DNA Repair and the DNA Damage Response

A. But first, a little background information...

1. how did we figure out that DNA (either directly or indirectly) was the principal target for the biological effects of ionizing radiation, including cell killing, mutagenesis and carcinogenesis?

a) the evidence includes:

- Radioactive nucleotides incorporated into cellular DNA produce cell killing, whereas this is not the case for radionuclides incorporated into other cellular proteins
- Bromodeoxyuridine incorporated into cellular DNA is a radiosensitizer.
- Selective irradiation of the cellular cytoplasm produces *much less* cell killing than selective irradiation of the nucleus.
- Mutant cell lines unable to repair some types of DNA or chromosomal damage are exquisitely radiosensitive.

B. OK, so DNA has been damaged by radiation exposure...what happens next?

1. the direct or indirect damage to DNA initially takes the form of **DNA·** ("radicalized" DNA), but this is an unstable structure that promptly decays into one or more of the following biochemical lesions:

Yields of Damaged DNA

Type of DNA Damage	Number of Lesions/Cell/Gy†
★ Base damage, loss or substitution	**1,000 - 2,000**
★ Sugar damage	**~1,000**
★ Single strand breaks (SSB)	**~1,000**
★ Double strand breaks (DSB)	**30 - 50**
★ DNA-protein crosslinks (CL)	**100 - 200**
★ DNA-DNA crosslinks (ISCL)	**~30**

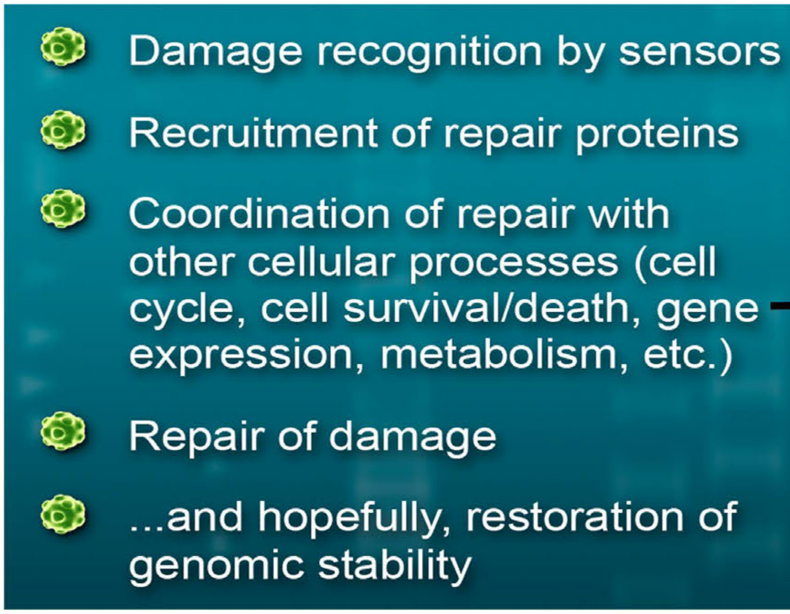
†For X-rays.

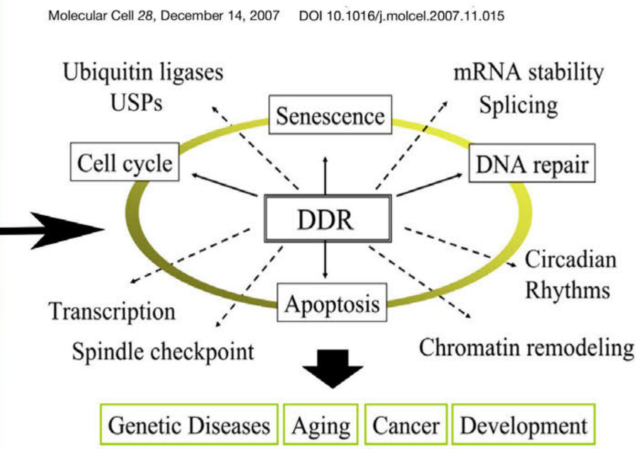
up to 500,000 DNA modification events per cell per day

By way of perspective. always remember that this is how many DNA damages there are per cell per day just from normal metabolism!

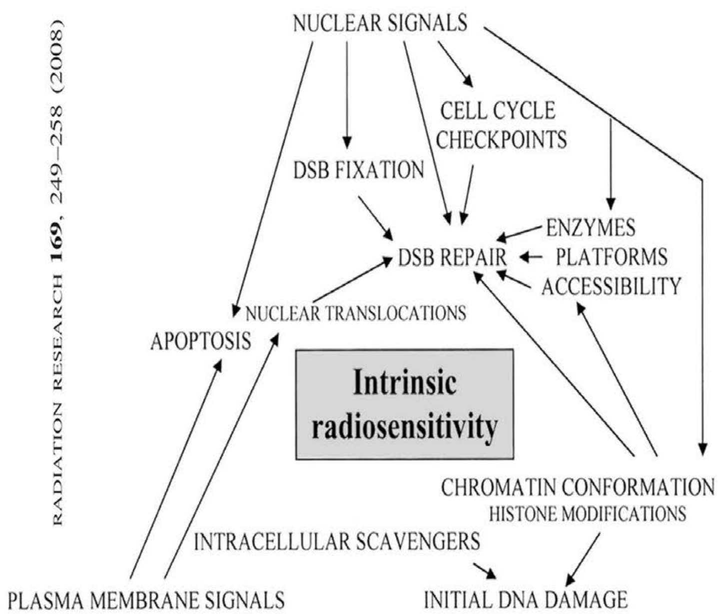
2. once the biochemical lesions are registered, this illicts (at least in normal cells), the ***DNA Damage Response***

   a. the DDR is a collective chain of molecular events that consists of:





3. The fidelity of the DDR largely determines - directly or indirectly - a cell's inherent radiosensitivity



4. it also follows that ***a loss or defect in one or more of the genes/proteins involved in the DDR will cause a DNA repair defect***, which in turn can cause:

   a. **genomic instability**, an early step in the carcinogenesis process

   b. a number of **clinical syndromes associated with radiation or drug sensitivity, cancer proneness, neurological or immunological abnormalities, or signs of premature aging**

5. Clinical correlates:

   a) **tumor cells are known to harbor DDR defects, and efforts are already underway to try to exploit these clinically**

   b) in addition, **drugs that inhibit DDR components are already in clinical trials**

Types of DNA Repair - which repair pathway handles what depends on:

1. ***there are six major DNA repair pathways, along with a few other minor ones*** - and yet, there are more than six kinds of DNA damage, meaning that some of these pathways are able to handle more than one type of lesion

The Six Major DNA Repair Pathways

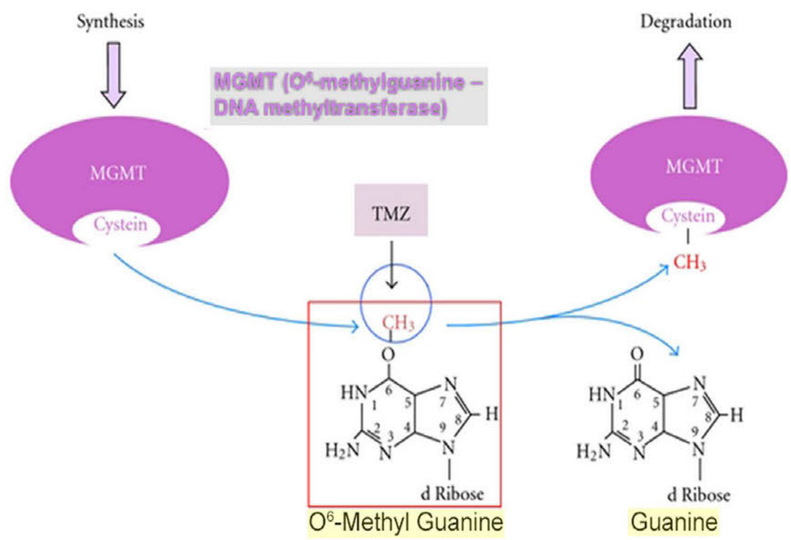
DNA Damage Repair Pathway	Function	Examples of Gene Mutation	Examples of Altered Expression of a Normal Gene	Effect of Loss of Pathway on Clinical Response
Base-excision repair (BER)	Repair of damaged bases or single-strand DNA breaks	None reported	None reported	None reported
Mismatch repair (MMR)	Repair of mispaired nucleotides	Mutation of *MSH2*, *MSH6*, and *MLH1* in Turcot syndrome (brain and colon tumors) and *HNPCC* (colon and gynecologic cancers)	Loss of expression of *MSH2* or *MLH1* in sporadic colon cancer	Resistance to DNA monoadducts Sensitivity to DNA crosslinks
Nucleotide-excision repair (NER)	Excision of a variety of helix-distorting DNA lesions	Mutation of *XPA*, *XPB*, *XPC*, *XPE*, *XPF*, or *XPG* in xeroderma pigmentosum (skin cancer) Variant expression of *ERCC1* or *XPD* in lung cancer	Loss of *XPA* expression in testicular germ-cell tumors	Sensitivity to DNA adducts
Homologous recombination (HR)	Repair of double-strand DNA breaks	*BRCA1/2* mutated in early-onset breast/ovarian, prostate, pancreas, and gastric cancers *FANC* genes mutated in Fanconi anemia	Loss of expression of *BRCA1/2* in ovarian and lung cancers Loss of *NBS1* expression in prostate cancer	Sensitivity to DNA double-strand breaks
Nonhomologous end joining (NHEJ)	Repair of double-strand DNA breaks	DNA ligase IV mutated in Lig4 syndrome (leukemia) Artemis mutated in Omenn syndrome (lymphoma)	Loss of *Ku70* expression in cervical, rectal, and colon cancers Loss of *Ku86* expression in rectal cancer	Sensitivity to DNA double-strand breaks
Translesional synthesis (TLS)	Bypass of DNA adducts during DNA replication	DNA pol E mutated in xeroderma pigmentosum variant (XPV; skin cancers)	Pol β overexpressed in uterus, ovary, prostate, and stomach cancers Pol iota overexpressed in breast cancer	Resistance to DNA adducts

2. which repair pathway is used in a particular situation depends on:

   - the kind of lesion
   - the lesion's physical location (e.g., in coding vs. non-coding DNA)
   - the functional/temporal location of the lesion (e.g., in actively-transcribing vs. non-transcribing DNA)
   - how well-equipped the cell is to repair that kind of lesion (i.e., with high fidelity vs. error-prone vs. defective)
   - the extent to which the different repair pathways share components and/or talk to each other

Direct Reversal of DNA Damage - a simple, one step chemical reaction that "undoes" specific types of base damage *(involves one protein)*

Transalkylation - one step removal of bulky adduct types of DNA damage (such as caused by UV or alkylating agents) using specific methyl or ethyl transferase enzymes



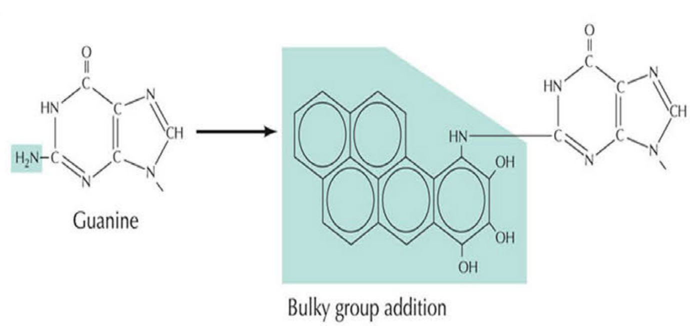
Clinical correlate - when the *MGMT* gene is silenced, cells are more sensitive to temozolamide (an alkylating agent), because the methylated DNA bases won't be repaired.

Excision Repair

Excision Repair – is a multi-enzyme process that handles base damage or loss, nucleotide loss and some sugar damage; different repair sub-systems, that often share some protein components, act on different types of lesions and in different locations

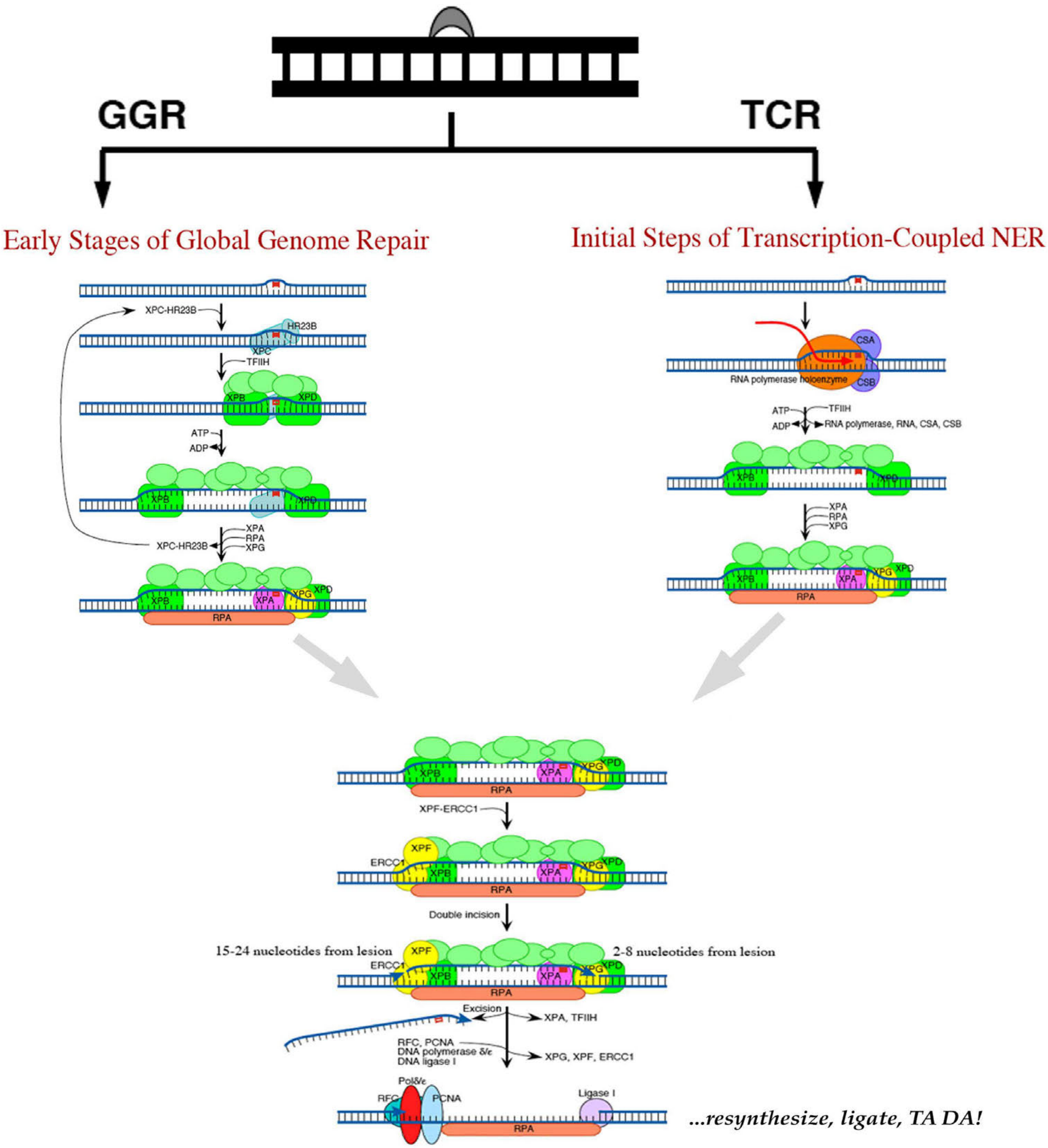
Nucleotide Excision Repair - the more common, generalized form of excision repair in which specific damaged bases aren't recognized *per se*, but rather, the physical distortions in the DNA structure *caused by* the damage serve as the recognition sites for repair proteins

