Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7714–7718, August 1995
Biochemistry

Eukaryotic methionyl aminopeptidases: Two classes of cobalt-dependent enzymes

(cotranslational processing/initiation of protein synthesis/evolution)

STUART M. ARFIN*, RICHARD L. KENDALL*†, LINDA HALL*, LARRY H. WEAVER‡, ALBERT E. STEWART*‖, BRIAN W. MATTHEWS‡, AND RALPH A. BRADSHAW*¶

*Department of Biological Chemistry, College of Medicine, University of California, Irvine, CA 92717-1700; and ‡Institute of Molecular Biology, Howard Hughes Medical Institute and Department of Physics, University of Oregon, Eugene, OR 97403

Contributed by Brian W. Matthews, May 24, 1995

ABSTRACT **Using partial amino acid sequence data derived from porcine methionyl aminopeptidase (MetAP; methionine aminopeptidase, peptidase M; EC 3.4.11.18), a full-length clone of the homologous human enzyme has been obtained. The cDNA sequence contains 2569 nt with a single open reading frame corresponding to a protein of 478 amino acids. The C-terminal portion representing the catalytic domain shows limited identity with MetAP sequences from various prokaryotes and yeast, while the N terminus is rich in charged amino acids, including extended strings of basic and acidic residues. These highly polar stretches likely result in the spuriously high observed molecular mass (67 kDa). This cDNA sequence is highly similar to a rat protein, termed p67, which was identified as an inhibitor of phosphorylation of initiation factor eIF2α and was previously predicted to be a metallopeptidase based on limited sequence homology. Model building established that human MetAP (p67) could be readily accommodated into the *Escherichia coli* MetAP structure and that the Co^{2+} ligands were fully preserved. However, human MetAP was found to be much more similar to a yeast open reading frame that differed markedly from the previously reported yeast MetAP. A similar partial sequence from *Methanothermus fervidus* suggests that this p67-like sequence is also found in prokaryotes. These findings suggest that there are two cobalt-dependent MetAP families, presently composed of the prokaryote and yeast sequences (and represented by the *E. coli* structure) (type I), on the one hand, and by human MetAP, the yeast open reading frame, and the partial prokaryotic sequence (type II), on the other.**

Protein synthesis is generally initiated with methionine (in eukaryotes) or *N*-formylmethionine (in prokaryotes). However, the products of this process are subject to one or more N-terminal modification reactions, occurring co- or posttranslationally, that often result in mature proteins with a variety of free (or N^{α}-acylated) N-terminal amino acid residues other than methionine (1–3). Although the physiological importance of these modifications is incompletely understood, they likely operate in functional regulation, intracellular targeting, and protein turnover.

Removal of the initiator methionine of nascent polypeptide chains (when it occurs) is catalyzed by a methionyl aminopeptidase (MetAP; methionine aminopeptidase, peptidase M; EC 3.4.11.18). In bacteria, the N-formyl group of the initiator *N*-formylmethionine is first removed by an appropriate deformylase. Analyses of the protein data base (2, 4–6) and studies of the processing of mutant proteins (6–8) or proteins with systematically modified N-terminal sequences (9, 10) suggest that MetAPs from diverse sources have quite similar substrate specificities. In addition, purified enzymes from bacteria (11, 12), yeast (13), and porcine liver (14) display similar substrate specificities that are largely consistent with that predicted from these analyses. In all cases, the penultimate amino acid is apparently the primary determinant of specificity; cleavage occurs when the penultimate residue is small (G,A,S,C,T,P,V) and not when it is large (D,E,N,Q,K,H,R, L,I,M,F,Y,W).

