Slx9p facilitates efficient ITS1 processing of pre-rRNA in Saccharomyces cerevisiae

RALPH BAX, HENDRIK A. RAUÉ, and JAN C. VOS
Section of Biochemistry and Molecular Biology, Department of Chemistry and Pharmaceutical Sciences, Faculty of Sciences, BioCentrum Amsterdam, Vrije Universiteit, Amsterdam, Amsterdam, The Netherlands

ABSTRACT

Slx9p (Ygr081cp) is a nonessential yeast protein previously linked genetically with the DNA helicase Sgs1p. Here we report that Slx9p is involved in ribosome biogenesis in the yeast Saccharomyces cerevisiae. Deletion of SLX9 results in a mild growth defect and a reduction in the level of 18S rRNA. Co-immunoprecipitation experiments showed that Slx9p is associated with 35S, 23S, and 20S pre-rRNA, as well as U3 snoRNA and, thus, is a bona fide component of pre-ribosomes. The most striking effects on pre-rRNA processing resulting from deletion of SLX9 is the accumulation of the mutually exclusive 21S and 27SA2 pre-rRNA. Furthermore, deletion of SLX9 is synthetically lethal with mutations in Rrp5p that block cleavage at either site A2 or A3. We conclude that Slx9p has a unique role in the processing events responsible for separating the 66S and 43S pre-ribosomal particles. Interestingly, homologs of Slx9p were found only in other yeast species, indicating that the protein has been considerably less well conserved during evolution than the majority of trans-acting processing factors.

Keywords: yeast; ribosome biogenesis; pre-rRNA processing

INTRODUCTION

In eukaryotes, biogenesis of ribosomes starts in the nucleolus with transcription by RNA polymerase I of a large precursor RNA molecule, called 35S pre-rRNA in yeast, in which the 18S, 5.8S, and 25S mature rRNAs reside, while RNA polymerase III transcribes a 3'-extended pre-5S rRNA. The 35S precursor also contains external transcribed spacer elements (5'-ETS and 3'-ETS) at either end as well as internal transcribed spacers (ITS1 and ITS2) that separate the mature sequences (Fig. 1A). During transcription many nonribosomal (trans-acting) factors as well as some ribosomal proteins start to associate with the nascent transcript, eventually forming a large pre-ribosomal complex called the small subunit processome (SSU) or 90S pre-ribosome (for reviews, see Fromont-Racine et al. 2003; Granneman and Baserga 2004; Raué 2004). The nonribosomal factors include small nucleolar RNAs (snoRNA), exo- and endonucleases, RNA helicases, and a host of other protein factors, whose exact role is still unclear. However, individual inactivation of any of these components in most cases inhibits one or more specific steps in the ordered removal of the ITS and/or ETS sequences from the 35S precursor, causing accumulation of intermediate pre-rRNAs at the expense of downstream products.

The first processing steps occurring within the 90S complex are cleavages at sites A0 and A1 that remove the 5'-ETS (Fig. 1B). Then, endonucleolytic cleavage of the resulting 32S precursor at site A2 splits the complex into a 43S and a 66S particle containing the 20S and 27SA2 pre-rRNA, respectively. The 43S particle is exported to the cytoplasm where its 20S precursor RNA is further matured by cleavage at site D to generate the mature 18S species. Within the 66S pre-ribosomal complex the 27SA2 pre-rRNA is further processed into 25S and 5.8S rRNA by an ordered series of exo- and endonucleolytic digestion steps. The majority of the 27SA2 molecules are cleaved at site A3, producing 27SA3 pre-rRNA, which is subsequently processed endonucleolytically to sites B1S and B2 to render the 27SB5 precursor. A minor portion of the 27SA2 pre-rRNA enters an alternative route involving endonucleolytic cleavage at site B1L that produces the 5'-extended 27SB5l pre-rRNA (Faber et al. 2006). The 27SB5 and 27SB5l precursor are then matured into 25S as well as 5.8S or 5.8Sl rRNA by endonucleolytic cleavage at site C1, followed by exonucleolytic digestion to sites C2 and E, respectively.

