Peroxisomal protein import

Azevedo, JE., Fransen, M., May, B., Van Veldhoven, PP.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

The contents of this document may be freely copied and distributed in any media, provided the authors, plus the institutions, are credited, as stated under the terms of Creative Commons Attribution 4.0 International (CC BY 4.0) License. For more information see our license.

04/07/2019
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 69

This document contains 2 pathways and 19 reactions (see Table of Contents)
Peroxisomal protein import

Stable identifier: R-HSA-9033241

Compartments: cytosol, peroxisomal matrix, peroxisomal membrane

Peroxisomes are small cellular organelles that are bounded by a single membrane and contain variable compositions of proteins depending on cell type. Peroxisomes function in oxidation of fatty acids, detoxification of glyoxylate, and synthesis of plasmalogens, glycerophospholipids containing an alcohol with a vinyl-ether bond (reviewed in Lohdi and Semenkovich 2014). All of the approximately 46 proteins contained in peroxisomal matrix are imported from the cytosol by a unique mechanism that does not require the imported proteins to be unfolded as they cross the membrane (Walton et al. 1995, reviewed in Ma et al. 2011, Fujiki et al. 2014, Baker et al. 2016, Dias et al 2016, Emmanoulidis et al. 2016, Erdmann 2016, Francisco et al. 2017). The incompletely characterized process appears to involve the transport of the proteins through a variably sized pore in the membrane comprising at least PEX5 and PEX14 (inferred from the yeast homologs in Meinecke et al. 2010, the yeast pore is reviewed in Meinecke et al. 2016). Oligomeric proteins are also observed to cross the peroxisomal membrane (Otera and Fujiki 2012) but their transport appears to be less efficient than monomeric proteins (Freitas et al. 2011, inferred from mouse homologs in Freitas et al. 2015, reviewed in Dias et al. 2016).

In the cytosol, receptor proteins, PEX5 and PEX7, bind to specific sequence motifs in cargo proteins (Dodt et al. 1995, Wiemer et al. 1995, Braverman et al. 1997). The long and short isoforms of PEX5 (PEX5L and PEX5S) bind peroxisome targeting sequence 1 (PTS1, originally identified in firefly luciferase by Gould et al. 1989) found on most peroxisomal matrix proteins; PEX7 binds PTS2 (originally identified in rat 3-ketoacyl-CoA thiolase by Swinkels et al. 1991) found on 3 imported proteins thus far in humans. The long isoform of PEX5, PEX5L, then binds the PEX7:cargo protein complex (Braverman et al. 1998, Otera et al. 2000). PEX5S,L bound to a cargo protein or PEX5L bound to PEX7:cargo protein then interacts with a complex comprising PEX13, PEX14, PEX2, PEX10, and PEX12 at the peroxisomal membrane (Gould et al. 1996, Fransen et al. 1998, inferred from rat homologs in Reguenga et al. 2001).
The ensuing step in which the cargo protein is translocated across the membrane is not completely understood. During translocation, PEX5 and PEX7 become inserted into the membrane (Wiemer et al. 1995, Dodt et al. 1995, Oliveira et al. 2003) and expose a portion of their polypeptide chains to the organellar matrix (Rodrigues et al. 2015). One current model envisages PEX5 as a plunger that inserts into a transmembrane barrel formed by PEX14, PEX13, PEX2, PEX10, and PEX12 (the Docking-Translocation Module) (Francisco et al. 2017).

