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

DNA strand breaks:
Natural cellular processes, exposure to radiation (cosmic, medical e.g. X-rays, radiation therapy) and some forms of chemotherapy
**Estimated rates of DNA damage per human cell per day:**

<table>
<thead>
<tr>
<th>Type of Damage</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single strand breaks</td>
<td>50,000</td>
</tr>
<tr>
<td>Depurination</td>
<td>10,000</td>
</tr>
<tr>
<td>Deamination</td>
<td>600</td>
</tr>
<tr>
<td>Oxidative base damage</td>
<td>2000</td>
</tr>
<tr>
<td>Alkylated bases</td>
<td>5000</td>
</tr>
<tr>
<td>Intrastand cross links</td>
<td>10</td>
</tr>
<tr>
<td>DNA double-strand break</td>
<td>10</td>
</tr>
</tbody>
</table>

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

$\sim 3 \times 10^{17}$ DNA damaging events per hour!

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**“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**

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**Base Excision Repair: BER**

Repairs DNA bases damaged by
- Alkylation
- Deamination
- Oxidation
- Lost bases (abasic sites)

Example: spontaneous deamination of Cytosine to Uracil

![Chemical structure of Cytosine to Uracil](image)
**Base Excision Repair (BER): a simple model:**

- Spontaneous Deamination
- Uracil DNA glycosylase
- AP-Endonuclease
- DNA Polymerase (fill) and DNA ligase (ligate)

Maizels Ann. Rev Genet, 2005

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**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?

**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,
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

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**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**

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**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)

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**Global NER:**

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

2. **DNA opening:**
   TFIH (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

**Transcription coupled NER:**

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

2. **DNA opening:**
   - TFIIH (XBP 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

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**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

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**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**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ku70/80:</td>
<td>heterodimer of 70 and 80 kDa subunits, binds DSB</td>
</tr>
<tr>
<td>DNA-PKcs:</td>
<td>catalytic subunit of DNA-dependent protein kinase</td>
</tr>
<tr>
<td></td>
<td>member of PIKK family of S/T protein kinases</td>
</tr>
<tr>
<td></td>
<td>interacts with Ku to form DNA-PK</td>
</tr>
<tr>
<td></td>
<td>protein kinase activity required for NHEJ</td>
</tr>
<tr>
<td>Artemis:</td>
<td>nuclease: interacts with DNA-PKcs</td>
</tr>
<tr>
<td>XRCC4:</td>
<td>scaffolding protein, interacts with DNA ligase IV</td>
</tr>
<tr>
<td></td>
<td>stabilizes and stimulates activity of DNA ligase IV</td>
</tr>
<tr>
<td>DNA ligase IV:</td>
<td>ligates DNA ends</td>
</tr>
<tr>
<td>XLF:</td>
<td>XRCC4-Like-Factor, interacts with XRCC4, stimulates activity of DNA ligase IV</td>
</tr>
<tr>
<td>DNA polymerases:</td>
<td>mu and lambda, gap filling</td>
</tr>
<tr>
<td>Polynucleotide kinase (PNK):</td>
<td>DNA phosphatase/kinase interacts with XRCC4</td>
</tr>
</tbody>
</table>

**Working model for Nonhomologous End Joining**

1. Detection of DSB by Ku
2. Recruitment of DNA-PKcs to form DNA-PK
3. Synapsis
4. DNA-PK activity is required for NHEJ
5. DNA ligase IV
6. Artemis
7. Polynucleotide kinase (PNK)
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)

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 VDJ and CSR may promote chromosomal translocations that are the defining characteristics of many human hematological malignacies.

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

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 homology recombination factors

<table>
<thead>
<tr>
<th>Name</th>
<th>Biochemical function(s)</th>
<th>Notable features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rad51</td>
<td>DNA binding, DNA-dependent ATPase</td>
<td>Member of the MRN protein family forms a complex with MRE11 and NBS1 involved in DNA damage checkpoint.</td>
</tr>
<tr>
<td>Mre11</td>
<td>DNA structure-specific endonuclease; 3’ → 5’ exonuclease</td>
<td>Forms a complex with RAD50 and NBS1 involved in DNA damage checkpoint.</td>
</tr>
<tr>
<td>Xrs2</td>
<td>DNA binding</td>
<td>Forms a complex with RAD50 and MRE11 involved in DNA damage checkpoint.</td>
</tr>
<tr>
<td>Rad50</td>
<td>DNA binding and annealing recombination mediator</td>
<td>Member of the RAD50 complex interacts with RAD51.</td>
</tr>
<tr>
<td>Rad52</td>
<td>DNA-dependent ATPase, DNA translocase, DNA helicase</td>
<td>Mediates the process of HR.</td>
</tr>
</tbody>
</table>

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

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**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


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)

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

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

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

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 to 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)


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