DNA damage and DNA repair

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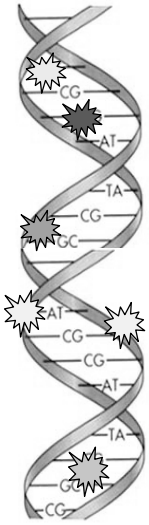
Commonly occurring types of DNA damage:

Spontaneous loss of bases

Alkylation of bases

Oxidation of bases



DNA strand breaks:
Natural cellular processes, exposure to radiation (cosmic, medical e.g. X-rays, radiation therapy) and some forms of chemotherapy

UV-light induced damage:
Cyclobutane dimers
6,4,-photoproducts

Estimated rates of DNA damage per human cell per day:

Single strand breaks	**50,000**
Depurination	**10,000**
Deamination	**600**
Oxidative base damage	**2000**
Alkylated bases	**5000**
Intrastrand cross links	**10**
DNA double-strand break	**10**

Total DNA damaging events per cell per day: 60,000

Total DNA damaging events per cell per hour: 2,500

Estimate 10^{13} - 10^{14} cells in human body
~ 3 x 10^{17} DNA damaging events per hour!

"Mutation is rare because of repair"

Over 200 human genes known to be involved in DNA repair

Major mammalian DNA repair pathways:

1. **Base excision repair (BER)**
2. **DNA Mismatch repair (MMR)**
3. **Nucleotide excision repair (NER)**
4. **DNA strand break repair pathways:**
   - **Single strand break repair (SSBR)**
   - **Double-strand break repair pathways (DSBR)**
     - **Homologous Recombination (HR)**
     - **Nonhomologous end joining (NHEJ)**

Common themes in all DNA repair pathways:

Detection of the lesion: protein or proteins that specifically detect and bind the particular DNA lesion

Removal of the damaged DNA: glycosylases, nucleases, etc

Resynthesis/Repair: DNA polymerases, DNA ligases

Regulatory proteins: protein kinases etc

Effects on other cellular processes: temporary halt in transcription, replication and/or cell division to allow more time for repair to take place

Consequences: accurate repair: **survival**

inability to repair: **cell death**

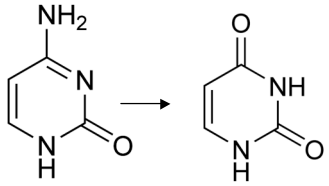
misrepair: **genomic instability**

Base Excision Repair: BER

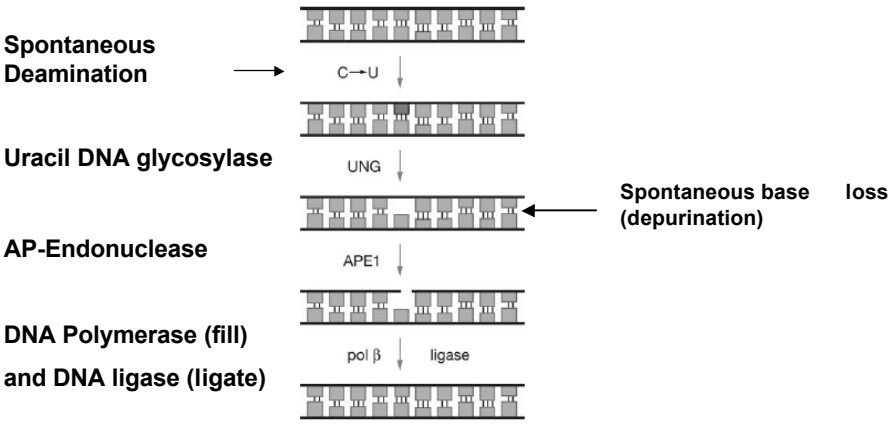
Repairs DNA bases damaged by

- **Alkylation**
- **Deamination**
- **Oxidation**
- **Lost bases (abasic sites)**

Example: spontaneous deamination of Cytosine to Uracil



Base Excision Repair (BER): a simple model:



Maizels Ann. Rev Genet, 2005

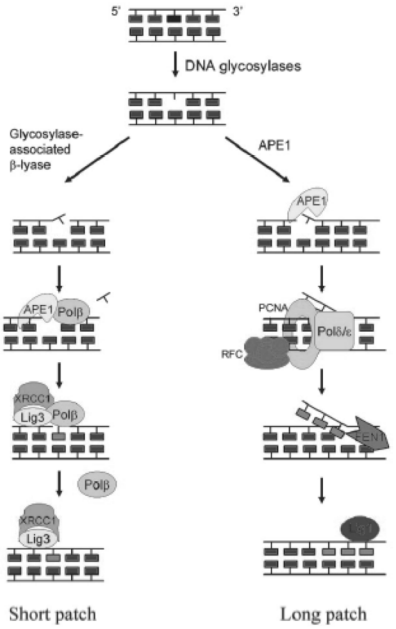
BER in more detail:

Involves multiple proteins

Different variations of the basic pathway depending on precise type of DNA damage

Different glycosylases detect different types of base damage

How do DNA glycosylases detect one damaged base in a 3 billion base pair human genome?



Ref: Sancar et al, 2004, Ann Rev Biochem

DNA Mismatch Repair (MMR):

Corrects errors introduced during DNA replication
(base mismatches, insertions/deletions)

Also required for the removal of bases damaged by:
Methylating agents (MNU, MNNG)
Antimetabolites (6-thioguanine)

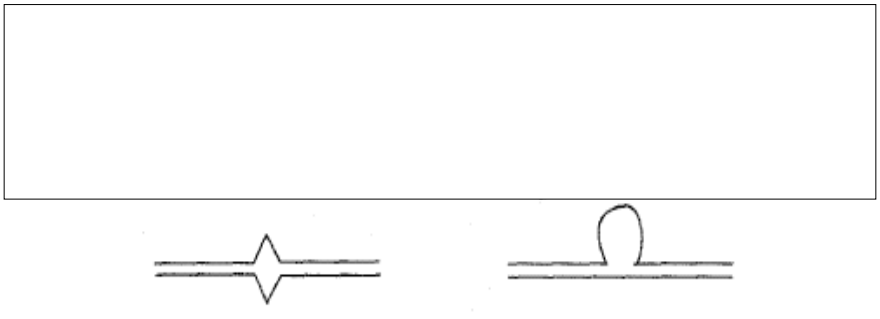
and possibly
Intrastrand crosslinking agents (cisplatin and MMC)

