MITOCHONDRIAL TARGETING OF NORMAL AND MUTANT PROTOPORPHYRINOGEN OXIDASE

Mikael von und zu Fraunberg¹, Tommi Nyrönen² and Raili Kauppinen¹

¹Department of Medicine, Division of Endocrinology, University of Helsinki, Biomedicum Helsinki, Helsinki, Finland
²CSC Scientific Computing Ltd., Espoo, Finland

Running title: Mitochondrial transport of normal and mutant PPOX

Keywords: Mitochondria, protein import, mitochondrial targeting, porphyria, protoporphyrinogen oxidase, mutation

*Address:
Dr. Mikael von und zu Fraunberg
Porphyria Research Center
Department of Medicine
University Central Hospital of Helsinki
Biomedicum Helsinki
P.O.Box 700
00029 HUS, Helsinki, Finland
tel. +358-9-47171910
fax. +358-9-47171921
email:mikael.fraunberg@hus.fi
SUMMARY

We have investigated the signal sequence for mitochondrial transport of mutants (I12T, 78insC, IVS2-2a→c, 338G→C, R152C, 470A→C and L401F) and the wild-type protoporphyrinogen oxidase (PPOX), which is the penultimate enzyme in the heme biosynthesis. We constructed the corresponding green fluorescent protein (GFP)-fusion proteins and studied their intracellular localization in COS-1 cells. We showed that 28 amino acids in the amino-terminus of PPOX contain an independently functioning signal for mitochondrial targeting. The experiments with amino-terminally truncated GFP-fusion proteins revealed that amino acids 25-477 of PPOX contained an additional mitochondrial targeting signal(s). We constructed a structural model for the interaction between the amino-terminal end of PPOX and the putative mitochondrial receptor protein Tom20. The model suggests that leucine and isoleucine residues Leu8, Ile12 and Leu15 forming an \( \alpha \)-helical hydrophobic motif LXXXIXXL were crucial for the recognition of the targeting signal. The validity of the model was tested using mutants L8Q, I12T and L15Q disrupting the hydrophobic surface of the LXXXIXXL helix. The results from \textit{in vitro} expression studies and molecular modeling were in accordance supporting the hypothesis that the recognition of the mitochondrial targeting signal is dependent on hydrophobic interactions between the targeting signal and the mitochondrial receptor.
INTRODUCTION

Protoporphyrinogen oxidase (PPOX, [E.C.1.3.3.4]) is the penultimate enzyme in the heme biosynthesis (1). PPOX catalyzes the six-electron oxidation of protoporphyrinogen IX to the planar, fully conjugated macrocycle protoporphyrin IX in the inner membrane of the mitochondrion and requires oxygen for its activity (2). Partial deficiency of PPOX causes a disease, variegate porphyria (VP [MIM 176200]), which is inherited as an autosomal dominant trait displaying incomplete penetrance (3). The biochemical abnormalities found in VP patients include overproduction and increased excretion of porphyrins and porphyrin precursors. VP manifests clinically with photosensitivity and acute attacks, which include various neuropsychiatric symptoms (4,5).

PPOX is anchored to the inner membrane of mitochondria in eukaryotes (6) with its active site facing the cytosolic side of the membrane (7). The anchoring may involve amphipathic helical domains inserting PPOX into the inner mitochondrial membrane. Alternatively, post-translational acylation may facilitate transient or permanent association of PPOX with the membrane (8). The majority of proteins imported to mitochondria contain a signal sequence of 20 to 60 amino acids in their amino-terminus that directs them into mitochondria (9). The mitochondrial targeting signals of different polypeptides show no amino acid sequence identity, but they have characteristic physico-chemical properties. They are enriched in positively charged, hydroxylated and hydrophobic residues, have no acidic residues and usually are able to form an amphiphilic secondary structure (10,11). There is evidence that several amino-terminal mitochondrial targeting signals interact with the general import receptor Tom20 (translocase of outer membrane), which is a part of the TOM complex (12,13) (for a review, see (14,15)).
The amino-terminus of PPOX contains a characteristic $\beta\alpha\beta$ dinucleotide-binding motif (Figure 1A), which is often found within flavine adenine dinucleotide-binding domains (16). The amino-terminus of PPOX is also a putative mitochondrial targeting domain, since it contains 3 basic residues and no acidic residues, and it is capable of forming an $\alpha$-helix (17). Of the more than 110 mutations reported in the PPOX gene worldwide, five are located in this domain and potentially interfere with the mitochondrial transport (18-22).

