

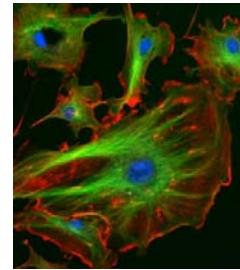
Lecture 7: From DNA Mutation & DNA Repair to Disease & Disorders

Section A: From Nature to Concepts

**Genes & Society
LSM '3201' -> '1302' / GEK 1527**

The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music.

- Lewis Thomas (Physician, Essayist)



Overview

- **DNA Mutation**
- **DNA Repair**
- **Mutation in DNA Repair Genes & Disorders**
- **Mutation in Cell Cycle Genes & Cancers**

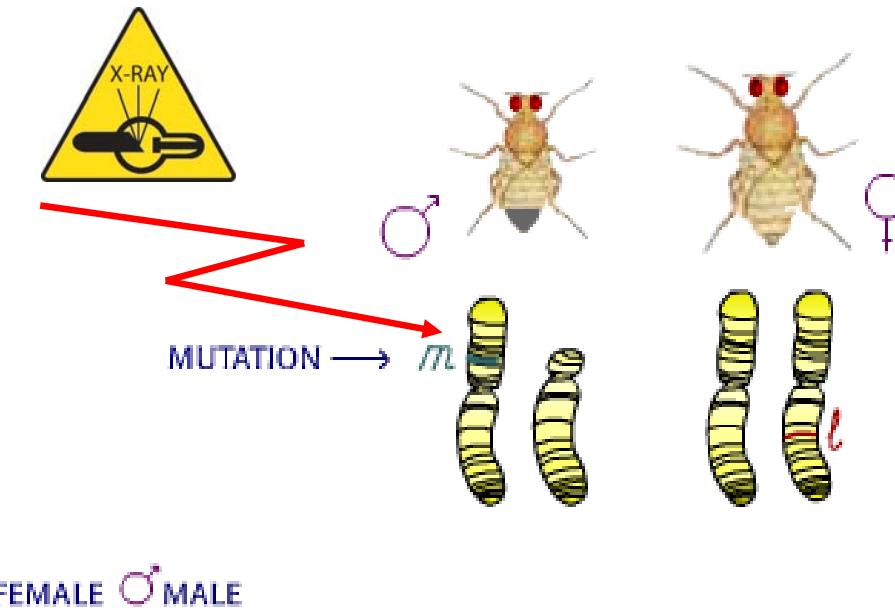


Mutated Human Beings Who Became Superheroes



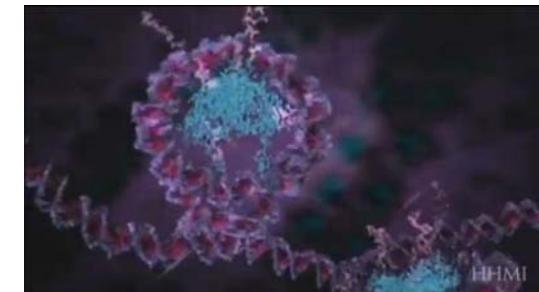
Muller and X-ray mutants

I designed the experiment to detect a mutation (*m*) induced by X-rays in the X chromosome, but mutations can occur anywhere.



In 1946 he was awarded nobel prize for his work on X-ray mutagenesis. He was the first to imagine the potential of genetic modification and proposed that radiation might be used to produce new agricultural varieties. He also campaigned for public awareness of the health risks of radiation especially to doctors administering X-rays.

Inner Life of the Cell:
<https://www.youtube.com/watch?v=wJyUtbn0O5Y>



Watch HHMI BioInteractive
“Damage to DNA leads to mutation”
<http://www.hhmi.org/biointeractive/damage-dna-leads-mutation>

DNA / Gene mutations: *Changes in the nucleotide sequence*

Examples of mutations made simple in three-letter word message:

**Wild Type
(normal)**

THE BIG FLY HAS ONE EYE

Substitution (Missense) THE PIG FLY HAS ONE EYE

THE BIG LYH ASO NEE YE (F)

**Insertion
(frame-shift)** THE BIN GFL YHA SON EEY E

**(Nonsense) THE BIG FLY (premature stop codon)
(can be a result of substitution/deletion/insertion)**

Examples of mutations seen in mRNA transcribed from mutated DNA (not shown):

Wild Type mRNA ...-GUU-GCU-GAU-AGC-...
Wild Type peptide ... -Val – Ala – Asp – Ser-...

Substitution (silent) ...-GUC-GCU-GAU-AGC-...
Wild Type peptide ... -Val – Ala – Asp – Ser-...

Substitution (missense) mutated peptide ...-**UUC**-GCU-GAU-AGC-... ... -**Phe** – Ala – Asp – Ser-...

**Deletion (frame-shift)
mutated peptide** ...-GUU-GCG-AUA-GCC...
... -Val - Ala - Ile - Ala-...

Insertion mutated peptide (frame-shift nonsense) ...-GUU-GGC-UGA-UAG-C...
... -Val – Gly – **STOP**...

A point mutation in the hemoglobin gene causes sickle cell anemia

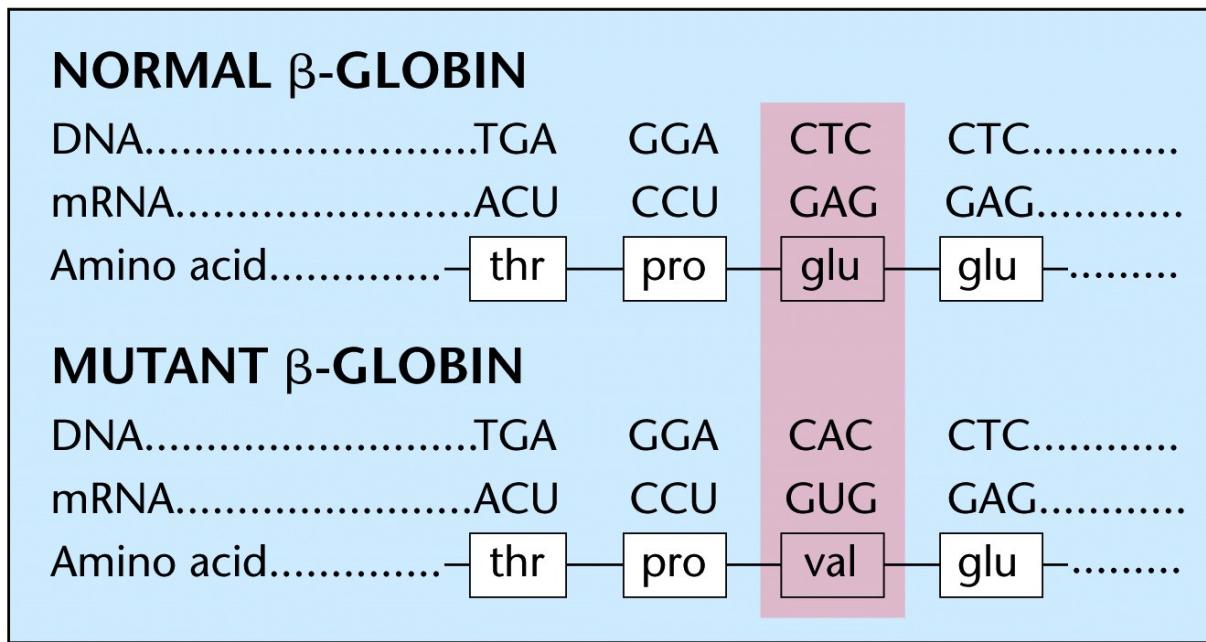
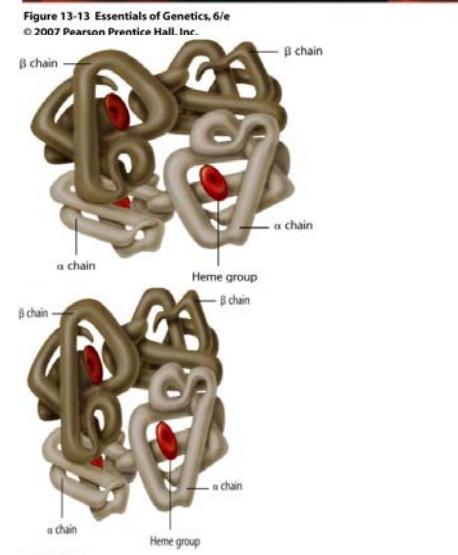
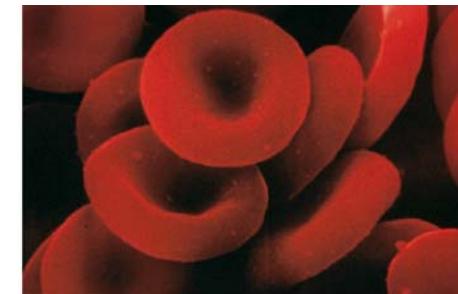


Figure 1-12 Essentials of Genetics, 6/e
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This subtle mutation alters the oxygen-transporting ability of hemoglobin and dramatically change the shape of the red blood cells to an abnormal sickled-shape.

