

# LSM1 over-expression in *Saccharomyces cerevisiae* depletes U6 snRNA levels

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Received May 19, 2009; Revised June 15, 2009; Accepted June 20, 2009

## ABSTRACT

Lsm1 is a component of the Lsm1-7 complex involved in cytoplasmic mRNA degradation. Lsm1 is over-expressed in multiple tumor types, including over 80% of pancreatic tumors, and increased levels of Lsm1 protein have been shown to induce carcinogenic effects. Therefore, understanding the perturbations in cell process due to increased Lsm1 protein may help to identify possible therapeutics targeting tumors over-expressing Lsm1. Herein, we show that *LSM1* over-expression in the yeast *Saccharomyces cerevisiae* inhibits growth primarily due to U6 snRNA depletion, thereby altering pre-mRNA splicing. The decrease in U6 snRNA levels causes yeast strains over-expressing Lsm1 to be hypersensitive to loss of other proteins required for production or function of the U6 snRNA, supporting a model wherein excess Lsm1 reduces the availability of the Lsm2-7 proteins, which also assemble with Lsm8 to form a complex that binds and stabilizes the U6 snRNA. Yeast strains over-expressing Lsm1 also display minor alterations in mRNA decay and demonstrate increased susceptibility to mutations inhibiting cytoplasmic deadenylation, a process required for both 5'-to-3' and 3'-to-5' pathways of exonucleolytic decay. These results suggest that inhibition of splicing and/or deadenylation may be effective therapies for Lsm1-over-expressing tumors.

## INTRODUCTION

Tumors of the pancreas pose a critical problem in eliminating mortality due to cancer in that incidence and morbidity rates for this disease are nearly equal (NCI SEER database, <http://seer.cancer.gov/statfacts/html/pancreas.html>). Even with recent technological advances in genomic analysis, the overall relative 5-year survival rate of pancreatic tumors from 1996 to 2004 was 5.1%, and

trend analysis of the period from 2003 to 2005 revealed no significant changes in mortality rate (NCI SEER database, <http://seer.cancer.gov/statfacts/html/pancreas.html>). Given that the current prognosis for patients with these tumors is dismal, it is vital that we search for novel therapeutics targeting this disease.

In 1997, Lsm1 was identified through subtractive hybridization cloning in pancreatic cancer cells (1) and was shown to be over-expressed in 87% of pancreatic cancers. Subsequently, its over-expression has been described in 40% of prostate cancers (2), a subset (15–20%) of breast cancers that are amplified at the 8p11-12 region (3,4) and most recently in lung cancers and mesotheliomas (5). The direct involvement of Lsm1 in carcinogenesis in these tissues has been demonstrated through analyses of Lsm1's effects on growth and anchorage dependence (2,6,7), contact inhibition (2), autocrine activity (7) and tumor establishment and metastases (2,6,8,9). The increase in Lsm1 levels in these tumors is moderate (about 2- to 5-fold) (7), suggesting that subtle changes in the levels of Lsm1 can affect the growth properties of mammalian cells. Thus, it is important to elucidate the processes affected by *LSM1* over-expression in order to provide new targets for therapeutic development against pancreatic and other Lsm1-over-expressing cancers.

Lsm1 over-expression could affect cellular metabolism in several manners. For example, Lsm1 over-expression has been suggested to destabilize certain tumor suppressor transcripts, allowing for carcinogenesis (2). This model is based on the fact that Lsm1 in yeast and humans assembles with the Lsm2-Lsm7 proteins to form a heteroheptameric Lsm1-7 complex that binds mRNAs, components of the decapping machinery, and promotes mRNA decapping and degradation (10–14). Alternatively, Lsm1 over-expression might inhibit the function of the related Lsm2-8 complex, wherein the Lsm1 protein is replaced by the Lsm8 protein. The Lsm2-8 complex binds the 3'-end of the U6 snRNA protecting it from degradation and thereby allowing normal rates of pre-mRNA splicing (15–17). Consistent with Lsm1 over-expression affecting the nuclear Lsm2-8 complex, over-expression of *LSM1*

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in budding yeast increased the cytoplasmic localization of Lsm7p (18). Hence, over-expression of *LSM1* may actually reduce U6 levels and selectively influence splicing, allowing for carcinogenesis.

To understand how Lsm1 over-expression influences cell processes, we took advantage of the conservation of Lsm1 function in both budding yeast and humans to determine how Lsm1 over-expression affects RNA metabolism in yeast. We found that over-expression of *LSM1* in the yeast *Saccharomyces cerevisiae* leads to defects in pre-mRNA splicing, which is caused by decreased levels of the U6 snRNA. The splicing defect causes yeast strains over-expressing Lsm1 to be hypersensitive to loss of other components required for maintaining levels of U6 snRNA. Moreover, yeast strains over-expressing Lsm1 are more susceptible to mutations inhibiting cytoplasmic deadenylation, which is normally a prerequisite for mRNA decay. These results suggest that inhibition of splicing and/or deadenylation may be effective therapies for *LSM1*-over-expressing tumors.

