Physical Links Between the Nuclear Envelope Protein Mps3, Three Alternate Replication Factor C Complexes, and a Variant Histone in *Saccharomyces cerevisiae*

Jared Haas, Amanda Lemoncelli, Christina Morozov, Karl Franke, John Dominder, and Lisa M. Antoniaci

Viability of cell progeny upon cell division require that genomes are replicated, repaired, and maintained with high fidelity. Central to both DNA replication and repair are Replication Factor C (RFC) complexes which catalyze the unloading/loading of sliding clamps such as PCNA or 9-1-1 complexes on DNA. Budding yeast contain four alternate RFC complexes which play partially redundant roles. Rfc1, Ctf18, Rad24, and Elg1 are all large subunits that bind, in a mutually exclusive fashion to RFC 2–5 small subunits. Ctf18, Rad24, and Elg1 are of particular interest because, in addition to their roles in maintaining genome integrity, all three play critical roles in sister chromatid tethering reactions that appear coupled to their roles in DNA replication/repair. Intriguingly, the nuclear envelope protein Mps3 similarly exhibits roles in repair and cohesion, leading us to hypothesize that Mps3 and RFCs function through a singular mechanism. Here we report that the nuclear envelope protein Mps3 physically associates with all three of these large RFC complex subunits (Ctf18, Elg1, and Rad24). In addition we report a physical interaction between Mps3 and the histone variant Htz1, a factor previously shown to promote DNA repair. In combination, these findings reveal a direct link between the nuclear envelope and chromatin and provide support for a model that telomeres and chromatin interact with the nuclear envelope during both DNA repair and sister chromatid pairing reactions.

Introduction

THE NUCLEAR ARCHITECTURE allows for the compartmentalization of chromatin within the nucleus that provides for spatial separation of processes that includes gene silencing, DNA repair, telomere extension, and ribosomal DNA replication. The nuclear periphery represents a key location within the nucleus where many of these chromatin activities take place (Therizols *et al.*, 2006; Akhtar and Gasser, 2007; Antoniaci *et al.*, 2007; Mekhail *et al.*, 2008; Oza *et al.*, 2009). Despite the importance of the nuclear periphery in these various forms of chromatin metabolism, the physical couplings that underlie each of these processes remain largely unknown. Growing evidence in yeast suggest that the highly conserved protein Mps3 may provide the critical link between the nuclear envelope and at least some of these chromatin-related processes. Mps3 is an integral inner nuclear membrane protein that has homology to the eukaryotic SUN domain containing proteins (Jaspersen *et al.*, 2006). SUN domain proteins identified in higher eukaryotic cells interact with both the nucleocytoskeleton and through interactions with KASH domain outer nuclear membrane proteins, tether chromatin processes, and dynamics to the cytoskeleton (Tzur *et al.*, 2006). In support of the central role

of Mps3 and the nuclear envelope orchestrating dynamics between the nucleoplasm and the cytoplasm are several studies documenting that Mps3 functions in spindle pole body duplication, sister chromatid cohesion, telomere clustering and anchoring, telomere bouquet formation during meiosis, and sequestration of double stranded DNA breaks to the nuclear periphery (Jaspersen *et al.*, 2002; Nishikawa *et al.*, 2003; Antoniaci *et al.*, 2004, 2007; Bupp *et al.*, 2007; Conrad *et al.*, 2007; Jaspersen and Hawley, 2009). While all of these functions for Mps3 involve chromatin metabolism, the mechanism through which Mps3 associates with chromatin or chromatin-associated factors to evoke these many roles remains unknown.

The Replication Factor C (RFC) complexes are chromatin-associated factors that load and unload sliding clamps such as PCNA or the 9-1-1 complex that promote DNA replication and repair (Bermudez *et al.*, 2003a, 2003b; Kanellis *et al.*, 2003; Majka and Burgers, 2003; Bylund and Burgers, 2005). Rad24-RFC functions as a checkpoint protein that is important for DNA processing, recombination partner choice, and survival of cells post-DNA repair (Aylon and Kupiec, 2003). Ctf18-RFC is important for S-phase DNA replication checkpoint and appears to promote Mrc1-dependent stabilization of stalled forks (Xu *et al.*, 2007; Kubota *et al.*, 2011). Insight

regarding Ctf18-RFC role in promoting sister chromatid cohesion comes from findings that Ctf18-RFC role in double stranded break (DSB) repair includes recombination between sister chromatids and homologous chromosomes (Skibbens *et al.*, 1999; Hanna *et al.*, 2001; Mayer *et al.*, 2001; Ogiwara *et al.*, 2007). Elg1-RFC also functions in sister chromatid cohesion, but its role is distinct from that of Ctf18-RFC in that Elg1 antagonizes Ctf7/Eco1-dependent sister pairing reactions and thus is termed an anti-establishment factor (Maradeo and Skibbens, 2009, 2010). Because of the roles of the alternate RFC complexes (Ctf18-RFC, Elg1-RFC, and Rad24-RFC) in sister chromatid cohesion and DNA damage repair, the molecular basis through which RFC's exert these diverse effects remains of tremendous interest.

