Mutations in the *Saccharomyces cerevisiae* LSM1 Gene That Affect mRNA Decapping and 3’ End Protection

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Manuscript received August 6, 2004
Accepted for publication January 20, 2005

ABSTRACT

The decapping of eukaryotic mRNAs is a key step in their degradation. The heteroheptameric Lsm1p–7p complex is a general activator of decapping and also functions in protecting the 3’ ends of deadenylated mRNAs from a 3’-trimming reaction. Lsm1p is the unique member of the Lsm1p–7p complex, distinguishing that complex from the functionally different Lsm2p–8p complex. To understand the function of Lsm1p, we constructed a series of deletion and point mutations of the Lsm1p–7p complex. In yeast, deadenylation is carried out by the Ccr4p Dhh1p, Lsm1p–7p complex, Edc1p, Edc2p, and Edc3p complex (also known as the “deadenylation-dependent decay pathway”). Several studies have shown that mRNA decay factors and decay pathways are well conserved in eukaryotes from yeast to humans (Seraphin 1992; Couttet et al. 1997; Gao et al. 2001; Wang and Kiledjian 2001; Decker and Parker 2002; Liu et al. 2002; Lykke-Andersen 2002; Van Dijk et al. 2002, 2003; Wang et al. 2002; Piccirillo et al. 2003; Tseng-Rogenski et al. 2003). In yeast, deadenylation-dependent decay pathways that involve 5’ to 3’ or 3’ to 5’ mode of degradation of the mRNA body. In yeast, deadenylation is carried out by the Ccr4p complex (Tucker et al. 2001). In the 5’ to 3’ decay pathway (also known as the “deadenylation-dependent decapping pathway”), this is followed by decapping by the decapping enzyme (Dcp1p–2p complex) that exposes the body of the message to 5’ to 3’ exonucleolytic decay by the exonuclease Xrn1p (Muhlrad and Parker 1992; Decker and Parker 1993; Hsu and Stevens 1993; Muhlrad et al. 1994, 1995; Beelman et al. 1996; Dunckley and Parker 1999; Tucker et al. 2001). In the 3’ to 5’ pathway, deadenylated mRNAs are degraded in a 3’ to 5’ exonucleolytic manner by the exosome (Muhlrad et al. 1995; Anderson and Parker 1998). Decapping is a crucial step in the 5’ to 3’ decay pathway because it is the site of several regulatory inputs (Coller and Parker 2004). Moreover, a large number of proteins affect decapping in addition to the decapping enzyme. The translation initiation machinery and the poly(A)-binding protein are antagonistic to decapping (Caponigro and Parker 1995; Coller et al. 1998; Schwartz and Parker 1999, 2000; Vilela et al. 2000; Wilusz et al. 2001; Ramirez et al. 2002; Khanna and Kiledjian 2004). Conversely, several other factors function as activators of decapping. They include Pat1p, Ddh1p, Lsm1p–7p complex, Edc1p, Edc2p, and Edc3p (Hatfield et al. 1996; Boeck et al. 1998; Bonnerot et al. 2000; Bouveret et al. 2000; Tharun et al. 2000; Wyers et al. 2000; Coller et al. 2001; Dunckley et al. 2001; Fischer and Weis 2002; Schwartz et al. 2003; Kshirsagar and Parker 2004).

The Lsm1p–7p complex is particularly interesting as a decapping activator for several reasons. First, it is a highly conserved component of eukaryotic mRNA decay machinery. Second, it physically interacts with several other factors involved in the major mRNA decay path-

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Genetics 170: 33–46 (May 2005)
way, including the decapping activators Pat1p and Dhh1p and the 5' to 3' exonuclease Xrn1p (BONNEROT et al. 2000; BOUVERET et al. 2000; THARUN et al. 2000; COLLER et al. 2001). Third, in vivo, it associates with a pool of deadenylated mRNPs that is bound to the decapping enzyme and targeted for decay but distinct from translating mRNPs (THARUN and PARKER 2001a). Fourth, several studies suggest that in addition to mRNA decapping, this complex is also involved in protecting mRNA 3' ends from trimming (BOECK et al. 1998; HE and PARKER 2001). Finally, mammalian cells overexpressing Lsm1p show a transformed phenotype, suggesting a possible role for Lsm1p in growth control (SCHWEINFEST et al. 1997; GUMBS et al. 2002).

The Lsm1p–7p complex, which is conserved in all eukaryotes, is a heptameric complex made of seven proteins, Lsm1p through Lsm7p. The Lsm (“Like Sm”) proteins are homologous to the Sm proteins and these proteins form a family of protein complexes that includes complexes found in eubacteria, archaea, and eukaryotes (ACHSEL et al. 1999, 2001; KAMBACH et al. 1999; COLLINS et al. 2001; MURA et al. 2001; TORO et al. 2001; MOLLER et al. 2002; SCHUMACHER et al. 2002). Individual members of this protein family are characterized by the presence of a bipartite sequence motif, referred to as the Sm domain. The Sm domain consists of two conserved segments of amino acids (Sm motifs I and II) separated by a nonconserved segment of variable length (COOPER et al. 1995; HERMANN et al. 1995; SERAPHIN 1995).

These Sm and Lsm proteins also show similarity in their tertiary structure and the quaternary structure of the complexes they form. Typically, they exist in cells in the form of ringshaped hexa or heptameric complexes (ACHSEL et al. 1999, 2001; KAMBACH et al. 1999; COLLINS et al. 2001; MURA et al. 2001; MOLLER et al. 2002; SCHUMACHER et al. 2002; ZARIC et al. 2005). For example, the seven Sm proteins conserved in all eukaryotes associate into a ringshaped heptameric “Sm complex” (KAMBACH et al. 1999) that assembles onto the U1, U2, U4, and U5 snRNAs to form the cores of the corresponding spliceosomal snRNPs (LEHRER and STEITZ 1981; LURHMANN et al. 1990). On the other hand, there are eight Lsm proteins (Lsm1p through Lsm8p) conserved in all eukaryotes and they appear to form two heptameric complexes (SALGADO-GARRIDO et al. 1999; BOUVERET et al. 2000; THARUN et al. 2000). One complex, consisting of Lsm1p through Lsm7p proteins, is an activator of mRNA decapping (BOECK et al. 1998; BOUVERET et al. 2000; THARUN et al. 2000), while the other made of Lsm2p through Lsm8p proteins functions in pre-mRNA splicing by forming the core of U6 snRNP (COOPER et al. 1995; PANNONE et al. 1998, 2001; ACHSEL et al. 1999; MAYES et al. 1999; SALGADO-GARRIDO et al. 1999). Thus, although involved in very different functions, the Lsm1p–7p and Lsm2p–8p complexes share six of their seven subunits and differ in only one subunit that is either Lsm1p or Lsm8p. However, it is not known what features of Lsm1p and Lsm8p dictate their respective roles.

