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Yeast Methionine Aminopeptidase Type 1 Is Ribosome-Associated and Requires Its N-Terminal Zinc Finger Domain for Normal Function In Vivo

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Abstract Methionine aminopeptidase type 1 (MetAP1) cotranslationally removes N-terminal methionine from nascent polypeptides, when the second residue in the primary structure is small and uncharged. Eukaryotic MetAP1 has an N-terminal zinc finger domain not found in prokaryotic MetAPs. We hypothesized that the zinc finger domain mediates the association of MetAP1 with the ribosomes and have reported genetic evidence that it is important for the normal function of MetAP1 in vivo. In this study, the intracellular role of the zinc finger domain in yeast MetAP1 function was examined. Wild-type MetAP1 expressed in a yeast *map1* null strain removed 100% of N-terminal methionine from a reporter protein, while zinc finger mutants removed only 31–35%. Ribosome profiles of *map1* null expressing wild-type MetAP1 or one of three zinc finger mutants were compared. Wild-type MetAP1 was found to be an 80S translational complex-associated protein that primarily associates with the 60S subunit. Deletion of the zinc finger domain did not significantly alter the ribosome profile distribution of MetAP1. In contrast, single point mutations in the first or second zinc finger motif disrupted association of MetAP1 with the 60S subunit and the 80S translational complex. Together, these results indicate that the zinc finger domain is essential for the normal processing function of MetAP1 in vivo and suggest that it may be important for the proper functional alignment of MetAP1 on the ribosomes. J. Cell. Biochem. 85: 678–688, 2002.

Key words: zinc finger; N-terminal protein processing; methionine aminopeptidase; ribosome association

Eukaryotes initiate the translation of endogenous cytosolic mRNA with a methionine-bound initiator tRNA (Met-tRNA$_i^{Met}$) [Housman et al., 1972]. As a result, most nascent polypeptides begin with an N-terminal methionine (Met$_{init}$). Methionine aminopeptidase (MetAP, product of the *MAP* gene, EC 3.4.11.18) cotranslationally removes Met$_{init}$ from nascent polypeptides, when the second residue in the primary structure is small and uncharged (e.g., Met–Ala–, –Cys–, –Gly–, –Pro–, –Ser–, –Thr–, –Val–) [Tsunasawa et al., 1985; Flinta et al., 1986; Ben-Bassat et al., 1987; Huang et al., 1987; Boissel et al., 1988; Hirel et al., 1989; Moerschell et al., 1990]. Prokaryotes, as well as mitochondria [Bianchetti et al., 1977] and chloroplasts [Lucchini and Bianchetti, 1980], initiate translation with an N^{α}-formylated methionine bound to an initiator tRNA (fMet-tRNA$_f^{Met}$) [Clark and Marcker, 1966; Leder and Bursztyn, 1966; Noll, 1966]. MetAP requires deformylation of fMet$_{init}$ before Met$_{init}$ can be removed [Adams, 1968; Solbiati et al., 1999].

MetAP activity is an essential cellular function. Deletion of the single *MAP* gene in *E. coli* [Chang et al., 1989] and *S. typhimurium* [Miller et al., 1989] or of both *MAP* genes (*MAP1* and *MAP2*) in yeast (*S. cerevisiae*) is lethal [Li and

Abbreviations used: MetAP, methionine aminopeptidase; *map1*Δ, yeast *map1* deletion strain; GST, glutathione S-transferase.

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Chang, 1995]. Deletion of *MAP1* [Chang et al., 1992] or *MAP2* [Li and Chang, 1995] alone in yeast results in a slow-growth phenotype. The *map1* knockout strain (*map1*Δ), however, displays a more severe slow-growth phenotype than the *map2*Δ strain [Li and Chang, 1995]. The more severe slow-growth phenotype of yeast *map1*Δ correlates well with recent evidence indicating that MetAP1 is primarily responsible for Met_{init} removal in yeast [Chen et al., 2002]. Together, these data suggest that the growth rate of yeast correlates with the level of Met_{init} processing of proteins in vivo.

Eukaryotic MetAPs, unlike prokaryotic MetAPs, possess an extended N-terminal region [Arfin et al., 1995; Li and Chang, 1995]. Within this N-terminal region, yeast MetAP1 has a zinc finger domain [Chang et al., 1990] consisting of a Cys_2–Cys_2 zinc finger motif (residues 22–40) similar to the RING finger family and a unique Cys_2–His_2 (residues 50–66) motif similar to zinc fingers involved in RNA binding (IY**C**^{22}SGLQ**C**^{27}-GRETSSQMK**C**^{37}PV**C**^{40}LKQGIVSIF**C**^{50}DTS**C**^{54}YENNYKA**H**^{62}KAL**H**^{66}NAK) [Zuo et al., 1995]. Furthermore, yeast MetAP1 chelates zinc in a 2:1 molar ratio of zinc: MetAP1 and deletion of the putative N-terminal zinc finger domain abolishes zinc binding [Zuo et al., 1995]. Thus, yeast MetAP1 is assumed to possess two functional zinc fingers. A similar domain is found in mammalian MetAP1 [Nagase et al., 1995] and in plant MetAP1A from *A. thaliana* [Giglione et al., 2000].

Zinc finger motifs have been reported to mediate protein–protein and protein–nucleic acid interactions for a variety of proteins [Berg, 1986; Struhl, 1989; Berg, 1990; Iuchi, 2001]. Thus, we proposed that the zinc finger domain might mediate the presumed association of MetAP1 with the ribosomes [Jackson and Hunter, 1970; Chang et al., 1992].

