Host Deadenylation-Dependent mRNA Decapping Factors Are Required for a Key Step in Brome Mosaic Virus RNA Replication†

Antonio Mas,1‡ Isabel Alves-Rodrigues,1‡ Amine Noueiry,2 Paul Ahlquist,3,4 and Juana Díez1*

Departamento de Ciencias Experimentales y de la Salud, Universitat Pompeu Fabra, Dr. Aiguader 80, 08003 Barcelona, Spain1; Division of Animal and Veterinary Sciences, West Virginia University, Morgantown, West Virginia2; and Institute for Molecular Virology3 and Howard Hughes Medical Institute,4 University of Wisconsin, Madison, Wisconsin 53706

Received 13 June 2005/Accepted 2 October 2005

The genomes of positive-strand RNA [(+)RNA] viruses perform two mutually exclusive functions: they act as mRNAs for the translation of viral proteins and as templates for viral replication. A universal key step in the replication of (+)RNA viruses is the coordinated transition of the RNA genome from the cellular translation machinery to the viral replication complex. While host factors are involved in this step, their nature is largely unknown. By using the ability of the higher eukaryotic (+)RNA virus brome mosaic virus (BMV) to replicate in yeast, we previously showed that the host Lsm1p protein is required for efficient recruitment of BMV RNA from translation to replication. Here we show that in addition to Lsm1p, all tested components of the Lsm1p-7p/Pat1p/Dhh1p decapping activator complex, which functions in deadenylation-dependent decapping of cellular mRNAs, are required for BMV RNA recruitment for RNA replication. In contrast, other proteins of the decapping machinery, such as Edc1p and Edc2p from the deadenylation-dependent decapping pathway and Upf1p, Upf2p, and Upf3p from the deadenylation-independent decapping pathway, had no significant effects. The dependence of BMV RNA recruitment on the Lsm1p-7p/Pat1p/Dhh1p complex was linked exclusively to the 3' noncoding region of the BMV RNA. Collectively, our results suggest that the Lsm1p-7p/Pat1p/Dhh1p complex that transfers cellular mRNAs from translation to degradation might act as a key regulator in the switch from BMV RNA translation to replication.

Positive-strand RNA [(+)RNA] viruses include important plant, animal, and human pathogens such as the severe acute respiratory syndrome coronavirus and hepatitis C virus. This large group of viruses replicate in the cytoplasm through negative-strand intermediates and share some fundamental features in their replication processes. A key common feature is the function of (+)RNA virus genomes as templates for both translation and replication. In contrast to other virus groups, (+)RNA viruses do not encapsidate viral polymerases required for viral replication, so upon virus entry into the cell, the genomic RNA must first be translated to produce viral replication factors. These replication factors then specifically recognize the viral RNA and recruit it from translation into the RNA replication complex. These two genomic RNA functions are mutually exclusive because 5'-to-3' ribosome trafficking blocks 3'-to-5' polymerase copying of viral (+)RNA (5, 17). Therefore, the switch from genomic RNA translation to replication must be highly regulated to allow sufficient translation but also efficient replication. The molecular features underlying this regulation are poorly understood. Other important common features in the replication of (+)RNA viruses are the assembly of replication complexes on intracellular membranes (31) and the requirement for host factors in multiple steps of the replication process (2). The identification of such host factors is important for a better understanding of fundamental issues in (+)RNA virus biology and to provide new targets for antiviral therapy.

Brome mosaic virus (BMV) is a member of the alphavirus superfamily of human, animal, and plant (+)RNA viruses. BMV has been a useful model for studying viral replication, encapsidation, and recombination. The genome of BMV consists of three genomic RNAs with 5' caps and tRNA-like 3' ends (1). RNA1 and RNA2 encode the essential RNA replication factors 1a and 2a. 1a contains a C-terminal helicase-like domain and an N-terminal capping domain required for viral RNA capping in vivo (3, 18, 25). 2a contains a central RNA-dependent RNA polymerase domain. RNA3 encodes cell-to-cell movement and coat proteins required for systemic infection in BMV’s natural host but dispensable for replication. The coat protein is translated from a subgenomic RNA.

