DNA Repair and Checkpoints

Cellular and Molecular Biology of Cancer
PATH G4500-001

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Goals

1. The link between DNA repair and Cancer biology
2. DNA repair pathways
3. DNA damage responses
4. Cell cycle checkpoints and cell death

DNA replication
Chromosome Segregation

5. Assays for DNA repair function and genomic instability

* Some key references for additional reading are included
Costmic: https://cancer.sanger.ac.uk/cosmic
Cbioportal: https://www.cbioportal.org/
UCSC genome browser: https://genome.ucsc.edu/

Functional information about Your Favorite Gene (YFG)
Uniprot: https://www.uniprot.org/
Overview

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways
4. DNA damage responses
5. Cell Cycle Checkpoints
6. Special cases
   1. Development DNA breaks - Lymphocyte, Meiosis, neuronal function etc.
   2. Oncogene – how do they induce instability
   3. Telomere, mitochondria and rDNA clusters
   4. Target DNA repair for cancer therapy
1.1 Cancer is a “genetic” disease

- Normal
- Pre-Cancer
- Cancer
- Residual
- Relapse

Oncogene ↑, Tumor Suppressor ↓, Treatment → Acquire Resistant mutations/"chromosomal changes"

"Replication Stress" → "DNA damages"

Bartkova et al. 2005 Nature 434, 864-870
1.2 Type of DNA lesions

Aneuploidy
Amplification
Translocation-(NOT) trans-splicing
Deletion (small or big)
Base substitution

Cytogenetics

1960’ ~
CGH
1990’ ~

Exon or WG seq
small

1990’ ~
RNA-seq

2000’s ~

## 1.2. Tissue type specificity

### Translocations (inter and intra)

**Trisome chr 21**  |  **Down Syndrome**
--- | ---
\(t(8;14)\)  |  c-Myc; IgH  
\(t(11;14)\)  |  CyclinD1; IgH  
\(t(14;18)\)  |  IGH; BCL2  
\(t(3;14)\)  |  BCL6; IgH  
\(t(1;14)\)  |  TAL1; TCRα/δ  

|  |  
|---|---
Burkitt’s lymphomas  |  Mantle cell lymphomas  
Follicular Lymphomas  |  Diffuse Large B cell lymphomas  
T-ALL  |  

---

**t(11;22)(q24;q11.2)**  |  EWS:FLI  
**t(21;21)(q22;q22)**  |  TMPRSS2:ERG  
**t(4;4)(p16;p16)**  |  FGFR; TACC  

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### Substitutions and indels

**Somatic mutations per case from WES**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Mutations per Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulloblastoma</td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
</tr>
</tbody>
</table>

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**UV - Signature 7**

CC>TT on untranscribed DNA strand.

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

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**Patterns of somatic mutation in human cancer genomes**


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

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

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

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

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

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

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

Sanger Signature Platform


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

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

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

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

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

Micro tubulin/mitotic blocker
• Vinca alkaloids: Vincristine, Vinblastine and Vinorelbine.
• Taxanes: Paclitaxel and Docetaxel.
• Antimicrotubule agent: Estramustine

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

Topo II inhibitors increase therapy induced AML
Overview

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3. DNA repair pathways
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   1. Development DNA breaks - Lymphocyte, Meiosis, neuronal function etc.
   2. Oncogene – how do they induce instability
   3. Telomere, mitochondria and rDNA clusters
   4. Target DNA repair for cancer therapy
2. Types of DNA Damages

- Replication Errors
  - Mismatch
- Mismatch Repair (MMS)
- Base modifications
  - Base Akylation
  - Base hydrolysis
  - Abasic site
  - Base Oxidation
- Oxygen radicals
  - Hydrolysis
  - Alkylation agents
- Base Excision Repair (BER)
- UV light, chemical agents
  - Thymidine Dimer
  - Cross Link
- Nucleotide Excision Repair (NER)
- Topo I inhibitors etc

- Repair intermediates
  - Single Strand breaks (nicks)
  - The Last PART of BER/NER
- Topo II inhibitors
  - Ionizing Radiation
  - X-ray

- Non-homologous end-joining (NHEJ)
- Homologous Recombination (HR)
- Development
- Ionizing Radiation
- X-ray
2.1 DNA Damage is very common

