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Post-transcriptional regulation through the *HO* 3′-UTR by Mpt5, a yeast homolog of Pumilio and FBF

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***Drosophila* Pumilio (Pum) and *Caenorhabditis elegans* FBF bind to the 3′-untranslated region (3′-UTR) of their target mRNAs and repress translation. Pum and FBF are members of a large and evolutionarily conserved protein family, the Puf family, found in *Drosophila*, *C.elegans*, humans and yeasts. Budding yeast, *Saccharomyces cerevisiae*, has five proteins with conserved Puf motifs: Mpt5/Uth4, Ygl014w, Yll013c, Jsn1 and Ypr042c. Here we report that Mpt5 negatively regulates expression of the *HO* gene. Loss of *MPT5* increased expression of reporter genes integrated into the *ho* locus, whereas overexpression of *MPT5* decreased expression. Repression required the 3′-UTR of *HO*, which contains a tetranucleotide, UUGU, also found in the binding sites of Pum and FBF. Mutation of UUGU to UACU in the *HO* 3′-UTR abolished Mpt5-mediated repression. Studies using a three-hybrid assay for RNA binding indicate that Mpt5 binds to the 3′-UTR of *HO* mRNA containing a UUGU sequence but not a UACU sequence. These observations suggest that the yeast Puf homolog, Mpt5, negatively regulates *HO* expression post-transcriptionally.**

Keywords: *HO*/*MPT5*/Puf/3′-UTR

Introduction

Post-transcriptional regulation plays an important role during development in a wide variety of organisms (Gray and Wickens, 1998). For example, the asymmetric patterns of gene expression in *Xenopus* and *Drosophila* embryos are determined by the differential localization, stabilization and translation of maternally synthesized mRNA. Translational regulation also plays a key role in germline sex determination in *Caenorhabditis elegans* (Goodwin *et al*., 1993; Zhang *et al*., 1997; Jan *et al*., 1999). In most cases studied in detail, post-transcriptional regulation is mediated by sites in the 3′-untranslated region (3′-UTR) of the regulated mRNAs. Although regulatory proteins have been identified that bind specifically to such elements, key questions about how their binding mediates regulation remain unanswered.

Drosophila Pumilio protein (Pum) and *C.elegans* FBF (*fem-3* mRNA-binding factor) are RNA-binding proteins which bind to the 3′-UTR of target mRNAs and repress their translation. In *Drosophila*, Pum binds to a pair of 32 nucleotide sequences (Nanos response elements, NREs) in the 3′-UTR of maternal *hunchback* (*hb*) mRNA and represses its translation in the posterior portion of the embryo (Wharton and Struhl, 1991; Murata and Wharton, 1995). In *C.elegans*, FBF binds to the 3′-UTR of the *fem-3* mRNA, thereby promoting the switch from spermatogenesis to oogenesis (Zhang *et al*., 1997). Pum and FBF are members of a large and evolutionarily widespread protein family, the Puf family (for Pumilio and FBF), found in *Drosophila*, *C.elegans*, humans and yeasts (Zhang *et al*., 1997; Zamore *et al*., 1999). Pum and FBF contain eight Puf repeats, each of ~36 amino acids (Zhang *et al*., 1997). RNA targets of Puf proteins other than Pum and FBF have not yet been identified. It is not known whether these other Puf proteins regulate translation or some other aspect of RNA metabolism.

The budding yeast, *Saccharomyces cerevisiae*, has five proteins that contain three to eight Puf repeats: Mpt5, Ygl014w, Yll013c, Jsn1 and Ypr042c (Zhang *et al*., 1997). *MPT5*, also known as *UTH4*, was first identified as a multicopy suppressor of *pop2* mutants, which are defective in glucose derepression (Hata *et al*., 1998). Mpt5/Uth4 and another Puf protein, Ygl014w, regulate relocalization of Sir3p and Sir4p from telomeres to the nucleolus and are required for the normal life span of yeast cells (Kennedy *et al*., 1995, 1997). Mpt5 interacts with the RGS (regulator of G-protein signaling) protein, Sst2, and plays a role in recovery from pheromone-induced G_1 arrest (Chen and Kurjan, 1997). *JSN1* was identified as a gene whose overexpression supresses a *tub2* mutation, which causes a defect in spindle elongation (Machin *et al*., 1995). Deletion of *JSN1* causes no discernible phenotype. *YPR042C* null mutants are viable and display increased resistance to cycloheximide and paramomycin, both of which inhibit translation (Waskiewicz *et al*., 1998). The molecular mechanisms by which these Puf homologs function in yeast are not known.

The *HO* gene codes for an endonuclease that stimulates mating-type switching in budding yeast (Herskowitz, 1988). It is transcribed only in cells that have budded previously (mother cells) but not in newly born cells (daughter cells). This asymmetric transcription of the *HO* gene results from the preferential accumulation of a negative regulatory protein, Ash1p, in daughter nuclei (Bobola *et al*., 1996; Sil and Herskowitz, 1996). *ASH1* mRNA is targeted to the distal tip of daughter buds in post-anaphase cells (Long *et al*., 1997; Takizawa *et al*., 1997). Localization of *ASH1* mRNA depends on an intact actin cytoskeleton and five *SHE* genes (Jansen *et al*., 1996).

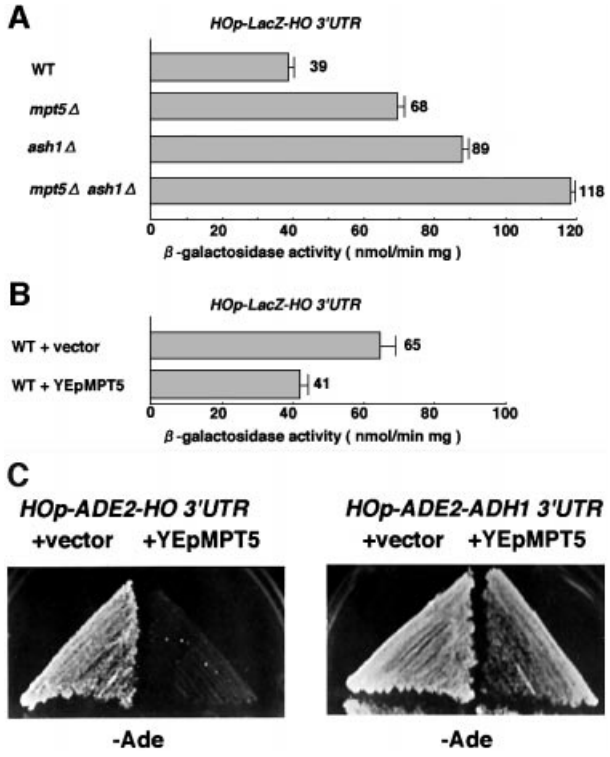

Fig. 1. *MPT5* negatively regulates *HO* expression. (**A**) Effect of the *mpt5*Δ mutation on expression of the *HOp-lacZ* reporter. Yeast strains K1107 (WT), TTC2 (*mpt5*Δ), TTC91 (*ash1*Δ) and TTC191 (*mpt5*Δ *ash1*Δ), harboring the *HOp-lacZ* reporter gene, were grown in YPD medium and assayed for β-galactosidase activity. Units shown are averages of five experiments. (**B**) Effect of *MPT5* overexpression on the *HOp-lacZ* reporter. Yeast strains K1107 carrying *HOp-lacZ* transformed with vector YEp13 or YEpMPT5 were cultured in SC-Leu medium and assayed for β-galactosidase activity. Units shown are averages of three experiments for independent transformants. (**C**) The *HO* 3′-UTR is required for *MPT5*-mediated repression of *HO*. Yeast strains 10B (*HOp-ADE2-HO 3′-UTR*) and TTC47 (*HOp-ADE2-ADH1 3′-UTR*) were transformed with YEp13 or YEpMPT5. The resulting transformants were streaked on SC-Ade Leu (–Ade) plates and incubated for 3 days at 30°C.

Here we describe the characterization of one of the Puf homologs, Mpt5, and show that it is involved in the regulation of *HO* expression. We found that Mpt5 binds to the 3′-UTR of *HO* mRNA and represses *HO* expression. Our results suggest that Mpt5 operates in the same manner as Pum and FBF, by binding to the 3′-UTR of its target mRNA. An *mpt5*Δ mutation allows mating-type switching in daughter cells, suggesting that Mpt5 provides a second mechanism for preventing synthesis of HO protein in daughter cells.

