

## Requirements for nuclear localization of Lsm2-8p and competition between nuclear and cytoplasmic Lsm complexes

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### Summary

Lsm proteins are ubiquitous, multifunctional proteins that are involved in the processing and/or turnover of many RNAs. In eukaryotes, a hetero-heptameric complex of Lsm proteins (Lsm2-8p) affects the processing of small stable RNAs and pre-mRNAs in the nucleus, while a different hetero-heptameric complex of Lsm proteins (Lsm1-7p) promotes mRNA decapping and decay in the cytoplasm. These two complexes have six constituent proteins in common, yet localize to separate cellular compartments and perform apparently disparate functions. Little is known about the biogenesis of the Lsm complexes, or how they are recruited to different cellular compartments. We show that in yeast, the nuclear accumulation of Lsm proteins depends on complex formation and that the Lsm8p subunit plays a crucial role. The nuclear localization of Lsm8p is itself most strongly influenced by Lsm2p and Lsm4p, its presumed neighbors in the Lsm2-8p complex. Furthermore, over-expression and depletion experiments imply that Lsm1p and Lsm8p act competitively with respect to the localization of the two complexes, suggesting a potential mechanism for co-regulation of nuclear and cytoplasmic RNA processing. A shift of Lsm proteins from the nucleus to the cytoplasm under stress conditions indicates that this competition is biologically significant.

### Introduction

The Lsm proteins were identified in *Saccharomyces cerevisiae* by their sequence similarity to the canonical Sm proteins (Cooper et al., 1995; Fromont-Racine et al., 1997; Séraphin, 1995). They are found throughout eukaryotes and related proteins are also present in archaeobacteria (Achsel et al., 1999; Mayes et al., 1999; Salgado-Garrido et al., 1999) and in eubacteria (Moller et al., 2002; Zhang et al., 2002). Eight Lsm proteins have been identified in *S. cerevisiae*. A ring-shaped hetero-heptameric complex of Lsm proteins 1 to 7 is involved in mRNA decapping and decay in the cytoplasm (Boeck et al., 1998; Bouveret et al., 2000; Tharun et al., 2000), whereas a different hetero-heptameric complex comprised of Lsm proteins 2 to 8 binds to the 3' end of U6 snRNA, and is required for its stability (Achsel et al., 1999; Mayes et al., 1999; Pannone et al., 1998; Salgado-Garrido et al., 1999) and nuclear accumulation in yeast (Spiller et al., 2007). In addition, the Lsm2-8p complex facilitates incorporation of U6 snRNPs into U4/U6 di-snRNPs and U4/U6.U5 tri-snRNPs and has been proposed to have a chaperone-like function in remodelling RNP particles (Verdone et al., 2004). The nuclear Lsm proteins were found to contribute to other RNA processing events, including the processing of pre-tRNAs, pre-snoRNAs and pre-rRNAs and the degradation of pre-mRNAs (Kufel et al., 2003b; Kufel et al., 2004; Kufel et al., 2002; Kufel et al., 2003a; Watkins et al., 2004), reviewed (Beggs, 2005). Furthermore, various nuclear Lsm proteins have been shown to interact with the U8 snoRNA in *Xenopus laevis* (Tomasevic and Peculis, 2002) and the snR5 snoRNA in *S. cerevisiae* (Fernandez et al., 2004). As these RNAs are all nuclear and, with the exception of the U6 snRNA, their associations with the Lsm

proteins are highly transient, this suggests that at least some of the Lsm proteins enter the nucleus separately from their target RNAs.

The mechanism of nuclear import of the Lsm2 to Lsm8 proteins has not been systematically studied, although we recently showed that nuclear accumulation of Lsm8p requires Kap95p (Spiller et al., 2007). Production of recombinant human LSm proteins in bacteria, followed by their injection into HeLa cells, showed that pre-assembled LSm2-8 complex localized to the nucleus, whereas LSm8 injected by itself accumulated in the cytoplasm (Zaric et al., 2005). These results suggest that LSm8 nuclear import involves an unidentified nuclear import signal that is only present when LSm8 interacts with other LSm2-8 subunits. In the case of the Sm proteins, the adjacent subunits, SmB, SmD1 and SmD3, are predicted to form a basic protuberance that may act as a nuclear localization signal in the yeast and human Sm complexes (Bordonne, 2000; Girard et al., 2004). As the three paralogous yeast Lsm proteins, Lsm8p, Lsm2p and Lsm4p, also contain basic C-termini, they may form a nuclear localization signal in a similar fashion. Genetic evidence supports an interaction between these three components, as mutations in *LSM8* are suppressed by over-expression of *LSM2* or *LSM4* (Pannone et al., 2001).

Lsm1-7p differs from Lsm2-8p by just one polypeptide, but is located in the cytoplasm (He and Parker, 2000). In *S. cerevisiae*, Lsm1 to 7 proteins and a wide range of RNA decapping and decay factors have been shown to accumulate in cytoplasmic foci named processing or P-bodies, but only under certain conditions (Sheth and Parker, 2003; Teixeira et al., 2005). In log phase yeast cells, P-body components, including Lsm1-7p, are spread diffusely throughout the cytoplasm, but localize to foci that increase in number and size with increased cell density (Teixeira et al., 2005). In addition, P-body formation is increased by glucose deprivation, osmotic stress and ultra-violet radiation, suggesting that they form in response to stress. In these circumstances, the transcriptome of the cell changes to cope with the new conditions, and increased mRNA degradation occurs. P-bodies appear to be sites of RNA decapping and degradation (Sheth and Parker, 2003; Teixeira et al., 2005), and they are probably also sites of nonsense mediated decay and translational repression (Bruno and Wilkinson, 2006; Sheth and Parker, 2006; Teixeira et al., 2005). Studies of *lsm1* $\Delta$  yeast indicate that Lsm1p is needed for accumulation of the other Lsm proteins in P-bodies (Tharun et al., 2005), but is not required for P-body formation (Sheth and Parker, 2003).

The existence of two Lsm complexes that differ by just one member, but which have different, even apparently opposing functions (promoting RNA stability versus degradation), is remarkable, especially as the protein sequences of Lsm1p and Lsm8p are highly similar. The different functions of the complexes may, therefore, be determined by their localization, as was suggested by Tharun et al. (2005). It is not currently known what determines the cellular localization of Lsm complexes, although it has been suggested that both the nuclear and the P-body localizations require Lsm complex formation (Ingelfinger et al., 2002; Tharun et al., 2005; Zaric et al., 2005). In the absence of Lsm1p, nuclear levels of Lsm2p and Lsm7p were slightly elevated (Tharun et al., 2005), indicating that Lsm1p may compete with Lsm8p for complex formation.

Here, we show that sequences within Lsm8p as well as formation of a heteromeric complex are important for the nuclear accumulation of Lsm2-8p complex,. Furthermore, we have investigated competition between Lsm1p and Lsm8p by over-expression and depletion of these proteins. Imbalance between these two factors affects localization of other Lsm complex members and under some conditions even shows deleterious effects on cell viability. Under normal physiological conditions, competition between Lsm1p and Lsm8p may provide a link between RNA processing events in the nucleus and mRNA degradation in the cytoplasm. We therefore investigated effects of stress on nuclear localization of Lsm7 and Lsm8 proteins. We observe a small shift of Lsm7p to the cytoplasm upon glucose deprivation, and a rapid and almost complete depletion of Lsm7p and Lsm8p from the nucleus after hyperosmotic shock. This

effect occurs independent from Lsm1p, however, deletion of *LSM1* inhibits subsequent recovery of nuclear Lsm protein levels. Taken together, our findings suggest that co-regulation of cytoplasmic and nuclear RNA processing events could be mediated through changes in sub-cellular Lsm protein levels.

## Results

### Lsm localizations

Yeast strains were constructed that produce 13-myc tagged Lsm1p, Lsm7p or Lsm8p (Fig. 1A) and immuno-fluorescence microscopy results (Fig. 1B) clearly show specific localization patterns for the Lsm proteins: Lsm1p was predominantly cytoplasmic (note the “holes” in the immunofluorescence, highlighted by arrows, and the lack of co-localization of immunofluorescence with DAPI staining of nuclear DNA in the merged image), Lsm8p localized exclusively to the cell nucleus, and Lsm7p was present throughout the cells. These results are consistent with the proposed existence of two Lsm complexes, a nuclear Lsm2-8p complex and a cytoplasmic Lsm1-7p complex.

Lsm proteins are actively imported through the nuclear pore

To investigate the mechanism of nuclear accumulation of Lsm proteins, a *nup49-313* strain (a nuclear pore mutant that affects protein import) (Doye et al., 1994) was constructed that produces 13-myc tagged Lsm1p, Lsm7p or Lsm8p. As this mutation causes temperature-sensitive growth, the cultures were grown at 23°C and then shifted to 37°C (restrictive temperature) for 5 hours prior to processing for microscopic analysis. Following the shift to 37°C, Lsm7-13myc displayed a reduced signal in the nuclei of the *nup49-313* cells compared to wild-type cells (Fig. 2A). This effect was clearer for Lsm8-13myc, which was no longer restricted to the nucleus in the *nup49-313* cells at 37°C (Fig. 2B). To control for a non-specific effect of the *nup49-313* mutation on the integrity of the nuclear membrane, U1 snRNA localization was investigated and showed no difference between wild-type and *nup49-313* cells (Fig. 2C). In combination with our previous report (Spiller et al., 2007) that the nuclear accumulation of Lsm8p is importin  $\beta$ /Kap95p-dependent, these results suggest that, although individual Lsm proteins are small (10 to 21 kDa), their nuclear accumulation likely depends on active import through the nuclear pores. This is in agreement with the report that the human LSm2-8 complex is actively imported into rat fibroblast nuclei (Zaric et al., 2005). In contrast to the effect of the *nup49-313* mutation on Lsm protein localization, the temperature-sensitive *xpo1-1* nuclear export mutation showed no effect on the localization of Lsm1p, Lsm7p or Lsm8p at the restrictive temperature in otherwise wild-type cells (Fig. 2D). Thus, it seems that the cytoplasmic localization of Lsm1p is not a consequence of nuclear exclusion by continual active