Recent experiments using mutant yeast strains have shown that the order of processing events described above is not a strictly obligatory one. For instance, processing of
32S pre-rRNA in ITS1 can start at site A3 instead of at A2, generating the normal 27SA3 intermediate and an alternative 21S precursor that is efficiently converted into 18S rRNA (Vos et al. 2004b). Processing of 35S pre-rRNA can skip the early cleavages at A0 and A1 and commence instead at site A3 (for review, see Venema and Tollervey 1999). This generates 27SA3 and a 23S precursor, whose fate is still in dispute. Although initially considered to be a dead-end product (Venema and Tollervey 1999; Allmang et al. 2000), recent evidence indicates that it may be further processed into 18S rRNA (Granneman and Baserga 2004). ITS1 also contains a further endonucleolytic processing site A4, that has so far been detected only in particular mutant yeast strains in which cleavage at site A3 is blocked (Eppens et al. 2002; Faber et al. 2004). It is as yet unclear whether this site is used in wild-type cells.

SLX9 was identified in a synthetic lethality (sl) screen searching for genes that interact with SGS1 (Ooi et al. 2003), a 3′ → 5′ DNA helicase that is involved in DNA damage repair and replication (Bennett et al. 1998). Double-mutant ΔgsiΔslx9 cells displayed a reduced growth rate. Although this demonstrates a genetic interaction between SGS1 and SLX9, their functional relationship remains to be clarified. In contrast, in large-scale affinity purifications Slx9p copurifies with Enp1p and Tsr1p (Gavin et al. 2002), two proteins that are involved in processing of precursor rRNA (Gelperin et al. 2001; Chen et al. 2003). Both Enp1p and Tsr1p associate with pre-rRNA as well as proteins previously characterized as components of 90S and 40S pre-ribosomal particles. Furthermore, GFP-tagged Slx9p was found to localize to the nucleolus (Ghaemmamghami et al. 2003). These observations suggest that, although Slx9p is a nonessential protein (Giaever et al. 2002; Steinmetz et al. 2002; Ooi et al. 2003; Deutschbauer et al. 2005), it may have a role in ribosome biogenesis.

The biochemical and genetic data reported in this paper demonstrate that Slx9p is present in pre-ribosomes from an early stage and implicate the protein in the processing events that remove the ITS1 spacer sequences.

RESULTS

SLx9p is specific to yeast species

SLX9 encodes a protein of 210 amino acids and has been identified as a nonessential gene in Saccharomyces cerevisiae by several genome-wide screens (Giaever et al. 2002; Steinmetz et al. 2002; Ooi et al. 2003; Deutschbauer et al. 2005). To assess its evolutionary conservation we performed a BLAST search in the SwissProt/TrEMBL proteome database using the amino acid sequence of S. cerevisiae Slx9p. Except for other yeast species, no homologs of Slx9p could be found in prokaryotes, archa, or higher eukaryotes. Figure 2 shows a sequence comparison of S. cerevisiae Slx9p with several of its counterparts in other yeast species. In addition to a conserved putative N-terminally located nuclear localization sequence (KRRxxLRxK), several highly conserved regions can be recognized. However, none of these regions are conserved in non-yeast species.
these domains shows any similarity to known functional protein motifs. Slx9p thus appears to be unique to yeast species, and its sequence does not give any hints regarding its precise function.

**Slx9p is associated with pre-rRNA species and U3 snoRNA**

In previous large-scale affinity purifications Slx9p was found to co-precipitate with Tsr1p and Enp1p, two proteins that are part of 90S and 43S pre-ribosomes (Gelperin et al. 2001; Gavin et al. 2002; Chen et al. 2003; Schafer et al. 2003). To determine whether Slx9p also is a component of these pre-ribosomes we tested its association with pre-rRNA molecules by performing co-precipitation experiments. To this end, we used the slx9::KAN strain (obtained from EUROSCARF) transformed with a plasmid encoding Slx9p that carried a ProtA tag at its N terminus. As a positive control, we used strain SC1413 that expresses TAP-tagged Rio2p, a trans-acting factor present in 43S pre-ribosomes (Vanrobays et al. 2003). Extracts were prepared from exponentially growing cells and treated with IgG-agarose beads. The bound material was eluted from the beads, after which the protein and RNA were separated by phenol extraction and the latter was analyzed by Northern hybridization. In agreement with earlier findings, Rio2p–TAP exclusively co-precipitates 20S pre-rRNA (Fig. 3A, lane 3). In the precipitate of ProtA–Slx9p, however, a broader spectrum of pre-rRNAs is present (Fig. 3A, B). Apart from 20S pre-rRNA, we also detected the 35S and 23S precursor species. Furthermore, ProtA–Slx9p co-precipitates substantial amounts of U3 snoRNA (Fig. 3C). Only trace amounts of 27SA2 are detectable (Fig. 3B). Northern analysis of the small pre-rRNA species demonstrated the absence of any significant amounts of 7S pre-rRNA (data not shown). Previously, we have high-level expression of ProtA–Rrp5p and shown a complete absence of 20S precursors in the immunoprecipitation (Vos et al. 2004a), which ensures further supports that the ProtA–Slx9p precipitation is specific. We conclude that Slx9p is a bona fide component of the 90S pre-ribosome, and upon ITS1 processing remains associated with 43S, but not the 66S, particle.

