Functions of Lsm proteins in mRNA degradation and splicing

Weihai He and Roy Parker*

Recent results have identified a family of Lsm (Like Sm) proteins that are related to the Sm protein family. Seven Lsm proteins form a complex, which interacts with the U6 snRNA and functions in splicing. In addition, a different complex of Lsm proteins interacts with cytoplasmic mRNA and promotes its turnover. These diverse functions of Lsm proteins suggest that they are important modulators of RNA biogenesis and function.

Addresses
Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, 1007 East Lowell Street, University of Arizona, Tucson, Arizona 85721, USA
*e-mail: rrparker@u.arizona.edu

Current Opinion in Cell Biology 2000, **12**:346–350

0955-0674/00/$ – see front matter
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Abbreviation
Lsm Like Sm

Introduction

The biogenesis and function of mRNA require a series of discrete events, including mRNA processing, transport, translation, and degradation. One major mechanism for mRNA degradation in eukaryotes takes place through deadenylation-dependent decapping, leading to 5′ to 3′ decay [1,2]. Examination of the proteins affecting the decapping step in this pathway identified a complex of proteins, referred to as Lsm proteins (Like Sm) that activates mRNA decapping [3,4••]. Interestingly, these Lsm proteins share sequence motifs with the Sm family of proteins and function in pre-mRNA splicing, suggesting that the Lsm proteins will be important modulators of RNA metabolism in both the cytoplasm and the nucleus. In this review, we summarize the characteristics of Lsm proteins, the functions of distinct Lsm complexes in pre-mRNA splicing and mRNA decay. We also discuss the possibility of other roles for these proteins.

What are Lsm proteins?

Lsm proteins were first identified as a conserved family of proteins that contain the 'Sm motif' found in the Sm proteins [5,6]. Sm proteins are a family of small proteins that assemble the core component of the U1, U2, U4 and U5 spliceosomal snRNPs. The Sm motif is relatively degenerate and consists of two parts, termed Sm1 and Sm2 domains, which are often separated by a large loop region [5,6]. Both the Lsm and Sm proteins can be divided into seven subtypes named B, D1, D2, D3, E, F and G (after the human Sm proteins) [7,8•,9••]. In *Saccharomyces cerevisiae*, there are nine Lsm proteins (Lsm1 to Lsm9). Lsm2 to Lsm7 are closely related to their Sm counterparts D1 to G respectively [8•], whereas Lsm1 and Lsm8 appear to be most closely related to the SmB subfamily [7,8•]. The Lsm family might even be more diverse and ancient, as the yeast Lsm9 is not clearly related to any subtype and one or two related proteins are found in some archaeal genomes [8•].

Biochemical and structural studies indicate that the Sm motif is a site of protein–protein interactions, which allows this family of proteins to assemble into seven-member ring structures that interact with RNA. For example, biochemical and genetic examination of interactions between individual Sm proteins demonstrated that the Sm motif is necessary and sufficient for the formation of the stable subcomplexes of B and D3, as well as a complex of E, F and G [6,10]. In addition, crystal structures of D1–D2 and D3–B Sm dimers revealed a common fold for the Sm motif, which contains a amino-terminal α helix, followed by an anti-parallel, five-stranded β sheet. The Sm1 domain contains the α-helix and β1 to β3, and the β4 and β5 strands reside in the Sm2 domain. The β4 strand in one Sm protein interacts with the β5 strand in another Sm protein [11••]. The results of the crystal structures of Sm subcomplexes, and knowledge of the interactions between Sm proteins [10,12,13], allowed the Sm proteins to be modeled into a seven-member ring through the interaction of their β-strands [11••].

