Regulation and Surveillance of Normal and 3′-Extended Forms of the Yeast Aci-reductone Dioxygenase mRNA by RNase III Cleavage and Exonucleolytic Degradation

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Aci-reductone dioxygenases are key enzymes in the methionine salvage pathway. The mechanisms by which the expression of this important class of enzymes is regulated are poorly understood. Here we show that the expression of the mRNA encoding the yeast aci-reductone dioxygenase ADI1 is controlled post-transcriptionally by RNase III cleavage. Cleavage occurs in a large bipartite stem loop structure present in the open reading frame region of the ADI1 mRNA. The ADI1 mRNA is up-regulated in the absence of the yeast orthologue of RNase III Rnt1p or of the 5′ → 3′ exonucleases Xrn1p and Rat1p. 3′-Extended forms of this mRNA, including a polycistronic mRNA ADI1-YMR010W mRNA, also accumulate in cells lacking Rnt1p, Xrn1p, and Rat1p or the nuclear exosome component Rrp6p, suggesting that these 3′-extended forms are subject to nuclear surveillance. We show that the ADI1 mRNA is up-regulated under heat shock conditions in a Rnt1p-independent manner. We propose that Rat1p cleavage targets degradation of the ADI1 mRNA to prevent its expression prior to heat shock conditions and that RNA surveillance by multiple ribonucleases helps prevent accumulation of aberrant 3′-processed forms of this mRNA that arise from intrinsically inefficient 3′-processing signals.

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§ The abbreviations used are: ARD, aci-reductone dioxygenase; ORF, open reading frame.

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Oligonucleotide-mediated RNase H mapping was performed according to (14). Oligonucleotides used for the RNase H mapping were as follow: oligo 1, 5′-GGCAATTAACCCCTAAAGGCGCTTGCGGACAMAATTACC-3′; oligo 2, 5′-CGCGGATCCGGCAATTAACCCCTAAAGGCGCTTGCGGACAMAATTACC-3′; oligo 3, 5′-CTGTGTGATGCGCTGCC-3′; oligo 4, 5′-GGCAATTAACCCCTAAAGGCGCTTGCGGACAMAATTACC-3′; oligo 5, 5′-GGCAATTAACCCCTAAAGGCGCTTGCGGACAMAATTACC-3′; and oligo 6, 5′-CTGTGTGATGCGCTGCC-3′. In vitro cleavage using recombinant Rnt1p or a catalytically inactive mutant, E320K, and mapping of the cleavage sites by primer extension were performed as described (8, 9). The ACAA or short stem loop

YMR010W translation initiation codon to position 200 of the ADI1 ORF; walk 3, ADI1 translation initiation codon to position 200 of the ADI1 ORF; walk 4, from position 200 to position 400 of the ADI1 ORF; walk 8, from 100 nucleotides downstream from the ADI1 ORF to 175 nucleotides upstream of the YMR010W initiation codon; YMR010W, from 250 nucleotides downstream from the ADI1 ORF to 175 nucleotides upstream of the YMR010W initiation codon; YMR010W, from 250 nucleotides downstream from the ADI1 ORF to 175 nucleotides upstream of the YMR010W translation termination codon.

MATERIALS AND METHODS

Strains used in this study have been described previously (9, 12, 13). RNA preparation and Northern analysis were performed according to (9, 12). [32P]dCTP-labeled probes were generated from PCR products spanning the following regions: walk 1, from 400 to 200 nucleotides upstream from the ADI1 ORF; walk 3, ADI1 translation initiation codon to position 200 of the ADI1 ORF; walk 4, from position 200 to position 400 of the ADI1 ORF; walk 8, from 100 nucleotides downstream from the ADI1 ORF to 175 nucleotides upstream of the YMR010W initiation codon; YMR010W, from 250 nucleotides downstream from the ADI1 ORF to 175 nucleotides upstream of the YMR010W translation termination codon.
deletion (ASL) mutations were generated in vivo using the delitto perfetto method (15). After insertion of these mutations in the ADI1 chromosomal locus, in vitro transcription templates for the mutant substrates were generated by PCR using primers spanning the mutated region.

