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NuA4 acetyltransferase is required for efficient nucleotide excision repair in yeast

Amelia J. Hodges[#], Dalton A. Plummer[#], and John J. Wyrick^{*,†,#}

^{*}School of Molecular Biosciences, Washington State University, Pullman, WA 99164

[†]Center for Reproductive Biology, Washington State University, Pullman, WA 99164

[#] These authors contributed equally to this work.

Abstract

The nucleotide excision repair (NER) pathway is critical for removing damage induced by ultraviolet (UV) light and other helix-distorting lesions from cellular DNA. While efficient NER is critical to avoid cell death and mutagenesis, NER activity is inhibited in chromatin due to the association of lesion-containing DNA with histone proteins. Histone acetylation has emerged as an important mechanism for facilitating NER in chromatin, particularly acetylation catalyzed by the Spt-Ada-Gcn5 acetyltransferase (SAGA); however, it is not known if other histone acetyltransferases (HATs) promote NER activity in chromatin. Here, we report that the essential Nucleosome Acetyltransferase of histone H4 (NuA4) complex is required for efficient NER in *Saccharomyces cerevisiae*. Deletion of the non-essential Yng2 subunit of the NuA4 complex causes a general defect in repair of UV-induced cyclobutane pyrimidine dimers (CPDs) in yeast; in contrast, deletion of the Sas3 catalytic subunit of the NuA3 complex does not affect repair. Rapid depletion of the essential NuA4 catalytic subunit Esa1 using the anchor-away method also causes a defect in NER, particularly at the heterochromatic *HML* locus. We show that disrupting the Sds3 subunit of the Rpd3L histone deacetylase (HDAC) complex rescued the repair defect associated with loss of Esa1 activity, suggesting that NuA4-catalyzed acetylation is important for efficient NER in heterochromatin.

Keywords

NER; Esa1; chromatin; DNA repair; histone acetylation

1. Introduction

Cellular DNA is damaged by a variety of endogenous and exogenous sources, inducing DNA lesions that must be efficiently repaired in order to maintain genomic stability and

[#]Corresponding author: Dr. John J. Wyrick, School of Molecular Biosciences, Biotechnology Life Sciences 241, Washington State University, Pullman, WA 99164, 509-335-8785, jwyrick@wsu.edu.

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avoid mutagenesis. The nucleotide excision repair (NER) pathway is responsible for the repair of DNA helix-distorting lesions, such as cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4PPs) induced by UV radiation [1, 2]. CPD lesions on the transcribed strand of genes are rapidly removed by the transcription-coupled NER (TC-NER) pathway, which is initiated when RNA polymerase II stalls at these lesions during transcription [3–5]. CPD lesions located elsewhere in the genome are repaired by the global genome NER (GG-NER) pathway [6]. CPD removal by the GG-NER pathway generally occurs more slowly than TC-NER, in part because damage recognition factors in GG-NER (i.e., DDB2 and XPC in humans, Rad7/Rad16 and Rad4 in yeast) must contend with lesions residing in chromatin [6–8].

The compaction of eukaryotic DNA into chromatin is a double-edged sword for DNA damage and repair. Chromatin structure can protect DNA from certain types of lesions (e.g., UV-induced 6–4PPs [9, 10]) and plays important roles in DNA damage signaling [11, 12]; however, this organization can also restrict accessibility of repair factors to DNA. The basic subunit of chromatin is the nucleosome, which is comprised of ~147 base pairs of DNA wrapped around an octamer of histone proteins [13]. Nucleosomes significantly reduce NER efficiency *in vitro* [7, 14] and *in vivo* [15–17], indicating that even the lowest level of chromatin organization presents a significant barrier to repair.

Chromatin structure is dynamically modulated by histone post-translational modifications, which have been implicated in regulating DNA repair in chromatin [18, 19]. The best-studied histone modification associated with NER is histone acetylation (reviewed in [8, 19, 20]). In *Saccharomyces cerevisiae*, histones H3 and H4 are rapidly acetylated in response to UV irradiation [21]. This response is mediated in part by the Gcn5 acetyltransferase, which is recruited to chromatin in response to UV irradiation [22] and has been shown to be required for efficient repair of UV damage at individual yeast loci (e.g., *MFA2* promoter [21, 23]). A recent genome-wide analysis indicates that a *gcn5* mutant alters the distribution of repair rates at many yeast genes [24].

