Nonhomologous end-joining (NHEJ)

Lessons from yeast

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Department of Pathology
University of Michigan
# Contributors

<table>
<thead>
<tr>
<th>NHEJ screens/ Two-hybrid</th>
<th>Tom Wilson laboratory</th>
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<tbody>
<tr>
<td></td>
<td>Elissa Karathanasis</td>
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<td>Phil Palmbos</td>
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<td>Leana Topper</td>
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<tr>
<th>3’ phosphatase</th>
<th>University of Montreal</th>
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<td>John Vance</td>
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<td>Rajashree Deshpande</td>
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<td>Aswathi Nair</td>
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<td>Dindial Ramotar</td>
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<td>Arshad Jilani</td>
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<th>Pol4/ Processed NHEJ</th>
<th>University of Sussex</th>
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<td>Jim Daley</td>
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<td>Renee Vander Laan</td>
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<td>Aidan Doherty</td>
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<td>Louise Tonkin</td>
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<td>Robert Pitcher</td>
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Cancer: a disease of DNA

D. Ferguson and F. Alt
Homologous recombination
Base excision repair

Nonhomologous end-joining

nuclease \ lyase  
polymerase  
ligase

Ape, Fen
Pol \( \beta \)
Lig3

PARP
XRCC1
PCNA

DNA-PK
XRCC4
Ku
Themes / conclusions

NHEJ evolved to repair DSBs when homologous recombination is disadvantageous.

NHEJ proteins act by re-annealing and joining short overhangs.

NHEJ yields efficient and accurate repair of DSBs.

End processing activities exist to deal with terminal damage - incompatible ends are a secondary phenomenon.

There is an inherent order to the NHEJ process.
What is a DSB?
Simple overhangs

Restriction enzymes
Rare damage-induced DSBs

“Simple Religation”
“Precise NHEJ” (a.k.a. “accurate”)

Result = genome restoration
The “suicide deletion” assay

I-SceI cleavage site

\( ADE2 \)

\( GAL1 \)

28 bp direct repeat

\( ADE2 \)

coding

\( URA3 \)

\( XV \)

\[ \text{SSA} = ade2 = \text{red (}\sim 50\%\text{)} \]

or

\[ \text{NHEJ} = ADE2 = \text{white (}\sim 5\%\text{)} \]
Suicide deletion: genome-wide screen

- 1,514 inviable or unavailable
- 3788 neg.
- 257 untestable
- 206 repeat failures
- 1,977 failures/untestable
- 4,182 screen neg.
- 4781 MATa mutants
- 993 pos. + failures
- 736 retested
- 394 neg.
- 136 screen pos.

Normalized NHEJ / SSA ratio

- Structural/catalytic rad52, srs2
- SSA ↓
- bud31, msn5
- Regulatory? nej1, ubi4, doa1, yaf9, fyv6, htx1, mck1, ard1, etc.
- NHEJ ↓
- Structural/catalytic yku70, yku80, dnl4, lif1, rad50, mre11, xrs2
Cell cycle-dependent stimulators of NHEJ efficiency

- **Doa1**: ubiquitin degradation
- **Mck1**: GSK-3 family kinase
- **Fyv6**: uncharacterized
- **Nej1**: Lif1 binding protein

1. NHEJ is stimulated in haploid cells.
2. NHEJ is stimulated in nutritionally deprived cells.
Yeast NHEJ varies as a function of the cell cycle

- **Doa1**: involved in ubiquitin-mediated degradation
- **Mck1**: GSK-3 family protein kinase
- **Fyv6**: largely uncharacterized
Suicide deletion: genome-wide screen

1,514 inviable or unavailable
~6,295 mutants
4781 MATa mutants
3788 neg.
993 pos. + failures
257 untestable
736 retested
206 repeat failures
394 neg.
1,977 failures/untestable
4,182 screen neg.
136 screen pos.

Normalized NHEJ / SSA ratio

log (ratiomut / ratiowt)

 SSA ↓

NHEJ ↓

Ku
Mre11/Rad50 complex
DNA ligase IV

Structural/catalytic
rad52, srs2

bud31, msn5

Regulatory?
nej1, ubi4
doa1, yaf9
fyv6, htt1
mck1, ardi
etc.