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

<table>
<thead>
<tr>
<th>Endogenous DNA Damage</th>
<th>DNA Lesions Generated</th>
<th>Number Lesions/Cell/Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depurination</td>
<td>AP site</td>
<td>10000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cytosine deamination</td>
<td>Base transition</td>
<td>100–500&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAM-induced methylation</td>
<td>3meA</td>
<td>600&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7meG</td>
<td>4000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>O&lt;sup&gt;6&lt;/sup&gt;meG</td>
<td>10–30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxidation</td>
<td>8oxoG</td>
<td>400–1500&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exogenous DNA Damage</th>
<th>Dose Exposure (mSv)</th>
<th>DNA Lesions Generated</th>
<th>Estimated Number Lesions/Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak hr sunlight</td>
<td>—</td>
<td>Pyrimidine dimers, (6–4) photoproducts</td>
<td>100,000/day&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cigarette smoke</td>
<td>—</td>
<td>aromatic DNA adducts</td>
<td>45–1029&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chest X-rays</td>
<td>0.02&lt;sup&gt;e,g,h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.0008&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dental X-rays</td>
<td>0.005&lt;sup&gt;e,g,h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.0002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mammography</td>
<td>0.4&lt;sup&gt;e,g,h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.016&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body CT</td>
<td>7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.28&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Head CT</td>
<td>2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.08&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coronary angioplasty</td>
<td>22&lt;sup&gt;h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.88&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tumor PET scan (&lt;sup&gt;19&lt;/sup&gt;F)</td>
<td>10&lt;sup&gt;h&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.4&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;sup&gt;131&lt;/sup&gt;I treatment</td>
<td>70–150&lt;sup&gt;j&lt;/sup&gt;</td>
<td>DSBs</td>
<td>2.8–6&lt;sup&gt;i&lt;/sup&gt;</td>
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<tr>
<td>External beam therapy</td>
<td>1800–2000&lt;sup&gt;k&lt;/sup&gt;</td>
<td>DSBs</td>
<td>72–80</td>
</tr>
<tr>
<td>Airline travel</td>
<td>0.005/hr&lt;sup&gt;f&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.0002/hr&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Space mission (60 days)</td>
<td>50&lt;sup&gt;k&lt;/sup&gt;</td>
<td>DSBs</td>
<td>2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chernobyl accident</td>
<td>300&lt;sup&gt;j&lt;/sup&gt;</td>
<td>DSBs</td>
<td>12&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hiroshima and Nagasaki atomic bombs</td>
<td>5–4000&lt;sup&gt;k&lt;/sup&gt;</td>
<td>DSBs</td>
<td>0.2–160&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
2.2 Replication Errors

- Up to 100,000 DNA replication origins are available per human cells. Among them, ~30-50,000 are activated in each cell.

- DNA replication requires, clean DNA template, sufficient nucleotide stock, “healthy” polymerase status and proper processing of difficult regions (telomere, centromere, rDNA..etc)

- Oncogene expression could active DNA replication prematurely, and increases conflicts between transcription and replication.
2.3 Polymerase by Numbers

<table>
<thead>
<tr>
<th></th>
<th>Mut/bp (replication)</th>
<th>Genome size (bp)</th>
<th>Mut/genome (replication)</th>
<th>Mut/Generation (Germline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>10^{-10}</td>
<td>3.3x10^9</td>
<td>~0.2-1</td>
<td>1-4x10^{-8}</td>
</tr>
<tr>
<td>Mouse</td>
<td>10^{-10}</td>
<td>2.8x10^9</td>
<td>~0.5</td>
<td>1x10^{-8}</td>
</tr>
<tr>
<td>Yeast</td>
<td>10^{-9}~10^{-10}</td>
<td>1.3x10^7</td>
<td>3x10^{-3}</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>10^{-9}~10^{-10}</td>
<td>5.0x10^6</td>
<td>5x10^{-3}~4</td>
<td></td>
</tr>
<tr>
<td>Virus*</td>
<td>10^{-3/4}~10^{-5/6}~10^{-7/8}</td>
<td>10^{0/1}~10^{-1/2}~10^{-3}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mito</td>
<td>~10^{-7}</td>
<td>1.7x10^4</td>
<td>0.5</td>
<td>3x10^{-5}/20 yr</td>
</tr>
</tbody>
</table>

* RNA virus has the highest mutation rate, followed by retrovirus and DNA virus (about 10 fold drop each step).