However, it is not known whether other histone acetyltransferases are important for efficient NER activity in yeast chromatin. Possible candidates include Sas3 and Esa1, which function as the catalytic subunits of the Nucleosome Acetyltransferase of histone H3 (NuA3) and Nucleosome Acetyltransferase of histone H4 (NuA4) complexes, respectively [25]. Esa1 is a particularly intriguing candidate, as it is the only histone acetyltransferase that is essential for yeast viability [26], and because partial loss of function mutants in Esa1, as well as other NuA4 subunits (e.g., Yng2), are hypersensitive to DNA damage and DNA replication stress [27–29]. Esa1 primarily acetylates lysine residues in the N-terminal tails of histones H2A, H2A.Z, and H4 [25], but can also acetylate non-histone proteins [30, 31]. In addition to NuA4, Esa1 also serves as the catalytic subunit of the Piccolo-NuA4 complex, which functions to maintain basal levels of histone acetylation throughout the genome [32]. Esa1 and its human homologue TIP60 have been shown to be important for double-strand break repair in yeast and human cells, respectively [27, 33, 34]. Additionally, the NuA4 complex has been shown to play a role in DNA damage bypass via translesion synthesis [35] and postreplication repair [36]. However, it is not known if NuA4 (or NuA3) is important for NER.

In this study, we investigated whether the NuA3 and NuA4 acetyltransferases are important for repair of UV-induced DNA lesions in yeast chromatin. Here, we show that deletion or conditional depletion of NuA4 subunits in yeast cause a striking decrease in repair of UV-induced lesions, particularly at heterochromatic loci. Furthermore, we demonstrate that this repair defect can be rescued by a mutant that disrupts the function of the Rpd3L histone deacetylase complex, highlighting the importance of NuA4-mediated histone acetylation dynamics in NER.

2. Materials and methods

2.1. Yeast strains and plasmids

Yeast cells were grown in YPD (yeast extract peptone dextrose) or SC (synthetic complete) media. Anchor-away strains were constructed using the CRISPR method [37] and PCR product derived from pfa6a-FRB-his3MX6 (gift from Dr. Frank Holstege). Detailed information about yeast strains and plasmids can be found in Supplemental Table S1 and S2.

2.2. Western Blotting

Yeast whole cell extracts were isolated from yeast cells using 0.1M NaOH and boiling in 1x SDS page buffer, as previously described [38], analyzed by SDS-polyacrylamide gel electrophoresis (typically 15% acrylamide), and transferred to PVDF membrane. Blots were probed using anti-acetyl H4 (Upstate), anti-GAPDH (ThermoFisher), with corresponding secondary antibodies.

2.3. Viability after rapamycin treatment

For spotting assays, cells were grown to mid-log phase in SC media. Serial dilutions of the culture were spotted onto SC media alone or SC media containing 1 μ g/mL rapamycin, and pictures were taken after 3–4 days. For the growth curves, cells were grown in YPD media and OD₆₀₀ measurements were taken at the indicated time points after addition of rapamycin (time 0). Concurrent with the growth curve assays, aliquots were taken at the indicated time points after rapamycin treatment to measure cell viability on plates. Using the OD₆₀₀ measurement, cell concentration was calculated, and approximately 100 cells were plated on complete media in the absence of rapamycin. Colony formation was counted after 3 days at 30°, and normalized to the 0 hr timepoint. Graphs indicate the mean and SEM of 3 independent replicates.

