HISTONE H3 LYSINE 14 ACETYLATION IS REQUIRED FOR ACTIVATION OF A DNA DAMAGE CHECKPOINT IN FISSION YEAST

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Running title: H3K14ac regulates DNA damage checkpoint
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Key words: histone, acetylation, H3K14, DNA damage, checkpoint, Mst2

Background: Histone acetylation regulates diverse cellular processes.

Results: The fission yeast Mst2 complex is a specific histone H3 lysine 14 acetyltransferase.

Conclusion: H3K14 acetylation is required for DNA damage checkpoint activation.

Significance: Understanding the in vivo functions of H3K14 acetylation.

SUMMARY
Histone lysine acetylation has emerged as a key regulator of genome organization. However, with a few exceptions, the contribution of each acetylated lysine to cellular functions is not well understood due to the limited specificity of most histone acetyltransferases and histone deacetylases. Here we show that the Mst2 complex in Schizosaccharomyces pombe is a highly specific H3 lysine 14 (H3K14) acetyltransferase, which functions together with Gen5 to regulate global levels of H3K14 acetylation (H3K14ac). By analyzing the effect of H3K14ac loss through both enzymatic inactivation and histone mutations, we found that H3K14ac is critical for DNA damage checkpoint activation by directly regulating the compaction of chromatin and by recruiting chromatin remodeling protein complex RSC.

Eukaryotic genomic DNA is folded with histones and non-histone proteins into chromatin, and covalent modifications of histones regulate the access of DNA by cellular proteins (1). One of the most extensively studied histone modifications, the acetylation of lysines, regulates diverse cellular processes such as gene expression, recombination, and DNA damage repair (2,3). Acetylation neutralizes the charge of lysine, which attenuates electrostatic interactions within and between nucleosomes, allowing other nuclear proteins to access the underlying DNA sequences. In addition, acetylated lysines serve as signals for the recruitment of bromodomain containing proteins, many of which are associated with other chromatin modifying activities (4). For example, the acetylation of H4K16 directly affects compaction of nucleosome arrays in vitro and stimulates the activity of chromatin-remodeling enzyme ACF (5).

Although the effect of histone acetylation on chromatin dynamics is clear in vitro, the contribution of each acetylated lysine to cellular functions is far less clear. Mutational analysis of four regularly acetylated lysines on the histone H4 tail in S. cerevisiae demonstrates that lysine 5, 8, 12 and 16 are partially redundant and cumulatively regulate gene expression, heterochromatin
assembly, maintenance of genome integrity, and cell cycle progression (6-10). However, it is difficult to apply similar analysis to other lysine residues because most, especially those on histone H3, are subjected to modifications other than acetylation (1). Thus mutation of histone lysine residues not only abolishes acetylation, but also blocks other histone lysine modifications, leading to difficulties in assigning phenotypes specifically associated with loss of acetylation.

Mutational analyses of the highly specific histone acetyltransferase Rtt109 established the role of H3K56ac in regulating the DNA damage response (11-14). However, the majority of histone acetyltransferases (HATs) and histone deacetylases (HDACs) have limited specificity (2,3). As a result, each enzyme usually modifies several lysine residues on histones, and each lysine is acetylated by several enzymes, making it difficult to attribute phenotypes to the loss of one specific acetylated residue. Thus, identification of highly specific histone acetyltransferases and deacetylases is critical for dissecting the functions of individual histone lysine acetylation.

Here we show that the fission yeast Mst2 complex is a highly specific nucleosomal H3K14 acetyltransferase and that it functions redundantly with another HAT, Gcn5, to regulate global H3K14ac levels in vivo. Examining the phenotypes of H3K14ac loss by means of both enzymatic inactivation and a H3K14 mutation reveal that H3K14ac plays a major role in regulating DNA damage checkpoint activation.

EXPERIMENTAL PROCEDURES:

Fission yeast strains and genetic analysis - Yeast strains containing deletion or epitope-tagged versions of Mst2 complex components were constructed using a PCR-based module method (15). Genetic crosses were used to construct all other strains. For DNA damage assays, serial dilutions of cells were plated onto YEA plates and YEA containing indicated concentrations of MMS or bleomycin. A 60Co source at a dose of 12.6 Gy/min and a UVP CL-1000 crosslinker (254nm) were utilized for IR and UV treatment, respectively. Cells were incubated for 3 days at 30°C.