The mechanism by which Lsm1p–7p complex activates decapping is not clear. It is also not known how the decapping activation function is related to the mRNA 3' end protection function. Given the structural similarity of the Lsm1p–7p complex to the Lsm2p–8p and the Sm complexes, a reasonable model would be that similarity also exists at a mechanistic level in the manner in which these complexes function. Importantly, given that the Sm complex and several Sm-like protein complexes (including the Lsm2p–8p complex) have been shown to directly bind to their RNA substrates to execute their functions (ACHSEL et al. 1999, 2001; RAKER et al. 1999; TORO et al. 2001; MOLLER et al. 2002; SCHUMACHER et al. 2002), it is likely that the Lsm1p–7p complex also directly binds to mRNAs to protect their 3' ends and to activate their decapping. This is consistent with the earlier co-immunoprecipitation analyses, which revealed that the Lsm1p–7p complex associates with deadenylated mRNAs in vivo (THARUN et al. 2000; THARUN and PARKER 2001a). If this is true, then, disrupting the interaction of the Lsm1p–7p complex with RNA should impair both mRNA 3' end protection and decapping activation functions.

Recent studies have shown that the decapping and 5' to 3' exonucleolysis of mRNAs occur in discrete cytoplasmic structures called processing bodies (P-bodies), where Xrn1p, the various decapping factors including the Lsm1p through Lsm7p proteins, and mRNA molecules targeted for decay are localized (INGELFINGER et al. 2002; VAN DIJK et al. 2002; SHETH and PARKER 2003; COUGOT et al. 2004). However, it is not known how the Lsm1p–7p complex and the other decay factors are recruited to the P-bodies.

To identify the critical regions of the Lsm1p protein and to determine how they are related to the functions of Lsm1p, we undertook a mutagenic analysis of Lsm1p. Structural and biochemical studies in the past have identified residues in the Sm domain of Sm proteins and archaeal Sm-like proteins that are involved in RNA and inter-subunit interactions (KAMBACH et al. 1999; MURA et al. 2001, 2003; TORO et al. 2001; ULRALFA et al. 2001). Therefore, we predicted that the homologous residues in Lsm1p could have similar roles, mutated such residues, and examined the effect of such mutations on the functions of Lsm1p. In addition, we also examined the roles of other residues within the Lsm1p, including those in the conserved C-terminal domain following the Sm domain.

These studies revealed the following:

i. Mutations affecting the predicted RNA-binding surface of Lsm1p lead to impairment of mRNA decay without significantly affecting the localization of the Lsm1p–7p complex to the P-bodies. Thus, interac-
tions with mRNA are critical for activation of decapping but not for recruitment of the Lsm1p–7p complex to P-bodies.

ii. Lesions in the predicted inter-subunit interaction domains also lead to significant impairment of mRNA decay indicating the importance of the Lsm1p–7p complex integrity for mRNA decay.

iii. All the mutants that are defective in mRNA-3’ end protection are also defective in mRNA decay, suggesting that 3’ end protection could be indicative of the Lsm1p–7p complex-mRNA interaction that leads to decapping activation.

iv. In addition to the Sm domain, the extended C-terminal domain of Lsm1p is also important for the function of the Lsm1p–7p complex.

MATERIALS AND METHODS

Yeasts strains, plasmids, and mutagenesis: All the mutant versions of LSM1 were made following the QuiK change mutagenesis protocol (Stratagene, La Jolla, CA) using plasmid pST11 (carrying the wild-type LSM1 gene) as the template. This generated the plasmids, pST21 through pST49 and pST18, each of which carried a different lsm1 allele (Tables 1 and 2). pST11 was made by amplifying the LSM1 gene (containing the ORF along with 619 and 543 nucleotides, respectively, of the 5’- and 3’-flanking regions) by polymerase chain reaction (PCR) from yeast genomic DNA and cloning it into pRS416 that is a CEN vector with URA3 marker (Sikorski and Hieter 1989). All the plasmids were sequenced to make sure that unintended mutations were not present in the LSM1 gene. Plasmids carrying wild-type (pST11) and mutant versions of LSM1 were transformed into the lsm1Δ strain yRP1365 (MATα, leu2, ura3 lys2 cdp1Δ::LEU2(PM) lsm1Δ::TRP1) (Tharun et al. 2000) and the resulting transformants were used for the experiments. yRP1365 carrying pRS416 served as the negative control.

For the experiments in Figure 5, an lsm1Δ strain expressing GFP fusions of LSM1 or lsm1-9 or lsm1-14 under LSM1’s native promoter from a CEN vector was used. Plasmid pRP1176 (J. Coller and R. Parker, unpublished results) is a CEN vector expressing LSM1-GFP from LSM1’s native promoter. Mutations of lsm1-9 and lsm1-14 were introduced into the LSM1-GFP cassette of this plasmid by QuiK change mutagenesis protocol (Stratagene) to generate plasmids pST91 and pST92, respectively. pRP1176, pST91, and pST92 were separately transformed into the Euroscarf lsm1Δ strain, ES11301 (MATα, his3, leu2, ura3, lys2, lsm1Δ::NEO’), to get the strains needed for the experiments shown in Figure 5. Strains used in experiments for Figure 6 were made by introducing each of the plasmids pST11, pST 29, or pST34 (coding for LSM1, lsm1-9, and lsm1-14, respectively; see Table 1) separately into the strain yRP2008 (genotype the same as ES11301 except that it is MATα and LSM7-GFP-HIS3) or yRP2010 (genotype the same as ES11301 except that it is MATα and LSM2-GFP-HIS3). yRP2008 and yRP2010 were made by crossing the LSM2-GFP and LSM7-GFP strains of Euroscarf with the Euroscarf lsm1Δ strain ES11301.

RNA and protein analyses and microscopy: Transcription shut-off experiments, RNA preparation, Northern analysis, determination of MFA2pG-MRNA half-life, quantitation of poly(G) fragment accumulation, preparation of yeast cell lysates, and Western analysis for Lsm1p were all performed as described earlier (Tharun and Parker 1999, 2001a; Tharun et al. 2000). For all experiments, cells were grown at 25°C in −ura medium and collected at log phase. For examination of P-bodies (experiments in Figure 5 and Figure 6), cells grown to log phase were collected, incubated in glucose-free medium for 10 min, and then observed using confocal microscope. Confocal microscopy was done as described (Seth and Parker 2003; Teixeira et al. 2005).

