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Authors: Spicakova, Tatiana, McCann, Kelly, and Brown, J. Martin

Source: Radiation Research, 170(4) : 411-421

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RR1477.1

RADIATION RESEARCH **170**, 411–421 (2008)
0033-7587/08 $15.00

A Role for Lsm1p in Response to Ultraviolet-Radiation Damage in *Saccharomyces cerevisiae*

Tatiana Spicakova, Kelly McCann and J. Martin Brown[1]

Division of Radiation and Cancer Biology, Department of Radiation Oncology, Stanford University School of Medicine, Stanford, California 94305

Spicakova, T., McCann, K. and Brown, J. M. A Role for Lsm1p in Response to Ultraviolet-Radiation Damage in *Saccharomyces cerevisiae*. *Radiat. Res.* 170, 411–421 (2008).

A genome-wide screen in *Saccharomyces cerevisiae* identified *LSM1* as a new gene affecting sensitivity to ultraviolet (UV) radiation. Lsm1p is a member of a cytoplasmic complex composed of Lsm1p–7p that interacts with the yeast mRNA degradation machinery. To investigate the potential role of Lsm1p in response to UV radiation, we constructed double mutant strains in which *LSM1* was deleted in combination with a representative gene from each of three known yeast DNA repair pathways. Our results show that *lsm1*Δ increases the UV-radiation sensitivity of the *rad1*Δ and *rad51*Δ mutants, but not the *rad18*Δ mutant, placing *LSM1* within the post-replication repair/damage tolerance pathway (PRR). When combined with other deletions affecting PRR, *lsm1*Δ increases the UV-radiation sensitivity of the *rev3*Δ, *rad30*Δ and *pol30-K164R* mutants but not *rad5*Δ. Furthermore, the UV-radiation sensitivity phenotype of *lsm1*Δ is partially rescued by mutations in genes involved in 3′ to 5′ mRNA degradation, and mutations predicted to function in RNA interactions confer the most UV-radiation sensitivity. Together, these results suggest that Lsm1p may confer protection against UV-radiation damage by protecting the 3′ ends of mRNAs from exosome-dependent 3′ to 5′ degradation as part of a novel *RAD5*-mediated, PCNA-K164 ubiquitylation-independent subpathway of PRR.

INTRODUCTION

Using a genome-wide screen in *Saccharomyces cerevisiae*, we have previously identified *LSM1* as a new gene affecting sensitivity to UV radiation (*1*). Lsm1p is part of a protein complex, Lsm1p–7p/Pat1p, that is involved in the regulation of mRNA degradation (*2*). In yeast, polyadenylated mRNAs are degraded by two general pathways, both of which require shortening of the 3′ end poly(A) tail (deadenylation). In the major pathway, deadenylation is followed by removal of the 5′ cap structure and subsequent 5′ to 3′ exonucleolytic degradation (*3–6*). In the minor pathway, deadenylation is directly followed by 3′ to 5′ exonucleolytic degradation mediated by the exosome complex (*6–10*).

The Lsm1p–7p/Pat1p complex localizes to discrete cytoplasmic structures called P-bodies where the 5′ to 3′ mRNA degradation process occurs (*11, 12*). The complex has been implicated in various mRNA degradation functions, including facilitating the decapping step of mRNA degradation (*13, 14*) as well as protecting the 3′ ends of mRNAs from partial degradation (*12, 15*). Consistent with our finding that deletion of *LSM1* causes sensitivity to UV radiation is that other proteins involved in mRNA turnover may play a role in response to UV radiation. For example, deletion of *DHH1*, a decapping activator that interacts with Lsm1p (*14*), causes decreased survival after UV irradiation (*16*). Deletion of *PAT1*, a gene encoding a protein that associates with the Lsm1p–7p complex (*13*), also results in decreased survival after UV irradiation (*17*). The role of *DHH1* in the UV-radiation damage response has been linked to recovery dependent on the G_1/S DNA damage checkpoint (*16*); however, the mechanism of action by which *LSM1* confers protection against UV radiation is currently unknown.

Because the human ortholog of *LSM1* was reported to play a possible role in carcinogenesis (*18, 19*), *LSM1* is an attractive gene for investigation in view of the known relationship between sensitivity to DNA-damaging agents and cancer (*20*). Genes that affect cell sensitivity to killing by UV radiation have classically been assigned to three major repair groups, each controlling a different type of DNA repair (*21*). The *RAD3* group mediates nucleotide excision repair (NER), a mechanism by which UV-radiation-induced thymine dimers, photoproducts and other bulky lesions are repaired (*22*). Mutants in this pathway are highly sensitive to UV radiation. The *RAD51* group mediates recombination repair, a mechanism by which DNA double-strand breaks and other forms of lesions are repaired using a homologous template (*23*). Mutants in this pathway are highly sensitive to ionizing radiation, and some are mildly sensitive to UV radiation. The *RAD6* group, the most complex and least characterized pathway, allows replication through UV-radiation lesions by mutagenic translesion synthesis, error-free translesion synthesis, and postreplication repair of discontinuities (*24*). Mutants in this pathway show variable sensitivity to many different DNA-damaging

[1] Address for correspondence: Stanford University School of Medicine, Department of Radiation Oncology, 269 Campus Dr, CCSR South Room 1255, Stanford, CA 94305-5152; e-mail: mbrown@stanford.edu.

agents, including UV radiation. Strains carrying mutations in two genes within the same repair group show UV-radiation sensitivity no greater than that of either single mutant (and are therefore in the same epistasis group), whereas strains carrying mutations in two genes in different groups show UV-radiation sensitivity greater than that of either single mutant.

In this study, we have used epistasis analysis to address the role of *LSM1* in response to UV radiation. Genetic analysis shows that *LSM1* is in the same epistasis group as *RAD18* and is specifically placed in a novel *RAD5*-dependent subpathway of PRR that does not require PCNA-K164 ubiquitylation. We also demonstrate that protection against UV-radiation damage is conferred by the whole Lsm1p–7p/Pat1p complex and is mediated via predicted RNA contact residues of Lsm1p, and the UV-radiation sensitivity phenotype of *lsm1*Δ is rescued by mutations in genes required for 3′ to 5′ mRNA degradation. Based on these results, we propose a model in which the Lsm1p–7p/Pat1p complex binds to the 3′ ends of transcripts involved in a novel *RAD5*-mediated, PCNA-K164 ubiquitylation-independent subpathway and protects them from exosome-mediated 3′ to 5′ degradation.

MATERIALS AND METHODS

Yeast Strains and Media

The *S. cerevisiae* strains used in this study are listed in Table 1. Yeast media were prepared according to standard protocols (*25*). For nonselective growth, cells were grown in YEP-rich medium consisting of 2% glucose, 1% bactopeptone, and 0.5% yeast extract. For selective growth, cells were grown in synthetic medium lacking uracil. *pol30-K164R lsm1*Δ::*KanMX4* was constructed by transforming an *lsm1*Δ::KanMX4 fragment into haploid *pol30-K164R* and selecting for G418-resistant clones. *lsm1*Δ::*KanMX4* disruption fragment was made by PCR amplification of the gene locus using template genomic DNA from YJL124C and LSM1A, LSM1D primers taken from the Saccharomyces Genome Deletion Project (http://sequence-www.stanford.edu/group/yeast_deletion_project/deletions3.html).

