DNA Repair and Cancer

RPN 530 Oncology for Scientists
Lecture

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DNA Repair and Cancer

Suggested additional resources:

Weinberg RA (2007) *The Biology of Cancer*. Garland Science, New York, (Chapter 12).

Friedberg EC, Walker GC, Siede W, Wood RD, Shultz RA, Ellenberger T (2006) *DNA Repair and Mutagenesis*, ASM Press, Washington, DC.

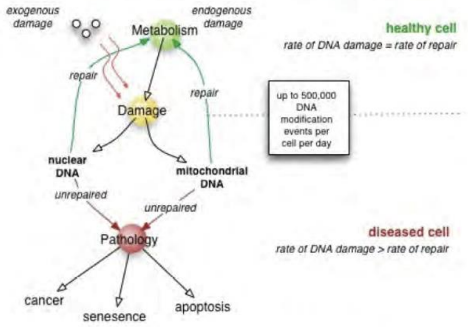
DNA Repair and Cancer

RNA and proteins can be totally degraded when damaged, and re-synthesized when needed

DNA is not totally degraded when damaged
- repaired at a nucleotide level or in small patches

DNA repair maintains integrity of the genetic information
- DNA -> RNA -> protein

All Cells need DNA Repair



Importance of DNA Repair

All organisms, from bacteria to yeast to humans, have multiple DNA repair mechanisms and pathways

Many of the genes, proteins and repair pathways are evolutionarily conserved

~ 150 human genes encode DNA repair proteins, and many more involved in DNA damage response (DDR)

Defects in DNA Repair can cause Cancer

DNA Repair

Types of DNA damage

Types of DNA repair pathways

DNA damage, repair, and cancer

DNA Repair

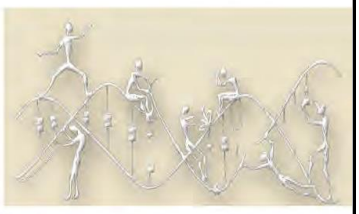
Types of DNA damage

- Errors in DNA synthesis
- Spontaneous/endogenous sources
- Exogenous sources

Endogenous Cellular Events threaten DNA Integrity

- Errors in DNA Replication
- Biochemical Processes

Errors cause Mutations if not Repaired



Replication Errors in the Genome

DNA polymerase delta (δ) copies DNA with high fidelity (proofreading mutant D400A)

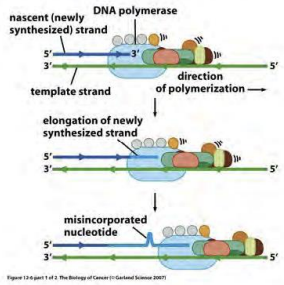
- Low error rate in copying: ~1 error/10^5 bp

Human genome has ~6×10^9 bp

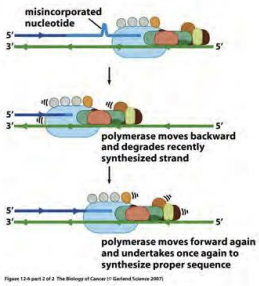
- 6×10^9 / 1×10^5 = ~60,000 errors!

How does the cell prevent/correct these errors to minimize the mutation rate?

Copying DNA by Polymerase δ

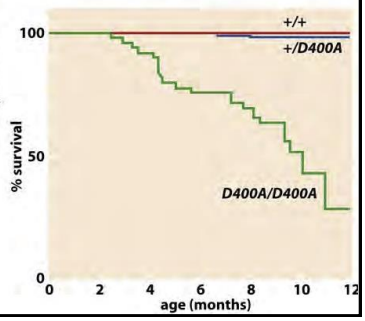


Proofreading DNA Polymerase δ



Defect in Replication Proofreading can cause Cancer

Homozygous point mutation D440A/D400A within the proofreading domain of DNA Polymerase δ causes cancer in mice and ultimately death



Proofreading Errors in the Genome

DNA polymerase δ + proofreading function
- ~1 error in 10^7 bp

Human genome has ~$6x10^9$ bp
- $6x10^9$ / $1x10^7$ = ~600 errors

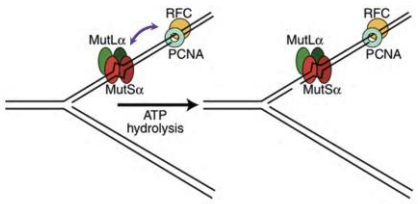
How does the cell deal with these errors?

