Supporting Online Material

Supporting Online Material Text

Budding index analysis of a *mec1*Δ strain was carried out in a *sml1-1* background to permit viablilty (*1*) (Fig. S1). Growth curves reveal that mutation of H3 K56 to an arginine residue causes a slight suppression of the growth defect of *rtt109*Δ cells (Fig. S2). A similar phenomenon has been reported for mutation of H3 K56 in *asf1*Δ cells (*2*). This result suggests that the K to R mutation does not perfectly phenocopy an unacetylated lysine residue although, alternatively, an additional modification on H3 K56 could result in growth defects in the absence of acetylation. However, mass spectrometry analyses did not reveal any other modifications on H3 K56 in a previous study (*3*).

Supporting Online Material Figure Legends

Supp. Figure 1

Asynchronous cultures of wild-type, *sml1-1*, *rtt109*Δ, *rtt109*Δ *sml1-1, rtt109*Δ *sml1-1, mec1*Δ *sml1-1* and *rtt109*Δ *mec1*Δ *sml1-1* were examined microscopically to determine the % of cells with a bud. Over 100 cells were counted for each analysis and the experiment was performed twice. The mean and standard deviation is plotted.

Supp. Figure 2

Growth curves were an average of 2 independent cultures for each strain indicated, diluted to 0.06 OD_{600} from mid-log cultures and measured at OD_{600} each hour during growth at 30°C in YPAD.

Materials and Methods

Yeast strains

Yeast strains used in this study are listed in Supplementary data table 1.

DNA damage hypersensitivity analyses

Ten-fold serial dilutions of overnight cultures diluted to an absorbance at 600 nm of 0.5 were plated onto medium containing the indicated concentrations of MMS, HU, CPT or phleomycin. MMS plates were used within 24 h of preparation. For IR treatment, cells were serially diluted (ten-fold), spotted onto YPAD plates and irradiated at 200 Gy. All plates were incubated at 30°C for 2-3 days.

Flow cytometry analysis

For cell cycle analysis, ~1 x 10^7 midlog cells from appropriate strains were fixed in 70% ethanol overnight, washed and resuspended in PBS, treated with RNase A and stained with propidium iodide. Approximately 10,000 cells were scanned with a Beckman Coulter FACSCalibur machine with CellQuest software.

Western blot analysis

Trichloroacetic acid extracts were prepared as described previously (*4*). Proteins were electrophoresed on the appropriate percentage SDS-polyacrylamide gel and transferred to nitrocellulose membrane. Immunoblot analysis was carried out with anti-HA (HA.11; Covance), anti-Pgk1 (Invitrogen), anti-histone H3 (Abcam), anti-histone H3 acetyl-lysine 56 (Upstate), anti-Clb2 (Santa Cruz Biotechnologies) and anti-Rad53p (kindly provided by Noel Lowndes).

Fluorescence microscopy

Strains of the desired genotype carrying the *RAD52-YFP* fusion were grown at 30°C to mid-log before being harvested for microscopy. Samples were examined with a DeltaVision Spectris microscope (Applied Biosystems, Seattle, WA) using a 100x objective lens as previously described (*5*). Over 100 cells were counted for each strain; each experiment was performed twice and the mean and standard deviation is plotted.

Recombination analysis

Recombination frequencies were calculated as described previously (*6*). Fluctuation test experiments were performed as described previously (*7*), with six independent colonies for each experiment and each strain studied. Each experiment was repeated three times and the mean and standard deviation are plotted.

GCR assays

GCR assays were performed as described before (*8*). Fluctuation tests of six colonies were performed twice.

