NuA4 and SAGA acetyltransferase complexes cooperate for repair of DNA breaks by homologous recombination

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Running title: NuA4 cooperates with SAGA to promote homologous recombination

ABSTRACT

Chromatin modifying complexes play important yet not fully defined roles in DNA repair processes. The essential NuA4 histone acetyltransferase (HAT) complex is recruited to double-strand break (DSB) sites and spreads along with DNA end resection. As predicted, NuA4 acetylates surrounding nucleosomes upon DSB induction and defects in its activity correlate with altered DNA end resection and Rad51 recombinase recruitment. Importantly, we show that NuA4 is also recruited to the donor sequence during recombination along with increased H4 acetylation, indicating a direct role during strand invasion/D-loop formation after resection. We found that NuA4 cooperates locally with another HAT, the SAGA complex, during DSB repair as their combined action is essential for DNA end resection to occur. This cooperation of NuA4 and SAGA is required for recruitment of ATP-dependent chromatin remodelers, targeted acetylation of repair factors and homologous recombination. Our work reveals a multifaceted and conserved cooperation mechanism between acetyltransferase complexes to allow repair of DNA breaks by homologous recombination.

Keywords: chromatin, acetylation, DNA double-strand break, NuA4; SAGA, homologous recombination, RPA

48 **Key Points:**

49 -NuA4-dependent acetylation modulates nucleosome dynamics to assist DNA end resection and
50 strand invasion during homologous recombination.

51 -Combined recruitment and local action of NuA4 and SAGA acetyltransferase complexes is
52 essential for DNA end resection.

53 -Cooperation between NuA4 and SAGA acetyltransferases is required for homologous
54 recombination in eukaryotes.

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INTRODUCTION

DNA double-strand breaks (DSBs) are one of the most detrimental types of DNA lesions that need to be handled properly to preserve genome integrity [1]. DSBs can be repaired by two major pathways, non-homologous end-joining (NHEJ) and homologous recombination (HR), while other types of repair also play important roles [2]. Repair by NHEJ tends to be error-prone due to its template-independent nature whereas repair by HR is mostly error-free [2]. Notably, DSB repair takes place in the context of chromatin. Consistent with this, several studies have gradually delineated the functional interplay between chromatin modifiers/remodelers and critical processes during DNA repair [3, 4].

The yeast 13-subunit NuA4 histone acetyltransferase (HAT) complex has been implicated in the repair of DNA breaks [5-9]. Highly conserved during evolution, the main activity of NuA4 is to acetylate nucleosomal histone H4 and H2A(Z/X) through its catalytic subunit KAT5 (Esa1/Tip60), the sole HAT protein essential for cell viability in *Saccharomyces cerevisiae* [10, 11]. Initial evidence for NuA4 involvement in repair came from yeast experiments showing that cells with mutations of NuA4 subunits or its lysine targets on the H4 tail are sensitive to DNA-damaging agents, suggesting that NuA4 and its activity are required for efficient repair [6, 8, 12, 13]. Subsequent analysis of NuA4 functions in DSB repair found that it is physically recruited to an endonuclease-induced DSB [6]. This recruitment is performed through a direct interaction with the Mre11-Rad50-Xrs2 (MRX) complex and leads to spreading of NuA4 along with DNA end resection [5, 14]. Local acetylation of chromatin has been argued to help recruitment of ATP-dependent remodelers near the break site [6, 15]. NuA4 has also been implicated in DNA damage tolerance, post-replicative and nucleotide excision repairs [16-18]. In human cells, NuA4 (a.k.a. the TIP60 complex) regulates DNA damage signalling and

counteracts 53BP1 to favour repair by HR instead of NHEJ [19-21]. However, the functional consequences of NuA4 recruitment near DSBs in the repair process per se remain to be clearly defined.

In this study, we aimed to define NuA4 function and requirement during the repair of DNA DSB. Using a conditional mutant and a rapid depletion approach in the context of integrated DSB-induction system in budding yeast, we found that NuA4 participates in multiple steps during HR but do so through an essential collaboration with another HAT complex, SAGA, regulating chromatin dynamics, DNA end resection and non-histone substrates.

RESULTS

NuA4-dependent acetylation of chromatin around a DNA break affects end resection

In order to study NuA4 function in DNA DSB repair, we first used a thermosensitive (ts) mutant of Esa1 (*esa1-L254P*) [11] integrated in a well-established inducible DSB system (*pGAL-HO*, *hmlΔ/hmrΔ*) [22]. Although the non-permissive temperature compromised HO cleavage efficiency (**Fig. S1A**) potentially due to NuA4 involvement in transcription [10], semi-permissive temperature allows us to partially cripple NuA4 activity while still effectively generating DSBs *in vivo* (**Fig. S1B-C**). Accordingly, the increase of local H4 acetylation detected upon DSB formation is lost at semi-permissive temperature (**Fig. 1A-B, S1D**). In parallel, we also integrated the anchor-away system [23] into the aforementioned inducible DSB background, aiming to rapidly deplete proteins from the nucleus upon rapamycin treatment while importantly preserving robust DSB formation. Since C-terminal tagging of Esa1 with FRB leads to a decrease in global acetylation even without rapamycin treatment (**Fig. S1E**, potentially due

to the importance of an intact C-terminus), we created N-terminal FRB-tagging of endogenous Esa1, keeping it under control of its own promoter. Upon rapamycin treatment, FRB-Esa1 cells show dramatic defect in growth (**Fig. 1C**) and a rapid decrease in global H4 acetylation level (**Fig. 1D**), demonstrating that anchor-away of FRB-Esa1 is potent and efficient. Notably, the cutting efficiency of the HO endonuclease under these Esa1 anchor-away conditions is comparable to the respective WT (No-FRB) while H4 acetylation near the break is crippled (**Fig. S1F-I**). Interestingly, both systems indicate that the domain of NuA4-dependent increased H4 acetylation upon DSB formation is larger than the extent of NuA4 spreading in these conditions (3-5kb, see [5]), suggesting longer-range contact in the tri-dimensional space.