Results

Mpt5 represses expression of HO

Because asymmetric expression of *HO* is determined ultimately by localization of *ASH1* mRNA (Long *et al*., 1997; Takizawa *et al*., 1997), we carried out a systematic survey of the effect of different candidate RNA-binding proteins on expression of *HO*. We examined *HO* expression and *ASH1* mRNA localization in yeast mutants lacking each of the five genes coding for members of the Puf family of RNA-binding proteins: *MPT5*, *YGL014w*, *YLL013c*, *JSN1* and *YPR039c*. We constructed disruptants for each of these Puf genes in a strain harboring a *HOp-ADE2* reporter gene to monitor expression of *HO* (see Materials and methods). *HOp-ADE2* was constructed by replacing the *ho* open reading frame (ORF) with the *ADE2* ORF at the *ho* locus (as also described by Jansen *et al*., 1996). Expression of the reporter can be assayed in an *ade2*Δ background by growth on medium lacking adenine (SC-Ade). In these strains, the *ash1*Δ mutant grew faster than wild-type cells on SC-Ade plates, whereas cells overexpressing *ASH1* did not grow under these conditions (data not shown). Furthermore, inactivation of *SHE* genes (*SHE1*/*MYO4*, *SHE2*, *SHE3*, *SHE4* and *SHE5*), which leads to delocalization of *ASH1* mRNA, prevented growth of these strains on SC-Ade plates. These results demonstrate that *ASH1* negatively regulates the *HOp-ADE2* reporter. *mpt5* disruptants carrying this reporter grew faster than wild-type strains on SC-Ade plates; overexpression of *MPT5* in the *HOp-ADE2* strain completely inhibited growth on SC-Ade plates (see below). In contrast, disruptions of any of the four other Puf genes, *YGL014w*, *YLL013c*, *JSN1* or *YPR039c*, had no effect on growth on SC-Ade plates. Therefore, we further characterized the role of *MPT5* in the regulation of *HO*.

In order to quantitate the effect of *MPT5* on *HO* expression, we next used a *HOp-lacZ* reporter, in which the *ho* ORF was replaced with the *lacZ* ORF at the *ho* locus. Using this reporter, we observed a 2-fold increase in *HO* expression in *mpt5*Δ mutants compared with wild-type cells (Figure 1A). In contrast, *HO* expression decreased ~40% when *MPT5* was overexpressed from a multicopy plasmid (YEpMPT5) (Figure 1B). These results suggest that *MPT5* negatively regulates *HO* expression.

We next examined whether *MPT5* affects *HO* expression by acting on *ASH1* mRNA. Compared with wild-type cells, *ASH1* mRNA was found to be somewhat delocalized in an *mpt5*Δ mutant (Figure 2A): 22% of *mpt5*Δ cells and 87% of wild-type cells localized *ASH1* mRNA to the distal cortex of the bud; 62% of *mpt5*Δ cells and 12% of wild-type cells localized *ASH1* mRNA diffusely within the bud; and 16% of *mpt5*Δ cells and 1% of wild-type cells exhibited substantial *ASH1* mRNA in both mother and daughter cells. However, Ash1 protein was still localized asymmetrically in the *mpt5*Δ cells, similarly to that observed in the wild-type cells (84% for *mpt5*Δ cells versus 92% for wild-type cells; Figure 2B). Therefore, delocalized mRNA in the bud of the *mpt5*Δ strain does not affect the daughter-specific localization of Ash1 protein. Since delocalization of *ASH1* message in *she* mutants causes symmetric localization of Ash1 protein and a reduction in *HO* expression (Jansen *et al*., 1996), the partial delocalization of *ASH1* mRNA in *mpt5*Δ mutants cannot be responsible for enhancing *HO* expression. Furthermore, overexpression of *MPT5* had no effect on *ASH1* mRNA localization (data not shown).

To confirm that *MPT5* does not affect *HO* expression by acting on *ASH1* mRNA, we examined *HO* expression in an *mpt5*Δ *ash1*Δ double mutant using a *HOp-lacZ* reporter. The *mpt5*Δ *ash1*Δ double mutant showed a higher level of expression than either an *mpt5*Δ or *ash1*Δ single mutant (Figure 1A), indicating that *MPT5* and *ASH1* act on *HO* expression independently. Their independent action is supported further by the observation that overexpression

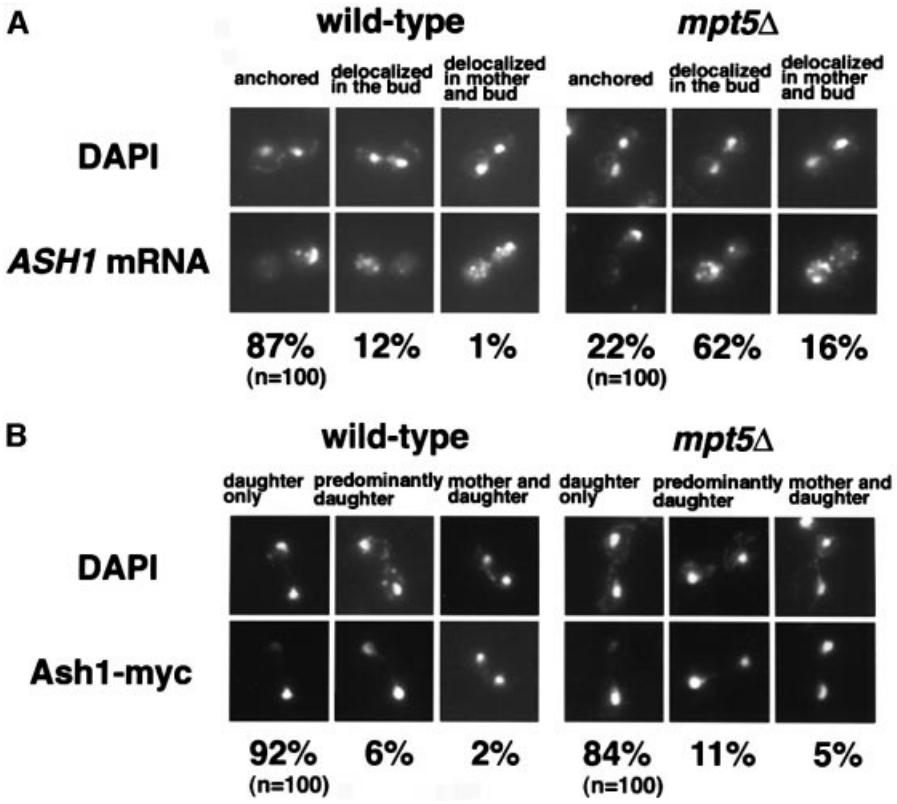

Fig. 2. Effect of the *mpt5*Δ mutation on the localization of *ASH1* mRNA and Ash1 protein. (**A**) Comparison of *ASH1* mRNA localization in K5552 (*ASH1-myc*; wild type) and in TTC219 (*ASH1-myc mpt5*Δ). The percentages of cells that showed each pattern of *ASH1* mRNA localization were determined by RNA *in situ* hybridization: anchored = tightly localized *ASH1* mRNA at the distal tip; delocalized in the bud = delocalized *ASH1* mRNA confined to the bud; delocalized in mother and bud = *ASH1* mRNA in both mother cell and bud. (**B**) Comparison of Ash1 protein localization in K5552 and TTC219. The percentages of cells that showed each pattern of Ash1 protein localization were determined by staining with anti-myc in cells expressing Ash1-myc: daughter only = visible Ash1 only in the daughter; predominantly daughter = Ash1 staining predominantly in the daughter nucleus with an intermediate amount of staining in the mother nucleus; mother and daughter = equivalent levels of Ash1 in both mother and daughter nuclei.

of *MPT5* still repressed *HO* in *ash1*Δ mutants (data not shown).

The HO 3′ UTR is required for MPT5-mediated repression of HO

The Puf proteins, Pum and FBF, bind to the 3′-UTR of their target mRNAs to repress translation (Zhang *et al*., 1997; Sonoda and Wharton, 1999). Mpt5 has a Puf motif, raising the possibility that it represses *HO* expression via the 3′-UTR of *HO*. To test this possibility, we used two reporters that differ only in their 3′-UTR, *HOp-ADE2-HO 3′-UTR* and *HOp-ADE2-ADH1 3′-UTR*. In the latter reporter, the *HO* 3′-UTR was replaced with the *ADH1* 3′-UTR (see Materials and methods). An *ade2*Δ strain harboring either *HOp-ADE2-HO 3′-UTR* or *HOp-ADE2-ADH1 3′-UTR* grew on SC-Ade plates in a manner similar to the *ADE2* strain, indicating that both reporters are expressed. We found that overexpression of *MPT5* from YEpMPT5 clearly inhibited growth of the *HOp-ADE2-HO 3′-UTR* strain and slightly inhibited that of the *HOp-ADE2-ADH1 3′-UTR* strain on SC-Ade plates (Figure 1C). The partial inhibition of the *HOp-ADE2-ADH1 3′-UTR* strain by YEpMPT5 apparently is due to non-specific inhibition of growth by *MPT5* overexpression, because growth of cells harboring YEpMPT5 was slightly inhibited on SC plates containing adenine when compared with that of cells carrying the empty vector (data not shown). These results indicate that the *HO* 3′-UTR is required for repression of *HO* mediated by Mpt5.