After delivering cargo to the matrix, PEX5 and PEX7 are recycled back to the cytosol by a process requiring mono-ubiquitination of PEX5 and ATP hydrolysis (Imanaka et al. 1987, Thoms and Erdmann 2006, Carvalho et al. 2007). PEX7 is not ubiquitinated but its recycling requires PEX5 mono-ubiquitination. A subcomplex of the Docking-Translocation Module comprising the RING-finger proteins PEX2, PEX10, and PEX12 conjugates a single ubiquitin to a cysteine residue of PEX5 (Carvalho et al. 2007, reviewed in Platta et al. 2016). The mono-ubiquitinated PEX5 and associated PEX7 are then extracted by the exportomer complex consisting of PEX1, PEX6, PEX26, and ZFAND6 (inferred from rat homologs in Miyata et al. 2012). PEX1 and PEX6 are members of the ATPases Associated with diverse cellular Activities (AAA) family, a group of proteins that use the energy of ATP hydrolysis to remodel molecular complexes. PEX1 and PEX6 form a hetero-hexameric ring, best described as a trimer of PEX1/PEX6 dimers (inferred from yeast in Platta et al. 2005, yeast homologs reviewed in Schwerter et al. 2017). Data on the yeast PEX1:PEX6 complex suggest that these ATPases use a substrate-threading mechanism to disrupt protein-protein interactions (Gardner et al. 2018). PEX7 is also then returned to the cytosol (Rodrigues et al. 2014). Once in the cytosol, ubiquitinated PEX5 is enzymatically deubiquitinated by USP9X and may also be non-enzymatically deubiquitinated by nucleophilic attack of the thioester bond between ubiquitin and the cysteine residue of PEX5 by small metabolites such as glutathione (Grou et al. 2012).


**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Author/Reviewed/Reviewed</th>
<th>Author/Reviewed/Reviewed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-15</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
PEX5S,L binds cargo proteins containing PTS1

Location: Peroxisomal protein import

Stable identifier: R-HSA-9033233

Type: binding

Compartments: cytosol

Inferred from: Pex5l binds Acox1 or Uox (Mus musculus)

It is unclear how the long isoform of PEX5 (PEX5L) and the short isoform of PEX5 (PEX5S) are generated. A current hypothesis suggests alternative mRNA splicing. Both isoforms can bind the peroxisome targeting signal 1 (PTS1) located at the C-terminus of most of the proteins that are targeted to the peroxisomal matrix (Dodt et al. 1995, Fransen et al. 1995, Wiemer et al. 1995, Gatto et al. 2000, Brocard et al. 2003, Gatto et al. 2003, Harper et al. 2003, Ghosh and Berg 2010, Freitas et al. 2011, Okumoto et al. 2011). PTS1 typically contains Ser-Lys-Leu (SKL) at the C-terminus but substantial variation in sequences and affinities for PEX5 are observed and upstream residues can modulate binding to PEX5 (Lametschwandtner et al. 1998, Ghosh and Berg 2010, reviewed in Brocard and Hartig 2006).

A minority of peroxisomal matrix proteins contain PTS2. While the PEX5S isoform binds proteins containing PTS1, the PEX5L isoform binds either proteins containing PTS1 or PEX7 bound to proteins containing PTS2 (Braverman et al. 1998). Some proteins appear to be imported as oligomers, however this is rather inefficient as PEX5 appears to have a preference for monomeric substrates (Otera and Fujiki 2012, Freitas et al. 2011, Freitas et al. 2015, also inferred from mouse homologs). Mutations in PEX5 cause defects in peroxisomal import and comprise complementation group 2 of peroxisomal biogenesis disorders (also called Zellweger spectrum disorders) (Dodt et al. 1995, Wiemer et al. 1995). A specific mutation affecting only the PEX5L isoform is the cause of rhizomelic chondrodysplasia punctate type 5 (Barøy et al. 2015).


