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SWI/SNF recruitment to a DNA double-strand break by the NuA4 and Gcn5 histone acetyltransferases

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Abstract

The DNA damage response to double-strand breaks (DSBs) is critical for cellular viability. Recent work has shown that a host of chromatin regulators are recruited to a DSB, and that they are important for the DNA damage response. However, the functional relationships between different chromatin regulators at DSBs remain unclear. Here we describe a conserved functional interaction among the chromatin remodeling enzyme, SWI/SNF, the NuA4 and Gcn5 histone acetyltransferases, and phosphorylation of histone H2A.X (γH2AX). Specifically, we find that the NuA4 and Gcn5 enzymes are both required for the robust recruitment of SWI/SNF to a DSB, which in turn promotes the phosphorylation of H2A.X.

Keywords

chromatin; homologous recombination; SWI/SNF; Gcn5; NuA4; histone acetyltransferase

1. Introduction

DNA damage repair is essential for cell viability and genomic stability. The most severe form of DNA damage, the double-strand break (DSB), can arise from exposure to damaging chemicals, ultraviolet or ionizing radiation, free oxygen radicals, or DNA replication errors, and failure to properly repair DSBs can lead to genome instability and cell death (for review see refs. 1,2). Upon DSB formation, the cell launches a complex network of signals to elicit cell cycle arrest and repair functions. In addition, the extensive compaction of eukaryotic DNA into chromatin necessitates the involvement of a diverse group of regulators to modify this structure and enable access for DNA repair.

A key first step of the DNA damage response is the activation of the checkpoint kinases (Mec1 and Tel1 in yeast, or ATM and ATR in mammals) that catalyze the phosphorylation of histone H2A.X at a C-terminal serine residue (S129 in yeast and S139 in mammals). This is a hallmark of the damage response and, although it does not directly recruit repair proteins

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or chromatin regulators, it does play an important role in stabilizing the binding of damage response factors to the DSB lesion [3,4]. In addition, several other chromatin modifications are formed in regions surrounding a break and have been shown to be important for DSB repair. Indeed, acetylation of H3 and H4 N-terminal domains has been shown to be important for DNA repair and facilitate factor accessibility to DNA [5–8]. In support of the importance of acetylation to DSB repair, the deletion of factors responsible for the acetylation and deacetylation of these residues, including the yeast histone acetyltransferases Gcn5 and Esa1, severely impacts repair and cell survival after damage [6–8].

Several ATP-dependent chromatin remodeling enzymes have been shown to be recruited to a DSB *in vivo*[4] and facilitate various aspects of repair. INO80 and SWR-C have been shown to be important for DNA end processing and checkpoint regulation [9–12], while RSC and SWI/SNF have been implicated in remodeling nucleosomes during repair [13–15]. However, the interplay between chromatin remodeling and histone modifications is still under investigation. Interestingly, a study has indicated that human SWI/SNF facilitates the phosphorylation of H2A.X by interacting with nucleosomes acetylated by Gcn5 [16]. Here we describe a similar functional interaction in yeast, although, in addition to Gcn5 activity, the Esa1-containing histone acetyltransferase, NuA4, plays a key role in recruiting SWI/SNF to the region surrounding a DSB. Furthermore, we find that the recruitment and subsequent function of SWI/SNF requires the bromodomain within the Swi2 subunit.

2. Materials and Methods

2.1 Yeast strains

All strains are derivatives of JKM139 [17], and were generated by one-step PCR disruption and confirmed by PCR analysis. Full genotypes are available in Supplementary Table S1. All strains were grown at 30°C as previously described [4], with the exception that *yng2*Δ cultures were grown overnight in YPD (1% yeast extract, 2% bactopeptone, 2% glucose), then washed and resuspended in warm YPRs (1% yeast extract, 2% bactopeptone, 2% raffinose, 0.2% sucrose (made and added fresh)) for 3-4 hours pre-induction. G2/M arrest was achieved using 30 mg/ml nocodazole for 4-5 hours. Degradation of Esa1 was achieved by addition of 1-naphthaleneacetic acid (NAA; Sigma) to a final concentration of 500 μm; an equal volume of solvent (100% ethanol) was added to control cultures. Cultures were concurrently transferred to a 22°C water bath.

