DNA Repair & Checkpoint

Cellular and Molecular Biology of Cancer
PATH G4500-001

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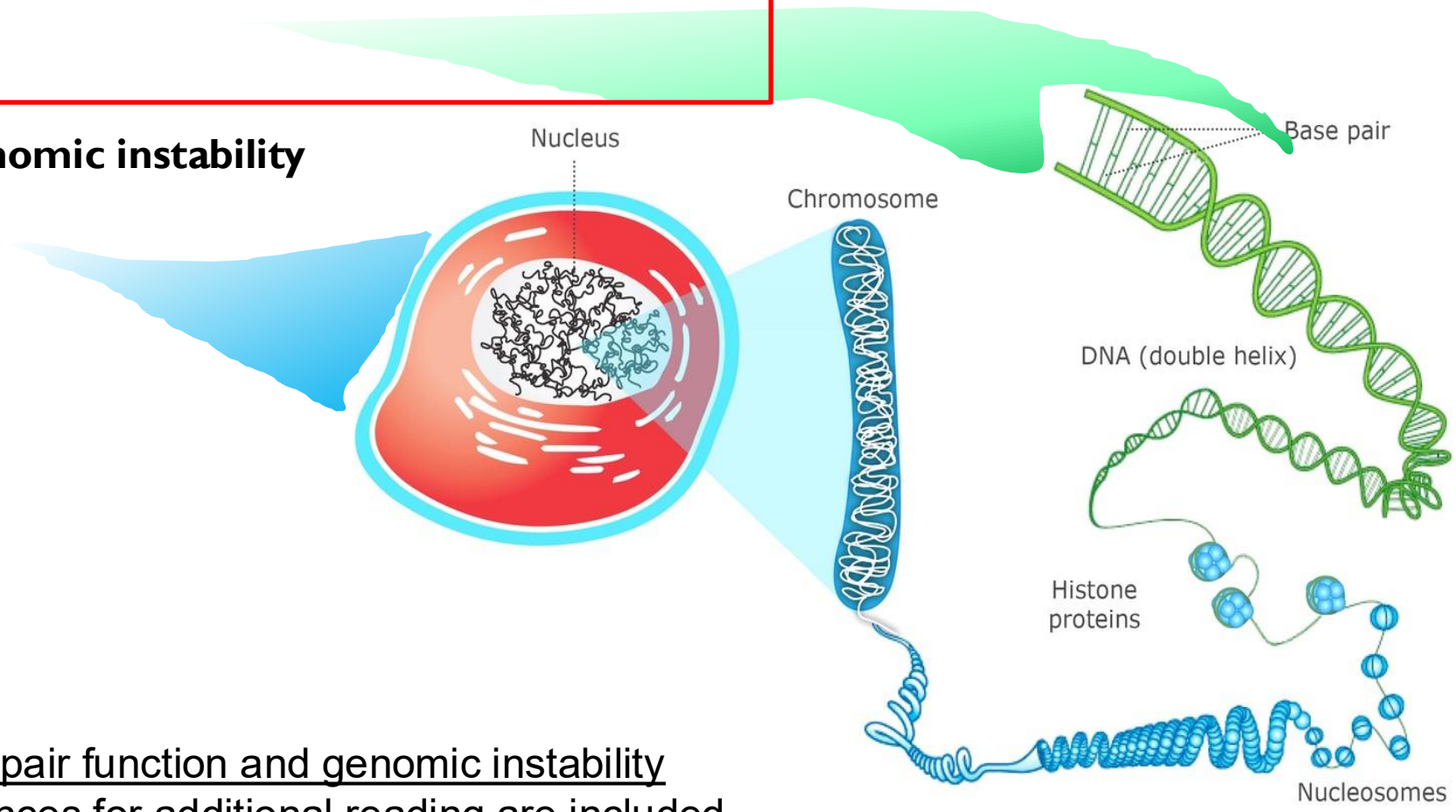
All about DNA

1. **Type of DNA damages and DNA repair pathways**

Dr. Shan Zha

2. **Aneuploidy/genomic instability**

Dr. Alison Tyler



Assays for DNA repair function and genomic instability

* Some key references for additional reading are included

Internet resources – a DNA centered view

Costmic https://cancer.sanger.ac.uk/cosmic

Cbioportal https://www.cbioportal.org/

UCSC genome browser https://genome.ucsc.edu/

Dependent Map https://depmap.org/portal/

Functional information about Your Favorite Gene (YFG)

Uniprot https://www.uniprot.org/

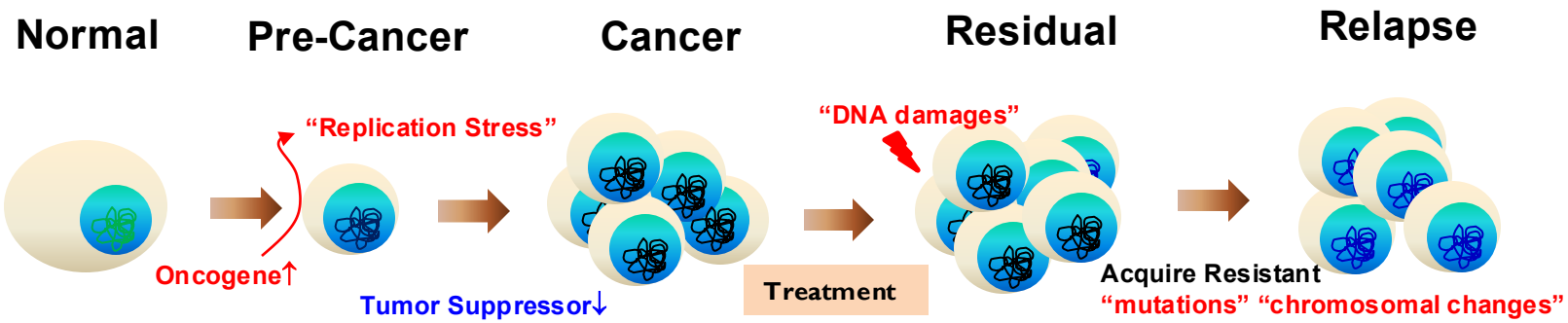
Structure (protein databank) https://www.rcsb.org/

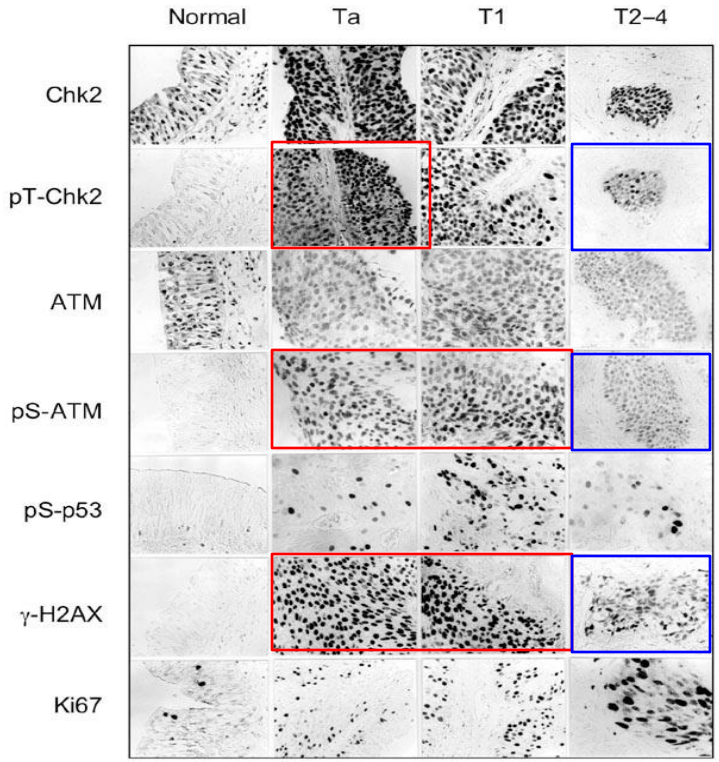
Alphafold3 https://alphafoldserver.com/about

Overview

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways and DNA damage response
4. Case Studies
    I. Developmental DNA breaks - Lymphocyte, Meiosis, Neuronal function etc.
    II. Telomere, mitochondria DNA, and rDNA clusters

1. Why DNA –Cancer is genetic disease



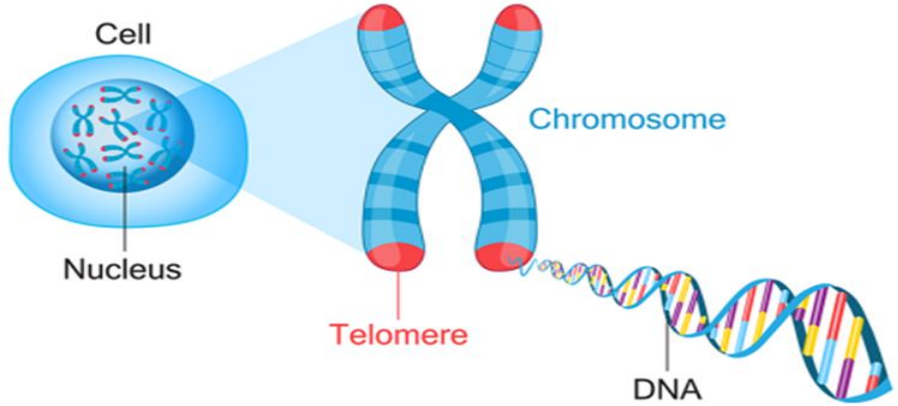


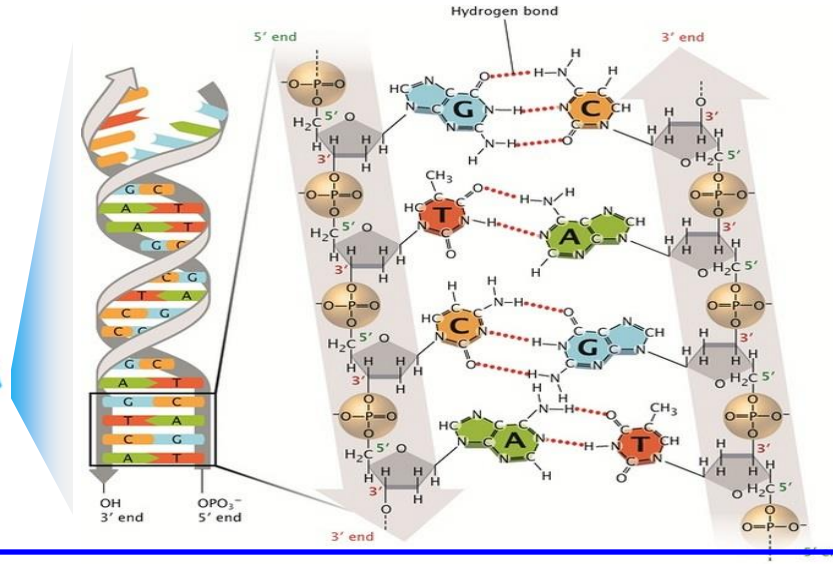
Bartkova et al. 2005 *Nature 434, 864-870*

Chemotherapy and radiation

Conventional chemotherapy and radiation – selectively target cancer cells (over normal cells), by inflicting DNA lesions (single strand breaks or base damage) that could be converted to double strand breaks in "proliferating" cells. Double stand breaks activate DNA damage checkpoints, which halt proliferation and induce cell death.

1.2 Type of DNA lesions- DNA





Chromosome changes

Mitotic/spindle error

Aneuploidy
(Dr. Alison Tylor)

Strand breaks
- single strand break/gaps
- double strand breaks

Base damages
- involving single base
- involving >1 adjacent bases
- mis-matches

Repair Errors or Defects

Strand breaks
Deletion/Insertion
Amplification/Translocation

Mutations

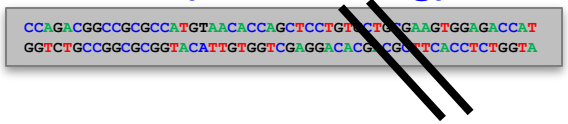
1.2 Type of DNA lesions - detection

Aneuploidy

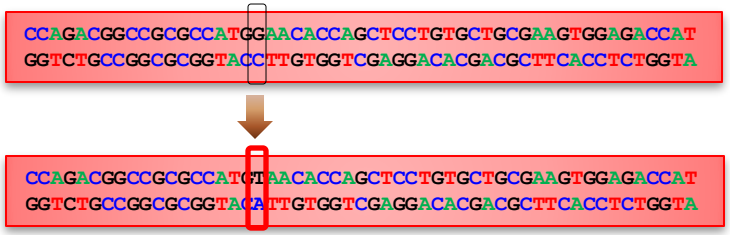
Amplification

Translocation-(NOT) trans-splicing

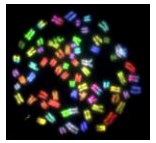
Deletion (small or big)

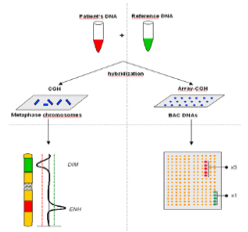


Base substitution



Cytogenetics



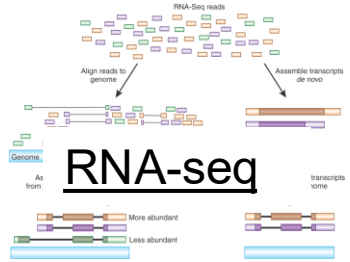


1960' ~

CGH

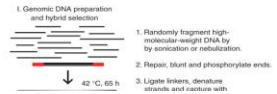
1990' ~

In frame

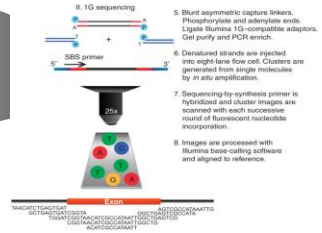


RNA-seq

small



Exon or WG seq



2000's~

Signatures of mutational processes in human cancer ***Nature*** *500, 415–421 (22 August 2013)*

1.2. Tissue type specificity

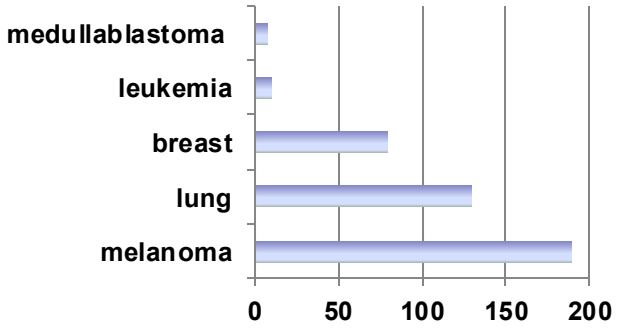
Translocations (inter and intra)

Trisome chr 21	Down Syndrome
t(8;14) c-Myc; IgH	Burkitt's lymphomas
t(11;14) CyclinD1; IgH	Mantle cell lymphomas
t(14;18) IgH;BCL2	Follicular Lymphomas
t(3;14) BCL6;IgH	Diffuse Large B cell lymphomas
t(1;14) TAL1; TCRα/δ	T-ALL
……	

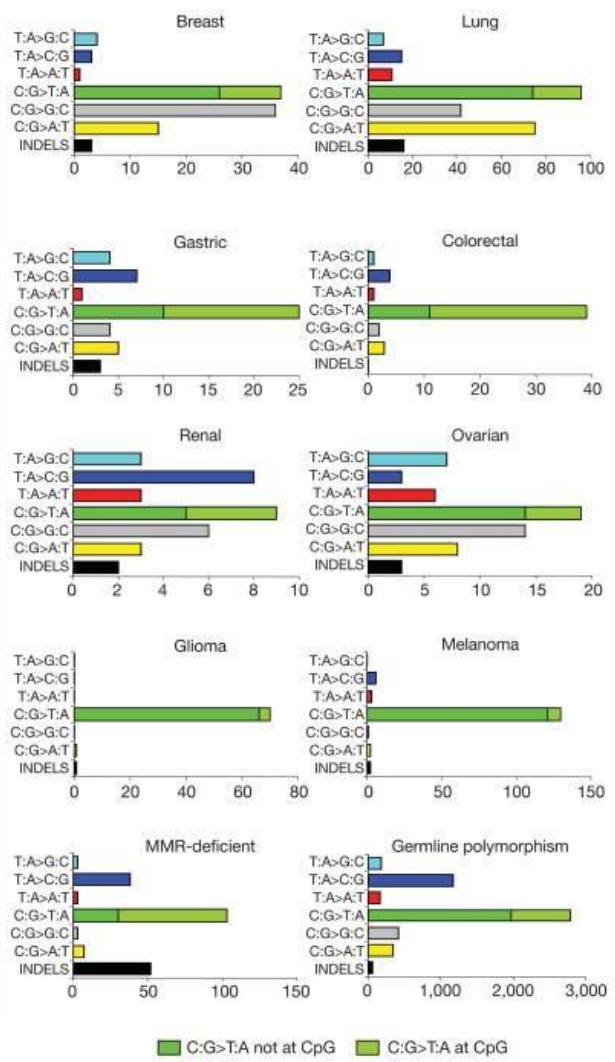
t(11;22)(q24;q11.2) EWS:FLI	**Ewing Sarcoma**
t(21;21)(q22;q22) TMPRSS2:ERG	**Prostate Cancer**
t(4;4)(p16;p16) FGFR:TACC	**Glioblastomas**
……	

Substitutions and indels

Somatic mutations per case from WES



WES – whole exon seq.
WGS - whole genome seq.
~2% of human genome are annotated as exons



UV - Signature 7 CC>TT on untranscribed DNA strand.

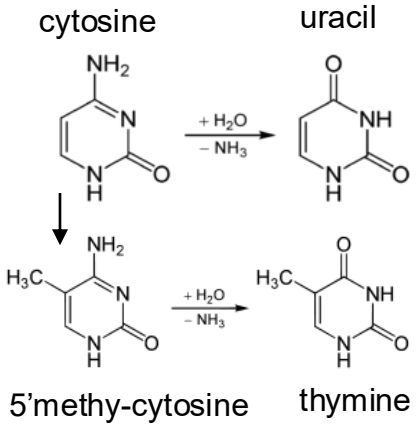
Tobacco - Signature 4 and 29, C>A on template strand (G>T on non-template strand)

……

Patterns of somatic mutation in human cancer genomes Nature. 2007 Mar 8; 446(7132): 153–158.

1.3. Repair pathway specificity

C:G
| |
T: A



Age-related mutagenesis
Signature 1 Np[C>T]G. Spontaneous deamination of 5meC.
Signature 5 T>C within ApTpN trinucleotide with transcriptional strand bias.

Homologous recombination (BRCA1/2) deficiency Signature 3 high indels (>5nt) with microhomology at the breakpoints.

APOBEC enzymes Signature 2 and Signature 13 are enriched for C>T and C>G substitutions and are thought to arise from cytidine deaminase activity of the AID/APOBEC enzymes family. C->U (T) substitutions due to cytidine deaminases. Signature 2 has a higher proportion of C[C>T]N substitutions and Signature 13 a higher proportion of T[C>G]N substitutions. Prefer lagging DNA strand during replication.

