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Wentian Luo · Hong Fang · Neil Green

Substrate specificity of inner membrane peptidase in yeast mitochondria

Abstract The inner membrane protease (IMP) cleaves intra-organelle sorting peptides from precursor proteins in mitochondria of the yeast *Saccharomyces cerevisiae*. An unusual feature of the IMP is the presence of two catalytic subunits, Imp1p and Imp2p, which recognize distinct substrate sets even though both enzymes belong to the same protease family. This nonoverlapping substrate specificity was hypothesized to result from the recognition of distinct residues at the P'_1 position (also termed $+1$ position) in the protease substrates. Here, we constructed an extensive series of mutations to obtain a profile of the critical cleavage site residues in IMP substrates and conclude that Imp1p, and not Imp2p, recognizes specific P'_1 residues. In addition to its specificity for P'_1 residues, Imp1p also shows substrate specificity for the P_3 (-3) position. In contrast, Imp2p recognizes the P_1 (-1) position and the P_3 position. Based on this new understanding of IMP substrate specificity, we conducted a survey for candidate IMP substrates in mammalian mitochondria and found consensus Imp2p cleavage sites in mammalian precursors to cytochrome c_1 and glycerol-3-phosphate (G-3-P) dehydrogenase. Presence of a putative Imp2p cleavage site in G-3-P dehydrogenase was surprising, as its yeast ortholog contains an Imp1p cleavage site. To address this issue experimentally, we performed the first co-expression of mammalian IMP with proposed mammalian IMP precursors in yeast and show that murine precursors to cytochrome c_1 and G-3-P dehydrogenase are cleaved by murine Imp2p. These results suggest, surprisingly, G-3-P dehydrogenase has switched from Imp1p in yeast to Imp2p in mammals.

W. Luo · H. Fang · N. Green (✉)
Department of Microbiology & Immunology,
Vanderbilt University Medical Center, 1161 21st Ave. S.,
A5217MCN, Nashville, TN 37232-2363, USA
E-mail: neil.green@vanderbilt.edu
Tel.: +1-615-3430453
Fax: +1-615-3437392

Introduction

The inner membrane protease (IMP) cleaves signal peptides from at least five proteins in mitochondria of the yeast *Saccharomyces cerevisiae*. These cleavage events are mediated by three nuclearly encoded IMP subunits (Imp1p, Imp2p, and Som1p) that are imported into mitochondria where they bind to each other and to the mitochondrial inner membrane (Schneider et al. 1991; Nunnari et al. 1993; Jan et al. 2000; Liang et al. 2004). Imp1p and Imp2p are catalytic subunits, and Som1p plays an auxiliary role by improving Imp1p cleavage efficiency. Imp1p and Imp2p belong to the type-I signal peptidase (SP) family of enzymes, which includes endoplasmic reticulum SP and many SPs found in eubacteria (Paetzel et al. 2002). In contrast to the functional conservation exhibited by most enzymes in the type-I SP family, Imp1p and Imp2p display nonoverlapping substrate specificity (Nunnari et al. 1993). Imp1p has four known substrates: the precursors to NADH-cytochrome (cyt) b_5 reductase, cyt b_2, FAD-dependent glycerol-3-phosphate (G-3-P) dehydrogenase, and cyt c oxidase (subunit II) of complex IV in the electron transport chain (Pratje and Guiard 1986; Hahne et al. 1994; Esser et al. 2004). Imp2p has only one known substrate: the precursor to cyt c_1 of complex III in the electron transport chain (Nunnari et al. 1993). Involvement of IMP substrates in electron transport probably accounts for the fact that the IMP is required for cell respiration in yeast.

Type-I SPs are generally thought to recognize small uncharged amino acids at the P_1 (-1) position and, to a lesser extent, P_3 (-3) position in the signal peptides that they cleave (von Heijne 1986). Imp1p substrate specificity violates this "$-1-3$ rule" as it tolerates a variety of structurally distinct residues at the P_1 position (Chen

et al. 1999). The P'_1 (+1) position has, instead, been implicated in Imp1p and Imp2p substrate specificity, although only one amino acid substitution at the P'_1 position has been examined to date (Luo et al. 2003). Here, we report the first identification of consensus Imp1p and Imp2p substrate recognition sequences and, with this information, present the first experimental evidence of probable substrates for mammalian Imp2p.