Watch Sickle Cell Anemia on IVLE animation



Sickle cell anemia

Mutation in the hemoglobin-beta gene on chromosome 11

Recessive trait (need 2 faulty alleles to be sick)

Sickle cell heterozygotes (have only one mutated allele – i.e. are not sick from the disease) have some protection from malaria

African american population in USA – 1 in 12 individuals is heterozygous for this mutation



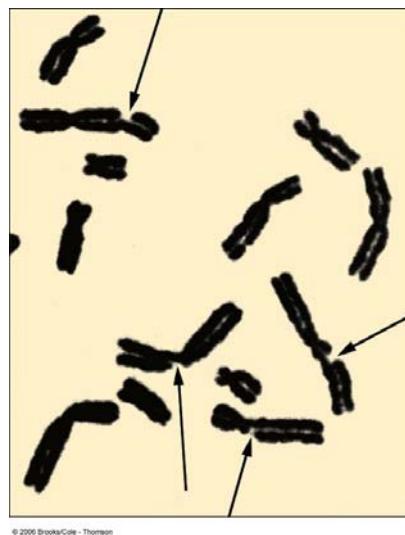
Watch The Making of the Fittest: Natural Selection Humans
<https://www.youtube.com/watch?v=Zsbhvl2nVNE&feature=youtu.be>

Additional Reading Material: *Blood, Genes and Malaria*

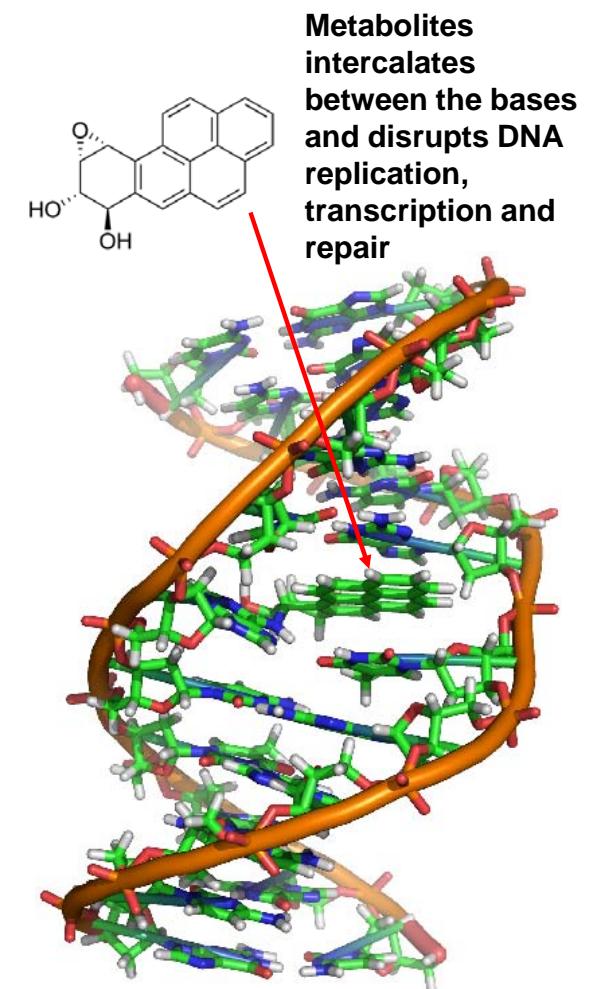
What if Something Goes Wrong?

DNA Proofreading, Damage and Repair

- RNA, protein, lipid, carbohydrate can be replaced, but the integrity of DNA sequence must be preserved.
- Cells require a means for repair of missing, altered or incorrect bases (analog), bulges due to insertion or deletion, UV-induced pyrimidine dimers, strand breaks or cross-links (by chemical carcinogens eg. Aflatoxin, BAP).



DNA repair enzymes repairing DNA damages

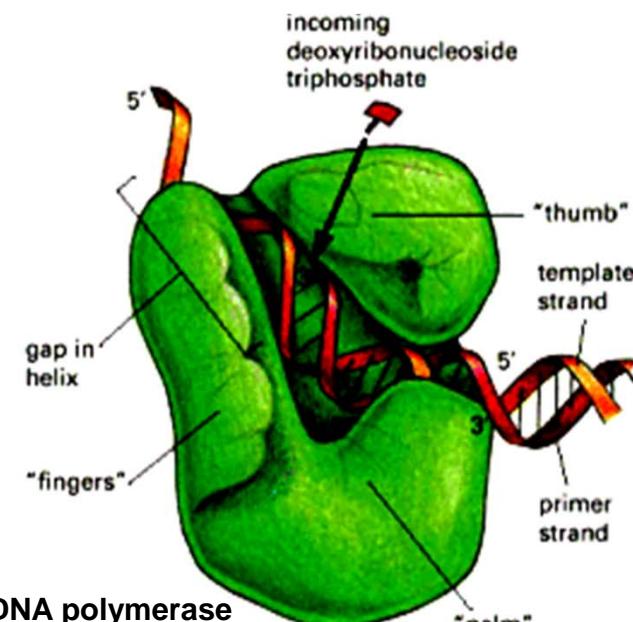
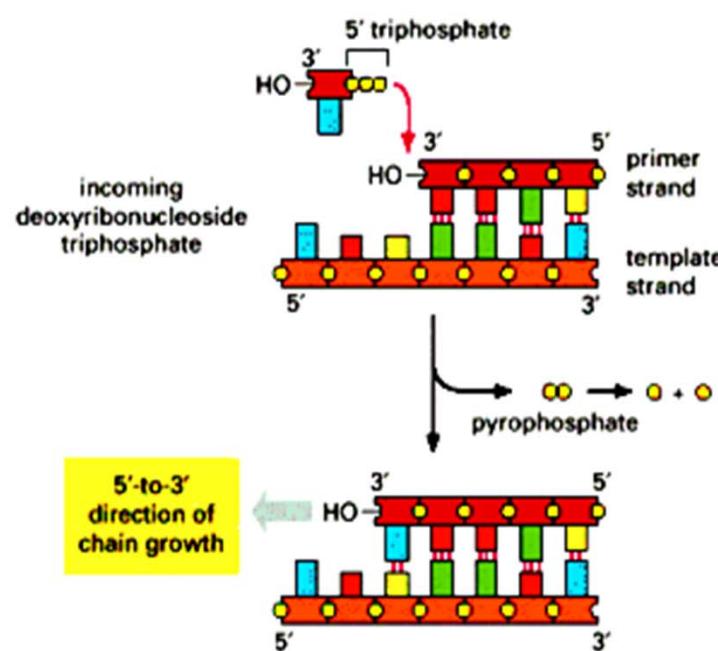


Benzopyrene, the major mutagen in tobacco smoke, intercalates between DNA bases.

Steps to Ensure High Fidelity DNA Replication

High-Fidelity Replication System Before Covalent-bonding:

- Correct base-pairing: correct nucleotide has a higher affinity for the moving polymerase than does the incorrect nucleotide.
- Polymerase undergoes conformational change to "double-check" the exact base-pair geometry before it catalyzes covalent bonding of the nucleotide. An incorrectly bound nucleotide is more likely to dissociate.