Numerous studies document that the processes of DNA damage repair and sister chromatid cohesion are coupled. Sister chromatid cohesion complexes are recruited to sites of double strand break and by bolstering sister chromatid pairing, ensure a proximal template for repair. Not surprisingly, cells diminished in cohesion pathways exhibit reduced capacities to repair DNA damage. In turn, DNA repair upregulates cohesion pathways providing a feedback loop to ensure high fidelity genome maintenance (Sjogren and Nasmyth, 2001; Ström *et al.*, 2004; Unal *et al.*, 2004, 2007; Ström *et al.*, 2007; Maradeo and Skibbens, 2009). Mps3 also plays a role in both sister chromatid cohesion and DNA damage repair. Mps3 plays a critical function in sister chromatid cohesion through its interaction with the cohesion establishment factor Ctf7/Eco1 (Antoniacci *et al.*, 2004). Ctf7/Eco1 is essential to establish cohesion between sister chromatids during S phase of the cell cycle (Skibbens *et al.*, 1999) and is also required to establish cohesion at the sites of DNA DSBs (Unal *et al.*, 2007). Ctf7/Eco1 associates with all three of the alternative RFC large subunits Rad24, Ctf18, and Elg1 (Kenna and Skibbens, 2003). Here we provide the first evidence that Mps3 physically associates with all three of the alternative RFC large subunits. We also report a physical interaction between Mps3 and the histone variant H2AZ (herein termed Htz1), the relevance of which is augmented by findings that the interaction between Mps3 and Htz1 is critical for DNA damage response by the cell. In combination these physical interactions provide new evidence that Mps3 functions in DNA repair and supports a model that the nuclear envelope forms an interaction surface upon which chromatin and cohesion establishment factors and DNA repair components can interact.

Materials and Methods

GST pull-downs

Escherichia coli cells harboring either pGEX4T-3 or pGEX4T-MPS3 were induced for 3 h at 37°C with IPTG and lysed by sonication (Supplementary Fig. S1). GST and GST-MPS3 proteins in bacterial extracts were coupled to glutathione Sepharose 4B beads (GE-Healthcare). Prior to exposure to yeast extracts the beads matrices were washed three times in 1 × phosphate-buffered saline (Supplementary Fig. S2). Yeast strains KSC1377 containing Rad24-FLAG, YJH40.4 containing Ctf18-9MYC, and YDD282 containing Elg1-13MYC (all epitope tagged strains generous gifts from R. Skibbens) were spheroplasted in 100T zymolyase (MP Biomedicals), lysed by swelling and mechanical disruption and centrifuged at 9500 rpm for 45 min. The supernatant was removed (S1), and the pellet was extracted with

lysis buffer containing 1 M NaCl. After incubation the salt extracted pellet was centrifuged at 9500 rpm for 45 min. The resulting supernatant (S2) along with the S1 were divided into four equal aliquots. One aliquot was precipitated using TCA and resuspended in Laemmli buffer. The remaining S1 aliquots were incubated with GST, GST-Mps3, or sepharose beads alone. The S2 aliquots were diluted 1:10 prior to incubation with bead matrices in lysis buffer. The bead matrices were washed several times and specifically bound proteins were eluted using reduced glutathione. Western blot analysis was performed. FLAG-tagged proteins were detected using anti-FLAG antibody, M2 (Sigma) and MYC-tagged proteins were detected using anti-MYC A-14 polyclonal antibody (Santa Cruz). HRP-conjugated goat anti-mouse antibody (Biorad) was used to detect the FLAG antibody and HRP-conjugated goat anti-rabbit antibody (Sigma) was used to detect the MYC antibody. ECL-Plus (GE Healthcare) was used for visualization.

Co-immunoprecipitation

Cells containing Mps3-MYC or *mps3-3*-MYC were grown to log phase and lysed with glass beads. Lysates were prepared in Buffer A (50 mM Tris (pH7.5), 50 mM NaCl, 0.2% Triton X-100) and centrifuged. The supernatant was incubated with A-14 anti-MYC (Santa Cruz biotechnology) at 4°C and then with Protein A Sepharose CL-4B beads at 4°C (Amersham Biosciences). A control using the same cell lines was also treated with Protein A Sepharose CL-4B without anti-MYC. After incubation beads were washed several times using Buffer A, low speed centrifugation, and gentle rocking. Bound proteins were removed from beads using SDS solubilization buffer. Western blot analysis was performed using Anti-Htz1 (Abcam) followed by HRP-conjugated goat anti-rabbit (Sigma) and ECL (GE-Healthcare).

Genetic analysis

YLA1203 *mps3-3* cells and *htz1Δ* (ATCC) cells were mated, sporulated, and dissected. Cells that contain both the *htz1Δ* and *mps3-3* genotype were selected for on-ADE plates, KAN plates and those that were dead at 37°C. Further determination of strain genotype was done using polymerase chain reaction. Primers GTTAGATTCTTTGATCAGGGCTACA and CTTTTGTAGGTGTCCTTAATTTCCA were used to confirm the *htz1Δ* and Primers GATAGAGTTACTGACC GACTCCAAA and AACTCTATGATTTAAAGGGAGTGCC were used to confirm *mps3-3*.

