Rpn11 cooperates with the remaining DUBs and ubiquitin receptors of the 19S RP. Despite these lingering questions, it indeed appears that the proteasome is slowly but surely revealing its secrets.

ACKNOWLEDGMENTS

We would like to thank P. da Fonseca for comments on the manuscript. Work in the authors' laboratory is supported by the UK Medical Research Council (U105192732), the European Research Council (309756) and the Lister Institute for Preventive Medicine.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper (doi:10.1038/nsmb.2800).

1. Finley, D. *Annu. Rev. Biochem.* **78**, 477–513 (2009).
2. Komander, D. & Rape, M. *Annu. Rev. Biochem.* **81**, 203–229 (2012).
3. Bhattacharyya, S., Yu, H., Mim, C. & Matouschek, A. *Nat. Rev. Mol. Cell Biol.* **15**, 122–133 (2014).
4. Förster, F., Unverdorben, P., Sledz , P. & Baumeister, W. *Structure* **21**, 1551–1562 (2013).
5. Beck, F. *et al. Proc. Natl. Acad. Sci. USA* **109**, 14870–14875 (2012).
6. da Fonseca, P.C.A., He, J. & Morris, E.P. *Mol. Cell* **46**, 54–66 (2012).
7. Lander, G.C. *et al. Nature* **482**, 186–191 (2012).
8. Matyskiela, M.E., Lander, G.C. & Martin, A. *Nat. Struct. Mol. Biol.* **20**, 781–788 (2013).
9. Worden, E.J., Padovani, C. & Martin, A. *Nat. Struct. Mol. Biol.* **21**, 220–227 (2014).
10. Pathare, G.R. *et al. Proc. Natl. Acad. Sci. USA* **111**, 2984–2989 (2014).
11. Yao, T. & Cohen, R.E. *Nature* **419**, 403–407 (2002).
12. Clague, M.J., Coulson, J.M. & Urbé, S. *J. Cell Sci.* **125**, 277–286 (2012).
13. McCullough, J. *et al. Curr. Biol.* **16**, 160–165 (2006).
14. Cooper, E.M. *et al. EMBO J.* **28**, 621–631 (2009).
15. Sato, Y. *et al. Nature* **455**, 358–362 (2008).
16. Sanches, M., Alves, B.S.C., Zanchin, N.I.T. & Guimarães, B.G. *J. Mol. Biol.* **370**, 846–855 (2007).
17. Inobe, T., Fishbain, S., Prakash, S. & Matouschek, A. *Nat. Chem. Biol.* **7**, 161–167 (2011).
18. Prakash, S. *Nat. Struct. Mol. Biol.* **11**, 830–837 (2004).
19. Verma, R. *et al. Science* **298**, 611–615 (2002).
20. Pruneda, J.N. *et al. Mol. Cell* **47**, 933–942 (2012).
21. Plechanovová, A., Jaffray, E.G., Tatham, M.H., Naismith, J.H. & Hay, R.T. *Nature* **489**, 115–120 (2012).
22. Dou, H., Buetow, L., Sibbet, G.J., Cameron, K. & Huang, D.T. *Nat. Struct. Mol. Biol.* **19**, 876–883 (2012).



# RPA puts the brakes on MMEJ

Mitch McVey

**Microhomology-mediated end joining (MMEJ) is a mechanism of DNA double-strand-break repair that creates deletions and promotes other types of genome instability. New *in vivo* and *in vitro* analyses demonstrate that the heterotrimeric replication protein A (RPA) complex prevents spontaneous annealing of microhomologies, thereby preventing genome-destabilizing MMEJ.**

DNA double-strand breaks are serious lesions that must be accurately repaired in order to promote cell survival and genome stability. Of the many break-repair mechanisms that have been characterized, MMEJ is one of the least conservative and therefore one of the most dangerous from a genomic perspective. MMEJ gives rise to deletions and has been implicated in the formation of translocations and other chromosome rearrangements[1,2]. It therefore appears that cells would try to avoid MMEJ repair at all costs. In this issue of *Nature Structural & Molecular Biology*, Deng *et al.*[3] demonstrate that the heterotrimeric RPA complex, which binds single-stranded DNA (ssDNA) and has multiple roles in DNA replication and repair, is a crucial negative regulator of MMEJ. This finding identifies a new mechanism for how cells prevent MMEJ and reveals how pathway choice in double-strand-break repair is regulated in yeast.

