Bi 8 Midterm Review

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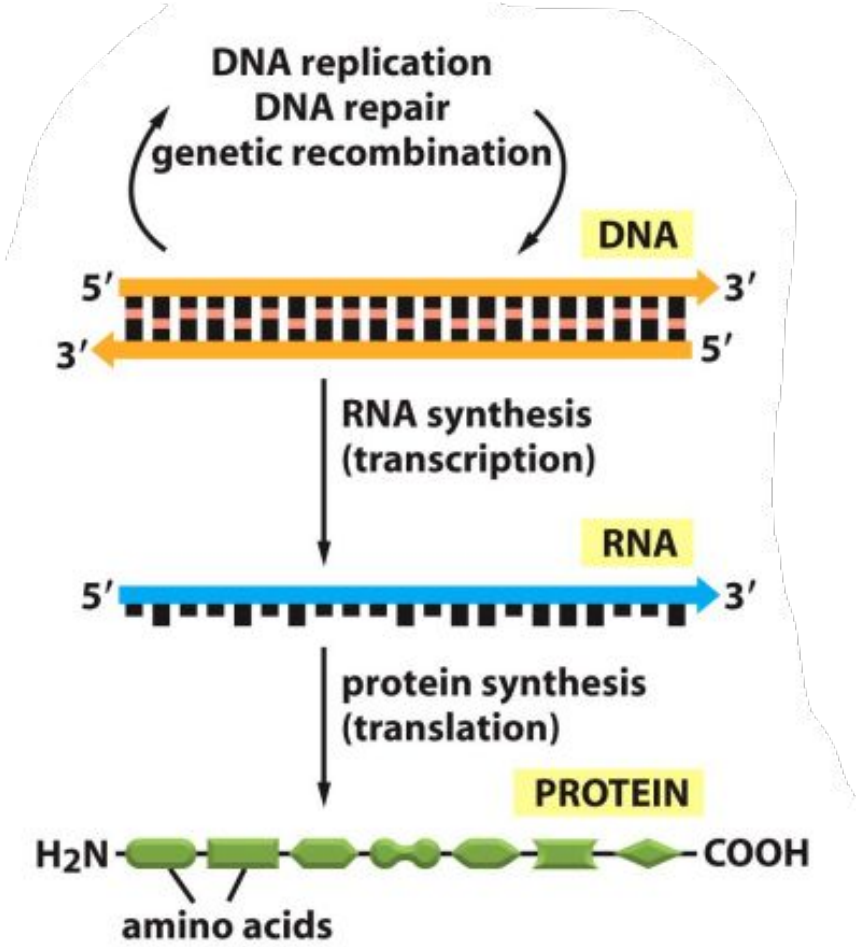
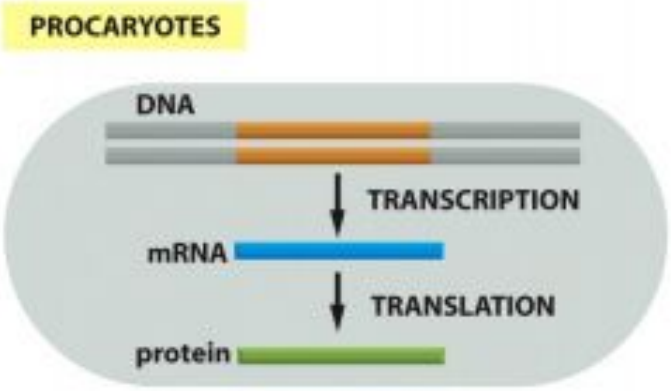
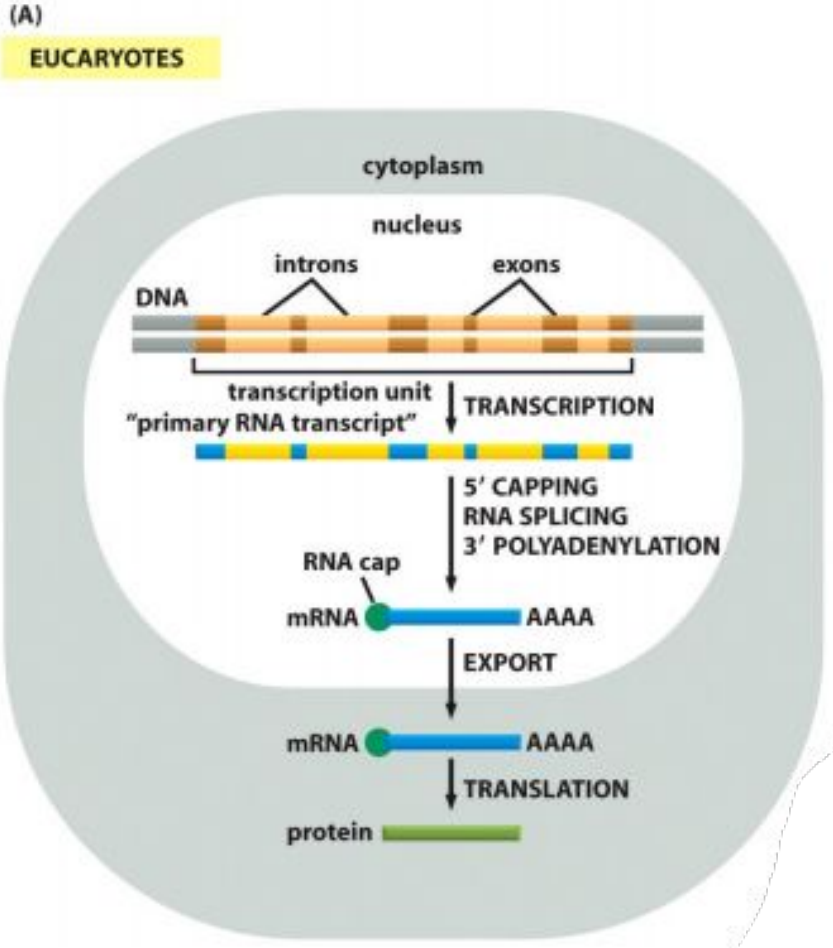
The Central Dogma

- Biology Fundamental!