Something like this, for example:



1] for this type of DNA repair processes, the cell is able to "prioritize" the damage depending on whether it has occurred in an inactive versus actively-transcribing gene; repair occurs more rapidly (and maybe with greater fidelity?) in the latter

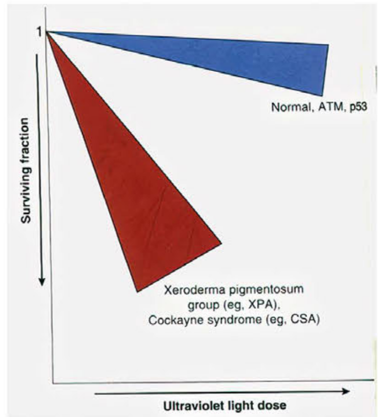
the "regular" type of repair is called **GLOBAL GENOME REPAIR (GGR)**, and the higher priority repair is called **TRANSCRIPTION-COUPLED REPAIR (TCR)**



Some of the Proteins Required for Eukaryotic Nucleotide Excision Repair

Human protein	Probable Function
DDB1	Binds damaged DNA (with XPE); component of E3 ubiquitin ligase (E3UL)
XPE (DDB2)	Binds damaged DNA (with DDB1); partner with DDB1 in some E3ULs
XPC	Binds damaged DNA (with HR23B); recruits other NER proteins
HR23B	Binds damaged DNA (with XPC); recruits other NER proteins
XPB	3' to 5' helicase; early and late DNA unwinding
p62 (GTF2H1)	?
p44 (GTF2H2)	Regulation of XPD
p34 (GTF2H3)	?
p52 (GTF2H4)	Regulation of XPB
GTF2H5 (p8;TTD-A)	Stimulates early unwinding by XPB
XPD	5' to 3' helicase; late DNA unwinding
MNAT1	CDK assembly factor; transcription only
Cdk7	CDK; C-terminal domain kinase; CAK; transcription only
CCNH	Cyclin; transcription only
XPA	Binds, stabilizes open complex; confirms damage; recruits RPA, ERCC1
RPA1, 2, 3	Binds undamaged strand in open complex
XPG	Endonuclease (3' incision); stabilizes full open complex
XPF	Part of endonuclease (5' incision)
ERCC1	Part of endonuclease (5' incision)

Some of the approximately 40 proteins involved in nucleotide excision repair



Cell survival curves for UV radiation derived from patients with NER defects (red) versus normal cells and cells with defects associated with radiosensitivity (blue)

These diseases each show multiple phenotypes (ranging from mild - severe, depending on which specific component(s) of NER is defective

***Clinical correlates*:**

Patients with the disease **xeroderma pigmentosum** cannot complete GGR.

Patients with the disease **Cockayne syndrome** cannot complete TCR.

Patients with the disease **trichothiodystrophy** cannot complete either GGR or TCR.

NER and Human Genetic Diseases

- **Xeroderma pigmentosum**
  1. Severe light sensitivity
  2. Severe pigmentation irregularities
  3. Frequent neurological defects
  4. Early onset of skin cancer at high incidence
  5. Elevated frequency of other forms of cancer

- **Cockayne's syndrome**
  1. Premature aging of some tissues
  2. Dwarfism
  3. Light sensitivity in some cases
  4. Facial and limb abnormalities
  5. Neurological abnormalities
  6. Early death due to neurodegeneration

- **Trichothiodystrophy**
  1. Premature aging of some tissues
  2. Sulfur deficient brittle hair
  3. Facial abnormalities
  4. Short stature
  5. Ichthyosis (fish-like scales on the skin)
  6. Light sensitivity in some cases

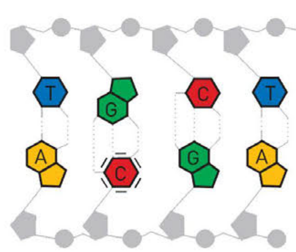
Human protein
XPC
HR23B
XPA
RPA p70, p32, p14
XPB
GTF2H1
GTF2H4
GTF2H2
GTF2H3
TFB5; TTD-A
XPD
MAT1
Cdk7
CycH
XPG
XPF
ERCC1

http://saturn.roswellpark.org/cmb/huberman/DNA_Repair.htm

Base Excision Repair - a more specific type of base damage repair in which enzymes called **DNA glycosylases** both recognize specific kinds of damaged bases and remove them from the DNA, leaving an abasic site (called an "*AP site*"); these sites are then processed further by other enzymes

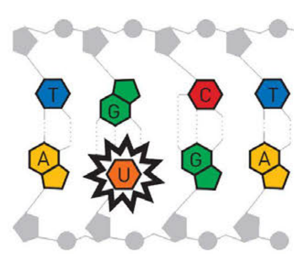
Base excision repairs DNA when a base of a nucleotide is damaged, for example cytosine.

1

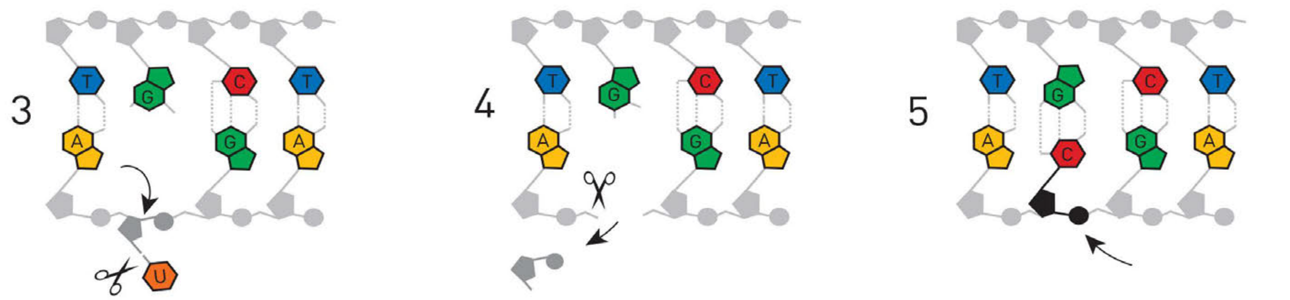


Cytosine can easily lose an amino group, forming a base called uracil.

2



Uracil cannot form a base pair with guanine.

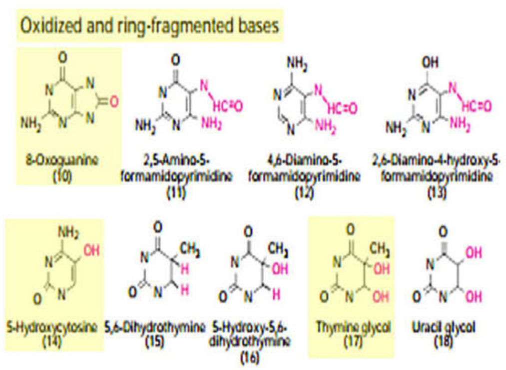


An enzyme, glycosylase, discovers the defect and excises the base of uracil.

Another couple of enzymes remove the rest of the nucleotide from the DNA strand.

DNA polymerase fills in the gap and the DNA strand is sealed by DNA ligase.

Illustration: © Johan Jarnestad/The Royal Swedish Academy of Sciences



Eleven human DNA glycosylases have been identified to date, and they can excise many different types of base damage, including assorted kinds of oxidized bases, which are the types most associated with ionizing radiation

1. **BER is also responsible for repairing most DNA-protein crosslinks (but not the DNA-DNA crosslinks) as well as single strand breaks in the DNA sugar-phosphate backbone**

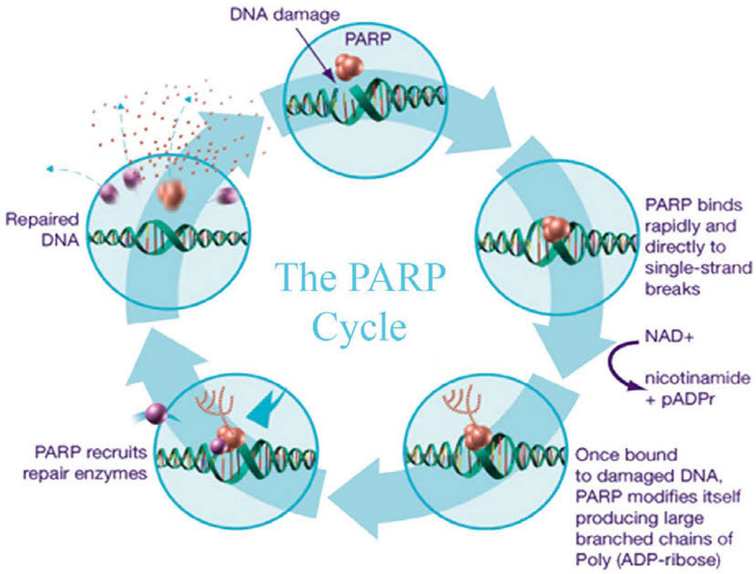
Strand Break Repair - the ability to repair/rejoin strand breaks in DNA is especially critical after exposure to ionizing radiation, as creating strand breaks is radiation's main claim to fame

- Each strand break repair pathway involves damage **sensors** (that locate and mark the sites of damage)...that in turn recruit **transducers** (that amplify the signal and recruit effectors)...and then **effectors** (that coordinate the repair process with other important cellular activities, and that do the actual repair)

- ***Strand break repair only occurs when the sensors, transducers and effectors are functioning properly - anything that goes wrong with any component of the system has the potential to dysregulate (or halt) the entire process***

Single Strand Break Repair

1. **in the case of DNA single strand breaks, the main sensor protein is PARP, Poly (ADP ribose) polymerase** - its job is to detect the breaks, bind to the DNA and synthesize a string of PAR proteins to mark the spot; the repair machinery then uses this signal to migrate to the site of the damage

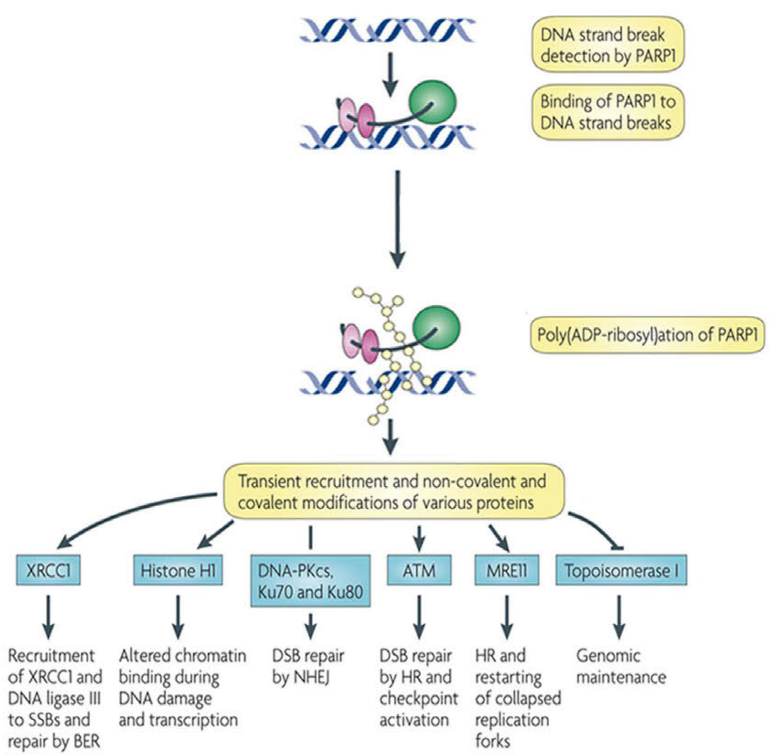


a) PARP typically sticks around until the repair is complete, and then the PAR chains are degraded by Poly(ADP-ribose) glycohydrolase (PARG)

b) **Too much PARP is Bad** - it consumes a lot of energy to operate, which, if the cell's NAD+ reserves run too low, will lead to ***programmed necrotic death***

c) **Too little PARP is Bad** - meaning that SSB's will remain unrepaired, which interferes with DNA synthesis and transcription, and can trigger ***apoptosis***

d) **PARP itself is inactivated by caspase 3 cleavage** (so that it doesn't run amok during apoptosis)



e) also of interest is that PARP participates in many different repair-related processes in addition to SSB repair

2. under normal conditions, ***SSBs are repaired quickly (repair half-time of <15 minutes) and with high fidelity***

a) "clean" breaks are reversed directly using DNA ligase

b) "dirty breaks" are where the SSB accompanies other, adjacent damage (frequently the case for ionizing radiation); these first require the pruning away of any ragged DNA ends and ***then the machinery of base excision repair does the rest***

Double Strand Break Repair

- arguably, the most important repair pathway(s) the mamalian cell posseses, and particularly important vis-a-vis radiation damage

