CMLS, Cell. Mol. Life Sci. 61 (2004) 1455–1474
1420-682X/04/121455-20
DOI 10.1007/s00018-004-3466-8

CMLS Cellular and Molecular Life Sciences

Review

Protein N-terminal methionine excision

C. Giglione, A. Boularot and T. Meinnel*

Protein Maturation Group, Institut des Sciences du Végétal, UPR2355, Centre National de la Recherche Scientifique, Bâtiment 23, 1 avenue de la Terrasse, 91198 Gif-sur-Yvette cedex (France), Fax: +33 1 69 82 36 07, e-mail: meinnel@isv.cnrs-gif.fr

Received 19 December 2003; received after revision 21 January 2004; accepted 4 February 2004

Abstract. N-terminal methionine excision (NME) is the major proteolytic pathway responsible for the diversity of N-terminal amino acids in proteins. Dedicated NME components have been identified in all organisms, in all compartments in which protein synthesis occurs: cytoplasm, plastids and mitochondria. Recent studies have revealed that NME is regulated at various levels and plays an important role in controlling protein turnover. NME is essential in Eubacteria and lower eukaryotes and is the target of many natural and synthetic inhibitors. Such inhibitors have considerable potential for use in the treatment of various human diseases, from cancer to bacterial and parasitic infections.

Key words. Peptide deformylase; aminopeptidase; drug; metal cation; metalloprotease; human pathogenesis; therapeutics.

Introduction: what is NME and what does it do?

The translation of messenger RNAs (mRNAs) to generate proteins generally starts at an AUG codon. The translation machinery interprets AUG codons as 'methionine' (Met) in all genetic codes described to date. GUG, CUG or even AUU (only one instance) codons may also be used to initiate translation in Eubacteria and account for ~12% of all translation start sites in *Escherichia coli*. Non-AUG translation initiation codons – although much rarer – have been described in eukaryotes. Nevertheless, it is generally believed that only the specialized transfer RNA (tRNA) for translation initiation – $tRNA^{fMet}$ in Eubacteria and $tRNAi^{Met}$ in other organisms – can be used to start translation. Given the known rules of amino acid identity for both tRNAs [1], the first residue incorporated in all newly synthesized proteins should be Met [2, 3]. However, recent data have suggested possible exceptions to this rule, in particular situations involving (i) viral mRNA translation after infection and inhibition of the usual translation initiation pathway or (ii) translation of small open reading frames. The resulting proteins start with Val, Leu or even Gln [4–6]. Similarly, although artificial, functional protein synthesis systems in which translation is initiated with such residues or others have already been described in bacteria [7, 8]. We need to investigate further these proteins that do not start with Met in eukaryotes if we are to understand their physiological relevance [9, 10].

Although Met is the first amino acid of newly synthesized proteins, it is usually removed from mature proteins to leave a nonbulky N-terminal residue such as Ala, Cys, Gly, Pro, Ser, Thr or Val [11]. The process by which Met is removed from proteins is called N-terminal Met excision (NME). Between 55 and 70% of proteins are subject to NME, depending on the organism and compartment considered. Plastid NME provides the best-documented example to date of NME in a given proteome (~80 proteins) as most (70%) of the natural N-termini of proteins produced in the plastid have been

* Corresponding author.

Table 1. N-terminal maturation of gene products synthesized in plastids.

N-terminal modifications and cleavages[a]	Protein number and corresponding gene names	(%) data available
(i) N-formyl retaining	**6**	**11**
f-Met-Arg	*psaJ*	2
f-Met-Asn	*atpH*	2
f-Met-Glu	*psbM psbT*	4
f-Met-Leu	*psbI*	2
f-Met-Thr	*ycf9*	2
(ii) *N*-formyl cleavages only	**14**	**25**
f⇓Met-Asn	*psbG*	2
f⇓Met-Asp	*petN, rpl23*	4
f⇓Met-Glu	*psaB*	2
f⇓Met-Ile	*ndhA, petG, psaI, rpl14*	7
f⇓Met-Leu	*rpl16, rpoB*	4
f⇓Met-Lys	*rpl36, rps15*	4
f⇓Met-Val	*rpoA, rps16*	4
(iii) *N*-formylMet cleavages	**30**	**55**
fMet⇓Ala	*atpA, psbH, psbJ, rpl2, rpl32, rpl33, rpoC2, rps11, rps14*	16
fMet⇓Gly	*petD, psbB, rpl22, rps3, rps8*	9
fMet⇓Pro	*clpP, rps12*	4
fMet⇓Ser	*atpB, atpE, petB, psaC, psbE, rps4, rps7*	13
fMet⇓Thr	*psbA, psbD, psbF, psbL, rpl20, rps2, rps19*	13
(iv) Extensive cleavages	**5**	**9**
fMet-Ser⇓Pro	*rbcL*	2
Δ(fMet1-Ser18)⇓Gly	*atpI*	2
Δ(fMet1-Ala35)⇓Tyr	*petA*	2
Δ(fMet1-Glu14)⇓Thr	*psbC*	2
Δ(fMet1-Gly22)⇓Lys	*psbk*	2

[a] ⇓ is the location of the cleavage observed. The corresponding references are not given due to space constraints. References and more complete data are available at http://www.isv.cnrs-gif.fr/tm/maturation/images/chloro.html [40]. If different N-termini have been identified for the same protein, we chose those originating from spinach or pea, the two plants for which data are most abundant. f is N-formyl.

determined (table 1). NME appears to be conserved from bacteria to eukaryotes, and the rules governing whether Met is removed or retained are well known and are thought to be similar in all organisms (reviewed in [2]). Whether the Met is removed depends mostly on the nature and bulkiness of the side chain of the second amino acid. NME requires at least Met aminopeptidase (MAP; EC 3.4.11.18) activity. This activity is the principal determinant of Met excision or nonexcision, as shown in various organisms (i) in vitro [12–14], (ii) in silico (table 1 and [15, 16]) and (iii) in vivo, based on analysis of overexpressed reporter proteins [17–20]. Eubacterial and organellar nascent proteins begin with an N-formylated Met. Peptide deformylase (PDF; EC 3.5.1.88) activity is required to unmask the first Met, facilitating the action of MAP [21, 22].

NME is an irreversible cotranslational mechanism, completed before the nascent polypeptide chains are fully synthesized [23]. It starts early in translation, as soon as the first residues emerge from the ribosomal exit tunnel, before the initiation of protein folding [24–26]. This early start to this process has resulted in the use of small polypeptides, often tripeptides, in in vitro assays of the enzyme activities associated with NME, these small polypeptides being considered to model nascent protein chains more accurately than longer peptides. In many full-length proteins, the N-terminus is likely to be unavailable for NME due to (i) protein location [27] or folding and/or (ii) burying of the N-terminal residues within the three-dimensional (3D) structure.

Pioneering work on NME began as early as the 1960s [28–31]. The number of publications in this field began to increase in the late 1980s (early reviewed in [2, 23, 32]), but has exploded in the last couple of years, probably because NME is now recognized to be an excellent therapeutic target and recent genomics data have provided further insight into this pathway. Topical reviews have recently appeared on the potential use of components of this pathway as therapeutic targets [33–37]. This review summarizes recent data in the field of NME, the huge effort made to develop specific inhibitors of this process and the therapeutic use of these inhibitors, and integrates the latest evidence for NME regulation in eukaryotes, not yet considered as a whole. Finally, we discuss the role of NME in controlling protein half-life.

NME, its main catalysts and their occurrence in living organisms

NME in proteomes

NME is generally not considered in most protein data libraries, but is nonetheless taken into account and well annotated at http://ca.expasy.org/ A bioinformatics tool for including NME annotation in protein databanks is available [38]. This tool could be particularly useful for mass spectrometry analysis coupled with Web-based proteome searches. Proteomics methods are now beginning to take the difficult issue of NME and N-terminal modifications into account [39]. Plant organelles give the most complete and 'unbiased' source of data on NME (table 1). Significant data on plant mitochondrial NME were compiled [40], with regularly updated data available at http://www.isv.cnrs-gif.fr/tm/maturation/images/table mito.html Both data sources are fully consistent with the combined action of PDFs and MAPs in the organelles. In *Escherichia coli*, more than 850 experimentally determined N-terminal sequences are available (http:// bmb. med.miami.edu/EcoGene/EcoWeb/CESSPages/VerifiedProts.htm), and these data are also consistent with the combined action of PDFs and MAPs. Most of the data for *E. coli* were obtained by overproducing proteins, which frequently leads to partial saturation of the NME machinery and somewhat biased results (see below). To our knowledge, data on cytoplasmic NME in higher eukaryotes have not been updated since the work carried out in von Heijne's laboratory [16]. Yeast mitochondria appear to be exceptional in that N-terminal sequencing data for these organelles suggests the absence of NME (see [35]).

Involvement of PDF in eubacteria and in the organelles of eukaryotes

General features

Newly synthesized bacterial and organellar proteins are tagged with a one-carbon unit called a formyl group. This group is added by the N-formylation of Met-tRNA^{fMet} by a specialized enzyme, Met-tRNA^{fMet} transformylase (FMT), N-blocking the Met already bound to the tRNA as a result. This small modification of Met-tRNA^{fMet} seems to increase the rate of protein synthesis, as it plays an important role in the recognition of major translation factors such as IF2 and EFTu [41]. The formyl group is later cleaved from almost all nascent peptides by peptide deformylase (PDF), a monomeric protein with a relative molecular weight (Mr) of 22 ± 3 kDa. PDF activity was originally described in the late 1960s [29, 42, 43], but its unusually high level of instability rendered further studies impossible for 25 years. Interest in this enzyme increased again following the cloning and identification of the *E. coli def* (formerly *fms*) gene as the gene encoding PDF [2, 44–46]. Thus, studies of the biology of bacterial PDFs and their structural and chemical characterization have been carried out over the last 10 years, greatly increasing our understanding (for reviews see [35, 40, 47]).

Organellar PDFs in most organisms

Large-scale in silico searches provided the first clues to possible PDF sequences in eukaryotic genomes [48]. These findings suggested that PDFs may occur in the nuclear genomes of most eukaryotes, including diblastic and triblastic metazoans. Plants have two PDFs (PDF1A and PDF1B), whereas only one, PDF1A, has been found in humans and other animals [40, 49]. All eukaryotic PDFs have a cleavable N-terminal extension composed of 50–100 residues [50]. Both classes of PDF in plants have been fully characterized [51, 52]. PDFs cleave most, but not all, N-formyl groups from very small substrates, dipeptides in some cases (see table 1 and discussion of possible reasons in [40, 52]).

PDF1As are targeted to the mitochondria [50, 51, 53]. In plants, PDF1B is targeted to both mitochondria and plastids. PDF1Bs have also been identified in several eukaryotic protists, including *Chlamydomonas reinhardtii* and apicomplexan parasites. *Plasmodium falciparum* PDF1B has been characterized [54]. PDF1A and PDF1B are thought to function to similar extents in plant mitochondria. Although both PDF1A and PDF1B are targeted to the plastid in vitro and in heterologous systems [40, 55], PDF1B is by far the most prevalent PDF in this organelle. This conclusion is supported by the sensitivities of the two plant *PDF1A* and *PDF1B* KO mutant lines to the specific plastid PDF inhibitor actinonin [56]. As the catalytic efficiency of PDF1B is only about one-seventh that of PDF1A, any PDF1A entering the plastid is likely to contribute only ~1% of the total deformylation potency of the organelle. In plant PDF mutants devoid of PDF1B, PDF1A is significantly induced, by a factor of 3–5, leading to incomplete but nonnegligible (~5% the wild-type level) plastid deformylation [56].

Mitochondrial PDFs have been identified in many eukaryotic protists, including *Dictyostelium discoideum,* but not in fungi (Ascomycota). This lack of PDF enzymes in fungi is probably due to the absence from fungal mitochondria of the major components of complex I proteins and of other important proteins encoded by the mitochondrial genomes of higher eukaryotes. Finally, no PDF homolog has been identified in the genome of the nematode *Caenorhabditis elegans,* whereas such homologs have been identified in insect genomes. This is surprising, given the high similarity between mitochondrial genomes in animals and the near-identity of the 13 mitochondrion-encoded proteins. We cannot yet exclude the possibility that another protease can compensate for PDF.

MAP1s vs MAP2

Ubiquitous character of MAPs

All organisms have MAPs. Two types of MAPs have been described to date [57]: MAP2s occur in Archaea [58] and in the cytoplasm of eukaryotes [59], whereas MAP1s have been found in Eubacteria (MAP1Bs), and in the cytoplasm (MAP1A) and organelles of eukaryotes (MAP1D) [49, 50]. A MAP2 gene has been identified in the extremely small genome (encoding ~2000 proteins) of the so-called 'amitochondrial' eukaryotic parasite *Encephalitozoon cuniculi* [60]. MAP1Ds are found in the same set of organisms as organellar PDFs and have been found in neither fungi nor nematodes [50].

N-terminal extensions of MAPs and the POEP activity of animal MAP2s

All eukaryotic MAPs have 50–100 residue-long N-terminal extensions, whereas no such extensions are present on prokaryotic MAPs. Organellar MAP1s are thought to have a cleavable N-terminal presequence, targeting the catalytic domain to the correct cell compartment [50]. The processed enzymes probably resemble bacterial MAP1s. Cytoplasmic MAP1As also have an extension, including a conserved zinc finger motif. Although not involved in catalytic activity, this additional domain is not removed from the mature form and is essential for the cellular function of MAP1As. It has been suggested that this N-domain may facilitate interaction with ribosomes [61, 62]. The N-terminal extensions of eukaryotic MAP2s are not required for aminopeptidase activity [63], and this domain was indeed found to be disorganized in the crystal structure of human MAP2 [64]. This extension is thought to be responsible for POEP activity – the protection of animal eIF2α from stress-induced phosphorylation. This protective activity is mediated by both an N-terminal lysine-rich domain I and an O-glycosylation site in animal MAP2s [65–67]. As at least one of these two domains is absent from the MAP2s of plants and fungi, these enzymes are unlikely to display POEP activity. Finally, although similar to MAP2s in terms of peptide chain length and monomeric behavior (i.e. ~380 vs 450 residues), eukaryotic cytoplasmic MAP1s have very different electrophoretic and chromatographic mobilities. MAP1As have a M_r of 38 ± 5 kDa [68, 69], whereas MAP2s have a M_r of 67 ± 5 kDa [70, 71]. This effect is not due to glycosylation of MAP2.

Evidence for various levels of control of NME activity

NME has long been considered a constitutive pathway. Interestingly, recent data suggest that NME is tightly regulated throughout development, tumorigenesis and in response to abiotic stress. Both transcriptional and post-transcriptional mechanisms have been demonstrated to be involved. We review here the current state of knowledge concerning this subject.