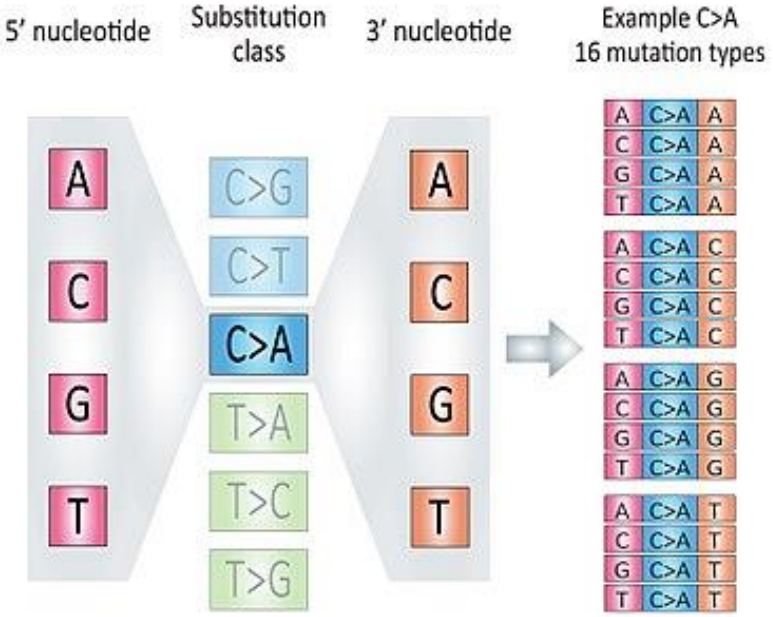
Mismatch repair deficiency - Signature 6, 15, 20 and 26, Loss of function MLH1, MSH2, MSH6 or PMS2 genes cause defective DNA mismatch repair.

Replication DNA polymerase proofreading-Signature 10 has a transcriptional bias and is enriched for C>A substitutions in the TpCpT context as well as T>G substitutions in the TpTpTp context.

***MUTYH* deficiency (Base excision repair).** Signature 18 more transversion mutations (G:C>T:A

……..

Sanger Signature Platform



Alexandrov LB, et al. (August 2013). "Signatures of mutational processes in human cancer" (PDF). *Nature*. **500** (7463): 415–21.

Sanger provides "Catalogue Of Somatic Mutations In Cancer (Cosmic)"
https://cancer.sanger.ac.uk/cosmic

1.4. Therapy induced lesions

Alkylating Agents - Base damage

•Mustard gas derivatives: Mechlorethamine, Cyclophosphamide, Chlorambucil, Melphalan, and Ifosfamide.
•Ethylenimines: Thiotepa and Hexamethylmelamine.
•Hydrazines and Triazines: Altretamine, Procarbazine, Dacarbazine and **Temozolomide.**
•Nitrosureas: Carmustine, Lomustine and Streptozocin. Nitrosureas(cross the blood-brain barrier)
•Metal salts: Carboplatin, Cisplatin, and Oxaliplatin.

Temozolomide, Signature 11
(see next page)

Topoisomerase Inhibitors and crosslinking agents

•Topoisomerase I inhibitors: Ironotecan, topotecan, other camptothecon analogs
•Topoisomerase II inhibitors: Amsacrine, etoposide, etoposide phosphate, teniposide
• Anthracyclines: Doxorubicin, Daunorubicin, Epirubicin, Mitoxantrone, and Idarubicin.
•Chromomycins: Dactinomycin and Plicamycin.
•Miscellaneous: Mitomycin and Bleomycin.

Topo II inhibitors increase therapy induced AML

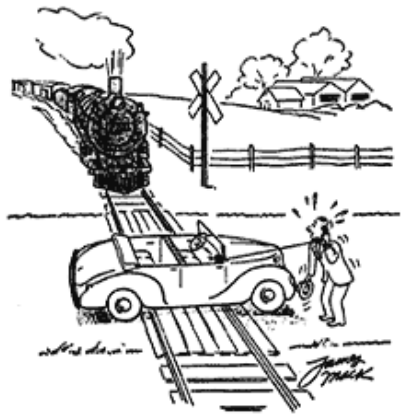
Anti-metabolites – Nucleotide homeostasis

•Folic acid antagonist: Methotrexate.
•Pyrimidine antagonist: 5-Fluorouracil, Foxuridine, Cytarabine, Capecitabine, and Gemcitabine.
•Purine antagonist: 6-Mercaptopurine and 6-Thioguanine.
•Adenosine deaminase inhibitor: Cladribine, Fludarabine, Nelarabine and Pentostatin.
•Ribonucleotide reductase inhibitor: Hydroxyurea.
•Enzymes: Asparaginase and Pegaspargase.

"DNA Replication"
Single stand/base lesion ==========> Double stand breaks
(do NOT activate checkpoints) (active checkpoints)

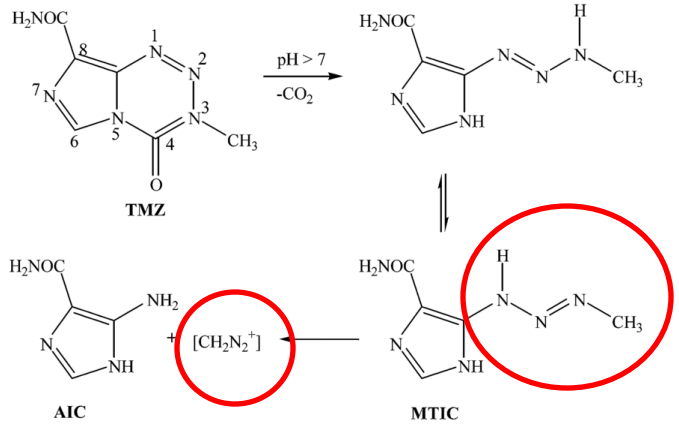
Micro tubulin/mitotic blocker

- Vinca alkaloids: Vincristine, Vinblastine and Vinorelbine.
- Taxanes: Paclitaxel and Docetaxel.
- Antimicrotubule agent: Estramustine



"Hello, Mr. Bunting, I've changed my mind— I'll take that accident policy!"

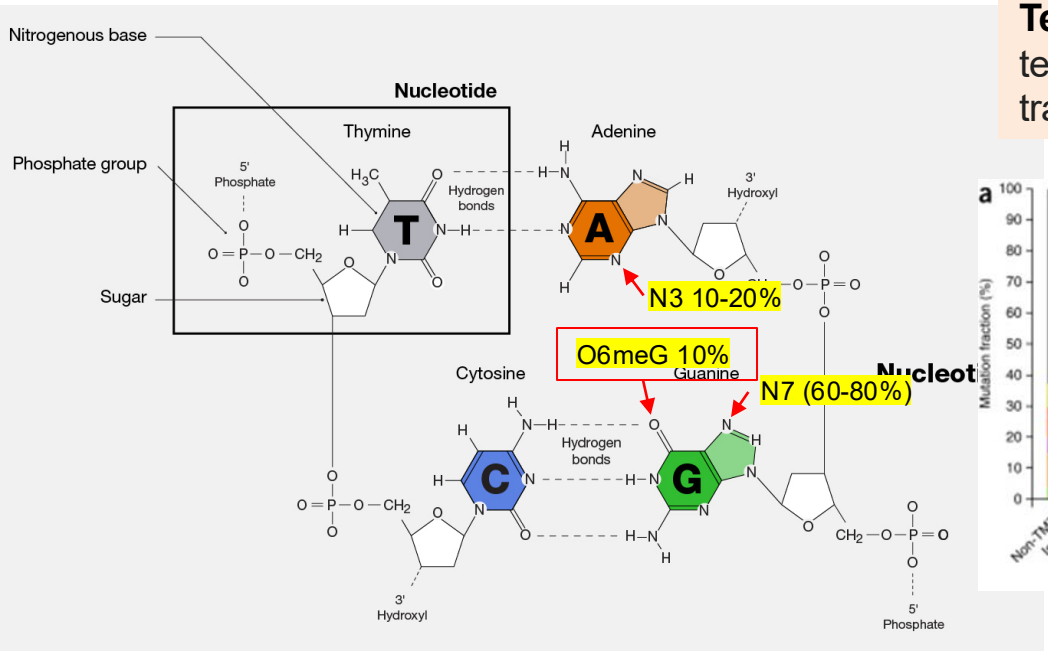
“Temozolomide – case study”



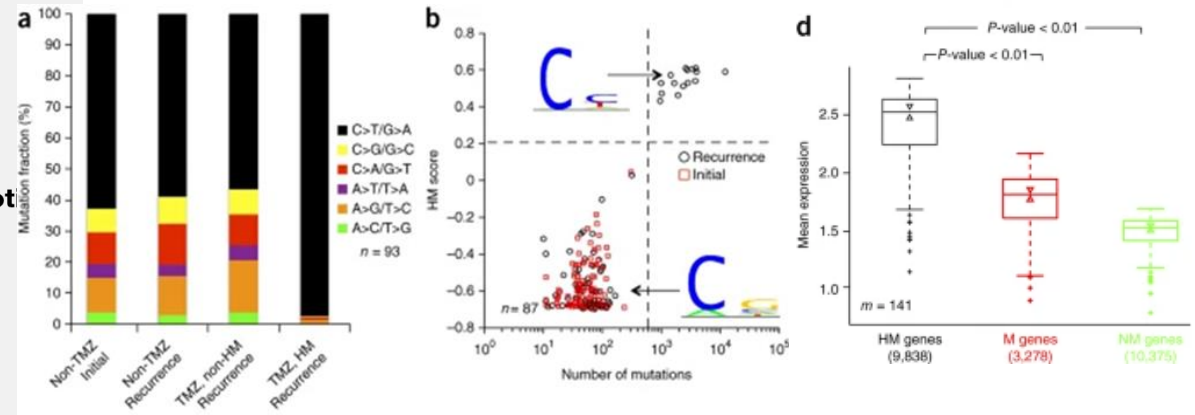
Temozolomide, is small, lipophilic (enter blood brain barrier effectively) and very stable in acid condition (stomach – oral effective). Top choice for Glioblastoma.

In PH>7, it converts to reactive MTIC with help of H2O.

MITC methylates G (O6-meG, and N7-meG), and A (N3meA)



Temozolomide, Signature 11 enriched for C>T substitutions on template strand (actually G -> A on the non-template strand in transcriptional active genes)



Nat Genet 2016 Jul;48(7):768-76. doi: 10.1038/ng.3590.

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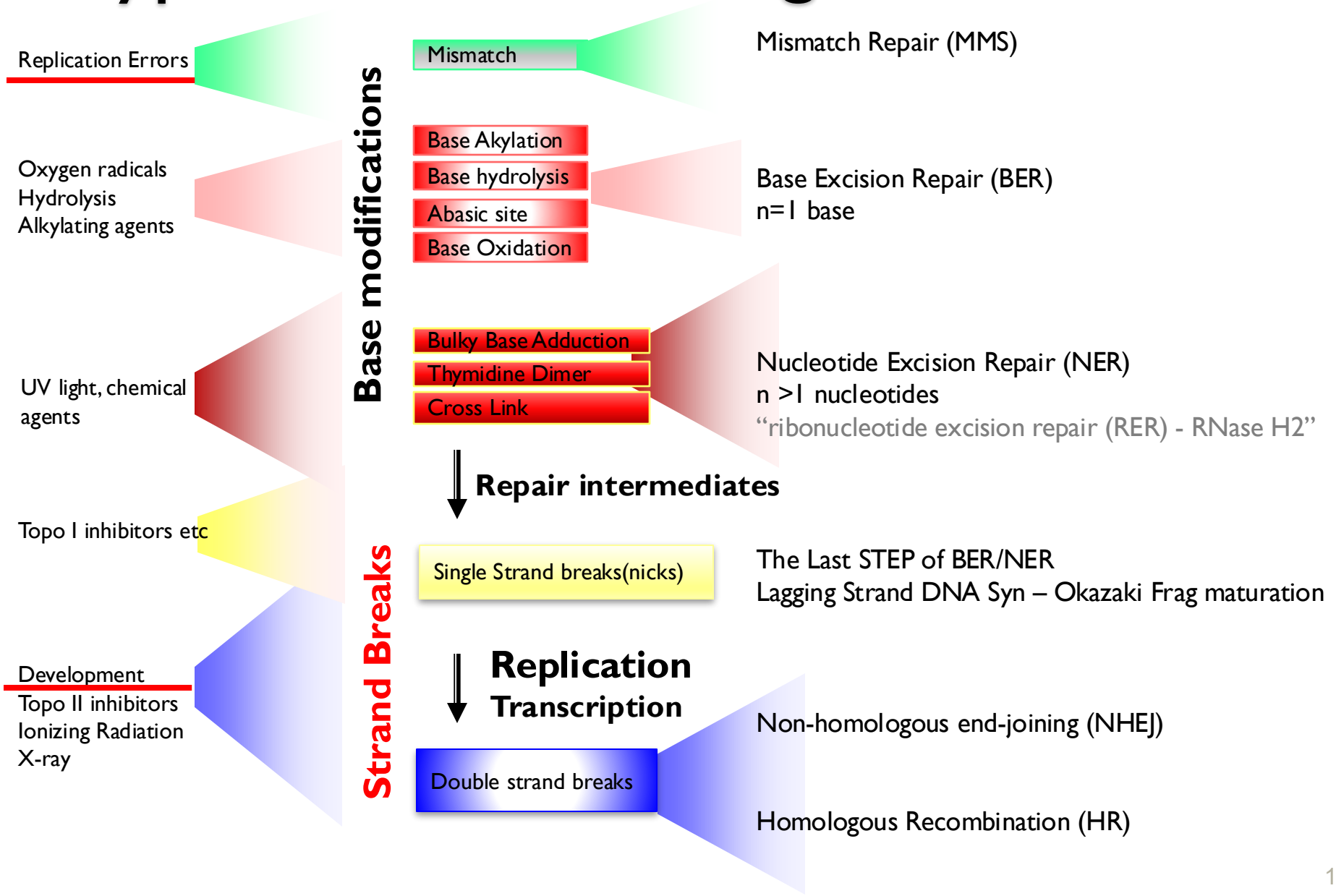
2. Types of DNA Damages



2.1 DNA Damage is very common

None dividing cells

Table 1. DNA Lesions Generated by Endogenous and Exogenous DNA Damage

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day	
Depurination	AP site	10000[a]	
Cytosine deamination	Base transition	100–500[a]	
SAM-induced methylation	3meA	600[a]	
	7meG	4000[a]	
	O^6meG	10–30[b]	
Oxidation	8oxoG	400–1500[c]	
Exogenous DNA Damage	**Dose Exposure (mSv)**	**DNA Lesions Generated**	**Estimated Number Lesions/Cell**
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

Ciccia A and Elledge SJ Mol Cell (Review) 2010

2.2 Replication Errors

- Up to 100,000 DNA replication origins are available per human cells. Among them, ~30-50,000 are activated in each cells.
- DNA replication requires, clean DNA template, sufficient nucleotide stock, “healthy” polymerase status and proper processing of difficult regions (telomere, centromere, rDNA.etc)
- Oncogene expression could active DNA replication prematurely, and increases conflicts between transcription and replication. (https://pubmed.ncbi.nlm.nih.gov/18323444/)

The debate over the “bad luck theory”

Variation in cancer risk among tissues can be explained by the number of stem cell divisions

Cristian Tomasetti, Bert Vogelstein

Science 02 Jan 2015:
Vol. 347, Issue 6217, pp. 78-81
DOI: 10.1126/science.1260825
https://science.sciencemag.org/content/347/6217/78

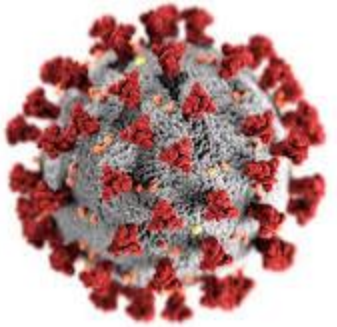
Scientific American
https://www.scientificamerican.com/article/most-cancer-cases-arise-from-bad-luck/

2.3 Polymerase by Numbers

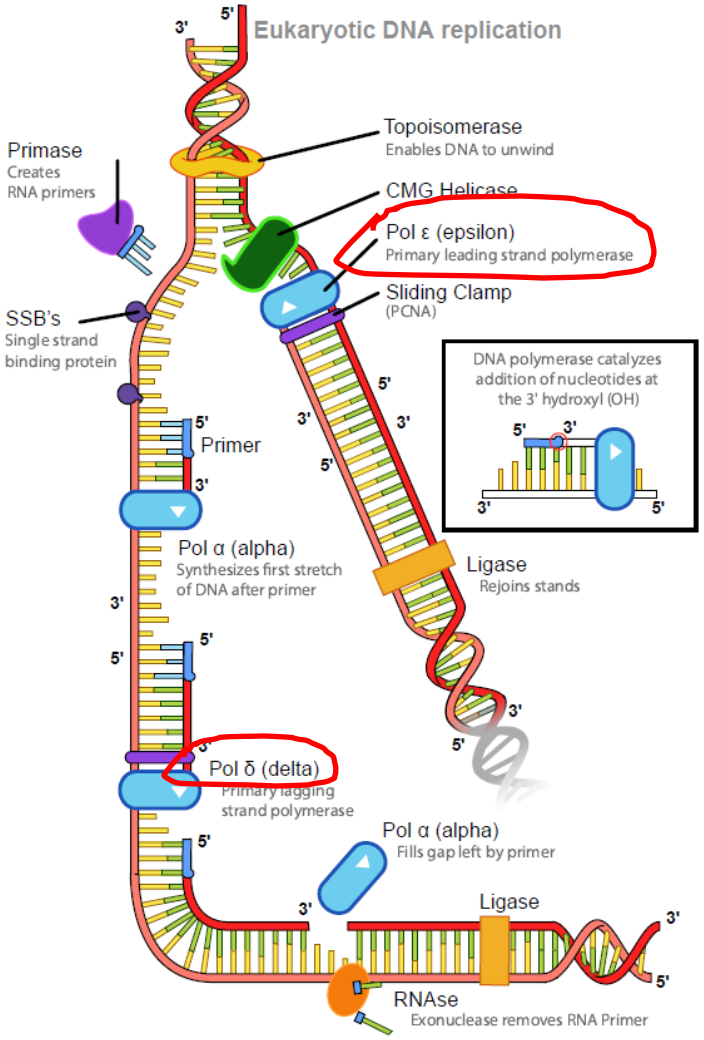
	Mut/bp (replication)	Genome size (bp)	Mut/genome (Mitotic replication)	Mut/ "Generation" (Germline)
Human	10^{-10}	3.3×10^{9}	~0.2-1	$1\text{-}4 \times 10^{-8}$
Mouse	10^{-10}	2.8×10^{9}	~0.5	1×10^{-8}
Yeast	$10^{-9} \sim 10^{-10}$	1.3×10^{7}	3×10^{-3}	
E. coli	$10^{-9} \sim 10^{-10}$	5.0×10^{6}	$5 \times 10^{-3 \sim -4}$	
Virus*	$10^{-3/4} \sim 10^{-5/6} \sim 10^{-7/8}$		$10^{0/1} \sim 10^{-1/-2} \sim 10^{-3}$	
* RNA virus has the highest mutation rate, followed by retrovirus and DNA virus (about 10 fold drop each step).				
Mito	$\sim 10^{-7}$	1.7×10^{4}	0.5	3×10^{-5}/20 yr

Coronavirus is unusually large single stranded RNA virus with a genome size of ~30Kb.

Fun video about Coronavirus replication by Dr. Britt Glaunsinger from UC Berkeley and HHMI
https://www.youtube.com/watch?v=8_bOhZd6ieM



2.3 Polymerase by Numbers



Polε and Polδ ($10^{-5\sim-6}$)

↓

Exo Nuclease($10^{-7\sim-8}$)

↓

Mismatch Repair($10^{-9\sim-10}$)
Ribo excision Repair

Genomic replication polymerase error rate 10^{-8} and the repair pathways fix 99% of the breaks.

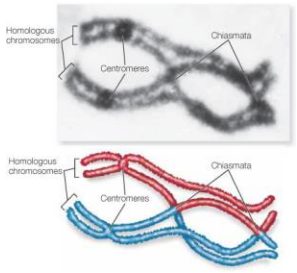
Mito Polymerase (polG/γ) has a base substitution rate ~$2x10^{-6}$

Taq	$2.3x10^{-5}$ /bp
Pfu	$2.8x10^{-6}$ /bp
Phusion	$4.4\text{-}9.5x10^{-7}$/bp (lower with GC buffer, higher with HF buffer)

Fun questions: How many E Coli per ml in overnight mini-prep?

