Lecture 25: DNA mutation, proofreading, and repair

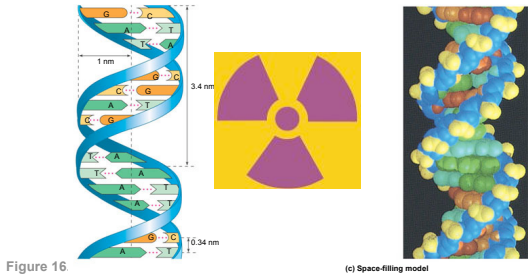


Figure 16.

(c) Space-filling model

Lecture Outline 11/2/05

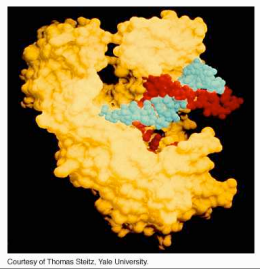
- Review DNA replication machine
- Fidelity of replication and proofreading
- Replicating the ends of chromosomes
- Mutation
  - Types of mutations
  - Repair mechanisms

Replication overview

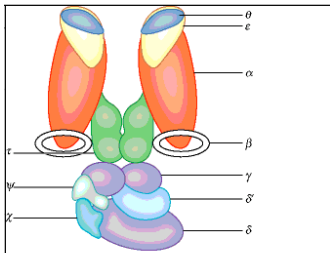
- Look at animations on your textbook CD
- Look again at the animation from DNAi
  - http://www.dnai.org
  - (go to the section on copying the code)

DNA Polymerase III

- A complex enzyme with many subunits
- one part adds the nucleotides
- another helps it slide along the template
- another checks for mis-pairing



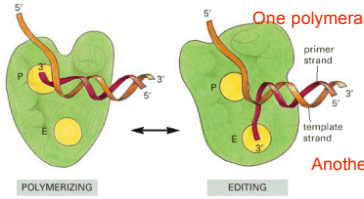
Courtesy of Thomas Steitz, Yale University



Figs. from http://www.mun.ca/biochem/courses/3107

Proofreading

- Even though bases preferentially pair G-C and A-T, the initial error rate is about 1 in 10,000.
- Many polymerases have “proofreading” ability. They can excise an mis-paired base and try again.
- This reduces the error rate to about 1 in a billion.



One polymerase subunit adds nucleotides

Another “edits” out incorrect bases

Fidelity of replication

Replication step	error rate
5′ →3′ polymerization	1×10^5
3′ →5′ proofreading	1×10^2
Strand-directed mismatch repair	1×10^2
Total error rate	1×10^9

What happens to the lagging strand at the end of the chromosome?

Leaves a gap when the RNA primer is removed



The ends of eukaryotic chromosomal DNA get shorter with each round of replication

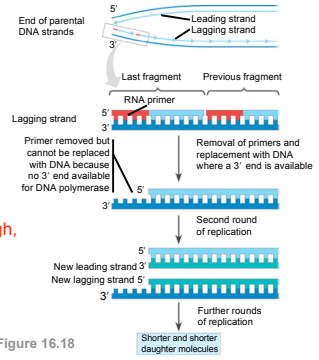
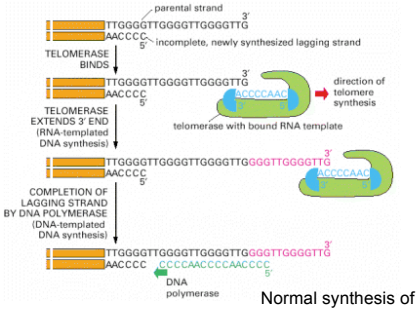