Evidence for stress-induced NME regulation in bacteria

In *E. coli*, neither PDF nor MAP1B was initially thought to be controlled [unpublished data and 2, 72]. For instance, the *def-fmt* operon is insensitive to metabolic control, which couples the concentration of components of the biosynthetic machinery to growth rate [2]. In other words, the higher the rate of translation, the higher the rate of production of components of the translation machinery. As the *def-fmt* operon is not subject to metabolic control, when the NME system in bacteria is saturated by protein overproduction or has to compete with other cotranslational systems such as protein targeting, NME is incomplete, resulting in the production of incorrectly processed or unprocessed proteins [27, 73–75]. The recurrent issue of incomplete NME induced by the overproduction of an exogenous protein may have major consequences in pharmaceutical applications involving the production of human components in bacteria, for example [76–80]. For such applications, the proteins produced must be similar to those naturally produced in human cells. As NME is a cotranslational process, it cannot be carried out after enzyme purification. Partial NME can usually be overcome by increasing both the expression of the gene concerned and the intracellular concentration of the two NME pathway components and/or by inhibiting the N-formylation of $tRNA^{fMet}$ [81–83].

However, proteomic analysis recently showed that *def* gene expression in *Streptococcus pyogenes* is decreased by a factor of three in conditions of exposure to fluoride stress [84]. A number of observations in other *Streptococcus* strains indicate that the *def* promoter responds to environmental stress [85, 86]. Transcription of *def* is induced by a factor of 5 under iron starvation in *Pseudomonas aeruginosa* [87]. Similarly, upon iron depletion during the stationary phase in *Staphylococcus aureus*, an increase in the amount of formylated peptides was observed [88].

Organellar NME regulation

The mouse PDF gene is strongly down- and then upregulated during muscle regeneration after injury, a process resembling myogenesis during embryogenic development [89]. In plants, in which PDF1B is routed to both organelles, an epigenetic increase in PDF1A levels was observed in a *pdf1b* genetic background. In contrast, PDF1B fully compensates for the lack of PDF1A in a *pdf1a* knockout (KO) mutant. Finally, promoter identification by activation tagging revealed that PDF1A is a

stress-responsive gene in the plant *Arabidopsis thaliana*. This is fully consistent with data showing that the major plant PDF, PDF1B, is inactivated by stress-induced reactive oxygen species, whereas PDF1A is not [51]. Finally, PDF1B and MAP1D expression is strongly induced in developing flowers and leaves [49].

Cytoplasmic NME regulation

MAP2 seems to accumulate in a cell- and tissue-specific manner. During embryonic development in *Drosophila*, MAP2 transcription is strongly regulated and induced in some tissues [90]. In mammalian cell cultures, MAP2 is abundant in mammary and prostate epithelial cells but present in only modest quantities in endothelial cells [91]. Moreover, MAP2 production appears to be strongly induced in growth factor-treated [92], germinal [93] and malignant [94] cells in mammals. Partners of MAP2 have recently been identified: a prion-like protein and a DNA replication factor in yeast (http://mips.gsf.de/genre/proj/yeast/searchEntryAction.do?text=YBL091c, complex number 532) and the calcium-binding protein S100A4 in mammals [92]. Interestingly, S100A4 regulates cell proliferation and tumor metastasis by interacting with various proteins, including p53 [95], which is involved in cell cycle control and apoptosis. Although the binding of S100A4 to MAP2 does not affect the NME activity of MAP2, it was suggested that S100A4 might be involved in the regulation of MAP2 activity by modifying its intracellular distribution [96]. In higher plants, it has also been suggested that MAP2 is developmentally regulated, with upregulation in the embryo [97]. A similar pattern of MAP2 regulation has been observed during asexual development of the eukaryotic parasite *Toxoplasma gondii* [98].

Few data are available concerning MAP1A. During the life cycle of *Plasmodium falciparum*, a cluster of genes encoding MAP1 and various ubiquitination pathway components have been shown to be coregulated [99]. Similarly, MAP1 is differentially expressed during the cell cycle of dinoflagellates [100]. There is a lack of data concerning MAP1A expression in the tissues of higher eukaryotes, although some data are available for some plant organs [49].

Structural and evolutionary data for the various NME components

Although PDF and MAPs belong to different enzyme families, they share several characteristics: (i) both bind an essential but labile metal cation, the nature of which may vary, (ii) both have well-defined 3D structures, (iii) both can be subdivided into smaller groups and (iv) both are closely related to larger protease families.

Essential role of metal cations in NME catalysis

Peptide deformylases

PDFs bind a metal cation, which is generally thought to be Fe^{2+} [101, 102]. This cation is very unstable as it has a low binding affinity – the dissociation constant (K_d) is in the micromolar range [103] – and is highly sensitive to oxidation by reactive oxygen species [102, 104]. For example, if Fe^{2+} is oxidized to generate Fe^{3+}, the protein is inactivated and Fe^{3+} readily dissociates. Oxidation of the cysteine side-chain ligand to generate cysteine-sulfinic acid often occurs [104, 105]. The physiological relevance of this conversion is unknown, but it has frequently been observed and in some cases shown to have a functional role in other enzyme systems [106]. Zinc – a natural contaminant of glassware – binds irreversibly to the enzyme in the place of iron, and acts as an inhibitor. Zinc-coordinated enzymes are usually less active than the corresponding iron forms (by a factor of 100–1000) but display similar substrate specificity [107–110]. The high level of instability of PDF makes it difficult to preserve the activity of this enzyme, but several protocols aiming to do this have been described (reviewed in [35]). Some PDF enzymes have never been described in their most active state, probably for this very reason. Ni^{2+}, Co^{2+} and Mn^{2+} ions have been described as possible replacements for ferrous cations, making it possible to preserve full activity [102, 108, 111]. Plant PDF1As present an exception to the iron-binding rule, as these enzymes seem to bind zinc preferentially and are highly stable in vitro [51]. The bacterial *Leptospira interrogans* PDF1B enzyme may be similar in this respect, as its catalytic performance with zinc coordination is good [112].

MAPs

Like PDFs, MAPs are metalloproteases that are strongly activated in vitro by several divalent cations such as Fe^{2+}, Co^{2+}, Mn^{2+} and Zn^{2+}. However, the nature of the metal cation present in physiological situations remains unclear for MAP1s [113–115] and MAP2s [63]. Early attempts at MAP purification and activity assays involved the systematic addition of millimolar amounts of cobalt salts to preserve enzyme activity [13, 69, 116, 117]. Hence, the crystal structures of both MAPs revealed the presence of two cobalt cations per polypeptide [64, 118]. The first cobalt-binding site in EcMAP1 has a K_d of 0.2 µM, whereas the second has a much higher constant: 2.5 mM [119]. However, prokaryotic MAPs (such as EcMAP1 and *Pyrococcus furiosis* MAP2) – like most PDFs – are now thought to be mononuclear iron enzymes [120, 121]. In contrast, human MAP2 produced in *E. coli* seems to be a manganese enzyme [122]. Thus, it is now thought that the nature of the bound cation may vary: (i) there may be a mixture of cations, depending on the metal ions available in a given set of physiological conditions or (ii) the nature of the bound cation may depend on the MAP con-

sidered (see discussion in [34]). The situation may be complicated as many bacteria have several *map* genes [123], and the enzymes they encode are unlikely to display similar metal preferences. PDFs are likely to display similar behavior, as they can be activated by different metal cations in vitro.

Structural data

MAP1 and MAP2 have a similar 3D folding pattern: the 'pita-bread'

MAPs belong to a large family of metalloenzymes, the 'pita-bread' family [34, 118, 124], also known as the 'clan MG' M24 protease family [125, 126]. The pita-bread fold of the enzymes of this family features a pseudo two-fold axis of symmetry, with the interface between the domains involved in catalysis and binding of the metal ion. The 3D structures of the enzymes of this family have overlapping 5-residue metal binding sites (1 His, 2 Asp and 2 Glu) and similar topology of the active site, including additional conserved His and Glu residues. This similarity of structure exists despite the low levels of amino acid sequence between the various members of the family [57, 64, 127]. The root mean square deviation value for 227 α-carbon pairs of EcMAP1 and *P. furiosis* MAP2 is only 1.4 Å, indicating similar folding [128]. The main structural difference between MAP1s and MAP2s is essentially an additional 60-residue α-helical domain within the C-domain of MAP2s.

The pita-bread family includes two other aminopeptidase subfamilies more closely related to MAPs: prolidase (peptidase D, imidodipeptidase or dipeptidase P; DPP; EC 3.4.13.9) and *E. coli* aminopeptidase P (APP; EC 3.4.11.9) 1 orthologs. Like human MAP2s, EcDPP and EcAPP1 are manganese cation-dependent proteases. DPP enzymes are ubiquitous, whereas no APP1 orthologs have been identified in Archaea. DPPs behave as dimers and APP1s oligomerize as homotetramers, with each monomer composed of 440–500 residues in both cases. EcAPP1 consists of two domains. The first 175 residues are involved in tetramerization, whereas the C-terminal 270 residues bear the catalytic domain featuring the typical pita-bread fold [129]. Unlike DPPs, which are very specific for dipeptides, both APP1s and MAPs cleave oligopeptides consisting of at least two amino acids [130–132]. Thus, APP1s and MAPs function similarly despite having different substrate specificities. APP1s preferentially interact with a number of rather large N-terminal amino acids containing Met [133], whereas MAP is restricted to Met only. Both APP1s and DPPs specifically interact with peptide substrates with a Pro in the second position, whereas MAPs preferentially interact with a large number of small amino acids containing Pro. Finally, both APP1s and MAPs have higher catalytic efficiencies with substrates that have large residues at position 3. This overlapping substrate specificity of these two types of enzyme accounts for the overproduction of human APP1 in *E. coli* being able to extend MAP action in vivo in the case of Met-Pro-polypeptides [134]. Nevertheless, it remains unclear whether APP1s are involved in NME in physiological conditions.

PDFs

Early investigations of PDFs showed poor conservation of the full-length amino acid sequences of the protein (~20–30%) [135, 136]. This low level of amino acid sequence conservation contrasts with the high degree of conservation of the 3D structures. The first high-resolution 3D structure analysis revealed that PDFs belong to the thermolysin-metzincin superfamily known as the 'clan MA and MB' metalloprotease family [125, 126]. All the enzymes belonging to this large family share a common superstructure, featuring a three-stranded β strand facing the catalytic metal cation and the HEXXH motif-containing α helix [137–139]. The nature of the third metal cation ligand of PDFs, the oxidation-sensitive Cys, defined PDF as the first member of a new subfamily, as thermolysins (clan MA) have a Glu and metzincins (clan MB) have a His. The structure of PDF is more similar to that of metzincins than to that of thermolysins, and PDFs preferentially use ferrous ions as the metal cation of the active site, whereas thermolysins-metzincins use zinc. This new subfamily, including PDFs, probably also includes the quorum-sensing protein S-ribosylhomocysteinase LuxS. This enzyme has a similar active site with identical metal ligands, uses iron as the native metal cation and has a glutamate side chain involved in water molecule activation [140–142]. It has also been suggested that the Cys residue undergoes side-chain oxidation, similar to that occurring in PDF.

A number of 3D structures for PDFs are now available, including four bacterial PDF1Bs and two PDF2s [105, 143, 144], and the PDF1B of the apicomplexan parasite *P. falciparum* [145]. Further 3D structures are likely to be released in the next few years (for instance, see [146]). Two types of active PDF (types 1 and 2; fig. 1) have been identified on the basis of structure and amino acid sequence [35, 147]. Only slight differences have been found between these two types of PDF, and these differences are not believed to have a significant impact on the active site crevasse. This similarity accounts for the similar substrate specificity of all PDFs. The main structural difference concerns the C-terminal domain, which is either floppy or folded as an α helix in type 1, and which is folded back as a supplementary β strand in type 2. Three short stretches of amino acids (motifs I, II, III or G, H, C) are perfectly conserved among all PDFs. These motifs build the whole active site. The G motif (GΦGΦAAXQ) is part of the first strand of the three-stranded β sheet, and this

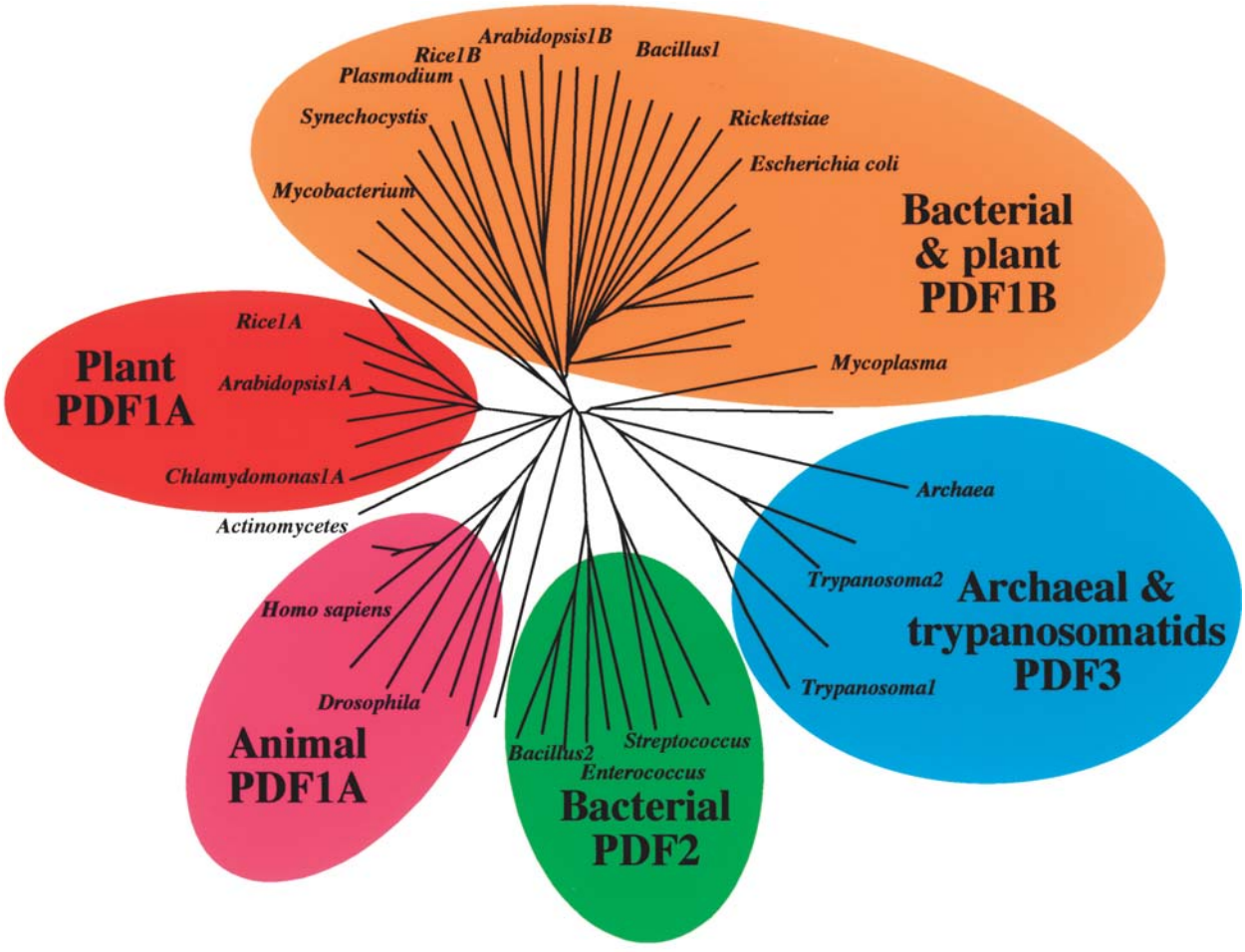

Figure 1. An updated phylogenic tree for PDFs. 50 PDF sequences were selected as representative of sequence diversity. These sequences were aligned, and the tree was constructed. The phylogenic tree was constructed with N-J Tree [246] and drawn with TreeView1.65 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html see [247]). The sequences were extracted from completely sequenced genomes or from genomes for which sequencing is almost complete. The three PDF types and classes are clustered.

structure is important as it aligns the peptide backbone of the substrate, creating an additional strand when the substrate is located in the active site. The Gln side chain is crucial for recognition of the formyl group. The C-motif (EGCXS) bears one of the three ligands of the metal cation, the Cys residue sensitive to oxidation. The Glu side chain makes a salt bridge with the conserved Asp of the H motif and a buried, conserved Arg. The H motif (QHEXDHLXG) bears the last two metal ligands, the two His residues. These two His residues are part of the HEXXH motif also present in the clan MA and MB families. The glutamate of the H motif is crucial for water activation. Note that in the enlarged family including LuxS, both the relative position of the conserved glutamate with respect to the two His residues and those of the G and C motifs with respect to the H motif are inverted. In all cases, the H motif is part of an α helix, whereas the C motif is located in a bend so that the catalytic metal is located at one extremity of the protein. The G-like motif is also involved in an antiparallel β sheet in LuxS.