Protein purification and Western blot analysis - Affinity purification of Pdp3-Flag and Mst2-Flag complexes and MudPIT Mass spectrometry analyses were performed as described previously (16). The following antibodies were used for western blot analyses: myc (Covance, MMP-150), Flag (Sigma, F7425), H3K14ac (Millipore 07-353), H3K9ac (Millipore, 07-352), H3 (Abcam, ab1791), GST (Sigma, G7781), phospho-H2A (Abcam, ab15083).

Histone acetyltransferase and kinase assays - HAT assays with purified protein complexes were performed as previously described (17). Rad3 kinase assays were performed as previously described (18). Nucleosomes containing recombinant fission yeast histones were assembled with the Chromatin Assembly Kit (Active Motif). Recombinant H3 containing acetylated K14 was obtained from Active Motif (31254).

ChIP and MNase assays - ChIP analysis of H3K14ac levels at an HO-induced break was performed as described previously (19). MNase digestion of chromatin was performed as described previously (20).

RNA analysis - RNA extraction, RT-PCR and microarray analysis were performed as described previously (21). The gene expression profile of gcn5Δ (22) was included for comparison.

RESULTS:

Identification of a Mst2-containing histone acetyltransferase complex - We previously discovered that the PWWP domain protein Pdp1 is essential for histone H4K20 methylation and activation of a DNA damage checkpoint (16). The fission yeast genome contains two additional uncharacterized PWWP domain proteins, Pdp2 and Pdp3. We performed affinity purification of Pdp3-Flag (Fig. 1A, B) and identified a number of interacting proteins by mass spectrometry: Mst2 is a putative MYST family histone acetyltransferase (23); Tfg3, Nto1 and Eaf6 are homologous to S. cerevisiae NuA3 histone acetyltransferase complex subunits (2,24); SPAC823.14 and SPBC16G5.13 are uncharacterized proteins that we named Ptf1 (Pdp three interacting factor 1) and Ptf2 (Fig. 1C, D). Purification of Mst2-Flag and mass spectrometry analysis identified identical components (Fig. 1D). Furthermore, co-immunoprecipitation analysis of Pdp3-Flag with myc-tagged Nto1, Mst2, Ptf1, Ptf2, Eaf6 and HA-
tagged Tfg3 demonstrated that these proteins indeed form a stable complex (Fig. 1E).

The Mst2 complex is a nucleosomal H3K14 acetyltransferase - To test whether Mst2 is an active HAT, we performed in vitro histone acetyltransferase (HAT) assays with affinity purified Mst2-Flag complex. Although Mst2 complex acetylated both H3 and H4 when histone octamers were used as substrates, it specifically acetylated H3 in a nucleosomal context (Fig. 2A and S1A). Pdp3-Flag complex purified from mst2Δ or a mutation (E274Q) in the putative active site of Mst2 resulted in complete loss of acetyltransferase activity, corroborating that Mst2 is the catalytic subunit (Fig. 2A and 2B). Edman sequencing of radio-labeled H3 indicates that lysine 14 is the major substrate of the Mst2 complex (Fig. 2C). Consistent with this, the enzymatic activity of the Mst2 complex was severely reduced when recombinant nucleosomes containing a H3K14R mutation or a H3K14ac peptide were used as substrates (Fig. 2D and S1B). These results demonstrate that the Mst2 complex is a highly specific nucleosomal histone H3K14 acetyltransferase.

However, Western blot analysis of histones extracted from mst2Δ cells showed that H3K14ac levels are only modestly reduced as compared to those in wild type cells (Fig. 2E), indicating the presence of additional HATs that modify this residue. One candidate is the GNAT family histone acetyltransferase Gcn5, because S. cerevisiae GCN5 has activity towards H3K14 and GCN5 genetically interacts with the NuA3 complex, which is similar in activity to the Mst2 complex (25,26). Indeed, gcna5 Δ mst2Δ or gcna5 Δ mst2-E274Q cells completely lack H3K14ac, suggesting that Mst2 functions redundantly with Gcn5 to acylate H3K14 in vivo (Fig. 2E, 2F, S2A and S2B) (27). Furthermore, two components of the Mst2 complex, Nto1 and Ptf2, are also required for H3K14ac in a gcna5Δ background (Fig. S2C). In vitro histone acetyltransferase assays showed that Mst2 complex purified from nto1Δ or ptf2Δ backgrounds completely lacked activity (Fig. S2D). Further mass spectrometry analysis revealed that Mst2-Flag complex purified from nto1Δ or ptf2Δ cells lost the majority of its components (Fig. S2E), indicating that proper assembly of the Mst2 complex is essential for its enzymatic activity.