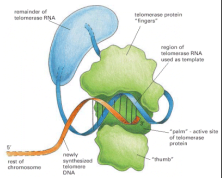
Figure 16.18

If they get short enough, essential genes will eventually be deleted

Telomerase



Normal synthesis of new DNA



Carries its own RNA template

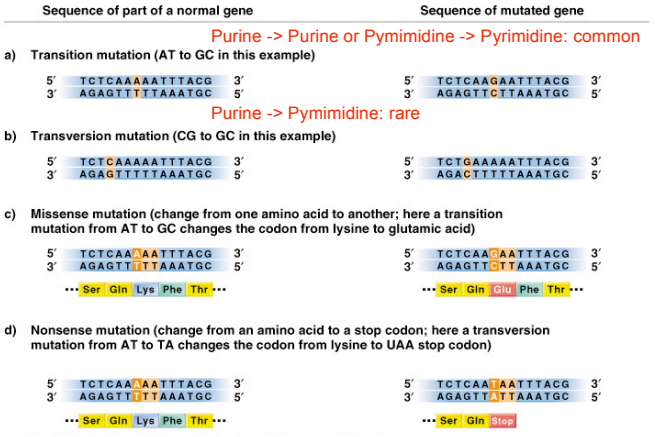
Extends the old (template) strand

What happens to the lagging strand that the end of the chromosome?

- Telomeres contain hundreds of simple tandem repeats.
  - In humans, the repeat sequence is TTAGGG
    TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG . . . . . .
- Cell lines with active telomerase live longer than those without telomerase.
  - That may be important in allowing cancer cells to continue to divide.

Mutations and repair

Various kinds of mutations:



Types of base pair substitutions and mutations.

	Sequence of part of a normal gene	Sequence of mutated gene
e) Neutral mutation (change from an amino acid to another amino acid with similar chemical properties; here an AT to GC transition mutation changes the codon from lysine to arginine)	5′ TCTCAAAATTTACG 3′ 3′ AGAGTTTTTAAATGC 5′ ···Ser Gln Lys Phe Thr···	5′ TCTCAAAGATTTACG 3′ 3′ AGAGTTTCTAAATGC 5′ ···Ser Gln Arg Phe Thr···
f) Silent mutation (change in codon such that the same amino acid is specified; here an AT-to-GC transition in the third position of the codon gives a codon that still encodes lysine)	5′ TCTCAAAAATTTACG 3′ 3′ AGAGTTTTTTAAATGC 5′ ···Ser Gln Lys Phe Thr···	5′ TCTCAAAAGTTTACG 3′ 3′ AGAGTTTTCAAATGC 5′ ···Ser Gln Lys Phe Thr···
g) Frameshift mutation (addition or deletion of one or a few base pairs leads to a change in reading frame; here the insertion of a GC base pair scrambles the message after glutamine)	5′ TCTCAAAAATTTACG 3′ 3′ AGAGTTTTTAAATGC 5′ ···Ser Gln Lys Phe Thr···	5′ TCTCAAGAAATTTACG 3′ 3′ AGAGTTCTTTAAATGC 5′ ···Ser Gln Glu Ile Tyr···

Mutations can be caused by:

- Chemical mutagens
- Ionizing radiation
- Slippage during DNA replication
- Spontaneous errors

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Chemical changes in one of the nucleotide bases