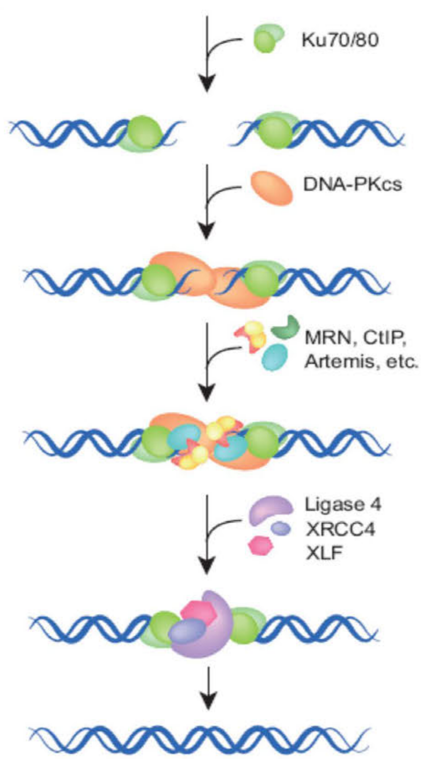
1. the cell has two main pathways for the repair of double strand breaks: ***non-homologous end joining (NHEJ),*** and ***homologous recombination (HR)***

a] **NHEJ predominates in G1/G0 cells** (that are pre-S phase) and therefore do not have another DNA copy to serve as a template for repair...as such **NHEJ is necessarily error-prone**, although less so than initially thought

b] **HR predominates in S and G2 phase cells** that do have a homologous chromosome to serve as a repair template; therefore, **HR is, in theory, error -free**

Non-Homologous End Joining (NHEJ)

- operates throughout the cell cycle, but is most active during G_0/G_1; involves ~20 proteins



Sensor(s): **Ku complex**

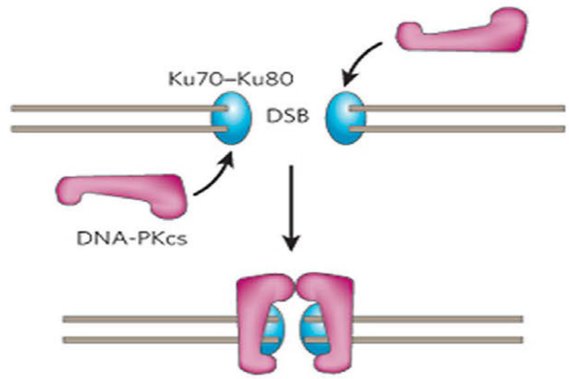
Transducers: **MRN complex (composed of proteins MRE11, NBS1 and RAD50)** and **DNA-PKcs, the catalytic subunit of the repair protein**, but that also acts to **amplify the damage signal by phosphorylating histone H2AX** (γ-H2AX - more on this below)

Effectors: **the Ku's, along with DNA-PK make up the main repair protein**; Artemis and other accessory proteins, plus DNA Ligase IV finish the job

Note: NBS1 has its own name, "nibrin"

a) the Ku proteins are evolutionary conserved all the way back to bacteria and serve multiple functions, but in this context are the first to recognize and bind to DSB's; **the Ku complex exists as heterodimers of two polypeptides, Ku70 (XRCC6) and Ku80 (XRCC5)**

1] *once bound the Ku complex can slide up and down the DNA as it is being repaired, serving as a mobile scaffolding for the other repair proteins*



Genes and Proteins Important for NHEJ

Mammalian gene name	Protein
ligase IV	ligase IV
XRCC4	In collaboration with Ku, targets DNA ligase IV to DNA ends
XRCC5	Ku80
XRCC6	Ku70; deficiency associated with elevated frequency of T-cell lymphoma
XRCC7	DNA-PKcs
ARTEMIS	Artemis; nuclease regulated by DNA-PKcs; important for preparing DNA ends to make them ligatable

Clinical correlate: defects in DNA-PKcs (in mice) or Artemis (in humans) lead to the **SCID** ("severe combined immunodeficiency") phenotype, characterized by extreme radiation sensitivity and severe immunodeficiency

Microhomology-Mediated (or Theta-Mediated) End Joining - a recently identified DSB repair pathway (at least 6 proteins involved) that is thought to account for about 10% of the total DNA repair in normal cells...and probably more in tumor cells

1. this pathway used to be termed "Alternate NHEJ", but that's really a misnomer because:

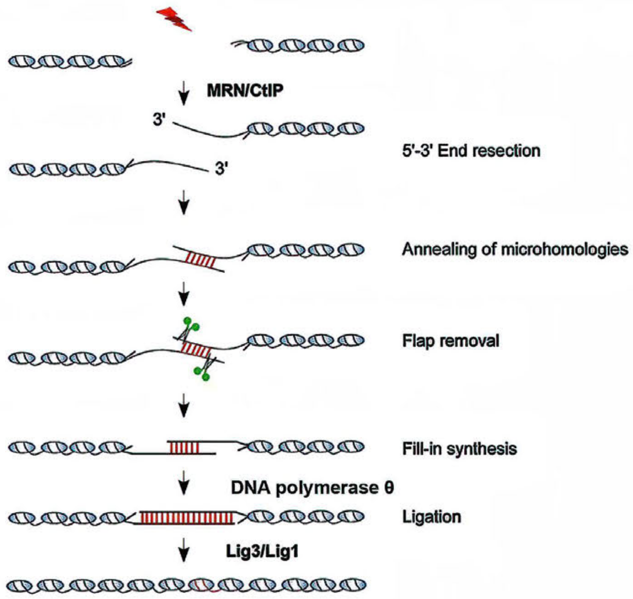
a) **MMEJ isn't really a substitute for NHEJ** - it *can* substitute for NHEJ when the latter is inhibited, however it also operates independently of NHEJ, and uses different sensors and repair proteins

1] **the main repair protein is DNA polymerase theta (Pol Θ)**, which is generating much buzz as a possible target for new drug development aimed at producing radiosensitization by inhibiting components of the DNA damage response

2. MMEJ depends on the presence of small (5-25 base pair) microhomologous DNA sequences to help align the broken DNA ends, with the non-homologous, overhanging regions cut out prior to ligation of the break

a) as such, **MMEJ always produces deletions flanking the original break, and is implicated in chromosomal rearrangements including translocations and inversions, potentially carcinogenic lesions**

b) because of this, **MMEJ is even more error-prone than classical NHEJ**, much more in many cases



Sensor: PARP1

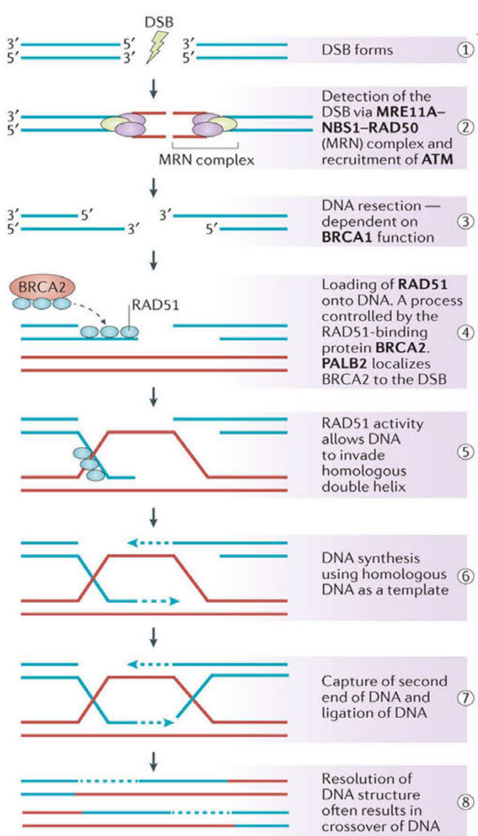
Transducer: MRN complex

Repair protein(s): CtIP endonuclease helps clean up the broken DNA ends and allows the annealing of microhomologous regions, DNA polymerase theta resynthesizes DNA (regardless of deletions), and DNA ligases 3 and 1 seal the open ends

MMEJ role in cancer?

- Several of its components tend to be up-regulated in many human tumors
- It may be able to fill in repair-wise when other DSB repair pathways are defective

Homologous Recombination – a long-recognized pathway for DSB repair in yeast, yet not considered all that important for mammalian cells until fairly recently



HR involves ~20 proteins, and increases in activity through S phase and into G_2

Sensor: MRN complex

Transducer: ATM (which phosphorylates itself and histone H2AX, and also signals through p53 to coordinate repair with other cellular processes)

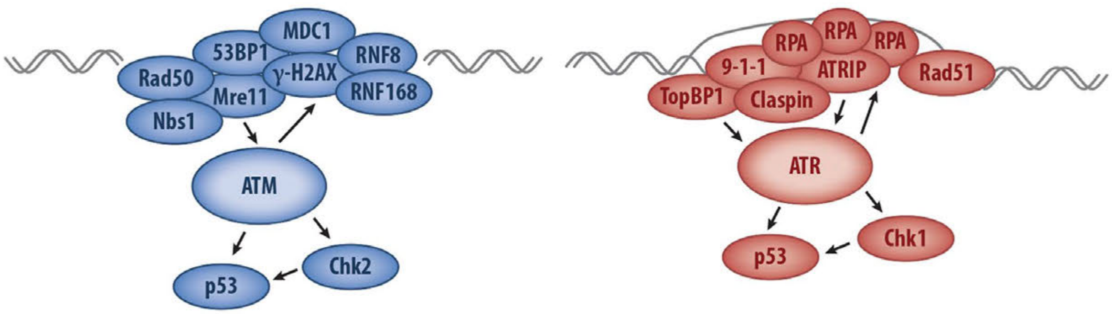
Effectors:

- BRCA1/2, RAD51 and PALB2 help prepare and match the area of the break to the corresponding area on the homologous chromosome
- DNA polymerase synthesizes new DNA to fill in the gap where the DSB is by using the homologous strand as a template
- DNA ligase seals the sugar phosphate backbone

1. arguably, the most important players in HR are ATM and BRCA 1/2 (via p53)

   a) ***ATM is the major tranducer and amplifier of the DSB signal, and ATR has a comparable role in the case of replication stress*** (often caused by persistent SSBs during S phase). These are responsible for coordination of the DDR with other critical cellular pathways, including those related to the activity of other repair processes, cell cycle regulation and cell survival/death

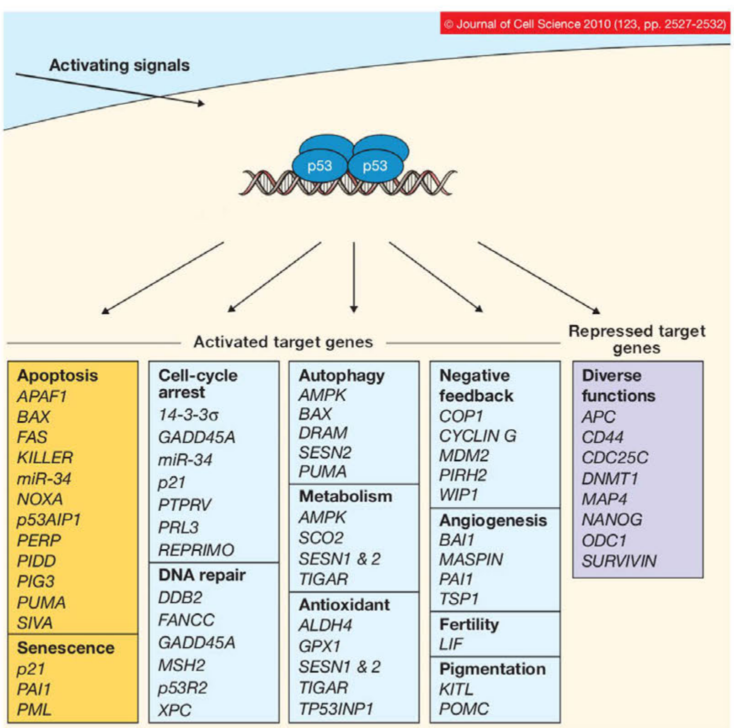
Annu. Rev. Virol. 2014. 1:605–25



Homologous Recombination (HR): Triggered by DNA DSBs when a homologous chromosome is available (Sensors: MRE11, Rad 50, NBS1 = "MRN complex")

Replication Stress Response: secondary to stalled replication forks, as would occur due to the presence of single-stranded DNA (sensors: RPA, ATRIP)

1. note that both ATM and ATR activate p53, which in turn regulates *many* different cellular activities



p53's critical role as a "central node" is how the DNA damage response coordinates with other celllar processes.

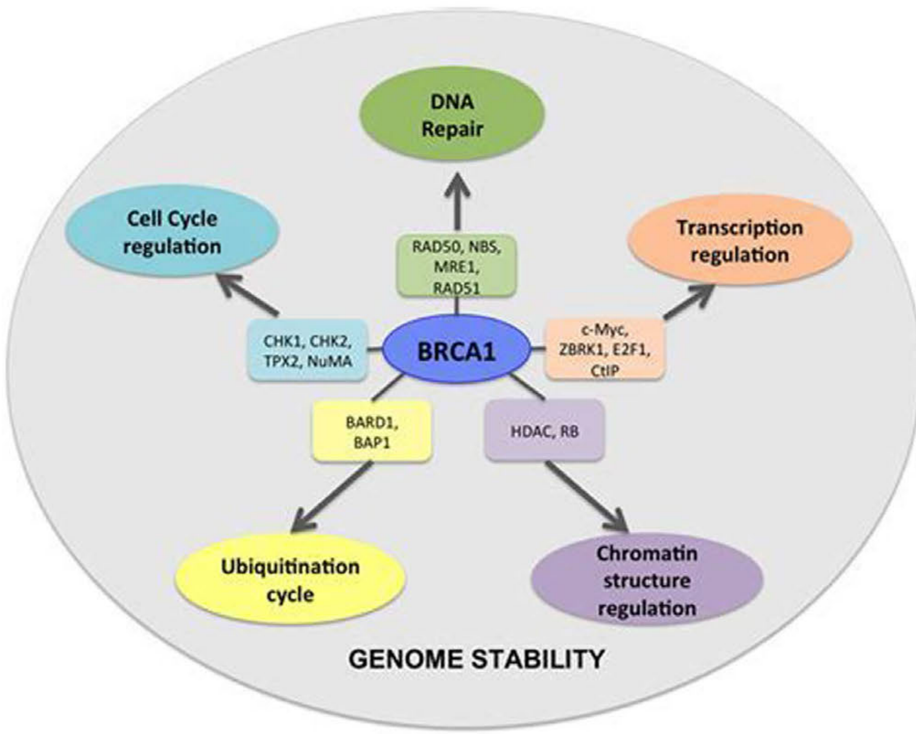
This in turn aids the cell with its decision-making in the face of DNA damage, (e.g., do I live or die, do I halt the cell cycle or keep proliferating, etc.)

b) meanwhile, **the BRCA1 and BRCA2 proteins are the main regulators of HR, plus they are participants in the repair process itself**