2.4. Developmental DNA double strand breaks

Meiosis



SPO11 is a Topoisomerase II related protein that initiated meiotic recombination by linking itself to DNA in prophase I. Mre11/NBS/RAD50/CtIP complex then cleaves the surrounding DNA to creates DNA double strand breaks, which are repaired by homologous recombination and meiotic specific proteins to result in crossover at the average of 1-2/chromosome.

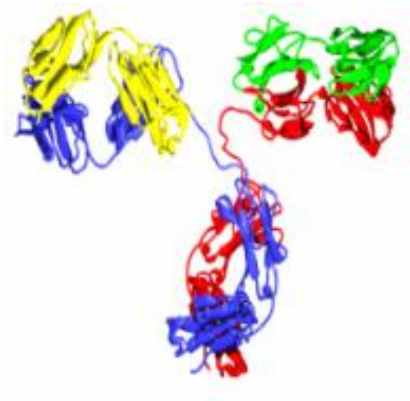
Meiosis Review: https://www.nature.com/articles/nrg3573
Formation of DSB during meiosis: https://link.springer.com/chapter/10.1007%2F7050_2007_026

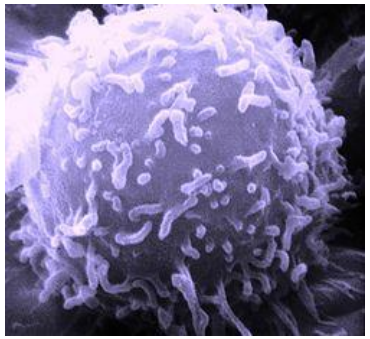
??? Neuron synapsis

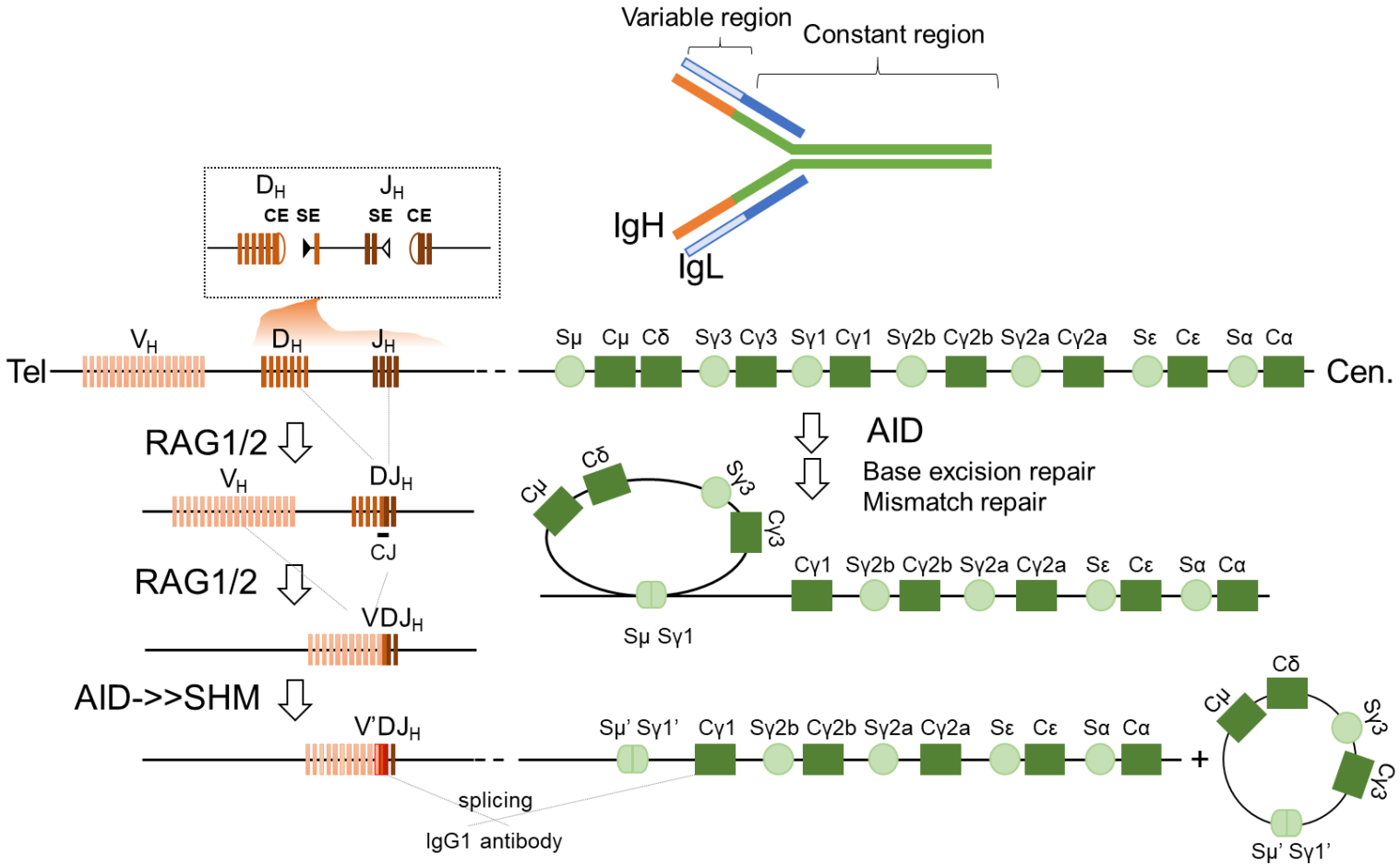
Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid-β
Nature Neuroscience 16, 613–621 (2013)

Activity-Induced DNA Breaks Govern the Expression of Neuronal Early-Response Genes.
Cell. 2015 Jun 18;161(7):1592-605.

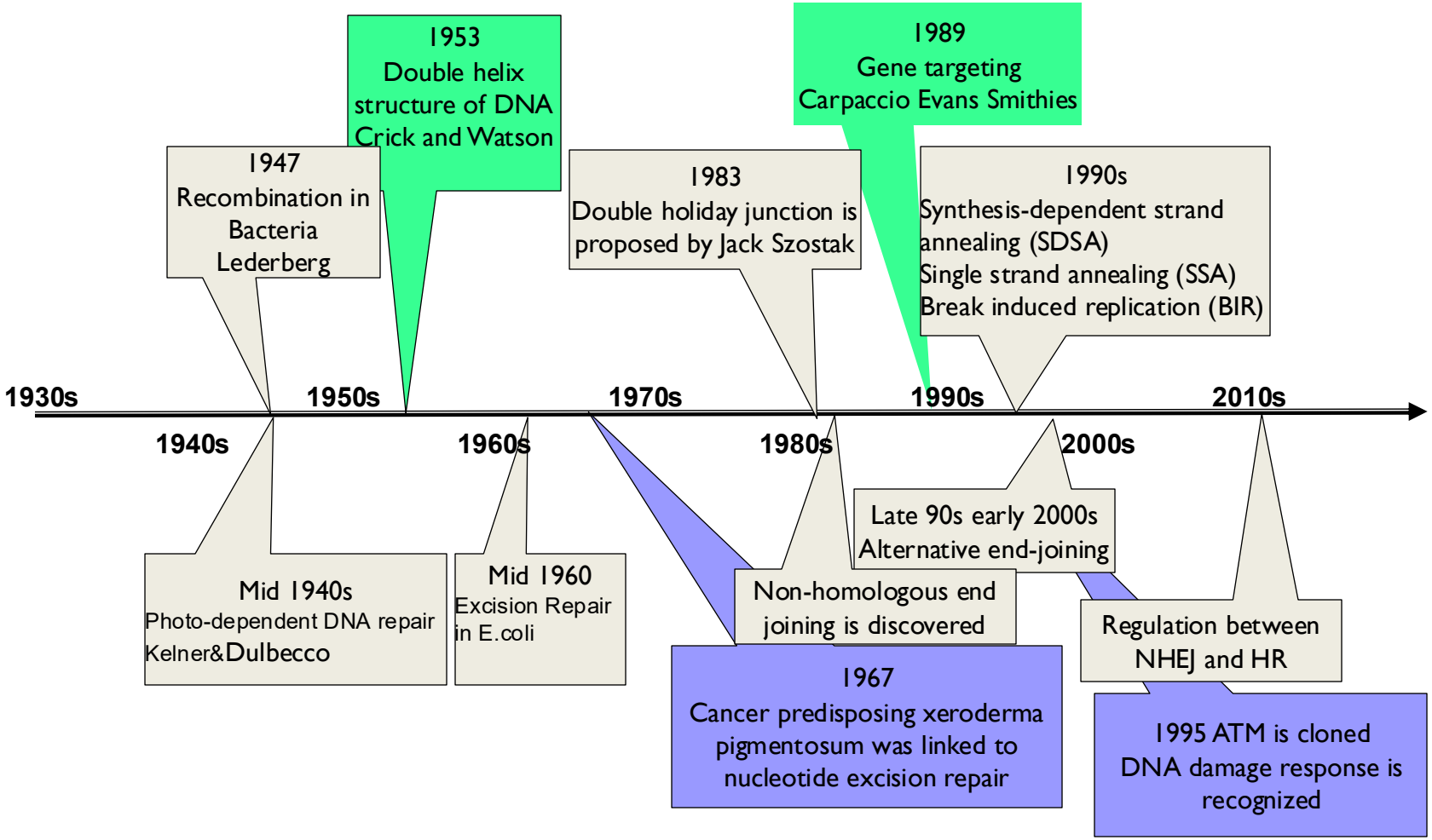
2.4. Developmental DNA double strand breaks and somatic hyper-mutations



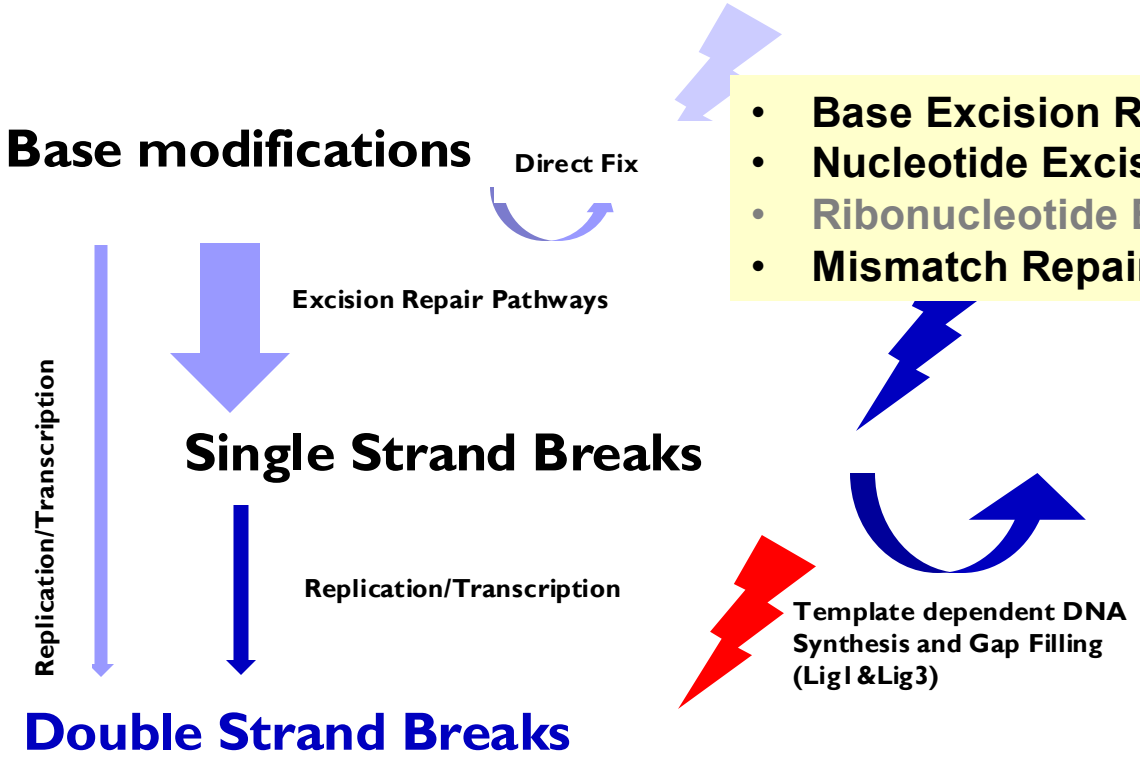




3. DNA repair pathways
A Brief History of DNA Repair



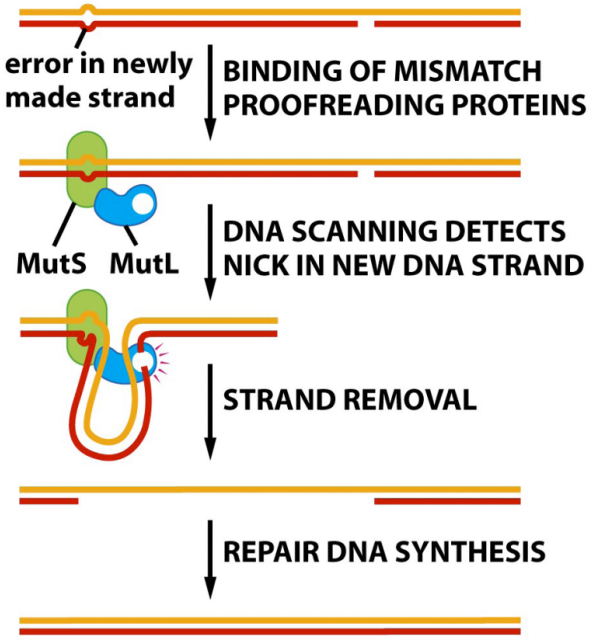
DNA repair pathways



- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- Ribonucleotide Excision Repair (RER)
- Mismatch Repair (MMR)

- Non-Homologues End Joining (NHEJ)
- Homologous Recombination (HR)
- Alternative End Joining (A-EJ)/ Micro-homology Mediated End Joining (MMEJ)

Mismatch Repair (MMR)



MMR is a highly conserved process from prokaryotes to eukaryotes. MMR is often coupled with DNA replication and loading with PCNA ring.

Sensing: travel with DNA polymerase
Strand identification: hemi-methylation in E.coli, potentially nicks in other eukaryotes.

Function : prokaryote gene: Eukaryotes :
Sensor: MutS = **Msh2**/Msh6 (MutSα) : base substitution/small loops
Msh2/Msh3 (MutSβ) : small/large loop

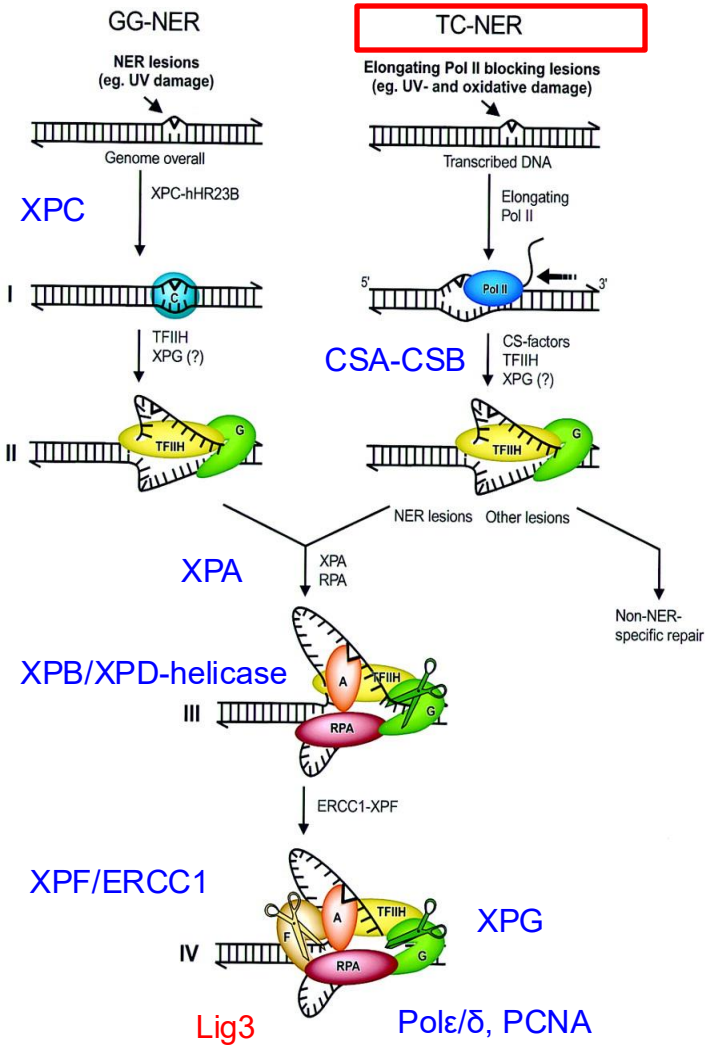
Helicase/regulator/endo: MutL =**Mlhl**/Pms1 (MutLα), MutLβ, MutLγ

Scissor: MutH (no eukaryote homology, MutLα is an endonuclease)

Germline mutations of MMR factors (dominant allele) cause **Lynch** syndrome and **microsatellite instability (MSI).** Patients have greatly increased risk for hereditary nonpolyposis colorectal cancers (HNPCC) (often MSH2 or MLH1 mutations), increased risk for endometrium cancers and sebaceous neoplasms of the skin and visceral malignancies with colonic carcinoma, known as Muir-Torre Syndrome (MTS).

https://www.cell.com/trends/molecular-medicine/pdf/S1471-4914(16)00033-2.pdf

Nucleotide Excision Repair (NER)



NER is also a highly conserved process from prokaryotes to eukaryotes.

NER is primarily responsible for repairing Thymidine Dimer formed following UV lesions.

In bacteria, it is initiated by scanning the DNA by UvrA-UurB, followed by UvrB loading and UvrC-mediated nicking.

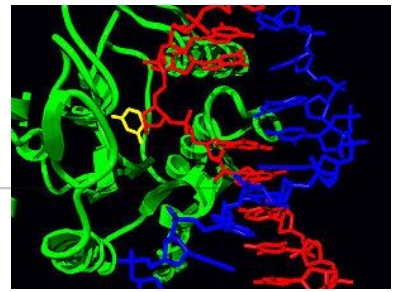
In humans, there are two kinds of NER pathways that differ in the recognition mechanism— Globe General NER and Transcription Coupled NER.

Homozygous germline mutations of NER proteins lead to Xeroderma Pigmentaosum (XPA~G), trichothiodystrophy (XPB,XPD, TTDA), and Cockayne Syndrome (CSA and CSB).

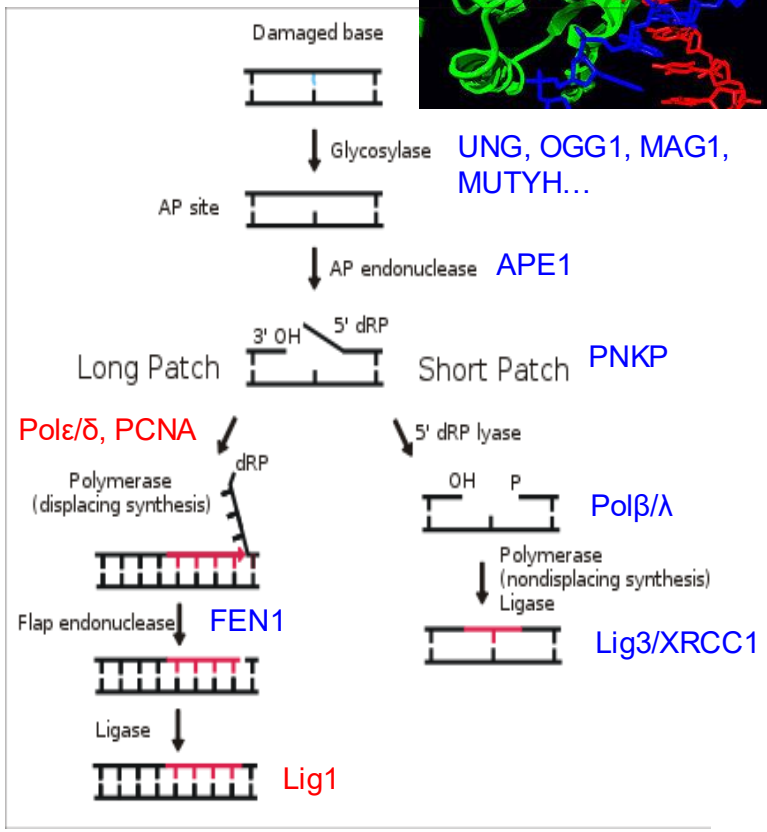
XP patients are extremely sensitive to sunlight and develop early onset basal cell carcinomas. Metastatic malignant melanoma and squamous cell carcinoma are the two most common causes of death in XP patients.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3783044/

Base Excision Repair (BER)



<-Uracil DNA glycosylase flips an uracil residue out of the duplex, shown in yellow.