Figure 1

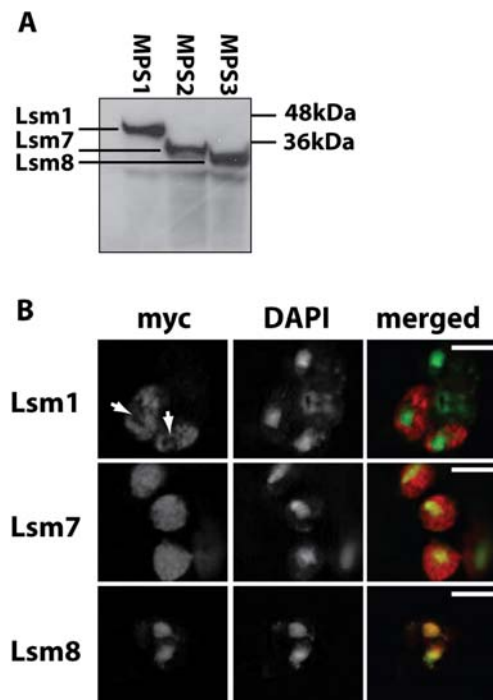


Fig. 1: Localization of Lsm1p, Lsm7p and Lsm8p. (A) Western blot showing 13-myc-tagged Lsm1p, Lsm7p and Lsm8p from yeast strains MPS1, MPS2 and MPS3 respectively. (B) Localization of Lsm1-13myc, Lsm7-13myc and Lsm8-13myc in strains MPS1, MPS2 and MPS3 respectively. Fixed cells were stained with anti-Myc antibodies followed by Cy3 conjugated secondary antibody (shown in red). Nuclei were stained with DAPI (shown in green). For clarity, single channel images of DAPI and of immunofluorescence are shown in grayscale. Arrows indicate “holes” in the immunofluorescence at the positions of nuclei. Scale bar, 10µm.

export from the nucleus, at least not through this export pathway. In contrast, the *xpo1-1* mutation did show a clear effect on the localization of a control protein (NLS-NES-GFP), both under permissive and restrictive conditions (Fig. S1 in supplementary material) (Stade et al., 1997))

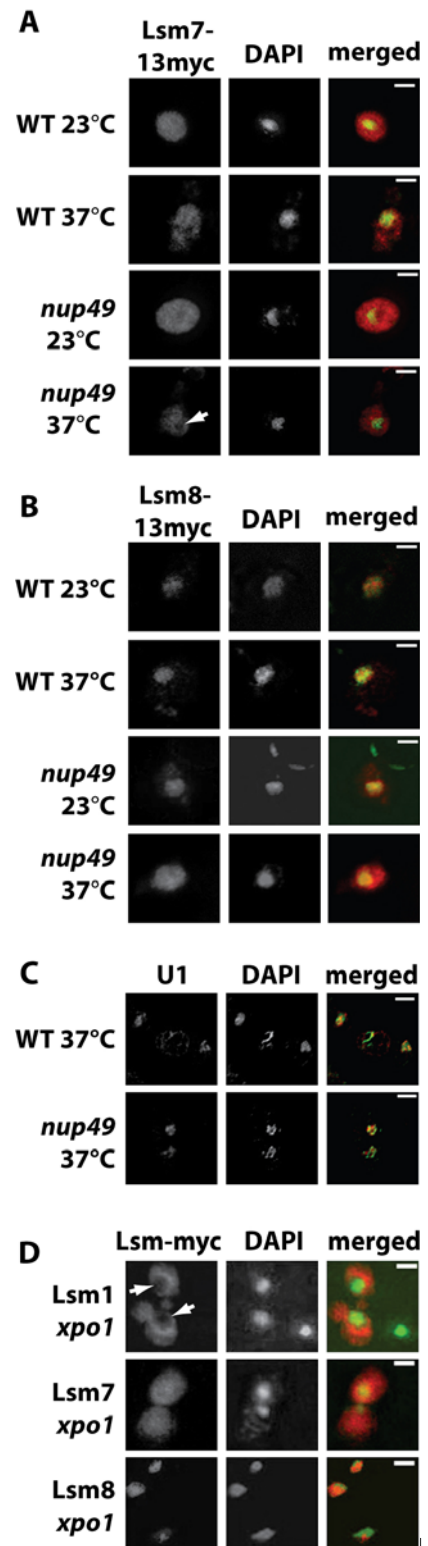
### Nuclear localization of Lsm7p requires other Lsm proteins

As the only member of the Lsm2-8p complex that is exclusively nuclear, Lsm8p may be required for nuclear uptake or retention of the Lsm2 to 7 proteins. To investigate this, yeast cells that produce 13-myc tagged Lsm7p and in which *LSM8* was under control of a galactose-inducible promoter ( $P_{GAL1}$ ) were grown to log phase in galactose, then switched to glucose medium for 12 hours (long enough to deplete Lsm8p but not long enough to significantly deplete U6 snRNA) (Mayes et al., 1999), and Lsm7p localization was examined. In cells depleted of Lsm8p, Lsm7p levels were decreased in the nuclei (Fig. 3A, fat arrow) and increased in cytoplasmic foci (probably P-bodies; thin arrows).

To test for the dependence of Lsm7p nuclear accumulation on the presence of other Lsm proteins, yeast strains were tested in which *LSM3*, *LSM4* or *LSM5* was expressed from the  $P_{GAL1}$  promoter (for unknown reasons it proved difficult or impossible to 13myc-tag Lsm7p in the  $P_{GAL1}$ -*LSM2* strain) and these were shifted from galactose to glucose medium for 12 hours to metabolically deplete these proteins (Mayes et al., 1999), while *lsm1* $\square$  and *lsm6* $\square$  cells, which are heat-sensitive, were grown in glucose at the permissive temperature of 30°C. The nuclear localization of Lsm7-13myc was unaffected by the absence of Lsm1p (Fig. 3B). Depletion of Lsm3p, Lsm4p, Lsm5p, or Lsm6p appeared to reduce the nuclear Lsm7p signal slightly, as seen by decreased co-localization of Lsm7p with the DAPI stain, with depletion of the essential Lsm4 protein having the strongest effect, as seen by the hole in the immunofluorescence (Fig. 3B, arrow). Thus, nuclear localization of Lsm7p requires the presence of Lsm8p and is more efficient when most or all of the other components of the Lsm2-8p complex are present, but it is not affected by the absence of cytoplasmic Lsm1p.

Fig. 2: Localization of the Lsm proteins in the *nup49-313* nuclear import mutant and the *xpo1-1* export mutant. Cultures were grown at 23°C to log phase and then at 37°C for a further four and a half hours. In each case, a representative cell is shown. (A) Localization of Lsm7-13myc in MPS2 (wild-type) and MPS12 (*nup49-313*) at 23°C and 37°C. (B) Localization of Lsm8-13myc in MPS3 (wild-type) and MPS13 (*nup49-313*) at 23°C and 37°C. (C) Fluorescent in situ hybridization showed that localization of U1 snRNA in *nup49-313* yeast at restrictive temperature was the same as in wild-type cells. (D) Localizations of Lsm1-13myc, Lsm7-13myc and Lsm8-13myc at 37°C in *xpo1-1* mutant strains MPS13, MPS14, MPS15 respectively were the same as for wild-type cells. Arrows indicate "holes" in the immunofluorescence at the positions of nuclei. Scale bar, 10µm.

Figure 2



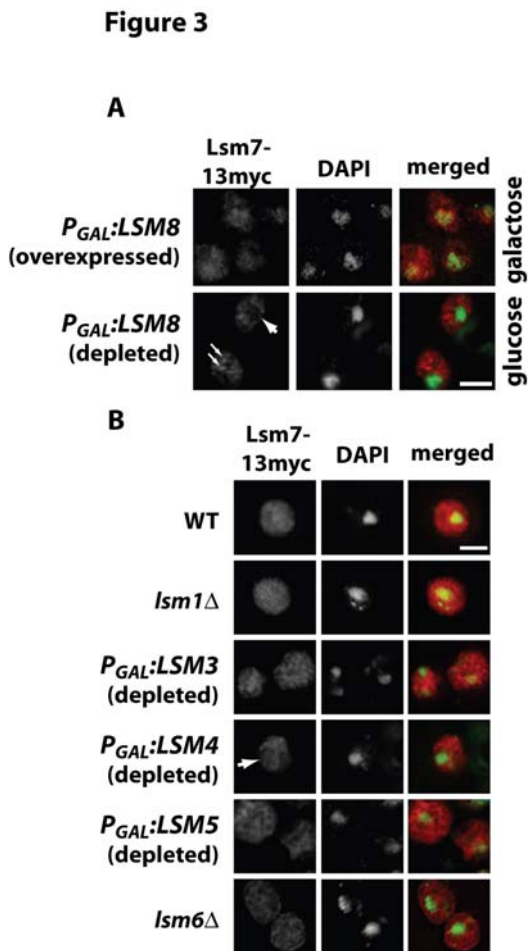


Fig. 3: Localization of Lsm7p upon depletion of other Lsm proteins.

(A) Localization of Lsm7p in the absence of Lsm8p, in strain MPS7. MPS7 (*P<sub>GAL</sub>-HA-LSM8*) cells were grown in galactose medium to mid-log phase and then shifted to glucose for 12 hours to deplete Lsm8p (confirmed by western blotting; data not shown). Nuclei depleted of Lsm7p are indicated by a fat arrow and cytoplasmic foci are indicated by thin arrows. (B) Localization of Lsm7p in strains lacking other Lsm proteins. *Ism1*Δ and *Ism6*Δ strains (MPS6 and MPS4) were grown at permissive temperature in glucose, while MPS8, MPS9 and MPS10 were depleted of Lsm3p, Lsm4p and Lsm5p respectively by growth in glucose for 12 hours. Representative cells are shown. Scale bar, 10μm.