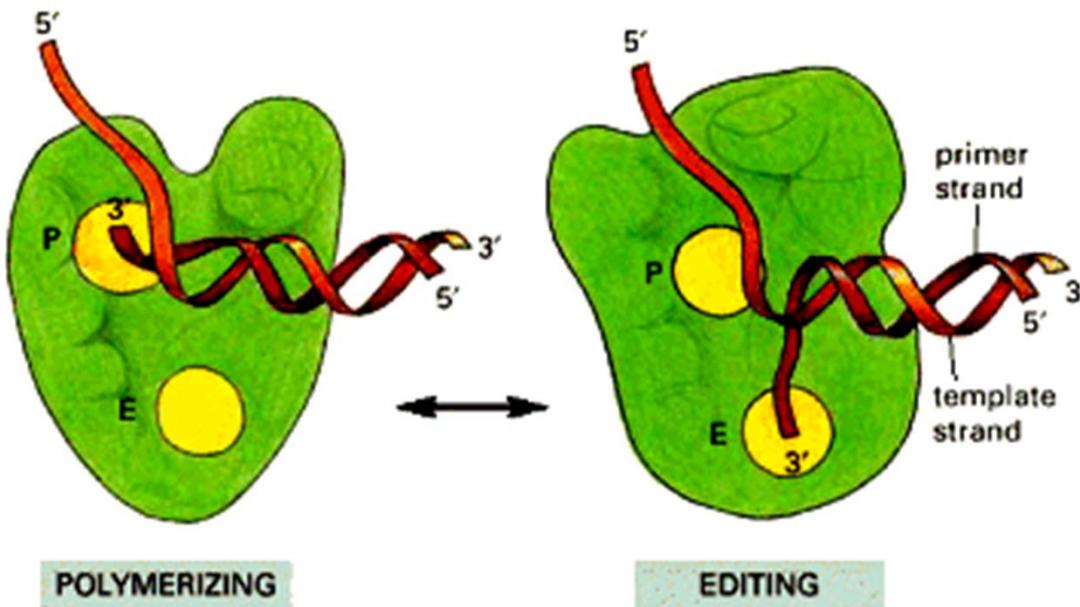


© 2002 Molecular Biology of the Cell 4ed.

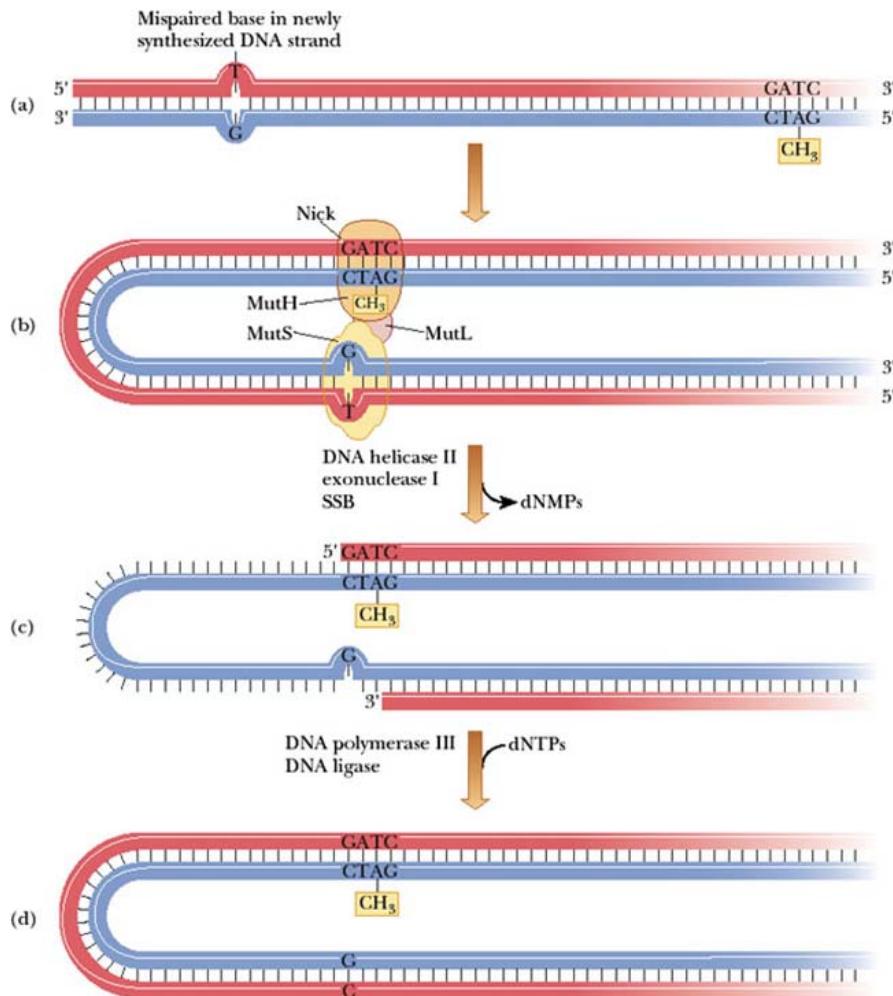
Steps to Ensure High Fidelity DNA Replication

Repair Systems After Covalent-bonding:

- *3'-to-5' proofreading exonuclease* clips off any improperly-paired nucleotide at the 3-OH end of the primer strand by using the 'Editing' (E) domain to cut the phosphodiester bond it has just made in the 'Polymerizing' (P) domain.
- (Strand-directed) Mismatch Repair system
- (Base- or Nucleotide-) Excision Repair system



Mismatch repair in *E. coli*

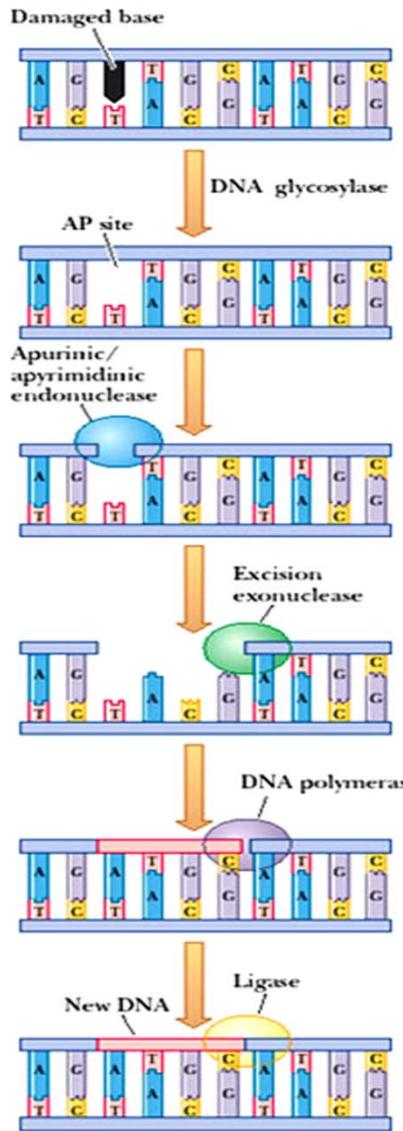


Newly synthesized DNA (red) has a mismatch (G-T). Parental strand (blue) is methylated.

MutH, MutS and MutL linked mismatch to nearest methylation site identifying parental (correct) strand (blue).

DNA helicase helps unwind the DNA and Exonuclease I removes the DNA region containing error between the proteins.

DNA polymerase fills the gap with dNTPs using methylated parental strand as template and DNA ligase seals the phosphodiester backbone.