Since histone acetylation can directly attenuate DNA-histone interactions and can be recognized by bromodomain-containing ATP-dependent chromatin remodelers [10, 15], its NuA4-dependent increase near the DSB is expected to affect nucleosome stability [9](**Fig. S1J**) and downstream events like HR-linked DNA end resection process. Nucleosomes represent physical barriers to resection, thus defects in nucleosome displacement generally impact repair of the break [3, 24-28]. Accordingly, in the *ts* mutant, we detect a small but significant decrease of DNA end resection near the break site as well as decreased single-strand DNA (ssDNA) binding RPA and Rad51 recombinase recruitment (**Fig. 1E-G**). These results are also confirmed using the anchor-away system with ssDNA detection using BrdU-labeling and ChIP-qPCR (**Fig. 1H-J, S1K-L**).

A functional link between NuA4 and resection is further supported by strong genetic interactions detected between a viable anchor-away NuA4 mutant (Eaf1-FRB) and key resection factors in the presence of DNA damage (**Fig. 2A-D**), while NuA4 does not affect DSB repair by resection-independent end-joining (**Fig. S2A**). On the other hand, there is no increased DNA

damage sensitivity with deletion of the Fun30 chromatin remodeler linked to DNA end resection [26], suggesting an epistatic relationship (**Fig. 2E, S2B**). If DNA damage sensitivities observed in NuA4 mutant cells are solely due to the impaired resection, one would expect boosting resection activity would rescue NuA4 mutant sensitivity to DNA damage. Overexpression of long-range resection factor Exo1 has been shown to rescue the *FUN30* deletion [26]. However, we did not observe suppression of the DNA damage sensitivity of *esal* mutant cells when overexpressing Exo1 (**Fig. 2F**). This suggests that NuA4 role in the repair of DNA breaks is not limited to resection. Related to this, decreased recruitment of the Rad51 recombinase in *Esal* depleted cells (**Fig. 1G, 1J**) and genetic interaction between NuA4 and recombination factor Rad52 (**Fig. 2G**) suggest that NuA4 may also play a role in the recombination step after resection.

NuA4 plays a role in strand invasion at the homologous donor sequence

To progress through HR, the Rad51 recombinase replaces RPA on the resected ssDNA and this Rad51 filament performs homology search, ultimately invading the homologous donor sequence to form a structure termed D-loop. Importantly, this donor sequence is also covered with nucleosomes that require disruption. To investigate whether NuA4 can directly participate in the later steps of HR such as recombination/D-loop formation, we again took advantage of the inducible DSB system in this case carrying an intact donor sequence (*HML/HMR*) in the genome (**Fig. 3A**). After break induction followed by removal of the HO endonuclease, we observed by ChIP the presence of both Rad51 and NuA4 at the invading strand, as previously reported (**Fig. 3B-C**) [5, 29]. Importantly, the association of Rad51 was also detected using primers specific for the donor sequence, confirming the formation of D-loop (**Fig. 3D**). Interestingly, the presence of NuA4 was also found at the donor locus (**Fig 3E**) (using an antibody for Eaf1, the only subunit

unique to NuA4 [12]). Importantly, a concomitant increase of H4 acetylation is also detected at the donor sequence (**Fig. 3F**). These results provide the first evidence that links NuA4 to D-loop formation, suggesting a role of NuA4 in modulating chromatin structure at the donor sequence to allow recombination.

To measure the importance of NuA4 in the recombination process *in vivo*, we used a yeast HR reporter assay in which an uncuttable *MAT* donor sequence (*MAT-inc*) is integrated in the genome of the inducible HO-DSB system. In this system, cells with DSBs repaired by HR will become resistant to HO cleavage, thus growing on the solid medium containing galactose [30]. Surprisingly, using both the *esal-L254P* mutant at semi-permissive temperature and the viable anchor-away NuA4 mutant (Eaf1-FRB), we did not observe a significant defect in homologous recombination using this assay (**Fig. S2C-D**). While this was also reported for another NuA4 mutant [13], one explanation may be that NuA4 itself is not essential for the repair of endonuclease-induced “clean-end” DSBs, similar to what has been reported for Sae2(CtIP) and Mre11 nuclease [31]. Another possibility is the relatively small effect on end resection seen in NuA4 mutants not being sufficient to affect HR in this assay over the long time of growth on plates. It could also be due to functional redundancy or cooperativity with another chromatin modifier (see below).

Combined recruitment of NuA4 and SAGA acetyltransferases is essential for DNA end resection

In a previous ChIP experiment coupled to mass spectrometry to identify proteins linked to DNA end resection (RPA) after HO-DSB formation, we could detect NuA4 components along with repair proteins [5]. Interestingly, beside ATP-dependent chromatin remodelers, a subunit of the

