Effect of Different CRISPR/Cas9 Variants on Repair Pathway Choice

Cecilia Cotta-Ramusino
Cas9 Stimulates the Endogenous Repair Pathways

WT Cas9 → DSB

C-NHEJ
- Locus Unaltered
- Small Deletions
- Small Insertions

Alt-NHEJ
- Blunt EJ: Deletions
- MMEJ: Deletions
- SD-MMEJ: Insertions

HDR
- SSA: Large Deletions
- HR: Correction

Recession 3’
Cas9 is a Flexible Tool

WT Cas9

N863A Nickases

D10A Nickases

5’ Overhang

Blunt

3’ Overhang

5’ Overhang

• Could we engage different pathways by using these different variants?

• Could we selectively stimulate HDR?
Sickle Cell Disease: Editing of the HBB locus
DSBs Generated by D10A are Predominantly Repaired by HDR

- Long insertions
- Repetitions of the overhang
- Microhomology mediated

Bothmer et al., Nat Comm 2017
DSBs Generated by D10A are Predominantly Repaired by HDR

- HDR
- Insertions
- Deletions

Modification (%)

WT

N863A

D10A

3'

5'
DSBs Generated by D10A are Predominantly Repaired by HDR

Bothmer et al., Nat Comm 2017
Do Gene Conversion and Gene Correction have the same Genetic Requirement?

Gene Correction

ssODN

Gene Conversion

HBB → HBD

Do they both dependent on the HR pathway?
Gene Conversion and Gene Correction have Different Genetic Requirements

Gene Correction

<table>
<thead>
<tr>
<th></th>
<th>Neg. Cont.</th>
<th>K.D. Rad51</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS ODN donor</td>
<td>10 ± 2</td>
<td>20 ± 3</td>
</tr>
</tbody>
</table>

 Gene Conversion

<table>
<thead>
<tr>
<th></th>
<th>Neg. Cont.</th>
<th>K.D. Rad51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous HBD</td>
<td>30 ± 4</td>
<td>30 ± 5</td>
</tr>
</tbody>
</table>
Gene Conversion and Gene Correction have Different Genetic Requirements

HR is required for repair from double stranded donors (endogenous homology tracks or plasmids) but not single stranded donors.
3’ Overhang is Required to Promote Gene Conversion and Gene Correction

Gene Conversion

Gene Correction

BRCA2 Rad51

HR

ss-ODN SST-R

Alt-NHEJ
Rad52 has a Role in Promoting SST-R

K.O. of Rad52

Overexpression of Rad52

Fold change in Gene Correction

K.O. of Rad52

Overexpression of Rad52

Anne Bothmer Poster 1019
Conclusions from the Dual Nick Analysis

- Different ends activate different DNA repair pathways

- Different donors stimulate different pathways

Gene Correction mediated by ssODN is not HR dependent but partially depends on Rad52

Bothmer et al., Nat Comm 2017
Characterization of the DNA Repair Pathway in Response to Single Nick
D10A Nick Results in More Frequent HDR Than N863A

- Gene Conversion
- Gene Correction
- Insertions
- Deletions
D10A but Not N863A Nick Repair Depends on HR Pathway

Gene Conversion
Gene Correction
Insertions
Deletions

% of Modifications

siRNA  N.C. Rad51

D10A
D10A but Not N863A Nick Repair Depends on HR Pathway

The diagram shows the percentage of modifications in the gene correction process. The siRNA treatment with N.C. Rad51 results in higher gene conversion and deletion rates compared to N863A. The gene correction rate is significantly lower in both conditions compared to insertions.
Working Model

D10A

Nick “Protected”
Removal of the Cas9/gRNA in S phase

Nick → DSB

c-NHEJ  
a-NHEJ  
HDR  
BRAC2 Rad51  
SST-R  
HR

N863A

Nick exposed

More easily repaired
• Single Nick Repair
• Simple Ligation
Working Model

**D10A**

Nick “Protected”

Removal of the Cas9/gRNA in S phase

Nick → DSB

- c-NHEJ
- a-NHEJ
- HDR
- SST-R
- HR

**N863A**

Nick exposed

More easily repaired
- Single Nick Repair
- Simple Ligation
Overall Conclusions

• Different lesions activate different repair pathways

• Different donors activate different repair pathways

• Understanding the differential pathway engagement allows for a deterministic approach in designing research and therapeutic genome engineering strategies
Thank you!