**Figure 4**

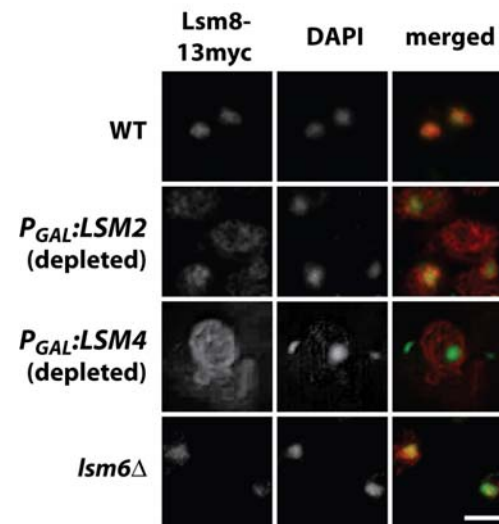


Fig. 4: Localization of Lsm8p in the absence of Lsm2p, Lsm4p or Lsm6p.

Localization of 13-myc-tagged Lsm8p (shown in red) in wild-type (MPS3) yeast, or after depleting Lsm2p or Lsm4p by growing MPS22 or MPS25 cells in glucose for 12 hours, or in strain MPS5 (which has the *LSM6* gene knocked out) grown at permissive temperature. Scale bar, 10μm.

#### Depletion of Lsm2p or Lsm4p disrupts Lsm8p localization

As Lsm8p is normally nuclear, a defect in its localization is more readily detected. Therefore, to confirm that complex formation is required for Lsm2-8p nuclear localization, Lsm8p localization was investigated in strains in which Lsm2p and Lsm4p, its proposed neighbors in the Lsm2-8p ring, were depleted. This assignment is based on the organization of the Sm protein complex, in which SmB (closest in structure to Lsm8p) is flanked by SmD1 and SmD3 (most similar to Lsm2p and Lsm4p respectively) (Kambach et al., 1999), and on genetic and yeast two hybrid interactions between *LSM2*, *LSM4* and *LSM8*

(Lehner and Sanderson, 2004; Pannone et al., 2001). 13-myc-tagged Lsm8p localization was examined in wild-type, *P<sub>GAL</sub>-LSM2*, *P<sub>GAL</sub>-LSM4* and *Ism6*Δ cells (Fig. 4). Following depletion of Lsm2p or Lsm4p by growth in glucose medium for 12 hours (Mayes et al., 1999), there was still some nuclear signal for Lsm8p, but there was a dramatic increase in the level of signal in the cytoplasm. In contrast, absence of Lsm6p, caused no visible change in Lsm8p localization. Overall, these results demonstrate that Lsm8p by itself does not accumulate in the nucleus, since loss of either of its proposed partners in the Lsm2-8p ring results in its delocalization. However, it seems that a complete Lsm2-8p complex is not essential as lack of Lsm6p has no effect, which might be expected as Lsm6p is a non-essential protein.

### Lsm7p and Lsm8p delocalization is not caused by defective splicing

Depletion of Lsm2 to 8 proteins leads to decreased levels of U6 snRNA and an accumulation of pre-mRNA (Mayes et al., 1999). To investigate whether delocalization of Lsm7p and Lsm8p from the nucleus upon depletion of other Lsm proteins may be due to an indirect effect on pre-mRNA splicing, cells were depleted of SmD1 and localization of myc-tagged Lsm7p and Lsm8p was investigated (Fig. S2A). Depletion of SmD1 had no effect on nuclear localization of either of these Lsm proteins, whereas Northern analysis showed decreased levels of U1 snRNA and an accumulation of pre-U3 RNA (Fig. S2B).

**Figure 5**

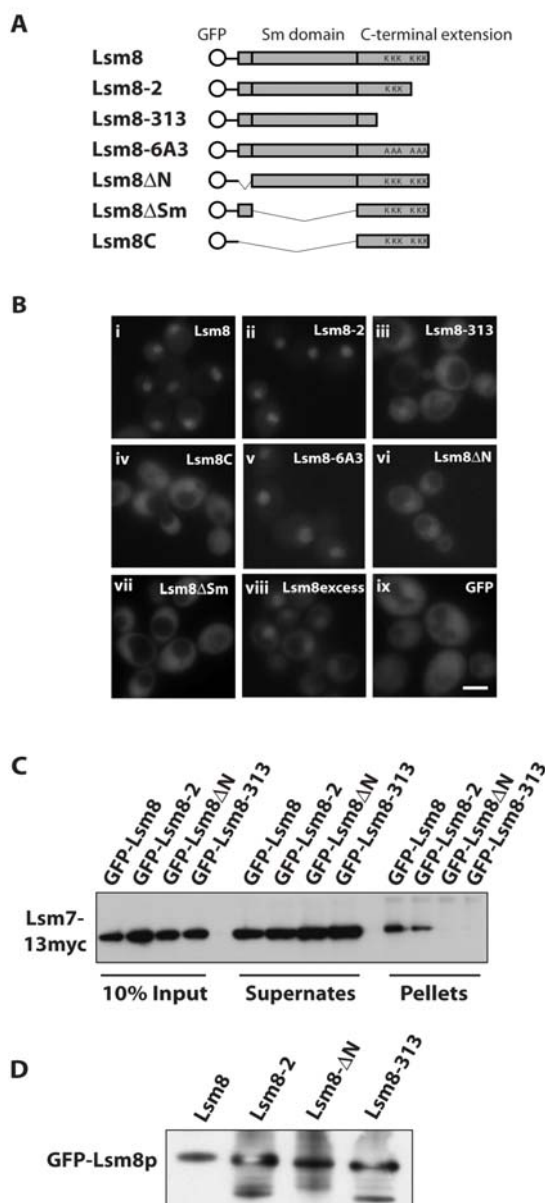


Fig. 5: Lsm8p nuclear localization is affected by C-terminal, N-terminal and Sm-site deletions

(A) Cartoon showing the structures of the Lsm8 deletion constructs that were tested as GFP-fusion proteins. (B) Localization of GFP-tagged Lsm8p constructs in yeast strain MPS26 plus (i) pMPS8 (GFP-Lsm8), (ii) pMPS8-82 (GFP-Lsm8-2), (iii) pMPS8-313 (GFP-Lsm8-313), (iv) pMPS8C (GFP-Lsm8Cterm), (v) pMPS8-6A3 (GFP-Lsm8-6A3), (vi) pMPS8-□N (GFP-Lsm8□N), (vii) pMPS8-□SM (GFP-Lsm8□Sm), or (viii) in BMA38a plus pMPS8 (i.e. GFP-Lsm8 produced in the presence of endogenous Lsm8p) or, (viii) pGFP-N-FUS (GFP). In all cases, GFP localization was examined in live cells after growth in SD-Ura-Met (to induce expression of the GFP construct). Scale bar, 10μm. (C) Co-immunoprecipitation of myc-tagged Lsm7p with GFP-Lsm8p. Lsm8p-containing complexes were precipitated with anti-GFP antibodies, and Lsm7p was detected with anti-myc antibodies. (D) All the GFP-Lsm8 constructs were stably expressed, as shown by western blotting (and data not shown) using anti-GFP antibodies.

### Lsm8p truncations affect its nuclear localization

As Lsm8p is important for nuclear accumulation of other Lsm proteins, we performed deletion analysis on N-terminally GFP-tagged Lsm8 protein to investigate sequences in Lsm8p that might be responsible for its nuclear localization. Plasmids encoding the GFP-Lsm8 variants (Fig. 5A) were tested for their ability to support growth of strain MPS11 ( $P_{GAL1}$ -LSM8) on glucose (Table 1), and for localization (Fig.5B). SmB, the member of the Sm protein family that is structurally most similar to Lsm8p, contains a functional nuclear localization signal in its C-terminus (Bordonne, 2000). The C-termini of both proteins are basic in nature, containing a high number of lysine residues which, in SmB as well as SmD1 and D3, are part of a nuclear localization signal (NLS)-like motif (Bordonne, 2000). The effect of C-terminal truncations of Lsm8p on its nuclear localization was therefore tested. GFP-Lsm8-2, which lacks the

extreme C-terminal 10 amino acids, (analogous to the *lsm8-2* mutation) (Pannone et al., 1998), showed normal nuclear accumulation (Fig. 5B (i and ii)), whereas Lsm8-313p, which lacks a further 14 residues at the C-terminus, was mislocalized (Fig. 5B (iii)). However, the Lsm8 C-terminus (aa65-109) alone, when fused to GFP failed to accumulate in the nucleus (Lsm8C; Fig. 5B (iv)). Thus, unlike the C-terminus of SmBp (Bordonne, 2000), the C-terminus of Lsm8p, although apparently required for nuclear localization, is not by itself sufficient to determine nuclear localization. Although the region of Lsm8p that is missing in Lsm8-313p contains 6 lysine residues, simultaneous mutagenesis of all 6 lysines to alanine (Lsm8-6A3; Fig. 5B (v)) did not significantly affect nuclear accumulation of GFP-Lsm8p supporting the conclusion that the C-terminus of Lsm8p does not behave like a classical NLS, nor a functional NLS-like motif. Truncation of 11 amino acids at the N-terminus, (Lsm8 $\square$ N; Fig. 5B (vi)), or deletion of residues 52 to 64, corresponding to the Sm2 motif which is required for Lsm complex formation (Lsm8 $\square$ Sm; Fig. 5B (vii)) caused these mutant proteins to localize throughout the cell, similar to GFP alone (Fig. 5B (ix)). This latter result emphasizes the importance of complex formation for nuclear localization of Lsm8p, as the Sm motif is critical for inter-subunit interactions within the Lsm complexes.