Genomic replication polymerase error rate $10^{-8}$ and the repair pathways fix 99% of the breaks.
Mito Polymerase (polG/γ) has a base substitution rate $~2x10^{-6}$

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

Fun FACTS: How many E Coli in per ml in overnight mini-prep? $~10^9$
2.4. Developmental DNA breaks

**Lymphocytes**

V(D)J Recombination

V
D
J
V
D
J
V
J
D

Class Switch Recombination

VDJ
C
µ
C
δ
C
γ
1
C
γ
3
s
s
s
IgM
VDJ
C
γ
1
IgG1

**Meiosis**

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

**Neuron synapsis**


3. DNA repair pathways
A Brief History of DNA Repair

1930s
1940s
1950s
1960s
1970s
1980s
1990s
2000s

1930s:
- Double helix structure of DNA is discovered (Crick and Watson)
- Recombination in Bacteria

1940s:
- Photo-dependent DNA repair
- Excision Repair in E. coli

1950s:
- Synthesis-dependent strand annealing (SDSA)
- Single strand annealing (SSA)
- Break induced replication (BIR)

1960s:
- Double holiday junction is proposed by Jack Szostak

1970s:
- Cancer predisposing xeroderma pigmentosum was linked to nucleotide excision repair

1980s:
- Gene targeting

1990s:
- Non-homologous end joining is discovered
- Alternative end-joining

2000s:
- Late 90s early 2000s Alternative end-joining

1995:
- ATM is cloned
- DNA damage response is recognized
DNA repair pathways

Base modifications
- Direct Fix
- Excision Repair Pathways
- Replication/Transcription

Single Strand Breaks
- Replication/Transcription

Double Strand Breaks
- Template dependent DNA Synthesis and Gap Filling (Lig1&Lig3)

- Non-Homologues End Joining (NHEJ)
- Homologous Recombination (HR)
- Alternative End Joining (A-EJ)/ Micro-homology Mediated End Joining (MMEJ)
- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- Mismatch Repair (MMR)
Mismatch Repair (MMR)

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

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

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

Helicase/regulator/endo: MutL = Mlh1/Pms1 (MutLα), MutLβ, MutLγ

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

Mutations in the human homologues of the Mut proteins affect genomic stability, which can result in microsatellite instability (MI). MI is implicated in most human cancers. Specifically the overwhelming majority of hereditary nonpolyposis colorectal cancers (HNPCC) are attributed to mutations in the genes encoding the MutS and MutL homologues MSH2 and MLH1 respectively, which allows them to be classified as tumour suppressor genes. A subtype of HNPCC is known as Muir-Torre Syndrome (MTS) which is associated with skin tumors.

Nucleotide Excision Repair (NER)

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

NER is primarily responsible to repair Thymidine Dimer formed following UV lesions.

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

In human, there are two kinds of NER pathway that differ at the recognition mechanism- termed as Globe General NER and Transcription Coupled NER.

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

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

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3783044/
Base Excision Repair (BER)

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

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

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

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

Deletion of BER genes increases the mutation rate in a variety of organisms. Somatic mutations in Pol β have been found in 30% of human cancers, and some of these mutations lead to transformation when expressed in mouse cells. Mutations in the DNA glycosylase MYH are also known to increase susceptibility to colon cancer.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4100245/
# Unique role of BER/NER/MMR

<table>
<thead>
<tr>
<th>MMR</th>
<th>BER</th>
<th>NER</th>
<th>RER</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>TC</td>
<td>rNTP</td>
<td></td>
</tr>
<tr>
<td>Lesion</td>
<td>W-C mismatch</td>
<td>Bulk base damage</td>
<td>UV</td>
</tr>
<tr>
<td>Condition</td>
<td>Base mismatch</td>
<td>Specific Glycosylase</td>
<td>Helix distortion</td>
</tr>
<tr>
<td>Committed lesion</td>
<td>Abase sites</td>
<td>XPF/XPG</td>
<td>RnaseH2 (A,B,C)</td>
</tr>
<tr>
<td>Features</td>
<td>Co-replication</td>
<td>nicks</td>
<td>Patch re-syn</td>
</tr>
</tbody>
</table>
# Features of BER/NER/MMR Defects

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

*Aicardi-Goutieres syndrome (AGS)*
DNA repair pathways

- **Base modifications**
  - Direct Fix
  - Excision Repair Pathways

- **Single Strand Breaks**
  - Replication/Transcription

- **Double Strand Breaks**
  - Replication/Transcription
  - Template dependent DNA Synthesis and Gap Filling (Lig1&Lig3)

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

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

HR is conserved in eukaryotes.

