A specialized processing body that is temporally and asymmetrically regulated during the cell cycle in *Saccharomyces cerevisiae*

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RNase mitochondrial RNA processing (MRP) is an essential ribonucleoprotein endoribonuclease that functions in the degradation of specific mRNAs involved in cell cycle regulation. We have investigated where this processing event occurs and how it is regulated. As expected, results demonstrate that RNase MRP is predominantly localized in the nucleolus, where it processes ribosomal RNAs. However, after the initiation of mitosis, RNase MRP localizes throughout the entire nucleus and in a single discrete cytoplasmic spot that persists until the completion of telophase. Furthermore, this spot was asymmetrically found in daughter cells, where the RNase MRP substrate, *CLB2* mRNA, localizes. Both the mitotic exit network and fourteen early anaphase release pathways are nonessential but important for the temporal changes in localization. Asymmetric localization was found to be dependent on the locasome. The evidence suggests that these spots are specialized processing bodies for the degradation of transcripts that are cell cycle regulated and daughter cell localized. We have called these TAM bodies for temporal asymmetric MRP bodies.

Introduction

RNase mitochondrial RNA processing (MRP) is an essential ribonucleoprotein endoribonuclease that cleaves RNA substrates in a site-specific manner and is highly conserved in eukaryotes in sequence and structure (Piccinelli et al., 2005). In the yeast *Saccharomyces cerevisiae*, RNase MRP consists of an RNA core, encoded by the nuclear gene *NME1*, and at least 11 protein subunits (Schmitt and Clayton, 1992; Salinas et al., 2005). Nine of these proteins are shared with a second highly conserved endoribonuclease called RNase P, which is involved in pre-tRNA processing (Chamberlain et al., 1998). The two protein subunits unique to RNase MRP are an RNA binding protein, encoded by the gene *SNM1*, and a recently identified protein, Rmp1p (Schmitt and Clayton, 1994; Salinas et al., 2005).

In *S. cerevisiae*, as in mammalian cells, RNase MRP RNA is found in at least two subcellular organelles. In mitochondria, RNase MRP cleaves RNA transcripts complementary to the origin of replication, forming the RNA primer required for transcription-driven mitochondrial DNA replication (Lee and Clayton, 1998). In the nucleolus, RNase MRP specifically cleaves 27SA preribosomal RNA at the A3 site, forming the 5.8S(s) ribosomal RNA (rRNA; Schmitt and Clayton, 1993; Lygerou et al., 1996). More recent research has uncovered a novel function for RNase MRP in cell cycle regulation (Cai et al., 2002). Mutations in RNase MRP components cause a cell cycle delay in late mitosis characterized by large budded cells, a dumbbell-shaped nucleus, and extended spindles. Analyses determined that RNase MRP directly cleaves the 5′-untranslated region (UTR) of the yeast B-type cyclin, *CLB2* mRNA, allowing for rapid degradation by the 5′ to 3′ exoribonuclease Xrn1p (Gill et al., 2004). Cleavage of the 5′-UTR of *CLB2* mRNA and subsequent degradation by Xrn1p is a unique mode of mRNA turnover in *S. cerevisiae*. The cell cycle delay observed in RNase MRP mutants can be explained by the elevated *CLB2* mRNA levels causing sustained levels of the Clb2p past anaphase, the stage at which Clb2p is normally degraded through ubiquitination by the anaphase promoting complex (APC; Yeong et al., 2000). This results in prolonged Clb2p/Cdk activity, delaying the completion of mitosis. Degradation of the *CLB2* mRNA by RNase MRP fine tunes the system, allowing for rapid simultaneous degradation of both the mRNA and the protein.

It is thought that RNase MRP is predominantly localized to the nucleolus; however, degradation of *CLB2* mRNA is presumed to occur in the cytoplasm. In addition, it is unknown how

RNase MRP activity against the *CLB2* mRNA is regulated, as the enzyme is required to be active throughout the cell cycle to process rRNAs. To investigate this, we examined the in vivo localization of RNase MRP in yeast. We find that its localization is cell cycle controlled, exiting the nucleolus during mitosis and localizing to a single punctate cytoplasmic foci in daughter cells. We propose that it is in these foci that the *CLB2* mRNA is being degraded.

Results

RNase MRP localizes to the nucleolus, the nucleus, and a punctate cytoplasmic spot

In previous experiments, a Pop3p-GFP fusion was used to visualize the localization of RNase MRP. This produced a diffuse nuclear staining pattern indicative of poor association with the RNase MRP complex (Cai et al., 2002), and similar results were seen with an Snm1p-GFP fusion (unpublished data). So that RNase MRP localization could be more clearly defined, a GFP-tagged version of the Pop1p subunit under control of the actin promoter (pTD125 GFP-Pop1p; Table I) was introduced as a reporter for RNase MRP. Because Pop1p is a protein component of both RNase MRP and RNase P, both complexes are visualized with this reporter. A wild-type strain carrying this plasmid had a strong fluorescent GFP signal, the majority of which localized in the nucleus with occasional punctate cytoplasmic staining. Within the nucleus, a more concentrated GFP fluorescence was observed that was presumed to be the nucleolus. This was confirmed by introduction of a second plasmid producing a DsRed1 fusion to the Nop1 protein (Fig. 1; Gadal et al., 2001). Nop1p is a protein known to localize to the nucleolus (Tollervey et al., 1991). This plasmid was used in all subsequent experiments to identify the nucleolus. Merging the GFP and DsRed images indicates that GFP-Pop1p and DsRed-Nop1p colocalized (Fig. 1), consistent with RNase MRP and RNase P localizing to the nucleolus (Bertrand et al., 1998). However, cytoplasmic GFP signal was never seen to overlap with the Nop1p signal.

The GFP-Pop1p fusion was able to complement the lethality of a deletion of the *POP1* gene. Indeed, yeast carrying this as their only version of the *POP1* gene grew at normal rate
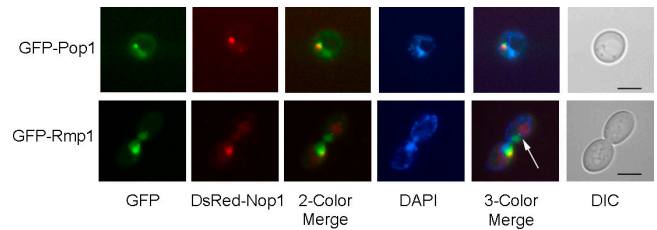

Figure 1. **Localization of the RNase MRP protein subunits, Pop1 and Rmp1, to the nucleolus.** GFP-Pop1 and -Rmp1 proteins predominantly localize to a discrete location in the nucleus. This is also the location of the nucleolar protein Nop1 as indicated by a DsRed-Nop1 protein. A small amount of signal is detected throughout the nucleus. In addition, punctate cytoplasmic staining was seen with both the Pop1 and Rmp1 proteins (arrow). DIC, differential interference contrast. Bars, 5 μm.

at a variety of temperatures and had no apparent defect in rRNA processing or tRNA processing. In addition, visualization of the GFP-Pop1p was identical with or without a wild-type copy of *POP1* being present. This indicates that the GFP-Pop1 is fully functional and is assembled into active RNase MRP and P complexes. Several fusions to the carboxy terminus of the Pop1 coding region were tested, but all of them were unable to complement a deletion of the *POP1* gene and were hence inappropriate for these studies (Lygerou et al., 1994; unpublished data).

Because Pop1p is also a subunit of RNase P, it was important to demonstrate that RNase MRP localized to all of the sites seen, as opposed to only a subset of them. To accomplish this, GFP was fused to Rmp1p, a protein component unique to RNase MRP. The resulting fluorescent Rmp1p localized to the nucleus, the nucleolus, and the punctate cytoplasmic spots in a fashion identical to the GFP-Pop1p (Fig. 1). However, as the fluorescent signal rapidly bleached, the GFP-Pop1p was used for all subsequent localization experiments. Like the GFP-Pop1p fusion, the Rmp1p fusion was found to fully complement a strain deleted for the corresponding wild-type gene (unpublished data).