## MATERIALS AND METHODS

### Yeast strains, growth conditions and plasmids

The genotypes of the strains used are listed in Supplementary Table S3. Cells were cultured in either yeast extract/peptone medium or synthetic medium supplemented with appropriate amino acids and 2% sugar (sucrose or galactose) and were grown at 30°C. Yeast strains were transformed as previously described (19) and maintained in the appropriate selective media. Over-expression studies were performed by culturing strains continuously in galactose. Plasmids utilized in this study are found in Supplementary Table S4.

### Plasmid construction

The *GAL LSM1* 2  $\mu$  plasmid was constructed by amplifying the *LSM1* coding region 64 nt prior to its start through 240 nt following the stop. A *Bam*HI restriction site immediately upstream of the 5'-end and a *Sal*I restriction site at the 3'-end of this product facilitated its ligation to a *GAL* 2  $\mu$  vector (pRP861) and placed the *LSM1* gene under transcriptional control of a *GAL* promoter. The *GAL LSM1 CEN* plasmid was constructed by amplifying the *LSM1* coding region 59 nt prior to its start through 240 nt following the stop. A *Sac*I restriction site immediately upstream of the 5'-end and a *Sal*I restriction site at the 3'-end of this product facilitated its ligation to a *GAL CEN* vector (pRP23) and placed the *LSM1* gene under transcriptional control of a *GAL* promoter.

### RNA analysis

RNA analyses were performed as previously described (20). Total RNA was isolated (21) from midlog cultures grown in appropriate media, and 20  $\mu$ g of each sample were electrophoresed on 6% acrylamide, 8M Urea gels. Northern blots were performed using the indicated oligonucleotides radiolabelled with  $^{32}$ P at the 5'-end.

Oligonucleotides used in this study are detailed in Supplementary Table S5.

For decay time course experiments, transcriptional shutoff was achieved by resuspending galactose-induced cultures in media containing 4% dextrose (22) and then collecting samples over a brief time course.

### Protein analysis

Midlog cultures were collected and harvested for protein analysis. Samples were lysed using 5 M urea, boiled, then vortexed in glass beads for 5 min. A solution of 125 mM Tris-Cl pH 6.8, 2% SDS was added at 2.5 $\times$  the volume of 5M urea used, and this was vortexed into the mixture, then samples were boiled a second time. Collected lysate was clarified by spinning at 16000 RCF, and the supernatant was resuspended in protein loading buffer (0.05 M Tris pH 6.5, 1% SDS, 0.01% bromophenol blue, 10% glycerol), boiled, and run on a 12% Tris-SDS acrylamide gel. Gels were transferred to nitrocellulose and probed using standard Western blotting protocols using an antibody to Lsm1 (a generous gift of Allen Sachs and Karsten Weis) and an anti-rabbit secondary coupled to HRP (Pierce). Lsm1 signal was revealed using Pierce SuperSignal West Dura and exposing the blots to film and developing in a film processor (Konica).

Films were scanned into .tif format using an HP Scanjet Pro flatbed scanner, and images were analyzed and quantitated in Adobe Photoshop following the method outlined at <http://www.lukemiller.org/journal/2007/08/quantifying-western-blot-without.html>. References from Supplementary Tables are Table S3 (23–25) and Table S4 (26–28).