Materials and methods

Site-directed mutagenesis

Two-step polymerase chain reaction (PCR) (Luo et al. 2003) was used to create mutations in this study. To mutagenize *CYB2* encoding *p*-cyt b_2, upstream primer (5′-AAA CTG CAG ATG CTA AAA TAC AAA CCT TTA-3′) and downstream primer (5′-CCG GAA TTC TGC ATC CTC AAA TTC TGT TAA-3′) were paired with a series of internal oligonucleotides whose sequences include the mutations employed in this study (sequences of internal oligonucleotides are available by request). The generated DNA fragments were employed in a second PCR with upstream and downstream primers. Each DNA fragment was restricted with *PstI* and *EcoRI* and introduced into PHF454 (2 μ *TRP1*) immediately downstream of *ADH1* promoter and upstream of DNA encoding a triple HA tag (Liang et al. 2004). To mutagenize *CYT1* gene encoding *p*-cyt c_1, a two-step PCR reaction included upstream primer (5′-AAA CTG CAG ATG TTT TCA AAT CTA TCT AAA CG-3′), downstream primer (5′- CCG GAA TTC CTT TCT TGG TTT TGG TGG ATT-3′), and internal oligonucleotides that contain the sequences of mutations employed in this study. Downstream primer (5′-CCG GAA TTC AAT GGA ACC ACC TGG GAA GTA-3′) was used in the construction encoding C-223, a truncation that contains the N-terminal 223 residues of *p*-cyt c_1.

Biochemical procedures

Pulse-chase methods were used as described previously (Luo et al. 2003) with the following modifications. Yeast cells were cultured overnight at 30°C to log-phase, and 4 OD_{600} equivalents of cells were used for each experiment. After washing cells in 1 ml distilled water, cells were cultured in media lacking methionine and cysteine for 60 min, and cells were labeled with 48 μCi of ^{35}S-methionine/cysteine. *CYB2* expressing cells were labeled for 10 min and chased for 30 min with excess unlabeled methionine/cysteine. *CYT1* expressing cells were labeled for 15 min, and murine G-3-P dehydrogenase expressing cells were labeled for 15 min. Collected cells were broken using glass beads in 200 μl 10% TCA. Protein pellets were resuspended in 20 μl SDS-PAGE sample buffer

and boiled for 5 min. After boiling, the material was mixed with a solution containing 0.75 ml phosphate-buffered saline with 1%Triton X-100 (PBS-T) and a protease inhibitor cocktail. Cell debris was cleared by centrifugation, and rat anti-HA high affinity monoclonal antibody (10-μl) was added. Following incubation at 4°C for 3 h, 20-μl agarose conjugated protein G beads were added, and this was incubated overnight at 4°C with rotation. The agarose beads were collected and washed twice with PBS-T and twice with distilled water. SDS-PAGE sample buffer (20 μl) was added, and the mixture was boiled for 5 min. The sample was subjected to a second immunoprecipitation as described above. Proteins were resolved on a 7% SDS-PAGE gel for cyt b_2, 10% SDS-PAGE gel for cyt c_1 and 12% SDS-PAGE gel for C-223.

To detect yeast-expressed murine cyt c_1, an immunoprecipitation/immunoblotting procedure was employed. Yeast cells were grown to log phase, and 20 OD_{600} cell equivalents were collected and treated with 0.3 μg/ml lyticase (Sigma) for 1 h at room temperature. Cells were subjected to centrifugation for 1 min in an eppendorf centrifuge. Pellet was resuspended with 0.2 ml 10% TCA. After vortexing and centrifugation, the supernatant was removed. The pellet was suspended in 20 μl of SDS-PAGE sample buffer, and proteins were subjected to the above-mentioned immunoprecipitation procedure. Proteins were then resolved on a 10% SDS-PAGE gel and transferred onto a Hybond-ECL nitrocellulose membrane (Amersham Bioscience, Germany). The membrane was treated in blocking solution with 5% nonfat dry milk in Tris-buffered saline with 1% Tween 20 for 1 h at room temperature. The membrane was then treated with high affinity anti-HA-peroxidase (3F10) (25 mU/ml) for 1 h at room temperature.

