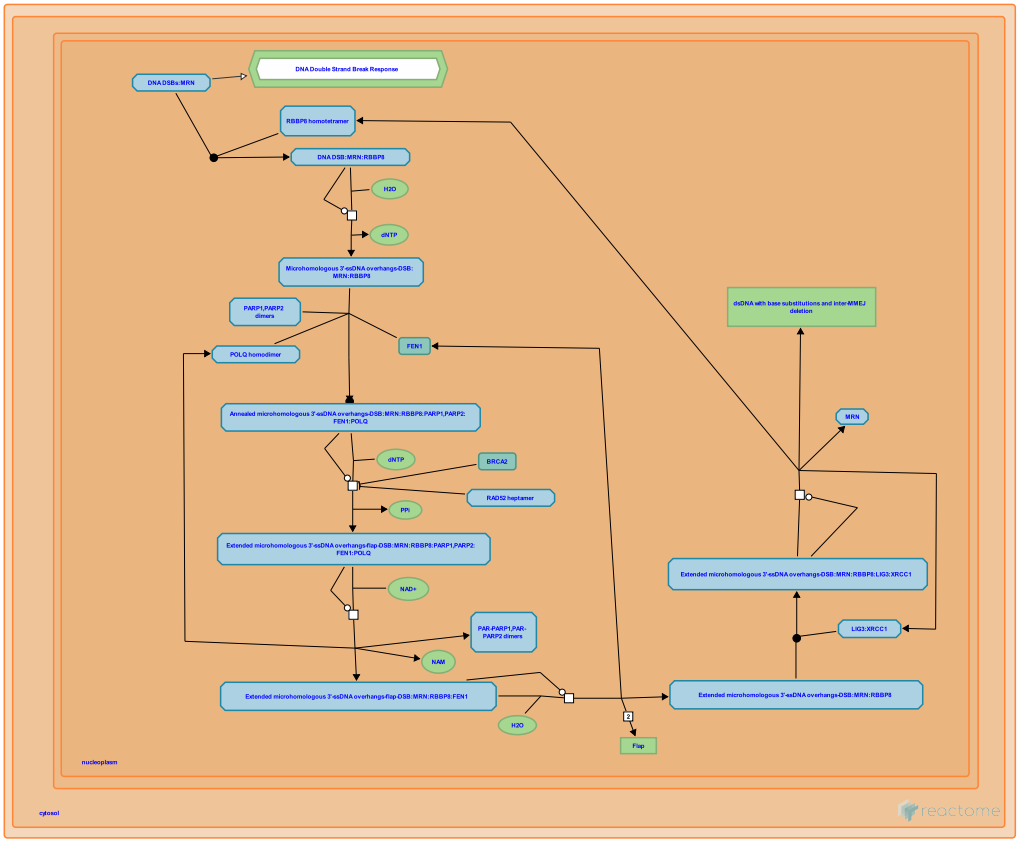
HDR through MMEJ (alt-NHEJ)



Borowiec, JA., Heyer, WD., Le, HP., Liu, J., Orlic-Milacic, M.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

07/05/2024

Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.
The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics, 18*, 142. ↗

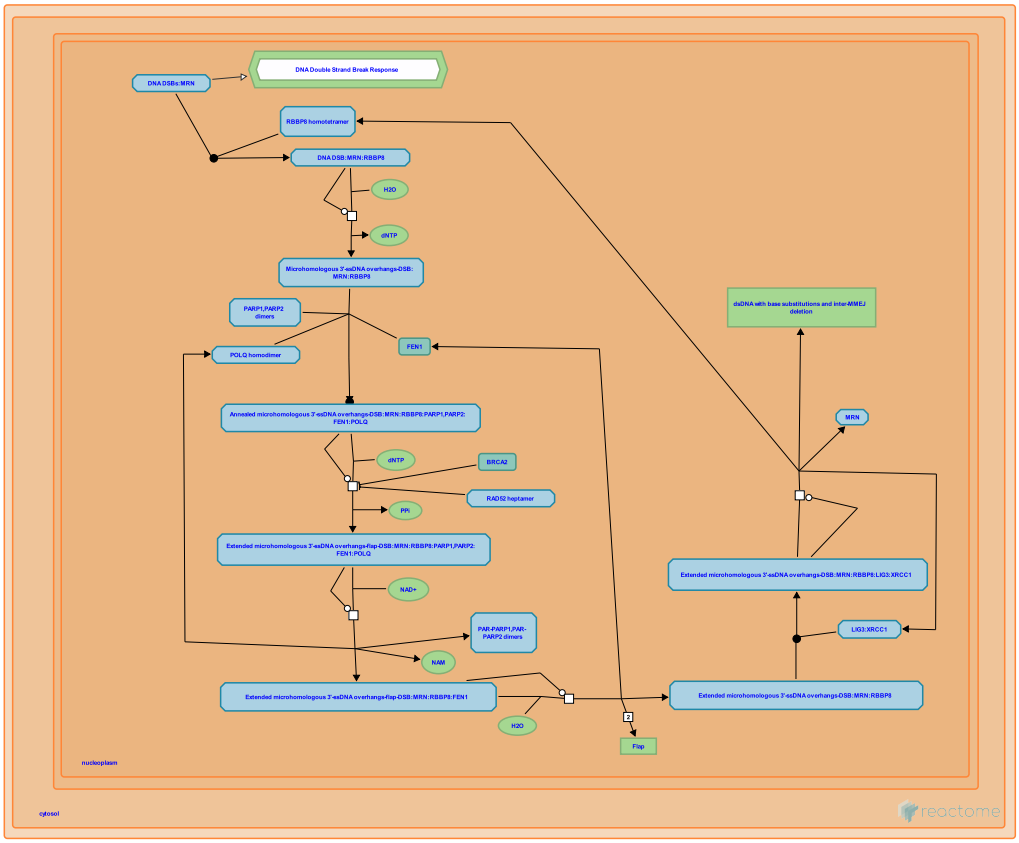
Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