172 SAGA histone acetyltransferase complex was also detected (**Fig. 4A**). In transcription regulation,
 173 NuA4 has been shown to collaborate with SAGA, which targets H3 and H2B tails in
 174 nucleosomes through its catalytic subunit Gcn5 [32-34]. Both NuA4 and SAGA have also been
 175 shown to acetylate non-histone substrates, some linked to the DNA repair process [5, 7, 35-38].
 176 NuA4 and SAGA share the large ATM/ATR-related Tra1 subunit which is implicated in their
 177 recruitment to gene promoters by activators [39]. Since NuA4 is recruited by the MRX complex
 178 potentially through a direct interaction with Tra1 [5], we asked if the SAGA complex is also
 179 recruited to DNA breaks and collaborates with NuA4, as in gene regulation. Indeed, using
 180 SAGA scaffold subunit Spt7 [40], we observe clear recruitment of SAGA by ChIP after DSB
 181 induction, covering 3-5kb next to the break (**Fig. 4B**). Importantly, this recruitment of SAGA is
 182 lost in *xrs2Δ* mutant cells, suggesting that it also involves MRX. To test whether this similar
 183 mode of recruitment leads to a functional collaboration between NuA4 and SAGA during DSB
 184 repair by homologous recombination, we examined resection levels in Esa1 and/or Gcn5 anchor-
 185 away conditions by measuring the exposure of ssDNA using BrdU and RPA binding by ChIP-
 186 qPCR (**Fig. 4C-D**; **Fig. S3A-D** for controls). Although anchor-away of a single HAT shows little
 187 difference compared to No-FRB cells, a complete loss in ssDNA exposure is observed when
 188 both HATs are depleted (**Fig. 4C**), correlated by a similar complete loss of RPA binding (**Fig.**
 189 **4D**), supporting an essential combined role of NuA4 and SAGA during DNA end resection. As
 190 the ssDNA generated by resection can activate DNA damage checkpoint through the Mec1-
 191 dependent pathway [41], we reasoned that the crippled resection in double anchor-away
 192 conditions should also impact checkpoint activation. Consistent with our hypothesis, checkpoint
 193 activation is highly defective in double anchor-away conditions as shown by measuring Rad53
 194 phosphorylation (**Fig. 4E**). It has been argued that NuA4 and SAGA acetyltransferase activities

can independently affect the recruitment of the SWI/SNF chromatin remodeler near DSBs through bromodomain-acetylated histones interactions [15]. Since the loss of acetylation correlates with increased nucleosome occupancy near the DSB in our *Esa1/Gcn5*-double depleted background (**Fig. S3E-F**), we investigated defects in the recruitment of chromatin remodelers. Recruitment of the SWI/SNF complex is indeed greatly affected by the combined loss of NuA4 and SAGA (**Fig. 4F-G**), but we also see defects in the recruitment of the essential remodeling complex RSC which contains multiple bromodomains (**Fig. 4H-I**), as well as the Rvb-containing remodeling complexes (Ino80/Swr1)(**Fig. 4J**).

NuA4 cooperates with SAGA to allow HR-mediated repair of DSBs in eukaryotes

Using qPCR to measure repair by HR in the inducible DSB system coupled to an uncuttable donor sequence (as above), a strong defect in HR efficiency is also observed in *Esa1/Gcn5* double anchor-away conditions (**Fig. 5A-B**). Depletion of the potential SAGA/NuA4 common recruiting interface Tra1 by anchor-away also shows reduction in RPA and Rad51 association on each side of the DSB, albeit not as much as the double *Esa1/Gcn5* depletion, suggesting other means of recruitment (**Fig. S4A-D**). Altogether, these results indicate that NuA4 collaborates with SAGA to promote repair of DNA breaks by HR, at least in part through combined acetylation of chromatin (histone H4/H2A/H2B/H3 tails) that promotes nucleosome removal/disruption by chromatin remodelers.

We next asked whether NuA4 and SAGA could also collaborate in targeting non-histone substrates implicated in DNA repair, such as key resection/ssDNA-binding factor RPA, which has been shown to be a substrate of NuA4 in yeast DSB repair [5] and Gcn5 in mammalian nucleotide excision repair [36] [38]. Using Acetyl-lysine immunoprecipitation (IP) followed by

RPA western blot (WB), we observe decreased RPA signal in Esa1 or Gcn5 anchor-away conditions (**Fig. 5C**). Importantly, RPA signal is further strongly decreased in the double anchor-away condition, indicating that RPA acetylation depends on both NuA4 and SAGA *in vivo*.

While mammalian NuA4, the TIP60 complex, has been implicated in break repair by HR for many years [19-21], the role of mammalian SAGA came to light more recently [42]. The well-conserved nature of both complexes led us to test whether they also function together to promote HR-mediated break repair in human cells. We used the DR-GFP HR reporter assay in U2OS cells by measuring GFP positive cells after I-SceI induction of a DSB (as in [19]). While single siRNA-mediated knock-down (KD) of either Tip60 or Gcn5 shows mild HR defect, double KD led to a strong defect in HR efficiency (**Fig. 5D**). KD of the Gcn5 paralog PCAF did not show this effect but a significant alteration of the cell cycle profile makes it difficult to interpret (**Fig. S5A-B**). Overall, our results support an essential conserved cooperation between two major HAT complexes, NuA4 and SAGA, to promote repair of DNA breaks by homologous recombination, through acetylation of both chromatin and non-histone substrates.

DISCUSSION

In this study, we discovered that the NuA4 complex is involved not only in the resection but also at the recombination step of repair by HR. The role of NuA4-dependent acetylation of histone H4 and H2A in a large domain surrounding the DNA break was expected to be linked to nucleosome destabilization to allow end resection but it is now also linked to strand invasion/D-loop formation at the donor sequence (**Fig. 3**). NuA4 mutants have been shown to affect recruitment of ATP-dependent chromatin remodelers INO80/SWR1 and bromodomain-containing SWI/SNF to the HO-induced break [6, 14], as well as the RSC complex to stabilize