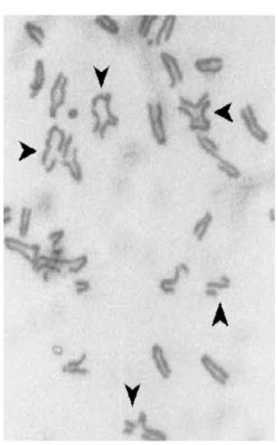
1] **BRCA2** – ***is the master controller of HR, and as such is in charge of delegating the DSB repair to either HR or NHEJ***

a. it has an accessory protein called **PALB2** ("partner and localizer of BRCA2"); if it loses function, the resulting phenotype would be similar to those bearing BRCA2 defects, i.e., ~35% likelihood of getting breast cancer by age 70, plus an increased risk of pancreatic cancer

2] **BRCA1** – ***also helps regulate HR, but has many other cellular functions in addition,*** including regulation of other DNA repair pathways, gene expression and the cell cycle, and protein degradation

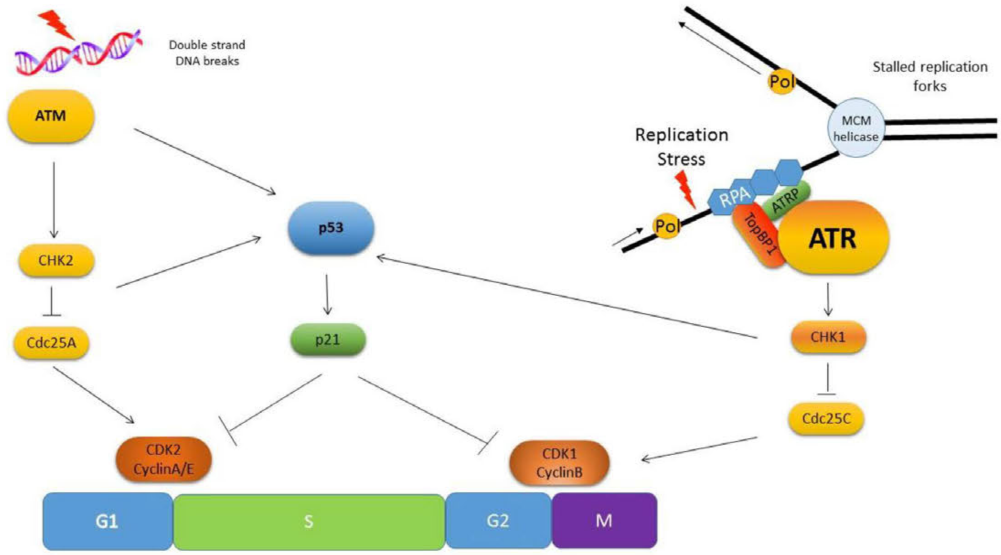


a) this is why, when BRCA1/2 are lost or mutated, the resulting phenotype becomes one of:



- spontaneous gross chromosomal abnormalities
- inability to undergo any kind of recombinational process
- immunostaining for the presence of repair complexes is absent
- variable X-ray sensitivity
- cancer proneness - at specific sites

Another aspect of the DDR: Activating cell cycle checkpoints once DNA damage is sensed



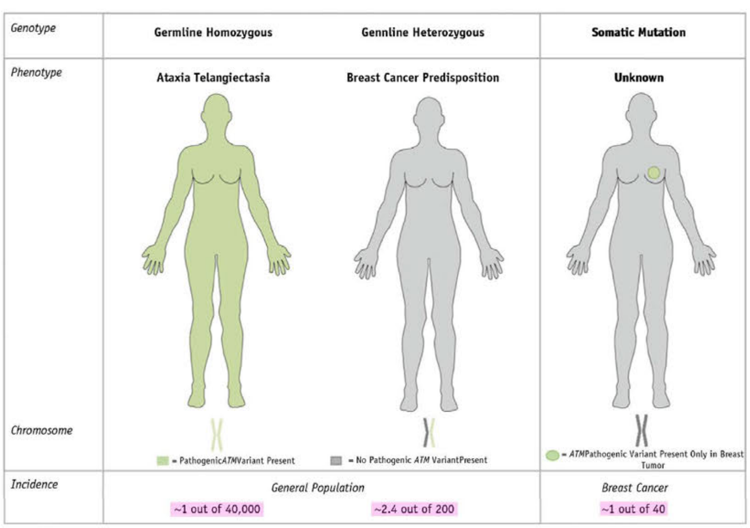
Curr Probl Cancer. 2017 Jul - Aug;41(4):302-315

Clinical Correlate!

Ataxia Telangiectasia (sometimes called "Louis-Bar syndrome") - **a rare, autosomal recessive, multisystem disorder characterized by cancer predisposition, radiosensitivity, and severe neurological and immunological abnormalities**; *the genetic basis is a defect in, or loss of, the ATM gene/protein, a serine-threonine protein kinase, causing cells to be unable to complete HR repair of DSBs or trigger cell cycle checkpoints*

1. Loss of function of ATM can also result in excessive apoptosis of otherwise normal cells, which accounts in part for the neurological and immunological problems associated with AT

2. What is meant by "defective ATM gene", exactly?



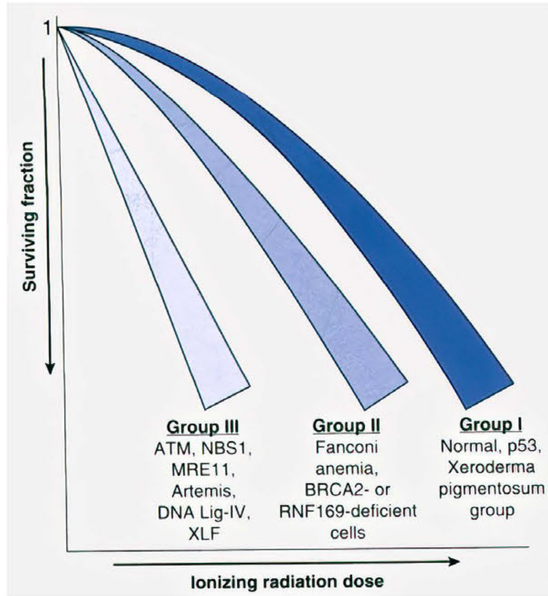
Incidence of germline versus somatic *ATM* variants. Roughly 1 in 40,000 individuals have ataxia telangiectasia through autosomal recessive inheritance, in which both copies of the *ATM* gene are characterized as possessing a pathogenic variant. However, heterozygous inheritance is much more common, with approximately 2.4 in 200 individuals harboring 1 copy of a variant allele. Recent evidence suggests these individuals have a higher risk of developing breast cancer. Additionally, somatic variants are more common, affecting 1 in 40 tumors. *Abbreviation*: *ATM* = ataxia telangiectasia mutated.

Int J Radiation Oncol Biol Phys, Vol. 110, No. 5, pp. 1373–1382, 2021

Not shown: If both copies of the ATM gene are intact in normal cells, but contain very small mutations (SNPs) of unclear significance, some of the features of AT *could* be present, but to varying degrees

2. the radiobiology of AT cells:

Steep, shoulderless survival curves
No SLD recovery
No dose rate effect for X-rays
No γH2AX repair foci
Contain residual, unrejoined DSBs



Adverse Reactions to Radiotherapy in AT Patients

Disorder	Age (y)/gender	RT (Gy)	Outcome
A-T	10.5/M	30	Died, 8 mo
A-T	9/M	27.5 (mediastinal); 27.5 (supraclavicular)	Died, 3 mo
A-T	3.9/M	3	Died, <1 mo
A-T	7/M	30	Died, 3 wk
A-T	3.8/F	30	Died, 9 mo
A-T	9/M	16	Severe mucosal ulceration
A-T	4.5/M	18	Leukoencephalopathy
A-T	7/M	24 (brain); 12 (spine)	Somnolescence syndrome
A-T	9/F	9	Died, 10 mo
A-T	15.2/M	15.5	Died, 1 mo
A-T	3.9/M	3	Died, 3 mo
A-T	1.5/M	18 (brain); 3 (chest)	No excessive toxicity
A-T	2.5/M	24 (brain); 6 (spine)	Leukoencephalopathy, 10 mo

Int. J. Radiation Oncology Biol. Phys., Vol. 74, No. 5, pp. 1323–1331, 2009

3. **AT heterozygotes** - they have an apparently normal phenotype compared to the homozygotes, but are there any hidden surprises lurking?

a) Answer: Yes and no...

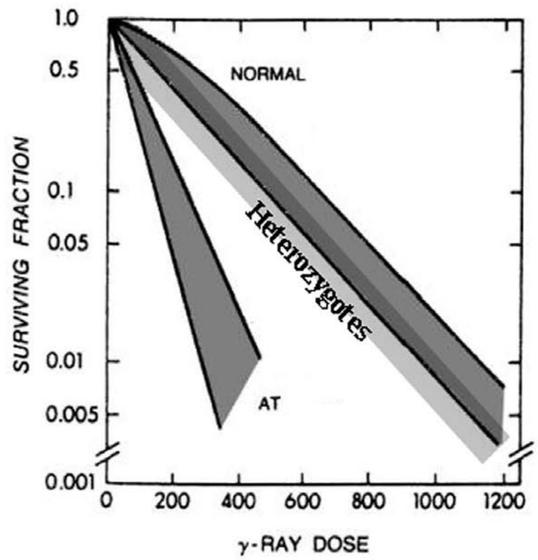
1) there is good reason to believe that AT heterozygotes ARE more prone to radiation carcinogenesis (although nowhere near as much as the homozygotes)

a. this could turn into a significant public heath concern given that 1-2:100 people could be heterozygotes - for example, for an AT heterozygote, screening mammography might constitute a greater risk than benefit!

1) **are AT heterozygotes also more radiosensitive, that is, prone to a higher incidence of normal tissue complications during and after radiotherapy?**

a) the clinical literature seems conflicted on this

b) however, cell lines derived from known heterozygotes fall into the "low-normal" or "slightly below normal" range of cellular radiosensitivities...but that doesn't mean the whole patient will be



4. **SNPs in the ATM gene -** more than a dozen SNPs have been characterized, many of which result in truncated or loss of function versions of the ATM protein

a) **all seem to confer a slightly higher carcinogenesis risk (1.5-2.5 fold), for breast cancer in particular**

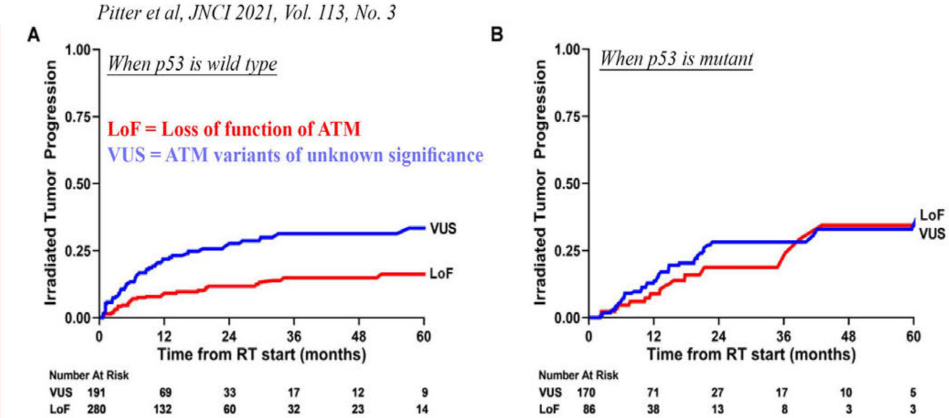
b) conflicting results on whether any of the SNPs confer increased normal tissue radiosensitivity, however **there are reports of increased radiosensitivity/improved clinical outcomes for *tumors* bearing SNPs in the ATM gene**

Among 357 pan-cancer patients, tumors bearing full ATM loss of function showed markedly improved tumor control (i.e., reduced rate of tumor progression at 2 years) following radiotherapy than for tumors bearing ATM variants of unknown significance.

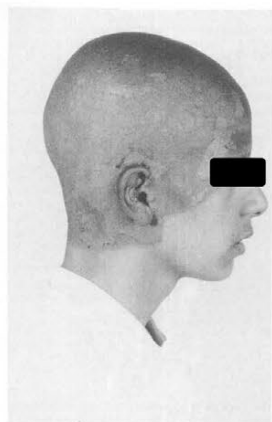
This was only true in patients whose tumors had wild-type p53, but not when p53 was also mutated.

This type of genetic signature associated with radiosensitivity across multiple cancer types shows potential for genomically-guided radiotherapy.

Pitter et al, JNCI 2021, Vol. 113, No. 3



Clinical outcomes stratified by *TP53* genotype and loss of *ATM* heterozygosity. **A)** Cumulative incidence of irradiated tumor progression stratified by *ATM* genotype among *TP53* wild-type tumors. **B)** Cumulative incidence of irradiated tumor progression stratified by *ATM* genotype among *TP53* mutant tumors. *ATM* loss-of-function (LoF) was associated with decreased tumor progression for *TP53* wild-type tumors ($P < .001$) but not *TP53* tumors ($P = .26$; Fine-Gray competing risk regression with clustering).



Lateral view of scalp erythema and skin desquamation immediately after radiotherapy.

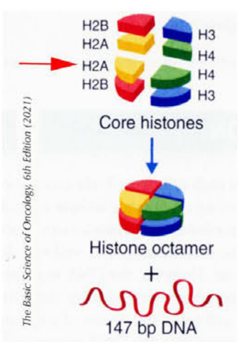
This patient (with ALL) received ***1800 cGy in 10 daily fractions*** of prophylactic cranial irradiation following chemotherapy, resulting in brisk scalp and skin desquamation by the end of treatment. He also experienced an extreme case of somnolence syndrome that resolved only very slowly. By 3-4 months post radiotherapy, he had developed bilateral osteitis and a necrotic, right mastoid ulcer. After 7 months, an EEG revealed diffuse radiation-induced encephalopathy, that lead to his death soon thereafter.

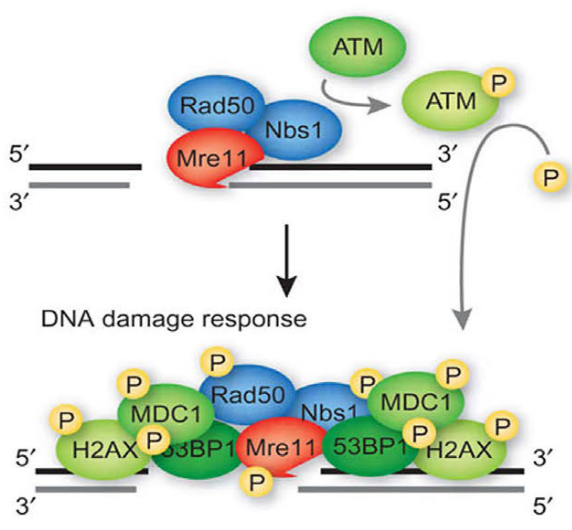
He was found to be neither an AT homozygote or heterozygote, yet even so turned out to be exquisitely (fatally) radiosensitive. Although never verified, suspicion was that he had SNPs in his AT genes.