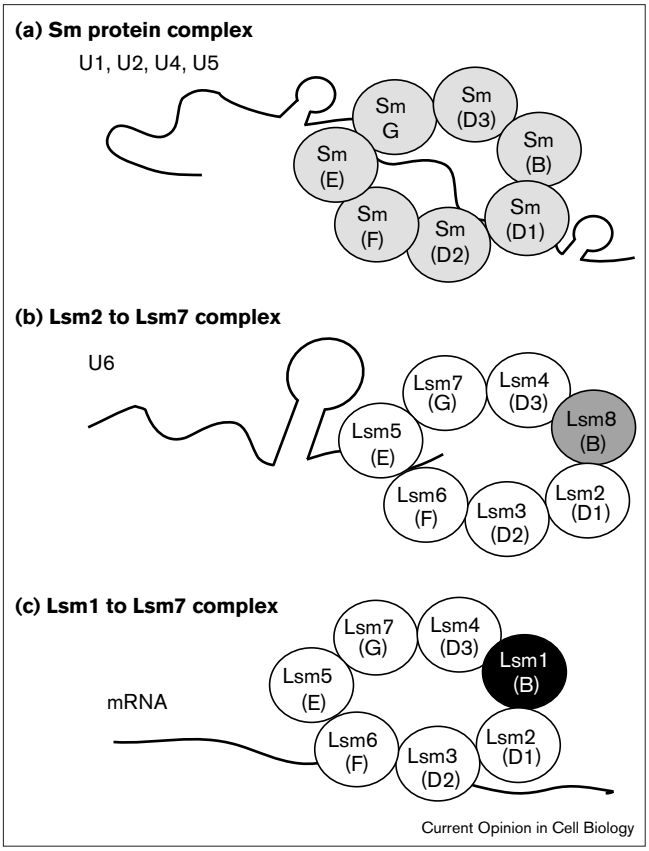
Several observations suggest that the Lsm proteins also form seven-member ring structures that interact with RNA. First, co-immunoprecipitation assays indicate that the Lsm proteins form complexes with each other [8•,14•]. Second, seven Lsm proteins (Lsm2 to Lsm8) co-immunoprecipitate with U6 snRNA [5,8•,9••,14•,15,16] and have been found to be present in the purified U4–U6•U5 tri-snRNP [9••,17,18]. Similarly, seven Lsm proteins (Lsm1 to Lsm7) have been found to co-immunoprecipitate with mRNA decay factors ([4••], and see below). Finally, on the basis of electron micrographs, purified human Lsm2 to Lsm8 proteins form a seven-member ring structure [9••]. Taken together, these observations suggest that Sm and Lsm proteins assemble into seven-member ring structures that interact with various RNA molecules (Figure 1).

Lsm proteins affect several aspects of RNA metabolism

The Lsm proteins function in pre-mRNA splicing

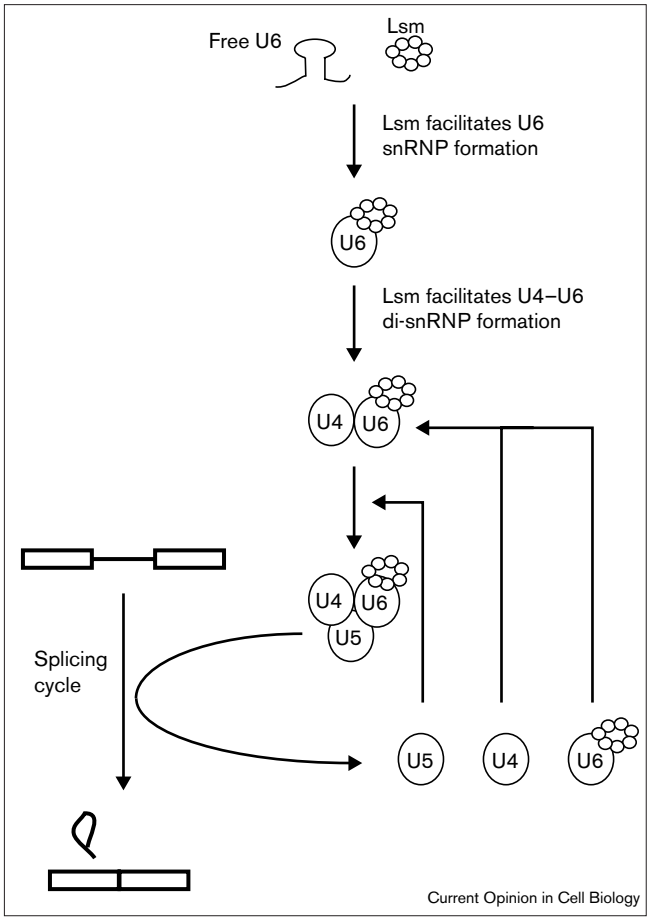
There are several pieces of evidence indicating that the Lsm proteins are involved in pre-mRNA splicing through interactions with the U6 snRNA. First, Lsm2 to Lsm8 proteins are found to co-immunoprecipitate with free U6, as well as with the U4–U6 di-snRNP and U4–U6•U5 tri-snRNP [5,8•,14•,15,16] and are present in purified tri-snRNPs [9••,17,18]. *In vitro* analysis suggests that the Lsm complex binds the U6 snRNA by specifically interacting with the single-stranded 3′-terminal U-tract of U6