We have previously shown that the zinc finger domain is not required for MetAP1 catalytic activity in vitro [Zuo et al., 1995]. However, unlike wild-type MetAP1, MetAP1 zinc finger mutants cannot fully complement the slow-growth phenotype of a yeast *map1*Δ strain [Zuo et al., 1995]. These findings suggest that the zinc finger domain is required for the normal function of MetAP1 within the cell.

In this report, we provide the first biochemical evidence of MetAP1 ribosome association and present evidence that the zinc finger domain is important for the normal processing function of MetAP1 in vivo. We also examined the role of the zinc finger domain in MetAP1 ribosome association and found evidence that suggests it may be important for the correct functional alignment of MetAP1 on the ribosomes.

MATERIALS AND METHODS

Materials

All chemicals were from Sigma (St. Louis, MO) unless otherwise stated. Two YPD reagents, yeast extract and peptone, came from Difco. Synthetic dropout media plus 2% dextrose (SD) contained yeast nitrogen base without amino acids/ammonium sulfate (Difco) and were supplemented with amino acids to give the desired dropout mixture as described [Ausubel et al., 1992]. DNA digests were carried out with restriction enzymes from Promega.

DNA Constructs

Construction of the *MAP1* (wt) and zinc finger mutants has been described [Zuo et al., 1995]. Wild-type *MAP1*, a zinc finger domain deletion mutant, *map1* (Δ2-69), and two *map1* zinc finger point mutants, *map1* (C22S) and *map1* (H62R), were subcloned from pRS415 into pRS416 [Sikorski and Hieter, 1989] using Xho I/Sac I for subsequent assay of MetAP1 activity in vivo. Each construct is under 1 kb of the wild-type *MAP1* promoter.

Yeast/Bacteria Culturing and Transformations

General handling and techniques for yeast and bacteria were followed as outlined [Ausubel et al., 1992]. Bacterial transformations were carried out using an *E. coli* transformation kit (Zymo Research, Orange, CA) according to the manufacturer's protocol. Yeast transformations were done by the alkali-cation method [Ito et al., 1983] using a yeast transformation kit (QBIO Gene, Carlsbad, CA).

Generation of Polyclonal Antibodies

A standard procedure was adapted [Harlow and Lane, 1988]. Oligopeptides corresponding to the amino acid sequence at the C-terminal end of the 40S subunit protein, RPS3 (CRPAE-ETEAQAEPVEA), the 60S subunit protein, RPL3 (CAEKHAFMGTLKKDL), and MetAP2 [Li and Chang, 1995] with an N-terminal cysteine added for column coupling were obtained. Each peptide was conjugated to the carrier protein maleimide-activated keyhole limpet

hemocyanin (KLH) at 2 mg peptide/2 mg KLH according to the manufacturer's protocol (Pierce Endogen). Each KLH-peptide immunogen was mixed with an equal volume of Freund's complete adjuvant, and 400 μl of the emulsion was injected into each rabbit. Two booster injections of the same amount of antigen emulsified in incomplete Freund's adjuvant were given at weeks 4 and 8. Blood was collected from the ear prior to the initial injection and 10 days after each boost. Blood samples were incubated at room temperature 4 h before being placed at 4°C overnight. Coagulated blood was cleared by centrifugation at 3,000*g* for 10 min. RPS3, RPL3, and MetAP2 polyclonal antibodies were purified using cyanogen bromide-activated thiol-sepharose coupled with the respective peptide antigen and eluted with 1 mg/ml free peptide.

Assay of MetAP1 Processing In Vivo

Each yeast *map1*Δ/*MAP1* and *map1*Δ/*map1* zinc finger mutant transformant was co-transformed with a mutant glutathione S-transferase (GST) construct (yeast vector p425*GPD*) [Christianson et al., 1992] containing a Ser to Gly substitution (S2G) at the second residue in the primary sequence (NH_2–Met–Gly–GST) [Chen et al., 2002]. Each doubly transformed strain was grown in 1.0 L SD/Leu^-/Ura^- to $ABS_{600} \sim 2.0$ and lysed with glass beads in 1 volume TPBS (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4 [pH 7.3], 1% Tween 20, 8 μl yeast protease inhibitor cocktail). The lysate was loaded onto an agarose bead-glutathione column (2 ml), washed 3 × with 15 ml PBS, and eluted with freshly prepared 10 mM reduced glutathione diluted in 50 mM HEPES (pH 7.5). Total purified GST (S2G) from each strain was subjected to electrophoresis then transferred to a polyvinylidene fluoride (PVDF) membrane for N-terminal sequencing by Edman degradation. The percentage of Met_{init} removed from the isolated GST protein (processing efficiency) was calculated by dividing GST with Met_{init} removed (processed GST) by total GST. For example, the processing efficiency for MetAP1 (Δ2-69) was calculated by dividing the second peak in the first cycle, G (5.4), which represents GST with Met_{init} removed, with the sum of the peaks representing total GST: G (5.4) from the first cycle and G (7.2) from the second cycle, which represents GST with Met_{init} retained (5.4/12.6). The same calculation was then made using each consecutive cycle (e.g., P, I, and L). Values are reported as the mean ± SD% (n = 4).