RESULTS

The YMR009W mRNA Encoding the Yeast Aci-reductone Dioxygenase Adi1p Is Expressed in a Complex Pattern and Is Up-regulated in the Absence of Yeast RNase III—We previously analyzed whole genome gene expression in cells lacking the yeast orthologues of RNase III Rnt1p by microarrays (11). Among the candidates identified through our microarray studies was the YMR009W mRNA, which was up-regulated 3-fold in cells lacking Rnt1p. The YMR009W gene encodes a protein with significant sequence similarity to the K. pneumoniae ARD and to the rodent ARD enzyme ALP1 (1, 4, 16). The ARD enzymes are conserved between bacteria, yeast, and mammals (4). The involvement of the gene product of YMR009W in methionine metabolism was demonstrated experimentally (17). We therefore decided to investigate the potential regulation of this member of an important class of enzymes by Rnt1p. Because the ARD or ALP acronyms were already used, we called the gene and protein product of YMR009W ADI1 and Adi1p, respectively, for aci-reductone dioxygenase.

To gain further insight into the potential control of the expression of Adi1p by Rnt1p, we analyzed the expression of the ADI1 mRNA in wild-type cells and in cells lacking Rnt1p (rnt1Δ) by Northern blot analysis. The ADI1 gene is located near a downstream gene, YMR010W (Fig. 2A). We first hybridized membranes containing RNAs extracted from both strains with a probe that hybridizes against the ADI1 ORF (Fig. 2A, walk 4 probe). In addition to the most abundant band, which corresponds to the ADI1 mRNA (see below), this probe also detected three additional minor species, two of which migrated slower and one that migrated faster. Note that the faster migrating species is found in equal abundance in wild-type and rnt1Δ strains, serving as an internal control. This analysis confirmed the higher abundance of the ADI1 mRNA in RNAs extracted from the rnt1Δ cells (Fig. 2A). To further characterize these different species, we synthesized additional probes derived from PCR products of different regions of the ADI1-YMR010W loci. The walk 1 probe hybridizes upstream from the ADI1 ORF, walk 3 hybridizes within the ADI1 ORF, and walk 8 hybridizes within the intergenic ADI1-YMR010W region (Fig. 2A). In addition, we also synthesized a probe hybridizing to the YMR010W mRNA. All of these probes detected the slower migrating species, suggesting that it corresponds to an extended species containing both the ADI1 and YMR010W ORFs (Fig. 2B). The profile of RNAs detected by the walk 3 probe was similar to that of the walk 4 probe (Fig. 2B). The walk 1 probe, which hybridizes upstream from the ADI1 ORF, detected all but the faster migrating species. Note that this probe hybridizes 400 to 200 nucleotides upstream from the ADI1 ORF, suggesting that the ADI1 transcripts have an unusually long 5'-untranslated region. Hybridization with the YMR010W probe revealed the YMR010W mRNA and also detected the largest species (Fig. 2B). The faster migrating species was detected only by the walk 3 and walk 4 probes (Fig. 2B), suggesting that it does not contain much additional sequence besides the ADI1 ORF.

To investigate the status of polyadenylation of species that accumulate in cells lacking Rnt1p, we purified polyadenylated RNAs using oligo(dT) affinity and compared the profiles of RNAs hybridizing to the walk 4 probe in total and poly(A) RNA samples (Fig. 2C). This experiment showed that all species that accumulate in the rnt1Δ strain are polyadenylated. The largest species was not as well enriched as other species in the poly(A)-purified RNAs. This might mean that these long species are heterogeneous polyadenylated or that they were somehow underrepresented during the poly(A) purification because of their long sizes.