**Slx9p is required for normal pre-rRNA processing in ITS1**

To identify potential defects in pre-rRNA processing in cells carrying an slx9 deletion, we performed Northern hybridization experiments on total RNA that was extracted from exponentially growing slx9::LEU2 cells. As controls, we used RNA from the slx9::LEU2 strain supplemented with a plasmid-borne copy of the SLX9 gene, as well as the wild-type parent strain. The slx9 deletion strain is sensitive to a high concentration of 600 ng/mL cycloheximide, which is rescued upon introduction of the plasmid-borne copy (data not shown).

As is evident from Figure 4A, the level of 25S rRNA in cells lacking Slx9p is very similar to that in cells expressing the protein, either from a genomic or a plasmid-borne gene. Figure 4B shows that the lack of Slx9p also has no significant effect on the relative level of the long and short forms of 5.8S rRNA. In contrast, the level of 18S rRNA in slx9::LEU cells is reduced by about 20%, as is evident from quantification of Northern blots. This effect is abolished by introduction of a plasmid-borne copy of the gene into the mutant cells (Fig. 4A; cf. lanes 2 and 3). These findings indicate that inactivation of SLX9 does not inhibit processing in the large subunit pathway, leading to mature 25S and 5.8S rRNA, but does have a negative effect on one or more of the processing steps required for formation of the small subunit. The limited reduction in 18S rRNA in slx9::LEU cells is reduced by about 20%, as is evident from quantification of Northern blots. This effect is abolished by introduction of a plasmid-borne copy of the gene into the mutant cells (Fig. 4A; cf. lanes 2 and 3). These findings indicate that inactivation of SLX9 does not inhibit processing in the large subunit pathway, leading to mature 25S and 5.8S rRNA, but does have a negative effect on one or more of the processing steps required for formation of the small subunit. The limited reduction in 18S rRNA in slx9::LEU cells is reduced by about 20%, as is evident from quantification of Northern blots. This effect is abolished by introduction of a plasmid-borne copy of the gene into the mutant cells (Fig. 4A; cf. lanes 2 and 3). These findings indicate that inactivation of SLX9 does not inhibit processing in the large subunit pathway, leading to mature 25S and 5.8S rRNA, but does have a negative effect on one or more of the processing steps required for formation of the small subunit.
in Figure 1A. Analysis with probe 1, complementary to the sequence shortly downstream from site D, revealed that 35S pre-rRNA is present at normal levels in Slx9p-deficient cells (Fig. 4C). However, we observed a strong accumulation of an intermediate that, on the basis of its mobility, could be either 20S or 22S/21S pre-rRNA. To distinguish between these possible products, we rehybridized the blot with probe 2, located just downstream from site A2. We obtained a strong signal at the same position (Fig. 4D), indicating the accumulation of substantial amounts of 22S/21S pre-rRNA in the slx9::LEU2 strain. Using probe 3 specific for RNA sequences between sites A0 and A1, we found only minor amounts of 22S pre-rRNA (Fig. 4F). Thus, the signal observed in the slx9 deletion strain predominantly represents the 21S species. Furthermore, we observed an increase in 23S pre-rRNA (also visible in Fig. 4C, E) as well as 27SA2 pre-rRNA (Fig. 4D) and 32/33S pre-rRNAs in these cells (best visible in Fig. 4D, E).