Ribosome Profiles of Yeast Crude Extracts

Ribosome profiles of a yeast *map1* (*map1*Δ) knockout strain, YHC001 [Klinkenberg et al., 1997], singly transformed with yeast *MAP1* (wt) or *map1* zinc finger mutants were performed with slight modifications [Eisinger et al., 1997]. A seed culture of each strain grown overnight in selective media was used to inoculate 300 ml SD/Ura^-. The culture was grown aerobically overnight at 30°C to $ABS_{600} \sim 1.0$. Cultures were incubated on ice for 5 min after the addition of cycloheximide (100 μg/ml final concentration) and harvested (5,000*g*, 6 min, 4°C, GS 3 rotor, Sorvall RC5C). Preparations without $MgCl_2$ did not receive cycloheximide treatment. Each pellet was resuspended in an equal volume (~600 μl) of breaking buffer (20 mM Tris-HCl [pH 7.5], 50 mM NaCl, 2 mM $MgCl_2$, 5 mM sodium molybdate, 5 mM sodium fluoride, 2 mM dithiothreitol) plus protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 5 μM leupeptin, 0.15 μM aprotinin, 0.025% [v/v] diethyl pyrocarbonate [DEPC]) and homogenized with glass beads (0.5 mm diameter, Biospec, Bartelsville, OK). Crude extracts of each transformant were cleared by centrifugation (1,000*g*, Jouan CR 422) and 15 ABS_{260} units loaded onto an 11.0 ml, 5–47% sucrose gradient (made in breaking buffer lacking DEPC). The gradient was centrifuged at 39,000*g* for either 3 h (2 mM Mg^{2+} samples) or 3 h 45 min (no Mg^{2+} samples) at 4°C (SW-41 rotor, Beckman L8-60M Ultracentrifuge). A Buchler Auto Densi-Flow IIC was used to remove the post-centrifugation gradient through a coupled FPLC sensor (Pharmacia), which simultaneously measured ABS_{254}. Fractions (600 μl) were collected sequentially from the top of the gradient (cytosolic proteins) to the bottom (polysomal fractions) with the FPLC fraction collector. Collected fractions were adjusted to 12.5% trichloroacetic acid (TCA) and allowed to precipitate overnight at −20°C. The next day, fractions were spun down (microcentrifuge, high speed) for 10 min at 4°C and the supernatant removed. The pellet was washed 2 × with 200 μl acetone, then resuspended in 100 μl 1 × SDS sample buffer [Laemmli, 1970] containing 5 mM dithiothreitol (DTT) and boiled for 5 min. Twenty microliters of each fraction were separated by 10% SDS–PAGE

[Laemmli, 1970] then transferred to nitrocellulose and immunoblotted with primary rabbit anti-MetAP1 (1:500) [Chang et al., 1992] or rabbit anti-MetAP2 (1:500) and secondary goat anti-rabbit IgG conjugated horse radish peroxidase (1:5,000). Gradient fractions were aligned by stripping the membrane (ECL protocol, Amersham) and probing for a cytosolic protein (polyclonal rabbit anti-glucose-6-phosphate dehydrogenase, 1:6,000; Sigma), a core protein of the 40S subunit (polyclonal rabbit anti-RPS3, 1:500) and a core protein of the 60S subunit (polyclonal rabbit anti-RPL3, 1:2,000). All antibodies were diluted in Tris-buffered saline containing 1% Tween 20 (TBST) plus 1% nonfat dry milk.

RESULTS

Yeast MetAP1 Zinc Finger Mutants With Normal Catalytic Activity In Vitro Are Dysfunctional In Vivo

We previously reported that zinc finger mutants of yeast MetAP1 have normal catalytic activity in vitro but, unlike wild-type MetAP1, are unable to fully complement the slow-growth phenotype of a yeast *map1* knockout strain (*map1*Δ) [Zuo et al., 1995]. These results suggest that the zinc finger domain is important for the normal intracellular function of MetAP1.

To investigate the importance of the zinc finger domain in MetAP1 function in vivo, we compared the Met_{init} processing efficiency of wild-type MetAP1 with three MetAP1 zinc finger mutants. A yeast *map1*Δ strain lacking endogenous MetAP1 was transformed with either the yeast wild-type *MAP1* gene, a zinc finger domain deletion mutant (Δ2-69), or *map1* mutants with a single point mutation in the first (C22S) or second (H62R) zinc finger motif (Table I). Each gene was in a single-copy vector under 1 kb of the wild-type *MAP1* promoter. The steady state expression levels of each MetAP1 protein construct are comparable in yeast *map1*Δ (Fig. 1) and the wild-type *MAP1* construct is able to fully complement the slow-growth phenotype of yeast *map1*Δ [Zuo et al., 1995].

TABLE I. Strains Used in This Study

Strain	Genotype	Reference
	S. cerevisiae	
YHC001	*MATα ade2-1 can1-100 ura3-1 leu2-3, 112 trp1-1 his3-11, 15 map1::HIS3*	
JAV01	YHC001/pRS416/*MAP1*	This study
JAV02	YHC001/pRS416/*map1* (Δ2–69)	This study
JAV03	YHC001/pRS416/*map1* (C22S)	This study
JAV04	YHC001/pRS416/*map1* (H62R)	This study