GAG-repeats during replication [17]. The Gcn5 HAT has also been shown to affect SWI/SNF recruitment at the break [15] and, recently, to collaborate with MRX at stalled replication forks, playing a role in resection of nascent DNA [43]. We now show that the Gcn5-containing SAGA complex is recruited to the DNA break in a Xrs2-dependent manner, similar to NuA4. Strikingly, we show that the combined action of the two HAT complexes is essential for DNA end resection to occur after appearance of the DSB, and therefore HR (**Fig. 4-5**). Several studies have highlighted the cooperation between NuA4 and SAGA in gene transcription through acetylation of chromatin [32-34]. It has been shown that NuA4- and SAGA-dependent local acetylation of chromatin stimulates the association of ATP-dependent chromatin remodelers SWI/SNF and RSC through an interaction of their bromodomains with acetylated histones both at the promoter and during transcription elongation [44-46]. Since SWI/SNF and RSC complexes are also involved in the repair of DSBs [47], it is not surprising that a similar mechanism can occur following NuA4- and SAGA-dependent acetylation of nucleosomes surrounding the damage site, and that loss of both complexes has a most detrimental effect. Since it was proposed that SWI/SNF, RSC and Ino80 function at different steps of the HR process [28, 48-50], it will be interesting to investigate whether the HAT complexes are implicated. Based on current and previous results, a model for NuA4 and SAGA function in HR-mediated repair of DSBs is depicted in **Fig. 5E**.

Important questions remain about NuA4 and SAGA involvement/mechanisms in different DNA repair pathways. NuA4-dependent acetylation of RPA has been suggested to destabilize RPA from ssDNA [5]. Our results indicate that NuA4 and SAGA again cooperate in that process, which in turn could favour RPA replacement by the Rad51 recombinase. This is difficult to judge in our system since NuA4 and SAGA are required for resection and therefore

RPA loading on the ssDNA. Since RPA antagonizes micro-homology mediated end-joining (MMEJ) [51], it is also possible that NuA4 and SAGA participate in MMEJ repair by regulating RPA dynamics. A study in mammalian cells reported that p400, a subunit of the TIP60/NuA4 complex, regulates a similar alternative end-joining pathway [52], supporting another possible conserved function in repair pathways.

Moreover, our preliminary data indicated that many other DNA damage response factors are substrates of both HATs, such as Exo1, Rad50 and Sae2 (data not shown)[37, 53]. NHEJ factors are also known to be acetylated, including Ku and Nej1 [54, 55]. It will be interesting to determine how NuA4 and SAGA may directly regulate these factors and differentially affect HR and NHEJ pathways. The literature and our data point to an essential role of histone acetyltransferase activities in stimulating DSB repair by the HR pathway, while histone deacetylases have been reported as major facilitators of NHEJ [56]. This highlights an important function of chromatin structure in regulating resection which is the key step in repair pathway selection. Since NuA4 and SAGA are highly conserved during evolution and involved in several human diseases [10, 57], it will be of interest to dissect the actions of each HAT, investigate the potential functional redundancy in these acetylation events and elucidate the multifaceted HAT collaboration from yeast to human. The development of HAT inhibitors with greater specificity will allow efficient synthetic lethal screens to target specific cancer types in order to cripple their repair potential during radio- and chemotherapy [58]. Interestingly, Tip60/KAT5 has recently been identified through a CRISPR screen as a key regulator of PARP inhibitor response in BRCA2-deficient cells [59].

METHODS

Yeast strains

Yeast strains used in this study are listed in Table S1 and were constructed based on conventional PCR-based transformation protocol. Detailed construction information is available upon request. For N-terminal tagging of proteins, 500bp upstream and 500bp downstream sequence from start codon was inserted in pFA6a-kanMX6 plasmid [60] after the kanMX6 cassette using Gibson assembly cloning (NEB, E2611). FRB tag [23] was then inserted after the start codon by using Gibson assembly cloning and used as template for PCR. Cells were transformed with PCR product using forward primer (40bp before start codon + GAATTCGAGCTCGTTTAAAC) and reverse primer (~20bp reverse complement sequence before +500bp) and selected for G418 resistance. The region corresponding to the N-terminal region of the protein was sequence verified. The yeast strain used for transformation was previously mutated (*tor1-1* mutation and *FPR1* deletion) to make the cells resistant to rapamycin, while Rpl13a was tagged with FKBP12, as described [23]. BrdU-inc strains were constructed using p405-BrdU-Inc as reported [61]. For *MAT*-inc strains used in Fig. S2C-D and 5A-B, *MATα* sequence was mutated at HO cut site and then inserted to the *HIS3* locus using *URA3* selection. Yeast growth/sensitivities by serial dilution spot assays and flow cytometry analysis of cell cycle were performed as before [5].

Chromatin immunoprecipitations (ChIP)

ChIP-qPCR was performed essentially as reported previously [5] except for the following modifications. For experiments using *esal-L254P* mutant, since cells tend to accumulate in G2/M [11], cells were synchronized in G2/M before induction of HO with galactose (to avoid potentially differed cell cycle population between WT and mutant). More specifically, cells were

grown overnight in YPR at 23°C until OD600 around 0.5. Nocodazole was then added (20µg/ml final in 1% DMSO) and cell culture was shifted to 32°C for 3hrs before addition of 2% galactose for another 3hrs at 32°C. Cells were cultured at 30°C for all the other ChIP-qPCR experiments performed. For experiments with anchor-away system, cells were grown overnight in YPR until OD600 around 0.5, and rapamycin was added to a final concentration of 1µg/ml for 20min followed by 2% final galactose induction for 3hrs. For experiment with BrdU, cells were grown overnight in YPR until OD600 around 0.5 and then BrdU was added (400µg/ml final) and grown for 6hrs. HO cleavage efficiency was calculated with the ratio from qPCR using primers across the DSB site over an uncut control locus (interV, long intergenic region on chromosome V). Lists of primers standardised on a LightCycler qPCR apparatus are available upon request. Antibodies used were against penta-H4 acetylation (Upstate, 06-946), H4 (Abcam, Ab7311), H2B (Abcam, Ab1790), Myc (9E10), Rad51 (SCBT, sc-33626), BrdU (GE, RPN202), Eaf1 [12], Swi2/Snf6, Sth1/Rsc6 and Rvb1 (kindly provided by Joe Reese, Brad Cairns and Yasutaka Makino, respectively). ChIP-qPCR data are presented as % of IP/input (or ratio of IP/input when correcting for nucleosome occupancy, i.e. H4ac/H3 or H4) and are from at least two independent yeast cultures in each experiment. Histone acetylation Anti-Rfa1 ChIP-coupled to mass spectrometry was described in [5].

