

UC Irvine

UC Irvine Previously Published Works

Title

Phospho-dependent recruitment of the yeast NuA4 acetyltransferase complex by MRX at DNA breaks regulates RPA dynamics during resection

Permalink

<https://escholarship.org/uc/item/6s88z9d0>

Journal

Proceedings of the National Academy of Sciences of the United States of America, 115(40)

ISSN

0027-8424

Authors

Cheng, Xue
Jobin-Robitaille, Olivier
Billon, Pierre
et al.

Publication Date

2018-10-02

DOI

10.1073/pnas.1806513115

Peer reviewed



Phospho-dependent recruitment of the yeast NuA4 acetyltransferase complex by MRX at DNA breaks regulates RPA dynamics during resection

Xue Cheng^{a,1}, Olivier Jobin-Robitaille^{a,1}, Pierre Billon^{a,2}, Rémi Buisson^{a,3}, Hengyao Niu^{b,4}, Nicolas Lacoste^{a,5}, Nebiyu Abshiru^{c,d}, Valérie Côté^a, Pierre Thibault^{c,d}, Stephen J. Kron^e, Patrick Sung^b, Christopher J. Brandl^f, Jean-Yves Masson^a, and Jacques Côté^{a,6}

^aSt. Patrick Research Group in Basic Oncology, Laval University Cancer Research Center, Centre de Recherche du Centre Hospitalier Universitaire de Québec-Axe Oncologie, Québec City, QC G1R 3S3, Canada; ^bDepartment of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06520; ^cInstitute for Research in Immunology and Cancer, Université de Montréal, Montréal, QC H3C 3J7, Canada; ^dDépartement de Pathologie et Biologie Cellulaire, Université de Montréal, Montréal, QC H3C 3J7, Canada; ^eDepartment of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637; and ^fDepartment of Biochemistry, The University of Western Ontario, London, ON N6A 5C1, Canada

Edited by Fred M. Winston, Harvard Medical School, Boston, MA, and approved August 21, 2018 (received for review April 16, 2018)

The KAT5 (Tip60/Esa1) histone acetyltransferase is part of NuA4, a large multifunctional complex highly conserved from yeast to mammals that targets lysines on H4 and H2A (X/Z) tails for acetylation. It is essential for cell viability, being a key regulator of gene expression, cell proliferation, and stem cell renewal and an important factor for genome stability. The NuA4 complex is directly recruited near DNA double-strand breaks (DSBs) to facilitate repair, in part through local chromatin modification and interplay with 53BP1 during the DNA damage response. While NuA4 is detected early after appearance of the lesion, its precise mechanism of recruitment remains to be defined. Here, we report a stepwise recruitment of yeast NuA4 to DSBs first by a DNA damage-induced phosphorylation-dependent interaction with the Xrs2 subunit of the Mre11-Rad50-Xrs2 (MRX) complex bound to DNA ends. This is followed by a DNA resection-dependent spreading of NuA4 on each side of the break along with the ssDNA-binding replication protein A (RPA). Finally, we show that NuA4 can acetylate RPA and regulate the dynamics of its binding to DNA, hence targeting locally both histone and nonhistone proteins for lysine acetylation to coordinate repair.

chromatin | lysine acetylation | DNA double-strand break | NuA4 | RPA

NuA4 is a highly conserved multisubunit histone acetyltransferase (HAT) complex responsible for the acetylation of nucleosomal histone H4 and H2A (and variants H2A.Z/X) through its KAT5 (Esa1/Tip60) catalytic subunit (1). As the only essential HAT in yeast, NuA4 regulates through chromatin modification the expression of specific genes from transcription activation to elongation (2, 3), but it also regulates lifespan and autophagy through acetylation of nonhistone proteins (4–6). In higher eukaryotes, NuA4/TIP60 is essential for the maintenance and renewal of stem cells, a key regulator of cell homeostasis and response to stress, and a haplo-insufficient tumor suppressor (1). NuA4 is also critical for genome stability, as it is rapidly recruited to chromatin surrounding a DNA double-strand break (DSB) to facilitate repair (7), regulate DNA damage signaling, and counteract 53BP1 to favor homologous recombination (HR) (8, 9).

The repair of DSBs is critical for genome stability, and several studies over the past decade have implicated chromatin modifiers and remodelers in that process throughout eukaryotes. Histone phosphorylation, methylation, and ubiquitination have been shown to play key roles in the association of repair factors to the DNA damage site (9–11). Since NuA4 can interact with DNA damage-induced phosphorylated H2A(X) [γ H2A(X)] around DSBs (7), we proposed that this interaction was important for NuA4 appearance at the break and that acetylation by NuA4 affects later steps of chromatin remodeling and repair (12, 13). In this study, we dissected mechanisms of NuA4 recruitment and function at DNA breaks, uncovering a stepwise process initiated by a critical phospho-dependent interaction with the Mre11-Rad50-Xrs2 (MRX) complex

followed by spreading during DNA resection and regulation of replication protein A (RPA) dynamics through lysine acetylation.

Results

The MRX Complex Is Required for Recruitment of NuA4 at a DNA Break. To understand the exact mechanism of NuA4 recruitment around DSBs, we performed chromatin immunoprecipitation (ChIP) experiments with an inducible DSB system (*pGAL-HO*, *hmlΔ/hmrΔ*) in different mutant backgrounds. Eaf1 antibody was used, since it is the only subunit unique to NuA4 (14). Consistent with previous results, we observed NuA4 enrichment on both sides of the DNA break in wild-type cells after

Significance

Double-strand breaks (DSBs) in the DNA are the most dangerous type of damage and must be repaired to maintain genome integrity, avoiding disease-linked mutations and cancer initiation/progression. The NuA4 histone acetyltransferase complex is recruited to DNA DSBs to facilitate repair through modification of the chromatin surrounding the damage, but its recruitment mechanism is poorly understood. Here, we report that the DNA damage sensor complex Mre11-Rad50-Xrs2 physically recruits NuA4 to the break sites followed by bidirectional spreading linked to the DNA end resection required for error-free repair by homologous recombination. During that process, NuA4 can acetylate ssDNA-binding replication protein A (RPA), leading to its displacement.