HDR through MMEJ (alt-NHEJ) ↗

Stable identifier: R-HSA-5685939



Homology directed repair (HDR) through microhomology-mediated end joining (MMEJ) is an error prone process also known as alternative nonhomologous end joining (alt-NHEJ), although it does not involve proteins that participate in the classical NHEJ. Contrary to the classical NHEJ and other HDR pathways, homologous recombination repair (HRR) and single strand annealing (SSA), MMEJ does not require ATM activation. In fact, ATM activation inhibits MMEJ. Therefore, MMEJ may be triggered when the amount of DNA double strand breaks (DSBs) overwhelms DNA repair machinery of higher fidelity or when cells are deficient in components of high fidelity DNA repair.

MMEJ is initiated by a limited resection of DNA DSB ends by the MRN complex (MRE11A:RAD50:NBN) and RBBP8 (CtIP), in the absence of CDK2-mediated RBBP8 phosphorylation and related BRCA1:BARD1 recruitment (Yun and Hiom 2009). Single strand DNA (ssDNA) at resected DNA DSB ends recruits PARP1 or PARP2 homo- or heterodimers, together with DNA polymerase theta (POLQ) and FEN1 5'-flap endonuclease. In a poorly studied sequence of events, POLQ promotes the annealing of two 3'-ssDNA overhangs through microhomologous regions that are optimally 10-19 nucleotides long. Using analogy with POLB-mediated long patch base excision repair (BER), it is plausible that PARP1 (or PARP2) dimers coordinate the extension of annealed 3'-ssDNA overhangs via POLQ-mediated strand displacement synthesis with FEN1-mediated cleavage of the resulting 5'-flaps (Liang et al. 2005, Mansour et al. 2011, Sharma et al. 2015, Kent et al. 2015, Ciccaldi et al. 2015, Mateos-Gomez et al. 2015). The MRN complex subsequently recruits DNA ligase 3 (LIG3) bound to XRCC1 (LIG3:XRCC1) to ligate the remaining single strand nicks (SSBs) at MMEJ sites (Della-Maria et al. 2011).

Similar to single strand annealing (SSA), MMEJ leads to deletion of one of the microhomology regions used for annealing and the DNA sequence in between two annealed microhomology regions. MMEJ, just like classical NHEJ, can result in genomic translocations (Ghezraoui et al. 2014). In addition, since POLQ is an error-prone DNA polymerase, MMEJ introduces frequent base substitutions (Ceccaldi et al. 2015).

Literature references

Srivastava, M., Javadekar, SM., Pandey, M., Sharma, S., Kumari, R., Raghavan, SC. (2015). Homology and enzymatic requirements of microhomology-dependent alternative end joining. *Cell Death Dis, 6*, e1697. ↗

Li, GC., Shao, C., Tischfield, JA., Deng, L., Chen, Y., Liang, L. (2005). Modulation of DNA end joining by nuclear proteins. *J. Biol. Chem., 280*, 31442-9. ↗

Sallmyr, A., Ghezraoui, H., Brunet, E., Jasin, M., Piganeau, M., Ruis, B. et al. (2014). Chromosomal translocations in human cells are generated by canonical nonhomologous end-joining. *Mol. Cell, 55*, 829-42. ↗

McDevitt, SM., Chandramouly, G., Kent, T., Ozdemir, AY., Pomerantz, RT. (2015). Mechanism of microhomology-mediated end-joining promoted by human DNA polymerase ?. *Nat. Struct. Mol. Biol., 22*, 230-7. ↗

Tsai, MS., Kuhnlein, J., Zhou, Y., Della-Maria, J., Carney, JP., Tomkinson, AE. et al. (2011). Human Mre11/human Rad50/Nbs1 and DNA ligase IIIalpha/XRCC1 protein complexes act together in an alternative nonhomologous end joining pathway. *J. Biol. Chem., 286*, 33845-53. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