2.2 Chromatin Immunoprecipitation

Lysates were prepared as previously described [4]. Briefly, mid-log phase cells were cross-linked with formaldehyde for 15 minutes, lysed, and sonicated to obtain an average DNA fragment size of 500 bp. Immunoprecipitations (IPs) were performed by adding 1-2 μl of antibody to diluted chromatin lysate and incubated overnight at 4°C. 1% sarkosyl was also added to SWI/SNF IPs. Sepharose protein A beads (50% slurry; Rockland) were added for 2 hours at 4°C, washed, and eluted at 65°C. IP and 10% input DNAs were purified and quantified by qPCR. Primer sequences are available in Supplementary Table S2. Fold enrichment represents the ratio of IP to input DNA at the break region, normalized to the same ratio obtained for the *ACT1* ORF. These values were corrected for DSB induction and

normalized to pre-induction (0 h) values. Error bars indicate s.e.m from at least two independent biological replicates.

2.3 Western blotting

Whole cell extracts were prepared by TCA extraction and proteins were separated by SDS-PAGE, blotted onto nitrocellulose membrane (GE) and probed with α-AID (BioRois) antibody using standard methods.

2.4 Antibodies

Rabbit polyclonal antibodies Arp5 (ab12099), Yaf9 (ab4468), Eaf3 (ab4467), and H2A-S129phos (γH2AX; ab15083) are commercially available from Abcam, as is the AID antibody from BioRois (APC004Am). Anti-Snf6 and anti-Swi2, and Anti-Sth1 antibodies were kind gifts from J. Reese (Pennsylvania State University) and B. Cairns (University of Utah), respectively.

3. Results

3.1 Gcn5 promotes recruitment of chromatin regulators to a DSB, H2A.X phosphorylation, and DSB resection

In order to study DSB responses in yeast, we used an established genomic system which places the endogenous HO endonuclease under control of the *GAL1* promoter which allows for galactose-dependent induction of a single double-strand break (DSB) at the *MAT* locus (Figure 1a)[17]. These strains also lack homologous donor loci at either end of the chromosome so the DSB cannot be repaired by homologous recombination, and continuous HO expression ensures that the DSB remains unrepaired throughout the experiment. Since the Gcn5 acetyltransferase is key for recruitment of SWI/SNF to a DSB in human cells, we tested whether Gcn5 plays a similar role in yeast. We first deleted the *GCN5* locus, and performed chromatin immunoprecipitation assays to measure the recruitment of chromatin regulators. Because it has been previously shown that the majority of chromatin regulators are recruited to a DSB during the G2/M phase of the cell cycle, rather than in G1[4], we synchronized cultures in G2/M with nocodazole and then induced a DSB by galactose addition. We monitored the recruitment of two subunits of SWI/SNF, Swi2 and Snf6, and found that their recruitment was dramatically lower in the absence of Gcn5 (Figure 1b), mirroring the behavior of human SWI/SNF[16]. We also monitored the levels of two other chromatin regulators: Yaf9 (a shared subunit between the NuA4 and SWR-C enzymes) and Arp5 (a subunit of INO80). Recruitment of Yaf9 was also decreased by the lack of Gcn5, whereas Arp5 was not significantly affected (Figure 1c). Taken together, these data suggest that Gcn5 has a role in the recruitment of several chromatin regulatory complexes to a DSB in yeast.

Recruitment of chromatin modifying enzymes is tightly linked to DNA end processing, where the 5’ strand is resected and produces a long 3’ ssDNA tail[4]. DSB resection is a hallmark of the homologous recombination pathway which is favored in G2/M[18–20]. To monitor DSB resection, quantitative PCR of ChIP input DNA was used to compare the DNA signal at the DSB site to an undamaged locus (*ACT1*). Since resection will eliminate one

strand of DNA, this leads to a 2-fold decrease in the relative PCR amplification signal. While the wild-type strain showed 60% resection of the DSB locus by 4 hours, resection in the *gcn5*Δ strain was decreased to about 30% (Figure 1d). Thus, one possibility is that the decrease in resection rates in the *gcn5* mutant may be responsible for the decrease in SWI/SNF and Yaf9 recruitment.