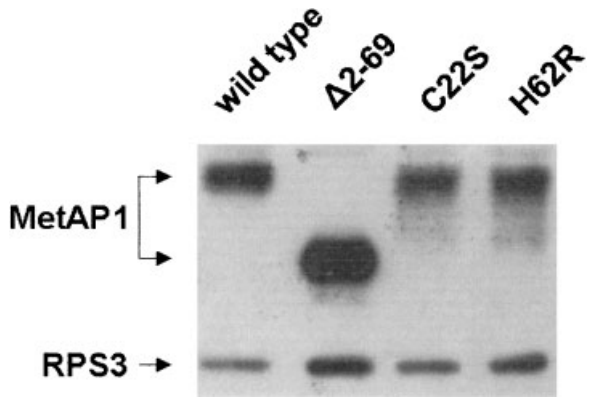

Fig. 1. Steady state levels of wild-type and MetAP1 zinc finger mutant constructs in *map1*Δ. Yeast *map1*Δ strains expressing wild-type MetAP1 (wt), truncated MetAP1 (Δ2-69), which lacks the entire zinc finger domain, or mutants of MetAP1 with a single point mutation in the first (C22S) or second (H62R) zinc finger motif were grown to $ABS_{600} \sim 1.0$ (10 ml) and crude extracts were obtained. Approximately 1.5 μg of total protein was loaded in each lane, resolved by SDS–PAGE, transferred to nitrocellulose, then simultaneously probed for yeast MetAP1 and a core protein of the 60S ribosome subunit, RPL3.

The *map1* zinc finger deletion mutant (Δ2-69) was chosen partly because of all the zinc finger mutants, it most effectively rescues the *map1*Δ slow-growth phenotype. The two zinc finger point mutants (C22S and H62R) were selected because they have the least effect on the *map1*Δ slow-growth phenotype [Zuo et al., 1995]. Considering that growth rate in yeast directly correlates with the level of Met_{init} processing in vivo [Chen et al., 2002], use of these mutants potentially allowed for examination of a range of Met_{init} processing efficiencies.

To provide a substrate for the measurement and comparison of MetAP1 processing efficiency in vivo, each *map1*Δ transformant strain was co-transformed with a reporter construct expressing a mutant gene of glutathione S-transferase (GST) containing a Ser to Gly substitution in the second codon (S2G) [Chen et al., 2002]. In the *map1*Δ strain, which expresses wild-type MetAP2 alone, approximately 5% of Met_{init} is removed from GST (S2G) as shown by electrospray ionization mass spectrometry (ESI-MS) analysis of total isolated GST (S2G) [Chen et al., 2002]. In contrast, GST (S2G) is almost

completely processed (95%) by a *map2*Δ strain that expresses only wild-type MetAP1 [Chen et al., 2002]. Thus, the processing of GST (S2G) in yeast relies almost entirely on MetAP1 function and, as such, is an ideal substrate for measuring yeast MetAP1 processing efficiency in vivo.

Total GST (S2G) was isolated from each *map1*Δ transformant strain and the extent of Met_{init} removal was determined by amino terminal sequencing (Table II). N^{α}-acetylation of GST (S2G) with and without Met isolated from yeast was not detected by electrospray ionization mass spectrometry (ESI-MS) [Chen et al., 2002]. Thus, N^{α}-acetylation is not present to interfere with the N-terminal sequencing reactions.

The percentage of Met_{init} removed from GST (S2G) isolated from *map1*Δ expressing wild-type MetAP1 was 100%, but significantly lower for GST (S2G) isolated from *map1*Δ expressing MetAP1 (Δ2-69) ($34.88 \pm 0.06\%$), MetAP1 (C22S) ($35.23 \pm 0.03\%$), or MetAP1 (H62R) ($31.53 \pm 0.03\%$) (Table II). Together with previous evidence, these results indicate that although there is no effect on MetAP1 catalytic activity in vitro, mutations in the zinc finger domain compromise the function of MetAP1 in vivo.

Surprisingly, no difference between the processing efficiencies of truncated and zinc finger motif point mutants was observed against GST (S2G). This was unexpected as MetAP1 (Δ2-69) complements the *map1*Δ slow-growth phenotype (4.0 ± 0.3 h doubling time) more effectively than MetAP1 (C22S) or MetAP1 (H62R) (6.0 ± 0.3 and 5.5 ± 0.3 h doubling time, respectively) [Zuo et al., 1995]. Thus, MetAP1 (Δ2-69) is expected to have a greater processing efficiency than C22S or H62R as the level of Met_{init} processing correlates with growth rate in yeast [Chen et al., in press]. From these data, however, it can only be concluded that the processing efficiencies of MetAP1 (Δ2-69), MetAP1 (C22S), and MetAP1 (H62R) are less than wild-type MetAP1 in vivo.

Ribosome Profile Distribution of Wild-Type Yeast MetAP1

Based on evidence that zinc finger motifs are involved in protein–protein and protein–nucleic acid interactions in other proteins, we hypothesized that the zinc finger domain mediates the presumed ribosome association of MetAP1 [Chang et al., 1992]. Considering that MetAP1 zinc finger mutants are dysfunctional in vivo (Table II), we investigated the possible role of the zinc finger domain in MetAP1 ribosome association.

Ribosome profiles of the *map1*Δ/*MAP1*, *map1* (Δ2-69), *map1* (C22S), and *map1* (H62R) transformant strains were compared. In the presence of 2 mM Mg^{2+}, MetAP1 (wt) was found in the cytosolic, 40S subunit, 60S subunit, and 80S translational complex fractions (Fig. 2). This finding is in contrast to the distribution of a known cytosolic protein, glucose-6-phosphate dehydrogenase (G6PDH), which was found only at the top of the gradient (Fig. 2, lanes 1–5). Thus, the co-migration of MetAP1 with the ribosome fractions is not a result of gradient tailing. Identical results were observed in ribosome profiles of the wild-type yeast strain, W303-1A (data not shown).

