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Processing of mitochondrial precursor proteins

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Summary

The mitochondrial processing enzyme consists of two components, the **m**itochondrial **p**rocessing **p**eptidase (MPP) and **p**rocessing **e**nhancing **p**rotein (PEP). MPP and PEP act cooperatively in proteolytic processing of mitochondrial precursor proteins. Most of the mitochondrial precursors possess aminoterminal presequences (also called "targeting sequences" or "signal sequences"), that do not display a common motif and that show only limited similarities of the cleavage sites. The mitochondrial processing peptidase is a metal-dependent endoprotease, sensitive to sulfhydryl-modifying reagents and appears to belong to a new class of proteases. MPP and PEP, together with the core 1 and core 2 proteins of the respiratory complex III, form a new protein family.

Introduction

The majority of mitochondrial proteins is encoded by nuclear genes. These genes are transcribed and translated into precursor proteins most of which possess cleavable amino-terminal presequences. The latter serve as targeting signals that are recognized by receptors on the mitochondrial surface. Most precursors are transported through contact sites of the outer and inner membrane into the mitochondrial matrix [1–3]. During or shortly after the translocation process, the presequences are proteolytically removed [4–8]. In further steps, newly imported proteins are sorted into the mitochondrial subcompartments and become assembled into macromolecular complexes. In the case of several intermembrane space proteins, a second proteolytic cleavage is performed either in the matrix or in the intermembrane space [1–3].

In this review we want to focus on the first proteolytic step by which the matrix targeting sequences are removed. This reaction takes place in the matrix compartment of the mitochondria. Several different names have been proposed in the literature for the enzyme catalyzing this reaction; we call it the mitochondrial processing enzyme. The enzyme has been analyzed from different sources such as yeast, N. crassa, rat and bovine liver [4, 9–13]. General properties are i) it is a soluble component; ii) it is dependent on divalent metal ions and is inhibited by metal chelators (EDTA or o-phenanthroline); and iii) it is not sensitive to known inhibitors of serine or carboxyl proteases.

The mitochondrial processing enzyme was initially purified from N. crassa. Two poly-peptides, named MPP (**mi**tochondrial **p**rocessing **p**eptidase) and PEP (**p**rocessing **e**nhancing

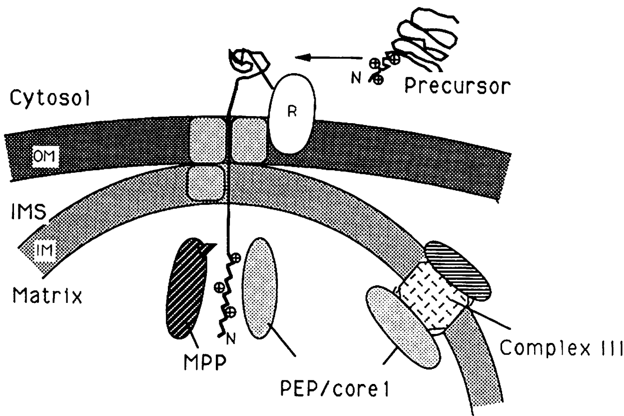

Fig. 1. Working model for the import of precursor proteins and cleavage of targeting sequences by the mitochondrial processing peptidase (MPP), and the processing enhancing protein (PEP).
R, receptor; OM, outer membrane; IMS, inter membrane space; IM, inner membrane; N, aminoterminus

protein) were found to be required for activity [14]. They are structurally related but functionally different [15].

The enzyme was subsequently purified from yeast and rat liver. In these organisms the mitochondrial processing enzyme consists also of two proteins [16, 17].

Results and discussion

Characteristics of MPP and PEP

Of the two components involved in processing, MPP bears the catalytic activity. It is a protein in the mitochondrial matrix and, in Neurospora, represents 0.03% of total mitochondrial protein [14]. MPP is encoded by a nuclear gene. The precursor form contains a cleavable presequence that becomes processed by the action of its own mature form together with PEP. This reflects a general rule of mitochondrial biogenesis, namely that preexisting structures are necessary for the formation of new mitochondria [18].