Although the expression of most of these forms is higher overall in the rnt1Δ strain, the same bands are observed in the wild-type strain (see Fig. 2B, walk 4 probe), suggesting that this complex pattern of expression is representative of species expressed in the wild-type strain. We further characterized...
these species in RNAs extracted from the rnt1Δ strain because their higher abundance made the analysis easier. We performed RNase H digestion using oligonucleotides hybridizing to different areas of the ADI1-YMR010W genomic region (Fig. 2A), followed by hybridization using three different probes. Oligo 1 hybridizes upstream from the ADI1 ORF, oligos 2 and 3 hybridize to the 5'- and 3'-ends of the ADI1 ORF, respectively, and oligos 4 and 5 hybridize to the intergenic ADI1-YMR010W region, whereas oligo 6 hybridizes to the YMR010W mRNA (Fig. 2A).

In agreement with the pattern of hybridization observed in Fig. 2B, band a in Fig. 2D is clearly downshifted with oligos 2 and 6, showing that it is a long RNA species that spans the ADI1 and YMR010W ORFs. When digested with oligos 2 or 3 and probed with the walk 1 probe, bands a, b, and c (Fig. 2D) disappeared and a single small species appeared, which showed that bands a, b, and c have the same 5'-end. However, bands b and c (Fig. 2D) do not have the same 3'-end. The difference in the 3'-end of these two RNAs most likely lies in the region between oligo 3 and oligo 4, as band b (Fig. 2D) is digested with oligos 4 and 5, whereas band c is unaffected (walk 1 and walk 4 probes). In contrast, oligo 3, which hybridizes upstream from oligo 4, digests these two bands completely.

In agreement with the previously described hybridization pattern (Fig. 2B), band d (Fig. 2D) is a very short RNA that contains mostly sequences of the ADI1 ORF. It is only detected by the walk 3 and walk 4 probes (which hybridize to the ADI1 ORF) and it is downshifted only when digested by oligos 2 and 3. Band e (Fig. 2D) is clearly the mRNA of YMR010W, as it is only digested by oligos 5 and 6.

Overall, these results show that at least five transcripts are generated from the ADI1-YMR010W genomic region (Fig. 2E).
as follows: (i) the canonical ADI1 mRNA (band c) with a rather long 5’-untranslated region; (ii) a shorter ADI1 mRNA with shorter 5’-untranslated region and 3’-end (band d); (iii) a 3’-extended ADI1 mRNA corresponding to band b that may partially extend onto the YMR010W ORF; (iv) the canonical YMR010W mRNA corresponding to band e; and (v) a dicistronic ADI1-YMR010W RNA corresponding to band a. Note that the walk 8 probe used in Fig. 2B detects a doublet of bands migrating faster than the dicistronic mRNA. Given the results of the hybridization with different probes and the RNase H mapping experiments, this doublet is likely to correspond to a mixture of bands b and e (Fig. 2, D and E), i.e., a mixture of 3’-extended ADI1 mRNAs and YMR010W mRNAs that migrate very closely. These results show that the ADI1 locus expresses a variety of transcripts and suggest that some of these transcripts arise from inefficient 3’-processing, which results in a fraction of transcripts that read through into the downstream ORF, YMR010W.

