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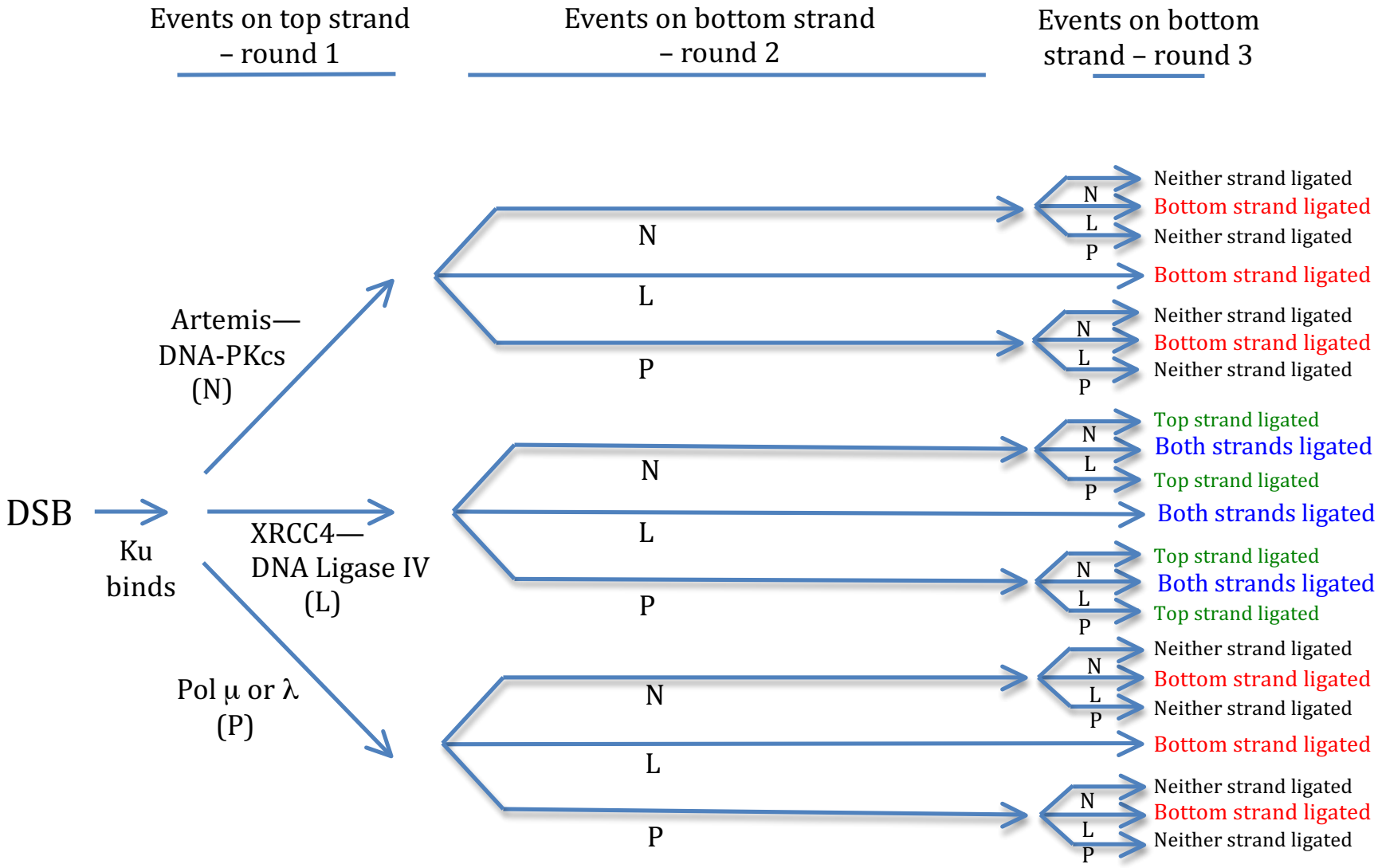

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**Supplemental Figure 2. The same DSB can be repaired in many different ways, depending on the order in which NHEJ proteins act.** This figure conveys the iterative nature of NHEJ and also illustrates that many different product junctions can form from the same starting DSB. Three rounds of enzymatic action are depicted (rounds 1, 2 and 3). More rounds of action would be needed for many of the pathways. After Ku binding to either DNA end or both ends of a double-strand break (DSB), the nuclease (N), polymerases (P), or the ligase complex (L) can bind in any order. In the simplest case of this illustration, the ligase IV complex would ligate the top strand, and then ligate the bottom strand, resulting in a fully repaired DSB site. This might occur at a DSB with two blunt DNA ends or two DNA ends with compatible overhangs. More typically, the nuclease or polymerases bind and modify either or both DNA ends. All of these pathways can eventually lead to a repaired DSB site, but yielding different amounts of nucleotide loss or addition. Therefore, all of the products shown on the right side are different from one other, even though the starting two DNA ends are the same. In other words, identical starting DNA ends give a variety of different possible products, depending on the order in which the enzymes act.