In N. crassa processing activity could be reconstituted from the separated components MPP and PEP. MPP is the component, which binds Mn^{2+} and is thus responsible for the metal ion requirement of the processing activity [18]. The sensitivity towards metal chelators, such as EDTA (ethylene diamine tetraacetate) or o-phenanthroline, is due to this requirement. Neurospora MPP is strongly inhibited by the sulfhydryl-modifying reagents N-ethylmaleimide (NEM) and iodoacetate. The apparent molecular mass of the mature MPP is 57 kD, when estimated on SDS-polyacrylamide, and 59 kD, when calculated from the amino acid composition [18].

PEP is the component that stimulates the catalytic activity of MPP [14]. PEP function does not require metal ions and is insensitive to sulfhydryl modifying reagents. The molecular mass of PEP in Neurospora is 52 kD according to SDS-polyacrylamide electrophoresis and 49.5 kD according to the amino acid composition [19]. PEP is 15 fold more abundant than MPP and represents 0.45% of total mitochondrial protein [14]. Seventy percent of the PEP detected in Neurospora mitochondria is attached to the inner face of the inner membrane; 30% are present in the matrix fraction. It turned out that in N. crassa the amino acid sequence of PEP is identical to that of the core 1 protein (or subunit 1) of the cytochrome reductase complex (or complex III) of the mitochondrial respiratory chain. A single gene has been found to code for PEP and core 1 protein. Core 1 protein isolated from purified complex III stimulates the catalytic activity of MPP with the same efficiency

as PEP. Thus, Neurospora PEP is a bifunctional protein involved in both electron transport and processing of precursor proteins [19].

In Saccharomyces cerevisiae, temperature sensitive mutants were characterized which accumulated uncleaved precursor proteins at non-permissive temperature. It turned out that the gene affected in one complementation group (mas 1/mif 1; mas = **mi**tochondrial **as**sembly; mif = **mi**tochondrial **i**mport **f**unctions) specifies the equivalent to Neurospora PEP [20]. The gene affected in the other mutant (mas 2/mif 2) is that for MPP, the catalytic component [14, 21]. Subsequently, the two proteins were purified from yeast and found to be necessary and sufficient for proteolytic cleavage of mitochondrial precursors. The yeast components have apparent molecular masses of 52 kD (MPP) and 48 kD (PEP). It was suggested that they form a complex with a molecular mass of about 100 kD [16]. In contrast to the situation in N. crassa, PEP is not identical to the core 1 protein, but has considerable sequence similarity to this protein [19].

From the matrix fraction of rat liver mitochondria also two proteins were purified which are responsible for processing of precursor proteins. Their apparent molecular masses are 55 kD and 52 kD [17]. It was shown that the 55 kD protein bears the catalytic activity, and sequence analysis proved its equivalence to Neurospora and yeast MPP [22]. Upon gel filtration both proteins eluted together with an estimated molecular mass of 105 kD indicating that they form a complex [17]. In contrast to yeast and rat, in Neurospora formation of a tight complex between MPP and PEP was not observed.

In summary, in Neurospora crassa, Saccharomyces cerevisiae and rat two proteins are necessary for processing of precursor proteins. In each case the protein with the higher molecular mass represents MPP, as judged on the basis of both functional and structural characteristics.

The MPP/PEP/core protein family

The two components of the matrix processing enzyme, MPP and PEP, and core 1 and core 2 protein of respiratory complex III form a protein family [18]. The MPP proteins of N. crassa and yeast show sequence identity of 43% [19]. The rat MPP has 36% identity with both Neurospora and yeast MPP [22] (Fig. 2A). A common feature of the MPPs from the various species is a box of 15 amino acids that have a very similar distribution of negatively charged residues (region 1, Fig. 2A). This region is found also in the PEP protein of Neurospora crassa.

Region 1 may be a putative binding site for the positively charged presequences of preproteins. Regions of similarity are distributed among the entire protein with the exception of a serine-rich stretch which is located in the middle of Neurospora MPP and which has no counterpart in the other members of the MPP/PEP/core protein family. This stretch has a high flexibility according to computer prediction and thus may form a hinge between an amino-terminal and a carboxyl-terminal domain. A stretch of 33 identical amino acid residues with four glycine residues in series is found within Neurospora and yeast MPP, and a very similar stretch is present in rat MPP (region 2, Fig. 2A). This conserved region is found only in the MPP proteins and not in the PEP and core proteins. Therefore, it might be a candidate for the active site of the peptidase.