Evolutionary boundaries

PDFs

PDF homologs have been detected in Archaea and protists such as Trypanosomatida [40, 148]. These homologs have been classified as a new group, type 3 PDFs (fig. 1), based on changes in conserved motifs. These changes suggest that type 3 PDFs are inactive in deformylation. However, animal PDF1As with substitutions in motifs G and C have been found to display PDF activity [50, 53, 149]. Plant PDF1Bs are related to bacterial type 1 PDFs, which are now also known as PDF1Bs (model EcPDF). Eukaryotic PDF1Bs are thought to result from the ancient endosymbiosis between a heterotrophic eukaryote and a photosynthetic cyanobacterium (fig. 1). Like most of the other genes encoding plastid proteins, the genes encoding PDF1B were probably transferred from the plastid to the nuclear genome [150]. PDF1As (animal type 1 PDFs) are encoded exclusively by genes in eukaryotic nuclear genomes and are transported to the mitochondria [49, 50]. PDF1As closely resemble the PDFs of actinobacte-

ria, a group of bacteria that naturally produce antibiotics, including *Streptomyces roseopallidus,* which produces actinonin (fig. 1). Thus, PDF1As may have arisen by horizontal transfer of genes between a *Streptomyces* sp. and a primitive eukaryote rather than by gene transfer from the mitochondrial genome [50].

MAP1s, MAP2s and their resemblance to aminopeptidase P

The significant sequence similarity between full-length EcMAP1 and the C-terminal domain of EcAPP1 (~23%) rapidly became evident (for an alignment, see fig. 4 in [2]), consistent with more recent 3D data [129]. In terms of amino acid sequence, DPPs and APP1s are more similar to each other than to MAPs (fig. 2). DPPs share significant similarity (~30%) with the C-terminal domain of APP1s, consistent with the occurrence in DPPs of a C-terminal pita-bread fold and an original fold of the N-terminal domain ensuring dimerization. APP1s are more similar to MAP1s than to MAP2s, whereas DPPs are more closely related to MAP2s than to MAP1s. MAP1s, MAP2s and APPs-DPPs are located on three different branches of the phylogenic tree (fig. 2). This suggests that the pita-bread family aminopeptidases were probably derived from a common ancestor but that they have since evolved separately.

MAP1Cs are found in mycoplasmas (mollicutes). MAP1As were clearly derived from MAP1Ds in eukaryotes, and MAP1Ds from MAP1Bs (fig. 2). Eukaryotic MAP1D, like PDF1As, strongly resembles an actinobacterial enzyme. We therefore cannot exclude the possibility that both PDF1As and MAP1Ds were derived from

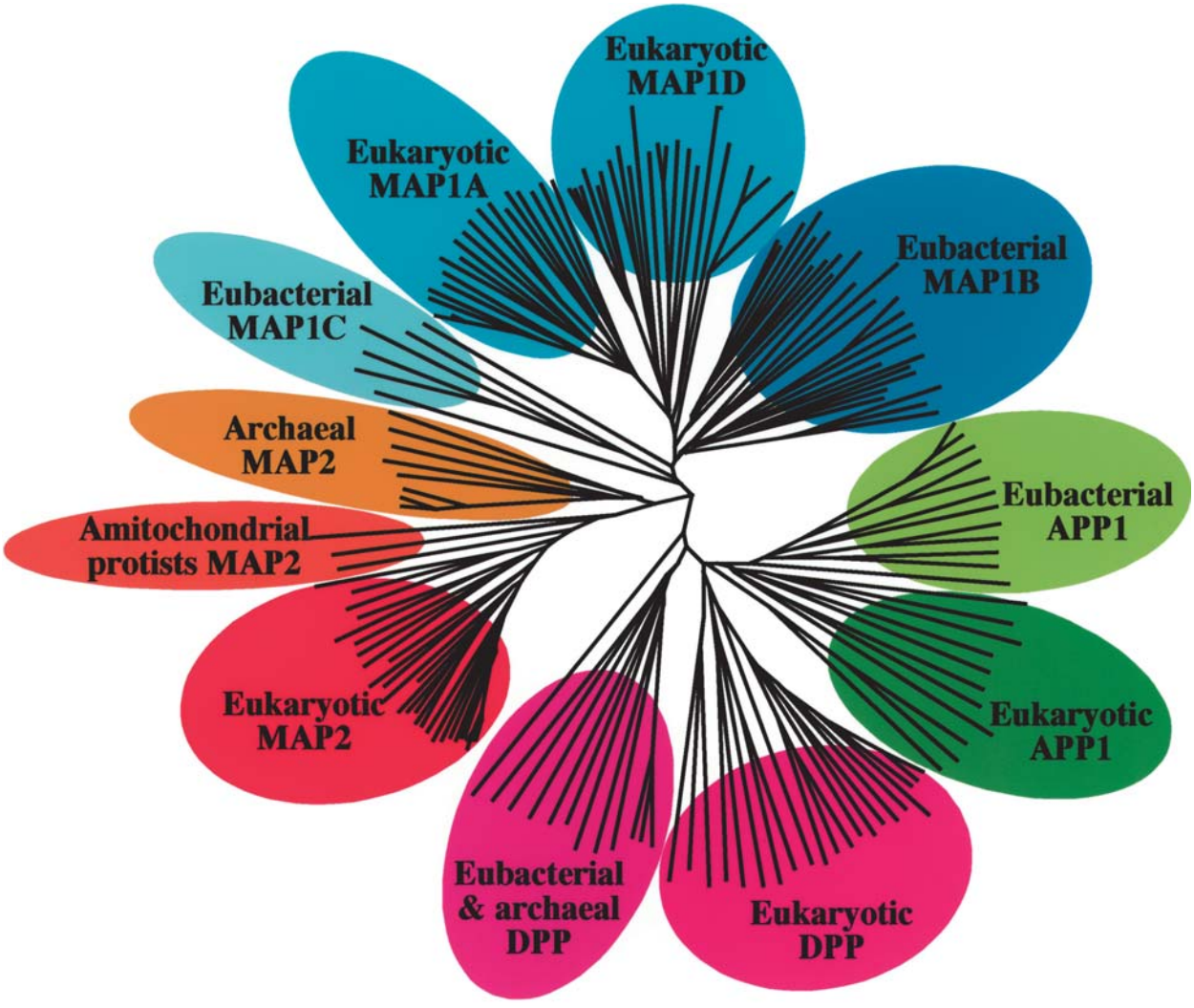

Figure 2. A phylogenic tree for MAPs, APPs and DPPs. 114 MAP, 23 APP1 and 34 DPP sequences were selected as representative of sequence diversity. These sequences were aligned, and the tree was constructed as in figure 1. N.B.: In constructing this tree, we deliberately excluded another class of ubiquitous APPs – APP2s – which belong to the APP-DPP subgroup but are only very distantly related to MAPs, with no significant similarity except for the five metal ligands. The subunits of APP2s are larger (600–800 amino acids), display significant similarity to DPPs and APP1s, and are also members of the M24 protease family.

an ancient horizontal transfer rather than from the migration of a mitochondria gene. Alternatively, the genome of an ancient actinobacterium may have captured part of the genome of an eukaryote, including both PDF and MAP1 genes. Eukaryotic MAP2s were clearly derived from archaeal MAPs (fig. 2). Amitochondrial protists such as *E. cuniculi* have no MAP1. Instead, they have only a MAP2, the sequence of which is intermediate between archaeal MAP2 and other eukaryotic MAP2 sequences (fig. 2). The nature and position of MAPs in the phylogenic tree (fig. 2) suggest that amitochondrial protists are ancient eukaryotes, originating before the integration of the bacterial precursor of mitochondria into a primitive eukaryotic cell. However, amitochondrial parasites such as *Giardia* are now thought to have evolved from a mitochondrion-containing organism [151, 152]. The lack of MAP1s in these organisms may therefore be due to gene deletion and genome compaction.

NME as a crucial pathway: therapeutic implications

NME is clearly essential for the following reasons: (i) PDF and MAP genes are both part of the minimal genome required for the viability of Eubacteria [153–155], and (ii) NME even occurs in organelles, even though organelle-encoded proteomes include fewer than 100 protein substrates [49]. The essential nature of NME and the fact that components of this pathway belong to diverse families of proteins that are the specific targets of natural, specific drugs (see also below) identify this process as a good candidate target for therapeutics.

PDFs

The *def* (formerly *fms*) gene encoding PDF is essential in Eubacteria [44, 156–160]. This may simply be because PDF unmasks the N-terminal Met, enabling MAP to act [21]. Strong evidence to support this hypothesis has been obtained for chloroplasts, in which PDF is also essential [40, 51, 55, 56].

As it has never been used as a target for antibiotics in human therapeutics and has therefore never been subject to selection pressure and the development of resistance, PDF has been proposed as a most attractive target for new antibiotics [35]. The identification of genes encoding PDFs resembling bacterial PDFs in the genomes of several pathogenic eukaryotic microorganisms such as apicomplexan (e.g. *P. falciparum*, the agent of malaria) and other similar agents (*Trypanosoma* and *Leishmania*) suggested that PDF inhibitors could be used for prophylaxis of the diseases caused by these agents [148, 161].

MAPs

The gene encoding MAP (*map*, formerly *pepM*) is essential in Eubacteria [154, 162, 163]. In yeast, these two genes are dispensable individually, but the disruption of both is lethal, indicating that cytoplasmic NME is essential in lower eukaryotes. In yeast, MAP1 seems to be the dominant isoform. Deletion of the *map1* gene results in a slow growth phenotype, whereas loss of the *map2* gene decreases the growth rate only slightly [164, 165]. These data suggest that MAP2 is redundant activity in fungi in contrast to MAP1. Recent studies have suggested that MAP1s and MAP2s may have different substrate efficiencies [166, 167]. In yeast, MAP1 was found to be the most efficient, whereas MAP2 increased its efficiency for some substrates only if overproduced. However, both MAPs were less efficient if the second residue in the substrate was Val, and MAP2 was less efficient than MAP1 if the second residue was Gly, Cys or Thr.

Nothing is yet known about whether cytoplasmic NME is essential in higher eukaryotes or about the relative contributions of the various enzymes. However, the few data available suggest that MAP2 plays a more important role in higher eukaryotes than in yeast. In *Drosophila*, *MAP2* gene disruption causes severe abnormal development phenotypes [90]. In malignant human cells, a reverse genetics strategy based on the use of MAP2 antisense oligonucleotides revealed that MAP2 inhibition induced apoptosis [94]. Moreover, blocking MAP2 activity with the specific drug fumagillin (see below) and its derivatives results in the selective inhibition of endothelial cell proliferation by arresting the cells in G1 phase. This effect seems to be mediated by a pathway depending on the pre-apoptotic transcription factors p53 and $p21^{WAF1}$ [168, 169]. Attempts have been made to explain the apparent discrepancy between fungi and animals in terms of the relative importance of MAP2. It has, for example, been suggested that MAP1 and MAP2, despite their common enzymatic functions, respond differently to metabolic conditions, such as Met concentration [165]. The rescue of the *map1* phenotype by MAP2 gene overexpression is prevented by adding Met to the culture medium. The addition of low millimolar amounts of Met has been shown to inhibit the activity of MAP2 [IC_{50} (concentration giving 50% inhibition) = 0.15 mM], but not MAP1A in vitro. Given the intracellular concentrations of Met in yeast (0.1–1 mM) and endothelial cells (5–30 μM), it has been suggested that MAP2 activity is fully inhibited in yeast when Met is not limiting, whereas this is not the case for MAP2 in animals. As yeast has an anabolic pathway for the synthesis of Met whereas animals do not, a coupling between NME and Met anabolism could be physiologically relevant. A link between MAP with Met salvage and metabolism has indeed been established in yeast [165]. Such a link may also exist in bacteria. We can speculate that, in conditions in which Met is limiting in yeast, MAP2 activity may in-

crease and Met may be recovered from the most fastidious substrates. MAP2 may also act posttranslationally, recovering Met from all polypeptide substrates with an N-Met in a favorable context – those generated by protein degradation – for example.