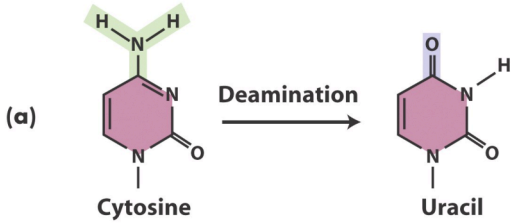
After replication, new strand has an A

Deamination changes C to U

--C--- → --U--- → --U---
--G--- --G--- --A---

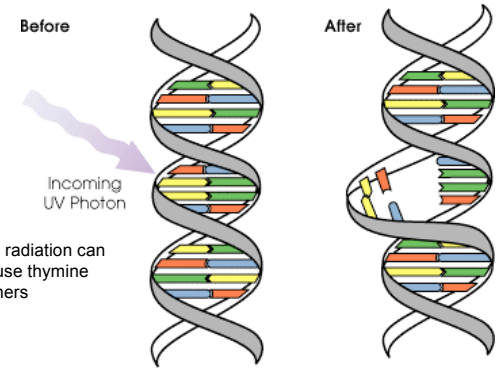
--T---
--A---

--G---
--C---



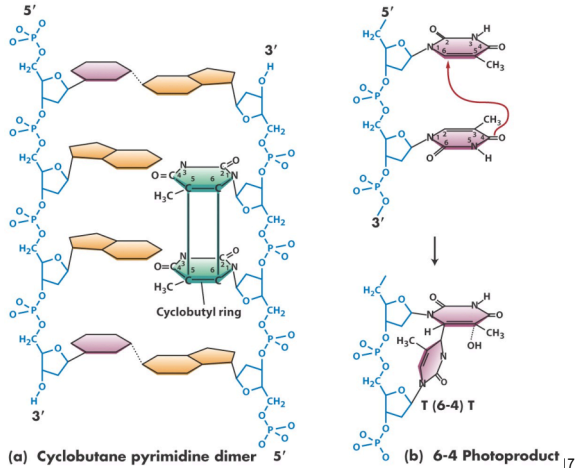
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UV damage (e.g. pyrimidine dimers)



UV radiation can cause thymine dimers

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- In nucleotide excision repair
  - Enzymes cut out and replace damaged stretches of DNA

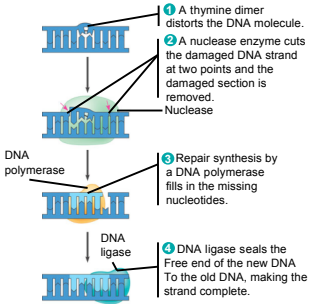


Figure 16.17

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Certain bacterial mutations cause increased mutation rates

Defect in:	Rif^r mutants per 10^8 cells
Wild-type (*mut*$^+$)	5-10
Pol III proofreading (mutD)	4000-5000
Mis-match repair *(mutS)*	760
Base excision repair *(mutY mutM)*	8200

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Mismatch repair

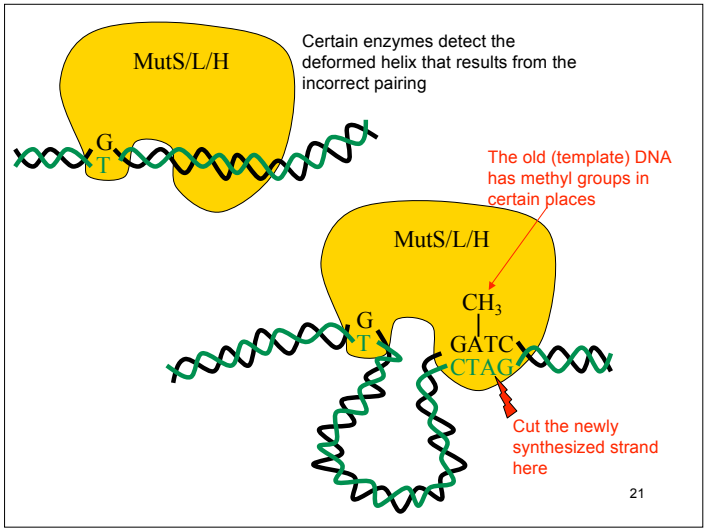
Here is a mis-paired base that must be repaired:

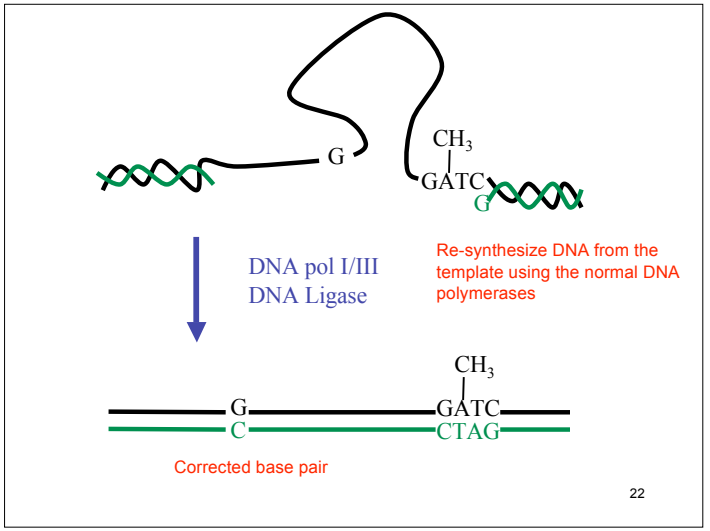


How is the mistake recognized?

How does the mismatch repair system know which strand is the new one and which strand is the old one?

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- Various similar mechanisms for other types of mutations