Interestingly, MetAP1 ribosome association was found to be sensitive to levels of Mg^{2+} (10 mM) traditionally used for ribosome profiles in yeast [Marcus et al., 1967]. At concentrations

TABLE II. Amino Terminal Sequence of GST (S2G) Isolated From a Yeast *map1*Δ Strain Expressing Wild-Type or Zinc Finger Mutants of MetAP1

Cycle	Literature sequence	JAV01	JAV02	JAV03	JAV04
1	M, G	G(9.9)	M(5.9), G(5.4)	M(10.9), G(6.2)	M(11.1), G(6.0)
2	G, P	P(4.6)	G(7.2), P(1.8)	G(9.6), P(5.0)	G(11.0), P(3.1)
3	P, I	I(3.9)	P(4.9), I(2.2)	P(10.4), I(3.4)	P(7.9), I(3.0)
4	I, L	L(6.0)	I(3.9), L(2.9)	I(6.8), L(5.6)	I(6.5), L(4.6)
5	L,G	G(4.6)	L(5.7), G(2.1)	L(10.0), G(3.7)	L(10.2), G(3.5)

Total glutathione S-transferase (GST) with a Ser to Gly substitution in the second residue (S2G) was isolated from yeast *map1*Δ co-expressing wild-type MetAP1 (JAV01), a zinc finger domain deletion mutant of MetAP1 (JAV02), a zinc finger point mutant of MetAP1 with a (C22S) point mutation in the first zinc finger motif (JAV03) or a (H62R) point mutation in the second zinc finger motif (JAV04). GST (S2G) isolated from each strain was separated by electrophoresis, blotted onto a PVDF membrane, and sequenced by Edman degradation. The picomole amount of amino acid present in each cycle is indicated.

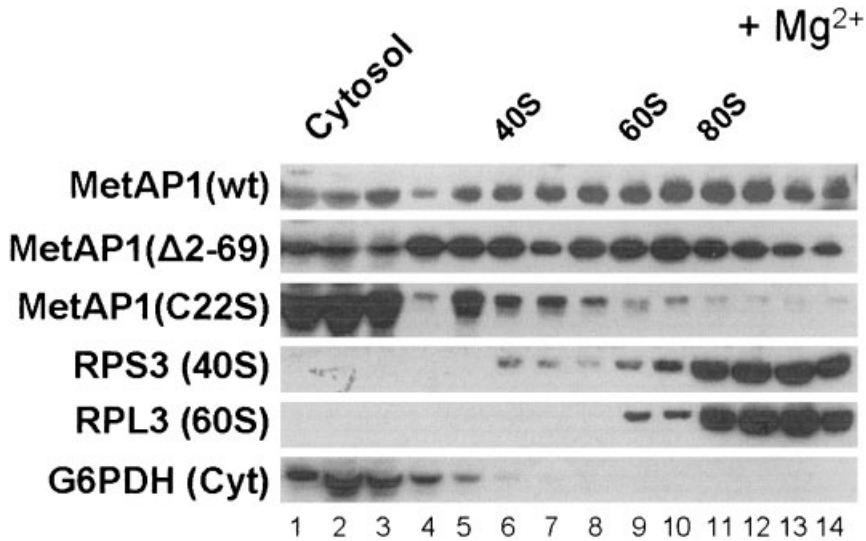

Fig. 2. Comparison of ribosome profile distributions of wild-type and zinc finger mutants of MetAP1 in the presence of Mg^{2+}. Crude extracts (CE) were isolated from yeast *map1*Δ strains singly expressing MetAP1 wild-type (wt), truncated MetAP1 (Δ2-69), which lacks the entire zinc finger domain, or mutants of MetAP1 with a single point mutation in the first (C22S) or second (H62R) zinc finger motif. Approximately 15 ABS_{260} units of CE were loaded onto 11.0 ml 5–47% sucrose gradient (2 mM Mg^{2+}) and centrifuged for 3 h at 4°C. Fractions (600 μl) were collected sequentially from the top (cytosolic proteins) to the bottom (polysomal fractions) of the gradient and aligned by stripping the membrane and probing for a cytosolic protein (glucose-6-phosphate dehydrogenase) and core proteins of the 40S (RPS3) and 60S (RPL3) ribosome subunit. Blots were aligned and stacked to facilitate comparison. Identical results were observed for MetAP1 (C22S) and MetAP1 (H62R).

of Mg^{2+} greater than 2 mM, MetAP1 was found to gradually shift from the ribosome fractions to the cytosolic fractions in a concentration-dependent manner (data not shown). The level of unbound cytosolic Mg^{2+} in eukaryotic cells, however, has been estimated to range from 0.1 to 1.0 mM [Romani and Scarpa, 1992]. Therefore, these results indicate that MetAP1 associates with the ribosomes and 80S translational complex near the estimated range of free Mg^{2+} in yeast.