NME inhibitors and their therapeutic use

MAP inhibition by natural and synthetic drugs and its use in anticancer therapy

A diversity of MAP inhibitors

Fumagillin – a sesquiterpene diepoxide compound produced by the fungus *Aspergillus fumigatus* – and its numerous derivatives (compounds **1**–**5** in fig. 3) such as TNP-470 (AGM-1470) [170], ovalicin [171], *cis*-fumagillin [172] and fumagalone [173], potently, specifically and covalently bind and inactivate MAP2s [174, 175]. Covalent binding involves an active site His involved in coordination of the catalytic metal cation [64, 176]. Fumagillin has no physiological effect on EcMAP1, although it also binds covalently to the enzyme with low affinity [177]. The structural basis of the selectivity of fumagillin for MAP has been analyzed in depth [178, 179]. Fumagillin acts specifically on MAP2 NME and does not impair the interaction of MAP2 with eIF2α binding or POEP activity (see also above and [176]). Fumagillin has a number of deleterious effects on angiogenic cells in animals and amoeba (*Entamoeba* sp.) [64, 174, 175, 180–182]. Several other antiproliferative effects have been also observed in animal liver [183]. Consistent with the fact that the minimal genome of the microsporidian parasite *E. cuniculi* contains a gene for MAP2, but not for MAP1 (see above and [60, 184]), fumagillin is active against the class of obligate human intracellular protists that includes *Enterocytozoon* and *Encephalitozoon* spp. These parasites are responsible for various clinical syndromes in immunocompromised patients, and fumagillin is one of the few drugs active against such diseases. Finally, fumagillin also seems to inhibit the growth of other major human parasites responsible for malaria and leishmaniasis [181]. However, these organisms do have an expressed MAP1A gene [50]. This suggests either that fumagillin inhibits the MAP1 of these parasites more strongly or that, as for *map1* disruption in *Saccharomyces cerevisiae* [164, 185], fumagillin-induced MAP2 inactivation significantly re-

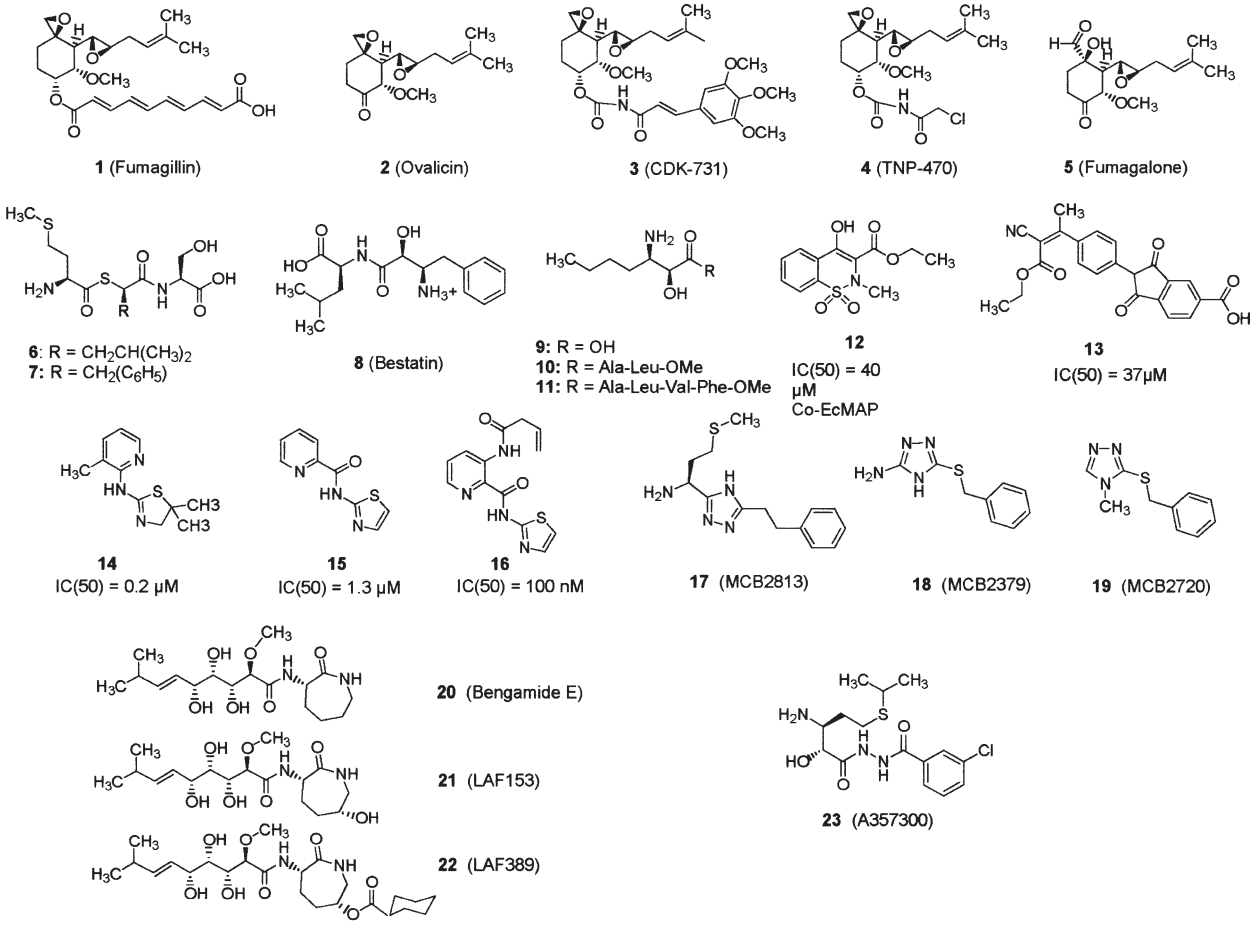

Figure 3. Chemical structures of drugs specifically targeting MAPs. The chemical structures of some compounds that inhibit MAPs are shown.

duces the growth rate of the protist, decreasing pathogenicity by generating an overall deficiency in MAP (i.e., MAP1 + MAP2) activity (see above).

Until recently, only weak inhibitors of MAP1s, such as fumagillin [177], phosphorus-based Met analogs [186] and bestatin-derived peptide inhibitors [187], had been described (compounds **6**–**13** in fig. 3). These inhibitors have K_d values > 5 μM and therefore cannot be used in therapeutics. New, more potent compounds have recently been discovered (**14**–**22** in fig. 3). They include triazole-based inhibitors (**17**–**19** in fig. 3), which bind well to *S. aureus* MAP1 [188] and Met mimetic compounds (**12**–**16** in fig. 3) [189]. In any case, drug potency appears to depend on the nature of the metal cation bound to the enzyme. This situation strongly resembles that for MAP2 inhibition by a similar series of inhibitors [122]. Finally, very potent inhibitors of both *E. coli* and *S. cerevisiae* MAP1 have also been described [190]: pyridine-2-carboxyl derivatives (**16** in fig. 3). The efficiency of these molecules for MAP2 inhibition is unknown. If they should prove to be inefficient inhibitors of MAP2, they could be used as the starting point for the design of new antibiotics as bacteria have only MAP1.

Bengamides are compounds (**20** in fig. 3) isolated from marine sponges that inhibit the growth of human tumors at concentrations in the low nanomolar range [191]. Synthetic, more soluble analogs of bengamides, such as **21**–**22** (fig. 3), have been produced [192, 193]. Recent proteomics analysis revealed that bengamides specifically bind and inhibit both MAP1 and MAP2 from humans, with similar K_d values for both enzymes [194]. They mimic a tripeptide substrate (Leu-Val-Asp), and the central hydroxyl groups coordinate the metal cation. However, although not specific for MAP2, bengamides have similar effects to fumagillin in vivo, with pronounced activity in endothelial cells. Bengamides are very promising molecules and could be used as the starting point for the design of improved drugs.

It is becoming clear that new inhibitors specific for one or other MAP will soon be discovered. Compound A-35700 – a new MAP2-specific, potent, reversible inhibitor with in vivo antitumor efficacy – is interesting as its chemical structure (**23** in fig. 3) was designed rationally based on that of bestatin [195]. Peptidyl hydroxamic acids were also described most recently as MAP inhibitors [196].

Toxicity and side effects of MAP2 inhibitors

Angiogenesis is the process by which new blood vessels are formed. It is essential for the growth of tumors and is important in many diseases, such as arthritis and psoriaris. The pharmacological inhibition of angiogenesis is now a major field in anticancer research [197, 198] following Judah Folkman's pioneering work [199]. Derivatives of fumagillin inhibit angiogenesis and tumor growth [182]; they are now undergoing clinical trials as promising anticancer drugs [200]. No resistance to TNP-470 was detected in trials of the use of this drug to inhibit angiogenesis [201], making TNP-470 a good candidate drug for use in future chemotherapy [202]. Phase II clinical trials have been performed, testing the effects of TNP470 against cancers, including Kaposi's sarcoma, retinoblastomas, hemangiomas, renal carcinoma, breast, brain and prostate cancers [200, 203]. TNP-470 has a short half-life in the serum and appears to be well tolerated by patients. Some side effects concerning wound healing and the female reproductive system have been reported. However, it should be borne in mind that fumagillin and derivatives have a long history of use in human therapeutics, over the last fifty years or so, in the treatment of parasitic diseases [204].

PDF inhibitors

PDFI as new antiinfection agents

The similarity between PDF and metalloproteases of the thermolysin-metzincin family led to the rational design and synthesis of the first PDFI. Analyses led to the prediction of the ideal structure for a potent PDFI: a di- or tripeptide with the formyl group replaced by a metal-chelating group, a hydroxamate for example [109, 205–209]. Actinonin, the most potent known natural PDFI, matches these requirements perfectly. Actinonin is a natural antibiotic produced by the Gram-positive actinobacterium *Streptomyces roseopallidus*. This pseudotripeptide hydroxamate is, however, not bioavailable and has therefore never been used as an antibiotic in clinical practice [157]. Two other natural molecules produced by *Streptomyces* sp. and targeting PDF activity have been described [210]. Actinonin has also been shown to block *P. falciparum* infection [211]. The crystal structures of the complex between actinonin and various representative members of eubacterial PDF (PDF1B and PDF2) were solved [147]. Such data now form the starting point for the structure-based design of PDFI.

Bioavailable derivatives of actinonin were recently described and shown to be active against *S. aureus* septicemia in mouse [212]. In the last 2 years, several companies have reported impressive progress on PDFI, with two molecules in particular – BB-83698 and VRC-4887 (also called NVP-PDF386) – showing great promise (see data reviewed in [213]). Research and preclinical studies on PDF inhibitors have shown them to be highly potent against bacteria responsible for serious upper tract respiratory diseases, including *Streptococcus pneumonia, Moraxella catarrhalis*, *Enterococcus* species and *Haemophilus influenzae*, and against strains resistant to current antibiotics. PDFI clearly constitute a new family of antibiotics. Vernalis has started human trials for BB-83698, and Vicuron recently began clinical trials in collaboration with Novartis for VRC-4887. Used at doses

toxic to bacteria, PDFI have not been found to be toxic to mammals in the short term. However, actinonin inhibits the proliferation of human cells if used at higher concentrations [214]. These data suggest that PDF is crucial for mitochondrial function but that the well-known low permeability of the mitochondrial membranes to antibiotics protects the organelle. This low permeability has made it possible to use several antibiotics with mitochondrial targets (macrolides and so on) successfully in human therapeutics. However, the fact that PDF can be inhibited in humans without any serious deleterious effects in the short term raises questions concerning the exact role of NME in the mitochondrion and its actual targets.

The issue of resistance to PDFI

At a recent meeting focusing on PDFI as a new class of antiinfection agents (see [215]), the possible emergence of resistance was identified as the major concern for PDFI use in human therapeutics. The rate of resistance development depends on the bacterium considered (for a review, see [47]). Resistance may involve mutations in the genes encoding PDF (*def*) or FMT (*fmt*), which adds the formyl group to the initiator Met-tRNA. Unlike *def*, *fmt* is not truly essential in some bacteria, such as *E. coli*. The inactivation of *fmt* drastically decreases the growth rate of the bacterium but circumvents the need for PDF, resulting in resistance [44, 46, 158, 216]. Indeed, *fmt*-, PDFI-resistant bacteria have such limited growth that they are not infectious [212, 216]. Thus, such bacteria are not thought to present a major problem. Mutations occur less frequently in *def* (10^{-8}–10^{-9}), than in *fmt* (10^{-6}–10^{-7}), so very few data are available concerning the molecular basis of resistance caused by *def* mutations, although such mutations appear to be the major concern with respect to PDFI resistance. In bacteria such as *S. pneumoniae*, in which *fmt* is an essential gene [158], a small number of substitutions in the product of the type 2 *defB* gene were recently reported [158, 217, 218]. These substitutions are restricted to amino acids outside the active site, in some cases at some distance from it, and the structural and biochemical bases of such resistance are not yet understood. Finally, it is unknown whether PDFI use can result in the induction of multi-drug resistance genes, although efflux from *acrAB-tolC* [219] is a natural cause of resistance to PDF inhibitors in Gram-negative bacteria. The minimal inhibitory concentration in vivo for actinonin is ~0.2 µg/ml in a *tolC* context and >400 µg/ml for the wild type [157].

N-terminal Met maintenance/removal as a signal controlling protein half-life

Until very recently, the physiological function of NME was unknown. It was first suggested some time ago that NME might be involved in controlling protein half-life [23]. Recent data have provided strong support for this hypothesis.

Bridging the gap between NME and protein half-life

The plastid has been used as a model system for investigating the role of NME, because the N-terminal modifications within plastids are diverse enough that this system can be used to mimic a bacterium, and almost a whole eukaryotic organism (table 1 and [40]). An integrated approach involving simplified proteome analysis of the higher plant *A. thaliana* as a model system has been used to elucidate the role of NME within the plant [40, 56]. This study dealt with the role of NME in the plant as a whole, and no assumptions were made concerning the role of NME. NME was inhibited by (i) disrupting the PDF gene and/or (ii) drug-specific PDF inactivation. Only one protein – the core photosystem II protein D2 – behaved abnormally, with a much shorter half-life, but no change in function. The decrease in steady-state level of this only protein induced further cascade mechanisms involving (i) destabilization of the major large protein complex to which it belongs, and (ii) further disorganization of the biogenesis of the whole compartment – the plastid – in which this protein is active. This effect was fully reproduced by site-directed mutations resulting in amino-acid substitutions altering the *N*-Met cleavage of the corresponding plastid target, provided that the N-Met was retained. Thus, (i) only very few proteins of a proteome are directly sensitive to NME inhibition and (ii) blocking NME destabilizes a normally stable key core protein of the major protein complex of the compartment.

Protein degradation and the role of the N-terminus in development

Regulated protein degradation is known to play a crucial role during development [220] and aging, often through programmed cell death in higher organisms, including higher plants [221, 222]. The ubiquitin-proteasome pathway is one of the major mechanisms of proteolysis in eukaryotes. Proteins destined for degradation are modified by a multi-ubiquitin chain anchored to the ε-NH_2 group of one or more lysine residues. However, various studies have shown that protein αN-termini may themselves also serve as ubiquitination signals [223]. The transcription factor MyoD, a DNA-binding protein required for muscle differentiation, and p21, a regulator of the cell division cycle, are degraded via this pathway, known as the N-terminus-dependent ubiquitination pathway [224, 225]. Recent data strongly suggest that the N-terminus-dependent ubiquitination pathway is used for the degradation of short-lived nuclear proteins [223]. The numerous protein substrates of this pathway are recognized by specific E3 ligases and degraded.

Motifs for protein degradation, the N-end rule and NME: is N-Met a destabilization signal?

