Published in final edited form as:

Radiat Res. 2008 November ; 170(5): 618–627. doi:10.1667/RR1420.1.

Radiosensitization of Yeast Cells by Inhibition of Histone H4 Acetylation

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Abstract

Deletion of genes for proteins involved in histone H4 acetylation produces sensitivity to DNA-damaging agents in both *Saccharomyces cerevisiae* and mammalian cells. In the present studies, we show that treating wild-type yeast cells with histone acetyl transferase (HAT) inhibitors, which are chemicals that cause a global decrease in histone H4 acetylation, sensitizes the cells to ionizing radiation. Using HAT inhibitors, we have placed histone H4 acetylation into the *RAD51*-mediated homologous recombination repair pathway. We further show that yeast cells with functionally defective HAT proteins have normal phospho-H2A (γ -H2A) induction after irradiation but a reduced rate of loss of γ -H2A. This argues that HAT-defective cells are able to detect DNA double-strand breaks normally but have a defect in the repair of these lesions. We also show that cells treated with HAT inhibitors have intact G₁ and G₂ checkpoints after exposure to ionizing radiation, suggesting that G₁ and G₂ checkpoint activation is independent of histone H4 acetylation.

INTRODUCTION

In eukaryotes, genomic DNA exists in a highly condensed form by being packaged into chromatin. This consists of 147 bp of DNA wrapped around histone octamers (two each of histones H2A, H2B, H3 and H4) and stabilized by linker histones (H1) and other factors. In its condensed form, chromatin makes DNA inaccessible to cellular processes that use DNA as substrates such as transcription, replication and DNA repair. Post-translational modifications of N-terminal tails of the core histones, notably by acetylation, phosphorylation, ubiquitination, sumoylation and methylation, have been implicated in regulating these cellular processes.

An active current area in cancer therapy is the use of agents that modify chromatin, particularly inhibitors of histone deacetylases (HDACs). By inhibiting deacetylation of histone tails, HDAC inhibitors promote the acetylation of these N-terminal tails, which tends to open up chromatin, making it more accessible to transcription. Consistent with this, it is postulated that one of the main mechanisms for the anticancer effects of HDAC inhibitors is the reactivation of silenced checkpoint or tumor suppressor genes such as p21 or p16 (1). However, in addition to these effects, a number of investigators have shown that HDAC inhibitors sensitize tumor cells to killing by ionizing radiation both *in vitro* and *in vivo* (2-7). The mechanism for this sensitization is not clear, but it is associated with abrogation of the G₂ checkpoint (3), diminished DNA repair assayed by loss of phosphorylated H2AX (5,7), and hyperacetylation of histone H4 (5). Paradoxically, a number of recent studies, outlined below, have also demonstrated that mutation, deletion or knockdown of genes involved in acetylation of histone H4 in both

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mammalian and yeast cells sensitizes to killing by ionizing radiation and/or inhibits the repair of DNA double-strand breaks (DSBs).

In the budding yeast *S. cerevisiae*, mutations in histone H4 that prohibit its acetylation and defects in components of the NuA4 histone acetyl transferase (HAT) complex, the HAT that predominantly acetylates specific lysine residues on histone H4, sensitize cells to DSB-producing agents such as ionizing radiation (8-10). After the induction of DSBs, HAT complexes are recruited to the site of DSBs, resulting in acetylation of histone H4 flanking the break, later followed by deacetylation of histone H4 after break repair (9). Studies in mammalian cells have shown that deletion of TRRAP, a core component of a number of HAT complexes, causes histone H4 hypoacetylation, a defect in recruitment of a subset of repair factors to the sites of DSBs, and compromised DSB repair (11,12). These studies suggest that the role of histone H4 acetylation in mediating DNA DSB repair is to relax a normally closed chromatin structure, allowing for the recruitment of DNA repair proteins and DNA damage signaling proteins to the site of DNA damage (13).