Excision Repair

Three types:
- Mismatch repair (MMR)
- Nucleotide excision repair (NER)
- Base excision repair (BER)

What do they have in common?
- Excise the damaged or mismatched DNA strand
- Synthesize based on the complementary strand
- Ligate the nick to complete synthesis

Mismatch Repair in Humans



- Nicked in same strand as first nick
- Excision by Exonuclease I
- Gap filling by Polymerase δ; Ligation by DNA ligase I

Proofreading Errors in the Genome

DNA polymerase δ + proofreading function
- ~1 error in 10^7 bp

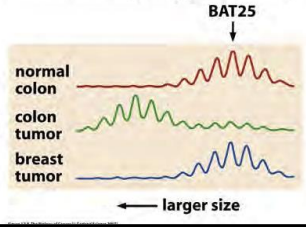
DNA polymerase δ + proofreading + MMR
- ~1 error in 10^9 bp

Human genome has $6x10^9$ bp
- $6x10^9$ bp / 10^9 bp = ~6 errors in genome

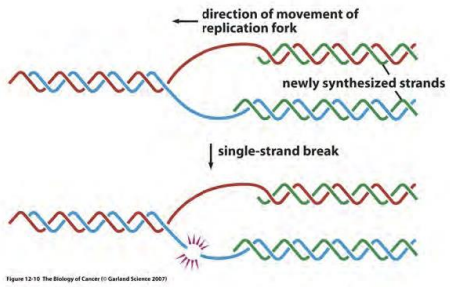
MMR Defects cause Microsatellite Instability (MIN)

- DNA Repeats, called Microsatelites, can expand or contract during DNA replication
- Microsatellite: $TTTTnTnTTTTnT_7nnT_{25}$ [named BAT25]

Expansion of BAT25 to a larger size in Colon Cancer (HNPCC)



DNA can Break during Replication



DNA can break during Replication

Breaks occur near replication forks

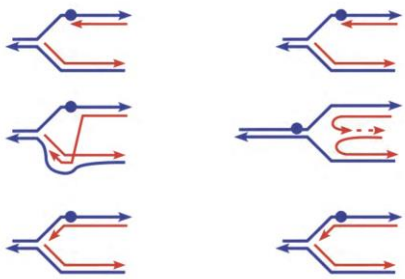
Up to ~ 10 breaks per cell per S phase

Breaks may result after trying to replication past a ssDNA break (nick) or DNA "damage"

Failure to properly repair breaks can lead to cell death, or to DNA translocations, which can lead to cancer

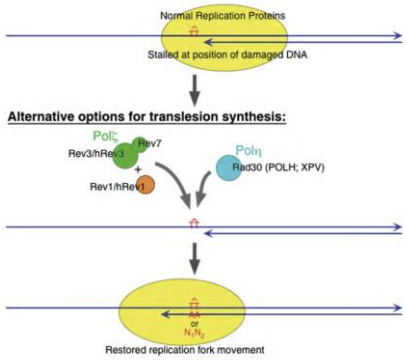
Bypass of DNA Damage by DNA Replication Forks

Template-Switch Damage Bypass Mechanisms

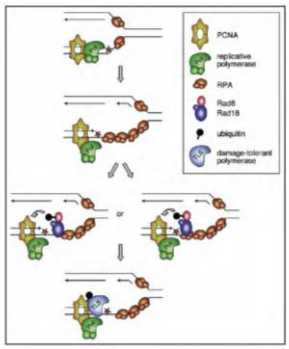


In both examples, information is read from the sister nascent strand.

