Supplementary Information

Plasmids construction

To construct pIT2069 (p416*GAL1p-AUG-FLAG-MPT4ΔN-PGK1-3'UTR-MS2*; *AUG-FLAG-MS2*), an *Xba*I-*Bam*HI *AUG-FLAG-MPT4ΔN* fragment and a *Bam*HI-*Eco*RI *3'UTR(PGK1)-MS2* fragment were inserted into the corresponding restriction sites of p416*GAL1p*. The *Xba*I-*Bam*HI *AUG-FLAG-MPT4ΔN* fragment was constructed as follows. A region of the *MPT4* ORF (380–822 nt) was PCR-amplified using the primers OTS017 and OIT934. The PCR fragment resulting from this amplification has an ATG codon and FLAG sequences inserted upstream of the ORF. This fragment was inserted into the *Xba*I-*Bam*HI sites of p416*GAL1*p (Mumberg et al., *Nucleic Acids Res,* 1994). The ATG sequence encoded by nucleotides 629–632 of the *MPT4* ORF was replaced by *Spe*I restriction site using site-directed mutagenesis and the two oligonucleotides OIT932 and OIT933. The *Bam*HI-*Eco*RI *3'UTR(PGK1)-MS2* fragment, which contains tandem MS2 coat protein binding sites was constructed as follows. The *Bam*HI-*Eco*RI *3'-UTR* of *a PGK1* fragment was PCR-amplified using the primers OIT557 and OIT558 and was inserted into the *Bam*HI-*Eco*RI site of p416*GAL1*p. An *Xho*I site was introduced 60 nts downstream of the *Bam*HI site using site-directed mutagenesis and the two oligonucleotides OKK130 and OKK131. Two oligonucleotides (OIT949 and OIT950) that contain MS2 coat protein binding sites were annealed and were then inserted into the *Xho*I site yielding *Bam*H1-*3'UTR(PGK1)-MS2-Eco*RI.

To construct pIT2071 (p416*GAL1p-No-AUG-FLAG-MPT4ΔN-3'UTR(PGK1)-MS2* (*No-AUG-FLAG-MS2*))*,* a region of the *MPT4* ORF (380-822 nt) was PCR-amplified using the primers OIT953 and OIT934, which also insert a TAC codon and a FLAG sequence upstream of this ORF. This *Xba*I-*Bam*HI fragment was then substituted for the *Xba*I-*Bam*HI fragment of pIT2069 (*AUG-FLAG-MS2*).

To construct pIT2074 (*AUG-MPT4ΔN-FLAG-MS2*), pIT2075 (*No-AUG-MPT4ΔN-FLAG-MS2*) or pIT2076 (*SL-AUG-MPT4ΔN-FLAG-MS2*) plasmids, a region of the *MPT4* ORF (633-822 nt) was PCR-amplified using the primers OIT933 and OTS10. This *Spe*I-*Bam*HI fragment was then substituted for the *Spe*I-*Bam*HI fragment of pIT2069 (*AUG-FLAG-MS2*) or pIT2071(*No-AUG-FLAG-MS2*), pIT2073 (*SL-AUG-FLAG-MS2*), respectively.

To construct plasmids that did not contain tandem MS2 binding sites, the *Bam*HI-*Eco*RI fragment encoding the *3'-UTR* of *PGK1* was directly inserted into plasmids containing the above *Bam*HI-*Eco*RI fragments.

