Lsm Proteins Are Required for Normal Processing and Stability of Ribosomal RNAs*

Joanna Kufel, Christine Allmang‡, Elisabeth Petfalski, Jean Beggs, and David Tollervey§

From the Wellcome Trust Centre for Cell Biology, Swann Building, King’s Buildings, University of Edinburgh, Edinburgh EH9 3JR, United Kingdom

Received for publication, August 29, 2002, and in revised form, October 30, 2002
Published, JBC Papers in Press, November 15, 2002, DOI 10.1074/jbc.M208856200

Depletion of any of the essential Lsm proteins, Lsm2–5p or Lsm8p, delayed pre-rRNA processing and led to the accumulation of many aberrant processing intermediates, indicating that an Lsm complex is required to maintain the normally strict order of processing events. In addition, high levels of degradation products derived from both precursors and mature rRNAs accumulated in Lsm-depleted strains. Depletion of the essential Lsm proteins reduced the apparent processivity of both 5′ and 3′ exonuclease activities involved in 5.8S rRNA processing, and the degradation intermediates that accumulated were consistent with inefficient 5′ and 3′ degradation. Many, but not all, pre-rRNA species could be coprecipitated with tagged Lsm3p, but not with tagged Lsm1p or non-tagged control strains, suggesting the absence of direct interaction with an Lsm2–8p complex. We propose that Lsm proteins facilitate RNA protein interactions and structural changes required during ribosomal subunit assembly.

The yeast 18S, 5.8S, and 25S rRNAs are transcribed by RNA polymerase I as a single precursor, the 35S pre-rRNA, which undergoes complex post-transcriptional processing to remove the external transcribed spacers (5′-ETS and 3′-ETS) and internal transcribed spacers (ITS1 and ITS2) to release mature rRNAs (see Fig. 1A). This process involves multiple endonucleolytic and exonuclease steps (see Fig. 1B) and is largely carried out in the nucleolus. In Saccharomyces cerevisiae, enzymes directly involved in these reactions include the endonucleases Rnase MRP and Rnt1p, the 5′ → 3′ exonuclease Rat1p, and 3′ → 5′ exonucleases, including the exosome complex, Rnx1p, and Rnx2p (1, 2; reviewed in Refs. 3 and 4). In addition to the RNA processing enzymes, around 110 other factors are known to be required for normal pre-rRNA processing in yeast. These include several small nucleolar ribonucleoprotein (snRNP) particles, putative RNA helicases, GTPases, and many other assembly factors (5). It is very likely that these act to promote correct folding of the pre-rRNA, assembly of the ~80 ribosomal proteins, and assembly/disassembly of the processing complexes, with processing inhibition arising as a secondary consequence of defects in the structure of the pre-ribosomal particles (see Refs. 3 and 4).

All of the enzymes known to process the pre-rRNA also process other RNA species. Rnt1p, the exosome, and Rex proteins generate the 3′-ends of small nuclear and small nucleolar RNAs (snRNAs and snoRNAs), whereas Xrn1p and Rat1p produce 5′-ends of intron-encoded and polycistronic snoRNAs (2, 6–13). Likewise, degradation of many RNAs, including cytoplasmic messenger RNAs (mRNAs) and nuclear pre-mRNAs, involves pre-rRNA processing exonucleases: the exosome, Xrn1p, and Rat1p (14–18).

Sm-like (Lsm) proteins have been identified in all kingdoms of life and participate in numerous RNA processing and degradation pathways. The Sm and Lsm complexes are all likely to form similar structures with seven-membered rings (or six in the case of Escherichia coli Hfp) with a central hole, through which the RNA may pass (19–24). An Lsm2–8p complex associates with U6 snRNA and is important for U6 accumulation, U6 snRNP biogenesis, and pre-mRNA splicing (19, 25–29). A complex of Lsm1–7p functions in cytoplasmic mRNA degradation, promoting mRNA decapping and 5′ degradation, probably via interactions with the decapping enzymes, Dcp1p and Dcp2p, and the 5′ → 3′ exonuclease Xrn1p (30–33). The Lsm1–7p complex also protects mRNA 3′-ends from premature degradation by the exosome complex of 3′ → 5′ exonucleases (34). Yeast Lsm proteins additionally associate with precursors to RNase P RNA, suggesting a direct role in its processing (28), and with tRNA precursors (35) but not with the mature RNase P RNA or the related MRP RNA (28). Depletion of the Lsm proteins does not reduce the accumulation of the mature MRP or P RNAs, or the accumulation of any small nucleolar RNA (snoRNA) tested (27).