In view of these studies suggesting that acetylation of histones, particularly histone H4, protects eukaryotic cells from ionizing radiation, we have explored the possibility of sensitizing cells to radiation using HAT inhibitors. We chose to do this with budding yeast for two reasons: first, because there are as yet no available inhibitors of acetylation of all four H4 lysine residues in mammalian cells, and second, because the availability of mutants in various HATs and DNA repair pathways makes yeast a powerful system to interrogate the specificity of any effects. The two chemicals that have been shown to have HAT-inhibitory activities at nontoxic concentrations in yeast are copper sulfate (CuSO_4) and nickel chloride (NiCl_2), both of which cause a decrease in acetylation at all four of the lysine residues on histone H4 (14,15). We hypothesized that wild-type yeast cells treated with HAT inhibitors would behave similarly to mutant cells lacking intact histone H4 acetylation machinery. Our data show that inhibition of HATs under conditions that produce hypoacetylation of H4 sensitizes yeast cells to radiation by inhibiting DNA repair by the homologous recombination pathway.

MATERIALS AND METHODS

Yeast Strains and Methods

Yeast strains used in this study are listed in Table 1. Information about the genotypes of the parental yeast strains BY4741 and BY4742 and construction of the deletion strains is available on the *Saccharomyces* Genome Deletion Project web site (http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html). All of these strains can be obtained from Research Genetics (Huntsville, AL) or EUROSCARF (Frankfurt, Germany). *ESA1* wild-type and *esa1-1851-ts*, a temperature-sensitive mutant, were generous gifts of Dr. Carl Mann (Département de Biologie Joliot-Curie, CEA/Saclay, Gif-sur-Yvette, France).

Standard genetic techniques were used for manipulating yeast strains. Yeast cells were grown in standard rich YPD (1% yeast extract, 2% peptone, 2% dextrose). All cells were grown at 30°C with shaking, except for temperature-sensitive (*ts*) mutants, which were grown at the permissive temperature of 24°C with shaking.

Clonogenic Survival Assays

Log-phase cells from overnight liquid YPD cultures were diluted to an OD₆₀₀ of 0.5 in either fresh YPD or YPD with HAT inhibitors added. In CuSO_4 -treated samples, cells were grown for two or three generations after addition of the chemical, or approximately 4-6 h; in NiCl_2 -treated samples, the cells were grown until they had reached stationary phase overnight. Cells

were collected by centrifugation, resuspended in cooled distilled water and placed on ice. Aliquots of cells were taken after exposure and irradiated using a ^{137}Cs source at a dose rate of $28.12 \text{ Gy min}^{-1}$. Serial dilutions were made from each aliquot, and three dilutions for each point were plated on YPD. Colonies were counted after incubation at 30°C for 2-3 days, and standard errors were generated from at least three independent experiments.

Protein Extraction and Immunoblotting

Whole-cell protein extracts were prepared by lysing cells by bead beating in extraction buffer (200 mM Tris, pH 8.0, 150 mM ammonium sulfate, 10% glycerol, 1 mM EDTA, 1% SDS) containing protease inhibitors (Protease Inhibitor Cocktail, Sigma, P8340) and 2 mM DTT. A total of 25 μg of total protein was electrophoresed for 35 min at 200 V on NuPAGE® Novex 12% Bis-Tris SDS-PAGE gels (Invitrogen, NP0341) in MES-SDS running buffer (50 mM Tris base, 50 mM MES, 0.1 mM EDTA, 0.1% SDS, pH 7.3). Protein was transferred to 2 μm nitrocellulose (BioRad, 162-0112) for 1 h at 30 V in NuPAGE® Transfer Buffer (Invitrogen, NP0006). Membranes were blocked for 1 h with 5% nonfat milk in TBST (2 mM Tris, 13.7 mM NaCl, 0.5% Tween-20, pH 7.6) except for membranes probed for H2A phosphorylation, which were blocked with 5% BSA in TBST. Proteins were probed overnight in blocking buffer at 4°C with primary antibodies and for 2 h in blocking buffer at room temperature with secondary antibodies. Rabbit pan histone H4 and rabbit hyper-acetylated H4 antibodies were purchased from Upstate (Lake Placid, NY). Rat anti-tubulin, rabbit histone H2A-phospho-Ser 129, and rabbit alkaline-phosphatase-conjugated anti-rat secondary antibodies were purchased from Abcam (Cambridge, MA). Goat alkaline-phosphatase-conjugated anti-rabbit secondary antibody was purchased from Zymed. Blots were incubated with ECF substrate (GE Healthcare, 1067873) for 5 min at room temperature prior to scanning with a Storm 860 fluoroiager (Molecular Dynamics, Sunnyvale).