DSB resection rates and the phosphorylation of histone H2A.X typically show an inverse relationship [4]. Thus, extensive resection generally leads to a decrease in γH2AX, and defects in resection are associated with increased levels of γH2AX. Notably, although the *gcn5* mutant showed decreased levels of resection, there was a significant reduction of γH2AX after 4 hours of DSB induction (Figure 1e). Taken together, these data suggest that Gcn5 has a broad impact on early events at a DSB where it appears to promote DSB resection, chromatin regulator recruitment, and γH2AX formation.

3.2 The NuA4-dependent acetylation promotes the recruitment of SWI/SNF to a DSB, as well as H2A.X phosphorylation

Next we examined the role of a second histone acetyltransferase, NuA4, which, along with its mammalian homolog, Tip60, has been shown to be important for DSB repair and γH2AX levels [21]. The catalytic subunit of NuA4, Esa1, is essential for yeast viability, and so to study its role in SWI/SNF recruitment to a DSB, we utilized an inducible degron system to degrade Esa1 before DSB induction[22]. We fused the auxin-inducible degron (AID) cassette to the C-terminus of *ESA1* in a strain where the

Arabidopsis thaliana (At) TIR1 gene is constitutively expressed. In these strains, the binding of *AtTIR1*, an F-box protein, to the highly conserved Skp1 protein is promoted by auxin, and forms an E3 ubiquitin ligase that targets Esa1-AID for degradation. Following 2.5 hours in nocodazole media, *ESA1-AID*-containing cells were treated with either synthetic auxin (1-naphthaleneacetic acid; NAA) to induce degradation, or ethanol as a control (Figure 2a). Cells were maintained at 22°C during the auxin treatment since AtTIR1 has optimal activity at this lower temperature. In addition, the time courses were extended to 6 hours of DSB induction. This protocol resulted in a robust depletion of Esa1 (Figure 2b).

After Esa1 degradation (+NAA), the DSB recruitment of Swi2 and Snf6 were significantly reduced compared to the ethanol control (Figure 2c). These results paralleled the effects seen in the absence of Gcn5 (Figure 1b). Importantly, there was no decrease in the levels of Arp5 surrounding the break, indicating that INO80 recruitment does not require NuA4 (Figure 2d). Likewise, Eaf3, a shared subunit of both NuA4 and the Rpd3S histone deacetylase complex, showed no defect in recruitment (Figure 2d). This may indicate that the antibody preferentially detects the Rpd3-bound form of Eaf3. We also monitored recruitment of Yaf9 (SWR-C and NuA4), and in this case, Esa1 depletion led to a reduction in DSB recruitment at 4 hours, although they returned to wild-type levels by 6 hours (Figure 2d). Similarly γH2AX was also reduced at the 4 hour time point, but returned to wild-type levels by 6 hours (Figure 2e). DSB resection was also monitored by quantitative PCR, but the extent of resection in both the Esa1-depleted and control cells was quite low, likely due to the lower growth temperature (Figure 2f). The lower amount of resection may explain the

overall low recruitment levels for each of the chromatin modifying enzymes as well. However, these data indicate that the NuA4 enzyme promotes recruitment of SWI/SNF.