RESULTS

Mutagenesis of LSM1: Structural and biochemical studies have been conducted on the human Sm subcomplexes and several archaeabacterial Sm-like protein complexes in the past (Kambach et al. 1999; Mura et al. 2001, 2003; Toro et al. 2001; Urlaub et al. 2001). Such studies revealed the crucial amino acid residues—involved in RNA contact and inter-subunit contacts—of the Sm and Sm-like proteins that form these complexes. Using this information and the homology of these proteins to yeast Lsm1p, we predicted the residues of Lsm1p that are likely to be involved in RNA contact and inter-subunit contacts and targeted them (Figure 1, a and b) in our first set of mutants (lsm1-6 through lsm1-9, lsm1-13, and lsm1-14).

Structural studies on several members of the Sm-like protein family have revealed that the Sm domain of these proteins folds into a conserved structure (“Sm fold”) consisting of an N-terminal α-helix followed by a five-stranded, strongly bent β-sheet (Kambach et al. 1999; Mura et al. 2001, 2003; Toro et al. 2001). Here the key residues involved in contacting the RNA are located in loops 3 (between β-strands 2 and 3) and 5 (between β-strands 4 and 5), while many of the residues involved in inter-subunit interactions are located in β-strands 2, 3, and 4. The 3D structure of the Sm domain of the yeast Lsm1p generated using the homology-based modeling program 3D-JIGSAW (Bates and Sternberg 1999; Bates et al. 2001; Contreras-Moreira and Bates 2002) supports the idea that the homologous residues in Lsm1p are organized similarly (Figure 1b).

The Lsm1p residues implicated (on the basis of homology) in inter-subunit contacts included both charged and hydrophobic amino acids. R59 and R62 located in loop 2 of the modeled 3D structure were mutated in the allele lsm1-6. L64 and G66 located in β-strand 2 and R62 were changed in the allele lsm1-13. Residues 101 to 104 (I, F, M, and I) located in β-strand 4 were changed in lsm1-13. The charged residues R59 and R62 were replaced with alanines. On the other hand, residues 101 to 104 (I, F, M, and I) and L64 were changed to charged residues, since hydrophobicity was predicted to be crucial for their function. G66 was replaced by a bulky tryptophan (Figure 1, a and b).

Lsm1p residues implicated in RNA binding are located in loops 3 and 5 of the modeled 3D structure (Figure 1b). The loop 3 residues Y74 and N76 are mutated in lsm1-9, while the loop 5 residues R105, G106, and E107 are mutated in lsm1-14. The loop 3 residue
Figure 1.—Regions of LSM1 targeted in our study. (a) Position of the mutated residues in the primary sequence. Regions of primary sequence targeted in the mutant alleles, lsm1-1 through lsm1-24, are underlined. The allele number is given directly below the part of primary sequence targeted in each of these mutant alleles. Residues replacing the wild-type sequence are indicated in italics above the appropriate wild-type residues. Triangles following (alleles 25 and 26) or preceding (27–29) allele numbers point to the last of the residues deleted from the N and C termini, respectively, in those deletion alleles. Amino acid residues shown in boldface and italicized boldface letters are predicted to be involved in inter-subunit or RNA contacts, respectively. Regions of Lsm1p predicted to form the secondary structural elements (N-terminal α-helix, α1, and the five β-strands, β1 through β5) characteristic of the Sm and Lsm proteins are also indicated. Primary sequence stretches indicated by long and short shaded areas are Sm motifs I and II, respectively, of the Sm domain. Amino acid residue numbers are indicated on the right of each line. (b) Location of the residues implicated in inter-subunit and RNA contacts in the predicted three-dimensional structure of the Sm domain of Lsm1p. Tertiary structure of the Sm domain of Lsm1p was generated using the homology-based modeling program 3D-JIGSAW (Bates and Sternberg 1999; Bates et al. 2001; Contreras-Moreira and Bates 2002) using the known tertiary structure of the archebacterial Sm-like protein, Smap1, from Pyrobaculum aerophilum as the template. Regions in green and pink contain residues implicated in inter-subunit contacts and RNA contacts, respectively. Residues targeted in our study are indicated in boldface letters. Various secondary structural elements like the N-terminal α-helix (α1), the five β-strands (β1 through β5), and the loop regions are also indicated. (c) Alignment of yeast Lsm1p with human Lsm1p using the ClustalW program. Long and short shaded areas indicate Sm motifs I and II of yeast Lsm1p. Residues in boldface or italicized boldface letters indicate those predicted to be involved in inter-subunit and RNA contacts, respectively. Portions of the primary sequence forming the Sm domain and the N- and C-terminal extensions are indicated above the primary sequence. The bottom line in each block indicates the yeast Lsm1p residues that are either identical (*) or replaced with a similar residue (o) in the human Lsm1p. Amino acid residue numbers are indicated on the left and right (for the lower block) of the primary sequence. For both proteins, the entire primary sequence is shown.

D72 was mutated along with R69 located in β-strand 2 in lsm1-8. N76, Y74, R105, and E107 were predicted to form the RNA-binding pocket while D72 and G106 were predicted to be important in holding N76 in the proper orientation. While G106 was changed to a tryptophan, other residues were replaced with alanines (Figure 1, a and b).

All the clusters of charged residues in Lsm1p whose function could not be readily predicted on the basis of sequence homology were replaced with alanines to generate the mutants lsm1-1 through lsm1-5, lsm1-10 through lsm1-12, and lsm1-15 through lsm1-24 (Figure 1a). These included residues in the regions flanking or inside the Sm domain. Changes were restricted to four or fewer neighboring residues in all the mutants.

Finally, to address the role of the regions of Lsm1p that are outside the Sm-domain, we also made five deletion mutants. These included lsm1-25 and lsm1-26 that had, respectively, 17 and 36 amino acids deleted from the N terminus and lsm1-27 through lsm1-29 that had 55, 43, and 28 amino acids, respectively, deleted from the C terminus (Figure 1a). Importantly, in these mutants, the Sm domain was left intact so that the mutant proteins are likely to fold into the proper tertiary structure that is characteristic of the Sm-like proteins.