1a and 2a direct BMV RNA replication in Saccharomyces cerevisiae (24), reproducing the known features of BMV replication in plants (24, 30, 34). In plant cells and yeast, 1a targets itself and 2a polymerase to the endoplasmic reticulum (30) and also induces the formation of membrane-enveloped spherules where the replication complex is assembled (32). 1a plays a key role in recruiting viral RNAs from translation to replication as well. 1a, independently of 2a, acts through specific sequences in the BMV RNAs to recruit them out of the cellular transla-
tion machinery and into the endoplasmic reticulum-associated replication complexes, where replication is then carried out by 1a and 2a (23, 32). The specific BMV RNA sequences that are necessary and sufficient for 1a-directed recruitment contain a tRNA-like TYC stem-loop. This motif is located in the 5′ noncoding region (NCR) of RNA1 and RNA2 and in the intercistronic region of RNA3 (9, 34). By screening UV-mutagenized yeast for reduced BMV RNA replication, we previously showed that the yeast protein Lsm1p is required for efficient 1a-directed BMV RNA recruitment to the replication complex (13). Lsm1p belongs to the Sm and Sm-like (Lsm) family of proteins that have been identified in all kingdoms of life (33). The Lsm proteins associate in heptameric rings (12) and have been associated with an expanding list of functions in RNA metabolism (7, 26–28, 36). In particular, Lsm1 associates with six other Lsm proteins, Lsm2p to Lsm7p, to form the Lsm1p-7p ring. This ring, together with Pat1p, a protein with unknown biochemical properties, and the DEAD box helicase Dhh1p, is required for efficient decapping in a major pathway of mRNA turnover, the deadenylation-dependent mRNA decay pathway (10). There is evidence that these proteins form an Lsm1p-7p/Pat1p/Dhh1p complex (6, 11, 14).

The Lsm1p requirement for both BMV RNA recruitment and cellular deadenylation-dependent mRNA decapping suggested that these two processes are linked. It was predicted that in both processes, the RNA, be it cellular mRNA or viral genomic RNA, is transferred from a translating to a nontranslating state such that decapping or replication can take place. If this hypothesis is true, then one would expect that besides Lsm1p, other cellular decapping proteins with similar functions to those of Lsm1p would also be required for BMV RNA3 recruitment to the replication complex. Here we show that several other components of the Lsm1p-7/Pat1p/Dhh1p activator of decapping complex are indeed required for efficient BMV RNA recruitment but that other proteins from the same or other decapping pathways have no significant role.

**MATERIALS AND METHODS**

**Yeast strains and yeast methods.** Standard yeast genetics and culture conditions were used (4, 19). All mutant yeast strains, including lso1Δ, lso6Δ, lso7Δ, pat1Δ, dhh1Δ, vps16Δ, edc1Δ, edc2Δ, upf1Δ, upf2Δ, and upf3Δ mutants, were derived from Saccharomyces cerevisiae strain YPH500 (MATa ura3-52 lys2-801 ade2-101 trp1-200 leu2-3-121 his3-Δ200 kan1Δ trp1Δ). The generation of the lso1Δ mutant was previously described (13). The rest of the deletion mutant strains were constructed by a one-step gene disruption procedure (19). Briefly, a disruption cassette consisting of a URA3 gene with 5′ flanking bases corresponding to 5′ and 3′ sequences of the open reading frame (ORF) of interest was generated by PCR and transformed into the wild-type (wt) strain YPH500. Ura− colonies were selected, and the correct gene disruption was verified by PCR, using primers that amplified across the recombination sites. For lso1Δ, the correct disruption was confirmed by Southern analysis. The primers used to generate the disruption cassettes and to confirm disruptions are described in the supplemental material. The yeast strain lsm1Δ carries a single frameshift mutation in the LSM1 gene and was described previously (13).

**Plasmids.** The BMV 1a protein was expressed from either pBCT19 or pBIY31H by using the ADH1 or GAL1 promoter, respectively (3, 24). BMV RNA3 was transcribed from pJD5D1U (13) by using the CUP1 promoter induced with 0.5 mM CuSO4. A BMV RNA3 derivative in which the 3′-terminal poly(A) triggers removal of the 5′ cap. In the second mRNA degradation pathway, the deadenylation-independent decapping pathway or nonsense-mediated decay (NMD), decapping occurs in aberrant polyadenylated mRNAs such as mRNAs containing pre-

**RESULTS**

**BMV 1a protein expression in wt and mutant yeast strains.** We have previously described that the cellular protein Lsm1p is required for efficient recruitment of BMV RNA to the replication complex (13). Lsm1p functions in cellular RNA degradation as an activator of mRNA decapping. To investigate whether Lsm1p is the sole cellular decapping factor required for viral RNA recruitment or whether other factors of the decapping machinery are also required, we have generated multiple additional deletion strains of the yeast Saccharomyces cerevisiae and examined their effect on BMV RNA3 recruitment.

