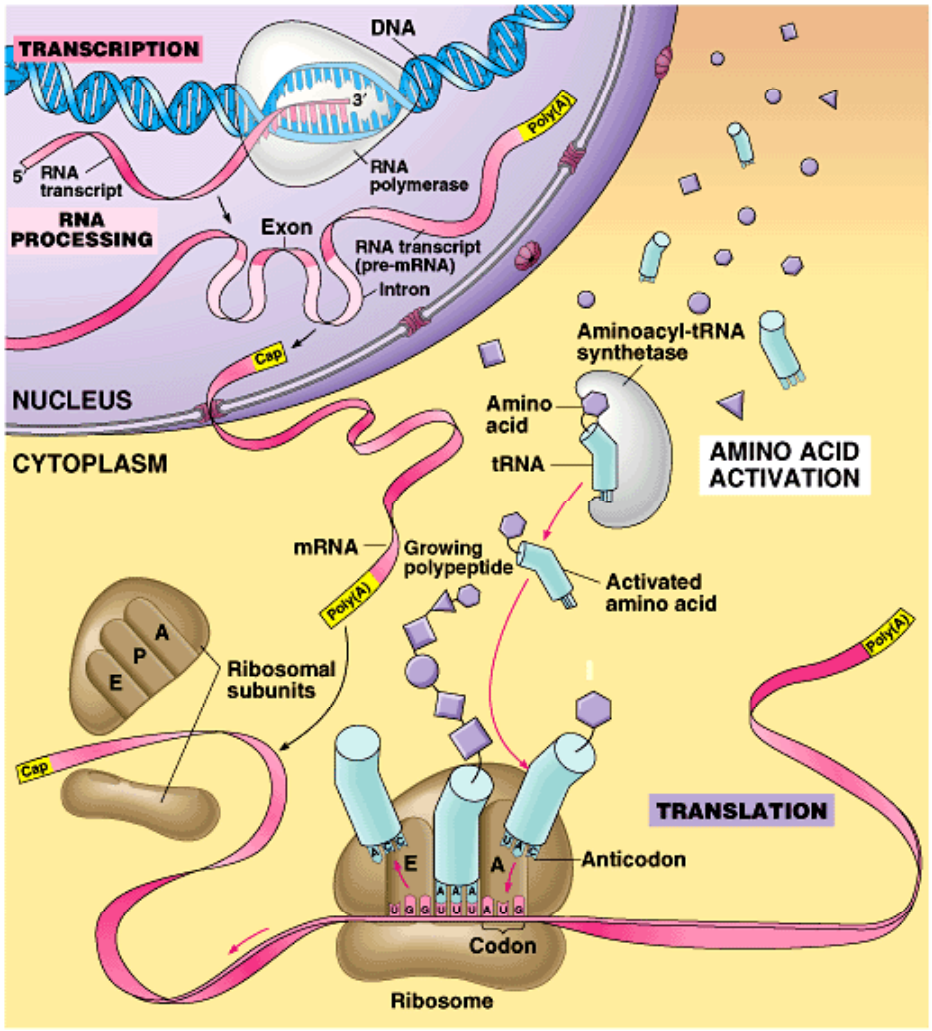
(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

- More often than not, in both eucaryotic and procaryotic cells and single mRNA contains many ribosomes simultaneously producing polypeptide