Nucleolar localization of RNase MRP is consistent with its known role in rRNA processing (Schmitt and Clayton, 1993). However, knowing that RNase MRP cleaves the 5′-UTR of the *CLB2* mRNA to promote its rapid degradation, it was difficult to speculate how this was occurring in the nucleolus (Gill et al., 2004). Because *CLB2* mRNA only needs to be degraded at a certain time in the cell cycle, we examined whether the localization of RNase MRP changes as a cell divides. Wild-type cells (TLG205) at various stages of the cell cycle were examined for their localization pattern (Fig. 2). The stages of the cell cycle were defined by bud size and location of the nucleus relative to the bud neck. RNase MRP remained in the nucleolus until the nucleolus began to move into the bud neck. At that time, RNase MRP localization appears homogeneous throughout the nucleus. After the nucleus had completely divided and the mitotic spindle had disassembled, RNase MRP moved back into the nucleolus, as indicated by colocalization with the DsRed-Nop1p.

While following RNase MRP localization throughout the cell cycle, we observed the consistent presence of a punctate spot localized in the cytoplasm of many, but not all, cells in an asynchronous culture. Hundreds of midlog, asynchronous,

Table I. **Plasmids used for localization studies**

Plasmid	Description	Source
pTD125	pTD125[*URA3 CEN ACT1p-GFP-MCS-ACT1t*]	D. Amberg
pTD125 GFP:POP1	pTD125[*URA3 CEN ACT1p-GFP-POP1-ACT1t*]	This study
pUN100DsRedNOP1	pUN100[*LEU2 CEN4 DsRED-NOP1*]	O. Gadal
pAH1	pRS305[*LEU2 SPC42-CFP*]	D. Amberg
pRP1085	pRS415[*LEU2 CEN6 LSM1-RFP-PGK1 3′UTR*]	R. Parker
pRP1152	pRS416[*URA3 CEN6 DCP2-RFP*]	R. Parker
pTG003	pRS315[*LEU2 CEN6 DCP2-RFP*]	This study
pTG005	pTD125[*URA3 CEN ACT1p-GFP-RMP1-ACT1t*]	This study

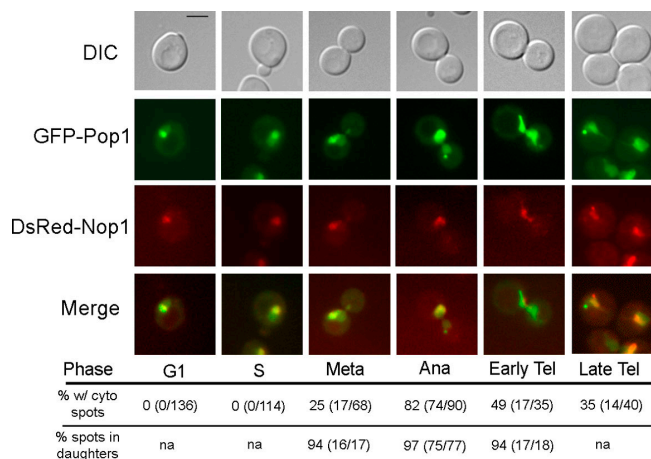| Phase	G1	S	Meta	Ana	Early Tel	Late Tel
% w/ cyto spots	0 (0/136)	0 (0/114)	25 (17/68)	82 (74/90)	49 (17/35)	35 (14/40)
% spots in daughters	na	na	94 (16/17)	97 (75/77)	94 (17/18)	na

Figure 2. **Intracellular localization of RNase MRP changes as cells pass through the cell cycle.** Wild-type exponentially growing yeast cells were examined for RNase MRP localization at different stages of the cell cycle (representative images are shown). The punctate cytoplasmic spot or TAM body is evident beginning in metaphase. The table indicates the percentage of cells with cytoplasmic spots at various stages of the cell cycle and the percentage of those spots that are found in daughter cells. The numbers in parentheses are the actual counts of random cells. DIC, differential interference contrast. Bars, 5 μm.

wild-type cells were examined, which revealed a pattern as to the appearance and localization of this foci. To rule out the possibility that the spots were random GFP aggregates, the localization of RNase MRP to cytoplasmic foci was quantitatively followed throughout the cell cycle. Cells in G1 phase, S phase, metaphase, anaphase, early telophase, and late telophase were scored for the presence of a cytoplasmic spot and the presence of the spot in the mother or daughter cell. The results, summarized in Fig. 2, confirm the observation that the localization of RNase MRP to cytoplasmic foci was a temporally regulated event. RNase MRP localization to a cytoplasmic spot was also seen in wild-type cells carrying the Rmp1p reporter for RNase MRP. The spot does not appear until the nucleus starts to move through the bud neck (metaphase) and can be seen through the end of the cell cycle. The spot was never seen in unbudded (G1 phase) or small budded (S phase) cells or cells arrested with hydroxyurea in S phase. Only a single spot was seen in nearly all cells. More than two spots were never seen in a wild-type strain. Interestingly, the spot localized to the daughter cell in >94% of cells counted. Because the RNase MRP–containing spot appears in a cell cycle–controlled manner and only in the daughter cell, we have named it the TAM body, for temporal asymmetric MRP body.

TAM bodies contain RNase MRP and not RNase P

Snm1 is a unique protein component of RNase MRP that is not shared with RNase P. Previous work has identified several temperature-conditional mutations in this essential gene (Cai et al., 1999). Two of the mutations, *snm1-172* and *-P18*, have been shown to have several genetic interactions with mitotic exit and cyclin mutations (Cai et al., 2002). In addition, these were the first RNase MRP mutants found to display a cell cycle

delay in telophase (Cai et al., 1999). To determine whether Snm1 has a role in localization of the complex to TAM bodies, we investigated the localization of RNase MRP in these mutations. As can be seen in Fig. 3, the *snm1-P18* mutant is defective in localization of the complex to both the TAM body and in concentration of the complex in the nucleolus. In this mutant, only a homogenous nuclear signal was seen and punctate cytoplasmic staining was never seen. This is in contrast to the *snm1-172* mutant, which displayed a pattern indistinguishable from wild type. Loss of TAM body localization in the *snm1-P18* mutant also indicates that RNase P does not localize with RNase MRP to these cytoplasmic spots. If RNase P also localized to the TAM bodies, its localization should not have been disrupted in the *snm1* mutant.

The TAM body is a processing body (P body)–like structure

The specific localization of the TAM body suggested that it might be the daughter cell spindle pole body. To determine whether the TAM body colocalized with the spindle pole body, a wild-type strain was transformed with a CFP-tagged version of the spindle pole component Spc42p (pAH1; Helfant, 2002). The TAM body was found to be a distinct focus from that of either of the two spindle pole bodies. This was consistently seen in all cells examined, indicating that the TAM body is not the daughter cell spindle pole (unpublished data).