Mutagenic and Non-Mutagenic Bypass Synthesis



TLS: Polymerase Switch



DNA Polymerases

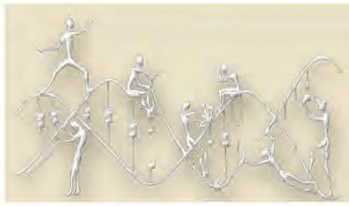
Polymerase	Gene	Catalytic subunit	Accessory subunits (kDa)	3′→5′ exonuclease	Fidelity	Primary function
Pol α	*POLA1*	1462 aa	49 *PRIM1*; 58 *PRIM2A*; 70 *PRIM2A*	No	10^{-4}–10^{-5}	RNA and/or DNA primers
Pol β	*POLB*	335 aa	None	No	5×10^{-4}	Base-excision repair
Pol γ	*POLG1*	1239 aa	55 *POLG2*	Yes	10^{-6}	Mitochondrial DNA replication and repair
Pol δ	*POLD1*	1107 aa	50 *POLD2*; 68 *POLD3*; 12 *POLD4*	Yes	10^{-6}–10^{-7}	Lagging-strand synthesis DNA repair
Pol ε	*POLE*	2286 aa	59 *POLE2*; 12 *POLE4*; 17 *POLE3*	Yes	10^{-6}–10^{-7}	Leading-strand synthesis

Polymerase	Gene	Family	Other names	Proposed function
η (eta)	*POLH*	Y	*RAD30A*, *XPV*	Bypass UV lesions
ι (iota)	*POLI*	Y	*RAD30B*	Bypass synthesis
κ (kappa)	*POLK*	Y	*DINB1*	Bypass synthesis
λ (lambda)	*POLL*	X	*POL4* (in *Saccharomyces cerevisiae*)	Base-excision repair, NHEJ
μ (mu)	*POLM*	X	–	NHEJ
θ (theta)	*POLQ*	A	*Mus308* (in *Drosophila melanogaster*)	DNA repair
ζ (zeta)	*POLZ*	B	*REV3*	Bypass synthesis
Rev 1	*REV1*	Y	*REV1L*	Incorporation of dC opposite abasic sites
ν (nu)	*POLN*	A	–	Unknown, but Pol ν has a unique error signature, G-dTMP mismatches[111]

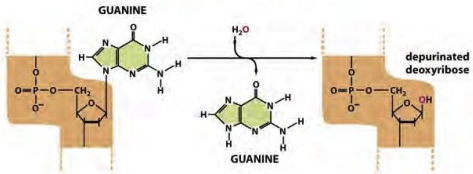
Endogenous Cellular Events threaten DNA Integrity

- Errors in DNA Replication
- Biochemical Processes

DNA Damage can cause Mutations if not Repaired

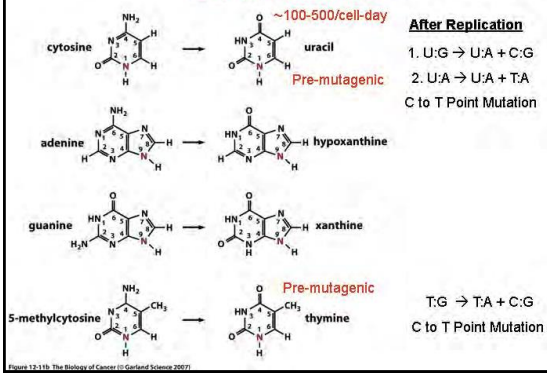


Depurination



- Spontaneous reaction
- Up to 10,000 Purine bases per cell in a day
- Up to 500 Pyrimidine bases per cell in a day
- Abasic sites are non-coding and block replication if not repaired

Deamination



After Replication

1. U:G → U:A + C:G
2. U:A → U:A + T:A

C to T Point Mutation

T:G → T:A + C:G

C to T Point Mutation

Figure 13-11b The Biology of Cancer (© Garland Science 2007)

Oxidation

Reactive Oxygen species:

- Hydrogen peroxide: HOOH
- Oxygen free radicals: O-O•, HO•, O•

May arise from:

- Mitochondria, peroxisomes, inflammation

Can damage DNA bases

- Oxidized bases
- Form abasic sites
- DNA breaks: ss- or ds-DNA break

Oxidation

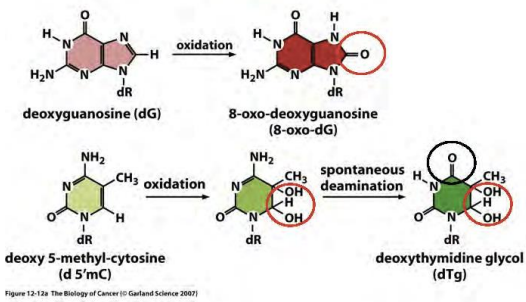
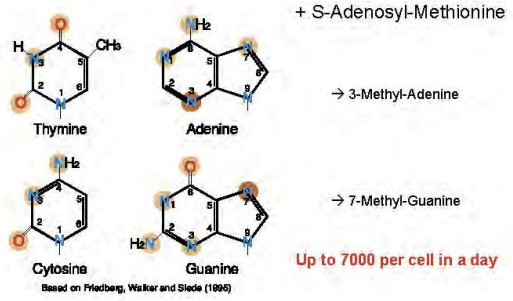


Figure 12-12a The Biology of Cancer (© Garland Science 2007)

Methylation



Based on Friedberg, Walker and Siede (1995)

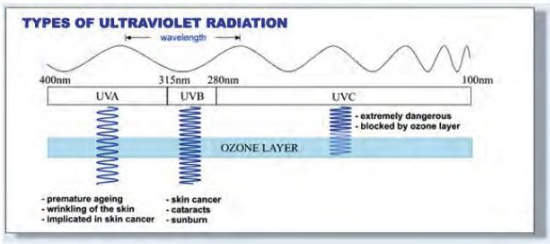
Exogenous agents can damage DNA

UV: ultra-violet radiation

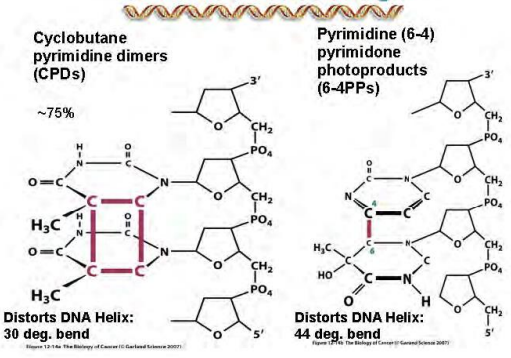
Alkylating agents

X-rays: ionizing radiation
- double-strand break (DSB)

Solar UV Radiation, Ageing & Skin Cancer



UV - DNA Damage



UV DNA damage: mutagen & carcinogen

Pyrimidine dimers:
- 60% T-T, 30% C-T, 10% C-C

Mutagenic: keratoses, basal skin carcinomas
- C-C dimers are the most mutagenic: C-C -> T-T
- T-T dimers are the least mutagenic, best repaired

Carcinogen: squamous cell carcinomas
- Incidence doubles every 10° decline in latitude
- Peaks at the Equator, where cumulative UV highest

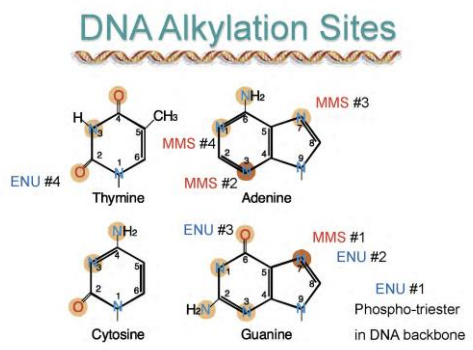
DNA alkylating agents

Many are known cytotoxics, mutagens and carcinogens
- Which interfere with DNA replication

Some are agents used in laboratory studies
- MMS, methyl methane sulfonate
- ENU, ethyl nitrosourea

Cytotoxic drugs used in chemotherapy
- Melphalan, chlorambucil, others

Environmental agents
- MeCl: microorganisms, algae, burning biomass
- Streptozotocin: MNU-derivative from resistant *S. achromogenes*



Impact of DNA alkylation

7-methyl-guanine is unstable
- Spontaneously depurinates -> abasic site
- Blocks replication, cytotoxic if not repaired

3-methyl-adenine blocks replication
- Cytotoxic if not repaired

6-ethyl-guanine is mutagenic, not cytotoxic
- Mispairs with T in replication, then T templates A
  - eG:C -> eG:T -> A:T mutant DNA
  - G:C -> G:C -> G:C wtDNA, if repaired
  - 1st 2nd :S phases