Sm-like proteins from Bacteria and Archaea have been shown to form homomeric ring structures (21, 23, 24) indicating that their general functions are universally conserved. The E. coli proteins are known to facilitate RNA-RNA interactions (23, 24), whereas an Archaeal Sm-like protein associates with the RNase P RNA, and its gene is located in an operon with a ribosomal protein, suggesting a role in ribosome synthesis or function (21). Here, we report that yeast Lsm2–8p are required for maintenance of the normal order of pre-rRNA processing steps and the stability of both the pre-rRNAs and rRNAs.

MATERIALS AND METHODS

Strains—Growth and handling of S. cerevisiae were by standard techniques. The transformation procedure was as described previously (36). Yeast strains used and constructed in this study are listed in Table

* This work was supported by the Wellcome Trust. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Present address: Institut de Biologie Moleculaire et Cellulaire, UPR 9002 du CNRS, 15, rue R. Descartes, 67084 Strasbourg Cedex, France.
§ To whom correspondence should be addressed. Tel.: 44-131-650-7092; Fax: 44-131-650-7040; E-mail: d.tollervey@ed.ac.uk.
1 The abbreviations used are ETS, external transcribed spacer; ITS, internal transcribed spacer; snoRNP, small nucleolar ribonucleoprotein; snRNP, small nuclear ribonucleoprotein; snRNA, small nuclear RNA; snRNA, small nuclear RNA; Lsm, Sm-like protein; Ab, antibody; HA, hemagglutinin; WT, wild-type; MRP, mitochondrial RNA processing; TAP, tandem affinity purification; PAP, peroxidase-anti-peroxidase; KB, kilobase.
I. Strain YJK34 was constructed by PCR strategy as described (37); construction was confirmed by PCR analysis, and expression of TAP-Lsm3p was tested by Western blotting using FAP antibodies.

RNA Extraction, Northern Hybridization, and Primer Extension—For depletion of the essential Lsm proteins, cells were harvested at intervals following a shift from RSG medium (1% Bacto-peptone, 2% galactose, 2% sucrose, 2% raffinose), or YPGal medium for 8.5 h. The isogenic WT strain (YJK53) was grown in the Lsm3p-depleted strain, leading to substantial accumulation of the 35S pre-rRNA (Fig. 2A, lanes 7–12). This was accompanied by a delay in the synthesis of the 27S and 20S pre-rRNAs. Little 27SApre-rRNA was synthesized in the mutant, so the 27SB pre-rRNA was presumably generated largely by cleavage at sites A3 and B11. Mature 18S and 25S rRNAs were synthesized with considerable retardation, but their relative ratio was not clearly altered. Synthesis of the mature 5.8S rRNA was also strongly retarded in the GAL::lsm3 strain (Fig. 2B). The aberrant 23S RNA was detected together with other aberrant RNA intermediates (marked with asterisks in Fig. 2, A and B; see Fig. 1C for the identities of aberrant pre-rRNA species seen in Lsm-depleted strains). We conclude that the depletion of Lsm3p inhibits pre-rRNA processing. The 35S pre-rRNA was present even after 60-min chase (Fig. 2A, lane 12) and in other experiments was shown to persist at 2-, 4-, 6-, and 12-h chase time points (data not shown). This is probably a consequence of degradation of the labeled pre-rRNA and rRNAs in the Lsm3p-depleted strain, with subsequent re- incorporation of the labeled nucleotides, which cannot be chased by exogenous uracil. As previously reported (35), synthesis of tRNAs was also retarded in the strain depleted of Lsm3p.