Cloning of murine genes encoding Imp2p, cyt c_1 precursor, and G-3-P dehydrogenase precursor for yeast expression

Murine genes were PCR amplified from a mouse liver cDNA library, Marathon Ready cDNA (Clontech). Upstream (5′-CGG AGG ATC CAT GGC ACA GTC ACA AAG CTG GGC GAG AAG ATG C-3′) and downstream (5′-CGG TGA ATT CTT ATT TCT CAC CAG TCT GGA GTG GAC AGC GCT C-3′) oligonucleotides were used to amplify the gene encoding Imp2p. Upstream (5′-CGG GAT CCA TGG CGG CGG CGG CGG CTT CG-3′) and downstream (5′-CGG AAT TCC TTG GGT GGC CGA TAA GCC AG-3′) oligonucleotides were used to amplify the gene encoding the cyt c_1 precursor. Upstream (5′-CGC GGA TCC ATG GCG TTT CAA AAG CA GTG-3′) and downstream (5′-CCG GAA TTC CAA TCC TCC ACA ACT ACG GTC-3′) primers were used to amplify the gene encoding G-3-P dehydrogenase. The amplified products were introduced into yeast expression vectors

pHF454 and pHF455 (13) between the *BamHI* and *EcoRI* restriction sites.

Results

Imp1p substrate recognition

A typical substrate for Imp1p is the cyt b_2 precursor (*p*-cyt b_2), a protein with a bipartite presequence in yeast (Guiard 1985). The N-terminal portion of the bipartite presequence is cleaved by mitochondrial processing peptidase, an enzyme that cleaves import signals from many mitochondrial proteins (Verner and Schatz 1988). Cleavage of *p*-cyt b_2 liberates intermediate cyt b_2 (*i*-cyt b_2), which is cleaved in turn by Imp1p to generate mature cyt b_2 (Guiard 1985). The *i*-cyt b_2 cleavage site contains P'_1E and P_3I residues. To assess importance of the P'_1 position, we employed site-directed mutagenesis (Materials and methods), substituting the natural glutamyl residue with a series of amino acids representing differences in charge, polarity, and size (Fig. 1). The mutant proteins (C-terminally tagged with HA) were expressed in yeast strain JNY34 (*Δimp2*)/pXC3, which has a functional Imp1p and a nonfunctional Imp2p (Chen et al. 1999). Log-phase cells (4 OD_{600} cell equivalents) were subjected to pulse chase as described in Materials and methods, and proteins were precipitated from cell extracts using anti-HA antibodies. As shown in Fig. 1, Imp1p cleaved *i*-cyt b_2 when glutamic acid (lane 1) or aspartic acid (lane 10) was present at the P'_1 position. Other residues strongly inhibited Imp1p cleavage of *i*-cyt b_2, thus revealing a strong preference for acidic residues at the P'_1 position and supporting the notion that the P'_1 position is important for Imp1p substrate specificity.

