Nucleic Acids

Lecture 27
(11/20/24)

TODAY
- Reading: Ch25, 916-921; Ch26, 960-964; Ch27, 1006-1013
- Homework #28
- Quiz #13-15 (11-12 for final)

A. Nucleotides
   1. parts
   2. nomenclature
   3. numbering
   4. properties

B. Nucleic Acids
   1. Polymer: **the phosphodiester bond**
   2. H-bonds
   3. Roles
      a. Nucleotides
      b. Nucleic acids

C. The 4 S's
   1. Size
      a. genomes
      b. RNAs
   2. Solubility
   3. **Shape**
      a. B-DNA; 10 aspects
      b. A-DNA
      c. Z-DNA
      d. Topology
         i. Packaging
         ii. Supercoiling
         iii. Topoisomerases
   4. **Stability**
      a. Nucleotides
         i. Tautomers
         ii. Acid/base
      b. Nucleic Acids
         i. Chemistry
         ii. Denaturation
         iii. Stability
         iv. Nucleases

D. Structure of the Information
   1. Exceptions to flow
   2. Structure
   3. Levels of Control

E. Recombinant DNA: Biochemical Basis of Biotechnology
   1. Restriction enzymes, DNA ligase
   2. Vectors and Inserts to make recombinant DNA (rDNA)
      a. Inserts
         i. cDNA
         ii. Genomic
      b. Vectors
   3. Transformation of hosts
   4. Selection of transformants
      a. Selectable marker/gene
      b. Distinguish empty plasmids
         i. Loss of resistance
         ii. Reporter gene
   5. Expression
      a. Special vectors
      b. Fusion proteins
         i. purification
         ii. labeling
   6. Site-directed mutagenesis

F. Replication
   1. Polymerases
   2. Fidelity
      a. Polymerase recognition
      b. Exonuclease
         i. 3'→5'

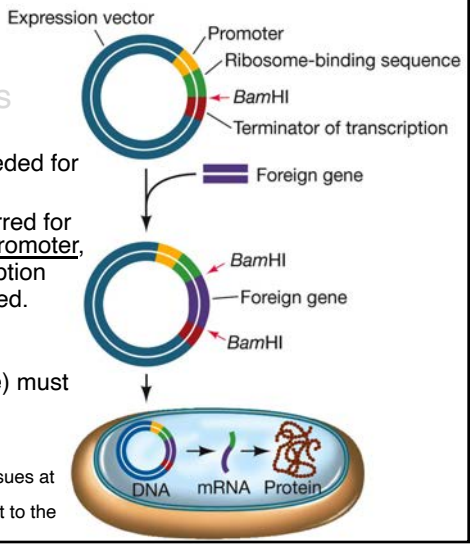
Recombinant DNA and Biotechnology

- Biochemical Basis of Biotechnology
  - Restriction enzymes, DNA ligase
  - Vectors and Inserts to make recombinant DNA (rDNA)
  - Transformation of hosts
  - Selection of transformants
  - Expression

Expression vectors include sequences needed for expression of a *transgene* in a host cell.

- For prokaryote host, which are preferred for making large amounts: A **bacterial** promoter, ribosome binding site, and a transcription termination signal, must all be included.
- For eukaryote (mostly yeast): The **eukaryotic** promoter/enhancer and terminator (poly-A addition signal/site) must be included.

These can also be:
- *Inducible promoters* which respond to a specific signal
- *Tissue-specific promoters* expressed only in certain tissues at certain times
- *Signal sequences*—e.g., a signal to secrete the product to the extracellular medium