Ref: *Jiricny, The multifaceted mismatch-repair system, Nat. Rev. Molec. Cell. Biol., 2006, 7, 335-340*

DNA Mismatch Repair (MMR):

Errors introduced by DNA replication

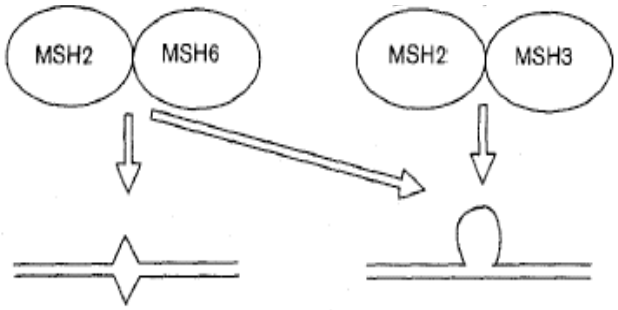
Mispaired bases ***(base pairing errors)*** **small insertions or deletions** ***(slippage of polymerase)***



DNA Mismatch Repair (MMR):

Corrects errors introduced during DNA replication

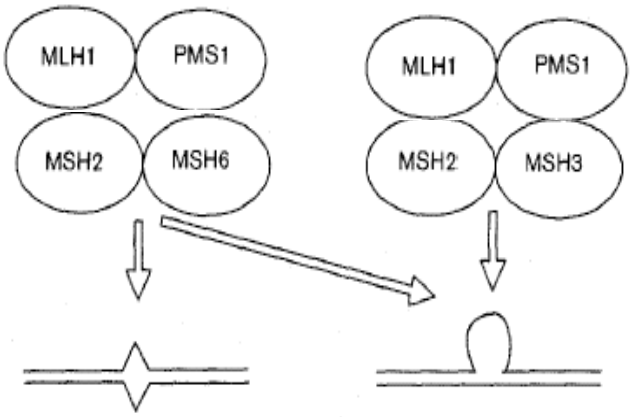
Mispaired bases **small insertions or deletions**



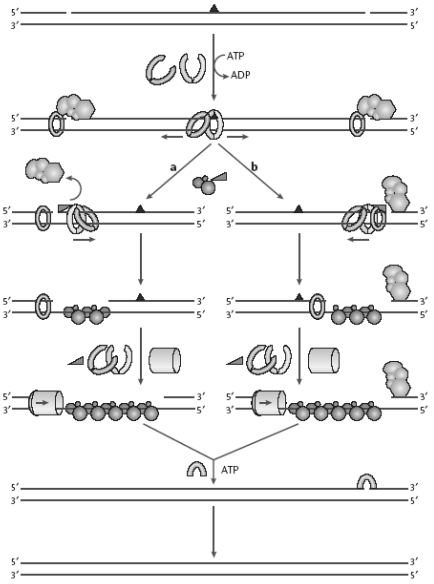
Mispaired bases are detected by the MSH2/MSH6 heterodimer (MutS-a)

Insertions or deletions are detected either by MSH2/MSH6 (MutS-a) OR by MSH2/MSH3 (MutS-b).

Binding of MLH1-PMS1/PMS2 (Mut L) stabilizes binding of MutS a and b to the DNA mismatch/insertion deletion



MMR in more detail:



Mismatch = red triangle

MutSα or MutSβ binds the mismatch and recruits MuLα

ATP-dependent conformational change releases the MutS/L complex from the mismatch.

The complex diffuses either upstream (a) or downstream (b) of the mismatch where exonuclease I, RFC, PCNA and RPA are involved in removal of the lesion

DNA polymerase delta fills the gap and DNA ligase 1 seals the ends

How the system knows to repair damage on the newly replicated strand in human cells is still unknown

Ref: Jiricny, Nat Rev Mol Cell Biol, 2006

DNA Mismatch Repair and Colon Cancer:

Hereditary nonpolyposis colon cancer (HNPCC)

the most common form of hereditary colorectal cancer.
accounts for 2-7% of all colorectal cancers
characterized by early onset (40-50 years), spontaneous colon cancer and increased cancer risk for endometrium, ovarian, stomach, and small intestine.

>90% HNPCC patients have mutations in MLH1 (40%) or MSH2 (40%)

Mutations in other MMS genes (e.g. PMS2, MSH6) are rare (1 -5% of patients)

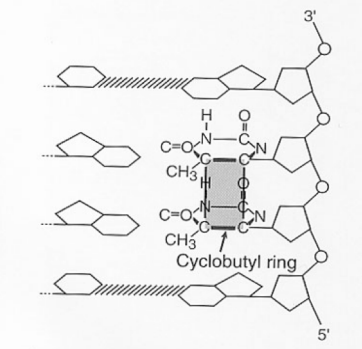
Cells with defects in MMR have 1000 X greater mutation rate than MMR proficient cells and are also characterized by microsatellite instability (MSI or MIN).

MIN is due to the inability of MMR defective cells to correct errors caused by DNA polymerase slippage at repetitive sequences in the genome.

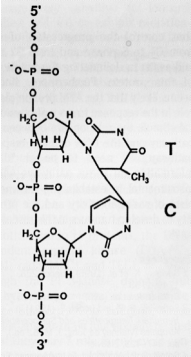
Nucleotide Excision Repair: NER

Repairs damage introduced by UV light

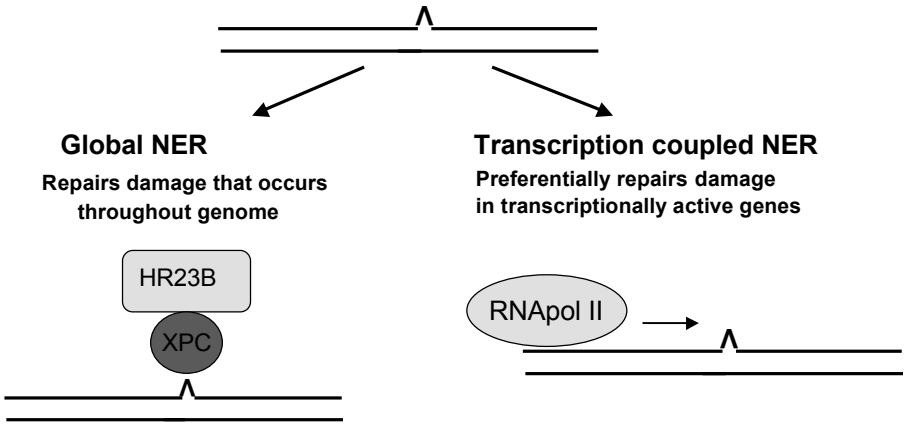
Cyclobutane dimers



6,4-photoproducts



Detection of UV-damaged DNA



Both branches converge into a common pathway involving over 20 different genes including XPA, XPB, XPD, XPF and XPG

