DNA damage, repair and diseases

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DNA synthesis
Growth
Offspring cells
Parental cell
Mitosis
Cell division
G_0: somatic cells
G_1
G_2
S
DNA synthesis
Prophase
High MPF
Anaphase
Low anaphase inhibitor
Improper spindle formation (M arrest)
Unreplicated DNA (S arrest)
START
S phase
DNA damage (G_2 arrest)
DNA damage (G_1 arrest)
 Genome integrity
2015: The Year of Repair (*Molecular Cell* vol.60)

The Nobel Prize in Chemistry 2015

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar "for mechanistic studies of DNA repair".

![Tomas Lindahl](Photo: Cancer Research UK)

Prize share: 1/3

![Paul Modrich](Photo: K. Wolf/AP Images for HHMI)

Prize share: 1/3

![Aziz Sancar](Photo: M. Englund, UNC-School of Medicine)

Prize share: 1/3

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**Tomas Lindahl**

Dept. Clinical Laboratory Sciences and Medical Biotechnology

National Taiwan University

College of Medicine

Prof. Jing-Jer Lin

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**Paul Modrich**

Department of Biochemistry and Molecular Biology

National Taiwan University

College of Medicine

Prof. Jing-Jer Lin

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**Aziz Sancar**

Department of Biochemistry and Molecular Biology

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Prof. Jing-Jer Lin
DNA damage response and signaling

Endogenous

- Cellular metabolism
- Replication errors
- Mitotic failure

Exogenous

- Ionization radiation
- Chemical damage
- UV light exposure

Cell cycle checkpoint control
Transcription program activation
DNA repair
* Direct reversal
* NER
* MMR
* HR .... etc.

Cell death
* Apoptosis
* Necrosis
.... etc.

Genome instability ➔ ➔ diseases & death
Local structure alteration created by DNA damage
--- potential sources/substrates for detection
DNA damage, corresponding signaling & responses

Sources
- Oxidants
- Radio- and chemotherapy
- Alkylating agents
- Topoisomerase errors
- Replication errors
- UV light
- X-rays
- Alkylating agents
- AID
- Telomere dysfunction

Impacts
- Chromatin alteration
- Bulky adduct
- SSB
- Protein-capped SSB
- Mismatch
- DSB
- Hiden DSB
- STOP

Activation
- Sensors
- Transducers
- Effectors
- Apoptosis
- DNA repair
- Transcription
- SOS

Processing
- exo or endo recognition
- transduction
- amplification
- Impacts
Types of DNA damage and corresponding repairs

Causes

- Oxidants
- Radio- and chemotherapy
- Alkylating agents
- Topoisomerase
- Replication errors
- AID
- UV light
- X-rays
- Telomere dysfunction

Damages

- Chromatin alteration
- Bulky adduct
- SSB
- Protein-capped SSB
- Mismatch
- NER
- BER removal
- SSA
- BER
- HR
- NHEJ
- Ub/proteasome
- DSB
- HR
- NHEJ
- Hiden DSB

Repair pathways

- HR
- NHEJ
In prokaryotic cells
Bacterial DNA damage responses
--- transcription activation and gene expression

**SOS response**

- **Uninduced Cells**
  - LexA Repressor
  - Promoter
  - SOS Gene
  - DNA damage
  - RecA filament
  - LexA synthesis
  - DNA repair

- **Induced Cells**
  - Promoter
  - SOS Gene
  - LexA Repressor

**Adaptive response**

- DNA repair and mutagenesis
  - (1995) Friedberg EC et al

**Oxidative response**

- H$_2$O$_2$ pretreatment
- Low concentrations (μM)
- High concentrations (mM)
- Cross-adaptive responses
- OxyR-dependent, or dependent on de novo protein synthesis
- OxyR-independent, or independent of de novo protein synthesis
- UVA, Menadione, UVC, Cumene hydroperoxide, MNNG

**SOS genes:**
- *uvrA/B/D*
- *recN/F*
- *ftsK, sulA*
The network of SOS response to DNA damage.
Recombination repair proteins in bacteria

<table>
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<tr>
<th>Protein</th>
<th>ORF</th>
<th>Function</th>
<th>Escherichia coli</th>
<th>Neisseria meningitidis</th>
<th>Best e-value</th>
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<td>RecA</td>
<td>CV1607</td>
<td>Coprotease, ATPase-dependent DNA</td>
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<td>RecC</td>
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<td>RecE</td>
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<td>5'-3' dsDNA exonuclease</td>
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<td>Escherichia coli</td>
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<td>RecF</td>
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<td>It assists RecA filamentation</td>
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<td>DNA repair DNA recombination zinc-finger protein</td>
<td>$2e^{-51}$</td>
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<td>RecT</td>
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<td>Recombinase, DNA renaturation enzyme</td>
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<td>SbcD</td>
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<td>dsDNA exonuclease</td>
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<td>-</td>
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Base excision repair (BER)