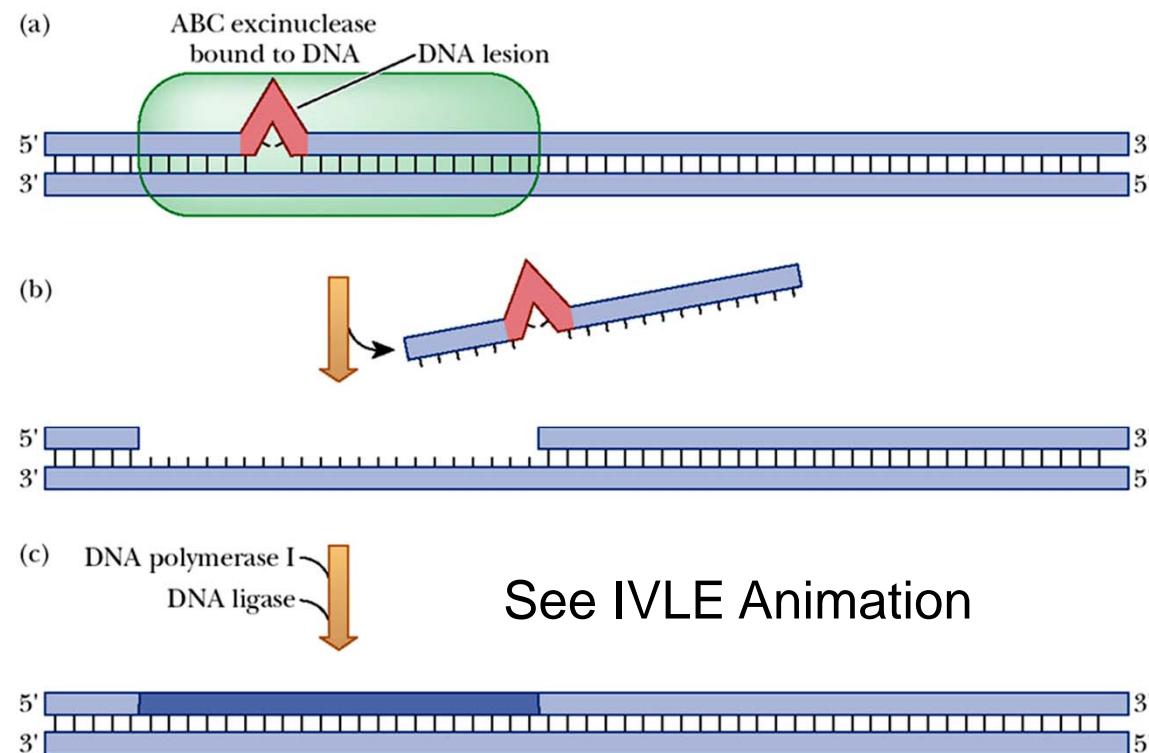
Base-excision Repair

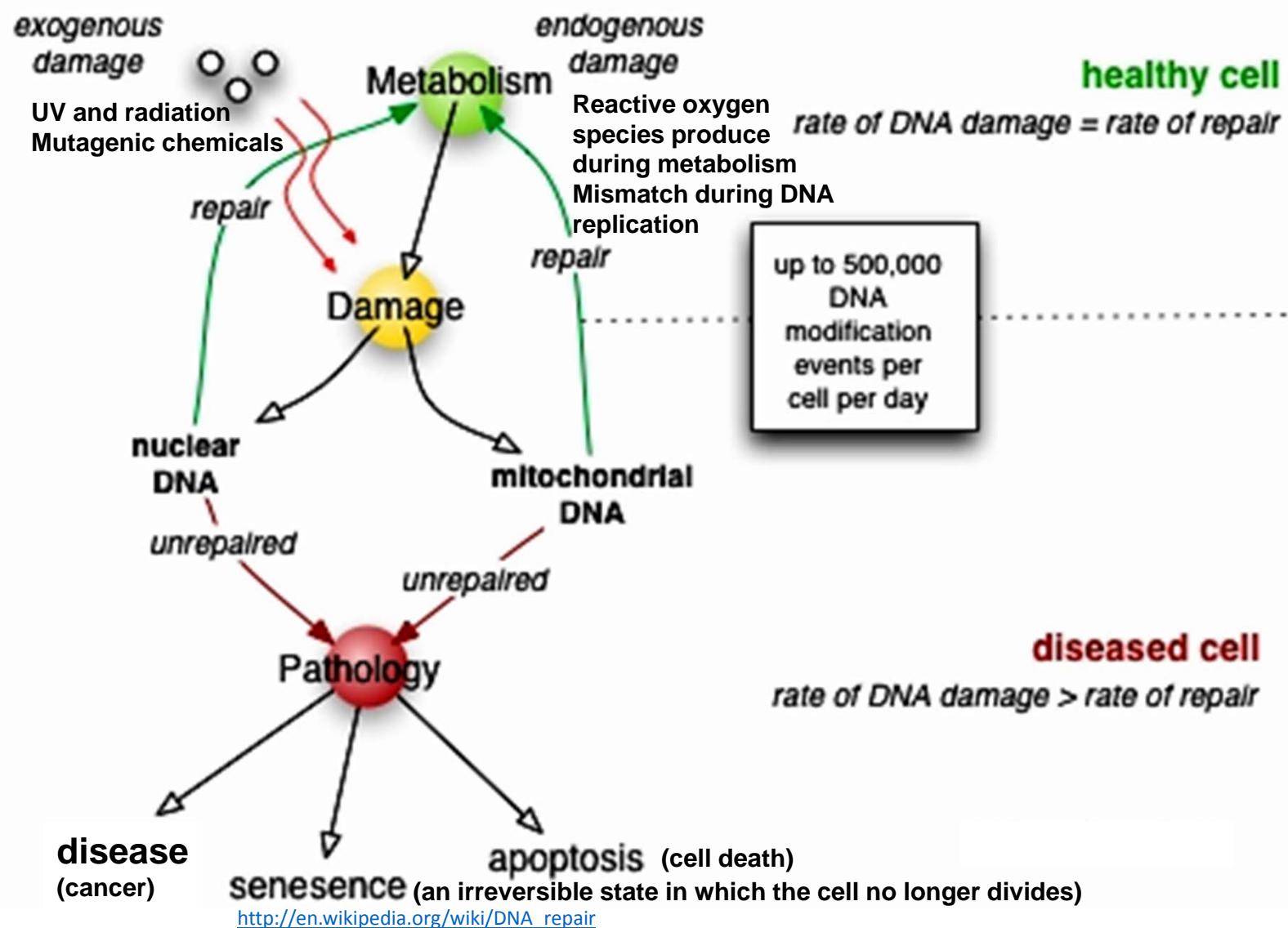
A damaged base is excised from the sugar-phosphate backbone by DNA glycosylase (cleaves glycosidic bonds), creating an apurinic/apyrimidinic (AP) site. Then, an AP endonuclease severs the DNA strand, and an excision exonuclease removes the AP site and several nucleotides. DNA polymerase and DNA ligase then repair the gap.

[Watch IVLE Animation](#)

Nucleotide-excision repair

When a serious lesion, such as a pyrimidine dimer, is detected, ABC excinuclease binds to the region and cuts out a large piece of DNA, including the lesion. DNA polymerase and DNA ligase then resynthesize and seal the DNA.





Inherited Syndromes with Defects in DNA Repair

NAME	PHENOTYPE	ENZYME OR PROCESS AFFECTED
MSH2, 3, 6, MLH1, PMS2	colon cancer	mismatch repair
Xeroderma pigmentosum (XP) groups A-G	skin cancer, cellular UV sensitivity, neurological abnormalities	nucleotide excision-repair
XP variant	cellular UV sensitivity	translesion synthesis by DNA polymerase δ
Ataxia-telangiectasia (AT)	leukemia, lymphoma, cellular γ-ray sensitivity, genome instability	ATM protein, a protein kinase activated by double-strand breaks
BRCA-2	breast and ovarian cancer	repair by homologous recombination
Werner syndrome	premature aging, cancer at several sites, genome instability	accessory 3'-exonuclease and DNA helicase
Bloom syndrome	cancer at several sites, stunted growth, genome instability	accessory DNA helicase for replication
Fanconi anemia groups A-G	congenital abnormalities, leukemia, genome DNA interstrand cross-link repair instability	
46 BR patient	hypersensitivity to DNA-damaging agents, DNA ligase I genome instability	

Xeroderma Pigmatosum caused damage in nucleotide excision repair



Xeroderma Pigmentosum, or XP, is a rare inherited disease affecting both males and females of every ethnic group, with an incidence of 1:250,000. XP is roughly six times more common in Japanese people than in other groups. It forbids any exposure to sunlight or any form of UV light, no matter how small. XP patients are trapped in darkness for life. Undiagnosed and untreated, XP can lead to the early onset of skin cancer and blindness. XP is an autosomal recessive disease caused by mutations in genes that are critical for DNA repair.