Supercoiling analysis

Supercoiling analysis of the 2μ plasmid was carried out essentially as described before (*9*). 30 ml of the indicated yeast strains were grown to a density of 2 x 10^7 cells/ml, harvested, resuspended in 400 μl of resuspension buffer (50 mM TrisCl, pH 8, 50 mM EDTA, 10 mM *N*-ethylmaleimide), 500 μl of phenol, and 200 μl of 0.5-mm glass beads, and then vortexed for 30 sec followed by 30 sec on ice. This was repeated three times. DNA was deproteinized by the addition of SDS to 0.5% final concentration and Proteinase K (50 μg/ml final concentration), and incubated at 37 °C for 3 h followed by phenol/chloroform extraction and ethanol precipitation. DNA was resuspended in 400 μl of Tris-EDTA buffer with RNase A (50 μg/ml final concentration) and incubated at 37 °C for 2 h. DNA was ethanol-precipitated, and 15 μg of DNA was electrophoresed on a 1.2% agarose gel containing 0.6 μg/ml chloroquine. Gels were transferred to nitrocellulose and probed with a ^{32}P-labelled 2-kb EcoRI fragment from the 2μ plasmid. Densitometric tracing was carried out using MacBas 2.5 software (Fujifilm).

HAT activity assay

HAT assays with recombinant Rtt109p (N-terminally His-tagged) were done in 30 μl with 50 mM Tris, pH 8.0, 0.1 mM EDTA, 80 mM NaCl, 2.53 μM ^{3}H acetyl

coenzyme A (Amersham Biosciences, 3.3 Ci/mmol). Assays contained 800 ng of recombinant human core histone octamers (a kind gift from Dr. Till Bartke), approximately 800 ng of recombinant Rtt109p, 600 ng of Asf1p or a similar dilution of a mock purification. Reactions were incubated at 30 °C for 45 min and then spotted onto Whatman p81 cation exchange paper and air-dried. The dried papers were washed three times for 5 min each with 50 mM sodium carbonate, pH 9.2 and once with acetone. Radioactivity was quantified in a liquid scintillation counter. The plasmid for Rtt109p expression was constructed by cloning the Rtt109p coding sequence as a PCR BamH1-EcoR1 fragment from genomic yeast DNA into pET28a (Novagen). The plasmid for Asf1p expression (pET28a) was a kind gift from Paul Kaufman. Both plasmids, as well as an empty vector were transformed into BL21 cells (Stratagene) and cells were grown to OD 0.8 at 37°C in an altered LB medium containing 0.2% w/vol NaCl, 0.7 M sorbitol, and 2.5 mM betaine before IPTG was added at final concentration of 0.1 mM. Cells were then incubated at 15°C overnight before being harvested and resuspended in seven volumes of 50 mM Na Hepes, pH 7.8, 10 mM EDTA, 1 M NaCl, 5% (vol/vol) glycerol, 10 mM 2-mercaptoethanol, 300 mg/ml lysozyme, 0.5 mM AEBSF, 1 mg/ml pepstatin, 1 mg/ml leupeptin for 60 min at 0°C, lysed by sonication, and clarified by centrifugation at 13,000 × g for 1 h. The supernatant was adjusted to 7 mM $MgCl_2$ and 10 mM imidazole (pH 7.0), applied to NiNTA agarose (Qiagen; 2 ml/g wet weight of cells), washed with 10 column vol. of buffer [20 mM Na Hepes, pH 7.8, 7 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 10% (vol/vol) glycerol, 0.5 mM AEBSF, 1 mg/ml each of leupeptin and pepstatin/0.5 M

NaCl] containing 10 mM imidazole, and eluted with 5 column vol. of the same buffer containing 200 mM imidazole. Protein concentration was estimated by comparing Coomassie Blue staining of samples with bovine serum albumin standards, analyzed by SDS-PAGE.

Cell cycle analysis

Log phase cultures were arrested in 10 μg/μl alpha factor for 2.5 h, washed extensively and released into fresh medium. Aliquots were taken at the indicated times for analysis by western blot and FACS.