Figure 1



Proposed structures of Sm and Lsm complexes. Sm and Lsm proteins may form analogous seven-member ring structures interacting with various RNAs. **(a)** Sm proteins complex with U1, U2, U4 and U5 snRNA. **(b)** Lsm2 to Lsm7 proteins complex with U6 snRNA. The exact nature of the interactions between the Lsm proteins is unclear but is speculated to be analogous to the order in the Sm complex. **(c)** Lsm1 to Lsm7 proteins complex with mRNA and may form a structure similar to the Sm complex.

Figure 2



A possible role of Lsm proteins in pre-mRNA splicing. Lsm proteins (Lsm2 to Lsm8) may function by facilitating U6 snRNP biogenesis and U4/U6 di-snRNP formation, possibly by rearranging RNA–protein structures.

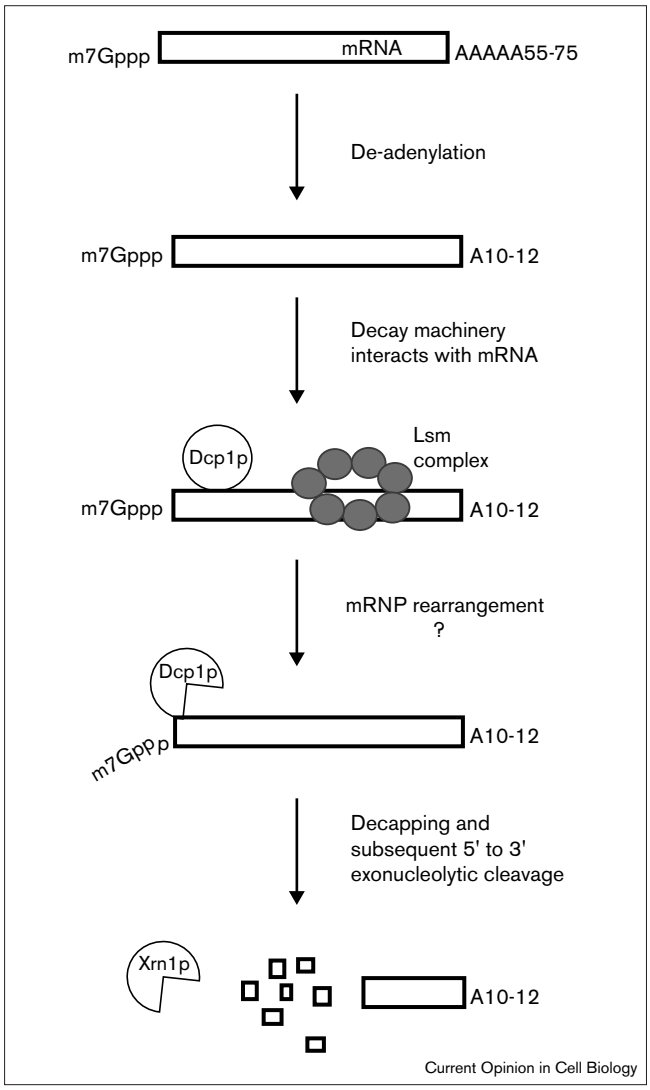
[9••]. Second, lsm2 to lsm8 mutants showed splicing defects [5,14•,15,16]. Third, Lsm proteins show several two-hybrid interactions with known splicing factors [7]. However, not all Lsm proteins are involved in splicing, as Lsm1 and Lsm9 proteins do not interact with U6 snRNA [8,14•], and mutations in these genes do not cause splicing defects [14•].