Previous research has demonstrated that *CLB2* mRNA is degraded by the exoribonuclease Xrn1p after the 5′-UTR
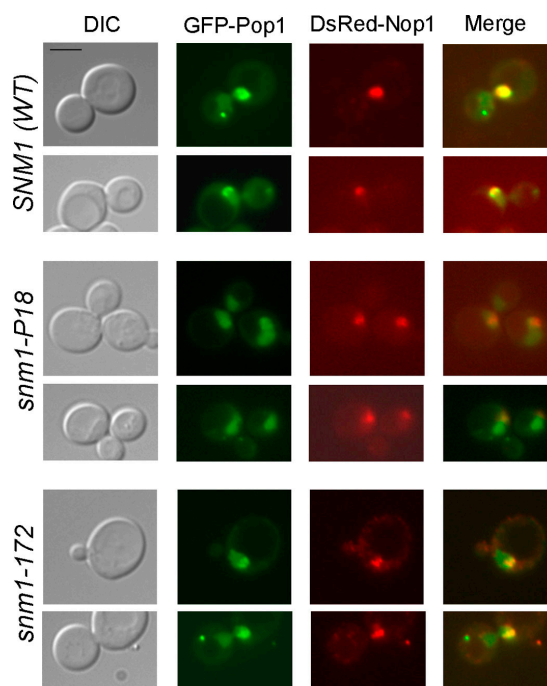

Figure 3. **RNase MRP is mislocalized in an *snm1* mutant.** RNase MRP was localized as in Fig. 2 in a wild-type strain, TLG254 (*SNM1 [WT]*), or the temperature-sensitive *snm1* mutant strains TLG256 (*snm1-172*) and TLG258 (*snm1-P18*). RNase MRP in the *snm1-172* carrying strain localized similar to wild type, but the *snm1-P18* mutation failed to give either nucleolar or TAM body localization. DIC, differential interference contrast. Bar, 5 μm.
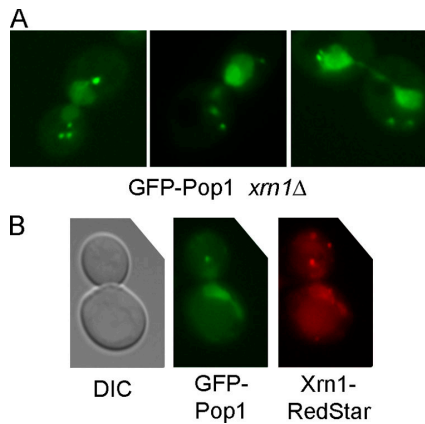
A

GFP-Pop1 *xrn1Δ*

B

DIC   GFP-Pop1   Xrn1-RedStar

Figure 4. **TAM bodies are P body like and colocalize with Xrn1.** (A) The number of TAM bodies increases in an *xrn1* deletion strain. The increase in the number of GFP fluorescing spots indicates that deletion of this exoribonuclease affects the number of TAM bodies per cell, similar to what is seen for P bodies. (B) Xrn1 localized with the TAM body. An Xrn1-RedStar2 fusion was used to localize the Xrn1 protein, whereas RNase MRP was localized using the GFP-Pop1p reporter. Xrn1 displayed multiple discrete cytoplasmic spots in both the mother and daughter cells, consistent with its role as a P body component. However, one of the daughter cell spots typically overlapped with the TAM body. DIC, differential interference contrast.

is cleaved by RNase MRP (Cai et al., 2002; Gill et al., 2004). An *xrn1Δ* strain accumulates RNase MRP cleavage products that are most likely incompetent for translation, as they have no 5′-cap. Many of the proteins involved in decapping mediated mRNA degradation, including Xrn1p, localize to cytoplasmic foci called P bodies. The decapping activator, Dhh1p, has been shown to display a significant increase in the size and number of P bodies that it localizes to in an *xrn1Δ* (Sheth and Parker, 2003). This too is caused by an accumulation of uncapped mRNAs that are incompetent for translation and are accumulating with the mRNA degradation machinery in P bodies. In an *xrn1Δ* strain examined with the reporter GFP-Pop1p, the same phenomenon occurs. There is a clear increase in the number of TAM bodies (two to five per cell; Fig. 4 A) compared with wild type, which has only one spot in most of the cells examined. Localization of the TAM bodies also differs from wild type in that the TAM bodies are present in both the mother and daughter cells. This result suggests that RNase MRP is processing mRNAs in the TAM bodies and that the degradation products are now accumulating in those sites.

These results led us to predict that RNase MRP may also localize to P bodies. Because Xrn1p works with RNase MRP to degrade the *CLB2* mRNA, we predicted that it would colocalize if mRNAs were degraded in the TAM body (Gill et al., 2004). Xrn1p has already been shown to be a P body component that degrades mRNAs after decapping. We generated an Xrn1-RedStar2 fusion and introduced it into a wild-type strain with the GFP-Pop1 reporter. As can be seen in Fig. 4 B, the Xrn1p localized to several discrete foci in cells. In addition, we saw clear colocalization of a single Xrn1p spot with the TAM body. This indicates that the TAM body is a form of P body.

We wanted to determine whether the TAM body was a discrete entity or a P body that adds certain degrading activities

(RNase MRP) at certain times in the cell cycle. To test this, the same *xrn1Δ* strain was transformed with RFP-Lsm1p (pRP1085), an activator of the Dcp1p–Dcp2p decapping complex known to localize to P bodies (Sheth and Parker, 2003). Interestingly, we observed that GFP-Pop1p and RFP-Lsm1p do not colocalize (unpublished data), suggesting that RNase MRP is not a P body component. To confirm these results, we also examined the localization of GFP-Pop1p and Dcp1p-RFP (pTG003) throughout the cell cycle in wild-type cells. As is evident (Fig. 5 A), the two proteins do not colocalize at any stage of the cell cycle, indicating that they are distinct cytoplasmic foci. These results indicate that the Xrn1p protein is associated with the TAM body and that the TAM body may represent a specialized form of a P body involved in degrading specific mRNAs. Yet, the TAM bodies are distinct from the P bodies involved in general mRNA decapping and degradation.

The RNase MRP mutation *nme1-p6* has genetic interactions with mutations in mRNA degradation

We have reported that the RNase MRP mutant, *nme1-p6* (a strong temperature-sensitive point mutation in the gene for the MRP RNA) is synthetically sick in combination with an *xrn1Δ* mutation. *CLB2* mRNA accumulates in an *xrn1Δ* mutant, and in an *nme1-p6/xrn1Δ* double mutant it rises to a level >15-fold higher than wild type (Gill et al., 2004). Because the TAM body appears to be a specialized type of P body, we investigated genetic interactions of the *nme1-p6* mutation with other genes encoding P body components and genes involved in mRNA degradation. The yeast strain yJA203, in which the chromosomal copy of *NME1* is replaced with the *nme1-p6* mutant allele, was mated to strains deleted in genes for *LSM1*, *LSM6*, *LSM7*, *POP2*, *PAT1*, and *CCR4*. Haploid double mutants were selected and tested for synthetic lethality or sickness as indicated by a lack of growth or compromised growth compared with each of the single mutants. As can be seen in Fig. 5 B, the *nme1-p6* mutant displayed synthetic genetic interaction with all of the P body components tested, including *lsm1Δ*, *lsm6Δ*, *lsm7Δ*, *pat1Δ*, *pop2Δ*, and *ccr4Δ*. These results suggest that both pathways are potentially interdependent and interconnected. Cytoplasmic exosome components displayed no synthetic interactions with the MRP RNA component, whereas a deletion of the nuclear exosome component *rrp6Δ* was synthetic lethal with the *nme1-P6* mutation (unpublished data). This result was not unexpected because Rrp6p is involved in 5.8S rRNA processing.