Sensitivity to DNA damaging agents

Yeast strains yLA1061, yLA1203, yLA1169, and ATCC 4011703 (Table 1) were grown to log phase and then normalized. 10-fold serial dilutions were made in Yeast Extract, Peptone, Dextrose (YPD) and spotted onto YPD plates, YPD plates containing 2 M Hydroxyurea (HU) or 0.01% Methyl Methanesulfonate (MMS). Plates were incubated at 23°C for 4 days and then photographed.

Results

Mps3 associates with Ctf18

Both Mps3 and Ctf18 promote sister chromatid cohesion reactions and both can associate with the cohesion

TABLE 1. YEAST STRAINS USED IN THIS STUDY

Yeast strain	Characteristics	Source
YJH40.4	MATa, CTF18::9MYC-TRP1	J. Hanna
KSC1377	MATa RAD24-FLAG::URA3	K. Sugimoto
YDD282	MATa ELG1::13myc-his5	D. Durocher
YLA1203	MATa <i>mps3-3::ADE, mps3::KAN</i>	L. Antoniaci
YLA1061	MATa MPS3::ADE, <i>mps3::KAN</i>	L. Antoniaci
YLA1062	MATa <i>mps3-3::13MYC-HIS3</i>	L. Antoniaci
YLA1169	MATa <i>htz1::KAN, mps3-3::ADE, mps3::KAN</i>	L. Antoniaci
SLJ924	MATa MPS3::13MYC-URA	S. Jaspersen
4011703	MAT alpha <i>htz1::KAN</i>	ATCC

establishment factor Ctf7/Eco1 (Kenna and Skibbens, 2003; Antoniaci *et al.*, 2004). From this we hypothesized that Mps3 might provide a platform from which Ctf18-RFC could impact cohesion. To test whether Mps3 physically associates with Ctf18, we bacterially expressed GST-Mps3 and a GST vector and incubated each separately with glutathione-sepharose beads. Yeast lysates expressing Ctf18-9MYC as the sole source of Ctf18 were prepared as previously described (Kenna and Skibbens, 2003). In the first strategy, soluble proteins was obtained from centrifugation of whole cell extract to obtain the primary supernatant sample (herein termed S1) In the second strategy, the pellet remaining after harvest was salt extracted and then spun to generate a secondary supernatant (herein termed S2). Western blot analysis revealed that the majority of Ctf18-MYC fractionated into S2. The S2 lysates were divided into four aliquots and incubated with either sepharose beads, beads coupled to GST, or beads coupled to GST-Mps3. A fourth aliquot was used as a fiduciary marker and loading control during Western blot analysis. Following copious washing tightly bound proteins were eluted from the bead matrices and then analyzed by Western blot. Results from the GST pull-down reveal that Ctf18 specifically associates with GST-Mps3, but that no interaction occurred between Ctf18 and GST or Ctf18 and the sepharose beads alone (Fig. 1). These results document a physical interaction between the nuclear envelope protein Mps3 and the RFC subunit Ctf18.

Mps3 associates with Rad24

Based on the interaction between Mps3 and Ctf18, we wanted to explore the possibility that Mps3 also may interact with other subunits of the RFC complex. Rad24 functions in the DNA damage checkpoint, DNA damage repair, and appears to promote Ctf7/Eco1-dependent DNA repair activities (Paulovich *et al.*, 1997; Aylon and Kupiec, 2003; Maradeo and Skibbens, 2009). More recently, Mps3 was shown to recruit double stranded DNA breaks to the nuclear periphery and that this motility was critical for efficient repair (Oza and Peterson, 2010). To determine if Mps3 physically associates with Rad24, GST-Mps3 was bacterially expressed and coupled to glutathione-sepharose beads. Yeast lysates expressing Rad24-FLAG as the sole source of Rad24 were prepared as previously described above. The resulting yeast lysates confirmed the presence of Rad24 in both S1 and S2 samples. Aliquots from both S1 and S2 lysates were incubated with either sepharose beads, beads coupled to GST,

FIG. 1. Ctf18 physically associates with Mps3. Lane 1: Ctf18-MYC S2 beads coupled with GST-Mps3, Lane 2: Ctf18-MYC S2 with beads, Lane 3: Ctf18-MYC S2 with beads coupled with GST, Lane 4: S2 extract from cells expressing Ctf18-MYC. GST-Mps3 or GST expression was induced in *E. coli* cells using IPTG. S2 extracts of Ctf18-MYC (YJH40.4) were prepared according to Kalinich and Douglas 1989. The supernatants were then harvested and divided into three equal aliquots. The three aliquots were incubated with one of the three bead matrices (glutathione-Sepharose beads, beads coupled to GST, or beads coupled to GST-Mps3). Incubations were performed at 4°C for 2 h. The treated beads were washed several times before bound proteins were removed using SDS-containing solubilization buffer. Samples were analyzed by Western blot using rabbit antibodies raised against MYC to show the presence of Ctf18-MYC in these extracts.

or beads coupled to GST-Mps3. A fourth aliquot from each was used as fiduciary marker and loading control to identify Rad24 during Western blot analysis. Following copious washings proteins tightly bound to bead matrices were eluted and analyzed by Western blot. The results reveal that Rad24 specifically binds GST-Mps3, but not to either GST or the sepharose beads alone (Fig. 2). These results show that Mps3 physically associates with a second RFC subunit Rad24.