Author contributions: X.C., O.J.-R., P.B., R.B., V.C., P.T., S.J.K., C.J.B., J.-Y.M., and J.C. designed research; X.C., O.J.-R., P.B., R.B., N.L., N.A., V.C., C.J.B., and J.C. performed research; H.N., N.L., S.J.K., P.S., C.J.B., and J.-Y.M. contributed new reagents/analytic tools; X.C., O.J.-R., P.B., R.B., N.A., V.C., P.T., C.J.B., and J.C. analyzed data; and X.C. and J.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Published under the PNAS license.

¹X.C. and O.J.-R. contributed equally to this work.

²Present address: Department of Genetics and Development, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY 10032.

³Present address: Department of Biological Chemistry, University of California, Irvine, CA 92697.

⁴Present address: Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN 47405.

⁵Present address: Institut NeuroMyoGene, Ecole Normale Supérieure de Lyon, 69364 LYON cedex 07, France.

⁶To whom correspondence should be addressed. Email: Jacques.Cote@crhdq.ulaval.ca.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1806513115/-DCSupplemental.

Published online September 17, 2018.

induction of the HO cleavage at the *MAT* locus (Fig. 1*A* and *SI Appendix, Fig. S1A*). However, when we used a mutant deficient for the DNA damage-induced phosphorylation of local H2A (H2AS129A), we still detected very efficient recruitment of NuA4 around the DSB even in the absence of γ H2A(X), as it was previously reported for chromatin remodelers (Fig. 1*B* and *SI Appendix, Fig. S1B and C*) (15). These results indicate that NuA4 interaction with γ H2A is not responsible for NuA4 initial recruitment at DSBs, but more likely for its local stabilization/accumulation. Thus, another molecular interaction is required for NuA4 early association with DNA breaks.

The Tra1 subunit of yeast NuA4 and its human homolog TRRAP have similar domain arrangements as the pioneer DNA damage-sensing kinases Mec1 and Tel1 in yeast and ATR, ATM, and DNA-PKcs in humans (Fig. 1*C*). These kinases are members of the phosphoinositide 3-kinase-related protein kinase (PIKK) family and function as damage signal transducers, with Tel1/ATR and DNA-PKcs being important for DSB repair and with Mec1/ATR responding to ssDNA generated by lesion processing (16). Importantly, the activation of these transducer proteins requires their recruitment to the damage sites by sensor proteins. For example, while the Xrs2/Nbs1 subunit of the MRX/N complex recruits Tel1/ATR, Mec1/ATR recruitment requires Lcd1/ATRIP, and DNA-PKcs recruitment is mediated by the Ku70-Ku80 dimer (*SI Appendix, Fig. S1D*) (17, 18). Consistent with the notion of PIKK recruitment for activation, Tra1, which lacks kinase activity, has been linked to the

recruitment of NuA4 to promoters through its interaction with the Gal4 and Gcn4 transcription activators (19–21). Thus, it was tempting to speculate that the factors known to recruit PIKK proteins could be responsible for NuA4 recruitment (*SI Appendix, Fig. S1D*). To test this possibility, we performed ChIP in different mutants of the candidate recruitment factors. We observed that, in the *yku80* Δ background, there is still recruitment of NuA4 at the break (Fig. 1*D* and *SI Appendix, Fig. S1E*). In parallel, the deletion of *LCD1*/ATRIP did not significantly affect NuA4 recruitment (Fig. 1*E* and *SI Appendix, Fig. S1E*). By contrast, the deletion of *XRS2* totally abolished the appearance of NuA4 around the DSB (Fig. 1*F* and *SI Appendix, Fig. S1F*), which was confirmed with the two other subunits of the MRX complex, Mre11 and Rad50 (Fig. 1*G* and *H* and *SI Appendix, Fig. S1G*). These results indicate that NuA4 recruitment is specifically dependent on the MRX complex, perhaps through an interaction between MRX and Tra1.

DNA Damage Induces a Phospho-Dependent Interaction of NuA4 with Xrs2. To test whether there could be a direct interaction between Tra1 and different PIKK recruitment factors, we performed GST pull-down assays using recombinant proteins. We observed a direct interaction between Tra1 PI3K-FATC domain and the N terminus of Xrs2 as well as Lcd1 (Fig. 2*A* and *SI Appendix, Fig. S2A*). To verify these results in the context of the native NuA4 complex, recombinant recruitment factors were used to pull down NuA4 complex purified from yeast cells (*SI Appendix, Fig. S2A*).

