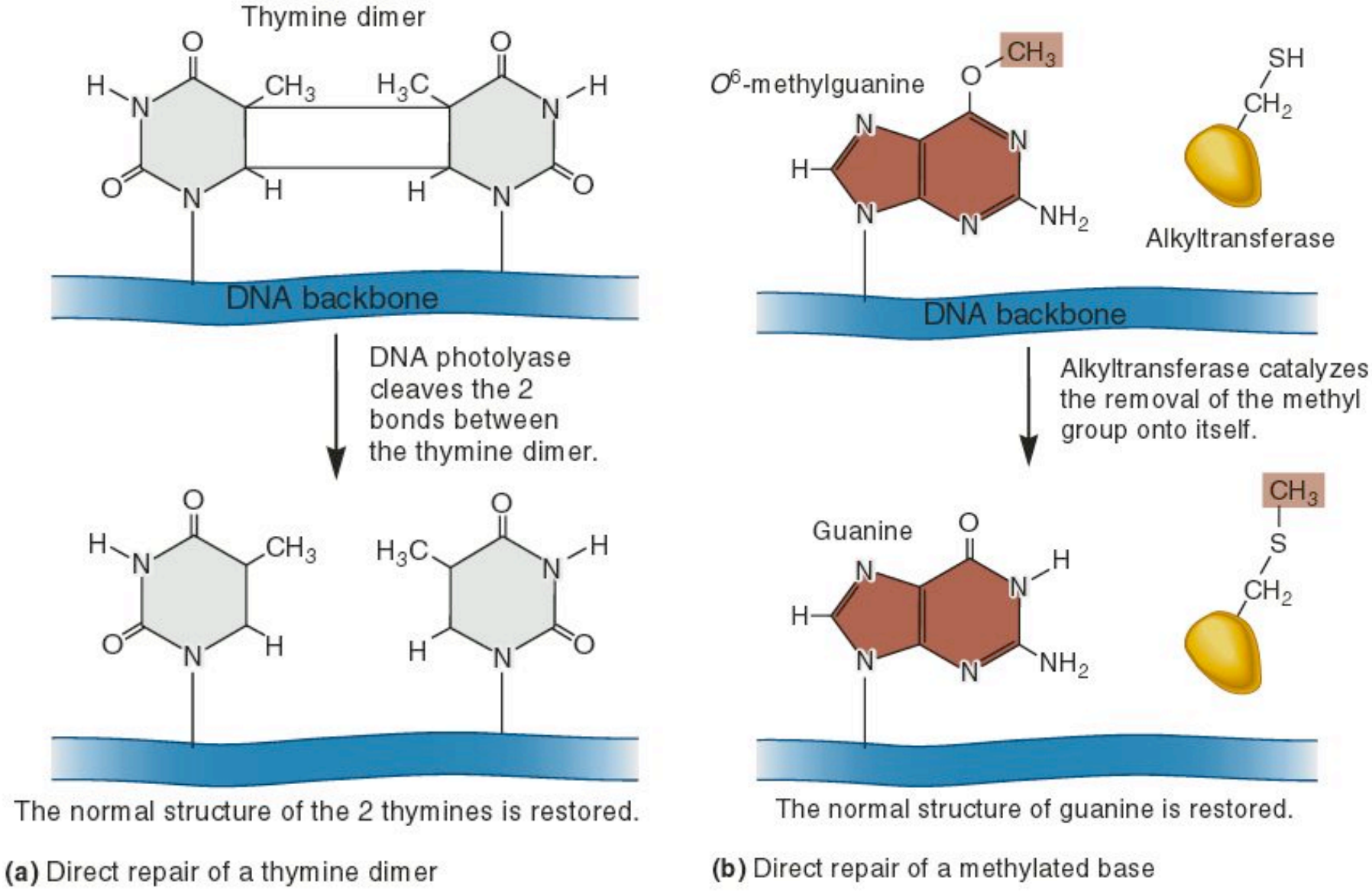
DNA Repair

- Since many mutations are deleterious, DNA repair systems are vital to the survival of all organisms
  - Living cells contain several DNA repair systems that can fix different type of DNA alterations
- DNA repair mechanisms fall into 2 categories
  - Repair of damaged bases
  - Repair of incorrectly basepaired bases during replication
- In most cases, DNA repair is a multi-step process
  - 1. An irregularity in DNA structure is detected
  - 2. The abnormal DNA is removed
  - 3. Normal DNA is synthesized

Damaged Bases Can Be Directly Repaired

- Called DIRECT REPAIR
- In a few cases, the covalent modifications of nucleotides can be reversed by specific enzymes
  - Photolyase can repair thymine dimers induced by UV light
    - It splits the dimers restoring the DNA to its original condition
  - O^6-alkylguanine alkyltransferase repairs alkylated bases
    - It transfers the methyl or ethyl group from the base to a cysteine side chain within the alkyltransferase protein