Furthermore, four cysteine residues are conserved in the MPPs from yeast and Neurospora. In rat MPP only two cysteine residues of these four are found, namely those in the amino-terminal half (shaded, Fig. 2A). These cysteine residues might be involved in the

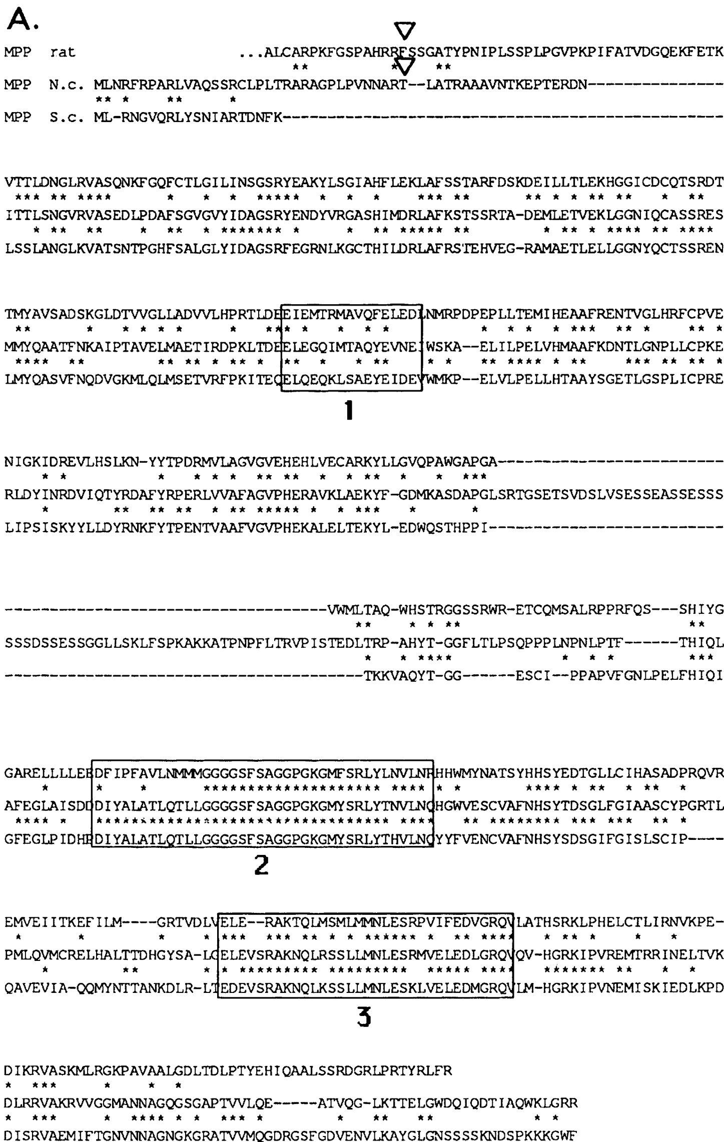

Fig. 2A

catalytic activity. A further highly conserved region (region 3, Fig. 2B) is present in all members of the protein family.

PEP/core 1 protein from N. crassa shares 23% and 26% sequence identity with MPP from yeast and N. crassa (Fig. 2B). On the other hand, N. crassa PEP/core 1 has 51% identity to yeast PEP and 32% to yeast core 1 protein. Furthermore, PEP and core 1 from yeast are only 24% identical (Fig. 2C). These sequence similarities are in line with the finding, that PEP/core 1 is bifunctional in Neurospora, whereas there are two proteins with separate