2.4. Global DNA repair assay

Alkaline gel analysis was conducted as previously described [39]. For anchor-away experiments, early log phase cells were treated with 1 μ g/ml rapamycin for 3 hours. The cell cultures were spun down and resuspended in water and then exposed to 100 J/m² of UV light (primarily 254nm) or mock treated for “No” UV control. Following UV irradiation, cells were resuspended in YPD (with 1 μ g/mL rapamycin if applicable) and incubated at 30°C for repair. Aliquots were taken at each repair time point and frozen at –80°C. Genomic DNA was isolated by bead beating the cell pellets in 200 μ L lysis buffer (2% Triton-X 100, 1% SDS, 100mM NaCl, 10mM Tris-Cl pH8, 1mM Na₂EDTA) and 300 μ L Phenol-Chloroform-Isoamyl alcohol (25:24:1). After addition of 300 μ L TE pH8, samples were

centrifuged and DNA from the aqueous layer was precipitated in ethanol. DNA pellets were resuspended in TE pH 8 +0.2mg/mL RNaseA and incubated at 37° for 15 minutes before further processing [40]. Equivalent aliquots of DNA were treated or not treated with T4 endonuclease V (Epicentre or gift of Dr. Steven Roberts) and resolved by alkaline gel electrophoresis. After neutralization and staining with Sybr Gold (Invitrogen), gels were imaged using the Typhoon FLA 7000 (GE Healthcare) and analyzed using ImageQuant 5.2 (Molecular Dynamics) by calculating the ensemble average of each lane corrected by the no enzyme control lane to determine the number of CPDs/kb [41]. Percent repair was calculated by normalizing the number of CPDs/kb to the 0 hr time point (0%). Graphs represent the mean and SEM of at least 3 independent experiments.

2.5. Site-specific DNA repair assay

Yeast cultures were grown, UV-irradiated (100 J/m²), and harvested as for the global assay. Genomic DNA was isolated as described above, then treated with restriction enzymes (EcoRI+EcoRV for *GAL10*, PvuII+BsaHI for *HML*, DraI for *RPB2*) in supplied buffer (NEB) to release the DNA fragment corresponding to each genomic locus. DNA was purified using Phenol-Chloroform-Isoamyl alcohol (25:24:1) and ethanol precipitated before being digested with T4 endonuclease V. DNA fragments were resolved by alkaline gel electrophoresis, then transferred to a nylon membrane. Regionspecific probes were created using region-specific PCR products amplified from yeast genomic DNA and were radiolabeled using the Prime-It II Random Primer Labeling Kit (Stratagene) and α -³²P dATP (Perkin-Elmer). Oligonucleotide primers to amplify *HML* probe were 5'-CACTGCTCTTTTCTGTGTTCCA-3' and 5'-GCATTAGAGAAATTTTCGCATAGCAA-3'; primers for the *GAL10* and *RPB2* probes were the same as previously described [42]. Autoradiograms were detected using the Typhoon FLA 7000 (GE Healthcare) and quantified using ImageQuant as previously described [42]. We analyzed repair at 0, 1, 2, and 3 hr time points for each locus, with the exception of *RPB2*. There was a similar trend in repair at *RPB2* for the WT and Esa1-AA strains at 3 hr repair, but this time point often had lower overall signal, and so was not analyzed further. Graphs represent the mean and SEM of at least 3 independent replicate experiments.

3. Results

3.1. Deletion of a non-essential subunit of the NuA4 complex impairs NER in yeast

To investigate whether the NuA3 or NuA4 acetyltransferase complexes are required for efficient NER, we analyzed repair of UV-induced CPD lesions in yeast mutants that compromised the activity of either complex. We first tested whether deletion of *SAS3*, which encodes the catalytic subunit of NuA3, affected repair of CPD lesions in yeast. The *sas3* mutant and wild-type (WT) control strains were irradiated with 100 J/m² dose of UV-C light (primarily 254 nm), and allowed to repair for various times. Repair of CPD lesions in isolated genomic DNA at various repair times was monitored using the published T4 endonuclease V digestion and alkaline gel electrophoresis assay [39, 43]. Using this assay, we found no significant difference in repair of CPDs in bulk genomic DNA at any repair time point (i.e., 1, 2, or 3 hours (hr) following UV irradiation) in the *sas3* mutant relative to WT (see Figure 1A,B). These results indicate that NuA3 activity is largely dispensable for

NER activity in yeast chromatin. Deletion of *Gcn5*, which encodes the catalytic subunit of the SAGA complex, causes a significant defect in the repair of CPD lesions at the final 3 hr repair time point (Figure 1C, $P < 0.05$), but not at early time points. A previous study has indicated that Gcn5 is required for efficient NER activity in yeast chromatin [24]; our data suggest that Gcn5 may be particularly important for slow-repairing regions of genomic chromatin.