To examine the association of MetAP1 with individual ribosome subunits, profiles of wild-type MetAP1 were performed in the absence of Mg^{2+}, which prevents the 40S and 60S ribosome subunits from forming the 80S translational complex in vitro [Zinker and Warner, 1976]. Under these conditions, the majority of wild-type MetAP1 is found in the 60S subunit fractions as levels of MetAP1 closely paralleled levels of a core protein of the 60S ribosome subunit, RPL3 (Fig. 3, lanes 7–13). Lower levels of MetAP1 were also observed in the 40S subunit fractions that did not closely reflect levels of a core protein of the 40S ribosome subunit, RPS3 (Fig. 3, lanes 5–7). Together, these results indicate that MetAP1 associates primarily with the 60S ribosome subunit and suggest a lower affinity or non-specific interaction with the 40S ribosome subunit.

Removal of the Zinc Finger Domain Does Not Affect the Association of MetAP1 With the 60S Ribosome Subunit

To test whether the zinc finger domain alone mediates MetAP1 ribosome association, profiles of yeast *map1*Δ expressing the zinc finger domain deletion mutant, MetAP1 (Δ2-69), were performed. The ribosome profile distribution of MetAP1 (Δ2-69) in the presence of 2 mM Mg^{2+} was similar to wild-type MetAP1 (Fig. 2). Profiles of MetAP1 (Δ2-69) in the absence of Mg^{2+} showed levels comparable to wild-type MetAP1 in the 60S ribosome subunit fractions (Fig. 3, lanes 7–13). Together, these results indicate that ribosome interaction sites remain in MetAP1 (Δ2-69).

Higher levels of MetAP1 (Δ2-69) were also detected in the 40S subunit fractions compared to wild-type MetAP1 (Fig. 3, lanes 4–7). However, the increase of MetAP1 (Δ2-69) observed in the 40S subunit fractions could be the result of a difference in gradient loading between wild-type and truncated MetAP1. Thus, it is unclear whether removal of the zinc finger domain leads to an increase in 40S subunit association.

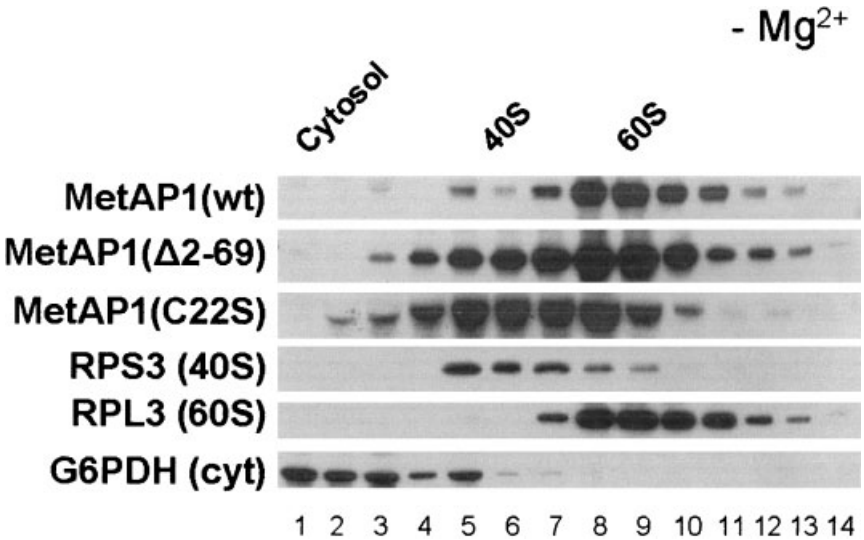

Fig. 3. Comparison of ribosome profile distributions of wild-type and zinc finger mutants of MetAP1 in the absence of Mg^{2+}. Crude extracts (CE) were isolated in breaking lacking Mg^{2+} from yeast *map1*Δ strains singly expressing MetAP1 wild-type (wt), truncated MetAP1 (Δ2–69), which lacks the entire zinc finger domain, or mutants of MetAP1 with a single point mutation in the first (C22S) or second (H62R) zinc finger motif. Approximately 15 ABS_{260} units of CE were loaded onto 11.0 ml 5–47% sucrose gradient lacking Mg^{2+} and treated as described in Figure 2. Blots were aligned and stacked to facilitate comparison. Identical results were observed for MetAP1 (C22S) and MetAP1 (H62R).

MetAP1 Zinc Finger Point Mutants Have Altered Ribosome Profile Distributions

Ribosome profiles of *map1*Δ expressing MetAP1 (C22S) or MetAP1 (H62R) were compared with wild-type and truncated MetAP1 profile distributions. In contrast to wild-type MetAP1 or MetAP1 (Δ2-69), significantly less MetAP1 (C22S) was observed in the 40S, 60S, and 80S fractions in the presence of 2 mM Mg^{2+} (Fig. 2). Higher levels of MetAP1 (C22S) were also observed in the cytosolic fractions compared to wild-type and truncated MetAP1 (Δ2-69) (Fig. 2).

Profiles in the absence of Mg^{2+} revealed that MetAP1 (C22S) is found primarily in the 40S subunit fractions with lower levels in the 60S subunit fractions compared to wild-type and truncated MetAP1 (Fig. 3). This suggests that MetAP1 (C22S) is primarily associated with the 40S subunit. Similar results were observed in the profiles of MetAP1 (H62R) (data not shown). Together, these results indicate that the association of MetAP1 (C22S) and MetAP1 (H62R) with the 60S ribosome subunit is disrupted and suggest that the majority of both MetAP1 (C22S) and MetAP1 (H62R) is dissociated from the ribosomes/80S translational complex during protein synthesis.