Excision Repair

Mismatch repair

Nucleotide excision repair

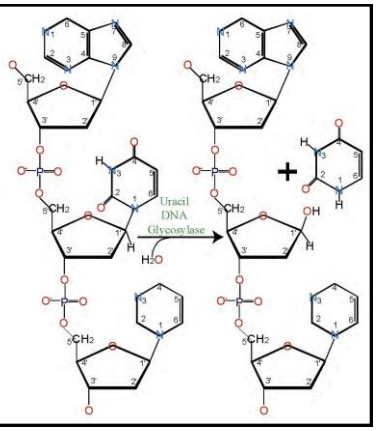
Base excision repair

Base Excision Repair

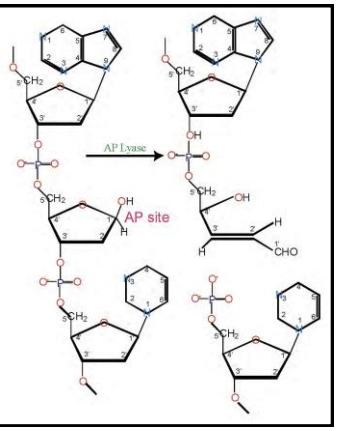
Many variations, depending on the nature of the damage, glycosylase, and nature of DNA polymerase

All have the following steps in common:
- Removal of the incorrect base by an appropriate DNA N-glycosylase to create an AP site
- An AP endonuclease nicks on the 5' side of the AP site to generate a 3'-OH terminus
- Extension from the 3'-OH by a DNA polymerase, which replaces the AP site with correct nucleotide
- Ligation of the DNA nick

An example of a DNA N-glycosylase



Some DNA N-glycosylases have AP lyase activity.



Examples of Human DNA Glycosylases

Acronym	Full Name	Size (aa)	AP Lyase	Substrates
UNG	Uracil DNA N-Glycosylase	313	No	ssU>U:G>U:A, 5-FU
TDG	Thymine DNA Glycosylase	410	No	U:G>ethenocytosine:G>T:G
UDG2	Uracil DNA Glycosylase 2	327	No	U:A
SMUG1	Single-strand-selective Monofunctional Uracil-DNA Glycosylase 1	270	No	ssU>U:A, U:G
MBD4	Methyl-CpG-Binding Domain 4	580	No	U or T in U/TpG:5-meCpG
MPG	Methyl Purine DNA Glycosylase	293	No	3-meA, 7-meA, 3-meG, 7-meG
MYH	MutY Homolog	535	Yes (±)	A:G, A:8-oxoG
OGG1	8-Oxo-Guanine Glycosylase 1	345	Yes	8-oxoG:C
NTH1	Endonuclease Three Homolog 1	312	Yes	T-glycol, C-glycol, formamidopyrimidine

Base Excision Repair & Cancer

MutYH: the first BER gene associated with a human cancer syndrome

MutYH excises A across from 8-oxoG:A bp

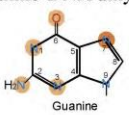
Bialleic germline mutations predispose to colorectal adenomas and carcinomas

Association with APE1, PCNA, RPA, & replication foci suggests MutYH has a role in long-patch BER

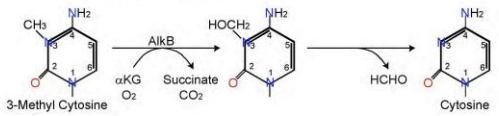
Direct Repair
aka
Direct Reversal of DNA Damage

More Examples of Direct Repair

- O^6-alkylguanine-DNA alkyltransferase



- Demethylation of 1-methyl adenine and 3-methyl cytosine



Excision Repair

Mismatch repair

Nucleotide excision repair
- Fixes a wide variety of DNA damage: UV & bulky adducts distorting the DNA helix