To determine the effects of some of these Lsm8 mutations on complex formation, tagged proteins were precipitated with anti-GFP antibodies and co-precipitation of 13myc-Lsm7 (as a representative of the other members of the Lsm2-8p complex) was analyzed by western blotting. Lsm7-13myc was co-precipitated with GFP-Lsm8-2p, albeit less efficiently than with full-length GFP-Lsm8p, whereas GFP-Lsm8 $\square$ N and GFP-Lsm8-313 did not pull down any Lsm7p (Fig. 5C), although the GFP-Lsm8 proteins were present in similar amounts in the respective extracts (Fig. 5D). Also, over-expression of the Lsm8 $\square$ N and Lsm8-313 proteins (which do not by themselves support growth; Table 1) did not affect growth of cells containing wild-type Lsm8p (data not shown), compatible with these mutant proteins being incapable of competing with wild-type Lsm8p for assembly into Lsm2-8p complexes. Thus, the nuclear accumulation of the GFP-Lsm8 (mutant) proteins correlated with their ability to associate with Lsm7p which likely reflects their incorporation into a complete Lsm2-8p complex. Furthermore, in the presence of chromosomally encoded wild-type Lsm8p, plasmid-encoded GFP-Lsm8p shows weaker nuclear accumulation (Fig. 5B (viii)). This is likely due to the Lsm8 protein being in excess over other members of the Lsm2-8p complex, and indicates that GFP-Lsm8p by itself does not accumulate in the nucleus. Taken together, these results strongly support the conclusion that the Lsm8 protein needs to interact with other Lsm proteins for its nuclear accumulation.

**Figure 6**

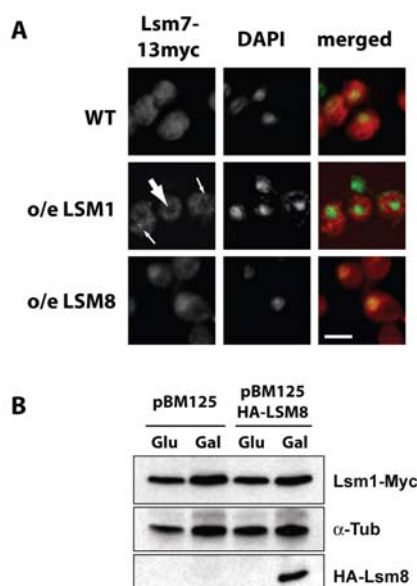


Fig. 6: Over-production of Lsm1p or Lsm8p has opposing effects on Lsm7p localization.

(A) HA-tagged Lsm1p or Lsm8p was over-produced from plasmid pAEM80 or pAEM76 respectively in strain MPS2 (Lsm7-13myc) grown in SDGal-Ura. Lsm7-13myc localization (red) in WT (MPS2 carrying pBM125 empty vector), or with *LSM1* (*o/e LSM1*) or *LSM8* (*o/e LSM8*) over-expression. Over-production of Lsm1p caused reduction of nuclear Lsm7p (fat arrow) and its accumulation in foci (thin arrows). (B) Lsm8p over-production does not affect Lsm1p levels. MPS1 was grown with pBM125 or pBM125-HA-LSM8 in SD-Ura (Glu) or SDGal-Ura (Gal). Total protein was separated by SDS-PAGE and the western blot was probed for Lsm1-13myc, HA-Lsm8 and  $\alpha$ -Tubulin.

Over-production of Lsm1p or Lsm8p has opposing effects on Lsm7p localization

To investigate the effect of overproducing Lsm1p or Lsm8p, HA-tagged *LSM1* or *LSM8* was over-expressed from the *P<sub>GAL1</sub>* promoter in yeast cells in which *LSM7* was myc-tagged. Over-production of Lsm8p (confirmed by western blot; data not shown) caused an increase in the nuclear fraction of Lsm7p compared with control cells (Fig. 6A and 3A), whereas over-production of Lsm1p had the opposite effect, namely a reduced level of nuclear Lsm7p and accumulation of Lsm7p in cytoplasmic foci (Fig. 6A), similar to depletion of Lsm8p (Fig. 3A). The level of Lsm1p does not change with *LSM8* over-expression compared to no over-expression as shown by western blot analysis (Fig. 6B).

**Figure 7**

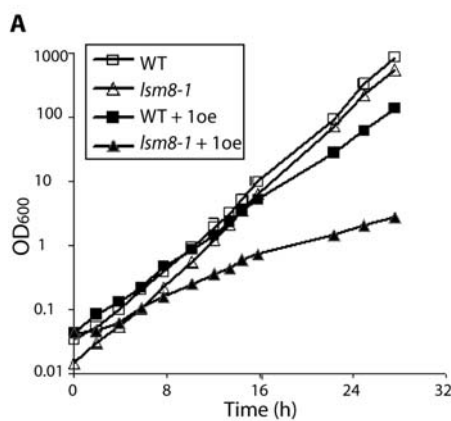
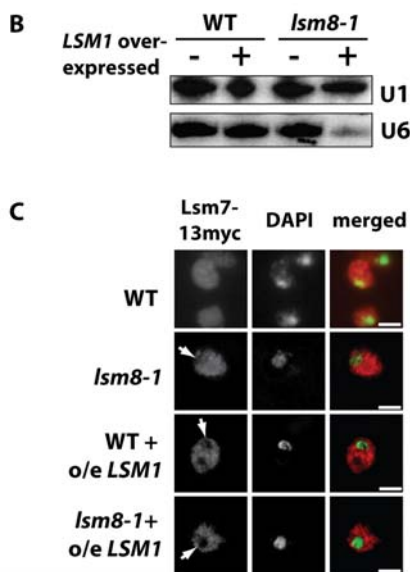


Fig. 7: Over-expression of *LSM1* in an *Ism8-1* background results in a severe slow-growth phenotype

(A) Growth curve of wild-type (MPS2) and *Ism8-1* (MPS17) strains with or without over-production of Lsm1p (1oe). Wild-type yeast with Lsm7p myc-tagged (MPS2), or *Ism8-1* yeast with Lsm7p myc-tagged (MPS17) were transformed with either pBM125 (empty vector), or pAEM80 (*P<sub>GAL1</sub>-HA-LSM1*). All strains were grown in galactose-based media. (B) Northern blot showing the levels of U1 and U6 snRNAs in wild-type or *Ism8-1* cells combined with over-production of Lsm1p. (C) Localization of Lsm7-13myc in wild-type and *Ism8-1* strains, with or without over-production of Lsm1p. All strains were grown in galactose, and the localization of Lsm7p was detected with anti-Myc antibodies. Representative cells are shown. Arrows indicate "holes" in the immunofluorescence at the positions of nuclei. Scale bar, 10µm.



Although Lsm8p is essential for viability, the *Ism8-1* mutation, which causes dramatically decreased levels of Lsm8p, is not lethal, resulting only in a weak growth phenotype (Pannone et al., 1998). Intriguingly, Pannone et al. (1998) showed that the *Ism8-1* mutation is synthetic lethal with deletion of *LHP1* (which encodes the yeast homolog of La, another U6 RNA binding protein), and that the requirement for Lhp1p in an *Ism8-1* strain can be suppressed by low-copy over-expression of *LSM2* (Pannone et al., 2001). In contrast to the effect of *LSM2* over-expression, we found that over-expression of *LSM1* in *Ism8-1* cells (strain MPS17) resulted in a severe slow-growth phenotype, but had only a minor effect with wild-type cells (Fig. 7A). These results further indicate that Lsm1p and Lsm8p act in an antagonistic fashion. As the Lsm2-8p complex is required for stability of the U6 snRNA, the level of U6 was examined in these yeast strains. With *LSM1* over-expressed in the *Ism8-1* strain the U6 RNA

level was reduced to only 10% of the wild-type level (normalized to the level of U1 snRNA; Fig. 7B), indicating that a high level of Lsm1p interferes directly or indirectly with the function of Lsm8p (in this case Lsm8-1p). Lsm7p localization was examined in the *Ism8-1/LSM1* over-expression strain and was found to be almost entirely cytoplasmic, much of it in foci, and it was undetectable in the nucleus (Fig. 7C). Thus, the effect on the level of U6 snRNA of over-producing Lsm1p was most likely a consequence of reduced nuclear accumulation of the Lsm2-8p complex.

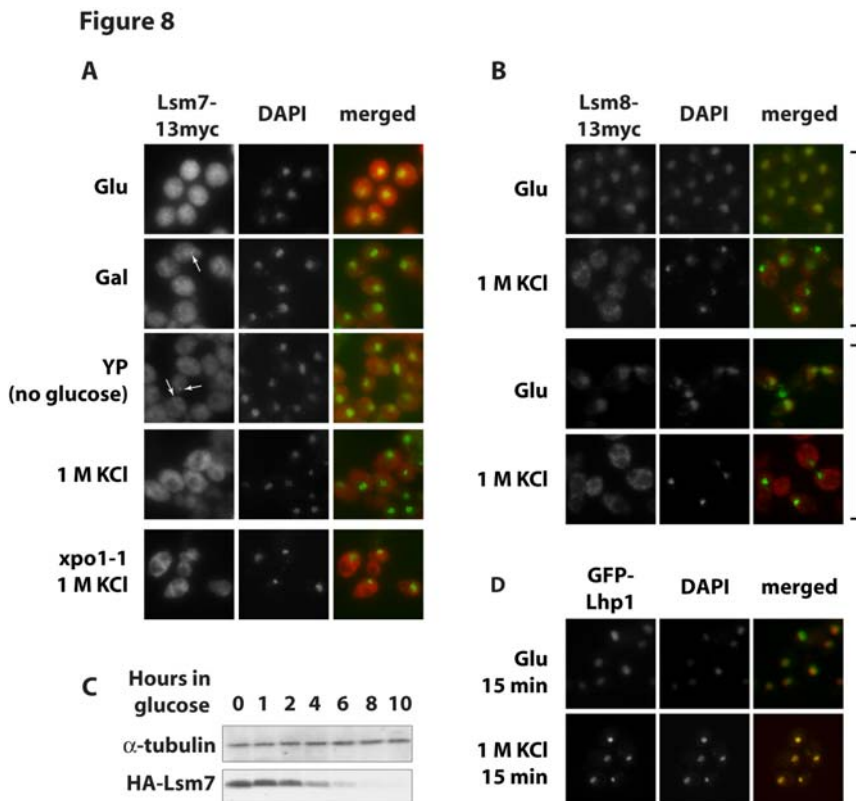


Fig. 8: Lsm7p and Lsm8p de-localize after hyperosmotic shock, but not after glucose deprivation. MRY74 (*LSM7-13myc*), MPS3 (*LSM8-13myc*), MPS15 (*xpo1-1, LSM7-13myc*) and MPS16 (*xpo1-1 LSM8-13myc*) cells were grown at 30°C in YPDA to log phase and shifted to medium containing galactose (Gal), no glucose (YP) or 1 M KCl. Cells were fixed after 15 minutes of incubation at room temperature and examined by immunofluorescence (A) Localization of Lsm7-13myc (red); P-bodies indicated by arrows (B) Localization of Lsm8-13myc (red) (C) To measure the decay rate of Lsm7p, extract from AEMY35 (*P<sub>GAL1</sub>-HA-LSM7*) cells harvested at 1, 2, 4, 6, 8, and 10 hours after shifting from galactose to glucose medium was tested by western analysis with anti-HA antibodies. (D) BMA38a with pMPS2 (GFP-Lhp1) was grown in SD-Ura-Met to log phase and shifted to 1 M KCl. Cells were fixed before and after 15 min in 1 M KCl. GFP-Lhp1 is shown in green, DAPI in red.

