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 Cib2p past anaphase, the stage at which Cib2p is normally degraded through ubiquitination by the anaphase promoting complex (APC; Yeong et al., 2000). This results in prolonged Cib2p/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 spots. However, cytoplasmic GFP signal was never seen to overlap with the Nop1p signal. A small amount of signal is detected throughout the nucleus. In addition, punctate cytoplasmic staining was seen with both the Pop1p and Rmp1 proteins (arrow). DIC, differential interference contrast. Bars, 5 μm.

![Figure 1.](image)

**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 Pop1p 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-Pop1p 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 1. Plasmids used for localization studies |
|-----------------------------------------------|
| **Plasmid** | **Description** | **Source** |
| pTD125 | pTD125[URA3 CEN ACT1p-GFP-MCS-ACT1]\(\beta\) | D. Amberg |
| pTD125 GFP-Pop1 | pTD125[URA3 CEN ACT1p-GFP-Pop1-ACT1]\(\beta\) | This study |
| pUN100DsRedNop1 | pUN100[LEU2 CEN4 DsRed-Nop1]\(\beta\) | O. Gadal |
| pAH1 | pRS305[LEU2 SPC42-CFP]\(3'UTR\) | D. Amberg |
| pRP1085 | pRS415[LEU2 CEN6 LSM1-RFP-PGK1 3'UTR] | R. Parker |
| pRP1152 | pRS416[URA3 CEN6 DCP2-RFP]\(3'UTR\) | R. Parker |
| pTG003 | pRS315[LEU2 CEN6 DCP2-RFP]\(3'UTR\) | This study |
| pTG005 | pTD125[URA3 CEN ACT1p-GFP-RMP1-ACT1]\(\beta\) | This study |
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.

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 delay in telophase (Cai et al., 1999). To determine whether Smn1 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 colocalize 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.

![Image](image_url)
This indicates that the TAM body is a form of P body.

clear colocalization of a single Xrn1p spot with the TAM body.

localized to several discrete foci in cells. In addition, we saw the GFP-Pop1 reporter. As can be seen in Fig. 4 B, the Xrn1p

degrades mRNAs after decapping. We generated an Xrn1-

Xrn1 has already been shown to be a P body component that

degrades the mRNA.

localize to P bodies. Because Xrn1p works with RNase MRP to

decay the P body-like complexes. 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 synthetically 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.
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, daughter cell. The results are summarized in Fig. 6 A. 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 S-FOA. Strains are directly compared with the single mutants. Failure to grow indicates a synthetic lethality or synthetic growth defect. DIC, differential interference contrast.

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 S-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 cohesion 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. Cdc5p 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 Smn1 (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...
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 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 cdc2Δ (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 Cib2 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. Cdh1 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 two-fold. 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 relocation. TAM bodies were never seen in cells arrested with hydroxyurea 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 Myp4–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 Ssn1 protein component of RNase MRP and the cell cycle phosphatase, Cdc14, appear to be important for the changes in localization.](Image 308x143 to 548x268)
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
proport localization in mutations in the RNase MRP–specific protein Smn1 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 Smn1p 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 mRNAs 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.
TCC ATG AGC GGG ATTT GTG TGC TAG AG-3'. The resulting 2.7-kb fragment containing POP1 was cloned into the BamHI and Sall 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/pmES145RMP1 [Salinas et al., 2005] using the primer ORM1/Bam[for] (5'-ATG GAG TCC ATG GAT GAG ATG AAT G-3') and the reverse primer ORM1-BP12 (5'-CAA AGC TIA TCC GAA TAT GCC ATC AAC 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/ml) 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 again 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 × 10⁷ 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) capture single-focal plane images. All images were processed using Openlab software (Improvis). 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 × 10⁷ 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 μg/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 mutation cdc5 (TLG206), cdc14 (TLG208), cdc15 (TLG285), and esp31 (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 × 10⁷ 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 × 10⁷ 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, transferred to a nylon membrane (Sambrook and Russell, 2001). Northern blots were probed with the nuclear RNase MRP RNA probe for 1 h. The blot was washed, and the hybridized mRNA was visualized by autoradiography.

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 NME1Δ::nme1-pHAT. The RNase MRP mutation in this strain is masked by a plasmid-borne wild-type copy. 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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