Pre-rRNA processing was analyzed in more detail by Northern hybridization (Figs. 3–5). The essential Lsm proteins, Lsm2–5p and Lsm8p, were placed under GAL control (strains GAL::lsm2, GAL::lsm3, GAL::lsm4, GAL::lsm5, and GAL::lsm8) (27) and depleted by transferring the strains from permissive RSG medium (0-h samples) to repressive glucose medium. The genes encoding non-essential Lsm proteins, Lsm1p and Lsm6–7p, were deleted, giving rise to temperature-sensitive strains (lsm1Δ, lsm6Δ, and lsm7Δ) (27), which were grown in glucose medium at 23°C (0-h samples) and transferred to the non-permissive temperature of 37°C. Depletion of the essential Lsm proteins leads to depletion of the U6 snRNA and the inhibition of pre-mRNA splicing (19, 25–29). Inhibition of RNA processing in lsm strains can potentially arise as a result of the splicing defect. Many r protein genes contain introns, and reduced r protein synthesis leads to the inhibition of ribosome synthesis (42, 43). We therefore compared the Lsm-depleted strain to strains depleted of known pre-mRNA splicing factors Prp45p (data not shown) (45) and Syf3p (Figs. 3 and 5) (44). Clear pre-rRNA processing defects were seen in the GAL::lsm2 to lsm5 and GAL::lsm8 strains at early times of depletion (shown for GAL::lsm3 in Fig. 3, the complete set of strains are shown for low molecular weight pre-rRNAs in Fig. 5).
5). Even on galactose medium some elevation was seen in the level of the 35S pre-rRNA in the \textit{GAL}::\textit{lsm3} strain, but other processing intermediates were present at wild-type levels. Processing was clearly defective 6 h after transfer to glucose medium, before the appearance of any detectable growth defect (27, 35, and data not shown). The 35S primary transcript was further elevated, accompanied by the appearance of the aberrant 23S RNA and depletion of the 27SA2 and 20S pre-rRNAs (Fig. 3, A–C). This phenotype is characteristic of the inhibition of pre-rRNA processing at sites A0 to A2. The level of the 27SB pre-rRNA was less strongly reduced at 6 h but was clearly reduced at later time points (Fig. 3D), as were the mature 25S and 18S rRNAs (Fig. 3E).

In addition to the loss of the normal pre-rRNA processing intermediates, there was a dramatic accumulation of aberrant pre-rRNAs in the \textit{Lsm3p} and other \textit{Lsm}-depleted strains. These included the 23S and 21S RNAs, which have been seen in several other pre-rRNA processing mutants, as well as many unusual intermediates, some of which are indicated in Fig. 1C.
21S species and reduced levels of 27S 20S pre-rRNAs. How-
abundant truncated products (Fig. 4, B and D). In contrast, much less depletion of the 5.8S rRNA was seen (Fig. 4E). Because 25S and 5.8S are cosynthesized and base-paired, this shows that the loss of mature 25S is due largely to post-synthesis degradation, rather than impaired synthesis.

The identities of the 18S rRNA breakdown products were examined in more detail using probes against different regions of the 18S rRNA (Fig. 4D, lanes 11–14). The most 5’ probe (008) detects products that are 3’-truncated but not 5’-truncated. Probes located at positions 186 and 668 from the 5’-end of 18S (011 and 030) detect both 5’- and 3’-truncated RNAs. This analysis shows that the majority of the 18S rRNA is degraded 3’→5’ with minor degradation occurring in the 5’→3’ direction.

The exosome complex functions in the degradation of pre-rRNA spacers and aberrant pre-rRNA intermediates (51, 52) and is a likely candidate to also degrade the rRNAs. Processing of the 3’-end of the 5.8S rRNA involves the exosome (53), and the mature 5.8S rRNA may therefore be a poor substrate for degradation. We conclude that depletion of any essential Lsm protein provokes the degradation of both pre-rRNAs and ma-

Fig. 4. Deletion of Lsm3p leads to degradation of the mature rRNAs. The wild-type strain (WT, lanes 1–3) and GAL-lsm3 strain (lanes 4–11) were grown in permissive RSG medium (0 h) and transferred to repressive, glucose medium at 30 °C for the times indicated. Probe names are shown in parentheses. RNA species are indicated. A and B, hybridization with the 25S 5’ probe (oligonucleotide 007: position +40). C and D, hybridization with the 18S rRNA 5’ probe (oligonucleotide 008: position +34). Oligonucleotides 011, 030, and 029 are complementary to the 18S rRNA at positions further 3’ (+178, +668, and +1785, respectively). These are shown in panel D as lanes 12–14 for the GAL-lsm3 strain 30 h after transfer to glucose medium. E, hybridization with 5.8S rRNA probe (oligonucleotide 017). Comigrating bands are indicated by dashes between lanes.