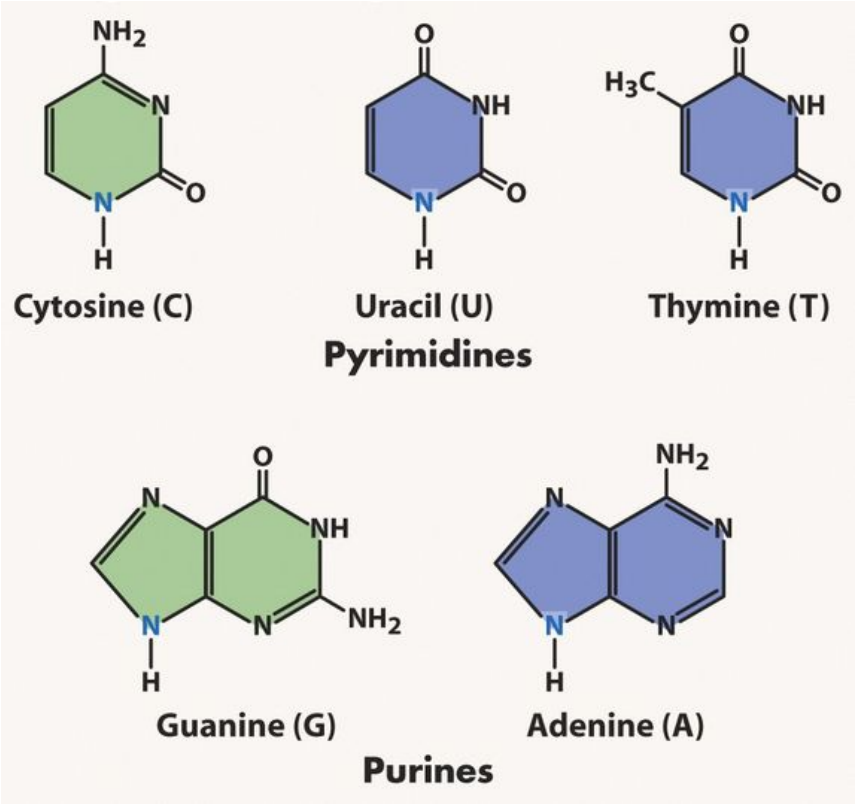


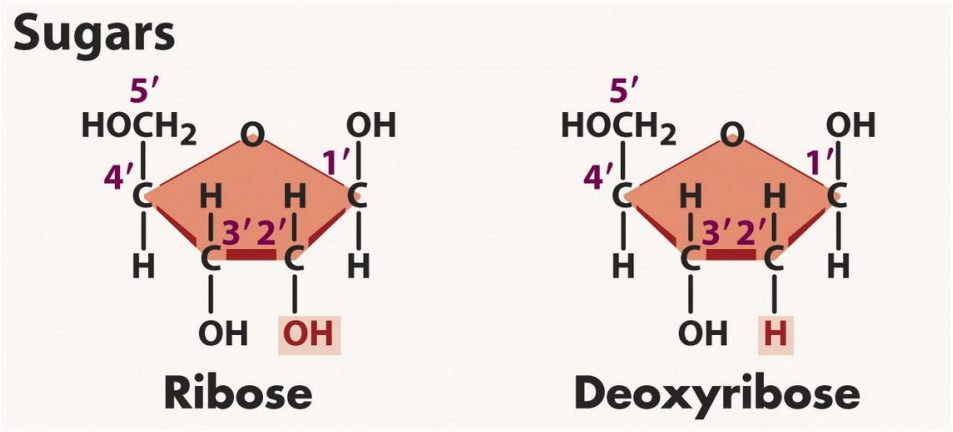
Prokaryotes and Eukaryotes



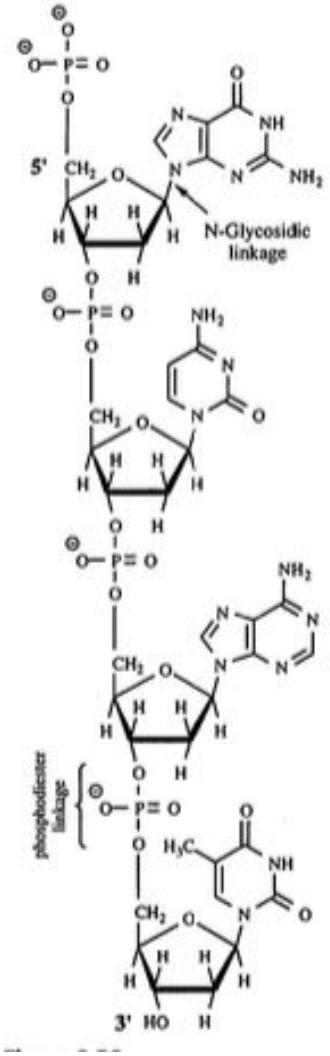


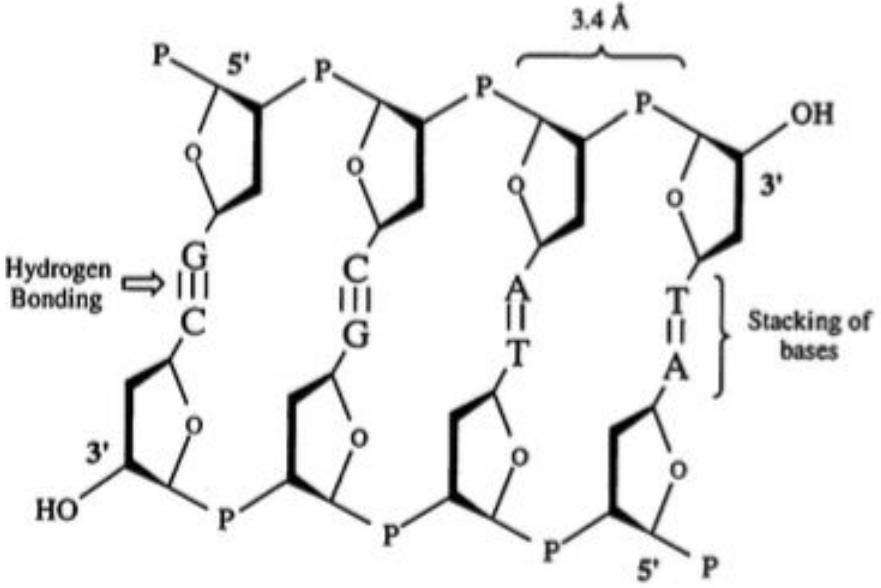
Nucleic Acid Components



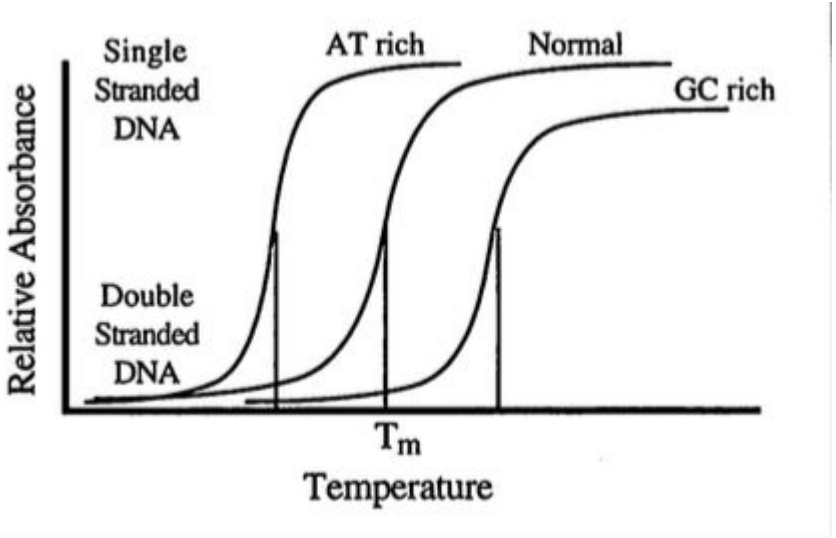


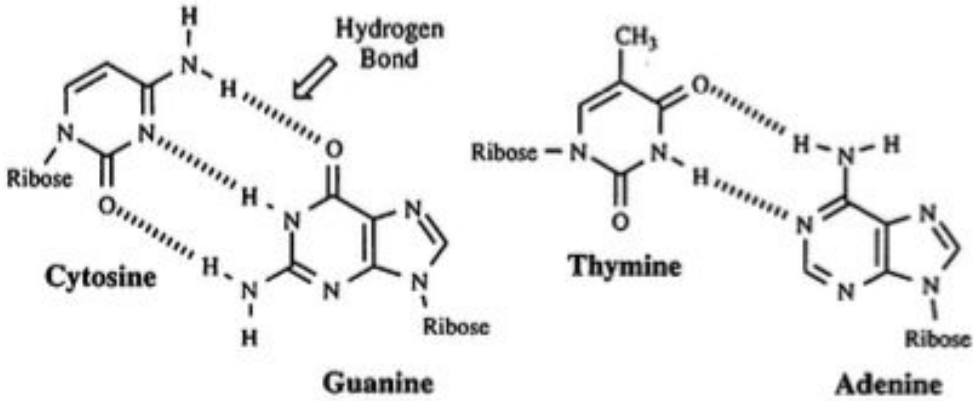
Nucleic Acid Structure



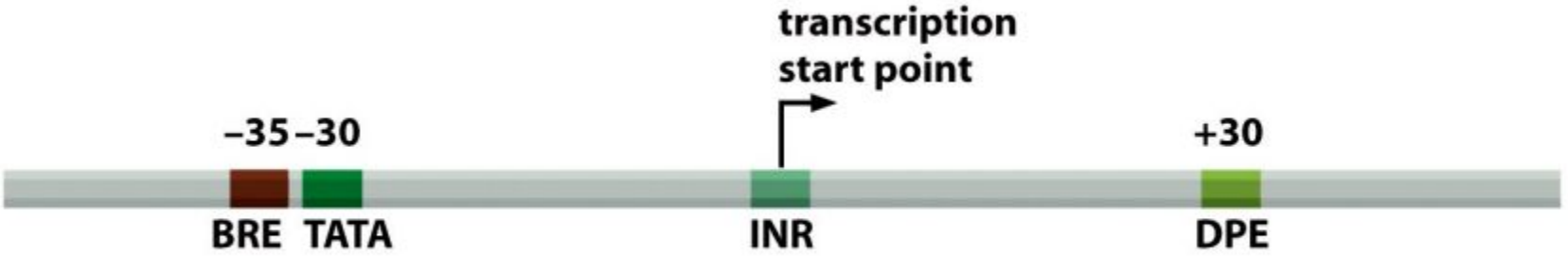


DNA Base Pair Interactions

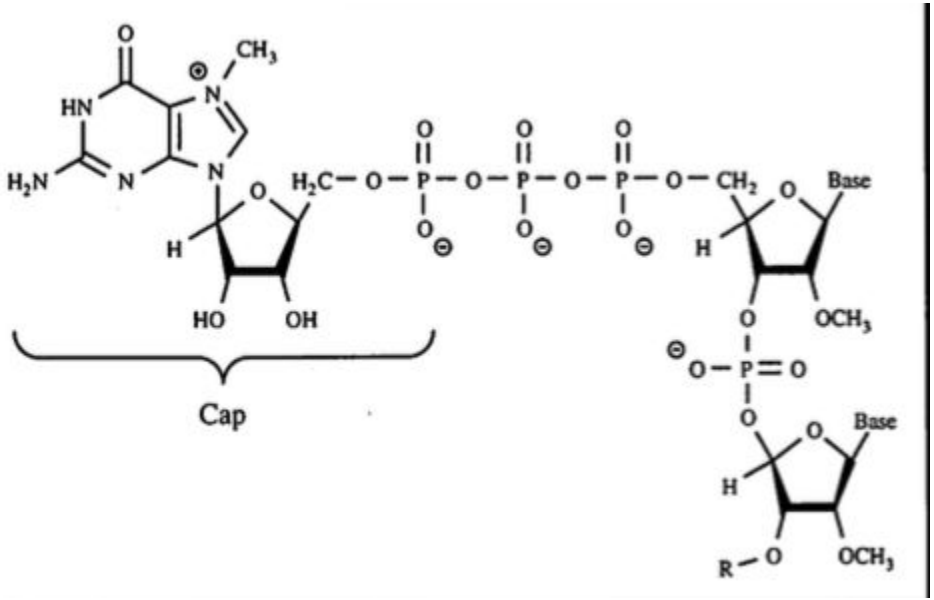