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

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

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

NHEJ is partially conserved in eukaryotes and evolved extensively in vertebrates.

Expressed in all cell types and throughout cell cycles.

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

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

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

Mutations in the NHEJ pathway is rare in human cancers.
Alternative End-Joining Pathway (A-EJ) or Micro-homology Mediated End Joining (MMEJ)

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

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

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

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

The canonical Ku-dependent NHEJ pathway CAN join DSBs with short MH (usually <4 nucleotides)!!
## Assays for NHEJ/A-EJ/HR Defects

<table>
<thead>
<tr>
<th></th>
<th>NHEJ</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Junction</td>
<td>Direct or 1-4nt homology</td>
<td>seamless</td>
</tr>
<tr>
<td>Hypersensitive</td>
<td>IR,</td>
<td>IR, CPT, UV, PARPi, crosslink agents</td>
</tr>
<tr>
<td>Accumulate</td>
<td>Chromosome translocations,</td>
<td>Replication defects, chromatid breaks</td>
</tr>
<tr>
<td></td>
<td>chromosome break</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>Lymphomas,</td>
<td>Br, Colon, Pancreatic, Ovarian</td>
</tr>
<tr>
<td>Neuronal</td>
<td>Neuronal apoptosis</td>
<td>Not common</td>
</tr>
<tr>
<td>Immunology</td>
<td>SCID</td>
<td>Not common</td>
</tr>
<tr>
<td>Others</td>
<td>Infertile, often required for</td>
<td></td>
</tr>
<tr>
<td></td>
<td>embryonic development</td>
<td></td>
</tr>
</tbody>
</table>
What to do? Pathway choice?
What to do? Pathway choice?

- **Resection**
  - **NHEJ**
  - **Annealing of MH seqs**
    - **MMEJ (A-EJ)**
      - 1 double SB
        - => 2 Single SBs
      - **HR**
    - **Homology search**
Cross Talks before HR and NHEJ

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

1. DNA damage and Cancer
2. Types and Sources of DNA Damages
3. DNA repair pathways
4. DNA damage responses
5. Cell Cycle Checkpoints
6. Special cases
   1. Development DNA breaks - Lymphocyte, Meiosis, neuronal function etc.
   2. Oncogene – how do they induce instability
   3. Telomere, mitochondria and rDNA clusters
   4. Target DNA repair for cancer therapy
4.1. DNA “Damage” Responses

Base modifications

Excision Repair Pathways

Single Strand Nicks

Replication/Transcription

Double Strand Breaks

Base modification and single strand nicks do NOT directly activate DNA damage responses.
PI3K related kinases (PI3KK)

- Inactivated at the basal level and activated by DNA doubles stand breaks through their specific “sensing proteins”
  - ATM – MRE11/NBS/RAD50 + dsDNA Breaks
  - ART- RPA/ATRIP+ ssDNA
  - DNA-PKcs – KU70/80 + dsDNA Breaks

- Activated ATM/ATR/DNA-PKcs phosphorylate targeted proteins (>800) at conserved SQ or TQ motifs to modulate checkpoints and DNA repair.

- Mutations in
  - ATM - Ataxia -Telangiectasia Syndrome
  - ATR-Seckel Syndrome
  - DNA-PKcs- SCID with neurological defects.

- Only ATM is inactivated in human cancers at significant levels.

- ATR is essential for normal DNA replication and cellular viability.

---


ATR: an essential regulator of genome integrity Nature Reviews Molecular Cell Biology 9, 616-627
DNA Damage Response Pathways

Damage -> Sensor

Sensor

PARP

PI3KKs

Mediators!