To determine whether the *HO* 3′-UTR is sufficient for repression by Mpt5, we replaced the *ADE2* 3′-UTR of the chromosomal *ADE2* locus with the *HO* 3′-UTR or *ADH1* 3′-UTR. We also added three copies of the hemagglutinin (HA) epitope to the 3′ end of the *ADE2* coding sequence to monitor the levels of Ade2 protein by western blotting. Replacement of the *ADE2* allele with the *ADE2-3HA-HO 3′-UTR* or *ADE2-3HA-ADH1 3′-UTR* constructs did not affect growth on SC-Ade plates, indicating that replacement of the 3′-UTR and addition of the HA tag did not inactivate the *ADE2* gene. When *MPT5* was overexpressed from the *GAL1* promoter in these strains, growth of the *ADE2-HO 3′-UTR* strain, but not of the *ADE2-ADH1 3′-UTR* strain, was inhibited on SG-Ade plates (Figure 3A). Thus, the *HO* 3′-UTR confers *MPT5*-mediated repression.

We confirmed that this growth inhibition by *MPT5* reflected a decrease in Ade2 protein levels. Western blotting analysis revealed that the levels of Ade2-3HA protein were reduced in the *ADE2-3HA-HO 3′-UTR* strain but not in the *ADE2-3HA-ADH1 3′-UTR* strain after *MPT5* was overexpressed (Figure 3B). Overexpression of *MPT5* also altered the levels of *ADE2-3HA* mRNA in a manner dependent upon the *HO* 3′-UTR (Figure 3C). The decrease

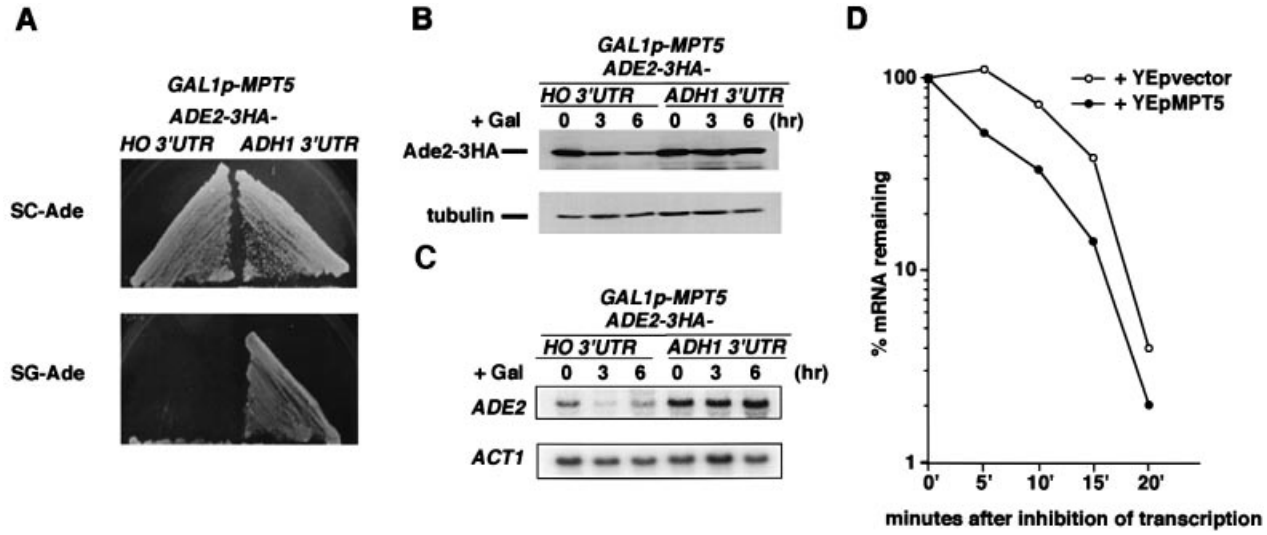

Fig. 3. The *HO* 3′-UTR is sufficient for *MPT5*-mediated repression of *HO*. (**A**) Effect of *MPT5* overexpression on *ADE2-3HA* reporter strains. Yeast strains TTC59 (*ADE2-3HA-HO 3′-UTR, GAL1p-MPT5*) and TTC62 (*ADE2-3HA-ADH1 3′-UTR, GAL1p-MPT5*) were streaked on the SC (glucose)-Ade or SG (galactose)-Ade plates and incubated for 3 days at 30°C. (**B**) Effect of *MPT5* overexpression on Ade2-3HA protein levels. Yeast cells were cultured in 2% raffinose medium at 30°C and treated with galactose (2%) to induce *MPT5* expression from *GAL1p-MPT5*. At the times indicated, cells were harvested and western blot analysis was performed to assay the level of Ade2-3HA protein (top). Tubulin protein (bottom) was included as a quantity control. (**C**) Effect of *MPT5* overexpression on *ADE2-3HA* mRNA levels. Yeast cells were harvested as in (B), and northern blot analysis was performed to assay the level of *ADE2-3HA* mRNA (top). *ACT1* mRNA (bottom) was included as a quantity control. (**D**) Effect of *MPT5* overexpression on degradation of mRNA. Yeast strain TTC181 (*GAL1p-ADE2-3HA-HO 3′-UTR*) harboring YEp195-MPT5 or empty vector was incubated in SR-Ura. Transcription was induced by adding galactose to a final concentration of 2%. The culture was then transferred to medium containing 2% glucose. Aliquots were removed at various times, and the amounts of the *ADE2* transcript were quantitated by dot blotting as described in Materials and methods. Decay rates were determined from semilog plots of the percentage of hybridizing material remaining at different times after the inhibition of transcription.

of *ADE2-3HA-HO 3′-UTR* mRNA in the cells overexpressing *MPT5* suggested that *MPT5* affects mRNA turnover. To test this possibility, we measured the decay rates of mRNA by performing transcriptional pulse–chase experiments using the regulated *GAL1* promoter. For this purpose, we constructed a reporter, *GAL1p-ADE2-HO 3′-UTR*. Transcription was first induced in the presence of galactose and then repressed by shifting the medium from galactose to glucose. The mRNA produced by *GAL1p-ADE2-HO 3′-UTR* showed a faster decay rate in the strain harboring YEpMPT5 as compared with the strain harboring the control vector (Figure 3D). The decay rates of mRNA produced by *GAL1p-ADE2-ADH1 3′-UTR* were not affected by *MPT5* overexpression (data not shown). These results suggest that overexpression of *MPT5* promotes degradation of mRNA carrying the *HO* 3′-UTR.

A region within the HO 3′-UTR required for repression by Mpt5

To determine which region of the *HO* 3′-UTR is required for repression by Mpt5, we first analyzed the 3′ end of *HO* mRNA using the 3′-RACE method (see Materials and methods). The *HO* transcript extends 67 nucleotides from the stop codon (Figure 4B). This segment contains a positioning element (+32 to +37 from the stop codon), which is involved in the addition of poly(A), and an efficiency element UAUAUA (+7 to +12 from the stop codon), which enhances the efficiency of downstream positioning elements (Guo and Sherman, 1996).

Pum binds to a pair of 32 nucleotide sequences, called NREs, in the 3′-UTR of *hb* mRNA (Wharton and Struhl, 1991; Murata and Wharton, 1995). Binding of FBF to the 3′-UTR of *fem-3* mRNA requires a five nucleotide segment in the center of the 3′-UTR, called the point mutation element (PME) (Zhang *et al*., 1997). Although NREs and the PME have little overall similarity to each other, they both contain the tetranucleotide UUGU (Figure 4A), suggesting that this tetranucleotide might be a conserved binding sequence for Puf proteins. The 3′-UTR of *HO* mRNA contains only one UUGU site, which is located 24 nucleotides downstream of the stop codon (Figure 4B). No UUGU site is present in the *ADH1* 3′-UTR region, which is not repressed by Mpt5. We thus examined whether this UUGU is important for repression by Mpt5 by changing its sequence to UACU. In NREs, this change eliminates Pum regulation (Murata and Wharton, 1995; Sonoda and Wharton, 1999). Changing UUGU to UACU in the *HOp-ADE2* reporter greatly reduced its repression by *MPT5* (Figure 5), suggesting that the UUGU sequence in the *HO* 3′-UTR is indeed required for repression by Mpt5.