The actual function of the Lsm complex in splicing is unclear but appears to be primarily related to the stability and/or function of the U6 snRNP. For example, lsm2 to lsm8 mutants show a defect in splicing that correlates with a reduced level of U6 snRNA [14•,15,16]. Moreover, overexpression of U6 snRNA can partially compensate for the loss of the Lsm2 to Lsm8 proteins, suppressing the growth defects of lsm2 to lsm8 conditional mutants [14•]. Similarly, the reduced levels of U6 snRNA with an lsm8-1 point mutant can be increased, and the splicing defect reduced, by extra copies of Lhp1p, a protein that stabilizes U6 RNA [16]. How the Lsm proteins affect U6 snRNP function is unclear. One possibility is that the Lsm complex facilitates various conformational rearrangements (Figure 2) that occur during the splicing cycle, wherein the U6 snRNP assembles into the U4–U6 and U4–U6•U5 snRNPs, interacts with mRNA and U2 snRNA, dissociates from the spliced mRNA as a free U6 snRNP, and then reassembles into the U4–U6 and U4–U6•U5 snRNPs [19]. In support of this view, Achsel *et al.* have shown that the Lsm2 to 8 complex can facilitate the formation of U4–U6 duplexes *in vitro* [9••].

The Lsm proteins activate mRNA decapping

Several pieces of evidence indicate that the Lsm1 to Lsm7 proteins function in cytoplasmic mRNA degradation by interacting with both the mRNA and the mRNA degradative machinery. First, strains defective in Lsm1 to Lsm7 function, but not Lsm8 or Lsm9, show decreased rates of mRNA turnover due to a defect in mRNA decapping ([3,4••], D Mulhrad, R Parker, unpublished data). Second, based on two-hybrid interactions and co-immunoprecipitations the Lsm proteins physically

Figure 3



Lsm proteins function in mRNA decapping. This figure illustrates a general pathway of yeast mRNA degradation. Lsm proteins (Lsm1 to Lsm7) may activate decapping by rearranging mRNA–protein structures, thereby activating the decapping machinery. It should be noted that it is unclear when during the decay pathway the Lsm complex and Dcp1p interact with the mRNA.

interact with mRNA decay factors [4••,7], including the Dcp1p decapping enzyme, the exonuclease Xrn1p, and two other proteins required for efficient decapping, Dcp2p [20] and Pat1/Mrt1p [4••,21]. Third, consistent with their functions in mRNA decay in the cytoplasm, Lsm1p and Lsm7p, like Dcp1p, are found in the cytoplasm [4••]. Fourth, the Lsm1 and Lsm5 proteins have been shown to co-immunoprecipitate full length CYH2 mRNA indicating that the affect on decapping may occur through interactions with the substrate [4••]. Interestingly, the Dcp1p decapping enzyme can also co-immunoprecipitate the CYH2 mRNA and the co-immunoprecipitation of Dcp1p and the Lsm proteins is sensitive to RNase [4••]. This suggests that the Lsm1p to Lsm7p complex and the Dcp1p decapping enzyme may be assembling on the mRNA prior to the actual decapping reaction.

The observation that Dcp1p and Lsm proteins both interact with mRNA suggests two possible functions for the Lsm complex in decapping. In one view, the Lsm proteins may first bind the mRNA and then function to recruit the decapping enzyme to the transcript. Alternatively, Lsm proteins may facilitate decapping by promoting rearrangements in the mRNP to allow the decapping enzyme access to the cap structure. This possibility is suggested by observations that efficient translation initiation is in competition with decapping *in vivo* [22–24] and that the cap-binding protein, eIF4E, is a potent inhibitor of decapping *in vitro* (DC Schwartz, R Parker, unpublished data). Given this, one speculative model is that the Lsm proteins directly or indirectly facilitate disassembly of the cap-binding complex and thereby activate the decapping reaction (Figure 3).