Mutants were made as described in materials and
Therefore, as a first assay for the function of the mutant of the alleles lsm1-27, we carried out experiments and referred to as lsm1 alleles affected in predicted inter-subunit contact residues

<table>
<thead>
<tr>
<th>LSM1 allele</th>
<th>Plasmid borne</th>
<th>Amino acid residue changes</th>
<th>Growth at 36°C</th>
<th>mRNA decayb</th>
<th>mRNA 3′ end protectionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>lsm1-6</td>
<td>pST26</td>
<td>R59, D60, and R62 to A’s</td>
<td>−</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>lsm1-7</td>
<td>pST27</td>
<td>R62, L64, and G66 to A, D, and W, respectively</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lsm1-13</td>
<td>pST33</td>
<td>I101, F102, M103, and I104 to R, E, K, and D, respectively</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

b. lsm1 alleles affected in predicted RNA-contact residues

<table>
<thead>
<tr>
<th>LSM1 allele</th>
<th>Plasmid borne</th>
<th>Amino acid residue changes</th>
<th>Growth at 36°C</th>
<th>mRNA decayb</th>
<th>mRNA 3′ end protectionb</th>
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<tr>
<td>lsm1-8</td>
<td>pST28</td>
<td>R69 and D72 to A’s</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lsm1-9</td>
<td>pST29</td>
<td>Y74 and N76 to A’s</td>
<td>−/+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>lsm1-14</td>
<td>pST34</td>
<td>R105, G106, and E107 to A, W, and A, respectively</td>
<td>−</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

c. lsm1 alleles affected in other Sm domain residues

<table>
<thead>
<tr>
<th>LSM1 allele</th>
<th>Plasmid borne</th>
<th>Amino acid residue changes</th>
<th>Growth at 36°C</th>
<th>mRNA decayb</th>
<th>mRNA 3′ end protectionb</th>
</tr>
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<tbody>
<tr>
<td>lsm1-5</td>
<td>pST25</td>
<td>D51, R52, and K53 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-11</td>
<td>pST31</td>
<td>E90, E91, and K93 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-12</td>
<td>pST29</td>
<td>E96, E97, D98, and R99 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-15</td>
<td>pST35</td>
<td>E114 and D116 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

a. Growth, no growth, and growth that is significantly slower than wild-type cells are indicated by +, −, and −/+ symbols, respectively.

b. Strong (comparable to lsm1Δ) and moderate block to these functions are indicated by + and ++, respectively. + ++ indicates normal (wild type) function.

c. Mutant Lsm1p protein bank as very weak or undetectable (by Western analysis) in the lysates of these alleles.

Methods by introducing the changes in the LSM1 gene (with its native promoter and 3′-UTR sequences) cloned in a CEN vector with URA3 marker. The plasmids carrying the wild-type and the various mutant versions of LSM1 were transformed into the lsm1Δ strain yRP1365 so that the plasmid-borne gene is the only source of Lsm1p protein in the cell. The lsm1Δ strain transformed with the empty vector pRS416 and the recombinant vector carrying the wild-type LSM1 gene were used as the negative and positive controls, respectively, in all experiments and referred to as lsm1Δ and wild type, respectively. The mutants were studied for temperature sensitivity, mRNA decay, and mRNA 3′ end protection as described below.

**LSM1 mutations resulting in temperature sensitivity of growth:** lsm1Δ cells are temperature sensitive for growth at higher temperatures (Mayes et al. 1999). Earlier studies had suggested that the temperature sensitivity of the lsm1Δ cells is likely to be related to their mRNA 3′-end-trimming phenotype (He and Parker 2001). Therefore, as a first assay for the function of the mutant Lsm1p proteins, we studied their ability to support growth at high temperature. To this end, growth of the mutant and the wild-type cells on −ura plates was examined at 25°C and 36°C. As expected, at 25°C both of the control strains LSM1 and lsm1Δ were able to grow, while at 36°C, lsm1Δ cells were unable to grow. Analysis of the growth of the mutants revealed the following (Tables 1 and 2):

First, we observed that among the three lsm1 alleles bearing mutations in the predicted RNA-binding residues (i.e., lsm1-8, lsm1-9, and lsm1-14), lsm1-8 and lsm1-14 were unable to grow at 36°C, while lsm1-9 was able to grow very slowly. These results provide the first indication that these residues are important for Lsm1p function. However, the ability of lsm1-9 cells to grow at 36°C, albeit slowly, suggests that at least some parts of the RNA-binding surface can undergo mutation without affecting viability at high temperature.

Second, we observed that all the three lsm1 alleles bearing mutations in the predicted inter-subunit contact residues, lsm1-6, lsm1-7, and lsm1-13 were unable to grow at 36°C just like lsm1Δ. These results support the idea that these residues are required for Lsm1p function.

Third, we observed that large deletions in the region of Lsm1p that is C-terminal to the Sm domain lead to temperature-sensitive growth as shown by the inability of the alleles lsm1-27 and lsm1-28 to grow at 36°C. The mutant lsm1-29, which bears the shortest deletion of this C-terminal region, grew slowly at 36°C. These observations indicate that residues outside the Sm domain are also likely to be important for Lsm1p function.

Fourth, we observed that mutations in or deletion of the stretch of amino acids N-terminal to the Sm domain did not affect growth at any temperature, sug-
TABLE 2
Description of the changes introduced in the various alleles and their
associated phenotypes: mutations outside the Sm domain

<table>
<thead>
<tr>
<th>LSM1 allele</th>
<th>Plasmid borne</th>
<th>Amino acid residue changes</th>
<th>Growth at 36°c</th>
<th>mRNA decayd</th>
<th>mRNA 3’ end protectiond</th>
</tr>
</thead>
<tbody>
<tr>
<td>lsm1-1</td>
<td>PST21</td>
<td>K6, D7, and R8 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-2</td>
<td>pST22</td>
<td>D14, K16, and R17 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-3</td>
<td>pST23</td>
<td>K24 and K25 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-4</td>
<td>pST24</td>
<td>E28, E30, and D32 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-16</td>
<td>pST36</td>
<td>D118, K119, E120, and D121 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>lsm1-17</td>
<td>pST37</td>
<td>E125, E128, and R129 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-18</td>
<td>pST38</td>
<td>K133 and E134 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-19</td>
<td>pST39</td>
<td>K139 and K141 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-20</td>
<td>pST40</td>
<td>D143, E144, K145, and R146 to A’s, respectively</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-21</td>
<td>pST41</td>
<td>K148, E149, and E150 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-22</td>
<td>pST42</td>
<td>H152, K153, K155, and K156 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-23</td>
<td>pST43</td>
<td>R159 and H160 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-24</td>
<td>pST44</td>
<td>D165, H167, K168, and D170 to A’s</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-25</td>
<td>pST45</td>
<td>N terminus to R17 deleted</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-26</td>
<td>pST46</td>
<td>N terminus to D36 deleted</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lsm1-27</td>
<td>pST47</td>
<td>D118 to C terminus (55 residues) deleted</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lsm1-28</td>
<td>pST48</td>
<td>I139 to C terminus (43 residues) deleted</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lsm1-29</td>
<td>pST49</td>
<td>K145 to C terminus (28 residues) deleted</td>
<td>–/+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lsm1-31</td>
<td>pST18</td>
<td>2× FLAG epitope fusion at the C terminus</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a Growth, no growth, and growth that is significantly slower than wild-type cells are indicated by +, −, and −/+ symbols, respectively.
b Strong (comparable to lsm1Δ) and moderate block to these functions are indicated by + and ++, respectively. +++ indicates normal (wild type) function.
c Mutant Lsm1p protein band was very weak or undetectable (by Western analysis) in the lysates of these alleles.

