Cadmium acts as a mutagen by inhibiting mismatch repair. 
(Genotoxicity caused by inhibition of a mutation avoidance system)

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Environmental factors can cause genome instability

- Normal DNA → Error
- Damage
- Error → Repair
- Mutant DNA

Normal DNA → Repair → Mutant DNA
Can hyper-mutability be caused by environmental factor inhibiting repair?
High Risks of Genome Instability

Combination of MMR and proofreading defects cause catastrophic mutability

(Morrison & Sugino; Schaaper)

- lethality (error catastrophe) in haploids
- synergy between mutators
At-Risk Motifs (ARMs) -- long homonucleotide runs

3' → 5' Exo

DNA - Pol

Long homonucleotide runs are hyper-mutable in MMR-deficient cells
Long homonucleotide runs are hyper-mutable in MMR-deficient yeast

<table>
<thead>
<tr>
<th>Homonucleotide run</th>
<th>Mutation rate (x10^-9)</th>
<th>msh2 mutator</th>
</tr>
</thead>
<tbody>
<tr>
<td>A14 (-1 nt)</td>
<td>164</td>
<td>x 9,756</td>
</tr>
<tr>
<td>A5 (+1 nt)</td>
<td>1</td>
<td>x 34</td>
</tr>
<tr>
<td>A8 (+1 nt)</td>
<td>10</td>
<td>x 344</td>
</tr>
<tr>
<td>A12 (+1 nt)</td>
<td>190</td>
<td>x 444</td>
</tr>
</tbody>
</table>

Mutation reporter - frameshifts in lys2-A14

- At-risk motif hyper-mutable in MMR-mutants
- Allows to detect as little as 0.1% unrepaired mismatches
Cadmium is hyper-mutagenic in a yeast long homonucleotide run (lys2-A14)
Cadmium (Cd++) in nature (IARC, Vol. 58, 1993)

112 µg=1µmole

• Natural occurrence: 100-500 µg/kg of the Earth's crust (mainly associated with zinc)

• Air: 0.05-0.5 µg/m³; occupational - mining, battery, paint, metal industries.

• Water: <0.005 µg/L - 405 µg/L

• Soil and plants: <1,000 µg/kg - 800,000 µg/kg

• Cigarette smoke: A smoker can accumulate 500 µg/year.

• Food: 10µg/day to 500 µg/day

• Animal and human tissues: Liver, kidney, prostate (0.1-500 mg/kg).
  (The half-life of cadmium in human kidneys is around 10-20 years.)
Cadmium carcinogenicity and genotoxicity

Carcinogenicity:

• lung cancer and prostate cancer (limited evidence) in humans
• lung, testicular, adrenal, liver, prostate tumors as well as lymphomas in experimental animals

Genotoxicity (mostly from acute short-term treatment):

• chromosomal aberrations in lymphocytes of exposed workers
• chromosomal aberrations and strand breaks in cultured mammalian cells
• gene mutations in cultured mammalian cells (*hppt, gpt*)
• intra-chromosomal recombination in yeast
Multiple Potential Mechanisms of Metal Genotoxicity

- inhibiting repair
- suppressing fidelity
- oxidative damage to DNA
- oxidative damage to proteins
- complexes with DNA
- competing with "physiological" metals for metallothioneins
Cadmium caused hyper-mutability in yeast, unlike other ions tested.

<table>
<thead>
<tr>
<th></th>
<th>H$_2$O</th>
<th>CdCl$_2$</th>
<th>CoCl$_2$</th>
<th>CuSO$_4$</th>
<th>MnCl$_2$</th>
<th>NiSO$_4$</th>
<th>ZnSO$_4$</th>
</tr>
</thead>
</table>

*Mutation reporter - frameshifts in *lys2-A14*

*At-risk motif hyper-mutable in MMR-mutants*
Multiple Potential Mechanisms of Cadmium Genotoxicity

- inhibiting repair
- suppressing fidelity
- oxidative damage to DNA
- oxidative damage to proteins
- complexes with DNA
- competing with "physiological" metals for metallothioneins
Hyper-mutability is not due to general oxidative damage.
**In vitro MMR assay system**

(Umar et al., Cell 1996)
Cadmium inhibits DNA mismatch repair in extract from human cells
Cadmium is hyper-mutagenic (as much as x2,000) to wild type yeast cells.

Cadmium is not mutagenic to yeast cells that are deficient in MMR (msh2).

Interaction between mutator effects of cadmium and MMR-null resembles epistatic interactions between two mutator defects in the same pathway.
How to prove that yeast MMR is \textit{in vivo} target for cadmium?