To further investigate the role of NuA4, we performed ChIP analyses in a *yng2* strain. Yng2 is a subunit of NuA4 that is essential for nucleosomal HAT activity[23,24]. Since *yng2* mutants are hypersensitive to microtubule destabilizing agents [25], the *yng2*Δ mutant could not be synchronized in G2/M by treatment with nocodazole, so experiments were performed in asynchronous cultures. Similar to the Esa1 depletion studies, recruitment of SWI/SNF to areas surrounding the induced DSB was dramatically decreased in the *yng2*Δ mutant (Figure 3a). However, there was also a moderate effect on Arp5 (INO80) recruitment, although only after 4 hours of break induction (Figure 3b). Meanwhile, Yaf9 (SWR-C & NuA4) exhibited only a marginal defect at farther distances from the break (Figure 3b). In addition, the *yng2*Δ mutant showed a defect in apparent resection rates (Figure 3d), but also only after 4 hours of break induction. This coincides with the defects seen in Arp5 and Yaf9 recruitment, suggesting that Arp5 and Yaf9 defects may be due to the resection deficiency. In contrast, the recruitment of SWI/SNF subunits was affected by 2 hours after break induction (Figure 3a). Furthermore, H2A.X phosphorylation was again negatively affected, although the contributions from very high levels of γH2AX in G1 cells may obscure a greater defect. Importantly, while only having minor effects on INO80 and SWR-C, both NuA4 mutants produced major defects in the recruitment of SWI/SNF to a DSB, indicating that NuA4 dependent acetylation plays a significant role in SWI/SNF recruitment.

3.3 The Swi2 bromodomain is necessary for SWI/SNF recruitment to a DSB

The ATPase subunit of SWI/SNF, Snf2/Swi2, contains a bromodomain which recognizes acetylated histone lysine residues [26], and this domain has previously been shown to stabilize the interaction of SWI/SNF with acetylated chromatin targets [27,28]. We asked if the Swi2 bromodomain could also stabilize SWI/SNF interaction with the chromatin surrounding a DSB. To address this point, we inserted a stop codon after Swi2 residue 1554, effectively removing the bromodomain of Swi2 (*swi2*Δ*br*). Notably, previous studies have shown that a similar C-terminal truncation has no impact on SWI/SNF assembly [29]. Consistent with previous work, recruitment of SWI/SNF to the *GAL1* promoter was decreased in the absence of the bromodomain (Figure 4a) [30–32], although this defect did not alter the induction of the galactose-induced DSB (data not shown). Importantly, the recruitment of both Swi2 and Snf6 to the DSB region was defective, particularly close to the break after 4 hours (Figure 4b). In contrast, other chromatin regulators were unaffected (Figure 4c). Notably, removal of the Swi2 bromodomain did not affect DSB resection (Figure 4e). But significantly, there was a significant defect in γH2AX levels (Figure 4d), emulating the effect seen in human cells. This indicates that SWI/SNF has an important role in promoting the high levels of γH2AX surrounding a DSB in G2/M.

4. Discussion

In this study, we have investigated the effects of two histone acetyltransferases on the recruitment of several chromatin regulators to a DSB. We found that the removal of the histone acetyltransferase Gcn5 negatively effects the recruitment of at least three chromatin remodeling complexes (SWI/SNF, INO80, and SWR-C), while also significantly affecting

DNA resection rates and levels of γH2AX. We further found that, even though removal of the histone acetyltransferase NuA4 also affected resection rates, it primarily affected SWI/SNF recruitment to DSBs with only minor effects on the other complexes tested. Finally, we showed that the acetyl-binding bromodomain of SWI/SNF is required for association of SWI/SNF with chromatin flanking a DSB. These results are consistent with early studies demonstrating that Gcn5 and Esa1 are recruited to an HO-induced DSB and that histones H3 and H4 are acetylated following DSB formation[8]. Furthermore, the defect in SWI/SNF recruitment led to a defect in the phosphorylation of H2A.X, indicating that SWI/SNF plays an important role in promoting the creation or maintenance of this important histone mark.