For quantification, the digital autoradiographic grayscale-image-density data obtained from each lane of each experiment was subjected to Gaussian deconvolution followed by nonlinear peak fitting using ImageQuant. The peak area of each lane was first adjusted for differential loading based on tubulin control, then was plotted as the relative increase over the peak area of the control lane. Quantifications represent the means from at least two independent experiments.

Flow Cytometry

For G_1 checkpoint arrest experiments, cultures were grown to mid-log phase and then split, with 5 mM CuSO_4 added to one culture. The cells were grown for two or three cell cycles, synchronized in G_1 phase with 50 μM synthetic α -factor for 150 min, irradiated (500 Gy) or sham irradiated, and then released from arrest by washing once with sterile water before dilution into medium without α -factor. Aliquots were harvested from each culture at designated times. For each time, 10^7 cells were fixed overnight in 70% ethanol. Cells were washed with 50 mM sodium citrate (pH 7.0), sonicated for 5 s, and resuspended in 50 mM sodium citrate (pH 7.0) with 0.25 mg/ml RNase A. The samples were incubated at 50°C for 1 h. Samples were incubated at 4°C overnight in 0.032 mg/ml PI in 50 mM sodium citrate. Each sample was sonicated for 5 s and then analyzed on a Beckman Coulter Elite flow cytometer.

Fluorescence Microscopy

For G_2 checkpoint experiments, cells were grown in YPD with or without 5 mM CuSO_4 for 4 h at 30°C , then incubated with 15 $\mu\text{g}/\text{ml}$ nocodazole for 2.5 h to arrest cells in the G_2 phase of the cell cycle. Arrested cultures were exposed to 0, 250 or 500 Gy γ radiation and placed immediately on ice. Cells were released from nocodazole arrest by washing twice with sterile water before resuspending in fresh YPD medium to be shaken at 30°C . Aliquots were removed at 0-, 30-, 60-, 90-, 120- and 150-min intervals and fixed in 70% ethanol. Fixed cells were

pelleted and resuspended in PBS, sonicated briefly, and stained with DAPI for visualization by fluorescence microscopy.

RESULTS

HAT Inhibitors Sensitize Wild-Type Yeast Cells to Radiation at Concentrations Producing Hypoacetylation of Histone H4

Treatment of haploid wild-type yeast with CuSO_4 or NiCl_2 at concentrations that produced hypoacetylation of histone H4 (Fig. 1B and D) sensitized the cells to radiation (Fig. 1A and C) but did not affect cell growth (data not shown). Treatment with lower concentrations of CuSO_4 or NiCl_2 that were insufficient to produce a substantial loss of H4 acetylation failed to sensitize under similar conditions (Fig. 1A-D).

An increase of haploid cells in G_1 could also cause sensitization of non-synchronized cells because haploid yeast cells in G_1 lack a sister chromatid with which to perform homologous recombination repair. However, in unirradiated, unsynchronized wild-type cells, we observed no effect of HAT inhibition on the distribution of cells in the cell cycle (Fig. 2A and B), suggesting that the radiosensitization by HAT inhibitors is not due to arrest of cells in G_1 . This was further verified by irradiating diploid yeast cells, for which a homologous chromosome is readily available for homologous recombination repair. Diploid BY4743 cells show sensitization to irradiation with CuSO_4 or NiCl_2 (Fig. 2C, D) similar to that seen for haploid cells.