[Diagram showing the process of BER]

1. Damaged base
2. DNA glycosylase
3. AP site
4. AP endonuclease
5. DNA polymerase I
6. DNA ligase
Nucleotide excision repair (NER)
Mismatch repair (MMR)

- Mismatch
- New Strand
- Template
- MutS, MutL, MutH

Exo VII or RecJ
SSB
Helicase II

DNA polymerase III
DNA ligase

DNA-binding proteins
MutS search clamp (1 s)

MutS sliding clamp (10 min)

b

MutS
ATP
ADP
3 s

MutL

DNA replication
The adaptive response to alkylating agents

Modified from www.mun.ca/.../4103/topics/mutagenesis.html
Induction of the *OxyR* regulon
In eukaryotic cells
Local structure alteration created by DNA damage: --- sources/substrates for damage detection
Local chromatin alteration created by DNA damage: --- detection and repair accessibility

X-Ray Structure Of The Nucleosome Core Particle At 2.5 Å Resolution [mmdb id:13235] (PDB: 1EQZ)

DNA

Nucleosome contains octamer wrapped twice around with DNA

Histone octamer

Isoforms & tails
Histone modification after DNA damage: accessibility & signaling for downstream events

DNA damage ➔ Histone modification

- **H3 K56 acetylation**
- **H4K methylation**
- **H2A & H2AX ubiquitination**

Enhanced P-P interactions
--- specificity

- H2AX phosphorylation
  - ATM, ATR, DNA-PK ➔ Localization of 53BP1, BRCA1, Nbs1, Crb2, SMC1, NuA4, Ino80, Swr1

- H4 Acetylation
  - Esa1, Tip60 ➔ DNA repair gamma-H2AX exchange

- H3-K79 methylation
  - Dot1 ➔ Localization of 53BP1 Rad53 activation

- H4-K20 methylation
  - Set9 ➔ Localization of Crb2 Chk1 activation

Cancer Science 97 (10), 984-989
The general outline of DNA damage response -- signal-transduction pathway in eukaryotic cells
Organization of DNA damage response/pathway in mammalian cells

DNA damage

Replication stress

DNA-PK

ATM + ATR

Rad17

Rad9, Rad1, Hus1

Chk1

Chk2

Mdm2

p53

BRCA1

Nbs1

cAbl

Cdc25

p21

14-3-3σ

Cdk

Cell cycle arrest

Apoptosis

Transcription

DNA repair
Conserved recruitment of ATM, ATR and DNA-PK$_{cs}$ to sites of DNA damage
ssDNA/RPA acts as a ligand for activation of the ATR-CHK1 checkpoint pathway.

lesion processing generates 3' overhang

ssDNA adopts folded conformation

RPA "melts" 2° structure

recruitment of 911 complex/ ATRIP

5' → 3' resection

ssDNA and RPA act as an activation ligand

focus formation: cytological marker

Key kinase molecule for DDR

Claspin?
Formation of DNA damage foci
--- an important cytological DNA damage marker ---

recombination repair*

DNA damage signaling
Watching the DNA repair ensemble dance
Model for recruitment of checkpoint and recombination proteins to a DSB

--- Choreography of checkpoint activation and DNA repair ---
Driving forces:
--- phosphopeptide-binding molecules

**FHA domain**
Rad53 - pThrXXAsp

**BRCT domain**
pS/TQ
BRCA1 - γH2AX (pSXXF)
DNA damage:

--- ubiquitin marks the damaging spot on chromatin

*Initiating sensor complex*

*Ubiquitin-mediated signal transduction complex*

*Late effector complex*

Turning off DNA damage checkpoints

1. Reversal of activating phosphorylations: Protein phosphatases could act to reverse the DNA damage induced phosphorylations.

2. Proteolysis of activated checkpoint proteins: Ubiquitin-dependent proteolysis of activated checkpoint proteins --- phosphorylation/activation acts as a mark for ubiquitination

3. Checkpoint antagonists: Independent pathways could antagonize the cell cycle arrest induced by checkpoint activation.

Modification of the ubiquitin signal: not yet finished off

DeUb Enzyme USP28 in Control of the DNA-Damage Response, Cell 126, 529–542, 2006
TAP-53BP1 IP USP28

USP28

DeUb Enzyme USP28 in Control of the DNA-Damage Response, Cell 126, 529–542, 2006
TAP-53BP1 IP USP28

Molecular Cell
USP4 Auto-Deubiquitylation Promotes Homologous Recombination

DSB induction

DDR factor recruitment

USP4 ubiquitylation & loss interaction

Impaired CtiP-recruitment

DNA-end resection & homologous recombination

Wijnhoven et al., 2015, Molecular Cell 60, 362–373 November 5,
Activation of repair pathway