RNase MRP localization in fourteen early anaphase release (FEAR) and mitotic exit network (MEN) mutants

Localization of RNase MRP to distinct cytoplasmic foci occurs during the late stages of the cell cycle. To fine tune the appearance and duration of TAM bodies during mitosis and to potentially identify cell cycle components regulating its localization, we examined GFP-Pop1p localization in various FEAR and MEN mutant strains (Dumitrescu and Saunders, 2002). These strains arrest at slightly different points late in mitosis.
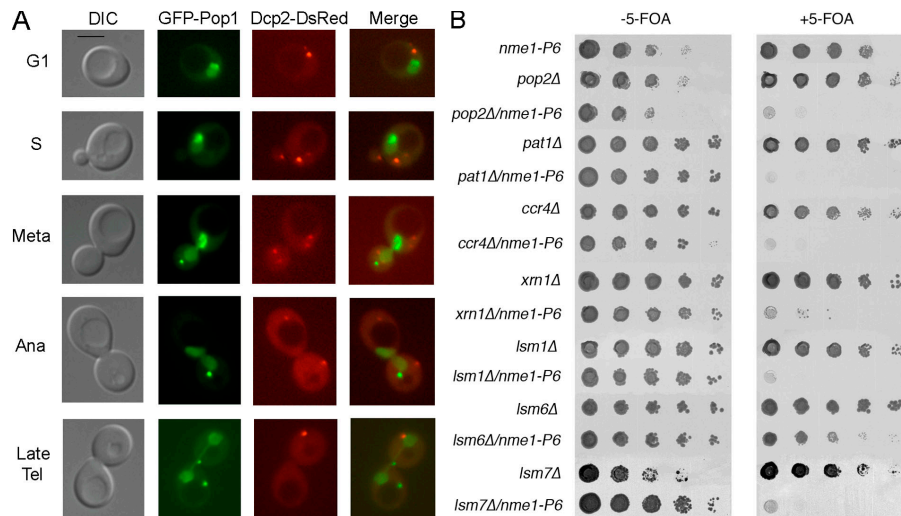
Figure 5. **The TAM body is a specialized P body.** (A) Simultaneous localization of both GFP-Pop1p and Dcp2p-RFP (the catalytic subunit of the decapping enzyme) indicate that they do not overlap. Micrographs of cells at various stages of the cell cycle were taken to determine that the TAM and P bodies are distinct cytoplasmic foci. Bar, 5 μm. (B) An RNase MRP mutant is synthetic lethal with mutations in P body components. A strain carrying an integrated copy of the *nme1-P6* mutation and a wild-type *NME1* gene on a *URA3* plasmid was crossed to a variety of strains deleted for different P body components. Haploid strains derived from the crosses that contained both mutations were tested for the ability to lose the wild-type *NME1*-containing plasmid by plating on 5-FOA. Strains are directly compared with the single mutants. Failure to grow indicates a synthetic lethality or synthetic growth defect. DIC, differential interference contrast.

In addition, wild-type strains were arrested using hydroxyurea to provide an S phase arrest and nocodazole to provide a premitotic arrest. Yeast strains with mutations in *cdc5-1* (TLG208), *cdc14-1* (TLG285), *cdc15-1* (TLG206), and *esp1-1* (TLG277) were grown at 25°C to midlog phase and shifted to 34°C for 2–3 h. Arrested cells were counted and scored for the presence and localization of a TAM body in the mother or daughter cell. The results are summarized in Fig. 6 A. As expected, there were no TAM bodies in cells arrested with hydroxyurea. This is consistent with a previous examination of asynchronous cultures. Nocodazole-arrested cells with a nucleus still in the mother did not have TAM bodies, whereas those that had a nucleus in the daughter cell or trapped between the two cells usually did (unpublished data). Because of the difficulty in separating the two phenotypes, quantitation was not done. Because the mother cell–localized nucleus indicates a premitotic cell and the others indicate a failed mitosis with the addition of nocodazole, the results are consistent with the appearance of the TAM bodies immediately or soon after the beginning of mitosis.

We postulated that Esp1p might be important for RNase MRP release from the nucleolus. Esp1p is a protease that cleaves the cohesions to initiate chromosome segregation and the FEAR pathway. Esp1p and the FEAR pathway are very important in the initial release of Cdc14p from the nucleolus (Dumitrescu and Saunders, 2002). The *esp1-1* mutant arrests between metaphase and the onset of anaphase. In this strain, 40% of the arrested cells had a TAM body, 94% of which localized to the daughter cell. This is comparable to what is seen in the wild-type strain (Fig. 2). This result indicates that RNase MRP is released before initiation of the FEAR pathway. Cdc15p is a protein kinase necessary for the MEN pathway. Cdc5p is also a protein kinase that plays a role in the FEAR

and MEN pathways. Previously, overexpression of Cdc5p was shown to suppress some RNase MRP mutations, whereas the *cdc5-1* mutation was synthetically lethal or sick with other mutations (Cai et al., 2002). Yeast strains with the *cdc5* or *-15* mutations arrest late in mitosis upon a shift to the nonpermissive temperature. Both *cdc5* and *-15* mutants arrested with a high percentage of cells having TAM bodies, similar to what is seen in wild-type telophase cells. In addition, the TAM bodies were restricted to daughter cells. The only cell cycle mutant we tested that differed from wild type was *cdc14*. Cdc14 is a protein phosphatase located at the end of both the FEAR and MEN pathways. Previously, the *cdc14-1* mutant was shown to be synthetically lethal with a mutation in the RNase MRP protein component Snm1 (Cai et al., 2002). In the *cdc14-1* mutant, there was a minor decrease in the number of TAM bodies and a small change in the asymmetry, with 83% of the TAM bodies localizing to the daughter cell as compared with 94% in a wild-type strain (Fig. 6 A).

The *cdc14*, *-15*, and *-5* mutations were also examined for levels of *CLB2* mRNA. The presence of significant amounts of *CLB2* mRNA would indicate that RNase MRP is hampered in TAM body localization or is unable to promote *CLB2* mRNA degradation. As can be seen in Fig. 6 B, levels of *CLB2* mRNA are elevated in an RNase MRP mutant as has been shown previously (Cai et al., 2002). *CLB2* mRNA levels were found to be elevated in the *cdc14* mutant compared with the *cdc5* and *-15* mutants, which arrest at similar points in the cell cycle. This is consistent with it playing a role in the activation of RNase MRP for mRNA degradation and localization. The *cdc5* and *-15* mutants displayed low levels of *CLB2* mRNA. To ensure that sufficient levels of nucleolar RNase MRP remain during mitosis, we examined the processing of the 5.8S rRNA (Fig. 5 C). Movement of RNase MRP to the TAM body may
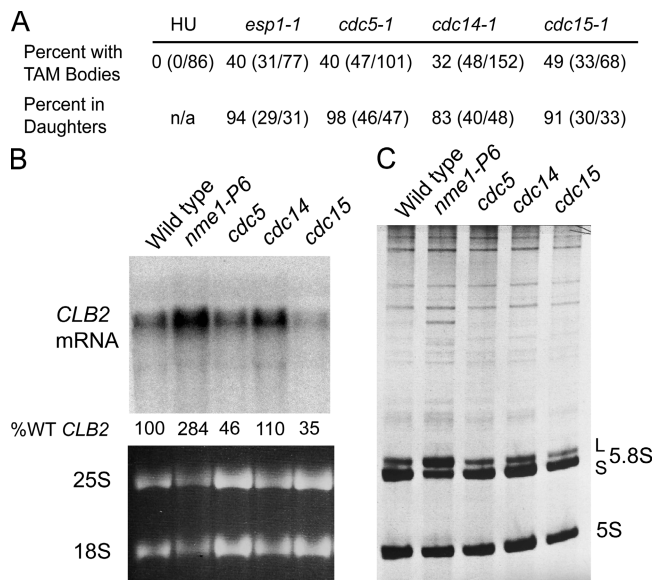
	HU	esp1-1	cdc5-1	cdc14-1	cdc15-1
Percent with TAM Bodies	0 (0/86)	40 (31/77)	40 (47/101)	32 (48/152)	49 (33/68)
Percent in Daughters	n/a	94 (29/31)	98 (46/47)	83 (40/48)	91 (30/33)

Figure 6. **Analysis of RNase MRP in mitotic exit mutant strains.** Complete strain genotypes can be found in Table II. Wild-type (WT), nme1-P6, cdc5, cdc14, and cdc15 strains were grown in rich medium to a density of 0.5–1.0 × 10⁷ cells/ml at 25°C. (A) Presence of TAM bodies at various cell cycle arrest points. Strains carrying the indicated mutation and a GFP:Pop1 reporter were arrested at the nonpermissive temperature or with hydroxyurea (HU). Cells indicative of the arrest point were scored for the presence of TAM bodies and for daughter cell localization. Numbers indicate percentage with TAM bodies and total cell counts. (B, top) Northern analysis for CLB2 mRNA. (bottom) Ethidium bromide staining of the gel before transfer, indicating the RNA loading. The mutant from which the RNA was derived is indicated above each lane. Percentage of wild-type CLB2 mRNA was calculated from the intensity of bands in the northern analysis and standardizing against the 18S rRNA. (C) Analysis of steady-state levels of the 5.8S rRNA. Total cellular RNA was separated on a 6% acrylamide gel and visualized with ethidium bromide. A change in the ratio of the 5.8S small (S) and large (L) RNAs results from a deficiency in RNase MRP activity.

lead to a reduction in processing of the nucleolar substrate. However, no changes in processing of the 5.8S rRNA were seen in any of the cell cycle mutants, even after 2 h at the nonpermissive temperature, indicating that sufficient RNase MRP enzyme remains in the nucleolus to continue rRNA processing.