The recognition of a protein substrate for degradation involves the recognition of specific structural motifs within the protein that do not necessary lead to the constitutive degradation of the protein. Such motifs may be unmasked by other, secondary mechanisms, such as DNA binding for the N-terminus of MyoD. The primary signal for degradation studied in most detail is the N-terminal residue itself. If the side chain of this residue is bulky, as for Arg or Leu for example, the protein is short lived. This rule of thumb is known as the N-end rule, and relates the in vivo half-life of a protein to the identity of its N-residue [226]. Unlike ubiquitination, this rule is universally conserved. Surprisingly, although Met is a bulky, and therefore theoretically destabilizing residue, studies with a single reporter protein have suggested that Met is a stabilizing residue in yeast, animals and plants [226–228]. However, further studies with other reporter proteins, such as GST in yeast [167], β-galactosidase in *E. coli* [19], β-glucuronidase [229] and the D2 component of photosystem II [56] in plants, have shown that the maintenance of the N-terminal Met residue destabilizes the protein. Thus, an N-terminal Met may act as a signal, targeting a protein for degradation. However, an N-terminal Met is not enough in itself to confer a short half-life on a given protein. Instead, other features of the protein close to the N-Met residue, such as phosphorylation of serine 2 of the protooncogene protein *c*-mos [230], may participate in fine-tuning protein degradation. In addition, recurring N-terminal motifs such as M-K-Φ-Φ-X-Φ are involved in cleavage by the bacterial ClpXP protease, an enzyme with orthologs in organelles [231].

Hypothetical targets and physiological mechanism for NME-sensitive regulation

The data obtained for plastid NME suggest that downregulation of the process may be involved in the degradation of key subsets of proteins, not only in the plastid but also in the other two NME-sensitive proteomes: those encoded by the mitochondrial and nuclear genomes. Other data suggest that regulation of cytoplasmic and mitochondrial NME is an important mechanism in the life of the cell. As in the plastid, identification of the most sensitive, destabilized targets in both the mitochondrion and cytoplasm should provide important insight into the physiology of development and aging.

Mitochondrial targets

Mitochondrial protein production is a major factor in apoptosis, which involves cytochrome *c* release and protein degradation [232]. Several signals may induce the process, including development, oxidative, abiotic or biotic stress [233, 234]. Mitochondrial NME is likely to be one such signal. A comprehensive study of the predicted N-termini of mitochondrial proteins from several animal genomes in which NME occurs has been carried out [50]. This study indicated that 7 of the 13 proteins of the animal mitochondrial proteome would be expected to undergo systematic NME. These proteins include two subunits of cytochrome *c* oxidase (COX2 and COX3) – a major complex involved in cytochrome *c* recycling. Cytochrome *c* release is known to be the primary signal for apoptosis in mammals. In plants, which have a mitochondrial proteome twice the size of that in animals, seven ribosomal proteins and six other proteins – the *nad7*, *nad9* and *cob* products, and three of the four cytochrome *c* chaperones (the *ccb* gene products) – are predicted to be substrates for NME. Blocking mitochondrial NME would probably render one or several of these proteins unstable. For instance, rapid degradation of one of its specific chaperones could lead to incomplete maturation of cytochrome *c*, resulting in its release into the cytoplasm and the induction of cell death.

Cytoplasmic NME and nuclear targets

As about two-thirds of the proteins of the proteome undergo NME, it is currently difficult to make reliable predictions concerning cytoplasmic NME targets. However, given the effects of MAP2 inhibition, developmentally regulated major nuclear transcription factors such as *ets-1* [235] might be expected to be sensitive to NME-regulated degradation. This regulation might involve the free amino group of a retained Met being unmasked in conditions of NME inhibition, whereas that of the penultimate amino acid is unmasked if Met is removed. This regulation may be direct or indirect, involving specific N-acylation. It should be noted that (i) several transcription factors in plants and mammals that undergo N-myristoylation (a modification entirely dependent on NME, [236]) were recently identified and (ii) the N-myristoylated α subunit of the major heterotrimeric G protein that regulates cell differentiation in yeast and plants [237] is degraded according to the N-end rule [238]. N-acetylation may provide a means of altering the accessibility of the N-terminus to nuclear ubiquitination. The impairment of NME might also be expected to result in the production of new ubiquitin-conjugating machinery components (E2 or E3) specific for N-termini. These components may form part of the so-called nuclear signalosome.

Conclusion: future prospects

Specific inhibitors of the various components of the NME pathway (MAP1, MAP2 and PDF), such as fumagillin and actinonin, have been described in the last few years. Much remains to be done before NME inhibitors enter the human health market, but NME is clearly a

highly promising target for future therapeutic intervention. Gene knockout experiments in higher eukaryotic organisms and cells are required (i) to increase our understanding of the basic physiology of the process and (ii) to evaluate the consequences or side effects due to long-term inhibition or exposure to inhibitors. The nature of the metal cation present in enzymes of the NME pathway also requires investigation, as this issue appears to be of particular importance in the design and activity of new drugs. This approach was recently followed for MAP [122, 189]. NME seems to be regulated in both prokaryotes and eukaryotes. Bridging the gap between protein half-life and NME is clearly of key importance, as NME appears to be involved in many crucial cell events, including development and programmed cell death.

This review has dealt with few data concerning the consequences of Met removal except as relates to protein half-life. However, the posttranslational modifications induced by NME must be taken into account. Few posttranslational modifications involving the N-terminus have been described in bacteria, whereas such modifications occur frequently in eukaryotes. Bacterial elongation factor EFTu [239] and some ribosomal proteins (S5, S18, L7 and L12) have been shown to be N-acetylated following NME, by the products of the *rimI*, *rimJ* and *rimL* genes (see data in [240]), none of which is essential for cell growth. Our genetic and biochemical understanding of N-acetylating enzymes is currently very good for fungi [240] but only partial for higher eukaryotes [241]. A large proportion of proteins appear to undergo N-acetylation – up to 90% in higher eukaryotes – but this modification does not necessarily depend on Met removal [57, 241]. For eukaryotic proteins translated in the cytoplasm, a cotranslational modification affecting a very small fraction of proteins, all beginning with a glycine unmasked by NME – N-myristoylation – is known to occur [236, 242]. This modification appears to be essential in yeast [243] and *Drosophila* [244]. In plant organelles, N-acetylation and N-methylation frequently occur, but the catalysts involved have yet to be identified [40, 241]. The O-phosphorylation of N-terminal Ser or Thr residues has also been reported to occur in plastids (table 1 and [40]).

The production of proteins or peptides of medical interest in plant plastids is emerging as a valuable tool in biotechnology [245]. Mastering NME in this organelle will enable us to control protein production in plastids, making it possible to produce drugs with the most active N-terminal configurations possible.

Acknowledgments. This work was supported by an Action Thématique et Incitative sur Programme grant from the Centre National de la Recherche Scientifique (CNRS) (to T. M.) and by grants #4477 and #3363 from the Association pour la Recherche sur le Cancer (ARC, Villejuif). A.B. was supported by a Bourse de Docteur Ingénieur from the CNRS.

1 Mechulam Y., Meinnel T. and Blanquet S. (1995) A family of RNA-binding enzymes: the aminoacyl-tRNA synthetases. Subcell. Biochem. **24:** 323–376
2 Meinnel T., Mechulam Y. and Blanquet S. (1993) Methionine as translation start signal: a review of the enzymes of the pathway in *Escherichia coli*. Biochimie **75:** 1061–1075
3 Kozak M. (1983) Comparison of initiation of protein synthesis in procaryotes, eucaryotes and organelles. Microbiol. Rev. **47:** 1–45
4 Barends S., Bink H. H., van den Worm S. H., Pleij C. W. and Kraal B. (2003) Entrapping ribosomes for viral translation: tRNA mimicry as a molecular Trojan horse. Cell **112:** 123–129
5 Sasaki J. and Nakashima N. (2000) Methionine-independent initiation of translation in the capsid protein of an insect RNA virus. Proc. Natl. Acad. Sci. USA **97:** 1512–1515
6 Schwab S. R., Li K. C., Kang C. and Shastri N. (2003) Constitutive display of cryptic translation products by MHC class I molecules. Science **301:** 1367–1371
7 Meinnel T., Sacerdot C., Graffe M., Blanquet S. and Springer M. (1999) Discrimination by *Escherichia coli* Initiation Factor IF3 against initiation on non-canonical codons relies on complementarity rules. J. Mol. Biol. **290:** 825–837
8 RajBhandary U. L. (2000) More surprises in translation: initiation without the initiator tRNA. Proc. Natl. Acad. Sci. USA **97:** 1325–1327
9 Kozak M. (2001) Constraints on reinitiation of translation in mammals. Nucleic Acids Res. **29:** 5226–5232
10 Kozak M. (2001) New ways of initiating translation in eukaryotes? Mol. Cell. Biol. **21:** 1899–1907
11 Sherman F., Stewart J. W. and Tsunasawa S. (1985) Methionine or not methionine at the beginning of a protein. Bioessays **3:** 27–31
12 Tsunasawa S., Stewart J. W. and Sherman F. (1985) Amino-terminal processing of mutant forms of yeast iso-1-cytochrome *c*. The specificities of methionine aminopeptidase and acetyltransferase. J. Biol. Chem. **260:** 5382–5391
13 Ben-Bassat A., Bauer K., Chang S.-Y., Myambo K., Boosman A. and Chang S. (1987) Processing of the initiation methionine from proteins: properties of the *Escherichia coli* methionine aminopeptidase and its gene structure. J. Bacteriol. **169:** 751–757
14 Boissel J. P., Kasper T. J. and Bunn H. F. (1988) Cotranslational amino-terminal processing of cytosolic proteins. Cell-free expression of site-directed mutants of human hemoglobin. J. Biol. Chem. **263:** 8443–8449
15 Boissel J. P., Kasper T. J., Shah S. C., Malone J. I. and Bunn H. F. (1985) Amino-terminal processing of proteins: hemoglobin South Florida, a variant with retention of initiator methionine and N alpha-acetylation. Proc. Natl. Acad. Sci. USA **82:** 8448–8452
16 Flinta C., Persson B., Jornvall H. and von Heijne G. (1986) Sequence determinants of cytosolic N-terminal protein processing. Eur. J. Biochem **154:** 193–196
17 Moerschell R. P., Hosokawa Y., Tsunasawa S. and Sherman F. (1990) The specificities of yeast methionine aminopeptidase and acetylation of amino-terminal methionine in vivo. Processing of altered iso-1- cytochromes c created by oligonucleotide transformation. J. Biol. Chem. **265:** 19638–19643
18 Huang S., Elliott R. C., Liu P. S., Koduri R. K., Weickmann J. L., Lee J. H. et al. (1987) Specificity of cotranslational amino-terminal processing of proteins in yeast. Biochemistry **26:** 8242–8246
19 Hirel P. H., Schmitter M. J., Dessen P., Fayat G. and Blanquet S. (1989) Extent of N-terminal methionine excision from *Escherichia coli* proteins is governed by the side-chain length of the penultimate amino acid. Proc. Natl. Acad. Sci. USA **86:** 8247–8251
20 Dalboge H., Bayne S. and Pedersen J. (1990) In vivo processing of N-terminal methionine in *E. coli*. FEBS Lett. **266:** 1–3

21 Solbiati J., Chapman-Smith A., Miller J. L., Miller C. G. and Cronan J. E. Jr (1999) Processing of the N termini of nascent polypeptide chains requires deformylation prior to methionine removal. J. Mol. Biol. **290:** 607–614

22 Ramesh V., Kohrer C. and RajBhandary U. L. (2002) Expression of *Escherichia coli* methionyl-tRNA formyltransferase in *Saccharomyces cerevisiae* leads to formylation of the cytoplasmic initiator tRNA and possibly to initiation of protein synthesis with formylmethionine. Mol. Cell Biol. **22:** 5434– 5442

23 Arfin S. M. and Bradshaw R. A. (1988) Cotranslational processing and protein turnover in Eukaryotic cells. Biochemistry **27:** 7979–7984

24 Housman D., Gillespie D. and Lodish H. F. (1972) Removal of formyl-methionine residue from nascent bacteriophage f2 protein. J. Mol. Biol. **65:** 163–166

25 Pine M. J. (1969) Kinetics of maturation of the amino termini of the cell proteins of *Escherichia coli*. Biochim. Byophys. Acta **174:** 359–372

26 Ball L. A. and Kaesberg P. (1973) Cleavage of the N-terminal formylmethionine residue from a bacteriophage coat protein in vitro. J. Mol. Biol. **79:** 531–537

27 von Heijne G. (1989) Control of topology and mode of assembly of a polytopic membrane protein by positively charged residues. Nature **341:** 456–458

28 Waller J.-P. (1963) The NH2-terminal residue of the proteins from cell-free extract of *E. coli*. J. Mol. Biol. **7:** 483–496

29 Adams J. M. (1968) On the release of the formyl group from nascent protein. J. Mol. Biol. **33:** 571–589

30 Fry K. T. and Lamborg M. R. (1967) Amidohydrolase activity of *Escherichia coli* extracts with formylated amino acids and dipeptides as substrates. J. Mol. Biol. **28:** 423–433

31 Vogt V. M. (1970) Purification and properties of an aminopeptidase from *Escherichia coli*. J. Biol. Chem. **245:** 4760–4769

32 Miller C. G. (1987) Protein degradation and proteolytic modification. In: *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology, pp. 680–691, Neidhardt F. C., Ingraham J. L., Low K. B., Magasanik B., Schaechter M. and Umbarger H. E. (eds.), American Society for Microbiology, Washington, DC

33 Bradshaw R. A. and Yi E. (2002) Methionine aminopeptidases and angiogenesis. Essays Biochem. **38:** 65–78

34 Lowther W. T. and Matthews B. W. (2002) Metalloaminopeptidases: common functional themes in disparate structural surroundings. Chem. Rev. **102:** 4581–4608

35 Giglione C., Pierre M. and Meinnel T. (2000) Peptide deformylase as a target for new generation, broad spectrum antimicrobial agents. Mol. Microbiol. **36:** 1197–1205

36 Pei D. (2001) Peptide deformylase: a target for novel antibiotics? Emerg. Therap. Targets **5:** 23–40

37 Yuan Z., Trias J. and White R. J. (2001) Deformylase as a novel antibacterial target. Drug Discov. Today **6:** 954–961

38 Demirev P. A., Lin J. S., Pineda F. J. and Fenselaut C. (2001) Bioinformatics and mass spectrometry for microorganism identification: proteome-wide post-translational modifications and database search algorithms for characterization of intact *H. pylori*. Anal. Chem. **73:** 4566–4573

39 Hayter J. R., Robertson D. H., Gaskell S. J. and Beynon R. J. (2003) Proteome analysis of intact proteins in complex mixtures. Mol. Cell Proteomics **2:** 85–95

40 Giglione C. and Meinnel T. (2001) Organellar peptide deformylases: universality of the N-terminal methionine cleavage mechanism. Trends Plant Sci. **6:** 566–572

41 Schmitt E., Guillon J. M., Meinnel T., Mechulam Y., Dardel F. and Blanquet S. (1996) Molecular recognition governing the initiation of translation in *Escherichia coli*. A review. Biochimie **78:** 543–554

42 Takeda M. and Webster R. E. (1968) Protein chain initiation and deformylation in *B. subtilis* homogenates. Proc. Natl. Acad. Sci. USA **60:** 1487–1494

43 Livingston D. M., and Leder P. (1969) Deformylation and protein synthesis. Biochemistry **8:** 435–443

44 Mazel D., Pochet S. and Marliere P. (1994) Genetic characterization of polypeptide deformylase, a distinctive enzyme of eubacterial translation. EMBO J. **13:** 914–923