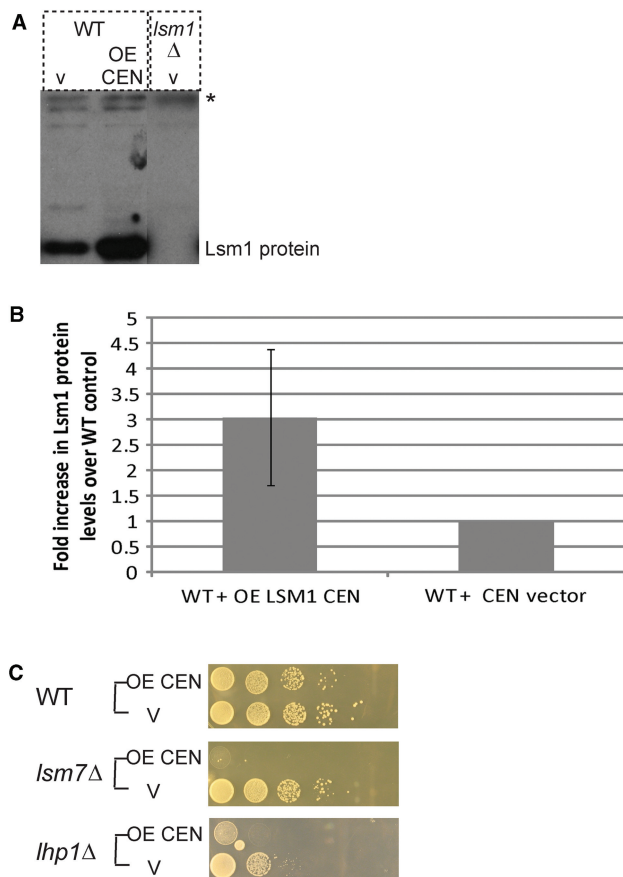
## RESULTS

### *LSM1* over-expression in yeast can affect cell growth

In order to understand the effects of *LSM1* over-expression in budding yeast, we first expressed *LSM1* from the *GAL* promoter on a *CEN* plasmid in a wild-type yeast strain. To determine the degree of over-expression, we utilized antisera against Lsm1 to determine the increase in the Lsm1 protein levels as compared to a vector only control strain. We observed that strains transformed with a *GAL-LSM1* centromere plasmid showed  $\sim 3\times$  the levels of wild-type yeast strains (Figure 1A and B), an increase in levels similar to what is seen in various human tumor cell lines. We observed that cells carrying this plasmid exhibited a slight decrease in growth as compared to the same strain carrying the vector backbone, although the strains were still able to grow at some rate (Figure 1C, uppermost panel). Thus, over-expression of *LSM1* inhibits, but does not completely prevent, the growth of wild-type yeast strains.

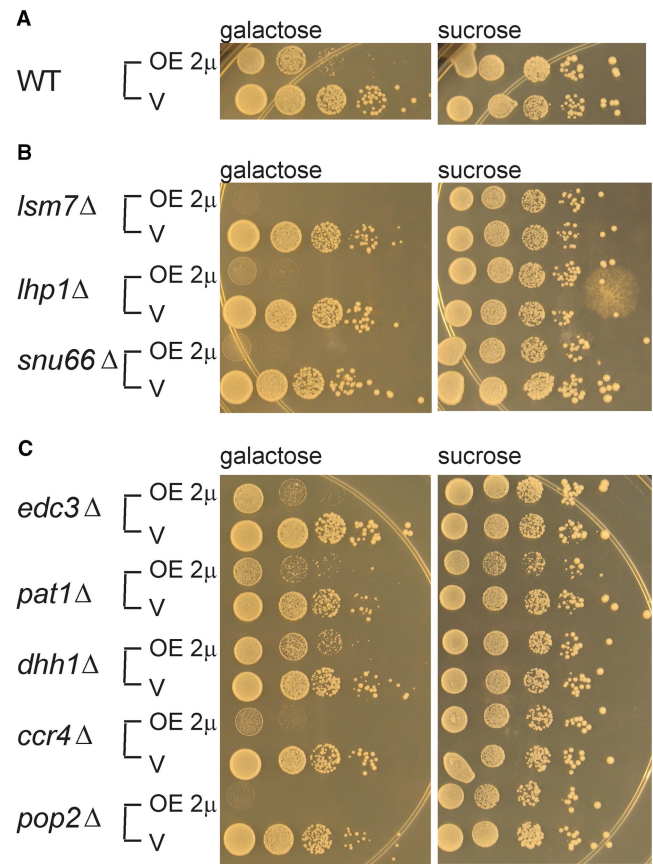
### *LSM1* over-expression shows genetic interactions with proteins affecting the U6 snRNP and mRNA deadenylases

There are two likely possibilities for how *LSM1* over-expression might affect cell function. First, since the Lsm1-7 complex functions in the control of mRNA



**Figure 1.** Moderate levels of Lsm1 affect cell growth. (A) *LSM1* over-expression on a *GAL CEN* plasmid yields 2- to 4-fold protein expression. (B) Quantitative analysis of Lsm1 over-expression. (C) *LSM1* over-expression on a *GAL CEN* plasmid affects growth and yields synthetic growth defects with strains mutant for U6 snRNP function. (A, B, C) OE CEN = Lsm1 expressed on *GAL CEN* plasmid (pRP1851). V = *CEN* vector (pRP23). (A) Western blot. Strains were transformed as indicated. 'Lsm1 protein' labels the position of Lsm1 band that was quantitated. Asterisk marks a cross-reacting band used for normalization. WT = yRP841, *lsm1Δ* = yRP1365. (B) Quantitation of protein levels. Relative intensities were compared to yield fold increase in Lsm1 protein levels over wild-type control. Four comparisons were made of OE CEN to v. Error bars represent standard deviation (see 'Materials and Methods' section, protein analysis for details on protocols used in A and B). (C) BY4741(WT) and isogenic deletion strains transformed as indicated and were frog ponded by 10-fold dilutions and plated on plates containing galactose and grown at 30°C.

decapping, over-expression of *LSM1* might alter the stoichiometry of components of the decapping complex, enhancing or inhibiting mRNA decapping and thereby affecting growth. Alternatively, increased levels of Lsm1p could deplete the nuclear Lsm2-8 complex, thus affecting cell growth through inhibition of splicing. To consider these possibilities, we first examined the effects of Lsm1 over-expression from the *GAL CEN* plasmid in *lsm7Δ* and *lhp1Δ* strains, which are both compromised for U6 levels (15, 29–32). We observed that over-expression of Lsm1 strongly limited the growth of both *lsm7Δ* and *lhp1Δ* strains, with the strongest effect seen with *lsm7Δ* (Figure 1C, lower panels).