The dramatic effect of Gcn5 on DNA resection surrounding the break (Figure 1d) was unexpected, but consistent with results from two recent studies. In one, inactivation of the fission yeast homolog of Gcn5 led to a significant defect in DSB resection as well as a reduction in chromatin accessibility[33]. The authors associated this effect with the acetylation of histone H3 at lysine 36, catalyzed by Gcn5, and in direct opposition to the methylation of the same residue, whose inhibition had similarly opposite effects[33]. In a second study, the authors find that inhibition of histone deacetylases caused impaired resection, as well as the rapid degradation of resection machinery. Both of these defects were partially recovered when Gcn5 was also removed[34]. From this data we can expect that the effects of acetylation on repair are complex and that a balance of Gcn5-dependent acetylation and deacetylation is required for proper DNA resection and repair[34]. Furthermore, we have previously shown that defects in resection substantially impact the association of chromatin remodeling complexes with a DSB [4], and so the observed defect in resection due to the loss of Gcn5 likely contributes to the negative effect on chromatin remodeling complex recruitment to the DSB in that mutant (Figure 1b and c). However, it remains possible that Gcn5's histone acetyltransferase activity may play a direct role in recruitment through acetylation of H3, as seen in human cells [16], particularly because histone acetylation by Gcn5 is known to stabilize the interaction of SWI/SNF with DNA [27,28].

Similarly to Gcn5, disruption of NuA4 by either Esa1 depletion or inactivation of the Yng2 subunit resulted in a defect in SWI/SNF recruitment and DNA resection. In contrast, removal of the NuA4 acetyltransferase produced only minor effects on the recruitment of INO80 and SWR-C subunits to a DSB, which may be due to the concurrent defects in end processing. On the other hand, the drastic effect on SWI/SNF recruitment is likely due to histone H4 or H2A acetylation by NuA4. Indeed, SWI/SNF is likely to interact with one or more of these residues directly, as several *in vitro* studies have shown interaction between SWI/SNF and NuA4 acetylated nucleosomes [27,35,36]. Moreover, deletion of the Swi2 bromodomain significantly impacted SWI/SNF's ability to bind and remodel a NuA4 acetylated nucleosomal template *in vitro* [36]. It will be interesting to investigate which of the acetylated residues SWI/SNF specifically interacts with during the DNA damage response.

In all experiments where SWI/SNF recruitment was affected, we also saw a striking defect in histone H2A.X phosphorylation near to a DSB. By removing just the bromodomain of Swi2, we were able to show that this effect is directly linked to SWI/SNF recruitment to the break site. A similar effect is observed in human cells [16], demonstrating a conserved role for

SWI/SNF in promoting this important histone mark during the DNA damage response. We propose a model where, in response to a DSB, acetylation by NuA4 and Gcn5 allows for increased DNA end processing and thus increased recruitment of NuA4 and other chromatin modifying enzymes. Acetylation also promotes SWI/SNF recruitment to the break site, in turn promoting the phosphorylation of H2A.X by the checkpoint kinases Mec1 and Tel1 (Figure 5). How SWI/SNF promotes γH2AX formation or maintenance is not yet known, but promises to be an interesting topic for future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

5. Acknowledgements

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Highlights

- Gcn5 HAT is required for SWI/SNF recruitment to a DSB
- Gcn5 HAT is required for optimal DSB resection
- NuA4 HAT is required for SWI/SNF recruitment to a DSB
- The Swi2 bromodomain regulates SWI/SNF recruitment to a DSB
- SWI/SNF recruitment regulates H2AX-phosphorylation