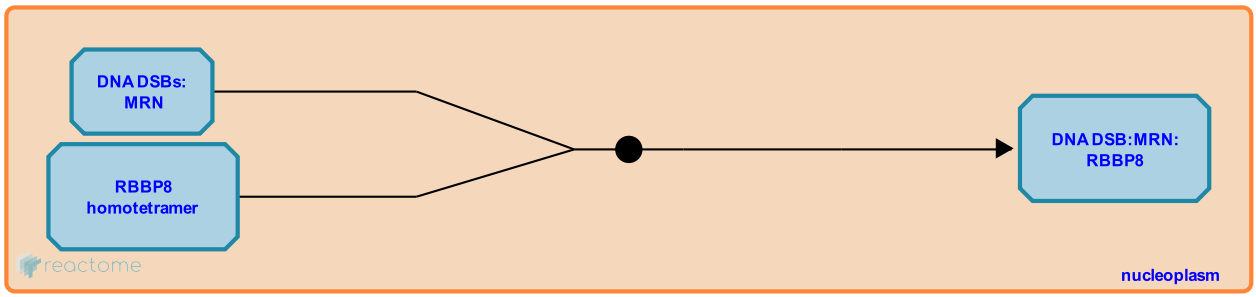
MRN complex binds RBBP8 ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687465

Type: binding

Compartments: nucleoplasm



In G1 phase, RBBP8 (CtIP) homotetramer associates with the MRN complex (MRE11A:RAD50:NBN) at DNA double strand breaks (DSBs) but does not undergo CDK2-mediated phosphorylation and is therefore unable to recruit BRCA1 (Yun et al. 2009). The activation of ATM DNA damage checkpoint is not needed for microhomology-mediated end joining (MMEJ or alt-NHEJ) (Rahal et al. 2010). MMEJ can also be triggered at other stages of the cell cycle, besides G1, when the amount of DNA DSBs overwhelms high fidelity DNA repair machinery (Liang et al. 2005).

Followed by: MRN and RBBP8 resect DNA DSBs in MMEJ

Literature references

Li, GC., Shao, C., Tischfield, JA., Deng, L., Chen, Y., Liang, L. (2005). Modulation of DNA end joining by nuclear proteins. *J. Biol. Chem., 280*, 31442-9. ↗

Tainer, JA., Henricksen, LA., Dixon, K., Williams, RS., Rahal, EA., Li, Y. (2010). ATM regulates Mre11-dependent DNA end-degradation and microhomology-mediated end joining. *Cell Cycle, 9*, 2866-77. ↗

Yun, MH., Hiom, K. (2009). CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature, 459*, 460-3. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

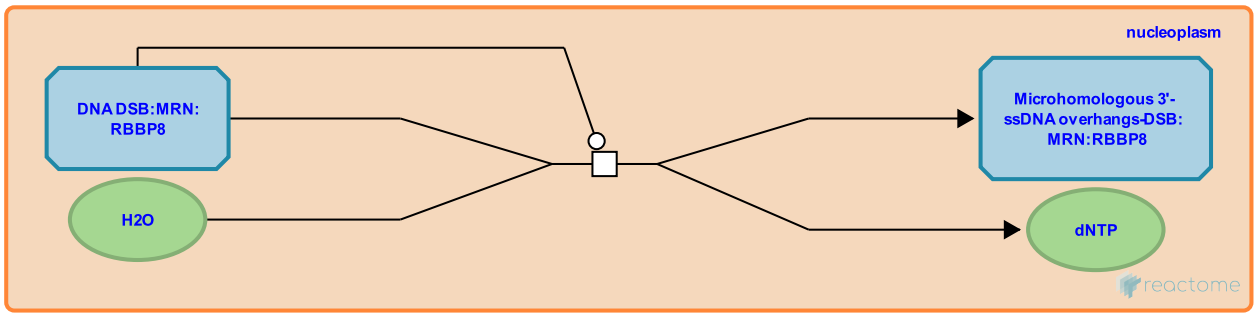
MRN and RBBP8 resect DNA DSBs in MMEJ ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687464

Type: transition

Compartments: nucleoplasm



The complex of MRN (MRE11A:RAD50:NBN) and RBBP8 (CtIP) performs a limited resection of DNA double strand breaks (DSBs) in the process of microhomology-mediated end joining (MMEJ) (Yun et al. 2009). ATM activation inhibits MMEJ-related resection of DNA DSBs by MRN, possibly through MRN phosphorylation (Rahal et al. 2010).