HAT Inhibitors do not Further Sensitize Cells Deleted for a Gene Encoding Part of the NuA4 HAT Complex

To examine the possibility that CuSO_4 and NiCl_2 cause sensitization through interfering with cellular processes other than histone H4 acetylation, we tested their effects on the sensitivity of yeast cells deleted for Vid21p/Eaf1p, a structural core component of the NuA4 HAT complex (16) that acetylates the first four lysine residues in the N-terminal tail of histone H4. Previous studies have shown that *vid21* deletion mutants are sensitive to radiation (10,17), presumably from the cells' defect in histone H4 acetylation. If CuSO_4 and NiCl_2 cause sensitization to radiation by inhibition of H4 acetylation, we would expect no further sensitization of *vid21* Δ to radiation. Consistent with this, we found that the *vid21* Δ mutant was not further sensitized to radiation after treatment with CuSO_4 and NiCl_2 at concentrations that radiosensitize wild-type cells (Fig. 3A and B).

HAT Inhibitors Radiosensitize by Inhibition of Rad51-Mediated Homologous Recombination Repair

After induction of a DNA DSB, H4 histones surrounding the DSB become acetylated, thereby relaxing the chromatin and facilitating the recruitment of homologous recombination (HR) repair proteins to the site of the DSB (8). This would predict that inhibition of histone H4 acetylation would not sensitize mutants deleted for crucial HR repair proteins such as Rad51p. Our data (Fig. 4A and B) shows that *rad51* Δ strains cannot be further sensitized to radiation by treatment with HAT inhibitors, arguing that histone acetyl transferases play important roles in homologous recombination. Consistent with this, the *rad51 vid21* double deletion mutant has the same radiation sensitivity as the *rad51* single deletion mutant (J. C. Game, personal communication, 2007), arguing that Vid21p/Eaf1p functions in the same pathway as Rad51p.

In addition, we show that histone H4 acetylation is not in the Rad6p- and Rad18p-mediated postreplication repair (PRR) pathway, because HAT inhibitors sensitize *rad18* Δ mutants to the same extent as they do wild-type cells (Fig 4 A and B). Similarly, deletion of *VID21* in *rad18* mutants sensitizes the cells to radiation (J. C. Game, personal communication, 2007).

HAT-Inhibited Cells Have Persistent Levels of γ -H2A after Irradiation

After induction of DNA DSBs, the checkpoint kinases Tel1 and Mec1 phosphorylate serine 129 of histone H2A in large tracts on either side of the break (18). This modification is important for recruitment of chromatin remodeling and repair proteins. As DNA repair progresses, the phosphorylated form of histone H2A (γ -H2A) is lost from chromatin surrounding the DSB, making it possible to use γ -H2A levels as a marker for the presence of DNA DSBs. Thus, to investigate the effect of HAT inhibition or loss of functional NuA4 HAT complexes on DNA repair, we measured γ -H2A levels as a function of time after irradiation. In wild-type cells, γ -H2A levels are high at 15 min after irradiation and decrease to near background levels after 2 h (Fig. 5A, C and D). In addition, initial levels of γ -H2A doubled with a doubling of the radiation dose from 250 to 500 Gy (Fig. 5B and E). In contrast, γ -H2A levels, although similar to wild-type levels at 15 min after 250 Gy, remained high in *vid21 Δ* and *esal-ts* mutants, both of which lack a functional NuA4 complex (Fig. 5A, C and D). In addition, there was a blunting of the increase in γ -H2A levels with increasing radiation dose (Fig. 5B and E). Similarly to NuA4 mutants, wild-type cells treated with HAT inhibitors show little decay in γ -H2A over time and lack the dose response observed in wild-type cells (Fig. 5A-E). These results argue that in cells that lack functional NuA4 HAT complexes, although detection of DSBs is largely intact, as seen by the initial induction of γ -H2A after irradiation, there is a defect in DSB repair, as seen by the failure to lose γ -H2A signal over time.