While glycosylase and APE homologous are widely spread, the BER pathway is not fully conserved in prokaryotes. Most short patch repair factors were not even found in yeast. BER functions throughout the cell cycle to repair small, non-helix-distorting base lesions (bulky -> NER).

Sensing: Glycosylases
? Long (2-10 nt) vs short (1-2 nt) patch

Members
Sensor: Glycosylase - UNG, OGG1, MAG1, MYH…
Scissor: APE1 (some glycosylase has nickase function)
Polymerases: Polβ, Polλ Polε, Polδ

Somatic mutations in Pol β have been found in 30% of human cancers, and some of these mutations lead to transformation when expressed in mouse cells.

DNA glycosylase **MUTYH** specifically recognizes this **8-oxoG mismatch** and removes the **adenine** that is incorrectly paired with 8-oxoG.

Germline Mutations in the MUTYH cause **MUTYH-associated polyposis (MAP), an autosomal recessive disorder that predisposes individuals to colorectal cancer.**

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4100245/

Unique roles of BER/NER/MMR

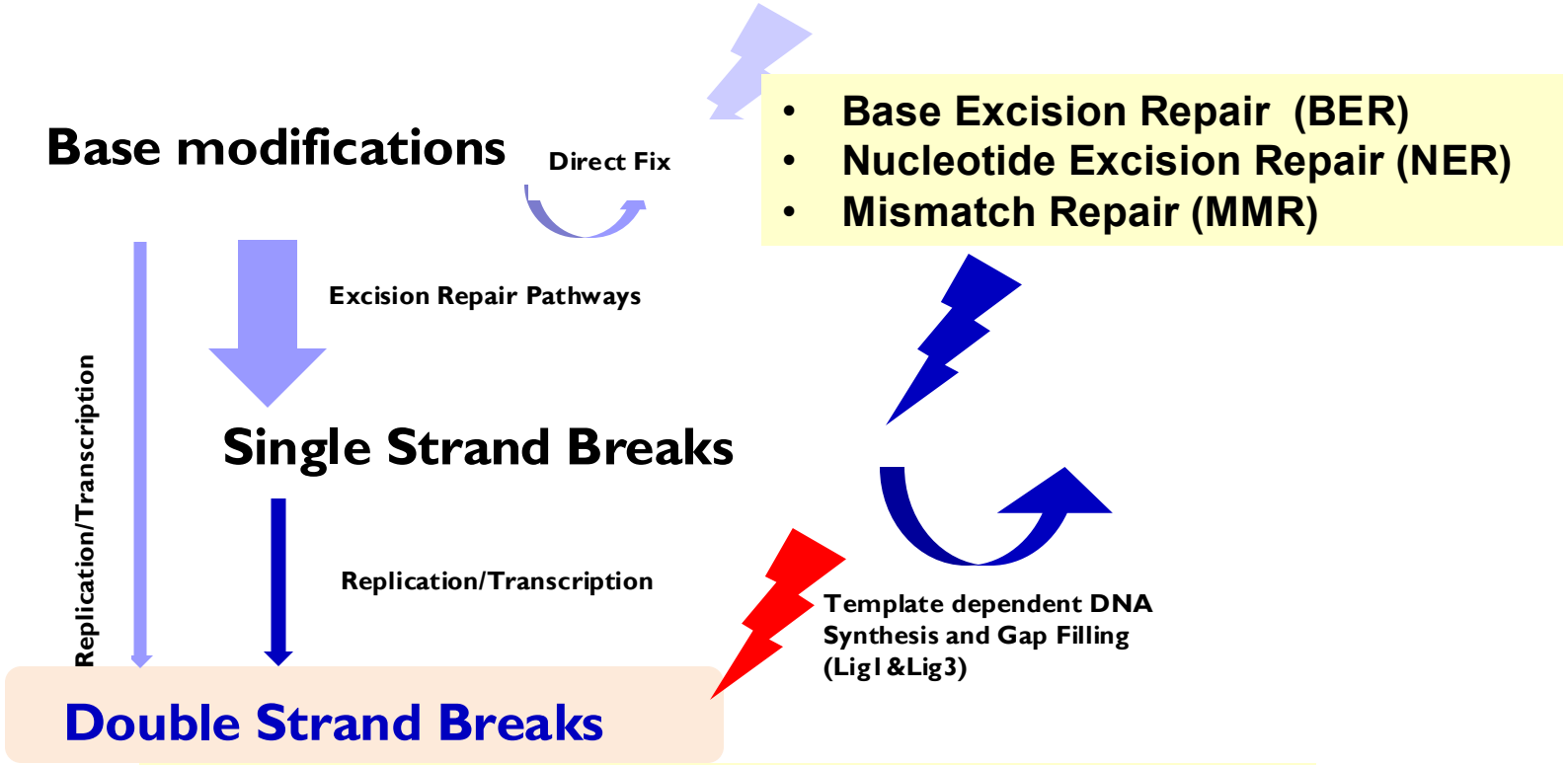
	MMR	BER	NER		RER
			General	TC	
Lesion	W-C mis-match	Bulk base damage	UV		rNTP
Condition	Base mis-match	Specific Glycosylase	Helix distortion	Transcription	replication
Committed lesion		Abase sites	XPF/XPG		RnaseH2 (A,B,C)
Features	Co-replication	nicks	Patch re-syn		Remove ribo

Features of BER/NER/MMR Defects

	BER	NER	MMR	RER
Hypersensitive	H2O2, Alkylation agents,	UV, Cross linking agents	Nitro, methylating agents	
Accumulate	8-oxo-G, Uracil…	Pyrimidine dimmers	Microsatellite instability	rNTP
Cancer	Colon	Skin	Colon/endometria/gastric/ovarian	overexpressed
Neuronal	Ataxia, microcephaly	Not common	Not common	neurological disorder*
Immunology	Antibody defects	mild	Antibody defects	auto-immuno
Others			infertile	

*Aicardi-Goutieres syndrome (AGS)

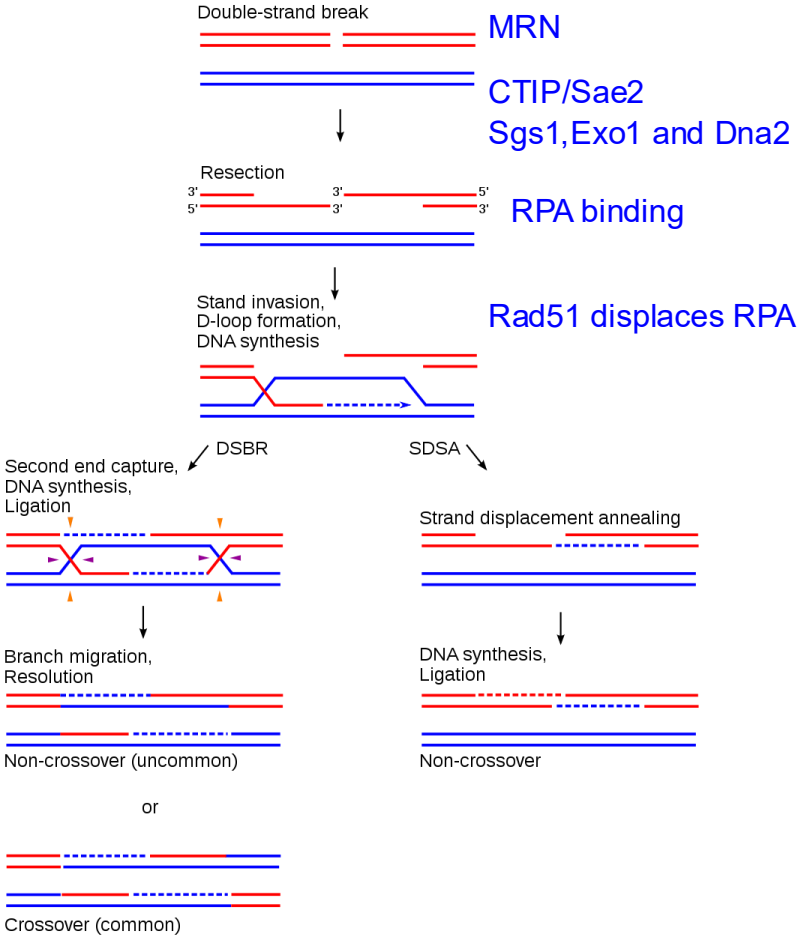
DNA repair pathways



- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- Mismatch Repair (MMR)

- Non-Homologues End Joining (NHEJ)
- Homologous Recombination (HR)
- Alternative End Joining (A-EJ)/ Microhomology Mediated End Joining (MMEJ)

Homologous Recombination (HR)



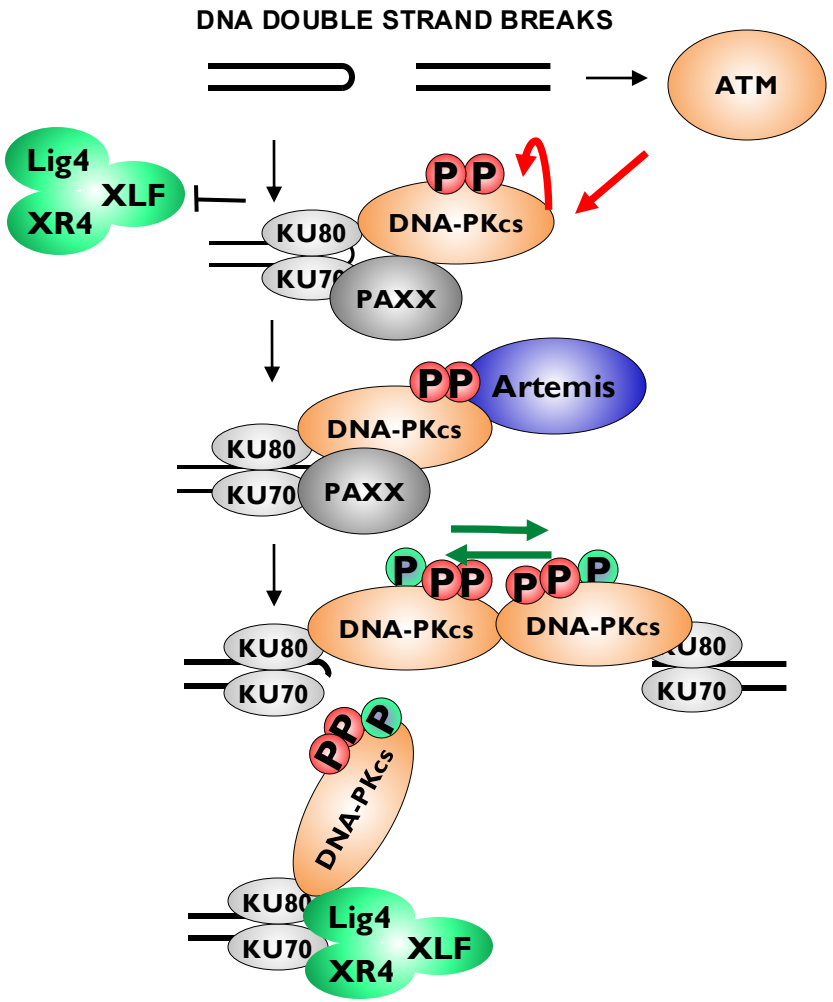
HR is conserved in eukaryotes.

Deficiencies in a subset of homologous recombination have been strongly linked to cancer.

Bloom's syndrome, Werner's syndrome and Rothmund-Thomson syndrome are caused by malfunctioning copies of **RecQ helicase** genes involved in the regulation of homologous recombination: **BLM, WRN** and **RECQ4**, respectively. In the cells of Bloom's syndrome patients (loss of BLM protein), there is an elevated rate of homologous recombination. Experiments in mice deficient in BLM suggested that the mutation gives rise to cancer through a loss of heterozygosity caused by increased homologous recombination.

Decreased rates of homologous recombination cause inefficient DNA repair, which can also lead to cancer. This is the case with **BRCA1** and **BRCA2**, two tumor suppressor genes whose malfunctioning has been linked with increased risk for breast and ovarian cancer. Cells missing BRCA1 and BRCA2 have a decreased rate of homologous recombination and increased sensitivity to ionizing radiation, suggesting that decreased homologous recombination leads to increased susceptibility to cancer.

Non-homologues end joining



NHEJ is partially conserved in eukaryotes and evolved extensively in vertebrates

Expressed in all cell types and throughout cell cycles.

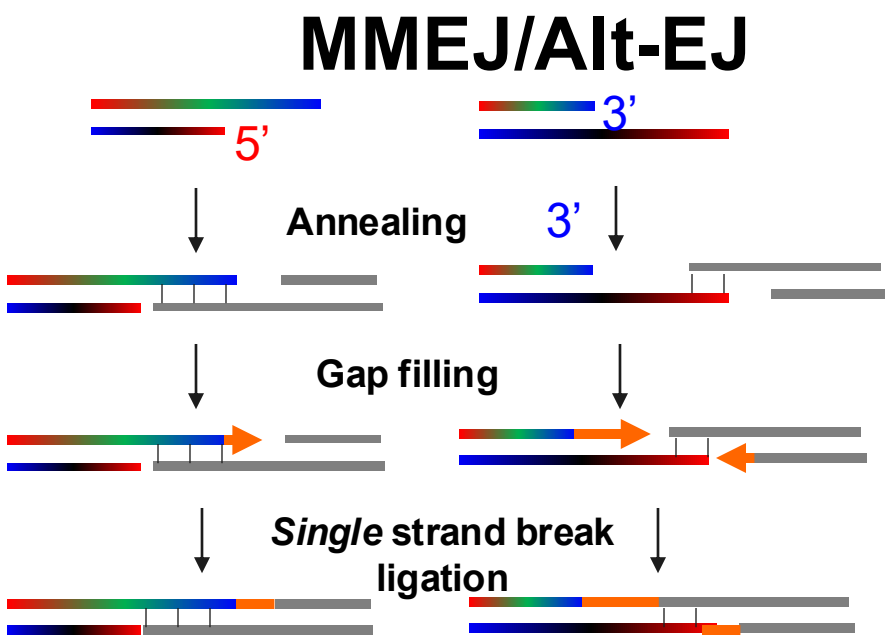
Members:
Ligation: Ku70/86, Lig4/XRCC4/XLF, PAXX
End-processing: DNA-PKcs, Artemis

Germ line mutations in NHEJ factors lead to microcephaly and severe combined immunodeficiency owing to the requirement of this pathway in V(D)J recombination.

On p53 deficient background, NHEJ deficient mice develop aggressive B cell lymphomas with clonal translocations involving IgH and c-Myc oncogene.

Mutations in the NHEJ pathway is rare in human cancers.

Alternative End-Joining Pathway (A-EJ) or Micro-homology Mediated End Joining (MMEJ)



A-EJ and MMEJ are two overlapping pathways that have been implicated in normal DNA repair and in chromosomal translocations.

A-EJ = end joining in cells lacking essential components of the NHEJ pathway (*e.g.*, XRCC4 or KU). MMEJ = end joining events that yield junctions with MH.

The degree of MH at the junctions varies dramatically depending on the sequence context and on the nature of the missing NHEJ factor, suggesting that there might be more than one A-EJ (and likely MMEJ) pathways.

Factors (mostly unknown): CtIP, MRE11, Lig1, PARP and ……

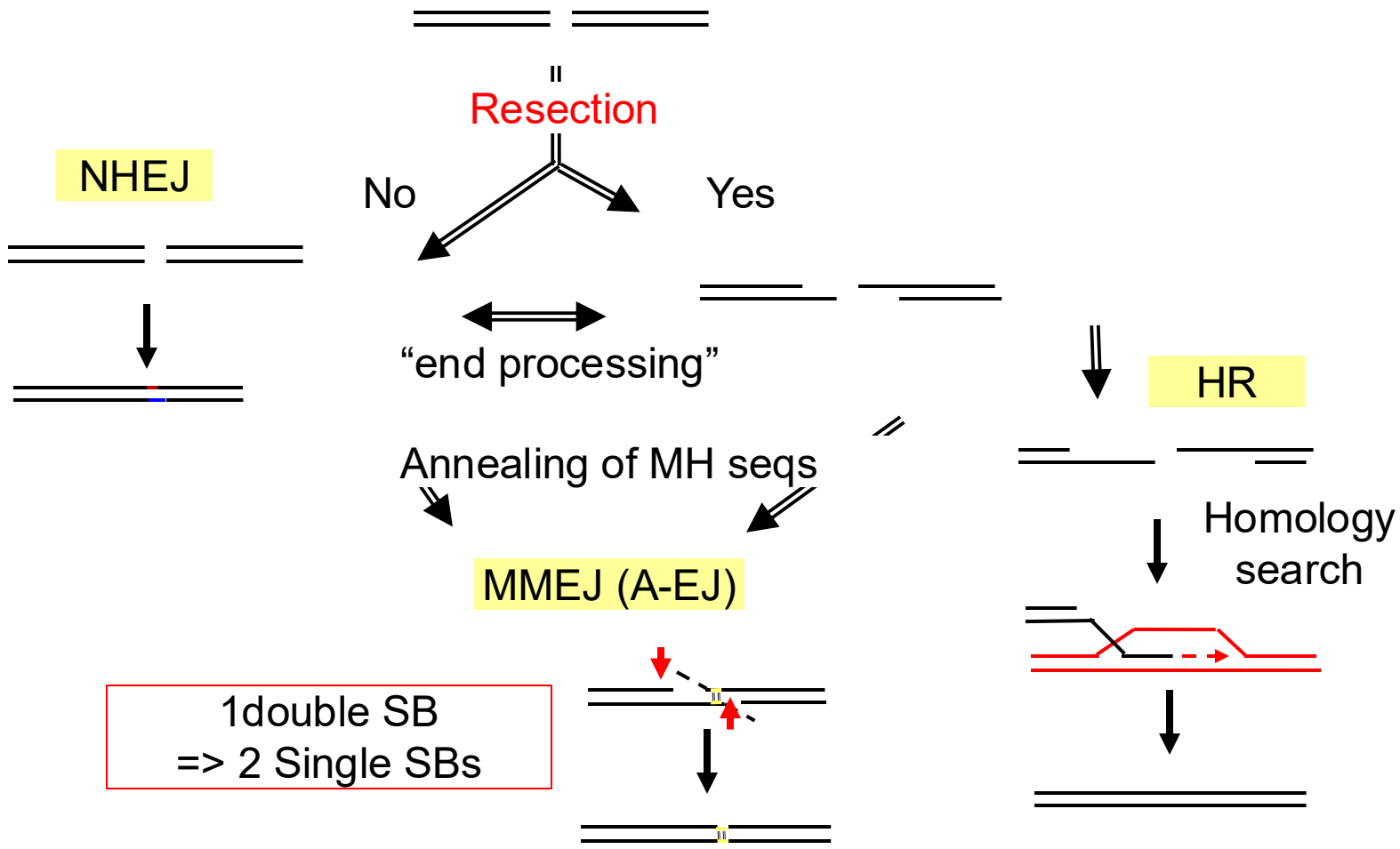
The canonical Ku-dependent NHEJ pathway CAN join DSBs with short MH (usually <4 nucleotides)!!