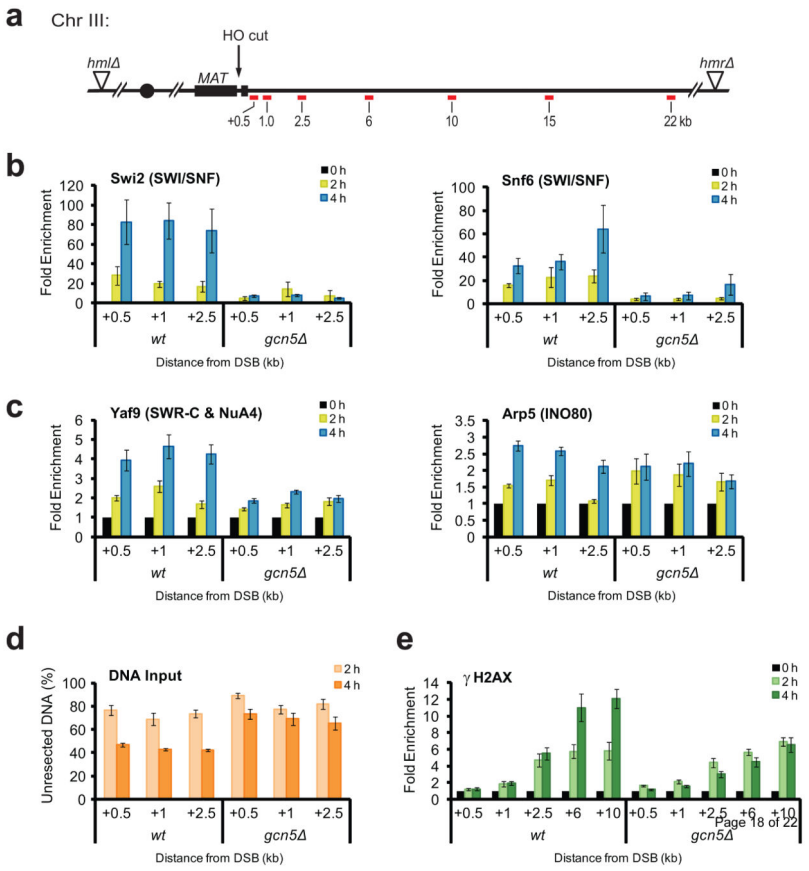

Figure 1. Gcn5 promotes H2A.X phosphorylation and the recruitment of multiple chromatin regulators to a DSB

(a) Schematic of the donorless yeast strain harboring a galactose inducible HO endonuclease, which cuts at the MAT locus of chromosome III. The approximate location of regions amplified for ChIP analyses to the right ("+") of the break are shown in red and labeled according to their distance from the DSB in kilobases (kb). (b, c) Wild-type (*wt*), and *gcn5*Δ strains were arrested in G2/M using nocodazole, and analyzed by ChIP for recruitment of the indicated chromatin modifying enzyme subunits to the DSB region at the specified time points after DSB induction. (d) Input DNA at the break site relative to an unbroken locus (*ACT1*) and normalized to pre-induction levels. (e) As in (b), γH2AX levels determined by ChIP at the indicated time points after break induction.