Wild-Type Cells Treated with HAT Inhibitors Show an Intact G₁ Checkpoint

In budding yeast, radiation-induced DNA damage activates cell cycle checkpoints including the G₁/S, intra-S and G₂/M checkpoints. To investigate the effect of HAT inhibition on activation of these checkpoints, we measured the effect of HAT inhibition on the G₁/S checkpoint in response to 500 Gy. Cells were treated with 5 mM CuSO₄, arrested in G₁ by treatment with α -factor, irradiated, released from α -factor arrest, and examined for progression through the cell cycle by flow cytometry. We observed no effect of HAT inhibition on the cell cycle progression of unirradiated cells (Fig. 6A and B) or any detectable abrogation of G₁/S arrest in irradiated cells (Fig. 6C and D).

Wild-Type Cells Treated with HAT Inhibitors Show an Intact G₂ Checkpoint

To investigate the effect of HAT inhibition on the G₂ checkpoint, cells were treated with 5 mM CuSO₄, arrested in G₂/M by treatment with nocodazole, irradiated, released from nocodazole arrest, and collected at 15, 30, 60, 90, 120 and 150 min. Cells were stained with DAPI, and the number of binucleate cells was counted at each time for evidence of progression through mitosis. Immediately after release, untreated, unirradiated wild-type cells showed progression from large budded cells (G₂/M arrest, Fig. 7A), to binucleate cells (undergoing mitosis, Fig. 7A), to single cells (mitosis complete, Fig. 7A), to small budded cells (Fig. 7B), back to large-budded cells (pre-mitosis) over the course of 60-90 min (Fig. 7C and D). Similarly to wild-type cells, HAT inhibitor-treated cells have an intact G₂ checkpoint after 250 Gy or 500 Gy irradiation (Fig. 7C and D), and the length of the checkpoint was dependent on dose.

DISCUSSION

The present investigation was undertaken to determine whether pharmacological inhibition of histone H4 acetylation would sensitize yeast cells to ionizing radiation. In both yeast and mammalian cells, mutations, deletions or knockdown of components of HAT complexes responsible for acetylation of histone H4 sensitizes cells to radiation and/or inhibits the repair of DNA DSBs (10,17,19). Our results show that both copper sulfate and nickel chloride, at non-toxic concentrations, produce hypoacetylation of histone H4 and moderate sensitization to radiation. Further, we show that the radiation sensitization in the treated cells correlates with loss of histone H4 acetylation. Consistent with inhibition of histone acetyltransferases as the

mechanism of radiosensitization, we also show that the NuA4 HAT mutant *vid21* is not further sensitized by treatment with HAT inhibitors. Because the deletion mutant has a complete knockdown of the relevant gene and its protein product, whereas inhibition from chemical compounds is transient, reversible and dependent on dose, it is not entirely surprising that *vid21* mutants are more sensitive to radiation than wild-type cells treated with HAT inhibitors.

We show that mutants in homologous recombination (HR) fail to be further sensitized by HAT inhibition. This is consistent with the finding that histone acetylation at sites of DSBs promotes homologous recombination repair (11). Cells with functionally defective NuA4 HAT complex have an intact DSB detection pathway, as seen in the induction of γ -H2A after radiation-induced DNA damage. However, these cells have a reduced rate of loss of γ -H2A over time compared to wild-type cells. This argues that histone H4 acetylation is not involved in the initial detection of DSBs but is required to mediate DSB repair. Recent reports in mammalian cells show that the involvement of the HAT-associated proteins Tip60 and TRRAP in the early detection of DNA DSBs is not associated with their HAT activity (12,20). It is possible that the decrease in γ -H2A levels is not dependent on the completion of DSB repair but rather on an intact NuA4 HAT complex. This possibility has been suggested by Kusch *et al.*, who examined the *Drosophila melanogaster* Tip60 chromatin remodeling complex, which is homologous to the yeast NuA4 complex (21). They showed that Tip60 acetylates the phosphorylated form of the fly histone variant H2Av, which becomes phosphorylated upon damage in a manner analogous to H2AX, and this acetylation of the phosphorylated form of H2Av promotes its removal. It is still unknown whether removal of the phospho-H2Av depends on repair of the DNA lesion.