A Model Transcript with Coaxially Stacked RNA Helices Present in the ADI1 mRNA Is Specifically Cleaved in Vitro by Recombinant Rnt1p—Rnt1p usually cleaves double-stranded RNAs capped by tetraloop structures with the sequence AGNN (18, 19). We searched the sequence of ADI1 mRNA for features that would resemble Rnt1p cleavage sites. Analysis of the theoretical secondary structure of the YMR009W mRNA using MFold (20, 21) predicted the existence of two sequential stem loop structures (Fig. 3A). The first stem loop is a small hairpin containing an AGAA tetraloop (gray letters in Fig. 3A) followed by a second longer hairpin with no apparent AGNN-type tetraloop. Stems capped by AGNN-type tetraloop structures are the major determinants of Rnt1p binding and cleavage (18, 22, 23). We hypothesized that this bipartite structure could reconstitute an RNase III cleavage site by stacking the small AGAA-containing stem onto the longer neighboring stem (Fig. 3A). This situation would be reminiscent of some processing signals present in small nucleolar RNA precursor substrates of Rnt1p (7, 8) or of some substrates of bacterial RNase III (24). To test if this bipartite stem loop could be cleaved efficiently in vitro using recombinant Rnt1p, we generated a model substrate containing these stem loops and incubated this substrate in the presence of recombinant Rnt1p and a catalytically inactive mutant (E320K). This experiment showed that Rnt1p cleaves this substrate efficiently in vitro, whereas no significant cleavage was observed with the catalytically inactive E320K mutant (Fig. 3B). Strikingly, the major cleavage generated by the wild-type protein was mapped 9–11 bp from the base of the long stem (Fig. 3, A and B, black double arrows). When the length of the helical region of the short stem loop (4 bp) was added to this...
For yeast RNase III, we generated two mutant substrates. In the first substrate, the AGAA tetraloop is mutated to ACAAn (Fig. 3A). This mutation strongly inhibits Rnt1p cleavage in vitro on canonical stem loop substrates (25). The second substrate carried a deletion of the short stem loop (Fig. 3A, ΔSL), which would be predicted to inhibit Rnt1p activity if the short AGNN-type stem loop directs the catalytic site onto the second stem. As shown in Fig. 3C, both mutations strongly inhibited Rnt1p activity, demonstrated by the large fraction of uncleaved substrate remaining, whereas >80% of the wild-type substrate was cleaved. This result shows that the short stem loop is important for cleavage in the second stem, as suggested by the mapping of the location of the cleavage site in the wild-type substrate. Surprisingly, some residual cleavage activity remained in these mutants. Although most cleavage at the normal site was inhibited, some cleavage was observed at a second site that was also observed in minor amounts with the wild-type substrate (labeled by a gray double arrow in Fig. 3, A and C). In the ΔSL mutant we also observed numerous inefficient cleavages in the double-stranded region, suggesting that the AGNN short stem loop is not only required for efficient cleavage of the long stem but is also needed to dictate the specificity of cleavage within the second stem. In the absence of the short AGNN stem loop, the enzyme probably binds the double-stranded RNA with poor efficiency and cleaves it indiscriminately at multiple sites.

The ADI1 mRNA Is Subject to Degradation by the Xrn1p and Rat1p Exonuclease, whereas 3'-Extended Species Are Degraded by Xrn1p, Rat1p, and Rrp6p—To further investigate the post-transcriptional regulation of the ADI1 mRNA, we analyzed its expression in strains carrying various exoribonuclease mutations. The 5'→3' exonucleases Xrn1p and Rat1p have been shown to cooperate with Rnt1p in the processing or degradation of multiple RNA species (8, 9). Xrn1p is not essential, whereas Rat1p is encoded by an essential gene (26). Therefore we used a double mutant xrn1Δ rat1-1 strain (13) in which both exonuclease activities are inactivated after a shift to 37 °C. The nuclear exosome, a complex of 3'→5' exonucleases, also cooperates with Rnt1p in the processing of multiple RNAs (27, 28). To investigate a potential function for the nuclear exosome in ADI1 mRNA regulation, we used a mutant strain in which the nuclear exosome component Rrp6p is absent (rrp6Δ). We studied the expression of the ADI1 mRNA by Northern blot using RNAs extracted from these strains grown at 25 °C or shifted to 37 °C to inactivate the Rat1p exonuclease. Strikingly, in the wild-type strain we observed an increased expression of the ADI1 mRNA at 37 °C (Fig. 4), suggesting that the expression of the ADI1 gene might be regulated by heat shock conditions. The faster migrating species did not show an increased expression at 37 °C. Given that this species has a shorter 5'-end than the regular mRNA (see Fig. 2), it is likely that this species is expressed from an alternative promoter that is not controlled by heat shock. We observed a stronger accumulation of the ADI1 mRNA in the xrn1Δ rat1-1 strain than in the wild-type strain or in the individual mutant strains, suggesting that Xrn1p and Rat1p cooperate to degrade the ADI1 mRNA (Fig. 4). This increase in the level of the ADI1 mRNA was also apparent in the xrn1Δ strain at 25 °C, showing that this exonuclease plays a major role in controlling the steady-state level of this mRNA (Fig. 4). In addition, we also observed an accumulation of the dicistronic species in the xrn1Δ and xrn1Δrat1-1 strains at both 25 and 37 °C, suggesting that these exonucleolytic activities also participate in the degradation of the polycistronic species that arise from poor 3'-end processing or transcription termination of the ADI1 gene. We did not observe an induction of the ADI1 mRNA at 37 °C in the rrp6Δ strain (Fig. 4), which may be due to the fact that this strain is thermosensitive and that some indirect effect might prevent the induction of this gene at 37 °C. However, we observed a significant accumulation of dicistronic species in this strain, showing that the nuclear exosome also contributes to degradation of these species. Overall, these results show that multiple exonucleases cooperate in the degradation of normal and 3'-extended forms of the ADI1 mRNA. Because the 3'-extended forms are present in the wild-type strain but accumulate only at low levels, we speculate that these species arise from intrinsically poor 3'-processing signals present downstream from the ADI1 mRNA, which results in read-through transcripts in the downstream open reading frame. Multiple exoribonucleolytic pathways therefore contribute to the discarding of these aberrantly terminated or processed mRNAs.