Analysis of Mpt5 by the three-hybrid system

To determine whether Mpt5 acts through the *HO* 3′-UTR, we used the three-hybrid RNA-binding assay (described in Figure 6A) (SenGupta *et al*., 1996). The reporter strain, L40c, contains a *HIS3* gene with LexA-binding sites inserted in place of the normal upstream promoter elements, and expresses a fusion bewteen LexA and the MS2 coat protein (LexA-CP). This strain was transformed with plasmids coding for two additional hybrids: plasmid pGAD-MPT5 codes for a fusion of Mpt5p to the Gal4 transcriptional activation domain; plasmid pMS2-HO 3′-UTR produces a chimeric nuclear RNA containing 134 bp

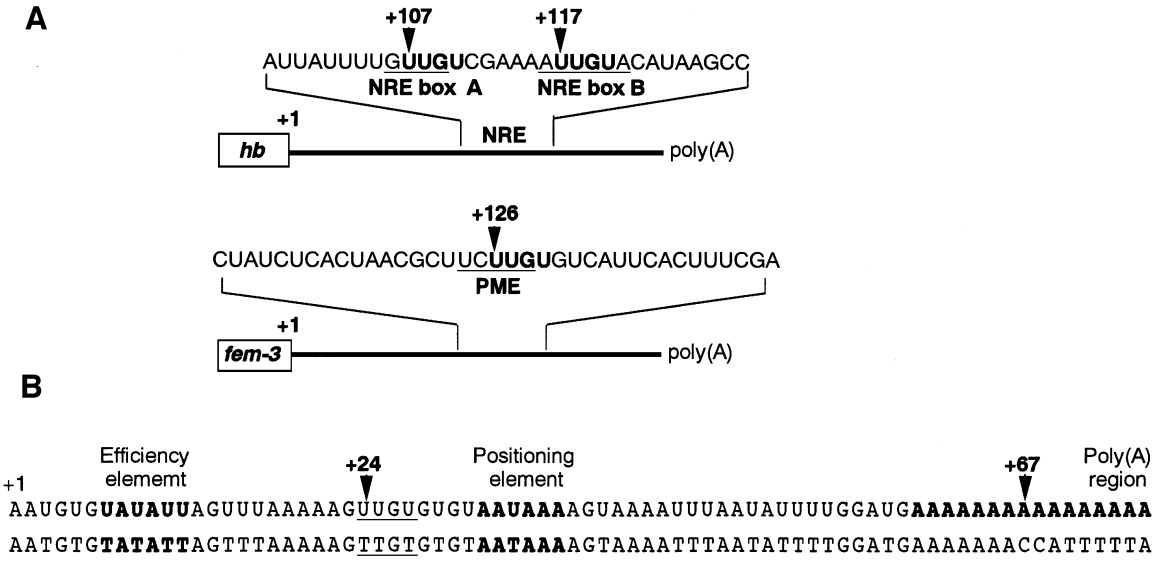

Fig. 4. Sequence of the 3′ end of *HO* mRNA. (**A**) Schematic drawing of the 3′-UTR structures of *hunchback* (*hb*) and *fem-3* mRNAs. Numbers indicate the number of nucleotides between the stop codon and UUGU sites. (**B**) The 3′ end of *HO* mRNA was determined by 3′-RACE analysis. The putative efficiency element, positioning element and poly(A) region are shown in bold as indicated. The UUGU site is underlined. Numbers indicate the number of nucleotides from the stop codon.

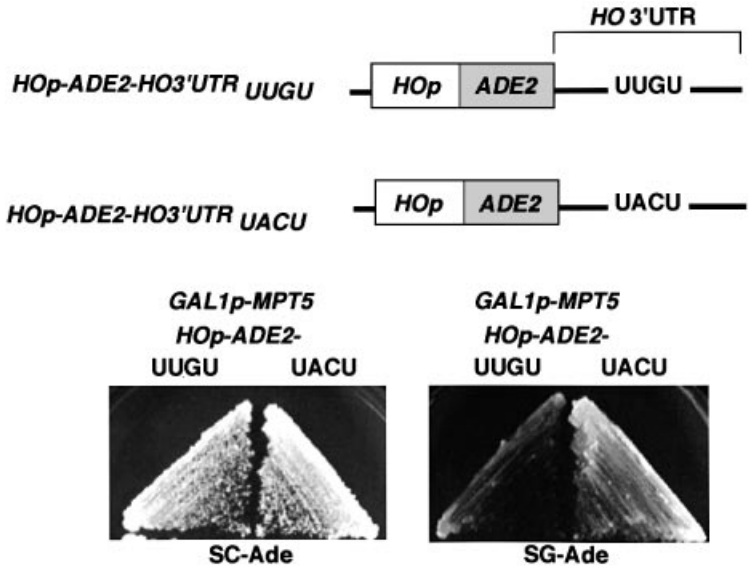

Fig. 5. The UUGU sequence in the *HO* 3′-UTR is required for *MPT5*-mediated repression of *HO*. Yeast strain TTC74 (*GAL1p-MPT5*) was transformed with YCplac33 (*HOp-ADE2-HO 3′-UTR*$_{UUGU}$) or YCplac33 (*HOp-ADE2-HO 3′-UTR*$_{UACU}$). Resulting transformants were plated on SC (glucose)-Ade Leu or SG (galactose)-Ade Leu plates and incubated for 3 days at 30°C.

of the region 3′ of the *HO* stop codon and a binding site for the bacteriophage MS2 coat protein. The resulting transformants grew on SC-His plates. In contrast, transformants carrying pGAD-MPT5 and a control plasmid (MS2) that contains only the MS2 RNA segment failed to grow on SC-His plates (Figure 6B). Similarly, transformants carrying the pGAD control vector and pMS2-HO 3′-UTR were also unable to grow on SC-His plates (data not shown). These results suggest that Mpt5 binds to the *HO* 3′-UTR. As described above, mutation of a single UUGU site in the 3′-UTR of *HO* abolishes repression by Mpt5. We thus generated a derivative of plasmid pMS2-HO 3′-UTR containing the UUGU to UACU change and tested it in the three-hybrid system. This mutation did not affect the expression of MS2-HO 3′-UTR reporter RNA (data not shown). These transformants were unable to grow on SC-His plates (Figure 6B), suggesting that the UUGU site is indeed essential for Mpt5 binding.

MPT5 affects mating-type switching in daughter cells

The ability of cells in a cell lineage to undergo mating-type switching has a very precise pattern in which mother cells switch efficiently (typically ~65% of cell divisions), but daughter cells do not (<0.1% of cell divisions) (Strathern and Herskowitz, 1979). The basis for this difference is the

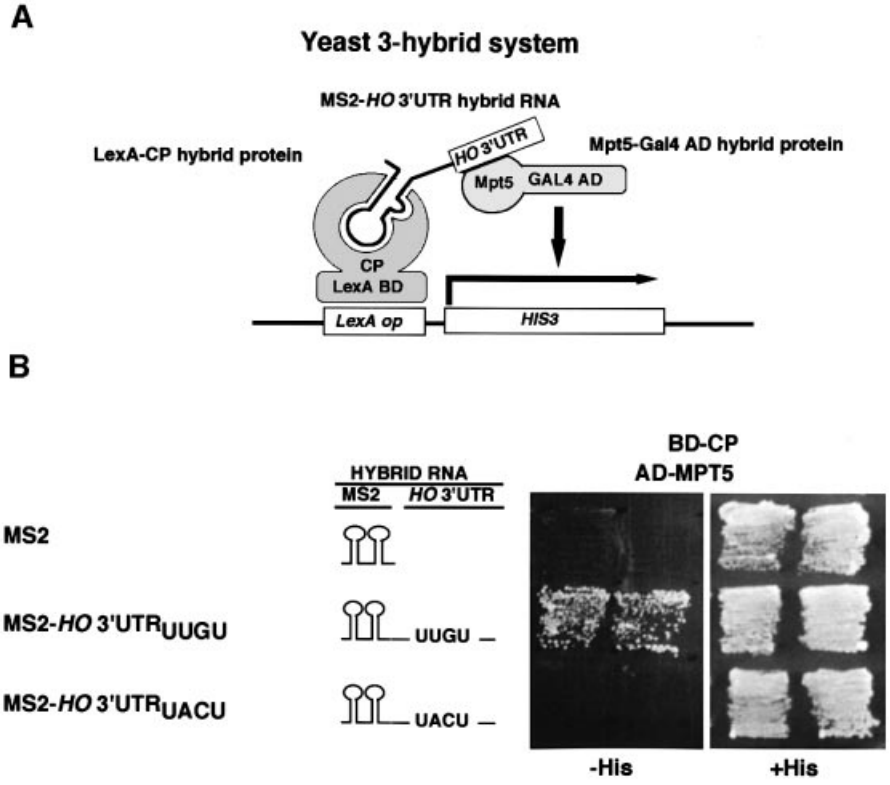

Fig. 6. Requirement for the *HO* mRNA 3′-UTR for functioning of Mpt5 in the three-hybrid RNA-binding assay. (**A**) Schematic drawing of the yeast three-hybrid assay. The LexA-CP hybrid protein (BD-CP) contains the LexA DNA-binding domain joined to the MS2 coat protein and binds to the *LexA* operator of the *HIS3* reporter gene. The Gal4 AD-Mpt5 hybrid protein (AD-MPT5) is a fusion of Mpt5 and the yeast Gal4 activation domain. The hybrid RNA (MS2-*HO* 3′-UTR) contains the binding site for MS2 coat protein and the *HO* 3′-UTR. If the Mpt5 segment of the Gal4 AD-Mpt5 hybrid binds to the *HO* 3′-UTR, then the MS2-*HO* 3′-UTR hybrid RNA will link the two hybrid proteins to each other and activate the *HIS3* reporter gene. (**B**) Yeast strain L40c, which expresses a LexA-MS2 coat protein fusion, was transformed with the indicated plasmids, and the transformants were streaked on SC-Ura Leu His (–His) and SC-Ura Leu (+His) plates and incubated for 2 (+His plates) or 3 days (–His plates) at 30°C. Plasmids were MS2, MS2-*HO* 3′-UTR$_{UUGU}$, MS2-*HO* 3′-UTR$_{UACU}$ and pGAD-*MPT5*.