Basic Steps in NER:

Detection of lesion:
Stalled RNA pol II for non- transcriptionally active genes (TC-NER) or XPC-HR23B for transcriptionally active genes (Global -NER)

Common steps:

Assembly of protein complex at site of DNA damage

Opening of DNA strands (DNA bubble): DNA helicases

Removal of DNA damage:

Cut DNA strand about 12-16 bases either site of lesion (endonucleases)
Release of 25-32bp fragment ssDNA containing the lesion

Resynthesize:

new DNA strand using undamaged strand as template (DNA polymerases)

Seal phosphodiester backbone (DNA ligases)

Global NER:

1. **Damage recognition:**
   XPC-hHR23B binds the lesion

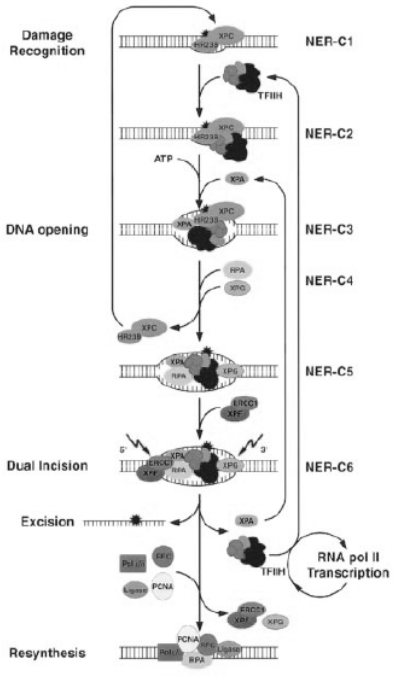
2. **DNA opening:**
   TFIIH (XPB and XPD: DNA helicases; p62, p52, p44, p34 and others)
   XPA
   RPA

3. **Incision and excision:**
   XPG and XPF-ERCC1 (structure specific endonucleases):
   XPF-ERCC1 cleaves 5' to lesion
   XPG cleaves 3' to lesion

   24-32 bp piece of DNA containing the lesion is excised

4. **Repair synthesis and DNA ligation:**
   DNA polymerases delta (d) and epsilon (e), RFC, PCNA, RPA and DNA ligase 1

Ref: Park and Choi, FEBS Lett, 273, 1600-1608 (2006).



Transcription coupled NER:

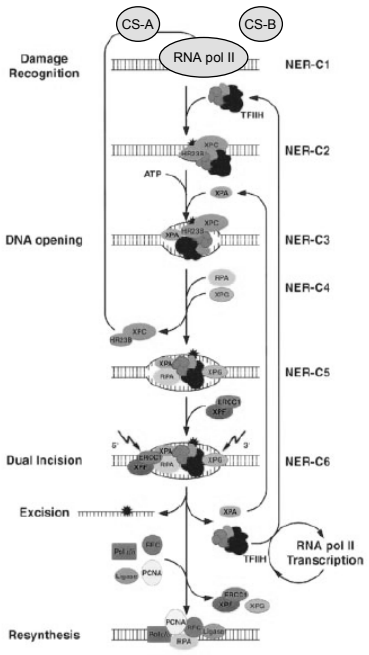
1. **Damage recognition:**
   Stalled RNA pol II
   Cockayne Syndrome A and B

2. **DNA opening:**
   TFIIH (XPB and XPD: DNA helicases; p62, p52, p44, p34 and others)
   XPA
   RPA

3. **Incision and excision:**
   XPG and XPF-ERCC1 (structure specific endonucleases):
   XPF-ERCC1 cleaves 5' to lesion
   XPG cleaves 3' to lesion

   24-32 bp piece of DNA containing lesion is excised

4. **Repair synthesis and DNA ligation:**
   DNA polymerases delta and epsilon, RFC, PCNA, RPA and DNA ligase 1

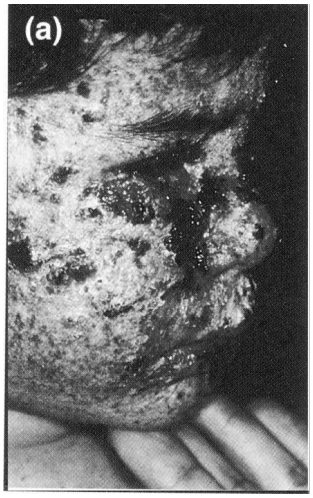


Nucleotide Excision Repair and Cancer

Xeroderma Pigmentosum (XP):
Rare genetic syndrome caused by mutation in XPA and other XP genes

Characterized by UV-induced skin cancer on skin exposed to sunlight

Sunlight:
90% UVA
10% UVB
trace UVC



Other diseases associated with defects in NER:
Cockayne's syndrome and Trichothiodystrophy

DNA strand breaks repair pathways

Causes of DNA strand breaks:

Reactive oxygen species (ROS):generated by normal metabolic/cellular processes or external agents

Errors during normal cellular processes: DNA replication, mitosis, meiosis

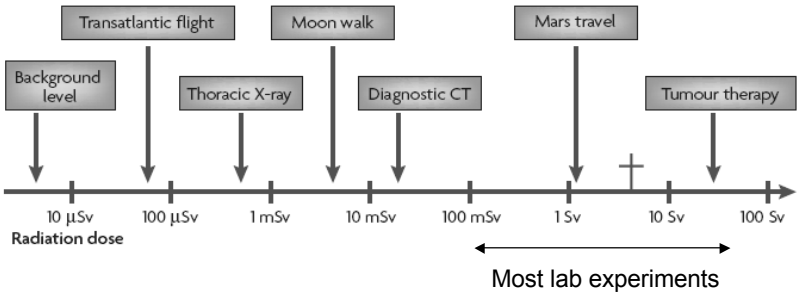
Induced as part of naturally occurring processes: V(D)J recombination, class switch recombination