Effects of Heat Shock and the Short Stem Loop Deletion on the Expression of the ADI1 mRNA—The previous experiment showed that the ADI1 mRNA is up-regulated at 37 °C in the wild-type strain, suggesting that this mRNA might be induced under mild heat shock conditions. To further investigate this potential regulation, we shifted wild-type and rat1Δ cells to different temperatures and monitored the expression of the different ADI1 transcripts (Fig. 5). This experiment confirmed the previous observation that the ADI1 mRNA is induced at 37 °C and showed that this induction is also visible after a 15-min exposure to 42 °C (Fig. 5). This result shows that the ADI1 mRNA is generally induced under heat shock conditions. This observation fits previous microarray data (29). We also noticed the appearance of the polycistronic ADI1-YMR010W transcript in wild-type cells that were heat-shocked at 42 °C (Fig. 5). The level of the ADI1 mRNA also increased when the rat1Δ strain was shifted to 37 or 42 °C, showing that the increased expression upon heat shock occurs independently from Rnt1p activity. We also studied a strain expressing an in-frame chromosomal deletion of the short stem loop in the endogenous ADI1 gene. This deletion is identical to the one studied in vitro in Fig. 3C. Strikingly, this deletion resulted in an increase of the expression of the ADI1 mRNA and of the dicistronic ADI1-YMR010W RNA at 42 °C compared with the wild-type strain, but there was no
significant increase at 25 °C compared with the wild-type strain. This result shows that deletion of the short stem loop partially phenocopies the absence of Rnt1p, at least at high temperatures. The lack of phenotype observed at 25 °C should be interpreted in the light of the result showing that deletion of this short stem loop does not completely abolish cleavage in vitro and results in a loss of specificity of the enzyme, which cleaved inefficiently and indiscriminately in the long stem structure of this substrate (Fig. 3C). Thus, it is possible that the residual nonspecific cleavage activity observed with this substrate in vitro is sufficient to provide the enzyme with sufficient activity at 25 °C to cleave this RNA and prevent its expression at normal temperatures. The stronger effect observed at higher temperatures might be due to the fact that a smaller fraction of the transcripts folds into the predicted secondary structure, abolishing the residual cleavage activity. In conclusion, these data show that the short stem loop deletion partially phenocopies the absence of Rnt1p and suggests that Rnt1p cleavage may help down-regulate the expression of the ADI1 mRNA under conditions where the expression of the Adi1p enzyme is unnecessary. In addition, Rnt1p cleavage eliminates the accumulation of 3′-unprocessed species, as shown by the accumulation of 3′-extended and -dicistronic forms of the ADI1 transcripts observed both in cells lacking Rnt1p or expressing a deletion of the short stem loop structure.