Direct repair of damaged bases in DNA



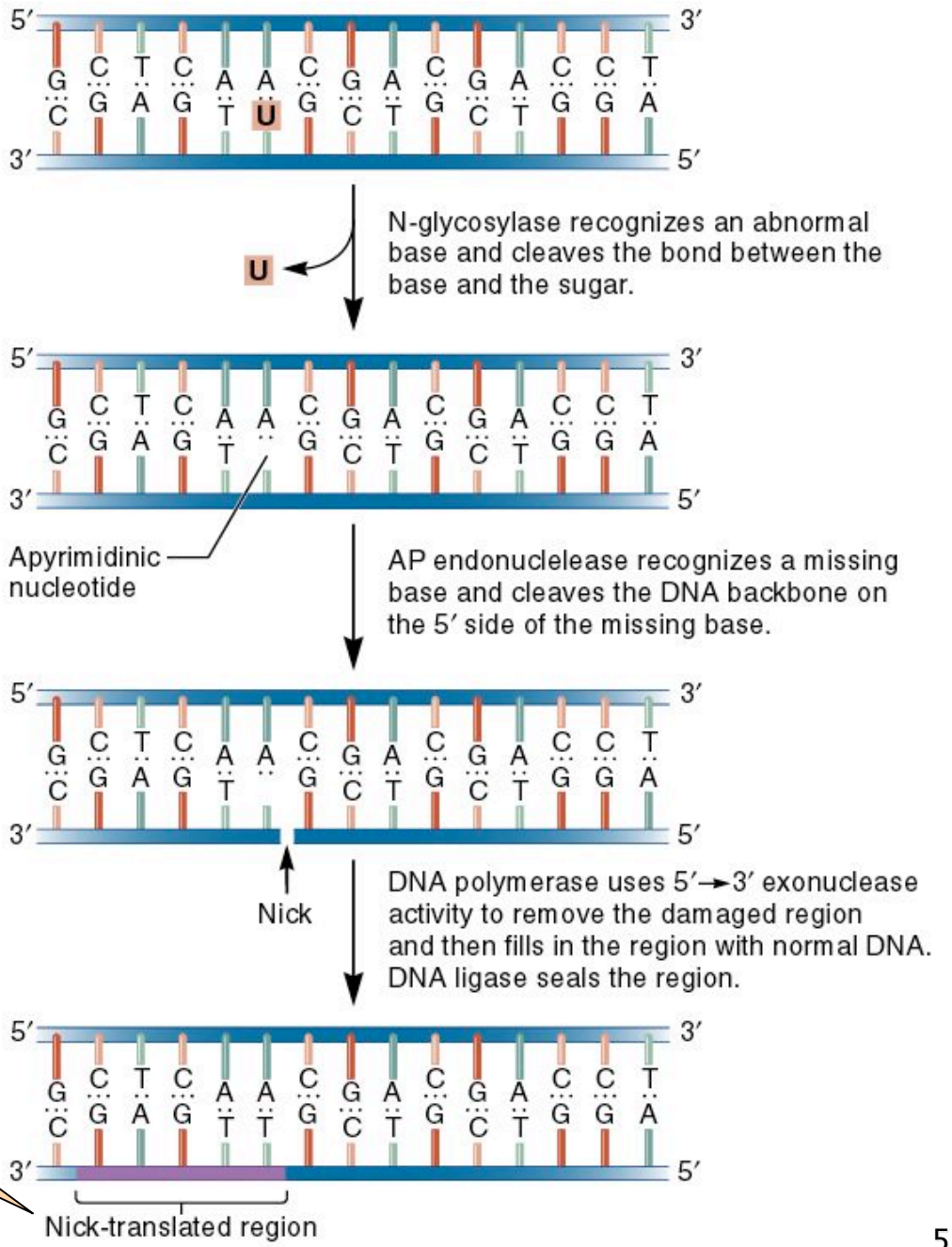
Base Excision Repair System

- Base excision repair (BER) involves a category of enzymes known as DNA-N-glycosylases
  - These enzymes can recognize a single damaged base and cleave the bond between it and the sugar in the DNA
  - Removes one base, excises several around it, and replaces with several new bases using Pol adding to 3' ends then ligase attaching to 5' end

- Depending on the species, this repair system can eliminate abnormal bases such as
  - Uracil; Thymine dimers
  - 3-methyladenine; 7-methylguanine

Base Excision Repair System

Depending on whether a purine or pyrimidine is removed, this creates an apurinic and an apyrimidinic site, respectively