Basic Science/Translational/Clinical/Commercial Correlate!

The activation/deactivation of proteins associated with the DNA damage response is now being used as a biomarker for the presence of DSBs...

- their relative numbers after a given radiation dose is an indicator of radiosensitivity
- their relative numbers can be used for dosimetric purposes, e.g., during a radiation emergency when the doses received are unknown
- their disappearance over time is an indicator of the cell's repair rate and overall capacity
- residual DSBs after repair is complete can be indicative of a DNA damage response defect, or that the cell is already dead or destined to die





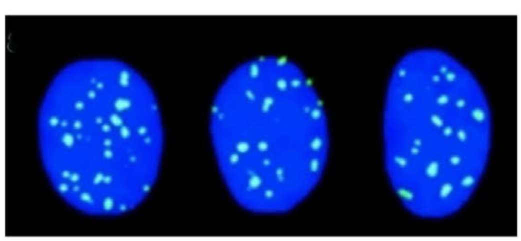
For DSBs, the earliest steps in the DNA damage response (for the HR pathway) are:

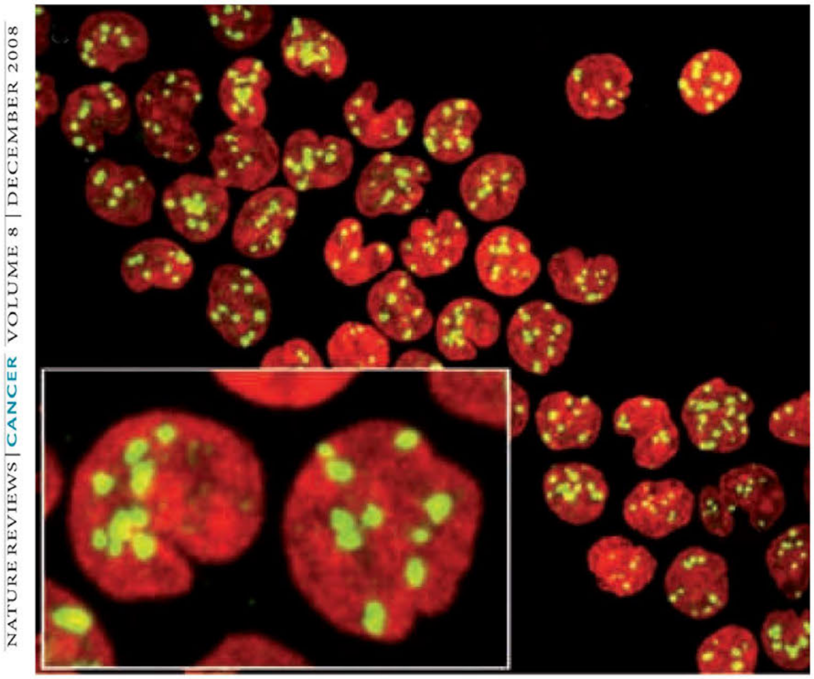
- ***binding of the MRN complex*** (MRE-11, RAD50 and NBS1), that serves as a tether to hold both broken strands and the repair proteins in the proper orientation
- ***self-phosphorylation of ATM***
- ***phosphorylation by ATM of several other proteins, including histone H2AX***, (phosphorylated form called "***γH2AX***")

Note: For the NHEJ pathway, Ku70/80 substitutes for the MRN complex (initially), and DNA-PKcs substitutes for ATM.

Antibodies have been raised against several of these early-DDR proteins, allowing them to be visualized in cell nuclei at the sites of DSBs, creating "***repair foci***"

The most robust and best studied of these repair foci assays visualizes γH2AX



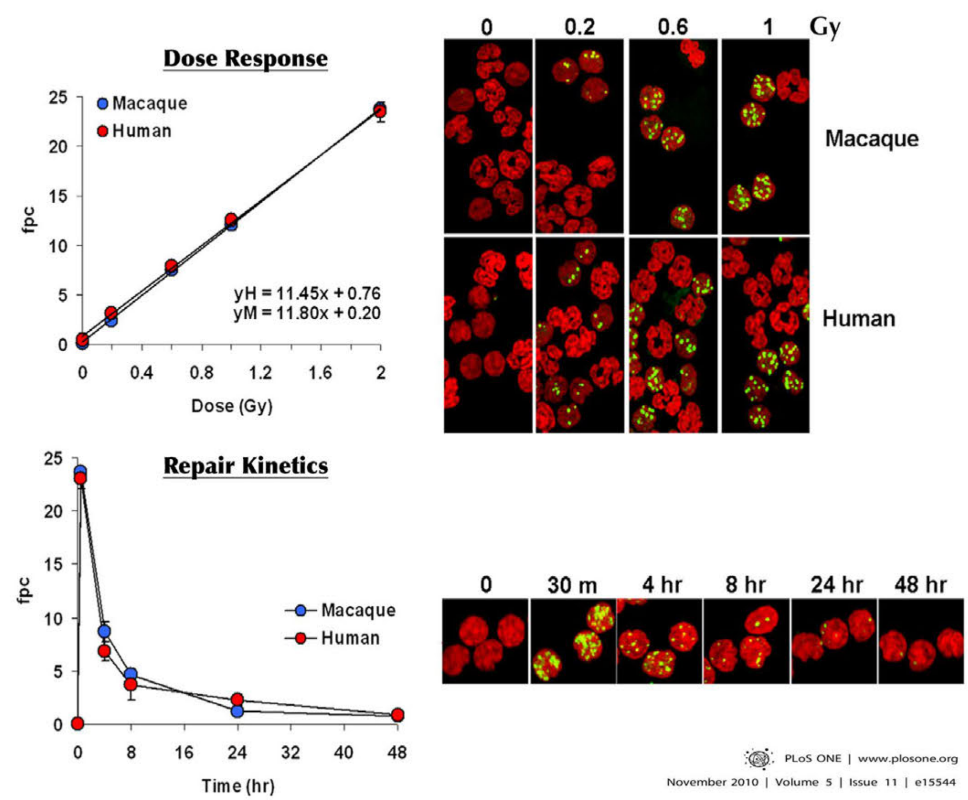


Nuclei stained for the presence of γH2AX foci that appear within minutes of irradiation.

In repair-competent cells, these will disappear over time (hours) as the DSB's are rejoined.

γ-H2AX foci in macaque and human peripheral blood white cells exposed to IR *ex vivo*.

γH2AX may be everybody's favorite marker these days, however there are lots of others to choose from (RAD50 or 51, 53BP1, etc.)



Clinical Syndromes not otherwise discussed

cancer disposition syndromes associated with defects in the other components of the HR machinery

AT-like disorder (ATLD) - mutation in MRE11, damage sensor component

MRN complex

Nijmegen Break Sydrome (NBS) - mutation in NBS1, damage sensor component

Li-Fraumeni Syndrome: extreme cancer proneness due to the inheritance of a germline mutation in the p53 and/or CHK2 tumor supressor genes that link DNA repair porcesses and cell cycle regulation

downstream of ATM

Werner's Syndrome - mutation in WRN, a DNA helicase

Bloom Syndrome - mutation in BLM, also a DNA helicase

unwind DNA to facilitate access of sensing and repair-related proteins

Radiation Sensitivity Syndromes Summarized

- note that not all the syndromes associated with cellular radiosensitivity also confer clinical radiosensitivity

Int J Radiation Oncol Biol Phys, Vol. 105, No. 4, pp. 698−712, 2019

Known rare syndromes associated with sensitivity to radiation

Syndrome	Mutated gene(s)	Associated with
Ataxia telangiectasia	*ATM*	Clinical and cellular radiosensitivity, cancer predisposition
Ataxia telangiectasia-like disorder	*MRE11*	Cellular radiosensitivity
Cornelia de Lange syndrome	*SMCL1A*	Variable radiosensitivity, cellular radiosensitivity in G2, chromosome instability
Cowden syndrome	*PTEN*	One report of severe toxicity in a patient with breast cancer with a heterozygous nonsense mutation at K322
Fanconi anemia	Numerous genes	Cellular and clinical sensitivity in some
Gorlin syndrome (nevoid basal cell carcinoma syndrome)	*PTCH1*	Cellular radiosensitivity in patients with severe PATCHED1 protein deficiency, cancer predisposition, risk of second malignancy
Li-Fraumeni syndrome	*TP53*	Risk of second malignancy
Ligase IV syndrome	*LIG4*	Clinical and cellular radiosensitivity
Neurofibromatosis type 1	*NF1*	Risk of second malignancy
Nijmegen breakage syndrome	*NBN*	Clinical and cellular radiosensitivity, cancer predisposition
Nijmegen breakage syndrome−like syndrome	*RAD50*	Cellular radiosensitivity
Radiosensitive SCID	*DCLRE1C (Artemis), PRKDC*	SCID associated with NHEJ defects, cellular radiosensitivity
Retinoblastoma	*RB1*	Moderately radiosensitive with increased chromosomal G2 radiosensitivity, risk of second malignancy
RIDDLE syndrome	*RNF168*	Cellular radiosensitivity

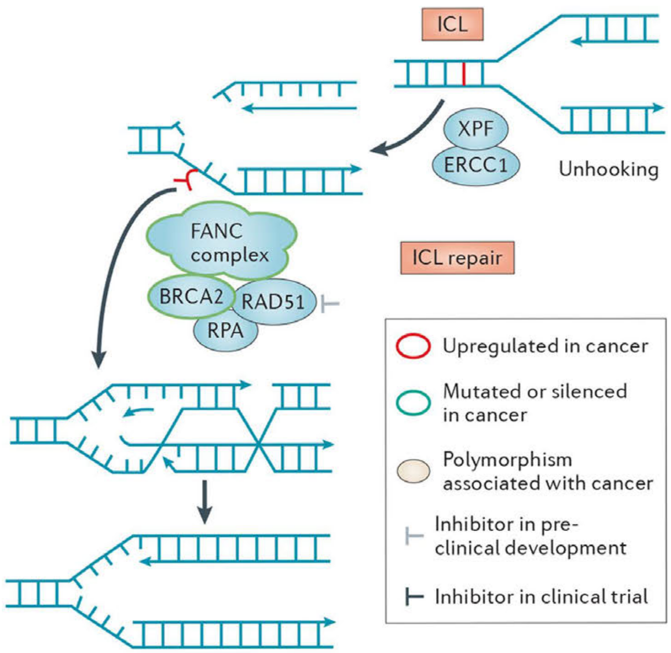
Abbreviations: NHEJ = non-homologous end joining; SCID = severe combined immunodeficiency.

Repair of DNA Crosslinks

1. **DNA-protein crosslinks are handled by the base excision repair pathway when possible, but what about the DNA-DNA crosslinks (ICL)?**

a. these are more dangerous because the two strands would not be able to separate for DNA replication, which would ultimately collapse the replication fork...which is why they have their own system, with some unique components, and others scavenged from other repair pathways

1] the unique components are assembled into the **FANC complex**, which can unhook the crosslink (creating a DSB), and then funnel the DSB into the HR pathway



Note how the proteins of the FANC family tend to be downregulated or silenced in cancer...meaning that this pathway might not be working in some tumor cells. This in turn could cause increased sensitivity to crosslinking agents

2. **Clinical correlate**: ***loss of the FANC pathway is the cause of the disease Fanconi's anemia***

a. prevalence: very rare, except in Ashkenazi Jews (approx 1:100 are carriers)

b. clinical presentation: characterized by progressive hematological impairment from a young age, chromosomal instability with frequent breakage, diverse congenital abnormalities, pancytopenia, skin pigmentation changes and cancer proneness, particularly nonlymphocytic leukemia; variable sensitivity to physical (UV, X-rays) and chemical (e.g., mitomycin C) agents that produce DNA crosslinks

Note the presence of tri- and quadriradial chromosome aberrations (arrows), which are commonly seen in patients with Fanconi's anemia

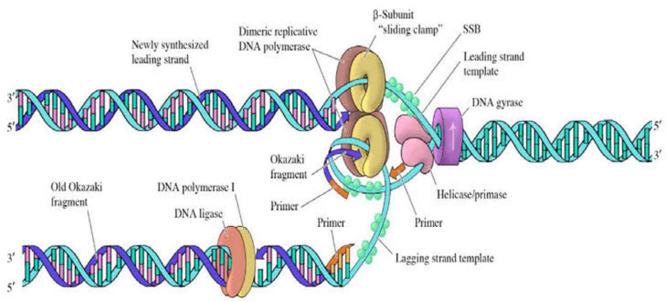
Another DNA Repair Strategy: Tolerate the Damage

1. Mammalian cells are able to tolerate the presence of DNA damage temporarily and to varying extents, although they risk permanent, heritable mutations if the damage is left in place permanently.

a. This damage comes both from external sources (e.g., radiation exposure) and from the inherently error-prone processes of DNA replication and some types of DNA repair (e.g., NHEJ)

Under what conditions would damage be tolerated?

a. usually, in a "crisis situation" when the presence of a DNA lesion interferes with DNA sythesis or repair such that prompt death would likely occur otherwise

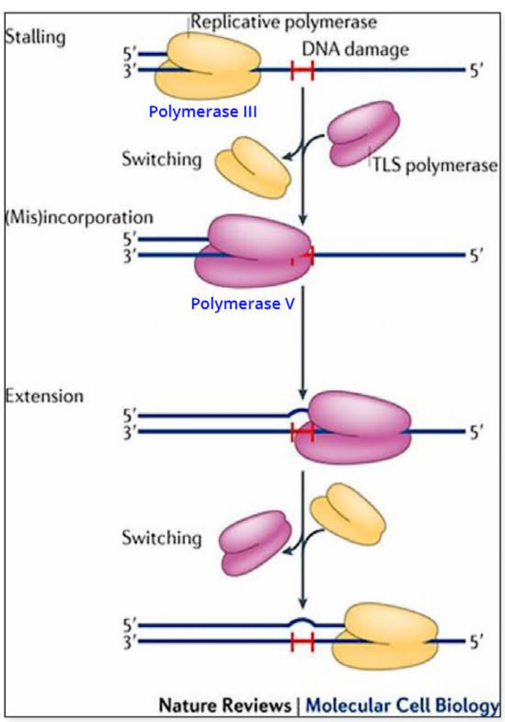


Do you really want to be encountering a DSB or crosslink at a time like this? I didn't think so.