gesting that this region is not critical for Lsm1p function. Similarly, several other mutations affecting small clusters of charged residues distributed throughout the protein were all tolerated, suggesting that none of these regions by itself is of major importance for the function of Lsm1p.

Finally, although all the strains were able to grow in −ura plates at 25°, differences in growth rates could be observed. As expected, lsm1Δ cells grew much more slowly than the wild-type cells. Further, a subset of the mutants unable to grow at 36° showed slow growth at 25°, similar to lsm1Δ. They were lsm1-7, lsm1-8, lsm1-13, and lsm1-27 (not shown). These strains and lsm1-9 and lsm1-14 were also slow growing in liquid cultures at 25°.

Effect of LSM1 mutations on mRNA decay: A key set of experiments examined the effect of the lsm1 mutations on mRNA decay. For this experiment, we utilized the MFA2pG reporter mRNA system. This mRNA is expressed under the control of the GAL promoter from a chromosomal location in our strains (Hatfield et al. 1996). The MFA2pG mRNA decays by deadenylation-dependent decapping followed by 5’ to 3’ exonucleolytic digestion by Xrn1p (Muhrad et al. 1994). The MFA2pG mRNA also contains a poly(G) insertion in its 3’-UTR that forms a strong secondary structure in vivo and blocks Xrn1p in cis. This results in the stable accumulation of the degradation intermediate called poly(G) fragment in vivo. As a result, in strains where decapping is defective, the accumulation of the poly(G) fragment is decreased. Thus, the relative levels of the poly(G) fragment and the full-length mRNA at steady state in a strain can be used as a first approximation of the efficiency of decapping of this reporter mRNA in that strain (Hatfield et al. 1996; Cao and Parker 2001). Therefore, we first identified the lsm1 mutants defective in mRNA decay on the basis of their inability to accumulate the poly(G) fragment at sufficient levels. This was followed by a direct measurement of the MFA2pG mRNA half-life in such mutants to fully confirm the mRNA decay phenotype.
As shown in Figure 2, fragment levels relative to the full length (quantitated using the PhosphorImager) were very low in \( \text{lsm1}\Delta \) cells (\(~15\%\) of total signal) compared to the wild-type cells (\(~40\%\) of total signal). It should be noted that because both the \( \text{MFA2pG} \) full-length mRNA and the poly(G) fragment accumulate 3’-trimmed species in addition to the normal species (see below) in \( \text{lsm} \) mutants (He and Parker 2001), the intensities of normal and trimmed species were added up to determine the levels of the full-length mRNA and the fragment.

Figure 2 also reveals the strong accumulation in the \( \text{lsm1}\Delta \) strain of the deadenylated full-length \( \text{MFA2pG} \) mRNA. This is characteristic of strains defective in decapping (Beelman et al. 1996; Dunckley and Parker 1999; Tharun et al. 2000).

Examination of the various mutants revealed the following:

First, mutants with lesions in the predicted RNA-binding surface, \( \text{lsm1-8}, \text{lsm1-9}, \) and \( \text{lsm1-14} \) all showed defects in mRNA decay. Each mutant showed a decreased accumulation of the mRNA decay intermediate relative to the full-length mRNA. In addition, all three alleles accumulated deadenylated full-length mRNAs, consistent with a defect in decapping (Figure 2). Moreover, measurement of \( \text{MFA2pG} \) mRNA decay rate in these mutants showed a prolonged half-life, indicating a clear defect in mRNA decapping (Figure 3).

While \( \text{lsm1-8} \) showed a strong phenotype (comparable to \( \text{lsm1}\Delta \)), \( \text{lsm1-9} \) and \( \text{lsm1-14} \) showed a moderate phenotype. These results argue that RNA binding is required for Lsm1p’s role in mRNA decapping.

Second, we observed that mutations in the predicted inter-subunit contact residues, \( \text{lsm1-6}, \text{lsm1-7}, \) and \( \text{lsm1-13} \) all showed defects in decapping as assessed by both the levels of mRNA decay fragment and the accumulation of deadenylated full-length mRNA (Figure 2). Again, measurement of the \( \text{MFA2pG} \) mRNA decay rate in these mutants revealed a prolonged half-life, indicating a clear defect in mRNA decapping (Figure 3). The \( \text{lsm1-7} \) and \( \text{lsm1-13} \) alleles were almost as strong as a null allele, whereas the \( \text{lsm1-6} \) allele showed a moderate phenotype. Thus, these results support the idea that maintenance of inter-subunit contacts is of critical importance for Lsm1p’s function.

Third, we observed that deletions within the C-terminal extension (\( \text{lsm1-27}, \text{lsm1-28}, \) and \( \text{lsm1-29} \)) resulted in a clear inhibition of mRNA decapping as assessed by both the poly(G) fragment levels and the accumulation of deadenylated mRNA (Figure 2). In fact, even fusing a FLAG-peptide at the C terminus of otherwise wild-type Lsm1p (\( \text{lsm1-31} \); Table 2) led to a partial defect in mRNA decapping (Figure 2). These observa-
ever, the alleles lsm1-16 through lsm1-24, which carry lesions in different clusters of charged amino acids residing in the C-terminal domain, failed to show any significant growth or mRNA decay defects, indicating that smaller perturbations in this domain can be tolerated (Table 2).