Plasmids

Plasmids pST11 (*LSM1*), pST26 (*lsm1-6*), pST33 (*lsm1-13*), pST28 (*lsm1-8*), pST29 (*lsm1-9*), pST34 (*lsm1-14*), pST25 (*lsm1-5*), pST21 (*lsm1-1*), pST36 (*lsm1-16*), and pST45 (*lsm1-25*) have been described (*26*). Plasmids pGal and pGal-RAD5 and pRP1000 (pSKI4) have been described (*10, 27*).

Genetic Procedures

Genetic crosses, sporulation and tetrad dissection were performed according to standard protocols (*28*). The genotype of each constructed strain was confirmed by PCR with gene-specific primers. The sequences of gene-specific primers A and D for each locus were designed as instructed from the Saccharomyces Genome Deletion Project.

Ultraviolet-Radiation Survival Curves

Cells in logarithmic growth (2.2×10^7 cells/ml) were serially diluted in sterile water, and different dilutions were plated on solid YEPD medium for different irradiations. Each plate was irradiated at the indicated dose using a Sankyo Denki UVC germicidal lamp giving most of its radiation at 254 nm at a rate of 1 J/m^{-2} or 10 J/m^{-2}, depending on the

TABLE 1
Strains Used in this Study

Strain	Source	Genotype
BY4741	Research genetics	Wild-type *MAT*a
BY4742	Research genetics	Wild-type *MAT*α
YJL124C-a	Research genetics	*MAT*a *lsm1*Δ::*kanMX*
YJL124C-α	Research genetics	*MAT*α *lsm1*Δ::*kanMX*
YJL124C-L4	This study	*MAT*α *lsm1*Δ::*LEU2*
YDR378C	Research genetics	*MAT*a *lsm6*Δ::*kanMX*
YNL147W	Research genetics	*MAT*a *lsm7*Δ::*kanMX*
TS 16D	This study	Wild-type *MAT*a
TS 16B	This study	*MAT*α *rad1*Δ::*kanMX*
TS 16C	This study	*MAT*α *lsm1*Δ::*kanMX*
TS 16A	This study	*MAT*a *lsm1*Δ::*kanMX rad1*Δ::*kanMX*
TS 3D	This study	Wild-type *MAT*α
TS 3A	This study	*MAT*a *rad51*Δ::*kanMX*
TS 3C	This study	*MAT*α *lsm1*Δ::*kanMX*
TS 3B	This study	*MAT*a *lsm1*Δ::*kanMX rad51*Δ::*kanMX*
TS 12D	This study	Wild-type *MAT*a
TS 12A	This study	*MAT*a *pat1*Δ::*kanMX*
TS 12C	This study	*MAT*α *lsm1*Δ::*kanMX*
TS 12B	This study	*MAT*α *lsm1*Δ::*kanMX pat1*Δ::*kanMX*
JMB 510B	This study	Wild-type *MAT*α
JMB 510D	This study	*MAT*α *rad18*Δ::*kanMX*
JMB 510A	This study	*MAT*a *lsm1*Δ::*LEU2*
JMB 510C	This study	*MAT*a *lsm1*Δ::*LEU2 rad18*Δ::*kanMX*
JMB 532C	This study	Wild-type *MAT*a
JMB 532A	This study	*MAT*α *rev3*Δ::*kanMX*
JMB 532D	This study	*MAT*α *lsm1*Δ::*LEU2 MAT*a
JMB 532B	This study	*MAT*a *lsm1*Δ::*LEU2 rev3*Δ::*kanMX*
JMB 514D	This study	Wild-type *MAT*a
JMB 514B	This study	*MAT*a *rad5*Δ::*kanMX*
JMB 514C	This study	*MAT*α *lsm1*Δ::*LEU2*
JMB 514A	This study	*MAT*α *rad5*Δ::*kanMX lsm1*Δ::*LEU2*
JMB 523D	This study	Wild-type *MAT*α
JMB 523C	This study	*MAT*α *rad30*Δ::*kanMX*
JMB 523A	This study	*MAT*a *lsm1*Δ::*LEU2*
JMB 523B	This study	*MAT*a *lsm1*Δ::*LEU2 rad30*Δ::*kanMX*
yRP840	Roy Parker	*MAT*a *trp1 leu2-3, 112 his4-539 ura3-52 cup1*Δ::*LEU2/PGKpG/MFA2pG*
yRP1195	Roy Parker	yRP840 *ski2*Δ::*LEU2*
yRP1410	Roy Parker	yRP840 *lsm1*Δ::*TRP1*
yRP1424	Roy Parker	yRP840 *lys2-201 lsm1*Δ::*TRP1ski2*Δ::*LEU2*
yRP841	Roy Parker	*MAT*a *trp1 leu2-3, 112 lys2-201 ura3-52 cup1*Δ::*LEU2/PGKpG/MFA2pG*
yRP1365	Roy Parker	yRP841 *lsm1*Δ::*TRP1*
yRP1540	Roy Parker	yRP840 *ski4-1*
yRP1555	Roy Parker	yRP840 *lsm1*Δ::*TRPski4-1*

Note. All strains in the background BY4741 or BY4742 carried the following genetic markers in addition to the ones listed above: BY4741 *his3*Δ*1, leu2*Δ*0, ura3*Δ*0, met15*Δ*0*; BY4742 *his3*Δ*1, leu2*Δ*0, ura3*Δ*0, lys2*Δ*0.*

TABLE 2
Primers Used in this Study

Primer	Sequence (5′-3′)
Rad 5 forward	TCCCACGCTTATATGATG
Rad 5 reverse	GCCAATTCTATGCAGTCTA
Rad 18 forward	GGGAACCGAGGATCAG
Rad 18 reverse	GTCAGCATCAGTTGAATC
Rad 6 forward	CCACCGGGTGTATCTG
Rad 6 reverse	TATGGAGTATCGGCTGG
Rad 30 forward	TCGTGGACTGTATTTCTTG
Rad 30 reverse	TCTTGGTATTACGATCTTGT
Rev 3 forward	GGAAAGCAAGGACAACTG
Rev 3 reverse	TCTCACCTTCTAAGAACTC
Lsm1 forward	AGCGACAACAGCAGAA
Lsm1 reverse	AGCGGTGGTAGTGAAGT

distance of the plate from the source. Plates were incubated in the dark for 4 days, and survival was calculated by counting visible colonies. UV-radiation sensitivity of *lsm1* point mutants was measured by spotting 5 μl of fivefold serial dilutions of cells onto plates containing synthetic medium lacking uracil. The plates were irradiated at 80 J/m^2, and viability was assessed after 3 days of incubation at 30°C.

RNA Isolation

Total RNA was isolated from yeast cells with a ToTALLY RNA Isolation Kit (Ambion, Inc.) according to the manufacturer's instructions. Total RNA was reverse transcribed into cDNA using a SuperScriptIII First-Strand Synthesis Kit with Oligo d(T)$_{20}$ primers (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The final cDNA product was stored at −20°C until further analysis.