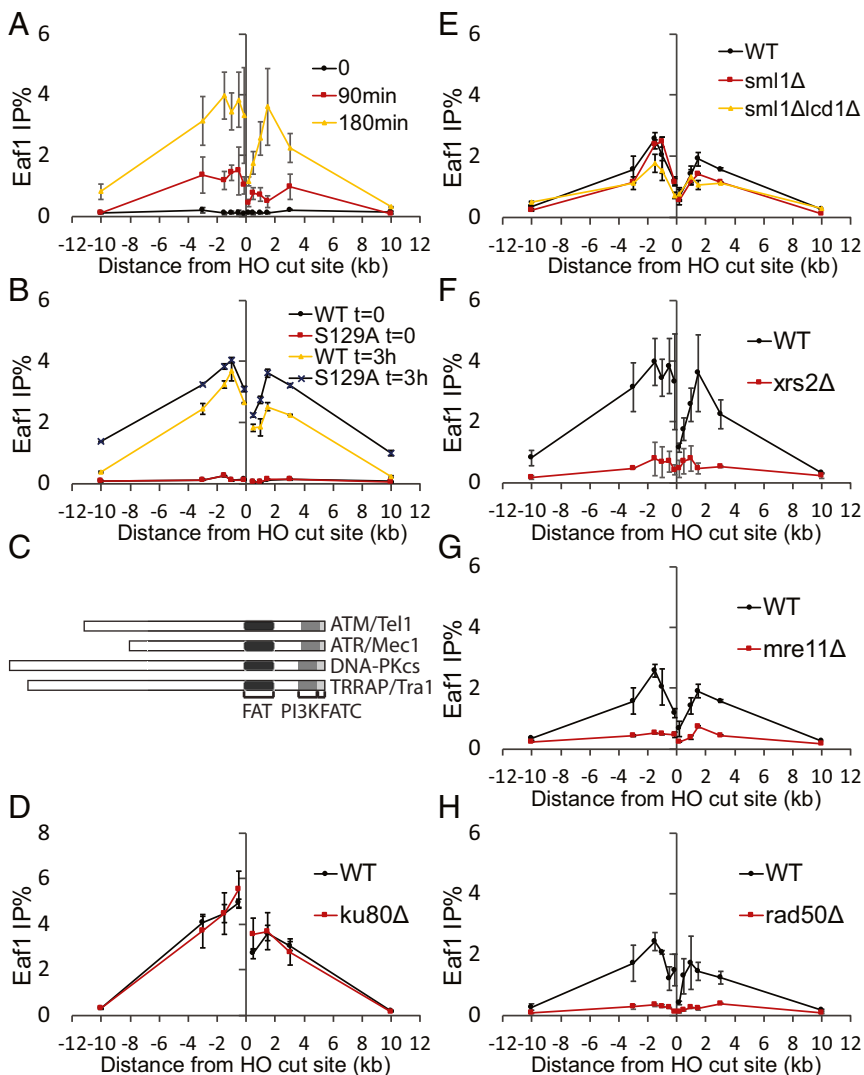


Fig. 1. NuA4 recruitment around DNA breaks depends on MRX. (A) ChIP assay of Eaf1 with time course DSB induction at *MAT* locus (0, 90, 180 min). Precipitated DNA was analyzed by qPCR with primers scanning both sides of the break up to 10 kb. (B) ChIP-qPCR assay as in A in the wild type (WT) and H2AS129A mutant with no damage or on 3 h of HO induction. (C) Schematic representation of domain arrangement of TRRAP/Tra1 and PIKK family kinases ATM/Tel1, ATR/Mec1, and DNA-PKcs. (D–H) ChIP-qPCR assay as in A in the WT and *yku80* Δ (D), *sml1* Δ , *sml1* Δ *lcd1* Δ (E), *xrs2* Δ (F), *mre11* Δ (G), and *rad50* Δ (H) strains on 3-h DSB induction. Error bars represent the range from two biological replicates (B, E, G, and H) or SE from three biological replicates (A, D, and F).

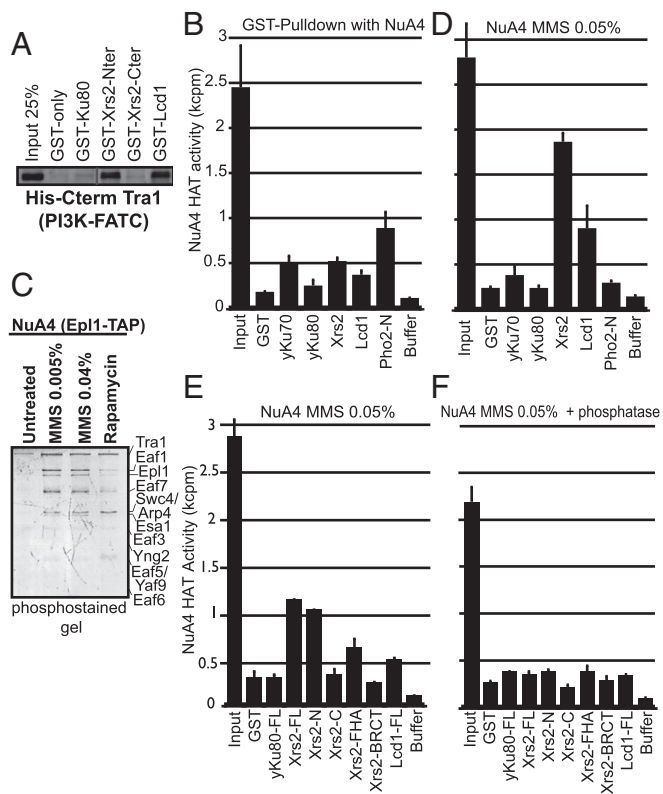


Fig. 2. DNA damage-induced phosphorylation of NuA4 is required to interact with Xrs2. (A) Pull-down assay with indicated recombinant proteins was visualized by Western blot. (B) Pull down of NuA4 with recombinant GST-yKu70, yKu80, Xrs2, Lcd1, and Pho2 was measured by HAT assay. NuA4 was purified from undamaged cells. (C) Purified NuA4 from untreated, MMS-treated, or rapamycin-treated cells (2 h) was stained with Pro-Q diamond phosphoprotein gel stain (P33300; Invitrogen) following the manufacturer's instruction. (D–F) Pull-down assay as in A with NuA4 purified from MMS-treated cells. In F, 5 μ l of λ -phosphatase (P0753; NEB) was added to the reaction, incubated at 30 °C for 30 min, and repeated once. Error bars represent the range from two technical replicates.

The Pho2 transcription factor served as a positive control, since it directly interacts with NuA4 (22). As none of the damage-sensing recruitment factors pulled down NuA4 activity (Fig. 2B), we considered the possibility that the interaction could be mediated by DNA damage-induced phosphorylation events. Indeed, NuA4 purified from cells treated with the DNA damaging agent methylmethane sulfonate (MMS) showed a distinct phosphorylation pattern for several subunits (such as Eaf1, Epl1, Eaf7, and Esa1) compared with untreated or treated with rapamycin (Fig. 2C). This NuA4 complex modified in conditions of DNA damage response can now be efficiently pulled down by Xrs2 and to a lesser extent, by Lcd1 (Fig. 2D). Additional analysis identified the N-terminal FHA phospho-binding domain of Xrs2 as the interaction interface (Fig. 2E). Pretreating purified NuA4 with λ -phosphatase abolished interaction with Xrs2, confirming that it depends on NuA4 phosphorylation induced by DNA damage (Fig. 2F). We attempted to identify the DNA damage-induced phosphorylation sites on NuA4 subunits responsible for the interaction with Xrs2. Unfortunately, mutation of the Mec1/Tel1-dependent phosphorylation sites that have been mapped on NuA4 subunits, either alone or in combination, failed to render cells sensitive to DNA damaging agents (*SI Appendix, Fig. S2 B–D*) (23–26). Nevertheless, our results support a phospho-dependent recruitment of NuA4 at DNA breaks by the Xrs2 FHA domain, potentially through the Tra1 subunit. Although the FHA domain of Xrs2/Nbs1 is important for phospho-dependent interactions with several repair factors (18)

and NuA4, the phosphorylation sites in the NuA4 complex that are germane for DNA damage recruitment remain to be identified. Interestingly, the FHA domain of Xrs2/Nbs1 interacts with CK2 phosphorylation motifs (27, 28), and Tra1 contains related consensus CK2 phosphorylation sites, offering a potential avenue for future investigation.