\textit{Compare wild type yeast grown on cadmium with MMR-deficient yeast mutants.}
High Risks of Genome Instability

Combination of MMR and proofreading defects cause catastrophic mutability

(Morrison & Sugino; Schaaper)

- lethality (error catastrophe) in haploids
- synergy between mutators
Cadmium Causes Synergistic Hyper-mutability Combined with Defects in Proofreading
Cadmium reduces viability of Pol δ Exo-deficient haploid (Note: viability of isogenic diploids is not reduced)

“Synthetic lethality” of cadmium with Pol δ Exo-deficiency can be due to catastrophic rate of recessive lethals.
Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

- MMR deficiency
- Cadmium

Synergy with Proofreading Defects
- hyper-mutability
- synthetic lethality
Cadmium Can Induce Base Substitutions and Frameshifts

Mutations in the yeast CAN1 gene
Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

- **MMR deficiency**
- **Cadmium**

- Synergy with Proofreading Defects
  - hyper-mutability
  - synthetic lethality

- Base substitutions and frameshifts

- yes
At-Risk Motifs (ARMs) -- long homonucleotide runs and other microsatellites are poor substrates for the 3'→5' Exo of DNA Pol

Consequences:

• homonucleotide runs are hyper-mutable in MMR-deficient cells

• mutation rate depends on the size of run and type of frameshift
- homonucleotide runs are hyper-mutable in MMR-deficient cells
- mutation rate depends on the size of run and type of frameshift

<table>
<thead>
<tr>
<th>Homonucleotide run (frameshift in run)</th>
<th>Mutation rate (x10^-9)</th>
<th>Mutator effect of the msh2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>msh2</td>
</tr>
<tr>
<td>A4 (-1 nt)</td>
<td>0.4</td>
<td>31</td>
</tr>
<tr>
<td>A7 (-1 nt)</td>
<td>4</td>
<td>1,550</td>
</tr>
<tr>
<td>A10 (-1 nt)</td>
<td>47</td>
<td>314,000</td>
</tr>
<tr>
<td>A14 (-1 nt)</td>
<td>164</td>
<td>1,600,000</td>
</tr>
<tr>
<td>A5 (+1 nt)</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td>A8 (+1 nt)</td>
<td>10</td>
<td>3,440</td>
</tr>
<tr>
<td>A12 (+1 nt)</td>
<td>190</td>
<td>84,000</td>
</tr>
</tbody>
</table>
Size of a run and a type of frameshift:
Relative mutability of runs mimics MMR-deficiency

A14, -1 nt mutation
A10, -1 nt mutation
A12, +1 nt mutation
Relative mutability of G-, C-, T-, and A- runs mimics MMR-deficiency

mutation rates in MMR-null (msh2) mutants (from Harfe and Jinks-Robertson, 2000)
Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

**MMR deficiency**

- Synergy with Proofreading Defects
  - hyper-mutability
  - synthetic lethality

- Base substitutions and frameshifts

- Mutator signature in homonucleotide runs:
  - type of frameshift
  - size of a run
  - sequence of a run

**Cadmium**

- yes

- yes

- yes
Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

MMR deficiency = Cadmium

Wild type yeast grown on cadmium behave as if they are deficient in mismatch repair.
Hyper-mutability is caused by cadmium via inhibiting post-replication mismatch repair (MMR)

- Very low (micromolar) concentrations of Cd$^{2+}$ ions, similar to those present in the environment and accumulated in organisms, inhibit MMR in yeast and human cell extract leaving about 20-50% mismatches unrepaired.

- Cadmium is a new kind of mutagen that causes hyper-mutability by inhibiting mutation avoidance DNA repair system, rather than by damaging DNA.

Specific notes of relevance:
- Cadmium is common in the environment.
- MMR system prevents mutations and cancer.
What is the target for cadmium inside eukaryotic MMR?

Mismatch recognition

Mismatch removal and gap filling

Exo1; Pol δ; Pol ε

MutL homologs

MutS homologs

G

T

1 nt

≥2 nt

MutL homologs

MutS homologs

Mlh1

Pms1

Mlh1

Mlh3

Msh2

Msh6

Msh2

Msh3

PCNA; RPA; RFC
OTHER QUESTIONS:

- Effect of cadmium on MMR in other species
  (pathogenic fungi, adaptive changes, evolution)

- Effect of cadmium on other functions of MMR proteins
  (meiosis, recombination, apoptosis, damage recognition,
tolerance to alkylation damage)

- Effect of cadmium on mammalian MMR
  (species, cell types, tissues, genotypes, relation to carcinogenesis).

- Other environmental factors and drugs that could inhibit MMR

- Mutagenesis caused by inhibiting other repair and fidelity systems
  with environmental factors and drugs
Mutagenesis caused by inhibiting repair and fidelity systems by environmental factors and drugs

Where to look for targets?

- Identify sensitized motifs in protein structures
- Identify repair components present at the "threshold" level
Identify repair components present at the "threshold" level

- at-risk?

- 100% repair

fold-excess over amount required for 100% repair

Repaid fraction of mismatches at different concentrations of cadmium.

(~40% mismatches were not repaired in wild type at 5 \( \mu M \) CdCl\(_2\))

Repaired fraction of mismatches

\( \mu M \) CdCl\(_2\)

Rx (fraction repaired)
Cadmium acts as a mutagen by inhibiting mismatch repair.
(Genotoxicity caused by inhibition of a mutation avoidance system)

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