Chapter 2 – Lecture 1

Molecular Mechanisms of DNA and Chromosome Damage and Repair

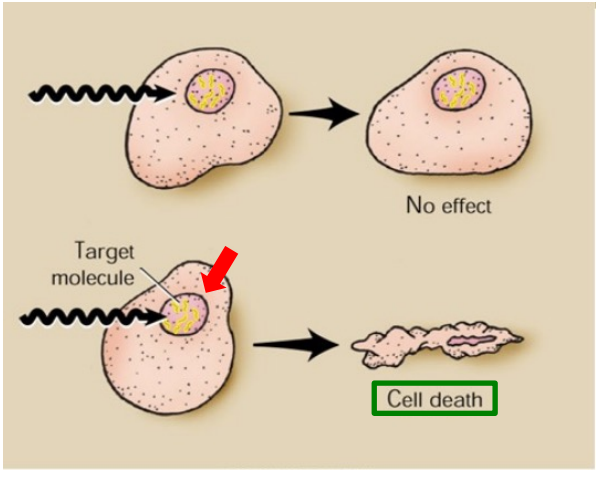
9/9/2024

Outline

- **General Overview of DNA Strand Breaks**
- Measuring DNA Strand Breaks
- DNA Repair Pathways
- Chromosomes and Cell Division
- Radiation-Induced Chromosome Aberrations
- Chromosome Aberrations in Human Lymphocytes

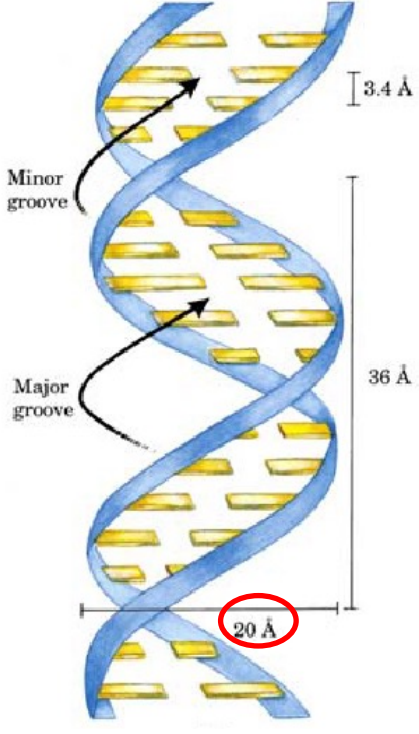
DNA as the Target

- It is long thought that radiation had to deposit its energy in the cell nucleus and that DNA was the target for the observed biological effects



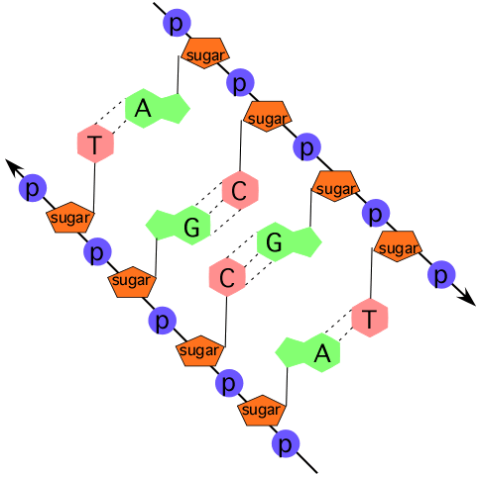
DNA is a Double Helix

- DNA = deoxyribonucleic acid
- It is a large molecule with a double helix structure
- The diameter of the DNA helix is 2 nm and the vertical rise per base pair is 0.34 nm
- 10 base per turn

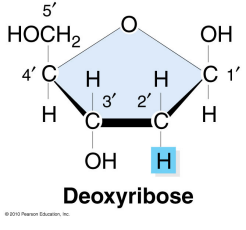


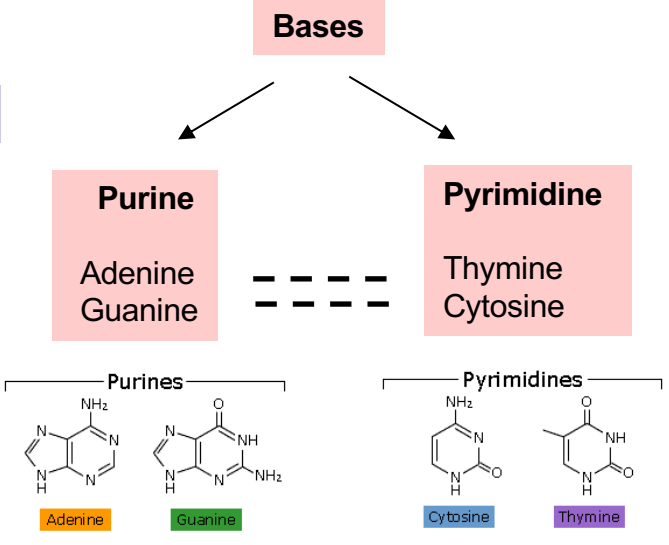
The Sugar Phosphate Backbone

- The **backbone** of each strand consists of alternating sugar and phosphate groups
- Attached to the backbone are **4 bases**
- The 2 strands are held together by H bonds between the bases

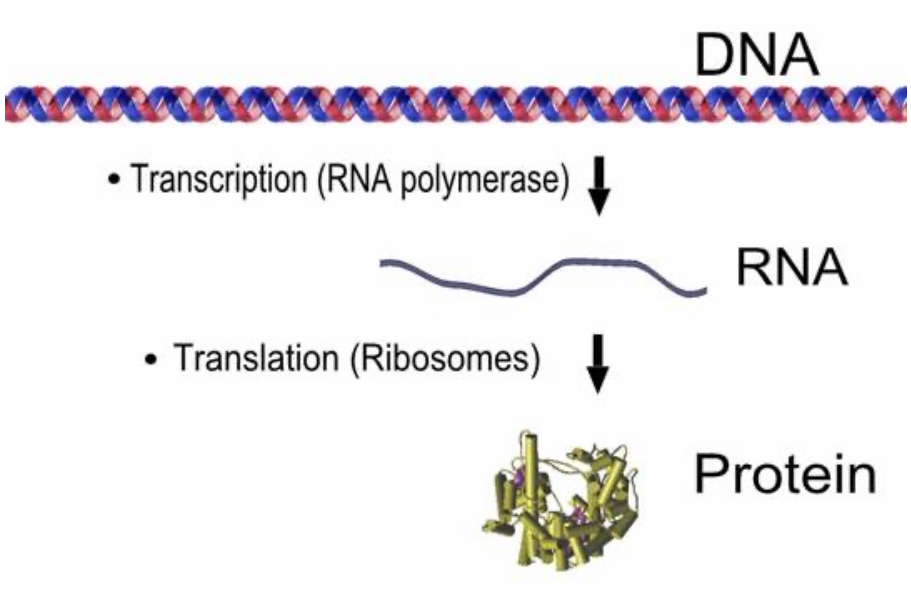


Sugar = deoxyribose



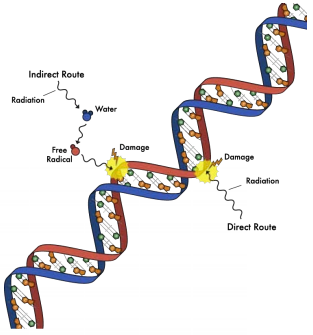


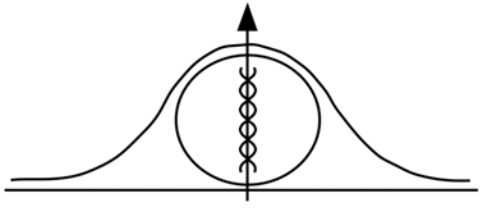
Flow of Genetic Information



“The Central Dogma”

DNA as the Target





Radiation

High Fidelity DNA Repair

Chapter 2

DNA Damage

Senescence

Death
- Apoptosis
- Necrosis
- Mitotic Linked

Most important mechanism of radiation-induced cell death

Chapter 3

Mutation(s) and/or Chromosome Rearrangements

Somatic

Germline

Fetal

Carcinogenesis

Chapter 10

Heritable Genetic Effects

Chapter 11

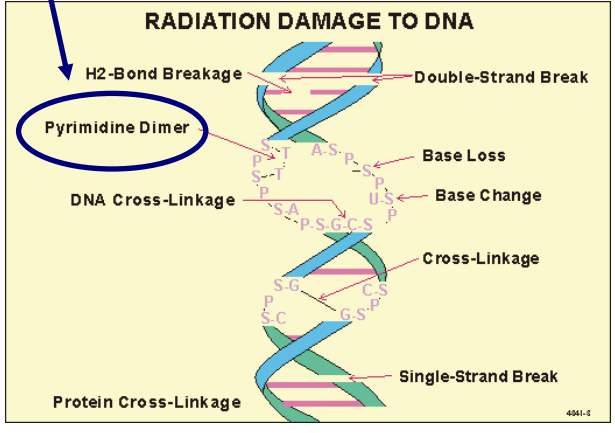
Developmental Effects

Chapter 12

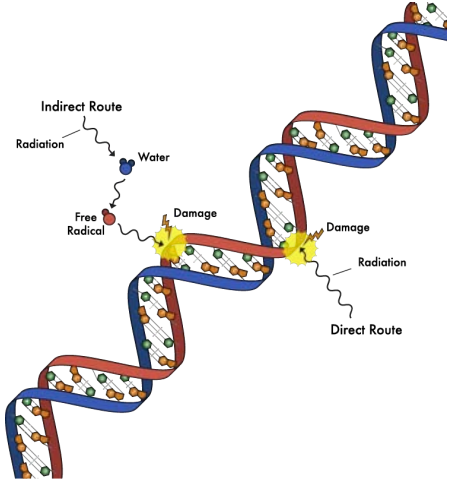
DNA Damage

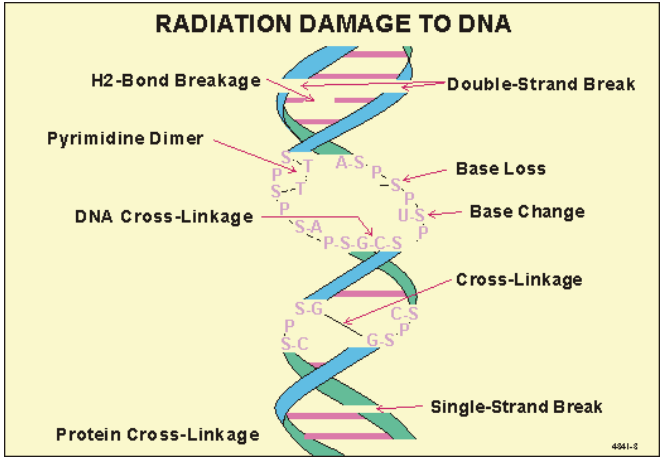
- A variety of DNA lesions can be induced by radiation
  - Base damage
  - Sugar damage
  - Single-strand breaks (SSB)
  - Double-strand breaks (DSB)
  - DNA-protein crosslinks
  - DNA-DNA crosslinks

Primarily induced by UV



Radiation Damage to DNA





What is the distribution and yield of DNA damage from ionizing radiation?

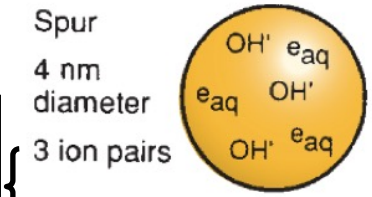
Energy Deposit

- The energy from ionizing radiation is deposited unevenly in the absorbing medium
- It tends to locate along the tracks of the charged particles set in motion
- “Spurs”, “blobs”, and “short tracks” are used to describe the **size** of these energy event sizes

“Spurs” & “Blobs”

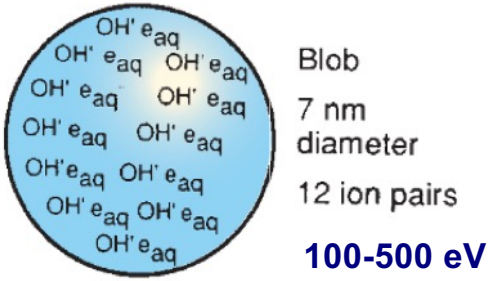
Spur

Recall that avg energy dissipated per ionizing event ≈ **33 eV**



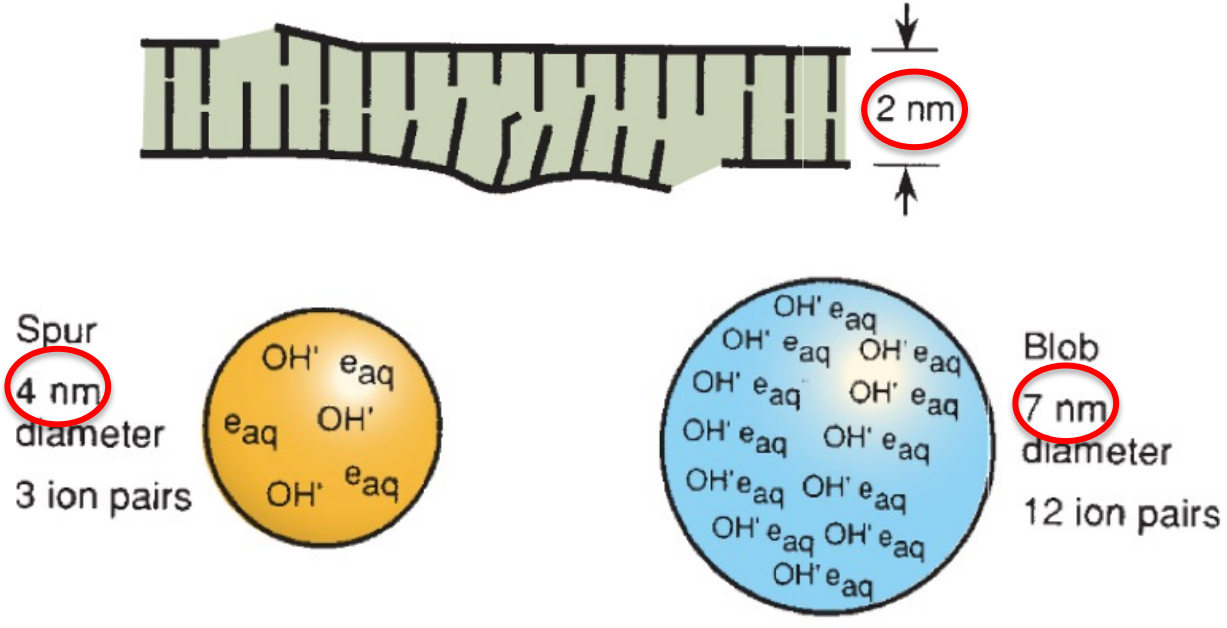
Sparsely ionizing – low LET
95% of the energy deposition of x- or γ-rays

Blob



Densely ionizing – high LET
Less frequent for x- or γ-rays

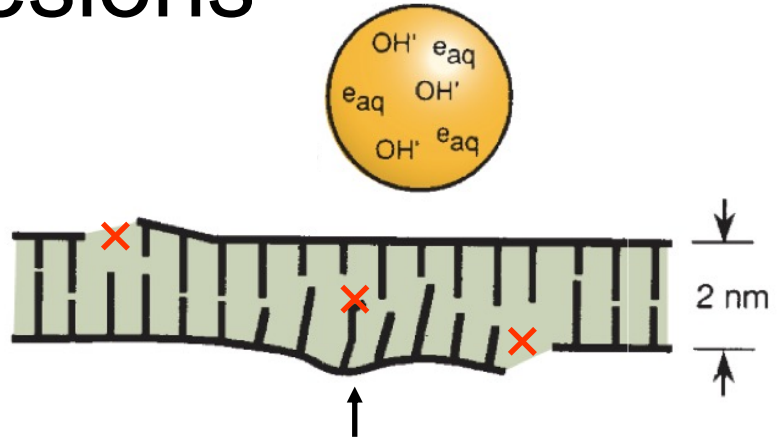
“Spurs” & “Blobs”



Spurs and blobs have dimensions similar to the DNA double helix

Clustered Lesions

OH• can diffuse

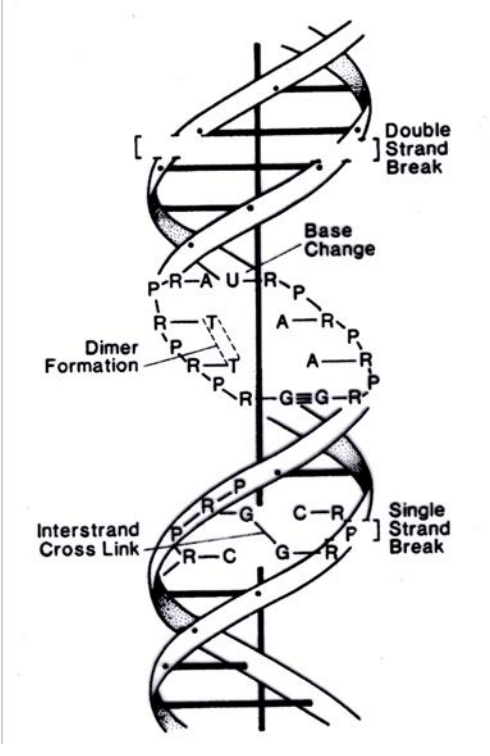


Strand breaks are accompanied by base changes

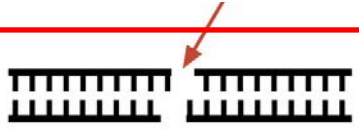
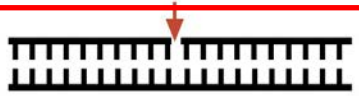
- Because spurs and blobs have dimensions similar to the DNA double helix, multiple radical attack can occur to cause a variety of complex lesions = **clustered lesions** (aka **locally multiply damaged site**)
- Given the size of the spur and the diffusion distance of OH free radicals, the multiple damage could be spread out up to **20 base pairs**