The role of H3K14ac in regulating gene expression - We reasoned that the functions of H3K14ac could be studied by analyzing common phenotypes associated with both gcna5Δ mst2Δ and H3K14R mutant cells, which are devoid of H3K14ac. As histone acetylation and HATs are generally associated with transcription regulation (1,3), we first analyzed by microarray the gene expression profiles of gcna5Δ mst2Δ and H3K14R mutants (Fig. 3A). To our surprise, the gene expression profiles of gcna5Δ mst2Δ and H3K14R are considerably different. This is consistent with the fact that Gen5 and Mst2 have other substrates in addition to H3K14 and that the H3K14R mutation also blocks modifications other than acetylation. Supporting this idea, the gcna5Δ mst2Δ H3K14R triple mutant grows considerably more slowly than either gcna5 Δ mst2Δ or H3K14R (Fig. 3B). Genes down-regulated in both mst2Δ gcna5Δ and H3K14R cells only account for less than 3% of genes examined, which is consistent with a recent microarray analysis of histone acetyltransferase mutants (27). The gene expression signature of gcna5Δ mst2Δ is similar to that of gcna5Δ (Fig. 3A) (22). Given that Gcn5 acetylates other residues of histones and Gcn5 also regulates gene expression independently of histone acetylation (2,22), it is likely that the majority of the effect of gcna5Δ on gene expression is independent of H3K14ac. This is consistent with earlier studies showing that the correlation of H3K14ac with transcription rate at euchromatic regions in fission yeast is weaker than that with H3K9ac (28).

The role of H3K14ac in DNA damage checkpoint activation - By systematically analyzing the phenotypes associated with gcna5Δ mst2Δ and H3K14R, we found that H3K14ac plays a critical role in the DNA damage response (Fig. 4), but has relatively little effect on cell cycle progression or heterochromatic silencing (data not shown and (21)). Cells containing mutations that abolish H3K14 acetylation, such as gcna5 Δ mst2Δ, gcna5 Δ mst2-E274Q, gcna5 Δ nto1Δ, gcna5 Δ ptf2Δ, are highly sensitive to a variety of DNA damage inducing agents such as ultraviolet light (UV), bleomycin, MMS, and ionizing radiation (IR) (Fig. 4A and S3). In addition, the H3K14R mutation (29), which blocks acetylation, resulted in DNA damage sensitivity similar to that of mst2Δ gcna5Δ (Fig. 4A). In contrast, the H3K14A mutation, which mimics the effect of acetylation in
neutralizing the charge on histones (29), showed much less DNA damage sensitivity as compared with H3K14R (Fig. 4A). These results suggest that defective H3K14 acetylation is the cause of high degree of sensitivity to DNA damage.

The sensitivity of gcn5Δ mst2Δ and H3K14R cells to diverse DNA damage treatments indicates that H3K14ac contributes to a common process underlying the DNA damage response, such as the activation of a DNA damage checkpoint (30,31). In fission yeast, DNA damage checkpoint activation requires the sensor kinase Rad3-Rad26 complex (ATR-ATRIP in mammals), whose activation leads to the phosphorylation of H2A (H2A.X in mammals). Phosphorylated H2A together with methylated H4K20 recruits and activates adaptor protein Crb2 (homologue of 53BP1) to promote phosphorylation of Chk1 kinase by Rad3, which triggers a delay in G2-M transition to allow time for DNA repair (31). Wild type cells subjected to DNA damage treatments activate a DNA damage checkpoint causing cells to transiently accumulate at G2 phase. These cells are elongated, and the septation index, the percentage of cells showing septa indicative of cell division, decreases significantly during the checkpoint delay (Fig. 4B and 4C). However, in checkpoint defective mutants such as chk1Δ, cells keep dividing in the presence of damaged DNA. The septation index remains constant and cells undergo lethal mitosis (Fig. 4B). Neither gcn5Δ mst2Δ nor H3K14R cells show significant reduction of septation index upon growth in MMS or after UV treatment, indicating failure of checkpoint-induced cell cycle delay (Fig. 4B and 4C).