Procaryotic cells can couple transcription and translation



DNA → RNA → protein



- Transcription
- RNA processing
- Translation

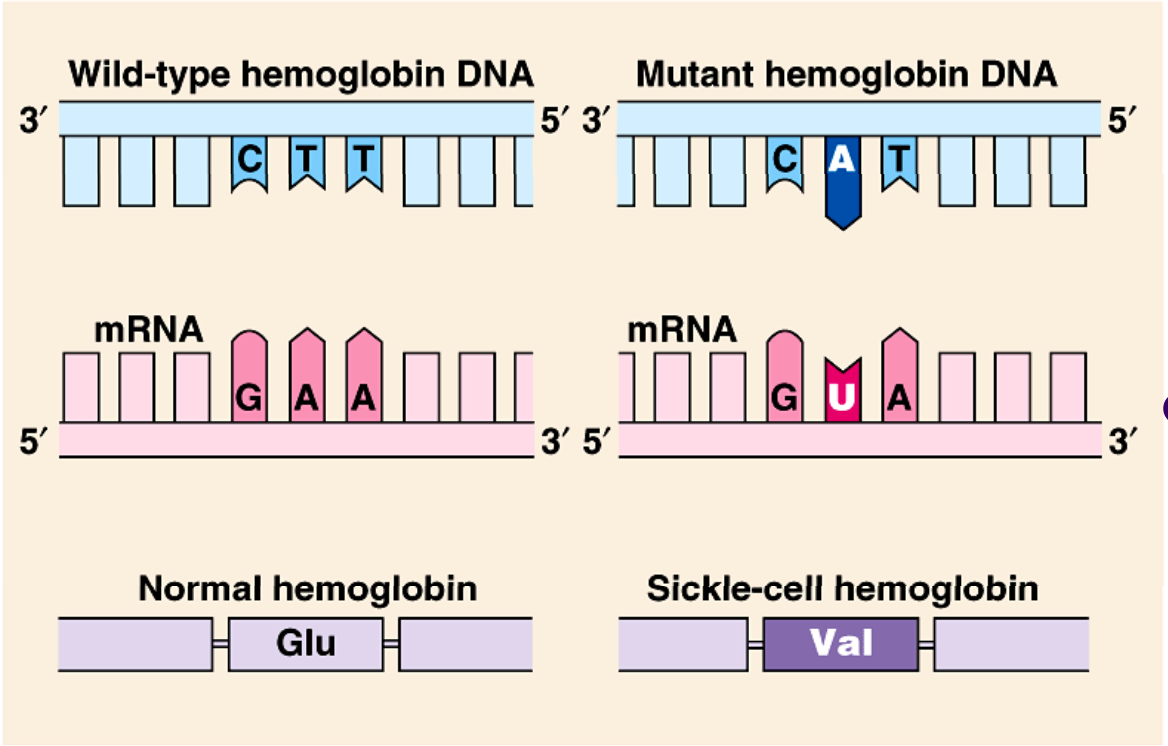
Cytoplasmic and ER bound ribosomes

- Ribosomes start in the cytoplasm
- A signal sequence in the N terminus of the protein, termed the signal peptide will target a protein for the ER to become part of the Endomembrane System (as discussed earlier in the course)
- The Signal-Recognition Particle, a multi-protein RNA complex facilitates binding of the ribosome to the ER and entry of the synthesizing protein into the ER where it can then proceed to the Golgi apparatus via a transport vesicle

Types of RNAs

- Messenger RNA
  - Codes for polypeptide
- Transfer RNA
  - Functions in translation by bringing amino acids to the mRNA using an anticodon
- Ribosomal RNA
  - Enzymatic RNAs that make up a portion of the Ribosome
- Small nuclear RNA (snRNA)
  - RNAs that are part of the Spliceosome
- SRP RNA
  - RNAs that are part of the Signal Recognition Particle
- snoRNA
  - Process ribosomal RNAs
- siRNA, miRNA
  - Involved in Gene Regulation

Point Mutation



- Single changes in the DNA sequence
- Can have drastic effects

Types of Point mutations

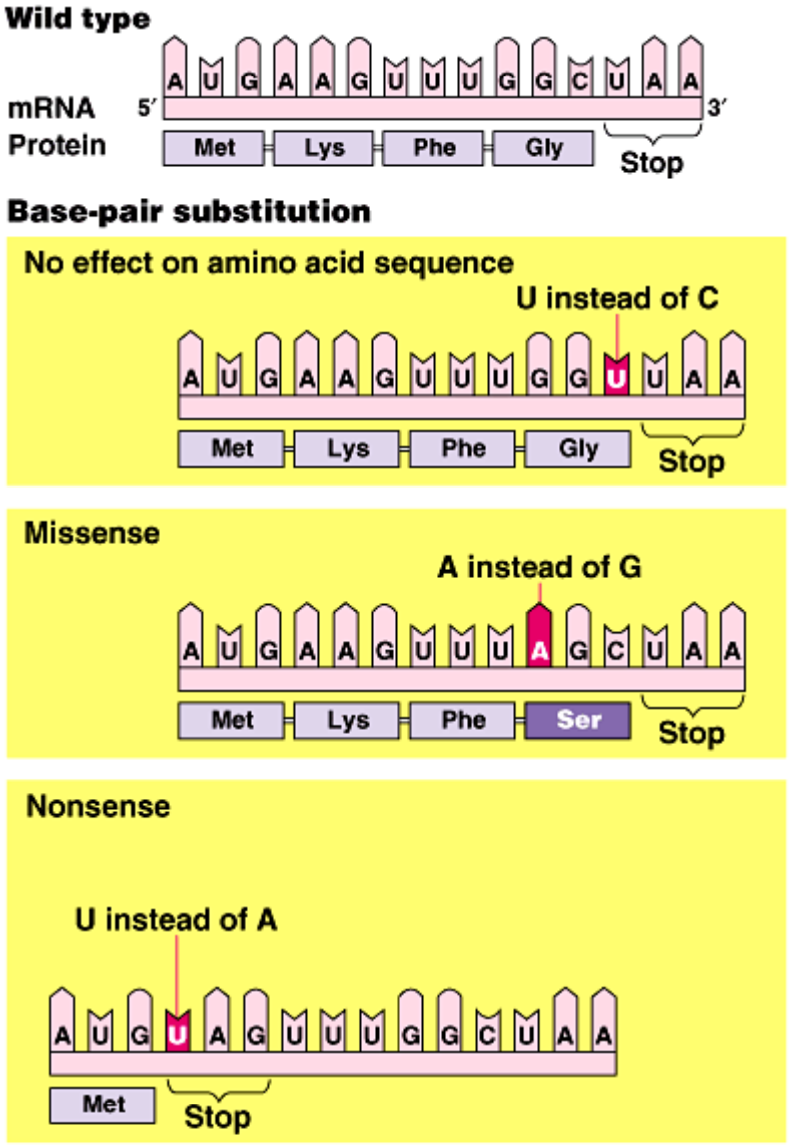
- **Base-Pair substitution**
  - A change in composition or nucleotide type at a single location
    - **Silent**: The substitution codes for the SAME amino acid
    - **Missense** Mutation: the substitution codes for another amino acid
    - **Nonsense** Mutation: the substitution codes for a STOP codon causing premature termination of the polypepetide