Plasmid-based NHEJ assay

Plasmid-based NHEJ assay was performed essentially as described previously [62]. After transformed with cut or uncut plasmid, cells were plated on URA- plates containing 1µg/ml rapamycin and let grow around 3 days before the colony numbers were quantified. *LEU2* ORF was completely removed from HHY168 genome to prevent potential repair through HR.

Acetyl-lysine IP

Immunoprecipitation with acetyl-lysine antibody was performed essentially as described previously [5]. Cells were grown in YPD until OD600 around 0.5 followed by addition of rapamycin (1ug/ml final) and DMSO or MMS (0.05% final) for 3hrs. Antibody used were acetyl-lysine (ImmuneChem, ICP0380), RPA (Agrisera, AS 07-214) and Pgk1 (Abcam, ab113687).

DR-GFP HR assay

DR-GFP HR assay was performed with DR-GFP-integrated U2OS cells, essentially as reported previously [19]. Knockdown (KD) was carried out with siRNA transfection using Lipofectamine RNAimax (ThermoFisher, 13778075) following manufacture's instruction. siTip60 was a single siRNA from the reported Smartpool siRNA pool [19] and validated for KD efficiency (slightly weaker KD than the Smartpool). siGcn5 and siPCAF used were as reported previously [42]. Specific sequences of siRNA are available upon request.

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Author contributions

XC and VC performed experiments. XC and JC designed experiments, analyzed the data and prepared the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Figure 1. NuA4 is required for H4 acetylation upon DSB induction and affects resection. A-B) ChIP-qPCR of H4ac signal near the DSB site (H4ac/total H3 ratio of %IP/input) in WT and *esal ts* strains during 0, 90 and 180min of galactose induction of HO nuclease. Values at the different locations near the DSB at time 0 was normalized to 1, showing the increase of H4 acetylation upon HO cut in WT conditions but not in mutant at 32°C (error bars are range of biological replicates). **C)** 10-fold serial dilutions of indicated FRB-tagged strains were spotted on YPD or YPD containing 1µg/ml rapamycin and grown at 30°C, showing loss of viability by depletion of *Esa1* and RNA PolII largest subunit for comparison (*Rpo21*). **D)** WB of WCE from indicated strains treated with 1µg/ml rapamycin for 0, 20 or 40min, showing loss of H4 acetylation by depletion of *Esa1* and *Yng2*, another subunit of NuA4 (not used further as it affects Gal induction). **E)** Resection assay of WT and *esal-L254P* cells after 3hr of galactose induction at 32°C. DNA content around the break was measured by qPCR with genomic DNA and normalized on uncut locus (*interV*), showing higher level of DNA remaining near the DSB in mutant cells. **F-G)** ChIP-qPCR assay of 13myc-*Rfa1* (F) and *Rad51* (G) next to the DSB site in WT and *esal-L254P* after 0, 90 and 180min of HO induction at 32°C, showing less accumulation in mutant conditions. **H)** ChIP-qPCR assay of BrdU in No-FRB and FRB-*Esa1* strains after 3hr of galactose induction in presence of rapamycin, showing slightly less exposed ssDNA near the break after *Esa1* depletion. BrdU enrichment was normalized on control locus (*interV*). **I-J)** ChIP-qPCR assay of 13myc-*Rfa1* (I) and *Rad51* (J) in No-FRB and FRB-*Esa1* cells after 3hr galactose induction in presence of rapamycin, showing less accumulation in *Esa1*-depleted conditions. Error bars in ChIP-qPCR represent range from biological replicates.

Figure 2. NuA4 shows genetic interaction with resection and recombination factors. A-E, G) 10-fold serial dilutions of indicated strains were spotted on solid medium without or with 1µg/ml rapamycin supplemented without or with indicated amounts of MMS and grown at 30°C. Another subunit of NuA4 was used with the FRB tag, scaffold subunit Eaf1[12], as its depletion does not affect cell viability on rich media (A). Genetic interaction (sickness/growth defect in presence of DNA damage induced by MMS) is seen with genes for resection factors Sae2 (B), Exo1 (C), Sgs1 (D) but not Fun30 (E), and with recombination factor Rad52 (G). F) DNA damage sensitivity of *esal ts* cells cannot be overcome by over-expression of potent resection factor Exo1. Spot assays as above with the indicated strains. The *fun30* deleted strain is used as control as it was shown to be rescued by Exo1 expression but not its catalytic mutant form (D173A) [26].

Figure 3. NuA4 functions at the donor homologous sequence during HR, implicating chromatin acetylation during D-loop formation. A) Schematic representation of HR donor strain genome arrangement and primer positions for ChIP-qPCR. B-E) ChIP-qPCR assay (% of Ip/input) of Rad51 (B, D) and Eaf1 (C, E) in HR donor strain after 0, 2hr galactose induction or 2hr galactose induction followed 1hr glucose (2%) treatment to block HO nuclease expression. Rad51 and NuA4 are clearly recruited at both the invading strand from the HO cut and at the donor sequence during HR. Error bars represent range from biological duplicates. F) ChIP-qPCR of NuA4-dependent H4 acetylation at the donor sequence (H4ac/total H2B ratio of %IP/input), showing a clear increase during HR. Error bars represent standard error from biological triplicates.