Yield of DNA Damage

- For mammalian cells, the # of DNA lesions <u>per cell</u> by **1 Gy** of x-ray is approximately
  - Base damage – > 1,000
  - SSB – 1,000
  - DSB – 40
- The yield of DSB is ~ 0.04x of SSB



Yield of DNA Damage by X-ray

Type of lesion		Number/Gy
Double strand breaks		40
Single strand breaks		500-1000
Base damage		1000-2000
Sugar damage		800-1600
DNA crosslinks		30
DNA-protein crosslinks		150

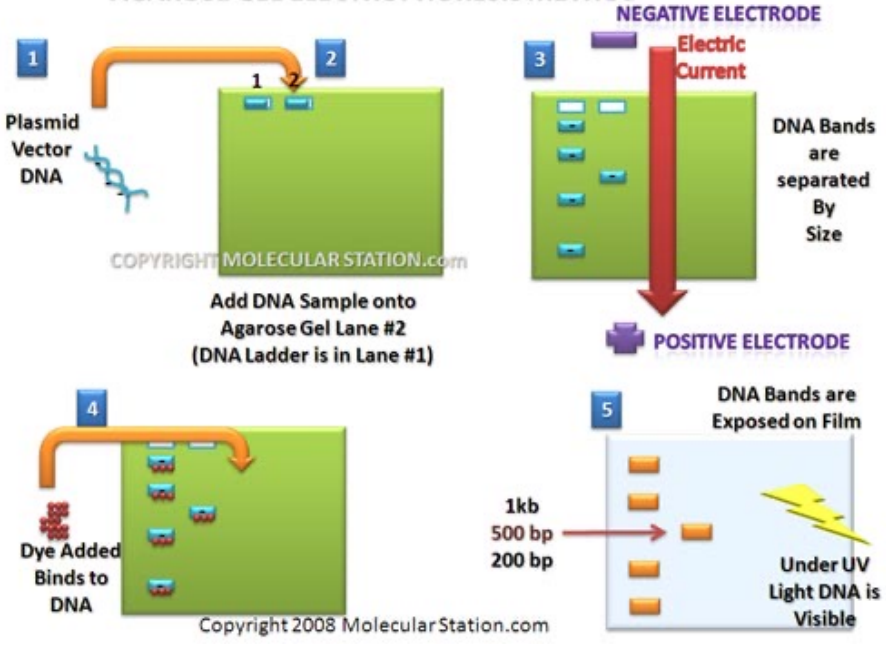
Outline

- General Overview of DNA Damages
- **Measuring DNA Strand Breaks**
- DNA Repair Pathways
- Chromosomes and Cell Division
- Radiation-Induced Chromosome Aberrations
- Chromosome Aberrations in Human Lymphocytes
- Summary

Measuring DNA Breaks

- Both SSB and DSB can be measured
- A variety of techniques have been developed
  - Sucrose gradient sedimentation
  - Alkaline and neutral filter elution
  - Nucleoid sedimentation
  - Pulsed field gel electrophoresis (PFGE)
  - Single-cell gel electrophoresis (aka comet assay)
  - DNA damage-induced nuclear foci assay

Principle of Agarose Gel Electrophoresis



DNA is negatively charged

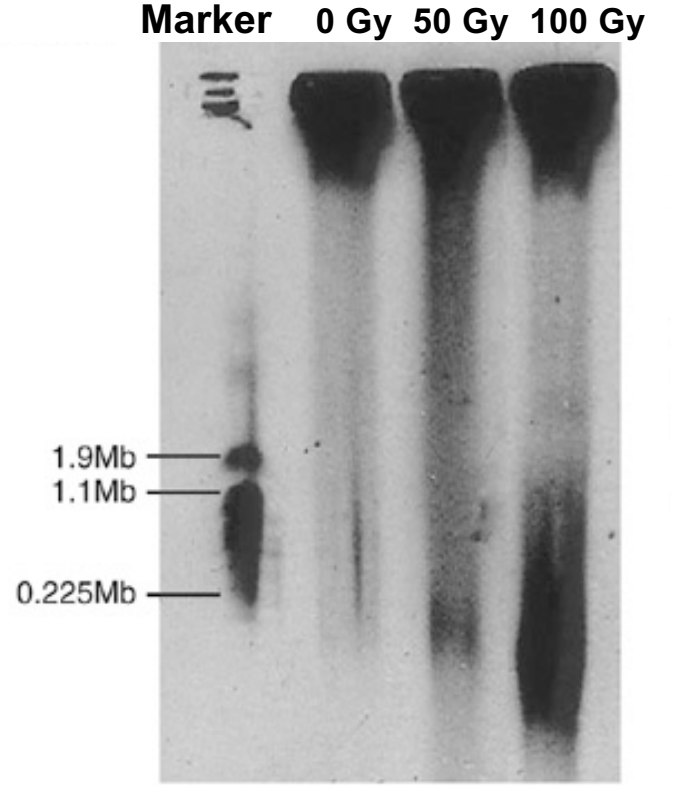
Smaller pieces move faster and farther than larger pieces of DNA

DSB are measured in **neutral** preparation

SSB are measured by denaturing and lysing the DNA in an **alkaline** preparation

By introducing an alternating voltage gradient (**pulsed field**), larger molecules in mega base pair range can be separated (**Pulsed Field Gel Electrophoresis**)

Measuring Radiation-Induced DSB by PFGE



Cells are irradiated with ↑ doses of IR

↓

DNA isolated in neutral solution

↓

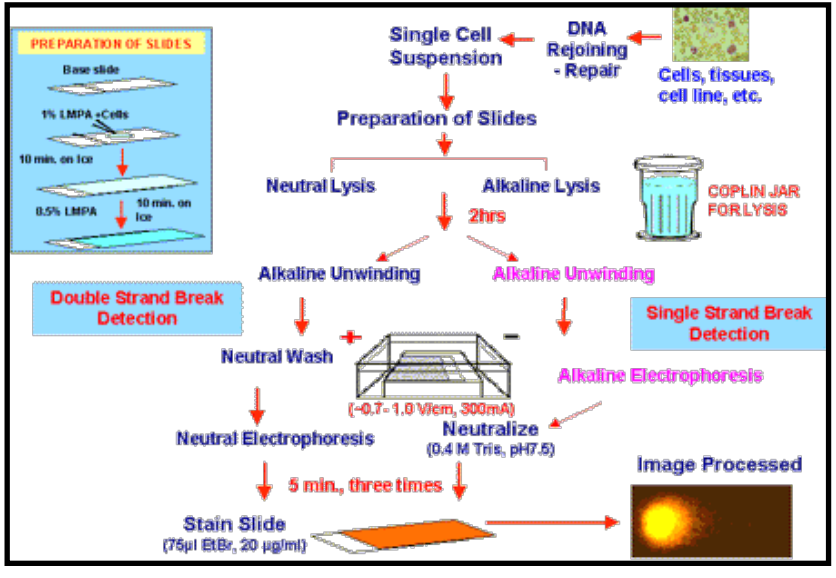
DNA pieces are separated by PFGE

The higher the radiation dose, the more the DNA is broken up into smaller pieces

The fraction of DNA released from the agarose plug is directly proportional to the dose

Comet Assay (Single-Cell Gel Electrophoresis)

- Measures DNA damage and repair at **the single-cell level**
- Cells are exposed to IR, embedded in agarose, lysed under neutral (for DSB) or alkaline (for SSB) buffer, and subjected to electrophoresis



Comet Assay

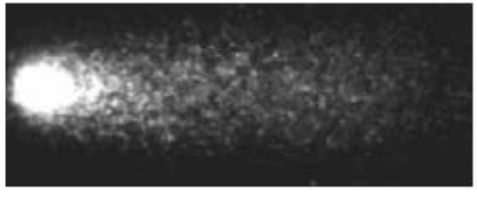
If cells are undamaged, the DNA remains compact

Fragmented DNA in irradiated cells that migrates takes the appearance of a comet

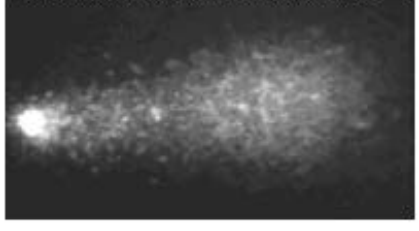
No significant damage

Small amount of damage

Large amount of damage



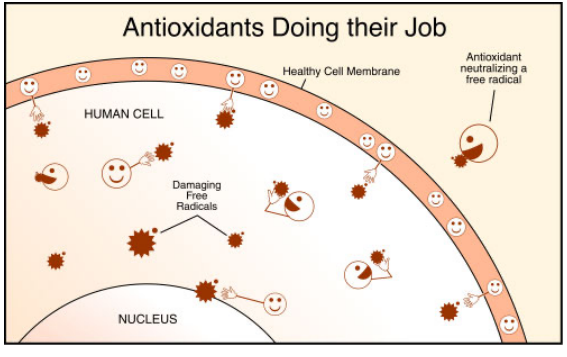
Very large amount of damage

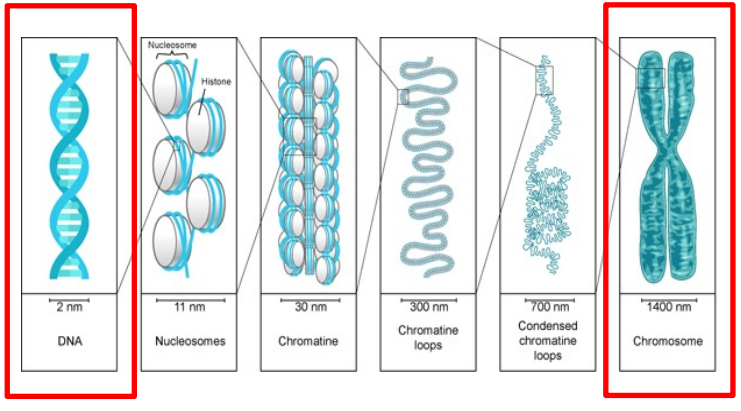


The length and fragment content of the tail is directly proportional to the amount of DNA damage

Cellular DNA vs. Free DNA

- In both assays, intact cells were irradiated
- DNA in cell is much more resistant to radiation damage than would be expected from free DNA in solution, because
  - Low molecular weight scavengers in the cells can mop up some free radicals
  - DNA packaging provides physical protection



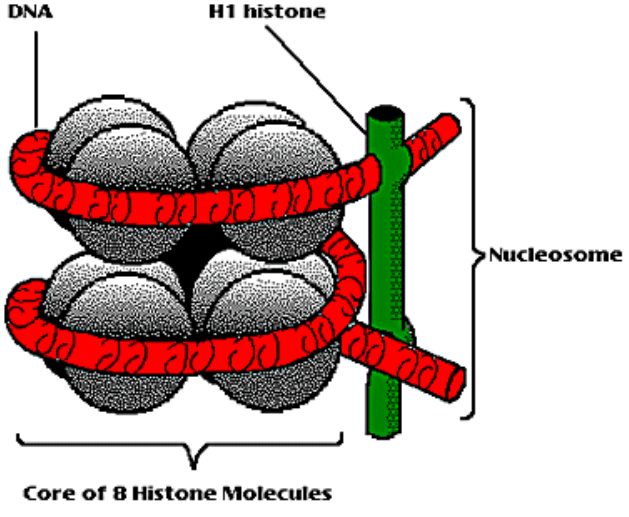


DNA Damage-Induced Nuclear Foci Assay

- In response to ionizing radiation, complexes of signaling and repair proteins localize to the sites of DNA strand breaks within the nucleus
- The appearance and disappearance of repair-related proteins recruited to sites of DSBs can be used both as dosimeters, and as a means of measuring DNA repair kinetics
- γ-H2AX and 53BP1 are commonly assayed proteins for foci formation

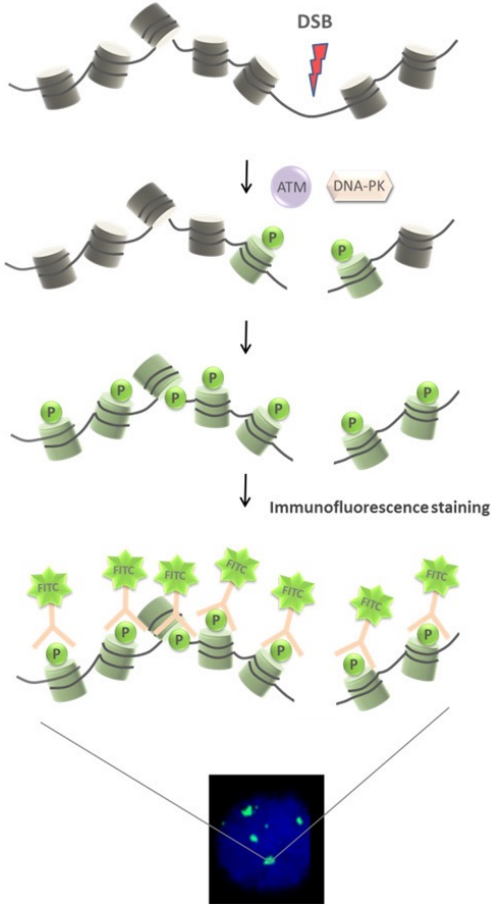
Histone Octamers

- The nucleosome contains an octamer of core histones: H2A, H2B, H3, H4
- Histone H2A has nine subtypes, among them the H2AX variant, which is involved in the response to DNA damage



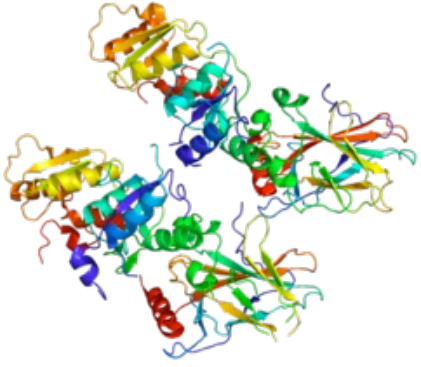
γ-H2AX Focus Assay

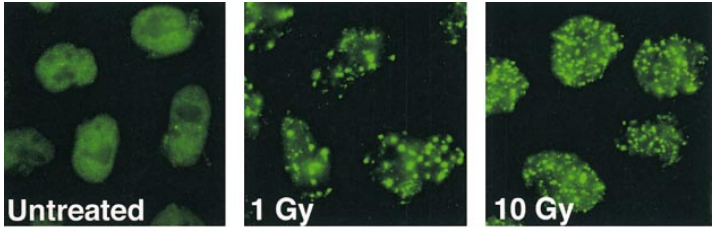
- Production of DNA DSBs by ionizing radiation leads to the rapid phosphorylation of histone H2AX on serine 139 (γ-H2AX)
- The specificity of this reaction provides a reliable yardstick for DSBs and the means to spatially localize DSBs within the nuclei of cells
- This is known as the **γ-H2AX focus assay**



Muslimovic 2012

53BP1 Focus Assay





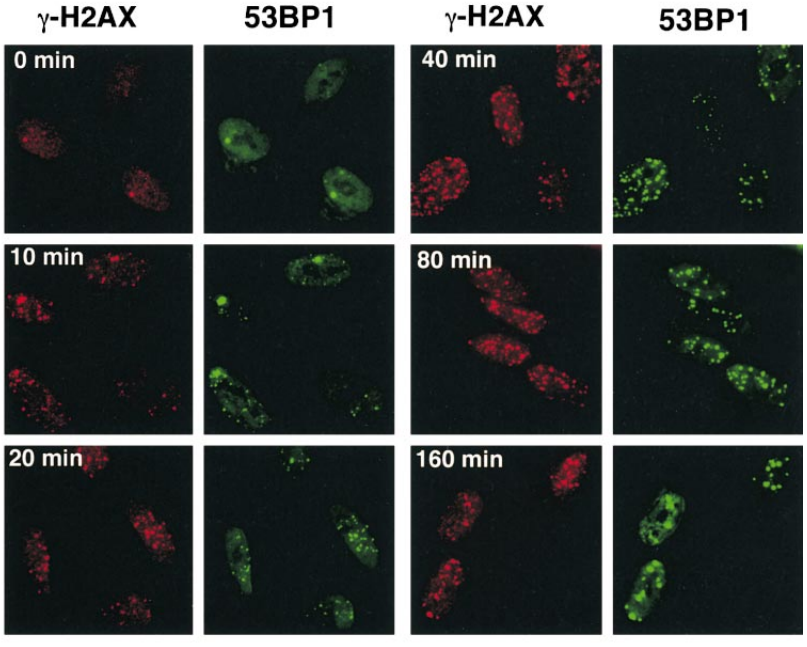
Hela cells irradiated with 1 Gy or 10 Gy

Note that p53BP1 becomes phosphorylated and forms nuclear foci in response to DNA damage

- **p53BP1** = p53 binding protein 1
- Interacts with the central DNA-binding domain of p53
- Enhances p53-depedent transcription
- **Involved in DNA damage-signaling pathways** and is regulated by ATM
- Under-expressed in most cases of triple negative breast cancer