Base excision repair

Nucleotide Excision Repair

In all organisms, NER involves the following steps:
- Damage recognition
- Binding of a multi-protein complex at the damage site
- Double incision of the damaged strand several nt away from the damaged site, both 5' and 3' sides
- Removal of the damage-containing oligonucleotide from between the two nicks
- Filling of the resulting gap by a DNA polymerase
- Ligation of the nick in the DNA

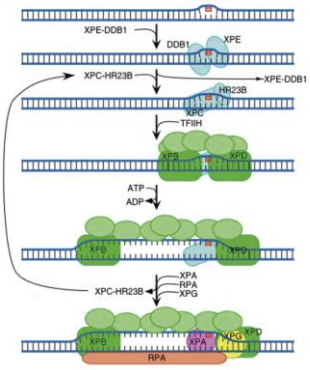
Nucleotide Excision Repair

Two Types:

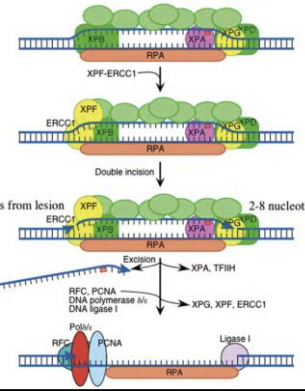
Global Genome NER
- Works on both DNA strands, except actively-transcribed strands

Transcription-coupled NER
- Works on actively-transcribed DNA strands only

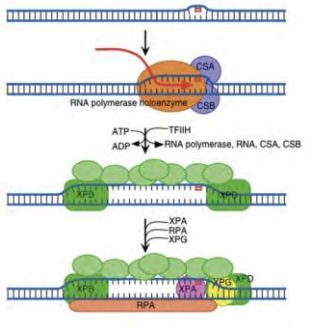
Early Stages of Global Genome NER



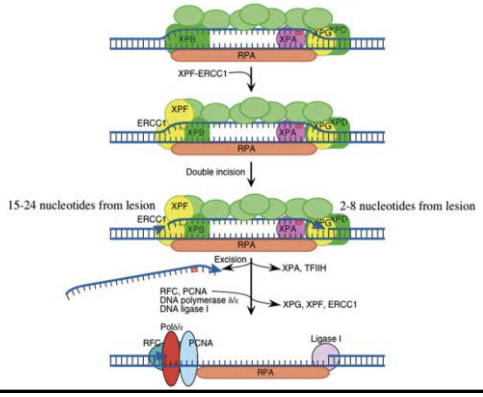
Final Steps of NER



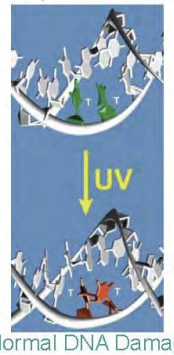
Initial Steps of Transcription-Coupled NER



Final Steps of NER

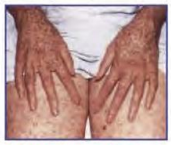


Sun, UV Damage & DNA Repair



Normal DNA Damage

XP Patients



Defective Repair

DNA double-strand breaks (DSBs)

Unrepaired DSBs are lethal or mutagenic

Sources: X-rays, chemicals, free radicals, replication across from a DNA SSB

Triggers recruitment of repair factors
- Homology-directed or End-joining

Triggers cell-cycle arrest via checkpoint kinase ATM (ataxia telangiectasia-mutated)

DSBs lead to GENOMIC INSTABILITY!

DNA strand break repair

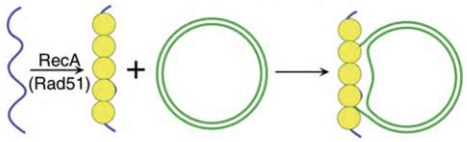
Essential for cell survival and genome stability

Multiple pathways

- DSB repair by Homologous Recombination
- DSB repair by Non-Homologous End Joining
- Non-ligateable SSB repair

Homologous Recombination is Based on the Ability of Single DNA Strands to Find Regions of Near-Perfect Homology Elsewhere in the Genome

Facilitation of Homology Searching by RecA and its Eukaryotic Homologs



- Eukaryotic proteins important in this process include Rad51, Rad52, Rad54, Rad55, Rad57 and Rad59. Brca1 mediates HR, and Rad52 / Brca2 interacts with Rad51.