The genes encoding the *Escherichia coli* (11), *Salmonella typhimurium* (12), *Bacillus subtilis* (15), and *Saccharomyces cerevisiae* (16) MetAPs have been cloned and sequenced. The prokaryotic enzymes are similar in size (≈30 kDa), whereas the yeast enzyme contains a 125-amino acid N-terminal extension with sequences resembling zinc-finger domains. The remainder of the sequence shares ≈40% amino acid identity with the bacterial enzymes. All MetAPs studied to date are activated by Co^{2+} and appear to require this ion for activity. The x-ray structure of *E. coli* MetAP has been determined to 2.4-Å resolution (17). The enzyme contains a distinctive fold that displays internal two-fold symmetry of the first and second halves of the sequence and appears to represent another class of proteolytic enzyme. Protein data base searches using both sequence- and structure-based profiles identified several other proteins likely to share the same fold (18). These proteins included creatinase (19), aminopeptidase P (20, 21), proline dipeptidase (22, 23), and p67 (24, 25), a eukaryotic initiation factor 2 (eIF2)-associated protein proposed to regulate protein synthesis by protecting the eIF2 α subunit from phosphorylation by eIF2 kinases.

With a panel of substrates that reflect the precise specificity associated with cotranslational processing, a porcine liver MetAP was isolated and characterized (14). The enzyme has an apparent M_r of 67,000, is monomeric, and requires Co^{2+} ions for activity. Using partial sequence data obtained from endoprotease Lys-C fragments, we report here the sequence of a full-length clone of the homologous human protein. The deduced amino acid sequence of the human protein is highly similar to the rat p67 protein (eIF2-associated protein). Model building suggests that substantial segments of the C-terminal domain are fully compatible with the three-dimensional structure of *E. coli* MetAP, confirming the prediction (18) that p67 may function as a metal-dependent aminopeptidase. The subsequent identification of a yeast open reading frame (ORF) coding for a putative protein 55% identical to human MetAP (p67) (although lacking most of the N-terminal extension) (26)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MetAP, methionine aminopeptidase; RACE, rapid amplification of 5′ cDNA ends; ORF, open reading frame.

†Present address: Department of Biochemistry, Merck Research Laboratories, Rahway, NJ 07065–0900.
§Present address: Imperial Cancer Research Fund, Structural Molecular Biology Unit, Department of Crystallography, Birkbeck College, Malet Street, London WCIE 7HX, United Kingdom.
¶To whom reprint requests should be addressed.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. U29607).

supports the view that this protein (and the yeast homolog) represent a second class of MetAPs that are distantly related to the previously described prokaryote/yeast enzymes and share specificity, metal requirements, and general backbone fold (in the catalytic domain).

MATERIALS AND METHODS

Determination of Partial Amino Acid Sequences. For peptide analyses, 200 μg of pure porcine MetAP was incubated with endoprotease Lys-C (Boehringer Mannheim) for 6 hr at 37°C. The resulting peptides were separated by HPLC on an Aquapore C_8 column. Peptides were eluted using a gradient from 10 to 70% (vol/vol) acetonitrile in 0.1% trifluoroacetic acid (Applied Biosystems model 140A). The column effluent was monitored at 212 nm with an ISCO model CV^4 capillary electrophoresis detector. Selected peaks were analyzed by automated liquid pulse sequencing on an Applied Biosystems model 477A sequencer equipped with an online phenylthiohydantoin analyzer. The N-terminal sequences of four peptides were determined: peptide 1, TVPIV; peptide 2, GGEATRMEEGEVYAIETFG; peptide 3, NLXDLGIVDPYPPL; and peptide 4, KALDQASEEIWNDFREAAEAXXQV (see also Fig. 1).