After Initial Recruitment, NuA4 Spreads on Each Side of the Break During End Resection. The MRX complex promotes HR, which mainly takes place in the S/G2 phases of the cell cycle (29). To test whether NuA4 recruitment at DNA breaks is similarly cell cycle dependent, NuA4 enrichment was measured in cells arrested in G1 or G2/M. We observed strong NuA4 recruitment at the HO DSB in G2/M cells as well as in asynchronous cells, while little recruitment was detected in G1 blocked cells (Fig. 3A and *SI Appendix, Fig. S3A*). Furthermore, since NuA4 recruitment is increased in *yku70* Δ or *yku80* Δ cells in G1 (Fig. 3B), and Ku inhibits the resection of DNA ends (30), we asked whether the increase of NuA4 is due to elevated resection in these mutants. Indeed, inhibiting resection by overexpression of the CDK1 inhibitor Sic1 (31) greatly compromised NuA4 recruitment (Fig. 3B).

To further determine the relationship between DNA end resection and NuA4 recruitment, we measured NuA4 enrichment in cells mutated for resection factors. Individually deleting *EXO1*, *SGS1*, or *SAE2* did not significantly diminish recruitment (Fig. 3C). However, by deleting both *EXO1* and *SGS1*, which mediate distinct resection mechanisms in parallel, we observed a loss of NuA4 recruitment at 1 kb from the DSB (Fig. 3C and *SI Appendix, Fig. S1F*). This was accompanied by a clear retention of nucleosomes and strong accumulation of γ H2A in close proximity to the break in contrast to the usual depletion seen in wild-type conditions (*SI Appendix, Fig. S3 B and C*). An extended analysis of the region surrounding the DNA break revealed that NuA4 recruitment still occurred but became confined to the close proximity to the DSB instead of the several kilobases recruitment domain observed in wild-type cells (Fig. 3D). Together, these results indicate that NuA4 recruitment at DNA breaks occurs in a two-step mechanism, whereby NuA4 is first recruited by MRX on its DNA damage-induced phosphorylation and then spreads during DNA end resection on each side of the DSB.

The single-stranded DNA (ssDNA) structure generated during resection serves as the recruitment platform for the ssDNA-binding protein RPA, which is subsequently replaced by the

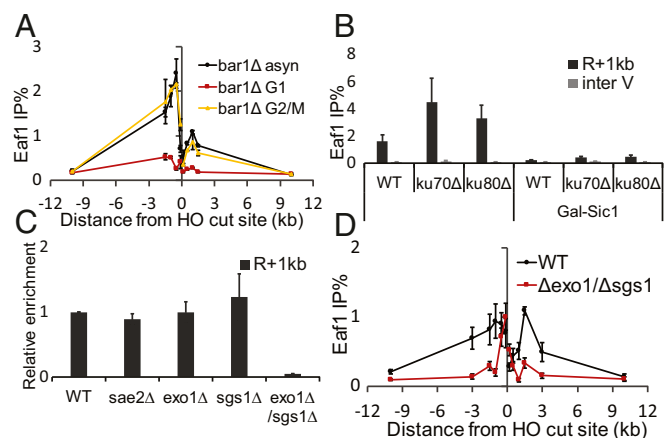


Fig. 3. NuA4 recruitment at DNA breaks occurs mainly in G2 and is followed by spreading during resection. (A) ChIP-qPCR of Eaf1 after 3-h HO induction in *bar1* Δ cells with/without synchronization. (B) ChIP-qPCR of Eaf1 in wild-type (WT), *yku70* Δ , *yku80* Δ , Gal-Sic1, Gal-Sic1 *yku70* Δ , and Gal-Sic1 *yku80* Δ cells. Cells were synchronized in G1 with 15 μ g/mL α -factor for 3 h followed by 3 h of 2% galactose induction before cross-linking. (C) ChIP-qPCR of Eaf1 in the WT and indicated resection mutants. WT value was normalized to one. (D) ChIP-qPCR of Eaf1 in the WT and *exo1* Δ *sgs1* Δ strain. Error bars represent the range from two biological replicates.

recombinase Rad51 for the catalysis of DSB repair by HR (32). We considered the possibility that NuA4 spreading during resection might occur through direct binding to ssDNA. The NuA4 subunit Swc4 contains a SANT/Myb-like DNA-binding domain related to the one present in telomere-binding protein Tbf1 (14, 33). Using a gel shift assay with recombinant Swc4, we detected some binding to ssDNA and a DNA molecule with a 3' overhang mimicking a resected DNA end (*SI Appendix, Fig. S4A*). Furthermore, yeast cells carrying a point mutation in the Swc4 SANT domain (*swc4-23*, thermosensitive) (34) are hypersensitive to DNA damage (*SI Appendix, Fig. S4B*) and show a significant decrease in NuA4 enrichment near the HO break (*SI Appendix, Fig. S4 C and D*). These results suggest that NuA4 may spread on ssDNA generated during DNA end resection through direct binding. Additional detailed studies will be required to verify this hypothesis.