### Lsm7p and Lsm8p are de-localized upon hyperosmotic shock

The existence of an apparent equilibrium between the Lsm1-7p and Lsm2-8p complexes raises the possibility that this balance may be altered in response to changing physiological conditions. Therefore, Lsm7-13myc localization was examined after glucose deprivation (galactose or YP medium) or osmotic shock (exposure to 1 M KCl). As expected, Lsm7p accumulated in P-bodies 5 to 15 minutes after glucose deprivation or hyperosmotic shock (Fig. 8A). There was also an apparent reduction in nuclear Lsm7p that was most striking following hyperosmotic shock. Hyperosmotic shock also led to quick and almost complete depletion of Lsm8p from the nucleus (Fig. 8B). To determine whether these effects were due to active export of Lsm proteins from the nucleus, their localization was examined after hyperosmotic shock in the temperature-sensitive *xpo1-1* strain (Fig. 8A,B). The *xpo1-1* strains showed an identical shift of Lsm7-13myc and Lsm8-13myc to the cytoplasm, indicating that these proteins are not actively exported by Xpo1p after osmotic shock. As the half-life of Lsm7p is more than 2 hours (Fig. 8C; tested in a *P<sub>GAL1</sub>-LSM7* strain, AEMY35) (Mayes et al., 1999), the reduced nuclear Lsm7p signal within 15 minutes of stress most likely represents movement of Lsm7p from the nucleus to the cytoplasm rather than failure of newly synthesized Lsm7p to enter the nucleus.

To rule out a non-specific effect of osmotic stress on nuclear protein localization, GFP-Lhp1 localization was examined before and after exposure to 1 M KCl (Fig. 8D). GFP-Lhp1 localizes exclusively to the nucleus, and concentrates in the nucleolus in some cells. No increase in cytoplasmic localization was detected 15 minutes after exposure to 1 M KCl. It did appear to localize to a smaller, more compact area, just like the DAPI-stained nuclear DNA. This is presumably due to an effect of osmotic stress on nuclear morphology and recovers after longer periods of time. In contrast, Lsm8-GFP showed de-localization similar to Lsm8-13myc,

although somewhat less severe; interestingly, some cells showed accumulation of Lsm8-GFP at the nuclear periphery (Fig. S3 in supplementary material).

**Lsm de-localization does not depend on Lsm1p, whereas re-localization does**

De-localization of Lsm7 and Lsm8 proteins is reversible as nuclear levels return to normal after longer periods of incubation (Fig. 9A; Fig. S3 in supplementary material and data not shown). Recovery was more complete for lower salt concentrations and started around 30 minutes after first exposure (Fig. 9A). Exposure to 1 M sorbitol showed a similar effect on Lsm8 and Lsm7-13myc localization (data not shown). Other effects of hyperosmotic stress, including those on translation initiation, have previously been shown to occur within a similar time-frame and recovery was shown to depend on the Hog1p protein kinase (Uesono and Toh, 2002). We therefore investigated Lsm8 and Lsm7-13myc localization after hyperosmotic shock in *hog1Δ* strains (Fig. S4 in supplementary material and data not shown). Both de-localization and recovery were identical to that seen for the isogenic *HOG1* strains, indicating that these effects are independent of this particular protein kinase. As P-body localization of Lsm1-7p depends on Lsm1p (Tharun et al., 2005) and osmotic shock leads to accumulation of Lsm proteins in these cytoplasmic foci, we investigated whether nuclear de-localization of Lsm proteins is dependent on Lsm1p (Fig. 9B). Surprisingly, nuclear localization of Lsm8-13myc was slightly reduced in an *lsm1Δ* strain under normal growth conditions. This effect was even more apparent for GFP-Lsm8 expressed in the same strain background (data not shown). De-localization of Lsm8-13myc from the nucleus was even stronger after hyperosmotic shock, and failed to recover after up to 2 hours in YPDA with 0.6 M NaCl, whereas some (incomplete) recovery occurred when cells were shifted back to YPDA. These results may indicate that in the *lsm1Δ* strain Lsm8p may move to the cytoplasm to replace Lsm1p.

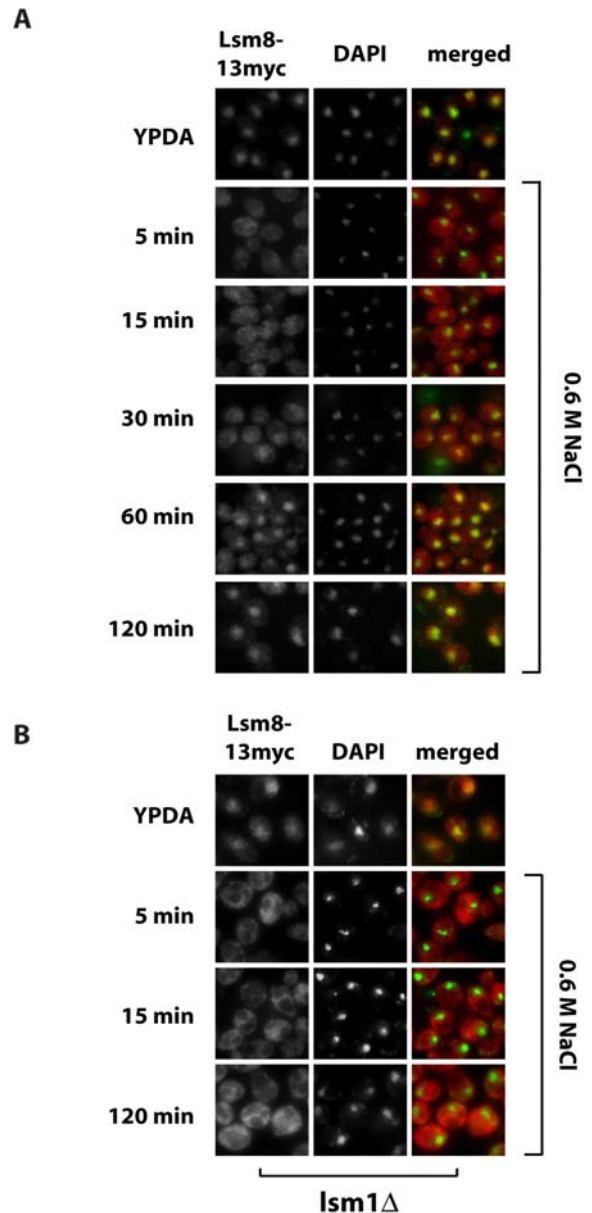


Fig. 9: Lsm protein delocalization upon osmotic shock is reversible and Lsm1p independent. (A) MPS3 (Lsm8-13myc) cells grown in YPDA were fixed before and 5, 15, 30, 60 or 120 minutes after shifting to YPDA with 0.6 M NaCl. Lsm8-13myc is shown in red, DAPI in green. (B) MRY83 (*lsm1Δ LSM8-13myc*) cells grown in YPDA were fixed before and 5, 15 or 120 minutes after shifting to YPDA with 0.6 M NaCl. Lsm8-13myc is shown in red, DAPI in green.

## Discussion

In this work, factors that affect the localization of the Lsm7 and Lsm8 proteins were investigated in order to try to understand what determines the nuclear localization of the Lsm2-8p complex. The results show that Lsm8p is a determinant for the nuclear accumulation of Lsm7p, although they do not clearly distinguish between nuclear import and nuclear retention. However, considering the effect of the *nup49-313* nuclear pore mutation as well the previously demonstrated dependence of Lsm8p nuclear accumulation on importin  $\beta$  (Spiller et al., 2007), Lsm8p may function as a nuclear import determinant for the rest of the Lsm2-8p complex. The deletion studies show that most of the Lsm8p sequence seems to be essential, including the N-terminus, the Sm domain and the C-terminus. In particular, a deletion of part of the Sm domain caused a dramatic mislocalization of Lsm8p, strongly suggesting the importance of interaction with other Lsm proteins for nuclear accumulation of Lsm8p. Unexpectedly, a relatively small N-terminal deletion outside the Sm core motif also affected complex formation. In principal, this could be due to a direct or indirect effect on complex formation or complex stability..