Preceded by: MRN complex binds RBBP8

Followed by: PARP1 or PARP2, FEN1 and POLQ are recruited to MMEJ site

Literature references

Tainer, JA., Henricksen, LA., Dixon, K., Williams, RS., Rahal, EA., Li, Y. (2010). ATM regulates Mre11-dependent DNA end-degradation and microhomology-mediated end joining. *Cell Cycle, 9*, 2866-77. ↗

Yun, MH., Hiom, K. (2009). CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature, 459*, 460-3. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

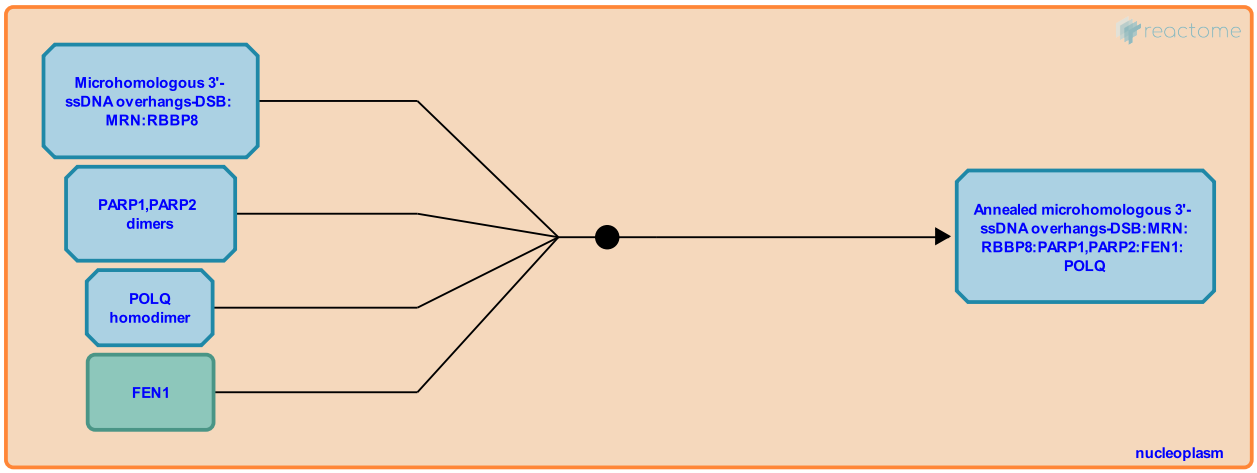
PARP1 or PARP2, FEN1 and POLQ are recruited to MMEJ site ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687484

Type: binding

Compartments: nucleoplasm



Flap endonuclease FEN1, DNA polymerase theta (POLQ) and PARP1 or PARP2 homo- or heterodimers are recruited to DNA double strand breaks (DSBs) resected by MRN and RBBP8 (CtIP) in the process of microhomology-mediated end joining (MMEJ). The mechanism of recruitment of FEN1, PARP1 (or PARP2) and POLQ, which are all necessary for MMEJ progression (Liang et al. 2005, Mansour et al. 2010, Sharma et al. 2015, Mateos-Gomez et al. 2015, Ceccaldi et al. 2015, Kent et al. 2015), is poorly defined. PARP1 (or PARP2) recognizes ssDNA. In the DNA polymerase beta (POLB)-dependent long patch base excision repair (BER), PARPs form ternary complexes with FEN1 and POLB (Prasad et al. 2001, Lavrik et al. 2001, Cistulli et al. 2004), and it is possible that a similar mechanism involving PARPs, FEN1 and POLQ operates in MMEJ. POLQ functions as a homodimer and facilitates annealing of two 3'-ssDNA overhangs through their microhomology regions. POLQ requires <20 nucleotide (nt) long resected overhangs (Kent et al. 2015). Microhomology regions are optimally 10-19 nt long (Sharma et al. 2015), and the annealing is facilitated if the microhomology region is GC-rich (Kent et al. 2015).

Preceded by: MRN and RBBP8 resect DNA DSBs in MMEJ

Followed by: POLQ extends annealed 3'-ssDNA overhangs in MMEJ

Literature references

Li, GC., Shao, C., Tischfield, JA., Deng, L., Chen, Y., Liang, L. (2005). Modulation of DNA end joining by nuclear proteins. *J. Biol. Chem., 280*, 31442-9. ↗

Srivastava, M., Javadekar, SM., Pandey, M., Sharma, S., Kumari, R., Raghavan, SC. (2015). Homology and enzymatic requirements of microhomology-dependent alternative end joining. *Cell Death Dis, 6*, e1697. ↗

McDevitt, SM., Chandramouly, G., Kent, T., Ozdemir, AY., Pomerantz, RT. (2015). Mechanism of microhomology-mediated end-joining promoted by human DNA polymerase ?. *Nat. Struct. Mol. Biol., 22*, 230-7. ↗

Wilson, SH., Ackerman, EJ., Sobol, RW., Horton, JK., Lavrik, OI., Prasad, R. (2001). Photoaffinity labeling of mouse fibroblast enzymes by a base excision repair intermediate. Evidence for the role of poly(ADP-ribose) polymerase-1 in DNA repair. *J. Biol. Chem., 276*, 25541-8. ↗

Vande Berg, BJ., Wilson, SH., Kim, SJ., Kedar, P., Yang, XP., Lavrik, OI. et al. (2001). DNA polymerase beta -mediated long patch base excision repair. Poly(ADP-ribose)polymerase-1 stimulates strand displacement DNA synthesis. *J. Biol. Chem., 276*, 32411-4. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