Figure 4. Essential collaboration of NuA4 and SAGA HAT complex at DSBs to allow DNA end resection and recruitment of ATP-dependent chromatin remodelers. **A)** List of protein components of chromatin remodeling/modifying proteins and number of total peptides detected in ChIP of Flag-Rfa1 followed by MS analysis from cells after 0 or 90 min of HO induction [5], showing increased association of remodelers but also a component of the SAGA HAT complex. **B)** ChIP-qPCR assay of Myc-Spt7 (SAGA scaffold subunit) in WT and *xrs2Δ* cells after 3hr of HO induction with galactose. % of IP/input is shown at different locations near the DSB after subtraction of the signal in untagged strain. **C)** ChIP-qPCR assay of BrdU in indicated FRB-tagged strains after 3hr galactose induction in the presence of rapamycin to measure ssDNA production (as in Fig 1H). BrdU enrichment was normalized on control locus (inter V). **D)** ChIP-qPCR as in (B) but measuring resection through anti-RPA, showing again full loss of resection when Esa1 and Gcn5 are both depleted. **E)** DNA damage checkpoint activation measured by Western blotting of Rad53 (Santa cruz, sc-6749) from whole cell extracts after 0, 2, 3, 4hr of galactose induction in presence of rapamycin, showing slower migrating signal upon activation by DNA damage, which is lost after depletion of both Esa1 and Gcn5. **F-J)** Recruitment of ATP-dependent chromatin remodeling complexes at locations near the HO DSB as measured by ChIP-qPCR with specific antibodies after 3hr of galactose induction in presence of rapamycin. The Esa1/Gcn5 double depletion shows clear defects in recruitment of these factors. Error bars represent range from biological duplicates.

Figure 5. NuA4 and SAGA also cooperate for acetylation of RPA and are together required of DSB repair by HR from yeast to humans. **A)** Schematic representation of uncuttable-donor (*MAT-inc*) strain genome arrangement and primer positions to detect repair by HR. **B)** HR assay

by qPCR on genomic DNA with primers shown (A) after 3hr of HO induction in presence of rapamycin and normalization on control locus (InterV). Double depletion of Esa1 and Gcn5 shows strong defect of repair by HR. C) Measurement of *in vivo* RPA acetylation by Western blotting after acetyl-lysine IP from whole cell extracts corresponding to the indicated strains, incubated with rapamycin plus DMSO or MMS (0.05%) for 3hr. Inputs signals before Kac IP are shown for control as long as PGK1 for loading. Both Esa1 and Gcn5 affect the level of RPA acetylation in the cell. D) HR reporter assay in human U2OS cells containing an inducible I-SceI endonuclease and an integrated DR-GFP reporter. After siRNA transfection, I-SceI DSB induction and repair, cells were subject to fluorescence-activated cell sorting (FACS) analysis for GFP expression (reflecting the level of repair by HR). Results represent the percentage of GFP-positive cells normalized on control siRNA. Double knock-down of Tip60 and hGcn5 shows stronger defect in repair by HR. PALB2 knock-down is shown as positive control. Error bars represent standard error from three biological replicates. E) Model depicting the roles of NuA4 and SAGA HAT complexes for the repair of DNA breaks by homologous recombination. It shows the recruitment to the break by MRX, acetylation of chromatin to allow resection with recruitment of remodelers, followed by acetylation of RPA on resected ssDNA and acetylation of chromatin at the homologous donor sequence during strand invasion.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Using NuA4 mutants in the Gal-HO system to study DSB repair. Related to Figure 1. **A)** Percentage of HO cutting efficiency in WT and *esal-L254P* mutant after 2hr 37°C treatment followed by 3hr galactose induction at 37°C. **B)** Western blot (WB) of whole cell extract (WCE) from WT and *esal-L254P* cells treated with 23°C or 32°C for 3hr. Antibodies used are penta-H4 acetylation (Upstate, 06-946) and H4 (Abcam, Ab7311). **C)** Percentage of HO cutting efficiency in WT and *esal-L254P* cells after 3hr galactose induction at 32°C (based on qPCR across the cut site normalised to control locus). **D)** ChIP-qPCR of H4ac signal at the *RPS11B* control locus in WT and *esal ts* strains during galactose induction of HO nuclease, showing no increase (ratio of H4ac/total H3 %IP/input, error bars are range of biological replicates). **E)** Western blot (WB) of H4 acetylation and H3 with whole cell extract (WCE) from indicated strains grown in YPD without rapamycin treatment, showing the non-specific effect of Esa1 tagging at its C-terminus. **F)** HO cutting efficiency as above in No-FRB and FRB-Esa1 cells after 30min of galactose induction in presence of rapamycin (depleted conditions, no change is seen after 3hrs either). **G)** ChIP-qPCR of H4ac signal as above at the *RPS11B* control locus in No-FRB and FRB-Esa1 strains during galactose induction of HO nuclease. **H)** Relative H4ac/total H4 level by ChIP-qPCR (ratio of % IP/input) in No-FRB cells after 3hr galactose induction in presence of rapamycin. Values at the different locations near the DSB at time 0 was normalized to 1, showing the increase of H4 acetylation upon HO cut. **I)** Relative H4ac/total H4 level by ChIP-qPCR (ratio of % IP/input) in No-FRB and FRB-Esa1 cells after 3hr galactose induction in presence of rapamycin. Values at the different locations near the DSB in No-FRB cells were normalized to 1, showing the loss of H4 acetylation induction in FRB-Esa1 cells. **J)** Relative total H4 level (nucleosome occupancy) by ChIP-qPCR in No-FRB and FRB-Esa1 cells