These results strongly suggest that Lsm8p must interact with other Lsm proteins to form a nuclear localization signal that is unlikely to be of the basic SV40 type of NLS. Whereas depletion of Lsm2p and Lsm4p, the presumed neighbors of Lsm8p in the Lsm2-8p complex resulted in delocalization of Lsm8p, lack of the non-essential Lsm6p had no apparent effect. In contrast, the complete Lsm2-8p complex is required for nuclear accumulation of U6 snRNA (Spiller et al., 2007). Lsm6p and Lsm7p are non-essential, thus, in the absence of Lsm6p or Lsm7p, alternative Lsm complexes must form in which there are fewer subunits or in which Lsm6p and Lsm7p are replaced by other proteins (Verdone et al., 2004). However, as U6 snRNA is unstable and mislocalized in *lsm6 $\Delta$*  and *lsm7 $\Delta$*  strains, these alternative complexes are probably less stably associated with U6 snRNA. Thus, nuclear retention of U6 snRNA requires its stable association with a complete Lsm2-8p complex. The normal occurrence of nuclear Lsm complexes that have different subunit compositions and that associate with other RNAs is possible. For example, the processing of pre-tRNAs, pre-snoRNAs and pre-rRNAs is unaffected by lack of Lsm6p or Lsm7p, but depends on Lsm2, 3, 4, 5 and 8 (Kufel et al., 2003b; Kufel et al., 2002; Kufel et al., 2003a). The proposed existence of an Lsm2-7p complex that associates with snR5 in the nucleolus (Fernandez et al., 2004) is in apparent contradiction with our finding of Lsm8p requirement for nuclear localization. It seems possible that the full Lsm2-8p complex may interact with snR5, but that the Lsm8p epitope tag may be masked in the snR5 RNP.

Our results are compatible with the observation of Zaric et al. (2005) that an RNA-free LSm2-8 complex which had been pre-assembled from recombinant human Lsm proteins and injected into the cytoplasm of rat fibroblast cells accumulated in nuclei, whereas LSm8p alone did not. Thus, the Lsm2-8p assembly pathway may be conserved throughout eukaryotes.

As all the Lsm proteins are small enough to diffuse into the nucleus there may be a mechanism to retain Lsm1-7p complex in the cytoplasm. In this scenario, if Lsm1p is present in excess, Lsm2 to 7 proteins will be cytoplasmic unless incorporated into Lsm2-8p complex. This may explain why depletion of Lsm8p had the most dramatic effect on nuclear localization of Lsm7p, as lack of any of the other Lsm proteins likely affects formation of the cytoplasmic Lsm1-7p complex as well, thereby allowing some diffusion of free Lsm7p into the nucleus. This can also explain the strong antagonistic effect of over-producing either Lsm1p or Lsm8p, if each is competing for the same pool of cytoplasmic Lsm proteins rather than interacting with the available pools of cytoplasmic or nuclear Lsm proteins respectively.

Although no increased nuclear accumulation of Lsm7p was observed in an *lsm1* deletion strain, nuclear accumulation of Lsm7p may be limited by the level of Lsm8p. One of the major functions of Lsm2-8p is in U6 snRNP biogenesis and stability, and repression of *LSM8* transcription stops cell growth (Mayes et al., 1999). However, the *lsm8-1* mutant strain, which

has a low level of Lsm8 protein, has only a moderately-reduced U6 snRNA level and no growth defect at 30°C, suggesting that the yeast cell requires only a low level of Lsm8p for survival. This could explain why over-expression of Lsm1p does not cause a significant growth defect despite the effect on Lsm7p localization. The fact that it requires both mutation of *LSM8* and over-expression of *LSM1* to see a severe effect on cell growth indicates that the yeast cell can absorb large reductions in Lsm2-8p complex without significant deleterious effects. Thus the ability to survive severe alterations in the levels of Lsm complexes in these mutant strains suggests that wild-type cells may significantly alter the equilibrium in response to various stimuli and stresses without detrimental effects.

As shown here, causing stress by hyperosmotic shock, or to a lesser extent by glucose deprivation, leads to a rapid shift of Lsm proteins from the nucleus to the cytoplasm and localization of Lsm1-7p to P-bodies. However, this delocalization depends neither on P-body formation (no effect of *hog1Δ*), nor on the presence or action of Lsm1-7p in these foci (no effect of *lsm1Δ*). In contrast, Lsm1p appears to be essential for re-localization when cells recover from hyperosmotic shock, indicating that an intact Lsm1-7p complex is required. The activity of Lsm1-7p in RNA turnover may be required for cells to efficiently re-program to cope with a sudden change in conditions.

As the Lsm1 and Lsm8 proteins are rather similar in sequence, it is remarkable that they effect the different localizations of their constituent complexes. A more extensive analysis of the mechanism of Lsm complex localization will require a detailed examination of the roles of individual amino acids in these and other Lsm proteins, and is a focus of our ongoing investigations. In addition, there is the interesting question of why the family of Lsm proteins has evolved to form two competing complexes. The multiple functions of the Sm-like proteins and their conservation throughout all organisms suggests that they evolved as general RNA/RNP chaperones, and that gene amplification produced the many Sm and Lsm proteins now found in eukaryotes, with their more specialized functions possibly being determined by their localization in the cell. Interestingly, no *LSM1* gene has been identified in the genomes of *Trypanosoma brucei* or *T. cruzi*, and RNA silencing experiments in *T. brucei* indicated that Lsm8p functions in both U6 stabilization and mRNA decay, suggesting that a single Lsm protein performs the roles of Lsm1p and Lsm8p (Liu et al., 2004). Thus, it seems likely that Lsm1p and Lsm8p have evolved from a common ancestral protein by gene duplication, and developed separate intracellular locations and functions. Having two or more Lsm complexes, with overlapping composition, involved in different metabolic processes, such as pre-mRNA splicing and degradation in the nucleus and mRNA degradation in the cytoplasm allows scope for a regulatory link between these processes. For example, by transiently varying the relative levels of each Lsm complex, nuclear RNA degradation could be reduced while cytoplasmic RNA turnover is enhanced. This would allow a quick and efficient change in genome expression in response to a sudden change in environmental conditions. Our over-expression experiments show that such effects can be artificially created, and similar effects in cells under stress show that cells are able to respond by changing sub-cellular localizations of the Lsm1-7p and Lsm2-8p complexes, thus allowing co-regulation of nuclear and cytoplasmic RNA processing events.

## Materials and methods

### Yeast media, plasmids and strains

Yeast media and manipulations were as described (Sherman, 1991); YPGalA is YPDA with 2% glucose replaced by 2% galactose. For over-expression in yeast and for GFP fusions, complete synthetic drop-out medium (SD) was used. A list of plasmids used is given in Table S1 in supplementary material, and yeast strains are described in Table S2 in supplementary material.

### Construction of epitope-tagged proteins

Thirteen copies of coding sequence for the c-myc epitope were integrated at the 3' end of the *LSM* genes by homologous recombination in BMA38a or a mutant derivative, using PCR product amplified from

pFA6a-13Myc-kanMX6 (Longtine et al., 1998) or pFA6a-13Myc-HphMX6 (derived from pFA6a-13Myc-kanMX6, this laboratory). Each PCR-amplified DNA contained the last 45 base pairs of *Lsm* coding sequence, thirteen copies of the *c-myc* epitope, hygromycin or kanamycin resistance sequence, and 45 base pairs of downstream sequence. All wild-type tagged strains exhibited wild-type growth at 14, 23, 30, and 37°C.

#### GFP-tagged constructs.

GFP-tagged genes were constructed by PCR amplification of the relevant part of *LSM8*, using primers containing XbaI and ClaI sites at their 5' ends. The PCR products were cut with XbaI and ClaI and ligated into pGFP-N-FUS or pGFP-C-FUS (Niedenthal et al., 1996). Site-directed mutagenesis was performed (Costa et al., 1996) to delete the part of the *LSM8* gene encoding the N-terminal Sm motif. Details of the GFP-*Lsm8p* fusions are given in Table 1.

#### Immuno-fluorescence and fluorescent in situ hybridization

For immuno-fluorescence, yeast cells grown to logarithmic phase in liquid medium were fixed for 40 minutes in 3.7% formaldehyde and spheroplasted for 30 minutes with Lyticase. Cells were incubated with anti-myc antibody (Abcam) overnight in 5% milk, and then for two hours with Cy3-conjugated anti-mouse antibody (Jackson ImmunoResearch). Mounting media containing DAPI (Vectashield) was added and cells were viewed using a Leica FW400 microscope.

For in situ hybridization, yeast cells in logarithmic growth phase in liquid culture were fixed, probed with fluorescently labeled oligodeoxynucleotides, stained with DAPI, and imaged as described previously (Long et al., 1995; Samarsky et al., 1998).

Imaging was performed essentially as described (Carter et al., 1993; Samarsky et al., 1998) and images were captured using LeicaFW4000 software (Scanalytics, Fairfax, VA) with a CH-250 16-bit, cooled CCD camera (Photometrics, Tucson, AZ) mounted on a Leica FW4000 fluorescence microscope (Leica). When images were restored, a three-dimensional data set, composed of 20 images separated by 200 nm in the axial direction, was acquired and deconvolved with an acquired point spread function using Leica Deblur software (Leica). Oligonucleotide probes for the U1 snRNA were labeled with a single fluorescein attached to the 5' end and had the sequences

5'-ACACCAATTTGAATTTGGGTGTCAAACTTCTCCAGGCAGAAGAAACAAAGGGCCCCAAAAATCAGTTTAA and 5'-AATCTCCGTCAAAACTAAAATGGCAGCTAGAGAAAAGTAGTCAAAAAGAATGCCTCTACAAAG.

#### Yeast extracts, immunoprecipitation and western blotting

Whole cell yeast extracts were prepared as described (Lin et al., 1985) from strain MPS11 carrying plasmid pMPS8, pMPS8-82, pMPS8-313 or pMPS8-□N, after growth in SD-Ura-Met plus glucose for 12 hours. Immunoprecipitations were performed as described (Teigelkamp et al., 1995) using anti-GFP antibodies (Invitrogen). Pellets were resuspended in 20 µL loading buffer and run on a denaturing 4-12% SDS-PAGE gel, alongside 10% of the supernates. Proteins were transferred to a nitrocellulose membrane and detected with HRP-conjugated mouse anti-Myc or anti-HA (Santa Cruz Biotechnology), rabbit anti-GFP (Invitrogen) or anti-α-tubulin antibodies (Sigma).