As the NuA4 catalytic subunit Esa1 is essential for yeast viability [26], we characterized a deletion of the non-essential NuA4 subunit Yng2, which is required for NuA4/Esa1 histone acetyltransferase activity in chromatin [32, 44, 45]. Western blot analysis using a pan-H4 acetylation antibody confirmed that histone H4 acetylation levels are lower in the *yng2* mutant relative to WT (Figure S1), consistent with a defect in NuA4-catalyzed histone acetylation. Using our repair assay, we found that CPD repair in bulk genomic DNA was lower in the *yng2* mutant relative to WT at every repair time point (i.e., 1, 2, and 3 hr following UV irradiation). There was an overall ~2fold reduction in repair in the *yng2* mutant relative to WT after 3 hr (Figure 1D), similar in magnitude to the repair defect observed in the *gcn5* mutant at the same time point. These data suggest that the NuA4 acetyltransferase activity is required for efficient repair of UV lesions in yeast chromatin.

3.2. Rapid nuclear depletion of Esa1 using a chemical genomics approach confirms that NuA4 activity is important for NER

Since NuA4 is an important regulator of yeast gene expression, it is possible that the *yng2* mutant might indirectly affect repair by decreasing the expression of key NER genes. However, none of the genes involved in NER are significantly decreased in expression in a published *yng2* gene expression data set [46], indicating that the repair defect is unlikely to be due to expression changes in the NER machinery.

To confirm the role of the NuA4 complex in regulating NER, and avoid potential secondary effects due to disrupting NuA4 activity, we employed a chemical genomics approach known as anchor-away [47] to rapidly deplete NuA4 activity from the nucleus in yeast. Esa1 was tagged on its N-terminus with FRB (FKBP rapamycin-binding domain) in a yeast strain with the ribosomal subunit RPL13A tagged with FKBP (FK506- and rapamycin-binding protein). Addition of rapamycin causes the FRB and FKBP tags to dimerize, leading to rapid nuclear depletion of the FRB-tagged protein, due to its export to the cytoplasm (Figure 2A).

Rapamycin treatment of a yeast strain containing anchor-away tagged Esa1 (AA-Esa1) caused a rapid and severe decrease in cellular histone H4 acetylation levels. Western blot analysis indicated that pan-H4 acetylation was significantly decreased following 3 hr of rapamycin treatment in the AA-Esa1 strain (Figure 2B). In contrast, rapamycin treatment had no effect on H4 acetylation in the isogenic untagged strain (WT, see Figure 2B). The decrease in H4 acetylation due to anchor-away depletion of Esa1 could be rescued by expression of untagged wild-type Esa1 (Figure 2B), but not by expression of a catalytically inactive Esa1 mutant (Esa1-E338Q), indicating that histone H4 acetylation is decreased because of Esa1 depletion. Since Esa1 is essential for growth, we also measured cell growth and viability following anchor-away depletion of Esa1. Chronic incubation with rapamycin caused a significant growth defect in the AA-Esa1 strain (Figure 2C). However, there was no

difference in cell growth or viability in AA-Esa1 relative to the WT control over a 6 hr time window following rapamycin treatment (Figure 2D-E), indicating that the decrease in H4 acetylation in the AA-Esa1 strain following 3 hr rapamycin treatment was not a secondary effect of inhibition of cell growth or cell death.