presence of *HO* mRNA in mother cells but not in daughter cells (Nasmyth, 1983). Ash1 protein, located in daughter cells, limits *HO* transcription (Bobola *et al*., 1996; Sil and Herskowitz, 1996). To test whether Mpt5-mediated repression of *HO* affects the regulation of mating-type switching, we disrupted the *MPT5* gene in an *HO* background and measured its effect on mating-type switching by pedigree analysis. In wild-type cells, we observed that 59% of mother cells and <1.5% of daughters switched mating type. In contrast, in the *mpt5*Δ mutant, 60% of mother cells and 27% of daughters switched mating type. Thus, the *mpt5*Δ mutation greatly increased the frequency of mating-type switching in daughter cells, whereas it had no effect in mothers. Although the effect of *mpt5*Δ on mating-type switching is not as strong as that of the *ash1*Δ mutation, in which 85–95% of daughter cells switch mating type (Bobola *et al*., 1996; Sil and Herskowitz, 1996), Mpt5 is clearly required for proper regulation of mating-type switching. The *mpt5*Δ mutation had no effect in mothers, suggesting that Mpt5-mediated repression might function only in daughter cells (see Discussion).

The observation that the *mpt5*Δ mutation allows mating-type switching in a substantial fraction of daughter cells suggests that some residual expression of *HO* may occur in daughter cells even in the presence of Ash1p and that this expression is blocked by Mpt5. To test this possibility, we examined the effect of *MPT5* on *HO* expression in *myo4*Δ mutants, in which Ash1p is delocalized and is thus present in both mother and daughter nuclei (Bobola *et al*., 1996). *myo4*Δ mutants containing the *HOp-ADE2* reporter failed to grow on SC-Ade plates (Figure 7). In contrast, the *mpt5*Δ *myo4*Δ double mutant containing the *HOp-ADE2* reporter grew on SC-Ade plates (Figure 7). These results suggest that Mpt5p inhibits the utilization of *HO* mRNA that has escaped from repression by Ash1.

Discussion

We show that one of the Puf homologs of *S.cerevisiae*, Mpt5, is involved in the regulation of *HO* expression. *mpt5*Δ mutants showed increased *HO* expression compared with wild-type cells, whereas cells overexpressing *MPT5* from a multicopy plasmid showed a decrease in *HO* expression. We have presented several pieces of evidence suggesting that Mpt5, like Pumilio and FBF, binds to the 3′-UTR of the *HO* mRNA and regulates *HO* expression post-transcriptionally. First, the *HO* 3′-UTR is required for Mpt5-mediated repression of *HO* expression. Secondly, the 3′-UTR of *HO* mRNA contains a UUGU site which might be a conserved binding sequence for Puf proteins. This site is essential for repression by Mpt5. Finally, studies using the three-hybrid assay indicate that Mpt5 binds to the *HO* 3′-UTR and that the UUGU site is

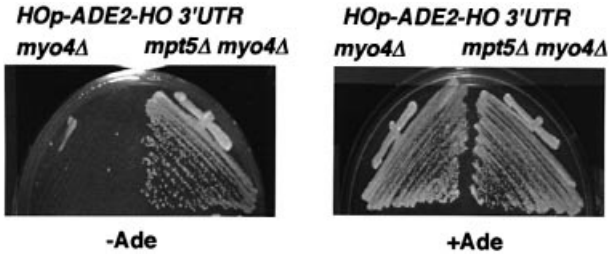

Fig. 7. Effect of *MPT5* on Ash1-mediated repression of *HO*. Yeast strains TTC120 (*myo4Δ HOp-ADE2-HO 3′-UTR*) and TTC121 (*mpt5Δ myo4Δ HOp-ADE2-HO 3′-UTR*) were streaked on SC-Ade (–Ade) or SC (+Ade) plates and incubated for 3 days at 30°C.

required for its binding. We thus propose that Mpt5 regulates *HO* expression post-transcriptionally by binding to the 3′-UTR of *HO*.

Mpt5 is a Puf protein with functional similarity to Pum and FBF

Mpt5 has structural and functional similarities to the founding members of the Puf family. Like Pum and FBF, which contain eight Puf repeats, Mpt5 contains seven or eight repeats. All three of these proteins are negative regulators that are known or inferred to act by binding to specific elements in the 3′-UTRs of their respective RNA targets (Murata and Wharton, 1995; Zhang *et al*., 1997; Sonoda and Wharton, 1999). The structural similarities of these proteins suggest that they may recognize related RNA sequences, and indeed their binding to 3′-UTRs appears to require the UUGU sequence. Changing UUGU to UACU eliminates regulation by Pum and Mpt5, indicating that UUGU is a conserved binding sequence for Puf family proteins. Mpt5, Pum and FBF all regulate expression post-transcriptionally. It is clear that Pum represses translation in early embryos (Wreden *et al*., 1997). In some cases, it appears to do so by promoting deadenylation of poly(A) RNA, leading to mRNA turnover (Wickens *et al*., 1997). The observation that overexpression of *MPT5* promotes degradation of *ADE2-3HA-HO 3′-UTR* mRNA suggests that Mpt5 may affect mRNA stability or turnover via binding to the *HO* 3′-UTR. Recently, Olivas and Parker reported that another Puf protein, Puf3/Yll013c, specifically promotes deadenylation and degradation of *COX17* mRNA (Olivas and Parker, 2000). Thus, Puf proteins regulate mRNA turnover in yeast, although previous work with Puf proteins from *Drosophila* and *C.elegans* has demonstrated a role in translational inhibition. Pum and FBF function with companion proteins, Nanos and Nos-3, respectively (Wreden *et al*., 1997; Wharton *et al*., 1998; Kraemer *et al*., 1999; Sonoda and Wharton, 1999; Subramaniam and Seydoux, 1999). It would not be surprising if Mpt5 also functioned with a companion protein, although there is no obvious homolog of Nanos in *S.cerevisiae*. The observation that inactivation of *MPT5* leads to only a 2-fold increase in *HO* expression could reflect the limits of regulation by Mpt5, or it could suggest the existence of a functionally redundant product. Inactivation of other Puf proteins, Ygl014w and Yll013c, in an *mpt5Δ* background did not increase *HO* expression further, indicating that Ygl014w and Yll013c are not redundant with Mpt5 for this function.

Mpt5 may provide a second mechanism to prevent synthesis of HO protein in daughter cells

Prior studies of mating-type interconversion focused on the regulation of *HO* by the transcriptional activator proteins Swi1–Swi6 and the negative regulators Sin1, Sin3 and Ash1. The Ash1 protein was shown recently to play a key role in generating the asymmetric pattern of mating-type switching as it is a daughter cell-specific repressor of *HO* transcription (Bobola *et al*., 1996; Sil and Herskowitz, 1996). Our studies on Mpt5 reveal an unexpected aspect of *HO* regulation, in particular that *HO* expression is also regulated post-transcriptionally by Mpt5 through the 3′-UTR of the *HO* transcript. Surprisingly, in *mpt5Δ* mutants, a substantial fraction (27%) of daughter cells switched mating type, whereas in wild-type strains, daughter cells are never observed to switch mating type. Thus, Mpt5-mediated repression is required for proper regulation of mating-type switching. Why does the *mpt5Δ* mutation allow mating-type switching in daughter cells, where Ash1p is thought to repress *HO* transcription? One possibility is that *HO* is still transcribed at a low level in daughter cells, even in the presence of Ash1p, and that use of this residual *HO* transcript is prevented by Mpt5. This explanation is supported further by the observation that expression of *HO* in *myo4Δ* mutants, in which Ash1p localizes to both mother and daughter, is enhanced by deletion of *MPT5*.

Does Mpt5 inhibit *HO* expression in mother cells as well as in daughter cells? Because the *mpt5Δ* mutation does not noticeably affect the frequency of mating-type switching in mother cells, Mpt5-mediated repression might, in principle, function only in daughter cells. However, using an Mpt5–Myc fusion protein, we have observed that Mpt5p is present in both mother and daughter cells (data not shown). Perhaps Mpt5 function requires a companion protein whose expression is restricted to daughter cells.