**Figure 2.** *LSM1* 2 $\mu$  over-expression affects cell growth. (A) *LSM1* over-expression on a *GAL* 2 $\mu$  plasmid affects growth of wild-type yeast. (B) *LSM1* over-expression on a *GAL* 2 $\mu$  plasmid yields synthetic growth defects with strains mutant for U6 snRNP or (C) deadenylase functions. BY4741(WT) (A) and isogenic deletion strains (B,C) transformed with either the *GAL-LSM1* 2 $\mu$  plasmid construct (pRP1840, OE) or *GAL* 2 $\mu$  plasmid control (pRP861, V) were frog ponded by 10-fold dilutions and plated on plates containing the indicated sugar and grown at 30°C.

In order to extend this genetic analysis, we decided to over-express Lsm1 from the *GAL* promoter on a multi-copy 2 $\mu$  plasmid, thereby increasing the probability of us detecting additional genetic interactions. We observed that over-expression from the *GAL* 2 $\mu$  plasmid gave a stronger inhibition of growth than the *GAL* centromere plasmid (compare Figure 1C uppermost panel and Figure 2A), although the cells do still grow at a reduced rate (Figure 2A). Using this system of expressing *LSM1* from the *GAL* 2 $\mu$  plasmid, we then examined Lsm1 over-expression in a variety of yeast strains lacking non-essential proteins that function in pre-mRNA splicing or mRNA degradation. A complete list of these mutants and their phenotypic effects observed with *LSM1* over-expression is provided in Supplementary Tables S1 and S2.

Our broader genetic analysis revealed that factors interacting with U6 snRNA were indeed important for growth when Lsm1 was over-expressed. Expressing *LSM1* from the *GAL* 2 $\mu$  plasmid still demonstrated strong synthetic growth defects with strains deleted for *LSM7* or *LHP1* (Figure 2B). Furthermore, *snu66Δ*, which removes a

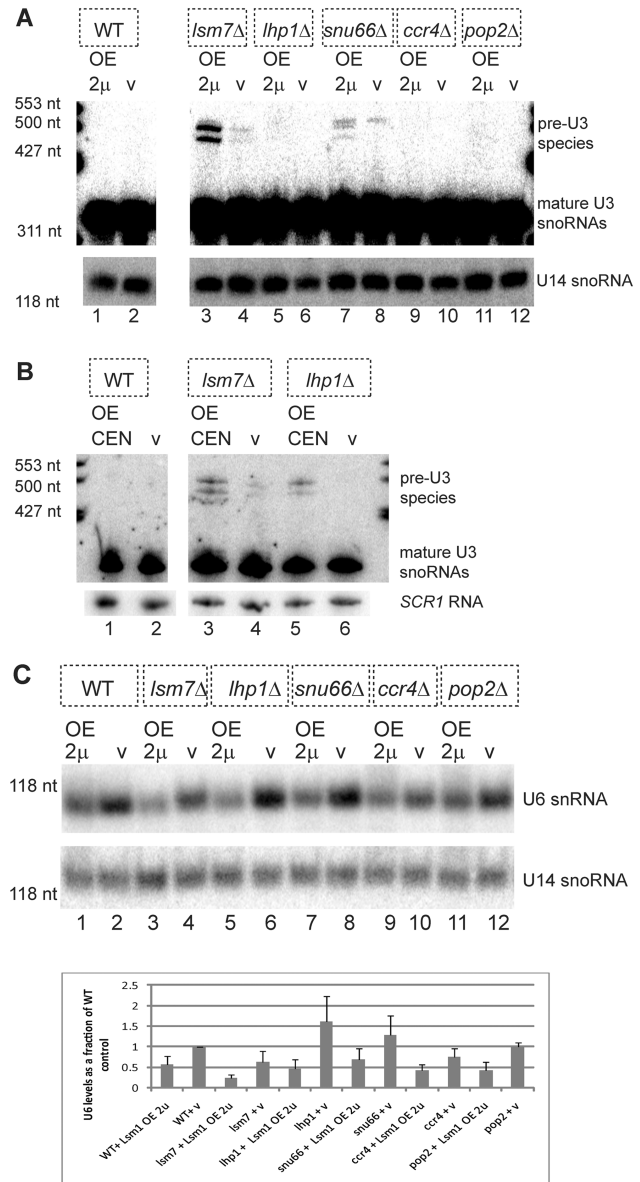
component of the U4/U6/U5 tri-snRNP (33,34), also showed a strong exacerbation of the growth defect observed when *LSM1* was over-expressed from the *GAL* 2  $\mu$  plasmid (Figure 2B). Taken together, these results suggest that *LSM1* over-expression may interfere with growth by inhibiting U6 snRNA biogenesis or function.

We also observed that over-expressing *LSM1* displayed strong synthetic growth defects with deletions of the *CCR4* and *POP2* genes, which are key components of the predominant mRNA deadenylase (35). As seen in Figure 2C, expression of *GAL-LSM1* on a 2  $\mu$  plasmid in strains deleted for *CCR4* or *POP2* inhibited growth more strongly than over-expression in a wild-type strain (Figure 2A). Significant synthetic growth defects were not demonstrated with deletions in many other decay factors (Supplementary Table S1), including strains deleted for *PAT1*, *DHH1*, and *EDC3* (Figure 2C), which encode factors that enhance decapping and/or translational repression (13,22,36,37). The enhanced toxicity of *Lsm1* over-expression in the *ccr4 $\Delta$*  and *pop2 $\Delta$*  strains suggests that mRNA decay might be altered when *Lsm1* is over-expressed, increasing the relative requirement for deadenylation.