In this communication we have investigated the mitochondrial transport of seven PPOX mutants by expressing the green fluorescent protein (GFP)–fusion proteins in COS-1 cells and studying their intracellular localization. The mitochondrial targeting signal in the amino-terminus of PPOX was characterized and a model for the interaction between the targeting signal and the mitochondrial receptor Tom20 was proposed.

MATERIAL AND METHODS

Seven mutations selected for this study were previously identified from Finnish VP patients and expressed in E.Coli and COS-1 cells (Table 1) (21,22).

Normal and mutant PPOX-GFP constructs - The normal and mutant PPOX-cDNAs were expressed as CT-GFP fusion proteins in COS-1 cells using the pcDNA3.1/CT-GFP-vector (Invitrogen, CA, USA). The human PPOX-pUC18 (kindly provided by Prof. S. Taketani, Kyoto Institute of Technology, Kyoto, Japan) was digested with EcoRI and XbaI and the fragment including exons 1-7 was ligated as a cassette to the pcDNA3.1/CT-GFP–vector with T4 DNA ligase (The New England Biolabs, Beverly, MA, USA). Exons 7-13 were amplified using PPOX-pUC18 as a template and primers #1 and #2, which abolished the stop codon (Table 2). The PCR-fragment was digested with XbaI and ligated as a cassette into
PPOX-(ex1-7)-pcDNA3.1/CT-GFP to obtain a full length PPOX-GFP. The constructs for the mutations I12T, R152C and 470A→C were made by digesting the mutated PPOX-pUC18 constructs (21,22) with PpuMI and ligating each fragment with a mutation as a cassette into the corresponding sites of the wild type PPOX-GFP. For the 78insC mutation, the mutated PPOX-pUC18 was amplified with primers #3 and #4. The PCR-fragment was digested with EcoRI and XbaI and ligated as a cassette into the PPOX-GFP to replace the wild type cDNA resulting in PPOX(1-28)-GFP. The constructs for the mutations IVS2-2a→c and 338G→C were made similarly using anti-sense primers #5 and #6, respectively. cDNA of each construct was sequenced to confirm their authenticity.

**PPOX-GFP constructs with deletions or missense mutations in the amino-terminus** - For PPOX(Δ5-11)-GFP, exons 2-7 were amplified using PPOX-pUC18 as a template and primers #7 and #8. The PCR-fragment was digested with EcoRI and Eco47III and ligated as a cassette into PPOX-GFP. PPOX(Δ5-24)-GFP was created similarly using primers #8 and #9. For PPOX(1-28)-L8Q-GFP, exons 2-7 were amplified using PPOX-pUC18 as a template with primers #4 and #10. The PCR-fragment was digested with EcoRI and XbaI and ligated as a cassette into PPOX-GFP to replace the wild type cDNA. PPOX(1-28)-L15Q-GFP was created similarly using primers #3 and #11 and PPOX(1-28)-I12T-GFP with primers #3 and #4 together with PPOX-I12T-GFP as a template.

**COS-1 cell culture and DNA transfection** - COS-1 cells were maintained in Dulbecco’s modified Eagle’s medium (Gibco BRL, U.K.) supplemented with 10% fetal heat-inactivated bovine serum and penicillin (10000 U/ml, Gibco BRL, U.K.) and streptomycin (10000 U/ml). For transfection, the cells were seeded on a 3 cm 6-well plate at 400 000 cells/well and grown overnight. The 50-70% confluent cells were transfected with 1.5 μg of the PPOX-GFP or
mutated constructs by lipofection using FuGENE6 transfection reagent (Boehringer Mannheim, Germany). The pcDNA3.1/CT-GFP-vector alone was used as a negative control in all experiments.

GFP fluorescence, mitochondrial staining and confocal laser scanning microscopy - For GFP fluorescence studies, COS-1 cells were trypsinized and replated on glass coverslips 24 hours after transfection. For mitochondrial staining, 40 nM MitoTracker Red CMXRos (Molecular Probes, Eugene, OR) was added to media for 45 minutes at 48 hours post-transfection. The cells were fixed immediately with 3% paraformaldehyde for 30 min in a room temperature. After fixation, the cells were washed three times in PBS and mounted in Mowiol 4-88 mounting medium (Calbiochem, Germany). The cells were observed on a Leica TCS confocal laser scanning microscope (Leica Microsystems GmBH, Heidelberg, Germany). Fluorescence of GFP was excited using a 488 nm argon/krypton laser, and emitted fluorescence was detected with a 500-530 nm band pass filter. For Mitotracker Red, a 543 nm helium-neon laser was used for excitation and fluorescence was detected with a 565-699 nm band pass filter. For 3D imaging, a stack of 30 images with 0.20 µm distance was taken and the 3D picture was constructed using the Leica Confocal Software 2.00.