Plasmids expressing various FLAG-MS2-Pab1p fusion proteins were constructed as follows. To construct pIT2077 (p415*TEF1p-PAB1*), the *Xba*I-*Sal*I fragment of *PAB1* was PCR-amplified using the primers OIT627 and OIT884, and was inserted into the *Xba*I-*Sal*I sites of p415*TEF1p* (Mumberg *et al*., *Gene,* 1995). The *Xba*I-*Xba*I fragment of the FLAG-MS2 coat protein was then PCR-amplified using the primers OIT670 and OIT630, and was inserted into the corresponding site of pIT2077 yielding pIT2078 (*p415TEF1p-FLAG-MS2-PAB1 LEU2 CEN*). To construct pIT2079 (*415TEF1p-FLAG-MS2-Pab1-34C LEU2 CEN*)*,* the *Xba*I-*Sal*I fragment of *Pab1-34C* was PCR-amplified using the primers GTD029 and OIT884 and was substituted for the *Xba*I-*Sal*I fragment of pIT2078. To construct pIT2080 (*p415TEF1p-FLAG-MS2-Pab1-4C LEU2 CEN*)*,* the *Xba*I-*Sal*I fragment of *Pab1-4C* was PCR-amplified using the primers OIT883 and OIT884 and was substituted for the *Xba*I-*Sal*I fragment of pIT2078. To construct pIT2081 (*p415TEF1p-FLAG-MS2-Pab1-C LEU2 CEN*)*,* the *Xba*I-*Sal*I fragment of *Pab1-4C* was PCR-amplified using the primers GTD038 and OIT884 and was substituted for the *Xba*I-*Sal*I fragment of pIT2078. The FLAG-MS2-Pab1p fusion proteins, expressed from PIT2079, pIT2080 and pIT2081 consisted of Pab1p residues 207–577, 309–577 and 431–577, respectively (Kessler *et al*., *Mol Cell Biol,* 1998). To construct pIT2086, pIT2087, pIT2088, pIT2089 plasmids that did not contain FLAG sequences, primers without FLAG sequences were used to amplify the fragment.

The *Bam*HI-*Eco*RI *3'UTR(PGK1)* fragments containing a hammerhead ribozyme sequence

were constructed as follows. A *Spe*I site was introduced 77 nts downstream of the *Xho*I site within the *Bam*HI-*Eco*RI *3'UTR(PGK1)* fragment using site-directed mutagenesis and the two oligonucleotides OTS034 and OTS035. Two oligonucleotides (OIT1181 and OIT1182) containing the hammerhead ribozyme sequence (Rz) were annealed and were then inserted into the *Spe*I site to construct the *3'UTR(PGK1)-Rz* and *3'UTR(PGK1)-MS2-Rz.* To construct pIT2082 (*p416GAL1p-No-AUG-FLAG-MPT4ΔN-PGK1(3'UTR)-Rz URA3 CEN*) and pIT2083 (*p416GAL1p-No-AUG-FLAG-MPT4ΔN-PGK1(3'UTR)-MS2-Rz URA3 CEN*) that contain the ribozyme sequence, the *Bam*HI-*Eco*RI *3'UTR(PGK1)-Rz* or *3'UTR(PGK1)-MS2-Rz* fragments were substituted for the *Xba*I-*Sal*I fragment of pIT2071 (*No-AUG-FLAG-MS2*) or pIT2072 (*No-AUG-FLAG*). To construct pIT2084 (*p416GAL1p-Rz-No-AUG-FLAG-MPT4ΔN-PGK1(3'UTR)-MS2-Rz URA3 CEN*) and pIT2085 (*p416GAL1p-Rz-No-AUG-FLAG-MPT4ΔN-PGK1(3'UTR)-Rz URA3 CEN*) that contain the ribozyme sequence in 5'-UTR, the two oligonucleotides (OIT1181 and OIT1182) that contain the hammerhead ribozyme sequence (Rz) were annealed and inserted into the *Xba*I site of the pIT2082 or pIT2083 plasmid, respectively.

Mumberg, D., Müller, R., Funk, M. (1994) *Nucleic Acids Res* **22**(25), 5767-5768
Mumberg, D., Müller, R., Funk, M. (1995) *Gene* **156**(1), 119-122
Kessler, S. H., and Sachs, A. B. (1998) *Mol Cell Biol* **18**(1), 51-57