Quantitative Real-Time PCR

Quantitative real-time PCR was carried out in duplicate in two independent experiments using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on a TaqMan Real-Time PCR Instrument (Applied Biosystems). A PCR reaction mixture of 20 μl contained 10 μl of 2× SYBR Green PCR Master Mix, 1 μl of 10 μ*M* gene-specific forward and reverse primer, 6 μl cDNA (diluted 1:8), and 2 μl of water. The amplification program consisted of one cycle at 95°C for 2 min followed by 40 cycles of 95°C for 30 s and 55°C for 1 min. A negative control (water) was included in each run. The oligonucleotide sequences used for gene-specific detection are summarized in Table 2.

RESULTS

Epistasis Analysis of the lsm1Δ

We previously identified *lsm1*Δ as sensitive to UV radiation (*1*) but with wild-type sensitivities to other damaging agents, including ionizing radiation (data not shown). To determine whether the UV-radiation sensitivity of *lsm1*Δ was due to a functional defect in any one of the three major yeast repair pathways, we crossed *lsm1*Δ with isogenic strains carrying a representative deletion in each repair group and compared the UV-radiation sensitivity of double mutants to that of the corresponding single mutants.

To analyze the relationship between *LSM1* and nucleotide excision repair, we measured survival after UV irradiation in *lsm1*Δ, *rad1*Δ and *lsm1*Δ *rad1*Δ strains. The UV-radiation sensitivity of the double mutant was dramatically increased compared to the *rad1*Δ single mutant (Fig. 1A), suggesting that *LSM1* affects a pathway other than nucleotide excision repair, and both pathways compete for a common substrate.

To analyze the relationship between *LSM1* and recombination repair, we measured survival after UV irradiation in *lsm1*Δ, *rad51*Δ and *lsm1*Δ *rad51*Δ mutants. The UV-radiation sensitivity of the double mutant was significantly increased compared to the *rad51*Δ single mutant (Fig. 1B), suggesting that *LSM1* affects a pathway other than recombination repair. Cells defective in recombination repair primarily exhibit a failure to repair double-strand breaks caused by agents such as ionizing radiation; thus this result is consistent with the finding that *lsm1*Δ is not hypersensitive to ionizing radiation (data not shown).

To analyze the relationship between *LSM1* and postreplication damage tolerance, we measured survival after UV irradiation in *lsm1*Δ, *rad18*Δ and *lsm1*Δ *rad18*Δ mutants. Our results show that the UV-radiation sensitivity of the *lsm1*Δ *rad18*Δ mutant was not increased compared to the *rad18*Δ single mutant (Fig. 1C), suggesting that *LSM1* affects the function of some aspect of the postreplication damage tolerance pathway. It is possible that the effect of *lsm1*Δ in the *rad18*Δ background was not detectable because of the extreme UV-radiation sensitivity of *rad18*Δ; however, there is clearly a difference between the *rad1*Δ *lsm1*Δ interaction and the *rad18*Δ *lsm1*Δ interaction at similar doses. The assignment of *LSM1* to the *RAD6* epistasis group is further supported by the finding that *lsm1*Δ *rad6*Δ is not any more sensitive than *rad6*Δ (data not shown).

lsm1Δ is Defective in a RAD5-Dependent Subpathway of Postreplication Repair

Rad18p forms a heterodimer with Rad6p, and the Rad6p/Rad18p complex promotes replication through DNA lesions via three different subpathways: error-free translesion synthesis, mutagenic translesion synthesis, and Rad5-dependent postreplication repair (PRR) of discontinuities (*24*). Since deletion of *RAD18* blocks the activity of all three subpathways (*24*), we sought to determine whether *LSM1* affects any particular sub-branch of the *RAD6* epistasis group. We examined the UV-radiation sensitivity of the *lsm1*Δ strain in combination with deletion of *RAD30* (a gene affecting the error-free translesion synthesis subpathway), deletion of *REV3* (a gene affecting the error-prone translesion synthesis subpathway), and deletion of *RAD5* (a gene affecting the error-free postreplicative repair of discontinuities). Deletion of *LSM1* enhanced the UV-radiation sensitivity of *rev3*Δ (Fig. 2C) and *rad30*Δ (Fig. 2B) but not *rad5*Δ (Fig. 2A). These results suggest that *RAD5* is epistatic to *LSM1*, thus placing *LSM1* in the *RAD5*-dependent subpathway of PRR.

UV-Radiation Sensitivity Phenotype of Other Members of the Lsm1p–7p/Pat1p Complex

Our yeast deletion pool study also identified *pat1*Δ as being in the top 100 strains sensitive to UV radiation (*1*).