cDNA Cloning of Porcine and Human MetAP. Pairs of sense and antisense degenerate oligonucleotides corresponding to regions of peptides 2 and 4 were synthesized by using phosphoramidite chemistry on an Applied Biosystems model 391A instrument and used in all combinations for PCR amplification of oligo(dT)-primed first-strand cDNA synthesized from template porcine liver RNA. The combination of the sense oligonucleotide from peptide 2, 5′-ATG GAR GAR GGN GAR GTN TT-3′ and the antisense oligonucleotide 5′-GG RTA NGG RTC NCA DAT NCC-3′ (where D is T, G, or A) from peptide 4 yielded a 273-bp product that was purified and ligated into the pCRII vector (TA cloning kit, Invitrogen). The translated nucleotide sequence contained the C-terminal sequence of peptide 2 and the N-terminal sequence of peptide 4. The PCR fragment was ^{32}P-labeled by the random-primer method and used to probe a porcine liver λgt10 cDNA library (Clontech). Forty positive clones were obtained from screening 1.5×10^6 recombinant phage. A 2.1-kb clone, p71A1, was subsequently used to screen a cDNA library prepared from human fetal brain poly(A)-selected RNA in the λZAP II vector (Stratagene). Sequencing of the human cDNA clone (pMB) with the largest insert (≈2.4 kb) revealed that this clone did not contain an in-frame stop codon upstream of the first AUG. An internal primer and a library-specific primer were then used to amplify the 5′ ends of all positive clones. None of these clones proved to be longer at their 5′ ends than clone pMB. Therefore, rapid amplification of 5′ cDNA ends (RACE) (27) was done with first-strand cDNA prepared by reverse transcription of human HepG cell RNA as template to determine the 5′ sequences. The product was cloned into pBluescript II. Five independent clones were isolated and sequenced.

MODEL BUILDING

Using the known crystal structure of *E. coli* MetAP (17) as a reference, the model-building program FRODO (28) was used to determine whether the sequence of the human enzyme, aligned as in Fig. 2, was compatible with a similar three-dimensional fold. The backbone of the protein was kept constant, and the side chains corresponding to the human MetAP sequence were built to have standard geometry and, so far as possible, to avoid steric interference with neighboring parts of the structure. Particular attention was given to substitutions involving glycine and proline because these residues can be associated with specific conformations of the polypeptide backbone.

RESULTS

Human MetAP cDNA and Predicted Protein Sequence. Using the highly selective, nonprocessive activity attributed to the N-terminal processing of the initiator methionine residue on nascent chains during eukaryotic protein synthesis, a 67-kDa protein was isolated and characterized from porcine liver (14). The enzyme is monomeric and is strongly stimulated by cobalt ions, similar to other MetAPs that have been identified (11–13). Treatment of 200 μg of porcine MetAP with endoprotease Lys-C, followed by fractionation of the digest by HPLC and automated peptide sequencing, yielded four peptide sequences (see *Materials and Methods* and Fig. 1). These data were used to generate oligonucleotides for PCR, yielding a fragment that was suitable for the identification of a partial porcine clone. This sequence was used to identify a nearly complete human cDNA sequence (pMB). The 5′ sequence was generated by the RACE procedure (27).

Fig. 1 shows the nucleotide sequence of the putative full-length cDNA of human MetAP, reconstituted from the RACE and pMB sequences, and the predicted amino acid sequence. The cDNA sequence contains a total of 2569 nt and a single ORF extending from nt 1 to nt 1439. There is no poly(A) tail, although several possible polyadenylylation signals can be found in the 3′ untranslated region. The 5′ end of the cDNA lies within a (GC)-rich region, suggesting that it is a GpC island and probably the start of transcription. Although there is no in-frame stop codon preceding the first ATG, the nucleotide sequence surrounding it agrees well with the consensus sequence proposed for translation initiation in higher eukaryotes (29).

The ORF encodes a protein of 478 amino acids with a calculated molecular mass of 52,832 Da. The N-terminal portion of MetAP is rich in charged amino acids, 53 of the first 110 residues consisting of arginine, lysine, glutamic, and aspartic acids. These residues include runs of 9 (residues 36–44) and 10 (residues 98–107) basic amino acids and a run of 11 (residues 77–87) charged amino acids, of which 9 are acidic and 2 are basic. Comparison of the deduced amino acid sequence to that of the MetAPs from *E. coli* (11), *S. typhimurium* (12), *B. subtilis* (15), and *S. cerevisiae* (13) shows that the yeast and human enzymes have N-terminal extensions of 125 and 160 amino acids, respectively, relative to the prokaryotic forms (Fig. 2). There is no obvious sequence similarity between the two extensions. In the portion of the protein shared by human MetAP and the yeast and prokaryotic enzymes, there is 20–24% sequence identity. In contrast, the yeast enzyme is 40% identical to the bacterial enzymes in this same region.