Effector

Repair

Check points

Recognizing DNA Damages

- ssDNA ends (nicks) are recognized by PARP1
- Extensive single strand DNA – RPA, SSB
- Single stand double stand DNA junction – 9-1-1 complex
- dsDNA ends (15nt) are recognized by Ku70/80, PARP1
- Extensive dsDNA (>100bp) with an end is recognized by MRN(X)
- Other – structural specific nucleases
- Coupling with transcription (NER) or DNA replication (MMR)
- Base alternations – cause strand distortion during globe NER
Damage Responses

DNA-PKcs
ATM
ATR

p53
Chk2
......

MDC1
S3BP1
NBS
MRE11
Rad50
ATM
ATM
H2AX
H2AX
H2AX
H2AX
H2AX
H2AX

4128
3056
2644

PI3K
PI3K
PI3K
ATM mutations lead to cancer

- Mutations of ATM and its downstream checkpoint components increased the risk for cancer

- Mutation of the repair specific substrates of ATM are not common in cancers.

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

- **G0 Phase**: Cells are quiescent and not cycling.
- **G1 Phase**: Cells prepare for DNA replication.
- **S Phase**: DNA replication occurs.
- **G2 Phase**: Cells grow and prepare for mitosis.
- **M Phase**: Cell division occurs.

**Regulatory Factors**

- **Growth Factors**
- **Rb**
- **E2F**
- **CCRE**

**Cyclin/Cdk Complexes**

- **↑ Cyclin B/Cdk1 (Cdc2)**
- **↑ Cyclin A/Cdk2**
- **↑ Cyclin D1/Cdk4/6**
- **↑ Cyclin E/Cdk2**

**Key Proteins**

- **Wee1**
- **Cdc25**

**Phases of the Cell Cycle**

- **G**: Growth
- **S**: Synthesis
- **G2**: Growth
- **M**: Mitosis
Cell cycle and Checkpoints

**G2/M checkpoint**: licensing for mitotic entry.
Exp: accumulation of G2
fraction, reduce mitotic cells

**Spindle checkpoint**: permit division – all chromosomes are aligned correctly.

**G1/S checkpoint**: Just before entry into S phase, making the key decision of whether the cell should divide, delay division, or enter a resting stage.
Exp: reduce S phase content, reduce DNA synthesis

---

**Intra-S phase “checkpoint”**: Availability of nucleotides, progression of DNA replication
4.1. DNA “Damage” Responses

**Base modifications**

- **Excision Repair Pathways**
- **Single Strand Nicks**
- **Replication/Transcription**

**Double Strand Breaks**

- **Single strand breaks do NOT activate DNA damage response.**
- But **extended single strand DNA will!** (through ATR)

- **Repair**
  - chromatin
  - transcription

**Cell cycle Checkpoints**

**Cell Death**
Aneuploidy – many copies
Spindle Check Point-Aneuploidy

Leaky spindle checkpoint lead to 1) anaphase bridge; 2) increased aneuploidy.

- Activation of Spindle Checkpoint is achieved by Cdc20 that usually prevents cyclin B degradation and keeps securin inactive.

Nature Reviews Cancer 7, 911-924 (December 2007)
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6.1. Lymphocytes Development

Pro B → Pre B → Immature B → Mature B cell

- **Pro B**: D-J<sub>H</sub>
- **Pre B**: V-DJ<sub>H</sub>
- **Immature B**: V-J<sub>κ/λ</sub>
- **Mature B cell**: IgM

- **Antigen Stimulation** by Helper T-cell

- **Somatic Hyper Mutation**

- **Class Switch Recombination**

**Bone Marrow**

- **V(D)J Recombination**
  - **RAG1/2**

**Germinal Center**

- **Class Switch Recombination and Somatic Hypermutation**
  - **IgM**
  - **IgG, IgA, IgE**
  - **Somatic Hyper Mutation**
  - **Class Switch Recombination**
### 6.1.2 Lymphocyte to Lymphoma

#### Pro B
- D-J<sub>H</sub>

#### Pre B
- V-DJ<sub>H</sub>
- V-J<sub>κ</sub>/λ

#### Immature B
- V-J<sub>κ</sub>/λ

#### Mature B cell
- IgM

### Class Switch Recombination
- Non-homologous end joining
- DNA damage response (ATM)
- Mistakes -> Translocations

- IgH (IgL) -cMyc (Burkett's Lymphomas)
- IgH- Bcl2 – Follicular Lymphomas
- IgH- CyclinD1 – Mantle Cell Lymphomas

### Class Switch Recombination
- Non-homologous end joining
- Alternative – end joining
- DNA damage response (ATM)
- Mistakes -> Translocation

- IgH – Bcl6 (DLBCL)

### Somatic Hypermutation
- Mismatch Repair/BER
- Base excision Repair
- Mistakes-> Mutation of other genes

- Myc
- Bcl-6
- .....
6.1.3 Translocation – risk factors

- Breaks!
- Reduced repair fidelity
- Rapid proliferation and/or accumulation of several “oncogenic” events
- Defective “checkpoints”
6.1.4 Translocation –where to go?