### *LSM1* over-expression induces defects in splicing

Since strains lacking *LSM7*, *LHP1*, and *SNU66* are defective for U6 or U4/U6/U5 snRNP function (29,32,34), we hypothesized that *LSM1* over-expression might further interfere with splicing in these deletion strains. To assess the effects of *Lsm1* over-expression on splicing we examined the accumulation of the intron-containing precursor to the U3 snoRNA, which is a sensitive measure of splicing in budding yeast (29). In this analysis, we also included the *ccr4 $\Delta$*  and *pop2 $\Delta$*  strains, in case these mutations had some previously unobserved effect on splicing. For this experiment, cells containing the *GAL-LSM1* 2  $\mu$  plasmid, or a vector control, were continuously grown in galactose, cells were harvested at mid-log, and the RNA was analyzed on northern blots for the U3 snoRNA.

We observed that over-expression of *Lsm1* in a wild-type strain did not lead to accumulation of the intron-containing precursor to the U3 snoRNA (pre-U3 snoRNA). However, over-expression of *Lsm1* in an *lsm7 $\Delta$*  strain, increased the accumulation of the pre-U3 snoRNA seen in this strain (Figure 3A, compare lanes 3 and 4). Similarly, a *snu66 $\Delta$*  strain accumulated small amounts of the pre-U3 snoRNA, and these levels increased with *LSM1* over-expression (Figure 3A, compare lanes 7 and 8). In an *lhp1 $\Delta$*  strain, no pre-U3 snoRNA was evident, and small amounts accumulated with *Lsm1* over-expression, which were visible on longer exposures (Figure 3A, compare lanes 5 and 6). Finally, *ccr4 $\Delta$*  and *pop2 $\Delta$*  strains failed to accumulate pre-U3 snoRNA with or without *LSM1* over-expression (Figure 3A, examine lanes 9 through 12). Moreover, in a similar analysis, over-expressing *LSM1* on the *GAL CEN* plasmid demonstrated similar synthetic splicing defects in strains deleted for *LSM7* or *LHP1* as compared to vector only controls (Figure 3B, compare lane 3–4 and lane 5–6). These results indicate that *Lsm1* over-expression can lead



**Figure 3.** *LSM1* over-expression inhibits splicing by affecting U6 snRNA. (A) *LSM1* over-expression (*GAL* 2  $\mu$ ) inhibits splicing of U3 snoRNA in some U6 snRNP defective strains. (B) *LSM1* over-expression (*GAL CEN*) inhibits splicing of U3 snoRNA in some U6 snRNP defective strains. OE 2  $\mu$  = *GAL-LSM1* 2  $\mu$  plasmid construct (pRP1840). OE CEN = *GAL-LSM1 CEN* plasmid (pRP1851). V = appropriate vector control (pRP861 or pRP23). (A, B, C) Total RNA (20  $\mu$ g) from BY4741(WT) and isogenic deletion strains transformed as indicated were electrophoresed on 8 M Urea, 6% acrylamide gels and blotted by northern using a probe for U3 snoRNA. U14 snoRNA or *SCR1* RNA is included as a normalization control. Marker sizes are indicated. (C) *Upper panel.* *LSM1* over-expression depletes levels of U6 snRNA. Separate blot, processed as in (A), but probed for U6 snRNA. *Lower panel.* Histogram of U6 levels (normalized to U14 levels) expressed as a fraction of levels for wild-type expressing the control plasmid. Results are an average of three experiments with error bars representing the standard deviation.

to defects in splicing, even at moderate levels of over-expression as observed in tumors (7), which are most easily revealed in strains lacking proteins affecting U6 snRNA biogenesis or function.

### LSM1 over-expression leads to a decrease in U6 snRNA levels

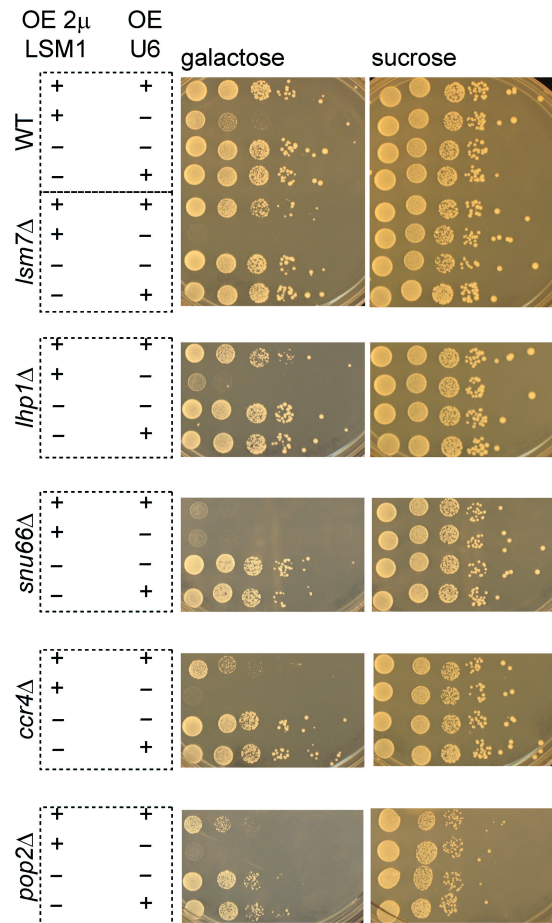
A simple hypothesis for how Lsm1 over-expression leads to defects in splicing is that Lsm1 over-expression reduces the levels of the Lsm2-8 complex, which binds to and stabilizes the U6 snRNA (17,29). This hypothesis predicts that the levels of U6 snRNA should be reduced by Lsm1 over-expression. To test this prediction, we examined the levels of U1, U2, U4, U5 and U6 snRNAs in both wild-type and various mutant strains over-expressing Lsm1.