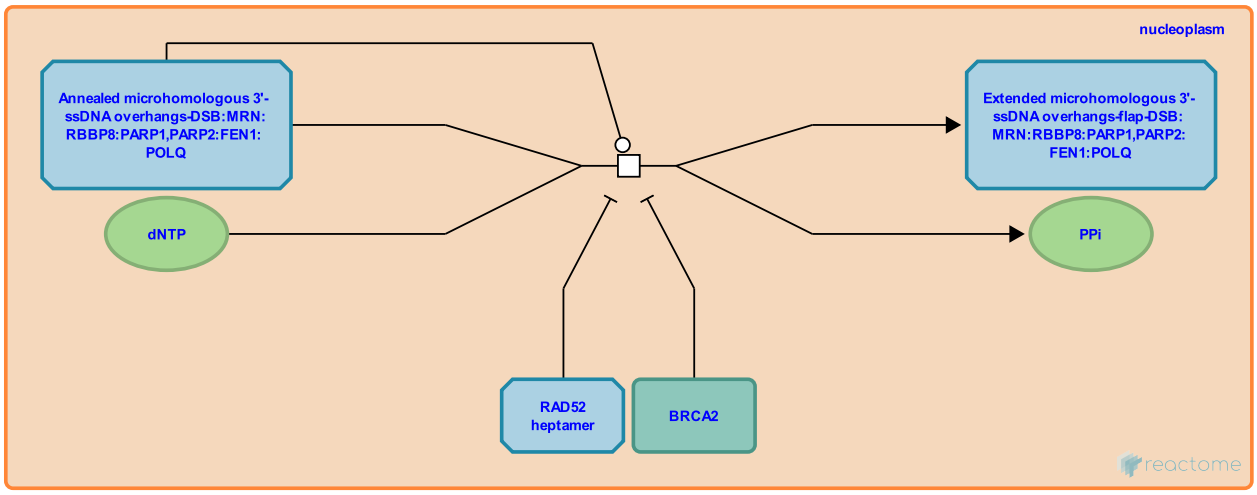
POLQ extends annealed 3'-ssDNA overhangs in MMEJ ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687640

Type: transition

Compartments: nucleoplasm



DNA polymerase theta (POLQ) extends annealed microhomologous 3'-ssDNA overhangs at DNA double strand breaks (DSBs), using opposing overhangs as templates. POLQ can perform strand displacement synthesis, extending the overhangs beyond ssDNA-dsDNA junction point, which leads to the formation of displaced strand flaps (Kent et al. 2015). PARP1 (or possibly PARP2) is necessary for the recruitment of POLQ to DNA DSBs. POLQ-mediated DNA synthesis during microhomology mediated end joining (MMEJ) (also known as alternative nonhomologous end joining or alt-NHEJ or theta-mediated end joining - TMEJ) counteracts homologous recombination repair (HRR) and promotes survival of cells with a compromised HR pathway (Mateos-Gomez et al. 2015). POLQ is error-prone and introduces single nucleotide substitutions during DNA synthesis. HRR-deficient epithelial ovarian cancers frequently overexpress POLQ, which correlates with an increased frequency of somatic point mutations in these tumors (Ceccaldi et al. 2015).

BRCA2 regulates DSB repair pathway choice independent of RAD51 (Han et al. 2017) by inhibiting DNA polymerase theta-mediated end-joining (TMEJ) during the S and G2 phases of the cell cycle until M phase (Llorens-Agost et al. 2021). In BRCA2-deficient cells, TMEJ is inhibited by RAD52. Loss of RAD52 in BRCA2-deficient cells, leads to inappropriate DSB repair by TMEJ and cell death providing a rationale for the synthetic lethality of BRCA2 and RAD52 deficiencies (Llorens-Agost et al. 2021).

Preceded by: PARP1 or PARP2, FEN1 and POLQ are recruited to MMEJ site

Followed by: PARP1,PARP2 dimers bound to MMEJ sites autoPARylate

Literature references

McDevitt, SM., Chandramouly, G., Kent, T., Ozdemir, AY., Pomerantz, RT. (2015). Mechanism of microhomology-mediated end-joining promoted by human DNA polymerase ?. *Nat. Struct. Mol. Biol., 22*, 230-7. ↗

Heyer, WD., Wood, RD., Löbrich, M., Cruz-García, A., Le, HP., Gawai, A. et al. (2021). POLθ-mediated end joining is restricted by RAD52 and BRCA2 until the onset of mitosis. *Nat Cell Biol, 23*, 1095-1104. ↗

Huen, MSY., Huang, J., Liu, T., Xie, A., Han, J., Fu, C. et al. (2017). BRCA2 antagonizes classical and alternative non-homologous end-joining to prevent gross genomic instability. *Nat Commun, 8*, 1470. ↗

Lazzerini-Denchi, E., Mateos-Gomez, PA., Miller, KM., Sfeir, A., Nair, N., Gong, F. (2015). Mammalian polymerase ? promotes alternative NHEJ and suppresses recombination. *Nature, 518*, 254-7. ↗

O'Connor, KW., Liu, JC., Elledge, SJ., Petalcorin, MI., Ceccaldi, R., Boulton, SJ. et al. (2015). Homologous-recombination-deficient tumours are dependent on Pol?-mediated repair. *Nature, 518*, 258-62. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.
2022-01-26	Reviewed	Liu, J., Heyer, WD., Le, HP.
2022-02-03	Revised	Orlic-Milacic, M.