On the surface, MMEJ repair of a double-strand break is conceptually straightforward (**Fig. 1**). It begins by resection of DNA ends to expose single-stranded, microhomologous sequences of between 8 and 20 nucleotides in length, and is followed by annealing of these microhomologies[4]. Repair is then completed by trimming of the unpaired DNA ends, fill-in synthesis and ligation[5]. Because the DNA between the microhomologous sequences is lost during this process, MMEJ results in deletions of tens to thousands of base pairs.

One well-studied mechanism that prevents such deletions involves binding of the Ku70–Ku80 complex to DNA breaks and recruitment of other classical nonhomologous end joining (C-NHEJ) proteins[4,6]. In the absence of the Ku proteins or when C-NHEJ is unsuccessful, resection may occur via a number of mechanisms[7], thus providing cells with at least two repair options. Loading of Rad51 onto the ssDNA can channel the break into homologous recombination (HR) repair. However, the presence of extensive ssDNA also makes MMEJ a viable option. It is now apparent that MMEJ is used at an appreciable frequency even when more-accurate repair pathways such as homologous recombination are available[8,9], and this apparent paradox reflects the limited understanding of the rules that govern MMEJ usage.

To specifically study factors that affect repair-pathway preference and prevent MMEJ, Deng *et al.*[3] used a clever genetic system in *Saccharomyces cerevisiae*. They used two inverted I-SceI–cleavage sites located within a chromosomal *ADE2* coding sequence to induce noncomplementary DNA breaks. The break sites were flanked by 12-bp direct-repeat sequences that could anneal during MMEJ and thus restore *ADE2* function and change the colony color from red to white. As expected, most repair in this system occurred by C-NHEJ, but proximal MMEJ using the 12-bp direct repeats was observed at similar rates in cells proficient or deficient in C-NHEJ. Interestingly, distal MMEJ repair using imperfect 16-bp repeats located 5 kb apart was also observed. This is consistent with previous reports that MMEJ can occur across great distances and that its efficiency depends on both the length and extent of microhomology as well as on the distance between the complementary sequences[5]. Distal MMEJ was abolished in cells lacking the resection-promoting proteins Exo1 and Sgs1.

Intriguingly, the frequency of proximal MMEJ was unaffected in cells that could not carry out extensive resection. This suggested that other factors, such as the rate of microhomology annealing, might be limiting. The heterotrimeric RPA complex, encoded in *S. cerevisiae* by the *RFA1*, *RFA2* and *RFA3* genes, has previously been shown to prevent annealing between complementary ssDNA molecules *in vitro*[10] and might therefore be expected to regulate this stage of MMEJ. Because RPA is essential, Deng *et al.*[3] tested this hypothesis by using hypomorphic mutant alleles of *RFA1* with point mutations in the DNA-binding domain[11–13]. Strikingly, these *rfa1* mutations increased proximal MMEJ repair to levels 85-fold to 350-fold higher than those of wild type and also enhanced distal

*Mitch McVey is at the Department of Biology, Tufts University, Medford, Massachusetts, USA.*
*e-mail: mitch.mcvey@tufts.edu*