Nick replication would be a more accurate term

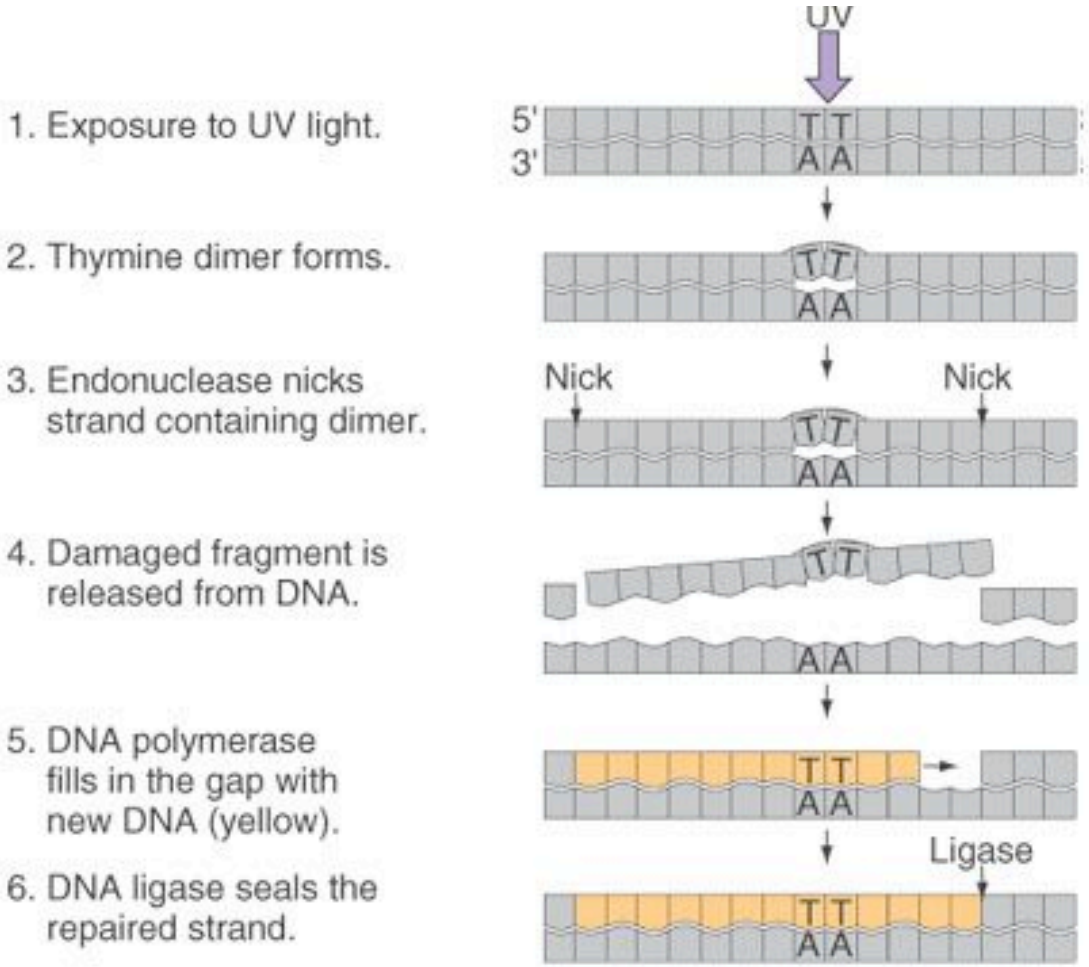
Nucleotide Excision Repair System

- An important general process for DNA repair is nucleotide excision repair (NER)
  - Nicks DNA around damaged base and removes region
  - Then fills in with Pol on 3'ends, and attaches 5' end with ligase

- This type of system can repair many types of DNA damage, including
  - Thymine dimers and chemically modified bases

- NER is found in all eukaryotes and prokaryotes
  - However, its molecular mechanism is better understood in prokaryotes

DNA REPAIR of damaged base:
Nucleotide Excision Repair fixes errors created by mutagens