b. or when a cell is being positively bombarded with DNA damage, such as from a chemotherapy agent (tolerating such damage is one mechanism for the development of drug resistance)

2. luckily, the cell has two pathways that can allow it to carry on in the presence of damage, and to go back and screen for the residual damage after the fact, and repair it then

Translesion DNA Synthesis (TLS) - allows the cell to continue with DNA replication by bypassing lesions that could stall the process or collapse the replication fork, which would otherwise be fatal to the cell

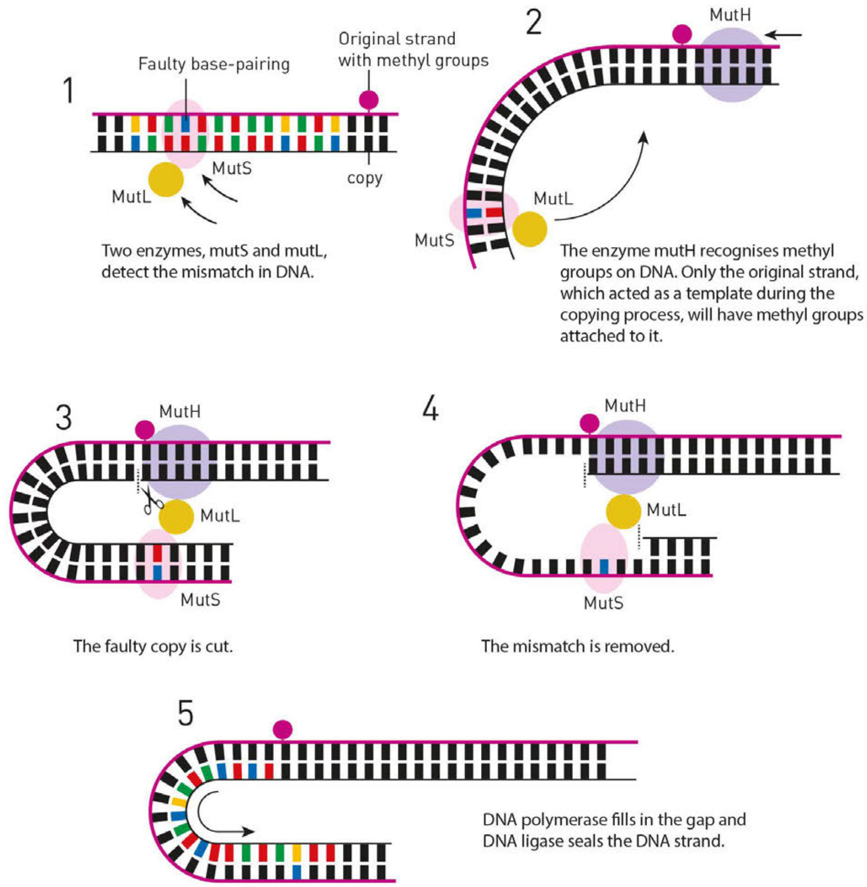


the way TLS works:

- during S phase, the DNA polymerase complex encounters a lesion near the replication fork and stalls there
- the RecA protein gloms onto single-stranded portion of the DNA around the site of the lesion to protect it from degradation
- a different, less stringent, DNA polymerase (pol V) is swapped out for the original (pol III), which *can* bypass the lesion
- then, the original polymerase swaps back in to complete DNA synthesis...but note that ***the lesion stays behind***, and the opposite strand probably has a random nucleotide inserted at that location
- ***this would then become a permanent mutation, unless there is a back-up system to remove the damage after the fact***

Mismatch Repair (MMR) – a DNA proofreading and editing system that corrects the inherent errors of replication and repair, but can also go back and correct errors left over from prior tolerance of damage

(About 25 proteins are involved in MMR)



MMR is not a radiation damage repair pathway per se, and yet, defects in some of its proteins also leads to genomic instability and cancer proneness

In humans, **the key proteins are MLH1 and MSH 2** (these are the equivalents of the MutL and MutS proteins shown in the figure for *E. coli*)

What sort of phenotype is obtained when one or more of the MMR components are mutated or lost?

NO INCREASE IN RADIATION SENSITIVITY, unlike many of the other DNA repair deficiency syndromes

the "**MUTATOR PHENOTYPE**" is expressed, i.e., the cell becomes hyper-mutable compared to the spontaneous mutation frequency

"**MICROSATELLITE INSTABILITY**", the tendency for DNA to keep accumulating more and more small insertions and deletions

Clinical correlate: *Hereditary non-polyposis colon cancer (HNPCC)* syndrome is an autosomal dominant disorder characterized by early onset colon cancer.

Most sufferers are defective in either MLH1 or MSH2 (although other, rarer defects are also possible).

	MutS	MSH1	?	DNA repair in mitochondria
35%	"	MSH2	MSH2	Single mismatch and small loop repair (with MSH6 to form MutSα); loop repair (with MSH3 to form MutSβ)
	"	MSH3	MSH3	Loop repair (with MSH2 to form MutSβ)
	"	MSH4	MSH4	Meiotic recombination (with MSH5)
	"	MSH5	MSH5	Meiotic recombination (with MSH4)
	"	MSH6	MSH6	Single mismatch and small loop repair (with MSH2 to form MutSα)
60%	MutL	MLH1	MLH1	Forms heterodimeric complexes with the other 3 MutL homologs
	"	PMS1	PMS2	Mismatch repair, especially in S phase
	"	MLH2	PMS1	Minor role in small loop and mismatch repair
	"	MLH3	MLH3	Promotes recombination during meiosis; Small loop repair during mitosis

Inhibition of DDR Proteins as a Clinical Strategy? *Hot topic!*

1. historically, the idea of trying to inhibit some type of DNA repair was considered asking for trouble in that it would be expected to cause damage to both tumors and normal tissues...*unless there was a way to accomplish it selectively*, or effectively so

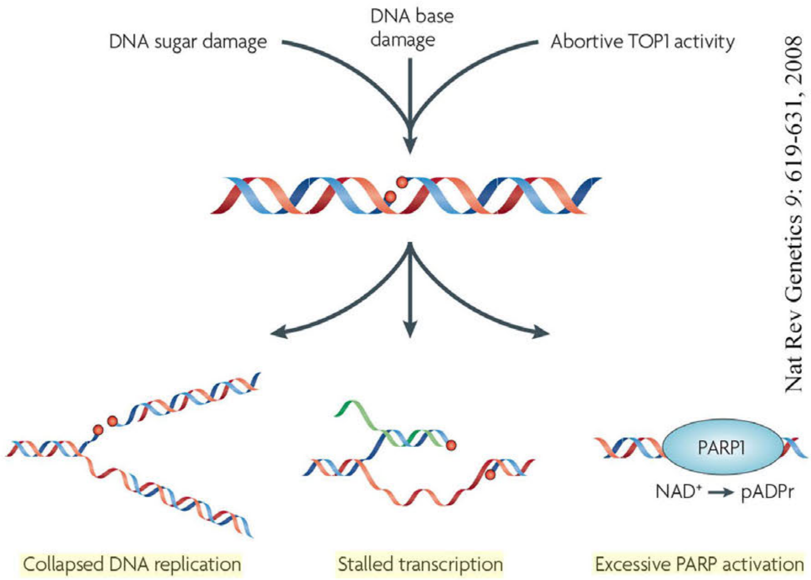
2. for example, *what would happen if PARP were inhibited in otherwise normal cells?*

Initially, a mess...because SSBs wouldn't close properly, and PARP would get "trapped" on the DNA.

This interferes with BER, which only creates even more SSBs. SSB's also collapse replication forks, which converts them to DSBs, and this impasse to replication *can only be resolved through HR*.

Transcription becomes stalled, which can be lethal. Further, too much PARP production, functional or not, can also lead to cell death.

However ultimately, so long as other repair pathways were intact (especially HR), the cell could still survive this.



Nat Rev Genetics *9*: 619-631, 2008

| **Single-strand breaks and cell fate.** Single-strand breaks (SSBs) can arise in a variety of ways, including directly from disintegration of deoxyribose, indirectly as normal intermediates of base-excision repair (BER), or as abortive intermediates of topoisomerase 1 (TOP1) activity. If they are not repaired rapidly or appropriately, SSBs can collapse replication forks, block transcription or promote excessive activation of the SSB sensor protein poly(ADP-ribose) polymerase 1 (PARP1). Red circles denote damaged DNA termini. pADPr, poly(ADP-ribose).

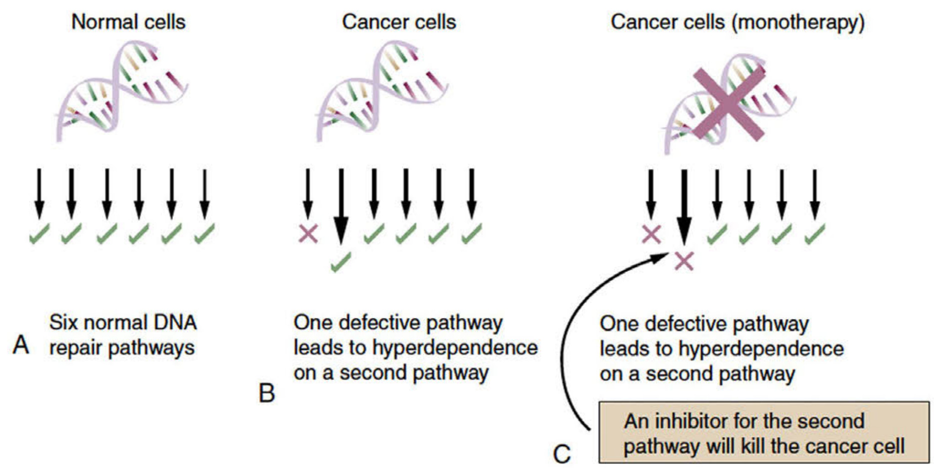
*But what if one or more other DNA repair pathways **weren't** intact?*

One strategy for (effectively) tumor-specific DNA repair inhibition takes advantage of a process known as SYNTHETIC LETHALITY

Synthetic lethality takes advantage of the fact that most tumors have at least one DNA repair defect, and that "synthetically" creating a second defect can be enough to kill the cell

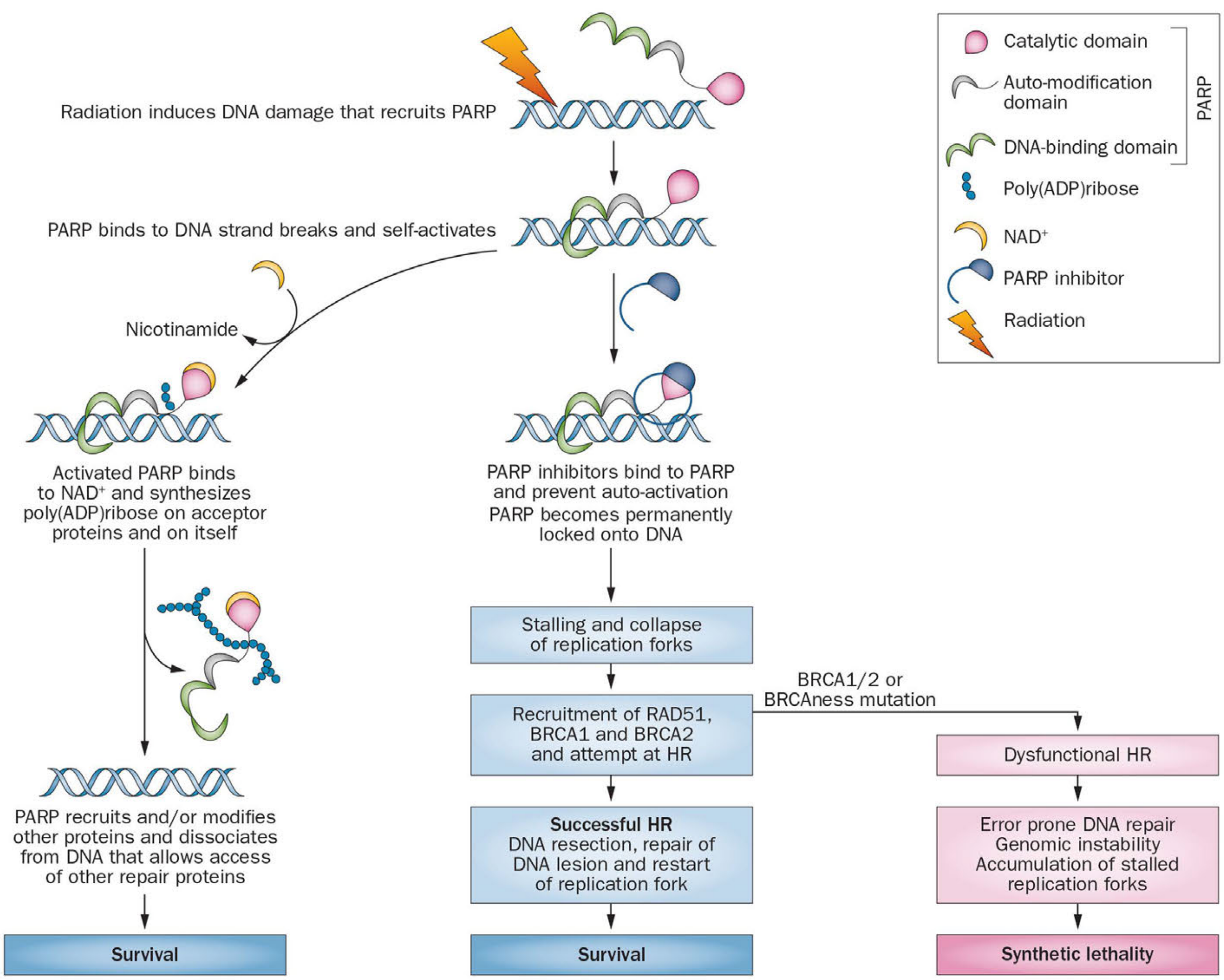
1} in theory, *synthetically knocking out a repair system in normal cells should have less impact, because all the cell's other repair systems are presumably intact* (there are assorted salvage pathways as well, which might also be absent in tumors)

PRINCIPLE OF DNA INHIBITOR MONOTHERAPY (A). Normal human cells have six DNA repair pathways. **(B)** Tumor cells, in contrast, have disrupted one DNA repair pathway through somatic mutation, loss of heterozygosity (LOH), or epigenetic silencing of a DNA repair gene in that pathway. The tumor cell has genomic instability and has partially compensated for its DNA repair defect by upregulating a second pathway. **(C)** The tumor is hyperdependent on this second pathway, and a specific inhibitor kills the tumor cells but has little effect on the normal cells.