Literature references


Location: Peroxisomal protein import

Stable identifier: R-HSA-9033236

Type: binding

Compartments: peroxisomal membrane


Preceded by: PEX5S,L binds cargo proteins containing PTS1

Followed by: Cargo of PEX5S,L translocates from the cytosol to the peroxisomal matrix

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Authors/Editors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-19</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
Cargo of PEX5S,L translocates from the cytosol to the peroxisomal matrix

Location: Peroxisomal protein import

Stable identifier: R-HSA-9033235

Type: uncertain

Compartments: peroxisomal membrane

After binding the Docking and Translocation Module comprising PEX14, PEX13, PEX2, PEX10 and PEX12, PEX5S or PEX5L bound to a cargo protein becomes localized to the membrane (Dodt et al. 1995, Wiemer et al. 1995, Alencastre et al. 2009, Francisco et al. 2013, Dias et al. 2017). In a reaction that is not yet fully characterized, the cargo protein is released into the peroxisomal matrix while PEX5S or PEX5L remains in the membrane (Dodt et al. 1995, Wiemer et al. 1995, Alencastre et al. 2009, Francisco et al. 2013). One model for the reaction hypothesizes that PEX13:PEX14 (associated with PEX2:PEX10:PEX12) forms a barrel in the peroxisomal membrane while PEX5S or PEX5L acts as a plunger to guide the cargo through the barrel (Dias et al. 2017, Francisco et al. 2017). Notably, the reaction does not require a source of energy such as ATP (Oliveira et al. 2003). Mutations in PEX5 cause defects in import of PTS1-containing proteins or PTS2-containing proteins or both (Eberrink et al. 2009, Barøy et al. 2015).


Followed by: PEX2:PEX10:PEX12 binds PEX5S,L (in PEX5S:PEX13:PEX14) and Ub:UBE2D1,2,3

Literature references


### Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Role</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-19</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
PEX2:PEX10:PEX12 binds PEX5S,L (in PEX5S:PEX13:PEX14) and Ub:UBE2D1,2,3

Location: Peroxisomal protein import

Stable identifier: R-HSA-8953917

Type: binding

Compartments: peroxisomal membrane

A RING E3 ubiquitin ligase complex containing PEX10, PEX12, and PEX2 ubiquitinates PEX5L. The PEX2:PEX10:PEX12 complex is believed to bind an activated E2-ubiquitin conjugate (one of Ub:UBE2D1, Ub:UBE2D2, Ub:UBE2D3) and PEX5L in a complex that also contains PEX13 and PEX14 (Chang et al. 1999, Carvalho et al. 2007, Grou et al. 2008, Grou et al. 2009, Okumoto et al. 2011). The short isoform of PEX5, PEX5S, is inferred to undergo the same reaction.

Preceded by: Cargo of PEX5S,L translocates from the cytosol to the peroxisomal matrix

Followed by: PEX2:PEX10:PEX12 monoubiquitinates PEX5S,L at cysteine-11

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Details</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-01-01</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
PEX2:PEX10:PEX12 monoubiquitinates PEX5S,L at cysteine-11

**Location:** Peroxisomal protein import

**Stable identifier:** R-HSA-8953946

**Type:** transition

**Compartments:** peroxisomal membrane

The RING-type E3 ubiquitin ligase sub-complex PEX2:PEX10:PEX12 catalyzes the transfer of ubiquitin from an E2-ubiquitin conjugate (one of Ub:UBE2D1, Ub:UBE2D2, or Ub:UBE2D3) to the cysteine-11 residue of the substrate PEX5L, the peroxisomal matrix protein shuttling receptor (Carvalho et al. 2007; Grou et al. 2008, Okumoto et al. 2011, Sargent et al. 2016, inferred from yeast in Dodt and Gould 1996). The thiol ester bond between ubiquitin and the cysteine residue of PEX5 is unusual among ubiquitin substrates, which usually have isopeptide bonds between ubiquitin and a lysine residue. Monoubiquitination of PEX5 at cysteine-11 is an integral and mandatory step in the PEX5-mediated peroxisomal protein transport pathway; in its absence, PEX5 cannot be extracted from the peroxisomal membrane docking/translocation machinery (the peroxisomal protein translocon), and thus transport of newly synthesized peroxisomal matrix proteins to the organelle matrix stops (Grou et al. 2009). In addition to monoubiquitinating PEX5 during peroxisomal protein import, the PEX2:PEX10:PEX12 complex has also been implicated in pexophagy, a type of selective autophagy targeting peroxisomes. Pexophagy seems to be triggered mainly by ubiquitination of PEX5, which, in this case, can occur either at its cysteine-11 or lysine-209 residues, but ubiquitination of ABCD3 (also known as PMP70) and other peroxisomal membrane proteins may also be involved (Zhang et al. 2015, inferred from mouse in Nordgren et al. 2015, Sargent et al. 2016).