Recombinant DNA and Biotechnology

Typical Expression Vector

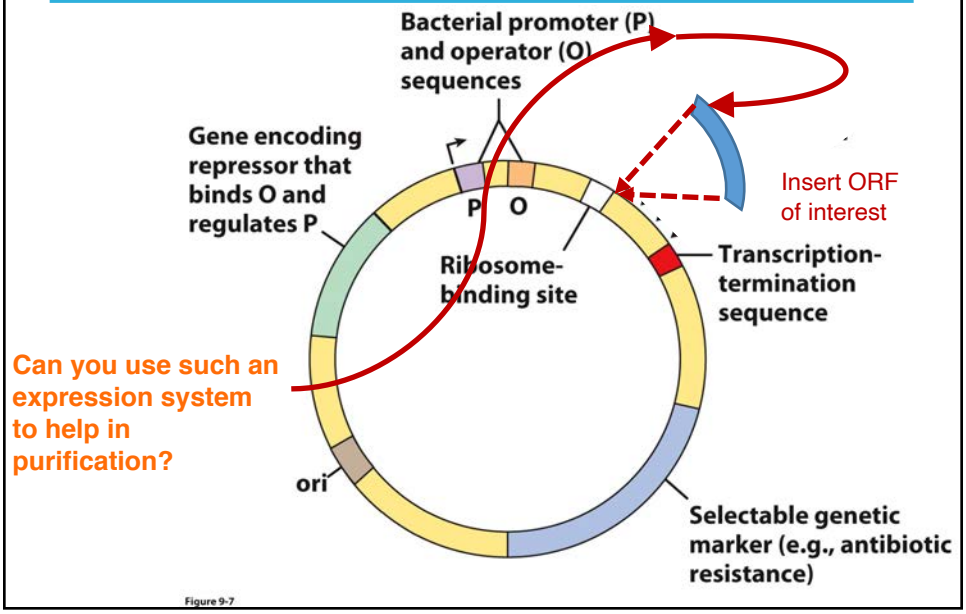


Figure 9-7

Can you use such an expression system to help in purification?

Recombinant DNA and Biotechnology

Purification of Recombinant Proteins

- Purification of natural proteins is difficult.
- Recombinant proteins can be tagged for purification.
- The tag binds to the affinity resin, binding the protein of interest to a purification column.

TABLE 9-3 Commonly Used Protein Tags

Tag protein/peptide	Molecular mass (kDa)	Immobilized ligand
Protein A	59	Fc portion of IgG
$(His)_6$	0.8	Ni^{2+}
Glutathione-*S*-transferase (GST)	26	Glutathione
Maltose-binding protein	41	Maltose
β-Galactosidase	116	*p*-Aminophenyl-*β*-D-thiogalactoside (TPEG)
Chitin-binding domain	5.7	Chitin

Recall Lecture 6: **Affinity Chromatography** Basis = function

Biotechnology (recombinant DNA technology) has revolutionized protein purification.

At the level of the DNA sequence, the DNA sequence encoding such binding proteins or "tags" can be "fused" to the sequence encoding YFP. In this way, a chimeric protein is produced that has the binding function, which allows the use of affinity chromatography.

Common "tags" are:
- Maltose-binding protein
- Chitin-binding protein
- Glutathione-S-transferase (GST)
- His-His-His-His-His-His

Column beads have attached:
- Maltose
- Chitin
- Glutathione (γ-Glu-Cys-Gly)
- Ni-chelate

ATGCATCATCATCATCATCATATGCCCGCATAT.....
TACGTAGTAGTAGTAGTAGTATACGGGCGTATA.....

His — *Fau*ND I — MetProAlaTyr.....your protein of interest

Recombinant DNA and Biotechnology

Purification of Recombinant Proteins

Can you use such an expression system to help visualize proteins INSIDE of cells?