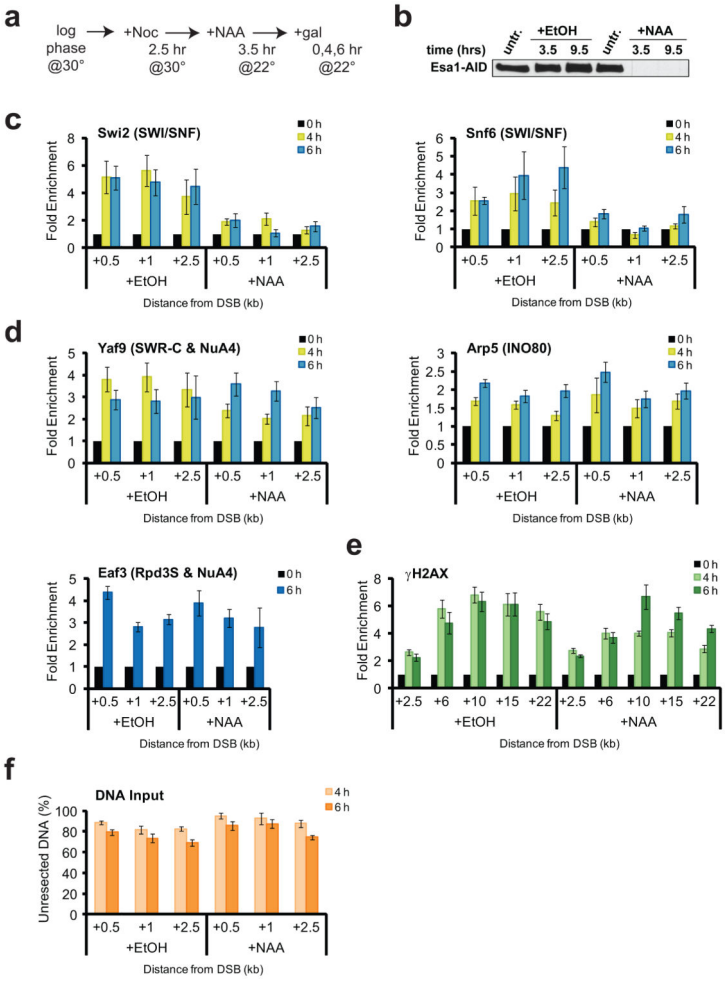

Figure 2. NuA4 promotes SWI/SNF recruitment to a DSB and H2A.X phosphorylation

(a) Schematic representing the yeast culture growth and treatments. (b) Western blot of samples from *ESA1-AID* containing yeast strains treated with either ethanol or 500μm NAA. untr. = untreated samples. (c,d) The *ESA1-AID* yeast strain treated with ethanol or NAA and analyzed by ChIP for the indicated chromatin modifying enzyme subunits after DSB induction. (e) γH2AX levels determined by ChIP at the indicated time points after break induction. (f) Input DNA at the break site relative to an unbroken locus (*ACT1*) and normalized to pre-induction levels.

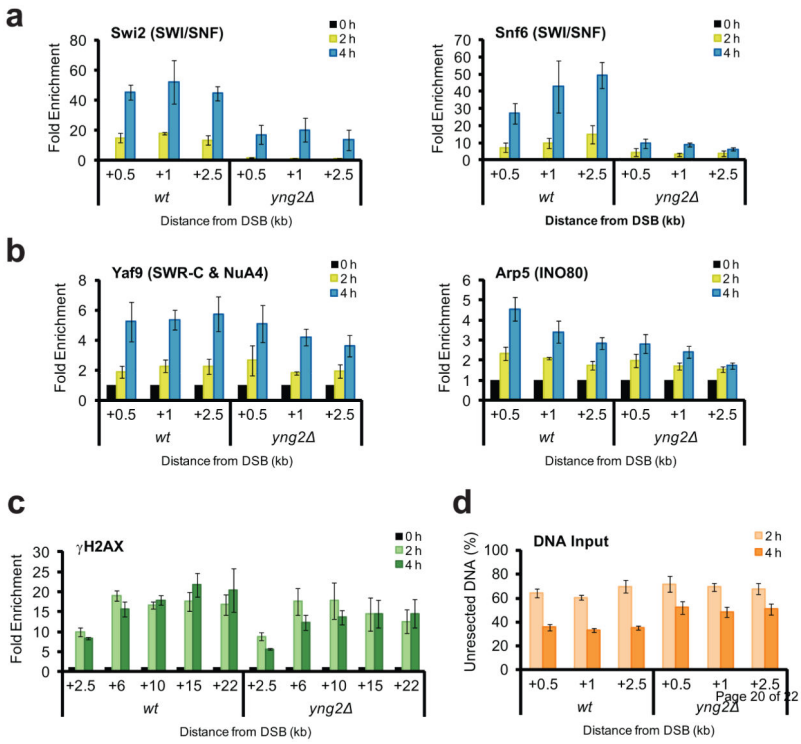

Figure 3. NuA4 acetylation of chromatin specifically promotes SWI/SNF recruitment to a DSB

(a,b) ChIP analyses of the indicated chromatin modifying enzyme subunits after an induced break in isogenic *wt* and *yng2*Δ yeast strains. (c) γH2AX levels determined by ChIP at the indicated time points after break induction. (d) Input DNA in at the break site relative to an unbroken locus (*ACT1*) and normalized to pre-induction levels.