To identify residues tolerated at the P_3 position in *i*-cyt b_2, the natural isoleucine residue was substituted with amino acids listed in Fig. 2. The mutant constructs (C-terminally tagged with HA) were introduced into strain JNY34 (*Δimp2*)/pXC3, and cells were analyzed by pulse chase. Imp1p was able to efficiently cleave *i*-cyt b_2 when isoleucine (lane 1) and valine (lane 5) were present at the P_3 position. Remaining substitutions inhibited
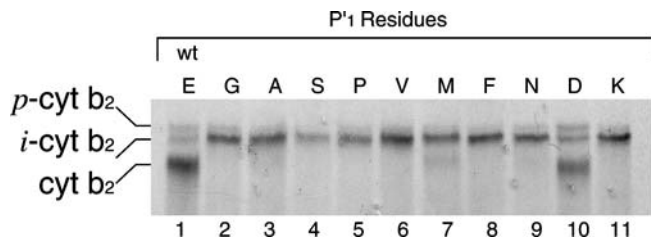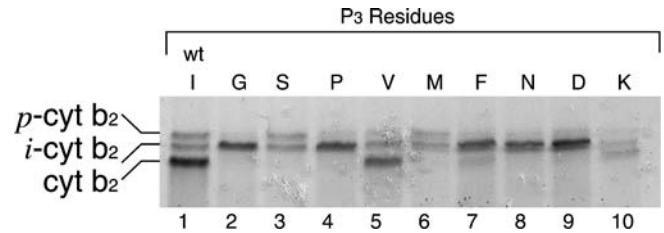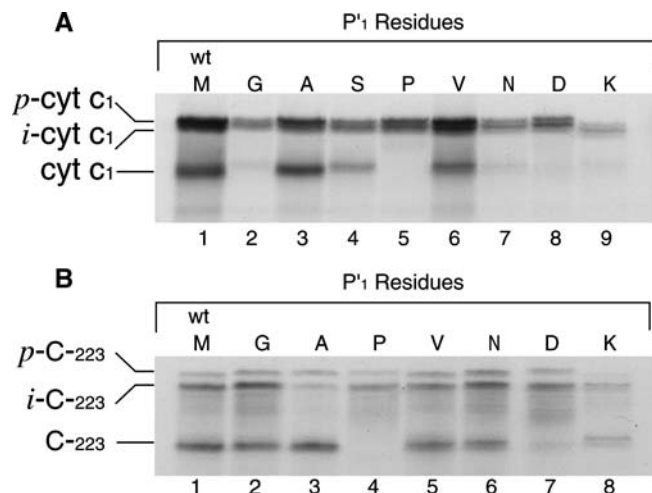

Fig. 2 Amino acid substitutions at the P_3 position of cyt b_2 precursor. Strain JNY34/pXC3 was subjected to a pulse-chase analysis as described in Materials and methods. The *lane 1* depicts wild-type *p*-cyt b_2 (3HA). Amino acid substitutions are indicated at the *top of lanes 2–10*

substrate cleavage, indicating Imp1p prefers hydrophobic (nonaromatic) residues at the P_3 position in its substrates. The P_3 lysyl substitution reduced expression of *i*-cyt b_2 in yeast and produced a protein band with reduced molecular mass (lane 10). While this band could result from Imp1p cleavage of *i*-cyt b_2, a more likely interpretation is that poor expression resulted from instability of mutated *i*-cyt b_2 and generated distinct degradation product.

Imp2p substrate recognition

The *p*-cyt c_1 precursor is the only known Imp2p substrate in yeast mitochondria (Nunnari et al. 1993). As with *p*-cyt b_2, *p*-cyt c_1 contains a bipartite signal peptide (Sadler et al. 1984). The Imp2p cleavage site consists of P'_1M, P_1A, and P_3A. To determine whether P'_1M is important for Imp2p substrate recognition, a series of structurally distinct amino acid residues was placed at the P'_1 position of *i*-cyt c_1. DNA constructs encoding these mutant proteins (HA-tagged) were introduced into yeast strain XCY101 (*Δimp1*) that expresses Imp2p but lacks Imp1p (Chen et al. 1999). As shown by pulse labeling, *i*-cyt c_1 was cleaved efficiently when P'_1M residue was present (Fig. 3a, lane 1) and when methionine was replaced with alanine (lane 3), serine (lane 4), and valine (lane 6). This result suggests that Imp2p does not recognize a specific P'_1 residue, although five P'_1 residues (glycine, proline, asparagine, aspartic acid, and lysine) inhibited Imp2p processing of *i*-cyt c_1 (Fig. 3a).