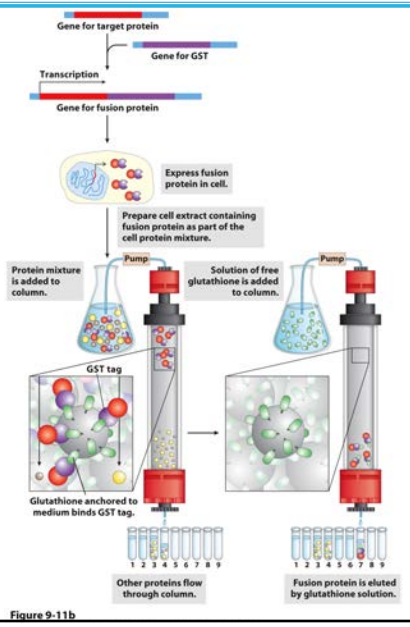


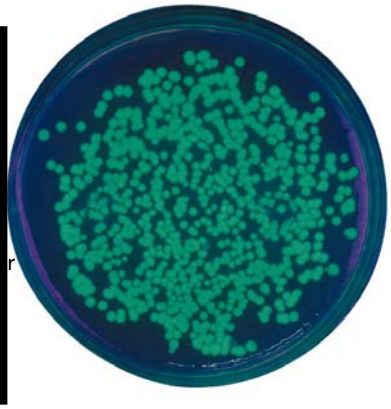
Figure 9-11b

Recombinant DNA and Biotechnology

Expression

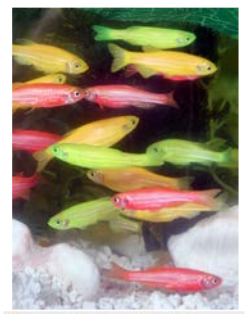
- *EXAMPLE*: Green fluorescent protein, which normally occurs in a jellyfish, emits visible light when exposed to UV light. The gene for this protein has been isolated and incorporated into vectors as a reporter gene

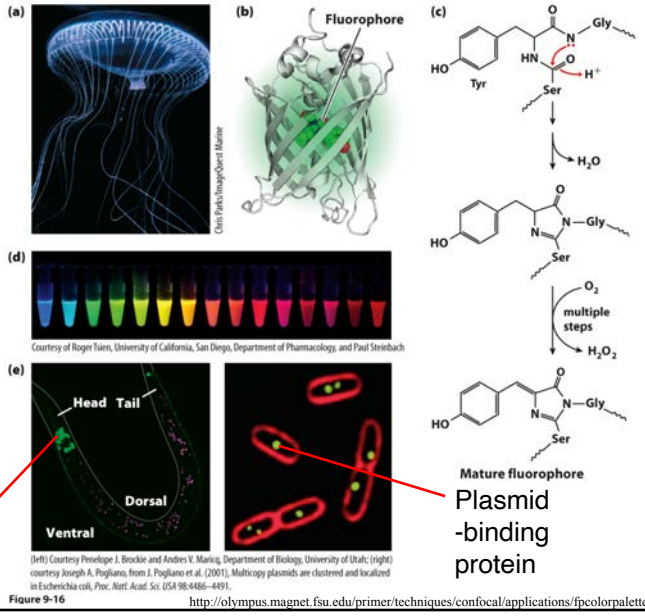
r



Recombinant DNA and Biotechnology

GFP–Tagged Protein Localization





Glu receptor in round worm

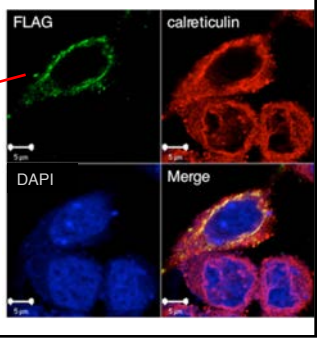
Plasmid -binding protein

http://olympus.magnet.fsu.edu/primer/techniques/confocal/applications/fpcolorpalette.html

Recombinant DNA and Biotechnology

YES! Fluorescence Can Be Used to Determine Protein Location *In Vivo*

- Green fluorescent protein (GFP)
  - use recombinant DNA technologies to attach GFP to protein of interest
  - visualize with a fluorescent microscope
- Immunofluorescence
  - tag protein with primary antibody and detect with secondary antibody containing fluorescent tag
  - Protein can also be fused to a short "epitope" (e.g., myc-tag [EQKLISEEDL], HA-tag [YPYDVPDYA] or Flag-tag [DYKDDDDK]), and the primary antibody detecting the epitope can be fluorescently labeled.