--- ssDNA and RPA act as a substrate for HR ---

RPA “melts” 2° structure

recruitment of BRCA2/ DSS1

Rad51 polymerization displaces RPA

Rad51 nucleoprotein filament

Strand invasion and homologous pairing

Oncogene (2005) 24, 2871–2876
Double strand break (DSB) repair

Homologous recombination (HR)

Non-homologous end joining (NHEJ)
Mismatch Repair (MMR)
Nucleotide excision repair (NER)
Transcription-coupled repair (TCR)
TFIIH Is a multi-functional protein complex
--- Linking transcription and repair ---
Poly ADP-ribose polymerase-1 (PARP-1) in transcription and repair
DNA damage, genome instability, cancer development and aging

Colorectal cancer as an example

- MLH1, MSH2, MSH6 Mutations
- TGFBR2, JGFIR, BAX (Known growth or apoptosis control genes with MSI in their coding regions)
- APC Gene Mutation
- K-ras Mutation
- 18q Loss (DCC/SMAD4 Mutation)
- 17p Loss (p53 Mutation)
- HNPPCC (Microsatellite Instability in Growth Control Genes (15% of colorectal cancer cases))
- FAP (Chromosomal Instability and Aneuploidy (25% of colorectal cancer cases))

Normal Epithelium → Early Adenoma → Intermediate Adenoma → Late Adenoma → Cancer

Daf-2 (IGF receptor)
- Age-1 (P38 kinase)
- Daf-12 (nuclear hormone receptor)
- Akt (tumor suppressor gene)
- Daf-16 (transcription factor)
- SGK-1 (guanethidine inducible kinase)
- FOXO3a (forkhead transcription factor)

Sirt-2 (histone deacetylase)
- HSF-1 (heat shock factor)
- Ku70 (DNA repair factor)
- Indy (neurochemical substrate transporter) mutant not as efficient

DNA repair
- oxidant neutralization
- DNA repair

Tert (telomerase reverse transcriptase)
- reduction in oxidant production

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Parallel pathways of tumorigenesis

--- Hallmarks of cancer/where we stand in the fight against cancer

A

<table>
<thead>
<tr>
<th>Component</th>
<th>Acquired Capability</th>
<th>Example of Mechanism</th>
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<tbody>
<tr>
<td>Self-sufficiency in growth</td>
<td>Self-sufficiency in growth signals</td>
<td>Activate H-Ras oncogene</td>
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<tr>
<td>signals</td>
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<td></td>
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<tr>
<td>Insensitivity to anti-growth signals</td>
<td>Insensitivity to anti-growth signals</td>
<td>Lose retinoblastoma suppressor</td>
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<tr>
<td>apportionment</td>
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<td>Sustained angiogenesis</td>
<td>Sustained angiogenesis</td>
<td>Produce VEGF inducer</td>
</tr>
<tr>
<td>Tissue invasion &amp; metastasis</td>
<td>Tissue invasion &amp; metastasis</td>
<td>Inactivate E-cadherin</td>
</tr>
<tr>
<td>Limitless replicative potential</td>
<td>Limitless replicative potential</td>
<td>Turn on telomerase</td>
</tr>
</tbody>
</table>

B

Hanahan and Weinberg, Cell (2000) 100:57-70
Emerging hallmarks & enabling characteristics

Cancer arises from a series of genetic alterations that promote resistance to apoptosis, self-sufficiency in growth, cellular immortalization and escape from cell-cycle-exit.

Hanahan and Weinberg, Cell (2011) 144:646-74
Genome instability and Cancer development

Genome instability \(\Rightarrow\) Mutations \(\Rightarrow\) Dysfunctions \(\Rightarrow\) Diseases

On and off \(\Rightarrow\) Mis-regulation \(\Rightarrow\) Genome instability \(\Rightarrow\) Cancer development

\(\Rightarrow\) Cancer Types \(\Rightarrow\) Inter-tumor heterogeneity \(\Rightarrow\) Intra-tumor heterogeneity

\(\Rightarrow\) Many oncopgenic mutations confer a cell-autonomous fitness + micro... h) ???

Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity

Andriy Marusyk, Doris P. Tabassum, Philipp M. Altrock, Vanessa Almendro, Franziska Michor & Kornelia Polyak

ARTICLE  2 OCTOBER 2014 | VOL 514 | NATURE | 55

doi:10.1038/nature13556
Transcription-Coupled Nucleotide Excision Repair Factors Promote R-Loop-Induced Genome Instability

Julie Sollier,1 Caroline Townsend Stork,1 María L. García-Rubio,2 Renee D. Paulsen,1 Andrés Aguilera,2 and Karlene A. Cimprich1,*

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