**DISCUSSION**

In this study, we show that the expression of the ADI1 gene encoding the yeast ARD enzyme is regulated post-transcriptionally by RNase III cleavage and that multiple ribonucleolytic pathways contribute to the control of the steady-state levels of this mRNA. In addition, we show that the ADI1 mRNA is induced in heat shock conditions. These results provide important advances in our understanding of the regulation of the expression of this class of enzymes. The regulation of the expression of ARD enzymes is an important biomedical problem, as the mammalian ARD gene ALPI has been shown to be down-regulated in prostate cancer cells, and its overexpression inhibits prostate cancer cell proliferation (4). In yeast, the ADI1 mRNA is cleaved by Rnt1p in a bipartite structure found in the open reading frame (Fig. 3). Rnt1p cleavage is normally followed by exonuclease digestion (8, 9, 30). In some cases, Rnt1p cleavage products can be detected when exonuclease mutants are inactivated (8, 9, 30). We did not detect such cleavage products in the case of ADI1 (Fig. 4). It is possible that the overall low levels of expression of this gene makes the detection of cleavage intermediates difficult.

Our results show that Rnt1p cleavage of the ADI1 mRNA serves two purposes. First, cleavage and degradation reduces steady-state levels of the ADI1 mRNA to avoid expression of the enzyme when unnecessary. Because the ADI1 mRNA is shown in this study to be highly expressed in heat shock conditions, it is likely that Rnt1p cleavage occurs at normal growth temperatures and that a strong transcriptional induction under heat shock conditions overrides the steady-state levels of cleavage by Rnt1p. In addition, Rnt1p cleavage plays a role in the surveillance of incorrectly processed species. For example, 3′-extended and dicistronic ADI1-YMR010W species accumulate in the absence of Rnt1p. These species are also observed, but at lower abundance, in wild-type strains (Fig. 2B), suggesting that the 3′-processing of this gene is intrinsically inefficient. Therefore, cells have adopted ways to eliminate RNA species that contain aberrantly processed species. Rnt1p cleavage contributes to the elimination of these aberrant species, but exoribonucleases are involved as well, because these species are observed both in 5′ → 3′ and 3′ → 5′ exonuclease mutants (Fig. 4). Thus, these enzymes act as surveillance enzymes for unprocessed species.

Our study provides a functional framework of the regulation of the ADI1 mRNA, where expression of the gene is prevented by ribonuclease degradation until a burst of expression occurs under heat shock conditions. This heat shock induction occurs in a mechanism that is independent from Rnt1p cleavage, because an increase of expression is also observed in cells lacking Rnt1p (Fig. 5) in heat shock conditions. Thus, it is likely that this burst of induction occurs by transcriptional induction. Because most of the ribonucleases that are involved in the surveillance of the ADI1 mRNA are nuclear (Rnt1p, Rat1p, Rrp6p), it is likely that the strong transcriptional induction is followed by a rapid export out of the nucleus, leaving most of the ADI1 transcripts unaffected by nuclear degradation. Some of these transcripts are however cleaved in heat shock conditions, as the absence of Rnt1p or the deletion of the stem loop structure increases ADI1 mRNA levels during heat shock (Fig. 5). At this point, we do not know what is the precise status of export of the ADI1 mRNA and whether its export mechanism is similar to that of heat shock mRNAs.

Finally, it is interesting to consider why more Adi1p enzyme may be required in heat shock conditions. The major source of methylthioadenosine is polyamine synthesis, from which it is generated as a side product (Fig. 1); thus, if polyamines are synthesized more rapidly under heat shock conditions, then more methylthioadenosine would be generated that would need to be regenerated by the salvage pathway (Fig. 1). An additional source of methylthioadenosine is the non-enzymatic degradation of S-adenosylmethionine (31). Elevated temperatures, such as a switch from 25 to 37 or 42 °C would be expected to increase the non-enzymatic rate of formation of methylthioadenosine from S-adenosylmethionine and might also result in the need to up-regulate the salvage pathway. In either case, if the steps catalyzed by the ARD enzymes are rate-limiting in the salvage pathway, increasing the amount of ARD enzyme would respond to the increased demand for this metabolic pathway. Another possibility is that the ARD proteins, which are activated or more active during heat shock conditions, are involved in other metabolic pathway(s). Further studies on the regulation of the methionine salvage pathway and on the metabolic role(s) of ARD enzymes should answer these questions.