after 0 and 3hr galactose induction in presence of rapamycin. t=0 values of respective strains were normalized to 1 to show the loss of nucleosomes upon DSB induction, which is less important after depletion of Esa1. Error bars represent range from biological duplicates. **K-L)** BrdU assay validation for measurement of end resection. **K)** Dot blot of BrdU-incorporation strains (BrdU-inc +) or non-modified strains (BrdU-inc -) grown in YPD without (BrdU -) or with (BrdU +) 400µg/ml BrdU for 4hrs. Genomic DNA was spotted on Amersham Hybond-N+ membrane and blotted with BrdU antibody (GE, RPN202). **L)** ChIP assay of BrdU (%IP/input) in No-FRB strain after 0 (No damage) or 3hr (+damage) galactose induction, showing clear detection of ssDNA generated by resection within 3kb of the HO break with background signal at 10kb and control locus and in cells not incubated with BrdU (no BrdU).

Figure S2. NuA4 *esal* mutant or depletion through Eaf1 does not affect NHEJ and HR in reporter assays measuring growth on plates. Related to Figure 2 and 3. **A)** Plasmid-based NHEJ assay using indicated strains transformed with cut or uncut plasmids, plated on 1µg/ml rapamycin-containing SCSM-URA solid medium and grown at 30°C. Error bars represent standard errors from biological triplicates. **B)** 10-fold serial dilutions of indicated strains spotted on solid medium without or with indicated amounts of MMS and grown at 32°C, showing synthetic sickness/interaction between *esal ts* mutant and *fun30* deletion. **C-D)** 10-fold serial dilutions of indicated strains were spotted on YPD or YP containing 2% galactose to induce HO break and recombination with the mat-inc donor locus (measured by survival, see system schematic in Fig 5A). The *esal ts* mutant shows no defect in HR at 32°C (*rad52* deletion is used as positive control) (C). The Eaf1-FRB strain does not show HR defect either in presence of 1µg/ml rapamycin grown at 30°C (D).

733

734 **Figure S3. Effects of anchor-away depletion of Esa1, Gcn5 and Esa1/Gcn5 together.** Related
735 to Figure 4. **A)** Western blot analysis of bulk histone acetylation marks in No-FRB, FRB-Esa1,
736 Gcn5-FRB and FRB-Esa1/Gcn5-FRB cells after 0, 60, 120, 180 and 240min in Galactose to
737 induce the HO break as well as in the presence of rapamycin (added 20min before time 0 of
738 galactose). A no rapamycin control before galactose is also shown. Esa1 depletion shows rapid
739 loss of H4ac (penta) while Gcn5 depletion shows a decrease of H3K9ac. **B)** Percentage of HO
740 cutting efficiency in No-FRB, FRB-Esa1, Gcn5-FRB and FRB-Esa1/Gcn5-FRB strains after
741 30min of induction in galactose. Error bars represent standard error from biological triplicates.
742 Error bars represent standard errors from biological triplicates. **C-D)** Cell cycle analysis of cells
743 from the same indicated strains by cell cytometry after fixing and staining with PI, before (C)
744 and after 3hr induction of HO (D) in presence of rapamycin, showing no major changes in cell
745 cycle profiles between strains and conditions. Error bars represent range from biological
746 duplicates. **E)** ChIP-qPCR of H4ac signal (ratio of H4ac/total H4 %IP/input) next to the HO
747 break in No-FRB, FRB-Esa1 and FRB-Esa1/Gcn5-FRB strains after 3hr of galactose induction
748 of HO in presence of rapamycin, showing the expected loss of H4ac. **F)** ChIP-qPCR of relative
749 H4 level (nucleosome occupancy) in the indicated strains after 3hr galactose induction in
750 presence of rapamycin. Values (% IP/input) near the HO break were compared to the No-FRB
751 samples set to 1, showing an increase in Esa1/Gcn5-depleted cells. Arp4-FRB is shown as
752 control as it is a shared subunit not only of NuA4 but also INO80/SWR1 chromatin remodeling
753 complexes. Error bars represent range from biological duplicates.

754

Figure S4. The ATM/ATR-related Tra1 subunit shared by NuA4 and SAGA affects DSB repair but does not recapitulate combined Esa1/Gcn5 function. Related to Figures 4 and 5. **A)** 10-fold serial dilutions of indicated FRB-tagged strains were spotted on solid medium without or with 1µg/ml rapamycin supplemented without or with 0.03% MMS and grown at 30°C. Depletion of the Tra1 subunit shared by NuA4 and SAGA leads to much decreased viability, while not as much as Esa1/Rpo21, but also clear sensitivity to DNA damage. **B)** Percentage of HO cutting efficiency in No-FRB and FRB-Tra1 strains after 30min of induction in galactose. Error bars represent standard error from biological triplicates. **C-D)** ChIP-qPCR assay of Rfa1-13myc (C) and Rad51 (D) (% of IP/input at different locations around the HO DSB) in No-FRB and FRB-Tra1 strains after 3hr of galactose induction. Error bars represent range from biological duplicates.

Figure S5. Knockdown of PCAF accumulates cells in S/G2. Related to Figure 5D. **A)** DR-GFP as in Fig. 5D with indicated siRNAs. **B)** Cell cycle analysis of cells shown in (A) and Fig. 5D by cell cytometry after fixing and staining cells with PI. Error bars represent range from biological duplicates. Note that siPCAF cells show accumulation in S/G2, potentially accounting for elevated HR repair efficiency observed in these cells.