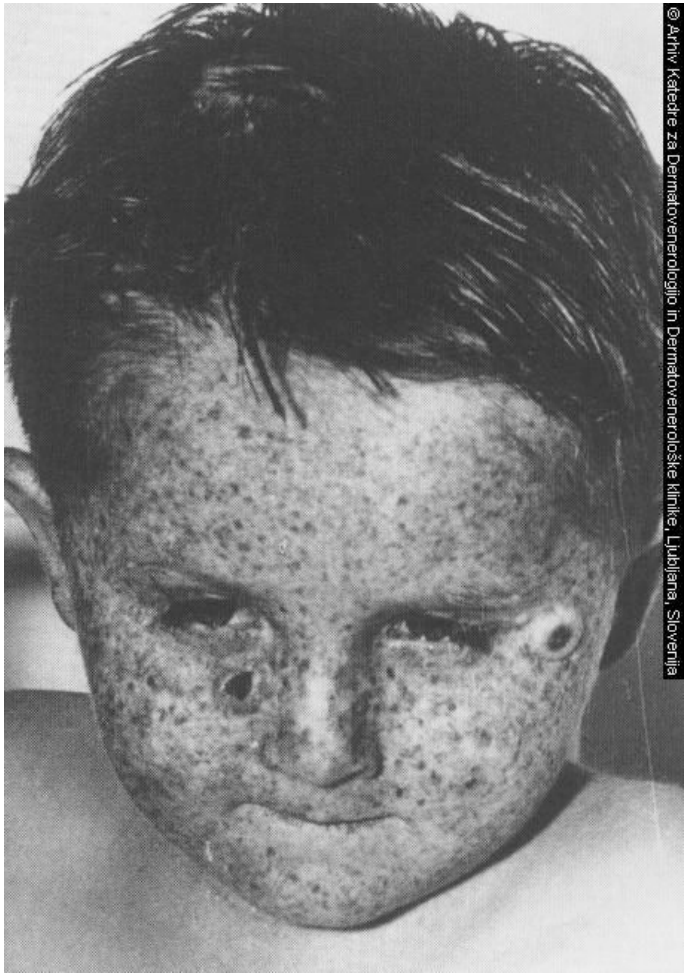


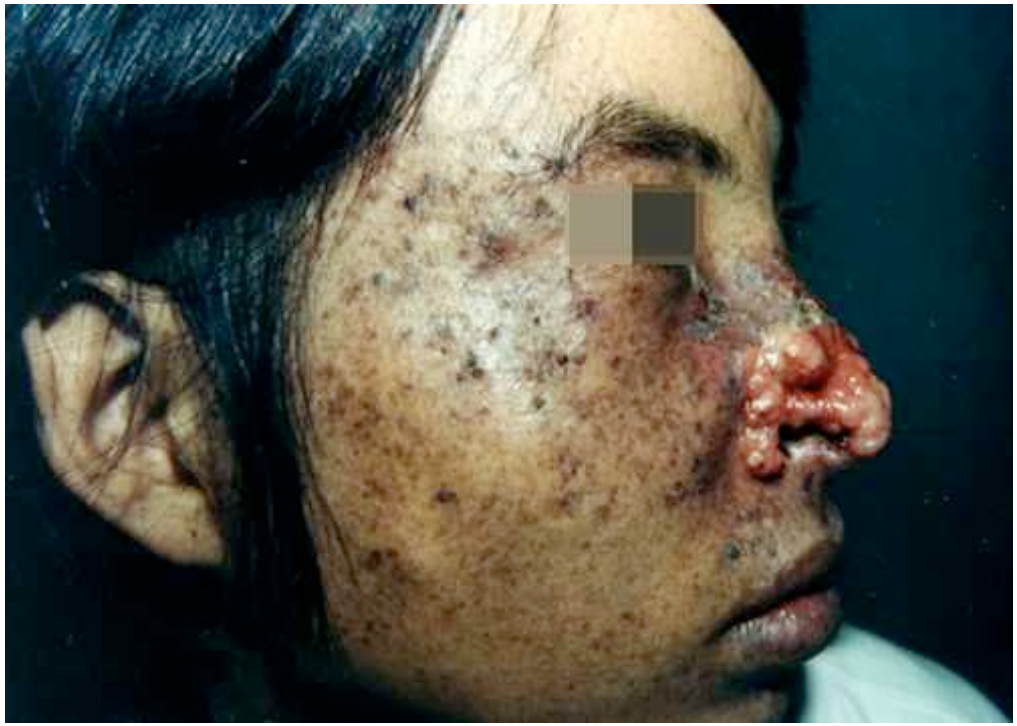
- Excision repair enzymes release damaged regions of DNA.
- Single strand released
- Repair is then completed by DNA polymerase and DNA ligase

Nucleotide Excision Repair Removes Damaged DNA Segments

- Several human diseases have been shown to involve inherited defects in genes involved in NER
  - These include xeroderma pigmentosum (XP) and Cockayne syndrome (CS)
    - A common characteristic of both syndromes is an increased sensitivity to sunlight
  - Xeroderma pigmentosum can be caused by defects in seven different NER genes