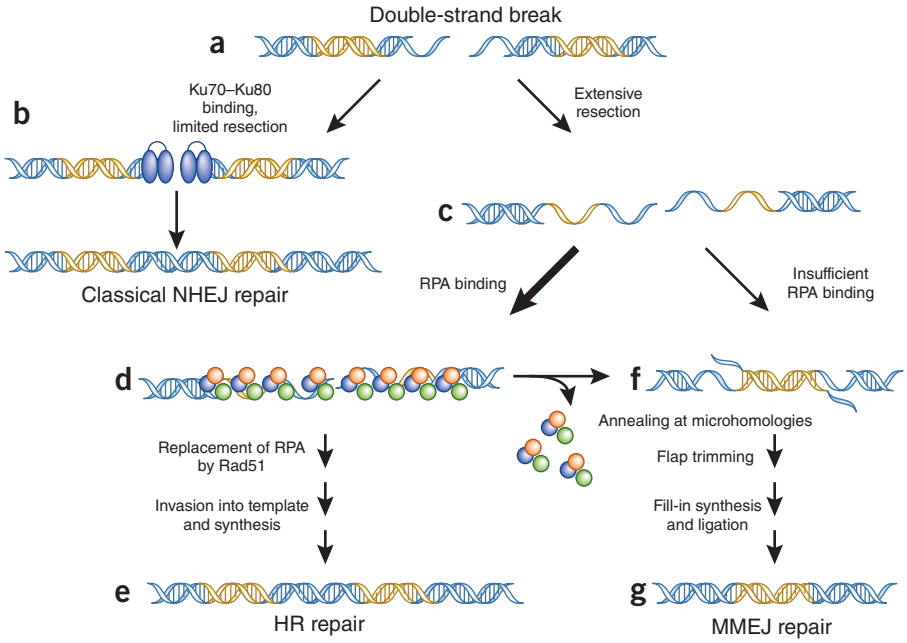


**Figure 1** RPA has a central role in pathway choice in double-strand-break repair. (**a**,**b**) Once a double-strand break is formed (**a**), binding of the Ku70–Ku80 heterodimer and other C-NHEJ proteins prevents resection and promotes rejoining with minimal loss of DNA sequence (**b**). (**c**) In the event that C-NHEJ is unavailable or fails, resection creates single-stranded tails. (**d**) RPA binding to ssDNA prevents intermolecular annealing at microhomologies. (**e**) Subsequent replacement of RPA with Rad51 promotes HR repair. (**f**) If there is insufficient RPA binding, spontaneous annealing at microhomologies can occur. (**g**) Further processing results in deletion formation by MMEJ repair.

MMEJ repair. The mutations had only minor effects on Rfa1 protein levels and did not affect DNA resection, thus indicating that the reduced ability of RPA to interact with ssDNA was responsible for the observed increase in MMEJ. In support of this, Deng *et al.*[3] purified mutant Rfa1–containing RPA complexes from *Escherichia coli* and showed that the mutant proteins interacted poorly with ssDNA in a DNA curtain assay. Moreover, the mutant RPA complexes were defective in preventing strand annealing *in vitro* and were also impaired in their ability to remove ssDNA structures.

An alternative explanation for the increased frequency of MMEJ observed in the *rfa1* mutants is that the inability of mutant RPA complexes to promote efficient recombination results in more MMEJ at the expense of HR. However, further testing of one of the *rfa1* mutants showed that it did not confer an HR defect. Furthermore, *rad51* and *rad52* mutants that were defective for HR displayed only a small increase in MMEJ, and this increase was not epistatic to the pronounced MMEJ increase observed in *rfa1* mutants. Therefore, the ability of RPA to suppress MMEJ repair is apparently independent of its function in homologous recombination.

These findings led Deng *et al.*[3] to propose that annealing at microhomologies during MMEJ is spontaneous and that RPA acts to inhibit this step. There is certainly supporting evidence for this conclusion in the literature. Prior studies showed that RPA binds tightly to ssDNA, at intervals of approximately 30 nucleotides per RPA heterotrimer, and prevents the formation of intramolecular secondary structures[14]. In this regard, RPA binding to two independent ssDNAs may also prevent the formation of intermolecular secondary structures during MMEJ.

Another important role of RPA is to recruit proteins required for particular steps of DNA replication and repair. In yeast, Rfa1 and Rfa2 collectively interact with many proteins involved in DNA metabolism[15]. Many of these interactions are dependent on the phosphorylation status of RPA, thus adding another layer of regulatory complexity. Similarly, human RPA1 interacts with many proteins crucial for DNA-damage signaling and repair, including p53, ATRIP, MRE11, RAD9 and the annealing helicase HARP (also known as SMARCAL1)[16]. Thus, an alternative (though not mutually exclusive) hypothesis is that a parallel function of RPA might be to recruit additional proteins that preclude or impair the annealing, trimming and/or fill-in–synthesis steps of MMEJ.