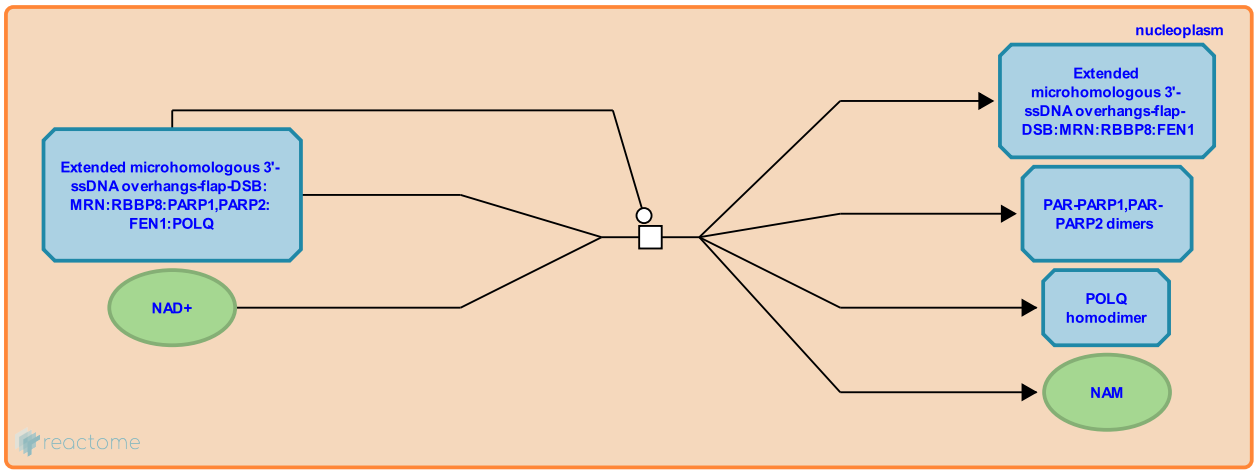
PARP1,PARP2 dimers bound to MMEJ sites autoPARylate ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687653

Type: transition

Compartments: nucleoplasm



PARP inihibitors that block catalytic activity of PARP1 (or PARP2) bound to single-stranded DNA (ssDNA), including PARP1 and PARP2 autoPARylation (auto-polyADPribosylation), also inhibit microhomology-mediated end joining (MMEJ). Thus, the catalytic activity of PARP1 (or PARP2), related to autoPARylation or PARylation of other proteins at MMEJ site, is necessary for the progression of MMEJ (Mansour et al. 2010, Ceccaldi et al. 2015). By analogy with the DNA polymerase beta (POLB)-dependent long patch base excision repair (Satoh et al. 1994, Prasad et al. 2001), autoPARylated PARPs dissociate from the repair site, thereby coordinating the termination of strand displacement DNA synthesis and the cleavage of displaced strand flaps by FEN1.

Preceded by: POLQ extends annealed 3'-ssDNA overhangs in MMEJ

Followed by: FEN1 cleaves displaced ssDNA flaps during MMEJ

Literature references

Vande Berg, BJ., Wilson, SH., Kim, SJ., Kedar, P., Yang, XP., Lavrik, OI. et al. (2001). DNA polymerase beta -mediated long patch base excision repair. Poly(ADP-ribose)polymerase-1 stimulates strand displacement DNA synthesis. *J. Biol. Chem., 276*, 32411-4. ↗

Rhein, T., Mansour, WY., Dahm-Daphi, J. (2010). The alternative end-joining pathway for repair of DNA double-strand breaks requires PARP1 but is not dependent upon microhomologies. *Nucleic Acids Res., 38*, 6065-77. ↗

O'Connor, KW., Liu, JC., Elledge, SJ., Petalcorin, MI., Ceccaldi, R., Boulton, SJ. et al. (2015). Homologous-recombination-deficient tumours are dependent on Pol?-mediated repair. *Nature, 518*, 258-62. ↗

Poirier, GG., Satoh, MS., Lindahl, T. (1994). Dual function for poly(ADP-ribose) synthesis in response to DNA strand breakage. *Biochemistry, 33*, 7099-106. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