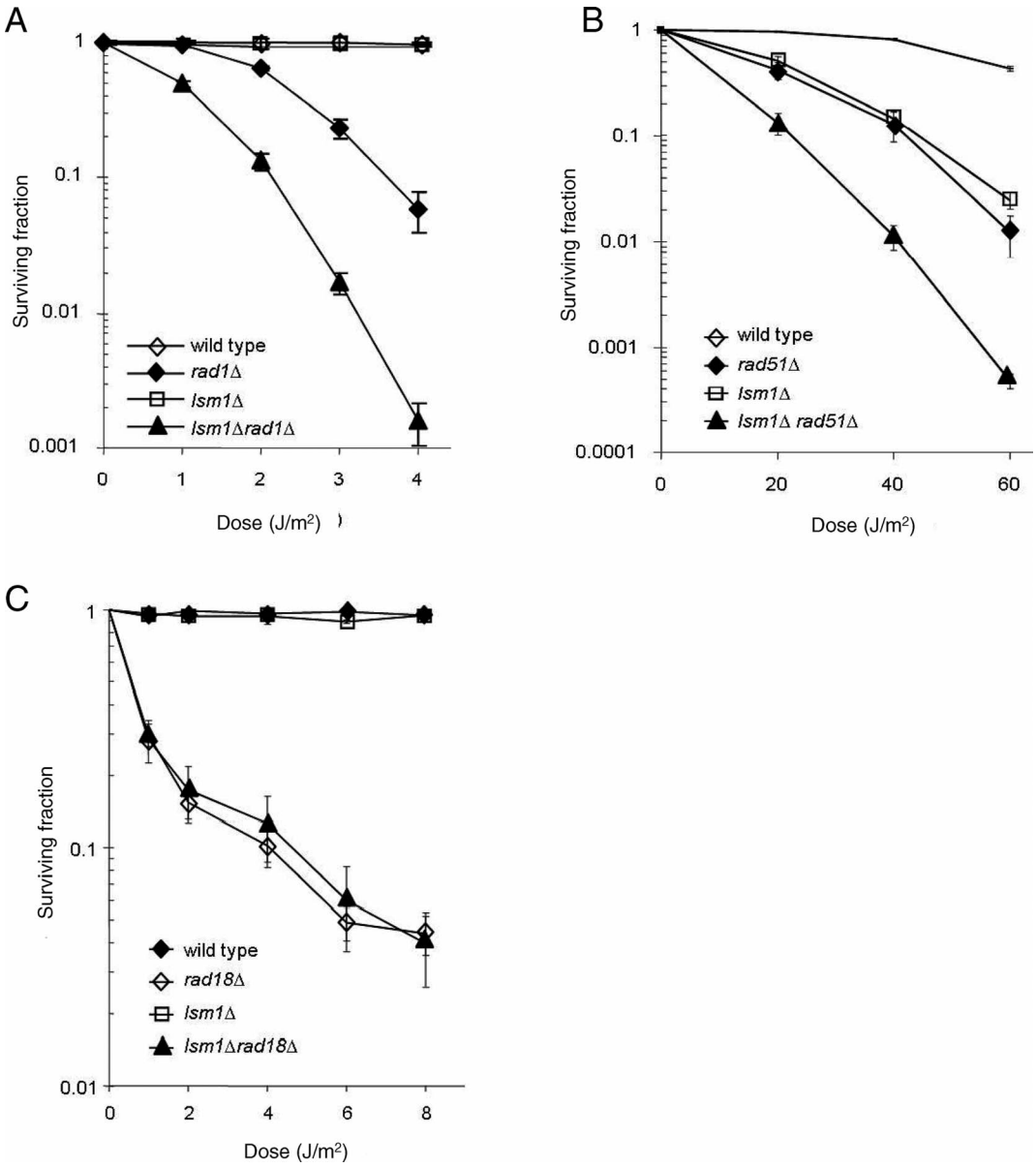

FIG. 1. *LSM1* and *RAD18* are epistatic in response to UV irradiation. Cells from logarithmically growing cultures were plated on YPD plates, irradiated with increasing doses of UV radiation, and incubated in the dark to determine viability. *lsm1*Δ mutant in combination with (panel A) *rad1*Δ (average of three independent spores), (panel B) *rad51*Δ (average of three independent spores), and (panel C) *rad18*Δ (average of three independent spores). Error bars indicate SEM.

This finding suggests that Lsm1p does not act alone in conferring protection against UV radiation but functions as a member of the cytoplasmic Lsm1p–7p complex, which interacts with Pat1p. To test this hypothesis, we examined the UV-radiation sensitivity of *pat1*Δ, *lsm6*Δ and *lsm7*Δ strains, which are deleted in the nonessential genes of the complex. Figure 3 shows that all three mutants display similar UV-radiation sensitivities to *lsm1*Δ. To test our hypothesis further, we constructed the *lsm1*Δ *pat1*Δ double mutant and compared its UV-radiation sensitivity to corresponding single mutants. The double deletion strain is no more sensitive to UV radiation than each of the single deletion strains (Fig. 3). Together, these results suggest that Lsm1p confers protection against UV radiation as a member of the Lsm1p–7p/Pat1p complex and is consistent with the finding that *lsm1* alleles defective in predicted inter-subunit contacts are also sensitive to UV radiation (Fig. 4A).

RNA Contact Residues of Lsm1p Mediate Protection against UV Radiation

Recently, several *lsm1* point mutants were generated whose mutated residues are predicted to be defective in RNA binding and inter-subunit contacts. Each of these mu-

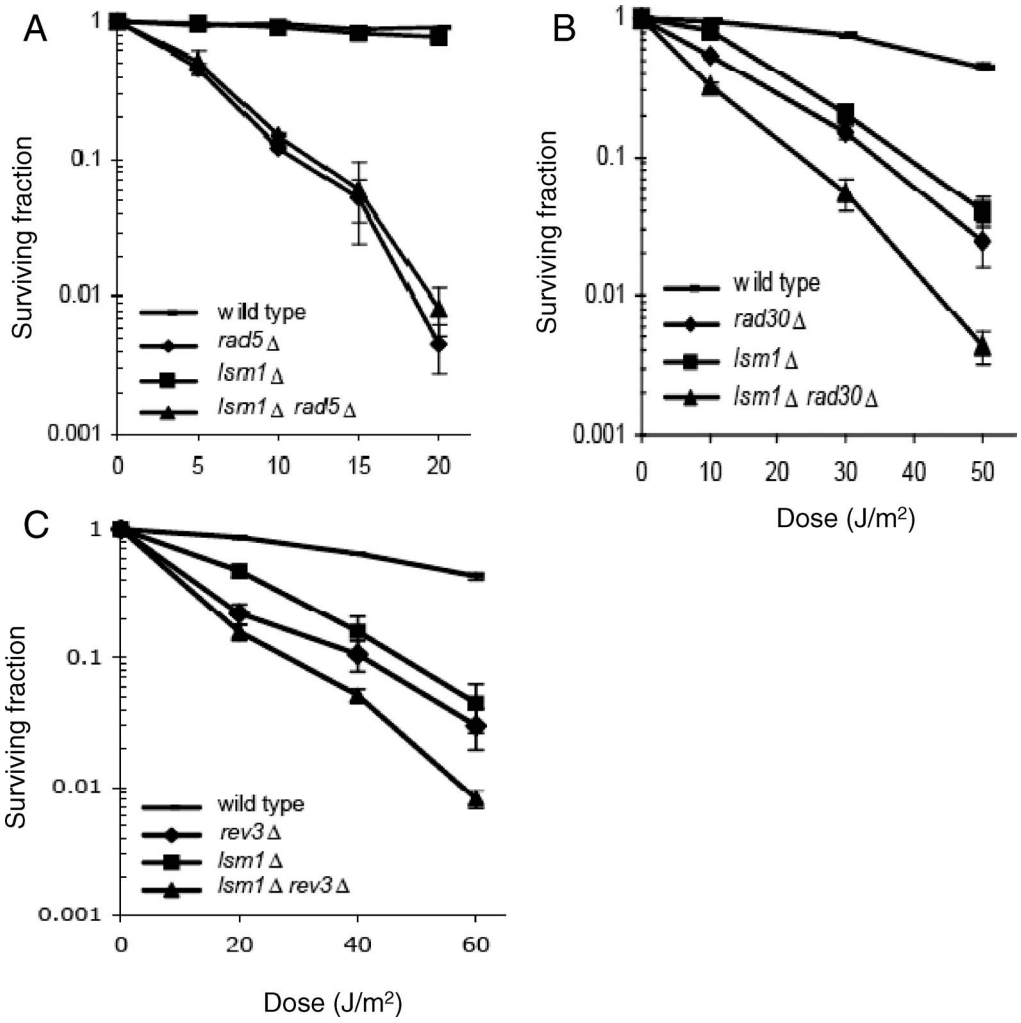

FIG. 2. *LSM1* and *RAD5* are epistatic in response to UV irradiation. Cells from logarithmically growing cultures were plated on YPD plates, irradiated with increasing doses of UV radiation, and incubated in the dark to determine viability. *lsm1*Δ mutant in combination with (panel A) *rad5*Δ (average of three independent spores), (panel B) *rad30*Δ (average of three independent spores), and (panel C) *rev3*Δ (average of three independent spores). Error bars indicate SEM.

tants demonstrated a different degree of deficiency in mRNA decay and 3′ end mRNA protection (*26*). We tested the UV-radiation sensitivity of these mutants to gain better insight into which residues may be implicated in mediating protection against UV radiation and whether UV-radiation sensitivity correlates with the defect in 3′ end mRNA protection and mRNA decay. Our results show that *lsm1* alleles with mutated RNA contact residues, *lsm1-8* (pST28), *lsm1-9* (pST29), and *lsm1-14* (pST34), and alleles with mutated inter-subunit contacts residues were indeed sensitive to UV radiation (Fig. 4). All other *lsm1* alleles that did not exhibit UV-radiation sensitivity (Fig. 4) also did not show any significant defects in mRNA metabolism (*26*). All together, the results suggest that the RNA binding property of Lsm1p as well as its ability to form a functional Lsm1p–7p complex are important for protection against UV-radiation-induced damage.