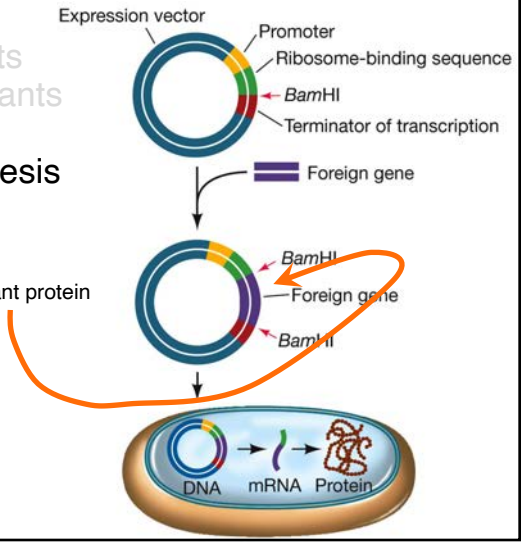


Example of Flag-tagged Ceramide Synthase, in which an expression plasmid for the fusion protein was transfected into SW480 cells (lymphoma). The counter stains are fluorescent dyes specific for DNA (4',6-diamidino-2-phenylindole, DAPI) and the ER (calreticulin).

Recombinant DNA and Biotechnology

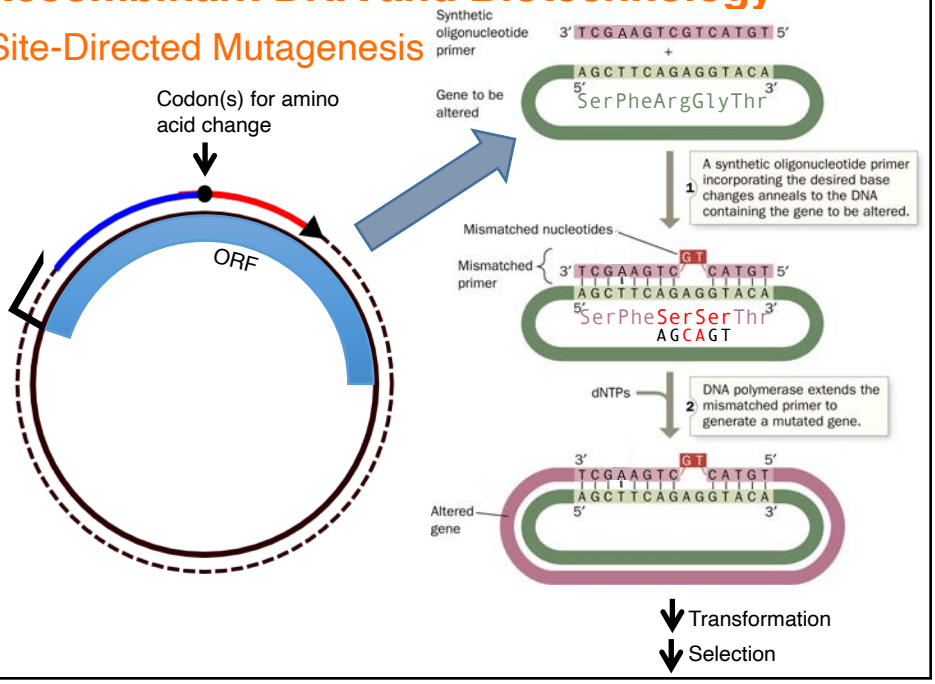
- Biochemical Basis of Biotechnology
  - Restriction enzymes, DNA ligase
  - Vectors and Inserts to make recombinant DNA (rDNA)
  - Transformation of hosts
  - Selection of transformants
  - Expression
  - Site-directed mutagenesis