Structural/catalytic
yku70, yku80, dni4, lif1
rad50, mre11, xrs2
The NHEJ puzzle
Yku80

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<th>β-barrel core</th>
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<td>217</td>
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<tr>
<td>1</td>
<td>265</td>
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<tr>
<td>1</td>
<td>539</td>
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<td>577</td>
</tr>
<tr>
<td>1</td>
<td>217</td>
<td>629</td>
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Systematic NHEJ 2-hybrid analysis

Glu-His, 3 day

Glu-Ade, 5 day
NHEJ – our current view
What is a DSB?
Simple overhangs

Restriction enzymes
Rare damage-induced DSBs

“Simple Religation”
“Precise NHEJ” (a.k.a “accurate”)

Result = genome restoration
Oligonucleotide Modified Plasmid Assay (OMP)

Reconstitution of ADE2 requires:
1) Ligation of both oligonucleotide pairs
2) Repair by NHEJ
DSB rejoining as a function of overhang length

3' overhang

5' overhang
DSB rejoining at long overhangs is NHEJ independent but Rad52 dependent
NHEJ as a function of overhang GC content

# of GCs: 1       2       3
in 4 nt overhang

-AAAACTA-
TTTGAT-

-AGACTA-
TCTGAT-

-AGACGA-
TCTGCT-

(Ade⁺/Leu⁺)
DSB rejoining as a function of overhang length: summary

- 0-4 Base Overhangs
  - Ku
  - Dnl4

- >6 Base Overhangs
  - Cdc9

- >10 Base Overhangs
  - Cdc9

- Rad52

- Nonproductive Annealing
What is a DSB?
Overhang misalignment

Mispairing of compatible overhangs
“Processed NHEJ”
“Imprecise NHEJ”
Result = frameshift mutation
NHEJ is fundamentally accurate (but not quite)  
- a “worst case” scenario

<table>
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<th>join</th>
<th>inferred alignment</th>
<th>wild type</th>
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<tr>
<td>M(+2)</td>
<td>AA _CGCGTT _TTGCGC _AA</td>
<td>0.19 %</td>
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<tr>
<td>M(0)</td>
<td>AACCGCGTT _TTGCGCAA</td>
<td>1.1 %</td>
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>5-fold bias
What is a DSB?
Incompatible overhangs

Two incorrectly paired DSBs (Single resected DSB)

“Processed NHEJ”
NOT “Imprecise NHEJ”!

Result = Rearrangement
Rejoining of long overhangs is very precise

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<th>bp</th>
<th>Accuracy</th>
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<tr>
<td>4</td>
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<tr>
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<td>0</td>
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What is a DSB?
Missing and damaged terminal nucleotides

Typical damage-induced DSB
AP endonucleases, lyase etc.

“Processed NHEJ”
“Precise NHEJ”

Result = genome restoration
NOT mutagenic!
The PolX Family of DNA Polymerases

- yPOL4
- hPolβ
- hPolλ
- hPolμ
- hTdT

- BRCT
- HhH (dRP lyase)
- 8 kDa
- Polymerase
- Fingers
- Palm
- Thumb
Pol4 mediates a subset of processed NHEJ events

Pol4:
PolX family polymerase
homology to hPol \( \lambda > \mu > \beta \)
Modular Evolution of Polynucleotide Kinase Domains

- Orange = 3’ phosphatase
- Green = 5’ kinase

Human, S. pombe, etc.

Arabidopsis
- homology to P65P, U1G3

S. cerevisiae
- homology to YOR238W

Dictyostelium
- tRNA ligase

AcNPV
- homology to tRNA ligase

T4
Tpp1 is a robust and specific 3’ phosphatase

Yeast (and most organisms) have 2 AP endos:

Apn1 (Endo IV-like)
Apn2 (Exo III-like)
Tpp1 is not required for NHEJ at 3’ phosphates
M. tuberculosis NHEJ operon

Mt-Ku  
Rv0937c

Mt-Lig  
Rv0938  
LigD

Polymerase  Nuclease  Ligase

*  
D137A  
H373A  
K481A

homodimeric Ku  multifunctional polypeptide
Reconstitution of simple religation NHEJ
Ku and ligase are species-specific partners
Mtb NHEJ is very precise
HA! WE'RE THE SUPER EFFECTIVE TEAM AROUND HERE! YO U CAN DO EVERYTHING IT TAKES YOUR WHOLE EUKARYOTIC REPAIR TEAM TO DO!

RUBBISH! WE'RE AN INTEGRATED, HIGHLY SPECIALISED, SKILLED TEAM, EVOLVED AND STREAM-LINED FOR MAXIMAL EFFICIENCY...

GARBAGE! I'VE NEVER SEEN SUCH FlagRANT OVER-MANNING! YOUR SO-CALLED 'TEAM' IS JUST A BUNCH OF WARY, SOFT SPINSTERS...

SHUT IT, BEAN-BRAIN! YOUR SYSTEM IS DANGEROUSLY SHORT-STAFFED! WHERE'S THE COVER IF ANYTHING GOES WRONG?

DON'T YOU LECTURE ME ABOUT STAFFING LEVELS, YOU WEASLING, ARROGANT...

...STUPID...

IGNORANT...

A!? DO!

TODAY!
NON-HOMOLOGOUS END-JOINING
DOUBLE STRAND BREAK REPAIR COMPLEXES.
UNION

WELCOME 2003
TRades UNION
CONGRESS

NHEJ UNION
JOINT NEGOTIATING
DELEGATES

Drawn for Tabs de Tab