Exposure to radiation: cosmic radiation, radiation during medical procedures (X-rays, CT scans, radiation therapy)

Chemotherapy: many chemotherapeutics (etoposide, doxorubicin, camptothecin derivatives etc) act as topoisomerase poisons, which induce DNA strand breaks

Exposure to radiation:

from Lobrich and Jeggo, Nat. Rev. Cancer 2007



Background dose (sea level):	**5 µSv (higher at higher elevations)**
Transatlantic flight:	**~80 µSv**
Chest X-ray:	**~800 µSv**
CT scan:	**30 µSv**
Radiation therapy:	**1-2 Sv per day for 30-50 days (~ 50 Sv cumulative dose)**
Lethal single body dose	**5 Gy (Sv)**

IR induces complex DNA lesions:

- **IR-induced damage caused by direct interaction of energy with DNA (direct effects) as well as by ionization of water in vicinity of DNA (indirect effects)**

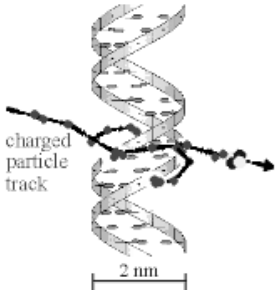
•IR induces damage to bases, sugars and DNA backbone

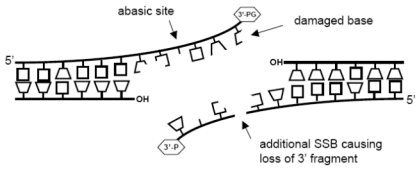
- **Produces complex DNA lesions that are lethal to the cell if not repaired**

•Examples of types of damage: Oxidative damage (bases, sugars)

•Single strand breaks (SSBs): frequently with non-ligatable end groups (3'P and 3'-Phosphoglycolate)

Double strand breaks (DSBs): occur when two SSBs occur on opposite strands





Repair of IR induced DNA damage

Base damage: BER

Single strand breaks (SSBs): break in phosphodiester bond of one DNA strand

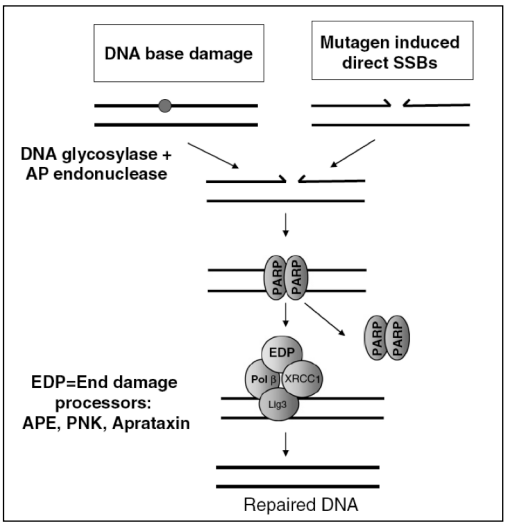
SSBR pathway:

SSBs detected by Poly-ADP ribose (PARP)

Repaired by SSB Repair pathway:

XRCC1, DNA ligase III, DNA pol beta and various end damage processing enzymes (EDP in figure) such as APE, PNK and aprataxin.

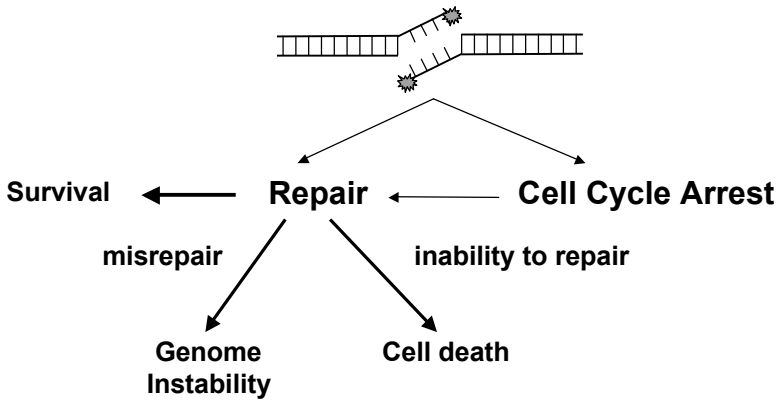
Sharma and Dianov, Mol Asp Med, 28, 2007, 345-374



Repair of IR induced DNA damage:

Double strand breaks (DSBs)

Occur when have 2 SSBs 10-20 bp apart on opposite DNA strands



Two major pathways for the repair of DSBs in human cells

Nonhomologous end joining (NHEJ):

DNA-PKcs, Ku70/80, XRCC4, DNA ligase IV, XLF
Artemis, PNK, DNA polymerases mu and lambda
53BP1? Tdp1? WRN? Others?

- **Major pathway in human cells for repair of IR-induced DSBs**
- **Active throughout the cell cycle, predominant pathway in G0, G1**
- **Does not require DNA template**
- **Potential to be error prone**
- **Required for V(D)J recombination and class switch recombination**

Homologous recombination repair (HRR):

Mre11-Rad50-Nbs1 (*Xrs2* in yeast), RPA, Rad51, Rad52, XRCC2, XRCC3, BRCA1, BRCA2 and others

- **Predominant pathway in yeast**
- **Active in late S and G2**
- **Requires undamaged DNA template**
- **Accurate, template directed repair**

Main players in NHEJ

Ku70/80: heterodimer of 70 and 80 kDs subunits, binds DSB

DNA-PKcs: catalytic subunit of DNA-dependent protein kinase
member of PIKK family of S/T protein kinases
interacts with Ku to form DNA-PK
protein kinase activity required for NHEJ

Artemis: nuclease: interacts with DNA-PKcs

XRCC4: scaffolding protein, interacts with DNA ligase IV
stabilizes and stimulates activity of DNA ligase IV