Fourth, we observed that mutations (lsm1-1 through lsm1-4) or deletions (lsm1-25 and lsm1-26) in the Lsm1p primary sequence that is N-terminal to the Sm domain did not lead to any significant decapping defect as assessed from the accumulation of the poly(G) fragment or deadenylated full-length mRNA (Figure 2 and Table 2). This observation was consistent with these lesions not affecting growth at high temperature (Table 2). Similarly, mutations in the nonconserved region that is located between the Sm motifs I and II (lsm1-11 and lsm1-12) also did not lead to any significant phenotype (Table 1). This suggests that the residues affected in these mutants do not play a major role in Lsm1p function.

**Effect of LSM1 mutations on mRNA-3′ end protection:** We also examined the effect of the lsm1 alleles on the protection of the mRNA 3′ end from a trimming reaction. Specifically, earlier studies (Boeck et al. 1998; He and Parker 2001) showed that in lsm1 through lsm7 mutants several mRNAs accumulate species that are truncated at their 3′ ends by ~10 to 20 nucleotides (“trimmed species”) in addition to the normal full-length species in vivo. This indicates that the Lsm1p–7p complex is involved in protecting mRNA 3′ ends in vivo.

In the case of MFA2pG and PGK1pG mRNAs, poly(G) fragments generated from these mRNAs also accumulate as normal and trimmed species in the lsm mutants (He and Parker 2001). While the trimmed and normal species of full-length mRNA run very close together in the gel, the trimmed poly(G) fragment and normal poly(G) fragment are more easily separable. Therefore, determining the ratio of the two species of the poly(G) fragment is a convenient way to assess the degree of mRNA-3′ end protection occurring in a given strain. This was quantitated using the PhosphorImager from Northern blots containing steady state RNA samples. As seen in Figure 2, wild-type cells had very little trimmed fragment (~10% of total fragment). However, lsm1Δ cells had high levels of trimmed fragment (~55 to 60% of the total fragment levels).

An important result was that all of the lesions that affected mRNA decapping also affected mRNA trimming. Specifically, all the mutants affecting the residues predicted to be involved in RNA and inter-subunit contacts showed significant accumulations of trimmed fragment, with lsm1-7, lsm1-8, lsm1-9, lsm1-13, and lsm1-14 showing strong defects. The mutant lsm1-6 showed a moderate accumulation of the trimmed fragment that is consistent with the moderate decapping defect seen in this mutant. Further, the C-terminal deletion mutants lsm1-27, lsm1-28, and lsm1-29 were further supported by the longer half-life of MFA2pG mRNA in these mutants (Figure 3). These results indicate that the C terminus has an important function in Lsm1p’s role in mRNA decapping.

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**Figure 3.**—MFA2pG mRNA half-life in lsm1 mutants. Cells were grown as described for Figure 2. At log phase, they were collected and resuspended in fresh –ura medium containing glucose but no galactose. Aliquots of culture were removed at specified time intervals (indicated above the lanes) following shift to glucose medium and cells were collected from them. RNA was extracted from these cells and subjected to Northern analysis after separation on polyacrylamide gels as described for Figure 2. Bands were visualized and the intensity of full-length MFA2pG mRNA bands quantitated using the PhosphorImager. Blots were stripped and reprobed for 7S RNA. 7S RNA bands were also quantitated in each lane with the PhosphorImager and those values were used for normalizing the MFA2pG full-length mRNA levels. Calculated half-life values are indicated on the right. lsm1 alleles carried by the strains used for the experiments are indicated on the left.
28, and lsm1-29 and the C-terminal FLAG-tagged allele lsm1-31 also showed higher levels of trimmed fragment. These results demonstrate a strong correlation between the impairment of mRNA decapping and the inability to protect mRNA 3' end from trimming, suggesting that these two functions are related (see discussion).

Expression of mutant Lsm1p proteins: It is possible that some of the mutants described above show a strong phenotype due to their inability to accumulate the mutant Lsm1p adequately. To test this possibility, we compared Lsm1p expression in the lsm1 mutants that are defective in mRNA decay and wild-type cells, by Western analysis of the lysates prepared from the corresponding strains using anti-Lsm1p antiserum. Cells grown in -ura liquid medium at 25°C and collected at log phase were used for making the lysates. As seen in Figure 4, the decay-defective mutants, lsm1-6, lsm1-8, lsm1-9, lsm1-13, and lsm1-14 expressed the mutant protein at levels comparable to wild-type protein. On the other hand, the results showed that the Lsm1p band was very weak or undetectable in the lysates of mutants lsm1-7, lsm1-27, and lsm1-28. At present, it is not clear whether this is due to low accumulation of the mutant Lsm1p in these cells or due to loss of reactivity of the mutant protein with the antibodies as a result of the mutations/deletions (especially in the case of lsm1-27 and lsm1-28 that have large deletions).

Effect of mutations affecting the predicted RNA-contacting surfaces of Lsm1p on the localization of the Lsm proteins to P-bodies: Recent studies have suggested that decapping and the 5' to 3' degradation of mRNA occur in discrete cytoplasmic structures called P-bodies, where the Lsm1p through Lsm7p proteins and many other decay factors including the decapping enzyme are concentrated in both yeast and mammalian cells (Ingelfinger et al. 2002; Van Dijk et al. 2002; Sheth and Parker 2003; Cougnot et al. 2004). It is not known how the Lsm1p through Lsm7p proteins are recruited to the P-bodies, although studies in mammalian cells have suggested that these proteins need to be a part of the Lsm1p–7p complex to be efficiently recruited to the P-bodies. To test this possibility, we performed experiments to determine if lesions in the predicted RNA-binding surfaces of Lsm1p affect the recruitment of either Lsm1p or the other Lsm proteins to the P-bodies.

First, we examined the intracellular localization of the GFP-tagged forms of the mutant proteins Lsm1-9p and Lsm1-14p (which bear lesions in the predicted RNA-binding surface of Lsm1p) or wild-type Lsm1p. We performed the studies with cells subjected to stress (incubation in glucose-free medium for 10 min) since P-bodies are larger and more easily visualized in stressed cells than in cells that are not subjected to stress (Teixeira et al. 2005). We observed that both Lsm1-9p and Lsm1-14p localized to P-bodies just like the wild-type Lsm1p protein (Figure 5). This indicates that the RNA-binding surface of Lsm1p is not absolutely required for the recruitment of Lsm1p to the P-bodies.