Decapping is a critical step in two cytoplasmic mRNA degradation pathways that are conserved between yeast and mammals (see reference 10 and references within) (Fig. 1). In the major pathway, the deadenylation-dependent decapping pathway, shortening of the 3′-terminal poly(A) triggers removal of the 5′ cap. In the second mRNA degradation pathway, the deadenylation-independent decapping pathway or nonsense-mediated decay (NMD), decapping occurs in aberrant polyadenylated mRNAs such as mRNAs containing pre-
mature stop codons. Both mRNA decapping pathways need shared and pathway-specific proteins. The Lsm1p-7p/Pat1p/Dhh1p complex and the Edc1p and Edc2p set of proteins specifically function in deadenylation-dependent decapping, whereas the proteins Upf1p, Upf2p, and Upf3p specifically function in NMD. Both pathways also require the decapping holoenzyme Dcp1p/Dcp2p, the 5′-to-3′ exonuclease Xrn1p, and Vps16p. Based on these functional assignments, we constructed deletion mutant yeast strains in which the corresponding nonessential genes of common (VPS16) and pathway-specific (LSM6, LSM7, PAT1, DHH1, EDC1, EDC2, UPF1, UPF2, and UPF3) proteins were replaced by the selectable marker URA3. The replacements were verified by PCR analysis, which amplified the 5′ and 3′ recombination sites. Similar to the lsm1Δ strain (13), lsm6Δ, lsm7Δ, pat1Δ, dhh1Δ, and vps16Δ yeast strains showed a temperature-sensitive growth defect at 37°C. However, all constructed yeast strains showed robust growth at 30°C, the temperature at which all subsequent studies were carried out.

The recruitment of BMV RNA templates to the replication complex requires expression of the BMV 1a protein but occurs in the absence of BMV 2a polymerase (23). Thus, to study the effect of the newly generated gene deletions on RNA recruitment, it was important to obtain similar 1a expression levels in wt and mutant yeast strains. Only then would it be ensured that the possible effects observed were due to the deleted host gene and not to variations in 1a protein expression. However, we expected that the relevant mutations would alter 1a expression from wt BMV RNA1, since the translation of BMV genomic RNAs depends on components of the Lsm1p-7p/Pat1p/Dhh1p complex (29). Further studies with BMV RNA2 showed that this dependence is due to the combined effects of sequences in the 5′ and 3′ NCRs and the 2a polymerase open reading frame (29). Accordingly, to achieve similar levels of 1a protein accumulation in wt and mutant strains, we modulated 1a expression levels by combining the replacement of the 5′ and 3′ NCRs of RNA1 by cellular mRNA sequences with the use of promoters of different strengths. For that purpose, we expressed 1a from two plasmids by using the weaker ADH1 or the stronger GAL1 promoter to generate 1a mRNA

\[ \text{ADH1} \] and 1a mRNA

\[ \text{GAL1} \] transcripts (Fig. 2A). In these transcripts, the 5′ and 3′ NCRs of the BMV RNA1 were replaced by the ADH1 or GAL1 5′ leader sequence and the yeast ADH1 terminator-derived polyadenylation signal, respectively. The wt and mutant yeast strains expressing 1a from the ADH1 or GAL1 expression plasmids were grown, and the levels of 1a proteins were analyzed. ADH1-promoted 1a expression produced similar levels of 1a protein in the wt and the lsm6Δ, lsm7Δ, upf1Δ, upf2Δ, upf3Δ, edc1Δ, and edc2Δ mutants (Fig. 2B). In contrast, very low or undetectable 1a protein levels were observed in lsm1Δ, pat1Δ, and dhh1Δ strains (data not shown). However, in these strains the expression of 1a from the GAL1 expression plasmid fully restored the 1a protein to wt levels. This could be due to the combined effect of the stronger GAL1 promoter and the fact that, in contrast to the ADH1 expression plasmid, the complete 5′ NCR of BMV was replaced by the GAL1 5′ leader (Fig. 2A). The expression of 1a protein in strain vps16Δ was extremely toxic, producing growth arrest and cell death. The cause of this toxicity was not investigated, and this mutant was not further analyzed. Thus, except for vps16Δ, similar levels of 1a protein accumulation were obtained in wt and mutant yeast strains.