45 Meinnel T. and Blanquet S. (1993) Evidence that peptide deformylase and methionyl-tRNA(fMet) formyltransferase are encoded within the same operon in *Escherichia coli*. J. Bacteriol. **175:** 7737–7740

46 Guillon J. M., Mechulam Y., Schmitter J. M., Blanquet S. and Fayat G. (1992) Disruption of the gene for Met-tRNA(fMet) formyltransferase severely impairs growth of *Escherichia coli*. J. Bacteriol. **174:** 4294–4301

47 Giglione C. and Meinnel T. (2001) Resistance to anti-peptide deformylase drugs. Expert Opin. Ther. Targets **5:** 415–418

48 Meinnel T. (2000) Peptide deformylase of eukaryotic protists: a target for new antiparasitic agents? Parasitol. Today **16:** 165–168

49 Giglione C., Serero A., Pierre M., Boisson B. and Meinnel T. (2000) Identification of eukaryotic peptide deformylases reveals universality of N-terminal protein processing mechanisms. EMBO J. **19:** 5916–5929

50 Serero A., Giglione C., Sardini A., Martinez Sanz J. and Meinnel T. (2003) An unusual peptide deformylase features in the human mitochondrial N-terminal methionine excsision pathway. J. Biol. Chem. **278:** 52953–52963

51 Serero A., Giglione C. and Meinnel T. (2001) Distinctive features of the two classes of eukaryotic peptide deformylases. J. Mol. Biol **314:** 695–708

52 Dirk L., Williams M. and Houtz R. (2002) Specificity of chloroplast-localized peptide deformylases as determined with peptide analogs of chloroplast-translated proteins. Arch. Biochem. Biophys. **406:** 135–141

53 Nguyen K. T., Hu X., Colton C., Chakrabarti R., Zhu M. X. and Pei D. (2003) Characterization of a human peptide deformylase: Implications for antibacterial drug design. Biochemistry **42:** 9952–9958

54 Bracchi-Ricard V., Nguyen K. T., Zhou Y., Rajagopalan P. T., Chakrabarti D. and Pei D. (2001) Characterization of an eukaryotic peptide deformylase from *Plasmodium falciparum*. Arch. Biochem. Biophys. **396:** 162–170

55 Dirk L. M., Williams M. A. and Houtz R. L. (2001) Eukaryotic peptide deformylases. Nuclear-encoded and chloroplast- targeted enzymes in *Arabidopsis*. Plant Physiol. **127:** 97–107

56 Giglione C., Vallon O. and Meinnel T. (2003) Control of protein life-span by N-terminal methionine excision. EMBO J. **22:** 13–23

57 Bradshaw R. A., Brickey W. W. and Walker K. W. (1998) N-terminal processing: the methionine aminopeptidase and N alpha-acetyl transferase families. Trends Biochem. Sci. **23:** 263–267

58 Tsunasawa S., Izu Y., Miyagi M. and Kato I. (1997) Methionine aminopeptidase from the hyperthermophilic Archaeon *Pyrococcus furiosus*: molecular cloning and overexpression in *Escherichia coli* of the gene, and characteristics of the enzyme. J. Biochem. (Tokyo) **122:** 843–850

59 Arfin S. M., Kendall R. L., Hall L., Weaver L. H., Stewart A. E., Matthews B. W. et al. (1995) Eukaryotic methionyl aminopeptidases: two classes of cobalt-dependent enzymes. Proc. Natl. Acad. Sci. USA **92:** 7714–7718

60 Katinka M. D., Duprat S., Cornillot E., Metenier G., Thomarat F., Prensier G. et al. (2001) Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. Nature **414:** 450–453

61 Zuo S., Guo Q., Ling C. and Chang Y. H. (1995) Evidence that two zinc fingers in the methionine aminopeptidase from *Saccharomyces cerevisiae* are important for normal growth. Mol. Gen. Genet. **246:** 247–253

62 Vetro J. A. and Chang Y. H. (2002) Yeast methionine aminopeptidase type 1 is ribosome-associated and requires its N-terminal zinc finger domain for normal function in vivo. J. Cell Biochem. **85:** 678–688
63 Yang G., Kirkpatrick R. B., Ho T., Zhang G. F., Liang P. H., Johanson K. O. et al. (2001) Steady-state kinetic characterization of substrates and metal-ion specificities of the full-length and N-terminally truncated recombinant human methionine aminopeptidases (type 2). Biochemistry **40:** 10645–10654
64 Liu S., Widom J., Kemp C. W. and Clardy J. (1998) Structure of human methionine aminopeptidase-2 complexed with fumagillin. Science **282:** 1324–1327
65 Datta R., Choudhury P., Bhattacharya M., Soto Leon F., Zhou Y. and Datta B. (2001) Protection of translation initiation factor eIF2 phosphorylation correlates with eIF2-associated glycoprotein p67 levels and requires the lysine-rich domain I of p67. Biochimie **83:** 919–931
66 Datta R., Tammali R. and Datta B. (2003) Negative regulation of the protection of eIF2alpha phosphorylation activity by a unique acidic domain present at the N-terminus of p67. Exp. Cell Res. **283:** 237–246
67 Datta R., Choudhury P., Ghosh A. and Datta B. (2003) A glycosylation site, 60SGTS63, of p67 is required for its ability to regulate the phosphorylation and activity of eukaryotic initiation factor 2alpha. Biochemistry **42:** 5453–5460
68 Walker K. W., Yi E. and Bradshaw R. A. (1999) Yeast (*Saccharomyces cerevisiae*) methionine aminopeptidase I: rapid purification and improved activity assay. Biotechnol. Appl. Biochem. **29 (Pt 2):** 157–163
69 Chang Y. H., Teichert U. and Smith J. A. (1990) Purification and characterization of a methionine aminopeptidase from *Saccharomyces cerevisiae*. J. Biol. Chem. **265:** 19892–19897
70 Kendall R. L. and Bradshaw R. A. (1992) Isolation and characterization of the methionine aminopeptidase from porcine liver responsible for the co-translational processing of proteins. J. Biol. Chem. **267:** 20667–20673
71 Li X. and Chang Y. H. (1996) Evidence that the human homologue of a rat initiation factor-2 associated protein (p67) is a methionine aminopeptidase. Biochem. Biophys. Res. Commun. **227:** 152–159
72 Meinnel T., Guillon J. M., Mechulam Y. and Blanquet S. (1993) The *Escherichia coli fmt* gene, encoding methionyl-tRNA(fMet) formyltransferase, escapes metabolic control. J. Bacteriol. **175:** 993–1000
73 Milligan D. L. and Koshland D. E. Jr (1990) The amino terminus of the aspartate chemoreceptor is formylmethionine. J. Biol. Chem. **265:** 4455–4460
74 Dong M. S., Bell L. C., Guo Z., Phillips D. R., Blair I. A. and Guengerich F. P. (1996) Identification of retained N-formylmethionine in bacterial recombinant mammalian cytochrome P450 proteins with the N-terminal sequence MALLLAVFL...: roles of residues 3–5 in retention and membrane topology. Biochemistry **35:** 10031–10040
75 Wolfe P. B., Wickner W. and Goodman J. M. (1983) Sequence of the leader peptidase gene of *Escherichia coli* and the orientation of leader peptidase in the bacterial envelope. J. Biol. Chem. **258:** 12073–12080
76 Adinolfi B. S., Cafaro V., D'Alessio G. and Di Donato A. (1995) Full antitumor action of recombinant seminal ribonuclease depends on the removal of its N-terminal methionine. Biochem. Biophys. Res. Commun. **213:** 525–532
77 Proudfoot A. E., Power C. A., Hoogewerf A. J., Montjovent M. O., Borlat F., Offord R. E. et al. (1996) Extension of recombinant human RANTES by the retention of the initiating methionine produces a potent antagonist. J. Biol. Chem. **271:** 2599–2603
78 Endo S., Yamamoto Y., Sugawara T., Nishimura O. and Fujino M. (2001) The additional methionine residue at the N-terminus of bacterially expressed human interleukin-2 affects the interaction between the N- and C-termini. Biochemistry **40:** 914–919
79 Hogset A., Blingsmo O. R., Gautvik V. T., Saether O., Jacobsen P. B., Gordeladze J. O. et al. (1990) Expression of human parathyroid hormone in *Escherichia coli*. Biochem. Biophys. Res. Commun. **166:** 50–60
80 Smith S. P., Barber K. R. and Shaw G. S. (1997) Identification and structural influence of a differentially modified N-terminal methionine in human S100b. Protein Sci. **6:** 1110–1113
81 Warren W. C., Bentle K. A., Schlittler M. R., Schwane A. C., O'Neil J. P. and Bogosian G. (1996) Increased production of peptide deformylase eliminates retention of formylmethionine in bovine somatotropin overproduced in *Escherichia coli*. Gene **174:** 235–238
82 Sandman K., Grayling R. A. and Reeve J. N. (1995) Improved N-terminal processing of recombinant proteins synthesized in *Escherichia coli*. Biotechnology (N Y) **13:** 504–506
83 Shen T. J., Ho N. T., Zou M., Sun D. P., Cottam P. F., Simplaceanu V. et al. (1997) Production of human normal adult and fetal hemoglobins in *Escherichia coli*. Protein Eng. **10:** 1085–1097
84 Thongboonkerd V., Luengpailin J., Cao J., Pierce W. M., Cai J., Klein J. B. et al. (2002) Fluoride exposure attenuates expression of *Streptococcus pyogenes* virulence factors. J. Biol. Chem. **277:** 16599–16605
85 Wilkins J. C., Homer K. A. and Beighton D. (2001) Altered protein expression of *Streptococcus oralis* cultured at low pH revealed by two-dimensional gel electrophoresis. Appl. Environ. Microbiol. **67:** 3396–3405
86 Marra A., Asundi J., Bartilson M., Lawson S., Fang F., Christine J. et al. (2002) Differential fluorescence induction analysis of *Streptococcus pneumoniae* identifies genes involved in pathogenesis. Infect. Immun. **70:** 1422–1433
87 Palma M., Worgall S. and Quadri L. E. (2003) Transcriptome analysis of the *Pseudomonas aeruginosa* response to iron. Arch. Microbiol. **180:** 374–379
88 Somerville G. A., Cockayne A., Durr M., Peschel A., Otto M. and Musser J. M. (2003) Synthesis and deformylation of *Staphylococcus aureus* delta-toxin are linked to tricarboxylic acid cycle activity. J. Bacteriol. **185:** 6686–6694
89 Yan Z., Choi S., Liu X., Zhang M., Schageman J. J., Lee S. Y. et al. (2003) Highly coordinated gene regulation in mouse skeletal muscle regeneration. J. Biol. Chem. **278:** 8826–8836
90 Cutforth T. and Gaul U. (1999) A methionine aminopeptidase and putative regulator of translation initiation is required for cell growth and patterning in drosophila. Mech. Dev. **82:** 23–28
91 Wang J., Lou P. and Henkin J. (2000) Selective inhibition of endothelial cell proliferation by fumagillin is not due to differential expression of methionine aminopeptidases. J. Cell Biochem. **77:** 465–473
92 Endo H., Takenaga K., Kanno T., Satoh H. and Mori S. (2002) Methionine aminopeptidase 2 is a new target for the metastasis-associated protein, S100A4. J. Biol. Chem. **277:** 26396–26402
93 Kanno T., Endo H., Takeuchi K., Morishita Y., Fukayama M. and Mori S. (2002) High expression of methionine aminopeptidase type 2 in germinal center B cells and their neoplastic counterparts. Lab. Invest. **82:** 893–901
94 Catalano A., Romano M., Robuffo I., Strizzi L., and Procopio A. (2001) Methionine aminopeptidase-2 regulates human mesothelioma cell survival: role of bcl-2 expression and telomerase activity. Am. J. Pathol. **159:** 721–731
95 Grigorian M., Andresen S., Tulchinsky E., Kriajevska M., Carlberg C., Kruse C. et al. (2001) Tumor suppressor p53 protein is a new target for the metastasis-associated Mts1/S100A4 protein: functional consequences of their interaction. J. Biol. Chem. **276:** 22699–22708
96 Mazzucchelli L. (2002) Protein S100A4: too long overlooked by pathologists? Am. J. Pathol. **160:** 7–13

97 Langland J. O., Langland L., Zeman C., Saha D. and Roth D. A. (1997) Developmental regulation of a plant encoded inhibitor of eukaryotic initiation factor 2 alpha phosphorylation. Plant J. **12:** 393–400

98 Cleary M. D., Singh U., Blader I. J., Brewer J. L. and Boothroyd J. C. (2002) *Toxoplasma gondii* asexual development: identification of developmentally regulated genes and distinct patterns of gene expression. Eukaryot. Cell **1:** 329–340

99 Florens L., Washburn M. P., Raine J. D., Anthony R. M., Grainger M., Haynes J. D. et al. (2002) A proteomic view of the *Plasmodium falciparum* life cycle. Nature **419:** 520–526

100 Taroncher-Oldenburg G. and Anderson D. M. (2000) Identification and characterization of three differentially expressed genes, encoding S-adenosylhomocysteine hydrolase, methionine aminopeptidase and a histone-like protein, in the toxic dinoflagellate *Alexandrium fundyense*. Appl. Environ. Microbiol. **66:** 2105–2112

101 Rajagopalan P. T. R., Yu X. C. and Pei D. (1997) Peptide deformylase: a new type of mononuclear iron protein. J. Am. Chem. Soc. **119:** 12418–12419

102 Groche D., Becker A., Schlichting I., Kabsch W., Schultz S. and Wagner A. F. (1998) Isolation and crystallization of functionally competent *Escherichia coli* peptide deformylase forms containing either iron or nickel in the active site. Biochem. Biophys. Res. Commun. **246:** 342–346

103 Groche D. (1995) Charakterisierung des Eisenzentrums und des Katalysemechanismus von Peptid-Deformylase aus *Escherichia coli*, Thesis, Universität Heidelberg, Germany

104 Rajagopalan P. T. and Pei D. (1998) Oxygen-mediated inactivation of peptide deformylase. J. Biol. Chem. **273:** 22305–22310

105 Kreusch A., Spraggon G., Lee C. C., Klock H., McMullan D., Ng K. et al. (2003) Structure analysis of peptide deformylases from *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Thermotoga maritima* and *Pseudomonas aeruginosa*: snapshots of the oxygen sensitivity of peptide deformylase. J. Mol. Biol. **330:** 309–321

106 Claiborne A., Mallett T. C., Yeh J. I., Luba J. and Parsonage D. (2001) Structural, redox and mechanistic parameters for cysteine-sulfenic acid function in catalysis and regulation. Adv. Protein Chem. **58:** 215–276

107 Rajagopalan P. T., Datta A. and Pei D. (1997) Purification, characterization and inhibition of peptide deformylase from *Escherichia coli*. Biochemistry **36:** 13910–13918

108 Ragusa S., Blanquet S. and Meinnel T. (1998) Control of peptide deformylase activity by metal cations. J. Mol. Biol. **280:** 515–523