Daughter cell localization of the TAM body is dependent on the locasome

The locasome is a complex of proteins that utilizes the actin cytoskeleton to localize specific mRNAs to the daughter cell (Gonsalvez et al., 2005). Asymmetrically localized mRNAs include CLB2 and ASH1 mRNAs (Gonsalvez et al., 2005). To determine whether TAM body localization is dependent on the locasome, we examined the localization of the GFP-Pop1p reporter in strains with deletion mutations for two of the proteins of the locasome, myo4Δ and she2Δ. Cultures were grown to midlog phase and examined for the presence and localization of TAM bodies. In each strain, RNase MRP properly localized to the nucleus and nucleolus, as can be seen in the myo4Δ strain in Fig. 7 A. RNase MRP also localized to the TAM bodies at the correct time in the cell cycle. However, the TAM bodies no longer localized asymmetrically to the daughter. In myo4Δ and she2Δ strains, the TAM body localized to the daughter cell in
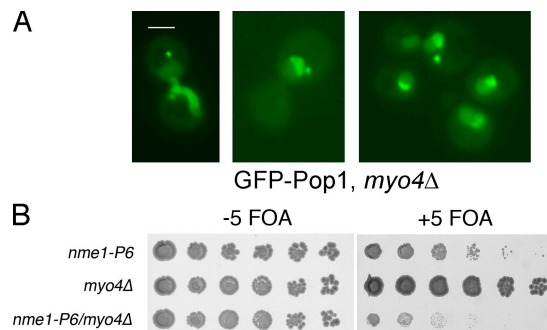

Figure 7. **The locasome is required for the asymmetry of TAM bodies.** (A) RNase MRP was localized as in Fig. 2 in a strain deleted for the myo4Δ gene. The GFP-Pop1 protein was still localized to the nucleolus and to a punctate cytoplasmic spot during mitosis. However, the TAM bodies were found in both the mother and daughter cells. Similar results were found with the she2 mutant. Bar, 5 μm. (B) The nme1-P6 mutation was combined with the myo4Δ, as in Fig. 5 B, to examine genetic interactions. Slow growth of the double mutant on media with 5-FOA indicates a synthetic sick interaction between the two genes.

only 29.7 and 41.4% of cells, respectively, as compared with 94% in wild type, indicating that localization has become random. Interestingly, a small number of unbudded cells (∼5%) also contained TAM bodies.

Because the CLB2 mRNA is both an RNase MRP substrate and an asymmetrically localized RNA, we examined whether daughter cell localization of RNase MRP was dependent on the presence of the CLB2 mRNA. RNase MRP may be using the CLB2 mRNA to carry it out to the daughter cell. However, we saw no changes in daughter cell localization or timing of TAM bodies in a clb2Δ (unpublished data).

The RNase MRP mutation nme1-P6 was also combined with the myo4Δ mutation to test for genetic interactions. As can be seen in Fig. 7 B, the double nme1-P6/myo4Δ strain grew considerably slower than either of the individual mutations. This genetic interaction indicates that localization of TAM bodies to daughter cells is not essential, but it is important for efficient mRNA degradation, especially when RNase MRP activity is limiting. However, we saw no change in levels of the CLB2 mRNA in a strain carrying a myo4Δ (unpublished data).

Discussion

RNase MRP is cell cycle regulated

RNase MRP is an evolutionarily conserved ribonucleoprotein endoribonuclease that performs several RNA processing events. We recently described a role for this endoribonuclease in degrading the CLB2 mRNA. A failure to degrade the CLB2 mRNA leads to persistent mRNA levels and consequently sustained Clb2 protein levels. This keeps the yeast Cdk active and delays exit from the cell cycle (Cai et al., 2002; Gill et al., 2004). We have demonstrated here that the activity of RNase MRP against mRNAs appears to be regulated during the cell cycle by changes in intracellular localization. Because RNase MRP must be active and localized to the nucleolus throughout the cell cycle to process rRNAs, changes in its localization are a simple and efficient means to regulate the activity of this enzyme against specific substrates.

The data indicate that RNase MRP localizes to a discrete spot in the cytoplasm. The earliest signs of this spot are immediately after the initiation of mitosis. The spot often appears to emanate from the nucleus as it pushes into the daughter cell but then clearly becomes a separate entity. The spot can be seen very late into the cell cycle in telophase cells. However, once septation has occurred, cytoplasmic localization is never seen. Occurrence of the discrete spot coincides with the disappearance of *CLB2* mRNA (Spellman et al., 1998), suggesting that the TAM body is the site of *CLB2* mRNA degradation. Clb2 protein appears to be degraded in two waves, the first wave being at the initiation of mitosis by Cdc20p and the APC and the second wave coming at the end of mitosis by Cdh1 and the APC (Yeong et al., 2000). Degradation of the *CLB2* mRNA during the first wave would be essential for proper regulation of Clb2 protein levels.

Late in the cell cycle, RNase MRP loses its concentrated nucleolar localization and localizes homogenously throughout the nucleus. The nucleolus never breaks down in yeast as indicated by Nop1p staining and the continuation in ribosome biogenesis (Bernstein and Baserga, 2004), so the reason behind the changes in nuclear staining are unclear. It may correspond to an unknown processing event by RNase MRP or P, as we cannot differentiate between RNase P and MRP in the nuclear fluorescence.

Specialized P bodies

In yeast, most mRNA is very efficiently exported out of the nucleus and is found in the cytoplasm. Hence, localization of RNase MRP to the cytoplasm is consistent with it degrading the *CLB2* mRNA and other mRNAs in that locale (Gill et al., 2004). Several recent reports have identified similar discrete cytoplasmic spots in several organisms from yeast to humans (Sheth and Parker, 2003; Kedersha et al., 2005; Liu et al., 2005). These spots, called P bodies, contain much of the machinery for mRNA degradation and are believed to be the site of removal of mRNAs. Exponentially growing yeast cells typically contain between 4 and 10 P bodies that are seen throughout the cell cycle. We found that although RNase MRP localizes in a spot with the Xrn1 nuclease, it does not localize with the decapping-enzyme Dcp2 or the decapping-accessory protein Lsm1. Xrn1p has been directly shown to participate with RNase MRP in mRNA degradation. These results indicate that P bodies may be specialized for various functions. Some P bodies that perform traditional decapping and constitutive degradation of mRNA may be present throughout the cell cycle, whereas other P bodies that initiate degradation through mRNA-specific endoribonucleases are present only during certain times or conditions. We propose that one such specialized P body contains the RNase MRP nuclease and is present only in single copy during mitosis. The initial study that identified P bodies in yeast demonstrated that localization with different P body components gave different numbers of P bodies per cell (Sheth and Parker, 2003). The significance of this was not clear at the time but may indicate further specialization of these particles. Likely there are other types of specialized P bodies that may perform other regulated mRNA degradation functions.