Efficient RNA3 recruitment to the site of replication requires the activator of decapping complex Lsm1p-7p/Pat1p/Dhh1p. First, we analyzed the role of individual proteins from the Lsm1p-7p/Pat1p/Dhh1p decapping complex in BMV RNA3 recruitment. In the absence of the 2a polymerase, 1a selectively transfers BMV (+) RNA templates to a membrane-associated state. As a result, 1a dramatically increases the stability and accumulation of BMV (+) RNAs. Multiple observations imply that this increase in the total accumulation of BMV RNA reflects the recruitment of viral RNA templates away from the cellular translation machinery and into RNA replication (9, 23, 32, 34). Accordingly, in lsm1Δ, a previously described mutant yeast strain with a single frameshift mutation in the LSM1 ORF that inhibits BMV RNA recruitment (13), a 75% reduction of the 1a-directed total RNA3 accumulation parallels a similar one in membrane-associated RNA3 (Fig. 3A). Therefore, and because the total RNA accumulation assay is reliable and easy to quantify, it was chosen for measuring BMV RNA recruitment as done before (13).
The wt, lsm1Δ, lsm6Δ, lsm7Δ, pat1Δ, and dhh1Δ yeast strains were transformed with a plasmid transcribing wt RNA3 from the CUP1 promoter plus another plasmid expressing the BMV 1a protein or the corresponding empty vector. In the absence of 1a, RNA3 accumulated to similar levels in wt and mutant yeast strains (Fig. 3B). However, important differences were observed when 1a was coexpressed. As described previously, in wt yeast 1a massively increased RNA3 accumulation (13). This increase was about 50-fold. Importantly, in all mutant strains analyzed, 1a stimulation of RNA3 accumulation was inhibited when 1a was coexpressed. As described previously, in wt yeast in the presence of 1a protein, RNA3 accumulation is similar to that previously described for the Lsm1p-7p/Pat1p/Dhh1p complex. For the lsm1Δ, lsm6Δ, lsm7Δ, pat1Δ, and dhh1Δ strain, RNA3 accumulation levels were unchanged compared to that in the wt (Fig. 4A). However, when the 1a protein was expressed, RNA3 accumulated in the edc2Δ strain to the same level as that in the wt, and in the edc1Δ strain, RNA3 accumulation levels were inhibited by only 30% relative to the wt. These observations are in marked contrast to the large inhibition of RNA3 accumulation seen with deletions of genes coding for components of the Lsm1p-7p/Pat1p/Dhh1p complex. For the upf1Δ, upf2Δ, and upf3Δ yeast strains, in the absence of 1a RNA3 accumulated to similar levels to those in wt yeast (Fig. 4B). However, when the 1a protein was expressed, RNA3 accumulation was not inhibited. In fact, we observed a small but reproducible 1.5- to 2-fold increase.

The 3′ NCR of BMV RNA3 is linked to the requirement of the Lsm1p-7p/Pat1p/Dhh1p complex for BMV RNA3 recruitment to the site of replication. To test whether other proteins of the decapping machinery besides the Lsm1p-7p/Pat1p/Dhh1p complex might also function in BMV RNA3 recruitment to the replication complex, we examined additional yeast strains with deletions in the EDC1, EDC2, UPF1, UPF2, and UPF3 genes. The corresponding Edc1p and Edc2p proteins are specific for the deadenylation-dependent decapping pathways; however, in contrast to the Lsm1p-7p/Pat1p/Dhh1p complex, they are not required for general decapping but possibly for decapping of specific mRNAs (10). The Upf1p-3p proteins function in the NMD pathway (Fig. 1).