To characterize the role of Esa1 in NER, we measured the repair of UV-induced CPDs following rapid anchor-away depletion of Esa1, using the repair assay described above. The AA-Esa1 strain was pretreated with rapamycin for 3 hr prior to UV irradiation, in order to deplete Esa1 acetyltransferase activity from the nucleus. Following UV-irradiation, cells were allowed to repair UV-induced DNA damage in rapamycin-containing media. As a control, repair of CPDs was measured in the untagged WT strain that had been treated in a similar manner. We observed a striking global defect in repair of CPD lesions in the rapamycin-treated AA-Esa1 strain (Figure 3A-B). There was a significant decrease in CPD repair at the 2 and 3 hr repair time points relative to the untagged WT control, with an overall ~2-fold decrease in repair after 3 hours. The repair defect due to Esa1 anchor-away depletion was very similar to the repair defect in the *yng2* strain, confirming that NuA4 activity is required for efficient NER. There was no significant change in repair activity in the AA-Esa1 strain in the absence of rapamycin (Figure 3C), indicating that this repair defect was due to rapamycin-mediated nuclear depletion of AA-Esa1.

3.3. NuA4 is important for repair of CPD lesions in the silent *HML* locus

To determine whether Esa1 is required for NER in different chromatin contexts, we analyzed repair of CPD lesions in yeast at the *RPB2* and *HML* loci [42, 48, 49]. The *RPB2* locus, which encodes the second largest subunit of RNA polymerase II, is constitutively expressed in yeast, while the *HML* locus is comprised of silent heterochromatin that is hypoacetylated [50]. To examine repair at these loci in the absence of nuclear Esa1, we pre-treated the AA-Esa1 strain with rapamycin for 3 hr prior to UV irradiation, and continued treating with rapamycin during the subsequent repair time course.

Anchor-away depletion of Esa1 caused a striking defect in repair at the silent *HML* locus. CPD repair was significantly decreased at the 2 and 3 hr time points (Figure 4A-B), with a ~3-fold decrease in repair relative to the WT control at 2 hr ($P = 0.019$). In contrast, there was no significant effect of Esa1 depletion on repair at the transcriptional active *RPB2* locus (Figure 4C-D). These data suggest that Esa1 may play a particularly important role in facilitating NER in silent heterochromatin, which is normally hypoacetylated. We also tested whether Esa1 is required for repair of UV lesions in the *GAL10* locus, which is comprised of repressed chromatin when yeast are grown in glucose media [42, 48, 49]. Upon anchor-away Esa1 depletion, CPD repair at the repressed *GAL10* locus was decreased relative to the WT control at the 3 hr repair time point (Figure S2, $P = 0.0029$). These results indicate that Esa1 is important for repair in repressed and silent chromatin in yeast.

3.4. Holo-NuA4 and Piccolo-NuA4 complexes both contribute to NER

Esa1 and Yng2 are integral subunits of two distinct histone acetyltransferase complexes: the NuA4 complex (i.e., holo-NuA4) and the smaller Piccolo-NuA4 complex, which contains only the Esa1, Yng2, Epl1, and Eaf6 subunits (Figure 5A). To characterize the contributions

of these distinct Yng2- and Esa1-containing complexes, we measured repair of CPD lesions in a yeast strain lacking the NuA4-specific subunit Eaf1. The *eaf1* mutant specifically disrupts the integrity of the NuA4 complex, but has no effect on Piccolo-NuA4 structure or activity [45, 51, 52]. There was a decrease in repair of CPD lesions in the *eaf1* mutant relative to WT, with a ~1.5-fold reduction in repair at 3 hr (Figure 5B-C). The decrease in repair at 3 hr was not statistically significant using a conventional unpaired t-test ($P = 0.11$), in part because of the relatively high variability among replicates. Using a paired t-test to account for this variability, we found that repair at 3 hr was consistently decreased in the *eaf1* mutant relative to WT ($P = 0.03$). These results indicate that the NuA4-specific Eaf1 subunit has a marginal role in the repair of CPD lesions in yeast, to a lesser extent than Yng2 and Esa1.