Rappold 2001; JCO 153 (3): 613-620

53BP1 Co-localizes with γ-H2AX



Hela cells were treated with 1 Gy γ-irradiation

Co-immunostained with anti-53BP1 antibody and anti-γ–H2AX antibody

Other proteins forming foci in response to DNA damage include **ATM**, **RPA**, **RAD51**, **BRCA1**

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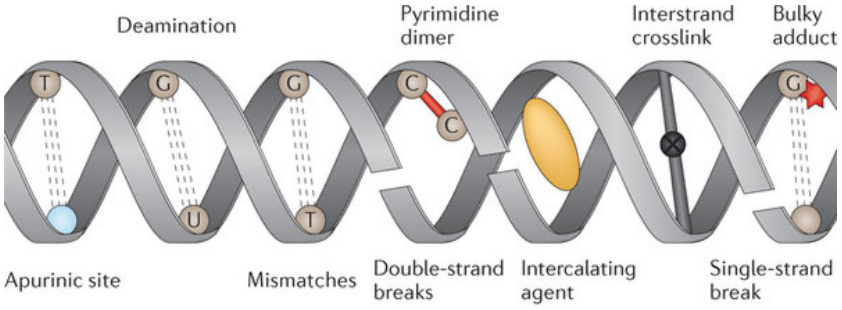
Sources of DNA Damage



Mammalian cells on average experience over 100,000 DNA lesions per day

Endogenous Sources

Replication error
Chemical decay of bases
Oxidative damage by free radicals

Exogenous Sources

UV (sunlight)
Pollution (hydrocarbon)
Smoking
Food
Ionizing radiation (background & therapy)
Chemotherapy

Carcinogen


CARCINOGENIC
NON-CARCINOGENIC
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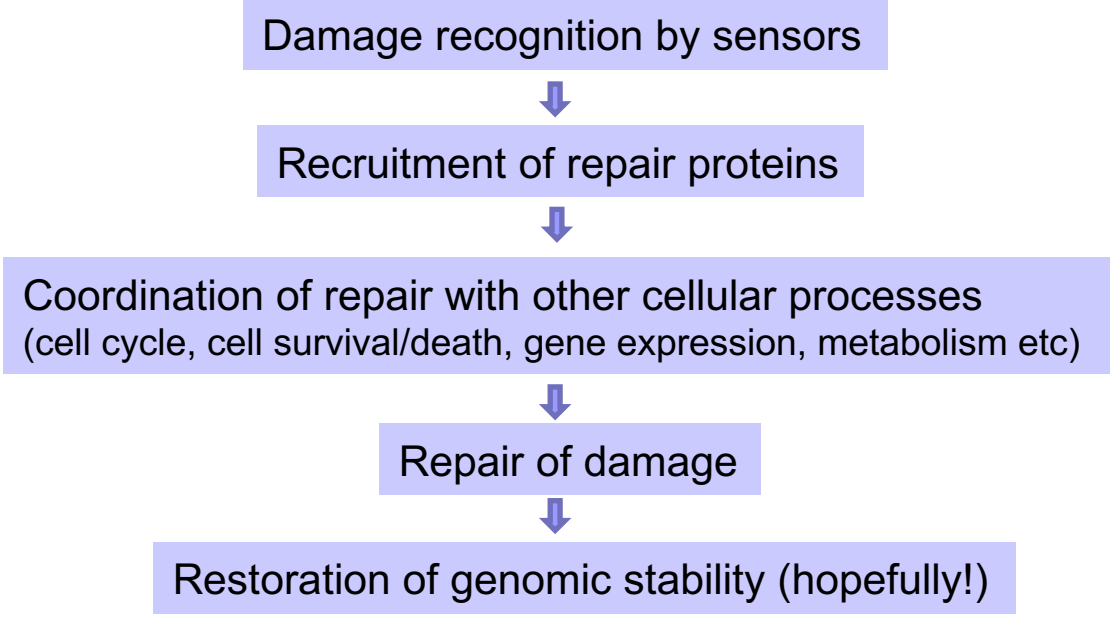
The DNA Damage Response (DDR)

- The presence of broken DNA initiates a chain of molecular events collectively known as the **DNA damage response (DDR)**, that consists of:



DNA Repair Pathways

- Reversal of Damage
- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- Cross-Link Repair
- Microhomology-Mediated End Joining (MMEJ)
- Mismatch Repair (MMR)
- Translesion Synthesis
- DSB Repair
  - Non-homologous End Joining (NHEJ)
  - Homologous Recombination Repair (HRR)

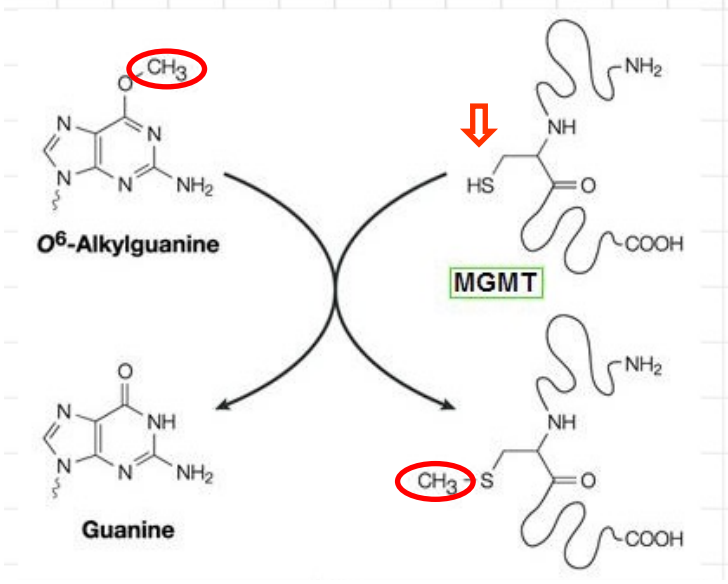
Repair Pathway Cheat Sheet

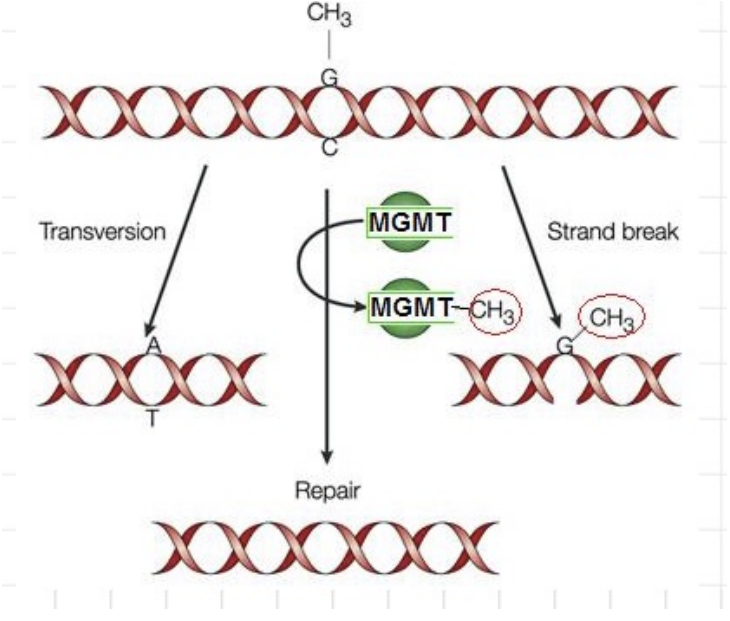
Repair Pathway	Major Players	Major Lesions Repaired
BER **Base Excision Repair**	Glycosylase Endonuclease XRCC1	Oxidative damaged bases
NER **Nucleotide Excision Repair**	XPA-XPG	UV pyrimidine dimers "Bulky" lesions
MMR **Mismatch Repair**	MSH2, MLH1	DNA replication error (Mismatches, Frameshifts)
NHEJ **Non-Homologous End Joining**	Ku70, Ku80, DNAPKcs	DNA double strand breaks (Error prone)
HRR **Homologous Recombination Repair**	ATM, RAD51, MRN	DNA double strand breaks (Error free)
Cross-Link Repair	FANC Proteins	DNA interstrand cross links

Physics students – Focus on key players and Key lesions. Use "Word Association". No need to memorize the details of the pathways.

Reversal of Damage

- Single step reaction to restore the genome
- Specific enzymes for specific damage, e.g., O^6-methylguanine-DNA methyltransferase **(MGMT)**





MGMT can remove the abnormal methyl group by transferring it to the sulfhydryl group of a cysteine in the active site.

Base Excision Repair (BER)

- Base excision repair pathway repairs **damaged bases** in DNA

Lesions Processed by BER

Oxidized Bases	▪ 8-oxoguanine ▪ 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG, FapyA)
Alkylated bases	▪ 3-methyladenine ▪ 7-methylguanine
Deaminated bases	▪ Hypoxanthine formed from deamination of adenine ▪ Xanthine formed from deamination of guanine
Uracil	▪ Inappropriately incorporated in DNA ▪ Formed by deamination of cytosine

BER Pathway

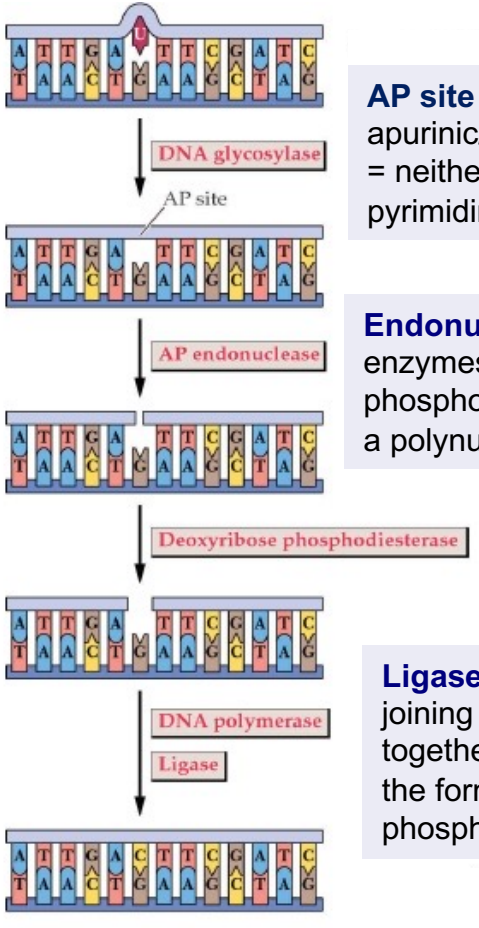
BER repairs Base Damages

Step 1:
Removal of damaged base by a **DNA glycosylase**

Step 2:
Cleaving of its deoxyribose phosphate in the backbone by an **AP endonuclease**, producing a gap

Step 3:
Removal of the sugar-phosphate lacking the base by **Deoxyribose phosphodiesterase (dRpase)**

Step 4:
Incorporation of a specific deoxyribonucleotide by the **DNA polymerase** and a **ligase**

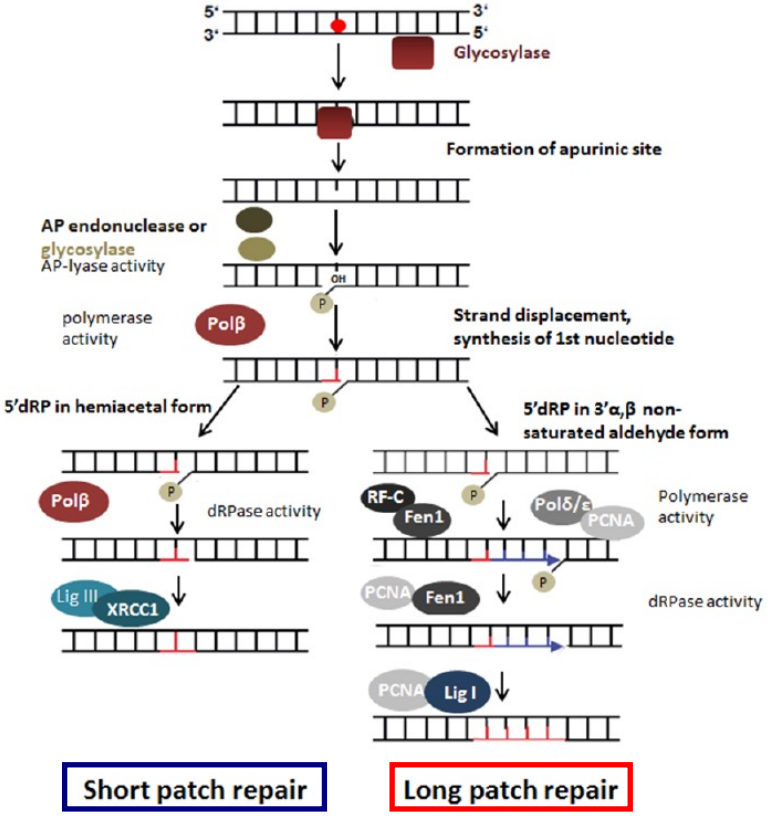


AP site = apurinic/apyrimidinic site) = neither a purine nor a pyrimidine base is present

Endonucleases are enzymes that cleave the phosphodiester bond within a polynucleotide chain.

Ligase facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond

BER



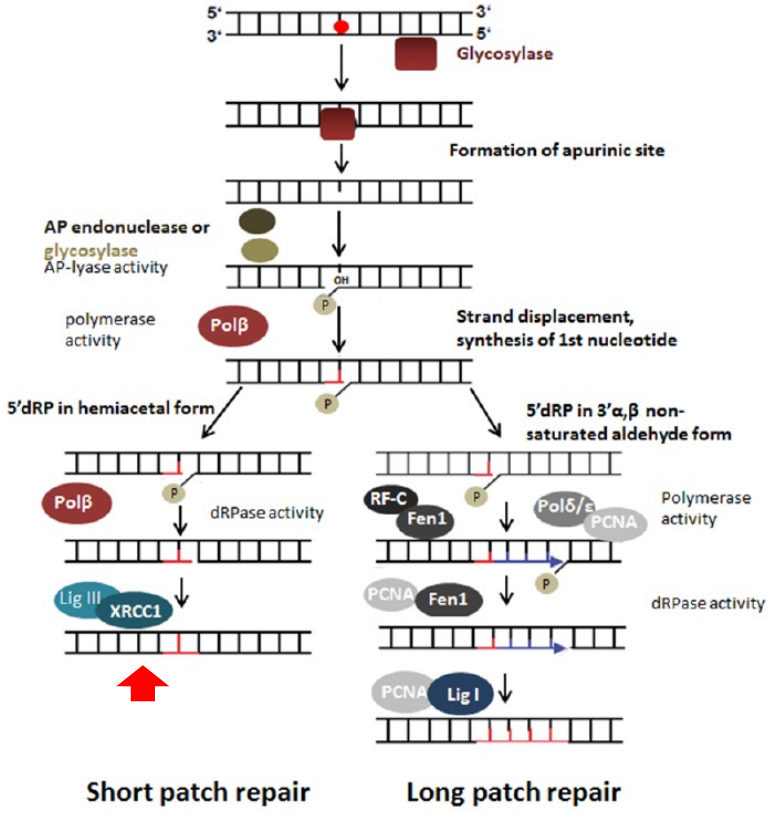
Short Patch Repair

- Repair synthesis = Polymerase β
- Ligation – DNA Ligase III-XRCC1

Long Patch Repair

- Repair synthesis – replication factor C (RFC)/proliferating cell nuclear antigen (PCNA)/DNA pol δ/ε
- Overhang flap removal – flap endonuclease I (FENI)
- Ligation – DNA Ligase I

BER



BER repairs damaged bases

Defects in BER may lead to an increased mutation rate

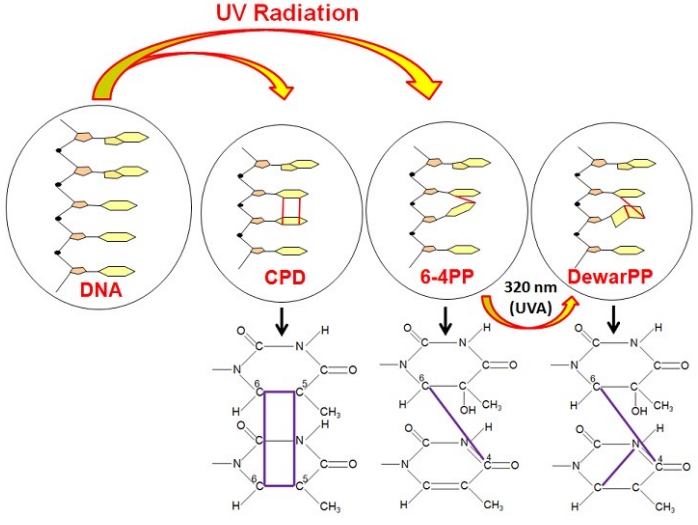
Defects in BER usually do not result in radiosensitivity (i.e, cell death)

Exception – **XRCC 1**

- Cells with *XRCC1* mutation are 1.7x more radiosensitive
- Likely due to XRCC1s' involvement in SSB repair

Nucleotide Excision Repair (NER)

- NER repairs **“bulky” lesions,** e.g., UV products



Cyclobutane pyrimidine dimer (**CPD**) & pyrimidine-pyrimidone (6-4) photoproduct (**64PP**) are major DNA lesions induced by UV light

- Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage

NER

NER repairs "bulky" lesions

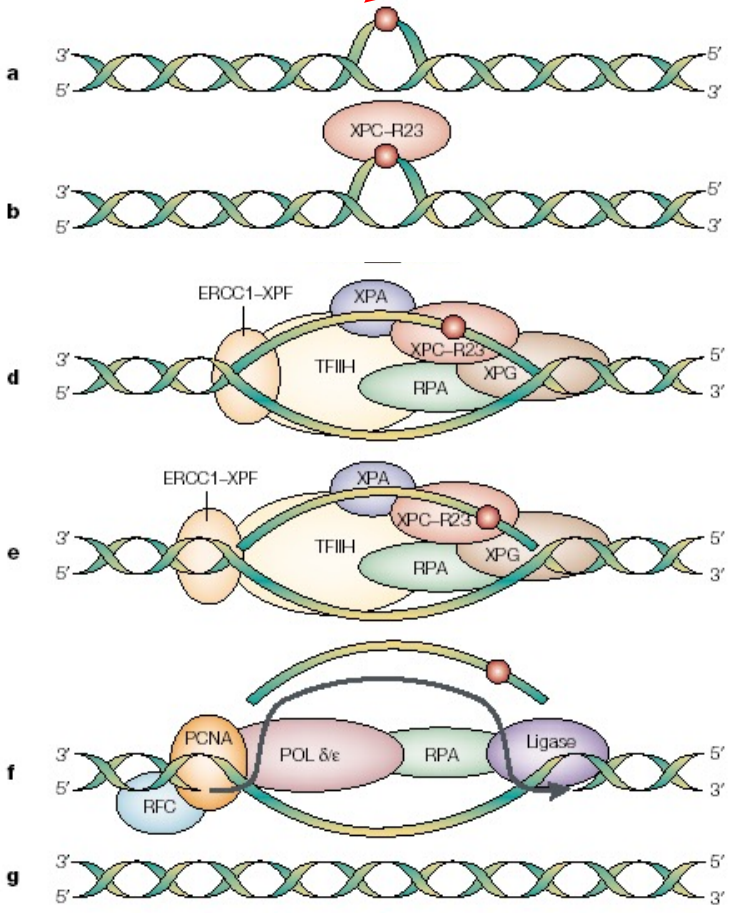
Step 1: Damage recognized by one or more protein factors that assemble at the location

Step 2: The DNA is unwound, producing a **"bubble"** . The enzyme system that does this is **Transcription Factor IIH**, TFIIH, which also functions in normal transcription

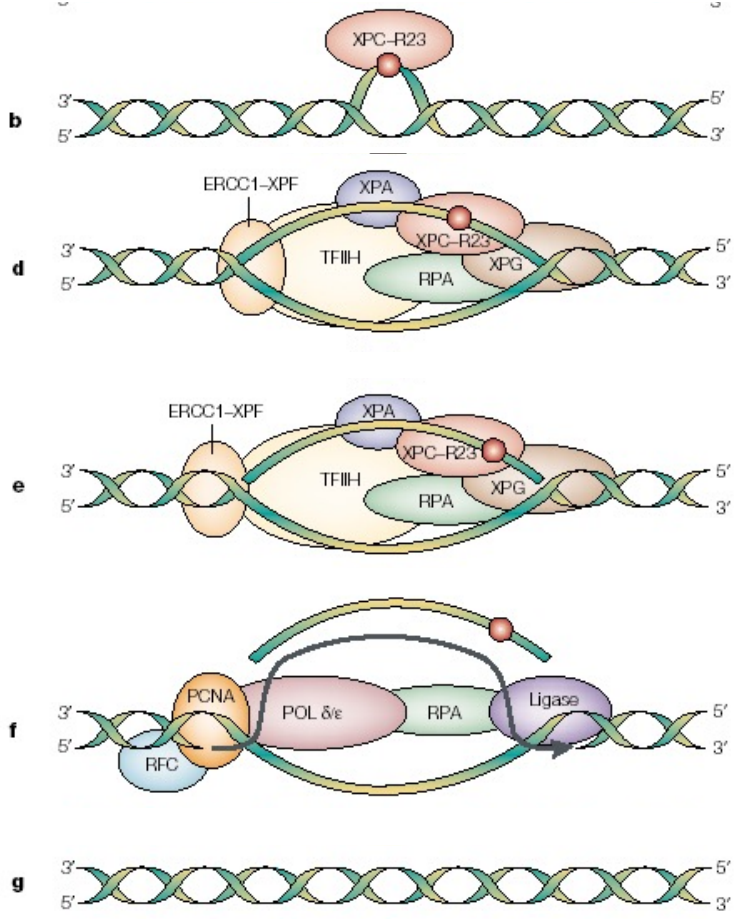
Step 3: **Cuts are made** on both the 3' side and the 5' side of the damaged area so the tract containing the damage can be removed

Step 4 : A fresh burst of DNA synthesis fills in the correct nucleotides. The **DNA polymerases** responsible are Pol δ and ε

Step 5 : A **DNA ligase** covalently binds the fresh piece into the backbone



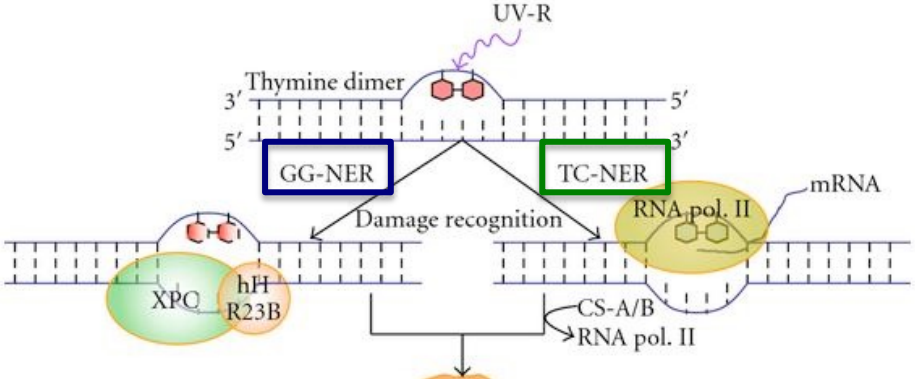
NER



- Damage is recognized by **XPC** & **RAD23B**, followed by binding of XPA, RPA, TFIIH, XPG
- **XPA** and **RPA** facilitate specific recognition of base damage
- **TFIIH** is a subcomplex of RNA polymerase II transcription initiation machinery
- TFIIH consists of 6 subunits, two of which (**XPB** and **XPD**) unwind the DNA duplex
- **XPG** is a DNA endonucleases that cuts the damaged strand at 3' site
- **ERCC1-XPF** cuts the damaged site at 5' site
- Incision generates an oligonucleotide **24-32 nucleotides** in length
- Repair synthesis requires **DNA polymerase δ or ε**
- Also requires several accessory replication proteins **PCNA, RPA, RFC**
- DNA is restored by **DNA ligase**

GG-NER & TC-NER

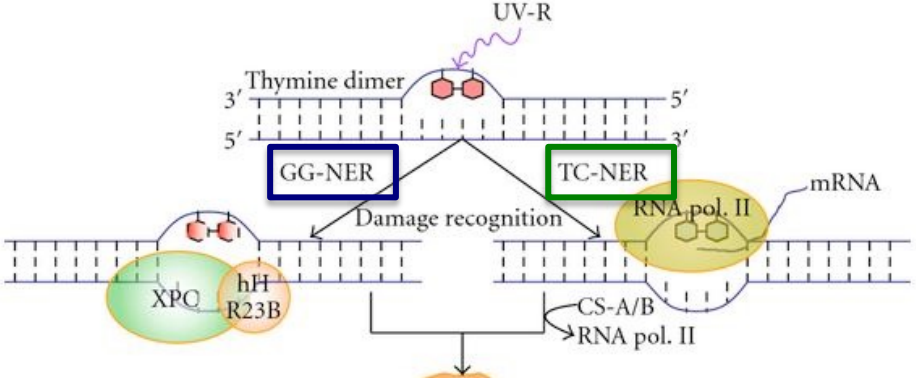
- NER can be divided into two subpathways
  - Global genome repair (**GG-NER**)
  - Transcription-coupled repair (**TC-NER**)



Repairs damage in both transcribed and non-transcribed DNA strands **throughout the genome**

Repairs lesions in the DNA strands of **actively transcribed genes**

GG-NER & TC-NER



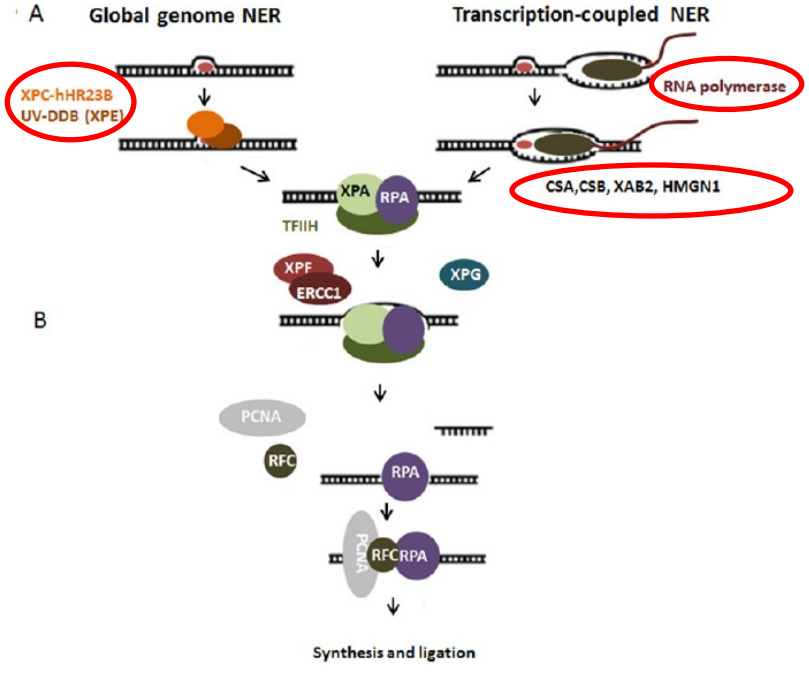
Cockayne Syndrome A and B

Damage recognition by **XPC-Rad23B** complexes

NER proteins are recruited by the **stalled RNA polymerase** in cooperation with **CSA and CSB**

- The two pathways differ only at the initial damage recognition step; the remainder of the pathways is the same for both
- NER proceeds most rapidly in cells whose genes are being actively transcribed

GG-NER & TC-NER



The two pathways differ only at the initial damage recognition step; the remainder of the pathways is the same for both

NER

- Mutation in NER genes does NOT lead to IR sensitivity
- Defective NER increases sensitivity to **UV-induced DNA damage** and anticancer agents such as alkylating agents that induce **bulky adducts**
- Germline mutations lead to several human DNA repair deficiency syndromes
  - Xeroderma Pigmentosum
  - Cockayne Syndrome
  - Trichothiodystrophy

Xeroderma Pigmentosum (XP)

- XP is a rare inherited disease of humans which, among other things, predisposes the patient to pigmented lesions on areas of the skin exposed to the sun and an elevated incidence of skin cancer

- XP can be caused by mutations in *XPA* through *XPG* plus a variant *XPV* – all of which have roles to play in NER

An eight-year old girl from Guatemala with Xeroderma Pigmentosum.

https://commons.wikimedia.org/w/index.php?curid=12628941

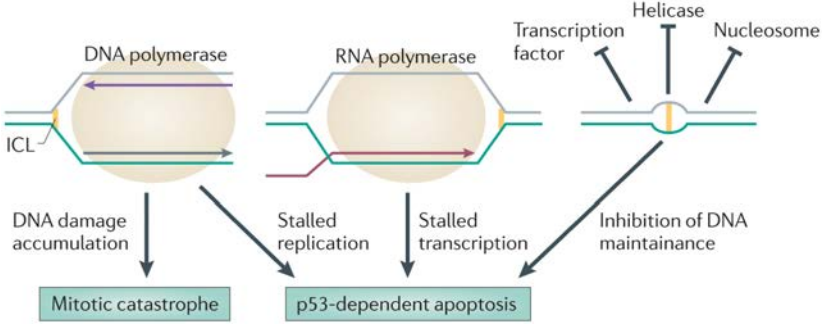
Cockayne Syndrome

- A rare autosomal recessive disorder characterized by short stature, premature aging, impaired development of nervous system and photosensitivity

- Caused by mutations in either the *ERCC6* gene (also known as the ***CSB*** gene) or the *ERCC8* gene (also known as the ***CSA*** gene)

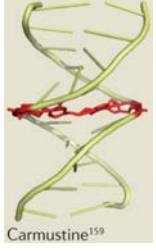
http://cockaynesyndrome.org/news/cs-drug-treatment-on-the-horizon/

Cross-Link Repair

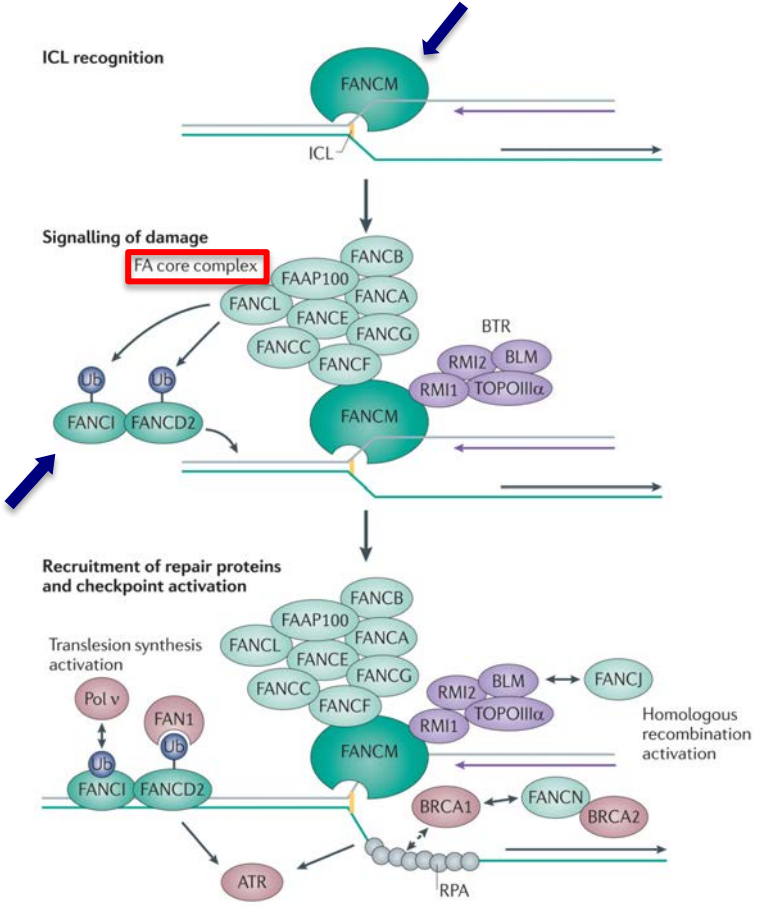


DNA interstrand crosslinks (ICL) are extremely toxic to cells because they prevent separation of the two strands of a DNA double helix, which is essential for cellular processes such as **replication** and **transcription**

Crosslinking agents used in the clinic

Drug	Clinical application	Dose-limiting toxicity	Example solution structure
Platinums			
Cisplatin	Testicular, ovarian and non-small-cell lung cancer	Central nervous system, renal and gastrointestinal toxicity[153]	 Cisplatin[152]
Carboplatin	Ovarian cancer	Neutropenia[154]	
Oxaliplatin	Colorectal cancer	Neuropathy[135,155]	
Satraplatin	Prostate and breast cancer	Thrombocytopenia and neutropenia[156,157]	
Picoplatin	Phase II trials for relapsed lung cancer and Phase I and II trials for the treatment of solid tumours, and prostate, colorectal and small-cell lung cancer	Thrombocytopenia and neutropenia[158]	
Nitrogen mustards			
Cyclophosphamide	Lymphoma	Neutropenia[160]	 Carmustine[159]
Melphalan	Multiple myeloma, melanoma and ovarian cancer	Leukopenia and thrombocytopenia[161]	
Chlorambucil	Chronic lymphocytic leukaemia	Pancytopenia and neurotoxicity[162]	
Ifosfamide	Non-small-cell lung cancer	Leukopenia, thrombocytopenia and renal toxicity[163]	
Others			
Mitomycin C	Oesophageal and bladder cancer	Leukopenia and thrombocytopenia[165,166]	 Psoralen[164]
Psoralen plus ultraviolet A radiation	Cutaneous T cell lymphoma	Dermatitis[167,168]	
Pyrrolobenzodiazepines	Phase II trial for solid tumours	Fatigue and thrombocytopenia[169]	