B.

```
MPP     N.  MLNRFRPARLVAQSSRCLPLTRARAGPLPVNNARTLATRAAAVNTKEPTERDNITTLSNGVRVASEDLP-DAFS
MPP     y.  ML-RNGVQRLYSNIARTDNFKL--------------------------------SSLANGLKVATSNTP-GHFS
PEP     y.  MFSRTAS--------------KFRNT------RRLLSTISSQIP-GTRT-----SKLPNGLTIATEYIPNTSSA
c.1/PEP N.  MASR----RLALNLAQGV---KARAGGVINPFRRGLATPHSGTGIKTQT-----ITLKNGLTVASQVSPYAQTS
c.1     y.  ML-RTVTSKTVS------------------NQFKRSLATAVATP--KAEV----TQLSNGIVVATEHNPSAHTA
c.2     y.  MLSAA---RLQFAQGSVRRLT-----------------------------------------VSARDAP-TKIS

GVGVYIDAGSRY--ENDYVRGASHIMDRLAFKSTSARTADEMLETVEKLGGNIQCASSRESMMYQAATFNKAIPTAVELMAETIRD
ALGLYIDAGSRF--EGRNLKGCTHILDRLAFRSTEHVEGRAMAETLELLGGNYQCTSSRENLMYQASVFNQDVGKMLQLMSETVRF
TVGIFVDAGSRA--ENVKNNGTAHFLEHLAFKGTQNRSQQGIELEIENIGSHLNAYTSRENTVYYAKSLQEDIPKAVDILSDILTK
TVGMWIDAGSRA--ETDETNGTAHFLEHLAFKGTTKRTQQQLELEIENMGAHLNAYTSRENTVYFAKALNEDVPKCVDILQDILQN
SVGVVF--GSGAANENPYNNGVSNLWKNIFLSKENSAVAAKE-------GLALSSNISRDFQSYIVSSLPGSTDKSLDFLNQSFIQ
TLAVKVHGGSRYATKD----GVAHLLNRFNFQNTNTRSALKLVRESELLGGTFKSTLDREYITLKATFLKDDLPYYVNALADVLYK
                1
PK---LTDEELEGQIMTAQYEVNEIW-SKAELILPELVHMAAFKDNTLGNPLLCPKERLDYINADVIQTYRDAFYRPERLV-VAFA
PK---ITEQELQEQKLSAEYEIDEVW-MKPELVLPELLHTAAYSGETLGSPLICPRELIPSISKYYLLDYRNKFYTPENTV-AAFV
SV---LDNSAIERERDVIIRESEEVD-KMYDEVVFDHLHEITYKDQPLGRTILGPIKNIKSITRTDLKDYITKNYKGDRMVLAGAG
SK---LEESAIERERDVILRESEEVE-KQLEEVVFDHLHATAYQHQPLGRTILGPRENIRDITRTELVNYIKNNYTADRMVLVGAG
QKANLLSSSNFEATKKSVLKQVQDFEENDHPNRVLEHLHSTAFQNTPLSLPTRGTLESLENLVVADLESFANNHFLNSNAVVVGTG
TA--FKPHELTESVLPAARYDYAVAE-QCPVKSAEDQLYAITFRKGL-GNPLLY--DGVERVSLQDIKDFADKVYTKENLEVSGEN

GVPHERAVKLAEKYFGDMKASDAPGLSRTGSETSVDSLVSESSEASSESSSSSSDSSESSGGLLSKLFSPKAKKATPNPFLTRVPI
GVPHEKALELTEKYLEDWQSTHPPI----------------------------------------------TKKV-----------
AVDHEKLVQTAQKYFGHVPKSESPVPLGSPRG---------------------------------------PL-PVFCRGERFIKE
GVPHEQLVEMADKYFSKLPAYA-PVSSASILS---------------------------------------KKKPDFIGSDIRIRD
NIKHEDLVNSIESKNLSLQTGTKPVL---------------------------------------------KKKAAFLDSEVRLRD
VVEADLKRFVDESLLSTLPAGKSLVSKSEPKF--------------------------------------------FLGEENRVRF
                                                                      2
STEDLTRPAHYTGGFLTLPSQPPPLNP-NLPTFTHIQLAFEGLAISDDDIY---ALATLQTLLGGGGSFSAGGPAKGMYSRLYTNV
--------AQYTGGESCI---PPAPVFGNLPELFHIQIGFEGLPIDHPDIY---ALATLQTLLGGGGSFSAGGPGKGMYSRLYTHV
NT-----------------------------------IALEGVSWSAPDYFV--ALAT-QAIVGNWDR--AIGTGTNSPSPL-AVA
DT-----------------------------------IAVEGVSWSDDDYFT--GLVT-QAIVGNYDK--ALGNAPHQGSKL-SGF
DT-----------------------------------LAVEGEPVNSPNYFVA-KLAA-Q-IFGSYNA--FEPASRLQGIKL-LDN
IGD----------------------------------VAAIGIPVNKASLAQYEVLAN---YLTSALSELSGLISSAKLDKFTDGG
                                                                           3
LNQHG-WVESCVAFNHSYTDSGLFGIAASCYPGRTLPMLQVMC--RELHALTTDHGYSA-LGELEVSRAKNQLRSSLLMNLESRMV
LNQYY-FVENCVAFNHSYSDSGIFGISLSCIP----QAVEVIA---QQMYNTTANKDLR-LTEDEVSRAKNQLKSSLLMNLESKLV
ASQNGSLANSYMSFSTSYADSGLWGM---YIVTDSNEHNVQLIVNEILKEWK--RIKSGKISDAEVNRAKAQLKAALLLSLDGSTA
VHKHD-LATSFMSFSTSYSDTGLWGI---YLVTDKLDRIDDLV-HFSLRENT--ALCSN-VSEAEVERAKAQLKASILLSLDGTTA
IQEYQ-LCDNFNHFSLSVKDSGLWGF---STATRNVTMIVDLI-HFTLKQWN--ALTIS-VTDTEVERAKSLLKLQLGQLYESGNP
LFTT--------LFVRD-QDSAVVSSNIKKIVADL----KKGK---DLSPAINYTKLKN-AVQNESVSSPIELNFDAVKDFKLGKF

ELED--LGRQVQVHGRKIPVREMTRRINELTVKDLRRVAKRVVGGMANNAGQGSGAPTVVLQE-----ATVQG-LKTTELGWDQIQ
ELED--MGRQVLMHGRKIPVNEMISKIEDLKPDDISRVAEMIFTGNVNNAGNGKGRATVVMQGDRGSFGDVENVLKAYGLGNSSSS
IVED--IGRQVVTTGKRLSPEEVFEQVDKITKDDIIMWANYRLQNKPVSMVA---------------------------LGNTSTV
VAED--IGRQIVTTGRRMSPAEIERIIDAVSAKDVMDFANKKIWDDDIAISA---------------------------VGSIEGL
VNDANLLGAEVLIKGSKLSLGEAFKKIDAITVKDVKAWAGKRLWDDDIAIAG---------------------------TGQIEGL
NYVAVGDVSNLPYLDEL¶--------------------------------------------------------------------

DTIAQWKLGRR¶
KNDSPKKKGWF¶
PNVSYIEEKLNQ¶
FDYARIRGDMSRNAF¶
LDYMRIRSDMSMMRW¶
```