#### RNA extraction and northern blotting

Total RNA was extracted (Schmitt et al., 1990) from BMA38a and *lsm8-1* cells carrying either pAEM80 or an empty vector, which had been grown in SDGal-Ura medium to OD<sub>600</sub> 0.5. Purified RNA was fractionated in an 8% (15:1) denaturing acrylamide urea gel. After transfer to nitrocellulose, the membrane was probed with labeled DNA complementary to the U1 and U6 snRNAs (Cooper et al., 1995). Total RNA was similarly extracted from MPS7, MRY76 and MRY77 cells before and after growth in YPDA. The same probes for U1 and U6 snRNAs were used, as well as probes against U3A (GGTTATGGGACTCATCA) and scR1 (ATCCCGGCCGCTCCATCAC)

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**Table 1 Effects of various mutations on Lsm8p localization and function**

Construct	Lsm8p residues	Localization	Supports viability
GFP alone	-	Non-specific	No
GFP-Lsm8	1- 109	Mostly nuclear	Yes
GFP-Lsm8-2	1-99	Mostly nuclear	Yes
GFP-Lsm8-313	1-85	As GFP	No
GFP-Lsm8C	65-109	As GFP	No
GFP-Lsm8-6A3	K87,90,92,102,107,109A	Mostly nuclear	Yes
GFP-Lsm8□N	12-109	Weakly nuclear	No
GFP-Lsm8□Sm	1-51/65-109	As GFP	No
GFP-Lsm8C	65-109	As GFP	No
GFP-Lsm8 plus endogenous Lsm8	1-109	Weakly nuclear	N/A

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## Supplementary Material

**Table S1: Plasmids used in this work**

Plasmid	Description	Source
pAEM80	pBM125 with <i>P<sub>GAL1</sub>-HA-LSM1</i> .	{Mayes, 1999 1462 /id}
pAEM76	pBM125 with <i>P<sub>GAL1</sub>-HA-LSM8</i> .	{Mayes, 1999 1462 /id}
pGFP-N-FUS	<i>CEN1, URA3, P<sub>MET25</sub>-GFP-MCS-CYC1<sub>TERM</sub></i>	{Niedenthal, 1996 2409 /id}
pGFP-C-FUS	<i>CEN1, URA3, P<sub>MET25</sub>-MCS-GFP- CYC1<sub>TERM</sub></i>	{Niedenthal, 1996 2409 /id}
pMPS8	pGFP-N-FUS, with <i>LSM8</i> fused in- frame	This work
pMR83	pGFP-C-FUS, with <i>LSM8</i> fused in-frame	This work
pMPS8-82	pMPS8 with G299A mutation resulting in a premature stop codon (aa1-99; as in {Pannone, 2001 2232 /id})	This work
pMPS8-313	pMPS8 with coding sequence 3' of base 313 deleted (aa1-85)	This work
pMPS8□N	pMPS8 with coding sequence 5' of 1st Sm site deleted (aa12-109)	This work
pMPS8-□SM	pMPS8 with Sm2 motif deleted (aa1-51 + 65-109)	This work
pMPS8-6A3	pMPS8 with the six most C-terminal lysine residues mutated to alanine residues	This work
pMPS8C	pGFP-N-FUS, with the region of <i>LSM8</i> encoding the C-terminus (aa65-109) fused in-frame	This work
pMPS2	pGFP-N-FUS, with <i>LHP1</i> fused in- frame	{Spiller, 2007 2503 /id}
pYX172	2μ, LEU2, SNR6	{Hu, 1994 833 /id}
pKW430	2μ, URA3, <i>P<sub>ADH</sub>-NLS-NES-GFP2</i>	{Stade, 1997 2413 /id}

**Table S2: Strains used in this work**

Strain	Genotype	Source
BMA38a	<i>MATa, trp1Δ1, his3Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100</i>	B. Dujon
AEMY35	<i>MATa, trp1Δ1, his3Δ200, ura3-1, leu2-3, -112, ade2-1, lsm7Δ::HIS3 [pAEM62 (<i>P<sub>GAL1</sub>-HA-LSM7</i>)]</i>	{Mayes, 1999 1462 /id}
MPS1	<i>MATa, trp1Δ1, his3Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100, Lsm1-13myc:KanMX6</i>	This work
MPS2	<i>MATa, trp1Δ1, his3Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100, LSM7-13myc:HphMX6</i>	This work
MRY74	<i>MATa, trp1Δ1, his3Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100, LSM7-13myc:KanMX6</i>	This work
MPS3	<i>MATa, trp1Δ1, his3Δ200, ura3-1, leu2-3,-112, ade2-1, can1-100, LSM8-13myc:HphMX6</i>	This work
MPS4	<i>MATa, ade2-1, his3Δ200, leu2-3, -112, trp1-1, ura3-1, can1-100, lsm6Δ::HIS3, LSM7-13myc:HphMX6</i>	This work
MPS5	<i>MATa, ade2-1, his3Δ200, leu2-3, -112, trp1-1, ura3-1, can1-100, lsm6Δ::HIS3, LSM8-13myc:HphMX6</i>	This work
MRY92	<i>MATa, ade2-1, his3Δ200, leu2-3, -112, trp1-1, ura3-1, can1-100, lsm1Δ::TRP1, LSM8-13myc:KanMX6</i>	This work
MPS7	<i>MATa, ade2-1, his3-11,-15, leu2-3, -112, trp1Δ, ura3-1, lsm8Δ::TRP1, LSM7-13myc:HphMX6 [pAEM76 (<i>pBM125, P<sub>GAL1</sub>-HA-LSM8</i>)]</i>	This work

MPS8	<i>MAT<math>\alpha</math></i> , <i>ade2-1</i> , <i>his3-11,-15</i> , <i>leu2-3</i> , <i>-112</i> , <i>trp1<math>\Delta</math></i> , <i>ura3-1</i> , <i>lsm3<math>\Delta</math>::TRP1</i> , <i>Lsm7-13myc:HphMX6</i> [pAEM64 (pBM125, <i>P<sub>GAL1</sub>-HA-LSM3</i> )]	This work
MPS9	<i>MAT<math>\alpha</math></i> , <i>ade1-101</i> , <i>his3-1</i> , <i>leu2-3,-112</i> , <i>trp1-289</i> , <i>ura3-52</i> , <i>LEU2-P<sub>GAL1</sub>-LSM4</i> , <i>LSM7-13myc:HphMX6</i>	This work
MPS10	<i>MAT<math>\alpha</math></i> , <i>ade2-1</i> , <i>his3-11,-15</i> , <i>leu2-3</i> , <i>-112</i> , <i>trp1<math>\Delta</math></i> , <i>ura3-1</i> , <i>lsm5<math>\Delta</math>::TRP1</i> , <i>LSM7-13myc:HphMX6</i> [pAEM75 (pBM125, <i>P<sub>GAL1</sub>-HA-LSM5</i> )]	This work
MPS11	<i>MAT<math>\alpha</math></i> , <i>ade2-1</i> , <i>his3-11,-15</i> , <i>leu2-3</i> , <i>-112</i> , <i>trp1<math>\Delta</math></i> , <i>ura3-1</i> , <i>lsm8<math>\Delta</math>::TRP1</i> , <i>LSM7-13myc:HphMX6</i> [pMPS1 (pRS313, <i>P<sub>GAL1</sub>-HA-LSM8</i> )]	This work
MPS12	<i>MAT<math>\alpha</math></i> <i>ade2</i> , <i>ade3</i> , <i>his3</i> , <i>leu2</i> , <i>lys2</i> , <i>ura3</i> , <i>nup49<math>\Delta</math>::TRP1</i> , <i>LSM7-13myc:HphMX6</i> [pUN90- <i>nup49-313</i> , <i>HIS3</i> ]	This work
MPS13	<i>MAT<math>\square</math></i> <i>ade2</i> , <i>ade3</i> , <i>his3</i> , <i>leu2</i> , <i>lys2</i> , <i>ura3</i> , <i>nup49<math>\Delta</math>::TRP1</i> <i>LSM8-13myc:HphMX6</i> [pUN90- <i>nup49-313</i> , <i>HIS3</i> ]	This work
MPS14	<i>MAT<math>\alpha</math></i> , <i>ade2-1</i> , <i>ura3-1</i> , <i>his3-11,15</i> , <i>trp1-1</i> , <i>leu2-3,112</i> , <i>can1-100</i> , <i>xpo1<math>\Delta</math>::LEU2</i> , <i>LSM1-13myc:KanMX6</i> [pKW457 ( <i>xpo1-1</i> , <i>HIS3</i> )]	This work, based on <i>xpo1-1</i> {Stade, 1997 2413 /id}
MPS15	<i>MAT<math>\alpha</math></i> , <i>ade2-1</i> , <i>ura3-1</i> , <i>his3-11,15</i> , <i>trp1-1</i> , <i>leu2-3,112</i> , <i>can1-100</i> , <i>xpo1<math>\Delta</math>::LEU2</i> , <i>LSM7-13myc:HphMX6</i> [pKW457 ( <i>xpo1-1</i> , <i>HIS3</i> )]	This work, based on <i>xpo1-1</i> {Stade, 1997 2413 /id}
MPS16	<i>MAT<math>\alpha</math></i> , <i>ade2-1</i> , <i>ura3-1</i> , <i>his3-11,15</i> , <i>trp1-1</i> , <i>leu2-3,112</i> , <i>can1-100</i> , <i>xpo1<math>\Delta</math>::LEU2</i> , <i>LSM8-13myc:HphMX6</i> [pKW457 ( <i>xpo1-1</i> , <i>HIS3</i> )]	This work, based on <i>xpo1-1</i> {Stade, 1997 2413 /id}
MPS17	<i>MAT<math>\alpha</math></i> , <i>ura3</i> , <i>lys2</i> , <i>ade2</i> , <i>trp1</i> , <i>his3</i> , <i>leu</i> , <i>lsm8-1</i> , <i>LSM7-13myc:HphMX6</i>	This work, based on BP4 {Pannone, 1998 1433 /id}
MPS22	<i>MAT<math>\square</math></i> , <i>ade2-1</i> , <i>his3-11,-15</i> , <i>leu2-3</i> , <i>-112</i> , <i>trp1<math>\Delta</math></i> , <i>ura3-1</i> , <i>lsm2<math>\Delta</math>::HIS3</i> , <i>LSM8-13myc:HphMX6</i> [pAEM68 (pBM125, <i>P<sub>GAL1</sub>-HA-LSM2</i> )]	This work
MPS25	<i>MAT<math>\alpha</math></i> , <i>ade1-101</i> , <i>his3-1</i> , <i>leu2-3,-112</i> , <i>trp1-289</i> , <i>ura3-52</i> , <i>LEU2-P<sub>GAL1</sub>-LSM4</i> , <i>LSM8-13myc:HphMX6</i>	This work
MPS26	<i>MAT<math>\alpha</math></i> , <i>ade2-1</i> , <i>his3-11,-15</i> , <i>leu2-3</i> , <i>-112</i> , <i>trp1<math>\Delta</math></i> , <i>ura3-1</i> , <i>lsm8<math>\Delta</math>::TRP1</i> , <i>LSM7-13myc:HphMX6</i> [pYX172 (2 $\mu$ , <i>LEU2</i> , <i>SNR6</i> )]	This work
MRY76	<i>MAT<math>\square</math></i> <i>leu2-3,112</i> <i>trp1-289</i> <i>ura3-52</i> <i>smd1<math>\Delta</math>::LEU2</i> <i>LSM7-13myc:KanMX6</i> [pGAL: <i>SMD1-HA</i> ]	This work, based on BZY16-D {Roy, 1995 997 /id}
MRY77	<i>MAT<math>\square</math></i> <i>leu2-3,112</i> <i>trp1-289</i> <i>ura3-52</i> <i>smd1<math>\Delta</math>::LEU2</i> <i>LSM8-13myc:KanMX6</i> [pGAL: <i>SMD1HA</i> ]	This work, based on BZY16-D {Roy, 1995 997 /id}
MRY83	<i>MAT<math>\alpha</math></i> <i>trp1<math>\Delta</math></i> <i>his3<math>\Delta</math>200</i> <i>ura3-1</i> <i>leu2-3,-112</i> <i>ade2-1</i> <i>can1-100</i> <i>LSM8-13myc:HphMX6</i> , <i>hog1<math>\Delta</math>::KanMX6</i>	This work