The substantial sequence differences, as well as the overall organizational differences distinguishing the human MetAP enzyme and the characterized MetAPs, raised the question of whether the human MetAP was truly homologous to this group, particularly because the extent of sequence similarity was marginal. Accordingly, the human MetAP sequence was fitted to the *E. coli* structure to ascertain the extent to which it could (or could not) be accommodated.

Significantly, none of the deletions or insertions occur within α-helices or β-sheets. Rather, they are located near residues 58, 84, 117, 163, 169, 176, and 220 of the *E. coli* sequence, all of which are within irregular or loop regions on the surface of the *E. coli* MetAP molecule, where insertions and deletions might be anticipated. Model building also suggests that the changes in sequence that occur within α-helices and β-sheets can be accommodated with only minor perturbations of the packing of these secondary-structure elements. This hypothesis is illustrated by the region surrounding resi-

FIG. 1. Nucleotide sequence and deduced amino acid sequence of human MetAP (p67). Differences in the predicted rat liver p67 amino acid sequence are indicated below the human MetAP sequence. Sequences corresponding to the endoprotease Lys C-derived peptides are underlined.

dues 240–250 (*E. coli* MetAP numbering) (Fig. 3). In this stereo projection, the model-built residues are shown with open bonds, and the side chains of the *E. coli* MetAP model are drawn in black circles. The backbone is retained exactly as in the *E. coli* structure. Clearly, the backbone conformation at Asp-242 is perfectly set up to accept the proline at this position in the human sequence. Gly-244 of the *E. coli* sequence can be replaced with the larger cysteine in the human molecule without introducing serious steric clashes. Cys-245 in the *E. coli* enzyme is largely buried, but it appears that a lysine at this position, as in the human, could extend toward the surface, so that the ε-amino group might reach the solvent. Thr-241 is on the surface, so that the arginine at this position can extend into the solvent.

Additional confirmation that human MetAP (p67) and the *E. coli* enzyme adopt similar conformations is provided by the retention of the Co^{2+} ligands (defined in the *E. coli* structure) (17). As shown in Fig. 2, these motifs are fully conserved for the human and *E. coli* MetAPs and, with the exception of a single Gln → Glu replacement, for the yeast MetAP as well. The requirement of the human enzyme for Co^{2+} for activity has been established (14).

Comparison to Other Sequences. A search of the GenBank data base revealed that human MetAP shared 92% sequence