The simultaneous increase in the levels of both the 27SA2 as well as the 21S and 23S pre-rRNAs is quite surprising considering that these two processing intermediates overlap each other (Fig. 1B). In previously studied mutants an increase in 21S and/or 23S pre-rRNA resulted from a defect in cleavage at site A2 (as well as A0 and A1 in the case of 23S), and was accompanied by a decrease in the level of the 27SA2 precursor (Jansen et al. 1993; Lee and Baserga 1997; Lafontaine and Tollervey 1999; Gallagher et al. 2004). Inactivation of SLX9, thus, appears to have complex consequences for processing in ITS1, resulting in a phenotype that has not been observed before.

To further refine our picture of the effect of the slx9 deletion on ITS1 processing, we performed a reverse transcription assay on total RNA extracted from slx9::LEU2 and slx9::KAN cells and their corresponding wild-type strains, using probe 3, which is complementary to a sequence between sites A3 and B1L. The results displayed in Figure 5 show an increase in the primer extension stop at A2 upon inactivation of SLX9 (cf. lanes 1, 3 and lane 1A). Several arguments support the conclusion that the reduction in the signal representing cleavage at site A3 is not due to inhibition of processing at this site. First, the Δslx9 mutant cells accumulate the 21S and 23S pre-rRNA products of this cleavage (Fig. 4D). Second, as shown in Figure 4B, inactivation of SLX9 does not cause the shift in the production of 5.8S rRNA toward the long form that invariably results from inhibition of processing at site A3 (Eppens et al. 2002; Henry et al. 1994). Finally, we failed to observe a primer extension stop corresponding to site A4 in the Δslx9 cells. This stop does appear as a consequence of mutations in either the RRP2 or RRP5 gene that lead to loss of cleavage at site A3 (Eppens et al. 2002; Faber et al. 2004).

Taken together, these data therefore suggest that lack of Slx9p does not have a major impact on the efficiency of the various processing cleavages taking place in ITS1, but affects the further maturation of the different precursor species resulting from these cleavages.

**Slx9p is essential when cleavage at either site A2 or A3 is impaired**

To try to clarify the possible role of Slx9p in ITS1 processing further, we decided to study the effect of combining the slx9 deletion with two mutants of the trans-acting factor Rrp5p that affect processing at sites A2 and A3, respectively. In the deletion, mutant rrp5-Δ6 processing of 32S
pre-rRNA at site A2 is completely inhibited while cleavage at site A3 proceeds unhindered to produce 21S and 27SA3 pre-rRNA. The 21S precursor behaves as a normal intermediate and is efficiently processed at site D to 18S rRNA. The growth rate of \textit{rrp5-}\textit{D}\textsubscript{6} mutant cells is not affected (Vos et al. 2004b). In the \textit{rrp5-}\textit{D}\textsubscript{3} deletion mutant, on the other hand, cleavage at site A3 is blocked and processing is redirected to site A4 (Eppens et al. 2002). To combine the \textit{D}\textit{slx9} allele with the respective \textit{rrp5} deletion mutations, we used yeast strain YJV154 in which the genomic \textit{RRP5} gene is under control of the \textit{GAL} promoter (Venema and Tollervey 1995). In this strain \textit{SLX9} was deleted by homologous recombination with an \textit{slx9::LEU2} construct. The resulting strain YRB154 was deleted by homologous recombination with an \textit{slx9::LEU2} construct. The resulting strain YRB154 was transformed with a plasmid carrying either the \textit{rrp5-}\textit{D}\textsubscript{3}, \textit{rrp5-}\textit{D}\textsubscript{6}, or the wild-type \textit{RRP5} allele under control of the wild-type \textit{RRP5} promoter. The strains were grown on YPD plates to deplete wild-type Rrp5p and to observe the effect of deletion of \textit{SLX9} on growth. Surprisingly, the absence of Slx9p results in a synthetic lethality with each of the two \textit{RRP5} mutants (Fig. 6A) due to a reduction in both 18S and 25S rRNA production. Moreover, 18S rRNA levels appear to be more strongly affected than 25S rRNA levels (Fig. 6B).