**Preceded by:** PEX2:PEX10:PEX12 binds PEX5S,L (in PEX5S:PEX13:PEX14) and Ub:UBE2D1,2,3


**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-01-01</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>

**Location:** Peroxisomal protein import

**Stable identifier:** R-HSA-9033533

**Type:** binding

**Compartments:** peroxisomal membrane

PEX1:PEX6:PEX26 (also known as the Receptor Export Module or peroxisomal AAA ATPase complex) extracts ubiquitinated PEX5S or PEX5L in the peroxisomal membrane (Tamura et al. 2006, Tamura et al. 2014). PEX1 and PEX6 are soluble proteins that form a hexameric ring bound to PEX26 in the peroxisomal membrane (Matsumoto et al. 2003, Weller et al. 2005). ZFAND6 (AWP1) probably binds to ubiquitinated PEX5 and PEX6 and acts as an export factor (Miyata et al. 2012).

**Preceded by:** PEX2:PEX10:PEX12 monoubiquitinates PEX5S,L at cysteine-11


**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Edition</th>
<th>Date</th>
<th>Authorship</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>

Location: Peroxisomal protein import

Stable identifier: R-HSA-9033505

Type: uncertain

Compartments: peroxisomal membrane


Ubiquitinated PEX5 isoform S or isoform L (Ub:PEX5S,L) is released from the peroxisomal membrane and interaction with the Docking and Translocation Module by PEX1:PEX6:PEX26:ZFAND6 (the peroxisomal AAA ATPase complex, receptor export module) (Tamura et al. 2014, Law et al. 2017, also inferred from yeast homologs). PEX1 and PEX6 form a cytosolic hexameric ring that is anchored to the peroxisomal membrane by PEX26. Hydrolysis of ATP by PEX1 and PEX6 appears to cause a conformational change in PEX1:PEX6:PEX26:ZFAND6 that releases Ub:PEX5S,L from the peroxisomal membrane and into the cytosol (reviewed in Saffert et al. 2017).


Followed by: USP9X binds Ub:PEX5S, USP9X hydrolyzes Ub:PEX5S yielding PEX5S and Ubiquitin, USP9X binds Ub:PEX5L

Literature references


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Authors/Editors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
The deubiquitinating enzyme USP9X binds cytosolic ubiquitinated PEX5S (Ub:PEX5S) and then hydrolyzes the thioester bond between the carboxyl terminus of ubiquitin and cysteine-11 of PEX5S (inferred from the large isoform of PEX5 in Grou et al. 2012).


**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Authors/Editors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
The deubiquitinating enzyme USP9X hydrolyzes the thioester bond between the carboxyl terminus of ubiquitin and cysteine-11 of PEX5S (inferred from the large isoform of PEX5L in Grou et al. 2012). The thioester bond is unstable and appears also to be spontaneously disrupted by nucleophilic attack of small metabolites such as reduced glutathione (Grou et al. 2009).


Literature references


Editions

<table>
<thead>
<tr>
<th>Edition Date</th>
<th>Action</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>

https://reactome.org
PEX7 binds cargo proteins containing PTS2

**Location:** Peroxisomal protein import

**Stable identifier:** R-HSA-9033232

**Type:** binding

**Compartments:** cytosol


**Followed by:** PEX5L binds PEX7:Cargo protein

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Author/Reviewer</th>
<th>Editors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-15</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
PEX5L binds PEX7:Cargo protein

Location: Peroxisomal protein import

Stable identifier: R-HSA-9033240

Type: binding

Compartments: cytosol

Inferred from: PEX5L binds PEX7:Acaa1a (Cricetulus griseus)

