Crystallisation and structure determination of mismatch specific uracil-DNA glycosylase (MUG) from *Deinococcus radiodurans*

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and

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Deinococcus radiodurans

- Radiation and desiccation resistant soil bacterium
- Withstands 5000-30000 grays of ionising radiation while most other organisms cannot survive doses above 50 grays
- Mechanisms of radiation resistance is not known
- Structural genomics project
Structural genomics of *D. radiodurans*

- Select protein targets with potential involvement in radiation resistance mechanism
- Increased number of specific protein families related to stress response and damage control is observed
- Proteins involved in DNA repair
- Five different uracil-DNA glycosylases identified
  - Uracil-DNA N-glycosylase (UNG) - drUNG
  - Mismatch specific uracil-DNA glycosylase (MUG) - drMUG
  - Family 4 UDG – dr1751
  - Hypothetic UDG – dr0022
  - Hypothetic UDG – dr1663
<table>
<thead>
<tr>
<th>Target</th>
<th>Cloned</th>
<th>Expressed (soluble)</th>
<th>Purified</th>
<th>Crystallisation</th>
<th>Crystals</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>drUNG</td>
<td>Yes</td>
<td>BL21(DE3) pLysS</td>
<td>Ni column</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>drMUG</td>
<td>Yes</td>
<td>BL21(DE3) pLysS</td>
<td>Ni Column</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>DR1751</td>
<td>Yes</td>
<td>BL21star</td>
<td>Ni column (presipitates)</td>
<td>Yes</td>
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<td></td>
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<tr>
<td>DR0022</td>
<td>Yes</td>
<td>BL21(DE3) pLysS</td>
<td>Ni column</td>
<td>Yes</td>
<td>Yes (small)</td>
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<tr>
<td>DR1663</td>
<td>Yes</td>
<td>BL21(DE3)</td>
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</table>
Mismatch-specific uracil-DNA glycosylase (MUG)

- Belongs to the uracil-DNA glycosylase (UDG) family in the base-excision repair pathway (BER)
- MUG are mismatch specific and is suggested to recognize the guanine on the complementary strand rather than uracil
- Specificity for G:U and G:T (weak) mismatches
- Ethenocytosine

Structure of *E. coli* MUG
(Barrett et al., 1998)
drMUG crystals

- Crystals were grown by mixing 1 µl drops of 10 mg/ml protein with 1 µl of a solution containing 0.2 M Sodium Acetate trihydrate, 0.1 M Sodium Cacodylate at pH 6.5 and 30% w/v Polyethylene Glycol (Peg) 8000
- The crystallisation reaction were equilibrated at 18°C
- Hexagonal crystals, suitable for data collection purposes appeared after one day
- The crystals had overall dimensions of about 80x80x80 µm³
drMUG structure

- 1.75 Å resolution by molecular replacement
- 25% identity and 40% similarity to *E.coli* MUG
- Consists of a 5-stranded β-sheet flanked on both sides by 6 α-helices with the general β-α-β topology
- Acetate in the active site as a result of crystallisation conditions
drMUG sequence
**drMUG activity**

<table>
<thead>
<tr>
<th>DNA-repair enzyme</th>
<th>Mean cpm value</th>
<th>Dilution</th>
<th>Protein conc. (mg/ml)</th>
<th>Specific activity (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>drMUG</td>
<td>1,160</td>
<td>1,000</td>
<td>3.5</td>
<td>10</td>
</tr>
<tr>
<td>drUNG</td>
<td>3,600</td>
<td>1x10^6</td>
<td>6</td>
<td>26,000</td>
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<tr>
<td>drMUGD93A</td>
<td>ND^2</td>
<td>0</td>
<td>43</td>
<td>0</td>
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</tbody>
</table>

**Diagram**

- ecMUG
- drMUG
- drMUGD93A
### drMUG specificity

<table>
<thead>
<tr>
<th>DNA substrate</th>
<th>ecMUG</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
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</tbody>
</table>

![Image of gel showing drMUG specificity]

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### drMUG

<table>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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</tbody>
</table>

![Image of gel showing drMUG]

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### drMUGD93A

<table>
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<tbody>
<tr>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

![Image of gel showing drMUGD93A]

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**Legend:**
- drMUG: DNA methyltransferase using adenosine as a donor in DNA synthesis.
- ecMUG: DNA methyltransferase using cytosine as a donor in DNA synthesis.
- ssU: Single-stranded uracil.
Conclusions

• The 3D-structure of the mismatch-specific uracil-DNA glycosylase (MUG) from *Deinococcus radiodurans* is overall the same as of *E.coli* MUG, however it possesses a unique catalytic residue (D93) and possess a broader substrate specificity – to increase the DNA repair repertoire of *Deinococcus radiodurans*?

• Work in progress to identify biological relevant substrate and look into the reaction mechanism

Why determine structures of proteins with already known structures?
New structures might reveal new information about the biological significance of the proteins
Acknowledgements

• NorStruct (University of Tomsø)
  – Arne Smalås
  – Nils Peder Willassen

• ESRF
  – Sean McSweeney
  – Ingar Leiros
  – All MX group people in the lab