Mps3 associates with Elg1

Given the physical association between Mps3 with both Rad24 and Ctf18, we also tested for a physical interaction between Mps3 and the third alternate large RFC subunit Elg1. Prior findings that Elg1 exhibits anti-establishment activity and thus antagonizes Ctf7/Eco1-dependent pairing reactions raised the possibility that Mps3 might not provide a platform conducive to Elg1 recruitment (Maradeo and Skibbens, 2009, 2010). To test Mps3 ability to physically associate with Elg1, GST-Mps3 and GST were expressed in bacterial cells as before and incubated with extracts of yeast cells harboring Elg1-MYC as the source of Elg1 function. Our fraction analysis revealed that, similar to Ctf18-RFC, the majority of Elg1-RFC appeared in the S2. The S2 lysate was divided into four aliquots and three of these were incubated with GST-Mps3, GST, or sepharose beads alone. A fourth aliquot was used as a fiduciary marker and loading control during Western blot analysis. Each of the bead matrices were copiously washed and tightly bound proteins eluted for Western blot analysis. Despite the role of Elg1 antagonizing Ctf7/Eco1 function, the results show that Elg1 specifically associates with GST-Mps3, but not GST or beads alone (Fig. 3). In combination these findings support prior studies that Elg1 binds to Ctf7/Eco1 and support a new model that Elg1 regulates Ctf7/Eco1 function at the nuclear periphery in association with Mps3.

FIG. 2. Rad24 physically interacts with Mps3. Lane 1: Rad24-FLAG S1 with glutathione-Sepharose beads coupled to GST-Mps3, Lane 2: Rad24-FLAG S1 with beads coupled with GST, Lane 3: Rad24-FLAG S1 with beads, Lane 4: Rad24-FLAG S2 with glutathione sepharose beads coupled to GST-Mps3, Lane 5: Rad24-FLAG S2 with beads coupled to GST, Lane 6: Rad24-FLAG S2 with beads, Lane 7: Extract from cells expressing Rad24-FLAG. GST-Mps3 or GST expression was induced in *E. coli* cells using IPTG. S1 and S2 extracts of Rad24-FLAG (KSC1377) were prepared according to Kalinich and Douglas 1989. The supernatants were then harvested and divided into three equal aliquots. The three aliquots were incubated with one of the three bead matrices (glutathione-Sepharose beads, beads coupled to GST, or GST-Mps3). Incubations were performed at 4°C for 2 h. The treated beads were washed several times before bound proteins were removed using SDS-containing solubilization buffer. Samples were analyzed by Western blot using monoclonal antibodies raised against FLAG to show the presence of Rad24-FLAG in these extracts.

Mps3 associates with Htz1 in vivo

While the above results provide novel information regarding an Mps3-dependent landing platform at the nuclear envelope for all three RFC complexes, we have yet to couple Mps3 directly to chromatin. In addition to Mps3, we decided to exploit prior findings that Mps3 may associate with Htz1, a histone variant that promotes DNA repair (Uetz *et al.*, 2000; Gardner *et al.*, 2011). We included in our analysis *mps3-3*, an allele that abrogates sister pairing activities at elevated temperatures. Epitope tagged Mps3-MYC and *mps3-3*-MYC cells were grown to log phase and lysed by mechanical

FIG. 3. Elg1 physically associates with Mps3. Lane 1: S2 extract from cells expressing Elg1-MYC, Lane 2: Elg1-MYC S2 with beads, Lane 3: Elg1-MYC S2 with beads coupled with GST, Lane 4: Elg1-MYC S2 beads coupled with GST-Mps3. GST-Mps3 or GST expression was induced in *E. coli* cells using IPTG. S2 extracts of Elg1-MYC (YDD282) were prepared according to Kalinich and Douglas 1989. The supernatants were then harvested and divided into three equal aliquots. The three aliquots were incubated with one of the three bead matrices (glutathione-Sepharose beads, beads coupled to GST, or beads coupled to GST-Mps3). Incubations were performed at 4°C for 2 h. The treated beads were washed several times before bound proteins were removed using SDS-containing solubilization buffer. Samples were analyzed by Western blot using rabbit antibodies raised against MYC to show the presence of Elg1-MYC in these extracts.

disruption. The lysates were centrifuged and the supernatants were incubated with MYC directed antibodies and Protein A Sepharose beads. After incubation the complexes were washed several times prior to Western blot analysis. Anti-Htz1 antibodies were used to probe the membrane for the presence of Htz1 in the immunoprecipitated samples. The results confirm that Mps3 and Htz1 physically associate with each other *in vivo* (Fig. 4A). In addition, we also show that the physical interaction between Htz1 and our *mps3-3* mutant strain still remains intact (Fig. 4B) in support of findings that the mutations present in this allele are positioned outside of the interaction domain required for Mps3 and Htz1 binding (Gardner *et al.*, 2011).