In a prior study, C-terminal cyt c_1 sequences were found to interfere with cleavage of mutated *i*-cyt c_1, probably through a conformational effect (Luo et al. 2003). We therefore prepared a *p*-cyt c_1 truncation (*p*-C-223) consisting of amino acids 1–223 including the bipartite signal peptide. When structurally distinct residues were introduced into the P'_1 position of *p*-C-223, we observed efficient cleavage of substrates that have P'_1M (Fig. 3b, lane 1), P'_1G (lane 2), P'_1A (lane 3), P'_1V (lane 5), and P'_1N (lane 6). There could also be a small amount of cleavage with the P'_1K substitution (lane 8). These data strongly support the notion that the P'_1 position is not critical for Imp2p substrate recognition.

Fig. 1 Amino acid substitutions at the P'_1 position of cyt b_2 precursor. Strain JNY34/pXC3 was subjected to a pulse-chase analysis as described in Materials and methods. The *lane 1* depicts wild-type *p*-cyt b_2 (3HA). Amino acid substitutions are indicated at the *top of lanes 2–11*

Fig. 3 Amino acid substitutions at the P'_1 position of cyt c_1 precursor and truncation C-223. Strain XCY101 was subjected to a pulse-labeling analysis as described in Materials and methods. *Lane 1* depicts wild-type *p*-cyt c_1-(3HA) (**a**) and a truncated form of *p*-cyt c_1-(3HA), termed *p*-C-223 (3HA) (**b**). Amino acid substitutions in *p*-cyt c_1 (**a** *lanes 2–9*) and *p*-C-223 (**b** *lanes 2–8*) are indicated at the *top of each lane*

Despite an apparent tolerance for structurally distinct amino acids at the P'_1 position, the prolyl substitution (Fig. 3b, lane 4), and the aspartyl substitution (lane 7) strongly inhibited cleavage of *i*-C-223. The prolyl substitution probably alters the conformation of the signal peptide cleavage site in a manner similar to that seen with eubacterial signal peptides (Shen et al. 1991). The basis for the inhibitory effect of the aspartyl substitution is unclear, although this effect is consistent with the previously observed cleavage defect when a glutamyl substitution was examined at the P'_1 position of *i*-cyt c_1 (Luo et al. 2003). Indeed, that substitution led us to suggest that the P'_1 position may be important for Imp2p substrate recognition. However, present data indicate that structurally distinct residues are tolerated at the P'_1 position.

Having eliminated a specific role for the P'_1 residue in Imp2p substrates, we next examined the P_1 and P_3 positions. Considering the above-described negative effect of C-terminal *p*-cyt c_1 sequences, we utilized *i*-C-223 for our analysis. All examined substitutions at the P_1 position inhibited cleavage of *i*-C-223 (Fig. 4, compare lane 1 to lanes 2–10), revealing a strict requirement for the naturally occurring alanyl residue. For P_3 substitutions, we observed efficient cleavage of the substrate with the natural alanyl residue (Fig. 5, lane 1). Substitutions of glycine (lane 2), serine (lane 3), and valine (lane 4) also permitted Imp2p cleavage, albeit with reduced efficiency. Remaining P_3 substitutions strongly inhibited Imp2p cleavage (lanes 5–10). These data reveal a preference for small uncharged residues, small polar residues, and at least one hydrophobic residue at the P_3 position, a pattern similar to that exhibited by most type-I SPs (von Heijne 1986).
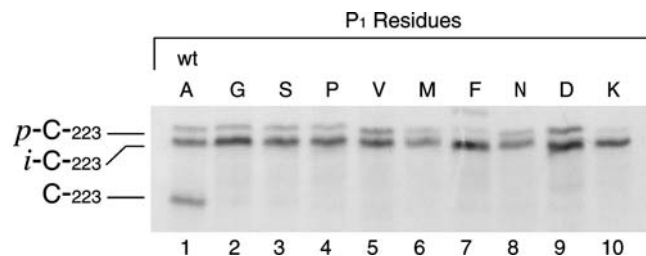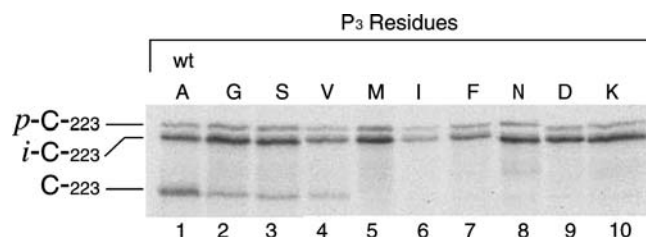