We observed that over-expressing *LSM1* on a *GAL* 2 $\mu$  plasmid had little effect on U1, U2, U4 and U5 snRNA levels (Figure 3C and data not shown), but U6 levels were decreased in all cells over-expressing *LSM1*. Specifically, in wild-type cells over-expressing *LSM1* on a *GAL* 2 $\mu$  plasmid, U6 snRNA levels decreased by an average of 2-fold (Figure 3C, compare lane 2 to 1 and histogram), and this level of U6 depletion was also observed when moderately over-expressing *LSM1* on a *GAL CEN* plasmid (data not shown). Moreover, and consistent with the genetic interactions, U6 levels were most depleted (4.5-fold less on average, Figure 3C histogram) in an *LSM7* deletion background over-expressing *LSM1* on a *GAL* 2 $\mu$  plasmid when compared to wild-type expressing a vector control (Figure 3C, examine lanes 3 and 2 and histogram). In these two instances, the results imply that *LSM1* over-expression exerts its inhibition of growth through a reduction of U6 levels, yielding an inhibition of splicing. However, the levels of U6 snRNA in *lhp1 $\Delta$* , *snu66 $\Delta$* , *ccr4 $\Delta$* , and *pop2 $\Delta$*  strains over-expressing *LSM1* on a *GAL* 2 $\mu$  plasmid were not consistently lower than that of a wild-type strain over-expressing *LSM1*. These results suggest that the consequences of Lsm1 over-expression on growth can be made more significant without further reductions in the U6 level. In the case of the *snu66 $\Delta$* , this effect may be due to the assembly of a defective U4/U6/U5 tri- snRNP, whereas the growth inhibition in the *ccr4 $\Delta$*  and *pop2 $\Delta$*  strains may be due to defects in mRNA decay (see 'Discussion' section).

### Depletion of U6 is responsible for synthetic growth and splicing defects in wild-type, *lsm7 $\Delta$* and *lhp1 $\Delta$* strains over-expressing *LSM1*

The decrease in U6 snRNA levels with Lsm1 over-expression suggests that the inhibition of growth in wild-type and possibly various mutant strains could be due to the decreased U6 snRNA levels. This hypothesis predicts that the growth and splicing defects due to Lsm1 over-expression should be reversed by increasing levels of U6. To test this possibility, we introduced a high-copy plasmid expressing U6 snRNA into each strain and examined its effects on growth and splicing in the presence and absence of Lsm1 over-expression from a *GAL* 2 $\mu$  plasmid.

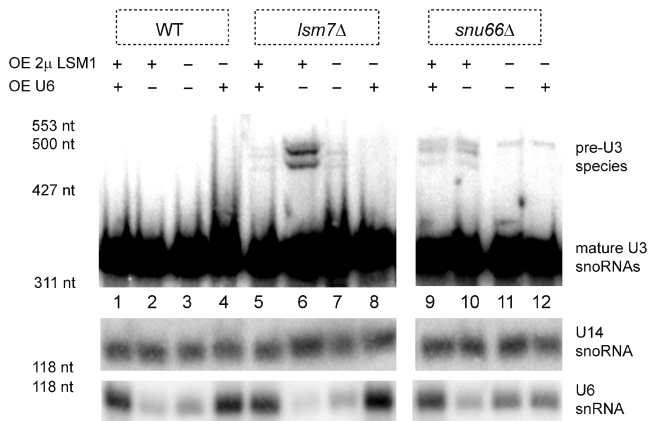
Our results revealed that at least some of the growth defects due to *LSM1* over-expression are attributable to low levels of U6 snRNA. First, over-expression of U6 snRNA suppressed the growth defect seen in wild-type cells due to Lsm1 over-expression (Figure 4, uppermost panel). Second, consistent with its severe growth defects



**Figure 4.** Growth defects due to *LSM1* over-expression can be rescued by increasing levels of U6. Increasing U6 levels rescues synthetic growth defects in wild-type, *lsm7 $\Delta$*  and *lhp1 $\Delta$*  strains. BY4741(WT) and isogenic deletion strains. Transformation of strains as indicated: '+' in a column indicates expression of either *GAL-LSM1* 2 $\mu$  (pRP1840, OE 2 $\mu$  *LSM1*) and/or pSNR6 (OE U6), '-' indicates that the control vector was used. Frog ponds are by 10-fold dilutions onto plates containing the indicated sugar, grown at 30°C.