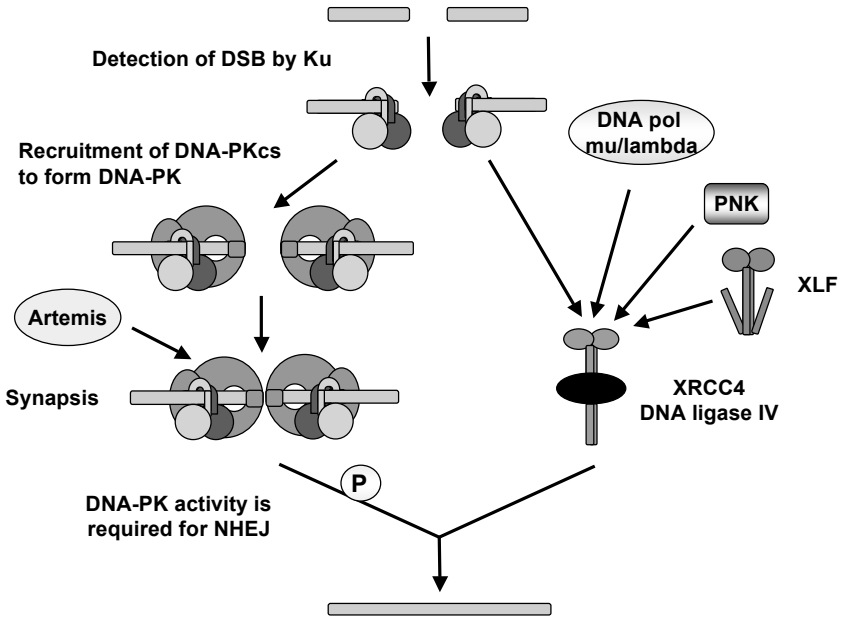
DNA ligase IV: ligates DNA ends

XLF: XRCC4-Like-Factor, interacts with XRCC4,
stimulates activity of DNA ligase IV

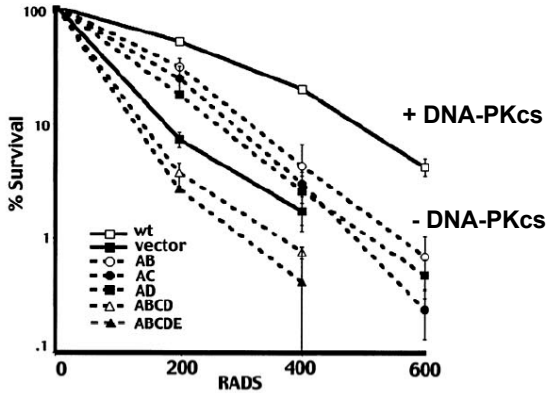
DNA polymerases: mu and lambda, gap filling

Polynucleotide kinase (PNK): DNA phosphatase/kinase interacts with XRCC4

Working model for Nonhomologous End Joining



Cells that lack any of the NHEJ components are radiation sensitive



Inhibitors of DNA-PK kinase activity radiosensitize cells

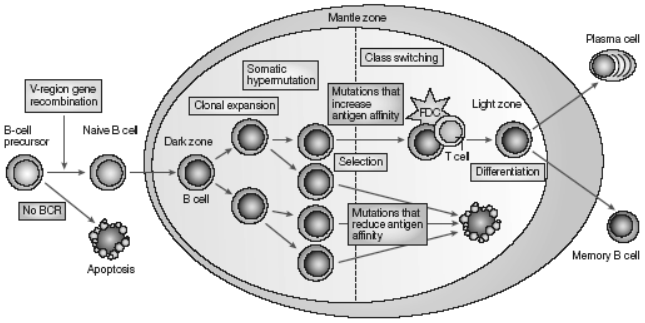
Being developed as potential radiation sensitizers for radiation therapy

Defects in NHEJ factors are also associated with defects in V(D)J recombination and Class Switch Recombination:

Sequence specific gene rearrangement processes that occur in B (and T) cells and are required for production of immunoglobulin genes, T Cell receptor genes and functional T and B cells

Inability to undergo V(D)J recombination results in lack of mature T and B cells

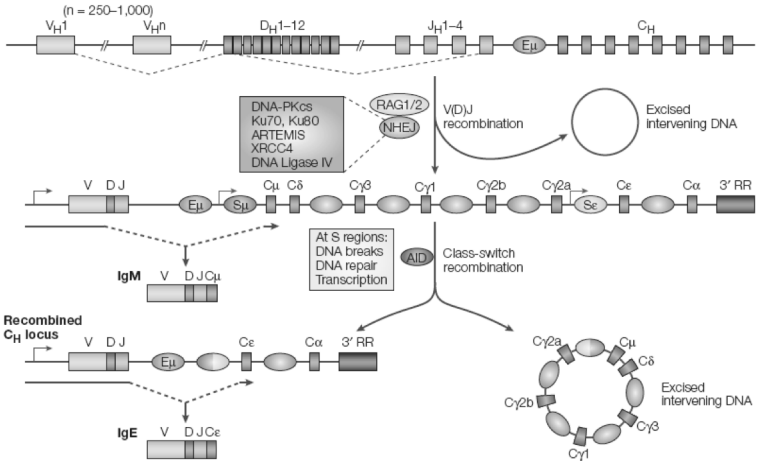
Animals lacking NHEJ factors suffer from Severe Combined Immune Deficiency (SCID)



Chaudhuri J, Alt FW. Nat Rev Immunol. 2004, 4(7):541-52).

NHEJ and DSB repair proteins are required for V(D)J and CSR:

Sequence specific gene rearrangement processes that occur in B (and T) cells and are required for production of immunoglobulin genes, T Cell receptor genes and functional T and B cells



Chaudhuri and Alt, Nat Rev Immunol. 2004

Defects in VDJ and CSR may be linked to chromosomal translocations in B cell malignancies:

Many B cell malignancies are characterized by translocation of Immunoglobulin gene promoter and proto-oncogene, leading to suggestions that defects in V(D)J and CSR may promote chromosomal translocations that are the defining characteristics of many human hematological malignancies