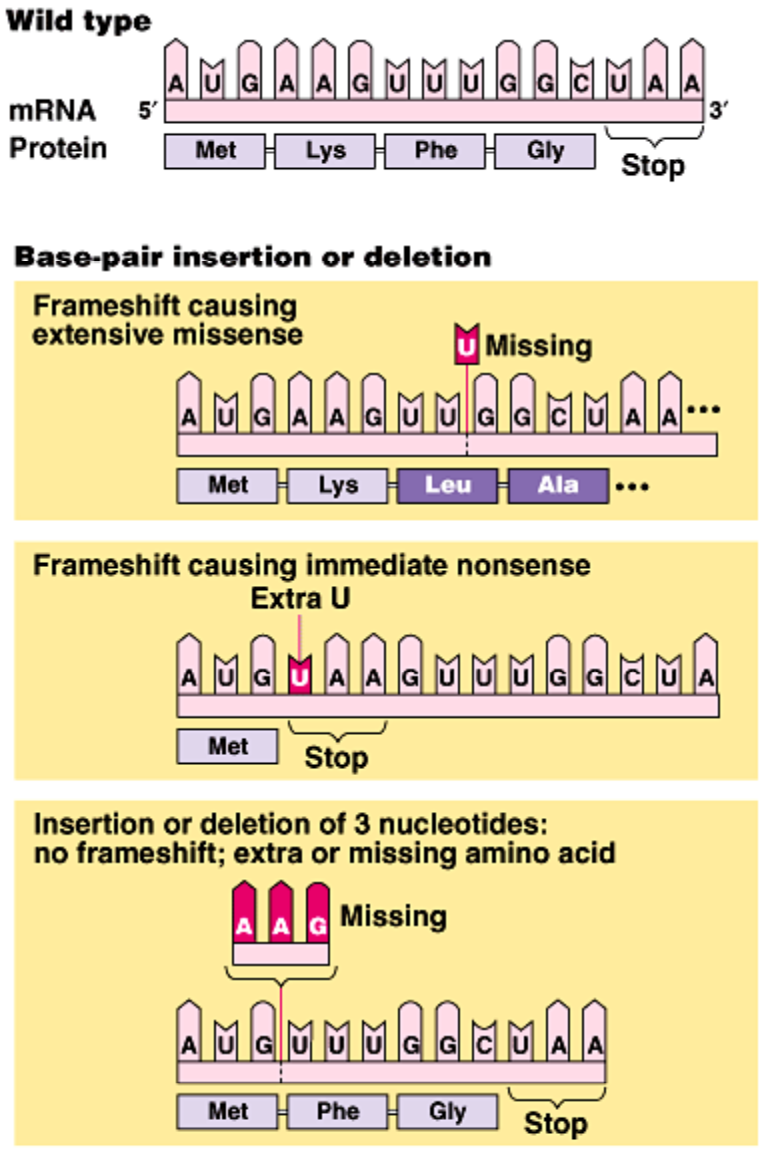
- **INDELs or Frameshift mutations**
  - The insertion or deletion of one or more base pairs
    - NO Frameshift: if 3 base pairs (or some multiple of 3) is added, then the reading frame will be the same
    - Frameshift: a change in all subsequent codons

  - THE CAT ATE THE DOG

  - THE CAT **CAT** ATE THE DOG

  - THE CAT **C**AT ETH EDO G





- Frameshift can cause
  - MISSENSE
  - NONSENSE