and U6 snRNA reduction, *lsm7 $\Delta$*  over-expressing *LSM1* was nearly completely rescued by the increased U6 expression (Figure 4) and U6 snRNA over-expression suppressed the splicing defect seen with Lsm1 over-expression in this strain (Figure 5, compare lanes 5, 6 and 7). Third, a similar complementation of synthetic growth defects was observed in *lhp1 $\Delta$*  over-expressing *LSM1* when U6 was over-expressed (Figure 4). These observations indicate that at least some of the growth and splicing defects in *LSM1*-over-expressing strains arise from depletion in the levels of U6.

However, the growth defects in the *snu66 $\Delta$*  strain were not rescued by over-expression of U6 snRNA (Figure 4). We speculate that the synthetic growth defects in the *snu66 $\Delta$*  strain may be due to the assembly of a U6 snRNA that is compromised in function due to loss of both the Snu66p and the Lsm2-8 complex. Consistent with that possibility, over-expression of U6 snRNA did not suppress the splicing defect seen in the *snu66 $\Delta$*  strain (Figure 5, compare lanes 9, 10 and 11). This suggests that



**Figure 5.** Synthetic splicing defects due to *LSM1* over-expression can be rescued by increasing levels of U6. Total RNA (20  $\mu$ g) from BY4741(WT) and isogenic deletion strains transformed as indicated: '+' in a row indicates expression of either *GAL-LSM1* 2 $\mu$  (pRP1840, OE 2 $\mu$  LSM1) and/or pSNR6 (OE U6), '-' indicates that the control vector was used. Samples were electrophoresed on 8 M Urea, 6% acrylamide gels and blotted by northern using a probe for U3 snoRNA. U14 snoRNA is included as a normalization control. U6 snRNA probe confirms its over-expression. Marker sizes are noted.

in the absence of the Snu66 protein, the Lsm2-8 complex may contribute directly to U6 snRNP function (see 'Discussion' section).

We observed that the *ccr4Δ* and *pop2Δ* strains were partially rescued by over-expression of U6 snRNA (Figure 4). This suggests that the growth defects in these strains is in part due to reduced U6 snRNA levels and in part due to alterations in deadenylation, which might affect some aspect of mRNA decay.

#### Lsm1 over-expression does not globally alter mRNA decay

The strong synthetic growth defects seen in the *pop2Δ* and *ccr4Δ* strain with over-expression of Lsm1 on a *GAL* 2 $\mu$  plasmid led us to hypothesize that Lsm1 over-expression could also alter mRNA decay in some manner. This was also supported by the fact that the strong synthetic growth defects in *ccr4Δ* and *pop2Δ* over-expressing *LSM1* was only partially rescued by increasing U6 levels. To test if Lsm1 over-expression affects decapping, we examined the effects of *LSM1* over-expression on mRNA decay in a wild-type strain using the *MFA2pG* mRNA, which is a commonly used reporter for mRNA decapping in yeast (38). In order to analyze the decay of *MFA2pG* mRNA, we expressed it as a low-copy, galactose-inducible promoter fusion along with our *GAL-LSM1* 2 $\mu$  plasmid. Cells were grown in galactose and transcription was repressed by the addition of 4% dextrose (22), with samples taken for RNA analysis over a brief time course to avoid impacting the pool of Lsm1 protein whose half-life has been reported to be 76 min (39). We observed no significant change in the decay rate of the *MFA2pG* mRNA in a wild-type strain over-expressing Lsm1 as compared to a vector control ( $t_{1/2}$  less than 5' for both). Similarly, over-expressing Lsm1 at moderate levels on a *GAL-LSM1 CEN* plasmid did not affect the decay profile of the *MFA2pG* mRNA (data not shown). This indicates

that Lsm1 over-expression does not have a strong effect on the decay of all mRNAs although it remains possible that Lsm1 over-expression affects the decay of a subset of mRNAs.

## DISCUSSION

### Lsm1 over-expression alters pre-mRNA splicing

In this work, we provide several lines of evidence that Lsm1 over-expression can inhibit cell growth in budding yeast by affecting the biogenesis and/or function of the U6 snRNA. First, over-expression of Lsm1 inhibited growth and decreased the levels of the U6 snRNA (Figures 1, 2 and 3). Second, the toxicity of the Lsm1 over-expression, and in certain strains its impact on splicing, was increased in the *lsm7Δ*, *lhp1Δ* and *snu66Δ* strains, all of which impact in some manner on the function/biogenesis of the U6 snRNP (Figures 1, 2 and 3). Third, over-expression of the U6 snRNA rescued growth and the splicing defect in some strains mutant for U6 snRNP function (Figures 4 and 5). The simplest interpretation of these observations is that Lsm1 over-expression impacts on U6 snRNA by depleting the levels of the Lsm2-8 complex due to competition between Lsm1 and Lsm8 for the Lsm2-7 complex members. This interpretation is also consistent with synthetic growth defects, as well as depletion of U6 snRNA levels, seen when *LSM1* was over-expressed in a *lsm8-1* mutant and with the observation that *LSM1* over-expression increases the concentration of Lsm7p in cytoplasmic foci, presumably reflecting an increased formation of the Lsm1-7p complex (18).