Fig. 4 Amino acid substitutions at the P_1 position of C-223. Strain XCY101 was subjected to a pulse-labeling analysis using methods described in Materials and methods. Amino acids present at the P_1 position are indicated at the *top of lanes 1–10*
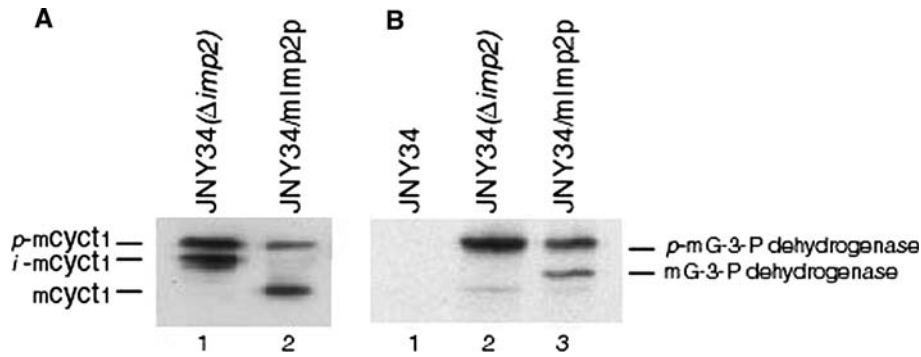
Candidate substrates for mammalian IMP

Our identification of IMP substrate motifs in yeast led us to search for IMP substrates in mammals. Obvious candidates include mammalian orthologs of the known IMP substrates in yeast. The mammalian cyt c_1 precursor has an 84-residue presequence that is similar in length to the 61-residue presequence of its yeast ortholog (Sadler et al. 1984; Suzuki et al. 1989). The cleavage site consists of P_1A and P_3V. As shown above, these residues are tolerated by yeast Imp2p. As the mammalian *IMP2* gene has been identified and shown to complement *Δimp2* mutant yeast (Petek et al. 2001; Burri et al. 2005), we expressed murine (m) *IMP2* in yeast strain JNY34 (*Δimp2*) and asked whether the corresponding protein could cleave the mcyt c_1 precursor. For this and subsequent analyses, mammalian genes were PCR amplified from a murine cDNA library (Materials and methods). Results depicted in Fig. 6a, lane 1, show that, as with its yeast counterpart, mcyt c_1 precursor (C-terminal HA tag) has a bipartite signal peptide (*p*-cyt c_1 and *i*-cyt c_1), which was seen in strain JNY34 (*Δimp2*) that lacked Imp2p and Imp1p as a consequence of the instability of Imp1p in cells that lack Imp2p (Nunnari et al. 1993). Importantly, mature cyt c_1 was present in strain JNY34/mImp2p (lane 2), indicating mImp2p cleaved m*i*-cyt c_1.

G-3-P dehydrogenase has a 37 residue presequence in yeast and a 42 residue presequence in mammals (Esser et al. 2004; Brown et al. 1994). Yeast G-3-P dehydrogenase has a conventional Imp1p substrate motif and is cleaved by Imp1p in yeast (Esser et al. 2004). The

Fig. 5 Amino acid substitutions at the P_3 position of C-223. Strain XCY101 was subjected to a pulse-labeling analysis using methods described in Materials and methods. Amino acids present at the P_3 position are indicated at the *top of lanes 1–10*

Fig. 6 Identification of probable substrates for mammalian Imp2p. **a**. Cells of strains JNY34 (*Δimp2*)/mcyt c_1 precursor (*lane 1*) and JNY34/mImp2p/mcyt c_1 precursor (*lane 2*) were immunoblotted with anti-HA antibodies as described in Materials and methods. **b**. Cells of strains JNY34 (*lane 1*), JNY34/mG-3-P dehydrogenase precursor (*lane 2*) and JNY34/mImp2p/mG-3-P dehydrogenase precursor (*lane 3*) were subjected to a pulse-labeling analysis using methods described in Materials and methods