Supporting the role of H3K14ac in DNA damage checkpoint activation, epistasis analysis showed that combining mutants involved in DNA damage checkpoint function, such as rad3Δ, crb2Δ and chk1Δ, with gcn5Δ mst2Δ resulted in little difference in DNA damage sensitivity as compared with gcn5Δ mst2Δ (Fig. S4). In contrast, combining gcn5Δ mst2Δ with mutations involved in DNA damage repair through homologous recombination such as rhp55Δ and rhp57Δ (32), resulted in much more sensitivity than that of gcn5Δ mst2Δ (Fig. S4). Analysis of DNA damage checkpoint markers also confirms the requirement of H3K14ac for proper checkpoint functions. For example, after bleomycin treatment, DNA damage induced H2A phosphorylation is significantly reduced in both gcn5Δ mst2Δ and H3K14R cells, indicating that H3K14ac is required for an early step during the activation of the DNA damage checkpoint (Fig. 4D).

H3K14ac levels increase at an HO-induced DNA break - DNA damage treatment increases histone acetylation levels in mammalian cells, which create a more accessible chromatin that is able to initiate DNA damage signaling, although the mechanism is unknown (33). However, we did not detect significant changes in global H3K14ac levels after different DNA damage treatment in fission yeast by Western blot analysis (Fig. S5A and data not shown). It is possible that changes in H3K14ac levels only occur at DNA break sites, and the high levels of H3K14ac across the fission yeast genome prevent the detection of small changes. To test this possibility, we examined H3K14ac levels at the site of a double strand break (DSB). We used a strain that contains the recognition sequence of the HO nuclease inserted at the arg3Δ locus, and the HO gene under the control of an inducible nmt1 promoter (19) (Fig. 5A). Induction of HO expression results in more than 90% cleavage at the HO site as indicated by real time PCR analysis, and the majority of cells contain a single dot of YFP-Crb2 (Fig. 5B and 5C) (19). Chromatin immunoprecipitation (ChIP) analysis demonstrated that H3K14ac levels increased at 2 kb from the DNA damage sites, but not at 16 kb away (Fig. 5D), suggesting that H3K14ac is directly involved in regulating the DNA damage response at the site of DNA damage.

In gcn5Δ mst2Δ cells, the efficiency of HO-induced breaks is extremely low (Fig. S5B), which prevents us from further examining whether H3K14ac at the DSB is dependent on Mst2 or Gcn5. Such low efficiency of HO-induced breaks likely results from reduced accessibility to DNA from changes in chromatin structure. Nonetheless, since gcn5Δ mst2Δ cells are devoid of H3K14ac even in the presence of DNA damage (Fig. S5A), it is highly likely that H3K14ac present at DSB is dependent on Mst2 and Gcn5.

H3K14ac regulates chromatin structure for efficient checkpoint activation - The result that H3K14R cells are much more sensitive to DNA damage treatment compared to H3K14A cells indicates that the function of H3K14ac is to
neutralize the charge of H3K14 during DNA damage checkpoint activation. Since H2A phosphorylation is an early event of DNA damage checkpoint signaling (31), which is partially dependent on H3K14ac (Fig. 4D), we examined whether H3K14ac can directly affect the activity of the H2A kinase Rad3. We used recombinant histone H3 that is uniformly acetylated at K14 to assemble nucleosomes as substrates for a Rad3 kinase assay. However, Rad3 activity on histone H2A is little affected by H3K14ac status (Fig. 5E). Thus it is more likely that H3K14ac regulates chromatin structure to affect the accessibility of Rad3 to modulate H2A phosphorylation. Confirming this idea, MNase digestion indicated that chromatin after DNA damage treatment is considerably more compacted in \textit{gcn5}^{-}\textit{mst2}^{-}\textit{∆} cells as compared to wild type cells (Fig. 5F). Chromatin compaction levels in H3K14\textit{R} and H3K14\textit{A} mutants are difficult to analyze due to reduced histone levels in these strain background and their effects on heterochromatin assembly independent of H3K14ac (21,29).