Assays for NHEJ/A-EJ/HR Defects

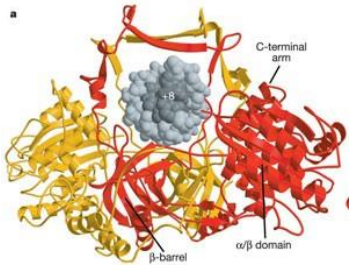
	NHEJ	HR
Junction	Direct or 1-4nt homology	seamless
Hypersensitive	IR,	IR, CPT, UV, PARPi, crosslink agents (decrease in Sister chromatid exchange)
Accumulate	Chromosome translocations, chromosome break	Replication defects, chromatid breaks
Cancer	Lymphomas,	Br, Colon, Pancreatic, Ovarian
Neuronal	Neuronal apoptosis	Not common
Immunology	SCID	Not common
Others		Infertile, often required for embryonic development

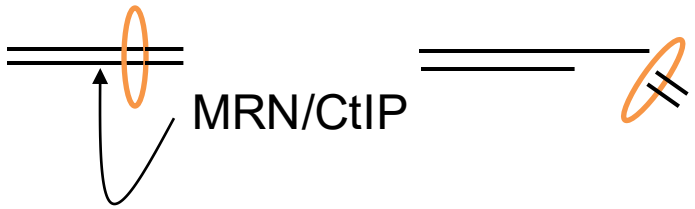
What to do? Pathway choice?

What to do? Pathway choice?



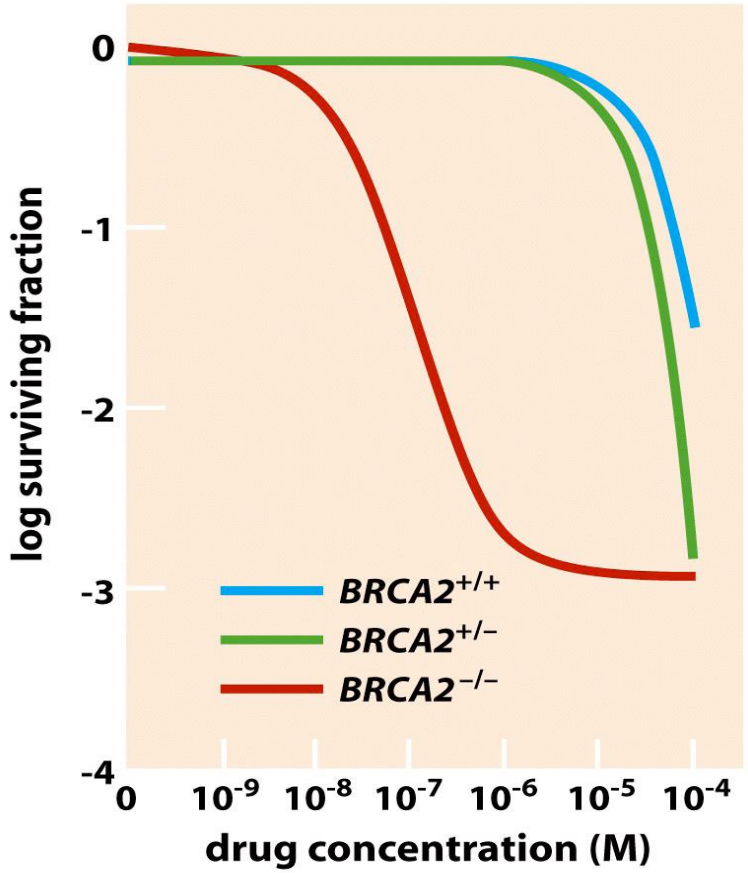
Cross Talks before HR and NHEJ





- They are not isolated events AND the pathway choice is not a permanent commitment.
- Share the substrates: DSBs that are not repaired by NHEJ in G1, can leak to S phase and get repaired by HR.
- **CDK1/2 mediated phosphorylation of CtIP in S/G2 phase plays an important role of regulating end-resection – the first step of HR.**
- Compete for ends: HR starts with end resection and resection (>4nt) will prevents Ku binding and NHEJ. Ku binding to the ends prevent resection by CtIP.
- Regulating each other: BRCA1 actively removes 53BP1 to promote HR. DNA-PKcs and Ku suppresses HR.

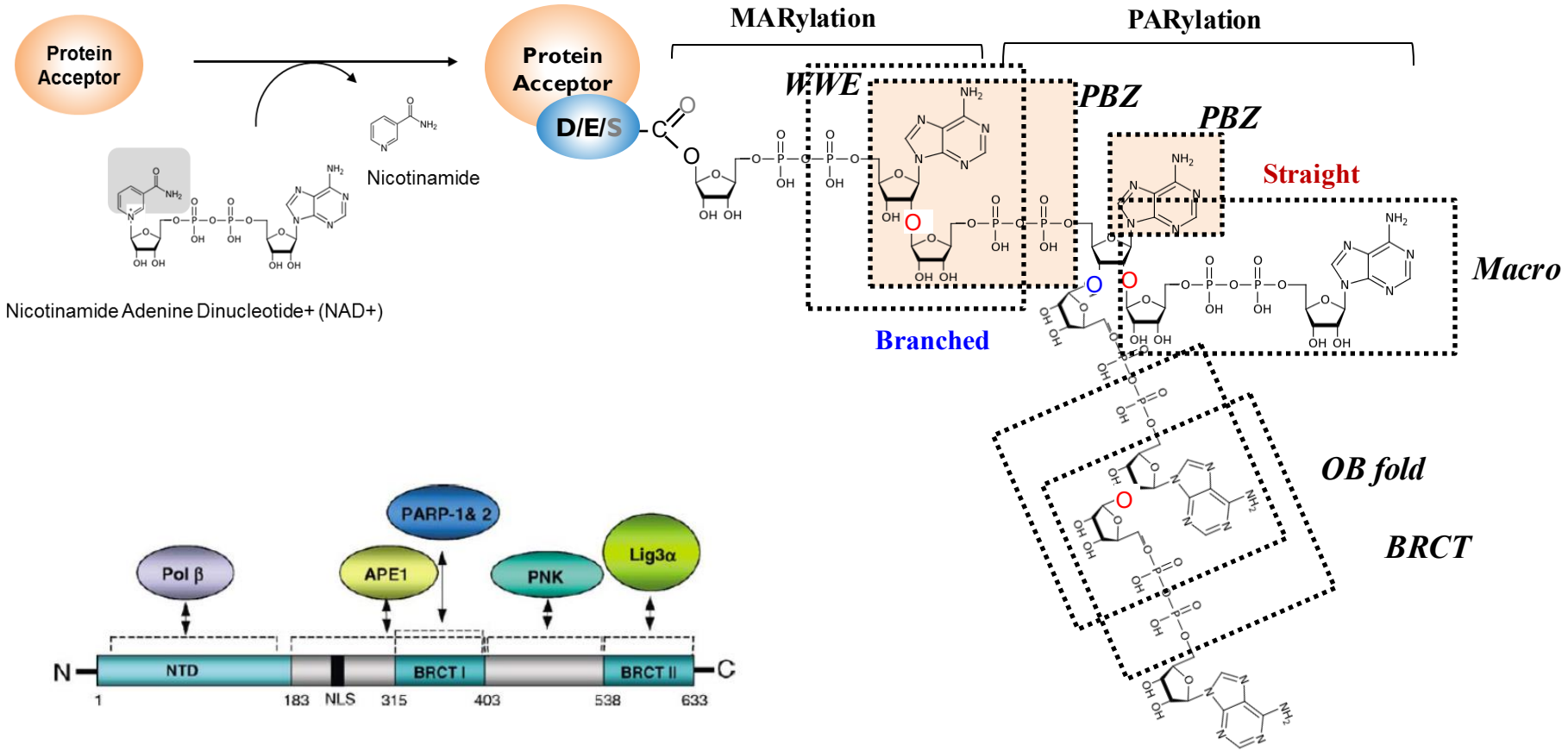
Target BRCAness cancer
Synergistic lethality with BRCA mutants



Agent	Tumor types	Company
Olaparib (Lynparza)	Breast, endometrial, gastric, glioblastoma, head and neck, lung, ovarian, pancreatic, prostate, sarcomas	AstraZeneca
Rucaparib	Breast, ovarian, pancreatic	Clovis Oncology
Niraparib (MK4827)	Breast, Ewing sacroma, ovarian	Tesaro
Veliparib (ABT-888)	Breast, cervical, colorectal, glioblastoma, head and neck, lung, leukemias, multiple myeloma, non-Hodgkin lymphoma, ovarian, pancreatic, prostate	AbbVie
Talazoparib (BMN-673)	Breast, endometrial, leukemias, ovarian, solid tumors	BioMarin Pharmaceutical

Farmer H ….Ashworth A. Nature. 2005 Apr 14;434(7035):917-21.
Bryant HE….Helleday T. Nature. 2005 Apr 14;434(7035):913-7.

The activity of PARP1&2

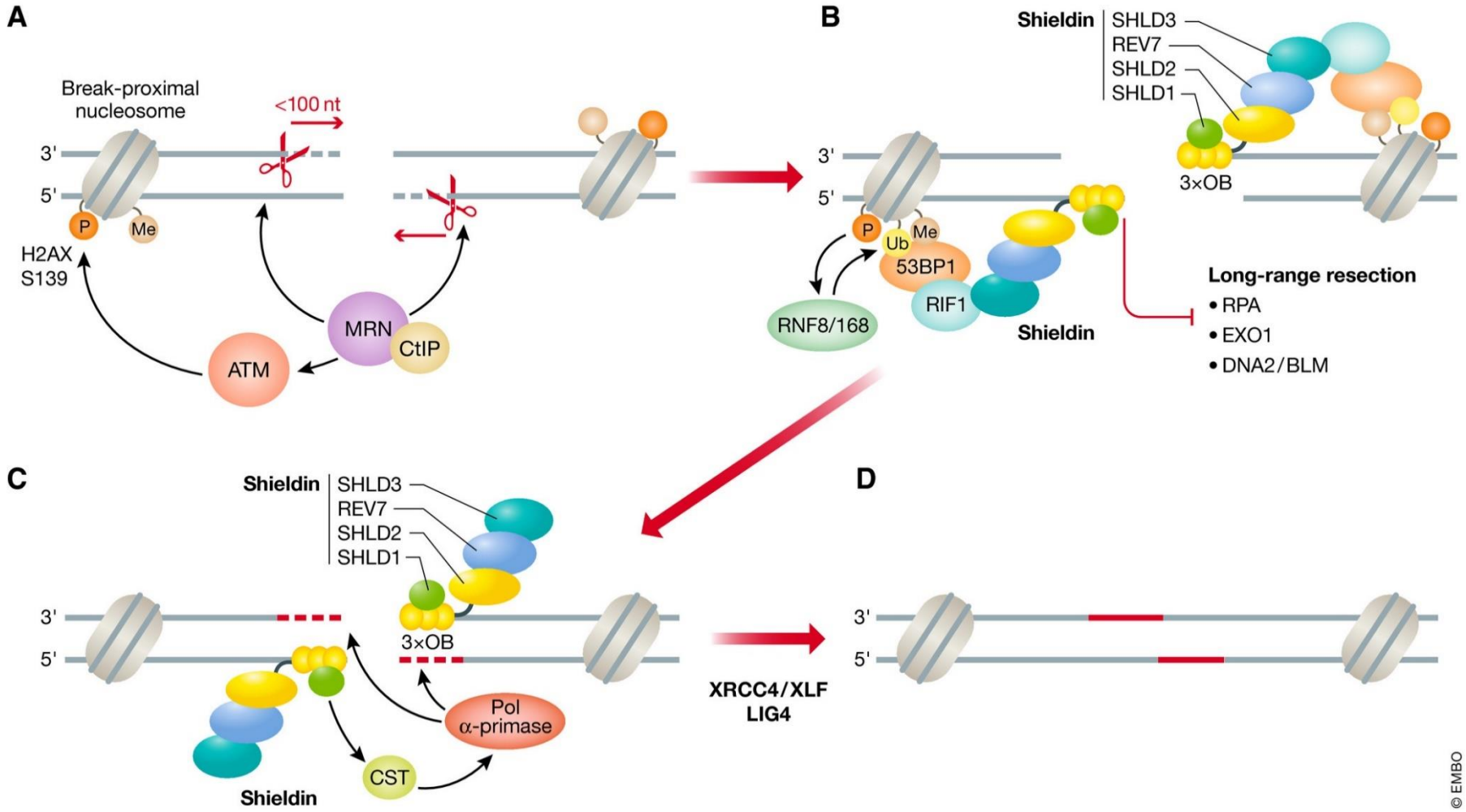


Nucleic Acids Research 2015, 43 (14) 6934–6944

Reader of PAR and MAR: WWE, PIN, BRCT, OB fold, Macrodomain, RRM, RGG, FHA, RS/KR

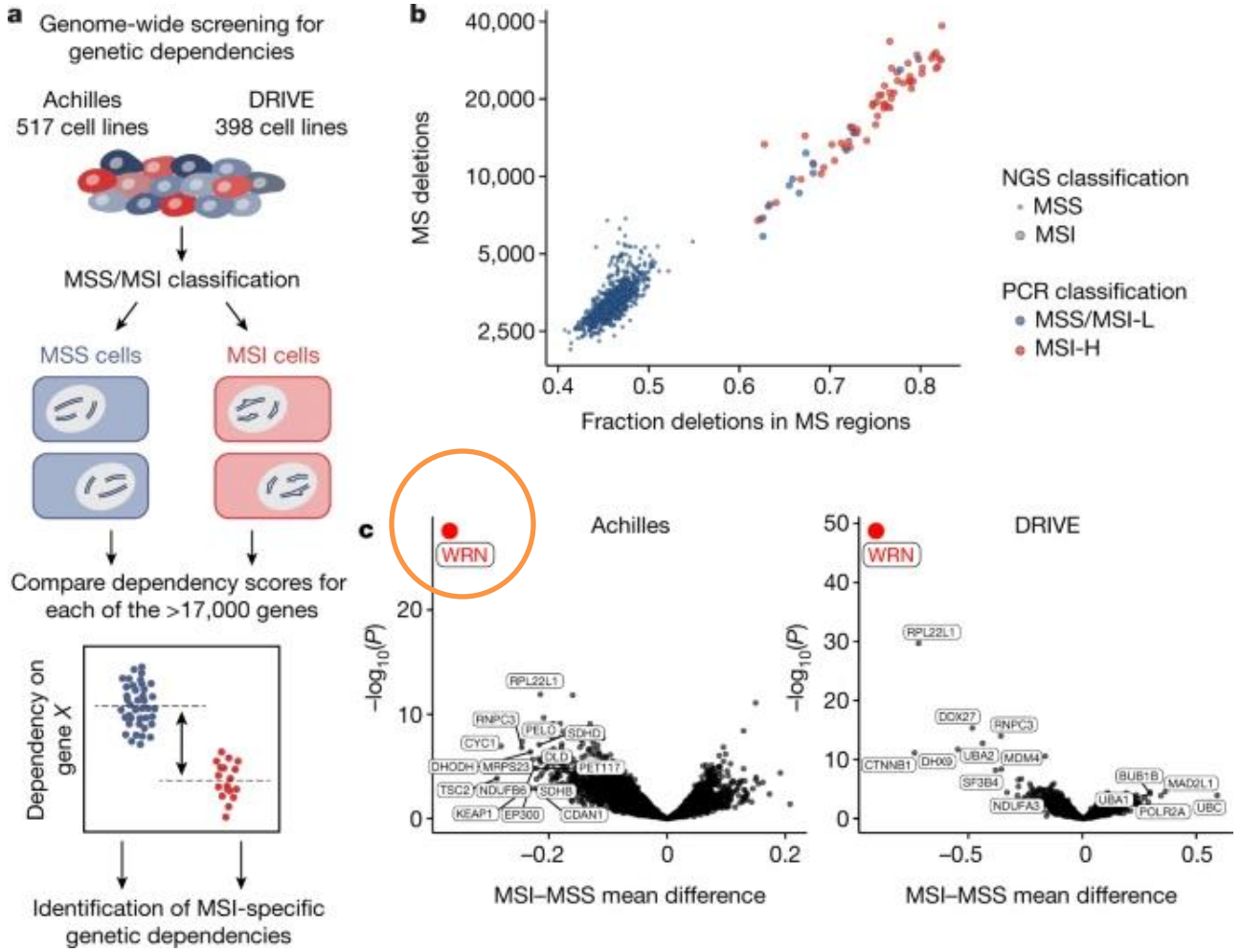
Overcome BRCAness

https://pubmed.ncbi.nlm.nih.gov/34503990/



https://www.embopress.org/doi/full/10.15252/embr.201847560

WRN helicase is a synthetic lethal target in microsatellite unstable cancers



Nature Article
Published: 30 September 2020
Repeat expansions confer WRN dependence in microsatellite-unstable cancers

https://www.nature.com/articles/s41586-019-1102-x

DNA "Damage" Response

Base modifications

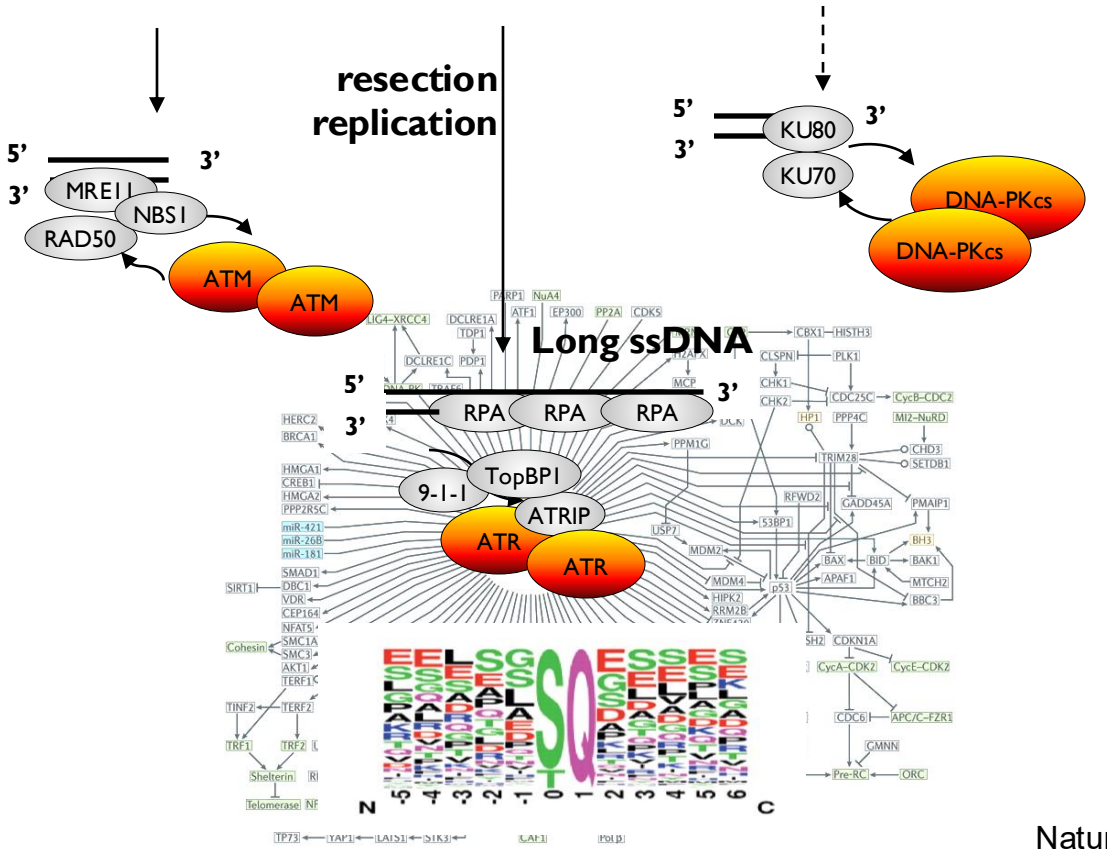
↓ Excision Repair Pathways

Single Strand Nicks

↓ Replication/Transcription

Double Strand Breaks 5' ═══ 3' / 3'

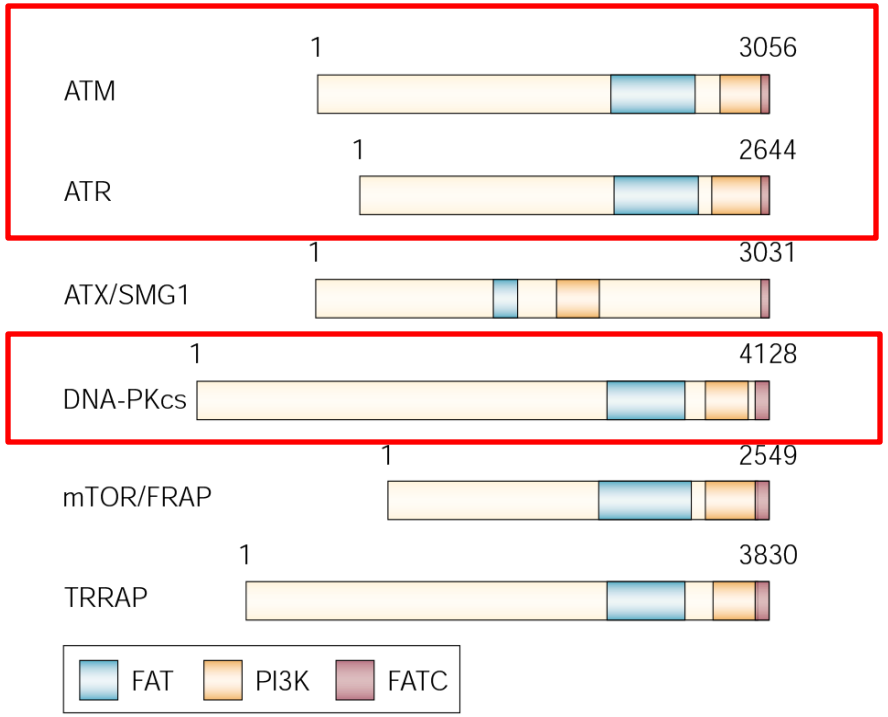
Base modification and single strand nicks do NOT directly activate DNA damage responses.