RESULTS

Model of the interaction of PPOX with Tom20 - Analysis of the amino-terminal sequence of PPOX using the Swiss-Model and MolMol programs predicts that a 11-residue polypeptide, PPOX(11-21), forms an α-helical structure, where hydrophobic residues are clustered together (Figure 1) ((23,24); http://www.expasy.org/swissmod/SWISS-MODEL.html, com-
pared with MAO-B structure (pdb-entry: 1GOS) with 42.7% amino acid identity as a template).

The model of PPOX(6-23) bound to Tom20 was constructed in the Bodil Modeling Environment (http://www.abo.fi/fak/mnf/bkf/research/johnson/bodil.html) using the coordinates of the NMR structure of rat Tom20 in a complex with a 11-residue recognition peptide from rat aldehyde dehydrogenase, pALDH(12-22), as a template (pdb-entry: 1OM2). pALDH(12-22) contains a hydrophobic LXXLL motif in an α-helical conformation, where leucines are in contact with a hydrophobic groove on the surface of Tom20 (Figure 2B) (25). Rat Tom20 shows a 98 percent amino acid identity with human Tom20 and the amino acids in the vicinity of the hydrophobic groove are fully conserved (25).

When the program Malign (26) in Bodil was used in comparison of PPOX(1-23) and pALDH(12-22), the sequence GPRLSRLLSYA in pALDH(12-22) aligns with the GGGISGLAASY part of the PPOX(1-23) sequence at best. The most important determinant of this comparison was alignment of the ISGLA motif in PPOX(1-23) with the LSRLL motif in pALDH(12-22). ISGLA is the only part in PPOX(1-23) that has a similar hydrophobic surface in the α-helical conformation as the α-helical LSRLL motif in pALDH(12-22) which is needed for the interaction with Tom20 (25). The model of the 18-residue PPOX(6-23) was constructed using Sybyl 6.8 (Tripos Inc., St. Louis, MO, USA) (Figure 2A).

The α-helical model of PPOX(6-23) was superimposed on the coordinates of pALDH(12-22) in a complex with Tom20 applying a constraint that the LXXLL motif in pALDH(12-22) must be aligned with the IXXLA motif in PPOX(6-23). Sterical clashes were removed manually by modifying the amino acid side chains using Bodil Modeling Environment. The α-helical structure of pALDH(12-22) bound to Tom20 ends amino-terminally to Pro13, whereas in our model of PPOX(6-23) (VVLGGISGLAASYHLSR) the α-helix (underlined) continues an additional turn to facilitate the contact of Leu8 with Tom20 (Figure
2C). Together the residues 8-15 (LXXXIXXL) of PPOX(6-23) form a continuous hydrophobic surface on one face of the α-helix (Figure 2A). The validity of the model was tested using mutants Leu8, Ile12 and Leu15 that disrupt the hydrophobic face of the LXXXIXXL helix.

The amino-terminus of PPOX contains a mitochondrial targeting signal - We investigated the transport of the wild-type and seven PPOX mutants to mitochondria by constructing green fluorescent protein (GFP)-fusion proteins, in which GFP located at the carboxyl-terminal end of PPOX (Figure 3). PPOX-GFP and GFP-construct alone were used as controls in all experiments. COS-1 cells were transfected with the constructs and GFP fluorescence was analyzed with confocal laser scanning microscopy to monitor subcellular localization of the polypeptides. The cells expressing the wild type PPOX-GFP demonstrated a typical filamentous mitochondrial pattern (27,28), which was clearly distinguishable from the cytosolic pattern of GFP-construct alone (Figure 3A-B). The localization was confirmed by counter-staining with a mitochondrion-specific dye (Figure 3C). A pattern indistinguishable from PPOX-GFP was observed for each of the seven mutants (Figure 3D) indicating that transport to mitochondria was not impaired in any of them. Since the truncated PPOX fusion protein corresponding to the mutation 78insC in exon 2 (PPOX(1-28)-GFP) contained only 28 first residues of PPOX, these amino acids must contain an independently functioning mitochondrial targeting signal.