mammalian ortholog has a cleavage site that, surprisingly, resembles an Imp2p substrate motif (P_1A P_3V), raising the possibility of Imp2p cleavage. To test this possibility, the gene encoding G-3-P dehydrogenase was PCR amplified from a murine cDNA library, and the protein (C-terminal HA tag) was expressed in yeast strains JNY34 (*Δimp2*) and JNY34 (*Δimp2*)/mImp2p (Materials and methods). Cells were then subjected to a 15-min pulse, and proteins were precipitated with anti-HA antibodies. As shown in Fig. 6b, G-3-P dehydrogenase was, indeed, cleaved by mImp2p.

Discussion

Based on the analysis of many amino acid substitutions in IMP substrate cleavage sites, Imp1p recognizes hydrophobic (non-aromatic) residues at the P_3 position and acidic residues at the P'_1 position in yeast cells, suggesting the P_3 and P'_1 positions provide the principal determinants of Imp1p substrate specificity. On the other hand, Imp2p recognizes small uncharged or small polar residues at the P_3 position and an alanyl residue at the P_1 position, indicating Imp2p displays substrate specificity similar to that of most type-I signal peptidases. Utilizing this information, we asked whether mammalian orthologs of known IMP substrates possess recognizable IMP cleavage sites. As expected, mammalian cyt c_1 precursor, an Imp2p substrate in yeast, has an Imp2p cleavage site motif (P_3V and P_1A). Indeed, murine cyt c_1 precursor was cleaved by murine Imp2p in a novel yeast assay employing co-expressed protease/substrate pairs. Unexpectedly, murine G-3-P dehydrogenase precursor, an Imp1p substrate in yeast, has an Imp2p substrate motif (P_3V and P_1A). Consistent with this prediction, G-3-P dehydrogenase was cleaved by murine Imp2p. This result strongly suggests that G-3-P dehydrogenase has switched from Imp1p to Imp2p in the evolution of mammalian mitochondria.

Study of the yeast Imp1p has demonstrated not only cleavage of G-3-P dehydrogenase precursor but also precursors to cyt c oxidase (subunit II), cyt b_2, and NADH-cyt b_5 reductase. Our survey for potential Imp1p substrate recognition motifs in the mammalian orthologs of these yeast proteins revealed (1) cyt c oxidase (subunit II) does not have a cleavable presequence in mammalian mitochondria (Steffens and Buse 1979; Anderson et al. 1982) (2) mammalian cells appear not to have an ortholog to cyt b_2, and (3) NADH-cyt b_5 reductase, although present in mammalian mitochondria, appears not to contain a cleavable signal peptide (Tomatsu et al. 1989; Borgese et al. 1996). As such, known Imp1p substrates in yeast, including G-3-P dehydrogenase, are probably not Imp1p substrates in mammalian mitochondria. Although mammalian *IMP1*-like gene has been identified, it does not complement the respiration defect in *Δimp1* mutant yeast (Burri et al. 2005). These issues lead us to question whether mammalian Imp1p has enzymatic activity or whether mammalian Imp1p exhibits substrate specificity distinct from that of yeast Imp1p. While the yeast system presents clear opportunities to monitor enzymatic properties of mammalian Imp2p, a functional understanding of additional IMP subunits is lacking and will probably require examination inside mammalian mitochondria or in vitro with purified protein.

References

Anderson S, de Bruijn MH, Coulson AR, Eperon IC, Sanger F, Young IG (1982) Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. J Mol Biol 156:683–717

Borgese N, Aggujaro D, Carrera P, Pietrini G, Bassetti M (1996) A role for N-myristoylation in protein targeting: NADH-cytochrome b_5 reductase requires myristic acid for association with outer mitochondrial but not ER membranes. J Cell Biol 135:1501–1513

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Brown LJ, MacDonald MJ, Lehn DA, Moran SM (1994) Sequence of rat mitochondrial glycerol-3-phosphate dehydrogenase cDNA. Evidence for EF-hand calcium-binding domains. J Biol Chem 269:14363–14366