NuA4 and RPA Interact on ssDNA During Resection. Since RPA is present on the ssDNA during resection, we next asked whether there are physical and functional interactions between RPA and NuA4. NuA4 coimmunoprecipitated with Rfa1, the largest subunit of RPA, from whole-cell extracts but only when cells are treated with MMS (Fig. 4A). Reciprocal immunoprecipitation (IP) confirmed this DNA damage-dependent interaction. To verify this result within the cellular setting, we used a ChIP-mass spectrometry (MS) approach to analyze proteins that colocalize with RPA after DSB induction. DNA repair factors and NuA4 components were clearly detected with RPA upon DSB induction (Fig. 4B). Interestingly, ChIP-qPCR analysis revealed increased NuA4 recruitment at the HO break in RPA mutant cells (*rfa1-D228Y*), a mutant with less affinity for ssDNA without affecting resection per se, which we also confirmed by ChIP assay (Fig. 4C and *SI Appendix, Fig. S4 E and F*) (35, 36). These results suggest that, while RPA is not required for NuA4 spreading during resection, there may be a functional incompatibility between NuA4 and RPA on the ssDNA. Importantly, repair factors Rad51 and Rad52, required for HR (both) or single-strand annealing (Rad52), did not significantly affect NuA4 binding around the HO break (Fig. 4D and E),

nor did the 9-1-1 clamp or clamp loader (*SI Appendix, Fig. S4 G and H*). Future studies will determine if NuA4 and ATP-dependent chromatin remodelers spread on resected DNA ends via a similar mechanism (15).

NuA4 Acetylates RPA and Modulates Its Binding to ssDNA. To further investigate the functional cross-talk between RPA and NuA4 during DNA end resection, we used a reconstituted in vitro system with purified NuA4 and RPA complexes (*SI Appendix, Fig. S5A*) and ssDNA bound to magnetic beads. RPA was first preloaded on the DNA, and NuA4 was then added to measure possible competition between the two complexes for the ssDNA (Fig. 5A). Just adding NuA4 complex to RPA-coated ssDNA did not affect the RPA (Rfa2) signal associated with the beads (Fig. 5B and C, lanes 1–3). Strikingly, the addition of AcCoA led to a clear NuA4-dependent displacement of RPA from the ssDNA (Fig. 5B and C, lanes 4–6). The extent of the effect correlates with the incubation time (Fig. 5B) and the amount of NuA4 in the reaction (Fig. 5C), but the displacement was not observed in *Esa1* catalytically dead mutants (*SI Appendix, Fig. S5B*) (37, 38). These results with a purified reconstituted system imply that NuA4 targets RPA for acetylation, which affects its interaction with ssDNA. To confirm that NuA4-dependent RPA acetylation occurs in vivo, we performed acetyllysine IP in extracts from wild-type and thermosensitive *esa1* mutant cells [*esa1-L254P* (39)] at nonpermissive temperature (Fig. 5D and *SI Appendix, Fig. S5C*). Less RPA signal was detected in the *esa1* mutant sample in the presence of DNA damage, arguing that NuA4 is indeed involved in RPA acetylation in vivo. Different studies, including our work, have identified multiple in vivo lysine acetylation sites in the heterotrimeric yeast and human RPA (*SI Appendix, Fig. S5 D–F*). To determine whether acetylation of RPA is functionally relevant, we generated mutants within residues that are acetylated in yeast or in human RPA on lysines that are conserved in yeast (Rfa1 K259, K463, K494) (40–42) (our work in *SI Appendix, Fig. S5 D–F*). Mutation of lysine to arginine blocks acetylation but conserves the positive charge, while glutamine residues mimic acetylated lysines. Substitution of all three aforementioned lysines with arginine did not affect cell growth or the cellular resistance to DNA damage (Fig. 5E and *SI Appendix, Fig. S5 G and H*). Similarly, substituting them for three glutamine residues does not affect normal cell growth or resistance to UV, indicating that RPA function on ssDNA during replication fork progression is not affected, and also, its role in nucleotide excision repair is not affected (Fig. 5E, *Top Left* and *SI Appendix, Fig. S5H*). In contrast, growth on media containing chemicals that cause DNA breaks is as strongly impaired as seen for *sae2Δ* mutant cells (Fig. 5E). The most striking sensitivity was detected in the presence of the topoisomerase I inhibitor camptothecin (CPT), which induces DSBs arising from the collapse of DNA replication forks (Fig. 5E, *Bottom Left*). We then analyzed the binding of these mutant RPA molecules during resection at the HO DSB in vivo and found a clear defect of Rfa1-QQQ association near the break, in agreement with the in vitro data (Fig. 5F and *SI Appendix, Fig. S5I*). Surprisingly, the Rfa1-RRR mutant was also affected but to a lesser extent, suggesting that dynamic acetylation/deacetylation is important for RPA function during resection. Altogether, our in vitro and in vivo data point to an important functional interaction between NuA4 and RPA, providing yet another regulatory mechanism of DNA damage response through the direct action of a chromatin-modifying enzyme on substrates beyond histones/chromatin.

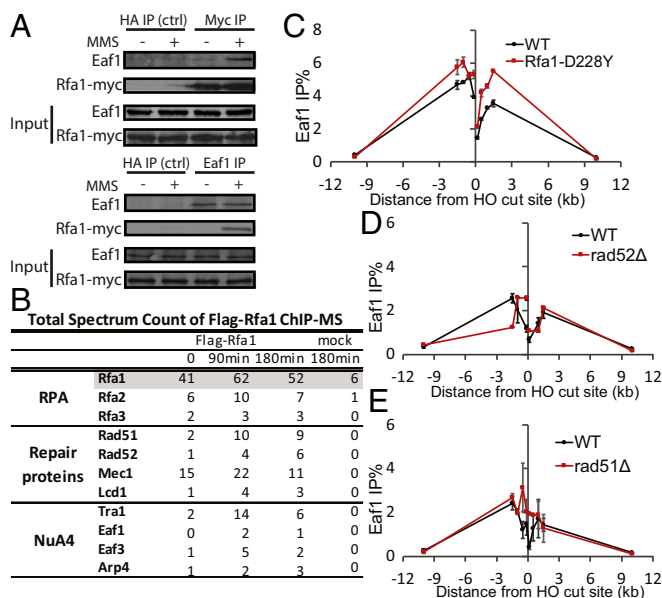


Fig. 4. NuA4 interacts with RPA on DNA damage. (A) Co-IP and reciprocal co-IP of 13myc-Rfa1 and Eaf1 from whole-cell extracts of cells untreated or treated with MMS 0.05% for 1 h. HA IP serves as the negative control. (B) List of proteins and number of total peptides detected in ChIP of Flag-Rfa1 followed by MS analysis from cells after 0, 90, or 180 min of HO induction. (C–E) ChIP-qPCR of Eaf1 after 3-h HO induction in the wild type (WT) and *rfa1-D228Y* (C), *rad52Δ* (D), and *rad51Δ* (E). Error bars represent the range from two biological replicates.