Table 2 | **Mechanisms of B-cell lymphoma pathogenesis**

Lymphoma	Chromosomal translocations	Tumour-suppressor gene mutations	Viruses	Other alterations
Mantle-cell lymphoma	*CCND1–IgH* (95)[107]	*ATM* (40)[108,109]		Deletion on 13q14 (50–70)[110*]
B-cell chronic lymphocytic leukaemia	–	*ATM* (30)[111,112], *TP53* (15)[113]	–	Deletion on 13q14 (60)[114*]
Follicular lymphoma	*BCL2–IgH* (90)[12–14]	–	–	–
Diffuse large B-cell lymphoma	*BCL6*–various (35)[115,116], *BCL2–IgH* (15-30)[117], *MYC–IgH* or *MYC–IgL* (15)[118]	*CD95* (10–20)[25], *ATM* (15)[119] *TP53* (25)[120,121]	–	Aberrant hypermutation of multiple proto-oncogenes (50)[18]
Primary mediastinal B-cell lymphoma	–	*SOCS1* (40)[122]	–	Aberrant hypermutation of multiple proto-oncogenes (70)[123]
Burkitt's lymphoma	*MYC–IgH* or *MYC–IgL* (100)[124,125]	*TP53* (40)[113], *RB2* (20–80)[126]	EBV (endemic, 95; sporadic, 30)[28]	–
Post-transplant lymphomas	–	–	EBV (90)[28]	–
Classical Hodgkin's lymphoma	–	*IKBA* (10-20)[127–129], *IKBE* (10)[130], *CD95* (<10)[131]	EBV (40)[28]	*REL* amplifications (50)[132]
Lymphocyte-predominant Hodgkin's lymphoma	*BCL6*–various (48)[133]	–	–	–
Splenic marginal-zone lymphoma	–	–	–	Deletion on 7q22-36 (40)[134*]
MALT lymphoma	*API2–MALT1* (30)[135], *BCL10–IgH* (5)[136,137],	*CD95* (5-80)[25,140,141‡]	Indirect role of *Helicobacter pylori* in	–

R Kuppers, Mechanisms of B cell lymphoma pathogenesis, Nature Reviews Cancer, 5, 2005,

Homologous recombination

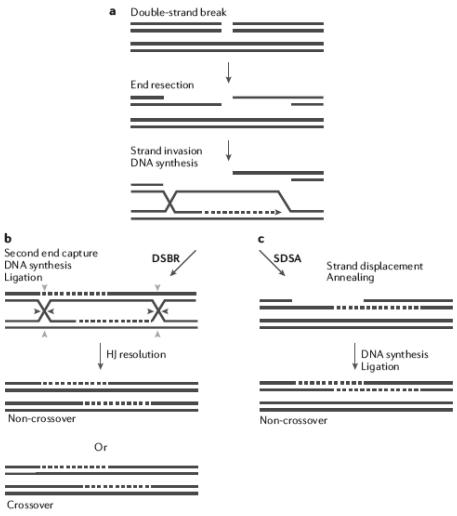
Analysis of DNA intermediates

Requires intact sister chromatid (red)
End resection to produce 3'overhangs
Strand invasion by 3' end
DNA synthesis (red dotted line)

DSBR:
Second end capture
Double Holliday junction
Resolution of double Holliday junction
Cross over or non-cross over possible

OR

Single strand annealing
Strand displacement
Annealing
no cross over
no Holliday junction



Sung and Klein, Nat Rev Mol Cell Biol 2006

Filippo et al, Ann. Rev Biochem. 2008

Protein factors involved in HR:

from biochemistry and genetics

Mre11, Rad50, Nbs1/Xrs2 (MRN complex): end binding, end resection

Rad51: protein-DNA filaments

RPA: regulates access of Rad51 to DNA

Rad52: interacts with Rad51 and RPA

BRCA2: helps load Rad51 on DNA

BRCA1: interacts with BRCA2

Table 1 | **Mitotic and meiotic homologous-recombination factors**

Saccharomyces cerevisiae	Human	Biochemical function(s)	Notable features
Factors that function with RAD51			
Rad50	RAD50	DNA binding; DNA-dependent ATPase	Member of the SMC protein family; forms a complex with MRE11 and NBS1; involved in DSB end resection; involved in DNA-damage checkpoints
Mre11	MRE11	DNA-structure-specific endonuclease; 3′→5′ exonuclease	Forms a complex with RAD50 and NBS1; involved in DSB-end resection; involved in DNA-damage checkpoints
Xrs2	NBS1	DNA binding	Forms a complex with RAD50 and MRE11; involved in DSB-end resection; involved in DNA-damage checkpoints
Rad52	RAD52	ssDNA binding and annealing; recombination mediator	Interacts with RAD51 and RPA
Rad54	RAD54, RAD54B	dsDNA-dependent ATPase; dsDNA translocase; induces superhelical stress in dsDNA; stimulates the RAD51-mediated D-loop reaction; chromatin remodeller	Member of SWI2/SNF2 protein family; interacts with RAD51; Yeast Rad54 strips Rad51 from dsDNA
Rdh54/Tid1	RAD54, RAD54B	dsDNA-dependent ATPase; dsDNA translocase; induces superhelical stress in dsDNA; stimulates the RAD51-mediated D-loop reaction	Member of SWI2/SNF2 protein family; interacts with RAD51
Rad55–Rad57	XRCC2, XRCC3, RAD51B, RAD51C, RAD51D	Binds ssDNA; recombination mediator (shown for Rad55–Rad57 and RAD51B–RAD51C complexes only)	The human proteins form complexes (see main text for details); might stabilize the presynaptic filament; interacts with RAD51; Human RAD51C associates with Holliday-junction-resolvase activity
Rad59	Unknown	ssDNA binding and annealing	Homology to Rad52; interacts with Rad52
Mer3	Unknown	ssDNA-dependent ATPase; 3′→5′ DNA helicase activity; unwinds the Holliday junction	Extends the DNA joint made by Rad51
Factors that function with DMC1			
Mei5–Sae3	Unknown	Predicted recombination-mediator activity	Interacts with Dmc1
Hop2–Mnd1	HOP2–MND1	Binds ssDNA and dsDNA; stimulates the DMC1-mediated D-loop reaction	Interacts with DMC1
Rdh54/Tid1	RAD54 RAD54B	DNA-dependent ATPase; DNA translocase; induces superhelical stress in dsDNA; predicted to stimulate the DMC1-mediated D-loop reaction; Human RAD54B stimulates the DMC1-mediated D-loop reaction	Yeast Rdh54 was identified by yeast two-hybrid interaction with Dmc1; Human RAD54B interacts with DMC1

Sung and Klein, Nat. Rev. Mol. Cell. Biol, 2006

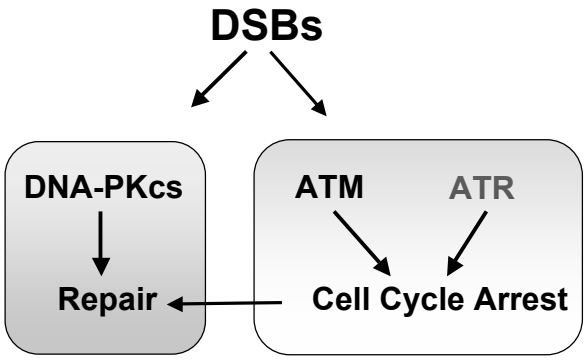
IR-induced cell signalling and cell cycle arrest pathways