The amino acids 25-477 of PPOX contain an alternative mitochondrial targeting signal(s) - After localization of the mitochondrial targeting signal in the amino-terminus of PPOX, two additional constructs were created, where this region was removed either totally (PPOX(Δ5-23)-GFP) or partially (PPOX(Δ5-11)-GFP) (Figure 4A). The experiments using fluorescence confocal microscopy demonstrated that these fusion proteins were associated with the mito-
chondria indicating that the amino acids 25-477 of PPOX must contain an additional mitochondrial targeting signal(s).

To make a hypothesis for the molecular recognition of the amino-terminal mitochondrial targeting signal of PPOX, we constructed an $\alpha$-helical model of PPOX(6-23), and a model of the interaction between PPOX(6-23) and the mitochondrial import receptor Tom20. The model places the LXXXIXXL (Leu8, Ile12 and Leu15) motif of PPOX(6-23) in contact with the hydrophobic groove on the Tom20 surface (Figure 2B). We tested our structural model by mutating the PPOX(6-23) residues Ile12 into threonine and residues Leu8 and Leu15 into glutamine. These amino acid substitutions disrupt the hydrophobic face of the LXXXIXXL helix model (Figure 4C) which should affect the capability of interaction with Tom20. Each mutation caused a disruption of PPOX transport into mitochondria (Figure 4B-C) confirming that each of these residues was essential for the PPOX targeting. In the amino-terminus of PPOX, the LXXXIXXL motif including residues Leu8, Ile12 and Leu15 appeared to be crucial for the putative interaction between PPOX and Tom20.

DISCUSSION

In this communication, we have investigated the signal sequence for the mitochondrial transport of the wild-type PPOX and mutants by constructing the corresponding GFP-fusion proteins. The intracellular localization of the seven mutants corresponding to clinically manifest VP showed no impairment of mitochondrial targeting. Because of the naturally occurring mutation 78insC, we were able to show that the first 28 amino acids in the amino-terminus of PPOX contained sufficient information for transporting a reporter protein into mitochondria.

The predicted secondary structure of the PPOX amino-terminus consists of a $\beta\alpha\beta$-motif (17), where the $\alpha$-helix is a common structure found in mitochondrial targeting
signals. Our structural model of interaction between the amino-terminus of PPOX and Tom20 and the experiments with amino-terminally mutated fusion proteins indicated that the critical residues for the recognition of the PPOX targeting signal include leucine and isoleucine residues that form a hydrophobic motif LXXXIXXL. Our findings support the hypothesis that recognition of the mitochondrial targeting signal is dependent on hydrophobic interactions with the mitochondrial receptor. The NMR structure of rat Tom20 in a complex with the mitochondrial presequence peptide revealed that an amphiphilic α-helical structure of the presequences was important for binding to the receptor (25). Subsequent mutagenesis studies showed that the hydrophobic residues were essential for binding to Tom20, while the hydrophilic residues, including two positively charged arginines in the presequence, were dispensable (25). Consistently, in the amino-terminal recognition sequence of PPOX, replacing a single hydrophobic leucine or isoleucine with a hydrophilic residue of the same size could prevent the mitochondrial transport.

The amino-terminus of PPOX contains only three positively charged residues, which is less than in most presequences (29). It has been postulated that the positively charged residues could be involved in subsequent ionic interactions between the targeting signal and Tom22, which is also known to bind amino-terminal recognition sequences, especially their carboxyl-terminal parts, in a salt-sensitive manner (13,30). In the case of PPOX, two positively charged residues Arg23 and Lys29, which are located in the carboxyl-terminal part of the recognition sequence, could facilitate this interaction.

Surprisingly, PPOX derivatives where the amino-terminal targeting signal was removed either totally or partially, were still located in the mitochondria. This implies that the residual part of PPOX must contain an additional mitochondrial targeting signal(s) (29). PPOX is a further example of mitochondrial proteins whose import is not strictly dependent on the presence of an amino-terminal presequence (29,31-33). Secondary structure prediction
of PPOX reveals several internal leucine-rich α-helical segments with a net positive charge. Such segments can putatively form hairpin-like structures that mimic a typical amphiphilic presequence and function as an internal mitochondrial targeting sequence (34). Without knowledge of the tertiary structure of PPOX it is, however, difficult to predict which of these segments could be accessible to the receptor and serve as an effective targeting signal.