Skin lesions of Xeroderma Pigmentosum

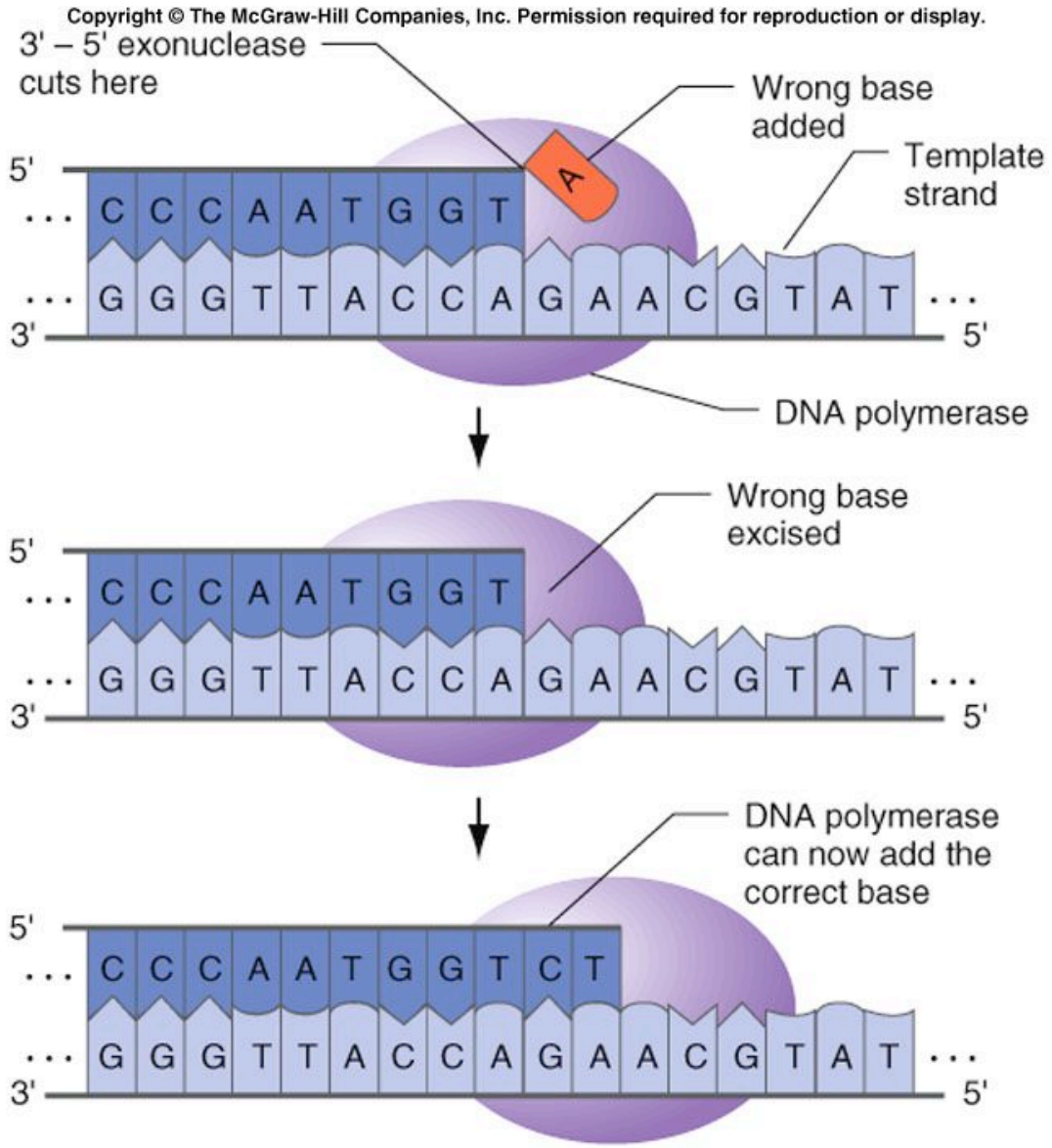




Mistakes during replication alter genetic information

- Errors during replication are exceedingly rare, less than once in 10^9 base pairs
- Proofreading enzymes correct errors made during replication
  - DNA polymerase has 3′ – 5′ exonuclease activity which recognizes mismatched bases and excises them
  - If errors slip through proofreading:
    - In bacteria, methyl-directed mismatch repair finds these errors on newly synthesized strands and corrects them
    - In euks, mismatch repair finds these errors on newly synthesized strands and corrects them

DNA polymerase proofreading

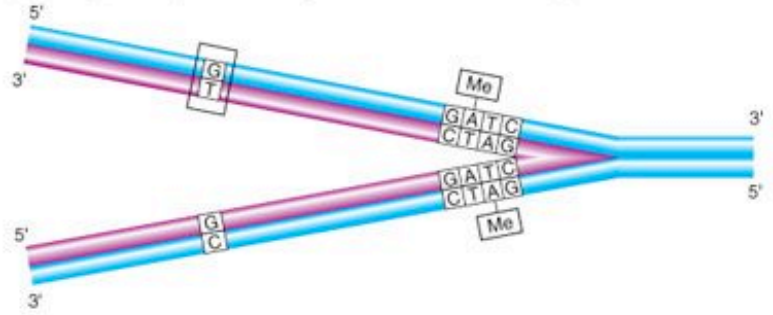


Mismatch Repair System

- If proofreading fails, the methyl-directed mismatch repair system comes to the rescue
- This repair system is found in all species
- In humans, mutations in the system are associated with particular types of cancer
- Methyl-directed mismatch repair recognizes mismatched base pairs, excises the incorrect bases, and then carries out repair synthesis.

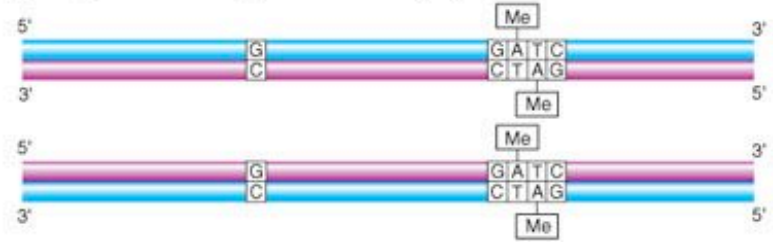
(a) Parental strands are marked with methyl groups.

(b) Enzyme system recognizes mismatch in replicated DNA.



(c) DNA on unmarked new strand is excised.

(d) Repair and methylation of newly synthesized DNA strand.



Methyl-directed mismatch repair in Prokaryotes

Mismatch Repair in Eukaryotes

- Eukaryotes also have mismatch repair, but it is not clear how old and new DNA strands are identified.
  - Four genes are involved in humans, *hMSH2* and *hMLH1, hPMS1,* and *hPMS2*
  - All of these are mutator genes
  - mutation in any one of them confers hereditary predisposition to hereditary nonpolyposis colon cancer (HNPCC: OMIM 120435).

Transpositions

- Cytologically invisible sequence rearrangement:
  - movement of a segment of DNA from one location to another in the genome.
  - Not a translocation….
- This may be a transfer of DNA or a duplication of DNA.
- The sequences that cause transpostions
  - are called transposable elements,
  - have specific characteristics,
  - notably the potential to propagate themselves.
- Transposable elements are found in virtually all organisms.

Types of Transposable Elements

- Transposons:
  - Move their DNA directly without the requirement of an RNA intermediate.

- Retroposons:
  - Copy and then move the copied DNA
  - via reverse transcription of an RNA intermediate.

Transposons

- Encode an enzyme called Transposase.
- Rather than converting RNA to DNA, this enzyme:
  - directly removes the DNA sequence and
  - inserts it in another location.
- Transposons usually have inverted repeats (IR) on either side upstream and downstream.