A temperature-sensitive mutation in the RNase MRP RNA, *nme1-P6*, is synthetic lethal or synthetic sick with several P body components. The explanation for this phenotype is twofold. First, compromising the regulated MRP-dependent mRNA degradation pathway may make certain messages completely dependent on the constitutive pathway for mRNA degradation. This is clearly the case for the genetic interactions with a deletion of *dcp2Δ* and *lsm1Δ*. The *lsm1Δ/nme1-P6* double mutant grew extremely slowly, and we were unable to make a strain heterozygous for the *nme1-P6* mutation and deleted for *dcp2Δ* (unpublished data). The second explanation for the phenotype is if some of these components participate with RNase MRP in degradation of certain mRNAs. This is clearly the case for the Xrn1 exonuclease, which has been shown to degrade RNase MRP cleavage products (Gill et al., 2004). The fact that there is an increase in the number of TAM bodies in an *xrn1Δ* strain and that Xrn1p localizes to the TAM bodies indicates that *CLB2* mRNA degradation occurs there.

The synthetic lethality of the *nme1-p6/ccr4Δ* double mutant may be caused by the accumulation of mRNAs that cannot be degraded because they have not been deadenylated or are degraded at a much slower rate by an alternate pathway. This may also hold true for the *nme1-p6/pop2Δ* because Pop2p is a regulator of deadenylation (Coller and Parker, 2004).

Cell cycle regulation

We examined several cell cycle arrest conditions to determine in detail the timing and control of RNase MRP relocalization. TAM bodies were never seen in cells arrested with hydroxy urea during S phase or with nocodazole before mitosis, consistent with RNase MRP release being a postmitotic event. Localization was also examined in strains with mutations in several cell division cycle regulators that work at the end of mitosis. These include *esp1* (the yeast separase), *cdc5* and *cdc15* (protein kinases in the FEAR and MEN pathways), and *cdc14* (the protein phosphatase at the end of both the FEAR and MEN pathways). TAM bodies were seen in all of these mutants, indicating that
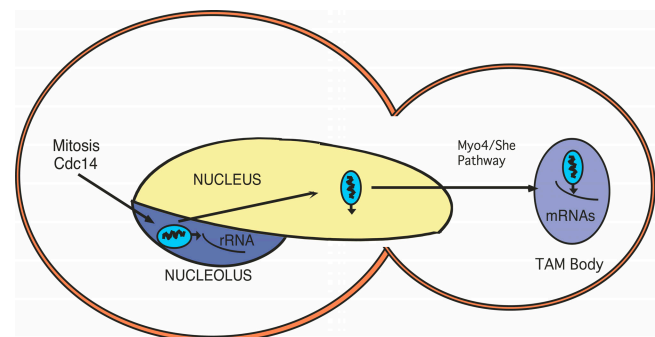

Figure 8. **Model for regulation of RNase MRP during the cell cycle.** RNase MRP is localized to the nucleolus through most of the cell cycle. During mitosis, RNase MRP localizes into the nucleoplasm and into the cytoplasm, where it collects asymmetrically in the daughter cell TAM body via the Myo4–She pathway. Localization to the TAM body allows for degradation of daughter cell–localized mRNAs, such as the *CLB2*, that need to be degraded during mitosis. Both the Snm1 protein component of RNase MRP and the cell cycle phosphatase, Cdc14, appear to be important for the changes in localization.

Table II. **S. cerevisiae strains used in this study**

Strain	Genotype	Source
DBY2006	MATα his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 ade2-1	Schmitt and Clayton, 1992
DBY2007	MATa lys2 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1	Schmitt and Clayton, 1992
MES111-140	MATα his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 nme1-Δ2::TRP1 pMES140[LEU2 CEN NME1]	Schmitt and Clayton, 1994
MES111-P6	MATα his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 nme1-Δ2::TRP1 pMES140-P6[LEU2 CEN nme1-P6]	Schmitt and Clayton, 1994
MES116	MATα lys2-801 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 nme1-Δ2::TRP1 pMES127[URA3 CEN NME1]	Schmitt and Clayton, 1992
MES117	MATa lys2-801 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 nme1-Δ2::TRP1 pMES127[URA3 CEN NME1]	Schmitt and Clayton, 1992
THR200	MATa ade2 his3-Δ200 leu2-3,112 trp1-Δ1 ura3-52 snm1-Δ1::HIS3 pTH101[URA3 CEN SNM1]	Cai et al., 1999
TLG105	MATα xrn1-Δ1::KANR nme1-Δ2::TRP1 ade2-1 leu2-3,112 trp1-Δ1 his3-Δ200 ura3-52pMES127[URA3 CEN NME1]	Gill et al., 2004
TLG153	MATα xrn1-Δ1::KANR nme1Δ2::TRP1 ade2-1 leu2-3,112 trp1-Δ1 his3-Δ200 ura3-52 pMES140[LEU2 CEN NME1]	Gill et al., 2004
TLG205	MATa ade2-1 leu2-1,112 trp1-Δ1 his3-Δ200 ura3-52 pUN100[LEU2 CEN4 DsRED-NOP1] pTD125[URA3 CEN GFP-POP1]	This study
TLG206	MATa ade2-1(or ade1) leu2-3,112 trp1-Δ1 his3-Δ200 ura3-52 cdc15-1 pUN100[LEU2 CEN4 DsRED-NOP1] pTD125[URA3 CEN GFP-POP1]	This study
TLG208	MATa ade2-1(or ade1) leu2-3,112 trp1-Δ1 his3-Δ200 ura3-52 cdc5-1 pUN100[LEU2 CEN4 DsRED-NOP1] pTD125[URA3 CEN GFP-POP1]	This study
TLG212	MATa ade2-1 leu2-1,112 trp1-Δ1 his3-Δ200 ura3-52 pTD125[URA3 CEN GFP-POP1]	This study
TLG216	MATa ade2 his3-Δ200 leu2-3,112 trp1-Δ1 ura3-52 snm1-Δ1::HIS3 pTC120[LEU2 CEN SNM1] pTD125[URA3 CEN GFP-POP1]	Cai et al., 1999
TLG217	MATa ade2 his3-Δ200 leu2-3,112 trp1-Δ1 ura3-52 snm1-Δ1::HIS3 pTC172[LEU2 CEN snm1-172] pTD125[URA3 CEN GFP-POP1]	Cai et al., 1999
TLG218	MATa ade2 his3-Δ200 leu2-3,112 trp1-Δ1 ura3-52 snm1-Δ1::HIS3 pTHR100-p18 [LEU2 CEN snm1-p18] pTD125[URA3 CEN GFP-POP1]	Cai et al., 1999
TLG254	MATa ade2 his3-Δ200 leu2-3,112 trp1-Δ1 ura3-52 snm1-Δ1::HIS3 pTC120[LEU2 CEN SNM1] pTD125[URA3 CEN GFP-POP1] pRS314[TRP1 CEN6 DsRED-NOP1]	This study
TLG255	MATa ade2 his3-Δ200 leu2-3,112 trp1-Δ1 ura3-52 snm1-Δ1::HIS3 pTC172[LEU2 CEN snm1-172] pTD125[URA3 CEN GFP-POP1] pRS314[TRP1 CEN6 DsRED-NOP1]	This study
TLG258	MATa ade2 his3-Δ200 leu2-3,112 trp1-Δ1 ura3-52 snm1-Δ1::HIS3 pTHR100-p18[LEU2 CEN snm1-p18] pTD125[URA3 CEN GFP-POP1] pRS314[TRP1 CEN6 DsRED-NOP1]	This study
TLG262	MATα ade2-1 leu2-1,112 trp1-Δ1 his3-Δ200 ura3-52 pTD125[URA3 CEN GFP-POP1] pAH1[pRS305(LEU2) SPC42-CFP]	This study
TLG265	MATα ade2-1 leu2-1,112 trp1-Δ1 his3-Δ200 ura3-52 pAH1[pRS305(LEU2) SPC42-CFP]	This study
TLG277	MATa esp1-1 leu2-3,112 his3-11,15 ura3 ade2-1 can1-100 CDC14-3HA pTD125[URA3 CEN GFP-POP1]	This study
TLG285	MATa ade2-1 leu2-1,112 trp1-Δ1 his3-Δ200 ura3-52 cdc14-1 pTD125[URA3 CEN GFP-POP1] pUN100[LEU2 CEN4 DsRED-NOP1]	This study
TLG287	MATa his3-Δ0 leu2-Δ0 met15-Δ0 ura3-Δ0 xrn1-Δ0 pTD125[URA3 CEN GFP-POP1]	This study
TLG289	MATα ade2-1 leu2-1,112 trp1-Δ1 his3-Δ200 ura3-52 pTD125[URA3 CEN GFP-POP1] pTG003[LEU2 CEN Dcp2p-RFP]	This study
TLG291	MATa his3-Δ0 leu2-Δ0 met15-Δ0 ura3-Δ0 xrn1-Δ0 pTD125[URA3 CEN GFP-POP1] pTG003[LEU2 CEN Dcp2p-RFP]	This study
TLG293	Mat α leu2-1,112 trp1-Δ1 his3-Δ200 ura3-52 pTG003[LEU2 CEN Dcp2p-RFP]	This study
TLG295	MATa his3-Δ0 leu2-Δ0 met15-Δ0 ura3-Δ0 xrn1-Δ0 pTG003[LEU2 CEN Dcp2p-RFP]	This study
TLG312	MATα ade2-1 leu2-3,112 trp1-Δ1 his3-Δ200 ura3-52 pTD125[URA3 CEN GFP-POP1] pRP1085	This study
TLG314	MATa his3-Δ0 leu2-Δ0 met15-Δ0 ura3-Δ0 xrn-Δ0 pTD125[URA3 CEN GFP-POP1] pRP1085	This study
TLG316	MATa lys2 ade2-1 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 pTD125[URA3 CEN GFP-POP1]	This study
TLG318	MATa lys2 ade2-1 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 pTD125[URA3 CEN GFP-POP1] pUN100[LEU2 CEN4 DsRED-NOP1]	This study
TLG321	MATa his3Δ1 ura3Δ0 leu2Δ0 lys2Δ0 or met15Δ0 she2::KanMX4	This study
TLG324	MATa his3Δ1 ura3Δ0 leu2Δ0 lys2Δ0 or met15Δ0 myo4::KanMX4	This study
TLG334	MATa his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 XRN1-RedStar2-natNT2r	This study
TLG336	MATa his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1 XRN1-RedStar2-natNT2r pTD125[URA3 CEN GFP-POP1]	This study
YJA203	MATa his3Δ1 ura3Δ0 leu2Δ0 lys2Δ0 can1^r mfa1-Pr$_{mfa1}$-SpHis3$^+$ NME1::nme1-p6:NAT pJA203 [URA3:NME1]	This study
BY4741	MAT α his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 orf::KanMX4	Euroscarf
KLS109	MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0ura3Δ0 met15Δ0/MET15 LYS2/lys2Δ0 YLR145W/YLR145W:KanMX pKLS109[CEN LEU2 YLR145W]	Salinas et al., 2005
TLG343	MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0ura3Δ0 met15Δ0/MET15 LYS2/lys2Δ0 YLR145W/YLR145W:KanMX pTG005[URA CEN GFP-RMP1]	This study
TLG348	MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0ura3Δ0 met15Δ0/MET15 LYS2/lys2Δ0 YLR145W/YLR145W:KanMX pTG005[URA CEN GFP-RMP1] pUN100[LEU2 CEN4 DsRed-NOP1]	This study