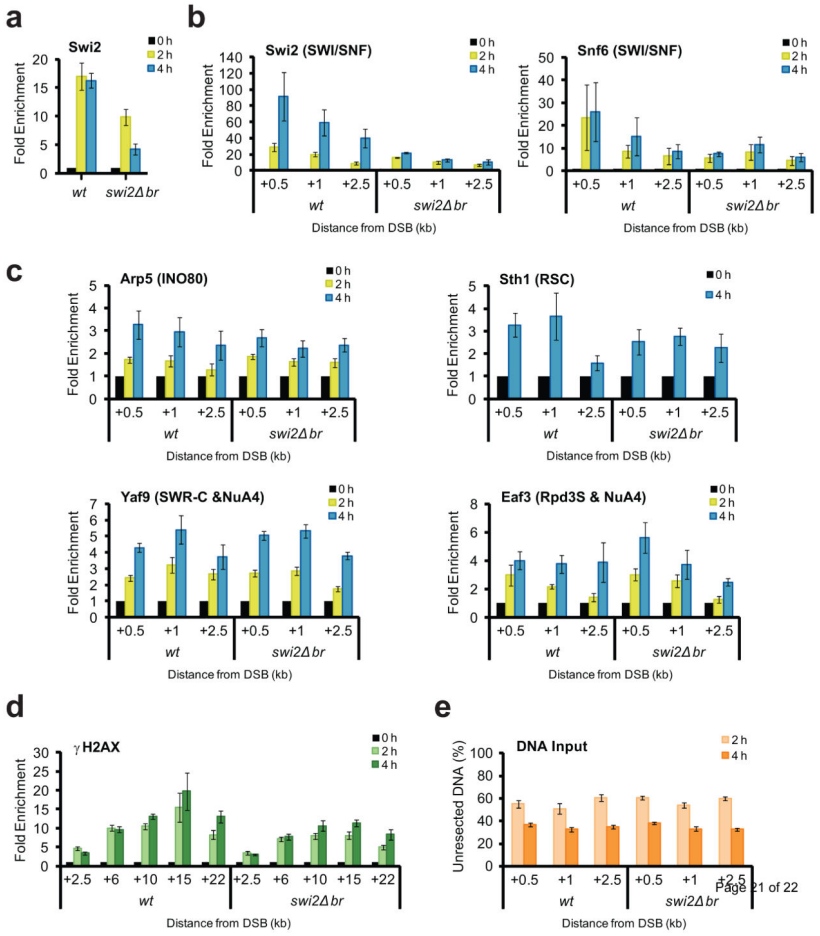

Figure 4. SWI/SNF's bromodomain is key for DSB recruitment and promotes H2A.X phosphorylation

(a) Isogenic *wt* and *swi2Δbr* yeast strains were synchronized in G2/M and analyzed by ChIP for the indicated chromatin modifying enzyme subunits at (a) the *GAL1/10* promoter region, and (b,c) the double-strand break site. (d) γH2AX levels determined by ChIP at the indicated time points after break induction. (e) Input DNA at the break site relative to an unbroken locus (*ACT1*) and normalized to pre-induction levels.

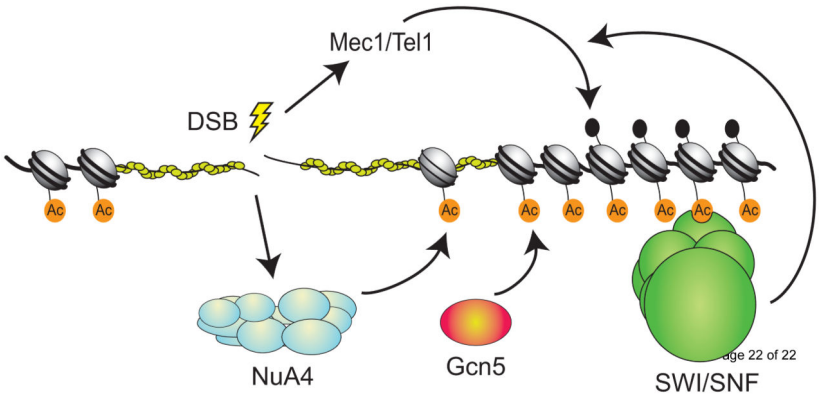

Figure 5. A model for the interaction of NuA4 acetylation, SWI/SNF DSB recruitment, and γH2AX during DSB damage response

See text for details