DISCUSSION

Yeast MetAP1 possesses an N-terminal zinc finger domain common to all eukaryotic MetAP1, but not found in bacterial or organellar MetAP1 [Giglione et al., 2000]. We previously proposed that the zinc finger domain mediates the presumed association of yeast MetAP1 with the ribosomes [Chang et al., 1992] and have reported genetic evidence suggesting that it is important for the normal function of yeast MetAP1 in vivo [Zuo et al., 1995]. In this study, we provide the first biochemical evidence of yeast MetAP1 ribosome association and demonstrate that the zinc finger domain is required for the normal processing function of MetAP1 in vivo. We also examined the role of the zinc finger domain in MetAP1 ribosome association and found evidence that suggests it may be important for the proper functional alignment of MetAP1 on the ribosomes.

Ribosomal Association of Yeast MetAP1

Much indirect evidence has suggested that eukaryotic MetAPs are ribosome-associated. Studies in rabbit reticulocyte lysates show that N-terminal methionine is cotranslationally removed from nascent polypeptides that are approximately 15–20 amino acids in length

[Jackson and Hunter, 1970], whereas ribosomes protect nascent polypeptides up to 30 amino acids in length from protease degradation [Rich et al., 1966; Malkin and Rich, 1967]. Also, proteins that cotranslationally modify N-termini (e.g., N^{α}-myristoyltransferase N^{α}-acetyltransferase) rely on the timely removal of methionine to reveal the second N-terminal residue for modification [Gordon et al., 1991; Polevoda and Sherman, 2000]. Evidence that human N^{α}-myristoyltransferase [Glover et al., 1997] and mammalian N^{α}-acetyltransferase [Pestana and Pitot, 1975] are associated with the ribosomes has been also reported. The present study is the first direct evidence of eukaryotic MetAP ribosome association. The association of bacterial MetAP with the ribosomes remains to be determined.

The current study demonstrates that, in addition to being a cytosolic protein, yeast MetAP1 primarily associates with the 60S ribosome subunit and associates with the 80S translational complex in the presence of 2 mM Mg^{2+}. Low levels of MetAP1 were also detected in the 40S ribosome subunit fractions in the absence of Mg^{2+}. This may suggest a lower-affinity or non-specific interaction of MetAP1 with the 40S subunit. Importantly, the association of MetAP1 with the 80S translational complex occurs near the range of free cytosolic Mg^{2+} estimated in eukaryotes (0.1–1.0 mM) [Romani and Scarpa, 1992]. It is important to note that rabbit reticulocyte lysate studies demonstrating the cotranslational removal of Met_{init} from nascent polypeptides were performed at low concentrations of Mg^{2+} (2.7 and 1.5 mM) [Jackson and Hunter, 1970; Yoshida et al., 1970]. These conditions could conceivably accommodate the apparent sensitivity of MetAP ribosome association to Mg^{2+} concentrations.

As a whole, these results suggest that MetAP1 is recruited from the cytosol to the 80S translational complex through an association with the 60S ribosome subunit (Fig. 4A). It is noteworthy that a channel exists for the nascent polypeptide in the 60S ribosome subunit [Morgan et al., 2000]. Thus, it is possible that MetAP1 is localized to the 60S subunit near the exit of the polypeptide channel, where it can cotranslationally remove Met_{init} from nascent polypeptides in a timely manner. The ribosomal subunit protein(s)/cytosolic association factors/rRNA that interact with MetAP1 remain to be determined.