mps3-3 and *htz1Δ* are conditionally lethal

We reasoned that if the physical association between Mps3 and Htz1 is physiologically relevant, then *mps3-3* should evoke novel phenotypes when expressed in cells devoid of Htz1 function. To test this prediction, cells harboring either *mps3-3* or *htz1Δ* were crossed and the resulting diploids sporulated. Dissection analysis produced the

FIG. 4. Mps3 physically associates with Htz1 *in vivo*. (A) Lane 1: Cell lysates expressing Mps3-MYC and Htz1 incubated without (–) MYC antibody and then precipitated with Protein A-sepharose beads, Lane 2: Cell lysate expressing Mps3-MYC and Htz1, Lane 3: Cell lysates expressing Mps3-MYC and Htz1 incubated with (+) MYC antibody and then precipitated with Protein A-sepharose beads (B) Lane 1 and Lane 2: Cell lysates expressing Mps3-MYC and Htz1 incubated without (–) or with (+) MYC antibody and then precipitated with Protein A-sepharose beads. Lane 3 and Lane 4: Cell lysates expressing *mps3-3*-MYC and Htz1 incubated without (–) or with (+) MYC antibody and then precipitated with Protein A-sepharose beads. Western blot analysis using antibodies against Htz1 reveal that both Mps3 and *mps3-3* co-immunoprecipitate with Htz1p.

expected frequencies of viable wild type and each class of single mutant *mps3-3* and *htz1Δ* cells. In addition, the expected frequency of double mutant *htz1Δ mps3-3* cells was recovered. To test for conditional phenotypes, *htz1Δ mps3-3* double mutant cells, *htz1Δ* and *mps3-3* single mutant cells, and wild type cells were plated on YPD rich-medium and grown at 28°C, 33°C, and 37°C. As expected, all cells robustly grew at 28°C, and any cell harboring the *mps3-3* temperature sensitive allele was inviable at 37°C. While *htz1Δ* and *mps3-3* single mutant cells, and wild type all exhibited growth at 33°C, *htz1Δ mps3-3* show significant decreased growth at 33°C (Fig. 5). The decreased growth kinetics displayed by the *htz1Δ mps3-3* double mutants reveal a conditional synthetic interaction and further support a functional link between these two genes.

Mps3 functions with *htz1* in response to DNA damage repair

All three large subunits of the alternative RFC complex have been shown to be involved in some aspect of DNA damage repair and exhibit severe sensitivities to genotoxic agents (Naiki and Sugimoto, 2001; Bellaoui *et al.*, 2003). Because both Mps3 and Htz1 have been identified in playing a role in DNA damage repair/response (Downs *et al.*, 2004; Oza *et al.*, 2009) and we have shown that Mps3 physically interacts with all three of the large alternative RFC subunits we wanted to test Mps3 and Htz1 sensitivities to these same DNA damaging agents. Wild type, *mps3-3*, *htz1Δ*, and *htz1Δ mps3-3* double mutants were grown to log phase, normalized, and then plated as serial dilutions onto YPD rich-media, YPD containing 30mM HU, and YPD containing 0.01% MMS. In response to MMS the *mps3-3* and *htz1Δ* single mutants grew the same as they did on YPD and with the same growth kinetics as wild type. The *htz1Δ mps3-3* double mutant cells exhibit no growth in the presence of MMS (Fig. 6). Similarly, when the same cells were grown in the presence of HU the *mps3-3* and *htz1Δ* single mutants show robust growth, while the *htz1Δ mps3-3* double mutant cells are completely inviable (Fig. 6). This reveals a new role for the interaction between Mps3 and Htz1 in the response to DNA damage and DNA damage repair mechanisms.

Discussion

In the current study we examined interactions between Mps3 and other known proteins involved in DNA damage repair. Previous reports identified Mps3 as an important factor for tethering slow-repairing double stranded DNA breaks to the nuclear envelope and suggesting that components of the telomerase machinery aid in this telomere-specific context of repair (Oza *et al.*, 2009). Here we report a novel physical interaction between Mps3 and the three large alternate RFC subunits Rad24, Ctf18, and Elg1. Because all three of these RFC subunits function in DNA damage repair, the interaction between Mps3 and these subunits broadens previous reports on Mps3 role in DNA damage repair. We speculate that Mps3 might not only be a tethering factor for double stranded DNA breaks but also a recruiting factor for additional repair machinery beyond telomerase needed to repair other forms of mutagenic challenges such as stalled forks and bulky alkyl DNA adducts. In addition previous data suggest that *de novo* telomere formation is an extremely

FIG. 5. Conditional lethality of *mps3-3/htz1Δ* strain. Wild type, single mutant *mps3-3*, knockout *htz1Δ*, and double mutant *htz1Δ mps3-3* were plated on YPD medium in 10-fold serial dilutions and placed at 23°C, 33°C, and 37°C. All strains grew at 23°C. At 33°C wild type and both single mutants are viable. The *htz1Δ mps3-3* double mutant growth is severely retarded at 33°C compared with wild type and both single mutants revealing a conditionally synthetic lethal genetic interaction between the two genes. The *mps3-3* single mutant and *htz1Δ mps3-3* double mutant were inviable at 37°C as expected due to *mps3-3* temperature sensitivity.