An outstanding question in the field of DNA repair is the relationship of MMEJ to other types of nonclassical end-joining repair observed in metazoans, collectively referred to as alternative end joining, or A-EJ[17–19]. Like MMEJ, the frequency of A-EJ increases in the absence of the Ku and DNA ligase 4 complexes. However, A-EJ does not always involve annealing at microhomologous sequences, especially those of the length observed in MMEJ in yeast[20]. Furthermore, A-EJ can produce repair junctions with no deletions and can even give rise to junctions containing insertions that appear to be templated from adjacent sequences[18]. Could RPA also prevent A-EJ repair, given that intermolecular annealing at long microhomologies seems to be less crucial for A-EJ? This intriguing possibility will require further experimentation in other systems.

As previously mentioned, one of the perplexing questions pertinent to MMEJ is why it is used in contexts in which the option of HR repair is available. The findings of Deng *et al.*[3] suggest that it may be the amount or configuration of RPA-coated ssDNA that regulates MMEJ. Exactly how much RPA needs to be present to prevent intermolecular annealing? Can differences in the local concentrations of RPA explain situations in which MMEJ occurs even when HR should be the preferred repair outcome? Addressing these questions will be necessary to fully understand the regulation of MMEJ and A-EJ frequency in yeast and other organisms. The study by Deng *et al.*[3] lays the groundwork for future investigations into how cells keep tight rein over error-prone double-strand-break repair and thereby prevent unnecessary genomic instability.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Zhang, Y. & Jasin, M. *Nat. Struct. Mol. Biol.* **18**, 80–84 (2011).
2. Povirk, L.F. *DNA Repair (Amst.)* **5**, 1199–1212 (2006).
3. Deng, S. *et al. Nat. Struct. Mol. Biol.* **21**, 405–412 (2014).
4. Ma, J.L. *et al. Mol. Cell. Biol.* **23**, 8820–8828 (2003).
5. Lee, K. & Lee, S.E. *Genetics* **176**, 2003–2014 (2007).
6. Fattah, F. *et al. PLoS Genet.* **6**, e1000855 (2010).
7. Symington, L.S. & Gautier, J. *Annu. Rev. Genet.* **45**, 247–271 (2011).
8. Truong, L.N. *et al. Proc. Natl. Acad. Sci. USA* **110**, 7720–7725 (2013).
9. Grabarz, A. *et al. Am. J. Cancer Res.* **2**, 249–268 (2012).
10. Sugiyama, T., New, J.H. & Kowalczykowski, S.C. *Proc. Natl. Acad. Sci. USA* **95**, 6049–6054 (1998).
11. Smith, J. & Rothstein, R. *Mol. Cell. Biol.* **15**, 1632–1641 (1995).
12. Smith, J. & Rothstein, R. *Genetics* **151**, 447–458 (1999).
13. Umezu, K. *et al. Genetics* **148**, 989–1005 (1998).
14. Prakash, A. & Borgstahl, G.E. *Subcell. Biochem.* **62**, 171–196 (2012).
15. Chen, S.H. *et al. J. Biol. Chem.* **285**, 12803–12812 (2010).
16. Oakley, G.G. & Patrick, S.M. *Front. Biosci.* (Landmark Ed.) **15**, 883–900 (2010).
17. Decottignies, A. *Front. Genet.* **4**, 48 (2013).
18. McVey, M. & Lee, S.E. *Trends Genet.* **24**, 529–538 (2008).
19. Taylor, E.M. *et al. Nucleic Acids Res.* **38**, 441–454 (2010).
20. Mansour, W.Y., Rhein, T. & Dahm-Daphi, J. *Nucleic Acids Res.* **38**, 6065–6077 (2010).