Freckles: varying in color and size, appear on the sun exposed areas first on the face and hands and later on other exposed parts, the neck and the lower legs, the lips and the conjunctiva where in severe cases the trunk is affected. The entire face mainly around nose and eyes shows pigmented spots of various tints of brown.

Breast and Ovarian Tumors (damaged to BRCA2 repair protein)



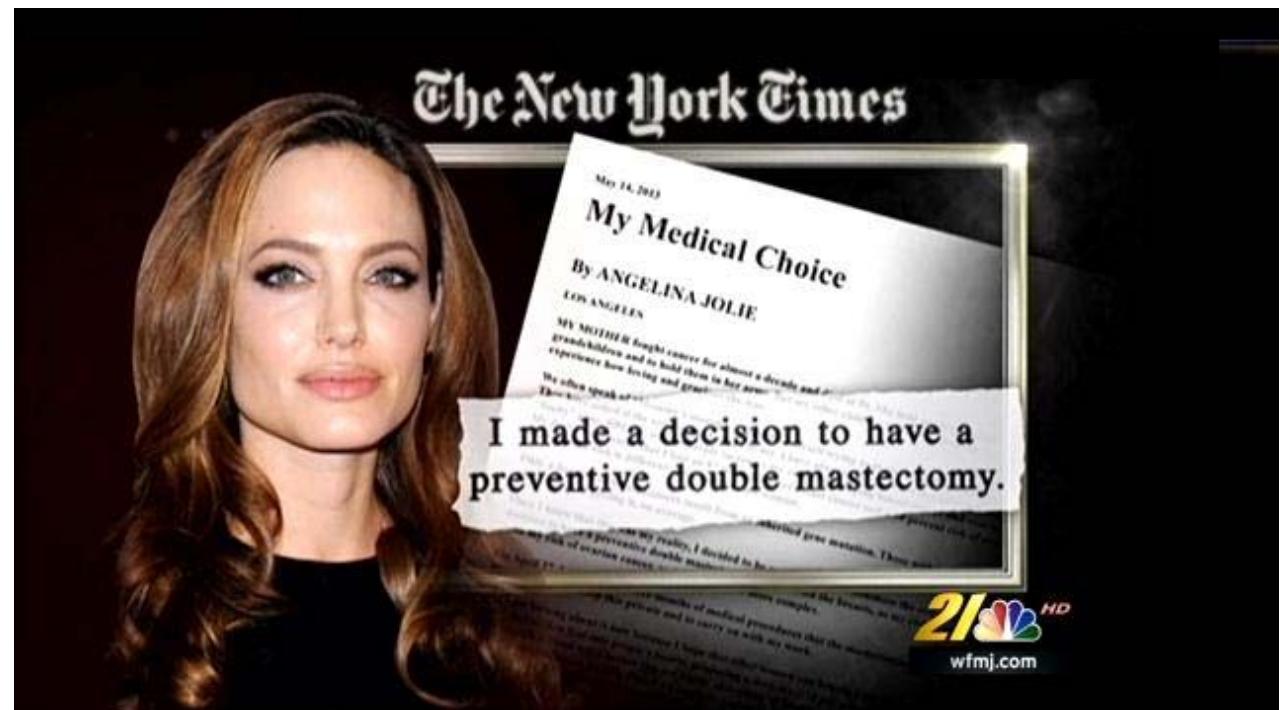
Normal mammogram



Cancer

Watch “Between You & Eternity” video on IVLE

<http://www.cancer.gov/cancertopics/screening/understanding-breast-changes/mammogram>



<http://www.wfmj.com/story/22263704/angelina-jolie-opts-to-remove-breasts-to-reduce-cancer-risk-raising-awareness>

“Life comes with many challenges. The ones that should not scare us are the ones we can take on and take control of.”

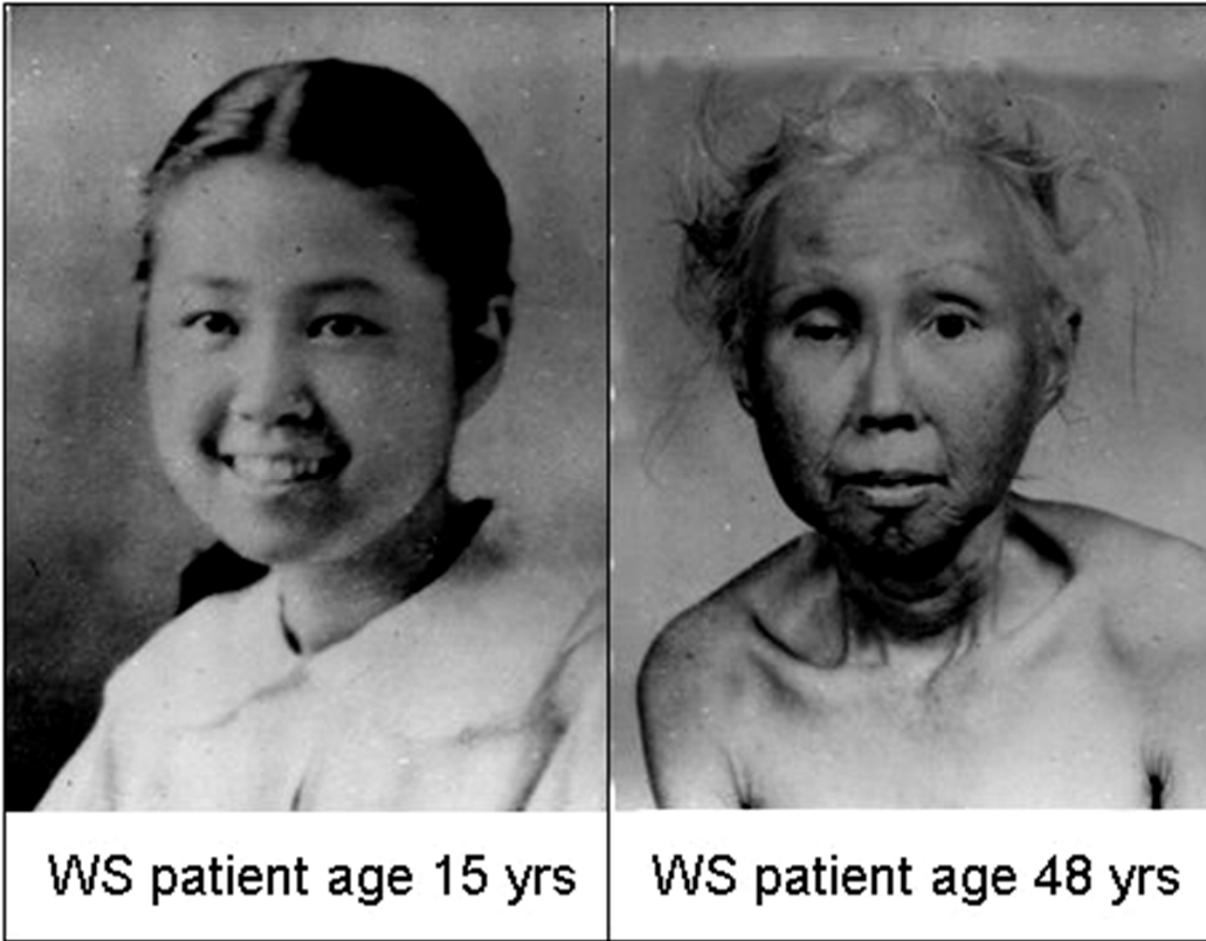
- Angelina Jolie (My Medical Choices)

[Read My Medical Choices in Additional Reading material]

Werner syndrome:

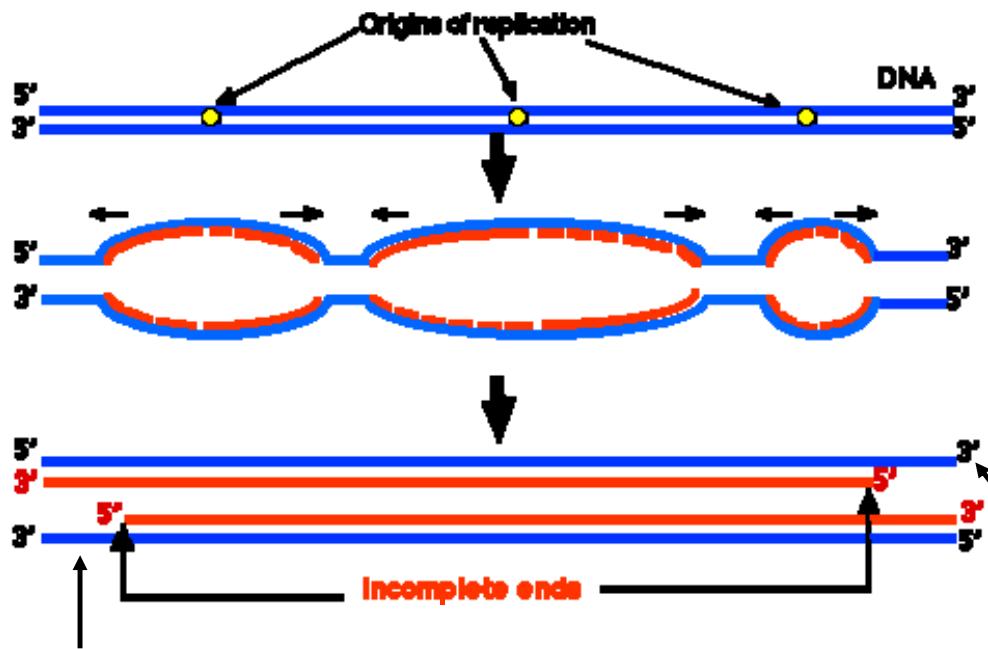
Damage to 3' exonuclease of WRN helicase involved in DNA repair by unwinding or digesting aberrant DNA structures accidentally generated during DNA activities, including maintenance of telomeres (end regions at chromosomal tips).

Mutations affecting WRN helicase activity result in replication-associated telomere loss and chromosomal fusion characteristic of Werner syndrome. Telomere loss has been suggested to accelerate cellular senescence, and may be one of the reasons why individuals with Werner syndrome develop premature aging symptoms



<http://wilsongen677s10.weebly.com/>

Telomeres and Ageing

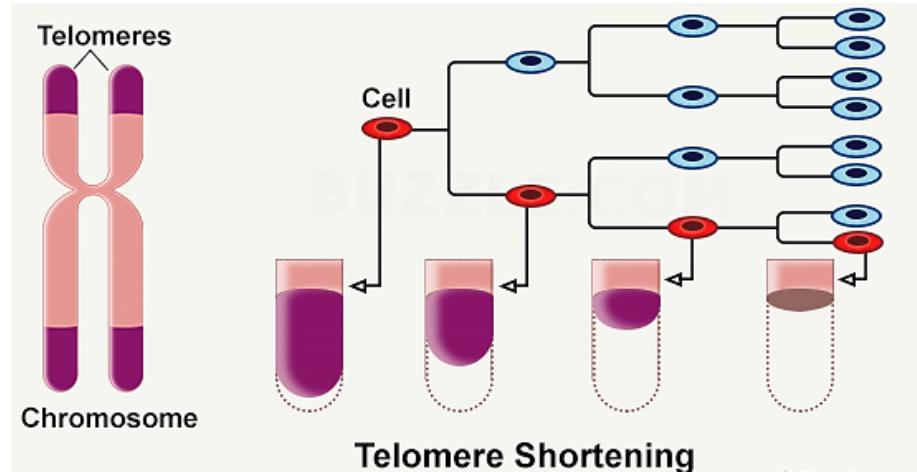


The telomere ends are complementary and if not removed, this can cause chromosomal fusion, hence have to be degraded resulting in shortening of the telomeres.

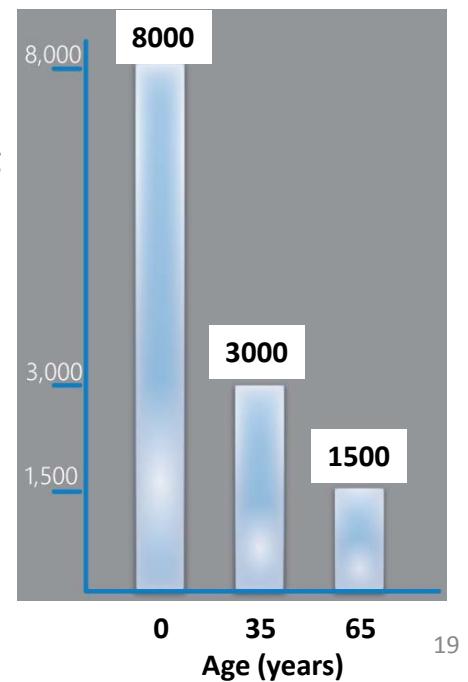
<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/Telomeres.html>

<http://www.buzzle.com/articles/is-there-a-connection-between-longevity-and-telomeres.html>

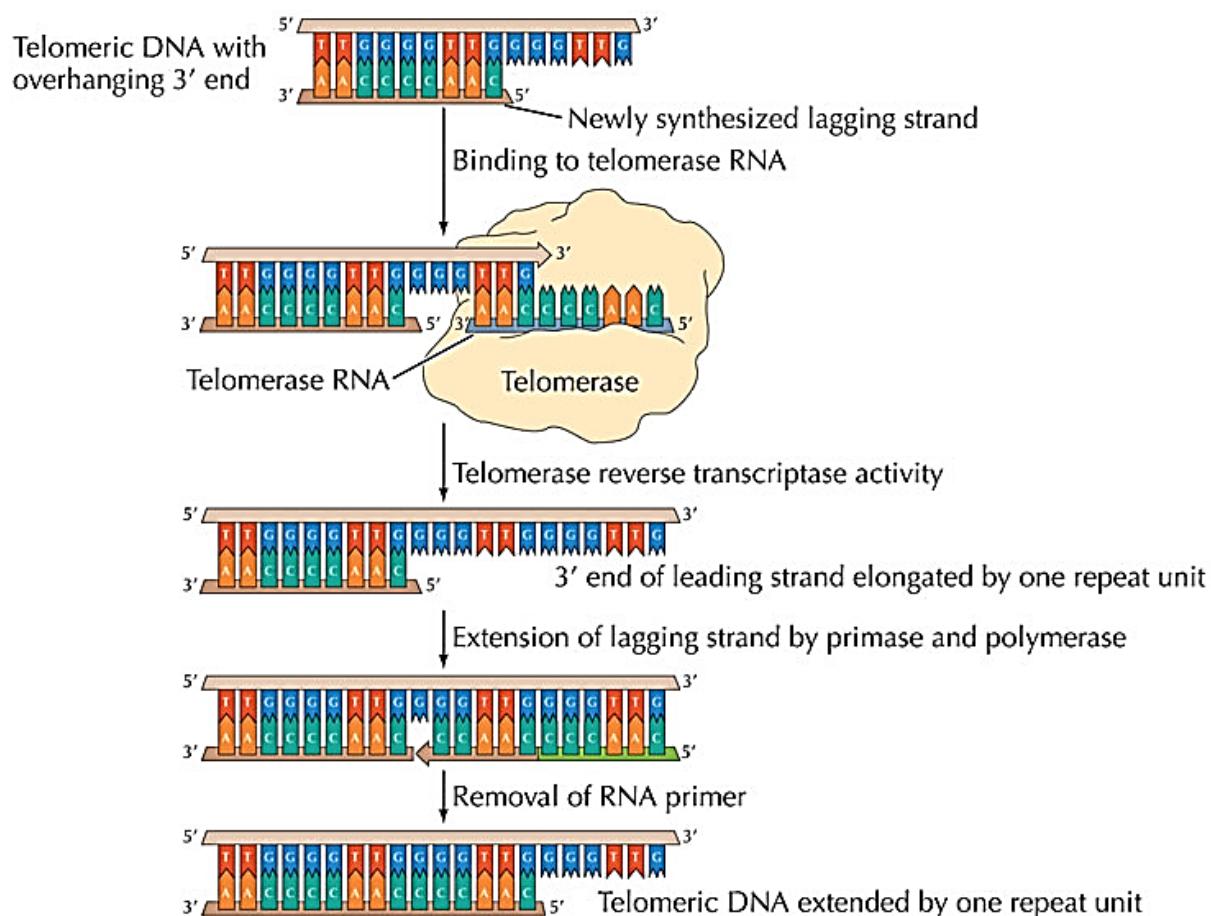
<http://learn.genetics.utah.edu/content/chromosomes/telomeres/>