Possible roles of Mpt5p in regulating other processes

MPT5, also known as *UTH4*, was first identified as a multicopy suppressor of the growth defect of *POP2*-deficient strains (Hata *et al*., 1998). *POP2* encodes a component of the Ccr4 transcription regulatory complex required for glucose derepression (Sakai *et al*., 1992; Liu *et al*., 1998). *pop2Δ* mutants exhibit temperature-sensitive growth, perhaps due to overproduction of some protein. Overexpression of Mpt5 might restore growth to *pop2Δ* mutants by reducing the level of this growth-inhibitory product. Although we do not know the mechanism by which Mpt5 negatively regulates utilization of *HO* and other mRNAs, it should be possible to identify Mpt5-regulated transcripts other than *HO* by use of DNA microarrays to assess the level of poly(A) RNA species or by isolation of RNA from polysomes in wild-type versus *mpt5Δ* strains (Diehn *et al*., 2000).

mpt5Δ mutants exhibit many defects, including temperature-sensitive growth, shortened life span, a slight increase in sensitivity to mating pheromone and a defect in recovery from pheromone arrest (Kennedy *et al*., 1995,

Table I. Strains used in this study

Strain		Genotype	Source
W303	**a**	*ade2 trp1 can1 leu2 his3 ura3 GAL psi+*	Sil and Herskowitz (1996)
K1107	**a**	*HOp-LacZ-HO 3′-UTR*	Nasmyth (1987)
K5552	**a**	*ASH1-myc*	Jansen *et al.* (1996)
10B	α	*HOp-ADE2-HO 3′-UTR*	this study
TTC2	**a**	*HOp-LacZ-HO 3′-UTR mpt5Δ::CgHIS3*	this study
TTC28	α	*HOp-ADE2-HO 3′-UTR* His3MX6*::GAL1p-MPT5*	this study
TTC47	α	*HOp-ADE2-ADH1 3′-UTR*	this study
TTC59	**a**	*ADE2-3HA-HO 3′-UTR::*His3MX6 kanMX6*::GAL1p-MPT5*	this study
TTC62	**a**	*ADE2-3HA-ADH1 3′-UTR::*His3MX6 kanMX6*::GAL1p-MPT5*	this study
TTC74	**a**	kanMX6*::GAL1p-MPT5*	this study
TTC85	**a**/α	*HO/HO*	Sil and Herskowitz (1996)
TTC87	**a**/α	*HO/HO mpt5Δ::CgHIS3 mpt5Δ::CgHIS3*	this study
TTC91	**a**	*HOp-LacZ-HO 3′-UTR ash1Δ::hisG URA3 hisG*	this study
TTC120	α	*HOp-ADE2-HO 3′-UTR myo4Δ::CgTRP1*	this study
TTC121	α	*HOp-ADE2-HO 3′-UTR mpt5Δ::CgHIS3 myo4Δ::CgTRP1*	this study
TTC181	**a**	kanMX6*::GAL1p-ADE2-3HA-HO 3′-UTR::*His3MX6	this study
TTC191	**a**	*HOp-LacZ-HO 3′-UTR mpt5Δ::CgLEU2 ash1Δ::hisG URA3HisG*	this study
TTC219	**a**	*ASH1-myc mpt5Δ::CgHIS3*	this study
L40c	**a**	*ura3 leu2 his3 trp1 ade2 LYS2::(lexAop)-HIS3 ura3::(lexAop)-LacZ TRP1-LexA-coat*	SenGupta *et al*. (1996)

All strains except L40c were isogenic derivatives of W303.

Table II. Plasmids used in this study

Plasmid	Relevant markers	Source
YEp13	*LEU2*, 2 μm	
YEp13MPT5	*MPT5* sequence in YEp13	Sakai *et al*. (1992)
YEp195	*URA3*, 2 μm	
YEp195MPT5	*MPT5* sequence in YEp195	this study
YCplac33	*URA3, CEN4*	
YCplac33*HOp-ADE2-HO 3′-UTR*$_{UUGU}$	*HOp-ADE2-HO 3′-UTR*$_{UUGU}$ in YCplac33	this study
YCplac33*HOp-ADE2-HO 3′-UTR*$_{UACU}$	*HOp-ADE2-HO 3′-UTR*$_{UACU}$ in YCplac33	this study
pIIIA/MS2-1	*URA3*, 2 μm, MS2 sequence behind pol III promoter	SenGupta *et al*. (1996)
pIIIA/MS2-1-HO 3′-UTR$_{UUGU}$	*MS2-HO* 3′-UTR$_{UUGU}$ sequence in pIIIA/MS2-1	this study
pIIIA/MS2-1-HO 3′-UTR$_{UACU}$	*MS2-HO* 3′-UTR$_{UACU}$ sequence in pIIIA/MS2-1	this study
pGAD-C1	*LEU2*, 2 μm, *GAL4-AD* sequence behind *ADH1* promoter	James *et al*. (1996)
pGAD-MPT5	*GAL4-AD-MPT5* sequence in pGAD-C1	this study
pFA6a-3HA-His3MX6	*3HA-ADH1 3′-UTR-His3* sequence	Longtine *et al*. (1998)
pFA6a-3HA-HO 3′ UTR-His3MX6	*3HA-HO 3′-UTR-His3* sequence	this study
pFA6a-kanMX6-GAL1p-3HA	*KanMX6-GAL1p-3HA* sequence	Longtine *et al*. (1998)
pCgHIS3	*Candida glabrata HIS3* sequence in pUC19	Sakumoto *et al*. (1999)

1997; Chen and Kurjan, 1997). Can this pleiotropic phenotype be explained by the ability of Mpt5 to negatively regulate gene expression post-transcriptionally? Mpt5 might down-regulate synthesis of proteins that control growth at high temperature, life span and response to mating pheromones. Accordingly, the 3′-UTRs of the encoding mRNAs might contain one or more UUGU sites. However, the finding that Mpt5 protein interacts with Sst2, Fus3, Kss1 and Cdc28 proteins, as assayed by the two-hybrid system and by co-immunoprecipitation (Chen and Kurjan, 1997), suggests that Mpt5 could also affect pheromone response by a mechanism unrelated to post-transcriptional regulation.

Materials and methods

Strains and general methods

Escherichia coli DH5α was used for DNA manipulations. The yeast strains used in this study are described in Table I. Standard procedures were followed for yeast manipulations (Kaiser *et al*., 1994). The media used in this study included rich medium, synthetic complete medium (SC), synthetic minimal medium (SD) and sporulation medium (Kaiser *et al*., 1994). SC lacking amino acids or other nutrients (e.g. SC-Leu lacks leucine, SC-Ade lacks adenine) was used to select transformants and to score *ADE2* reporter activity. SG and SR were identical to SC except that they contained 2% galactose and raffinose, respectively, instead of 2% glucose. Recombinant DNA procedures were carried out as described previously (Sambrook *et al*., 1989).

Plasmids

Plasmids used in this study are described in Table II. Plasmid YEpMPT5 (kindly provided by A.Sakai) is YEp13 carrying *MPT5* (Hata *et al*., 1998). Plasmid YEp195-MPT5 is YEplac195 carrying *MPT5*. Plasmid pFA6a-3HA-*HO* 3′-UTR-His3MX6 was constructed as follows. The *HO* 3′-UTR was amplified by PCR using a 5′ primer (5′-CTCGGCGCG-CCAATGTGTATATTAGTTTAAAAAG-3′, incorporating an *Asc*I site) and a 3′ primer (5′-CTCAGATCTGAATTCGACTTGAAGAACATC-CC-3′, incorporating a *Bgl*II site). The resulting fragment was inserted into the *Asc*I–*Bgl*II gap of pFA6a-3HA-His3MX6 (Longtine *et al*., 1998) to generate pFA6a-3HA-*HO* 3′-UTR-His3MX6. Plasmids YCplac33 (*HOp-ADE2-HO 3′-UTR*$_{UUGU}$) and YCplac33 (*HOp-ADE2- HO 3′-UTR*$_{UACU}$) are YCplac33 carrying *HOp-ADE2-HO 3′-UTR* reporter genes. YCplac33 (*HOp-ADE2-HO 3′-UTR*$_{UACU}$) contains two base changes, from TTGT to TACT. Plasmids pIIIA/MS2-1-*HO* 3′-UTR$_{UUGU}$ and pIIIA/MS2-1-*HO* 3′-UTR$_{UACU}$ express chimeric nuclear RNAs bearing binding sites for the MS2 coat protein and 134 bp 3′ of the

HO stop codon. pIIIA/MS2-1-*HO* 3′-UTR$_{UACU}$ contains two base changes, from TTGT to TACT. The DNA fragments of the *HO* 3′-UTR containing nucleotides +1 to +134 from the stop codon were amplified by PCR and inserted into the *Sma*I gap of pIIIA/MS2-1 vector (SenGupta *et al*., 1996) to generate pIIIA/MS2-1-*HO* 3′-UTR$_{UUGU}$ and pIIIA/MS2-1-*HO* 3′-UTR$_{UACU}$. The hybrid RNAs were transcribed by RNA polymerase III. Plasmid pGAD-MPT5 expresses *MPT5* fused to the *GAL4* transcriptional activation domain. The N-terminal portion of the *MPT5* coding sequence was amplified by PCR using a 5′ primer (5′-CTCGGATCCATGATCAATAACGAACCATTTCC-3′, incorporating a *Bam*HI site) and a 3′ primer (5′-GAGTTAGCAGAATTCGATG-3′). An ~0.3 kb *Bam*HI–*Eco*RI fragment generated by PCR and a 2.5 kb *Eco*RI–*Xho*I fragment containing the C-terminal portion of *MPT5* were inserted into the *Bam*HI–*Sal*I gap of pGAD-C1 vector (James *et al*., 1996) to generate pGAD-MPT5. Plasmid pCgHIS3 is pUC19 carrying the *Candida glabrata HIS3* gene (Sakumoto *et al*., 1999).