Nature Reviews Molecular Cell Biology 14, 197-210

PI3K related kinases (PI3KK)

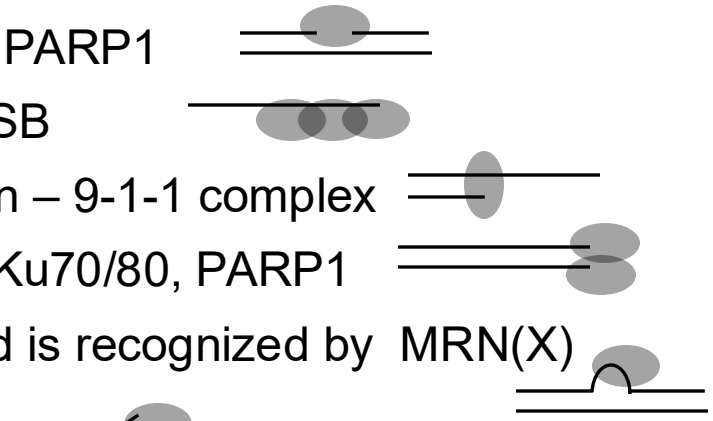
- **Inactivated at the basal level and activated by DNA doubles stand breaks through their specific "sensing proteins"**
  - ATM – MRE1 1/NBS/RAD50 + dsDNA Breaks
  - ART- RPA/ATRIP+ ssDNA
  - DNA-PKcs – KU70/80 + dsDNA Breaks
- **Activated ATM/ATR/DNA-PKcs phosphorylate targeted proteins (>800) at conserved SQ or TQ motifs to modulate checkpoints and DNA repair.**
- **Mutations in**
  - ATM - Ataxia -Telangiectasia Syndrome
  - ATR-Seckel Syndrome
  - DNA-PKcs- SCID with neurological defects.
- **Only ATM is inactivated in human cancers at significant levels.**
- **ATR is essential for normal DNA replication and cellular viability.**



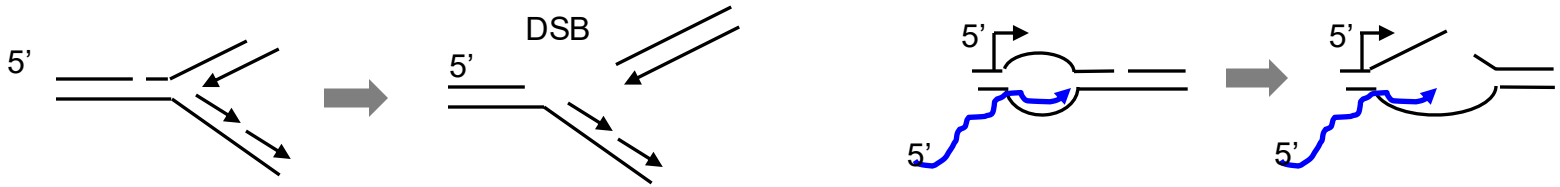
ATM and related protein kinases: safeguarding genome integrity. Nature Reviews Cancer 3, 155-168
ATR: an essential regulator of genome integrity Nature Reviews Molecular Cell Biology 9, 616-627

Recognizing DNA Damages

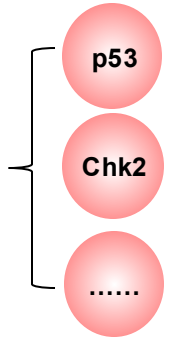
- ssDNA ends (nicks) are recognized by PARP1
- Extensive single strand DNA – RPA ,SSB
- Single stand double stand DNA junction – 9-1-1 complex
- dsDNA ends (15nt) are recognized by Ku70/80, PARP1
- Extensive dsDNA (>100bp) with an end is recognized by MRN(X)

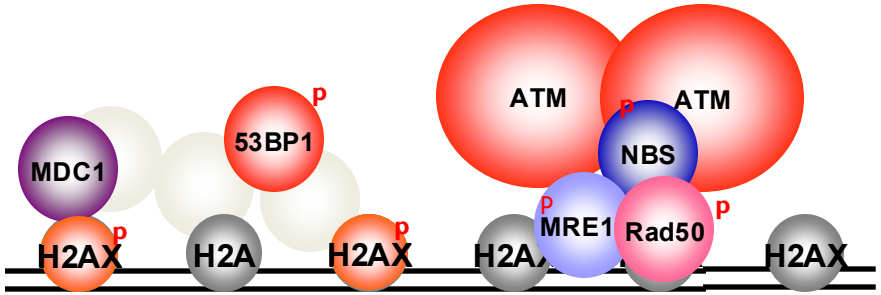


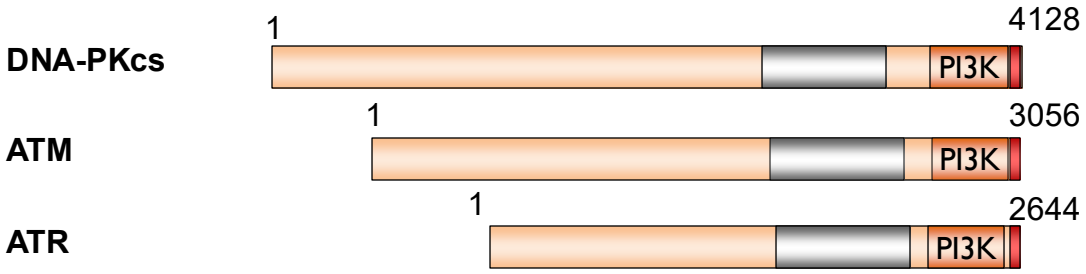
- Other – structural specific nucleases
- Coupling with transcription (NER) or DNA replication (MMR)
- Base alternations –cause strand distortion during globe NER



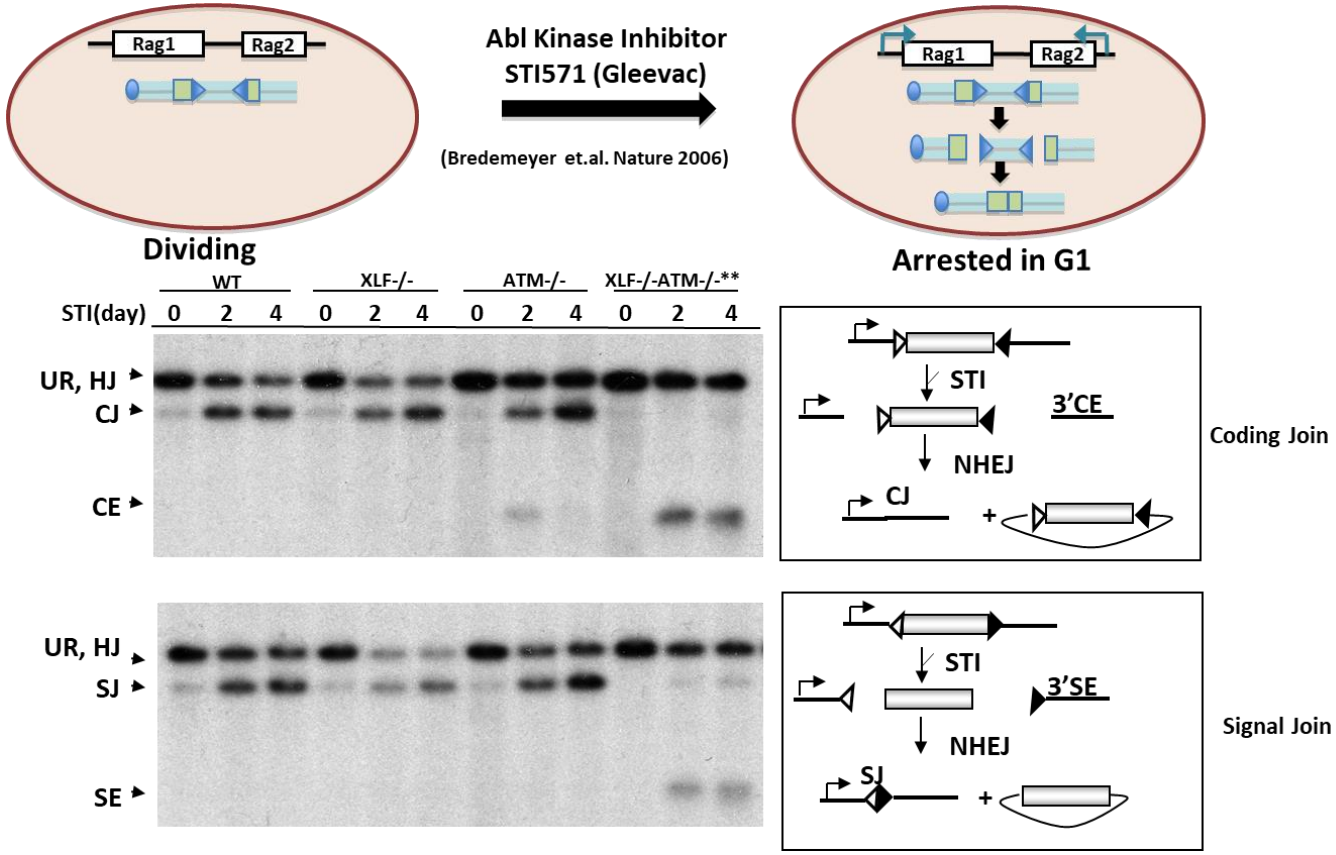
Damage Responses





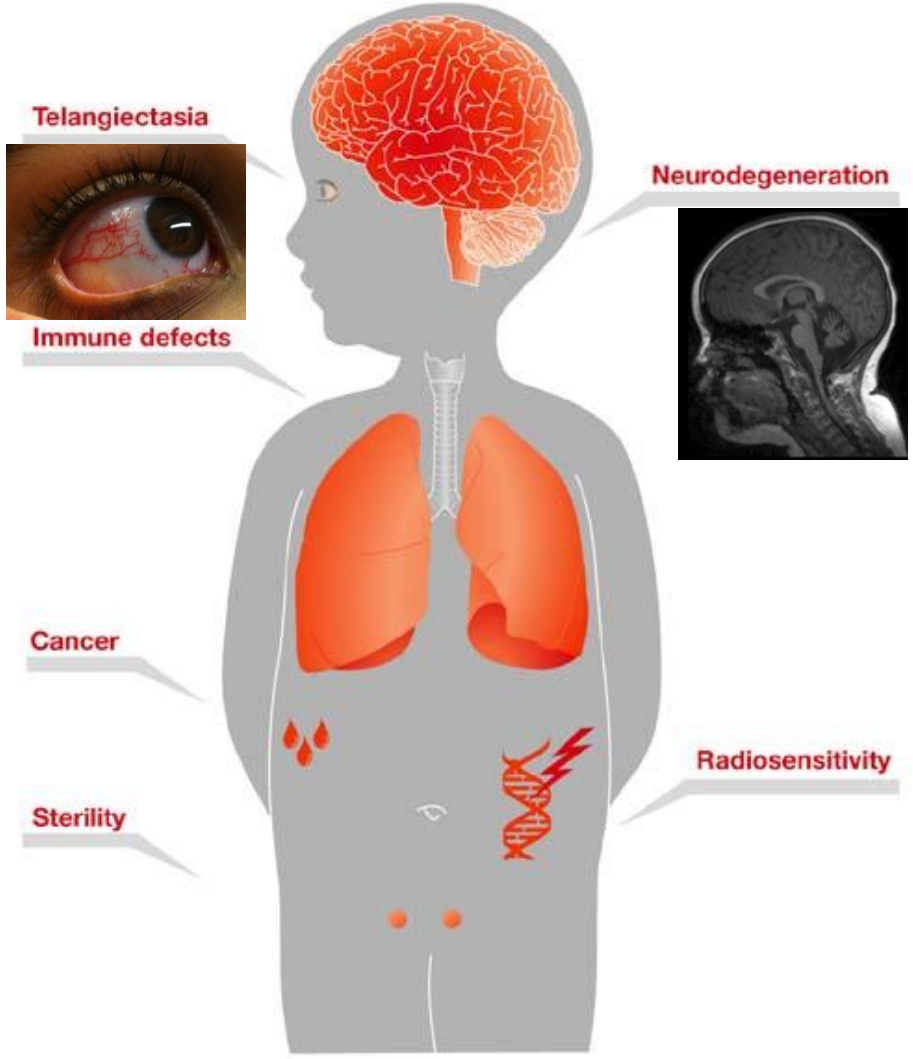


DNA damage responses promotes efficient repair



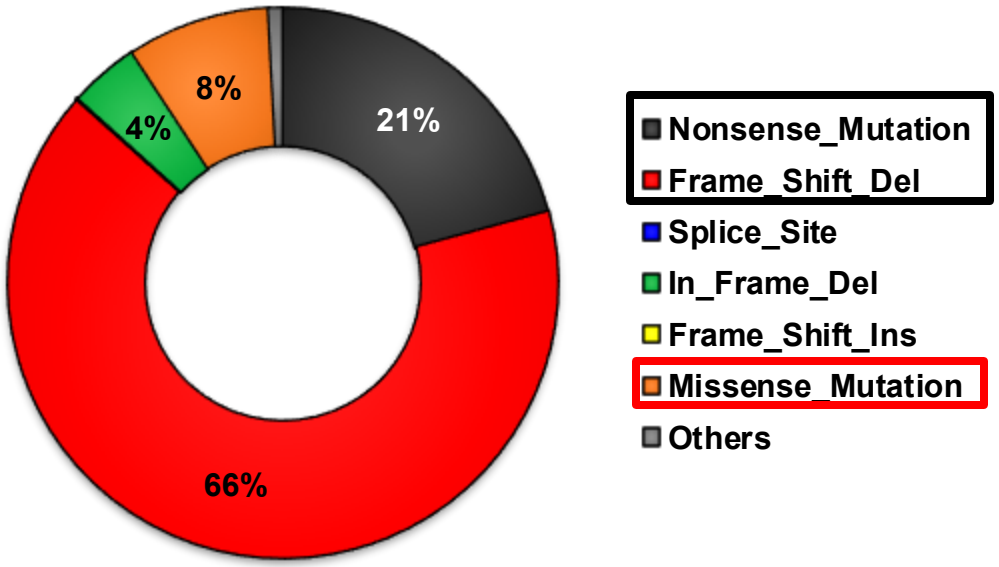
Ataxia-Telangiectasia Mutated (ATM)

- ATM is a Ser/Thr protein kinase and a master regulator of DNA damage response.
- ATM is activated by DNA double stand breaks through interaction with MRE11-RAD50-NBS1 complex.
- Homozygous germline inactivation mutation of ATM cause Atxia-Telangestasia (A-T syndrome).
- A-T syndrome is characterized by **cerebellum degeneration**, **primary immunodeficiency** and greatly increased risk for **leukemia and lymphomas**. Life time risk~ 25%. Yet ~50% of the patients die of lung diseases.
- Primarily an antibody defect
- T cell malignancies are especially increased.

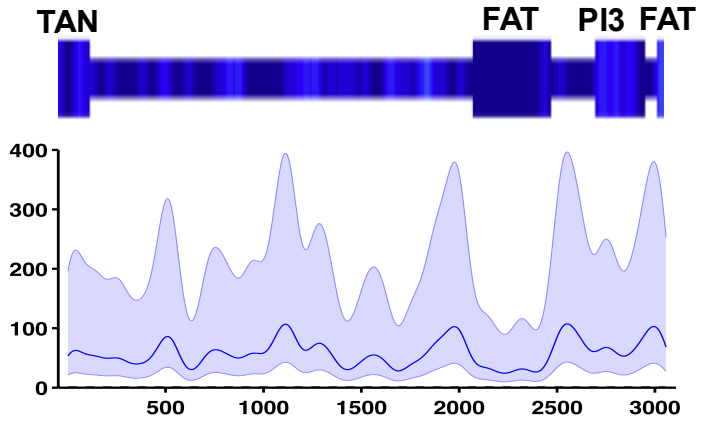


A-T like mutations of ATM

1116 A-T cases, 410 unique alterations



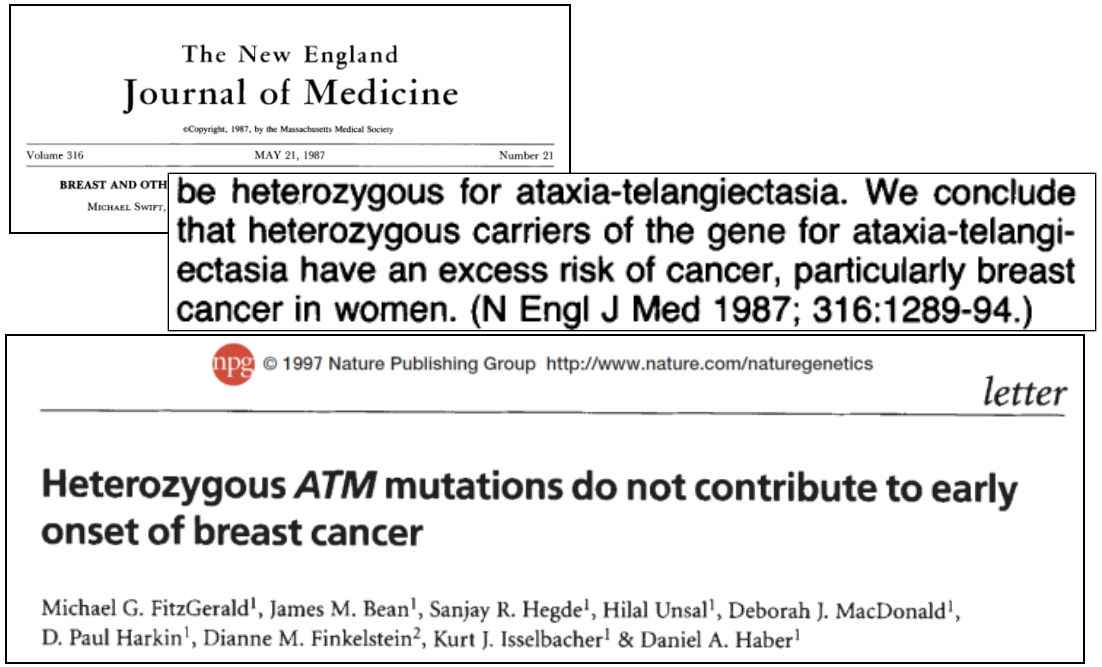
Distribution of A-T associated germline truncation mutations



Carrier of ATM mutations

It is estimated that ~1-2% of Caucasians carrying ATM mutations. As the diagnosis of A-T improves in Asian countries, more and more A-T cases have also been reported from Indian and Japan.