RNase MRP remains relocalized through the end of mitosis. However, the *cdc14* mutant displayed a clear reduction in the percentage of arrested cells with TAM bodies. The activity of the Cdc14 phosphatase is clearly not required for RNase MRP regulation, but it may be required for full localization and activity. This is consistent with elevated levels of the *CLB2* mRNA in an arrested *cdc14-1* mutant. Cdc14p could be involved in both release and relocalization of RNase MRP. Cdc14p and several other mitotic exit regulators display strong genetic interaction with mutations in RNase MRP components (Cai et al., 2002). Future studies to examine the exact cell cycle regulators that lead to RNase MRP relocalization will be interesting. Loss of

proper localization in mutations in the RNase MRP–specific protein Snm1 may indicate that this protein is key in regulating the relocalization pattern. Indeed, Rmp1pp, the other specific protein, may play an important role.

Asymmetric RNase MRP localization

Cytoplasmic localization of RNase MRP was reserved to a single spot. Interesting, that spot was exclusively localized to the daughter cell. A mother cell spot was seen in <6% of cells examined and was usually found in the rare cell that had two spots. Identical to the RNase MRP complex, *CLB2* mRNA is asymmetrically localized to daughter cells (Gonsalvez et al., 2005). Localization of *CLB2* mRNA is dependent on the locasome (Gonsalvez et al., 2005). The locasome is a complex of several proteins that uses the actin cytoskeleton to transport >30 different mRNAs into daughter cells. Our results indicate that this complex is also necessary for localization of RNase MRP to daughter cells. Deletion of the locasome components *myo4* or *she2* leads to a loss of asymmetric RNase MRP localization.

Whether RNase MRP localizes to the daughter cell by associating with the locasome or with locasome-associated mRNAs is not clear. We tested to determine whether localization of RNase MRP was dependent of the *CLB2* mRNA and found it was not. However, this does not exclude RNase MRP from traveling with a different RNA. The RNase MRP mutant *nme1-P6* was found to be synthetically sick with a deletion in the *myo4* gene, indicating that daughter cell localization of RNase MRP is important for its activity in mRNA degradation. Localization of RNase MRP to the site of its mRNA substrate adds an elegant mechanism of regulating and refining cell cycle control.

RNA degradation as an ancient form of cell cycle regulation

RNase MRP, like its homologue RNase P, is an ancient enzyme that is thought to be a vestigial leftover from the RNA world (Orgel, 1986). These enzymes perform ancient processing jobs, including the production of tRNAs, the initiation of DNA replication, and the production of ribosomes. In the world of today, proteins mainly control cell cycle regulation. These proteins often lead to posttranslational modifications of other proteins, producing a plethora of effects from enzymatic activation and inhibition to degradation or relocalization. However, before proteins evolved, cells required a means of cell cycle regulation. Enzymes that degraded or synthesized various RNAs that performed other enzymatic functions would be the simplest mechanism. The identification of RNase MRP functioning in regulating the cell cycle by degrading specific mRNAs adds credence to this hypothesis. Indeed, regulated localization of an RNA enzyme to its site of action or substrate adds an additional level of regulation that could have been performed in the RNA world.

Model for RNase MRP regulation

Three lines of evidence suggest that TAM bodies are the sites of RNase MRP–directed mRNA degradation. First, the temporal appearance of the TAM bodies coincides with the time of degradation of the *CLB2* mRNA. Second, deletion of the Xrn1p

nuclease leads to accumulation of RNase MRP–cleaved *CLB2* mRNA (Gill et al., 2004). The accumulation of RNase MRP products and, hence, Xrn1p substrates is probably what leads to the increase in TAM body numbers. Third, asymmetric localization of RNase MRP is consistent with previous reports of asymmetric localization of the *CLB2* mRNA. There are clearly other important mRNA substrates of RNase MRP (Cai et al., 2002; Gill et al., 2004). It will be interesting if they are also asymmetrically localized.