Burri L, Strahm Y, Hawkins C, Gentle IE, Puryer MA, Verhagen A, Callus B, Vaux D, Lithgow T (2005) Mature DIABLO/Smac is produced by the IMP protease complex on the mitochondrial inner membrane. Mol Biol Cell 16:2926–2933

Chen X, VanValkenburgh C, Fang H, Green N (1999) Signal peptides having standard and nonstandard cleavage sites can be processed by Imp1p of the mitochondrial inner membrane protease. J Biol Chem 274:37750–37754

Esser K, Jan PS, Pratje E, Michaelis G (2004) The mitochondrial IMP peptidase of yeast: functional analysis of domains and identification of Gut2 as a new natural substrate. Mol Genet Genomics 271:616–626

Guiard B (1985) Structure, expression and regulation of a nuclear gene encoding a mitochondrial protein: the yeast L (+)-lactate cytchrome c oxidoreductase (cytchrome b2). EMBO J 4:3265–3272

Hahne K, Haucke V, Ramage L, Schatz G (1994) Incomplete arrest in the outer membrane sorts NADH-cytochrome b_5 reductase to two different submitochondrial compartments. Cell 79:829–839

von Heijne G (1986) A new method for predicting signal sequence cleavage sites. Nucleic Acids Res 14:4683–4690

Jan P-S, Esser K Pratje E, Michaelis G (2000) Som1, a third component of the yeast mitochondrial inner membrane peptidase complex that contains Imp1 and Imp2. Mol Gen Genet 263:483–491

Liang H, Luo W, Green N, Fang H (2004) Cargo sequences are important for Som1p-dependent signal peptide cleavage in yeast mitochondria. J Biol Chem 279:39396–39400

Luo W, Chen X, Fang H, Green N (2003) Factors governing nonoverlapping substrate specificity by mitochondrial inner membrane peptidase. J Biol Chem 278:4943–4948

Nunnari J, Fox TD, Walter P (1993) A mitochondrial protease with two catalytic subunits of nonoverlapping specificites. Science 262:1997–2004

Paetzel M, Karla A, Strynadka NC, Dalbey RE (2002) Signal peptidases. Chem Rev 102:4549–4580

Petek E, Windpassinger C, Vincent JB, Cheung J, Boright AP, Scherer SW, Kroisel PM, Wagner K (2001) Disruption of a novel gene (IMMP2L) by a breakpoint in 7q31 associated with Tourette syndrome. Am J Hum Genet 68:848–858

Pratje E, Guiard B (1986) One nuclear gene controls the removal of transient presequences from two yeast proteins: one encoded by the nuclear the other by the mitochodrial genome. EMBO J 5:1313–1317

Sadler I, Suda K, Schatz G, Kaudewitz F, Haid A (1984) Sequencing of the nuclear gene for the yeast cytochrome c_1 precursor reveals an unusually complex aminoterminal presequence. EMBO J 3:2137–2143

Schneider A, Behrens M, Scherer P, Pratje E, Michaelis G, Schatz G (1991) Inner membrane protease 1, an enzyme mediating intramitochondrial protein sorting in yeast. EMBO J 10:247–254

Shen LM, Lee JI, Cheng SY, Jutte H, Kuhn A, Dalbey RE (1991) Use of site-directed mutagenesis to define the limits of sequence variation tolerated for processing of the M13 procoat protein by the Escherichia coli leader peptidase. Biochemistry 30:11775–11781

Steffens GJ, Buse G (1979) Studies on cytochrome c oxidase (IV): primary structure and function of subunit II. Hoppe-Seyler's Z Physiol Chem 360:613–619

Suzuki H, Hosokawa Y, Nishikimi M, Ozawa T (1989) Structural organization of the human mitochondrial cytochrome c_1 gene. J Biol Chem 264:1368–1374

Tomatsu S, Kobayashi Y, Fukumaki Y, Yubisui T, Orii T, Sakaki Y (1989) The organization and the complete nucleotide sequence of the human NADH-cytochrome b_5 reductase gene. Gene 80:353–361

Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, Vaux DL (2000) Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 102:43–53

Verner K, Schatz G (1988) Protein translocation across membranes. Science 241:1307–1313