Supplementary Data Table 1. Strains Used in This Study

Strain	Genotype	Background	Reference/Source
W303	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1ura3-1 RAD5*	W303	(*10*)
RDY009	*rtt109*Δ*::KANMX6*	W303	This study
RDY041	*rad52*Δ*::URA3*	W303	This study
RDY220	*sml1-1*	W303	This study
RDY221	*rtt109*Δ*::KANMX6 sml1-1*	W303	This study
RDY072	*mec1*Δ*::TRP1 sml1-1*	W303	This study
RDY116	*mec1*Δ*::TRP1 sml1-1 rtt109*Δ*::KANMX6*	W303	This study
RDY157	*asf1*Δ*::KANMX6*	W303	This study
RDY096	*rad53*Δ*::HIS3 sml1-1*	W303	This study
W3749-14C	*RAD52-YFP*	W303	(*5*)
RDY109	*RAD52-YFP rtt109*Δ*::KANMX6*	W303	This study
RDKY3615	*MATa ura3-52 leu2*Δ*1 trp1-63 his3-200 lys2*Δ *Bgl hom3-10 ade2-1 ade8 yel069c::URA3*	S288C	(*8*)
RDY012	*rtt109*Δ*::KANMX6*	RDKY3615	This study
RDY022	*sgs1*Δ*::KANMX*6	RDKY3615	This study
AYW31-B	*MAT***a** *leu2-k::URA3-ADE2::leu2-k ura3 ade2 his3 trp1*	AYW31-B	(*7*)
RDY013	*rtt109*Δ*::KANMX6*	AYW31-B	This study
RDY021	*sgs1*Δ*::KANMX*6	AYW31-B	This study
RDY056	*rtt109*Δ*::NATMX6*	W303	This study
RDY158	*cac1*Δ*::LEU2*	W303	This study
RDY146	*rtt109*Δ*::NATMX6 asf1*Δ*::KANMX6*	W303	This study
RDY149	*rtt109*Δ*::NATMX6 cac1*Δ*::LEU2*	W303	This study
RDY142	*asf1*Δ*::KANMX6 cac1*Δ*::LEU2*	W303	This study
RDY143	*rtt109*Δ*::NATMX6 asf1*Δ*::KANMX6 cac1*Δ*::LEU2*	W303	This study
HMY152	*MAT***a** *trp1-1 ura3-1 his3-11, 15 leu2-3, 112 ade2-1 can1-100 hht1-hhf1*Δ*::LEU2 hht2-hhf2*Δ*::KANMX3 trp1::HHT1/HHF1::TRP1*	W303	(*11*)
HMY140	*trp1::hht-K56R/HHF1::TRP1*	HMY152	(*11*)
RDY223	*rtt109*Δ*::NATMX6*	HMY152	This study
RDY226	*rtt109*Δ*::NATMX*6	HMY140	This study
yDPT144-6	*hst3*Δ*::HIS3*	HMY152	D.P. Toczyski
yDPT147-16	*hst4*Δ*::URA3*	HMY152	D.P. Toczyski
RDY230	*hst3*Δ*:HIS3 hst4*Δ*::URA3*	HMY152	This study
RDY231	*hst3*Δ*::HIS3 hst4*Δ*::URA3 rtt109*Δ*::NATMX*	HMY152	This study
RDY124	RTT109-HA3::HIS3MX6	W3031-A	This study

Supplementary Information References

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Supplementary Figure 1; Driscoll *et al*

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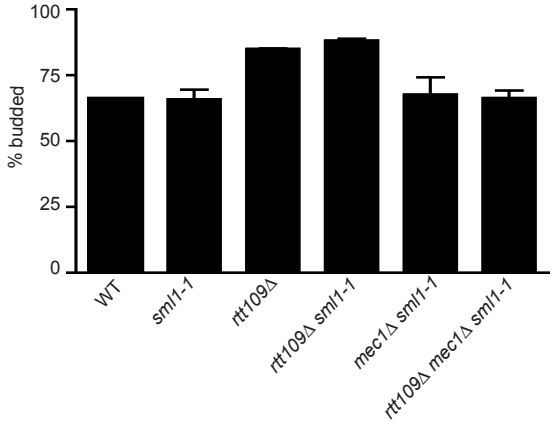

Supplementary Figure 2; Driscoll *et al.*