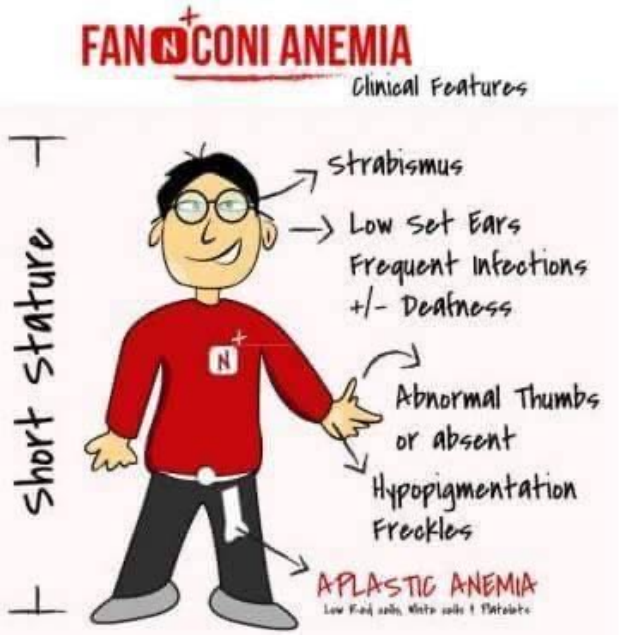
Cross-Link Repair



- The **Fanconi anemia (FA) pathway** is essential for the repair of DNA interstrand cross-links (ICL)
- At least 15 FA gene products constitute this pathway
- ICL at stalled replication fork is recognized by **FANCM**
- FANCM then recruits **FA core complex** (8 FA proteins)
- FA core complex monoubuquitinates FANCD2-FANCI, which then coordinates multiple DNA repair activities required for the resolution of ICLs
- Recent studies have demonstrated how the FA pathway coordinates three critical DNA repair processes, including **nucleolytic incision, translesion DNA synthesis (TLS)**, and **homologous recombination (HR)**

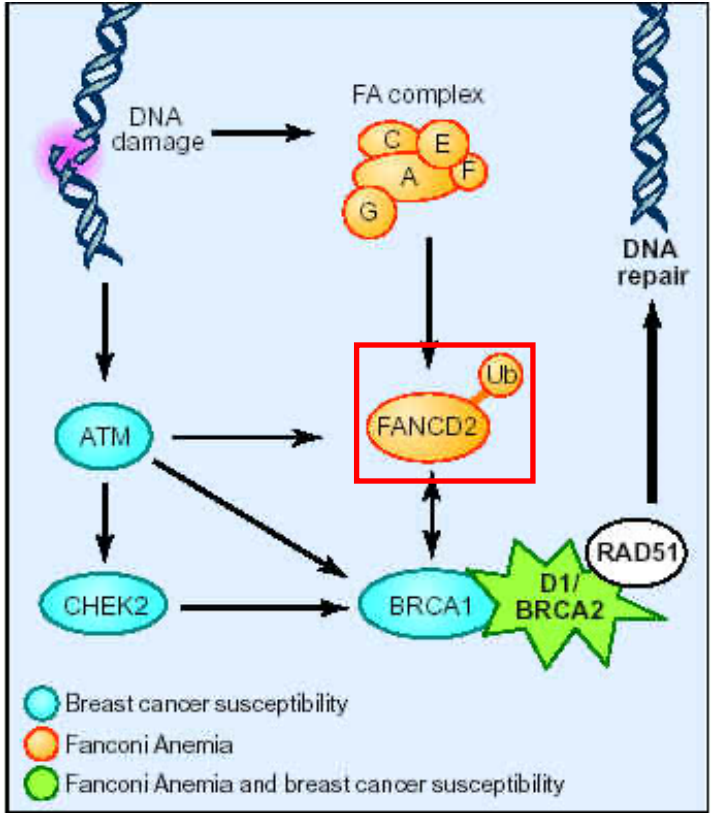
Fanconi Anemia

- An autosomal recessive disease
- Clinically FA is characterized by short stature, skeletal anomalies, increased incidence of solid tumors and leukemia, bone marrow failure
- At least 15 genes have been implicated in FA
- ***FANCD1*** = *BRCA2*
- ***FANCD2*** is a key player in the FA pathway



FANCD2

- FA core complex leads to ubiquitination of the D2 proteins
- FANCD2 is also a substrate of ATM
- FANCD2 co-localizes with **BRCA1/BRCA2/RAD51/MRE11** → implies a role in homologous recombination
- Cells from FA patients are **hypersensitive to DNA-crosslinking agents** (e.g. mitomycin C)
- Tumor cells from FA patients are hypersensitive to ionizing radiation

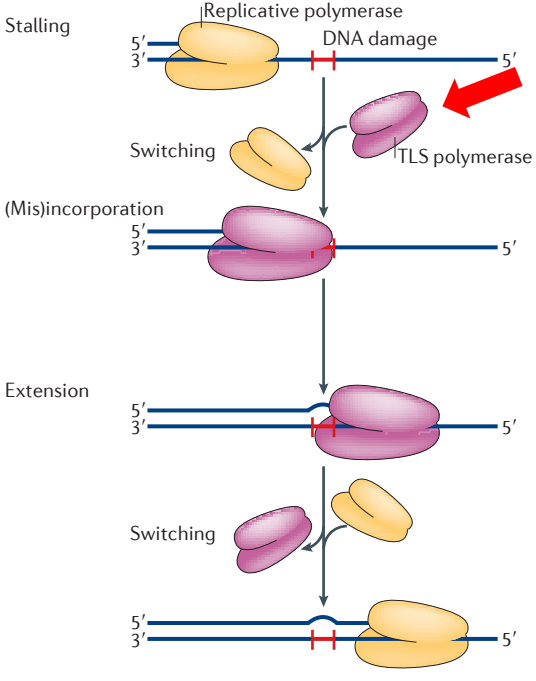


Translesion Synthesis

- Translesion synthesis can **bypass** a replication block caused by various DNA damage
- It accomplishes this by swapping out DNA Pol III with a less stringent version, a Y-family Polymerase, that can bypass the lesion
- Then the original polymerase swaps back in and continues with DNA replication…while damage remains behind

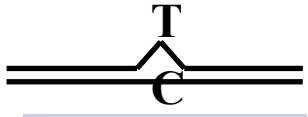
A mechanism of DNA Damage Tolerance

Polymerase Swapping



Mismatch Repair (MMR)

- Its function is to remove **base substitution** and **frameshift mismatches** that escape from DNA polymerase proofreading activity after DNA replication, increasing DNA replication fidelity 100- to 1000-fold
- Cells also use the MMR system to enhance the fidelity of recombination; i.e., assure that only homologous regions of two DNA molecules pair up to crossover and recombine segments (e.g., in meiosis).



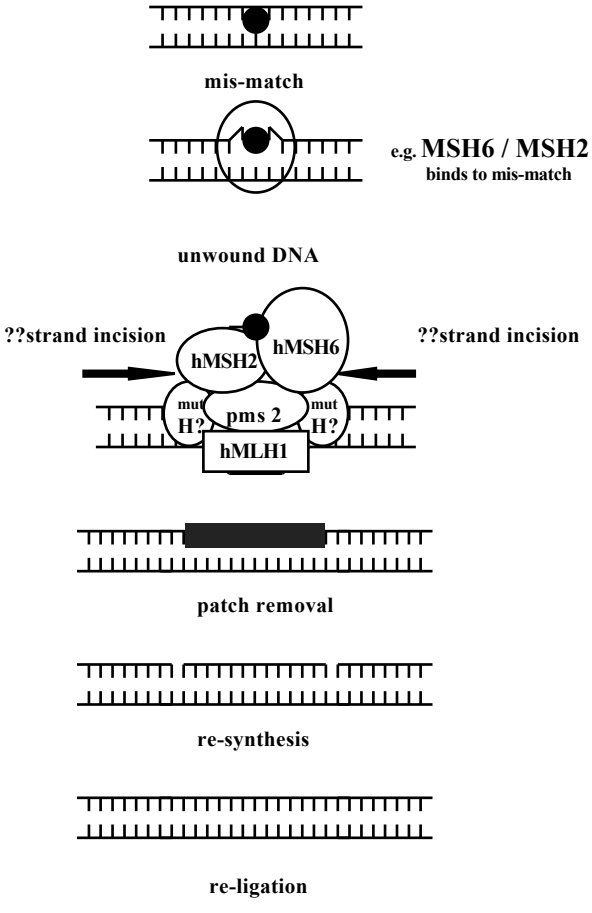
Base mismatch

Frameshift

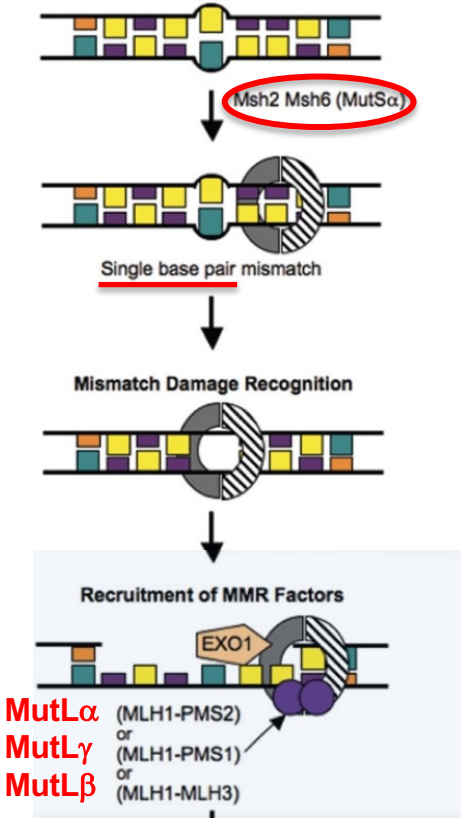
MMR

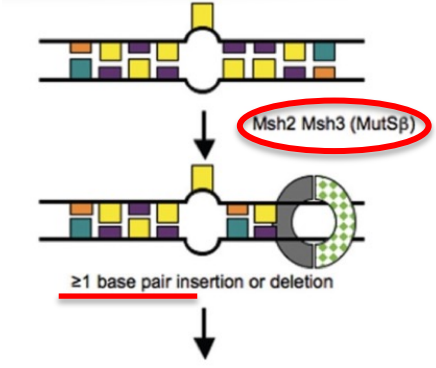
- Basic steps include
  - Recognition of mismatch
  - Recruitment of MMR factors
  - Excision of incorrect/altered nucleotides
  - Re-synthesis and ligation
- Recognition of a mismatch involves MutS homolog (in mammalian cells known as MSH proteins)
- Cutting the mismatch out involves MutL homolog (in mammalian cells known as MLH & PMS proteins)

MMR repairs mismatches in DNA



MMR



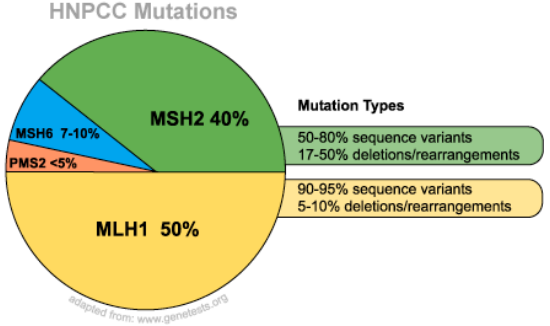


Eukaryotic equivalent of E *coli* MutS = **MSH proteins**

- MutL homologs (**MLH & PMS proteins**) are recruited to organize other proteins, such as PCNA, at the damaged site
- The MutL equivalent in humans exist in 3 heterodimeric forms, which have endonuclease activity

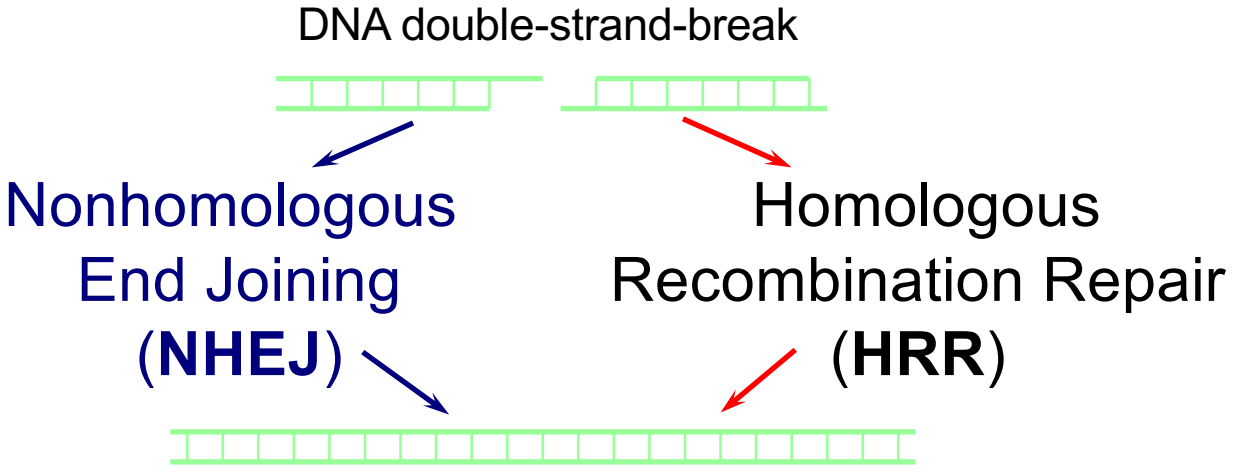
MMR

- Germline mutations in any one of five DNA MMR genes — *MSH2*, *MLH1*, *MSH6*, infrequently *PMS2* and, rarely, *PMS1,* are associated with **Lynch Syndrome** (**HNPCC** or Hereditary Non-Polyposis Colorectal Cancer)



- Affected individual has a high risk of **colon cancer**, as well as other cancers including **endometrium**, ovary, stomach, small bowel, hepatobiliary tract, upper urinary tract etc

Double Strand Break Repair

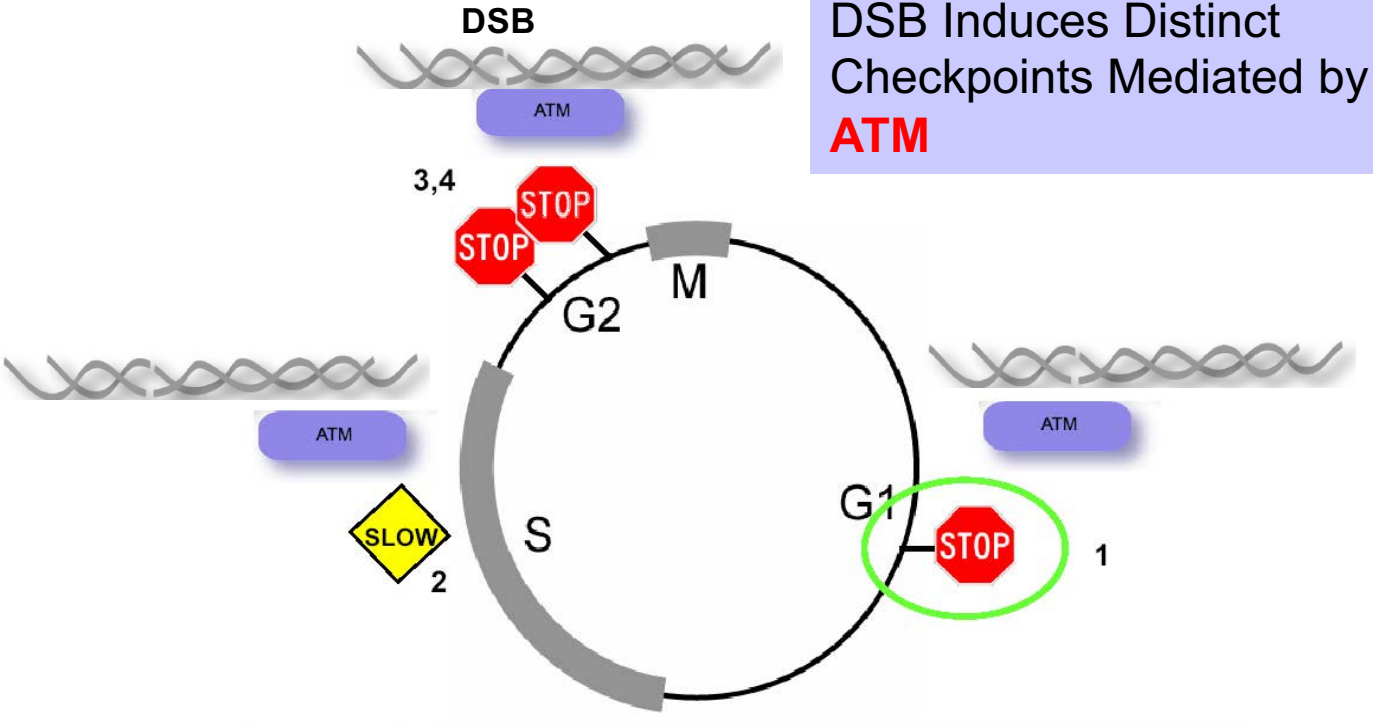


- No homology
- G_1 phase
- Error-prone

- Homologous template
- S/G_2 phase
- Error-free

Not Mutually Exclusive !!!