Telomere length
in base pairs
(human dividing
cells)

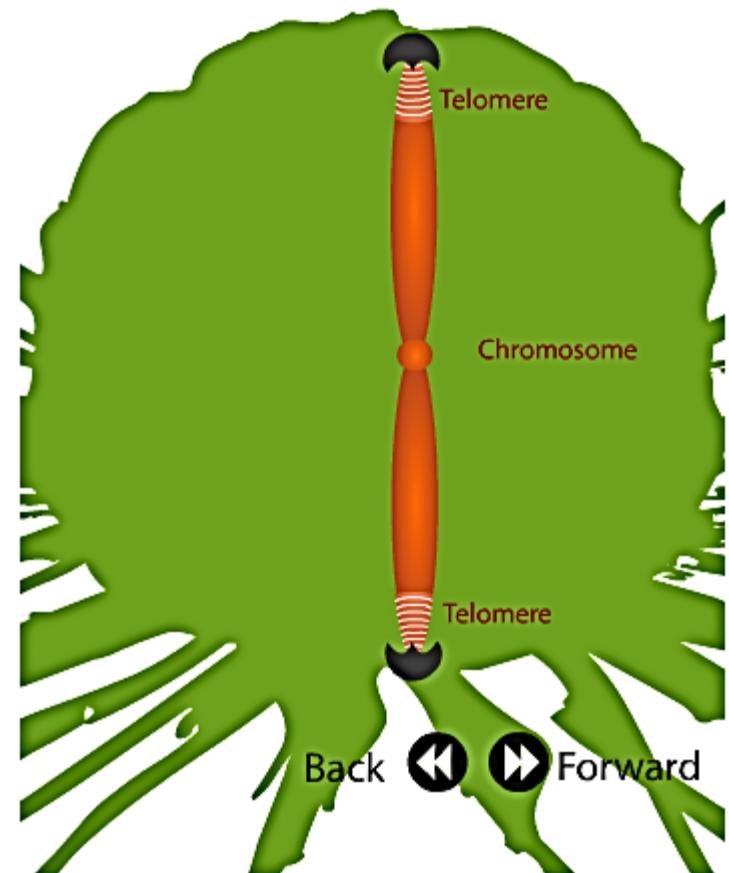


Telomerase maintains Telomeres



THE CELL, Fourth Edition, Figure 6.16 © 2006 ASM Press and Sinauer Associates, Inc.

Telomeres and Cancer



Check out Telomeres at Learn Genetics

<http://learn.genetics.utah.edu/content/chromosomes/telomeres/>

Mutation in Cell Cycle Genes and Cancer

Checkpoints and proliferation decision points monitor the progress of the cell through the cell cycle.
If genes coding for the proteins involved in cell cycle control are mutated, the risk of cancer will increase.

Watch Cell Cycle & Tumor Formation_Retinoblastoma IVLE Animation

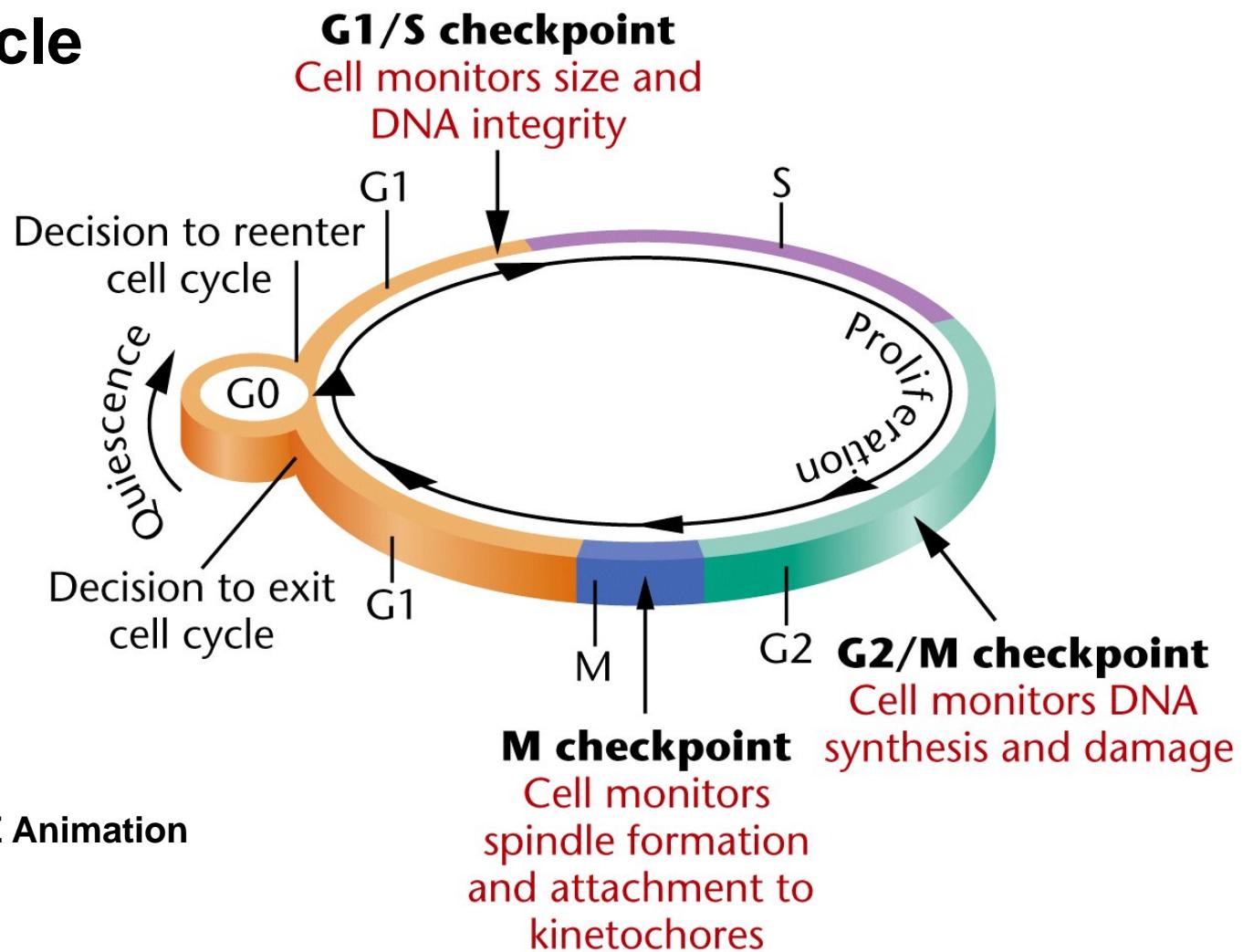


Figure 16-5 Essentials of Genetics, 6/e
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Mutation in Cell Cycle Genes and Cancer