Transposons encode transposase enzymes that catalyze events of transposition

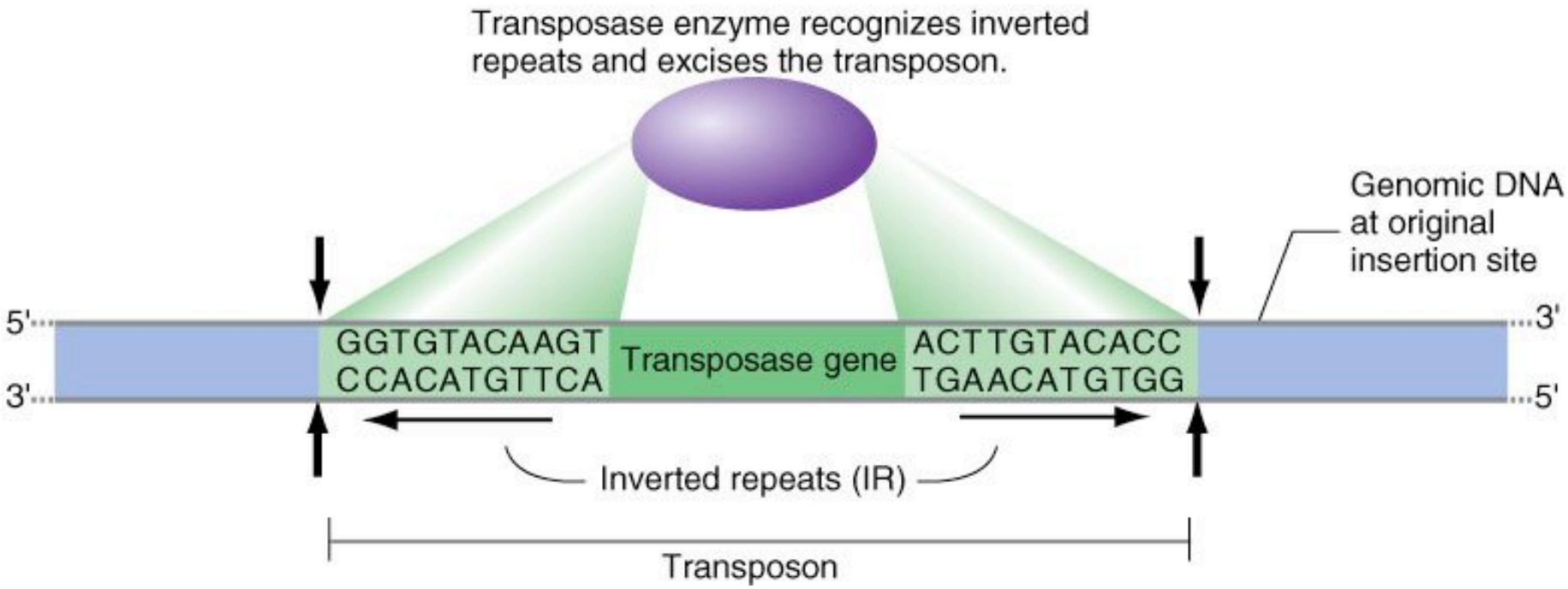


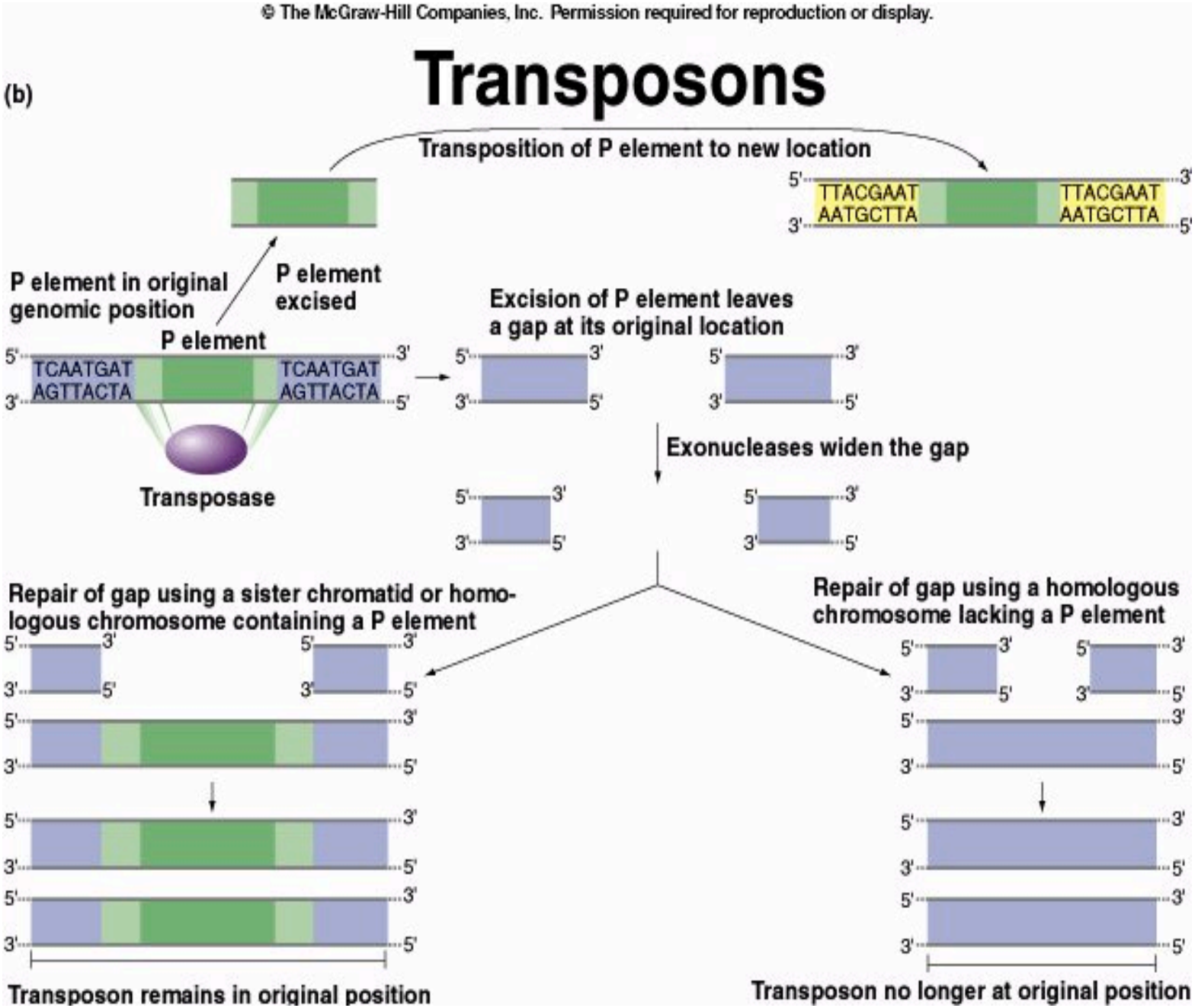
Fig. 13.24 a

Transposons

- Transposase excises the sequence between the inverted repeats and inserts it into another region of the genome

- The gap created is widened by exonucleases.

- The gap is filled in by repair enzymes that use the sister chromatid or homologous chromosome as a template to fill the gap.
  - If copying from a sister chromatid (also containing the transposon) it will reappear in the original location
    - And hence be copied in the genome