Fig. 2B.

functions in yeast [19]. The amino-terminal half of Neurospora PEP/core 1 and of yeast PEP have a higher similarity (63%) than those of Neurospora PEP/core 1 and yeast core 1 (23%). In contrast, the carboxyl-terminus of Neurospora PEP/core 1 is more closely related to that one of the yeast core 1 protein than to the carboxyl-terminus of yeast PEP (Fig. 2B). These findings support the hypothesis that the Neurospora PEP/core 1 protein has two domains. The amino-terminal domain might be responsible preferentially for the PEP function, whereas the carboxyl-terminal domain might bear the core function. The question arises as to whether the situation in Neurospora reflects an exceptional case, or whether,

C.

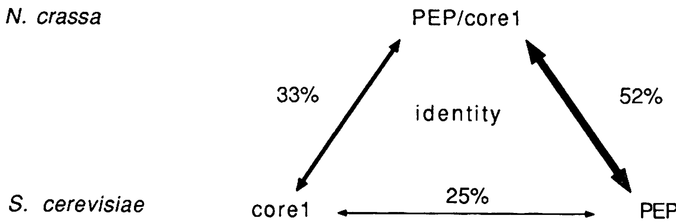

Fig. 2. A. Sequence alignment of rat, Neurospora and yeast MPP. Identical residues are marked by asterisks. Cleavage sites of the presequences of rat and Neurospora MPP are marked by arrows. The regions of similarity regarding a stretch rich in negatively charged amino acids (1) and the two strongly conserved regions (2, 3) are indicated by shaded boxes. The conserved cysteine residues are cross-hatched
B. Sequence alignment of Neurospora crassa (N.) MPP and PEP/core 1 and of yeast (y.) MPP, core 1 (c. 1) and core 2 (c. 2). Identical residues are shaded and highly conserved regions 1—3 are indicated by overlining.
C. Sequence identities among the PEP/core 1 polypeptides of N. crassa and yeast (S. cerevisiae)