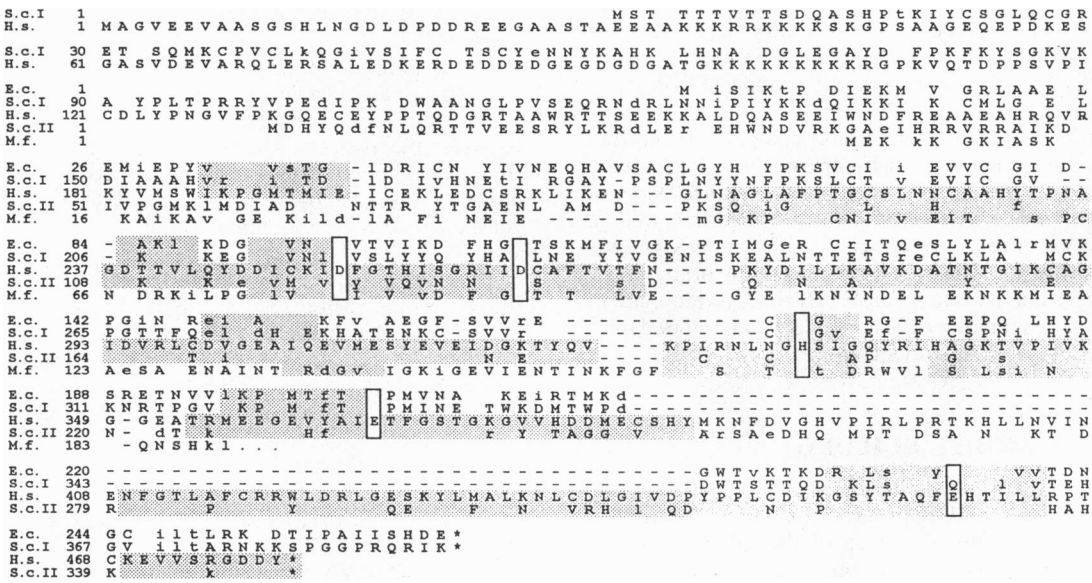

FIG. 2. Primary sequence alignment of *E. coli* MetAP (E.c.), *S. cerevisiae* MetAP-1 (S.c. I), human MetAP (H.s.), *S. cerevisiae* hypothetical protein YBL091c (S.c. II), and a partial ORF from *Methanothermus fervidus* (M.f.). Blank spaces indicate that the amino acid residues are identical to those in the human sequence; dashes indicate deletions introduced to maximize alignments. The three dots at the end of the *M. fervidus* sequence indicate that it is derived from a partial clone and that the sequence extends beyond this point for an unknown distance. Lowercase letters denote conservative substitutions. Residues that are cobalt ligands in the structure of *E. coli* MetAP and are conserved in the other sequences are boxed. Regions of substantial homology between human MetAP and one or more of the other sequences are indicated by shading.

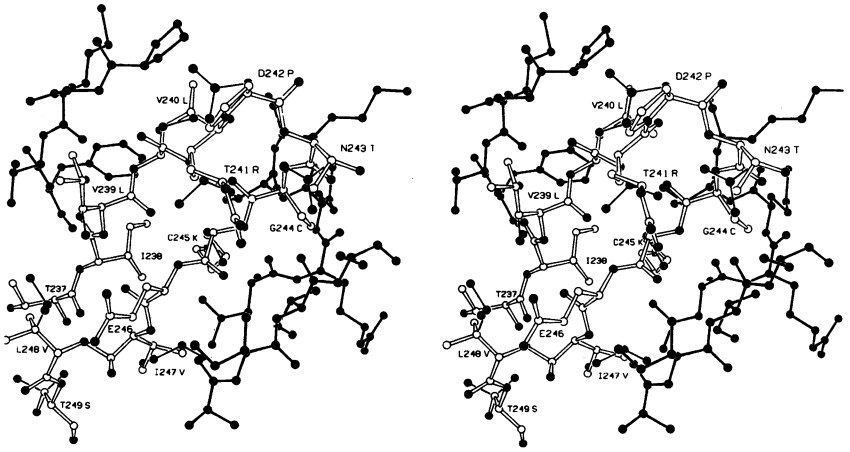

FIG. 3. Stereo drawing illustrating the compatibility of the amino acid sequence of human MetAP with the three-dimensional structure of *E. coli* MetAP (17). The figure shows the results of model building in which residues Thr-461–Ser-473 of the human sequence (open bonds) were model-built onto the backbone of the *E. coli* structure (solid bonds). [This is the region in which the alignment of the human vs. *E. coli* MetAP sequence has been revised relative to that previously proposed for the rat P67 sequence (18) (see text).] Numbering corresponds to the *E. coli* sequence. D242P, for example, indicates that Asp-242 in the *E. coli* sequence is replaced by a proline in the human enzyme. ○, Carbon atoms; ●, oxygen atoms. To show the overall context within the three-dimensional *E. coli* MetAP structure, neighboring residues are shown in solid black and are unlabeled.

identity at the amino acid level with that previously reported for a rat liver eIF2-associated protein designated p67 (Fig. 1). p67 copurifies with eIF2 and is postulated to regulate protein synthesis by protecting the eIF2 α subunit against phosphorylation. The amino acid differences occur almost exclusively in the N-terminal region and at the C-terminal end. The observed N-terminal differences are probably due to species variations (rat vs. human). However, at the C terminus the p67 sequence can be transformed into the MetAP sequence by a +1 frameshifting over 18 codons. The insertion of an extra nucleotide at position 1412 in the MetAP sequence is compensated for by a single-base deletion 50 nt further downstream. Interestingly, this revised sequence markedly improved alignment with prokaryotic MetAPs in this region (Fig. 2). Identity of the human MetAP and rat liver p67 proteins (excepting species variations) was, in fact, presaged by the recent suggestion that p67 belongs to a small family of structurally related proteins that includes the MetAPs of *E. coli, S. typhimurium, B. subtilis*, and *S. cerevisiae* and the prediction that it would function as a metal-dependent aminopeptidase (18).