TABLE 16.2 Some Proto-oncogenes and Tumor Suppressor Genes

Proto-oncogene	Normal Function	Alteration in Cancer	Associated Cancers
<i>Cyclins</i>	Bind to CDKs, regulate cell cycle	Gene amplification, overexpression	Lung, esophagus, many types
<i>CDK2, 4</i>	Cyclin-dependent kinases, regulate cell-cycle phases	Overexpression, mutation	Bladder, breast, many types
Tumor Suppressor	Normal Function	Alteration in Cancer	Associated Cancers
<i>p53</i>	Cell-cycle checkpoints, apoptosis	Mutation, inactivation by viral oncogene products	Brain, lung, colorectal, breast, many types
<i>RB1</i>	Cell-cycle checkpoints, binds E2F	Mutation, deletion, inactivation by viral oncogene products	Retinoblastoma, osteosarcoma, many types
<i>APC</i>	Cell-cell interaction	Mutation	Colorectal cancers, brain, thyroid
<i>Bcl2</i>	Apoptosis regulation	Overexpression blocks apoptosis	Lymphomas, leukemias
<i>BRCA2</i>	DNA repair	Point mutations	Breast, ovarian, prostate cancers

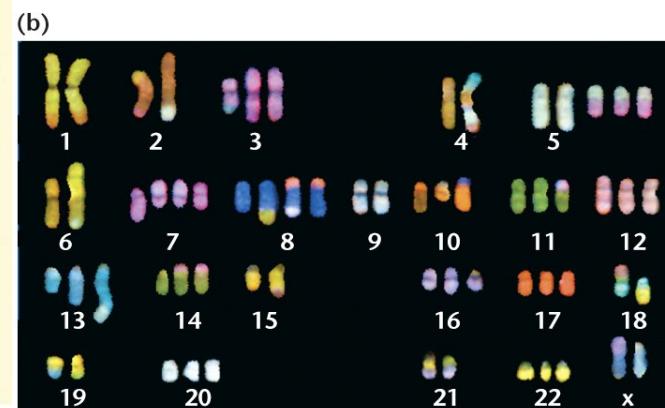
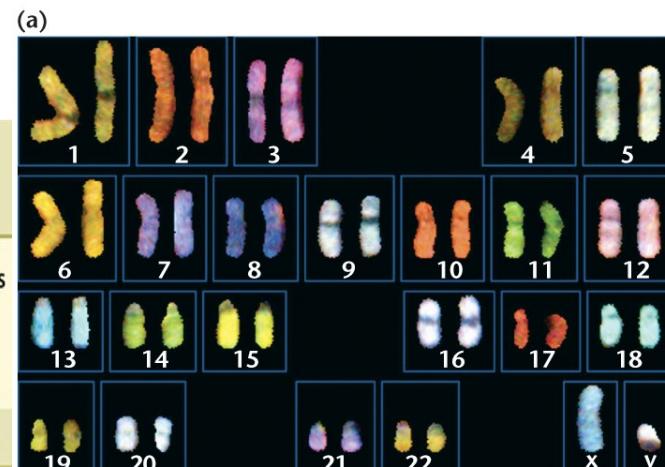


Figure 16-1 Essentials of Genetics, 6/e
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Chromosomal Abnormalities in Cancer Cells

Cancer's Random Assault

By DENISE GRADY JAN. 5, 2015



Some tissue types give rise to human cancers millions of times more often than other tissue types. Here, we show that the lifetime risk of cancers of many different types is strongly correlated (0.81) with the total number of divisions of the normal self-renewing cells maintaining that tissue's homeostasis... The majority is due to "bad luck," that is, random mutations arising during DNA replication in normal, noncancerous stem cells. – Tomasetti & Vogelstein, *Science* 347:78-81

TABLE 16.1 Cancer Probabilities in the United States

Cancer Site	Gender	Age			
		Birth to 39	40–59	60–79	Birth to Death
All sites	Male	1 in 62	1 in 12	1 in 3	1 in 2
	Female	1 in 52	1 in 11	1 in 4	1 in 3
Breast	Female	1 in 235	1 in 25	1 in 15	1 in 8
Prostate	Male	<1 in 10,000	1 in 53	1 in 7	1 in 6
Lung, bronchus	Male	1 in 3300	1 in 92	1 in 17	1 in 13
	Female	1 in 3180	1 in 120	1 in 25	1 in 17
Colon, rectum	Male	1 in 1500	1 in 124	1 in 29	1 in 18
	Female	1 in 1900	1 in 149	1 in 33	1 in 18

Source: American Cancer Society

Table 16-1 *Essentials of Genetics*, 6/e
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<http://www.nytimes.com/2015/01/06/health/cancers-random-assault.html>

It may sound flippant to say that many cases of cancer are caused by bad luck, but that is what two scientists suggested in an article published last week in the journal *Science*. The bad luck comes in the form of random genetic mistakes, or mutations, that happen when healthy cells divide. Random mutations may account for two-thirds of the risk of getting many types of cancer, leaving the usual suspects — heredity and environmental factors - to account for only one-third

Check out: Inside Cancer

(<http://www.insidecancer.org/> or hyperlink from DNA interactive)

The screenshot shows the homepage of the Inside Cancer website. At the top left is the logo "INSIDE CANCER" with a stylized cluster of black dots to the left. To the right of the logo is the text "Multimedia Guide to Cancer Biology". Below the header is a horizontal banner featuring four images: a blue-stained cell, two pink-stained cells, a grid of colored dots, and a complex network of lines and dots. Underneath the banner are four main categories: "HALLMARKS OF CANCER", "CAUSES AND PREVENTION", "DIAGNOSIS AND TREATMENT", and "PATHWAYS TO CANCER". Each category has a small icon to its left and a list of sub-topics below it.

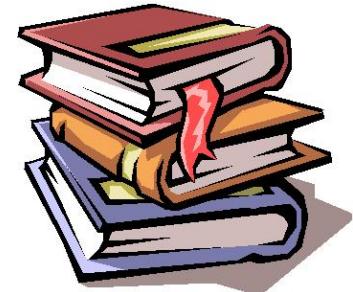
HALLMARKS OF CANCER	CAUSES AND PREVENTION	DIAGNOSIS AND TREATMENT	PATHWAYS TO CANCER
Overview	Overview	Pathology	Overview
Growing uncontrollably	Smoking	Pharmacogenetics	At the cell surface
Evading death	Inheritance	Targeted activators	Beneath the membrane
Processing nutrients	Diet	Blocking receptors	A bevy of interactions
Becoming immortal	Mold		To the nucleus
Invading tissues	Viruses		Inside the nucleus
Avoiding detection	Sunlight		Making the protein
Promoting mutations			Releasing the protein

Summary & Conclusion



- A gene mutation is a permanent change in one or more bases in the DNA sequence. The most common type are base-pair substitutions, insertions, and deletions.
- Mutation can be caused by chemicals and harmful radiation or can be ‘spontaneous’ (e.g. during DNA replication or metabolism).
- Several other mechanisms exist to repair damaged DNA after replication is over, including mismatch repair, base-excision repair, and nucleotide-excision repair.
- Mutation in DNA repair genes will result in increased accumulation of mutations leading to specific disorders.
- Shortening of telomeres will lead to cell senescence (ageing), however in stem cells and cancer cells are maintained by telomerase
- Mutation in cell cycle genes could lead to uncontrolled proliferation a hallmark of cancer cells

Additional Enrichment Materials



- IVLE Animations: Sickle Cell Anemia; Types of Mutation; Errors Corrected During DNA Replication; Base Excision Repair; Nucleotide Excision Repair; Cell Cycle & Tumor Formation_Retinoblastoma.
- Watch short movie “Between You & Eternity” on IVLE

Useful Weblinks:

- Inner Life of the Cell:
 - <https://www.youtube.com/watch?v=wJyUtn0O5Y>
 - https://www.youtube.com/watch?v=B_zD3NxSsD8
- Watch HHMI BioInteractive “Damage to DNA leads to mutation”
 - <http://www.hhmi.org/bioInteractive/damage-dna-leads-mutation>
- Watch The Making of the Fittest: Natural Selection Humans
 - <https://www.youtube.com/watch?v=Zsbhvl2nVNE&feature=youtu.be>
- Learn Genetics “Are Telomeres The Key To Aging And Cancer?”:
 - <http://learn.genetics.utah.edu/content/chromosomes/telomeres/>
- Check out Inside Cancer: <http://www.insidecancer.org/>