Phosphatidyl inositol 3 kinase like protein kinases (PIKKs):

DNA-PKcs: catalytic subunit of the DNA dependent protein kinase

ATM: Ataxia-Telangiectasia Mutated

ATR: ATM-, Rad-3, related



Ataxia-Telangiectasia Mutated (ATM):

Ataxia-telangiectasia (A-T):
Autosomal recessive; compound heterozygotes
Incidence 1 in ~ 40,000 to 1 in 100,000
Characterized by neurodegeneration, progressive loss of neuromuscular control, ataxia, telangiectasia, immune deficiencies, cancer predisposition (lymphoma), radiation sensitivity

Over 400 mutations identified to date
Mutations occur throughout the gene and are usually truncation or splicing; about 10% are mis-sense

Blood relatives of A-T patients have increased risk of developing breast cancer

ATM-deficient cell lines are characterized by:
Radiosensitivity, radiation resistant DNA synthesis, loss of check point control, chromosomal breakage, and genomic instability

ATM is also mutated in some tumour types (Mantle Cell Lymphoma)

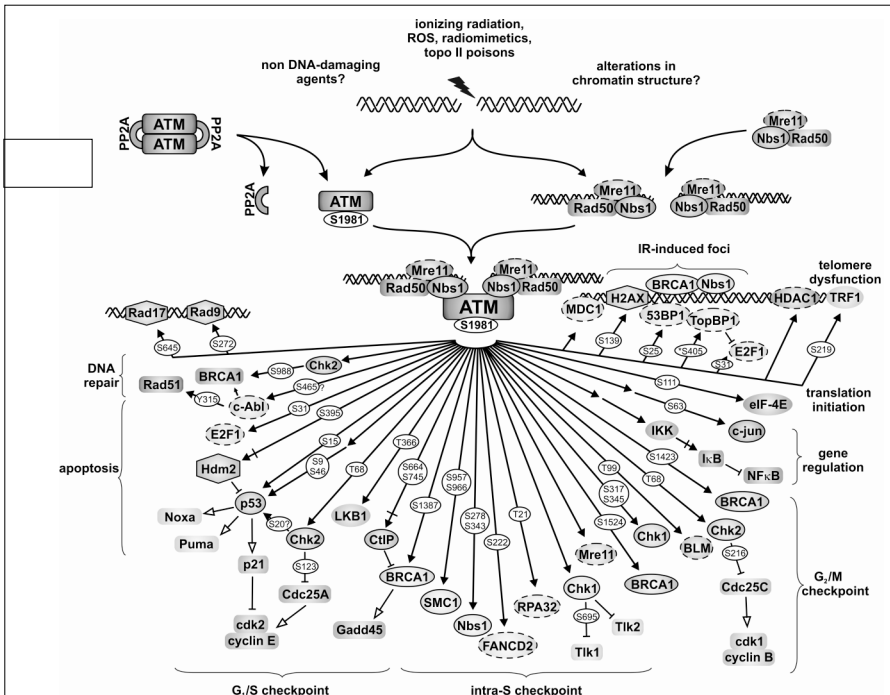
Activation of cell signalling pathways in response to DSBs:

ATM is activated in response to IR (exact mechanism of activation is still hotly debated)

In response to IR (and other DNA damaging agents), ATM phosphorylates many protein targets in the cell resulting in activation of cell cycle checkpoints that cause transient arrest of the cell cycle at G1 to S, during S or at G2 to M

Activation of cell cycle checkpoints may allow cells more time to detect and repair the DNA damage

ATM phosphorylates the tumour suppressor protein p53, which regulates cell cycle arrest at G1/S and cell death by apoptosis



DNA damage induced activation of p53

Chene, Nature Reviews Cancer, 2, 102 (2003)

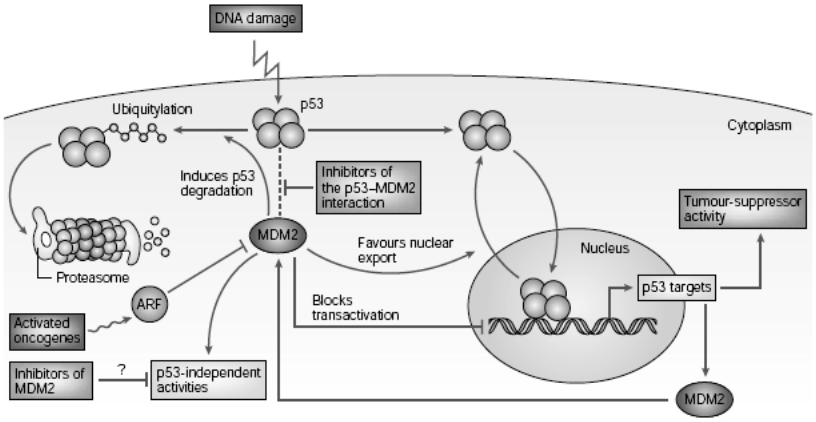


Figure 2 | **Regulation of p53 by MDM2.** p53 and MDM2 form an auto-regulatory feedback loop. p53 stimulates the expression of MDM2; MDM2 inhibits p53 activity because it blocks its transcriptional activity, favours its nuclear export and stimulates its degradation. Different cellular signals, such as DNA-damage or oncogene activation, induce p53 activation. DNA damage favours p53 phosphorylation, preventing its association with MDM2. Activated oncogenes activate the ARF protein, which prevents the MDM2-mediated degradation of p53. Similarly, inhibitors of the p53–MDM2 interaction should activate p53 tumour-suppressor activity in tumour cells that express wild-type p53. These compounds, because they bind to MDM2, could also affect the p53-independent activities of MDM2.

ATM substrates: BRCA1 and BRCA2

BRCA1 and BRCA2 genes: discovered in 1990s as Breast and ovarian cancer susceptibility genes

Mutations in BRCA1 and BRCA2 account for about 60% of hereditary breast cancer.

However, only 5 to 10 % of all breast cancers are hereditary: most are "sporadic"

The causes of sporadic breast cancer are not well understood.