Next, we examined how defects in Lsm1p affect the localization of other components of the Lsm1p–7p complex. We examined the localization of Lsm2p-GFP or Lsm7p-GFP in LSM1, lsm1-9, lsm1-14, and lsm1Δ cells that were subjected to stress. We observed that neither Lsm7p-GFP nor Lsm2p-GFP was localized to P-bodies in lsm1Δ cells (Figure 6). However, both Lsm2p-GFP and Lsm7p-GFP localized to P-bodies in the lsm1-9 and lsm1-14 mutants (Figure 6). Therefore, these results suggest that even when the direct contacts between Lsm1p and RNA are impaired, Lsm1p could be recruited to the P-bodies by virtue of its interactions with the other subunits of the Lsm1p–7p complex.

DISCUSSION

The mechanism by which the Lsm1p–7p complex activates mRNA decapping is not known. Since Lsm1p is the distinguishing subunit of this complex, we have analyzed the critical functional features of this protein. Our results support the idea that the functioning of the Lsm1p–7p complex is similar to the other Sm-like protein complexes at a mechanistic level. When the Lsm1p residues that are likely to function in inter-subunit contacts and RNA binding were predicted on the basis of the homology of Lsm1p to other Sm-like proteins (for whom such residues were identified experimentally) and mutagenized, it resulted in mRNA decay defects. This indicates that the organization of such crucial residues is similar between Lsm1p and other
the region C-terminal to the Sm domain led to significant mRNA decay defects (Table 2). Thus, the Lsm1p protein appears to have two critical regions for function.

Several observations indicate that the stretch of amino acids N-terminal to the Sm domain of Lsm1p (residues 1 to 26) is not required for Lsm1p function. First, deletions or point mutations in this region do not affect growth at high temperature (Table 2). Second, they also do not affect mRNA decapping as assessed by the ability to accumulate poly(G) fragment or deadenylated mRNA (Table 2 and Figure 2). Third, this region is almost completely absent in the human homolog of Lsm1p, although the region C-terminal to the Sm domain is reasonably well conserved in the human protein (Figure 1c). In fact, even in fungal species that are very closely related to *Saccharomyces cerevisiae*, the N-terminal extension is not highly conserved.

Our results argue that the C-terminal extension of Lsm1p is required for its function. Lsm1p has a 61-amino-acids-long region C-terminal to the Sm domain that is absent in other members of the Sm-like protein family. The following observations indicate that this region is important. First, deletions in this region (lsm1-27, lsm1-28, and lsm1-29) inhibited mRNA decay (Figures 2 and 3). Although the Western analyses indicate that the mutant (truncated) Lsm1p accumulation may be severely reduced in lsm1-27 and lsm1-28, they reveal normal accumulation of Lsm1-29p, indicating that at least the phenotype of lsm1-29 is attributable to the deletion (Figure 4). Second, the allele lsm1-31, which is a fusion of the FLAG peptide at the C terminus of an otherwise wild-type Lsm1p was also defective in mRNA decay and makes normal levels of the fusion protein (Figures 2, 3, and 4). Importantly, fusion of the FLAG epitope at the N terminus of Lsm1p has no effect on mRNA decay or mRNA 3' end protection (not shown). Finally, all the C-terminal deletion mutants were also defective in mRNA 3' end protection (Figure 2). The functional importance of Lsm1p’s C-terminal extension is also supported by the observation that it is reasonably well conserved in human Lsm1p (Figure 1c).

**Figure 5.**—Determination of the effect of mutations affecting the predicted RNA-binding surface of Lsm1p on the localization of Lsm1p to P-bodies. Yeast cells expressing GFP fusions of *LSM1* or *lsm1-9 or lsm1-14* (indicated on the left) were observed by confocal microscopy as described in MATERIALS AND METHODS.

Sm-like proteins. Further, this is also consistent with the idea that just like the other Sm-like protein complexes, the Lsm1p–7p complex also needs to directly bind to the RNA substrate to act on it (i.e., activate decapping).

Our studies indicate that most of the residues that are crucial for Lsm1p function reside in the conserved regions, Sm motifs I and II, of the Lsm1p protein. Mutations affecting any small set of neighboring residues located outside these regions did not lead to any significant mRNA decay defects, although large deletions in the region C-terminal to the Sm domain led to significant mRNA decay defects (Table 2).
Figure 6.—Determination of the effect of mutations affecting the predicted RNA-binding surface of Lsm1p on the localization of Lsm2p and Lsm7p to P-bodies. Wild-type (LSM1) or mutant (lsm1Δ, lsm1-9, or lsm1-14) cells (indicated on top) expressing GFP tagged versions of either Lsm2p (top) or Lsm7p (bottom) were observed by confocal microscopy as described in MATERIALS AND METHODS.

Our results indicate that the residues of Lsm1p that are predicted (on the basis of homology) to be involved in inter-subunit contacts are indeed crucial for Lsm1p function. This is shown by the significant mRNA decay defect exhibited by the mutants in which these residues are changed (lsm1-6, lsm1-7, and lsm1-13; see Figures 2 and 3). Although Western analyses revealed that the accumulation of the mutant Lsm1p may be severely impaired in lsm1-7, they showed normal levels of accumulation in the case of Lsm1–6p and Lsm1–13p. Since the residues mutated in these alleles are homologous to the experimentally determined inter-subunit contact residues in other Sm-like proteins, these observations are consistent with the idea that homologous regions serve similar functions in the different Sm-like proteins. Importantly, these results suggest that Lsm1p needs to be a part of the Lsm1p–7p complex to execute its function efficiently. Recent studies have identified cytoplasmic structures, referred to as P-bodies, which are the sites of decapping (Sheth and Parker 2003; Cougot et al. 2004). Mutations in human Lsm4p that are predicted to disrupt its interactions with other Lsm proteins inhibit its entry into P-bodies (Ingelfinger et al. 2002). Therefore, it is possible that in lsm1-6 and lsm1-13, the defect in decapping is due to the inefficient targeting of Lsm1p to P-bodies.

Despite the clear importance of the Sm domain for Lsm1p function, mutations in the region that separates the Sm motifs I and II failed to have any effect on mRNA decay. This region corresponds to loop 4 in the modeled tertiary structure of Lsm1p (Figure 1b). Both lsm1-11 and lsm1-12, which bear lesions in this region, were able to grow at 36°C and accumulate wild-type levels of poly(G) fragment (Table 1). This observation is consistent with the fact that neither the primary sequence nor the length of this region is conserved among the members of the Sm-like protein family. Given this, the primary function of this loop may be to provide a structural turn between the third and fourth β-sheets in the core structure.