Does A-T carrier have an increased risk for cancer as the BRCA1 carriers?



The New England
Journal of Medicine

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Volume 316 MAY 21, 1987 Number 21

BREAST AND OTH
MICHAEL SWIFT,

be heterozygous for ataxia-telangiectasia. We conclude that heterozygous carriers of the gene for ataxia-telangiectasia have an excess risk of cancer, particularly breast cancer in women. (N Engl J Med 1987; 316:1289-94.)

© 1997 Nature Publishing Group http://www.nature.com/naturegenetics

letter

Heterozygous *ATM* mutations do not contribute to early onset of breast cancer

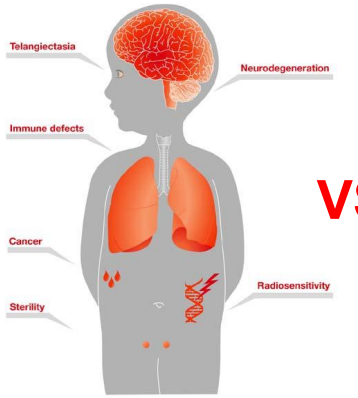
Michael G. FitzGerald[1], James M. Bean[1], Sanjay R. Hegde[1], Hilal Unsal[1], Deborah J. MacDonald[1], D. Paul Harkin[1], Dianne M. Finkelstein[2], Kurt J. Isselbacher[1] & Daniel A. Haber[1]

ATM inactivation in Cancer?

A-T causing mutation ≠ Cancer causing mutations

Gatti RA, Tward A, Concannon P. (1999). Mol Genet Metab 68: 419–423.

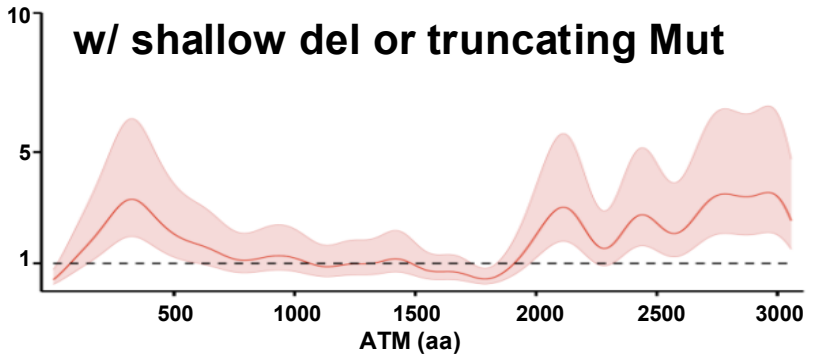
Kinase domain missense mutations are enriched in human cancer

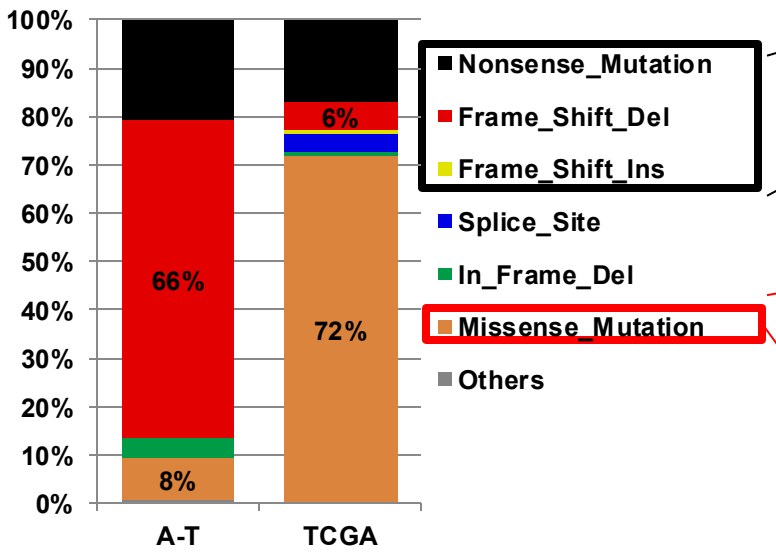


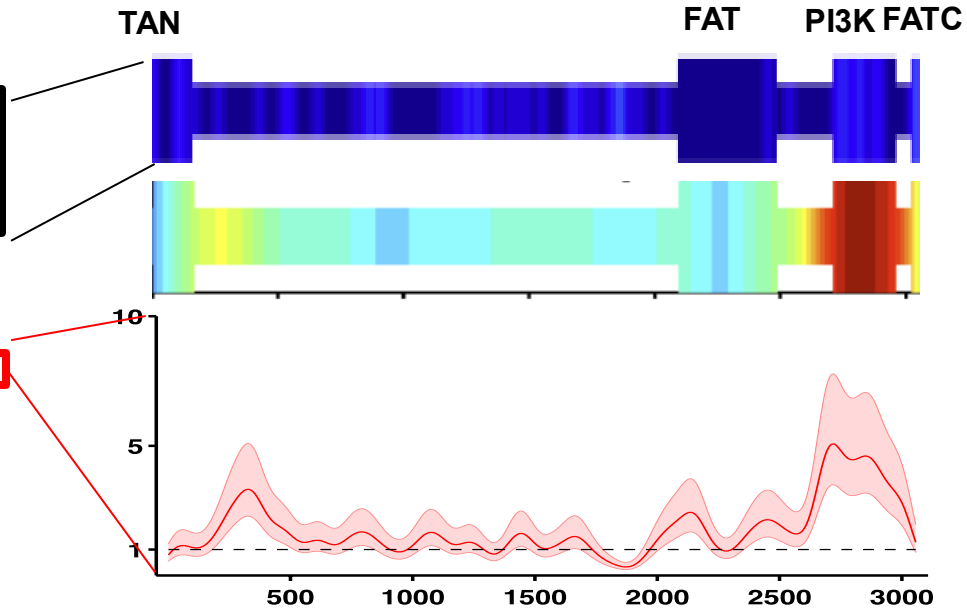
VS

1116 A-T cases
410 unique alterations

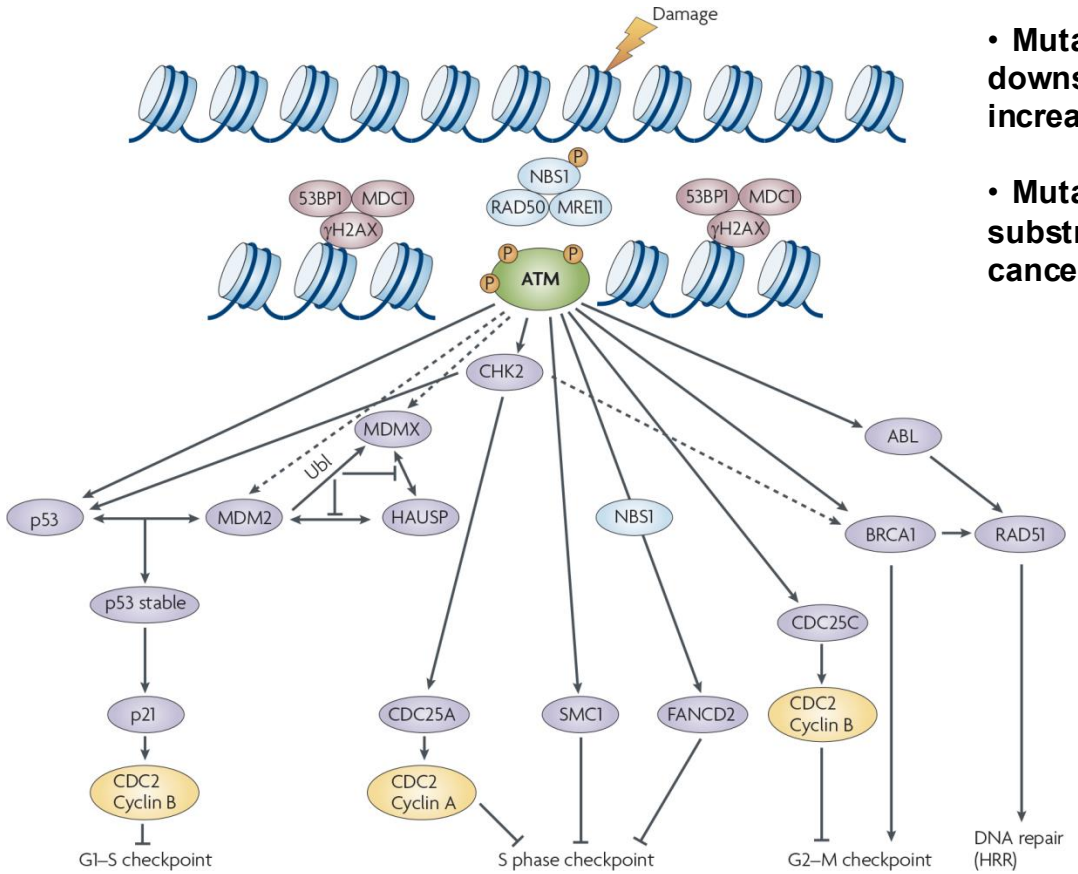
5,402 cases in TCGA,
332 alterations







ATM mutations lead to cancer



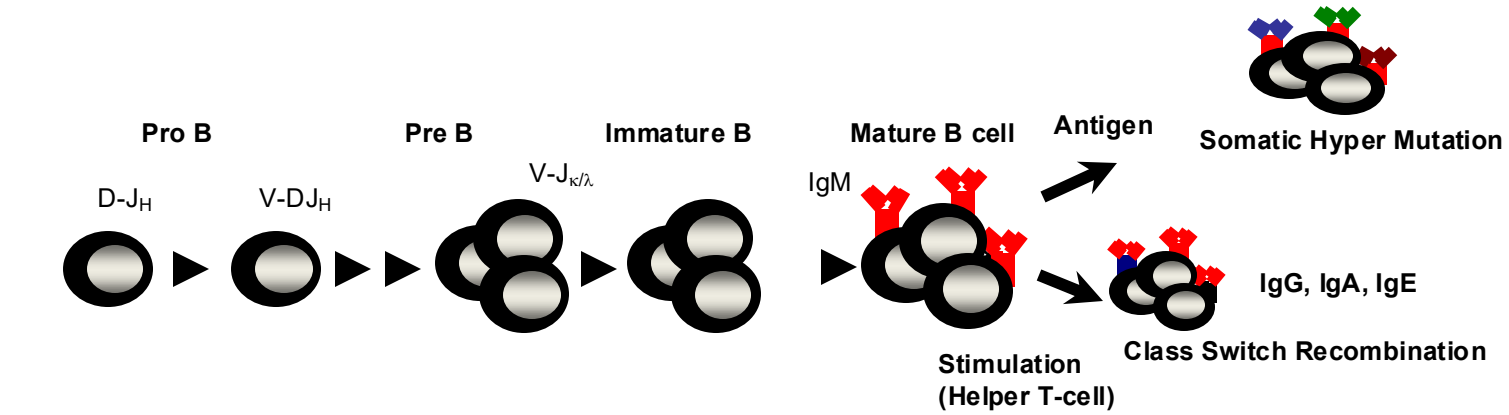
- **Mutations of ATM and its downstream checkpoint components increased the risk for cancer**
- **Mutation of the repair specific substrates of ATM are not common in cancers.**

Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signaling and cancer
Nature Reviews Molecular Cell Biology 9, 759-769 (October 2008)

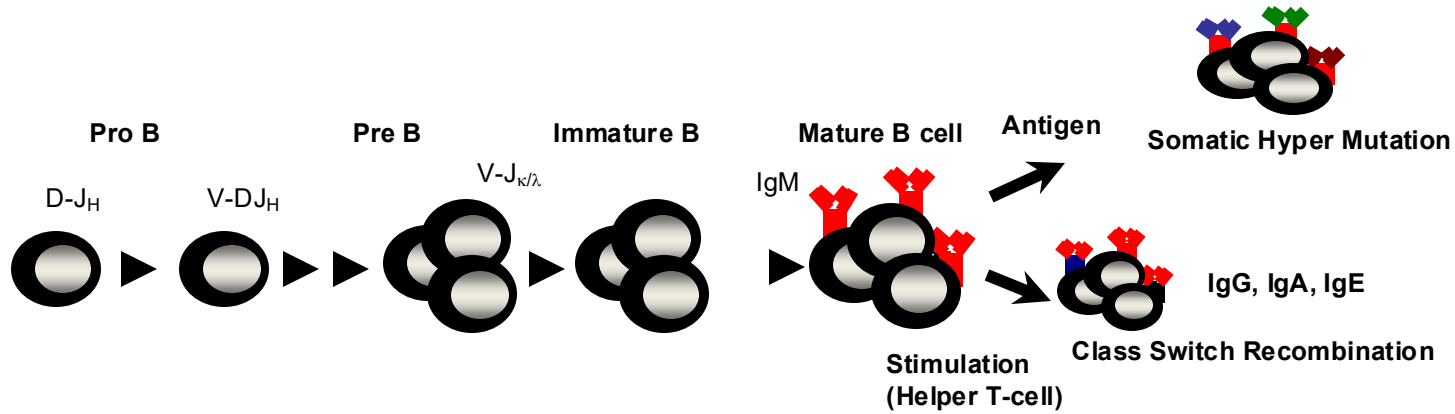
Overview

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways/DNA damage response
4. Case Studies
   I. Developmental DNA breaks - Lymphocyte, Meiosis, Neuronal function etc.
   II. Telomere, mitochondria DNA, and rDNA clusters

4.1.Lymphocytes Development



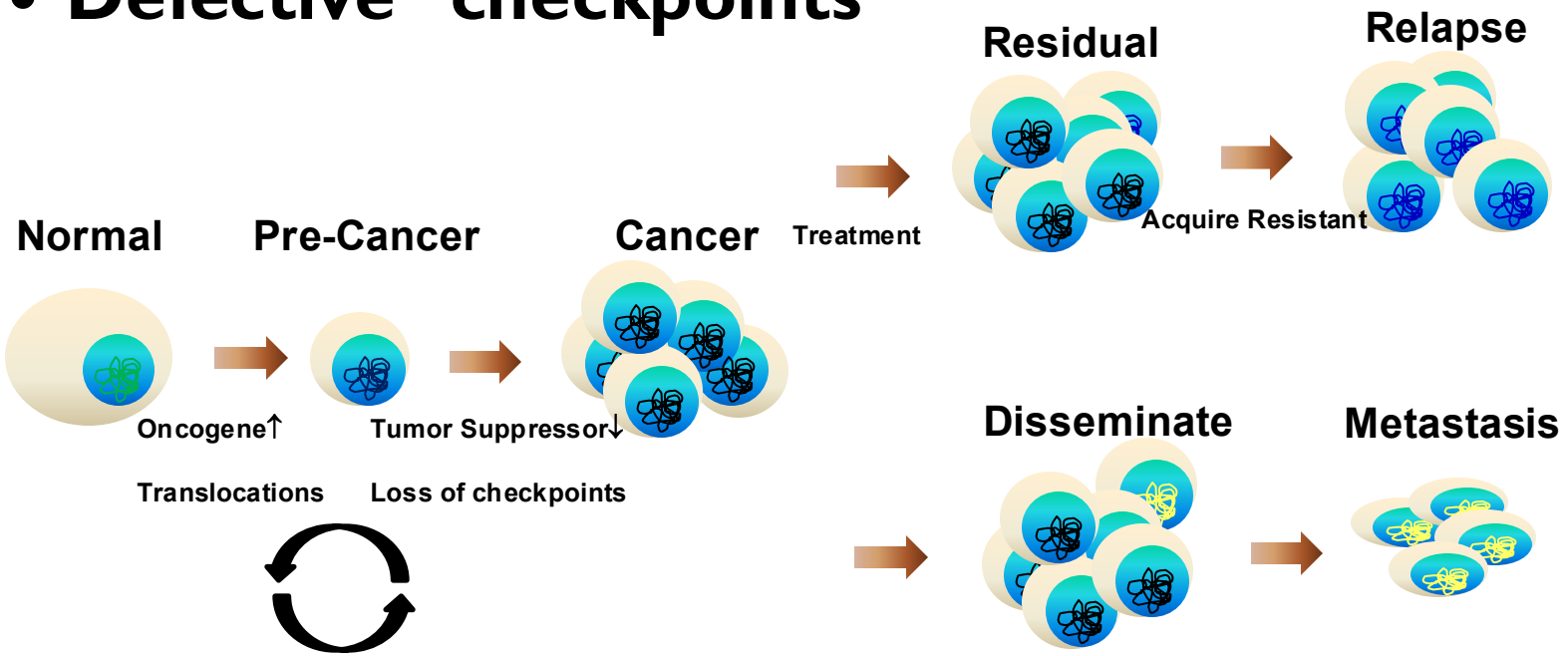
4.1.2 Lymphocyte to Lymphoma



V(D)J Recombination	Class Switch Recombination	Somatic hypermutation
• Non-homologous end joining	• Non-homologous end joining	• Mismatch Repair/BER
• DNA damage response (ATM)	• Alternative – end joining	• Base excision Repair
• Mistakes -> Translocations	• DNA damage response (ATM)	• Mistakes-> Mutation of other genes
	• Mistakes-> Translocation	
•IgH (IgL) -cMyc (Burkett's Lymphomas)		• Myc
•IgH- Bcl2 – Follicular Lymphomas	• IgH – Bcl6 (DLBCL)	• Bcl-6
•IgH- CyclinD1 – Mantle Cell Lymphomas	•	•…..

4.1.3 Translocation – risk factors

- **Breaks!**
- **Reduced repair fidelity**
- **Rapid proliferation and/or accumulation of several "oncogenic" events**
- **Defective "checkpoints"**



4.1.4 Translocation –where to go?

- Random translocation followed by functional selection
  - Passenger mutations/genomic instabilities
  - Why c-myc, not N-myc or L-myc?
- Targeted: Cryptic recombination site
- Other "influencing factors": transcription, physical distance, nuclear structure, etc.
  - Break first vs proximity first!

The emerging role of nuclear architecture in DNA repair and genome maintenance. Nat Rev Mol Cell Biol. 2009 Apr;10(4):243-54. Review.

Positional stability of single double-strand breaks in mammalian cells. Nat Cell Biol. 2007 Jun;9(6):675-82.