  - If the homologous chromosome is used to replace missing transposon (did not have transposon) it will not be replaced
    - And hence not be copied in the genome



Retroposons

- The DNA sequence in a retroposon codes for a reverse transcriptase,
  - which catalyzes the formation of DNA from an RNA template.
- They always copy DNA and cause a duplication
- Many retroposons also have other polypeptide coding sequences.
- Many retroposons have a poly A tail.
- Others have direct repeat sequences on either side,
  - these are generated because of the way the DNA sequence has been inserted.

Retroposons: The process of LTR transposition

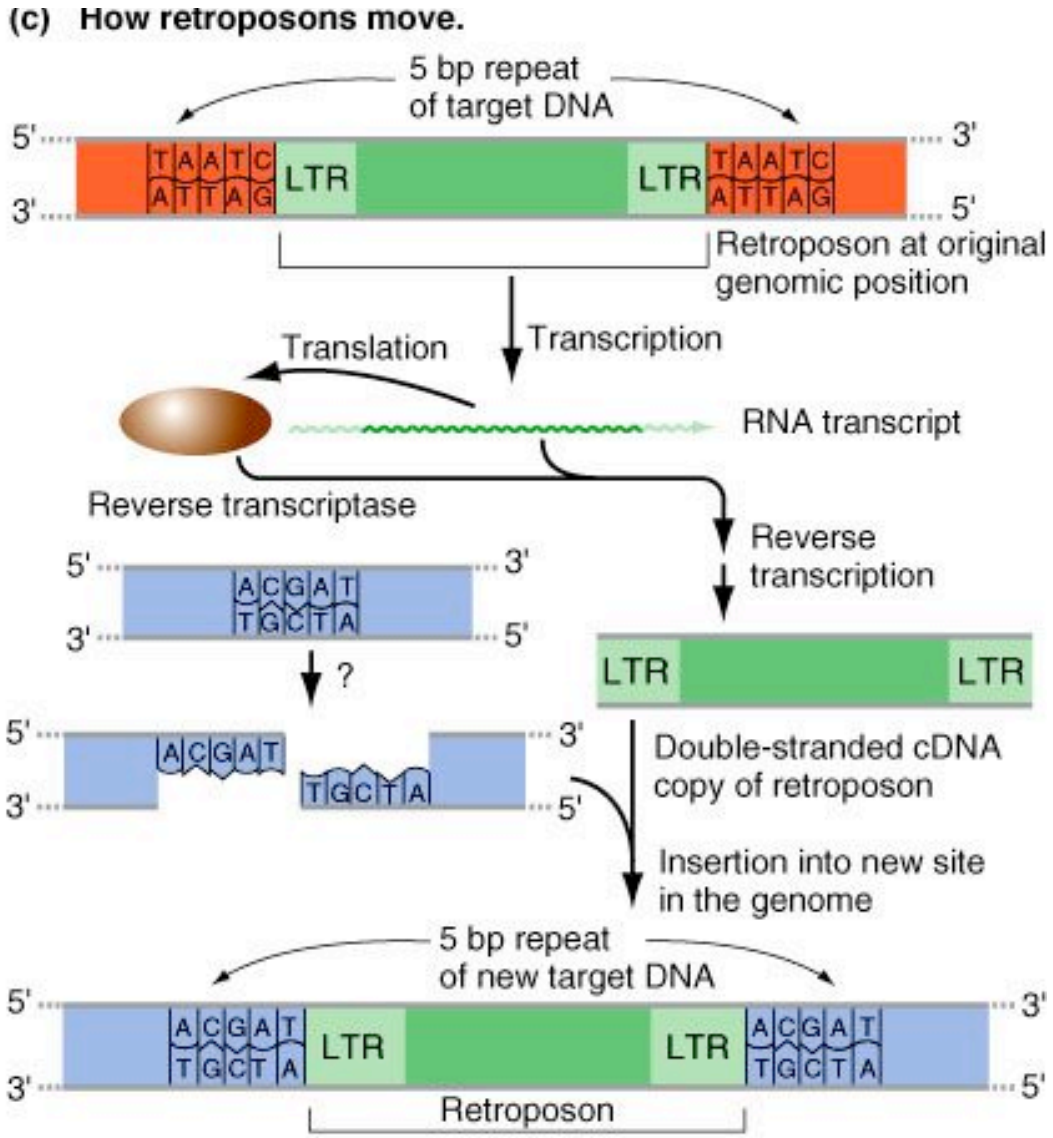
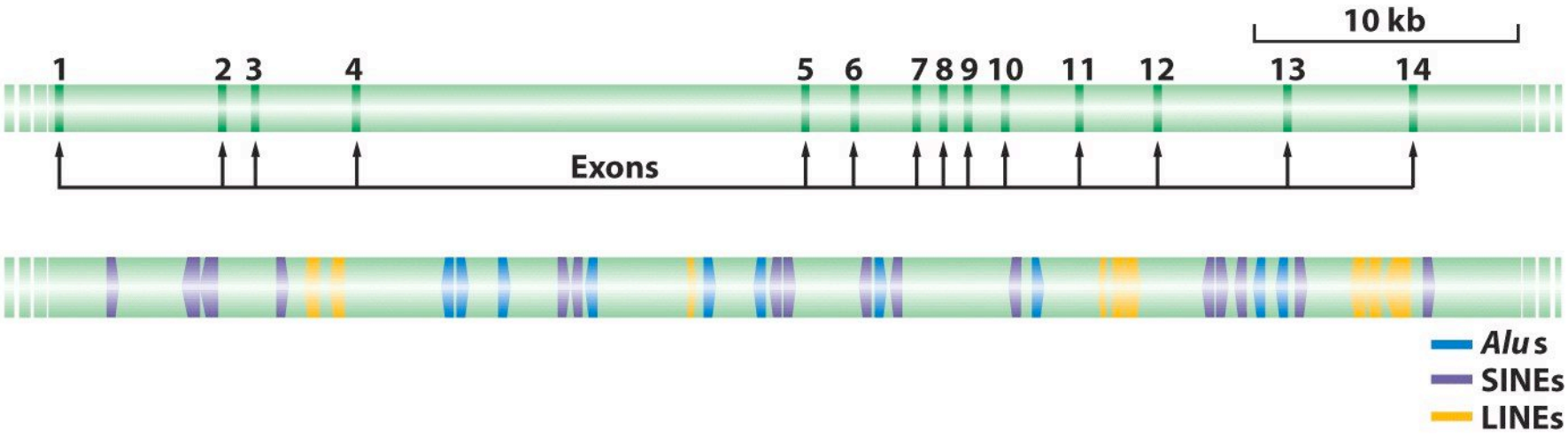