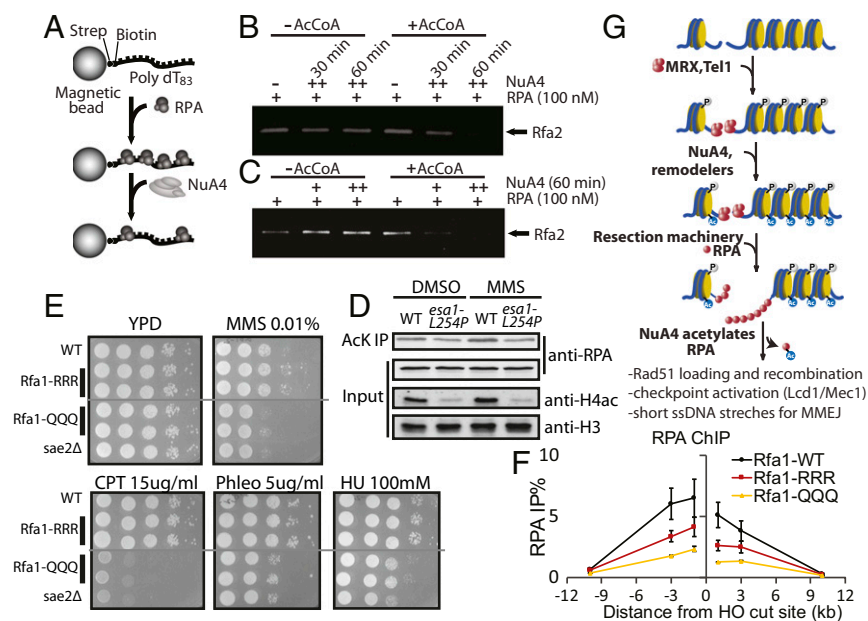


Fig. 5. NuA4 displaces RPA from ssDNA in an AcCoA-dependent manner. (A) Schematic representation of biotin-ssDNA pull-down assay. (B and C) Biotin-ssDNA pull-down assay under the indicated conditions was loaded onto 8% SDS/PAGE, stained by Sypro Ruby, and visualized under UV. B shows different incubation times (0/30/60 min), and C shows different amounts of NuA4 added to the reaction. (D) Western blot analysis with indicated antibodies of IPs with acetyllysine antibody (ICP0380; Immunechem) in whole-cell extracts (input) from wild-type (WT) and *esa1-L254P* cells treated with 0.05% DMSO or MMS at 37 °C for 1 h. (E) Tenfold serial dilutions of the WT, genome-integrated Rfa1 mutants (K259/463/494), and *sae2Δ* were spotted onto yeast extract peptone dextrose (YPD) or YPD containing MMS, CPT, phleomycin, or hydroxyurea (HU) at the indicated concentrations and grown at 30 °C. (F) ChIP-qPCR of RPA after 3-h HO induction in the WT and RRR/QQQ mutants. (G) Model for stepwise recruitment of NuA4 around DNA breaks.

acetylation around the break site to facilitate chromatin relaxation by ATP-dependent remodelers, like SWI/SNF (13) and Rvb1/2-containing complexes (7). Subsequently, NuA4 spreads on each side of the DSB concomitant with DNA end resection, where it regulates the dynamics of RPA binding to ssDNA via its acetylation. Future work will address the functional consequences of RPA regulation by NuA4 during the repair of DSBs. It is possible that NuA4 acetylates RPA to facilitate subsequent loading of Rad51 for the mediation of HR or Lcd1-Mec1 for DNA damage checkpoint regulation. Moreover, as RPA can antagonize microhomology-mediated end joining (MMEJ) (36), RPA displacement/destabilization by NuA4 may expose only short stretches of ssDNA that can channel the DSB repair pathway away from HR and more toward MMEJ. Based on recent analysis of RPA mutants with less affinity for ssDNA, another possibility is that NuA4-dependent acetylation may favor single-strand annealing and/or regulates break-induced replication (43). All of these different possible pathways will need to be analyzed in the context of RPA acetylation.

The understanding of how a DNA damage site is repaired by the cell has been a focus of intense research because of its link to genome stability and human disease. The discovery of the initial recruitment of a chromatin modifier complex by MRX to a DNA break is an important mechanistic insight. In gene regulation, NuA4-dependent acetylation is known to promote the association and action of ATP-dependent remodelers SWI/SNF, RSC, and SWR1 (1), and they are also implicated in DSB repair throughout eukaryotes (9, 44). Resection-dependent recruitment of NuA4 seems similar to what has been reported for a host of chromatin remodeling enzymes (15), while NuA4 activity has also been shown to regulate their recruitment (7, 13). Subsequent action of NuA4 during DNA end resection led us to uncover a regulatory step by targeting a nonhistone substrate at the heart of the DNA repair process. Based on the high degree of evolutionary conservation of these chromatin-modifying/remodeling complexes, repair proteins, and lysine acetylation sites, these mechanistic findings are expected to be conserved in higher eukaryotes.

Materials and Methods

SI Appendix, SI Materials and Methods provides details about ChIP-qPCR assay, GST pull down, gel shift assay, acetyllysine IP, and mutant *Esa1/NuA4*.

Yeast Strains, Materials, and MS. Yeast strains used in this study are listed in *SI Appendix, Table S1* and were constructed based on standard PCR-based transformation protocol. NuA4 and RPA complexes were purified from yeast cells as previously described (2, 45). Analysis of phosphorylation sites on

NuA4 purified from cell treated with MMS was performed after separation on SDS/PAGE followed by in-gel digestion of protein bands. Reduction and alkylation of cysteine residues were performed with 100 μ L of 20 mM tris(2-carboxyethyl)phosphine (TCEP) and 40 mM Iodoacetamide, respectively. After digestion with 2 μ g of trypsin in 50 mM ammonium bicarbonate, peptides were extracted with 70% acetonitrile, dried, resuspended in 0.2% formic acid, and analyzed on LTQ-Orbitrap XL MS coupled to an Eksigent nano-LC system. Raw liquid chromatography-MS/MS data were searched against a custom database with Mascot search engine v2.10 (Matrix science).