**Figure S1**

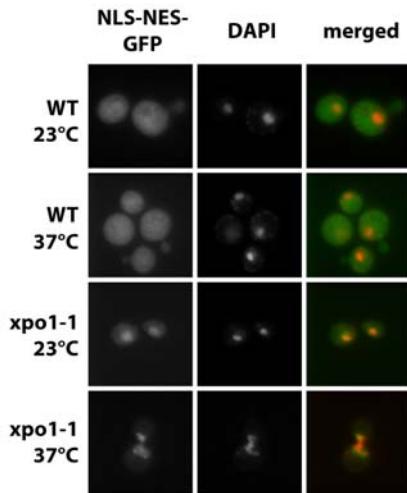


Figure S1: Mutant *xpo1-1* inhibits nuclear protein export of a control protein. BMA38a (WT) and MPS15 (*xpo1-1 LSM7-13myc*) with pKW430 (NLS-NES-GFP) were grown in SD-Ura-Met at 23°C and shifted to 37°C for 15 minutes, before fixing the cells. NLS-NES-GFP is shown in green, DAPI in red.

**Figure S2**

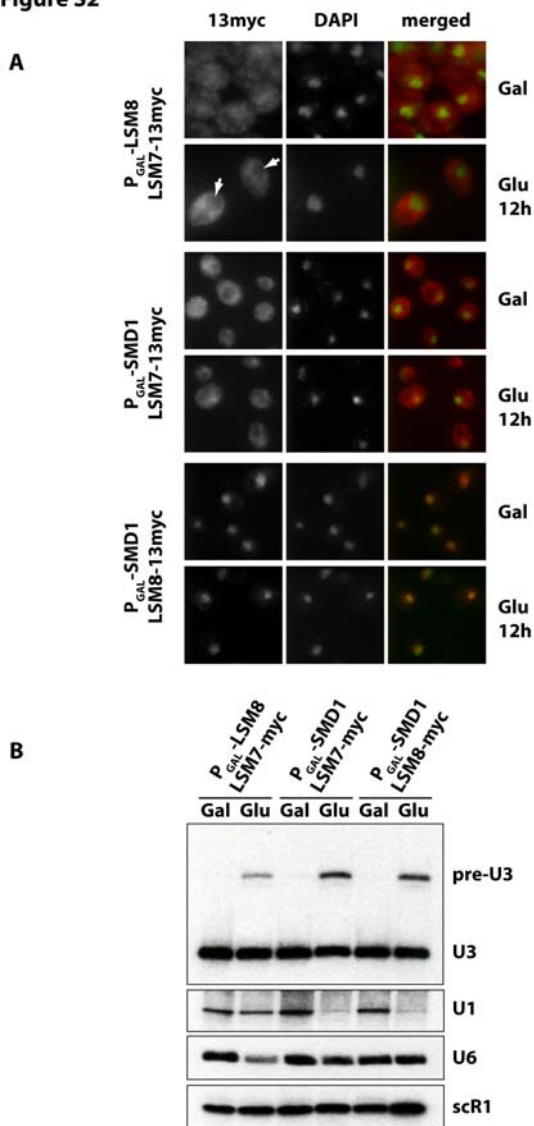


Figure S2: Lsm7p and Lsm8p localization is not affected by depletion of Smd1p. Cultures of MPS7, MRY76 and MRY77 were grown at 30°C in YPGalA to log phase and then for a further 12 hours in YPGA, while keeping the cells in log phase. (A) Localization of 13-myc-tagged Lsm7p or Lsm8p (shown in red) before (Gal) and after (Glu) growth in YPGA. (B) Total RNA isolated from the same cultures before and after growth in glucose was analyzed by northern blotting, probing for U3A, U1, U6 and scR1 RNAs.

Figure S3

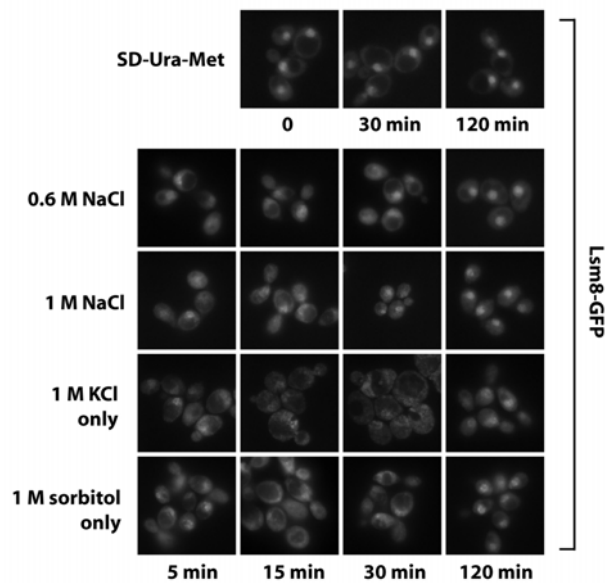


Figure S3: Lsm8-GFP is de-localized from the nucleus after hyperosmotic shock, but recovers after longer periods of time. MPS11 ( $P_{GAL}$ -LSM8) with pMR83 (Lsm8-GFP) was grown in SD-Ura-Met and shifted to SD-Ura-Met with 0.6 M or 1 M NaCl, or 1 M KCl or 1 M sorbitol only. Localization of Lsm8-GFP was examined in live cells after 5, 15, 30 and 120 minutes of incubation.

Figure S4

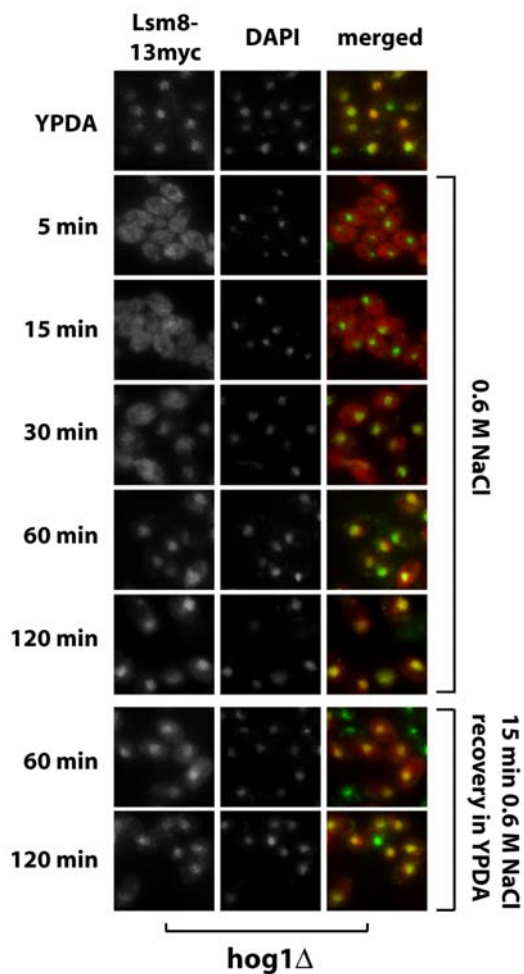


Figure S4: Recovery after hyper-osmotic shock is independent of Hog1p protein kinase. MRY83 ( $hog1\Delta$  LSM8-13myc) cells were grown in YPDA and shifted to YPDA with 0.6 M NaCl. Cells were fixed after 5, 15, 30, 60 and 120 minutes of incubation. Some cells were shifted back to YPDA (and incubated for 60 or 120 minutes) after incubation in YPDA with 0.6 M NaCl for 15 minutes. Lsm8-13myc is shown in red, DAPI in green.