3.5. Repair defect due to loss of Esa1 activity can be rescued by inactivating the Rpd3L complex

Previous studies have implicated the Rpd3L and Set3/Hos2 histone deacetylase complexes as opposing the activity of NuA4/Esa1. Deletion of the Sds3 subunit of Rpd3L histone deacetylase complex rescues the lethality of *esa1* mutant [53, 54], while deletion of Hos2 rescues many of the DNA damage sensitivities of the *esa1* partial loss-of-function mutants [55]. Hence, we tested whether the NER defect associated with mutations in NuA4 subunits could be rescued by a compensatory loss-of-function mutation in either *SDS3* or *HOS2*.

The *esa1 sds3* double mutant strain was viable, consistent with a previous report [54], so we tested whether the NER defect associated with loss of Esa1 acetyltransferase activity is rescued by a compensatory deletion of the Sds3 subunit of the Rpd3L HDAC complex. The *sds3* mutant alone did not significantly affect repair of CPD lesions relative to the WT control following UV irradiation (Figure S3). However, the *sds3* mutant rescued the repair defect associated with loss of Esa1 activity, as the *esa1 sds3* double mutant strain had similar repair efficiency of CPD lesions as the WT control strain (Figure 6A,B).

Since deletion of *HOS2* does not rescue *esa1* lethality [54], we tested whether a *hos2* mutant could rescue the repair defect observed when *YNG2*, a non-essential subunit of NuA4 that is required for Esa1 acetyltransferase activity, is deleted (see Figure 1). The *hos2* mutant alone caused a slight increase in repair of CPD lesions relative to the WT control (Figure S4), particularly at the 1 hr time point ($P < 0.05$), suggesting that Hos2 may function to repress NER in chromatin by deacetylating histones. However, the *yng2 hos2* double mutant had a significant repair defect following UV irradiation, as CPD repair was considerably lower in the *yng2 hos2* double mutant relative to the WT control (Figure 6C), particularly at the 2 hr time point ($P = 0.0044$). The repair defect in the *yng2 hos2* double mutant was very similar to the defect seen in the *yng2* mutant alone, indicating that deletion of the Hos2 HDAC does not rescue the repair defect caused by loss of NuA4 acetyltransferase activity.

4. Discussion

While histone acetylation by the Gcn5 subunit of the SAGA complex is known to play an important role in the repair of UV damage by the NER pathway [8, 20], the potential roles of

other yeast HAT complexes in NER were previously unclear. Here, we show that the essential NuA4 HAT complex is important for efficient repair of UV-induced CPD lesions in yeast chromatin. Deletion of the nonessential Yng2 subunit of NuA4, which is required for acetylation of nucleosomal histones [32, 44, 45], causes a global defect in NER of UV-induced CPD lesions. This defect in repair is unlikely to be due to Yng2-dependent changes in gene expression, since the *yng2* mutant does not downregulate the expression of known NER genes [46]. Moreover, rapid nuclear depletion of the Esa1 catalytic subunit of NuA4 using the anchor-away strategy causes a similar repair defect. Taken together, our data indicate that NuA4 likely plays a direct role in facilitating NER in chromatin; however, it will be important to test in future studies whether NuA4 is directly recruited in response to UV damage.

Yng2 and Esa1 are subunits of two distinct NuA4 complexes: the holo-NuA4 and Piccolo-NuA4 complexes, both of which can acetylate nucleosomal histones in vivo. Holo-NuA4 is targeted to specific genomic loci through its recruitment module, which interacts with transcriptional activators as well as DNA damage-associated signals [45, 56–58]. In contrast, Piccolo-NuA4 functions in an untargeted fashion to maintain basal levels of acetylated histones throughout the genome [32]. Our data indicate that an *eaf1* mutant, which specifically disrupts the holo-NuA4 complex, but does not affect Piccolo-NuA4 integrity or activity [45, 51, 52], causes a marginal decrease in repair of UV-induced CPD lesions. The *eaf1* strain has also been reported to have elevated sensitivity to UV damage [59], consistent with the model that holo-NuA4 is required for efficient NER of UV-induced CPD lesions. However, the repair defect in the *eaf1* strain is less severe than the *yng2* mutant, indicating the Piccolo-NuA4 may also function in NER.