ChIP and ChIP-MS. ChIP assay was essentially performed as described previously (2, 46) using anti-Eaf1 (14), anti-H3 (Ab1791; Abcam), anti-RFA (AS07 214; Agrisera), and anti- γ -H2A antibodies (07-745; Upstate). IP percentage is presented as the value of the IP sample over input. A list of primers used in PCR is presented in *SI Appendix, Table S2*. *SI Appendix* provides a more detailed ChIP protocol. For ChIP-MS, endogenous Rfa1 was tagged with Flag and subjected to ChIP modified as follows. One milligram of chromatin was incubated with 6 μ g of anti-Flag M2 antibody (F1804; Sigma) overnight at 4 °C; 200 μ L of dynabeads coated with protein G (10004D; Life Technologies) were added and placed on a rotating wheel at 4 °C for 4 h. After washing five times with formaldehyde (FA) lysis buffer, triple Flag peptide (F4799; Sigma) was added to a final concentration of 0.5 mg/mL to elute the bound fraction from the beads. Eluted fraction was loaded to 12% Bis-Tris precast polyacrylamide gel, Sypro Ruby (Bio-Rad) stained, and sent for MS analysis at the Quebec Genome Center.

GST Pull-Down Assay. GST pull-down assay was performed as described previously (46). GST expression plasmids were based on pGEX-4T3 backbone and constructed by conventional cloning containing full-length proteins or partial proteins: Xrs2-N-term (amino acids 1–423), Xrs2-C-term (amino acids 424–854), Xrs2-FHA (amino acids 1–117), Xrs2-BRCT (amino acids 116–423), and Swc4-N-term (amino acids 1–285); 6xHis-tag expression plasmid was based on pET15b backbone and constructed by conventional cloning containing Tra1-C-term (amino acids 3201–3744). *SI Appendix* provides a more detailed GST pull-down protocol.

Biotin-ssDNA Pull-Down and Gel Shift Assays. Biotin-ssDNA pull-down assay and gel shift assay with different ³²P-labeled DNA molecules were performed essentially as previously described (47). For biotin-ssDNA pull-down assay, magnetic beads containing 5'-biotinylated ssDNA poly dT 83-mer (corresponding to the final concentration of 1 μ M nucleotide) were resuspended in 16 μ L of reaction buffer (50 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM PMSF, 10 mM Na-Butyrate, 0.5 mM MgCl₂, 100 μ g/mL BSA with or without 0.15 mM AcCoA). One hundred nanomolar RPA (in 1 μ L of 20 mM KH₂PO₄, pH 7.4, 200 mM KCl, 0.5 mM EDTA, 1 mM DTT, 10% glycerol) was added, and the reaction was incubated for 5 min at 30 °C. Then, NuA4 complex (in 3 μ L of 20 mM HEPES, pH 7.5, 5% glycerol, 10 μ M ZnCl₂, 1 mM DTT, 1 mM PMSF, 100 mM KCl)

was added for 30 or 60 min at 30 °C. The beads were captured and washed twice with 20 μ L of washing buffer (50 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM PMSF, 10 mM Na-Butyrate, 0.5 mM MgCl₂, 40 mM KCl). Finally, 15 μ L of Laemmli buffer 1 \times was added followed by heating 5 min at 95 °C. The beads were spun down, and the supernatants were analyzed by SDS/PAGE (8%). The gel was stained with Sypro Ruby (Invitrogen) and visualized under UV.

NuA4-Rfa1 Co-IP. Cells were treated with 0.05% MMS (or DMSO in control) for 1 h. Cells were lysed in 10 mM Tris, pH 8.0, 150 mM NaCl, 10% glycerol, 0.1% Nonidet P-40, 2 μ g/mL leupeptin, 2 μ g/mL pepstatin A, 5 μ g/mL aprotinin, 1 mM PMSF, 10 mM β -glycerophosphate, 1 mM Na-Butyrate, 0.5 mM NaF, and 1 mM DTT. Soluble fraction was collected by centrifugation, and each IP was carried out using 1 mg of protein extract in addition to the antibody (anti-HA negative control, anti-myc, or anti-Eaf1 as indicated) overnight at 4 °C. Beads coated with protein G were added to the mixture for 4

additional hours. After extensive washes in 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 10% glycerol, 0.1% Nonidet P-40, and 1 mM PMSF, beads were resuspended in Laemmli buffer 1 \times buffer, boiled, and analyzed by SDS/PAGE followed by Western blotting using the indicated antibodies.

ACKNOWLEDGMENTS. We thank Joannie Roberge and Yan Coulombe for technical assistance. We also thank Alain Verreault and Craig Peterson for stimulating discussions and sharing reagents and Jef Boeke and Susan Gasser for sharing yeast strains. This work was supported by NIH Grants R00 ES021441 (to H.N.) and R01 GM60443 (to S.J.K. and J.C.) and Canadian Institutes of Health Research (CIHR) Grants MOP-14308 (to J.C.) and FDN-143314 (to J.C.). X.C., O.J.-R., and R.B. were supported by graduate scholarships from CIHR/Banting and Best, Desjardins/Luc Bélanger/Fonds de recherche du Québec-Santé (FRQS), and Fonds de recherche du Québec-Nature et technologies (FRQNT), respectively. J.C. holds the Canada Research Chair in Chromatin Biology and Molecular Epigenetics.