Fig. 5. Normal 5’ and 3’ processing of 5.8S rRNA requires Lsm proteins. A–F, Northern analysis of 5.8S rRNA processing in lsm mutant strains. RNA was separated on a 6% polyacrylamide gel and hybridized with oligonucleotide probes. Probe names are indicated in parentheses on the left. RNA species are shown between two columns. Strains carrying GAL-regulated constructs (GAL::lsm, lanes 3–17) and the BMA64 wild-type strain (WT, lanes 1–2) were grown in permissive RSG medium (0 h) and transferred to repressive, glucose medium at 30 °C for the times indicated. Strains deleted for Lsm1p (lanes 26–29), Lsm5p (lanes 20–22), and Lsm7p (lanes 23–25) and the BMA64 wild-type strain (WT, lanes 18 and 19) were pre-grown at 23 °C (0 h) and transferred to 37 °C for the times indicated. Probes are: A, oligonucleotide 033, complementary to 5’-ETS around position +278; B, oligonucleotide 002, complementary to ITS1 upstream of site A; C, oligonucleotide 003, complementary to ITS1 upstream of site A; D, oligonucleotide 001, complementary to ITS1 downstream of site A; E, oligonucleotide 020 complementary to the 5.8S-ITS2 boundary; F, oligonucleotide 017, complementary to the mature 5.8S. G–I, comparison of low molecular weight pre-rRNA processing and accumulation of unspliced pre-U3 RNA (U3-int) and mature U3 in GAL::lsm3 (lanes 1–5) and GAL::syf3 strains (lanes 6–10). Strains were grown and RNA was prepared as described for Fig. 4.
ture rRNAs. Degradation of mature tRNAs and tRNA precursors was also observed in the strains lacking Lsm proteins, particularly the essential Lsm2–5p or Lsm8p (35).

Depletion of the Essential Lsm Proteins Inhibits 5’ and 3’ Processing of 5.8S rRNA and Exosome-mediated Spacer Degradation—Consistent with the high molecular weight RNA analysis, dramatic accumulation of aberrant precursors to the 5.8S rRNA was seen in strains depleted for any of the essential Lsm proteins (Fig. 5). The 27S pre-rRNA is 5’-processed at two closely located sites, B11, and B18, prior to cleavage at site C2 to generate the 7Sb and 7Sa pre-rRNAs, the major 3’-extended precursor to 5.8S rRNA (see Fig. 1B). The 7S pre-rRNA is 3’-processed by the exosome complex (53–55). This reaction normally proceeds with high processivity but is multistep generating, successively, the 5.8S+30 pre-RNAs and 6S pre-rRNAs. Processing of 5.8S+30 specifically requires the Rrp6p component of the exosome.

Northern hybridization with a probe specific for the 3’-extended 5.8S species (probe 020; Fig. 5) showed that depletion of any essential Lsm protein (Fig. 5A, lanes 3–17) resulted in some decrease in the level of the 7S pre-rRNA and mature 5.8S (Fig. 5F), and the loss of the 5.8S+30 and 6S pre-rRNAs. Aberrant species intermediates in size between 7S and 5.8S+30 were also accumulated. The most abundant of these comigrated with RNAs observed in exosome mutants (data not shown) (11), consistent with inhibition of the exosome complex in the Lsm-depleted cells. Less striking effects were seen in strains lacking the non-essential proteins, Lsm1p, 6p, or 7p, but the 7S pre-rRNA was clearly reduced in lsm6Δ strain (Fig. 5A, lanes 20–28). Growth inhibition in the lsm6Δ and lsm7Δ strains at 37 °C is much more rapid than in the GAL::lsm3 and GAL::syf3 strains, and their growth had ceased by 10 h.

In addition to processing the 3’-end of the 5.8S rRNA, the exosome normally degrades the 5’-ETS region of the pre-rRNA from site A2 to the 5’-end of the transcript (51). In strains depleted of the essential Lsm proteins, the 5’-ETS-A2 fragment accumulated together with shorter fragments (Fig. 5A, lanes 3–17). Weaker accumulation of intermediates was seen in strains lacking Lsm6p or Lsm7p (Fig. 5B, lanes 20–25). The degradation remains predominately 3’→5’, as indicated by the cut-off in the signal at the fragment size corresponding to the position of the probe with respect to the 5’-end of the RNA. A fragment extending from the 5’-end of the transcript to site A2 also accumulated in the Lsm-depleted strain. We conclude that the activity of the exosome on both the 7S pre-rRNA and the excised 5’-ETS region is inhibited in Lsm-depleted strains.

In addition to the species below 7S, the Lsm-depleted strains also accumulated larger 5.8S-related RNA species. The largest species detected with probe 020 (Fig. 5A) has the gel mobility and hybridization pattern expected for a species that extends from site A2 in ITS1 to site C2, the 3’-end of the 7S pre-rRNA (see Fig. 1C for schematics of aberrant processing intermediates detected in lsm mutant strains). Northern analyses with probes hybridizing 5’ to 5.8S (003 and 001; Fig. 5, D and E), identified RNAs that extend from sites A2 and A3, within ITS1, to site E (the 3’-end of the 5.8S rRNA). Accumulation of the A2–C2, A2–E, and A3–E fragments was also observed in the strains deleted for the non-essential proteins Lsm6 and 7p (Fig. 5, D and E, lanes 20–25) even under the permissive conditions at 23 °C. Notably, the A3–E species did not visibly accumulate in the GAL::lsm8 strain (Fig. 5E, lanes 15–17) but was clearly accumulated in the lsm1Δ strain (Fig. 5E, lanes 26–28), indicating differences in Lsm protein requirements. It is therefore possible that some spacer fragments, including A3–E, are exported to the cytoplasm and degraded by a cytoplasmic pathway.