109 Ragusa S., Mouchet P., Lazennec C., Dive V. and Meinnel T. (1999) Substrate recognition and selectivity of peptide deformylase. Similarities and differences with metzincins and thermolysin. J. Mol. Biol. **289:** 1445–1457

110 Meinnel T. and Blanquet S. (1995) Enzymatic properties of *Escherichia coli* peptide deformylase. J. Bacteriol. **177:** 1883–1887

111 Rajagopalan P. T., Grimme S. and Pei D. (2000) Characterization of cobalt(II)-substituted peptide deformylase: function of the metal ion and the catalytic residue Glu-133. Biochemistry **39:** 791–799

112 Li Y., Chen Z. and Gong W. (2002) Enzymatic properties of a new peptide deformylase from pathogenic bacterium *Leptospira interrogans*. Biochem. Biophys. Res. Commun. **295:** 884–889

113 D'Souza V. M. and Holz R. C. (1999) The methionyl aminopeptidase from Escherichia coli can function as an iron(II) enzyme. Biochemistry **38:** 11079–11085

114 Walker K. W. and Bradshaw R. A. (1998) Yeast methionine aminopeptidase I can utilize either Zn2+ or Co2+ as a cofactor: a case of mistaken identity? Protein Sci. **7:** 2684–2687

115 D'Souza V. M., Swierczek S. I., Cosper N. J., Meng L., Ruebush S., Copik A. J. et al. (2002) Kinetic and structural characterization of manganese(II)-loaded methionyl aminopeptidases. Biochemistry **41:** 13096–13105

116 Miller C. G. (1975) Peptidases and proteases of *Escherichia coli* and *Salmonella typhimurium*. Annu. Rev. Microbiol. **29:** 485–504

117 Wingfield P., Graber P., Turcatti G., Movva N. R., Pelletier M., Craig S. et al. (1989) Purification and characterization of a methionine-specific aminopeptidase from *Salmonella typhimurium*. Eur. J. Biochem. **180:** 23–32

118 Roderick S. L. and Matthews B. W. (1993) Structure of the cobalt-dependent methionine aminopeptidase from *Escherichia coli*: a new type of proteolytic enzyme. Biochemistry **32:** 3907–3912

119 D'Souza V M., Bennett B., Copik A. J. and Holz R. C. (2000) Divalent metal binding properties of the methionyl aminopeptidase from *Escherichia coli*. Biochemistry **39:** 3817–3826

120 Cosper N. J., D'Souza V M., Scott R. A. and Holz R. C. (2001) Structural evidence that the methionyl aminopeptidase from *Escherichia coli* is a mononuclear metalloprotease. Biochemistry **40:** 13302–13309

121 Meng L., Ruebush S., D'Souza V M., Copik A. J., Tsunasawa S. and Holz R. C. (2002) Overexpression and divalent metal binding properties of the methionyl aminopeptidase from *Pyrococcus furiosus*. Biochemistry **41:** 7199–7208

122 Wang J., Sheppard G. S., Lou P., Kawai M., Park C., Egan D. A. et al. (2003) Physiologically relevant metal cofactor for methionine aminopeptidase-2 is manganese. Biochemistry **42:** 5035–5042

123 Atanassova A., Sugita M., Sugiura M., Pajpanova T. and Ivanov I. (2003) Molecular cloning, expression and characterization of three distinctive genes encoding methionine aminopeptidases in cyanobacterium *Synechocystis* sp. strain PCC6803. Arch. Microbiol. **180:** 185–193

124 Bazan J. F., Weaver L. H., Roderick S. L., Huber R. and Matthews B. W. (1994) Sequence and structure comparison suggest that methionine aminopeptidase, prolidase, aminopeptidase P and creatinase share a common fold. Proc. Natl. Acad. Sci. USA **91:** 2473–2477

125 Rawlings N. D. and Barrett A. J. (1995) Evolutionary families of metallopeptidases. Methods Enzymol. **248:** 183–228

126 Rawlings N. D., O'Brien E. and Barrett A. J. (2002) MEROPS: the protease database. Nucleic Acids Res. **30:** 343–346

127 Lowther W. T. and Matthews B. W. (2000) Structure and function of the methionine aminopeptidases. Biochim Biophys. Acta **1477:** 157–167

128 Tahirov T. H., Oki H., Tsukihara T., Ogasahara K., Yutani K., Ogata K. et al. (1998) Crystal structure of methionine aminopeptidase from hyperthermophile, *Pyrococcus furiosus*. J. Mol. Biol. **284:** 101–124

129 Wilce M. C., Bond C. S., Dixon N. E., Freeman H. C., Guss J. M., Lilley P. E. et al. (1998) Structure and mechanism of a proline-specific aminopeptidase from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **95:** 3472–3477

130 Yaron A. and Berger A. (1970) Aminopeptidase-P. Methods Enzymol. **19:** 521–534

131 Yoshimoto T., Orawski A. T. and Simmons W. H. (1994) Substrate specificity of aminopeptidase P from *Escherichia coli*: comparison with membrane-bound forms from rat and bovine lung. Arch. Biochem. Biophys. **311:** 28–34

132 Mars I. and Monnet V. (1995) An aminopeptidase P from *Lactococcus lactis* with original specificity. Biochim. Biophys. Acta **1243:** 209–215

133 Matos J., Nardi M., Kumura H. and Monnet V. (1998) Genetic characterization of *pepP*, which encodes an aminopeptidase P whose deficiency does not affect *Lactococcus lactis* growth in milk, unlike deficiency of the X-prolyl dipeptidyl aminopeptidase. Appl. Environ. Microbiol. **64:** 4591–4595

134 Yasueda H., Kikuchi Y., Kojima H. and Nagase K. (1991) In-vivo processing of the initiator methionine from recombinant methionyl human interleukin-6 synthesized in *Escherichia coli* overproducing aminopeptidase-P. Appl. Microbiol. Biotechnol. **36:** 211–215
135 Mazel D., Coic E., Blanchard S., Saurin W. and Marliere P. (1997) A survey of polypeptide deformylase function throughout the eubacterial lineage. J. Mol. Biol. **266:** 939–949
136 Meinnel T., Lazennec C., Villoing S. and Blanquet S. (1997) Structure-function relationships within the peptide deformylase family. Evidence for a conserved architecture of the active site involving three conserved motifs and a metal ion. J. Mol. Biol. **267:** 749–761
137 Meinnel T., Blanquet S. and Dardel F. (1996) A new subclass of the zinc metalloproteases superfamily revealed by the solution structure of peptide deformylase. J. Mol. Biol. **262:** 375–386
138 Dardel F., Ragusa S., Lazennec C., Blanquet S. and Meinnel T. (1998) Solution structure of nickel-peptide deformylase. J. Mol. Biol. **280:** 501–513
139 Chan M. K., Gong W., Rajagopalan P. T., Hao B., Tsai C. M. and Pei D. (1997) Crystal structure of the *Escherichia coli* peptide deformylase. Biochemistry **36:** 13904–13909
140 Hilgers M. T. and Ludwig M. L. (2001) Crystal structure of the quorum-sensing protein LuxS reveals a catalytic metal site. Proc. Natl. Acad. Sci. USA **98:** 11169–11174
141 Ruzheinikov S. N., Das S. K., Sedelnikova S. E., Hartley A., Foster S. J., Horsburgh M. J. et al. (2001) The 1.2 A structure of a novel quorum-sensing protein, *Bacillus subtilis* LuxS. J. Mol. Biol. **313:** 111–122
142 Zhu J., Dizin E., Hu X., Wavreille A. S., Park J., and Pei D. (2003) S-Ribosylhomocysteinase (LuxS) is a mononuclear iron protein. Biochemistry **42:** 4717–4726
143 Baldwin E. T., Harris M. S., Yem A. W., Wolfe C. L., Vosters A. F., Curry K. A. et al. (2002) Crystal structure of type II peptide deformylase from *Staphylococcus aureus*. J. Biol. Chem. **277:** 31163–31171
144 Smith K. J., Petit C. M., Aubart K., Smyth M., McManus E., Jones J. et al. (2003) Structural variation and inhibitor binding in polypeptide deformylase from four different bacterial species. Protein Sci. **12:** 349–360
145 Kumar A., Nguyen K. T., Srivathsan S., Ornstein B., Turley S., Hirsh I. et al. (2002) Crystals of peptide deformylase from *Plasmodium falciparum* reveal critical characteristics of the active site for drug design. Structure (Camb) **10:** 357–367
146 Li Y., Ren S., and Gong W. (2002) Cloning, high-level expression, purification and crystallization of peptide deformylase from *Leptospira interrogans*. Acta Crystallogr. D Biol. Crystallogr. **58:** 846–848
147 Guilloteau J. P., Mathieu M., Giglione C., Blanc V., Dupuy A., Chevrier M. et al. (2002) The crystal structures of four peptide deformylases bound to the antibiotic actinonin reveal two distinct types: a platform for the structure-based design of antibacterial agents. J. Mol. Biol. **320:** 951–962
148 Giglione C., and Meinnel T. (2001) Peptide deformylase as an emerging target for antiparasitic agents. Emerg. Therap. Targets **5:** 41–57
149 Lee M. D., Antczak C., Li Y., Sirotnak F. M., Bornmann W. G., and Scheinberg D. A. (2003) A new human peptide deformylase inhibitable by actinonin. Biochem. Biophys. Res. Commun. **312:** 309–315
150 Archibald J. M., Rogers M. B., Toop M., Ishida K. and Keeling P. J. (2003) Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga *Bigelowiella natans*. Proc. Natl. Acad. Sci. USA **100:** 7678–7683
151 Lloyd D. and Harris J. C. (2002) Giardia: highly evolved parasite or early branching eukaryote? Trends Microbiol. **10:** 122–127
152 Henze K. and Martin W. (2003) Evolutionary biology: essence of mitochondria. Nature **426:** 127–128
153 Hutchison C. A., Peterson S. N., Gill S. R., Cline R. T., White O., Fraser C. M. et al. (1999) Global transposon mutagenesis and a minimal Mycoplasma genome. Science **286:** 2165–2169
154 Kobayashi K., Ehrlich S. D., Albertini A., Amati G., Andersen K. K., Arnaud M. et al. (2003) Essential *Bacillus subtilis* genes. Proc. Natl. Acad. Sci. USA **100:** 4678–4683
155 Gerdes S. Y., Scholle M. D., Campbell J. W., Balazsi G., Ravasz E., Daugherty M. D. et al. (2003) Experimental determination and system level analysis of essential genes in *Escherichia coli* MG1655. J. Bacteriol. **185:** 5673–5684
156 Meinnel T. and Blanquet S. (1994) Characterization of the *Thermus thermophilus* locus encoding peptide deformylase and methionyl-tRNA(fMet) formyltransferase. J. Bacteriol. **176:** 7387–7390
157 Chen D. Z., Patel D. V., Hackbarth C. J., Wang W., Dreyer G., Young D. C. et al. (2000) Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. Biochemistry **39:** 1256–1262
158 Margolis P., Hackbarth C., Lopez S., Maniar M., Wang W., Yuan Z. et al. (2001) Resistance of *Streptococcus pneumoniae* to deformylase inhibitors is due to mutations in *def*B. Antimicrob. Agents Chemother. **45:** 2432–2435
159 Apfel C. M., Locher H., Evers S., Takacs B., Hubschwerlen C., Pirson W. et al. (2001) Peptide deformylase as an antibacterial drug target: target validation and resistance development. Antimicrob. Agents Chemother. **45:** 1058–1064
160 Haas M., Beyer D., Gahlmann R. and Freiberg C. (2001) YkrB is the main peptide deformylase in *Bacillus subtilis*, a eubacterium containing two functional peptide deformylases. Microbiology **147:** 1783–1791
161 Serero A., Giglione C. and Meinnel T. (2001) Seeking new targets for antiparasitic agents. Trends Parasitol. **17:** 7–8
162 Chang S. Y., McGary E. C. and Chang S. (1989) Methionine aminopeptidase gene of *Escherichia coli* is essential for cell growth. J. Bacteriol. **171:** 4071–4072
163 Miller C. G., Kukral A. M., Miller J. L. and Movva N. R. (1989) *pepM* is an essential gene in *Salmonella typhimurium*. J. Bacteriol. **171:** 5215–5217
164 Li X. and Chang Y. H. (1995) Amino-terminal protein processing in *Saccharomyces cerevisiae* is an essential function that requires two distinct methionine aminopeptidases. Proc. Natl. Acad. Sci. USA **92:** 12357–12361
165 Dummitt B., Micka W. S. and Chang Y. H. (2003) N-terminal methionine removal and methionine metabolism in *Saccharomyces cerevisiae*. J. Cell Biochem. **89:** 964–974
166 Turk B. E., Griffith E. C., Wolf S., Biemann K., Chang Y. H. and Liu J. O. (1999) Selective inhibition of amino-terminal methionine processing by TNP-470 and ovalicin in endothelial cells. Chem. Biol. **6:** 823–833
167 Chen S., Vetro J. A. and Chang Y. H. (2002) The specificity in vivo of two distinct methionine aminopeptidases in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **398:** 87–93
168 Zhang Y., Griffith E. C., Sage J., Jacks T. and Liu J. O. (2000) Cell cycle inhibition by the anti-angiogenic agent TNP-470 is mediated by p53 and p21WAF1/CIP1. Proc. Natl. Acad. Sci. USA **97:** 6427–6432
169 Yeh J. R., Mohan R. and Crews C. M. (2000) The antiangiogenic agent TNP-470 requires p53 and p21CIP/WAF for endothelial cell growth arrest. Proc. Natl. Acad. Sci. USA **97:** 12782–12787
170 Kusaka M., Sudo K., Fujita T., Marui S., Itoh F., Ingber D. et al. (1991) Potent anti-angiogenic action of AGM-1470: comparison to the fumagillin parent. Biochem. Biophys. Res. Commun. **174:** 1070–1076
171 Corey E. J., Guzman-Perez A. and Noe M. C. (1994) Short enantioselective synthesis of (-)-ovalicin, a potent inhibitor of