The Sm and Sm-like protein complexes are known to bind directly to their substrate RNA molecules as shown by several studies (Achsel et al. 1999, 2001; Raker et al. 1999; Toro et al. 2001; Moller et al. 2002; Schumacher et al. 2002). Our studies suggest that in an analogous manner, the Lsm1p–7p complex could also bind directly to mRNA. This is supported by the observation that all the three mutants affected in the residues predicted to play a key role in RNA binding (lsm1-8, lsm1-9, and lsm1-14) are impaired in mRNA decay. This indicates that the ability to directly interact with mRNA is important for the mRNA decapping function of the Lsm1p–7p complex. Further, these results also suggest that the Lsm1p–7p complex is similar to the Sm complex and the other Sm-like protein complexes in the organization of its RNA contacting surfaces and the manner in which it contacts RNA.

Studies in mammalian cells have shown that human Lsm4p cannot be recruited to P-bodies if its interactions with the other subunits of the Lsm1p–7p complex are disrupted by mutations (Ingelfinger et al. 2002). Our results in Figure 6 show that Lsm2p-GFP and Lsm7p-GFP are not concentrated in P-bodies in lsm1Δ cells, indicating that the recruitment of these proteins to P-bodies is dependent on Lsm1p. Thus, together these results support the idea that the recruitment of any of the subunits of the Lsm1p–7p is dependent on the ability of that subunit to be incorporated into the Lsm1p–7p
complex. Therefore, it is likely that the Lsm1p–7p complex is recruited as a unit to the P-bodies. On the other hand, our results also indicate that mutations disrupting the predicted RNA-binding surfaces of Lsm1p do not affect the recruitment of the mutant Lsm1p or the other subunits of the Lsm1p–7p complex to the P-bodies (Figures 5 and 6). This supports the idea that the mutations in \( lsm1-9 \) and \( lsm1-14 \) affect the inter-subunit interactions and, hence, the assembly of the Lsm1p–7p complex only minimally if at all. Since both \( lsm1-9 \) and \( lsm1-14 \) mutants are defective in mRNA decay and 3' end protection, these results further suggest that the Lsm1p can be recruited to the P-bodies by virtue of its interactions with the other subunits of the Lsm1p–7p complex, even when the direct interactions between Lsm1p and RNA are disrupted.

Our results document a strong correlation between the ability of Lsm1p to promote decapping and its ability to protect the 3' end from trimming. The key observation is that all the mutants that are defective in mRNA 3' protection are also defective in mRNA decay (Tables 1 and 2 and Figures 2 and 3). This is consistent with the model that an initial step in the activation of decapping is the interaction of the Lsm1p–7p complex with the 3' end of the mRNA substrate, and the 3' end protection is a consequence of such an interaction.

An unresolved issue is the binding specificity of the Lsm1p–7p complex and where on the mRNA this complex may interact. Several observations support the hypothesis that the Lsm1p–7p complex directly interacts with 3' ends of deadenylated mRNAs in vivo:

i. Co-immunoprecipitation studies indicate that Lsm1p and Lsm5p specifically associate with deadenylated mRNAs in vivo (THARUN AND PARKER 2001a).

ii. The poly(G) decay intermediate of MFA2 polyG mRNA, which lacks >50% of the 5' portion of the mRNA (including the whole of the ORF and a part of the 3'-UTR), also strongly co-immunoprecipitates with Lsm1p. This suggests that the 3'-UTR of this mRNA contains a possible Lsm1p–7p complex-binding site.

iii. The Lsm2p–8p and the Lsm2p–7p complexes that are closely related to the Lsm1p–7p complex bind to the 3' ends of their substrates, the U6 snRNA and snR5, respectively (ACHSEL ET AL. 1999; FERNANDEZ ET AL. 2004).

iv. The fact that several deadenylated mRNAs accumulate as 3' trimmed species (truncated at their 3' ends by ~10 to 20 nucleotides) in cells lacking any of the Lsm1p through Lsm7p proteins is consistent with a lack of protection to the 3' ends of deadenylated mRNAs in these cells.

v. The Sm-like protein complexes in general (including the Lsm2p–8p complex) are known to bind preferentially to U-rich regions of RNA (ACHSEL ET AL. 1999, 2001; RAKER ET AL. 1999; TORO ET AL. 2001; SCHUMACHER ET AL. 2002) and a significant percent-

age of yeast mRNAs contain a short stretch of U residues at their 3' end (GRABER ET AL. 1999). Given the fact that the Lsm1p–7p complex interacts with and affects the decay of multiple mRNAs (THARUN ET AL. 2000; THARUN AND PARKER 2001a), this supports the idea that the Lsm1p–7p complex binds to the 3' ends of deadenylated mRNAs in vivo.

On the basis of these observations, we propose that following deadenylation, the Lsm1p–7p complex directly interacts with the 3' end of the deadenylated mRNAs, which results in the protection of the mRNA 3' end. Further, this interaction also triggers mRNP rearrangement events that ultimately result in the activation of decapping. Importantly, mRNA 3' end protection by itself could be a crucial cellular function of the Lsm1p–7p complex, since it could potentially regulate the rate of 3' to 5' decay of the mRNA. Such a control could be very important in the case of mRNAs whose major mode of decay is the 3' to 5' decay pathway. Consistent with this, the studies of HE AND PARKER (2001) suggest that the temperature sensitivity of \( lsm1 \Delta \) cells is due to the increased susceptibility to (exosome and ski proteins mediated) 3' to 5' degradation of some mRNAs that are essential at high temperature. Thus, for such mRNAs, the Lsm1p–7p complex could have a protective/stabilizing (rather than decay-promoting) function. Although recent studies have suggested that mRNA decay occurs in P-bodies (INGELFINGER ET AL. 2002; VAN DIJK ET AL. 2002; SHETH AND PARKER 2003; COUGOT ET AL. 2004), it is not known what fraction of cellular mRNAs degrade outside the P-bodies. Interestingly, the ski and exosomal proteins (which mediate the 3' to 5' mRNA decay) are not concentrated in P-bodies but are uniformly distributed (ALLMANG ET AL. 1999; ZANCHIN AND GOLDFARB 1999; VAN HOOF ET AL. 2000; SHETH AND PARKER 2003) in the cytoplasm, suggesting that 3' to 5' decay is likely to occur outside the P-bodies. Thus, the major outcome of Lsm1p–7p binding could be different for mRNAs that degrade outside (stabilization) and inside (activation of decapping and decay) the P-bodies.

This work was supported by funds from the National Institutes of Health (GM45443) and the Uniformed Services University of the Health Sciences (C071G1).

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Communicating editor: S. Sandmeyer