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


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6.2. Oncogene induced genomic instability

1. Premature DNA replication - not enough transcription to clear out the inappropriate replication origin

2. Overall increased transcription (e.g. Myc)- more conflicts between transcription and replication.

3. Replication with limited recourse: reduced/unbalanced nucleotide pools. Especially excess ribonucleotides

4. Energy deprivation – lack of Glutamine and Glucose
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6.2.1 Telomere is the end of chromosome

Linearized eukaryotic chromosome present special “end” problems.

• A specialized mechanism of duplication – single replication origin

• To be protected from the cellular machinery that detects and repairs DNA breaks.
Telomere is protected from repair mechanisms by Shelterin complex

How telomeres solve the end-protection problem.

de Lange T.

Shelterin Protect Telomeres

A

Ku70/80
MRN
Loss of TRF2

MRN

t-loop opens

ATM kinase

G2

G1

M

S

Cell cycle arrest (apoptosis/senescence)

B

Loss of POT1

Exposed single-stranded DNA

RPA

NHEJ

Ku70/80

RPA

3'
6.2.2 Mitochondria and ROS

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

---

6.3.2 Mitochondrial DNA Damage

- Mitochondrial DNA (mtDNA) exists in multiple copies, and is tightly associated with a number of proteins to form a complex known as the nucleoid.

- Inside mitochondria, reactive oxygen species (ROS), or free radicals, byproducts of the constant production of adenosine triphosphate (ATP) via oxidative phosphorylation, create a highly oxidative environment that is known to damage mtDNA.

- A critical enzyme in counteracting the toxicity of these species is superoxide dismutase, which is present in both the mitochondria and cytoplasm of eukaryotic cells.

- Recent studies also identified the mitochondrion form of Lig3 as a critical component for mitochondrion DNA repair and survival.


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Genotoxic Drugs used in Cancer Chemotherapy

- **Alkylating agents**: The first class of chemotherapy agents used. These drugs modify the bases of DNA, interfering with DNA replication and transcription and leading to mutations.
- **Intercalating agents**: These drugs wedge themselves into the spaces between the nucleotides in the DNA double helix. They interfere with transcription, replication and induce mutations.
- **Enzyme inhibitors**: These drugs inhibit key enzymes, such as topoisomerase, involved in DNA replication inducing DNA damage.
BRCA2 deficient cells are hypersensitive to PARP inhibitors

6.4.2 New Ways......PARP inhibitors

- There are 17 member in the PARP super family.
- They share a conserved domain that presumably mediated NAD and ATP dependent poly ADP ribosylation activity.
- Only PARP1 and PARP2 activity were shown to be activated by DNA strand breaks – both single and double.
- The activation of PARP I/II contribute to DNA repair by at least three known mechanism
  - PARP I/II directly interacts with XRCCI-Lig3 complex to recruit them to DNA
  - Add PAR to H2A and H2B to physically open up chromatin
  - The PAR chains created at site of DSB could serve as an anchor to recruit DNA repair proteins, including NBS, BARD and others.

Reviewed in a special issue of Mol Cell in June 18, 2015
### 6.4.2 How does PARP “inh” work?

- Prevent PARylation mediated BER increase single stand nicks that are converted to double stand breaks during replication.
- PARP inhibitor traps PARP1 at the site of DNA damage, where it blocks DNA replication and transcription.