After DNA damage, cells initiate cell cycle arrest and DNA repair, which are largely separate but parallel pathways. Treating cells synchronized in G₁ with radiation induces a dose-dependent G₁ checkpoint delay before the onset of DNA replication. Irradiation of G₂-arrested HATi-treated or untreated cells induces activation of the G₂/M checkpoint. Both G₁ and G₂ checkpoints are dependent on Rad53p phosphorylation and Rad9p activation, which are mediated by Mec1 and Tel1 proteins. Our studies show that wild-type cells treated with HAT inhibitors have intact G₁ and G₂ checkpoints after irradiation. This suggests that inhibition of histone H4 acetylation, which leads to a closed chromatin structure, does not affect the initial DSB recognition pathway or the checkpoint activation pathway. We believe that the likely mechanism for the radiosensitization produced by HAT inhibition is that the more closed chromatin structure produced by HAT inhibition leads to reduced access of the DNA breaks to repair proteins, as suggested by recent studies (11). However, it is also possible that HAT inhibition leads to reduced levels of key proteins involved in homologous recombination repair.

Although several studies have demonstrated a radiosensitization effect of HDAC inhibitors on mammalian tumor cells *in vitro* and *in vivo* (2-7), we did not find a similar effect of HDAC inhibitors on wild-type yeast cells (unpublished data). A possible explanation for this is that in growing yeast cells, the steady-state pattern of histone acetylation is determined by HAT levels rather than HDAC levels, and inhibition of HDAC activities by HDAC inhibitors such as sodium butyrate or trichostatin A does not cause a perturbation in the global acetylation state (22). Another possible reason for the difference may be that yeast cells predominantly use homologous recombination to repair DSBs, while mammalian cells rely on NHEJ for repair. Because HDAC inhibition affects DNA DSB repair by NHEJ, this could also account for the lack of radiosensitization in yeast cells (23,24).

Our results have demonstrated the utility of using HAT inhibitors to study histone H4 acetylation-mediated DNA DSB repair in yeast cells. Wild-type cells treated with HAT inhibitors behave in ways similar to mutant cells without a functional NuA4 HAT complex.

Whether these results also occur in mammalian cells must await studies with specific HAT inhibitors in such cells.

ACKNOWLEDGMENTS

The authors gratefully acknowledge helpful discussions and sharing of unpublished data by Dr. J. C. Game. This work was funded by a National Institutes of Health Grant P01 CA82566 awarded to JMB. KEM was supported by a National Defense Science and Engineering Grant fellowship, and SS was supported by a Stanford Medical Scholars Award.

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FIG. 1.

Histone acetyl transferase inhibitors cause radiosensitivity of haploid wild-type yeast cells at concentrations that produce a decrease in histone H4 acetylation. Panel A: Radiation survival curves of haploid cells treated with CuSO_4 . Panel B: Histone H4 acetylation status in CuSO_4 -treated haploid wild-type yeast cells. Panel C: Radiation survival curves of haploid cells treated with NiCl_2 . Panel D: Histone H4 acetylation status in haploid NiCl_2 -treated cells.

FIG. 2.

HAT inhibitor treatment does not alter cell cycle distribution. Wild-type cells were treated with 5 mM CuSO₄ for 4 h, then subjected to flow cytometry analysis. Panel A: Wild-type cells untreated with HAT inhibitors. Panel B: Wild-type cells treated with 5 mM CuSO₄ for 4 h. Panel C: Radiation survival curves of diploid cells treated with CuSO₄. Panel D: Radiation survival curves of diploid cells treated with NiCl₂.

FIG. 3. Histone acetyl transferase inhibitors do not further radiosensitize *vid21*Δ mutants. Panel A: Radiation survival curves of *vid21*Δ mutants treated with CuSO₄. Panel B: Radiation survival curves of *vid21*Δ mutants treated with NiCl₂.

FIG. 4.