The wt, edc1Δ, and edc2Δ yeast strains were tested for the accumulation of CUP1-induced RNA3 transcripts in the absence and presence of the 1a protein. As with the strains lsm1Δ, lsm6Δ, lsm7Δ, pat1Δ, and dhh1Δ, in the absence of 1a the RNA3 levels were unchanged compared to that in the wt (Fig. 4A). However, when the 1a protein was expressed, RNA3 accumulated in the edc2Δ strain to the same level as that in the wt, and in the edc1Δ strain, RNA3 accumulation levels were inhibited by only 30% relative to the wt. These observations are in marked contrast to the large inhibition of RNA3 accumulation seen with deletions of genes coding for components of the Lsm1p-7p/Pat1p/Dhh1p complex. For the upf1Δ, upf2Δ, and upf3Δ yeast strains, in the absence of 1a RNA3 accumulated to similar levels to those in wt yeast (Fig. 4B). However, when the 1a protein was expressed, RNA3 accumulation was not inhibited. In fact, we observed a small but reproducible 1.5- to 2-fold increase.
lated tRNA-like 3' NCR of BMV RNA by a poly(A) tail (13). To further assess which BMV RNA sequence was linked to the requirement of the Lsm1p-7p/Pat1p/Dhh1p complex for RNA3 recruitment, we tested the accumulation of RNA3-poly(A) in wt, lsm1Δ, lsm6Δ, lsm7Δ, pat1Δ, and dhh1Δ yeast strains. RNA3-poly(A) was transcribed from the GAL1 promoter and corresponds to a wt BMV RNA3 in which the 3' NCR containing a tRNA-like structure was replaced by the yeast ADH1 terminator-derived polyadenylation signal. See the legend to Fig. 3 for abbreviations and a description of the layout of the figure.

FIG. 5. Requirement of Lsm1-7/Pat1/Dhh1 for RNA3 recruitment to the site of replication is linked to the 3' NCR. Northern blot analysis of 1a-directed RNA3-poly(A) accumulation was performed with wt, lsm1Δ, lsm6Δ, lsm7Δ, pat1Δ, and dhh1Δ yeast strains. RNA3-poly(A) was transcribed from the GAL1 promoter and corresponds to a wt BMV RNA3 in which the 3' NCR containing a tRNA-like structure was replaced by the yeast ADH1 terminator-derived polyadenylation signal. See the legend to Fig. 3 for abbreviations and a description of the layout of the figure.

**DISCUSSION**

Previously, we described that a single mutation in the LSM1 gene inhibited BMV RNA3 recruitment out of translation and into the replication complex (13). We now can show that besides Lsm1p, several other proteins of the Lsm1p-7p/Pat1p/Dhh1p decapping activator complex are necessary for this step as well, namely, Lsm6p, Lsm7p, Pat1p, and Dhh1p. Other proteins of this complex, such as Lsm2-5p, could not be tested in our assay because the respective yeast deletion strains are not viable. Taken together, these results suggest that the whole decapping activator complex, rather than only the Lsm1p-7p ring, is involved in this important early event in positive-strand viral RNA replication.

Since the Lsm1p-7p/Pat1p/Dhh1p complex is involved in RNA degradation of some cellular RNAs, one possibility to consider is that the deletion of the corresponding genes might affect RNA3 accumulation indirectly by influencing the turnover of the BMV RNA. However, two observations strongly argue against such a possibility. First, the levels of RNA3 accumulation in the absence of 1a were similar in the wt and mutant yeast strains. Second, any indirect effect of the deleted decapping genes would be expected to increase the level of RNA3. In contrast, in the presence of 1a, a reduction in RNA3 accumulation was observed when the LSM1, LSM6, LSM7, PAT1, and DHH1 genes were deleted (Fig. 3B). In line with this, a cellular function in mRNA decapping per se did not correlate with a major function in BMV RNA3 recruitment. Ecd1p and Ecd2p, two proteins involved with the Lsm1p-7p/Pat1p/Dhh1p complex in the deadenylating-dependent decapping pathway, had no large effects. The same was true for Up1p to -3p, three proteins from the NMD pathway. Interestingly, these last gene deletion mutants even showed a slight increase in BMV RNA3 recruitment. The reason for this is unknown, but it might be due to a direct competition of Up1p-3p with Lsm1p-7p/Pat1p/Dhh1p for limiting common factors in decapping (Fig. 1) or to an indirect effect.