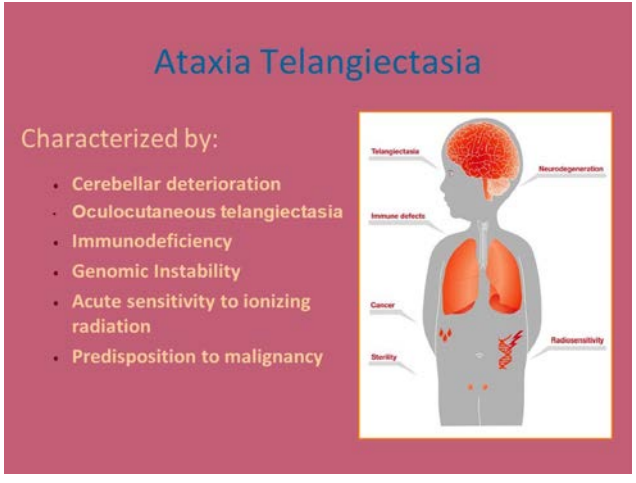
IR Induced Checkpoints

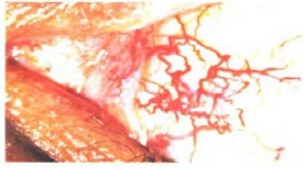


DSB Induces Distinct Checkpoints Mediated by **ATM**

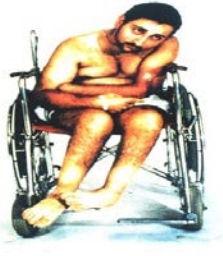
Ataxia -Telangiectasia Syndrome and ATM

- AT is a rare autosomal recessive disease
- AT patients exhibit a hypersensitivity skin reaction to IR and DNA breakage agents, but not UV light
- The genetic defect responsible for the AT phenotype is the ***ATM*** (ataxia telangiectasia mutated)





Oculocutaneous telangiectasia



Cerebellar ataxia

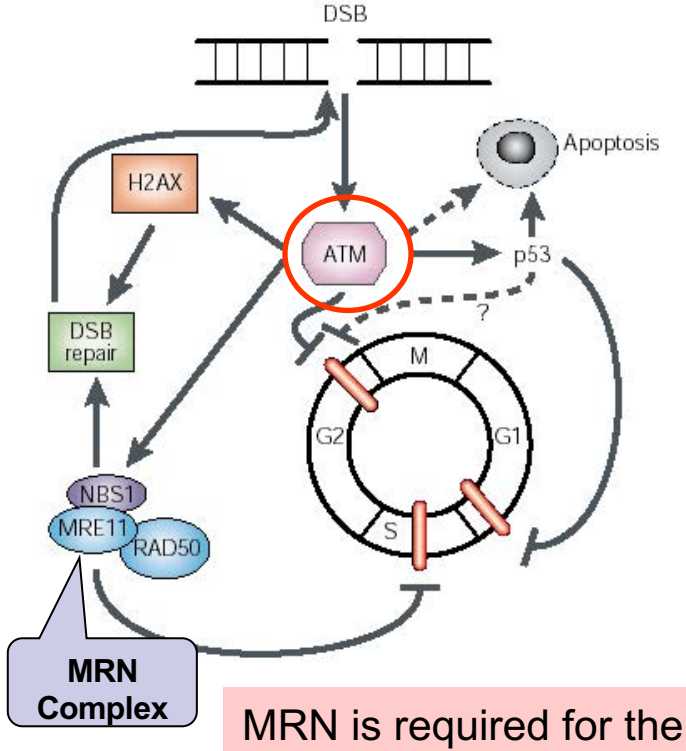
ATM

A kinase is an enzyme that transfers a phosphate group from ATP to target molecule

- ATM belongs to the phosphatidylinositol 3-kinase-related kinase **(PIKK) family**
- Other members of the PIKK family include **DNA-PKcs** (DNA-dependent protein kinase catalytic subunit) & **ATR** (AT and Rad3 related)
- **DNA-PKcs** plays an important role in **NHEJ**
  - Defect in DNA-PKcs results in **SCID** (Severe-Combined syndrome Immunodeficiency Syndrome) in mice
- **ATR** regulates DNA repair and cell-cycle control very much like ATM
  - ATR plays an important role in S-phase checkpoint
  - Individuals with reduced ATR levels develop Seckel's syndrome

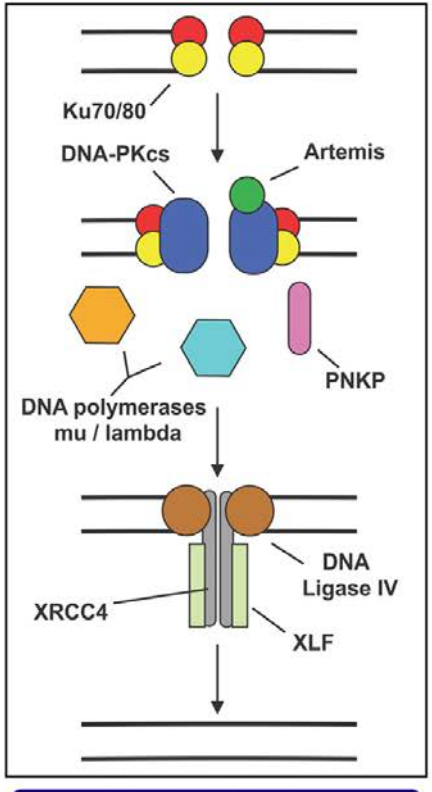
DSB Signaling and ATM

- The presence of DSB leads to activation of **ATM**
- ATM is involved in the rapid response of cells to DNA DSBs as well as **the activation of cell cycle checkpoints**
- ATM is a **kinase** (a kinase is an enzyme that transfers a phosphate group from ATP to target molecule)
- Many of the ATM functions are mediated by its downstream effectors, and the targets of ATM include **p53, NBS1, H2AX** etc



MRN is required for the full activation or functioning of ATM

NHEJ



Classical NHEJ
Active throughout the cell cycle

Step 1: End detection & Tethering

- **Ku70** & **Ku80** heterodimer detects and binds to the ends of DSB
- Ku70/80 recruits **DNA-PKcs** (DNA-dependent protein kinase catalytic subunit) which is a protein kinase

Step 2: Processing

- IR frequently produces DNA termini that are nonligatable
- Removal of these nonligatable end groups are processed by nucleases such as exonuclease 1 (Exo1), Mre11, **Artemis**
- DNA polymerase μ and λ fill in the gaps
- Processing leads to loss or modification of nucleotides, therefore NHEJ is inherently **error-prone**

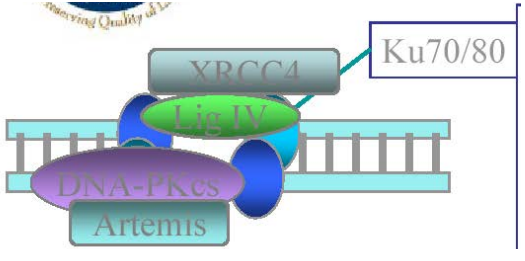
Step 3: Ligation

- DNA ends are re-ligated by **DNA ligase IV**, in complex with XRCC4 and XRCC4-like factor (XLF, also known as Cernunnos)

Artemis

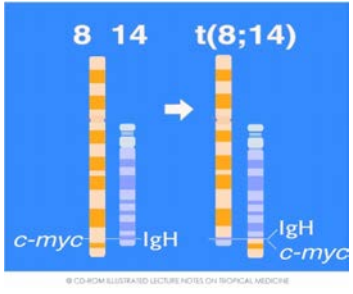
- Artemis is an endonuclease which processes DNA ends
- It is a substrate of DNA-PKcs, i.e., DNA-PKcs phosphorylates Artemis
- Mutations in Artemis is responsible for human SCID syndrome

The moon goddess

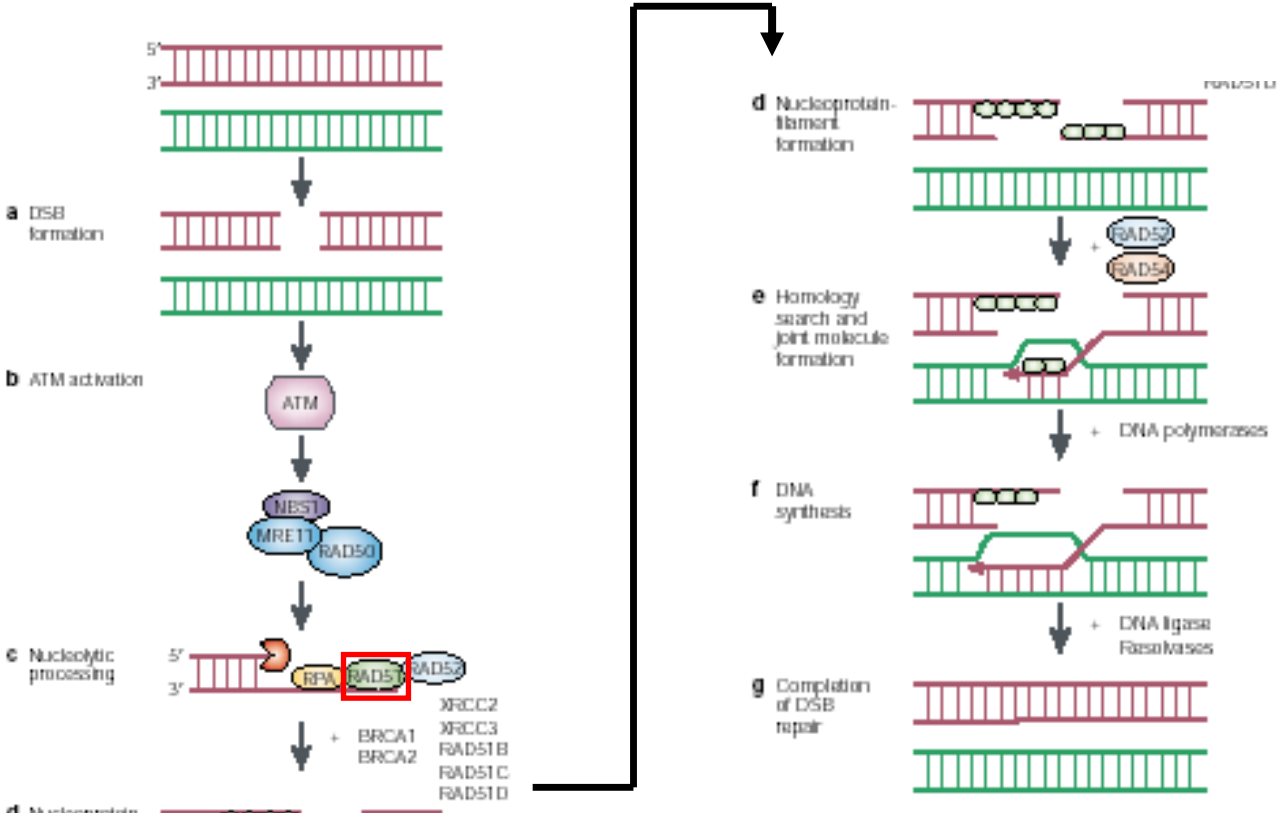


NHEJ

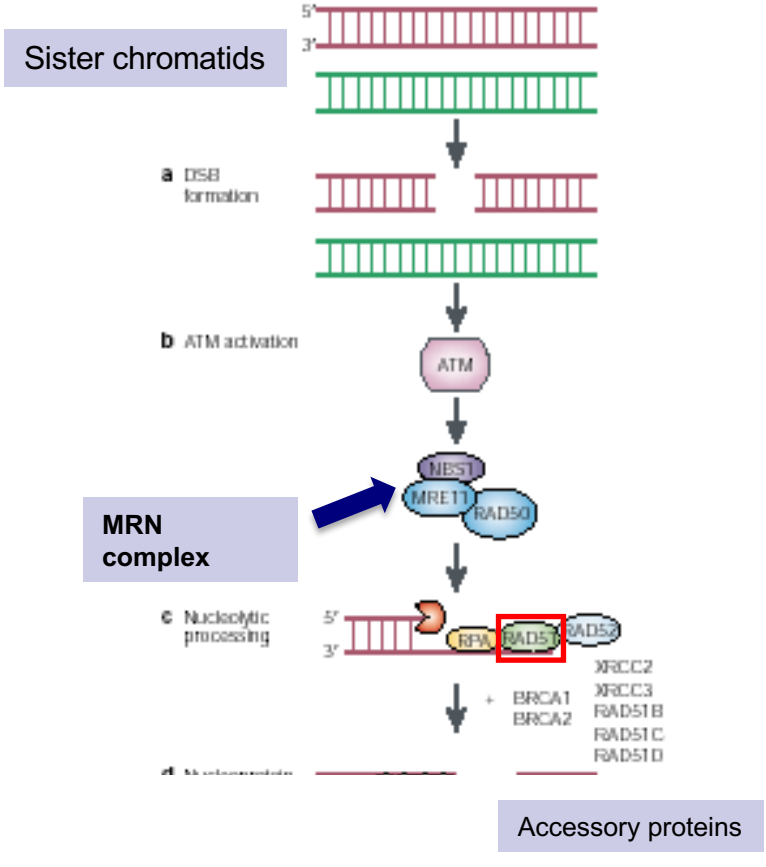
- **NHEJ is responsible for the generation of antibody diversity**
  - Some of the same enzymes used to repair DSBs by direct joining are also used to break and reassemble the gene segments used to make antibody variable regions; that is, to accomplish V(D)J joining
- **Errors in direct joining may be a cause of the various translocations that are associated with cancers**
  - Burkitt's lymphoma
  - The Philadelphia chromosome in chronic myelogenous leukemia (CML)
  - B-cell leukemia



HRR Pathway


5'
3'
a DSB formation
b ATM activation
ATM
NBS1
MRE11
RAD50
c Nucleolytic processing
5'
3'
RPA
RAD51
RAD52
+ BRCA1
BRCA2
XRCC2
XRCC3
RAD51B
RAD51C
RAD51D
d Nucleoprotein-filament formation
RAD52
RAD54
e Homology search and joint molecule formation
+ DNA polymerases
f DNA synthesis
+ DNA ligase
Resolvases
g Completion of DSB repair

HRR Pathway

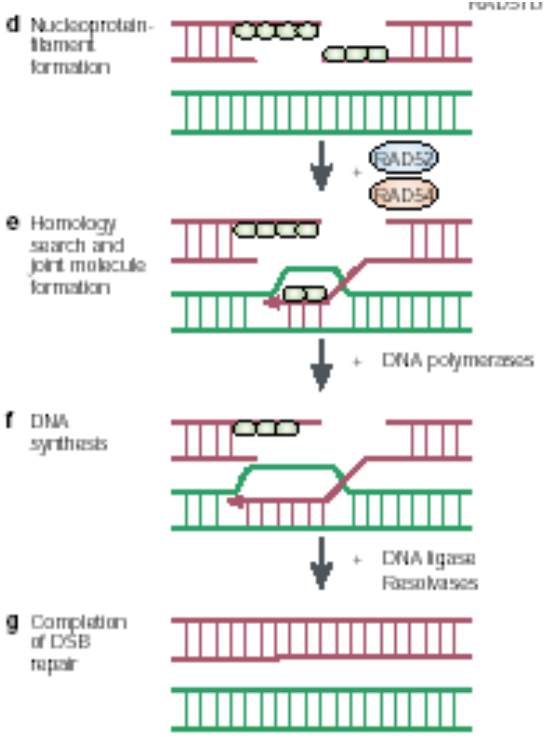


Unlike NHEJ, HRR requires physical contact with an undamaged chromatid to serve as a template for repair to occur

MRN (MRE11/NBS/Rad50) **complex** promotes sensing and repair of DNA ends
MRE11 is an endonuclease

RPA coats the SS DNA which is then bound by **RAD51**
Other proteins, including BRCA1, BRCA2, RPA, RAD52, RAD54, and several paralogous of RAD51 are thought to function as accessory proteins for RAD 51

HRR Pathway



RAD51 is a homologue of the E coli recombinase Rec A
RAD51 polymerizes onto the SS DNA to form nucleoprotein filament and searches for homologous duplex DNA

After the search is completed, DNA strand exchange is stimulated by RAD52 and RAD54, forming **Holliday junction** (4 DS arms joined)

DNA synthesis, which requires DNA polymerase and their accessory proteins, fills in the break in the strand

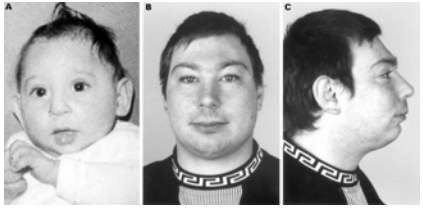
Ligation and resolution of recombination intermediates results in accurate repair of the DSB

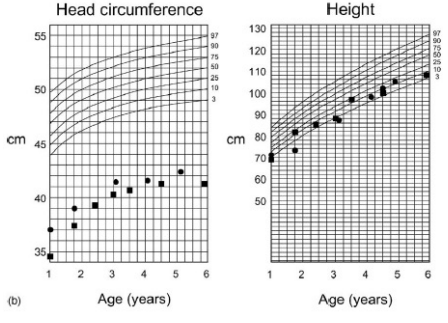
MRE11 and AT-Like Disorders (ATLD)

- Mutation in ***MRE11*** causes ATLD
- ATLD is an autosomal recessive disease which shares the clinical phenotype with AT, but milder
- Remember that MRE11 is required for functioning of ATM
- Cells derived from ATLD patients are radiosensitive
- ATLD cells are also defective in checkpoint response to DNA damage

NBS1 and Nijmegen Breakage Syndrome

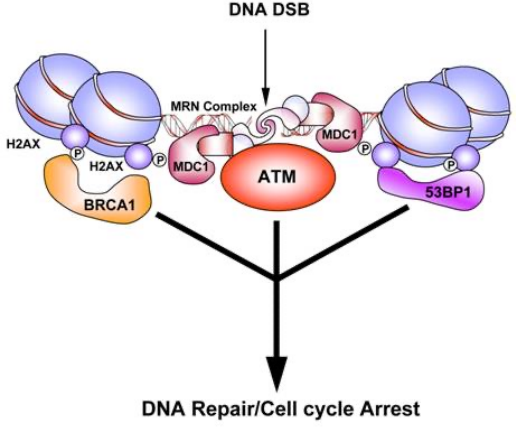
- NBS1 is a direct phosphorylation target of ATM
- Cells defective in NBS1 lack an S phase checkpoint and are radiosensitive
- Clinical features include microcephaly, radiosensitivity, immunodeficiency, increased cancer risk and growth retardation