Although H3K14\textit{A} cells are much less sensitive to DNA damage treatment as compared to H3K14\textit{R} cells, they are still more sensitive than wild type (Fig. 4A), indicating additional roles of H3K14ac other than neutralizing the charge of lysine in regulating DNA damage checkpoint activation. Studies in budding yeast suggest a role for H3K14ac in the recruitment of chromatin remodeling protein RSC (34,35) and RSC facilitates the recruitment of ATM/ATR sensor kinases to the break site and H2A phosphorylation (36,37). Since the composition of RSC complex in fission yeast is similar to that of budding yeast (38), it is likely that in fission yeast H3K14ac also regulates DNA damage checkpoint activation through RSC-dependent chromatin remodeling. Confirming this idea, we found that bromodomains of two RSC components, Snf21 and Rsc4, specifically bind an H3K14ac peptide (Fig. 6A and 6B). In addition, Rsc4 associates with histones in wild type cells but not in \textit{gcn5}^{-}\textit{mst2}^{-}\textit{∆} cells and only weakly in H3K14\textit{R} cells (Fig. 6C), suggesting that H3K14ac is critical for the recruitment of RSC to chromatin in vivo. Although both \textit{snf21}\textit{∆} and \textit{snf21}\textit{¬}\textit{ABD} are lethal, making it difficult to assess the role of RSC in DNA damage checkpoint control in vivo (data not shown) (38), we observed increased localization of Snf21 at HO-induced breaks in a pattern very similar to that of H3K14ac (Fig. 6D). Given the similarity of RSC composition and function between budding yeast and fission yeast, it is likely that H3K14ac also functions through RSC to regulate DNA damage checkpoint activation in fission yeast.

**DISCUSSION:**
Acetylation of histone tails is required for diverse cellular processes such as gene expression, recombination and DNA damage repair. However, with a few exceptions, the functions of individual lysine acetylation events are less clear. The major acetylation sites of histone H3 tails are H3K9 and H3K14. Genome-wide mapping studies demonstrate that both are enriched at gene promoters and correlate with transcription rate (39,40). However, whether they function redundantly or have distinct contributions to transcription or other cellular processes is not well understood. Mutations of either H3K9 or H3K14 affect the integrity of heterochromatin in fission yeast (29), which complicates analysis of their effects on gene expression. The lack of specificity of the majority of H3 tail acetyltransferases hinders detailed analysis of individual H3 tail lysine acetylation through enzymatic inactivation. Although budding yeast NuA3 is a highly specific H3K14 acetyltransferase (26,41), the lack of phenotypes associated with its loss hinders the analysis of the in vivo functions of H3K14ac. Here we describe the purification and characterization of a highly specific nucleosomal H3K14 acetyltransferase in fission yeast that allows us to dissect the in vivo functions of H3K14ac.

The enzymatic activity of Mst2 complex resembles that of \textit{S. cerevisiae} NuA3, with major activity towards H3K14 in a nucleosomal context (26,41). The subunit composition of fission yeast Mst2 complex is slightly different from that of NuA3 (Fig. 1D). Mst2, Nto1, Tfg3, and Eaf6 have clear homologues in NuA3 (24,41,42). Pdp3 is homologous to YLR455p, which has been shown to be associated with NuA3 (24,43). However, the Mst2 complex does not have a homologue of Yng1, a component of budding yeast NuA3 that binds to H3K4me3 to promote NuA3 activity as well as its chromatin association, but has no effect on the assembly of NuA3 complex (24,26,44). Although fission yeast contains two Yng1
homologues, Png1 and Png2, neither is associated with the Mst2 complex as indicated by both mass spectrometry and co-immunoprecipitation analysis (data not shown). On the other hand, the Mst2 complex contains two novel components, Ptf1 and Ptf2 (Fig. 1D). Ptf1 is a putative phosphatase with unknown substrates. However, loss of Ptf1 has little effect on the activity of Mst2 complex in vitro and in vivo (Fig S2C and data not shown). In contrast, loss of Ptf2 resulted in a complete loss of Mst2 activity (Fig. S2C and S2D). Unlike budding yeast Yng1, loss of Ptf2 resulted in the loss of most Mst2 complex components (Fig. S2E). Thus Ptf2 is not a functional substitute of Yng1 and it is interesting that budding yeast NuA3 does not have a Ptf2 homologue, yet functions well as a H3K14 acetyltransferase.

        In budding yeast, Yng1 provides a functional link between H3K4me3 and H3K14ac (24) and H3K14 mutation results in loss of H3K4me3 (45), suggesting a feedback mechanism between these two modifications associated with actively transcribed genes (39,40). In fission yeast, although the PWWP domain interacts with methylated histones (16,46), the PWWP domain of Pdp3 does not interact with H3K4me3 (data not shown), and pdp3Δ has little effect on Mst2-mediated H3K14ac in vitro and in vivo (Fig. S6A and S2C). In addition, unlike in budding yeast, loss of H3K14ac has no effect on H3K4me3 levels in fission yeast (Fig. S6B) (45). Thus, the decoupling of H3K4me3 and H3K14ac might contribute to the reduced correlation between H3K14ac and transcription levels in fission yeast.