Annual rates of breast cancer (US): 215,000 women; 1500 men

Gene and protein sequences had no distinguishing features that suggested what BRCA1 and BRCA2 actually do!

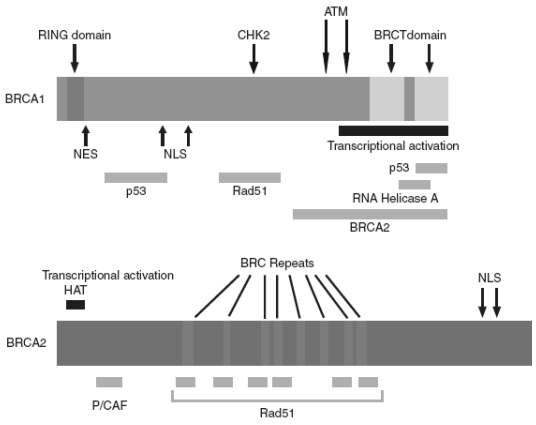
BRCA1 and BRCA2 are involved in the DNA damage response:

BRCA2:
interacts directly with Rad51
required for HR

BRCA1:
interacts with BRCA2, p53, Rad51:
involved in HR and possibly NHEJ

phosphorylated by ATM and Chk2 in response to DNA damage.
Phosphorylation required for cell cycle checkpoints

BRCA1 and BRCA2:
Recruited to sites of DNA damage after IR (IRIF)



Rev: Yoshida and Miki: Cancer Sci 2005

Summary

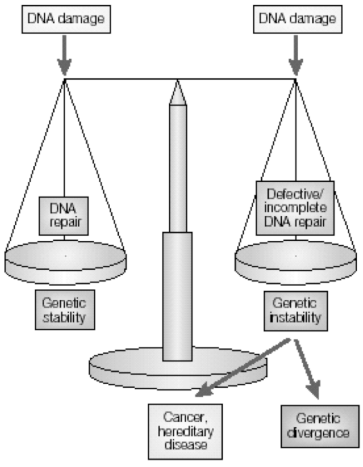
DNA damage happens: caused by endogenous and exogenous sources

Cells have multiple and complex pathways to detect and repair each specific type of DNA damage

Major repair pathways in human cells (BER, MMR, NER and the strand break repair pathways: SSBR, HR and NHEJ)

Perfect repair would result in genome stability

Imperfect repair can promote genetic divergence but also cause genomic instability



From Friedberg (2001) Nat. Rev. Cancer, 1, 22-33

Understanding DNA repair pathways has lead to greater understanding of some human diseases

DNA repair protein	DNA repair Pathway	Disease Syndrome
MSH2, MLH1	MMR	HNPCC
XP proteins	NER	XP skin cancer
ATM	DSB signalling	A-T; predisposition to breast cancer
Nbs1	DSB signalling, HR	Nijmegen Breakage Syndrome
Chk2	DSB signalling	Breast cancer
Artemis	NHEJ	RS-SCID
DNA-PKcs	NHEJ	RS-SCID in mice dogs and horses
DNA ligase IV	NHEJ	Lig4 syndrome
FA proteins	DNA crosslink repair, HR?	Fanconi Anemia
Apratxin	SSB repair	AOA1, microcephaly

Adapted from O'Driscoll M, Jeggo PA..Nat Rev Genet. 2006

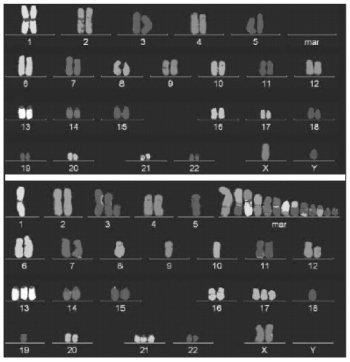
Understanding DNA repair pathways could lead to a better understanding of the causes of genomic instability:

Chromosome translocations are a hallmark of genomic instability
Cancer cells have highly unstable genomes characterized by chromosome duplication, chromosome loss, and chromosome translocations.

Spectral Karyotype analysis:

Karyotype of a normal cell

Karyotype of a cancer cell



How do chromosomal translocations occur?
Are these caused by aberrant DNA repair mechanisms?

Better Response to Radiation therapy?

Approximately half of all cancer patients are treated with radiation therapy

Some patients respond top treatment and survive

Others get same treatment but suffer from poor treatment outcomes

Understanding DNA repair pathways could also lead to ways to predict radiation response in cancer patients

Immunohistochemistry of protein levels of proteins involved in DNA repair, cell survival as well as hypoxia and angiogenesis etc could help predict whether tumours will respond to radiation (or other DNA damaging agents) or not

Similar for mRNA expression levels, microRNA profiles, SNPs

Reduced side effects of radiation therapy?

Understanding DNA repair pathways could also lead to development of novel radiosensitizers

Specific inhibitors of DNA-PK and ATM kinase activity sensitize human cell lines to IR and chemotherapeutics

Confirmed in animal models (Zhao et al, Cancer Research 2006)

Could they be of use as radiosensitizers in cancer patients?

Understanding DNA repair pathways could lead to novel cancer therapies

Some cancers are characterized by defects in DNA repair proteins

Examples:

Mutation of BRCA1 and BRCA2 in hereditary breast cancers

Mutation of loss of ATM in mantle cell lymphoma, B-CLL, and possibly some lung and gastric cancers

Hypothesis: One DNA repair pathway is compromised; so cancer cells rely more on other repair pathways

Prediction: If inhibit the alternative pathway will this kill the tumour cells?

Yes!

BRCA1/BRCA2 defective breast cancer cells (defective DSB repair; HR and ATM dependent pathways) are highly sensitive to inhibition of PARP (SSB repair)

Farmer et al, Nature 2005; Bryant et al, Nature, 2005

The Lees-Miller lab:

Effects of radiation and other DNA damaging agents on cells

Mechanism of NHEJ

Role for DNA-PK and ATM in the DNA damage response

Mechanisms of tumour radiation resistance and radio sensitivity: biomarkers of radiation response

Developing novel therapies based on understanding the molecular details DNA repair pathways

Web site:

http://www.ucalgary.ca/~leesmill

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

Alberta Cancer Board

UNIVERSITY OF CALGARY

National Cancer Institute of Canada / Institut national du cancer du Canada

Alberta Heritage Foundation for Medical Research

SOUTHERN ALBERTA Cancer Research Institute