We next analyzed the levels of different precursor intermediates in the double mutant cells by first growing them to mid-exponential phase on liquid-selective galactose-based medium and then shifting them to YPD medium for 24 h to make them dependent upon the mutant Rrp5p. Total RNA isolated from equal amounts of cells as determined by the OD\textsubscript{600} of the cultures was subjected to Northern analysis using probes 1 and 2 (cf. Fig. 1A). SLX9 cells dependent upon either Rrp5\textit{Δ3p} or Rrp5\textit{Δ6p} displayed the processing phenotypes observed previously (data not shown; Eppens et al. 2002; Vos et al. 2004b). In the first case, the cells still produce 27SA2 and 20S pre-rRNA, albeit at a reduced level in accordance with their reduced growth rate. The \textit{rrp5-}\textit{Δ6} mutation caused depletion of 27SA2 and 20S pre-rRNA on one hand, and increased production of 21S pre-rRNA on the other. Disruption of the SLX9 gene in the GAL::\textit{RRP5} strain shows only a mild increase of 21S pre-rRNA (Fig. 6D, lane 2) and considerably less decrease in 18S rRNA compared to the YCV711 strain used above (Fig. 6B), suggesting that the effects of loss of Slx9p depend upon genetic background, a conclusion stressed by the...
synthetic lethality shown. Inactivation of \textit{SLX9} in combination with either \textit{rrp5} deletion mutation resulted in loss of detectable levels of 20S and 27SA2 pre-rRNA in \textit{rrp5-\Delta 3} (Fig. 6C,D, lane 3) and 21S as well as 27SA2 in \textit{rrp5-\Delta 6} cells (lane 4). The relatively high levels of the 35S and 32S pre-rRNA species in both double mutants indicate that RNA polymerase I still transcribes the rDNA unit (cf. lanes 3,4 and lane 2). Thus, the lack of the 20S/21S and 27SA2 precursor species must be due to a complete failure of the processing machinery to cleave the primary precursor. We conclude that Slx9p, although not essential under normal conditions, plays a crucial role in ensuring the flexibility in ITS1 processing that guarantees production of both ribosomal subunits when cleavage at either site A2 or A3 is inhibited. Note that the ratio 18S to 25S rRNA is decreased, consistent with an initial shift from a high level of Rrp5p to a normal level of Rrp5p, followed by a complete dependence on the mutant protein and a complete block in ribosome biogenesis.

\section*{DISCUSSION}

The yeast gene \textit{SLX9} was first identified in a synthetic lethality screen with a null mutant of \textit{SGS1}, which encodes a 3'\rightarrow 5' DNA helicase that monitors movements of replication forks (Ooi et al. 2003). However, accumulating evidence generated by proteomics pointed toward an additional role for Slx9p in ribosome biogenesis: Slx9p (Fig. 3) show that the protein is associated with 35S, trans-acting factor whose N-terminal domain consists of 12 S1 RNA-binding motifs. Deletion of nonoverlapping sets of these motifs completely blocks processing at either site A2 (mutant \textit{rrp5-\Delta 3}) or A3 (mutant \textit{rrp5-\Delta 6}) (Eppens et al. 2002; Vos et al. 2004b), which would enable us to analyze the effect of the \textit{slx9} deletion in cells that carry either of two different mutant forms of Rrp5p, a trans-acting factor whose N-terminal domain consists of 12 S1 RNA-binding motifs. Deletion of nonoverlapping sets of these motifs completely blocks processing at either site A2 (mutant \textit{rrp5-\Delta 3}) or A3 (mutant \textit{rrp5-\Delta 6}) (Eppens et al. 2002; Vos et al. 2004b), which would enable us to analyze the effect of the \textit{Delta} \textit{slx9} mutation on each of the individual cleavages and further processing of the respective products. Surprisingly, however, both \textit{rrp5} deletion mutations proved to be synthetically lethal with the deletion of \textit{SLX9}, apparently because lack of Slx9p prevents the processing machinery from carrying out the cleavage in ITS1 still allowed by the mutant Rrp5p in question (Fig. 6). While these results defy the original purpose of the approach, they do provide additional strong support for a role of Slx9p in ITS1 processing. An attractive hypothesis is that in wild-type cells Slx9p acts as a chaperone, ensuring an architecture of the late 90S pre-ribosome that is optimal for ITS1 processing. However, because of the natural flexibility of this processing, lack of Slx9p does not have any drastic negative consequences for ribosome biogenesis. The need for Slx9p becomes compelling, however, when the normal architecture of the late 90S pre-ribosome is compromised of mutations in other components such as Rrp5p. Slx9p, therefore, resembles the Ssf1p/Ssf2p proteins that are important in determining the order of ITS1 and ITS2 processing in pre-60S complexes. Premature cleavage of 27SA2 pre-rRNA at site C2 within ITS2 in the absence of Ssf1p/Ssf2p, is suggested to result from an improper
transitional composition of the early large pre-ribosomal subunit (Fatica et al. 2002).