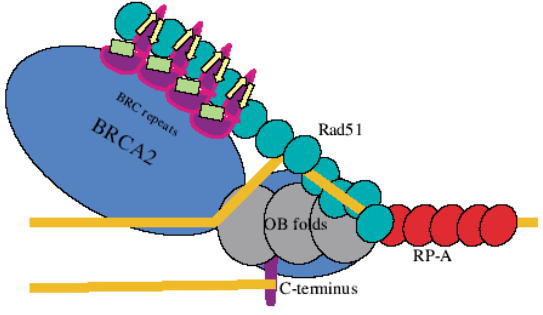




BRCA1 and BRCA2

- Individuals with mutations in BRCA1/2 are predisposed to breast and ovarian cancers
- BRCA1 and BRCA2 facilitate HRR



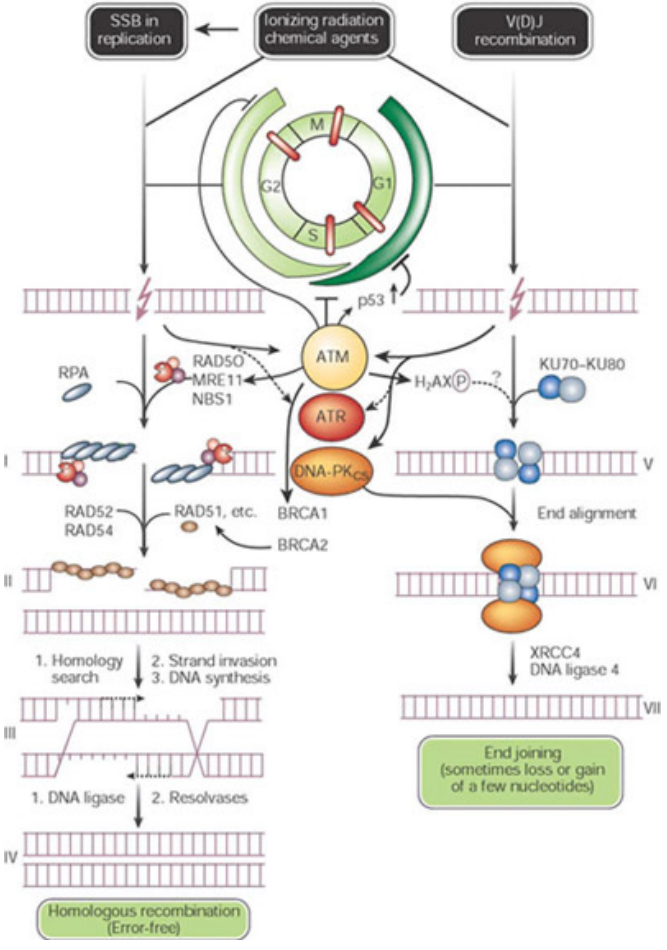


BRCA1 is recruited by H2AX to regulate the function of MRN
BRCA1 recruits BRCA2

BRCA2 primarily interacts with RAD51 to facilitate its assembly on the SSDNA

DSB Break Repair Summarized

- Homologous template
- S/G2 phase
- Error-free



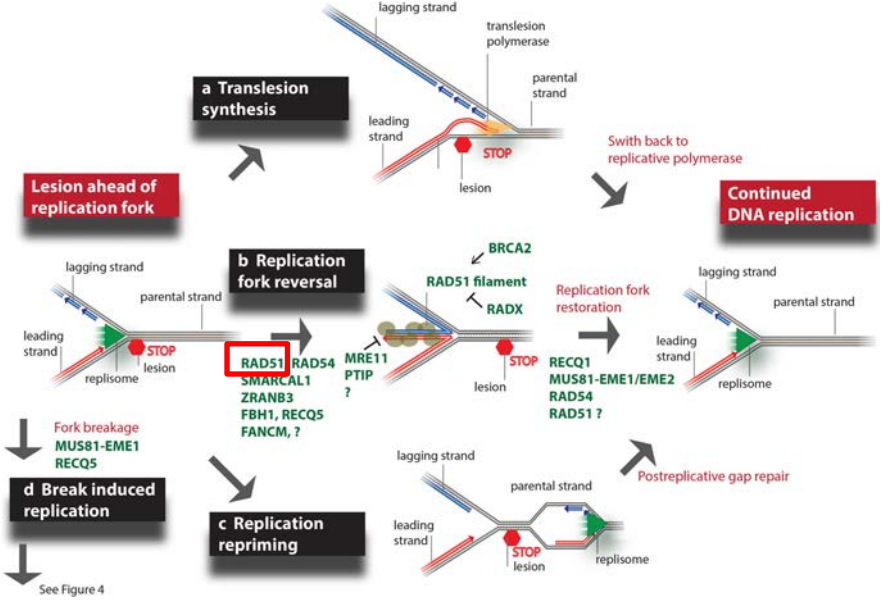
- No homology
- G1 phase
- Error-prone

Role of Recombination Proteins in Promoting the Stability of DNA Replication Forks

In addition to repairing DSBs, recombination contributes to the **repair of DNA cross-links** and **promotes the stability of replicating DNA**

Pathways that relieve DNA replication stress in human cells

Replication forks can break and become a substrate for homologous recombination



Ranjha 2018 – Chromosoma 127: 187-214

Micro-Homology Mediated End-Joining (MMEJ), aka Alternative NHEJ

- MMEJ works by identifying a small 5-25 bp homologies near the site of DSB, and using these to align the strands
- Any overhanging, non-homologous bases are pruned away before the break is ligated back together
- As such, this is an error-prone process – even more so than NHEJ – resulting in multiple small deletions and rearrangements
- MMEJ operates on its own (so aNHEJ is a misnomer) and is thought to account for ~ 10% of total DSB repair

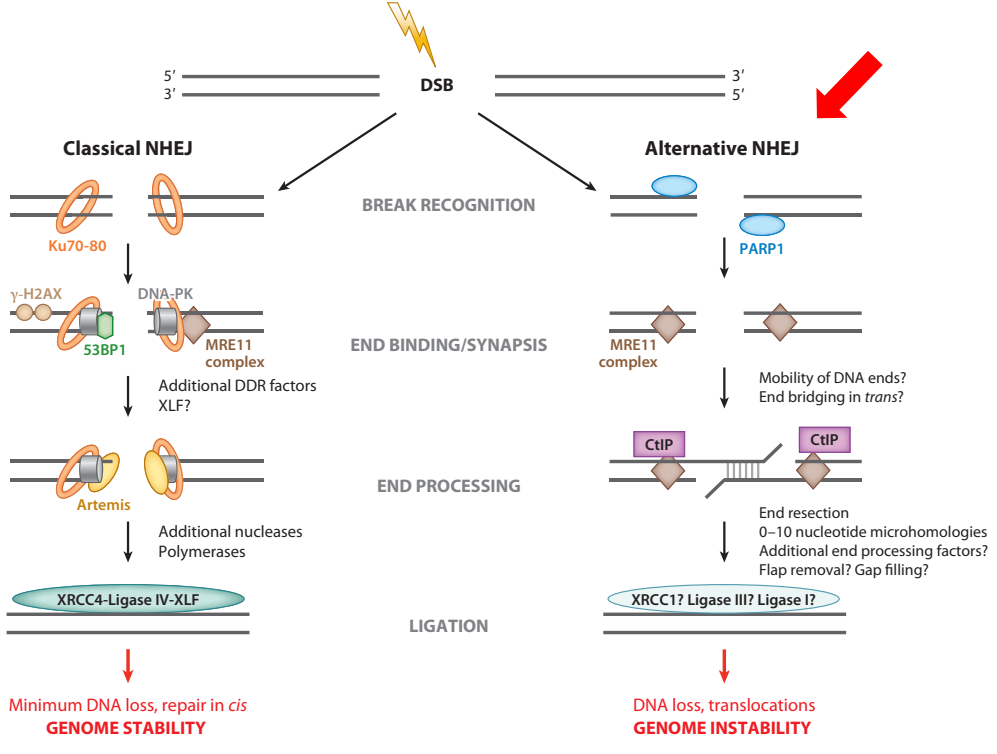


Figure 2

Classical and alternative nonhomologous end-joining (NHEJ) pathways. Abbreviations: DDR, DNA damage response.

Genes in Space

Vijayakumar, Sung, Li and Li's study will investigate the mechanisms of DNA repair in space. On Earth, DNA is shielded from radiation damage by the protective effects of the atmosphere and magnetic field. Astronauts traveling beyond Earth's protection are at serious risk of DNA damage from cosmic rays, including risk of double-strand breaks — a particularly harmful type of DNA lesion. Double-strand breaks are readily repaired by cells, but incorrect repair causes DNA mutations that may result in diseases such as cancer.

An astronaut on ISS will use the CRISPR/Cas9 gene editing tool in space for the first time to create targeted double-strand breaks in a yeast genome, which will then repair itself. Next, polymerase chain reaction and DNA sequencing will be used on the ISS to examine the repaired break sites for mutations caused by DNA repair. This experiment may provide insights on how cells repair their DNA in space, which could lead to better protection for astronauts' genomes. It will also enable the use of gene editing tools in space for the first time.

PLOS ONE

RESEARCH ARTICLE

A CRISPR-based assay for the study of eukaryotic DNA repair onboard the International Space Station

Sarah Stahl-Rommel[1], David Li[2☯], Michelle Sung[3☯], Rebecca Li[3☯], Aarthi Vijayakumar[3☯], Kutay Deniz Atabay[4,5☯], G. Guy Bushkin[4,5☯], Christian L. Castro[1], Kevin D. Foley[6], D. Scott Copeland[6], Sarah L. Castro-Wallace[7], Ezequiel Alvarez Saavedra[8], Emily J. Gleason[8☯], Sebastian Kraves[8☯]

1 JES Tech, Houston, Texas, United States of America, **2** Woodbury High School, Woodbury, Minnesota, United States of America, **3** Mounds View High School, Arden Hills, Minnesota, United States of America, **4** Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, **5** Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, United States of America, **6** Boeing Defense, Space & Security, Berkley, Michigan, United States of America, **7** Biomedical Research and Environmental Sciences Division, NASA Johnson Space Center, Houston, Texas, United States of America, **8** MiniPCR Bio, Cambridge, Massachusetts, United States of America

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Check for updates

Repair Pathway Cheat Sheet

Repair Pathway	Major Players	Major Lesions Repaired
BER	Glycosylase Endonuclease XRCC1	Oxidative damaged bases
NER	XPA-XPG	UV pyrimidine dimers “Bulky” lesions
MMR	MSH2, MLH1	DNA mismatches
NHEJ	Ku70, Ku80, DNAPKcs	DNA double strand breaks
HRR	ATM, RAD51, MRN	DNA double strand breaks
Cross-Link Repair	FANC Proteins	DNA interstrand cross links

Review Questions

Question 1

Repair Pathway	Major Players	Major Lesions Repaired
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DNA glycosylases:

A. rejoin strand breaks

B. act specifically on purine base damage

C. remove damaged bases

D. insert undamaged bases after the removal of damaged bases

E. are found in mammalian cells but not in bacterial cells

BER Pathway

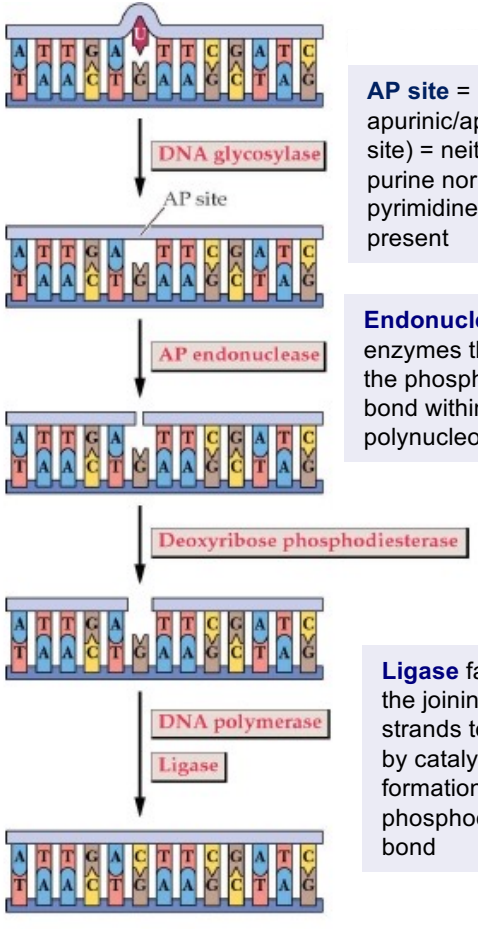
BER repairs Base Damages

Step 1:
Removal of damaged base by a **DNA glycosylase**

Step 2:
Cleaving of its deoxyribose phosphate in the backbone by an **AP endonuclease**, producing a gap

Step 3:
Removal of the sugar-phosphate lacking the base by **Deoxyribose phosphodiesterase (dRpase)**

Step 4:
Incorporation of a specific deoxyribonucleotide by the **DNA polymerase** and a **ligase**



AP site = apurinic/apyrimidinic site) = neither a purine nor a pyrimidine base is present

Endonucleases are enzymes that cleave the phosphodiester bond within a polynucleotide chain.

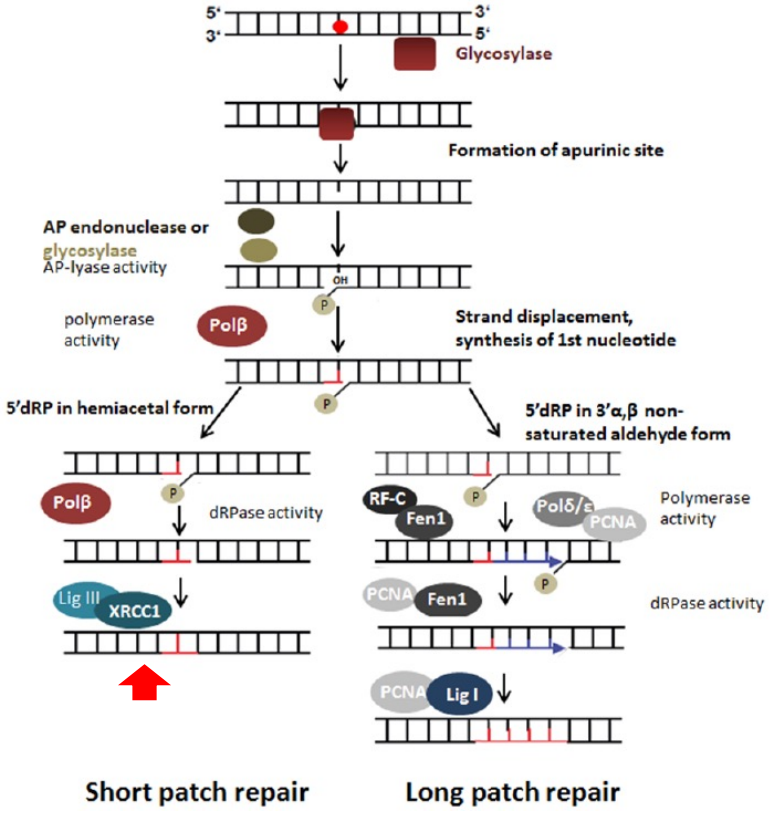
Ligase facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond

Question 2

Base excision repair (BER):

A. when defective, may increase mutation rate, but usually does not alter cell survival after X-irradiation
B. when defective, increases cell sensitivity to both UV and ionizing radiation
C. involves the *XP* and *CS* genes
D. acts on DNA lesions such as pyrimidine dimers, single-strand breaks and bulky adducts
E. is defective in patients with Li-Fraumeni Syndrome

BER



BER repairs damaged bases

Defects in BER may lead to an increased mutation rate

Defects in BER usually do NOT result in radiosensitivity

Exception – **XRCC 1**

- Cells with *XRCC1* mutation are 1.7x more radiosensitive
- Likely due to XRCC1s' involvement in SSB repair

Question 3

Cells derived from individuals diagnosed with xeroderma pigmentosum are deficient in:

A. nucleotide excision repair

B. methyl-guanine transferase

C. mismatch repair

D. base excision repair

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Cross-Link Repair	FANC Proteins	DNA interstrand cross links

Xeroderma Pigmentosum (XP)

- XP is a rare inherited disease of humans which, among other things, predisposes the patient to pigmented lesions on areas of the skin exposed to the sun and an elevated incidence of skin cancer

- XP can be caused by mutations in *XPA* through *XPG* plus a variant *XPV* – all of which have roles to play in nuclear excision repair (NER)

An eight year old girl from Guatemala with Xeroderma pigmentosum.

Question 4

Which of the following statements concerning UV-irradiation is FALSE?

A. UV causes activation of ATR.

B. UV usually induces pyrimidine dimers involving thymine bases

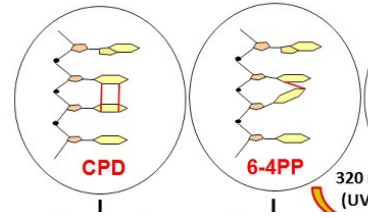
C. The damages caused by UV are repaired primarily by nucleotide excision repair.