Analysis of CPD repair at individual yeast loci indicates that NuA4 is particularly important for repair at the heterochromatic *HML* and repressed *GAL10* loci, but not at the actively transcribed *RPB2* locus. NuA4 normally acetylates histones associated with gene promoters and actively transcribed coding regions [57, 60–62], but this activity appears to be dispensable for efficient repair, at least at the actively transcribed *RPB2* locus. In contrast, NuA4 activity is essential for efficient repair at the silent *HML* locus, even though histones at the *HML* locus are not thought to be acetylated by NuA4 under normal conditions (i.e., in the absence of DNA damage). An implication of these findings is that NuA4 may play a particularly important role in facilitating NER in repressive and hypoacetylated chromatin, even though such chromatin domains are normally not targets of NuA4 activity.

This conclusion may explain our genetic data analyzing which HDACs can rescue the repair defects of NuA4 mutants. The Hos2 HDAC is a subunit of the Set3 complex that is important for deacetylation of histones located in 5' coding regions and certain promoters [63–65]. A *hos2* mutant can partially rescue H4 acetylation levels [55], but not the NER defect in NuA4 mutants (i.e., *yng2*). Presumably, this is because NuA4 is largely dispensable for repair at actively transcribed coding regions (such as *RPB2*), where Hos2 is targeted. In contrast, the *sds3* mutant, which disrupts the Rpd3L HDAC complex, results in WT levels of repair in an *esa1* mutant strain, even though it does not rescue cellular H4 acetylation levels in this strain (see Figure S1 [54]). This could be explained by the observation that the Sds3-containing Rpd3L complex has an important role in regulating

gene silencing at the yeast silent mating loci [66], since we observed the most severe repair defect in the *Esa1*-depleted strain at the silent *HML* locus. Alternatively, the Rpd3L complex could affect the acetylation state of non-histone proteins that may be an important target of NuA4 activity during NER.

Our finding that NuA4 is important for efficient NER in chromatin has potentially important implications for the role of the human NuA4/TIP60 complex in carcinogenesis and chemotherapeutic resistance. The catalytic subunit of the human NuA4 complex is TIP60, the human homolog of *Esa1*. TIP60 is a haploinsufficient tumor suppressor that is frequently down-regulated and associated with carcinogenesis in various cancer types, including malignant melanoma and breast cancer [67, 68]. Moreover, the Inhibitor of Growth 3 (*ING3*) gene, which encodes a subunit of the human NuA4/TIP60 complex and is homologous to the yeast *Yng2* subunit, is frequently decreased in expression in melanoma and head and neck cancers [69, 70]. Our data suggest that decreased NuA4 activity in human cells may stimulate carcinogenesis by down-regulating the activity of the NER pathway, which repairs potentially mutagenic DNA lesions. Tip60 overexpression has also been linked to cellular resistance to the chemotherapy drug cisplatin [71], which generates DNA lesions repaired by the NER pathway. We hypothesize that TIP60 overexpression may promote cisplatin resistance by directly stimulating NER of cisplatin DNA lesions. It will be important in future studies to test this mechanism, as well as to determine whether modulation of HDAC activity might compensate for TIP60-dependent alterations in NER activity and cisplatin resistance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights:

- NuA4 acetyltransferase complex is required for nucleotide excision repair (NER)
- Deletion or depletion of NuA4 subunits cause a global defect in NER in yeast
- NuA4 is particularly important for efficient NER in silent heterochromatin
- NER defect can be suppressed by a mutation in the Rpd3L deacetylase complex

Figure 1.

Effects of deletion mutants in non-essential subunits of SAGA (*gcn5*), NuA4 (*yng2*), and NuA3 (*sas3*) on repair of UV-induced CPD lesions in yeast. (A) Representative alkaline gels of genomic DNA isolated at the indicated repair time points following UV irradiation (100 J/m²), following treatment with or without (+/-) T4 endonuclease V. T4 endonuclease V creates single-stranded DNA nicks at CPD lesions, which were resolved on denaturing alkaline gels. (B-D) Quantification of CPD repair in mutant and wild-type (WT) yeast strains. The percentage of CPD lesions removed at each time point is plotted as the mean $\pm$ SEM of at least 3 independent experiments. P values were calculated using an unpaired t-test. *P $\leq$ 0.05.