rare event in persistent HO-induced DSBs (Kramer and Haber, 1993). Our data regarding the interactions between Mps3 and the three large RFC alternate subunits argue that nuclear envelope facilitated repair is a common strategy used by cells to maintain genome integrity. Based on these results, we hypothesize that Mps3 is recruiting these repair factors to

FIG. 6. Htz1 and Mps3 function in DNA damage response/repair. Wild type, *mps3-3*, *htz1Δ*, and *mps3-3 htz1Δ* were grown in 10-fold serial dilutions at 23°C on YPD rich medium, 30 mM HU, or 0.01% MMS. HU, hydroxyurea; MMS, methyl methanesulfonate.

the nuclear envelope during the cells response to DNA damage.

Recent data reveals that the mechanisms of DNA damage repair and sister chromatid cohesion within the cell are not mutually exclusive. In fact sister chromatid cohesion is a critical component required for efficient DSB DNA repair. This is demonstrated by the several cohesion factors recruited to DNA DSBs and defects in DNA damage repair when these cohesion factors are nonfunctioning (Sjogren and Nasmyth, 2001; Unal *et al.*, 2007; Maradeo and Skibbens, 2009). Mps3 and both RFC factors Ctf18 and Elg1 have documented roles in sister chromatid cohesion. In addition

Mps3, Ctf18, and Elg1 physically and genetically associates with the cohesion establishment factor Ctf7/Eco1 (Skibbens *et al.*, 1999; Kenna and Skibbens, 2003; Antoniaci *et al.*, 2004; Maradeo and Skibbens, 2010). This data continues to support a function for Mps3 in both sister chromatid cohesion and DNA repair where Mps3 at the nuclear envelope may recruit cohesion factors needed in the response to DNA damage.

The interplay between histones places Htz1 at the center of repair pathways. For instance, when a DSB is formed, the histone variant H2A.X is phosphorylated by ATM and ATR checkpoint kinases (Mec1/Tel1 in yeast) in areas immediately surrounding the DSB (Downs *et al.*, 2000; Redon *et al.*, 2003). In turn, Htz1 is recruited to double-stranded DNA breaks and replaces the phosphorylated H2A.X variant by the chromatin-remodeling enzyme Swr1 (Papamichos-Chronakis *et al.*, 2006). The replacement of H2A.X by Htz1 results in the creation of a less flexible structure in the region of the break which may be needed to repair the break (Foster and Downs, 2005). Htz1 is also functionally linked with the DNA damage checkpoint kinase Mec1, such that *htz1Δ mec1Δ* double mutant strains exhibit synthetic sensitivity to MMS (Bandyopadhyay *et al.*, 2010). Htz1 acetylation was strongly reduced in response to MMS suggesting that Htz1 acetylation may contribute to the DNA damage response.

Our findings identify a functional, physical, and genetic interaction between Mps3 and Htz1. Together these results provide the first link between Mps3 and Htz1 as functioning in DNA damage repair. The finding that double mutant strain *htz1Δ mps3-3* cells are extremely sensitive to DNA damaging agents raise an interesting conundrum: Why does the loss of Htz1 protein make cells that are deficient in Mps3 function inviable when challenged by stalled forks (HU) or bulky alkyl DNA adducts (MMS)? One of several explanations is that in the absence of Htz1, DNA damage is inefficiently marked for repair and fails to recruit to the nuclear envelope through Htz1 binding to Mps3. This reduction in both marking and recruitment, when coupled to diminished repair complex assembly and nuclear envelope recruitment may be the basis for the observed lethality. In summary this data documents a new and distinct shared physiological role between Mps3 and Htz1 and continues to support a model where Mps3 serves as a recruitment factor and landing platform at the nuclear envelope for chromatin and chromatin related factors.

Acknowledgments

We thank Robert V. Skibbens for generously sharing reagents and strains. We also thank Robert V. Skibbens for critical reading of the article and valuable suggestions. L. Antoniaci was supported by Marywood University research initiation funds, Marywood University Murray Award for Research and Development, and KISK (Keystone Innovation Starter Kit) Grant number C000026966.

Disclosure Statement

No competing financial interests exist.

References

Akhtar, A., and Gasser, S.M. (2007). The nuclear envelope and transcriptional control. *Nat Rev Genet* 8, 507–517.