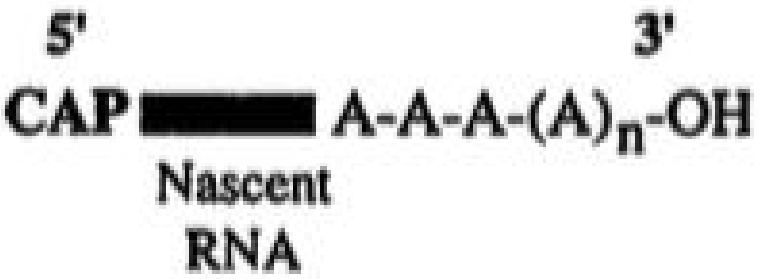


Transcription

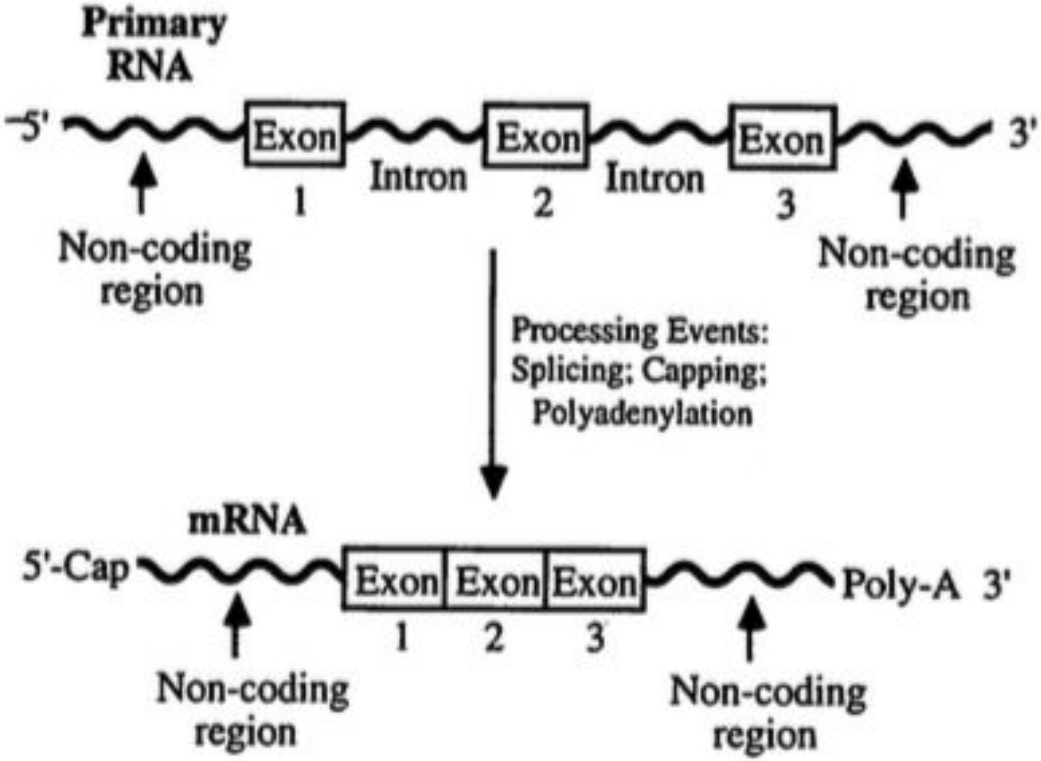


RNA Modification

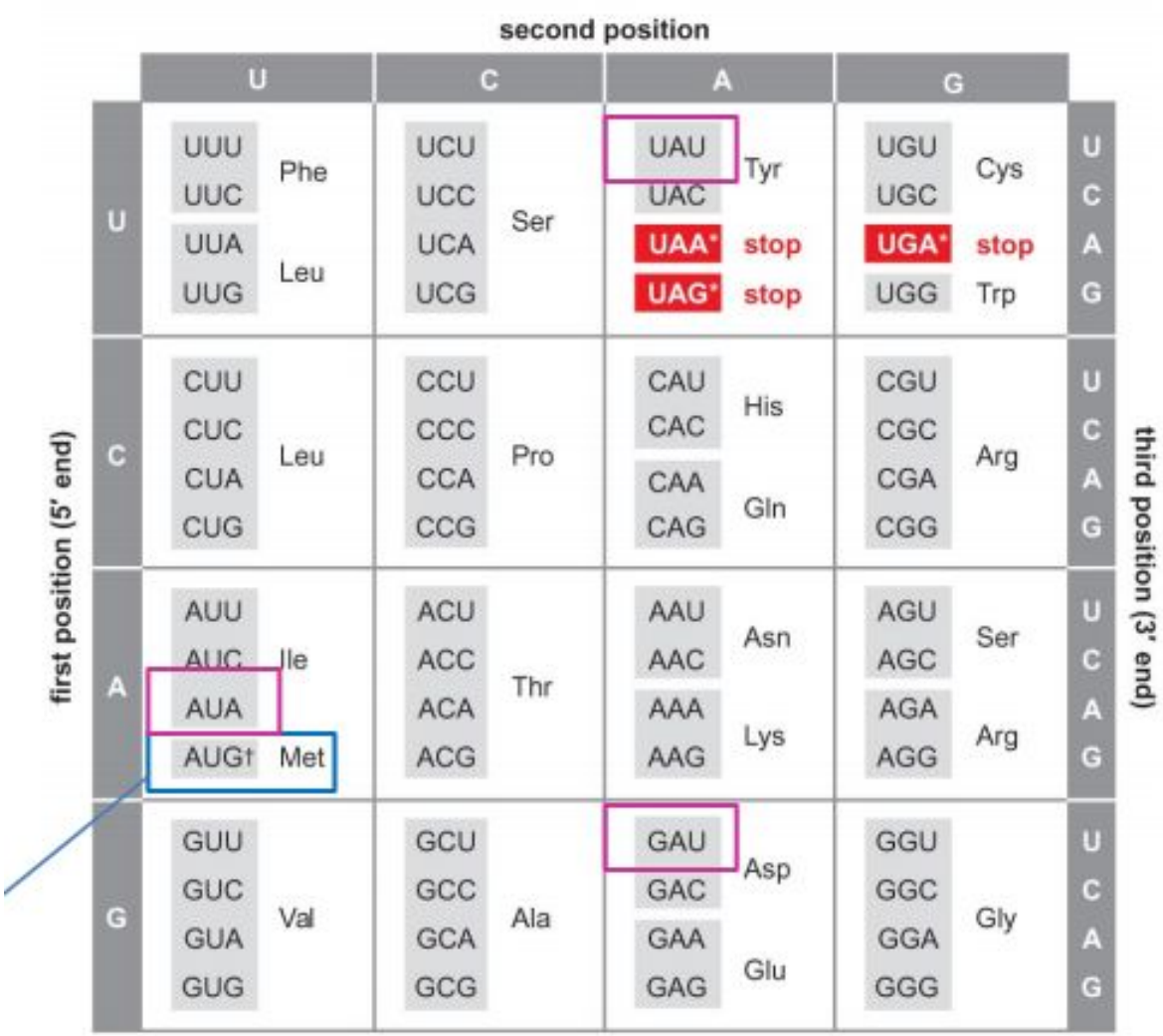




mRNA Splicing

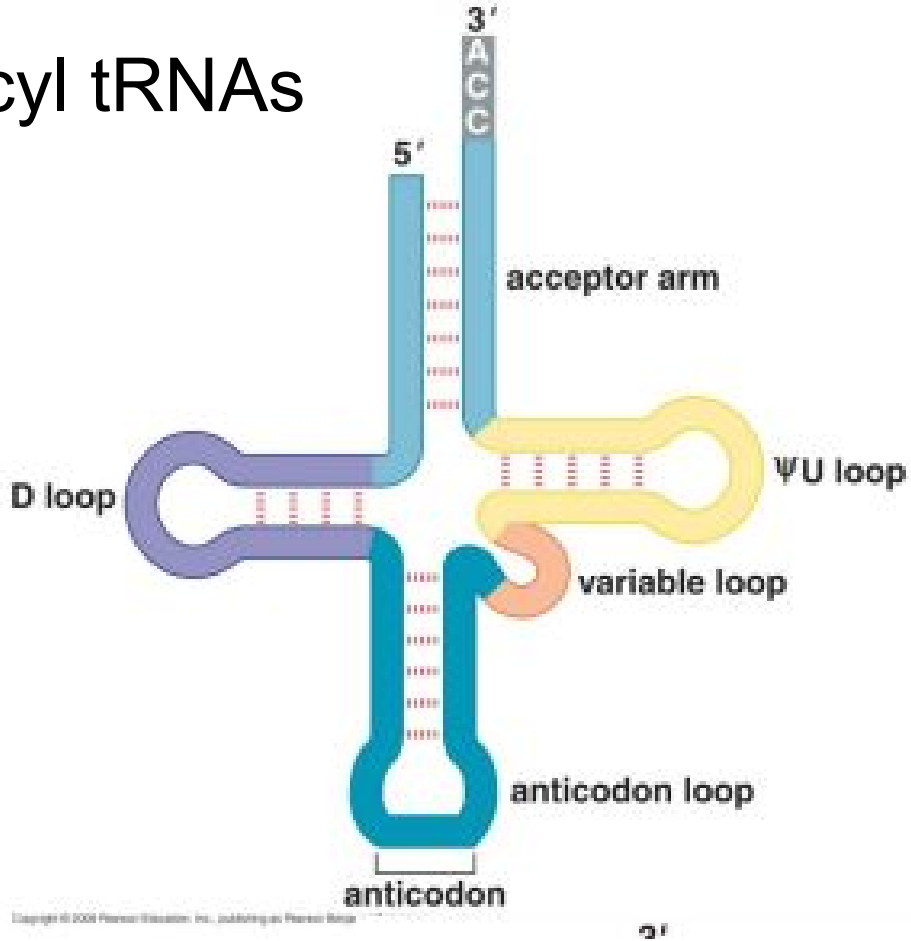


Codons

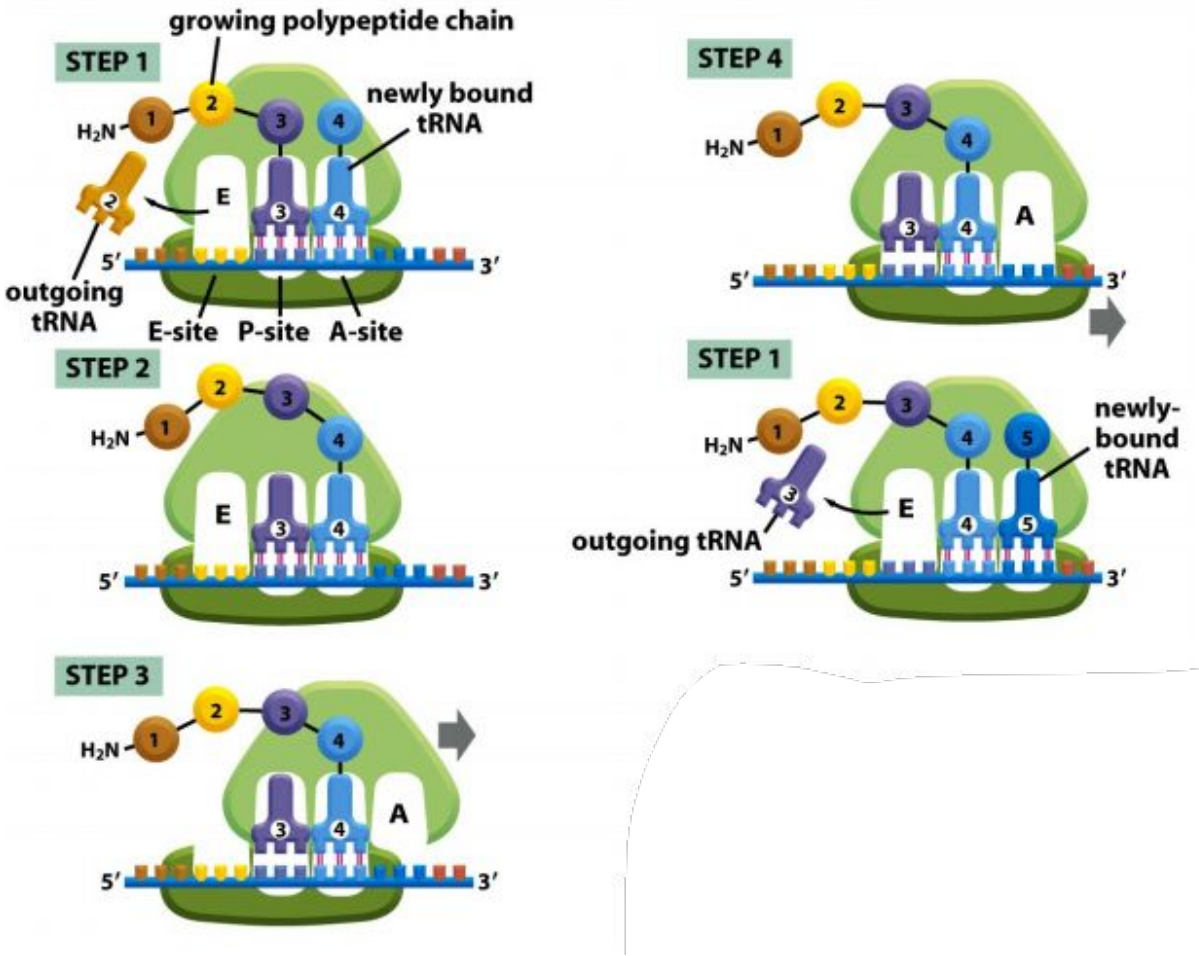

second position
U
C
A
G
first position (5' end)
third position (3' end)
U
C
A
G
UUU Phe
UUC
UUA Leu
UUG
UCU
UCC
UCA Ser
UCG
UAU Tyr
UAC
UAA* stop
UAG* stop
UGU Cys
UGC
UGA* stop