773 **Table S1: Yeast strains used in this study**

Strain	Genotype	Reference
JKM139	<i>MATa hoΔ hmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO</i>	(Lee et al. 1998)
JKM179	<i>MATa hoΔ hmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO</i>	(Lee et al. 1998)
QY3501	JKM139 <i>esa1-L254P::NatMX</i>	this study
QY3530	JKM139 <i>fpr1Δ::HphMX tor1-1::URA3 RPL13A-2*FKBP12::TRP1</i> (No-FRB)	this study
QY3572	Isogenic to QY3530 except <i>FRB-ESA1::KanMX</i>	this study
QY3534	Isogenic to QY3530 except <i>ESA1-FRB::KanMX</i>	this study
QY3562	Isogenic to QY3530 except <i>RPO21-FRB::KanMX</i>	this study
QY3531	Isogenic to QY3530 except <i>YNG2-FRB::KanMX</i>	this study
QY3535	Isogenic to QY3530 except <i>EAF1-FRB::KanMX</i>	this study
LSY-2900-13A	Symington donor strain wt	(Deng et al. 2014)
GA2321	JKM179 <i>MATa-inc-URA3</i>	(van Attikum et al. 2007)
GA2368	JKM179 <i>MATa-inc-URA3 rad52::LEU2</i>	(van Attikum et al. 2007)
QY3520	JKM179 <i>MATa-inc-URA3 esa1-L254P::NatMX</i>	this study
QY3668	JKM139 <i>fpr1Δ::HphMX tor1-1 RPL13A-2*FKBP12::TRP1 MATa-inc-HIS3::URA3</i>	this study
QY3672	Isogenic to QY3668 except <i>EAF1-FRB::KanMX</i>	this study
QY3669	Isogenic to QY3668 except <i>FRB-Esa1::KanMX</i>	this study
QY3670	Isogenic to QY3668 except <i>GCN5-FRB::KanMX</i>	this study
QY3671	Isogenic to QY3668 except <i>FRB-Esa1::KanMX GCN5-FRB::NatMX</i>	this study
QY3686	JKM139 <i>SPT7-13Myc::TRP1</i>	this study
QY3692	JKM139 <i>SPT7-13Myc::TRP1 xrs2Δ::KanMX</i>	this study
QY3608	JKM139 <i>fpr1Δ::HphMX tor1-1::URA3 RPL13A-2*FKBP12::TRP1 Leu2::BrdU-inc</i>	this study
QY3611	Isogenic to QY3608 except <i>FRB-Esa1::KanMX</i>	this study
QY3615	Isogenic to QY3608 except <i>Gcn5-FRB::KanMX</i>	this study
QY3616	Isogenic to QY3608 except <i>FRB-Esa1::KanMX Gcn5-FRB::NatMX</i>	this study
QY3596	Isogenic to QY3530 except <i>FRB-Tra1::KanMX</i>	this study
HHY168	K14708 <i>RPL13A-2×FKBP12::TRP1</i>	(Haruki et al. 2008)
QY3599	K14708 <i>RPL13A-2×FKBP12::TRP1 leu2Δ::HphMX</i>	this study
QY3621	Isogenic to QY3559 except <i>EAF1-FRB::KanMX</i>	this study
QY3607	Isogenic to QY3559 except <i>DNL4-FRB::KanMX</i>	this study
QY3622	Isogenic to HHY168 except <i>EAF1-FRB::KanMX</i>	this study
QY3625	Isogenic to HHY168 except <i>sae2Δ::HIS3</i>	this study
QY3633	Isogenic to HHY168 except <i>exo1Δ::HphMX</i>	this study
QY3624	Isogenic to HHY168 except <i>sgs1Δ::HIS3</i>	this study
QY3623	Isogenic to HHY168 except <i>fun30Δ::HIS3</i>	this study
QY3636	Isogenic to HHY168 except <i>rad52Δ::HIS3</i>	this study
QY3632	Isogenic to HHY168 except <i>EAF1-FRB::KanMX sae2Δ::HIS3</i>	this study
QY3634	Isogenic to HHY168 except <i>EAF1-FRB::KanMX exo1Δ::HphMX</i>	this study
QY3631	Isogenic to HHY168 except <i>EAF1-FRB::KanMX sgs1Δ::HIS3</i>	this study
QY3630	Isogenic to HHY168 except <i>EAF1-FRB::KanMX fun30Δ::HIS3</i>	this study

QY3637	Isogenic to HHY168 except <i>EAF1-FRB::KanMX rad52Δ::HIS3</i>	this study
QY3563	JKM139 +(pRS424 <i>TRP1</i> 2μ ori)	this study
QY3564	JKM139 +(pRS424 <i>EXO1 TRP1</i> 2μ ori)	this study
QY3565	JKM139 +(pRS424 <i>EXO1-D173A TRP1</i> 2μ ori)	this study
QY3566	JKM139 <i>esal-L254P::NatMX</i> +(pRS424 <i>TRP1</i> 2μ ori)	this study
QY3567	JKM139 <i>esal-L254P::NatMX</i> +(pRS424 <i>EXO1 TRP1</i> 2μ ori)	this study
QY3568	JKM139 <i>esal-L254P::NatMX</i> +(pRS424 <i>EXO1-D173A TRP1</i> 2μ ori)	this study
QY3569	JKM139 <i>fun30Δ::KanMX</i> +(pRS424 <i>TRP1</i> 2μ ori)	this study
QY3570	JKM139 <i>fun30Δ</i> +(pRS424 <i>EXO1 TRP1</i> 2μ ori)	this study
QY3571	JKM139 <i>fun30Δ</i> +(pRS424 <i>EXO1-D173A TRP1</i> 2μ ori)	this study

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Supp. Figure 1

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