Rescue of UV-Radiation Sensitivity Phenotype of lsm1Δ by Inactivating the Exosome

The temperature sensitivity phenotype of *lsm1*Δ was found to be suppressed by mutations in the exosome or the functionally related Ski proteins, which are required for efficient 3′ to 5′ mRNA degradation (*15*). To examine whether mutations in the exosome would also suppress the UV-radiation sensitivity phenotype of *lsm1*Δ, we assessed the UV-radiation survival of double mutants carrying *lsm1*Δ and a second lesion in *SKI2, SKI3, SKI4* and *SKI8* genes, all of which are required for 3′ to 5′ mRNA degradation (*7, 10*). Our results show that the *ski4-1* mutation rescued the UV-radiation sensitivity phenotype of *lsm1*Δ to almost wild-type levels (Fig. 5A), *ski2*Δ rescued the phenotype slightly less effectively (Fig. 5B), and deletions in *SKI3* and *SKI8* were able to only partially suppress the UV-radiation sensitivity of *lsm1*Δ (data not shown). The *ski4-1* allele is

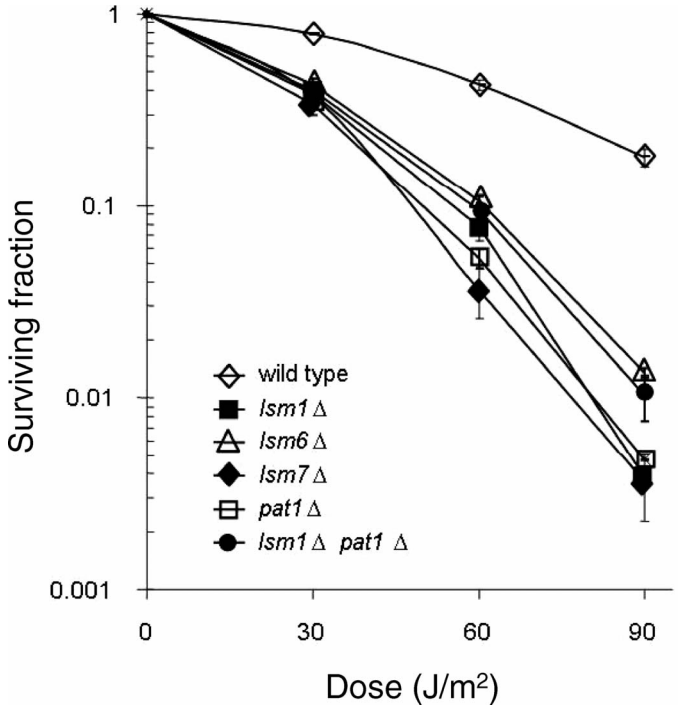

FIG. 3. The entire *LSM1-7/PAT1* complex provides protection against UV radiation. Logarithmically growing cultures of mutants deleted in members of the Lsm1–7/Pat1 complex were plated on YPD plates, irradiated with increasing doses of UV radiation, and incubated in the dark to determine viability. Results are the mean values of two independent experiments, with bars indicating SEM.

a point mutation in a core component of the exosome and has the strongest effect on mRNA turnover of all the *ski* mutations (*10*). This might explain why the *ski4-1* mutant has the strongest effect on suppression of UV-radiation sensitivity whereas the other *ski* mutants show only partial suppression. To confirm that the suppression of the phenotype was indeed due to the absence of Ski4p function in *lsm1*Δ, we transformed a plasmid encoding the wild-type *SKI4* gene into *lsm1*Δ *ski4-1* and tested its survival after UV irradiation. Replacing the wild-type Ski4p in *lsm1*Δ *ski4-1* resensitized the cells to UV radiation (Fig. 5C), thus confirming that Ski4p function is required to confer UV-radiation sensitivity in *lsm1*Δ cells. These results suggest that the UV-radiation sensitivity phenotype of *lsm1*Δ is at least partially due to 3′ to 5′ exosome-dependent degradation of unknown mRNAs and that the phenotype is alleviated by the absence of the exosome.

mRNA Transcript Levels in lsm1Δ

A simple interpretation of the above results is that the Lsm1p–7p complex binds to the 3′ ends of mRNAs and sterically inhibits their exosome-dependent 3′ to 5′ degradation, resulting in stabilization of the transcripts. It is currently not clear whether Lsm1p–7p complex can regulate a specific subset of mRNAs by binding to their 3′ ends, but it has been proposed that there may be some specificity for its substrates during growth at high temperatures (*15*). In view of the fact that *LSM1* is in the same epistasis group as *RAD18*, we sought to determine whether Lsm1p–7p might affect mRNA levels of genes downstream of *RAD18.* To assess the transcript levels, we isolated total RNA from wild-type and *lsm1*Δ growing cultures, reverse transcribed RNA to cDNA, and performed real-time quantitative PCR. The Ct value was calculated for each transcript in both strains, normalized to the actin housekeeping gene control and expressed as a ratio (*lsm1*Δ/wild type). Figure 6 shows that the Rad18, Rad5 and Rev3 transcripts are not differentially expressed in *lsm1*Δ mutants, whereas Rad6 and Rad30 transcript levels are slightly elevated. These results indicate that transcripts from the *RAD18* group do not appear to be preferentially degraded in the absence of Lsm1.

Overexpression of Rad5p and PCNA does not Rescue the UV-Radiation Sensitivity of lsm1Δ

Given the assignment of *LSM1* to the *RAD5*-mediated subpathway, we reasoned that overexpression of Rad5p

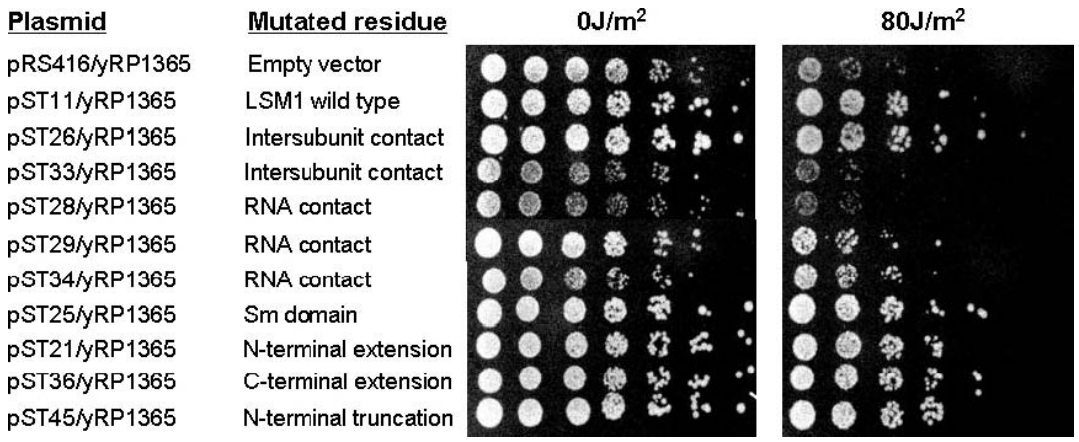

FIG. 4. RNA contact residues of Lsm1p mediate protection against UV radiation. Logarithmically growing cultures of *lsm1*Δ transformed with plasmids expressing *lsm1* point mutants were fivefold serially diluted, spotted onto plates containing synthetic drop out medium lacking uracil, and irradiated at 80 J/m^2.