as in yeast, the non-identity of PEP and core 1 is the general situation. An answer will be provided by sequencing the proteins of mammalian processing peptidases and cytochrome reductase complexes. The question remains whether the original function of this protein was that of a PEP-like molecule or was that of a core 1 protein. There are some indications that divergence started with the PEP function. Interestingly, the cytochrome bc_1 complex of photosynthetic bacteria and the homologous cytochrome b_6f complex of chloroplasts and cyanobacteria do not have core proteins [23, 24]. Several lines of evidence suggest that the role of mitochondrial core 1 and 2 proteins is to stabilize complex III and to facilitate assembly of this multicomponent systems [25]. One may speculate that a basic function of the core 1/PEP protein is exerted by protein-protein interactions. The role of PEP might be to interact with precursor proteins and to present them to MPP in such a conformation that the cleavage site becomes accessible.

Specificity of the mitochondrial processing peptidase

The presequences of imported precursor proteins are characterized by i) a high content of positively charged amino acid residues; ii) an almost complete lack of acidic amino acid residues; iii) presence of many hydroxylated residues and iv) by the absence of a common motif or consensus of the amino acid sequence.

Most precursor proteins have positively charged amino acids in the -2 position (the penultimate amino acid of the presequence). Out of 15 precursor proteins (yeast and N. crassa) which where proteolytically processed by Neurospora mitochondria, 12 possess an Arg, one a Lys, one a Leu and one a Val residue at this position [2]. With the purified processing peptidase of Neurospora crassa 11 precursors were tested. All of them contained an Arg residue in the -2 position and all of them were proteolytically processed (see table 1). When the arginine residue in the -2 position of a hybrid protein (containing an amino-terminal stretch of the precursor to cytochrome b_2 fused to dihydrofolate reductase

Table 1
Processing of precursor proteins by the purified processing peptidase. The arrows mark the cleavage sites: ↓ determined, **↓** putative; [] uncleavable by Neurospora enzyme; u, unpublished results. Basic (+) and acidic (−) amino acids are indicated

Neurospora crassa:				
F_1ATPase β	AlaLeuSerArg$^+$Phe	**↓**	AlaSerSerAlaGly	[26]
F_0ATPase 9	ValSerLys$^+$Arg$^+$Thr	↓	IleGlnThrGlySer	[12]
Rieske Fe/S protein	Arg$^+$AlaValArg$^+$Ala	↓	LeuThrThrSerThr	[27]
Cytochrome c_1	PheAlaLys$^+$Arg$^+$Ser	↓	AlaSerThrGlnSer	[28]
Cytochrome oxidase IV	ThrValValArg$^+$Cys	↓	AsnAlaGlu$^-$ThrLys$^+$	[29]
Cyclophilin	SerLys$^+$AlaArg$^+$Ala	**↓**	PheSerGlnThrSer	[30]
PEP	ProPheArg$^+$Arg$^+$Ala	↓	Lys$^+$AlaThrProHis	[14]
MPP	Asp$^-$Asp$^-$AlaArg$^+$Gly	↓	Lys$^+$AlaThrArg$^+$Ala	[18]
Saccharomyces cerevisiae:				
Cytochrome b_2	AspThrIleArg$^+$Ala	↓	ThrGlySerThrVal	[u]
ATP 11 protein	Lys$^+$LeuProArg$^+$Phe	**↓**	TyrSerLeuAsnAla	[31]
ATP 12 protein	ProValPheArg$^+$Phe	**↓**	TyrSerSerSerPro	[31]
Bovine:				
Phosphate carrier	AsnLeuAlaAlaAla	↓	AlaValGlu$^-$Glu$^-$Gln	[32]
Adrenodoxin (pBAdx-4)	SerGlyArg$^+$AlaGln	↓	SerSerSerGlu$^-$Asp$^-$	[33]
Human:				
Ornithine transcarbamylase	PheMetValArg$^+$Asn	↓	PheArg$^+$CysGlyGln	[6]
	−5 −4 −3 −2 −1		+1 +2 +3 +4 +5	[Ref.]

[pb2DHFR]) was exchanged against a glycine residue, processing did not occur with Neurospora mitochondria, nor with the purified Neurospora enzyme [unpublished results: ARRETZ, M., U. WIENHUES, B. GUIARD, W. NEUPERT].

The results suggest that the positively charged amino acid in the −2 position plays an important role in the processing of precursor proteins of Neurospora crassa and yeast. In addition to the nature of the amino acids, the cleavage site of the presequence and the mature part may be important.