UGG Trp
CUU
CUC
CUA Leu
CUG
CCU
CCC
CCA Pro
CCG
CAU His
CAC
CAA
CAG Gln
CGU
CGC
CGA Arg
CGG
AUU
AUC Ile
AUA
AUG† Met
ACU
ACC
ACA Thr
ACG
AAU Asn
AAC
AAA
AAG Lys
AGU
AGC Ser
AGA
AGG Arg
GUU
GUC
GUA Val
GUG
GCU
GCC
GCA Ala
GCG
GAU Asp
GAC
GAA
GAG Glu
GGU
GGC
GGA Gly
GGG

tRNAs and Aminoacyl tRNAs

- Degeneracy
- Quality Control
- Wobble



Ribosomes


growing polypeptide chain
STEP 1
newly bound tRNA
H2N
E
5′
3′
outgoing tRNA
E-site
P-site
A-site
STEP 2
STEP 3
A
STEP 4
STEP 1
newly-bound tRNA
outgoing tRNA

Proteins - The Basics

- Built from amino acids
  - N to C terminus in synthesis direction
  - Amide bonds between carboxy group to amino group
  - Amino acid side chains determine types of interactions being made
- Structure and function are TIED TOGETHER
- Multiple types of ways that amino acids/domains can contribute
  - Structural support/stability
  - Positioning
  - Effector functions