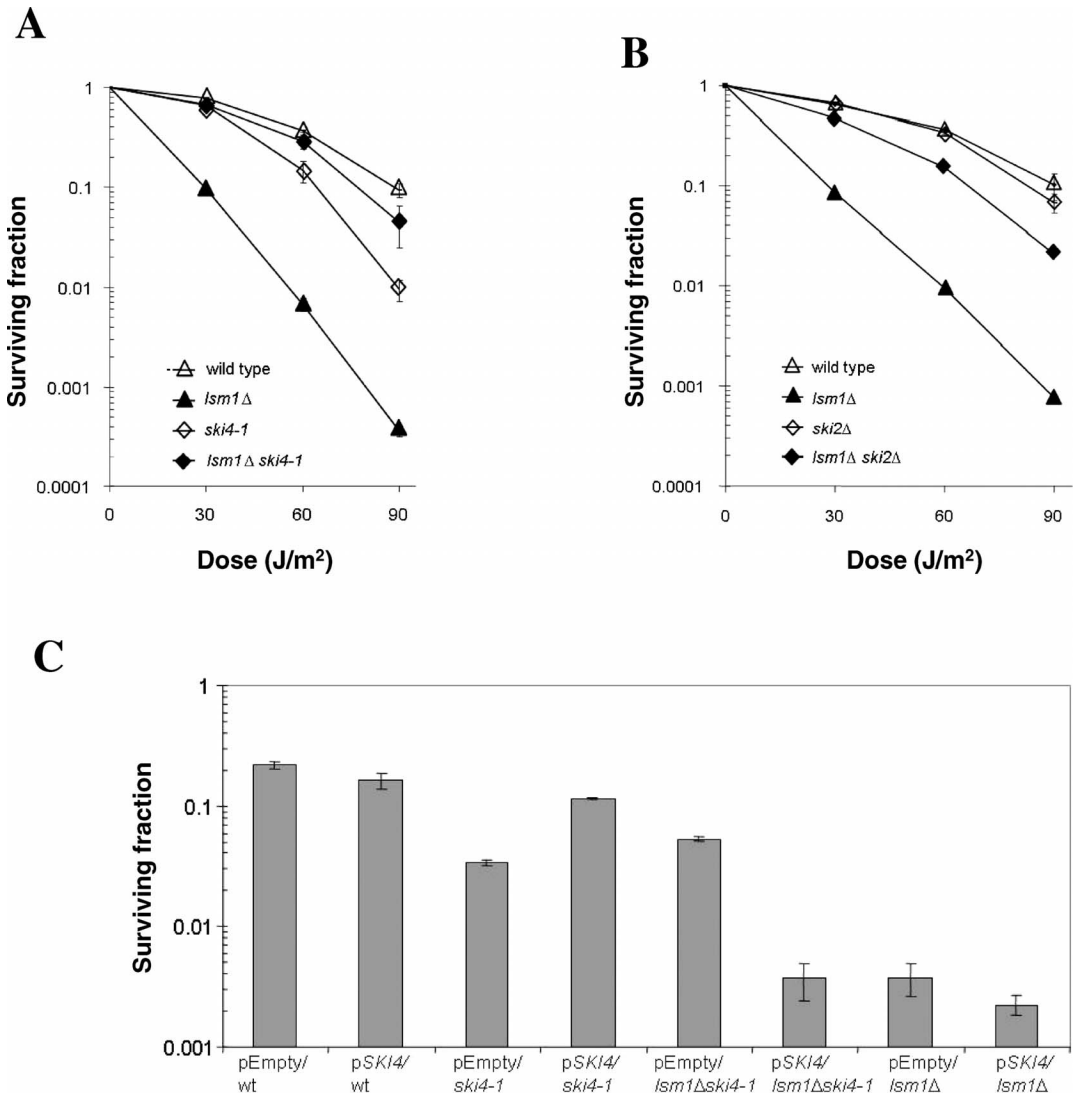

FIG. 5. Exosome mutants rescue the UV-radiation sensitivity phenotype of *lsm1*Δ. Cells from logarithmically growing cultures were plated on YPD plates, irradiated with increasing doses of UV radiation, and incubated in the dark to determine viability. *lsm1*Δ in combination with (panel A) *ski4-1* and (panel B) *ski2*Δ mutant. Panel C: Plasmid expressing *SKI4* was transformed into wild-type, *lsm*Δ, *ski4-1* and *lsm1*Δ*ski4-1*, and survival after UV irradiation was measured. Results are the mean values of three independent experiments, with bars indicating SEM.

and/or its downstream target PCNA might rescue the UV-radiation sensitivity phenotype of *lsm1*Δ. To test this hypothesis, we transformed wild-type, *lsm1*Δ and *rad5*Δ cells with a plasmid encoding Rad5p under a galactose-inducible promoter and a plasmid encoding PCNA under its native promoter. Cultures transformed with Gal-Rad5p plasmid were grown in nonrepressive raffinose medium or inducible galactose medium, plated on raffinose or galactose plates, and assessed for survival after UV irradiation. Our results show that overexpression of Rad5p rescued the UV-radiation sensitivity of *rad5*Δ whereas the empty plasmid did not, indicating that the plasmid encoding *RAD5* was functional (Fig. 7B). Overexpression of Rad5p, however, did not rescue the UV-radiation sensitivity of *lsm1*Δ, suggesting that protein levels of Rad5p are likely sufficient in *lsm1*Δ. Overexpression of PCNA partially rescued the UV-radiation sensitivity phenotype of *rad5*Δ (Fig. 7A), indicating that high levels of mono-ubiquitylated PCNA at lysine 164 can compensate for the lack of PCNA multi-ubiquitylation in *rad5*Δ. It did not, however, rescue the UV-radiation sensitivity of *lsm1*Δ (Fig. 7A), indicating that a high level of PCNA is not able to compensate for the defect in *lsm1*Δ.

LSM1 may Function in a Novel RAD5-Mediated, PCNA-K164 Ubiquitylation-Independent Subpathway of PRR

Since *rad5*Δ does not enhance the UV-radiation sensitivity of *pol30-K164R* (*29*) and overexpression of PCNA partially rescued the UV-radiation sensitivity of *rad5*Δ but not *lsm1*Δ (Fig. 7A), we wished to determine the effect of *lsm1*Δ on UV-radiation sensitivity in *pol30-K164R* background. Our results show that deletion of *LSM1* enhanced

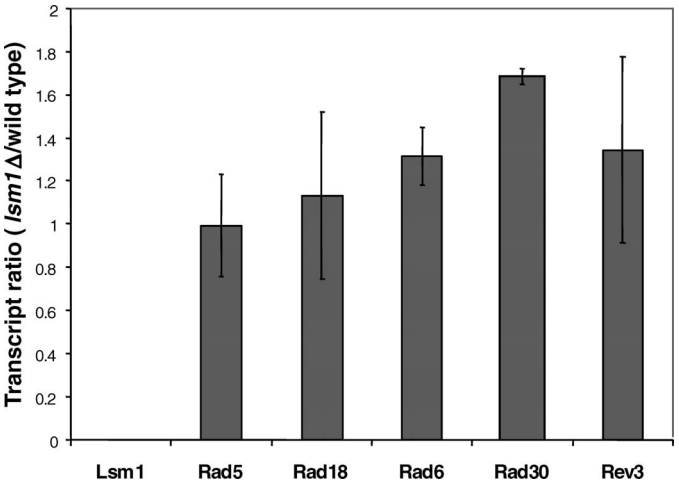

FIG. 6. mRNA levels of *RAD5* and *RAD18* transcripts. Total RNA was extracted from wild type and *lsm1*Δ, reverse transcribed and amplified using gene-specific primers. Ratio of transcripts between *lsm1*Δ and wild type was calculated after normalization to actin internal control. A value of 1 indicates no difference in mRNA expression between the two strains, value greater than 1 indicates an increase in expression in wild type, and value less than 1 indicates a decrease in expression in *lsm1*Δ. No value indicates that expression was not detectable in *lsm1*Δ.