Make a specific mutation to make a variant protein



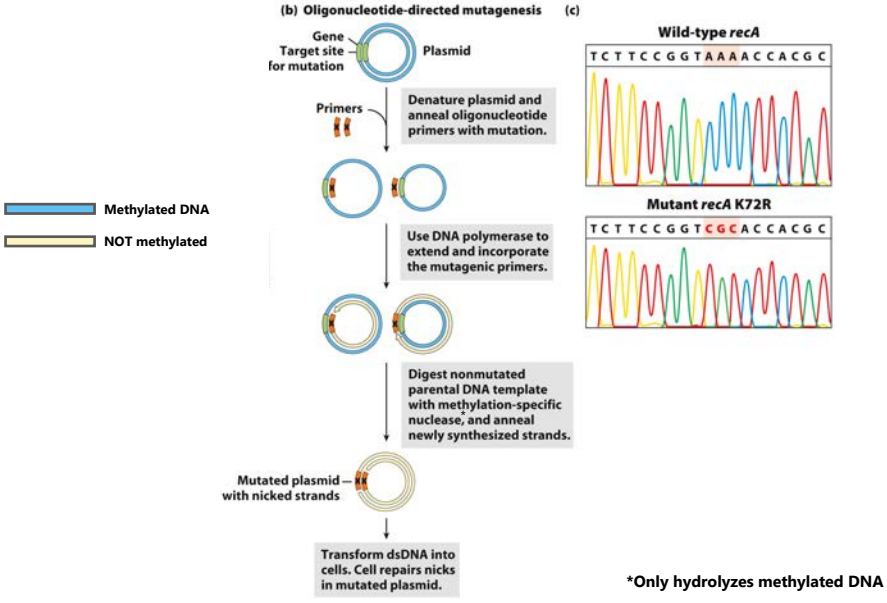
Recombinant DNA and Biotechnology

Site-Directed Mutagenesis

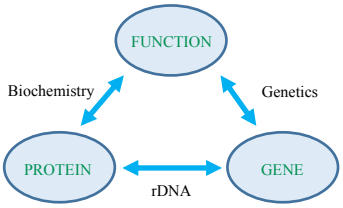


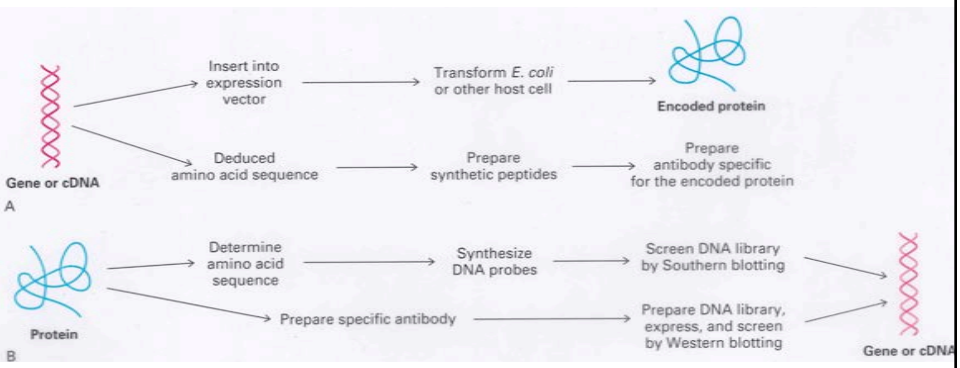
Recombinant DNA and Biotechnology

Site-Directed Mutagenesis



Recombinant DNA and Biotechnology





DNA Replication

- DNA Replication
  - Polymerases
    - Activities
    - Structure
    - Mechanism
- Replication/polymerase fidelity
  - Base-pairing
  - Exonuclease Activities
- DNA Sequencing
- PCR