- Antoniacci, L.M., Kenna, M.A., and Skibbens, R.V. (2007). The nuclear envelope and spindle pole body-associated Mps3 protein bind telomere regulators and function in telomere clustering. *Cell Cycle* **6**, 75–79.
- Antoniacci, L.M., Kenna, M.A., Uetz, P., Fields, S., and Skibbens, R.V. (2004). The spindle pole body assembly component mp3p/nep98p functions in sister chromatid cohesion. *J Biol Chem* **279**, 49542–49550.
- Aylon, Y., and Kupiec, M. (2003). The checkpoint protein Rad24 of *Saccharomyces cerevisiae* is involved in processing double-strand break ends and in recombination partner choice. *Mol Cell Biol* **23**, 6585–6596.
- Bandyopadhyay, S., Mehta, M., Kuo, D., Sung, M.K., Chuang, R., Jaehnig, E.J., Bodenmiller, B., Licon, K., Copeland, W., Shales, M., Fiedler, D., Dutkowski, J., Guénolé, A., van Attikum, H., Shokat, K.M., Kolodner, R.D., Huh, W.K., Aebersold, R., Keogh, M.C., Krogan, N.J., and Ideker, T. (2010). Rewiring of genetic networks in response to DNA damage. *Science* **330**, 1385–1389.
- Bellaoui, M., Chang, M., Ou, J., Xu, H., Boone, C., and Brown, G.W. (2003). Elg1 forms an alternative RFC complex important for DNA replication and genome integrity. *EMBO J* **22**, 4304–4313.
- Bermudez, V.P., Lindsey-Boltz, L.A., Cesare, A.J., Maniwa, Y., Griffith, J.D., Hurwitz, J., and Sancar, A. (2003a). Loading of the human 9-1-1 checkpoint complex onto DNA by the checkpoint clamp loader hRad17-replication factor C complex *in vitro*. *Proc Natl Acad Sci U S A* **100**, 1633–1638.
- Bermudez, V.P., Maniwa, Y., Tappin, I., Ozato, K., Yokomori, K., and Hurwitz, J. (2003b). The alternative Ctf18-Dcc1-Ctf8-replication factor C complex required for sister chromatid cohesion loads proliferating cell nuclear antigen onto DNA. *Proc Natl Acad Sci U S A* **100**, 10237–10242.
- Bupp, J.M., Martin, A.E., Stensrud, E.S., and Jaspersen, S.L. (2007). Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3. *J Cell Biol* **179**, 845–854.
- Bylund, G.O., and Burgers, P.M. (2005). Replication protein A-directed unloading of PCNA by the Ctf18 cohesion establishment complex. *Mol Cell Biol* **25**, 5445–5455.
- Conrad, M.N., Lee, C.Y., Wilkerson, J.L., and Dresser, M.E. (2007). MPS3 mediates meiotic bouquet formation in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* **104**, 8863–8868.
- Downs, J.A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., Kron, S.J., Jackson, S.P., and Côté, J. (2004). Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol Cell* **16**, 979–990.
- Downs, J.A., Lowndes, N.F., and Jackson, S.P. (2000). A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* **408**, 1001–1004.
- Foster, E.R., and Downs, J.A. (2005). Histone H2A phosphorylation in DNA double-strand break repair. *FEBS J* **272**, 3231–3240.
- Gardner, J.M., Smoyer, C.J., Stensrud, E.S., Alexander, R., Gogol, M., *et al.* (2011). Targeting of the SUN protein Mps3 to the inner nuclear membrane by the histone variant H2A.Z. *J Cell Biol* **193**, 489–507.
- Hanna, J.S., Kroll, E.S., Lunbald, V., and Spencer, F.A. (2001). *Saccharomyces cerevisiae* CTF18 and CTF4 are required for sister chromatid cohesion. *Mol Cell Biol* **21**, 3144–3158.
- Jaspersen, S.L., Giddings, T.H., Jr., and Winey, M. (2002). Mps3p is a novel component of the yeast spindle pole body that interacts with the yeast centrin homologue Cdc31p. *J Cell Biol* **159**, 945–956.
- Jaspersen, S.L., and Hawley, R.S. (2009). Pushing and pulling: microtubules mediate meiotic pairing and synapsis. *Cell* **139**, 861–863.
- Jaspersen, S.L., Martin, A.E., Glazko, G., Giddings, T.H., Jr., Morgan, G., Mushegian, A., and Winey, M. (2006). The Sad1-UNC-84 homology domain in Mps3 interacts with Mps2 to connect the spindle pole body with the nuclear envelope. *J Cell Biol* **174**, 665–675.
- Kalinich, J.F., and Douglas, M.G. (1989). *In Vitro* translocation through the yeast nuclear envelope. *The Journal of Biological Chemistry* **264**, 17979–17989.
- Kanellis, P., Agyei, R., and Durocher, D. (2003). Elg1 forms an alternative PCNA-interacting RFC complex required to maintain genome stability. *Curr Biol* **13**, 1583–1595.
- Kenna, M.A., and Skibbens, R.V. (2003). Mechanical link between cohesion establishment and DNA replication Ctf7p/Eco1p, a cohesion establishment factor, associates with three different replication factor C complexes. *Mol Cell Biol* **23**, 2999–3007.
- Kramer, K.M., and Haber, J.E. (1993). New telomeres in yeast are initiated with a highly selected subset of TG1-3 repeats. *Genes Dev* **7**, 2345–2356.
- Kubota, T., Hiraga, S., Yamada, K., Lamond AI, and Donaldson, A.D. (2011). Quantitative proteomic analysis of chromatin reveals that Ctf18 acts in the DNA replication checkpoint. *Mol Cell Proteomics* **10**, M110.005561.
- Majka, J., and Burgers, P.M. (2003). Yeast Rad17/Mec3/Ddc1: a sliding clamp for the DNA damage checkpoint. *Proc Natl Acad Sci U S A* **100**, 2249–2254.
- Maradeo, M.E., and Skibbens, R.V. (2009). The Elg1-RFC clamp-loading complex performs a role in sister chromatid cohesion. *PLoS One* **4**, e4707.
- Maradeo, M.E., and Skibbens, R.V. (2010). Replication factor C complexes play unique pro- and anti-establishment roles in sister chromatid cohesion. *PLoS One* **5**, e15381.
- Mayer, M.L., Gygi, S.P., Aebersold, R., Heiter, P. (2001). Identification of RFC (ctf18p, ctf8p, Dcclp): an alternative RFC complex Required for sister chromatid cohesion in *S. cerevisiae*. *Mol. Cell* **7**(5): 959–70.
- Mekhail, K., Seebacher, J., Gygi, S.P., and Moazed, D. (2008). Role for perinuclear chromosome tethering in maintenance of genome stability. *Nature* **456**, 667–670.
- Naiki, T., and Sugimoto, K. (2001). Role of the RFC-related Rad24 protein in the DNA damage checkpoint pathway. *Tanpakushitsu Kakusan Koso* **46** (8 Suppl), 1213–1219.
- Nishikawa, S., Terazawa, Y., Nakayama, T., Hirata, A., Makio, T., and Endo, T. (2003). Nep98p is a component of the yeast spindle pole body and essential for nuclear division and fusion. *J Biol Chem* **278**, 9938–9943.
- Ogiwara, H., Ohunchi, T., Ui, A., Tada, S., Enomoto, T., and Seki, M. (2007). Ctf18 is required for homologous recombination-mediated double-strand break repair. *Nucleic Acid Res* **35**, 4989–5000.
- Oza, P., and Peterson, C.L. (2010). Opening the DNA repair toolbox: localization of DNA double strand breaks to the nuclear periphery. *Cell Cycle* **9**, 43–49.
- Oza, P., Jaspersen, S.L., Miele, A., Dekker, J., and Peterson, C.L. (2009). Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. *Genes Dev* **23**, 912–927.
- Papamichos-Chronakis, M., Krebs, J.E., and Peterson, C.L. (2006). Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. *Genes Dev* **20**, 2437–2449.
- Paulovich, A.G., Margulies, R.U., Garvik, B.M., and Hartwell, L.H. (1997). RAD9, RAD17, and RAD24 are required for S