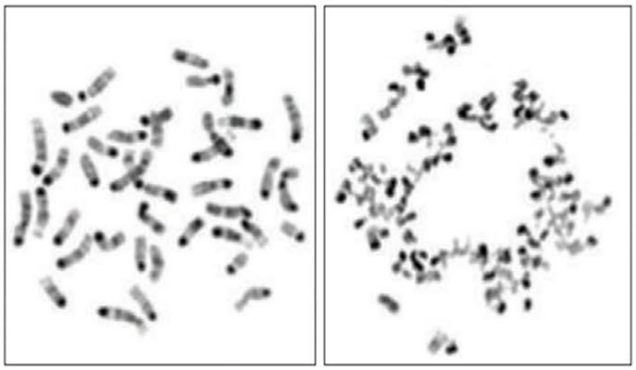
PARP inhibitors were developed with synthetic lethality in mind.

Schaue, D. & McBride, W. H. *Nat. Rev. Clin. Oncol.* **12**, 527–540 (2015)



Possible mechanisms by which PARP-1 inhibitors might interact with radiation-induced DNA damage for therapeutic benefit. PARP inhibitors cause synthetic lethality in cells that have a compromised HR apparatus

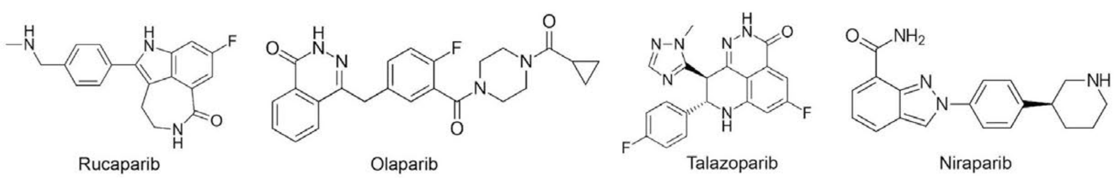
For women with BRCA1/2 gene mutations, their breast and/or ovarian cancer cells are probably already defective in the HR pathway (because it is controlled by the BRCA proteins). Adding a PARP inhibitor would knock out both SSB repair, base excision repair and MMEJ, which together should be enough to kill the cell.



Treatment with a PARP inhibitor in cells with a pre-existing BRCA2 mutation turns their chromosomes into mush.

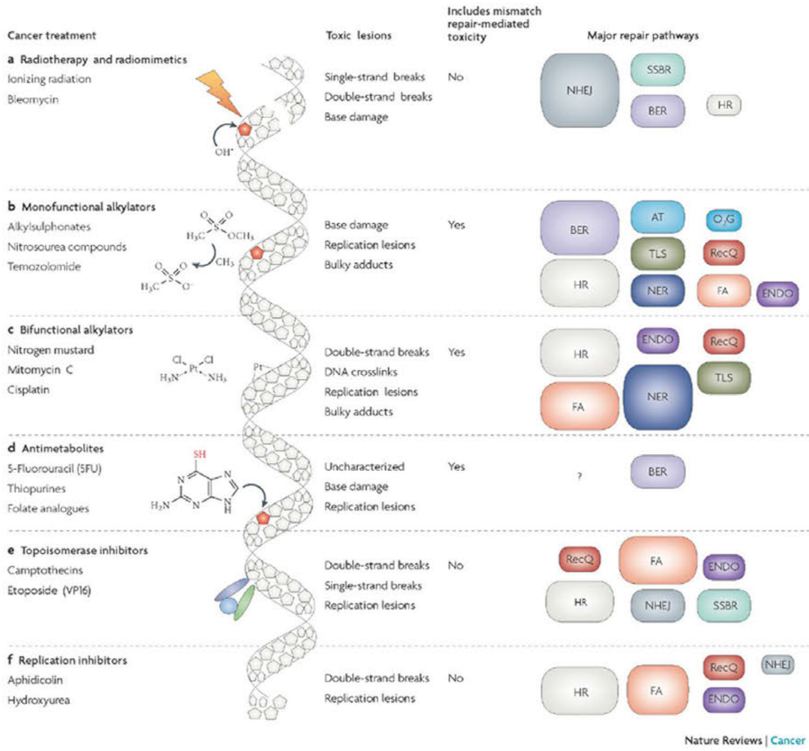
Effects of PARP inhibition on *BRCA2*-mutant cells. Untreated *BRCA2*-mutant mouse embryonic stem cells are shown on the left. *BRCA2*-mutant cells treated with a PARP inhibitor (KU-0058684, 1μM) for 24 h are shown on the right.

FDA Approved PARP Inhibitors

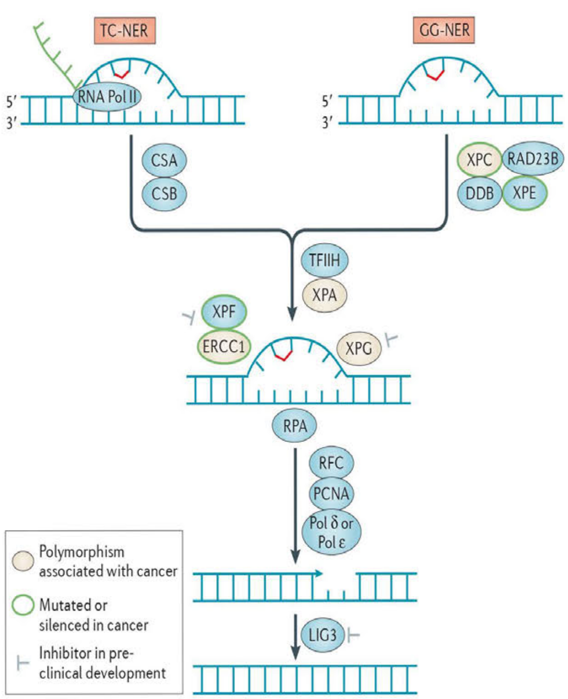


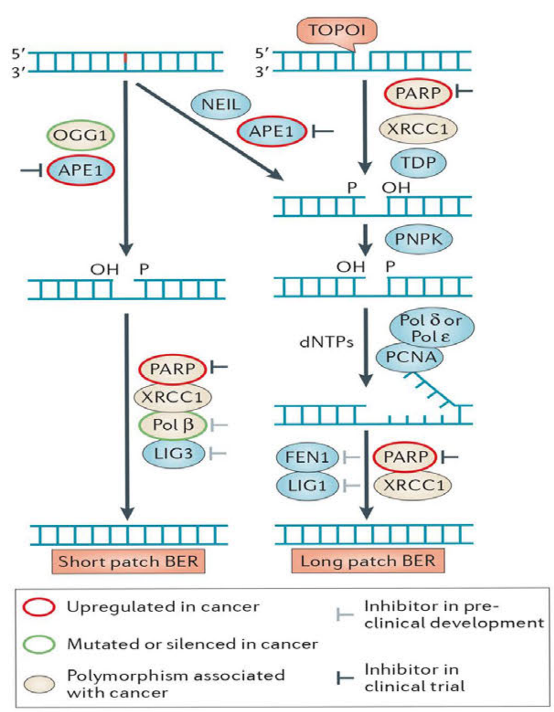
Appendix Materials

DNA Repair by Toxin and Lesion Type



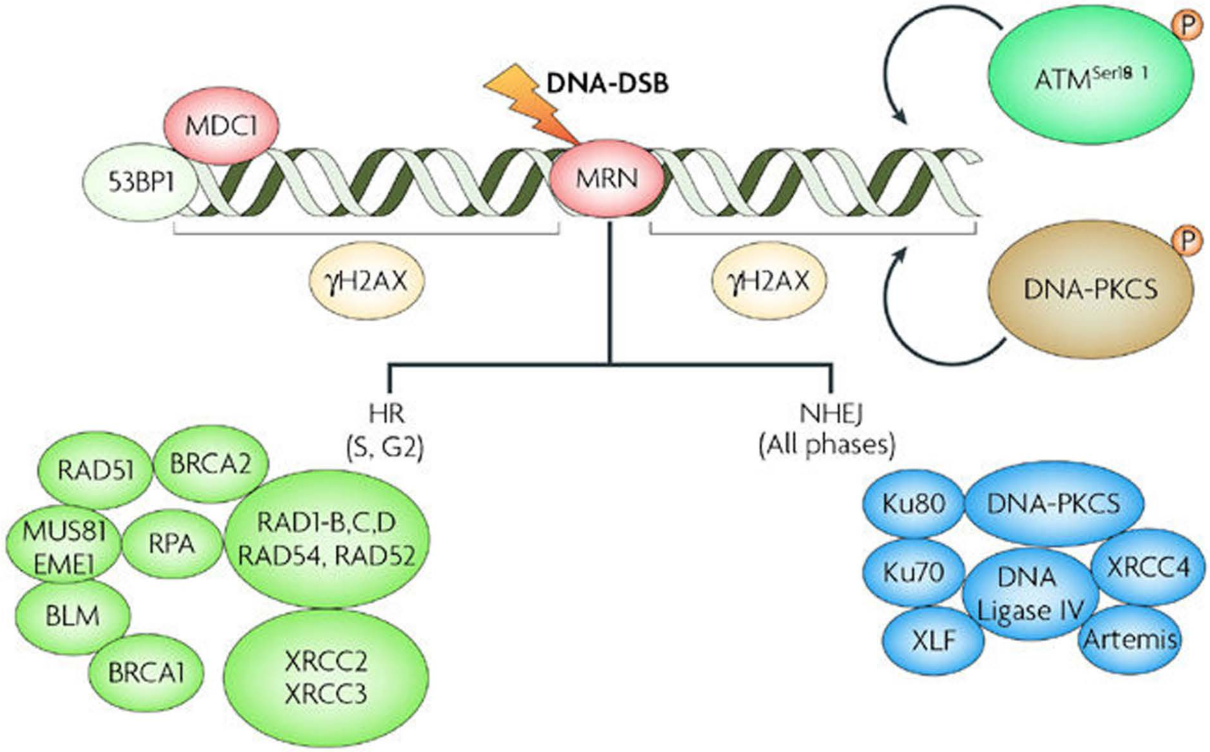
NER versus BER



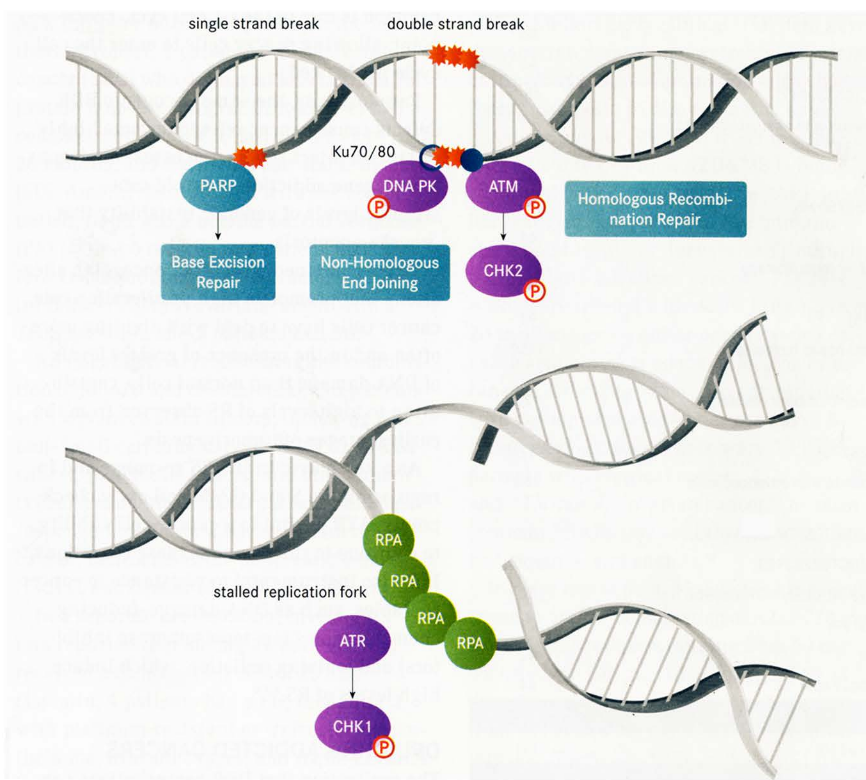


Curtin *et al.* NATURE REVIEWS | CANCER | DECEMBER 2012 | VOLUME 12

NHEJ and HR Compared



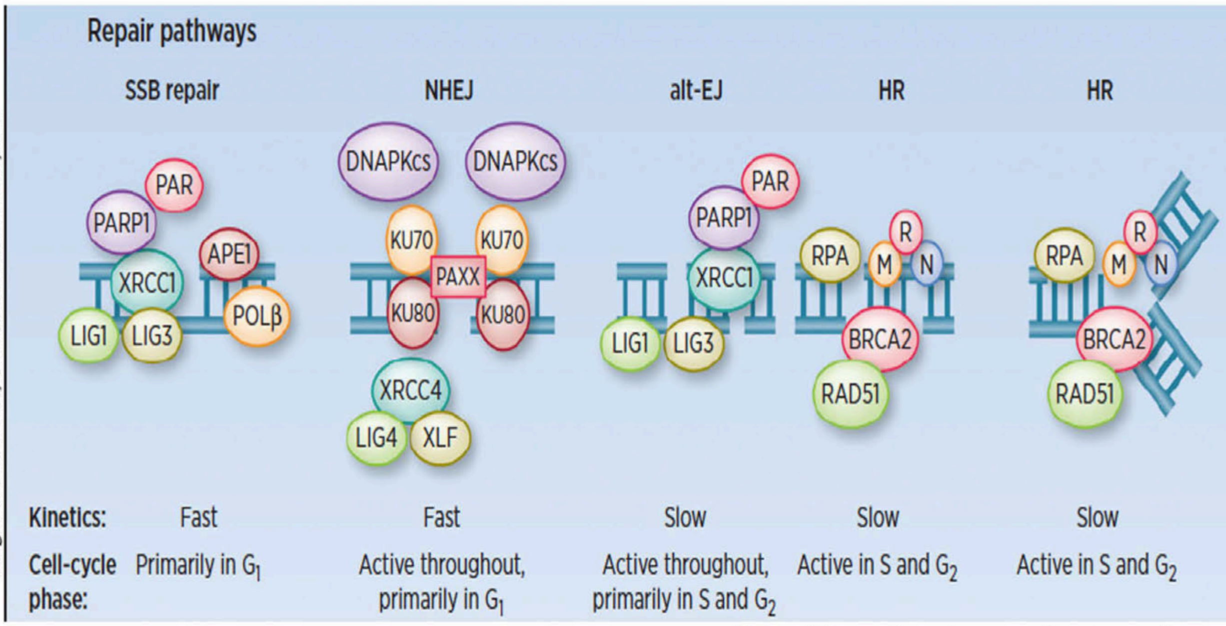
ATM versus ATR



Minchom A, Aversa C, Lopez J. *Ther Adv Med Oncol.* 2018;10:1758835918786658. doi: 10.1177/1758835918786658.