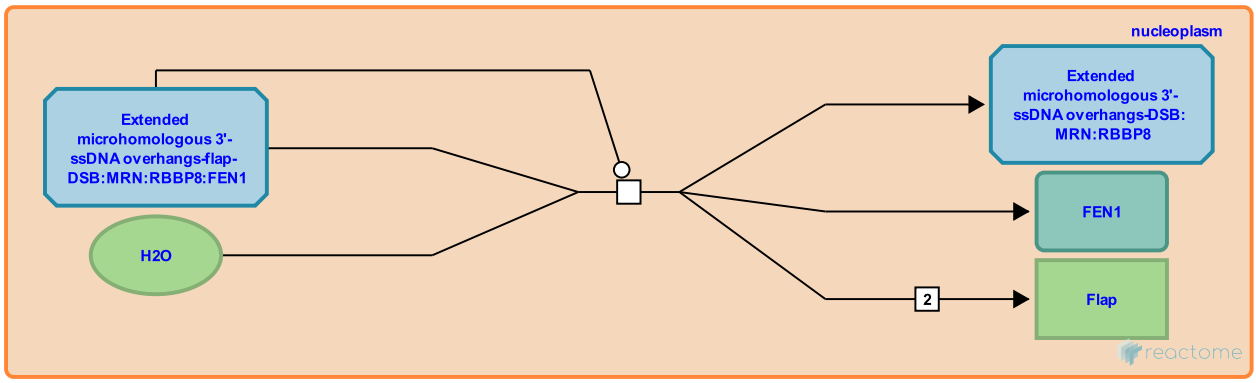
FEN1 cleaves displaced ssDNA flaps during MMEJ ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687664

Type: transition

Compartments: nucleoplasm



DNA polymerase theta (POLQ) performs strand displacement DNA synthesis during microhomology-mediated end joining (MMEJ) (Kent et al. 2015), which is expected to result in the formation of displaced 5'-ssDNA flaps. FEN1, a 5'-flap endonuclease, is a necessary participant of MMEJ (Liang et al. 2005, Sharma et al. 2015). By analogy with base excision repair (Klungland and Lindahl 1997, Liu et al. 2005), FEN1 is thought to cleave the 5'-flaps generated by POLQ-mediated DNA strand displacement synthesis during MMEJ, thus enabling the subsequent ligation step.

Preceded by: PARP1,PARP2 dimers bound to MMEJ sites autoPARylate

Followed by: MRN recruits LIG3:XRCC1 to MMEJ sites

Literature references

Liu, Y., Beard, WA., Wilson, SH., Prasad, R., Hou, EW., Shock, DD. (2005). DNA polymerase beta and flap endonuclease 1 enzymatic specificities sustain DNA synthesis for long patch base excision repair. *J. Biol. Chem., 280*, 3665-74. ↗

Srivastava, M., Javadekar, SM., Pandey, M., Sharma, S., Kumari, R., Raghavan, SC. (2015). Homology and enzymatic requirements of microhomology-dependent alternative end joining. *Cell Death Dis, 6*, e1697. ↗

Li, GC., Shao, C., Tischfield, JA., Deng, L., Chen, Y., Liang, L. (2005). Modulation of DNA end joining by nuclear proteins. *J. Biol. Chem., 280*, 31442-9. ↗

McDevitt, SM., Chandramouly, G., Kent, T., Ozdemir, AY., Pomerantz, RT. (2015). Mechanism of microhomology-mediated end-joining promoted by human DNA polymerase ?. *Nat. Struct. Mol. Biol., 22*, 230-7. ↗

Lindahl, T., Klungland, A. (1997). Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J, 16*, 3341-8. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

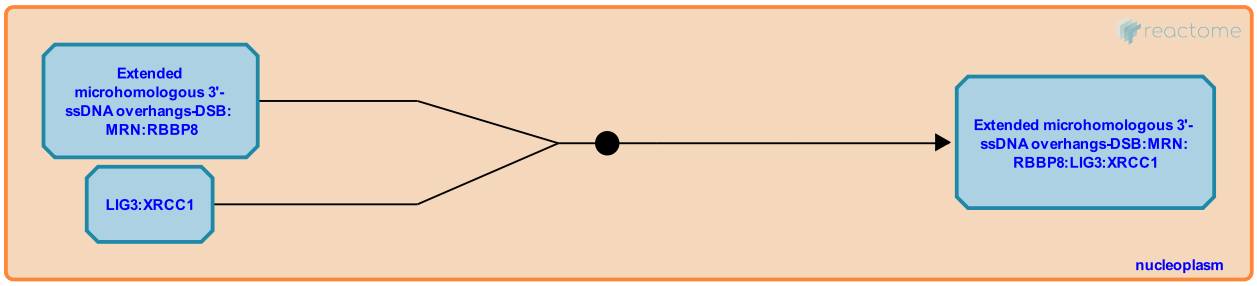
MRN recruits LIG3:XRCC1 to MMEJ sites ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687673

Type: binding

Compartments: nucleoplasm



The MRN complex recruits DNA ligase 3 (LIG3) bound to XRCC1 (LIG3:XRCC1) to microhomology-mediated end joining (MMEJ) sites through direct interactions of the MRN subunits RAD50 and NBN (NBS1) with LIG3 (Della-Maria et al. 2011).