The known function of the Lsm1p-7p/Pat1p/Dhh1p complex in cellular mRNA decapping suggests a role in the regulated transition of viral RNA from translation to replication. In the cell, mRNA translation and degradation are two inversely related processes, as are viral RNA translation and replication (10). Accordingly, an important step in mRNA degradation is the exit of the mRNA from active translation to a nontranslating state that allows the assembly of the decapping complex. In yeast and humans, this assembly occurs in discrete cytoplasmic foci named P bodies. The Lsm1p-7p/Pat1p/Dhh1p complex, the Dcp1p/Dcp2p decapping enzyme, and the Xrn1p exonuclease are located in these P bodies (10). The movement of mRNAs out of the translating polyosome pool into P bodies requires dramatic rearrangements in the state of the mRNA, including a loss of ribosomes and translation factors and the addition of mRNA decapping factors. It is exactly this rearrangement which the Lsm1p-7p/Pat1p/Dhh1p complex has been suggested to facilitate (10, 37). Positive-strand RNA genomes mimic cellular mRNAs. Like the case for the latter, the Lsm1p-7p/Pat1p/Dhh1p complex could mediate rearrangements in the BMV RNA that would facilitate the loss of ribosomes and translation factors and the recognition of the 1a replicase. This view fits with emerging functions of P bodies beyond mRNA degradation, namely, the maintenance of a proper balance between translating and nontranslating pools of mRNAs (8, 10, 35).

Besides the above-described role of the Lsm1p-7p/Pat1p/Dhh1p complex in BMV RNA recruitment, this complex is also required for the translation of BMV RNAs (29). Interestingly, only the genomic RNA1, RNA2, and RNA3 that are replicated in the viral life cycle and thus recruited to the replication complex were affected, and not the subgenomic RNA, which solely needs to be translated. This raises the question of how a single complex is able to function in apparently antagonistic processes, namely, virus RNA translation and recruitment to replication. While this question cannot be answered yet, such diverse functions are not without precedent. For example, the Pat1p component of the Lsm1p-7p/Pat1p/Dhh1p complex has been shown to work in the antagonistic processes of mRNA translation and mRNA degradation (6, 10, 39). An advantage of using such complexes with antagonistic functions seems to be a rapid switching between processes in response to different cellular requirements. A similar advantage might apply for (+)RNA viruses.

The mRNA signals mediating the cellular functions of the
Lsm1p-7p/Pat1p/Dhh1p complex have not been identified yet. However, the dependence on Lsm1p-7p/Pat1p/Dhh1p for BMV RNA recruitment was linked to sequences in the 3’ end, as described here, while the dependence for translation was mapped for RNA2 to the 5’ and 3’ NCRs and the 2a polymerase open reading frame (29). This shows that translation and recruitment are regulated by different and possibly overlapping viral signals. An involvement of 5’ and 3’ NCRs in the coordination of viral RNA translation and replication seems to be a common feature for other (+)RNA viruses (16, 20, 22).

In conclusion, the Lsm1p-7p/Pat1p/Dhh1p complex seems to be a key regulator in the switch from BMV RNA translation to replication. Because all (+)RNA viruses must regulate this essential step, other (+)RNA viruses, especially those with nonpolyadenylated genomic RNAs, may also rely on the Lsm1p-7p/Pat1p/Dhh1p complex or its homologs. Accordingly, with the exception of Pat1p, the human homologs of all the components of the yeast Lsm1p-7p/Pat1p/Dhh1p complex have been identified and shown to have similar functions to their yeast counterparts. Moreover, in bacteria the Lsm homolog Hfq forms a hexameric ring that is involved in regulating bacterial mRNA translation and degradation (38). Interestingly, Hfq is required for replication of the Qβ bacteriophage, a (+)RNA virus (15). Thus, Lsm1p-7p/Pat1p/Dhh1p has functions that are conserved from prokaryotes to eukaryotes and is utilized by a plant as well as a bacteriophage (+)RNA virus.

Future work will show whether these are exceptions or the norm.

ACKNOWLEDGMENTS

We thank Encarna Ulloa for technical assistance and Andreas Meyerhans for helpful discussions and comments on the manuscript. This work was supported by a grant from the Spanish Ministerio de Ciencia y Tecnologia. I.-R. was supported by Fundacao para a Ciencia e Tecnologia (SFRH/BD/9630/2002) through the GABBA program from the University of Porto, Porto, Portugal.

REFERENCES

6. Bovetti, E., R. Boec, and B. Laforee. 2000. The two proteins, Pat1p (Mr1p) and Shplp interact in vivo, are required for mRNA decay, and are functionally related to Pa1p. Mol. Cell. Biol. 20:9339–9346.
8. Bovetti, E., R. Boec, and B. Laforee. 2000. The two proteins, Pat1p (Mr1p) and Shplp interact in vivo, are required for mRNA decay, and are functionally related to Pa1p. Mol. Cell. Biol. 20:9339–9346.