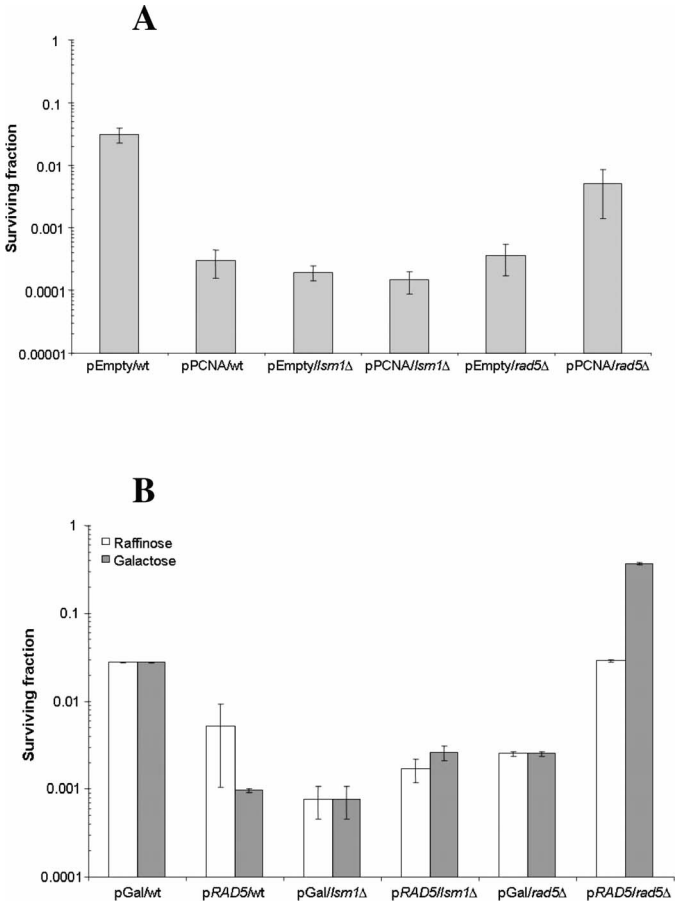

FIG. 7. Overexpression of *RAD5* and PCNA does not rescue the UV-radiation sensitivity phenotype of *lsm1*Δ. Logarithmically growing cultures transformed with plasmid expressing *PCNA* (panel A) or *RAD5* (panel B) were plated on YPD plates, irradiated (80 J/m^2 for wild type and *lsm1*Δ, 10 J/m^2 for *rad5*Δ), and incubated in the dark to determine viability. Results are the mean values of two independent experiments, with bars indicating SEM.

the UV-radiation sensitivity of *pol30-K164R* (Fig. 8), suggesting that Lsm1p has a function independent of PCNA-K164 ubiquitylation and that Rad5p may mediate additional pathways independently of PCNA-K164 ubiquitylation in which the Lsm1p–7p/Pat1p complex participates.

DISCUSSION

In this study, we show that deletion of the *LSM1* gene causes sensitivity to UV radiation, and several lines of evidence suggest that the UV-radiation sensitivity phenotype is linked to Lsm1p's role in mRNA metabolism: (1) *lsm6*Δ, *lsm7*Δ and *pat1*Δ mutants, the non-essential members of the Lsm1p–7p/Pat1p complex, display similar UV-radiation sensitivity to *lsm1*Δ, and the double-deletion *lsm1*Δ *pat1*Δ strain has the same UV-radiation sensitivity as each single deletion strain (Fig. 3); (2) mutations in predicted RNA contact residues of Lsm1p correlate with increased UV-radiation sensitivity of these mutants. (Fig. 4); and (3) the UV-radiation sensitivity phenotype of *lsm1*Δ is rescued by inactivating the core component of the exosome required for 3′ to 5′ mRNA degradation. Taken together, these observations suggest that the role of Lsm1p in conferring protection against UV radiation requires the integrity of the Lsm1p–7p/Pat1p complex and is linked to some aspect of mRNA metabolism.

One model for the role of Lsm1p in mRNA decay proposes that after deadenylation, the Lsm1p–7p complex binds to the 3′ end of the deadenylated mRNAs and triggers decapping activation and subsequent degradation by the major 5′ to 3′ mRNA pathway. The binding to 3′ ends of mRNA could, however, also result in protecting these ends from the minor 3′ to 5′ mRNA degradation pathway (*26*). The Lsm1p–7p complex could therefore have a dual function in the mRNA turnover processes: for mRNAs undergoing 5′ to 3′ degradation, binding of the Lsm1p–7p complex to their 3′ ends would promote decapping and subsequent decay, whereas for mRNAs undergoing 3′ to 5′ degradation, binding of the Lsm1p–7p complex to their 3′ ends would result in transcript protection and stabilization. Consistent with this model of protection of the 3′ ends of mRNAs is the accumulation of several deadenylated mRNAs truncated at their 3′ ends by ~10 to 20 nucleotides in cells lacking any of the Lsm1p–7p/Pat1p complex components. The truncation of these mRNAs is more severe at high temperatures, and the temperature sensitivity of *lsm1*Δ is thought to be due to the increased susceptibility of a subset of truncated mRNAs essential during high temperatures to 3′ to 5′ exosome-mediated degradation (*15*). Analogously, the lack of Lsm1p–7p/Pat1p complex might increase the susceptibility of a subset of trimmed mRNAs essential during the UV-radiation response to 3′ to 5′ ex-