The long isoform of PEX5, PEX5L, binds PEX7 that is already bound to a PTS2-containing cargo protein (Braverman et al. 1998, Dodt et al. 2001, Kunze et al. 2015, Rodrigues et al. 2015). The binding of PEX5L to PEX7 increases the affinity of PEX7 for cargo protein (Kunze et al. 2015). Mutations affecting the additional sequence present only in the long isoform of PEX5 cause rhizomelic chondrodysplasia punctata type 5 (Baroy et al. 2015).

Preceded by: PEX7 binds cargo proteins containing PTS2

Followed by: PEX5L:PEX7:Cargo binds PEX13:PEX14:PEX2:PEX10:PEX12 (Docking and Translocation Module)

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-15</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>

https://reactome.org
PEX5L:PEX7:Cargo binds PEX13:PEX14:PEX2:PEX10:PEX12 (Docking and Translocation Module)

**Location:** Peroxisomal protein import

**Stable identifier:** R-HSA-9033238

**Type:** binding

**Compartments:** peroxisomal membrane

**Inferred from:** Pex14 binds PEX5L (in PEX5L:PEX7:Acaa1a) (Cricetulus griseus)


**Preceded by:** PEX5L binds PEX7:Cargo protein

**Followed by:** Cargo of PEX5L:PEX7 translocates from the cytosol to the peroxisomal matrix

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-19</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>

https://reactome.org
Cargo of PEX5L:PEX7 translocates from the cytosol to the peroxisomal matrix

Location: Peroxisomal protein import

Stable identifier: R-HSA-9033514

Type: uncertain

Compartments: peroxisomal membrane

The cargo protein bound to PEX7 is released from PEX7 into the peroxisomal matrix in a reaction that does not require ATP (Purdue et al. 1997, Dodt et al. 2001, Rodrigues et al. 2014, Rodrigues et al. 2015). PEX7 may also be released into the matrix (inferred from yeast in Nair et al. 2004), however later research indicates that PEX7 remains with PEX5L in the peroxisomal membrane (Rodrigues et al. 2015) apparently in a proteinaceous cavity (Dias et al. 2017). Mutations in PEX5 cause defects in import of PTS1-containing proteins or PTS2-containing proteins or both (Eberrink et al. 2009, Barøy et al. 2015).

Preceded by: PEX5L:PEX7:Cargo binds PEX13:PEX14:PEX2:PEX10:PEX12 (Docking and Translocation Module)

Followed by: PEX2:PEX10:PEX12 binds PEX5L (in PEX5L:PEX7:PEX13:PEX14:PEX2:PEX10:PEX12) and Ub:UBE2D1,2,3

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Authorship</th>
<th>Reviewer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
PEX2:PEX10:PEX12 binds PEX5L (in PEX5L:PEX7:PEX13:PEX14:PEX2:PEX10:PEX12) and Ub:UBE2D1,2,3

Location: Peroxisomal protein import

Stable identifier: R-HSA-9033527

Type: binding

Compartments: peroxisomal membrane

A RING E3 ubiquitin ligase sub-complex containing PEX10, PEX12, and PEX2 ubiquitinates PEX5L. PEX10:PEX12:PEX2 is believed to bind an activated E2-ubiquitin conjugate (one of Ub:UBE2D1, Ub:UBE2D2, Ub:UBE2D3) and PEX5L in a complex that also contains PEX13 and PEX14 (Chang et al. 1999, Carvalho et al. 2007, Grou et al. 2008, Grou et al. 2009, Okumoto et al. 2011).