In our model, the initiation of mitosis produces a signal for the release of RNase MRP from the nucleolus (Fig. 8). Clearly, the Cdc14 phosphatase and Snm1p play some role in the relocalization or activation of RNase MRP for mRNA degradation. Substantial levels of RNase MRP must remain behind in the nucleolus to continue rRNA biogenesis, and this is indicated because neither nucleolar localization of RNase MRP nor RNase MRP–dependent rRNA processing are lost. Once RNase MRP localizes to the cytoplasm, daughter cell localization appears to be mediated by the locasome. It is not clear whether the locasome is specifically carrying RNase MRP into the daughter cell or if it is being transported with one of its substrates or the entire TAM body. Once in the TAM body, the evidence suggests that certain mRNAs, including the *CLB2* mRNA, are degraded. Degradation of these RNAs by RNase MRP and Xrn1p may actually lead to breakdown of the TAM body and relocalization of RNase MRP back into the nucleus and then the nucleolus.

Materials and methods

Strains
A list of yeast strains used in this study is provided in Table II. Strain construction and basic molecular biology techniques were performed as described previously (Sambrook and Russell, 2001). The *Escherichia coli* strain used for cloning, DH5α, has the genotype φ80dlacZΔM15 endA1 recA1 hsdR17 (r$_k$– m$_k$+) supE44 thi-1 λ⁻ gyrA96 relA1 Δ(lacIZYA-argF)U169 F⁻. The yeast strain TLG334 (Table II) was made by integrating RedStar2, a red fluorescent protein tag, at the *XRN1* chromosomal locus. The RedStar2 tag was amplified off the toolbox plasmid pYM-43 (Janke et al., 2004) using the primers OXRN/S2 (5′-TAT ACT ATT AAA GTA ACC TCG AAT ATA CTT CGT TTT TAG TCG TAT GTT CTA ATC GAT GAA TTC GAG CTG G-3′) and OXRN/S3 (5′-AAG TCA CAA AGC AAT GCT GCT GAC CGT GAT AAT AAA AAA GAC GAA TCT ACT CGT ACG CTG CAG GTC GAC-3′). The resulting PCR product was integrated into fresh, competent wild-type cells (DBY2006) using the Li Acetate method (Cai et al., 1999). After the transformation was complete, the cells were resuspended in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) and incubated at 30°C overnight. The cells were then concentrated, resuspended in TE, pH 7.5, and spread on medium containing 100 μg/ml of nourseothricin.

Plasmids
The plasmids used for fluorescence microscopy are listed in Table I. pTD125 and pUN100DsRedNOP1 were gifts from D. Amberg (State University of New York Upstate Medical University, Syracuse, NY) and O. Gadal (Institut Pasteur, Paris, France), respectively. To construct pTG003, pRP1152 [pRS416, *Dcp2:RFP:pgk$_{term}$*] (a gift from R. Parker, University of Arizona, Tucson, AZ; Sheth and Parker, 2003) was digested with the restriction enzymes HindIII and SacI to isolate the DNA fragment containing the *DCP2p:RFP:pgk$_{term}$* fusion and cloned into the HindIII and SacI sites of pRS315 (Sikorski and Hieter, 1989). Subsequent transformations and analyzes were performed as described previously (Sambrook and Russell, 2001).

To make plasmid pTD125 GFP:POP1, the *POP1* gene was amplified off the plasmid pTC101 using the primers OTC4 (5′-CTC GTC GAC CAT ATA ATA AGA TTT GTT GCC ACT-3′) and OTC5 (5′-CGC GGA

TCC ATG AGC GGGA GTTT GTC TAG AG-3′). The resulting 2.7-kb fragment containing *POP1* was cloned into the BamHI and SalI sites of pTD125 (Doyle and Botstein, 1996). Plasmid pTG105 was constructed by cloning the *RMP1* structural gene into the HindIII and BamHI sites of pTD125. *RMP1* was generated by PCR from the plasmid p33/pMES-145RMP1 (Salinas et al., 2005) using the primer ORMP1/Bam(for) (5′-AT ATA GGA TCC ATG GAT GAG ATG GAT AAT GTG-3′) and the reverse primer ORMP1-2 (5′-AA AAA AGC TTA TCC GAA TAT GCC ATC AAT GGC-3′).

Yeast cultures and microscopy
For fluorescence microscopy, strains were grown in SCD (2% dextrose, 0.5% ammonium sulfate, 0.17% yeast nitrogen base, 50 μg/ml phenylala-nine, 40 μg/ml tryptophan, 30 μg/ml lysine, 20 μg/ml methionine, 40 μg/ml adenine, 20 μg/ml histidine, 30 μg/ml leucine, and 20 μg/ml uracil) lacking leucine and uracil for plasmid selection and supplemented with adenine (20 μg/L) to reduce the fluorescence background that can occur in strains with an *ade2* mutation. A 3-ml aliquot of SCD (−leucine, −uracil, +adenine) media inoculated with a single colony was grown at 30°C for 20–24 h. A 10-μl aliquot of the overnight culture was passed into fresh media and grown another 20–24 h at 30°C. The second overnight culture was then used to inoculate 20 ml of SCD media and grown to a density of $0.5–1.0 \times 10^7$ cells/ml. Live cells with fluorescent-tagged proteins were examined on an Axioskop 2 (Carl Zeiss MicroImaging, Inc.) at 100× magnification using a digital camera (ORCA-ER; Hamamatsu) to capture single-focal plane images. All images were processed using Openlab software (Improvision). Final figures were generated using Photoshop software (Adobe).

Yeast cell arrest experiments
Wild-type strain TLG205 was used for hydroxyurea arrest experiments. In each experiment, cell cultures were grown to early log phase ($0.3–0.7 \times 10^7$ cells/ml) in defined SCD media (described in the previous section). Hydroxyurea was added directly to the 20-ml culture for a final concentration of 10 mg/ml. The culture was incubated at 30°C for an additional 3 h, at which time ∼90% of the cells were arrested as medium budded cells. Strains carrying the cell division cycle mutations *cdc5* (TLG206), *cdc14* (TLG208), *cdc15* (TLG285), and *esp1* (TLG277) were used to arrest cells in late mitosis. Cultures were grown as described in the previous section except at 25°C. When cultures reached a density of $0.5–1.0 \times 10^7$ cells/ml, they were shifted to 34°C for 2–4 h.

Northern blot analysis
Strains were grown in YPD at 25°C to a density of $0.7–0.8 \times 10^7$ cells/ml. Half of the culture was harvested and washed, and the RNA was extracted as previously described (Schmitt et al., 1990). The other half of the culture was shifted to 37°C for 3 h, after which time RNA was extracted. Equal amounts of whole-cell RNA were separated on a 1% agarose gel and 2.2 M formaldehyde gel and then transferred to a nylon membrane (Sambrook and Russell, 2001). Northern blots were probed using a 1,360-bp HindIII–SpeI fragment of the *CLB2* structural gene radio-labeled with α-[^{32}P]dCTP using the Prime-it kit (Stratagene). After a 12-h hybridization at 42°C, the membrane was washed three times at 45°C in 1× SSPE and 0.1% SDS and then three times at 45°C in 0.1% SSPE and 0.1% SDS. The hybridized blot was analyzed on a phosphoimager.

Synthetic genetic interactions
Yeast deletion strains were obtained from the Euroscarf gene knockout collection, derived from strain BY4741. These were crossed to the RNase MRP RNA mutant *nme1-P6* in the strain yJA203, in which the chromosomal *NME1* gene was replaced with *NME1::nme1-p6*-NAT. The RNase MRP mutation in this strain is masked by a plasmid-borne wild-type copy. Diploids were sporulated, and 20 tetrads were dissected for each cross. Three tetrads were selected from each verified interaction, and the level of growth defect was quantified by serial dilution and plating of haploid double-mutant cells alongside haploid single-mutant cells, both in the presence and absence of 5-fluoroorotic acid (5-FOA). Complete absence of growth for the haploid double-mutant cells compared with the haploid single-mutant cells on 5-FOA indicated a synthetically lethal genetic interaction. Reduced growth for the haploid double-mutant cells compared with the haploid single-mutant cells on 5-FOA indicated a growth-defective genetic interaction.

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