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REFERENCES

1. Dai, Y., Pochapsky, T. C., and Abeles, R. H. (2001) Biochemistry 40, 6379–6387
2. Wray, J. W., and Abeles, R. H. (1995) J. Biol. Chem. 270, 3147–3153
3. Verma, A., Hirsch, D. J., Glatt, C. E., Ronnett, G. V., and Snyder, S. H. (1993) Science 259, 381–384
4. Oram, S., Jiang, F., Cai, X., Haleem, R., Dincker, Z., and Wang, Z. (2004) Endocrinology 145, 1933–1942
5. Abou Elela, S., Igel, H., and Ares, M., Jr. (1996) Cell 85, 115–124
6. Chanfreau, G., Elela, S. A., Ares, M., Jr., and Guthrie, C. (1997) Genes Dev. 11, 2741–2751
7. Chanfreau, G., Legrain, P., and Jacquier, A. (1998) J. Mol. Biol. 284, 975–988
8. Lee, C. Y., Lee, A., and Chanfreau, G. (2003) RNA (N. Y.) 9, 1362–1370
9. Danin-Kreiselman, M., Lee, C. Y., and Chanfreau, G. (2003) Mol. Cell 11, 1279–1289
10. Ge, D., Lamontagne, B., and Elela, S. A. (2005) Curr. Biol. 15, 140–145
11. Lee, A., Henras, A. K., and Chanfreau, G. (2005) Mol. Cell. 19, 39–51
12. Chanfreau, G., Rotondo, G., Legrain, P., and Jacquier, A. (1998) EMBO J. 17, 3726–3737
13. Petfalski, E., Danekar, T., Henry, Y., and Tollervey, D. (1998) Mol. Cell. Biol. 18, 1181–1189
14. van Hoof, A., Lennertz, P., and Parker, R. (2000) EMBO J. 19, 1357–1365
15. Storici, F., Lewis, L. K., and Resnick, M. A. (2001) Nat. Biotechnol. 19, 773–776
16. Pochapsky, T. C., Pochapsky, S. S., Ju, T., Mo, H., Al-Mjeni, F., and Maroney, M. J. (2002) Nat. Struct. Biol. 9, 968–972
17. Subhi, A. L., Diegelman, P., Porter, C. W., Tang, B., Lu, Z. J., Markham, G. D., and Kruger, W. D. (2003) J. Biol. Chem. 278, 49868–49873
18. Chanfreau, G., Buckle, M., and Jacquier, A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3142–3147
19. Chanfreau, G. (2003) Eukaryotic Cell 2, 901–909
20. Zaker, M. (2003) Nucleic Acids Res. 31, 3406–3415
21. Mathews, D. H., Sabina, J., Zuker, M., and Turner, D. H. (1999) J. Mol. Biol. 288, 911–940
22. Nagel, R., and Ares, M., Jr. (2000) RNA (N. Y.) 6, 1142–1156
23. Wu, H., Henras, A., Chanfreau, G., and Feigon, J. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 8307–8312
24. Franch, T., Thisted, T., and Gerdes, K. (1999) J. Biol. Chem. 274, 26572–26578
25. Wu, H., Yang, P. K., Butcher, S. E., Kang, S., Chanfreau, G., and Feigon, J. (2001) EMBO J. 20, 7240–7249
26. Johnson, A. W. (1997) Mol. Cell. Biol. 17, 6122–6130
27. Allmang, C., Kufel, J., Chanfreau, G., Mitchell, P., Petfalski, E., and Tollervey, D. (1999) EMBO J. 18, 5399–5410
28. van Hoof, A., Lennertz, P., and Parker, R. (2000) Mol. Cell. Biol. 20, 441–452
29. Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., Botstein, D., and Brown, P. O. (2000) Mol. Biol. Cell 11, 4241–4257
30. Qu, L. H., Henras, A., Lu, Y. J., Zhou, H., Zhou, W. X., Zhu, Y. Q., Zhao, J., Henry, Y., Caizergues-Ferrer, M., and Bachelier, J. P. (1999) Mol. Cell. Biol. 19, 1144–1158
31. Hoffman, J. L. (1986) Biochemistry 25, 4444–4449