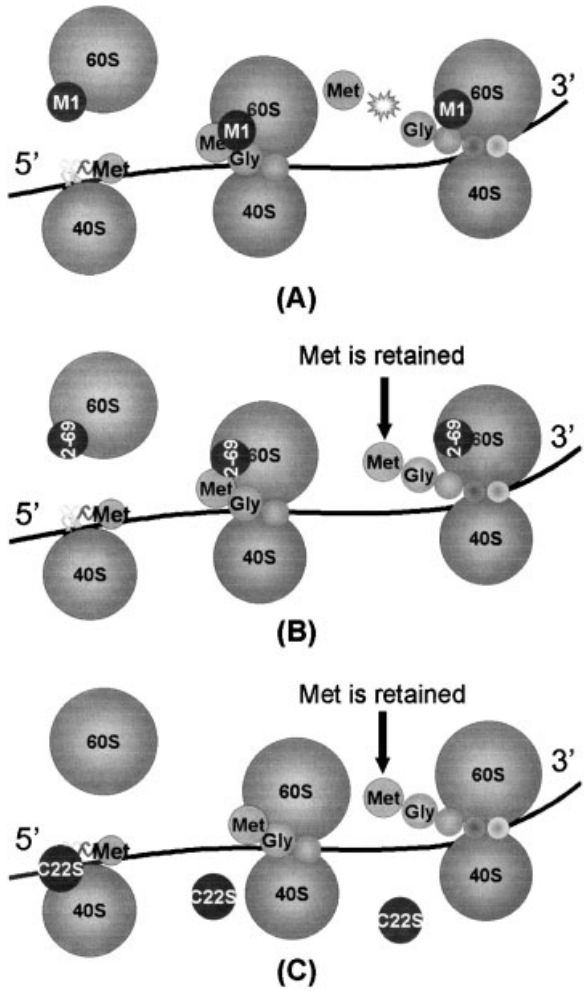

Fig. 4. Possible model of MetAP1 recruitment to the ribosomes. **A**: Under normal conditions, cytosolic MetAP1 (M1) associates with free 60S ribosomal subunits and is recruited to the 80S translational complex through this association. MetAP1 is then localized to the exit of the nascent polypeptide and correctly aligned so that Met_{init} is efficiently removed. **B**: Removal of the zinc finger domain does not affect the association of MetAP1 (Δ2-69) with the 60S subunit and subsequent recruitment to the 80S translational complex. The average alignment of MetAP1 (Δ2-69) on the ribosomes, however, is disrupted which leads to decreased processing efficiency. **C**: Single point mutations in the first or second zinc finger motif disrupt association of MetAP1 with the 60S ribosome subunit so that the majority of MetAP1 is found on the 40S ribosome subunit (e.g., C22S). A high percentage of the zinc finger point mutants may then dissociate as the 40S and 60S subunits come together to form the 80S translational complex. The decrease of MetAP1 (C22S) associated with the ribosomes then leads to a decrease in processing efficiency.

Role of the Zinc Finger Domain in MetAP1 Ribosome Association

Interestingly, deletion of the entire zinc finger domain up to residue 69 does not significantly alter the ribosome profile distribution of Met-

AP1 (Δ2-69) compared to wild-type MetAP1. This indicates that MetAP1 (Δ2-69) contains ribosome interaction sites downstream of its zinc finger domain.

The finding that MetAP1 (Δ2-69) is still ribosome-associated may also suggest that the zinc finger domain is not involved in MetAP1 ribosome association. However, although MetAP1 (Δ2-69) and wild-type MetAP1 have similar catalytic activity in vitro [Zuo et al., 1995] and are expressed at comparable levels in *map1*Δ, the processing efficiency of MetAP1 (Δ2-69) in vivo ($\sim 35\%$) is less than full length wild-type MetAP1 (100%) against the same target protein. This indicates that the interaction of MetAP1 (Δ2-69) with the ribosomes in the absence of the zinc finger domain is not the same as full-length MetAP1.

It is important to note that ribosome profiles demonstrate the association of a protein with the ribosomes, but give no information about the nature of the association. It is therefore possible that the orientation of truncated MetAP1 (Δ2-69) on the ribosomes differs from full length wild-type MetAP1 and leads to dysfunction in vivo (Fig. 4B). Thus, although MetAP1 (Δ2-69) is recruited to the ribosomes, it may have, on average, an incorrect orientation on the 60S ribosome subunit. This could compromise the function of MetAP1 as a population of MetAP1 (Δ2-69) associated with the ribosomes does not have a functional alignment that favors the cotranslational cleavage of Met_{init}. Taken as a whole, these data suggest that the zinc finger domain maybe involved in properly aligning MetAP1, once it has associated with the ribosomes.

It is curious that single point mutations in either zinc finger motif disrupt the association of MetAP1 with the ribosomes, while deletion of the entire zinc finger domain does not cause MetAP1 to dissociate from the ribosomes. It is possible that point mutations disrupt the secondary structure of the zinc finger motifs in such a way that interferes with or alters the ribosome interaction sites downstream of the zinc finger domain. Thus, it appears that although either C22S or H62R are able to associate with the 40S subunit, they are unable to remain associated with the ribosomes, once the 40S and 60S subunits come together to form the 80S translational complex (Fig. 4C).

The observation that C22S and H62R are found primarily in the cytosolic fractions and have decreased processing efficiency compared to wild-type MetAP1 further suggests that recruitment to the ribosomes is also required for normal MetAP1 function in vivo. Thus, at least two parameters are likely involved in the normal function of MetAP1 in vivo: (1) recruitment to the ribosomes and (2) correct functional align-ment on the ribosomes. The conclusion that ribosome association increases the processing efficiency of MetAP1 in vivo, however, is complicated by the finding that the processing efficiencies of GST (S2G) by MetAP1 (Δ2-69), C22S, and H62R are comparable in this study ($\sim 31-35\%$). This was unexpected as truncated MetAP1 (Δ2-69) complements the *map1*Δ slow-growth phenotype (doubling time 4.0 ± 0.3 h) to a much greater degree than C22S or H62R (doubling time 6 ± 0.3 h and 5.5 ± 0.3 h, respectively) [Zuo et al., 1995]. Considering that the level of processing efficiency is reflected in the growth rate in yeast [Chen et al., in press], MetAP1 (Δ2-69) was expected to have a greater processing efficiency against GST (S2G). It is possible that: (1) GST (S2G) does not accurately reflect the differences in processing efficiencies of other proteins between Δ2-69, C22S, and H62R. Considering that only a single protein was examined, we favor this explanation. It is more likely that MetAP1 (Δ2-69) processes proteins that affect cell growth and require Met_{init} removal for normal intracellular function more effectively than C22S and H62R. This is consistent with the finding that overexpression of wild-type MetAP2 in *map1*Δ increases both the growth rate and the overall Met_{init} processing efficency of *map1*Δ [Li and Chang, 1995; Chen et al., 2002]; (2) MetAP1 (Δ2-69) is ribosome-associated because these conditions are not stringent enough; thus, these results represent a steady state in vitro that may be more dynamic in the cell; (3) More extremely, the mere association of MetAP1 with the ribosomes is enough to increase the growth rate of *map1*Δ expressing Δ2-69 compared to C22S or H62R.

In summary, this study provides the first direct evidence of MetAP1 ribosome association and demonstrates that the zinc finger domain is important for normal MetAP1 function in vivo. Results from this study also suggest that the zinc finger domain maintains the proper functional alignment of MetAP1 on the ribosomes.

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