Fig. 13.23

Example of Transposable Elements Found in the Human Chromosome



Types of transposable elements in the human genome

Element	Transposition	Structure	Length	Copy number	Fraction of genome
LINEs	Autonomous	ORF1 ORF2 (*pol*) AAA	1–5 kb	20,000–40,000	21%
SINEs	Nonautonomous	AAA	100–300 bp	1,500,000	13%
DNA transposons	Autonomous	← transposase →	2–3 kb	300,000	3%
	Nonautonomous	← →	80–3000 bp		

Transposable elements move around the genome and are not susceptible to excision or mismatch repair

Why?

They are not damaged bases and they are not mismatches

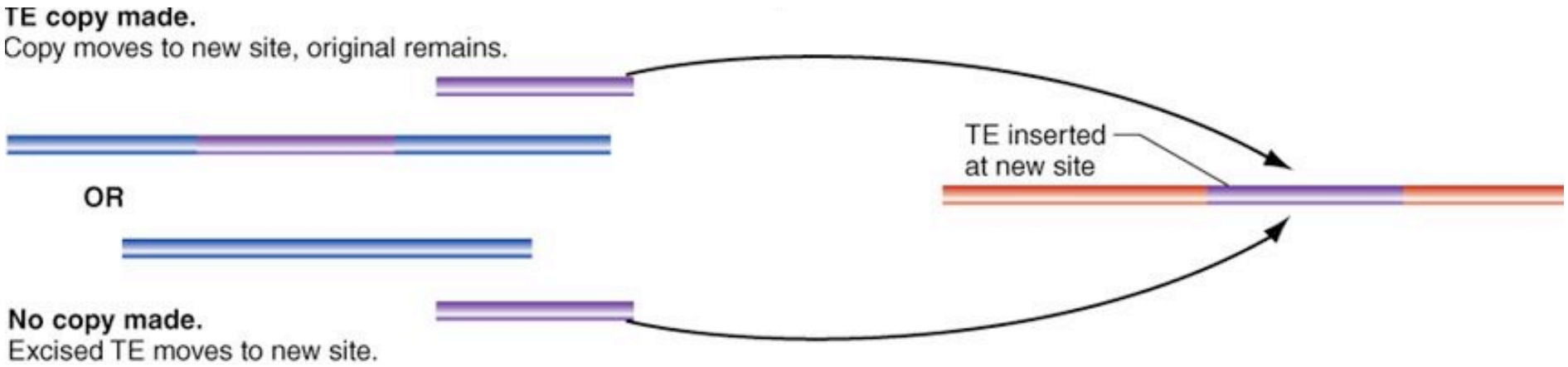


Fig. 7.10 e

Homework Problems

- Chapter 15

- # 15

- DON'T forget to take the online QUIZ
- DON'T forget to submit the online iActivity
  - "Overview B"