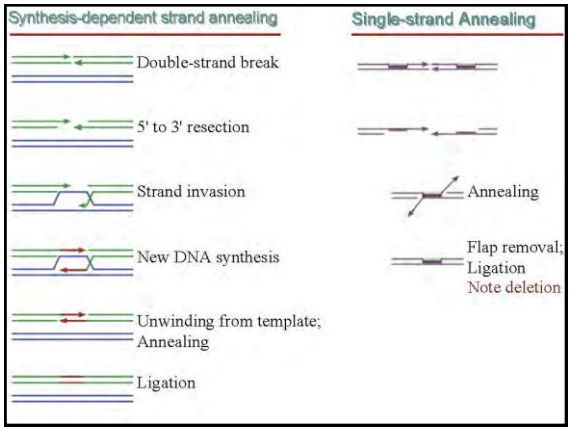
Homology-directed repair

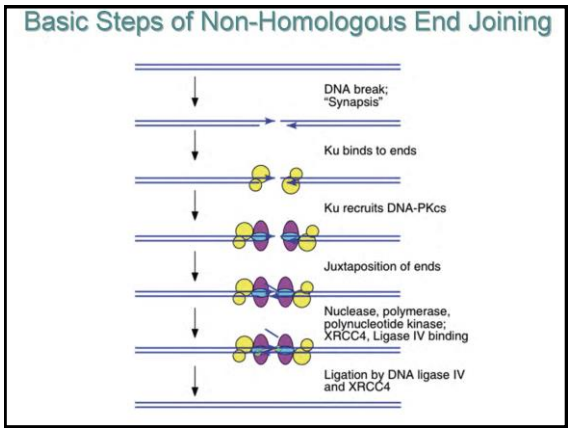
Non-mutagenic

- SDSA and DSB repair
- Require: Rad51, Rad52, and mediator proteins Rad54, Rad55, Rad57

Mutagenic

- Single-strand annealing
- Occurs at repeated DNA sequences
- Requires Rad52 and Rad59





DSB repair & cancer

Inherited predispositions to cancer

- ATM, MRE11
- NBS1: Nijmegen Breakage Syndrome
- BRCA1, BRCA2: homology-directed repair
- BLM, WRN: DNA helicases involved in repair

Null mutations: embryonic lethal (except ATM)

- Result in gross chromosomal rearrangements
  - SSB -> DSB during DNA replication

Repair defects in Familial Cancer

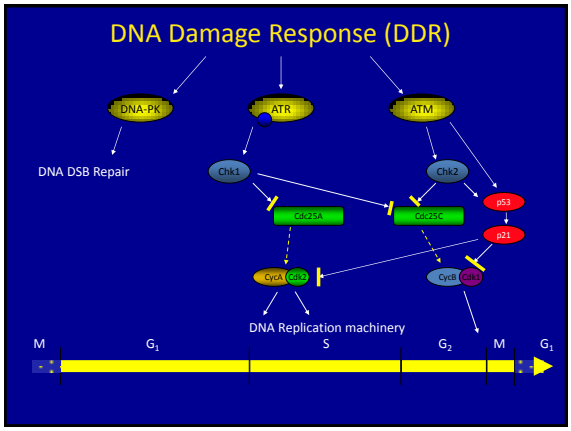
Table 12.1 Human familial cancer syndromes due to inherited defects in DNA repair