The appearance of the A2–C2, A2–E, and A3–E species shows that processing in ITS2 had occurred prior to the completion of processing in ITS1. We conclude that the normally strict order of processing is disorganized in the Lsm-depleted strains. Further evidence for disruption of the normal order of processing came from the detection of intermediates that appear to extend from site D (the 3’-end of the 18S rRNA) to site B1, encompassing the entire ITS1 region (Fig. 5, D–F). Fragments extending from site D to sites A9 and A9 within ITS1 also accumulated in the lsm2–8 mutants.

The processing of 5.8S precursors was also examined in the GAL::ppr45 (data not shown) and GAL::syf3 strains (Fig. 5, G and H). Depletion of Syf3p also affected the processing of 5.8S rRNA, with reduced levels of the 7S and 6S pre-rRNAs, but no accumulation of aberrant precursors was observed in Syf3p-depleted cells. The defects in splicing activity, as assessed by the level of pre-U3, were comparable in the GAL::lsm3 and GAL::syf3 strains (Fig. 5I).

Depletion of Lsm Proteins Inhibits 5’ Processing of 5.8S rRNA—The 5’-end of the major, short form of the 5.8S rRNA (5.8Sc) is generated by 5’→3’ digestion from site A9. This is carried out by two exonucleases, Xrn1p and Rat1p, with the major activity probably provided by Rat1p (46). Primer extension was performed on the GAL::lsm3 and GAL::lsm8 strains (shown for GAL::lsm3 in Fig. 6; identical results were seen for GAL::lsm8). Primer extension from within the 5.8S rRNA showed the existence of 5’-extended forms (Fig. 6E). The 3’-region of the ITS1 is stably base-paired to the 5’-region of the 5.8S rRNA (see Fig. 6F) (56), and the major stop observed in the Lsm3p-depleted strain lies 3 nucleotides away from the base of this stem (arrows in Fig. 6, E and H), consistent with a role for the Lsm complex in facilitating progression of the 5’ exonucleases into the base-paired region. A somewhat different pattern of stops was seen in the xrn1Δ::RAT1-1 strain (Fig. 6E, lane 6), but this pattern is partially allele-specific because the combination of another RAT1 allele, tap1-1, with xrn1Δ did not yield an identical pattern (46). As in the exonuclease mutants, the ladder extended to the A3 cleavage site in the Lsm-depleted strains. This site was accurate at the nucleotide level, as were other cleavage sites tested; A10, A1, A2, B11, and B18 (Fig. 6, A–F).

The pre-rRNA primary transcript is cleaved in the 3’-ETS by the endonuclease Rnt1p at sites 14 and 49nts 3’ to the mature 25S (site B0) generating the 35S pre-rRNA (1, 57). These cleavages can be detected by primer extension on the cleaved 3’-fragment and were unaffected in the Lsm3p and Lsm8p-depleted strains (Fig. 6G, lanes 2 and 3; and data not shown). The cleaved fragment is stabilized by mutation of Rat1p (Fig. 6G, lane 4) allowing the identity of the stops to be confirmed.

We conclude that the Lsm proteins are required for the normal, highly processive activities of both 5’→3’ and 3’→5’ exonucleases in 5.8S rRNA processing, but not for the specificity of endonucleases.

Lsm3p Is Associated with Pre-rRNAs—To test for a physical association of the Lsm complex with pre-rRNAs, immunoprecipitation was performed using a strain expressing Lsm3p with a C-terminal, tandem affinity purification (TAP) tag, under the control of the endogenous promoter (31, 37). As a control, precipitation was also performed using a previously described strain expressing HA-tagged Lsm1p (27).

Low molecular weight RNAs were separated on a polyacrylamide gel and visualized by Northern hybridization (Fig. 7, A–G). The 7S pre-rRNA was clearly recovered in the immunoprecipitate from the TAP-Lsm3 strain (Fig. 7A, lane 4) but not from an otherwise isogenic wild-type strain (Fig. 7A, lane 3) or from the HA-Lsm1 strain (Fig. 7A, lane 7). In contrast, the
Primer extension analysis through ITS1 in oligonucleotide 008, which hybridizes within mature 18S, activity but does not prevent accurate pre-rRNA cleavage.