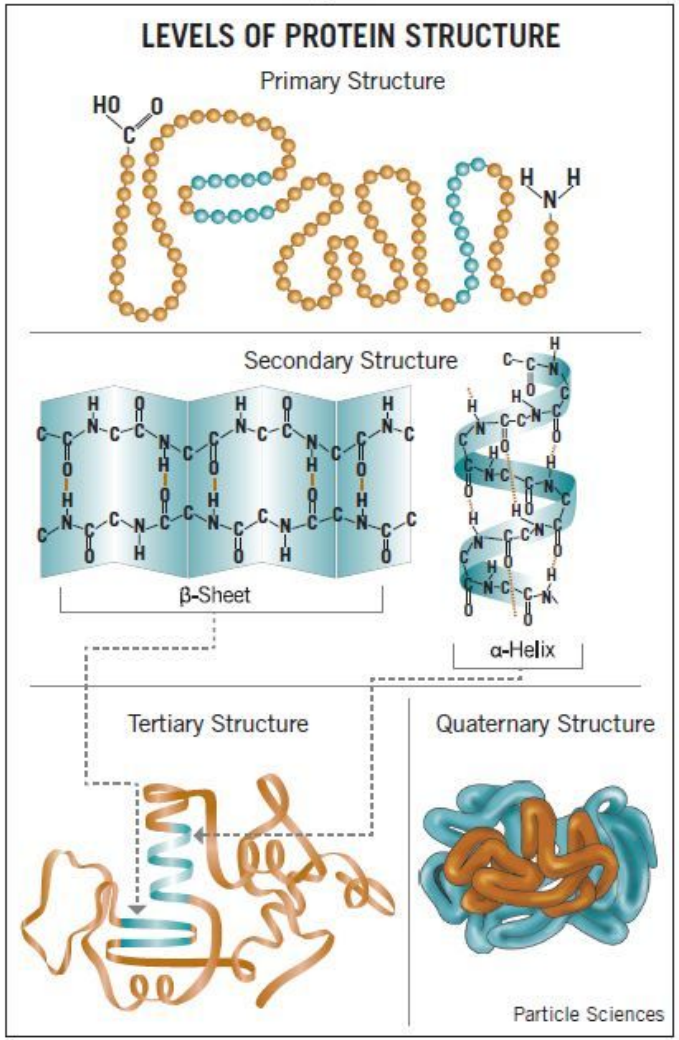
Protein Structure Analysis - Fundamental Qs

- What is the secondary/tertiary/quaternary structure looked at?
- Is the sequence examined part of an active interaction site or not?
  - If it is, what are the contacts being made/what residues are critical?
  - If not, is it structurally important?
- What is the conservation compared to a homologous protein (if there is one to be compared to) or mutant?
  - Where might they differ?
  - How important are the changes?
- How does the protein overall achieve its function?

Proteins - Structural Levels

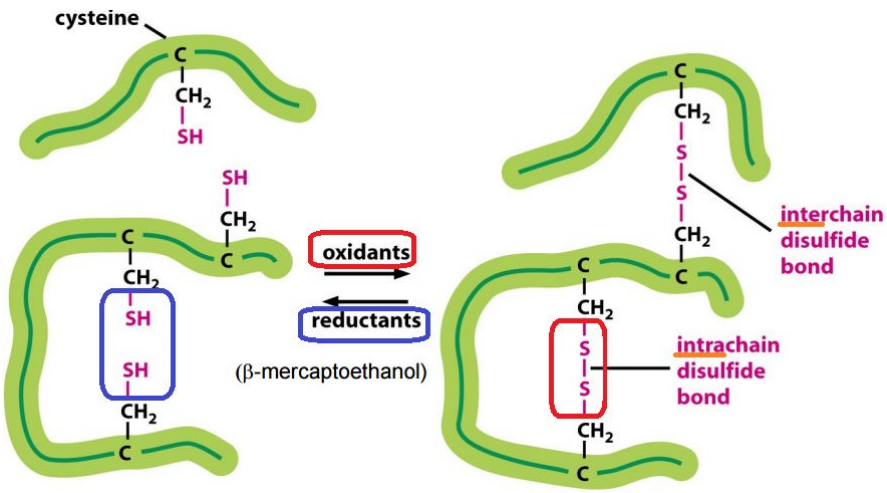
- Primary: more or less the amino acid sequence
  - Not 3D, not particularly informative on properties of prot.
- Secondary
  - *Alpha helices*
    - 3.6 aa per turn
    - Side chains bristle
      - Spatial constraints
      - Common properties often same side
    - O to H hydrogen bonds ~4th aa below
      - Many bonds; highly stable
  - *Beta-pleated sheets*
    - Hydrogen bonds between strands not inside strands
    - C=O --- H-N
    - Parallel vs antiparallel
      - Stronger when antiparallel b/c H bonds straight

Figure 2



Proteins - Structural Levels (cont.)

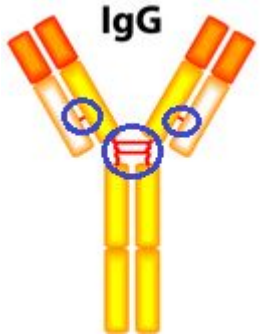
- Tertiary: OVERALL 3D shape
  - Conformation such that achieve lowest energy state/maximum stability
  - Hydrophobic side-chains buried inside (away from aqueous environment) and charged/polar will be exposed on surface (hydrophilic)
  - *Disulfide bonds* are formed by cysteines
    - True covalent bonds-- STRONG
    - Bridging = important stabilization of tertiary structure, links parts of chains
  - *Salt bridges*: ionic interactions via +/- aa side chains

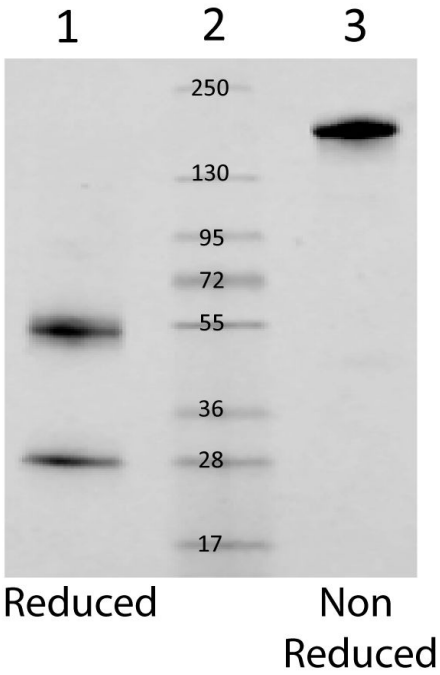


- Quaternary: subunit interactions of protein to form final complex
  - Final shape via subunit H-bonds, salt bridges, disulfide bridges
  - Often formation of unique epitopes composed of multiple protein chains

Protein Stability

- In a nutshell, proteins are held together by H-bonds mostly
  - Sensitive, multiple ways to mess w/3D structure
- Stressors:
  - pH, aqueous vs non-aqueous environment, salt/ions present, temp.
  - Essentially anything that would interrupt bonds
- Denature: unfolding of protein into misfolded/random shape
  - Result of loss of structure at secondary, tertiary, or quaternary
  - Can aggregate
  - Often will completely lose biological function compared to native form
- HELPFUL when dissecting structure!
  - Sizing w/ denaturing: reveals if individual or multimer
  - Sizing by disulfide reduction
  - Proteolysis to separate domains