Histone acetyl transferase inhibitors do not further radiosensitize *rad51Δ* mutants, indicating HATi act in the homologous recombination repair pathway but do cause further sensitization of *rad18Δ* control cells. Panel A: Radiation survival curves of *rad51Δ* and *rad18Δ* mutants treated with CuSO_4 . Panel B: Radiation survival curves of *rad51Δ* and *rad18Δ* mutants treated with NiCl_2 .

FIG. 5.

Cells with mutations in the NuA4 HAT complex or treated with HAT inhibitors can detect DSB breaks but have a defect in their repair. Panel A: NuA4 HAT mutants or HATi-treated cells were irradiated with 0, 250 or 500 Gy. Whole cell protein extracts were prepared 15 min, 1 h and 2 h after irradiation and immunoblotted with anti-phospho-H2A and anti-tubulin. Mutants and cells treated with HAT inhibitors have persistent levels of γ -H2A after irradiation. Panel B: 15 min after irradiation, γ -H2A signals are detectable in cells lacking a functional NuA4 complex, and levels are normal compared to wild-type cells. However, cells lacking a functional NuA4 do not show an increased response to 500 Gy compared to 250 Gy, as can be seen in wild-type cells. Panel C: γ -H2A signal strength over time for each yeast strain or treatment after irradiation with 250 Gy, represented as relative increases over 15 min recovery

time. Each bar represents the mean of at least three independent experiments. Panel D: γ -H2A signal strength over time for each yeast strain or treatment after irradiation with 500 Gy, represented as relative increases over 15 min recovery time. Each bar represents the mean of at least three independent experiments. Panel E: γ -H2A signal strength for each yeast strain or treatment 15 min after irradiation with 500 Gy, represented as relative increases over 15 min after irradiation with 250 Gy.

FIG. 6. HAT inhibition does not affect G₁ cell cycle arrest after irradiation. Panel A: Wild-type cells, untreated with HAT inhibitors, unirradiated. Panel B: Wild-type cells, treated with 5 mM CuSO₄, unirradiated. Panel C: Wild-type cells untreated with HAT inhibitors and irradiated with 500 Gy. Panel D: Wild-type cells treated with 5 mM CuSO₄ and irradiated with 500 Gy.

FIG. 7.

CuSO₄-treated cells have an intact G₂ cell cycle checkpoint. Panel A: Representative photos of cycling nonirradiated, nontreated, DAPI-stained BY4741 wild-type cells. From left to right: unbudded, large budded, large budded (with chromatin being pulled into daughter cell), and binucleate cells at 100 $\times$. All photographs were taken 120 min after release from nocodazole. Panel B: Bright-field (left) and fluorescence (right) photographs of small budded cells 60 min after nocodazole release. Panel C: Percentage of binucleate wild-type cells, with or without 5 mM CuSO₄ treatment, unirradiated or irradiated with 250 Gy, and released from nocodazole-mediated G₂ cell cycle arrest. Panel D: Percentage of binucleate wild-type cells, treated with

or without 5 mM CuSO₄, unirradiated or irradiated with 500 Gy, and released from nocodazole-mediated G₂ cell cycle arrest. Curves were generated from the averages of two experiments.

TABLE 1**Yeast Strains Used in this Study**

BY4741	MAT α his3 Δ 1 leu2 Δ 0 met15 Δ 0 ura3 Δ 0
BY4742	MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0
BY4743	MAT α /a his3 Δ 1/his3 Δ 1 leu2 Δ 0 /leu2 Δ 0 lys2 Δ 0/LYS2 MET15/met15 Δ 0 ura3 Δ 0 /ura3 Δ 0 (4741/4742)
ESA1	MAT α ade2-1 ura301 his3-11,15 trp1-1 leu2-3,112 can1-100 rDNA::ADE2
<i>esa1-ts</i>	MAT α esa1-1851::KIURA3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 rDNA::ADE2
<i>vid21</i>	MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0 vid21::Kan
<i>rad51</i>	MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0 rad51::Kan
<i>rad18</i>	MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0 rad18::Kan