Primer extension analysis through 5'ETS in oligonucleotide 013 (C), which hybridizes within ITS2.

Coligonucleotide 017 (E), which hybridizes within mature 5.8S and at sites B1L and B1S.

Position strain using oligonucleotide 053, which hybridizes downstream of lsm3 and transferred to 37°C and for 2 and 3 h, respectively.

Fig. 6. Depletion of Lsm proteins inhibits 5' exonuclease activity but does not prevent accurate pre-rRNA cleavage. A and B, primer extension analysis through 5'ETS in GAL-lsm3 strain using oligonucleotide 008, which hybridizes within mature 18S. A, primer extension stop at site A0. B, primer extension stop at site A1. C-F, primer extension analysis through ITS1 in GAL-lsm3 strain using oligonucleotide 017 (C-E), which hybridizes within mature 5.8S and oligonucleotide 013 (F), which hybridizes within ITS2. C, primer extension stop at site A0. D, primer extension stop at site A1. E, primer extension through ITS1 until stop at site A1. F, primer extension stops at sites B1L and B1S. Panel D represents a longer exposure of the same gel as panel E. G, primer extension analysis through 3'ETS in GAL-lsm3 strain using oligonucleotide 053, which hybridizes downstream of position +180. Rnt1p cleavage sites at positions +49 and +14 are indicated. H, predicted secondary structure of the stem-loop structure formed in the 3' region of ITS1. The major primer extension stop detected in the GAL-lsm3 strain is located three nucleotides away from the base of the stem and is indicated with an arrow (E and H). RNA from wild-type (WT) and GAL-lsm3 strains was prepared as described for Fig. 4. The rat1-1 and xrn1-1Δrat1-1 strains were pre-grown at 23°C and transferred to 37°C for 2 and 3 h, respectively.

High molecular weight RNAs were analyzed by primer extension (Fig. 7, G–P). Several pre-rRNAs tested (35S, 33S, 27SA0, 27SAp, 27SBp, and 26S) were detectably coprecipitated with TAP-Lsm3. Most of these species were not coprecipitated with HA-Lsm1 above the background in the wild-type control strains, although some coprecipitation was seen for the 35S pre-rRNA. The significance of this is unclear. The 20S pre-rRNA is reported to undergo late dimethylation at A1779 and A1780 after export to the cytoplasm (59), which can be detected by primer extension analysis (Fig. 7P). The dimethylated pre-rRNA was coprecipitated with TAP-Lsm3, but weaker coprecipitation was also seen for HA-Lsm1. Accumulation of the 20S pre-rRNA was also seen in the lsm1-Δ strain (data not shown). Taken together, these results suggest the association of Lsm1p with cytoplasmic pre-ribosomes.

The yield of coprecipitated pre-rRNA was low but comparable to results reported for other proteins associated with pre-ribosomal particles (60, 61) and to the efficiency of precipitation of deadenylated mRNAs (Ref. 32 and Fig. 7G). These results indicate that Lsm3p transiently associates with pre-ribosomal particles, presumably as a component of an Lsm complex.

DISCUSSION

Ribosome synthesis is a highly complex process that requires 80 r proteins and 5 kb of rRNA, as well as ~140 non-ribosomal protein factors and ~100 snoRNPs. Moreover, the very compact structures of the mature ribosomal subunits revealed by recent structural studies (62–64) appear to be incompatible with ribosomal protein assembly and, particularly, with binding of the many snoRNPs to the mature rRNA regions. Efficient assembly and disassembly of the ribosome synthesis machinery is therefore certain to involve extensive structural reorganization and to require many cofactors. Numerous yeast proteins, identified via defects in pre-rRNA processing and/or defects in export of ribosomal subunits to the cytoplasm, do not appear to function directly in pre-rRNA cleavage. In addition to the Lsm proteins discussed here, these include at least 17 putative ATP-dependent RNA helicases, four GTPases, and an AAA-ATPase (3, 4, 60, 65, 66). All of these are predicted to function in the organization of the RNP structure of the ribosomal subunits.