Interestingly, Slx9p does not have any readily identifiable orthologs in other eukaryotes apart from other yeast species. This is in clear contrast to the majority of trans-acting factors involved in ribosome biogenesis, which are generally found to be well conserved during evolution (Jansen et al. 1993; Kaser et al. 2001; Pestov et al. 2001; Wehner and Baserga 2002). The reason for the existence of such a yeast-specific factor could lay in the somewhat different manner in which ITS1 processing proceeds in yeast compared to higher eukaryotic cells (Borovjagin and Gerbi 2005).

The yeast strains that were used are listed in Table 1. The species. This is in clear contrast to the majority of orthologs in other eukaryotes apart from other yeast strains and plasmids

MATERIALS AND METHODS

Strains and plasmids

The yeast strains used are listed in Table 1. The oligonucleotides used are listed in Table 2.

The ORF of SLX9 was amplified by PCR, using genomic DNA as a template and the primers Slx9fw1 and Slx9rv1 to introduce flanking XhoI sites. After digestion with XhoI, the resulting fragment was cloned into the corresponding sites of pET15b (Invitrogen) and pBS(KS+) (Stratagene).

To construct the slx9::LEU2 allele, the BamHI fragment that contains the LEU2 marker from vector YDP-L (BCCM/LMBP) was cloned into the BglII-site located in SLX9 in pBS–SLX9 to give pBS–slx9::LEU2. The slx9::LEU2 allele on this plasmid was then amplified by PCR using primers Slx9fw1 and Slx9rv1. The PCR product was transformed into the strains, YJV140 and YJV154, to allow homologous integration into the genome. Transformants were selected on Leu plates and screened by PCR to check for disruption of the genomic wild-type SLX9 gene.

For complementation of the SLX9 disruption in YCV711, the SLX9 gene including its promoter region was amplified from wild-type genomic DNA using primers Slx9fw2 and Slx9rv1. The former primer introduced an XbaI site at the 5' end of the promoter region, the latter an XhoI site after the open reading frame of SLX9. The PCR product was cloned into the corresponding sites of pRS313 (Sikorski and Hieter 1989).

SLX9 was fused in-frame with the coding sequence of the two IgG-binding domains of Streptococcus aureus Protein A. To this end, an NcoI fragment containing the ProtA sequence was cloned into the NcoI site of pRS313 (Sikorski and Hieter 1989).

YJV140 MATa, ade2, his3, leu2, trp2, ura3
SC1413 Mat a, ade2, arg4, leu2–3, 112, trp1–289, ura3–52, YNL207w::TAP-K.I.URA3
Y14711 Mat a, his3, leu2, lys2, ura3, YGR081c::kanMX4
YCV711 Mat a, ade2, his3, leu2, trp2, ura3, YGR081c::LEU2
YJV154 MATa, ade2, his3, leu2, trp1, ura3, GAL::rrp5 (URA3)
YRB154 Mat a, ade2, his3, leu2, trp1, ura3, GAL::rrp5 (URA3)

Immunoprecipitation, isolation, and analysis of RNA

Immunoprecipitation was performed as described previously (Vos et al. 2004a). RNA isolation, and Northern and primer extension analyses were carried out as described previously (Venema and Tollervey 1996; Venema et al. 1998). The positions of the probes are indicated in Figure 1A.

ACKNOWLEDGMENTS

This work was supported by a grant from the Council for Chemical Sciences (CW) with financial aid from The Netherlands Foundation for Scientific Research (NWO).

Received May 24, 2006; accepted August 24, 2006.
REFERENCES


2012 RNA, Vol. 12, No. 11


Slx9p facilitates efficient ITS1 processing of pre-rRNA in
Saccharomyces cerevisiae

Ralph Bax, Hendrik A. Raué and Jan C. Vos

RNA 2006 12: 2005-2013 originally published online October 3, 2006
Access the most recent version at doi:10.1261/rna.159406

References
This article cites 43 articles, 14 of which can be accessed free at:
http://rnajournal.cshlp.org/content/12/11/2005.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the
top right corner of the article or click here.