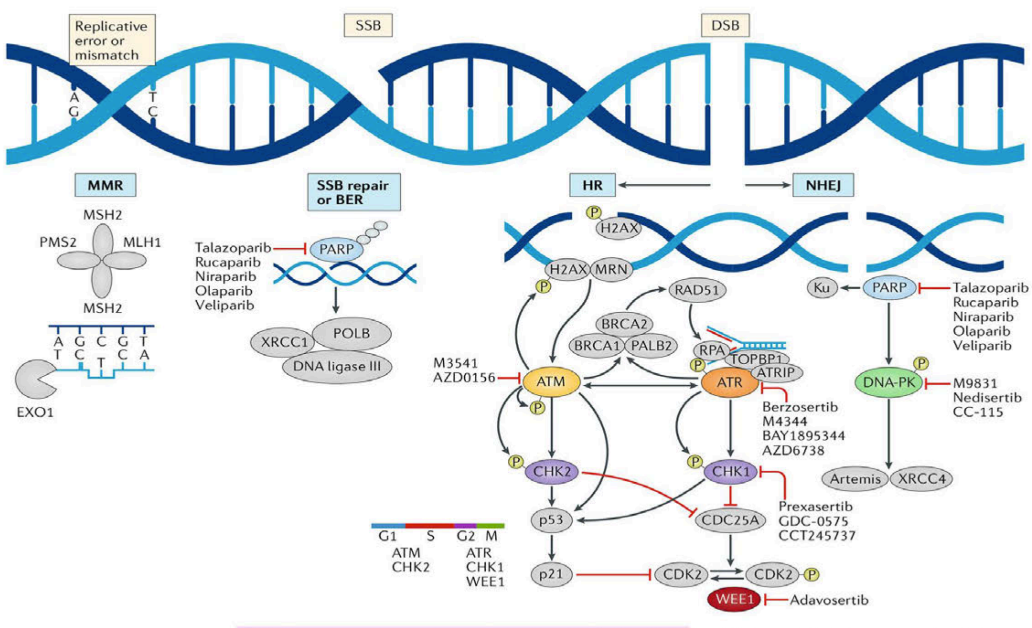
Time Course for Different Types of DNA Repair

Morgan and Lawrence, Clin Cancer Res 21: 2898, 2015



"Fast" = minutes to about an hour
"Slow" = hours to about a day

DDR Inhibitors of Clinical Interest



DNA damage response pathways being targeted in the clinic. Specific types of DNA damage — mismatches due to replication, single-strand DNA breaks (SSBs) or double-strand DNA breaks (DSBs) — result in the activation of specific signalling and repair cascades. DNA damage response (DDR) pathways mitigate replication stress and repair DNA; thus, deficiencies in these pathways result in the accumulation of SSBs and DSBs and increased immunogenicity owing to the generation of neoantigens from mutant proteins. Poly(ADP-ribose) polymerase (PARP) enzymes are key to activating a host of downstream repair mechanisms and are primary proteins involved in SSB repair or base-excision repair (BER). The repair of DSBs occurs predominately through the rapid, error-prone non-homologous end joining (NHEJ) repair pathway in conjunction with the much slower higher-fidelity, error-free homologous recombination (HR) repair pathway. DNA replication is a necessary component of DNA repair and thus cell cycle regulation and replication stress responses are intertwined with DDR pathways. The kinases ATR and ATM have crucial roles in DDR signalling and in maintaining replication fork stability, while also working together via their downstream targets, CHK1 and CHK2, respectively, to regulate cell cycle control checkpoints. The kinase activity of DNA-PK is essential for NHEJ and V(D)J recombination. WEE1 is a distinct nuclear kinase that regulates mitotic entry and nucleotide pools in coordination with DDR. Drugs targeting these key components of the DDR pathways that are undergoing clinical testing are indicated. ATRIP, ATR-interacting protein; EXO1, exonuclease 1; H2AX, histone H2AX; MRN, MRE11, RAD50 and NBS1 complex; POLB, DNA polymerase-β; RPA, replication protein A; TOPBP1, DNA topoisomerase 2-binding protein.

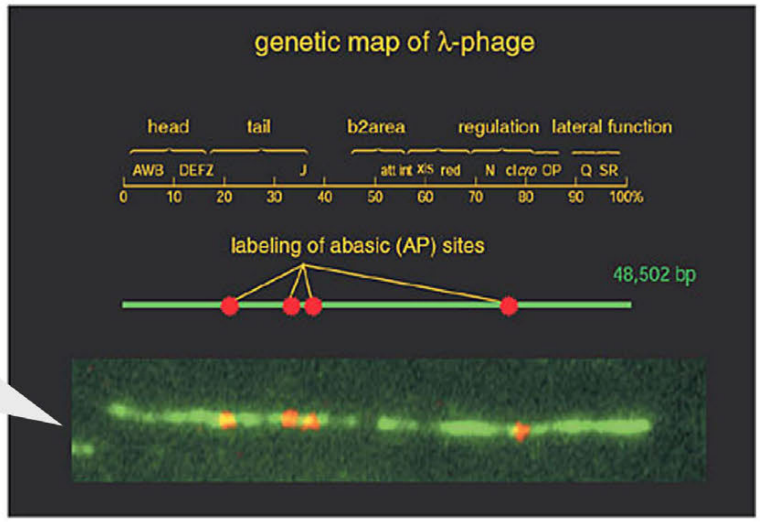
Pilié *et al.*
NATURE REVIEWS | CLINICAL ONCOLOGY
VOLUME 16 | FEBRUARY 2019

DNA Damage and Repair Assays

Base Damage

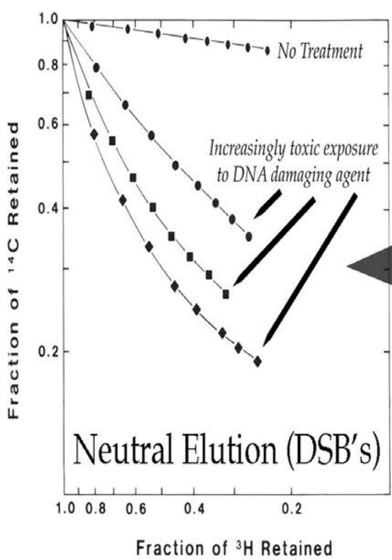
Some Techniques for Measuring Base Damage

- HPLC, GC-MS or GC-EC
- ^{3}H release from labeled thymine
- Phosphate release
- Enzyme sensitivity
- Immunological probes
- Fluorescent labeling of abasic sites



Photochem. Photobiol., 76(2), 123 (2002)

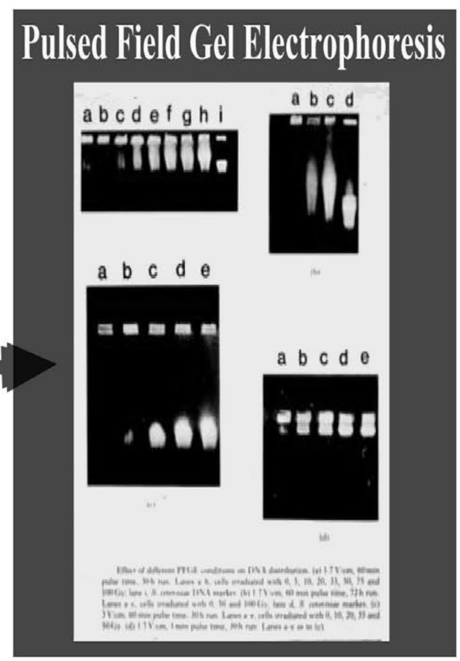
Strand Breaks
(alkaline conditions = SSBs; neutral conditions = DSBs)

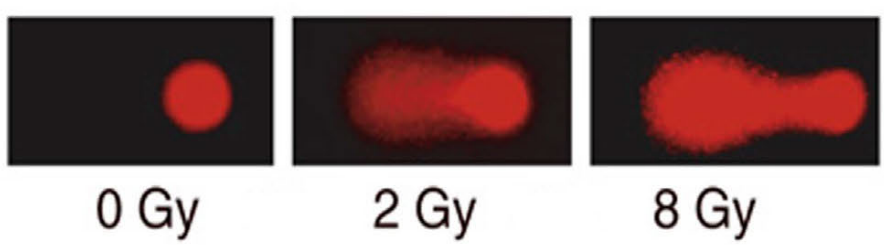


Some Techniques for Measuring Strand Breaks

- Sucrose gradients
- Alkali unwinding hydroxyapatite chromatography
- Filter elution
- Gel electrophoresis
- Nucleoid sedimentation
- Comet assay

Most of these can be alkaline for SSBs or neutral for DSBs





Nature Protocols 1, 23 - 29 (2006)

DNA Damage Assays Summarized: Sensitivity, Techniques and Limitations

Assay	Dose Range[a]	Technique	Limitations
1. Sucrose velocity sedimentation	ssb > 5 Gy dsb > 15 Gy	Larger DNA fragments sediment to a greater extent.	Insensitive to clinically-relevant low radiation doses
2. Filter elution	ssb > 1 Gy (alkaline elution) dsb > 5 Gy (neutral elution)	Smaller DNA fragments elute more quickly through a filter of defined pore size.	Uncertain effects of DNA conformation, cell cycle, cell number, and lysis
3. Nucleoid sedimentation	ssb 1–20 Gy	Irradiated cells show altered DNA supercoiling within nucleus.	Uncertain which DNA lesion(s) are being detected.
4. Pulse-field gel electrophoresis (PFGE)	dsb > 5–10 Gy	Allows for resolution of DNA-dsb, which can be quantified by relative migration within the gel.	Uncertain effects of DNA conformation. High number of cells in S phase may bias results of assay.
5. Comet assay	ssb > 1 Gy (alkaline lysis) dsb > 2 Gy (neutral lysis)	Following lysis, individual nuclei are subjected to agarose gel electrophoresis. The DNA that moves out of the nucleus (head) to form the "tail" of the comet is quantitated to provide a measure of DNA damage.	Requires image analysis system to quantify DNA damage. Increased numbers of cells in S phase may bias assay.
6. Fluorescence in situ hybridization (FISH)	Doses >1 Gy	Chromosome-specific probes, which can be detected with a fluorescent ligand, are used to identify radiation-induced translocations.	May be difficult to interpret in tumor cells that contain translocations prior to irradiation.
7. Premature chromosome condensation (PCC)	Doses >1 Gy	An irradiated interphase cell is fused to a mitotic cell. The chromosomes in the interphase cell undergo premature condensation, allowing radiation-induced chromosome damage to be scored.	May be difficult to interpret in tumor cells that contain chromosome aberrations prior to irradiation.
8. γ-H2AX Intranuclear Foci	Doses >0.05 Gy	Immunofluorescence microscopy or flow cytometry using an antibody to γ-H2AX phosphoprotein.	Requires image analysis system. No standard for size or type of foci to count as DNA breaks.

[a]ssb, single-strand breaks; dsb, double-strand breaks.

From: Tannock *et al.* *The Basic Science of Oncology, 4th Edition*, 2005

Mutations in many – but not all – DDR-related genes are associated with an increased risk of breast cancer

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Risk of Breast Cancer Overall Associated with Protein-Truncating Variants in 34 Genes

Gene	Population-Based Studies (48,826 patients and 50,703 controls)†			
	No. of Carriers of Protein-Truncating Variants		Odds Ratio (95% CI)	P Value
	Women with Breast Cancer	Controls		
ABRAXAS1	17	19	0.98 (0.50–1.94)	0.96
AKT1	3	6	0.47 (0.12–1.93)	0.29
ATM	294	150	2.10 (1.71–2.57)	9.2×10^{-13}
BABAM2	7	9	0.62 (0.23–1.71)	0.36
BARD1	62	32	2.09 (1.35–3.23)	0.00098
BRCA1	515	58	10.57 (8.02–13.93)	1.1×10^{-62}
BRCA2	754	135	5.85 (4.85–7.06)	2.2×10^{-75}
BRIP1	86	75	1.11 (0.80–1.53)	0.54
CDH1	11	12	0.86 (0.37–1.98)	0.72
CHEK2	704	315	2.54 (2.21–2.91)	3.1×10^{-39}
c.1100delC variant	548	245	2.66 (2.27–3.11)	1.1×10^{-33}
Other variants	156	70	2.13 (1.60–2.84)	3.0×10^{-7}
EPCAM	14	19	0.73 (0.36–1.49)	0.39
FANCC	71	65	1.26 (0.89–1.79)	0.20
FANCM	302	300	1.06 (0.90–1.26)	0.48
GEN1	31	43	0.66 (0.41–1.06)	0.088
MEN1	2	5	0.37 (0.07–1.97)	0.24
MLH1	5	9	0.58 (0.19–1.77)	0.34
MRE11	48	55	0.88 (0.59–1.32)	0.54
MSH2	13	13	1.06 (0.47–2.36)	0.89
MSH6	39	23	1.96 (1.15–3.33)	0.013
MUTYH	232	231	1.00 (0.83–1.21)	0.99
NBN	90	103	0.90 (0.67–1.20)	0.48
NF1	31	17	1.76 (0.96–3.21)	0.068
PALB2	274	55	5.02 (3.73–6.76)	1.6×10^{-26}
PIK3CA	3	12	0.21 (0.06–0.75)	0.016
PMS2	40	36	1.16 (0.73–1.85)	0.53
PTEN	14	6	2.25 (0.85–6.00)	0.10
RAD50	120	121	1.08 (0.83–1.40)	0.57
RAD51C	54	26	1.93 (1.20–3.11)	0.0070
RAD51D	51	25	1.80 (1.11–2.93)	0.018
RECQL	103	120	0.84 (0.64–1.10)	0.21
RINT1	32	49	0.72 (0.46–1.14)	0.17
STK11	6	5	1.60 (0.48–5.28)	0.44
TP53	7	2	3.06 (0.63–14.91)	0.17
XRCC2	15	18	0.96 (0.47–1.93)	0.90