} Please study slides/videos on website

DNA Replication

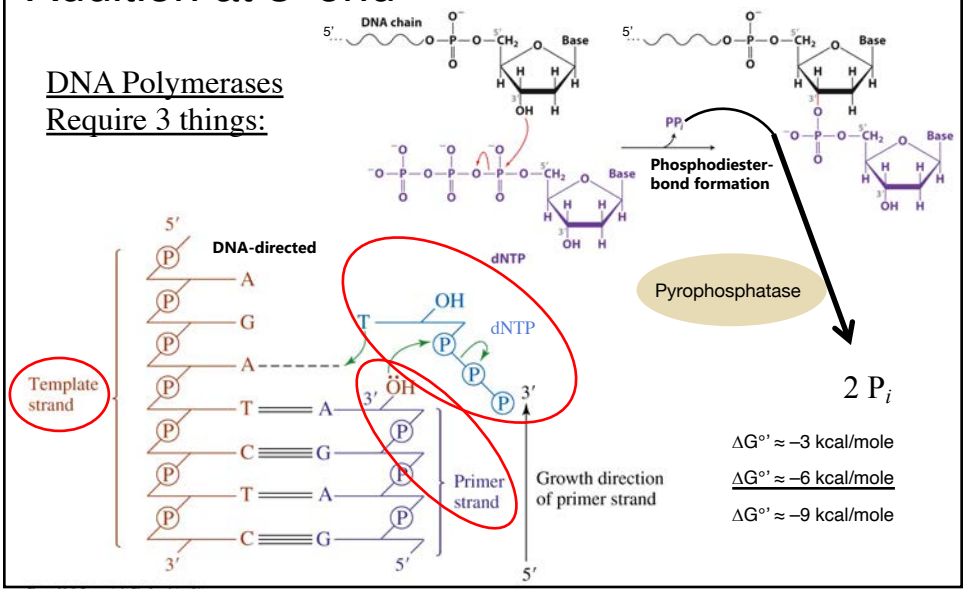
Nucleic acid function: Central Dogma

DNA Replication

DNA replication is catalyzed by DNA-directed DNA polymerase (DNA polymerase)

Addition at 3' end

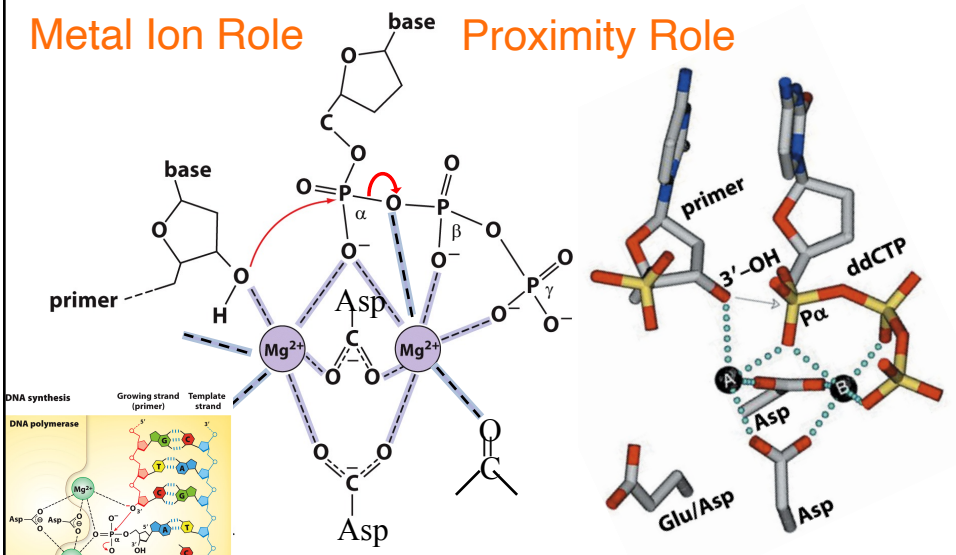
DNA Polymerases Require 3 things:



DNA/RNA polymerase mechanism

Metal Ion Role

Proximity Role



This is just the chemistry step, there are other more important enzymatic steps…

Comparison of Polymerases

TABLE 25-1 Properties of *E. coli* DNA Polymerases

	Pol I	Pol II	Pol III
Mass (kD)	103	90 (α_4)	130 *
Molecules/cell	400	?	10–20
Turnover number[a]	20	5	1000
Structural gene	*polA*	*polB*	*polC*
Conditionally lethal mutant	+	−	+
Polymerization: 5′ → 3′	+	+	+
? → Exonuclease: 3′ → 5′	+	+	+
Exonuclease: 5′ → 3′	+	−	−
Processivity	100	10,000	500,000[b]