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**Supplemental Table 1. Abundance of key proteins involved in nonhomologous DNA end joining (NHEJ), alternative end joining (a-EJ), single-strand annealing (SSA) and homologous recombination (HR).** Values have been compiled from quantitative proteomics study [1], western blot data summarized from various studies [2], or our own unpublished quantitative westerns in human Reh cells (asterisk).

Protein abbreviations: p53-binding protein 1 (53BP1); Aprataxin and PNKP-like factor (APLF); Bloom syndrome RecQ-like helicase (BLM); Breast cancer (BRCA); C-terminal binding protein interacting protein (CtIP); DNA protein kinase, catalytic subunit (DNA-PKcs); Excision repair cross-complementing 1 (ERCC1); Exonuclease 1 (Exo1); Meiotic recombination 11 (MRE11); Nijmegen breakage syndrome protein 1 (NBS1); Poly(ADP-ribose) polymerase (PARP); Paralog of XRCC4 and XLF (PAXX); Polymerase (Pol); RAD50 double strand break repair protein (RAD50); RAD51 recombinase (RAD51); DNA repair and recombination protein RAD54 (RAD54); Replication Protein A (RPA); Terminal deoxynucleotidyltransferase (TdT); XRCC4-like factor (XLF); Xeroderma pigmentosum group F (XPF); X-ray repair cross-complementing 4 (XRCC4).

## References

1. Beck, M. et al. The quantitative proteome of a human cell line. *Mol Syst Biol* 7, 549 (2011).
2. Anderson, C.W. & Carter, T.H. in Molecular Analysis of DNA Rearrangements in the Immune System (eds. Jessberger, R. & Lieber, M.R.) 91-112 (Springer-Verlag, Heidelberg, 1996).

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| Process | Proteins | Pathway | Abundance in U2OS cells (molecules/cell) [1] | Abundance from Western blots (molecules/cell) |
|---|---|---|---|---|
| **Pathway choice & DSB recognition** | 53BP1 | NHEJ | 11,000 | - |
| | Ku70 | NHEJ | 1,290,000 | 400,000 [2] |
| | Ku80 | NHEJ | 826,000 | 400,000 [2] |
| | DNA-PKcs | NHEJ | 238,000 | 50,000 – 100,000 [2] |
| | Mre11 | a-EJ, SSA, HR | 7,340 | - |
| | Rad50 | a-EJ, SSA, HR | 8,030 | - |
| | Nbs1 | a-EJ, SSA, HR | 5,480 | - |
| | CtIP | a-EJ, SSA, HR | Undetectable in U2OS | - |
| | RPA1 | SSA, HR | 246,000 | - |
| | RPA2 | SSA, HR | 133,000 | - |
| | RPA3 | SSA, HR | 435,000 | - |
| | PARP1 | a-EJ | 142,000 | - |
| | PARP2 | a-EJ | 12,800 | - |
| | PARP3 | a-EJ | Undetectable in U2Os | - |
| | RAD52 | SSA | Undetectable in U2OS | - |
| | RAD51 | HR | Undetectable in U2OS | - |
| | BRCA1 | HR | < 500 | - |
| | BRCA2 | HR | < 500 | - |
| | RAD54 | HR | < 500 | - |
| **Nucleases** | Artemis | NHEJ | Undetectable in U2OS | 70,000 * |
| | APLF | NHEJ | Undetectable in U2OS | - |
| | Exo1 | SSA, HR | < 500 | - |
| | BLM | SSA, HR | 603 | - |
| | XPF | SSA | 718 | - |
| | ERCC1 | SSA | 2,720 | - |
| **Polymerases** | Pol λ | NHEJ | 698 | - |
| | Pol μ | NHEJ | Undetectable in U2OS | - |
| | TdT | NHEJ | Undetectable in U2OS | - |
| | Pol θ | a-EJ | Undetectable in U2OS | - |

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| | | | | |
|---|---|---|---|---|
| **Ligase complex** | Ligase IV | NHEJ | 722 | - |
| | XRCC4 | NHEJ | 1,970 | - |
| | XLF | NHEJ | 1,130 | - |
| | PAXX | NHEJ | 6,200 | - |
| | Ligase I | a-EJ, SSA, HR | 25,600 | - |
| | Ligase III | a-EJ, SSA, HR | 9,260 | - |