DNA damage defines sites of recurrent chromosomal translocations in B lymphocytes. Nature. 2012 Feb 7;484(7392):69-74

Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. Cell. 2011 Sep 30;147(1):107-19. Erratum in: Cell. 2011 Dec 23;147(7):1640.

Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. Cell. 2012 Mar 2;148(5):908-21.

Overview

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways
4. Case Studies
    I. Developmental DNA breaks - Lymphocyte, Meiosis, Neuronal function etc.
    II. Telomere, mitochondria DNA, and rDNA clusters

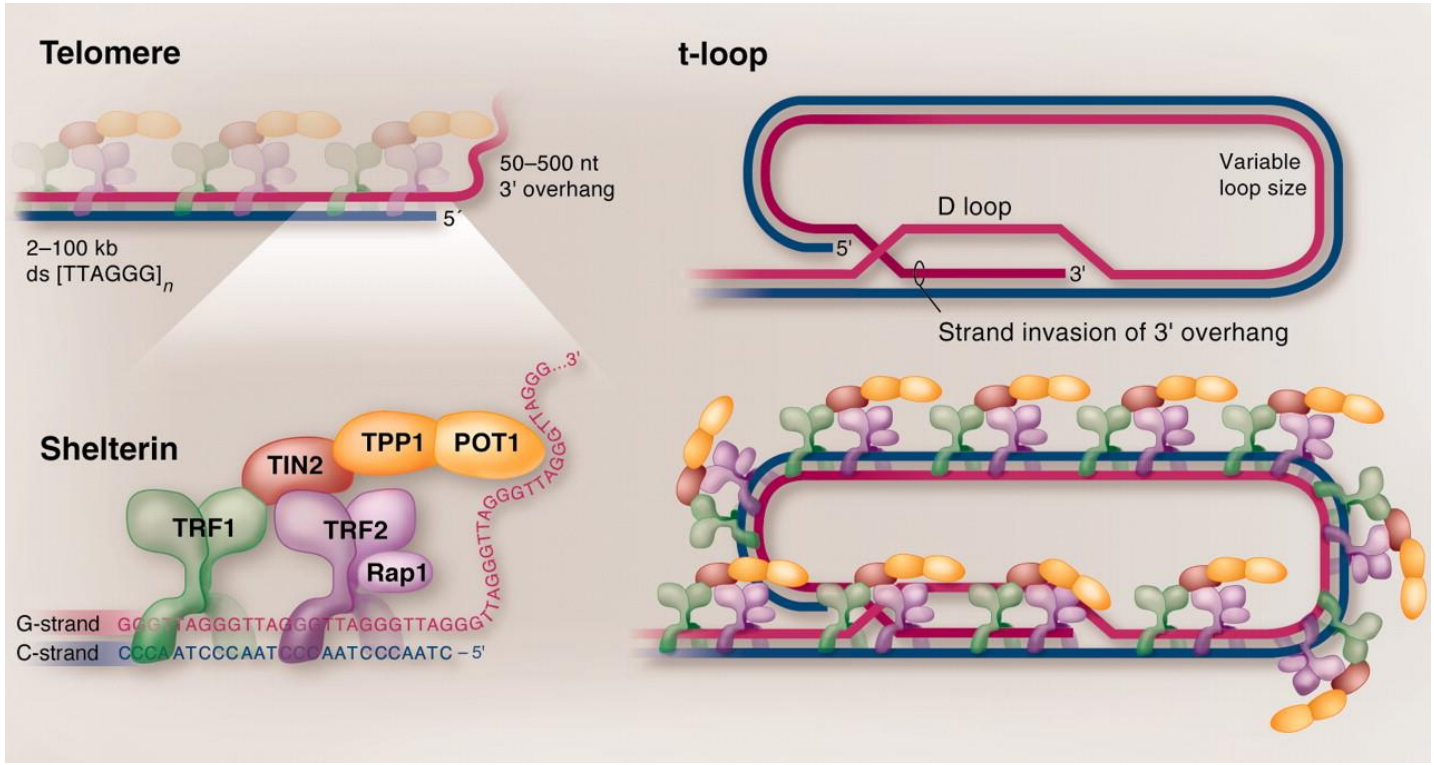
4.2.1 Telomere is the end of chromosome

Linearized eukaryotic chromosome present special "end" problems.

- A specialized mechanism of duplication – single replication origin

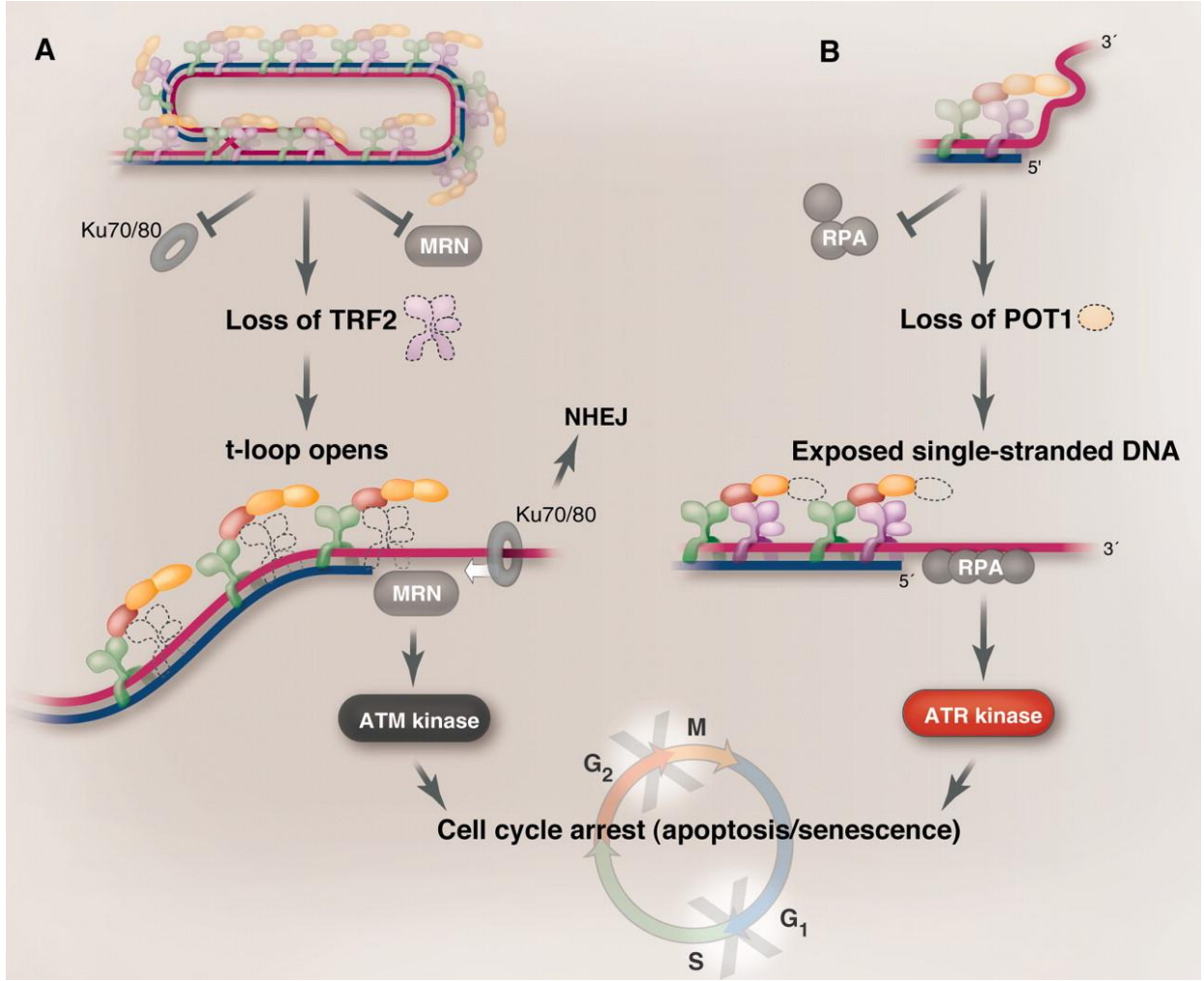
- To be protected from the cellular machinery that detects and repairs DNA breaks.

Telomere is protected from repair mechanisms by Shelterin complex

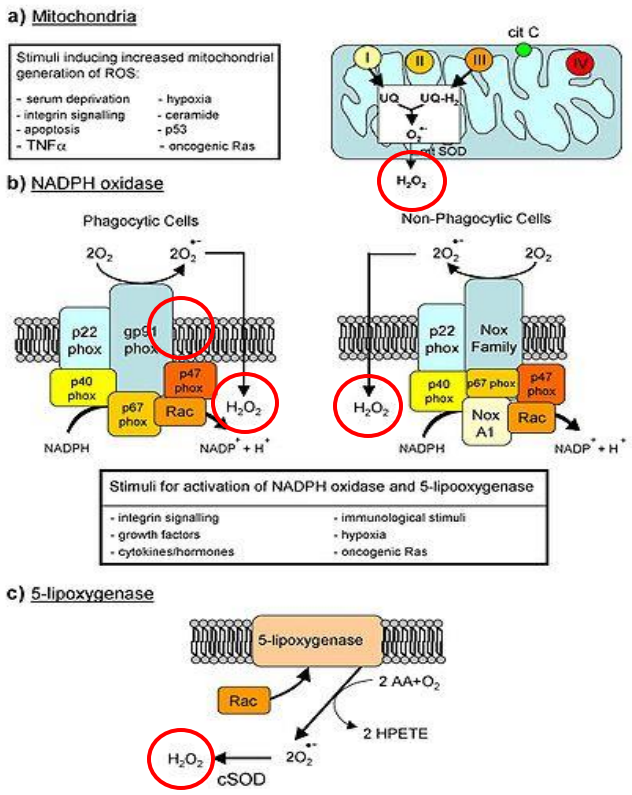


How telomeres solve the end-protection problem.
de Lange T.
Science. 2009 Nov 13;326(5955):948-52.

Shelterin Protect Telomere



4.2.2 Mitochondria and ROS



- **Reactive oxygen species** (ROS) are chemically-reactive molecules containing oxygen. Examples include oxygen ions and peroxides.
- ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling.
- ROS could directly modify DNA, RNA and proteins.
- It is also a mature source for mitochondrion DNA damage.
- ROS are also generated by exogenous sources such as ionizing radiation.

Nature. 2010 Mar 25;464(7288):610-4. **Heteroplasmic mitochondrial DNA mutations in normal and tumor cells.**

4.2.3 Mitochondrial DNA Damage

- Mitochondrial DNA (mtDNA) exists in multiple copies, and is tightly associated with a number of proteins to form a complex known as the nucleoid.
- Inside mitochondria, reactive oxygen species (ROS), or free radicals, byproducts of the constant production of adenosine triphosphate (ATP) via oxidative phosphorylation, create a highly oxidative environment that is known to damage mtDNA.
- A critical enzyme in counteracting the toxicity of these species is superoxide dismutase, which is present in both the mitochondria and cytoplasm of eukaryotic cells.
- Recent studies also identified the mitochondrion form of Lig3 as a critical component for mitochondrion DNA repair and survival.

- **Heteroplasmic mitochondrial DNA mutations in normal and tumour cells.** Nature. 2010 Mar 25;464(7288):610-4.
- **The maintenance of mitochondrial DNA integrity--critical analysis and update.** Cold Spring Harb Perspect Biol. 2013 May 1;5(5):a012641.
- **Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair.** Nature. 2011 Mar 10;471(7337):245-8.
- **DNA ligase III is critical for mtDNA integrity but not Xrcc1-mediated nuclear DNA repair.** Nature. 2011 Mar 10;471(7337):240-4.

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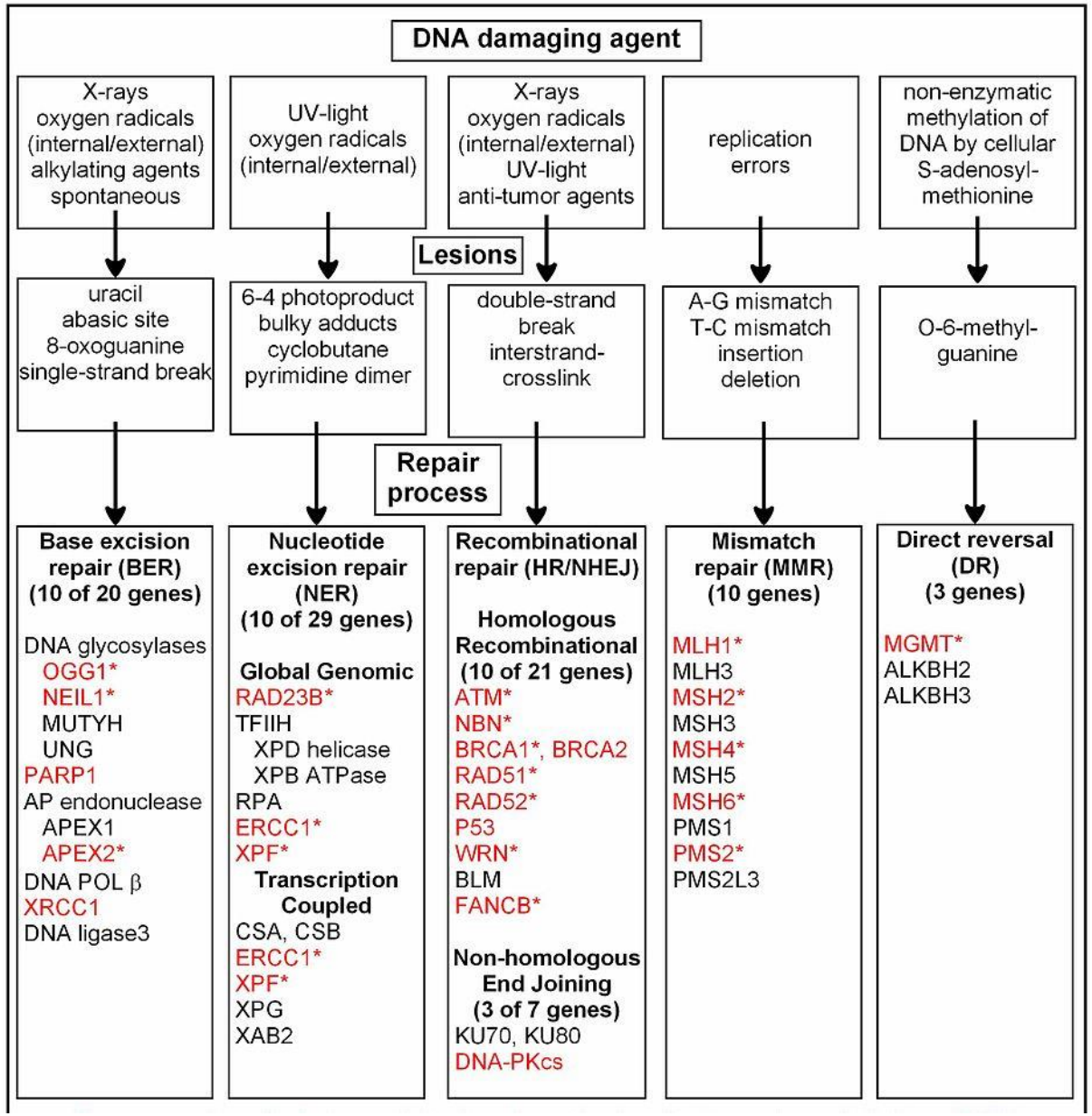
Take home…..

- **DNA damage and repair are constant battles in all living cells.**
- **DNA repair play important roles in the initiation, treatments and therapeutic responses of cancer.**
- **DNA damage response has a chromatin component which promotes DNA repair.**
- **DNA damage response also activates the cell cycle checkpoints.**
- **Checkpoints also act as the gate keeper to prevent damaged cells from further proliferation.**
- **Genomic instability can be targeted for Cancer therapy**

"The DNA damage response in tumorigenesis and cancer treatment"&
"Genomic instability in cancer"
Nature Review Cancer

DNA repair and DNA damage response defects

Problems In Responding to DNA Damage Or Stalled Replication Forks	
Ataxia telangiectasia	***ATM*** detects DNA damage & stalled forks
Seckel syndrome	***ATR*** detects DNA damage & stalled forks; other checkpoint/replication genes may also be involved
Li-Fraumeni syndrome	***CHK2***and ***TP53*** respond to DNA damage and stalled forks
Ataxia telangiectasia-like disorder	***MRE11***rescues stalled forks; repairs DNA damage
Nijmegen breakage syndrome	***NBS1***rescues stalled forks; repairs DNA damage
Bloom syndrome	*RECQ2/BLM* encodes a DNA helicases that rescues stalled forks
Werner syndrome	*RECQ3/WRN* encodes a DNA helicase
Rothmund-Thomson syndrome	*RECQ4/RTS* encodes a DNA helicase
Rapadillino Syndrome	*RECQ4* encodes a DNA helicase
Problems In Repair Of Damaged DNA	
Familial breast cancer, Ovarian,	***BRCA1*** and ***BRCA2*** repair radiation-induced breaks in double-stranded DNA
Fanconi anemia	Eleven **FA** genes, one of which (D1) is *BRCA2,* ICL repair
Xeroderma pigmentosum	***XPA, XPC, XPF, XPG*** repair nucleotide excisions ***XPD*** is a DNA helicase
Xeroderma pigmentosum variant	***POLH***/DNA polymerase-eta carries out trans-lesion DNA synthesis
Cockayne syndrome XP-Cockayne syndrome	***CSA*** and ***CSB*** repair DNA damage ***XPD*** encodes a DNA helicase
Trichothiodystrophy	***XPB*** and ***XPD*** encode DNA helicases
HNPCC, hereditary non-polyposis colon cancer	**MSH2, MLH1** (major), **MSH6, PMS2, PMS1** (minor) involved in mismatch repair (MMR)
LIG4 syndrome	***LIG4***/DNA ligase IV is required for non-homologous DNA end-joining
Radiosensitive severe combined immunodeficiency (RS-SCID), Omenn Syndrome	***ARTEMIS*** encodes a hairpin-specific nuclease that plays a subsidiary role in non-homologous end-joining, and V(D)J recombination.


DNA damaging agent
X-rays
oxygen radicals
(internal/external)
alkylating agents
spontaneous
UV-light
oxygen radicals
(internal/external)
X-rays
oxygen radicals
(internal/external)
UV-light
anti-tumor agents
replication
errors
non-enzymatic
methylation of
DNA by cellular
S-adenosyl-
methionine
Lesions
uracil
abasic site
8-oxoguanine
single-strand break
6-4 photoproduct
bulky adducts
cyclobutane
pyrimidine dimer
double-strand
break
interstrand-
crosslink
A-G mismatch
T-C mismatch
insertion
deletion
O-6-methyl-
guanine
Repair
process
Base excision
repair (BER)
(10 of 20 genes)
DNA glycosylases
OGG1*
NEIL1*
MUTYH
UNG
PARP1
AP endonuclease
APEX1
APEX2*
DNA POL β
XRCC1
DNA ligase3
Nucleotide
excision repair
(NER)
(10 of 29 genes)
Global Genomic
RAD23B*
TFIIH
XPD helicase
XPB ATPase
RPA
ERCC1*
XPF*
Transcription
Coupled
CSA, CSB
ERCC1*
XPF*
XPG
XAB2
Recombinational
repair (HR/NHEJ)
Homologous
Recombinational
(10 of 21 genes)
ATM*
NBN*
BRCA1*, BRCA2
RAD51*
RAD52*
P53
WRN*
BLM
FANCB*
Non-homologous
End Joining
(3 of 7 genes)
KU70, KU80
DNA-PKcs
Mismatch
repair (MMR)
(10 genes)
MLH1*
MLH3
MSH2*
MSH3
MSH4*
MSH5
MSH6*
PMS1
PMS2*
PMS2L3
Direct reversal
(DR)
(3 genes)
MGMT*
ALKBH2
ALKBH3