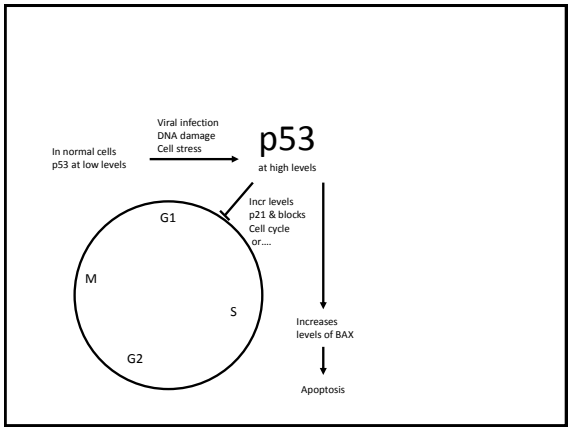
Name of syndrome	Name of gene	Cancer phenotype	Enzyme or process affected
HNPCC	(4–5 genes)[a]	colonic polyposis	mismatch repair enzymes
XP[b]	(8 genes)[b]	UV-induced skin cancers	nucleotide-excision repair
AT[c]	*ATM*	leukemia, lymphoma	response to dsDNA breaks
AT-like disorder[c]	*MRE11*	not yet determined	dsDNA repair by NHEJ
Familial breast, ovarian cancer	*BRCA1, BRCA2*[d]	breast and ovarian carcinomas	homology-directed repair of dsDNA breaks
Werner	*WRN*	several cancers	exonuclease and DNA helicase[e], replication
Bloom	*BLM*	solid tumors	DNA helicase, replication
Fanconi anemia	(9 genes)[f]	AML, HNSCC	repair of DNA cross-links and ds breaks
Nijmegen break[g]	*NBS*	mostly lymphomas	processing of dsDNA breaks, NHEJ
Li–Fraumeni	*TP53*	multiple cancers	DNA damage alarm protein
Li–Fraumeni	*CHK2*	colon, breast	kinase signaling DNA damage

[a]Five distinct MMR genes are transmitted as mutant alleles in the human germ line. Two MMR genes—*MSH2* and *MLH1*—are commonly involved in HNPCC; two other MMR genes—*MSH6* and *PMS2*—are involved in a small number of cases; a fifth gene, *PMS1*, may also be involved in a small number of cases.
[b]Xeroderma pigmentosum, at least eight distinct genes, seven of which are involved in NER. The seven genes are named *XPA* through *XPG*. An eighth gene, *XPV*, encodes DNA polymerase η.
[c]Ataxia telangiectasia, small number of cases.
[d]Mutant germ-line alleles of *BRCA1* and *BRCA2* together may account for 10–20% of identifiable human familial breast cancers.
[e]An exonuclease digests DNA or RNA from one end inward; a DNA helicase unwinds double-stranded DNA molecules.
[f]Nine genes have been cloned and at least eleven complementation groups have been demonstrated. Complementation group J encodes the BACH1 protein, the partner of BRCA1.
[g]The NBS1 protein (termed nibrin) forms a physical complex with the Rad50 and Mre11 proteins, all of which are involved in repair of dsDNA breaks. The phenotypes of patients with Nijmegen break syndrome are similar but not identical to those suffering from AT.

Adapted in part from B. Alberts et al., Molecular Biology of the Cell, 4th ed. New York: Garland Science, 2002; and from E.R. Fearon, *Science* 278:1043–1050, 1997.

Table 12-1 The Biology of Cancer (© Garland Science 2007)





DNA repair & cancer

Loss or mutagenesis of p53 has the devastating dual consequences of preventing cell cycle arrest due to DNA damage (resulting in accumulation of more DNA damage), and in preventing apoptosis of cells which have accumulated too much DNA damage. These two effects lead directly to genomic instability. This explains why p53 is the most commonly mutated protein in all human tumors.

Repair defects in Familial Cancer

Table 12.1 Human familial cancer syndromes due to inherited defects in DNA repair

Name of syndrome	Name of gene	Cancer phenotype	Enzyme or process affected
HNPCC	(4–5 genes)[a]	colonic polyposis	mismatch repair enzymes
XP[b]	(8 genes)[b]	UV-induced skin cancers	nucleotide-excision repair
AT[c]	*ATM*	leukemia, lymphoma	response to dsDNA breaks
AT-like disorder[e]	*MRE11*	not yet determined	dsDNA repair by NHEJ
Familial breast, ovarian cancer	*BRCA1, BRCA2*[d]	breast and ovarian carcinomas	homology-directed repair of dsDNA breaks
Werner	*WRN*	several cancers	exonuclease and DNA helicase[e], replication
Bloom	*BLM*	solid tumors	DNA helicase, replication
Fanconi anemia	(9 genes)[f]	AML, HNSCC	repair of DNA cross-links and ds breaks
Nijmegen break[g]	*NBS*	mostly lymphomas	processing of dsDNA breaks, NHEJ
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