Figure 2.

Rapid nuclear depletion of FRB-tagged Esa1 (AA-Esa1) using the anchor-away system. (A) Schematic diagram of the anchor-away system for rapidly depleting proteins from the nucleus. (B) Histone H4 acetylation is significantly diminished following anchor-away depletion of Esa1, based on western blot analysis of AA-Esa1 or isogenic wild type cells before and after 3 hours rapamycin treatment using a panH4acetyl antibody. Where indicated, a plasmid containing wild type Esa1 (+pEsa1) or mutant Esa1 (+pEsa1 E338Q) was co-expressed. GAPDH was used as a loading control. (C) Ten-fold serial dilutions of yeast spot onto synthetic complete (SC) or SC+ 1 μ g/mL rapamycin plates. (D) OD₆₀₀ measurements at the indicated time points following treatment with rapamycin. (E) Cultures treated with rapamycin for the indicated times were plated onto complete media and resultant colonies were counted and normalized to the untreated control. (D-E) represent the mean and SEM of 3 independent experiments.

Figure 3.

Anchor-away depletion of Esa1 causes a significant defect in NER of UV-induced CPD lesions. A) Representative alkaline gels of genomic DNA isolated at the indicated repair time points following UV irradiation (100 J/m^2), following treatment with or without (+/-) T4 endonuclease V. Cells were preincubated with $1 \mu\text{g/ml}$ rapamycin for 3 hours prior to UV irradiation to deplete Esa1 from the nucleus (AA-Esa1 strain), and were further incubated with rapamycin during the repair time course. A control strain (WT), in which Esa1 was not anchor-away tagged, was treated similar. (B-C) Quantification of %CPD repair from alkaline gels analyzing repair in the control (WT) or Esa1 anchor-away tagged (Esa1-AA) strain, treated with (B) or without (C) rapamycin. The mean $\pm$ SEM is depicted for a minimum of 3 independent replicates. P values were calculated for using an unpaired t-test. *P ≤ 0.05 .

Figure 4. Esa1 anchor-away depletion causes a significant repair defect at the heterochromatic *HML* locus, but not the actively transcribed *RPB2* gene. Southern blot analysis for site specific NER of UV-induced CPD lesions at *HML* (A, B) and *RPB2* (C, D) when Esa1 is conditionally depleted from the nucleus in the anchor-away system (see Figure 3 above). Quantification of % CPD repair at each locus is depicted as the mean and SEM for at least three independent replicates. P values were calculated using an unpaired t-test. Mean $\pm$ SEM is depicted. *P < 0.05.

Figure 5.

Deletion of the Eaf1 component of the NuA4 complex causes a marginal defect in repair of CPD lesions. (A) Schematic representation of the NuA4 complex with the Piccolo NuA4 subcomplex shown in orange and the scaffolding component of the NuA4 complex shown in green. The subunits analyzed in this study (Yng2, Esa1, Eaf1) are highlighted with darker coloring. Diagram adapted from [45]. (B-C) NER phenotype of the *eaf1* mutant. (B) A representative alkaline gel of CPD repair following UV irradiation. (C) Quantification of % repair for a minimum of 3 independent replicates, depicted as mean $\pm$ SEM.

Figure 6.

Disruption of the Rpd3L HDAC complex (i.e., *sds3*), but not the Hos2 HDAC, suppresses the repair defect in NuA4 mutant cells. (A) Representative alkaline gels analyzing repair of CPD lesions in WT, *esa1 sds3*, and *yng2 hos2* mutants. (B-C) Quantification of NER alkaline gel analysis of HAT/HDAC double mutants *esa1 sds3* and *yng2 hos2*. Quantification represents the mean $\pm$ SEM of at least three independent replicates. P values were calculated using an unpaired t-test. *P $\leq$ 0.05.