Preceded by: FEN1 cleaves displaced ssDNA flaps during MMEJ

Followed by: LIG3 ligates remaining SSBs in MMEJ

Literature references

Tsai, MS., Kuhnlein, J., Zhou, Y., Della-Maria, J., Carney, JP., Tomkinson, AE. et al. (2011). Human Mre11/human Rad50/Nbs1 and DNA ligase IIIalpha/XRCC1 protein complexes act together in an alternative nonhomologous end joining pathway. *J. Biol. Chem., 286*, 33845-53. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

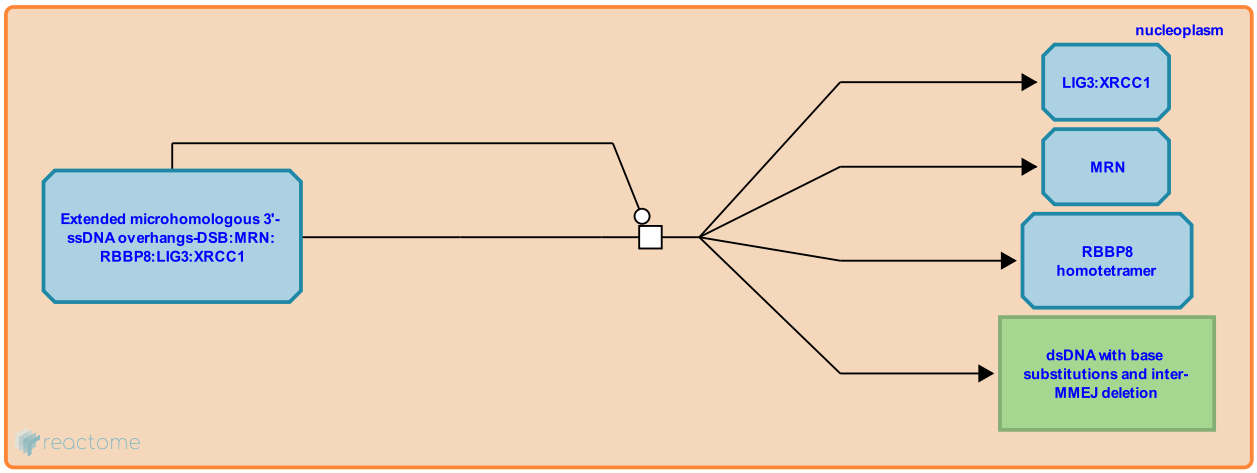
LIG3 ligates remaining SSBs in MMEJ ↗

Location: HDR through MMEJ (alt-NHEJ)

Stable identifier: R-HSA-5687675

Type: transition

Compartments: nucleoplasm



The complex of DNA ligase 3 (LIG3) and XRCC1 is necessary for the completion of microhomology-mediated end joining (MMEJ), although DNA ligase 1 (LIG1) may also be involved (Sharma et al. 2015). LIG3:XRCC1 is recruited to MMEJ sites by the MRN complex and ligates single strand nicks that remain after reparative DNA synthesis by DNA polymerase theta (POLQ) at DNA double strand break (DSB) sites (Della-Maria et al. 2011). The annealing of microhomology regions between two 3'-ssDNA overhangs of resected DNA DSBs during MMEJ leads to deletion of the intervening DNA sequence and one of the microhomology regions in repaired double strand DNA (dsDNA) (Ghezraoui et al. 2014). In addition, as POLQ is error-prone, repaired DNA contains base substitutions (Ceccaldi et al. 2015). Similar to nonhomologous end joining (NHEJ), MMEJ (also known as alternative-NHEJ) can also produce translocations by joining unrelated DNA molecules (Ghezraoui et al. 2014).

Preceded by: MRN recruits LIG3:XRCC1 to MMEJ sites

Literature references

Srivastava, M., Javadekar, SM., Pandey, M., Sharma, S., Kumari, R., Raghavan, SC. (2015). Homology and enzymatic requirements of microhomology-dependent alternative end joining. *Cell Death Dis, 6*, e1697. ↗

Sallmyr, A., Ghezraoui, H., Brunet, E., Jasin, M., Piganeau, M., Ruis, B. et al. (2014). Chromosomal translocations in human cells are generated by canonical nonhomologous end-joining. *Mol. Cell, 55*, 829-42. ↗

Tsai, MS., Kuhnlein, J., Zhou, Y., Della-Maria, J., Carney, JP., Tomkinson, AE. et al. (2011). Human Mre11/human Rad50/Nbs1 and DNA ligase IIIalpha/XRCC1 protein complexes act together in an alternative nonhomologous end joining pathway. *J. Biol. Chem., 286*, 33845-53. ↗

Editions

2015-05-12	Authored, Edited	Orlic-Milacic, M.
2015-06-12	Reviewed	Borowiec, JA.

Table of Contents