[a]dNTP polymerized sec^{-1} at 37 ° C. [b]not including Okasaki fragments

*In a complex with 10 proteins (26 subunits) of >900 kD. Core is trimer of α, θ, ε

DNA Replication

- DNA Replication
  - Polymerases
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    - Structure
    - Mechanism
- Replication/polymerase fidelity
  - Base-pairing
  - Exonuclease Activities
- DNA Sequencing
- PCR

} Please study slides/videos on website

Replication/polymerase Fidelity

Geometry of Base Pairing Accounts for High Fidelity

- Errors in replication for *E. coli*: $1/10^9 – 1/10^{10}$ bp
  - $3x10^6$ bp/genome x 10^{-10} mistakes/bp = 0.0003 mistakes/genome
  - 1 mistake per 1,000–10,000 replications
- DNA polymerase active site excludes base pairs with incorrect geometry
  - At BOTH the insertion site and the post insertion site
  - BUT, DNA polymerases still insert wrong base 1/10,000 times.
  - Repair mechanisms fix these errors.

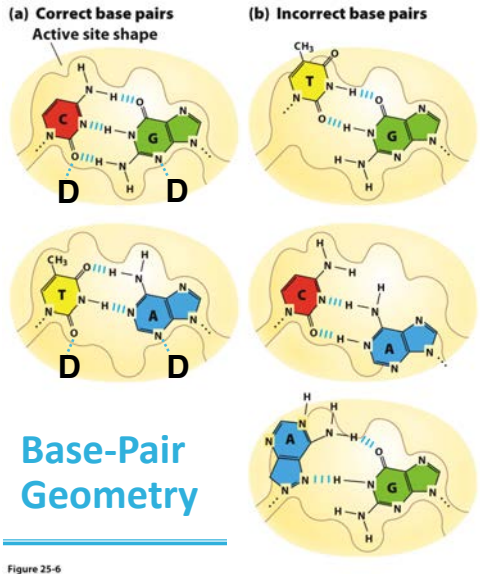
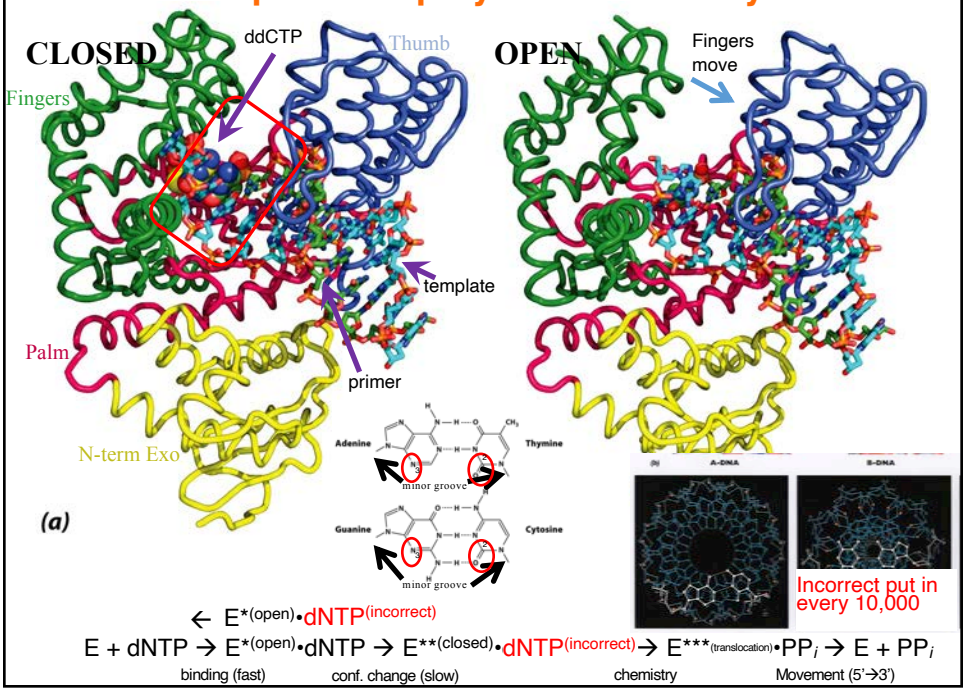