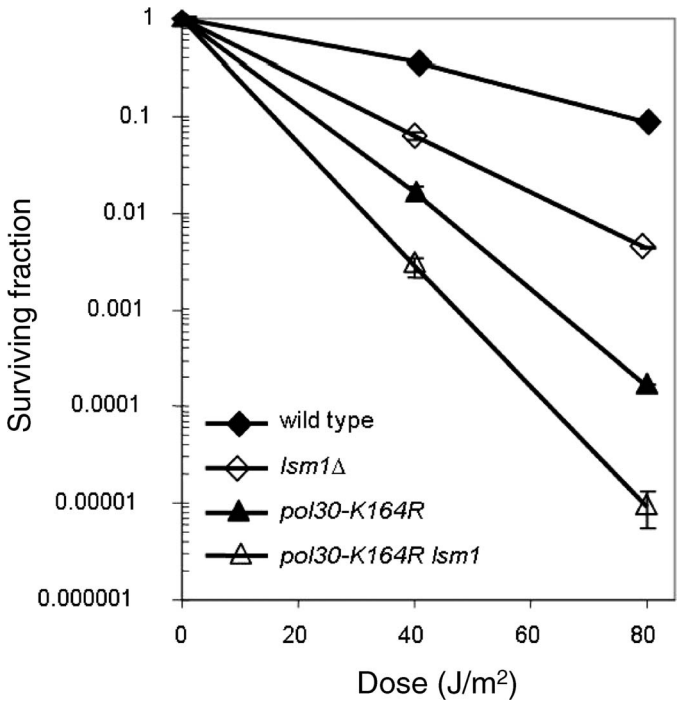

FIG. 8. *LSM1* is not epistatic with *PCNA* in response to UV irradiation. Cells from logarithmically growing cultures were plated on YPD plates, irradiated with increasing doses of UV radiation and incubated in the dark to determine viability. Results are the mean value of two independent experiments, with bars indicating SEM.

osome-dependent degradation. This model is consistent with the finding that the UV-radiation sensitivity phenotype is suppressed by mutation in *SKI4* (Fig. 5), the core component of the exosome complex required for efficient 3′ to 5′ mRNA degradation.

A recent study reported that deletion of *DHH1* also causes sensitivity to UV-radiation damage (*16*). Dhh1p physically interacts with the Lsm1p–7p complex (*14*), but decreased survival of *dhh1*Δ after UV irradiation was linked to a G_1/S DNA damage checkpoint recovery defect (*16*). We confirmed that *dhh1*Δ has a G_1/S checkpoint recovery defect in our genetic background, but we found no such defect in our *lsm1*Δ strain (data not shown). This suggests that the mechanism of action by which Lsm1p and Dhh1p provide protection against UV radiation is likely to be different. An intriguing possibility is that decapping activators might not only function at a global level during mRNA turnover, but each might also regulate the decay, stability and/or translation of a specific subset of mRNAs. For example, efficient recovery from G_1/S checkpoint arrest would require Dhh1p to regulate the decay, stability and/or translation of a specific subset of mRNAs important for the release from G_1 arrest, whereas Lsm1p might be required to regulate the decay, stability and/or translation of a specific subset of mRNAs in the *RAD5*-mediated subpathway of PRR during the UV-radiation damage response. Identifying the specific substrates of Lsm1p–7p/Pat1p complex will be crucial for understanding the complex interactions that occur during mRNA metabolism and UV-radiation response.

In yeast cells treated with UV radiation, yeast proliferating cell nuclear antigen (PCNA) becomes mono-ubiquitylated at the K164 residue by the Rad6p/Rad18p complex and subsequently becomes poly-ubiquitylated via a K63-linked chain by the Rad5p/Mms2/Ubc13p complex (*29–32*). Several analyses of PCNA have shown that a variant with a mutated K164 residue is sensitive to UV radiation, and PCNA mono-ubiquitylation at K164 is a prerequisite for Rad30- and Rev3-dependent translesion synthesis as well as subsequent Rad5-dependent poly-ubiquitylation (*29, 33, 34*). These findings suggest that all subpathways downstream of the Rad18p/Rad6p complex are regulated by mono-ubiquitylation of PCNA at K164, supported by the observation that deletions in *RAD5, RAD30* and *REV3* do not further enhance the UV-radiation sensitivity of the *pol30-K164* mutant (*29, 33, 34*). In this study, we present evidence that the Rad18p/Rad6p complex may mediate an additional subpathway that functions independently of PCNA-K164 ubiquitylation. We show that *LSM1* is in the same epistasis group as *RAD18* and *RAD5* but that deletion of *LSM1* in a *pol30-K164* background further enhances the UV-radiation sensitivity phenotype of the mutant. There is some evidence that PCNA may indeed function in a K164-independent manner. For example, the PCNA mutant *pol30-46* has four separate point mutations in charged residues with an intact K164 residue, yet it exhibits a UV-radiation sensitive phenotype (*35*). Genetic analysis of the *pol30-46* mutant placed it in the *RAD6* epistasis group with respect to UV-radiation sensitivity (*36*), and the *pol30-46 rad5*Δ double mutant showed a synergistic increase in UV-radiation sensitivity (*37*), as did the *pol30-46 rev3*Δ double mutant (*36*). In contrast, the *pol30-K164R* mutant is in the same epistasis group as *rad30*Δ, *rev3*Δ (*34*) and *rad5*Δ (*33*), indicating that separate pathways are blocked in the *pol30-K164R* and *pol30-46* mutants. The finding that *LSM1* functions independently of K164 ubiquitylation indicates that it might play a role in the subpathway that is blocked in the *pol30-46* mutant and that is distinct from the one blocked in the *pol30-K164R* mutant. Testing the UV-radiation sensitivity of *pol30-46 lsm1*Δ and *pol30-46 pol30-K164R* double mutants will be essential to confirm this hypothesis.

In addition to showing that *LSM1* functions in a PCNA-K164 ubiquitylation-independent pathway, we also show that *LSM1* is in the same epistasis subpathway as *RAD5.* These results suggest that *RAD5* may mediate more than one subpathway: one participating in poly-ubiquitylation of PCNA at K164 residue in conjunction with the Ubc13p/Mms2 complex and a second one participating in a yet-to-be-determined pathway to which *LSM1* belongs. Given the complexity of the cellular response to damaging agents, it would not be surprising if Rad5p had additional substrates and/or functions, and there are in fact several lines of evidence indicating that Rad5 affects at least two separate re-

pair subpathways in response to UV-radiation-induced damage (*24, 32, 38–41*).

In summary, we propose that *RAD18* is upstream of at least two major subpathways: one functioning in mono-ubiquitylation of PCNA-K164 leading to the activation of the known error-free and error-prone PRR subpathways and a second pathway involving a novel function mediated by *RAD5*. The Lsm1p–7p complex possibly regulates the stability of transcripts in this novel *RAD5* subpathway and protects them from exosome-mediated 3′ to 5′ degradation by binding to their 3′ ends. In the absence of Lsm1p–7p complex, these transcripts become susceptible to 3′ to 5′ exosome-dependent degradation and lead to the observed UV-radiation sensitivity. By inactivating the exosome complex, these transcripts become stabilized and the UV-radiation sensitivity phenotype is consequently suppressed. These transcripts most likely play a minor role in coping with the UV-radiation-induced stress since cells lacking any of the components of the Lsm1p–7p/Pat1p complex show only moderate UV-radiation sensitivity. However, the effect of this pathway becomes apparent in the absence of nucleotide excision repair, the major pathway dealing with UV-radiation damage, as shown by the synergistic increase in UV-radiation sensitivity in the *lsm1*Δ *rad1*Δ double mutant, indicating that both pathways compete for a common substrate resulting from UV-radiation damage.

ACKNOWLEDGMENTS

The authors thank Dr. Roy Parker for advice and generously providing strains and plasmids and Dr. John Game for many helpful comments and suggestions. This investigation was supported by PHS Training Grant CA09302 and Grants CA67166 and GM073879 awarded by the National Cancer Institute and the National Institute of General Medical Science, DHHS, respectively.

Received: May 22, 2008; accepted: June 26, 2008

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