Here we report that normal maturation of rRNAs also requires the activity of Lsm proteins. Depletion of the essential proteins, Lsm2–5p or Lsm8p, resulted in severe defects in pre-rRNA processing and stability. Milder effects were seen in strains lacking the non-essential proteins Lsm6p or Lsm7p, and it may be that these can be partially replaced by Lsm1p or other Sm-like proteins. The accumulation of some aberrant pre-rRNAs was clearer in the absence of Lsm1p than following smaller 6S and 5.8S+30 pre-rRNAs (Fig. 7B, lane 4), the pre-5S rRNA (Fig. 7D) and mature MRP RNA (Fig. 7F) were recovered at the same levels as in the non-tagged strain. The mature 5.8S and 55 rRNAs were coprecipitated with both TAP-Lsm3 and HA-Lsm1 (Fig. 7, C and E), presumably reflecting the reported association of Lsm1–7p with polysomes (58). The Lsm1–7p complex is reported to associate with deadenylated mRNAs to promote decapping and 5'→3' degradation (32, 33, 58). Consistent with this, both TAP-Lsm3 and HA-Lsm1 coprecipitated the SMX3 mRNA (Fig. 7G) with enrichment for the shorter, deadenylated form.
Lysates from (YJV140) were immunoprecipitated with rabbit IgG-agarose beads (Sigma) of RNAs from strain expressing TAP-tagged Lsm3p and HA-tagged Lsm1p. Lysates from (YJV140) were immunoprecipitated with rat monoclonal anti-HA Ab bound to Protein G-agarose. RNA was recovered from the lysate and the pellet fractions compared with the total fraction. Probes are: 266 complementary to the pre-5S; E, oligonucleotide 041 complementary to the mature 5S; F, oligonucleotide 031 complementary to the mature MRP RNA; G, oligonucleotide 471 complementary to SMX3 mRNA; H–J, oligonucleotide 008 complementary to the mature 18S; K and L, oligonucleotide 007 complementary to the mature 25S; M and N, oligonucleotide 006 complementary to ITS2; O, oligonucleotide 002 complementary to ITS1 upstream of site A2. The primer extension stops in panel P correspond to the 18S rRNA nucleotides m2A1779 and m2A1780, which are reported to be modified in the cytoplasmic 20S pre-rRNA.

Pre-rRNA Processing in lsm Mutants

An obvious question is the degree to which the observed effects are direct? Because the Lsm proteins are involved in both mRNA degradation and pre-mRNA splicing, indirect effects are certainly a possibility. However, strains carrying the lsm1-Δ mutation are impaired in mRNA decapping and degradation, whereas the Lsm6p and Lsm7p are required for the normal stability of U6, but these mutations do not strongly inhibit pre-rRNA processing, suggesting that the Lsm complex involved in pre-rRNA processing is distinct from these activities. Cells defective in pre-mRNA splicing are expected to show defects in pre-rRNA processing due to reduced synthesis of ribosomal proteins. Indeed, the original splicing mutants were identified on the basis of their reduced total RNA synthesis (67). It is very likely that reduced ribosomal protein synthesis contributes to the reduction in rRNA synthesis in the GAL::lsm strains. However, the accumulation of aberrant intermediates and degradation products appears specific for Lsm-depleted cells. Similar phenotypes were not seen on depletion of the splicing factors Syt3p and Prp45p or in strains carrying the temperature sensitive prp2-1 mutation. Cells mutant for cytoplasmic mRNA degradation factors, the decapping enzyme Dcp1p, the 5' → 3' exonuclease Xrn1p, and the exosome complex also failed to show similar defects in pre-rRNA processing. Moreover, pre-rRNA processing defects were observed in the GAL::lsm3 strain 6 h after transfer to glucose medium, well before growth was inhibited (27, 35).

Several pre-rRNA species were coprecipitated with tagged Lsm3p but not with tagged Lsm1p or in non-tagged control strains. These included the 35S, 33S, 27SA2, 27SA3, 27SB, and 7S pre-rRNAs but not the 5.8S+30 or 6S pre-rRNAs. These observations indicate the direct interaction of a nuclear Lsm2–6p complex with pre-ribosomes. Tagged Lsm1p coprecipitated the dimethylated, cytoplasmic form of the 20S pre-rRNA and the lsm1-Δ strain showed some 20S accumulation, suggesting that it may associate with a late cytoplasmic pre-40S particle. Direct roles for Lsm proteins in ribosome synthesis are supported by several two-hybrid interactions (50, 68). Lsm1p, Lsm2p, and Lsm8p each interacted with the exosome component Mtr3p. Lsm2–6p and 8p (but not Lsm6 or 7p) were each reported to interact with ribosomal proteins. In a recent proteomic analysis Lsm8p was found also to associate with another component of the exosome, Rrp42p (69).