Since the naturally occurring mutation I12T resides in the conserved amino-terminus of PPOX, the mutation could interfere with mitochondrial transport and modify the phenotype of the disease. Our study shows that although the I12T substitution is able to disrupt the mitochondrial transport of the truncated PPOX, the corresponding full-length PPOX is transported into mitochondria. *In vitro* expression studies of the I12T substitution have shown a dramatic loss of the enzyme activity both in prokaryotic and eukaryotic cells (21). A homozygous patient with the I12T substitution has been identified with around 10% residual PPOX activity measured from his lymphocytes (21). It would be intriguing to hypothesize that in this patient the secondary mitochondrial targeting signal(s) could serve as a backup system which directs the polypeptide into mitochondria if the primary signal fails. This transport may be, however, less specific and efficient (35) and lead to non-optimal mitochondrial compartmentalization. This could disrupt the final steps of the heme biosynthesis in the inner mitochondrial membrane, especially if the substrate channeling between the last three enzymes of the pathway, namely coproporphyrinogen oxidase, PPOX, and ferrochelatase, occurs through an enzyme complex as suggested by (7). Disruption of the enzyme complex would explain the low ferrochelatase activity (10% and 30-40% of normal) measured from the homozygous and heterozygous patients' erythrocytes, respectively.
ACKNOWLEDGMENTS

We thank Dr. Marc Baumann for excellent assistance in protein chemistry and Dr. Pekka Lehtovuori for assistance in molecular modeling. This study was supported by grants from the Finnish Cultural Foundation, Magnus Ehrnrooth Foundation, the Instrumentarium Research Foundation, Jalmari and Rauha Ahokas Foundation, the Research Funds and the Clinical Research Institute of the Helsinki University Central Hospital, the Biomedicum Helsinki Foundation and the University of Helsinki.

ELECTRONIC-DATABASE INFORMATION

Human Gene Mutation Database, http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html (for mutations in the PPOX gene)
REFERENCES


**Figure 1.** (A) Alignment of PPOX sequences. Sequences were aligned using the MaxHom dynamic multiple sequence alignment program (36). Residues are numbered according to the human sequence. Invariant residues are shaded in gray. Genbank database entries shown are: *Homo sapiens* (X99450); *Mus musculus* (U25114); *Schizosaccharomyces pombe* (Z68136); *Myxococcus xanthus* (M73709); *Arabidopsis thaliana* (AF104919); *Propionibacterium freudenreichii shermanii* (D85417); *Nicotiana tabacum* (Y13466); *Bacillus subtilis* (M97208); *Saccharomyces cerevisiae* (Z71381). (B) Tertiary structure of the first 87 amino acids of PPOX. The model was constructed using program Swiss-Model (23) (http://www.expasy.org/swissmod/SWISS-MODEL.html) with MAO-B structure (pdb-entry: 1GOS) as a template. Secondary structure calculation and figure drawing were carried out using MolMol (24). (C) α-helical wheel diagram of the first 18 amino acids of PPOX. Hydrophilic, hydrophobic and positively charged residues are shaded white, black and gray, respectively.

**Figure 2.** (A) Structure and solvent accessible surface of the α-helical rat aldehyde dehydrogenase presequence peptide, pALDH(12-22) (left), and the model of PPOX(6-23) (right). Hydrophobic and polar regions are colored blue and red, respectively. (B) Solvent accessible surface of the Tom20 receptor (pdb-entry: 1OM2). The boxed hydrophobic groove on the surface of the receptor is important for the interaction between Tom20 and pALDH(12-22) (25). (C) Model of the interaction between Tom20 (gray) and PPOX(6-23) (blue). The α–helixes in Tom20 are numbered according to Abe et al. (25). pALDH(12-22) in a complex with Tom20 (NMR structure, pdb-entry: 1OM2) is shown in orange. The hydrophobic L8, I12 and L15 residues are organized on one side of the helix in the model. The model locates the motif LXXXIXXL of PPOX(6-23) in contact with the hydrophobic groove on the surface of Tom20.
**Figure 3.** (A) 3D image of COS-1 cells transfected with PPOX-GFP or GFP alone. After transfection the cells were fixed and analyzed by fluorescence confocal microscopy. *Above* GFP alone showing a diffuse cytosolic pattern. *Below* PPOX-GFP demonstrating the filamentous network of mitochondria in the cell (28). (B) Schematic presentation of the wild type PPOX-GFP and mutants. *Above* Exon structure of human PPOX-cDNA. *Below* Translations for each fusion polypeptides are shown; open bars represent normally translated amino acids and solid bars represent nonsense amino acids. (C) Subcellular localization of the wild type PPOX-GFP and GFP alone. *Left* GFP fluorescence. *Center* MitoTracker Red fluorescence. *Right* Overlay image of GFP and MitoTracker fluorescence. (D) GFP fluorescence of cells transfected with seven different PPOX-GFP mutants.