The features determining cleavage sites of precursor proteins of mammalian cells are less clear. Only 8 out of 23 mammalian precursor proteins analyzed thus far have an arginine residue in the −2 position. In an *in vitro* system, where the processing was monitored with purified processing peptidase of rat, only one out of 7 precursor proteins had a positively charged amino acid in the −2 position [8, 17, 22]. This implies that the rat liver enzyme has a somewhat different specificity as compared to the fungal enzymes. In this context it is not surprising that some precursor proteins from mammalian organisms (adrenodoxin and phosphate carrier from bovine) were not processed by the purified Neurospora enzyme (see table 1), whereas the purified rat enzyme was observed to process precursors from Neurospora and yeast [22].

The mitochondrial processing peptidase is an endopeptidase; the enzyme in matrix fractions from rat liver mitochondria as well as the purified Neurospora enzyme makes a single cut, and the resulting prepeptide could be recovered [17, unpublished results M. ARRETZ and W. NEUPERT]. Despite that there is no common motif in the presequences, the enzyme is able to specifically cleave several hundred precursor proteins. We propose that the high specificity of cleavage is based on the cooperative action of the two components

MPP and PEP. Both may recognize certain structures in the presequence and/or the mature part of precursor proteins and thereby contribute to specific recognition of the cleavage site.

MPP is a member of a new protease class

In order to group MPP of Neurospora crassa into one of the four well characterized protease classes, i.e. serine proteases, metalloproteases, cysteine proteases and the carboxyl proteases, we analyzed the influence of several inhibitors on the catalytic activity of MPP; we also searched for sequence similarities of MPP to known proteases.

Phenylmethylsulfonylfluoride (PMSF), an inhibitor of serine proteases, has no effect on MPP. The metal chelators EDTA and o-phenanthroline abolished completely the processing of precursors by MPP (see Table 2). This led to the suggestion that MPP is a metalloprotease [4, 14, 16, 17]. However, by sequence comparison of Neurospora MPP with known metalloproteases we did not find any similarity [35].

MPP of Neurospora and yeast contain four cysteines at identical positions. We addressed the question if these cysteines are involved in processing. When Neurospora MPP was treated with the sulfhydryl-modifying reagent N-ethylmaleimide (NEM), processing activity was strongly reduced. With *p*-chloromercuribenzoate, MPP was also completely inhibited. These results indicated that at least one thiol group of MPP is necessary for catalytic activity. However, specific inhibitors of cysteine proteases, chicken cystatin [36] and E-64 [37] did not affect the catalytic activity of MPP (see Table 2). Therefore, it is more likely that the cysteine(s) are conformational determinants rather than being involved in the catalytic event. This is supported by the finding of NEM-insensitivity of the purified peptidase from rat liver mitochondria [22].

Attempts to detect sequence similarities to several members of the known classes of proteases were negative [18, 38].

Other proteases involved in protein topogenesis such as bacterial leader peptidase, eukaryotic signal peptidase of endoplasmatic reticulum or the thylakoidal peptidase of chloroplasts could also not be grouped into the known protease classes. They show a similar unusual specificity as mitochondrial processing peptidase, since they also cleave hundreds

Table 2
Effect of inhibitors on the catalytic activity of Neurospora MPP. The processing activity of MPP (25 ng, supplemented with 25 ng PEP) without addition of inhibitors was set to 100%. The assay was performed at 25 °C for 30 min with Neurospora $F_1\beta$ as a substrate

Additions	Concentration [mM]	Relative activity [%]
PMSF	1 – 5	100
EDTA	2 – 5	< 5
o-Phenanthroline	5.0	< 5
1,10-Phenanthroline	5.0	< 5
N-ethylmaleimide	10.0	< 5
Iodoacetate	10.0	33
Iodoacetamide	10.0	65
p-Chloromercuribenzoate	0.01	< 5
Chicken cystatin	0.001 – 0.5	100
E-64	0.02 – 0.5	100
Pepstatin	0.0001 – 0.1	100

of different preproteins which have little or no sequence similarity of the prepieces and (at least with the chloroplast enzyme) limited similarity of the cleavage sites. In summary, the mitochondrial processing enzyme appears to belong to a new class of proteases with an unknown, yet rather *intriguing* catalytic mechanism.

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