During synthesis of the 3'-end of 5.8S rRNA, the 5.8S+30 and 6S pre-rRNAs are generated by rapid, processive 3' → 5' exonuclease digestion by the exosome complex from site C3, the 3'-end of the 7S pre-rRNA. In strains depleted of any essential Lsm protein the 5.8S+30 and 6S pre-rRNAs were lost and a ladder of 3'-extended species appeared, with accumulation of the same species seen in exosome mutants. The ends of these RNAs correspond to sites that are required for ITS2 processing, which may therefore represent protein binding sites (70, 71). This suggests that the Lsm complex aids the displacement of bound proteins during 7S processing. Degradation of the excised 5'-ETS region by the exosome was also slowed by depletion of the essential Lsm proteins.
Processing of the 5′-end of the 5.8S rRNA by 5′ → 3′ exonucleases Rat1p and Xrn1p is normally highly processive, and intermediates in processing are not observed in wild-type strains. In contrast, processing intermediates were readily detectable by primer extension in strains depleted of Lsm3 or Lsm6p, showing that the processivity of 5′ processing was reduced. Northern hybridization indicated that this was also the case in strains depleted of the other essential Lsm proteins. The major nuclease pause site was positioned 3 nucleotides from the base of a stable stem-structure (56), suggesting that the exonucleases are less able to penetrate into the base-paired region in the absence of the Lsm complex.

In wild-type cells pre-rRNA processing sites are used in strict order, generating the predominant pathway shown in Fig. 1B. In Lsm-depleted cells a series of intermediates were detected that are absent or extremely rare in wild-type strains, some of which are shown in Fig. 1C. We interpret this as evidence that the Lsm complex helps maintain the normal order of use of the pre-rRNA processing sites, perhaps as a consequence of helping to establish the correct RNP structure of the pre-ribosomal particles. We cannot, however, exclude the possibility that at least some of these “aberrant” pre-rRNAs are normal products of the processing pathway, which have escaped detection in wild-type cells due to very rapid degradation. Indeed, the Lsm−E and Lsm−E species are detectable at very low levels in wild-type strains. These species also showed some elevation in the strain lacking Lsm1p, suggesting that they can be exported as components of a pre-60S particle and degraded in the cytoplasm. For none of the stable RNA species do we have accurate information on the discard rate during in vivo processing. However, the overexpression of several stable RNA species does have a significant effect during in vitro processing. Indeed, the Lsm−E and Lsm−E species are detectable at extremely low levels in wild-type strains. These species also showed some elevation in the strain lacking Lsm1p, suggesting that they can be exported as components of a pre-60S particle and degraded in the cytoplasm. For none of the stable RNA species do we have accurate information on the discard rate during in vivo processing. However, the overexpression of several stable RNA species does have a significant effect during in vitro processing. Indeed, the Lsm−E and Lsm−E species are detectable at extremely low levels in wild-type strains. These species also showed some elevation in the strain lacking Lsm1p, suggesting that they can be exported as components of a pre-60S particle and degraded in the cytoplasm.

Although the basis of the degradation of the mature rRNAs is not clear. The ribosomes may sustain damage in folding or RNP structure during normal use, which requires a chaperone function for its repair. Alternatively, the ribosomal subunits synthesized in the Lsm protein-depleted strains may be partially misassembled such that they are labile to subsequent degradation. The strain lacking the nuclear Lsm6p protein showed substantial rRNA degradation, whereas mild effects were seen in the strain lacking the largely cytoplasmic Lsm1p. We therefore favor the latter model. In either case, we assume that the strong accumulation of the degradation intermediates is enhanced by reduced degradation activity in the Lsm mutant strains.

Characterized functions of eukaryotic Lsm proteins in U6 snRNA stability and mRNA decapping are not conserved to Archaea and are most unlikely to represent their ancestral functions. An Archaeal Sm-like protein is encoded by a gene located in a putative operon with ribosomal protein L37e (21) and is associated with RNase P (22), which itself participates in pre-rRNA processing. The original Sm-like proteins are likely to have arisen as factors involved in the synthesis of rRNAs, tRNAs, and other small stable RNA species.

Acknowledgment—We thank Phil Mitchell for critical reading of the manuscript.

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Lsm Proteins Are Required for Normal Processing and Stability of Ribosomal RNAs
Joanna Kufel, Christine Allmang, Elisabeth Petfalski, Jean Beggs and David Tollervey

doi: 10.1074/jbc.M208856200 originally published online November 15, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M208856200

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