3′-RACE

Total RNA was isolated from wild-type yeast cells, W303, as described previously (Sambrook *et al*., 1989), and treated with RQ1 DNase (Promega). RT–PCR was performed using an Access RT–PCR kit (Promega) and 0.1 μg of total RNA sample as template. Primers were 5′-GGGAACCCGTATATTTCAGC-3′ (5′ primer) and 5′-GGCCACGCG-TCGACTAGTACTTTTTTTTTTTTTTTT-3′ (3′ primer). Cycling conditions were 45 min at 48°C, followed by 30 cycles of 30 min at 94°C, 1 min at 60°C, 2 min at 68°C, and then 15 min at 68°C. Nested PCR was performed using a 5′ primer (5′-CTGTCCGCGGGCCTCATAAGAG-3′), a 3′ primer (5′-GCCACGCGTCGACTAGTAC-3′) and Ex *Taq* polymerase (Takara). The resultant fragment (~150 bp) was subcloned into the pCR TOPO 2.1 vector (Invitrogen) and sequenced.

Deletion of the Puf genes

The deletions of five Puf genes, *MPT5*, *YGL014w*, *YLL013c*, *JSN1* and *YPR039c*, were constructed by the PCR-based gene deletion method (Baudin *et al*., 1993; Schneider *et al*., 1996; Sakumoto *et al*., 1999). Primer sets were designed such that 46 bases at the 5′ end of the primers were complementary to those at the corresponding region of the target gene, and 20 bases at their 3′ end were complementary to the pUC19 sequence outside the polylinker region in plasmid pCgHIS3 containing the *C.glabrata HIS3* gene as a selectable marker. Primer sets for PCR were designed to delete the ORF completely. The PCR products were used to transform the strains K1107 and 10B by selection for His$^+$. The disruption was verified by colony-PCR amplification (Huxley *et al*., 1990) to confirm that replacement had occurred at the expected locus.

Construction of GAL1p-MPT5 strains

The *GAL1p-MPT5* strain was constructed by the PCR-based gene modification method (Longtine *et al*., 1998). PCR was performed using pFA6a-kanMX6-GAL1p-3HA as template, a 5′ primer (5′-TCTACGC-AAATTTATAAATCAATTTCGATTTTTCCAGTTTCTCTTGAATTC-GAGCTCGTTTAAAC-3′) and a 3′ primer (5′-TCCATTGGCGAAG-AAAAATATTGGGAATTAAGAGATAATACATACGCACTGAGCA-GCGTAATCTG-3′). The resultant transformation module was used to transform a haploid strain by selection on YPD medium containing 200 μg/ml geneticin (Gibco-BRL). The resultant transformants were verified by colony-PCR amplification using a 5′ primer (5′-GGGTACTGTTGAGTCAACATC-3′, upstream of the *MPT5* ORF) and a 3′ primer (5′-GTTTAAACGAGCTCGAATTC-3′, annealing with the pFA6a-kanMX6-GAL1p-3HA module) to confirm that replacement had occurred at the expected locus.

Construction of ADE2-3HA-HO 3′-UTR and ADE2-3HA-ADH1 3′-UTR strains

The *ADE2-3HA-HO 3′-UTR* and *ADE2-3HA-ADH1 3′-UTR* strains were constructed as follows. *3HA-HO* 3′-UTR and *3HA-ADH1* 3′-UTR transformation modules were amplified by PCR using pFA6a-3HA-*HO* 3′-UTR-His3MX6 and pFA6a-3HA-GFP(S65T)-His3MX6 as templates, a 5′ primer (5′-CAAAAGTTAGAAACTGTCGGTTACGAAGCTT-ATCTAGAAAACAAGTGAGGCGCGCCACTTCTAAA-3′) and a 3′ primer (5′-ATTTTATAATTATTTGCTGTACAAGTATATCAATAA-ACTTATATAGAATTCGAGCTCGTTTAAAC-3′). The resultant PCR products were used to transform a haploid strain by selection for His$^+$. Yeast colony-PCR was carried out using a 5′ primer (5′-GTTTAAA-CGAGCTCGAATTC-3′, annealing with a portion of the templates) and a 3′ primer (5′-ATTGGAAGACCTTCCAAGGG-3′, annealing downstream of the *ADE2* ORF) to confirm that replacement had occurred at the expected locus.

Construction of GAL1p-ADE2-3HA-HO 3′-UTR strain

The *GAL1p-ADE2-3HA-HO 3′-UTR* strain was constructed by the PCR-based gene modification method (Longtine *et al*., 1998). PCR was performed using pFA6a-kanMX6-GAL1p as template, a 5′ primer (5′-TCTACGCAAATTTATAAATCAATTTCGATTTTTCCAGTTTCTC-GTTAATTCGAGCTCGTTTAAAC-3′) and a 3′ primer (5′-TCCATT-GGCGAAGAAAAATATTGGGAATTAAGAGATAATACATACG-CACTGAGCAGCGTAATCTG-3′). The resultant PCR product was used to transform the *ADE2-3HA-HO 3′-UTR* strain by selection on YPD medium containing 200 μg/ml geneticin (Gibco-BRL). The resultant transformants were verified by colony-PCR amplification using a 5′ primer (5′-GGGTACTGTTGAGTCAACATC-3′, upstream of the *MPT5* ORF) and a 3′ primer (5′-GTTTAAACGAGCTCGAATTC-3′, annealing with the pFA6a-kanMX6-GAL1p module) to confirm that replacement had occurred at the expected locus.

Mating-type switching assays

Pedigree analysis was performed as described previously (Strathern and Herskowitz, 1979) using homothalic strains TTC85 (*HO MPT5*) and TTC87 (*HO mpt5Δ::CgHIS3*).

β-galactosidase assays

β-galactosidase assays were performed as described previously (Kaiser *et al*., 1994).

Preparation of yeast extracts and western blot analysis

Yeast cells were grown to an optical density (600 nm) of 0.5–1.0 and treated with 2% galactose to activate the *GAL1* promoter. After treatment, yeast cultures were quickly chilled, and cells were collected by rapid centrifugation. The pellet was washed twice and then suspended in breaking buffer (4% SDS, 40 mM Tris–HCl pH 7.0, 8 M urea, 0.1 mM EDTA, 1% 2-mercaptoethanol). Glass beads (0.4–0.6 mm diameter) were added to this suspension, and cells were broken by vigorous vortexing for 5 min at room temperature. The beads and cell debris were removed by centrifugation at 14 000 r.p.m. at room temperature. Protein concentrations of cell extracts were measured at 280 nm. Cell extracts were subjected to SDS–PAGE on 7% acrylamide gels followed by electroblotting onto Hybond N+ membrane (Amersham). Blots were blocked by incubation for 15 min at room temperature in TBS-M (TBS with 4% non-fat dry milk). Blots were then incubated with monoclonal antibody HA11 (Babco) diluted 1:2000 (to detect Ade2-3HA) or anti-tubulin antibody diluted 1:1000 (to detect tubulin) in TBS-M overnight at 4°C. After three washes with TBS, blots were incubated for 2 h with peroxidase-conjugated secondary antibody (Calbiochem) diluted 1:3000 with TBS-M. After three final washes with TBS, blots were detected using an enhanced chemiluminescence detection kit (Amersham).

Nothern blot analysis

RNA preparation and sample analysis were performed as described previously (Sambrook *et al*., 1989). The probes used were all gel-purified, PCR-amplified DNA fragments labeled by random-prime labeling using the Random Primer DNA Labeling Kit Ver 2.0 (Takara). The primers used were as follows: *ADE2*, a 5′ primer (5′-CAGACTCTGACTCTG-ACTTGCCGGTAATG-3′) and a 3′ primer (5′-CTCGAATTCCTCGGC-GCGCCTTACTTGTTTTCTAGATAAGC-3′), amplifying 0.4 kb of *ADE2* coding sequence; *ACT1*, a 5′ primer (5′-GGAATCTGCCGGTAT-TGACC-3′) and a 3′ primer (5′-ACATACGCGCACAAAAGCAG-3′), amplifying 0.4 kb of *ACT1* coding sequence.

Yeast three-hybrid assays

Three-hybrid assays were performed as described previously (SenGupta *et al*., 1996). Yeast strain L40c (L40-coat of SenGupta *et al*., 1996) carrying the pGAD-MPT5 plasmid was transformed with pIIIA/MS2-1-*HO* 3′-UTR$_{UUGU}$, pIIIA/MS2-1-*HO* 3′-UTR$_{UACU}$ or pIIIA/MS2-1 vector. Transformants were tested for growth on SC-Ura Leu His plates.