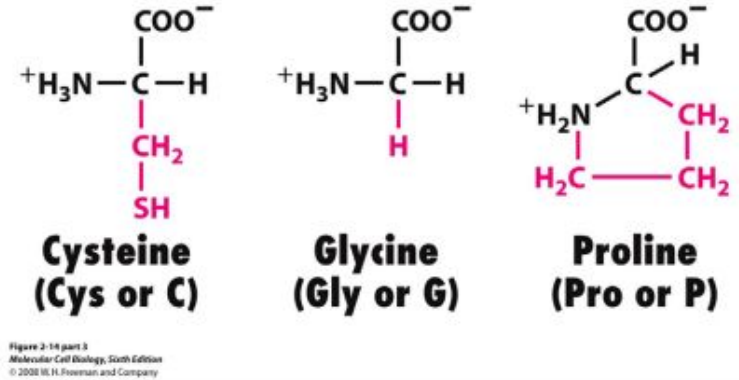


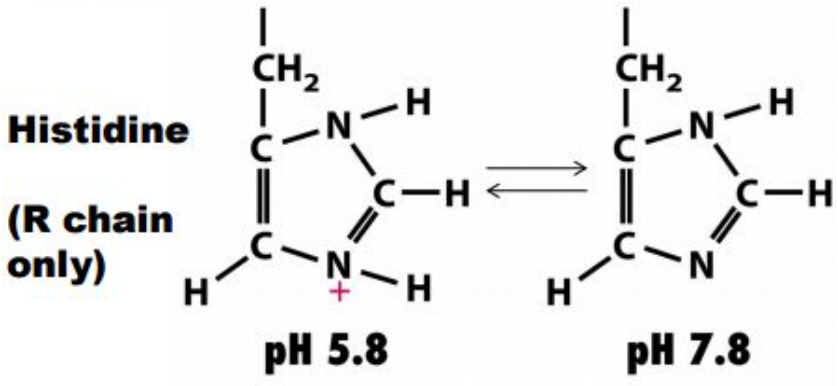
Proteins - Amino Acids

- All amino acids contribute in some way but some are more characteristic
- *Basic/acidic*
  - tend to be chemically highly active
- *Polar* amino acids
  - In general oftentimes functionally important, not just structurally
    - Can be catalytic in nature
    - OR overall make important electrochemical interactions (i.e. more H-bonds)
- *Nonpolar* amino acids
  - Often associated w/hydrophobic regions, internalized, and add to stability
  - Ring structures can stack, which are strong stabilizing interactions

Proteins - “Special” Amino Acids

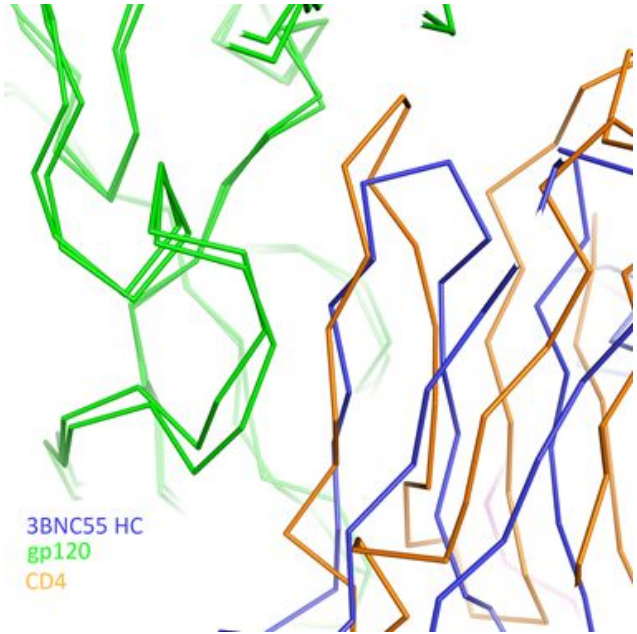
- Cysteine - R group has sulfhydrylgroup; forms disulfide bridges
  - Can DIMERIZE-- pairing w/another S
- Histidine - pH dependent proton exchange (@pH 5.8, gains H at a nitrogen, loses @ pH 7.8); useful in enzyme catalysis
- Glycine - smallest aa (H is its R group); can fit in tight parts of protein
  - Maximum flexibility
- Proline - ring involves central carbon; rigidity from phi bonds results in “helix breaker”, but in reality causes “kinks”/angles