DISCUSSION

Determination of the human MetAP sequence based on peptides derived from the homogeneous enzyme (14) established the predicted link between eIF2-associated protein (p67) and MetAP (18). The identity of these proteins is further established by their unusual behavior on SDS gels. Both proteins show apparent M_r values of 67,000 but have calculated molecular weights in the low 50,000 range. Datta *et al.* (30) attributed this difference to intracellular O-linked glycosylation (31), but because these modifications introduce only a single monosaccharide per site, a rather substantial number of sites would be required to account for the difference. A more likely explanation is the observation that elongated tracts of acidic and basic residues can produce anomalously high M_r values on SDS gels (32, 33). Yeast Fpr3 (also called yFKBP-47) is a protein of M_r 46,800 (calculated) but shows an apparent mass of 65 kDa on SDS gels; this yeast protein is characterized by similar stretches of basic and acidic residues (32).

Although it is not unusual for a protein to display more than one activity, the association of MetAP with the initiation complex rather than the ribosome was unexpected. Cotranslational excision of the initiator methionine occurs when the nascent chain is still quite short (20–40 residues) and presumably in conjunction with N^{α}-acetylation (3). Perhaps this apparent association with the initiation complex represents the mechanism by which MetAP in higher eukaryotes is recruited to the ribosome to insure stoichiometric interactions that lead to the highly selective (and apparently quantitative) removal of methionine from susceptible sequences (9).

Why the human enzyme has a significantly different organization than that of the yeast MetAP, including two major inserts (one of 63 amino acids) in the catalytic domain (as indicated by the prokaryotic forms) and an entirely different N-terminal extension, is also unclear. The N-terminal sequence of the yeast MetAP has two putative zinc-finger domains that may allow interactions with nucleic acid (13). The excision of these domains has little effect on the catalytic properties of the enzyme, but the construct expressing this truncated form is significantly less effective in rescuing the slow-growth phenotype of a MetAP mutant as compared with the wild-type MetAP (34). In contrast, the human enzyme lacks the consensus zinc-finger sequences and instead contains the extended runs of basic and acidic residues. The function of these sequences is unknown.

These differences are sufficiently great to suggest that human MetAP may be a member of a distinct subfamily of MetAPs, distantly related to but distinct from the prokaryotic and yeast MetAPs. Strong support for this view is provided by the recent report of a yeast ORF that is distinctly more closely related to human MetAP than is either the yeast or prokaryotic MetAPs (26). This protein (designated S.c. II in Fig. 2) con-

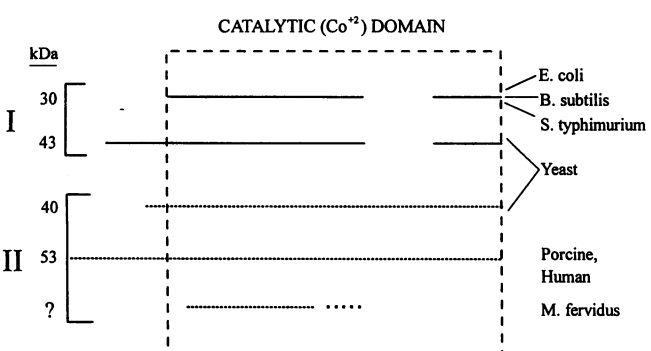

FIG. 4. Schematic representation of the two proposed subfamilies of MetAP.