        Although loss of Mst2 complex components only weakly affected H3K14ac in vivo, we found that mst2Δ gcna5Δ cells completely lost H3K14ac (Fig. 2E), suggesting that Mst2 and Gcn5 function redundantly to acetylate this residue. In budding yeast, simultaneous loss of Gcn5 and NuA3 activities results in lethality (26,42). However, mutations of H3K14 did not result in lethality (45,47), indicating that lethality is independent of H3K14ac. These results highlight the importance of comparing both enzymatic inactivation and histone mutations to deduce the in vivo functions of H3K14ac. The high selectivity of the Mst2 complex provides a unique opportunity to address this issue.

        The lack of specificity of histone acetyltransferases and histone deacetylases has previously hindered the detailed characterization of the functions of individual histone acetylations in regulating cellular processes. With specific H3K14 acetyltransferase and histone H3K14 mutants, we were able to demonstrate that H3K14ac functions in the activation of the G2-M DNA damage checkpoint through directly regulating chromatin compaction as well as the recruitment of chromatin remodeling complex RSC. Further analysis of the phenotypes associated with both gcna5Δ mst2Δ and H3K14R mutants will define more clearly the in vivo functions of H3K14ac.

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FOOTNOTES
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FIGURE LEGENDS:

Figure 1. Characterization of the Pdp3-Mst2 complex. (A) Purified Pdp3-Flag complex was subjected to SDS-PAGE and silver staining. (B) A portion of each sample from (A) was used for Western blot analysis with a Flag antibody. (C) Schematic representation of proteins identified in (A). (D) Mass spectrometry analyses of affinity purified Pdp3-Flag and Mst2-Flag complexes. The number of peptides and the percentages of each protein that these peptides cover are indicated. (E) Protein extracts prepared from indicated strains were immunoprecipitated with anti-Flag resin and analyzed by Western blot analyses with indicated antibodies. WCE, whole cell extract.

Figure 2. The Mst2 complex is an H3K14 acetyltransferase. (A, B) Purified Pdp3-Flag or Mst2-myc complexes from indicated strains were used in HAT assays with oligo-nucleosomes as substrates. A
portion of each reaction was subjected to SDS-PAGE followed by Coomassie staining to visualize histones and autoradiography to detect ³H labeled proteins. Western bloting with Flag or myc antibodies was performed to measure input protein levels. (C) The H3 band was excised for Edman sequencing analysis and the release of radioactivity in each cycle was measured by scintillation counting. (D) Mst2 complex was used for HAT assays with nucleosomes assembled with recombinant yeast histones containing either wild type histone H3 or a H3K14R mutation. (E, F) Whole cell extracts prepared from indicated strains were subjected to Western blot analysis with indicated antibodies.

Figure 3. Gene expression profiling of gcnaΔ mst2Δ and H3K14R mutants. (A) Hierarchical clustering analysis of gene expression profiles of gcnaΔ, mst2Δ, gcnaΔ mst2Δ and H3K14R. The H3K14R mutation was generated in a background in which only one of the three copies of histone H3/H4 gene pairs was present (29), and this control strain was used in all experiments with H3K14 mutations to avoid histone dosage effects. The gcnaΔ, mst2Δ, gcnaΔ mst2Δ and wild type strains all contain three copies of histone H3/H4 pairs. (B) Serial dilution analysis of indicated yeast strains.

Figure 4. H3K14ac is required for the activation of a DNA damage checkpoint. (A) Serial dilutions of the indicated cells were plated on the indicated medium (4 mU/ml Bleomycin, 0.01% MMS) or subjected to UV (120 J/m²) or IR (800 Gy) treatment. (B) Strains were grown in the presence of 0% or 0.05% MMS for 5 hours at 30°C, fixed and stained with DAPI. Images are DAPI+DIC. Arrows indicate cells that have undergone lethal mitoses. (C) Strains were irradiated with 100J/m² UV-C, and then septation index was determined as a marker of synchrony, and normalized to unirradiated cells. Checkpoint-dependent cell cycle arrest causes a temporary loss of septated (dividing) cells. (D) The indicated strains were treated with 3 mU/ml bleomycin and subjected to Western blot analysis with phosphorylated H2A and H3 antibodies.