**Figure 4.** (A) PPOX-GFP fusion proteins with modified amino-termini and GFP fluorescence of transfected COS-1 cells. The numbers refer to the amino acids of PPOX. (B) PPOX(1-28)-GFP fusion proteins with modified amino-termini and GFP fluorescence of transfected COS-1 cells. The mutated residues are shown in a black box. (C) Solvent accessible surfaces of the α-helical PPOX(6-23) models. Hydrophobic and polar regions are colored blue and red, respectively.
<table>
<thead>
<tr>
<th>Location</th>
<th>Mutation</th>
<th>Outcome</th>
<th>Residual activity (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>35T→C&lt;sup&gt;a&lt;/sup&gt;</td>
<td>I12T</td>
<td>3% 1%</td>
</tr>
<tr>
<td>Exon 2</td>
<td>78insC</td>
<td>Frameshift</td>
<td>0 0</td>
</tr>
<tr>
<td>IVS 2</td>
<td>IVS2-2 a→c</td>
<td>34 bp retention of intron 2</td>
<td>0 0</td>
</tr>
<tr>
<td>Exon 4</td>
<td>338G→C</td>
<td>Deletion of exon 4</td>
<td>5% 0</td>
</tr>
<tr>
<td>Exon 5</td>
<td>454C→T</td>
<td>R152C</td>
<td>5% 5%</td>
</tr>
<tr>
<td>Exon 5</td>
<td>470A→C</td>
<td>Deletion of exon 5 and 19 bp retention of intron 5</td>
<td>1% 0</td>
</tr>
<tr>
<td>Exon 11</td>
<td>1203A→C</td>
<td>L401F</td>
<td>N.D. N.D.</td>
</tr>
</tbody>
</table>

<sup>a</sup>The mutation I12T co-segregated with the polymorphism P256T (767C→G)

<sup>b</sup>(21,22)

N.D. not done
Table 2. Oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GGC TAC TCC GCC CTT CAC CCC</td>
</tr>
<tr>
<td>2</td>
<td>CAT GAA TGA GAG TTC TAG ATC CGC TGT TAG G</td>
</tr>
<tr>
<td>3</td>
<td>GAT TAC GAA TTC GGG GGG AGA ACA G</td>
</tr>
<tr>
<td>4</td>
<td>CTC TCC ACT AGG ACC TCT AGA GGG GGG GCA</td>
</tr>
<tr>
<td>5</td>
<td>CGA ATC CAG CCT TCT AGA CGC TCC CTG CTC</td>
</tr>
<tr>
<td>6</td>
<td>CTG TGC ACA GTC TCT AGA GGC TCT TTG CCC</td>
</tr>
<tr>
<td>7</td>
<td>GTG GAA TTC CGC ATG GGC CGG ACC ATC AGC GGC TTG GCC GCC AGT TAC CAC</td>
</tr>
<tr>
<td>8</td>
<td>CTT CCA GCG CCC TTC TGC CTG GAG</td>
</tr>
<tr>
<td>9</td>
<td>GTG GAA TTC CGC ATG GGC CGG ACC CCC TGC CCC CCT AAG GTG GTC</td>
</tr>
<tr>
<td>10</td>
<td>GTG GAA TTC CGC ATG GGC CGG ACC GTG GTC GTG CAG GGC G</td>
</tr>
<tr>
<td>11</td>
<td>CT AGG ACC TCT AGA GGG GGG GCA GGG GGC CCG GCT CAG GTG GTA ACT GGC GGC CTG GCC GC</td>
</tr>
</tbody>
</table>
Figure 3

A

<table>
<thead>
<tr>
<th>PPOX cDNA</th>
<th>5'</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11/12</th>
<th>13</th>
<th>3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I12T</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>78insC</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVS2-2a→c</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>338G→C</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R152C</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>470A→C</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L401F</td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

C

PPOX wt - GFP

MitoTracker

merged

GFP

MitoTracker

merged

D

I12T - GFP

78insC - GFP

IVS2-2a→c - GFP

338G→C - GFP

R152C - GFP

470A→C - GFP

L401F - GFP
Mitochondrial targeting of normal and mutant protoporphyrinogen oxidase
Mikael von und zu Fraunberg, Tommi Nyrönen and Raili Kauppinen

J. Biol. Chem. published online January 28, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M300151200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/early/2003/01/28/jbc.M300151200.citation.full.html#ref-list-1