angiogenesis, using substrate-enhanced catalytic asymetric dihydroxylation. J. Am. Chem. Soc. **116:** 12109–12110
172 Kwon J. Y., Jeong H. W., Kim H. K., Kang K. H., Chang Y. H., Bae K. S. et al. (2000) cis-fumagillin, a new methionine aminopeptidase (type 2) inhibitor produced by *Penicillium* sp. F2757. J. Antibiot. (Tokyo) **53:** 799–806
173 Zhou G., Tsai C. W. and Liu J. O. (2003) Fumagalone, a reversible inhibitor of type 2 methionine aminopeptidase and angiogenesis. J. Med. Chem. **46:** 3452–3454
174 Sin N., Meng L., Wang M. Q., Wen J. J., Bornmann W. G. and Crews C. M. (1997) The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. Proc. Natl. Acad. Sci. USA **94:** 6099–6103
175 Griffith E. C., Su Z., Turk B. E., Chen S., Chang Y.-H., Wu Z. et al. (1997) Methionine aminopeptidase (type 2) is the common target for angiogenesis AGM-1470 and ovalicin. Chem. Biol. **4:** 461–471
176 Griffith E. C., Su Z., Niwayama S., Ramsay C. A., Chang Y. H. and Liu J. O. (1998) Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2. Proc. Natl. Acad. Sci. USA **95:** 15183–15188
177 Lowther W. T., McMillen D. A., Orville A. M. and Matthews B. W. (1998) The anti-angiogenic agent fumagillin covalently modifies a conserved active-site histidine in the *Escherichia coli* methionine aminopeptidase. Proc. Natl. Acad. Sci. USA **95:** 12153–12157
178 Klein C. D. and Folkers G. (2003) Understanding the selectivity of fumagillin for the methionine aminopeptidase type II. Oncol. Res. **13:** 513–520
179 Brdlik C. M. and Crews C. M. (2004) A single amino acid residue defines the difference in ovalicin-sensitivity between type I and II methionine aminopeptidases. J. Biol. Chem. **279:** 9475–9480
180 McCowen M. C., Callender M. E. and Lawlis J. Jr (1951) Fumagillin (H-3), a new antibiotic with amebicidal properties. Science **113:** 202–203
181 Zhang P., Nicholson D. E., Bujnicki J. M., Su X., Brendle J. J., Ferdig M. et al. (2002) Angiogenesis inhibitors specific for methionine aminopeptidase 2 as drugs for malaria and leishmaniasis. J. Biomed. Sci. **9:** 34–40
182 Ingber D., Fujita T., Kishimoto S., Sudo K., Kanamaru T., Brem H. et al. (1990) Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature **348:** 555–557
183 Turk B. E., Su Z. and Liu J. O. (1998) Synthetic analogues of TNP-470 and ovalicin reveal a common molecular basis for inhibition of angiogenesis and immunosuppression. Bioorg. Med. Chem. **6:** 1163–1169
184 Weiss L. M., Costa S. F. and Zhang H. (2001) Microsporidian methionine aminopeptidase type 2. J. Eukaryot. Microbiol. **Suppl.:** 88S–90S
185 Chang Y. H., Teichert U. and Smith J. A. (1992) Molecular cloning, sequencing, deletion and overexpression of a methionine aminopeptidase gene from *Saccharomyces cerevisiae*. J. Biol. Chem. **267:** 8007–8011
186 Lowther W. T., Zhang Y., Sampson P. B., Honek J. F. and Matthews B. W. (1999) Insights into the mechanism of *Escherichia coli* methionine aminopeptidase from the structural analysis of reaction products and phosphorus-based transition-state analogues. Biochemistry **38:** 14810–14819
187 Keding S. J., Dales N. A., Lim S., Beaulieu D. and Rich D. H. (1998) Synthesis of (3*R*)-amino-(2*S*)-hydroxy amino acids for inhibition of methionine aminopeptidase 1. Synth. Commun. **28:** 4463–4470
188 Oefner C., Douangamath A., D'Arcy A., Hafeli S., Mareque D., Mac Sweeney A. et al. (2003) The 1.15A crystal structure of the *Staphylococcus aureus* methionyl-aminopeptidase and complexes with triazole based inhibitors. J. Mol. Biol. **332:** 13–21
189 Li J. Y., Chen L. L., Cui Y. M., Luo Q. L., Li J., Nan F. J. et al. (2003) Specificity for inhibitors of metal-substituted methionine aminopeptidase. Biochem. Biophys. Res. Commun. **307:** 172–179
190 Luo Q. L., Li J. Y., Liu Z. Y., Chen L. L., Li J., Qian Z. et al. (2003) Discovery and structural modification of inhibitors of methionine aminopeptidases from *Escherichia coli* and *Saccharomyces cerevisiae*. J. Med. Chem. **46:** 2631–2640
191 Quinoà E., Adamsczeski M., Crews P, and Bakus G. J. (1986) Bengamides, heterocyclic anthelmintics from a Jaspidae marine sponge. J. Org. Chem. **51:** 4494–4497
192 Kinder F. R. Jr, Versace R. W., Bair K. W., Bontempo J. M., Cesarz D., Chen S. et al. (2001) Synthesis and antitumor activity of ester-modified analogues of bengamide B. J. Med. Chem. **44:** 3692–3699
193 Kinder F. R. Jr, Wattanasin S., Versace R. W., Bair K. W., Bontempo J., Green M. A. et al. (2001) Total syntheses of bengamides B and E. J. Org. Chem. **66:** 2118–2122
194 Towbin H., Bair K. W., DeCaprio J. A., Eck M., Kim S., Kinder F. R. et al. (2003) Proteomics based target identification: bengamides as a new class of methionine aminopeptidase inhibitors. J. Biol. Chem. **278:** 52964–52971
195 Wang J., Sheppard G. S., Lou P., Kawai M., BaMaung N., Erickson S. A. et al. (2003) Tumor suppression by a rationally designed reversible inhibitor of methionine aminopeptidase-2. Cancer Res. **63:** 7861–7869
196 Hu X., Zhu J., Srivathsan S. and Pei D. (2004) Peptidyl hydroxamic acids as methionine aminopeptidase inhibitors. Bioorg. Med. Chem. Lett. **14:** 77–79
197 Taunton J. (1997) How to starve a tumor. Chem. Biol. **4:** 493–496
198 Matter A. (2001) Tumor angiogenesis as a therapeutic target. Drug Discov. Today **6:** 1005–1024
199 Folkman J. and Ingber D. (1992) Inhibition of angiogenesis. Semin. Cancer Biol. **3:** 89–96
200 Kruger E. A. and Figg W. D. (2000) TNP-470: an angiogenesis inhibitor in clinical development for cancer. Expert Opin. Investig. Drugs **9:** 1383–1396
201 Boehm T., Folkman J., Browder T. and O'Reilly M. S. (1997) Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature **390:** 404–407
202 Kerbel R. S. (1997) A cancer therapy resistant to resistance. Nature **390:** 335–336
203 Bergers G., Javaherian K., Lo K. M., Folkman J. and Hanahan D. (1999) Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. Science **284:** 808-812
204 Killough J. H., Magill G. B. and Smith R. C. (1952) The treatment of amebiasis with fumagillin. Science **115:** 71–72
205 Meinnel T., Patiny L., Ragusa S. and Blanquet S. (1999) Design and synthesis of substrate analogue inhibitors of peptide deformylase. Biochemistry **38:** 4287–4295
206 Meinnel T. (1999) Developing a rational strategy for new antibacterial agents. Pathol. Biol. **47:** 780–783
207 Hu Y. J., Rajagopalan P. T. and Pei D. (1998) H-phosphonate derivatives as novel peptide deformylase inhibitors. Bioorg. Med. Chem. Lett. **8:** 2479–2482
208 Huntington K. M., Yi T., Wei Y, and Pei D. (2000) Synthesis and antibacterial activity of peptide deformylase inhibitors. Biochemistry **39:** 4543–4351
209 Hao B., Gong W., Rajagopalan P. T., Zhou Y., Pei D. and Chan M. K. (1999) Structural basis for the design of antibiotics targeting peptide deformylase. Biochemistry **38:** 4712–4719
210 Chu M., Mierza R., He L., Xu L., Gentile F., Taerracciano J. et al. (2001) Isolation and structure elucidation of two novel deformylase inhibitors produced by *Streptomyces* sp. Tetrahedron Lett. **42:** 3549–3451
211 Wiesner J., Sanderbrand S., Beck E. and Jomaa H. (2001) Seeking new targets for antiparasitic agents. Trends Parasitol. **17:** 7

212 Clements J. M., Beckett R. P., Brown A., Catlin G., Lobell M., Palan S. et al. (2001) Antibiotic activity and characterization of BB-3497, a novel peptide deformylase inhibitor. Antimicrob. Agents Chemother. **45:** 563–570
213 Boularot A., Giglione C., Artaud I. and Meinnel T. (2004) Structure-activity relationship and therapeutic potential of peptide deformylase inhibitors. Curr. Opin. Invest. Drugs, in press
214 Xu Y., Lai L. T., Gabrilove J. L. and Scheinberg D. A. (1998) Antitumor activity of actinonin in vitro and in vivo. Clin. Cancer Res. **4:** 171–176
215 Giglione C. and Meinnel T. (2002) The situation on antimicrobial agents and chemotherapy in 2002: highlights of the 42nd ICAAC. Expert Opin. Ther. Targets **6:** 691–697
216 Margolis P. S., Hackbarth C. J., Young D. C., Wang W., Chen D., Yuan Z. et al. (2000) Peptide deformylase in *Staphylococcus aureus*: resistance to inhibition is mediated by mutations in the formyltransferase gene. Antimicrob. Agents Chemother. **44:** 1825–1831
217 Johnson I., Waller A., Clements J., Davies S., Palan S., Simpson A. et al. (2002) Characterisation of in vitro-generated *Streptococcus penumoniae* mutants with decreased sensitivity to peptide deformylase, Poster F-1681, American Society for Microbiology, San Diego, p. 223
218 Hackbarth C. J., Chen D. Z., Lewis J. G., Clark K., Mangold J. B., Cramer J. A. et al. (2002) N-alkyl urea hydroxamic acids as a new class of peptide deformylase inhibitors with antibacterial activity. Antimicrob Agents Chemother. **46:** 2752–2764
219 Sulavik M. C., Houseweart C., Cramer C., Jiwani N., Murgolo N., Greene J. et al. (2001) Antibiotic susceptibility profiles of *Escherichia coli* strains lacking multidrug efflux pump genes. Antimicrob. Agents Chemother. **45:** 1126–1136
220 Hellmann H. and Estelle M. (2002) Plant development: regulation by protein degradation. Science **297:** 793–797
221 Kuriyama H., and Fukuda H. (2002) Developmental programmed cell death in plants. Curr. Opin. Plant Biol. **5:** 568–573
222 Yoshida S., Ito M., Callis J., Nishida I. and Watanabe A. (2002) A delayed leaf senescence mutant is defective in arginyl-tRNA:protein arginyltransferase, a component of the N-end rule pathway in *Arabidopsis*. Plant J. **32:** 129–137
223 Breitschopf K., Bengal E., Ziv T., Admon A. and Ciechanover A. (1998) A novel site for ubiquitination: the N-terminal residue, and not internal lysines of MyoD, is essential for conjugation and degradation of the protein. EMBO J. **17:** 5964–5973
224 Floyd Z. E., Trausch-Azar J. S., Reinstein E., Ciechanover A. and Schwartz A. L. (2001) The nuclear ubiquitin-proteasome system degrades MyoD. J. Biol. Chem. **276:** 22468–22475
225 Bloom J., Amador V., Bartolini F., DeMartino G. and Pagano M. (2003) Proteasome-Mediated Degradation of p21 via N-Terminal Ubiquitinylation. Cell **115:** 71–82
226 Varshavsky A. (1996) The N-end rule: functions, mysteries, uses. Proc. Natl. Acad. Sci. USA **93:** 12142–12149
227 Worley C. K., Ling R. and Callis J. (1998) Engineering in vivo instability of firefly luciferase and *Escherichia coli* beta-glucuronidase in higher plants using recognition elements from the ubiquitin pathway. Plant Mol. Biol. **37:** 337–347
228 Lee P., Beem E. and Segal M. S. (2002) Marker for real-time analysis of caspase activity in intact cells. Biotechniques **33:** 1284–1287, 1289–1291
229 Sawant S. V., Kiran K., Singh P. K. and Tuli R. (2001) Sequence architecture downstream of the initiator codon enhances gene expression and protein stability in plants. Plant Physiol. **126:** 1630–1636
230 Sheng J., Kumagai A., Dunphy W. G. and Varshavsky A. (2002) Dissection of c-MOS degron. EMBO J. **21:** 6061–6071
231 Flynn J. M., Neher S. B., Kim Y. I., Sauer R. T., and Baker T. A. (2003) Proteomic discovery of cellular substrates of the ClpXP protease reveals five classes of ClpX-recognition signals. Mol. Cell **11:** 671–683
232 Balk J. and Leaver C. J. (2001) The PET1-CMS mitochondrial mutation in sunflower is associated with premature programmed cell death and cytochrome *c* release. Plant Cell **13:** 1803–1818
233 Tiwari B. S., Belenghi B. and Levine A. (2002) Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. Plant Physiol. **128:** 1271–1281
234 Lam E., Kato N. and Lawton M. (2001) Programmed cell death, mitochondria and the plant hypersensitive response. Nature **411:** 848–853
235 Wernert N., Stanjek A., Kiriakidis S., Hugel A., Jha H. C., Mazitschek R. et al. (1999) Inhibition of angiogenesis in vivo by *ets*-1 antisense oligonucleotides-inhibition of Ets-1 transcription factor expression by the antibiotic fumagillin. Angew. Chem. Int. Ed. Engl. **38:** 3228–3231
236 Boisson B., Giglione C. and Meinnel T. (2003) Unexpected protein families including cell defense components feature in the N-myristoylome of a higher eukaryote. J. Biol. Chem. **278:** 43418–43429
237 Ullah H., Chen J. G., Young J. C., Im K. H., Sussman M. R. and Jones A. M. (2001) Modulation of cell proliferation by heterotrimeric G protein in *Arabidopsis*. Science **292:** 2066–2069
238 Madura K. and Varshavsky A. (1994) Degradation of G alpha by the N-end rule pathway. Science **265:** 1454–1458
239 Arai K., Clark B. F., Duffy L., Jones M. D., Kaziro Y., Laursen R. A. et al. (1980) Primary structure of elongation factor Tu from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77:** 1326–1330
240 Polevoda B., Norbeck J., Takakura H., Blomberg A. and Sherman F. (1999) Identification and specificities of N-terminal acetyltransferases from *Saccharomyces cerevisiae*. EMBO J. **18:** 6155–6168
241 Polevoda B. and Sherman F. (2003) N-terminal acetyltransferases and sequence requirements for N-terminal acetylation of eukaryotic proteins. J. Mol. Biol. **325:** 595–622
242 Bhatnagar R. S., Ashrafi K., Futterer K., Waksman G. and Gordon J. I. (2001) Biology and enzymology of protein N-myristoylation. In: The Enzymes, vol. 21 (Protein Lipidation), pp. 241–286, Tamanoi F. and Sigman D. S. (eds.), Academic Press, San Diego
243 Duronio R. J., Towler D. A., Heuckeroth R. O. and Gordon J. I. (1989) Disruption of the yeast N-myristoyl transferase gene causes recessive lethality. Science **243:** 796–800
244 Ntwasa M., Aapies S., Schiffmann D. A. and Gay N. J. (2001) Drosophila embryos lacking N-myristoyltransferase have multiple developmental defects. Exp. Cell Res. **262:** 134–144
245 Daniell H., Khan M. S. and Allison L. (2002) Milestones in chloroplast genetic engineering: an environmentally friendly era in biotechnology. Trends Plant Sci. **7:** 84–91
246 Jeanmougin F., Thompson J. D., Gouy M., Higgins D. G. and Gibson T. J. (1998) Multiple sequence alignment with Clustal X. Trends Biochem. Sci. **23:** 403–405
247 Page R. D. M. (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. **12:** 357–358