Table 1. Amino acid identities in the catalytic domains of MetAPs

		Type I		Type II		
		E.c.	*S.c.* I	*H.s.*	*S.c.* II	*M.f.*
Type I	*E.c.*	—	104/244	50/234	48/234	41/189
	S.c. I	43	—	49/231	40/231	34/189
Type II	*H.s.*	21	21	—	185/314	52/189
	S.c. II	20	17	59	—	43/189
	M.f.	21	18	28	23	—

Entries above the diagonal refer to the number of identical residues per total number of residues compared based on the alignments in Fig. 2. Entries below the diagonal refer to percentage identities. The remainder of the *M. fervidus* sequence is unavailable. *E.c.*, *E. coli* MetAP; *S.c.* I, *S. cerevisiae* MetAP; *H.s.*, human MetAP; *S.c.* II, *S. cerevisiae* hypothetical protein YBLO91c; *M.f.*, partial ORF from *M. fervidus*.

tains the same organization as human MetAP with respect to the inserts in the catalytic domains but lacks a long N-terminal extension [it possesses an N-terminal segment of 25 amino acids beyond the catalytic domain that is unrelated to either human MetAP or the initially identified yeast MetAP (designated S.c. I in Fig. 2)]. This yeast protein is ≈80% identical to the human MetAP in the catalytic domain, including complete conservation of the putative Co^{2+} ligands. Thus, yeast apparently expresses both a prokaryotic-type and a human-type MetAP. We propose to designate the former as MetAP-I and to designate the latter as MetAP-II. In addition, a partial ORF from *M. fervidus*, previously identified as a potential homolog of rat p67 (18) (and, hence, MetAP-II type) (see Fig. 2), shows significant similarity to the human and yeast MetAP-II proteins (40% and 34% similarity, respectively, over the known segment), suggesting that the MetAP-II family extends across the eukaryotic–prokaryotic boundary. It is, therefore, possible that a human MetAP-I-type enzyme also exists.

The general structure of the two MetAP families is shown in Fig. 4, and their relationships in size and similarity are summarized in Table 1. Although all are likely Co^{2+} requiring (this remains to be shown for the *S.c.* II and *M. fervidus* sequences), the MetAP-I family differs from the MetAP-II family by significant structural features in the catalytic domain. However, more striking are the various N-terminal structures. Prokaryotic MetAPs of both families lack an N-terminal domain, as does yeast MetAP-II. Yeast MetAP-I and human MetAP-II have such extensions and are clearly distinct in this regard. Because the substrate specificity of both families is similar (and in keeping with that expected for cotranslational processing), it raises the interesting question whether one or both function in this regard and why two such families are necessary, at least in the case of yeast. At the least, these observations suggest that cotranslational processing is a more complex process than was originally thought.

We thank Mrs. Gale D. Trudeau for her expert assistance in the preparation of this manuscript. This work was supported by U.S. Public Health Service Grants DK32465 (S.M.A. and R.A.B.) and GM20066 (B.W.M.).