Figure 25-6

Replication/polymerase Fidelity



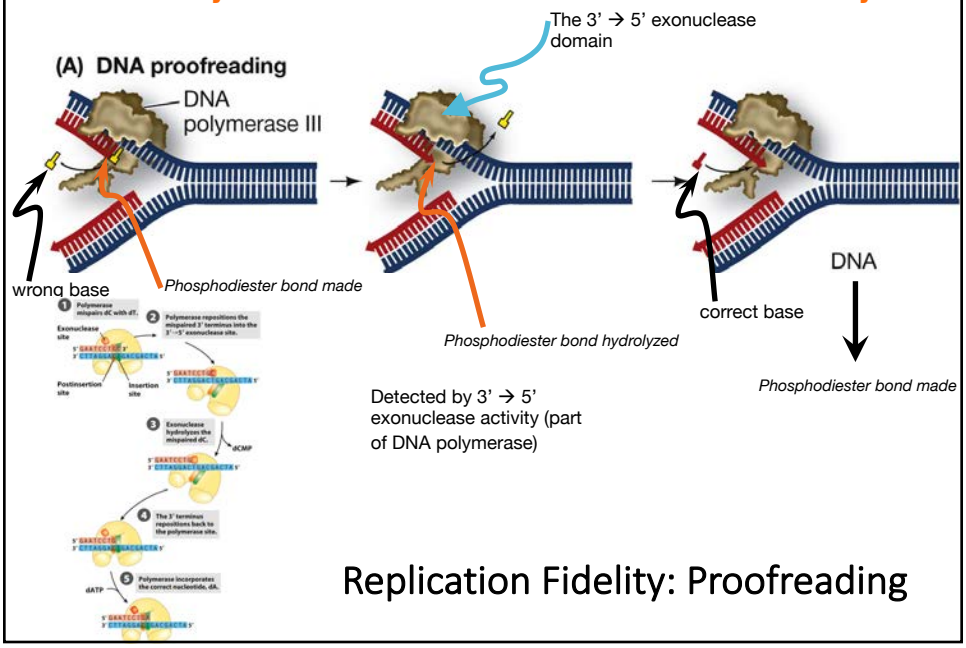
← $E^{*(open)}$•$dNTP^{(incorrect)}$

$E + dNTP \rightarrow E^{*(open)}$•$dNTP \rightarrow E^{**(closed)}$•$dNTP^{(incorrect)} \rightarrow E^{***}_{(translocation)}$•$PP_i \rightarrow E + PP_i$

binding (fast) | conf. change (slow) | chemistry | Movement (5'→3')

Replication/polymerase Fidelity

DNA Polymerases have 3' → 5' Exonuclease Activity



Replication Fidelity: Proofreading

Replication/polymerase Fidelity

Polymerases make mistakes in synthesis (polymerase 5'→3' activity) = 1/10,000

DNA polymerases have proofreading ability (3'→5' exonuclease activity) = 1/10,000

After replication fork, any mistakes are corrected by
① **mismatch repair** mechanism. This misses = 1/100

Overall for Replication = $1/10^{10}$

In humans with:
$3x10^9$ bp/haploid genome x $1/10^{10}$ mistakes/bp = 0.3 mistakes/haploid genome

Plus DNA can be damaged in living cells. These damages are repaired by other mechanisms called

Post-replication DNA Repair:

② • **Direct reversal of modification**
③ • **Base-excision repair**
④ • **Nucleotide excision repair**