Figure 5. H3K14ac directly regulates DNA accessibility after DNA damage. (A) Schematic diagram of the DSB induction system. (B) HO induction results in efficient generation of DSBs as indicated by quantitative PCR analysis of a DNA fragment flanking DSB before and after HO induction. (C) HO induction results in efficient formation of Crb2 foci. (D) H3K14ac levels at sites 0.2kb, 2kb, and 16kb from the HO cleavage site were determined by ChIP assay before and after induction of the HO endonuclease. Results shown are the average of three biological repeats and error bars represent standard deviation. (E) Purified Rad3-myc complex was examined for its activity to phosphorylate H2A. Recombinant nucleosomes with or without H3K14ac were used as substrates. (F) Chromatin from indicated strains treated with 3 mU/ml bleomycin were subjected to MNase digestion.

Figure 6. H3K14ac promotes recruitment of RSC to chromatin. (A) Schematic representation of RSC components containing bromodomains. (B) Peptide pull downs of the indicated GST-fusion proteins with biotinylated H3 tail (1-21) unmodified peptides and peptides acetylated at K14 or K9. (C) Cell extract from the indicated strains was immunoprecipitated with H3 antibody. Bound fractions were immunoblotted with myc and H3 antibodies. (D) ChIP analysis of Snf21 levels at an HO induced double strand break.
**Figure 1**

**A** and **B** show silver stain and α-Flag western analysis of control and Pdp3-Flag samples.

**C** illustrates the protein structures with indicated domains:
- **Nto1**
- **Pdp3**
- **Mst2**
- **Tfg3**
- **Ptf1**
- **Eaf6**
- **Ptf2**

**D** presents a table comparing protein expression levels:

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<th>Protein</th>
<th>Pdp3-Flag</th>
<th>Mst2-Flag</th>
<th>Sc. NuA3</th>
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<tr>
<td>Not1</td>
<td>71, 59.6%</td>
<td>45, 56.8%</td>
<td>Nto1</td>
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<td>Mst2</td>
<td>56, 70.0%</td>
<td>50, 74.2%</td>
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<td>Ptf1</td>
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<td>11, 45.4%</td>
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<tr>
<td>Eaf6</td>
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<td>Ptf2</td>
<td>14, 78.6%</td>
<td>12, 72.2%</td>
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**E** displays Western blot analysis for WCE and α-Flag conditions with representative samples:
- Pdp3-Flag
- Nto1-myc
- Mst2-myc
- Tfg3-HA
- Ptf1-myc
- Eaf6-myc
- Ptf2-myc

**Yng1**
**Wang Fig. 2**

**A**

<table>
<thead>
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**Fluorography**

**Coomassie**

**α-Flag**

**B**

**WT**

**E274Q**

**Fluorography**

**Coomassie**

**α-myc**

**C**

**Relative acetylation**

**D**

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**Fluorography**

**Coomassie**

**E**

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**F**

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A. Experimental conditions and results for wild-type (WT) and mutant strains. 

B. Microscopy images showing septation index under different conditions.

C. Graph illustrating the relative septation index over time for different strains.

D. Western blot analysis showing the expression levels of H2A-p and H3 for different strains and treatments.
A) HO site

B) 0.2 kb 2 kb 16 kb

C) YFP-Crb2 +DIC

D) H3K14ac levels

E) Rad3 + Rad3

F) MNase WT gcn5Δ mst2Δ
Figure 6

**A**

Gene interaction diagram showing Snf21, Rsc1, and Rsc4 with their respective domains (HSA, SNF2_NHelicase, BD, BD1, BD2, BAH).

**B**

Western blot analysis showing Snf21-BD, Rsc1-BD, Rsc4-BD1, Rsc4-BD2, and Rsc4-BD1+2 with input, beads, H3, H3K14ac, and H3K9ac.

**C**

A genetic cross-over experiment showing the effects of Snf21-Flag levels with HO treatment.

**D**

Graphical representation of Snf21-Flag levels at different genetic loci (0.2 kb, 2 kb, 16 kb) with and without HO treatment.
Histone H3 lysine 14 acetylation is required for activation of a DNA damage checkpoint in fission yeast

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