- phase regulation in *Saccharomyces cerevisiae* in response to DNA damage. *Genetics* **145**, 45–62.
- Redon, C., Pilch, D.R., Rogakou, E.P., Orr, A.H., Lowndes, N.F., and Bonner, W.M. (2003). Yeast histone 2A serine 129 is essential for the efficient repair of checkpoint-blind DNA damage. *EMBO Rep* **4**, 678–684.
- Sjogren, C., and Nasmyth, K. (2001). Sister chromatid cohesion is required for postreplicative double-strand break repaid in *Saccharomyces cerevisiae*. *Curr Biol* **11**, 991–995.
- Skibbens, R.V., Corson, L.B., Koshland, D., and Hieter, P. (1999). Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Gene Dev* **13**, 307–319.
- Ström, L., Karlsson, C., Lindroos, H.B., Wedahl, S., Katou, Y., Shirahige, K., and Sjögren, C. (2007). Postreplicative formation of cohesion is required for repair and induced by a single DNA break. *Science* **317**, 242–245.
- Ström, L., Lindroos, H.B., Shirahige, K., and Sjögren, C. (2004). Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. *Mol Cell* **16**, 1003–1015.
- Therizols, P., Fairhead, C., Cabal, G.G., Genovesio, A., Olivo-Marin, J.C., Dujon, B., and Fabre, E. (2006). Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. *J Cell Biol* **172**, 189–199.
- Tzur, Y.B., Wilson, K.L., and Gruenbaum, Y. (2006). SUN-domain proteins: ‘Velcro’ that links the -nucleoskeleton to the cytoskeleton. *Nat Rev Mol Cell Biol* **7**, 782–788.
- Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S., and Rothberg, J.M. (2000). A comprehensive analysis of protein-protein interactions *Saccharomyces cerevisiae*. *Nature* **403**, 623–627.
- Unal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J.E., and Koshland, D. (2004). DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol Cell* **16**, 991–1002.
- Unal, E., Heidinger-Pauli, J.M., and Koshland, D. (2007). DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7). *Science* **31**, 245–248.
- Xu, H., Boone, C., and Brown, G.W. (2007). Genetic dissection of parallel sister-chromatid cohesion pathways. *Genetics* **176**, 1417–1429.

Address correspondence to:
 Lisa M. Antoniaci, Ph.D.
 Science Department
 Marywood University
 Scranton, PA 18509

E-mail: lantoniacci@maryu.marywood.edu

Received for publication October 21, 2011; received in revised form December 13, 2011; accepted December 16, 2011.