Proteins - Conservation and Evolution

- Examination of protein sequences in alignment can reveal a lot
  - If same, can potentially infer function of unknown protein to be similar to what it's aligned to
  - If different, can hypothesize which amino acids are causing changes to function
- HOWEVER, protein structures can be similar WITHOUT having similar sequences!
  - Structures can be mimicked numerous ways
- What's crucial:
  - Conservation of PARTICULAR amino acids for particular structural function can be more important than OVERALL conservation
  - Prolonged conservation of certain residues over time despite drift can hint at necessity




```
CD4      QKKVEFKIDIVVLAFQKASSIVYKKEGEQVEFSFPLAFTVEKLTGSGELWWQAERASSSK
3BNC55   PSN--TKVDKXVE----------PKSCDK-THTCPPCPAPELLGGPSVFLFPPKPKDT--
          .:   *:*  *           *. ::  .: * . : * * * . : : :  .:

CD4      SWITFDLKNKEVSVKRVTQDPKLQMGKKL---PLHL--TLPQALPQYAGSGNLTLALEAK
3BNC55   LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR-EEQYNSTYRVVSV----
           *:   : . * *.   :**::::   :    :*   * *:   ** .: .:. .
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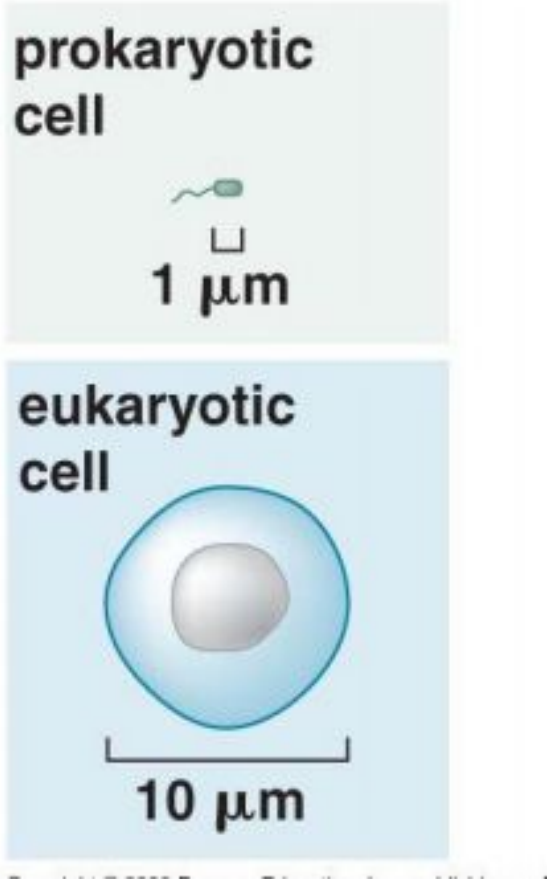
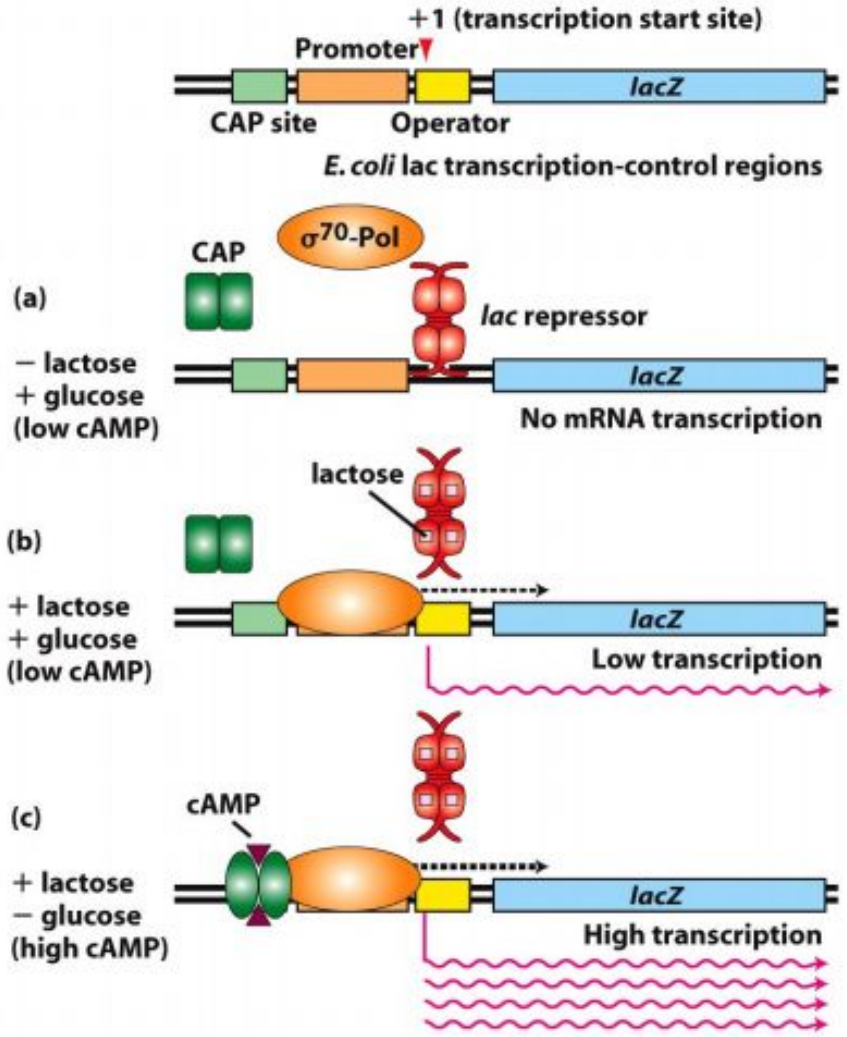
Bacteria

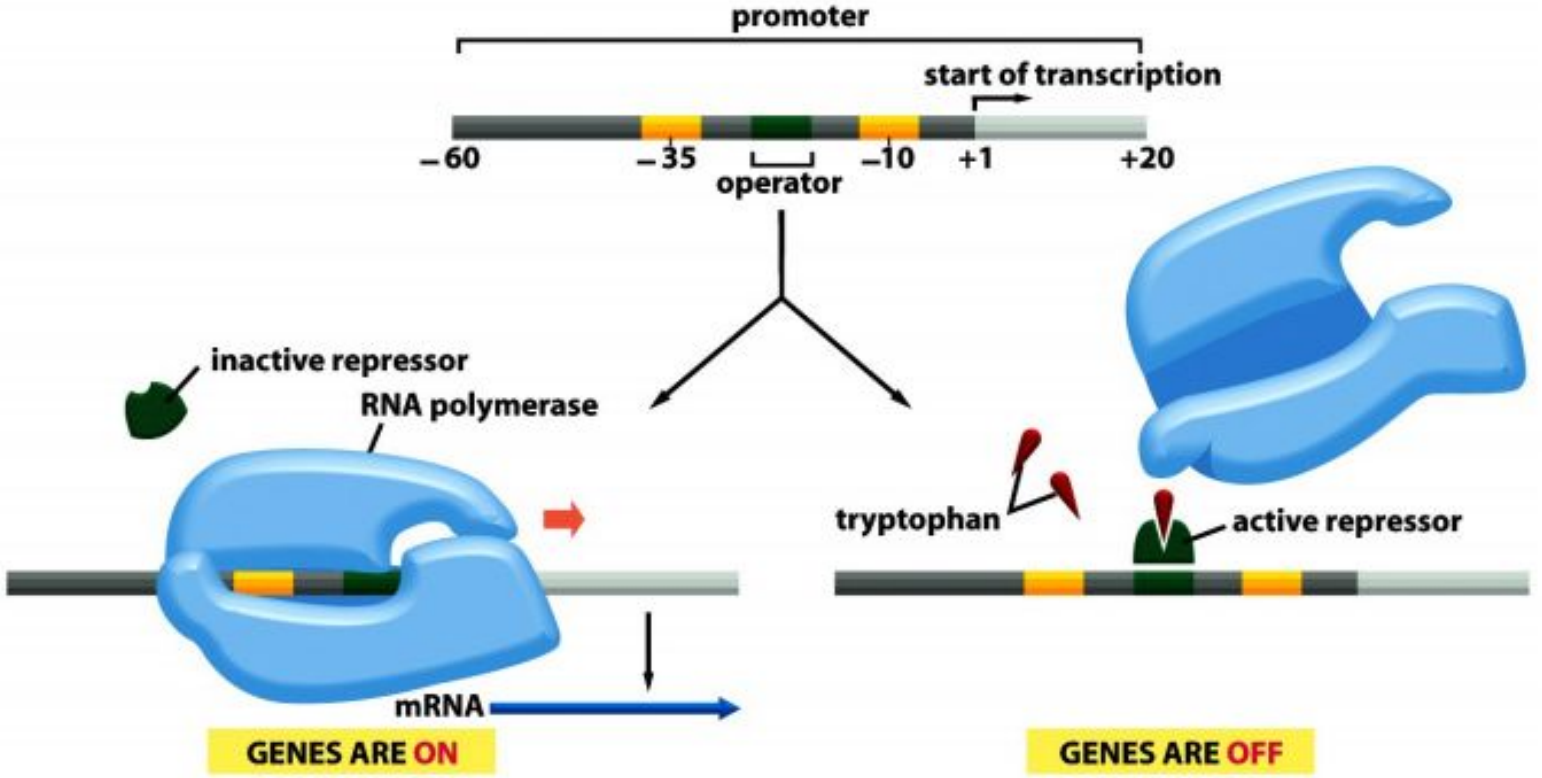
- Smaller, Faster, Simpler
- No separation of transcription/translation
- Double-use of DNA



Lac Operon


+1 (transcription start site)
Promoter
lacZ
CAP site
Operator
E. coli lac transcription-control regions
CAP
σ70-Pol
(a)
lac repressor
– lactose
+ glucose
(low cAMP)
lacZ
No mRNA transcription
lactose
(b)
+ lactose
+ glucose
(low cAMP)
lacZ
Low transcription
cAMP
(c)
+ lactose
– glucose
(high cAMP)
lacZ
High transcription

Trp Operon


promoter
start of transcription
–60
–35
operator
–10
+1
+20
inactive repressor
RNA polymerase
mRNA
GENES ARE ON
tryptophan
active repressor
GENES ARE OFF

Lambda Phage