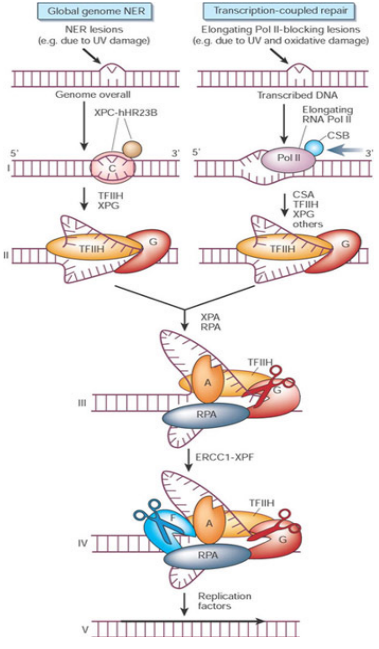
D. People with the syndrome xeroderma pigmentosum are very sensitive to UV irradiation.

E. UV plays an important role in the induction of skin cancer.

NER

- NER repairs **“bulky” lesions**
  - UV lesions – 6-4PP, CPD (pyrimidine dimers)
- Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage
- NER proceeds most rapidly in cells whose genes are being actively transcribed
  - Global genome NER vs. Transcription-coupled NER





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ATM

A kinase is an enzyme that transfers a phosphate group from ATP to target molecule

- ATM belongs to the phosphatidylinositol 3-kinase-related kinase **(PIKK) family**
- Other members of the PIKK family include **DNA-PKcs** (DNA-dependent protein kinase catalytic subunit) & **ATR** (AT and Rad3 related)
- **DNA-PKcs** plays an important role in **NHEJ**
  - Defect in DNA-PKcs results in **SCID** (Severe-Combined syndrome Immunodeficiency Syndrome) in mice
- **ATR** regulates DNA repair and cell-cycle control very much like ATM
  - ATR plays an important role in S-phase checkpoint
  - Individuals with reduced ATR levels develop Seckel's syndrome

Question 5

Which DNA repair process is used to repair replication errors?

A. nucleotide excision repair

B. base excision repair

C. single strand annealing

D. mismatch repair

E. transcription-coupled repair

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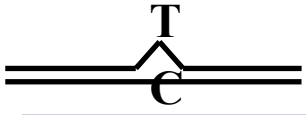
Cross-Link Repair	FANC Proteins	DNA interstrand cross links

Mismatch Repair

- Its function is to remove base substitution and frameshift mismatches that escape from DNA polymerase proofreading activity after DNA replication, increasing DNA replication fidelity 100- to 1000-fold

- Cells also use the MMR system to enhance the fidelity of recombination; i.e., assure that only homologous regions of two DNA molecules pair up to crossover and recombine segments (e.g., in meiosis).



Base mismatch

Frameshift

Question 6

Two of the main proteins involved in mismatch repair are:

A. MSH2/MLH1

B. DNA ligase IV (LIG4)/XRCC4

C. KU70 (XRCC6)/KU80 (XRCC5)

D. XPA/XPG (ERCC5)

E. DNA-PKcs (PRKDC)/Artemis

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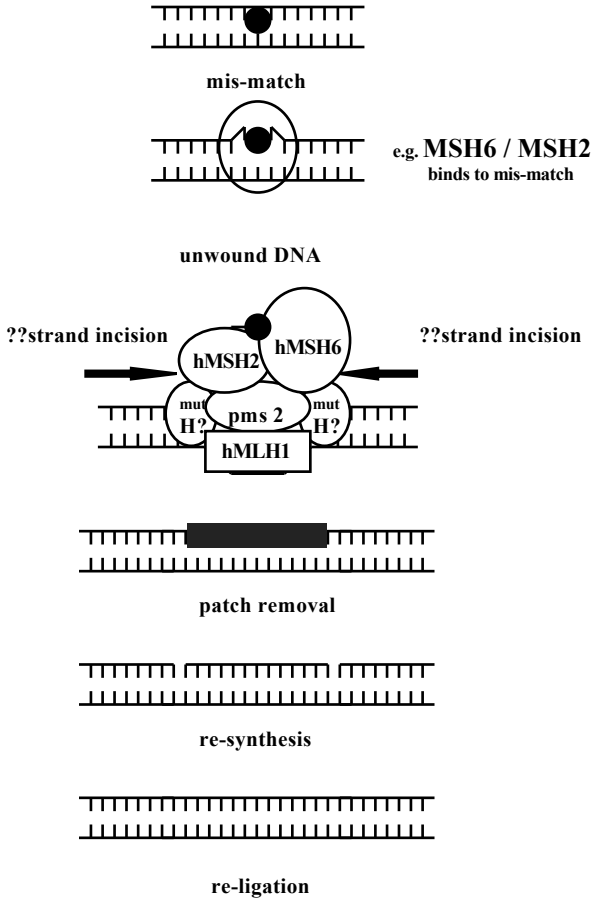
MMR

- Uses enzymes involved in both BER and NER as well as using enzymes specialized for this function
- Recognition of a mismatch requires several different proteins including one encoded by **MSH2**
- Cutting the mismatch out also requires several proteins, including one encoded by **MLH1**
- Mutations in either of these genes predisposes the person to an inherited form of colon cancer – Hereditary Non-Polyposis Colon Cancer (**HNPCC**)



Question 7

Which of the following proteins is not involved in non-homologous end joining (NHEJ)?

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A. Ku70

B. Ligase IV

C. DNA-PKcs

D. XRCC4

E. RAD51

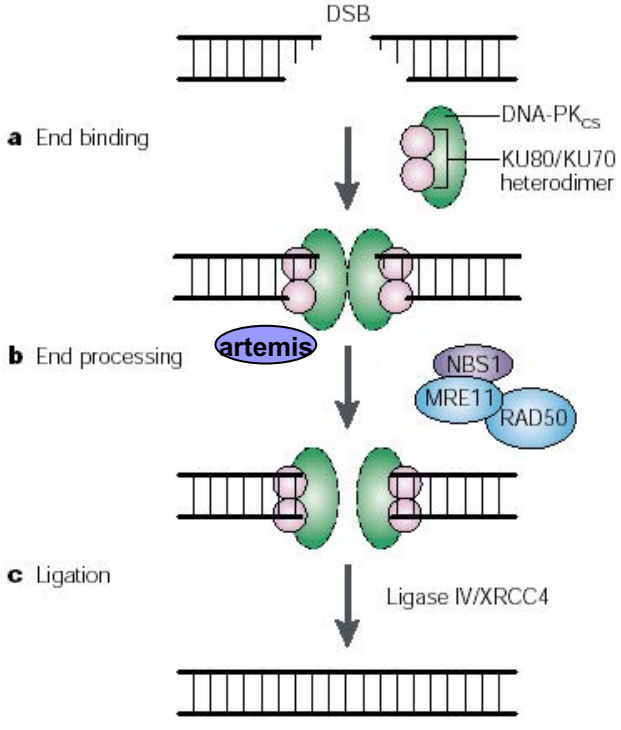
NHEJ

Step 1: End recognition by **Ku/DNA-PKcs**

Step 2: End processing

Step 3: Fill-in synthesis, or end bridging

Step 4: Ligation



Question 8

DNA-dependent protein kinase (DNA-PK) is known to have a role in the repair of DNA double strand breaks and:

A. base excision repair

B. removal of bulky adducts

C. single strand breaks ligation

D. repair of DNA-protein crosslinks

E. V(D)J recombination

NHEJ

- **NHEJ is responsible for the generation of antibody diversity**
  - Some of the same enzymes used to repair DSBs by direct joining are also used to break and reassemble the gene segments used to make antibody variable regions; that is, to **accomplish V(D)J joining**
- **Errors in direct joining may be a cause of the various translocations that are associated with cancers**
  - Burkitt's lymphoma
  - The Philadelphia chromosome in chronic myelogenous leukemia (CML)
  - B-cell leukemia

Question 9

Which of the following statements is FALSE?

A. DNA repair by homologous recombination occurs preferentially in the G1 phase of the cell cycle

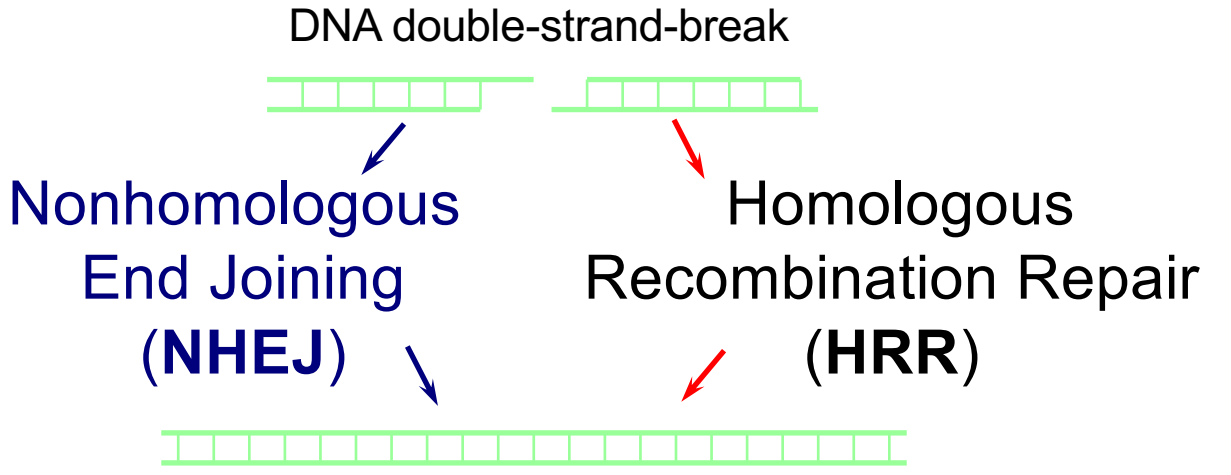
B. Non-homologous end joining is an error-prone repair pathway that involves DNA-PKcs-associated repair of DNA double-strand breaks

C. The DNA repair proteins MRE11, NBS1 (NBN) and RAD50, localize at nuclear foci corresponding to presumed sites of DNA damage following exposure to DNA-damaging agents

D. A defect in nucleotide excision repair is the basis for the hereditary disorder xeroderma pigmentosum, and can lead to increased rates of skin cancer

E. Following the production of DNA double-strand breaks, ATM is converted from an inactive dimer to an active monomer form

Double Strand Break Repair



- No homology
- G_1 phase
- Error-prone

- Homologous template
- S/G_2 phase
- Error-free

Not Mutually Exclusive !!!

Question 10 (Medical Resident Only)

Which of the following statements concerning the repair of radiation-induced DNA double strand breaks is INCORRECT?

A. The ATM protein initiates a signaling cascade that activates DNA strand break repair

B. Although the RAD50/MRE11/NBS1 (MRN) complex contributes to DNA repair, defects in either MRE11 or NBS1 do not result in a radiation sensitive phenotype

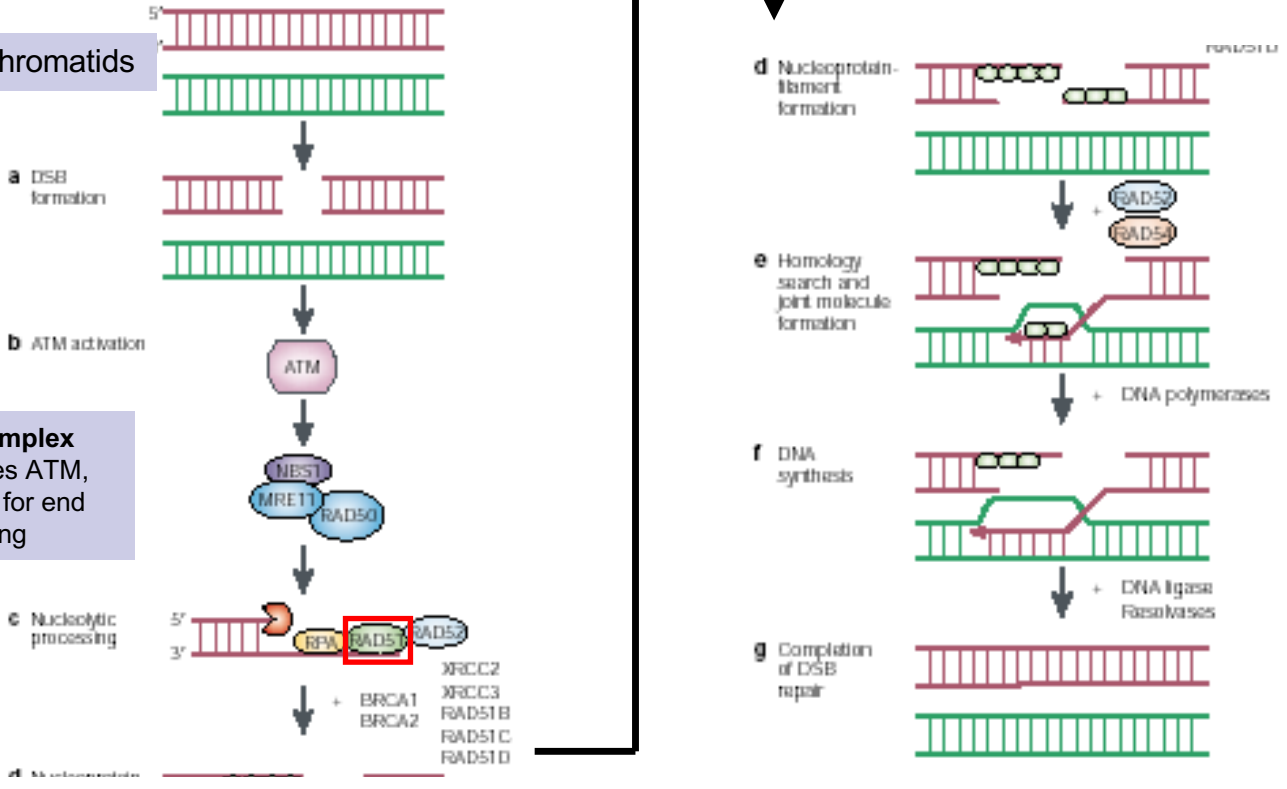
C. Inactivation of either BRCA1 or BRCA2 affects homologous recombination-dependent DSB repair

D. BRCA1 is required for RAD51 nuclear foci formation

E. In response to DNA-damaging agents, the RAD51 protein localizes to nuclear foci that are thought to represent sites of DNA repair

HRR Pathway

Sister chromatids



MRN complex stimulates ATM, required for end processing

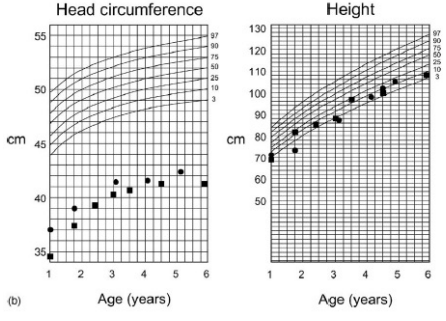
Accessory proteins

MRE11 and AT-Like Disorders (ATLD)

- Mutation in ***MRE11***causes ATLD
- ATLD is an autosomal recessive disease which shares the clinical phenotype with AT
- Cells derived from ATLD patients are radiosensitive
- ATLD cells are also defective in checkpoint response to DNA damage

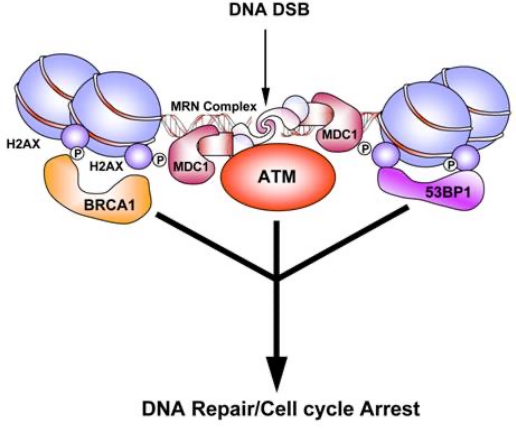
NBS1 and Nijmegen Breakage Syndrome

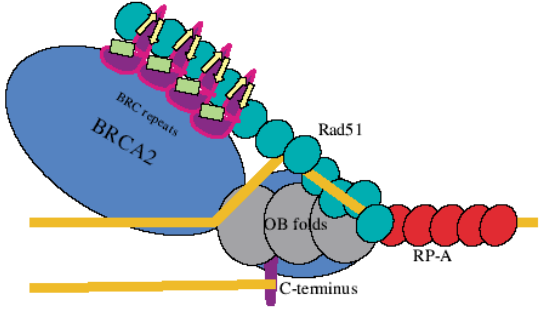
- NBS1 is a direct phosphorylation target of ATM
- Cells defective in NBS1 lack an S phase checkpoint and are radiosensitive
- Clinical features include microcephaly, radiosensitivity, immunodeficiency, increased cancer risk and growth retardation



BRCA1 and BRCA2

- Individuals with mutations in BRCA1/2 are predisposed to breast and ovarian cancers
- BRCA1 and BRCA2 facilitate HRR





BRCA1 is recruited by H2AX to regulate the function of MRN
BRCA1 recruits BRCA2

BRCA2 primarily interacts with RAD51 to facilitate its assembly on the SSDNA