Localization of ASH1 mRNA and Ash1 protein

In situ RNA hybridization with digoxigenin-labeled ASH1 antisense probe was performed as described previously (Takizawa *et al*., 1997). Indirect immunofluorescence microscopy of an Ash1-myc strain was described (Sil and Herskowitz, 1996) using anti-myc antibodies (9E10).

Analysis of mRNA decay rate

The *GAL1p-ADE2-3HA-HO 3′-UTR* strain harboring YEp195-MPT5 or empty vector was incubated in SR-Ura. Transcription was induced by adding galactose to a final concentration of 2%. An aliquot was removed at 10–15 min after addition of galactose. The culture was then transferred

to medium containing 2% glucose. Aliquots were removed at various times, and cells were collected by rapid centrifugation. RNAs were isolated from each sample and the amounts of the *ADE2* transcript and the *ACT1* transcript as a control were quantitated by dot blotting. Decay rates were determined from semilog plots of the percentage of hybridizing material remaining at different times after the inhibition of transcription.

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References

Baudin,A., Ozier,K.O., Denouel,A., Lacroute,F. and Cullin,C. (1993) A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **21**, 3329–3330.

Bobola,N., Jansen,R.P., Shin,T.H. and Nasmyth,K. (1996) Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells. *Cell*, **84**, 699–709.

Chen,T. and Kurjan,J. (1997) *Saccharomyces cerevisiae* Mpt5p interacts with Sst2p and plays roles in pheromone sensitivity and recovery from pheromone arrest. *Mol. Cell. Biol.*, **17**, 3429–3439.

Diehn,M., Eisen,M.B., Botstein,D. and Brown,P.O. (2000) Large-scale identification of secreted and membrane-associated gene products using DNA microarrays. *Nature Genet.*, **25**, 58–62.

Goodwin,E.B., Okkema,P.G., Evans,T.C. and Kimble,J. (1993) Translational regulation of *tra-2* by its 3′ untranslated region controls sexual identity in *C.elegans*. *Cell*, **75**, 329–339.

Gray,N.K. and Wickens,M. (1998) Control of translation initiation in animals. *Annu. Rev. Cell Dev. Biol.*, **14**, 399–458.

Guo,Z. and Sherman,F. (1996) Signals sufficient for 3′-end formation of yeast mRNA. *Mol. Cell. Biol.*, **16**, 2772–2776.

Hata,H., Mitsui,H., Liu,H., Bai,Y., Denis,C.L., Shimizu,Y. and Sakai,A. (1998) Dhh1p, a putative RNA helicase, associates with the general transcription factors Pop2p and Ccr4p from *Saccharomyces cerevisiae*. *Genetics*, **148**, 571–579.

Herskowitz,I. (1988) Life cycle of the budding yeast *Saccharomyces cerevisiae*. *Microbiol. Rev.*, **52**, 536–553.

Huxley,C., Green,E.D. and Dunham,I. (1990) Rapid assessment of *S.cerevisiae* mating type by PCR. *Trends Genet.*, **6**, 236.

James,P., Halladay,J. and Craig,E.A. (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics*, **144**, 1425–1436.

Jan,E., Motzny,C.K., Graves,L.E. and Goodwin,E.B. (1999) The STAR protein, GLD-1, is a translational regulator of sexual identity in *Caenorhabditis elegans*. *EMBO J.*, **18**, 258–269.

Jansen,R.P., Dowzer,C., Michaelis,C., Galova,M. and Nasmyth,K. (1996) Mother cell-specific *HO* expression in budding yeast depends on the unconventional myosin Myo4p and other cytoplasmic proteins. *Cell*, **84**, 687–697.

Kaiser,C.A., Adams,A. and Gottschling,D.E. (1994) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kennedy,B.K., Austriaco,N.J., Zhang,J. and Guarente,L. (1995) Mutation in the silencing gene *SIR4* can delay aging in *S.cerevisiae*. *Cell*, **80**, 485–496.

Kennedy,B.K. *et al.* (1997) Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S.cerevisiae*. *Cell*, **89**, 381–391.

Kraemer,B., Rittenden,S., Gallegos,M., Moulder,G., Barstead,R., Kimble,J. and Wickens,M. (1999) NANOS-3 and FBF proteins physically interact to control the sperm–oocyte switch in *Caenorhabditis elegans*. *Curr. Biol.*, **9**, 1009–1018.

Liu,H.Y., Badarinarayana,V., Audio,D.C., Rappsilber,J., Mann,M. and Denis,C.L. (1998) The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. *EMBO J.*, **18**, 1096–1106.

Long,R.M., Singer,R.H., Meng,X., Gonzalez,I., Nasmyth,K. and Jansen,R.P. (1997) Mating type switching in yeast controlled by asymmetric localization of *ASH1* mRNA. *Science*, **277**, 383–387.

Longtine,M.S., McKenzie,A.R., Demarini,D.J., Shah,N.G., Wach,A., Brachat,A., Philippsen,P. and Pringle,J.R. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast*, **14**, 953–961.

Machin,N.A., Lee,J.M. and Barnes,G. (1995) Microtubule stability in budding yeast: characterization and dosage suppression of a benomyl-dependent tubulin mutant. *Mol. Biol. Cell*, **6**, 1241–1259.

Murata,Y. and Wharton,R.P. (1995) Binding of pumilio to maternal hunchback mRNA is required for posterior patterning in *Drosophila* embryos. *Cell*, **80**, 747–756.

Nasmyth,K. (1983) Molecular analysis of a cell lineage. *Nature*, **302**, 670–676.

Olivas,W. and Parker,R. (2000) The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast. *EMBO J.*, **19**, 6602–6611.

Sakai,A., Chibazakura,T., Shimizu,Y. and Hishinuma,F. (1992) Molecular analysis of *POP2* gene, a gene required for glucose-derepression of gene expression in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **20**, 6227–6233.

Sakumoto,N. *et al*. (1999) A series of protein phosphatase gene disruptants in *Saccharomyces cerevisiae*. *Yeast*, **15**, 1669–1679.

Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning*: *A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schneider,D., Bruton,C.J. and Chater,K.F. (1996) Characterization of *spaA*, a *Streptomyces coelicolor* gene homologous to a gene involved in sensing starvation in *Escherichia coli*. *Gene*, **177**, 243–251.

SenGupta,D.J., Zhang,B., Kraemer,B., Pochart,P., Fields,S. and Wickens,M. (1996) A three-hybrid system to detect RNA–protein interactions *in vivo*. *Proc. Natl Acad. Sci. USA*, **93**, 8496–8501.

Sil,A. and Herskowitz,I. (1996) Identification of asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast *HO* gene. *Cell*, **84**, 711–722.

Sonoda,J. and Wharton,R.P. (1999) Recruitment of Nanos to hunchback mRNA by Pumilio. *Genes Dev.*, **13**, 2704–2712.

Strathern,J.N. and Herskowitz,I. (1979) Asymmetry and directionality in production of new cell types during clonal growth: the switching pattern of homothallic yeast. *Cell*, **17**, 371–381.

Subramaniam,K. and Seydoux,G. (1999) *nos-1* and *nos-2*, two genes related to *Drosophila nanos*, regulate primordial germ cell development and survival in *Caenorhabditis elegans*. *Development*, **126**, 4861–4871.

Takizawa,P.A., Sil,A., Swedlow,J.R., Herskowitz,I. and Vale,R.D. (1997) Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. *Nature*, **389**, 90–93.

Waskiewicz,S.B., Skala,J., Jasinski,M., Grenson,M., Goffeau,A. and Ulaszewski,S. (1998) Functional analysis of three adjacent open reading frames from the right arm of yeast chromosome XVI. *Yeast*, **14**, 1027–1039.

Wharton,R.P. and Struhl,G. (1991) RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen nanos. *Cell*, **67**, 955–967.

Wharton,R.P., Sonoda,J., Lee,T., Patterson,M. and Murata,Y. (1998) The Pumilio RNA-binding domain is also a translational regulator. *Mol. Cell*, **1**, 863–872.

Wickens,M., Anderson,P. and Jackson,R.J. (1997) Life and death in the cytoplasm: messages from the 3′ end. *Curr. Opin. Genet. Dev.*, **7**, 220–232.

Wreden,C., Verrotti,A.C., Schisa,J.A., Lieberfarb,M.E. and Strickland,S. (1997) Nanos and pumilio establish embryonic polarity in *Drosophila* by promoting posterior deadenylation of hunchback mRNA. *Development*, **124**, 3015–3023.

Zamore,P.D., Bartel,D.P., Lehmann,R. and Williamson,J.R. (1999) The PUMILIO–RNA interaction: a single RNA-binding domain monomer recognizes a bipartite target sequence. *Biochemistry*, **38**, 596–604.

Zhang,B., Gallegos,M., Puoti,A., Durkin,E., Fields,S., Kimble,J. and Wickens,M.P. (1997) A conserved RNA-binding protein that regulates sexual fates in the *C.elegans* hermaphrodite germ line. *Nature*, **390**, 477–484.

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