- Steunou A-L, Rossetto D, Côté J (2014) Regulating chromatin by histone acetylation. *Fundamentals of Chromatin*, eds Workman JL, Abmayr SM (Springer, New York), pp 147–212.
- Rossetto D, et al. (2014) Eaf5/7/3 form a functionally independent NuA4 submodule linked to RNA polymerase II-coupled nucleosome recycling. *EMBO J* 33:1397–1415.
- Steunou AL, et al. (2016) Combined action of histone reader modules regulates NuA4 local acetyltransferase function but not its recruitment on the genome. *Mol Cell Biol* 36:2768–2781.
- Yi C, et al. (2012) Function and molecular mechanism of acetylation in autophagy regulation. *Science* 336:474–477.
- Lin YY, et al. (2009) Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. *Cell* 136:1073–1084.
- Lu JY, et al. (2011) Acetylation of yeast AMPK controls intrinsic aging independently of caloric restriction. *Cell* 146:969–979.
- Downs JA, et al. (2004) Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol Cell* 16:979–990.
- Jacquet K, et al. (2016) The TIP60 complex regulates bivalent chromatin recognition by 53BP1 through direct H4K20me binding and H2AK15 acetylation. *Mol Cell* 62:409–421.
- Smeenk G, van Attikum H (2013) The chromatin response to DNA breaks: Leaving a mark on genome integrity. *Annu Rev Biochem* 82:55–80.
- Dantuma NP, van Attikum H (2016) Spatiotemporal regulation of posttranslational modifications in the DNA damage response. *EMBO J* 35:6–23.
- Wilson MD, Durocher D (2017) Reading chromatin signatures after DNA double-strand breaks. *Philos Trans R Soc Lond B Biol Sci* 372:20160280.
- Downs JA, Côté J (2005) Dynamics of chromatin during the repair of DNA double-strand breaks. *Cell Cycle* 4:1373–1376.
- Bennett G, Peterson CL (2015) SWI/SNF recruitment to a DNA double-strand break by the NuA4 and Gcn5 histone acetyltransferases. *DNA Repair* 30:38–45.
- Auger A, et al. (2008) Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. *Mol Cell Biol* 28:2257–2270.
- Bennett G, Papamichos-Chronakis M, Peterson CL (2013) DNA repair choice defines a common pathway for recruitment of chromatin regulators. *Nat Commun* 4:2084.
- Lempiäinen H, Halazonetis TD (2009) Emerging common themes in regulation of PIKKs and PI3Ks. *EMBO J* 28:3067–3073.
- Falck J, Coates J, Jackson SP (2005) Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* 434:605–611.
- Symington LS (2016) Mechanism and regulation of DNA end resection in eukaryotes. *Crit Rev Biochem Mol Biol* 51:195–212.
- Brown CE, et al. (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science* 292:2333–2337.
- Knutson BA, Hahn S (2011) Domains of Tra1 important for activator recruitment and transcription coactivator functions of SAGA and NuA4 complexes. *Mol Cell Biol* 31:818–831.
- Lin L, Chamberlain L, Zhu LJ, Green MR (2012) Analysis of Gal4-directed transcription activation using Tra1 mutants selectively defective for interaction with Gal4. *Proc Natl Acad Sci USA* 109:1997–2002.
- Nourani A, Utley RT, Allard S, Côté J (2004) Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. *EMBO J* 23:2597–2607.
- Breitkreutz A, et al. (2010) A global protein kinase and phosphatase interaction network in yeast. *Science* 328:1043–1046.
- Albuquerque CP, et al. (2008) A multidimensional chromatography technology for in-depth phosphoproteome analysis. *Mol Cell Proteomics* 7:1389–1396.
- Smolka MB, Albuquerque CP, Chen SH, Zhou H (2007) Proteome-wide identification of in vivo targets of DNA damage checkpoint kinases. *Proc Natl Acad Sci USA* 104:10364–10369.
- Bastos de Oliveira FM, et al. (2015) Phosphoproteomics reveals distinct modes of Mec1/ATR signaling during DNA replication. *Mol Cell* 57:1124–1132.
- Lloyd J, et al. (2009) A supramodular FHA/BRCT-repeat architecture mediates Nbs1 adaptor function in response to DNA damage. *Cell* 139:100–111.
- Williams RS, et al. (2009) Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. *Cell* 139:87–99.
- Ceccaldi R, Rondinelli B, D'Andrea AD (2016) Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol* 26:52–64.
- Zhang Y, et al. (2007) Role of Dnl1-Lif1 in nonhomologous end-joining repair complex assembly and suppression of homologous recombination. *Nat Struct Mol Biol* 14:639–646.
- Ira G, et al. (2004) DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* 431:1011–1017.
- Dubrana K, van Attikum H, Hediger F, Gasser SM (2007) The processing of double-strand breaks and binding of single-strand-binding proteins RPA and Rad51 modulate the formation of ATR-kinase foci in yeast. *J Cell Sci* 120:4209–4220.
- Aasland R, Stewart AF, Gibson T (1996) The SANT domain: A putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. *Trends Biochem Sci* 21:87–88.
- Lin YY, et al. (2008) A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. *Genes Dev* 22:2062–2074.
- Smith J, Rothstein R (1995) A mutation in the gene encoding the *Saccharomyces cerevisiae* single-stranded DNA-binding protein Rfa1 stimulates a RAD52-independent pathway for direct-repeat recombination. *Mol Cell Biol* 15:1632–1641.
- Deng SK, Gibb B, de Almeida MJ, Greene EC, Symington LS (2014) RPA antagonizes microhomology-mediated repair of DNA double-strand breaks. *Nat Struct Mol Biol* 21:405–412.
- Allard S, et al. (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *EMBO J* 18:5108–5119.
- Yuan H, et al. (2012) MYST protein acetyltransferase activity requires active site lysine autoacetylation. *EMBO J* 31:58–70.
- Clarke AS, Lowell JE, Jacobson SJ, Pillus L (1999) Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol Cell Biol* 19:2515–2526.
- Henriksen P, et al. (2012) Proteome-wide analysis of lysine acetylation suggests its broad regulatory scope in *Saccharomyces cerevisiae*. *Mol Cell Proteomics* 11:1510–1522.
- Mertins P, et al. (2013) Integrated proteomic analysis of post-translational modifications by serial enrichment. *Nat Methods* 10:634–637.
- Choudhary C, et al. (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 325:834–840.
- Ruff P, Donnianni RA, Glancy E, Oh J, Symington LS (2016) RPA stabilization of single-stranded DNA is critical for break-induced replication. *Cell Rep* 17:3359–3368.
- Jeggio PA, Downs JA, Gasser SM (2017) Chromatin modifiers and remodelers in DNA repair and signalling. *Philos Trans R Soc Lond B Biol Sci* 372:20160279.
- Niu H, et al. (2010) Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. *Nature* 467:108–111.
- Cheng X, et al. (2015) Eaf1 links the NuA4 histone acetyltransferase complex to Htz1 incorporation and regulation of purine biosynthesis. *Eukaryot Cell* 14:535–544.
- Buisson R, et al. (2010) Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. *Nat Struct Mol Biol* 17:1247–1254.