Our results suggest that Lsm1 over-expression can impact on the U6 snRNP in two manners depending on other alterations in the strains. In the *lsm7Δ* strain, the primary effect is on the levels of the U6 snRNA, and over-expression of the U6 snRNA can suppress the growth defect seen in these cases. This is consistent with other observations that in an otherwise wild-type strain the predominant role of the Lsm2-8 complex is to enhance the stability of the U6 snRNA (29,40). However, in the *snu66Δ*, over-expression of the U6 snRNA fails to suppress the Lsm1 growth and splicing phenotypes resulting from Lsm1 over-expression (Figures 4 and 5). The simplest interpretation here is that when Snu66, a component of the U4/U6/U5 tri-snRNP, is missing, the Lsm2-8 complex now plays a more important role in the tri-snRNP's function in splicing. Thus, even when U6 snRNA is over-expressed any resulting U4/U6/U5 complex lacking both the Snu66 and Lsm2-8 proteins would be defective for function.

A clear implication of these observations is that changes in Lsm1 levels might impact on splicing in pancreatic tumors. Our studies reveal that when Lsm1 is over-expressed in yeast at moderate levels comparable to that observed in cancer cells (7), splicing is altered due to a depletion in U6 snRNA levels. In this light, it is notable that alterations in the splicing machinery have been previously described in human pancreatic tumors and transgenic mouse models. For instance, a serine/arginine

protein kinase involved in splicing, *SRPK1*, has been shown to be upregulated in pancreatic tumors and its downregulation correlated to decreased proliferation and increased apoptosis in these tumors (41). In addition, an analysis of genomic changes in an *Ela-c-myc* transgenic mouse model for pancreatic cancer revealed that splicing factors and spliceosome-related genes were part of a major class of genes upregulated in primary tumors and liver metastatic regions as compared to normal pancreas (42). Hence, it is possible that *LSM1* over-expression also contributes to changes in splicing patterns by altering the stoichiometry of the spliceosomal machinery, allowing for carcinogenesis to occur in pancreatic cells. An implication of this analysis, and the increased toxicity of Lsm1 over-expression in yeast strains compromised for U6 snRNA function, is that therapies directed at reducing U6 snRNA biogenesis and/or function might be effective therapies for any tumor over-expressing Lsm1.

### ***LSM1* over-expression decreases cell viability when deadenylation is inhibited**

We also provide evidence that Lsm1 over-expression leads to a change in the cells' requirement for different mRNA decay factors. Specifically, we observed that the toxicity of the Lsm1 over-expression was increased in *ccr4Δ* and *pop2Δ* strains (Figure 1C), which are compromised for the predominant cytoplasmic deadenylase (35). Moreover, the synthetic growth defects in these deadenylase mutants could only be partially rescued by increasing U6 snRNA levels (Figure 4). Overall, these results indicate that some process of mRNA decay is altered by Lsm1 over-expression, perhaps due to the assembly of mRNA decay complexes that are defective in function. However, since we did not observe an alteration in the *MFA2pG* mRNA, any alterations in degradation must be limited to subsets of mRNAs.

Prior studies in human cells do suggest that alterations in the decay of subsets of mRNAs may exist in Lsm1 over-expressing cancers. Array analyses of how Lsm1 over-expression affects the transcriptome (2,7) have demonstrated that increased Lsm1 expression alters levels of certain transcripts, some of which encode factors important in carcinogenesis. However, only one example is published providing an mRNA decay analysis. The p21/Cip1 mRNA, encoding a cyclin-dependent kinase inhibitor (43), was shown to be stabilized by targeted reduction of Lsm1 in a cancer cell line, yet this did not correspond to an increase in its protein expression (2). Thus, it is still unclear whether therapeutic effects achieved with Lsm1 targeting in cancer cells are due to altering the decay of specific transcripts. Nevertheless, the synthetic lethality of Lsm1 over-expression in strains defective in mRNA deadenylation implies that therapeutic agents targeting deadenylase activity could be possible mechanisms for the treatment of tumors with over-expression of Lsm1.

### **SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

### **ACKNOWLEDGEMENTS**

We thank Dr Sandra L. Wolin for the generous gift of the pSNR6 plasmid, Dr Pamela A. Silver for providing the *GAL* 2 $\mu$  plasmid used in our studies (pRP861) and Drs Alan B. Sachs and Karsten Weis for providing the Lsm1 antibody. Finally, we thank all members of the Parker lab for their valuable feedback and useful discussions in the preparation of this manuscript.

### **FUNDING**

National Institutes of Health [R37 GM45443 to R.P.]; National Institutes of Health [T32 CA09213 to N.L.]. Funding for open access charge: Howard Hughes Medical Institute.

*Conflict of interest statement.* None declared.

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