  Inhibition ≠ loss of enzyme in DNA repair

<table>
<thead>
<tr>
<th></th>
<th>ATM</th>
<th>DNA-PKcs</th>
<th>ATR</th>
<th>PARP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>Viable</td>
<td>Viable</td>
<td>Lethal</td>
<td>Viable</td>
</tr>
<tr>
<td>Catalytic</td>
<td>Lethal</td>
<td>Lethal</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Inactivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibitors</td>
<td>Withdraw from Phase I due to toxicity</td>
<td>- (not specific)</td>
<td>Well tolerated</td>
<td>FDA approved</td>
</tr>
</tbody>
</table>

- PARP inhibitors block the recycle of NAD+ during replication and DNA damage responses.
- PARPi affects chromatin organization to impair DNA repair

Reviewed in a special issue of Mol Cell in June 18, 2015
6.4.3 Newest Ways......immunotherapy

Mismatch repair deficiency

Neo antigen

DSB repair defects

DNA/RNA fragments

cGAS-STING/ RIG pathway

https://www.nature.com/articles/s41576-019-0151-1
Take home.....

- DNA damage and repair are constant battles in all living cells.
- DNA repair play important roles in the initiation, treatments and therapeutic responses of cancer.
- DNA damage response activates the cell cycle checkpoints.
- Cell cycle checkpoints promote accurate repair of DNA damages.
- Checkpoints also act as the gate keeper to prevent damaged cells from further proliferation.
# DNA repair and DNA damage response defects

## Problems In Responding to DNA Damage Or Stalled Replication Forks

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td><strong>ATM</strong> detects DNA damage &amp; stalled forks</td>
</tr>
<tr>
<td>Seckel syndrome</td>
<td><strong>ATR</strong> detects DNA damage &amp; stalled forks; other checkpoint/replication genes may also be involved</td>
</tr>
<tr>
<td>Li-Fraumeni syndrome</td>
<td><strong>CHK2</strong> and <strong>TP53</strong> respond to DNA damage and stalled forks</td>
</tr>
<tr>
<td>Ataxia telangiectasia-like disorder</td>
<td><strong>MRE11</strong> rescues stalled forks; repairs DNA damage</td>
</tr>
<tr>
<td>Nijmegen breakage syndrome</td>
<td><strong>NBS1</strong> rescues stalled forks; repairs DNA damage</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td><strong>RECO2/BLM</strong> encodes a DNA helicase that rescues stalled forks</td>
</tr>
<tr>
<td>Werner syndrome</td>
<td><strong>RECO3/WRN</strong> encodes a DNA helicase</td>
</tr>
<tr>
<td>Rothmund-Thomson syndrome</td>
<td><strong>RECO4/RTS</strong> encodes a DNA helicase</td>
</tr>
<tr>
<td>Rapapadillo syndrome</td>
<td><strong>RECO4</strong> encodes a DNA helicase</td>
</tr>
</tbody>
</table>

## Problems In Repair Of Damaged DNA

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial breast cancer, Ovarian,</td>
<td><strong>BRCA1</strong> and <strong>BRCA2</strong> repair radiation-induced breaks in double-stranded DNA</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Eleven <strong>FA</strong> genes, one of which (D1) is <strong>BRCA2</strong>, <strong>ICL</strong> repair</td>
</tr>
<tr>
<td>Xeroderma pigmentosum</td>
<td><strong>XPA</strong>, <strong>XPC</strong>, <strong>XPF</strong>, <strong>XPG</strong> repair nucleotide excisions</td>
</tr>
<tr>
<td>Xeroderma pigmentosum variant</td>
<td><strong>XPD</strong> is a DNA helicase</td>
</tr>
<tr>
<td>Cockayne syndrome</td>
<td><strong>POLH/DNA</strong> polymerase-eta carries out trans-lesion DNA synthesis</td>
</tr>
<tr>
<td>XP-Cockayne syndrome</td>
<td><strong>CSA</strong> and <strong>CSB</strong> repair DNA damage</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td><strong>XPD</strong> encodes a DNA helicase</td>
</tr>
<tr>
<td>HNPPC, hereditary non-polyposis colon cancer</td>
<td><strong>XPB</strong> and <strong>XPD</strong> encode DNA helicases</td>
</tr>
<tr>
<td>LIG4 syndrome</td>
<td><strong>LIG4/DNA</strong> ligase IV is required for non-homologous DNA end-joining</td>
</tr>
<tr>
<td>Radiosensitive severe combined immunodeficiency (RS-SCID), Ommen Syndrome</td>
<td><strong>ARTEMIS</strong> encodes a hairpin-specific nuclease that plays a subsidiary role in non-homologous end-joining, and V(D)J recombination.</td>
</tr>
</tbody>
</table>