1. Brown, J. L. & Roberts, K. W. (1976) *J. Biol. Chem.* **251,** 1009–1014.
2. Driessen, H. P. C., deJong, W. W., Tesser, G. I. & Bloemendahl, H. (1985) *CRC Crit. Rev. Biochem.* **18,** 281–325.
3. Arfin, S. M. & Bradshaw, R. A. (1988) *Biochemistry* **27,** 7979–7984.
4. Persson, B., Flinta, C., von Heijne, G. & Jornvall, H. (1985) *Eur. J. Biochem.* **152,** 524–528.
5. Boissel, J.-P., Kasper, T. J., Shah, S. C., Malone, J. I. & Bunn, H. F. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8448–8452.
6. Sherman, F., Stewart, J. W. & Tsunasawa, S. (1985) *BioEssays* **3,** 27–31.
7. Tsunasawa, S., Stewart, J. W. & Sherman, F. (1985) *J. Biol. Chem.* **260,** 5382–5391.
8. Moerschell, R. P., Hosokawa, Y., Tsunasawa, S. & Sherman, F. (1990) *J. Biol. Chem.* **265,** 19638–19643.
9. Huang, S., Elliott, R. C., Liu, P. S., Koduri, R. K., Weickmann, J. L., Lee, J. H., Blair, L. C., Ghosh-Dastidar, P., Bradshaw, R. A., Bryan, K. M., Einarson, B., Kendall, R. L., Kolacz, K. H. & Saito, K. (1987) *Biochemistry* **26,** 8242–8246.
10. Boissel, J.-P., Kasper, T. J. & Bunn, H. F. (1988) *J. Biol. Chem.* **263,** 8443–8449.
11. Ben-Bassat, A., Bauer, K., Chang, S.-Y., Myambo, K., Boosman, A. & Chang, S. (1987) *J. Bacteriol.* **169,** 751–757.
12. Miller, C. G., Strauch, K. L., Kukral, A. M., Miller, J. L., Wingfield, P. T., Mazzei, G. J., Werlen, R. C., Garber, P. & Movva, N. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2718–2722.
13. Chang, Y.-H., Teichert, U. & Smith, J. A. (1990) *J. Biol. Chem.* **265,** 19892–19897.
14. Kendall, R. L. & Bradshaw, R. A. (1992) *J. Biol. Chem.* **267,** 20667–20673.
15. Nakamura, K., Nakamura, A., Takamatsu, H., Yoshikawa, H. & Yamane, K. (1990) *J. Biochem. (Tokyo)* **107,** 603–607.
16. Chang, Y.-H., Teichert, U. & Smith, J. A. (1992) *J. Biol. Chem.* **267,** 8007–8011.
17. Roderick, S. L. & Matthews, B. W. (1993) *Biochemistry* **32,** 3907–3912.
18. Bazan, J. F., Weaver, L. H., Roderick, S. L., Huber, R. & Matthews, B. W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2473–2477.
19. Hoeffken, H. W., Knof, S. H., Bartlett, P. A., Huber, R., Moellering, H. & Schumacher, G. (1988) *J. Mol. Biol.* **204,** 417–433.
20. Henrich, B., Monnerjahn, U. & Plapp, R. (1990) *J. Bacteriol.* **172,** 4641–4651.
21. Butler, M. J., Bergeron, A., Soostmeyer, G., Zimny, T. & Malek, L. T. (1993) *Gene* **123,** 115–119.
22. Yoshimoto, T., Tone, H., Honda, T., Osatomi, K., Kobayashi, R. & Tsuru, D. (1989) *J. Biochem. (Tokyo)* **105,** 412–416.
23. Endo, F., Tanoue, A., Nakai, H., Hata, A., Indo, Y., Titani, K. & Matsuda, I. (1989) *J. Biol. Chem.* **264,** 4476–4481.
24. Wu, S., Gupta, S., Chatterjee, N., Hileman, R. E., Kinzy, T. G., Danslow, N. D., Merrick, W. C., Chakrabarti, D., Osterman, J. C. & Gupta, N. K. (1993) *J. Biol. Chem.* **268,** 10796–10801.
25. Chakraborty, A., Saha, D., Bose, A., Chatterjee, M. & Gupta, N. K. (1994) *Biochemistry* **33,** 6700–6706.
26. Feldmann, H., Aigle, M., Aljinovic, G., André, B., Baclet, M. C., *et al.* (1994) *EMBO J.* **13,** 5795–5809.
27. Frohmann, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8998–9002.
28. Jones, T. A. (1982) in *Crystallographic Computing,* ed. Sayre, D. (Oxford Univ. Press, Oxford), pp. 307–317.
29. Kozak, M. (1991) *J. Biol. Chem.* **266,** 19867–19870.
30. Datta, B., Ray, M. K., Chakrabarti, D., Wylie, D. E. & Gupta, N. K. (1989) *J. Biol. Chem.* **264,** 20620–20624.
31. Hart, G. W. (1992) *Curr. Opin. Cell Biol.* **4,** 1017–1023.
32. Fuller, R. S., Brake, A. & Thorner, J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1434–1438.
33. Benton, B. M., Zang, J.-H. & Thorner, J. (1994) *J. Cell Biol.* **127,** 623–639.
34. Zuo, S., Guo, Q., Ling, C. & Chang, Y.-H. (1995) *Mol. Gen. Genet.* **246,** 247–253.