Distinct Lsm complexes function in different cellular events

Several observations indicate that there are at least two distinct Lsm complexes in cells: one complex consisting of Lsm1p to Lsm7p affecting mRNA decapping and a complex of Lsm2p to Lsm8p, which associates with U6 and functions in pre-mRNA splicing. First, although mutations in *LSM2* to *LSM7* affect both mRNA decay and pre-mRNA splicing, mutations in *LSM1* only affect mRNA decay and an lsm8 mutant affects splicing but not mRNA decay [3,4••,14•,16]. Second, Lsm2p to Lsm7p co-immunoprecipitate with both U6 snRNA [5,8•,14•,15] and with mRNA decay factors [4••], whereas Lsm1p does not immunoprecipitate U6 snRNA [8•,14•] but does pull down Dcp1p and Pat1p/Mrt1p [4••]. In contrast, Lsm8p does co-immunoprecipitate with U6 snRNA [8•,14•,16] but does not pull down Dcp1p or Pat1/Mrt1p [4••]. Third, immunoprecipitation of Lsm8p has been shown to co-immunoprecipitate Lsm2p to Lsm7p, but does not co-immunoprecipitate Lsm1p [8•]. Fourth, a pair of distinct complexes, each consisting of seven members would be consistent with the organization of Sm and Lsm proteins discussed earlier. This suggests that the Lsm proteins form at least two distinct complexes involved in different aspects of mRNA metabolism (Figure 1).

Do Lsm proteins have additional roles?

Several observations raise the possibility that the Lsm proteins function in other areas of RNA metabolism. One possibility is that an Lsm complex plays a role in the maturation or stability of nascent RNA polymerase III transcripts. For example, pre-RNase P RNA can be co-immunoprecipitated by Lsm2p to Lsm7p, but not by Lsm1p, Lsm8p or Lsm9p [8•], and Lsm2p-, Lsm5p- and Lsm8p-depleted strains show a small reduction in pre-RNase P RNA [14•]. Interestingly, the mature RNase P RNA cannot be co-immunoprecipitated with the same Lsm proteins that

co-immunoprecipitate the pre-RNase P RNA, suggesting that Lsm2p to Lsm7p may have a role in pre-RNase P RNA processing [8•]. In addition, the level of pre-5S RNA declined in lsm2 to lsm8 mutant strains and mature 5S rRNA was slightly reduced in lsm2, lsm5 and lsm8 mutants [14•]. These observations suggest that *LSM2* to *LSM8* may be involved in the processing of other RNA polymerase III transcripts, although this needs to be examined further.

Other possible functions for the Lsm proteins are suggested by multiple two-hybrid interactions between Lsm proteins and other proteins involved in RNA metabolism [7]. The Lsm proteins interact with proteins involved in mRNA 3′-end formation, 3′ to 5′ degradation and processing of RNA, and the modulation of translation, although the significance of these interactions has yet to be experimentally examined. Finally, some Lsm mutants show abnormalities in U5 RNA. The levels of a long form of U5 ($U5_L$) are slightly decreased when cells are depleted of Lsm2p or Lsm3p, whereas the levels of a shorter form ($U5_S$) increase in cells depleted of Lsm2p or Lsm8p [14•]. This raises the possibility that the Lsm complex has a role in U5 processing, although this could be an indirect alteration due to changes in U6 levels.

Conclusion

The roles of the Lsm proteins in U6 function, pre-mRNA splicing and mRNA decapping indicate that these proteins are a newly discovered important set of RNA-binding proteins that modulate various aspects of RNA metabolism. Given these functions, one can anticipate the clear definition of additional roles for these proteins in the near future. In addition, the presence of the Lsm9p in yeast suggests that there might be additional Lsm complexes that have, as yet, unknown functions. Important issues for future work include determining the specific role of these proteins in splicing and how they promote mRNA decapping. Finally, it will be important to determine how the differences in the composition of the Lsm complexes affect their functions.

Update

Several recent papers have added to our understanding of the Lsm proteins since this review was written. First, additional evidence for two distinct Lsm complexes has come from the biochemical purification and characterization of the two complexes and their associated proteins [25]. Insight into the interaction between Lsm complexes and RNA has come from *in vitro* reassembly and crosslinking experiments, which provide evidence that Lsm4, Lsm6 and Lsm7 proteins make direct contact with the U6 snRNA [26]. Finally, evidence that Lsm proteins have additional functions has come from the identification of a variety of two-hybrid interactions with Lsm proteins [27,28] and from the demonstration that Lsm1p is required for brome mosaic virus replication in yeast [29].

Acknowledgements

We thank Ambro van Hoof and Sandaresan Tharun for their comments on the manuscript. This work was supported by the Howard Hughes Medical Institute and by a grant to Roy Parker from the National Institutes of Health (GM45443).

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