Preceded by: Cargo of PEX5L:PEX7 translocates from the cytosol to the peroxisomal matrix

Followed by: PEX2:PEX10:PEX12 monoubiquitinates PEX5L at cysteine-11

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Authors, Editors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
The RING-type E3 ubiquitin ligase sub-complex PEX2:PEX10:PEX12 catalyzes the transfer of ubiquitin from an E2-ubiquitin conjugate (one of Ub:UBE2D1, Ub:UBE2D2, or Ub:UBE2D3) to the cysteine-11 residue of the substrate PEX5L, the peroxisomal matrix protein shuttling receptor (Carvalho et al. 2007; Grou et al. 2008, Okumoto et al. 2011, Sargent et al. 2016, inferred from yeast in Dodt and Gould 1996). In contrast to PEX5, PEX7 transiently associated with the docking and translocation module (which comprises PEX14, PEX13, PEX2, PEX10, and PEX12) is not ubiquitinated. The thiol ester bond between ubiquitin and the cysteine residue of PEX5 is unusual among ubiquitin substrates, which usually have isopeptide bonds between ubiquitin and a lysine residue. Monoubiquitination of PEX5 at cysteine-11 is an integral and mandatory step in the PEX5-mediated peroxisomal protein transport pathway; in its absence, PEX5 and PEX7 cannot be extracted from the peroxisomal membrane docking-translocation machinery (the peroxisomal protein translocon), and thus transport of newly synthesized peroxisomal matrix proteins to the organelle matrix stops (Grou et al. 2009). In addition to monoubiquitinating PEX5 during peroxisomal protein import, the PEX2:PEX10:PEX12 sub-complex has also been implicated in pexophagy, a type of selective autophagy targeting peroxisomes. Pexophagy seems to be triggered mainly by ubiquitination of PEX5, which, in this case, can occur either at its cysteine-11 or lysine-209 residues, but ubiquitination of ABCD3 (also known as PMP70) and other peroxisomal membrane proteins may also be involved (Zhang et al. 2015, inferred from mouse in Nordgren et al. 2015, Sargent et al. 2016).

**Preceded by:** PEX2:PEX10:PEX12 binds PEX5L (in PEX5L:PEX7:PEX13:PEX14:PEX2:PEX10:PEX12) and Ub:UBE2D1,2,3

**Followed by:** PEX2:PEX10:PEX12:Ub:PEX5L:PEX7:PEX13:PEX14 binds PEX1:PEX6:PEX26 and ZFAND6

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Authors/Editors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-01-01</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
PEX1:PEX6:PEX26 (known as the Receptor Export Module) extracts ubiquitinated PEX5L from the peroxisomal membrane Docking and Translocation Module (Tamura et al. 2006, Tamura et al. 2014). PEX1 and PEX6 are soluble proteins that form a hexameric ring bound to PEX26 in the peroxisomal membrane (Matsumoto et al. 2003, Welle et al. 2005). ZFAND6 (AWP1) probably binds to ubiquitinated PEX5 and PEX6 and acts as an export factor (Miyata et al. 2012).

**Preceded by:** PEX2:PEX10:PEX12 monoubiquitinates PEX5L at cysteine-11

**Followed by:** PEX1:PEX6:PEX26:ZFAND6 dissociates Ub:PEX5L and PEX7 from PEX14:PEX13:PEX2:PEX10:PEX12 and translocates PEX5L and PEX7 from the peroxisomal membrane to the cytosol

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Authors/Editors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
PEX1:PEX6:PEX26:ZFAND6 dissociates Ub:PEX5L and PEX7 from PEX14:PEX13:PEX2:PEX10:PEX12 and translocates PEX5L and PEX7 from the peroxisomal membrane to the cytosol

Location: Peroxisomal protein import

Stable identifier: R-HSA-9033499

Type: uncertain

Compartments: peroxisomal membrane

Ubiquitinated PEX5 isoform L (Ub:PEX5L) is released from the peroxisomal membrane Docking and Translocation Module by PEX1:PEX6:PEX26 (the peroxisomal AAA ATPase complex, receptor export module) (Tamura et al. 2014, Law et al. 2017, also inferred from yeast homologs). PEX1 and PEX6 form a cytosolic hexameric ring that is anchored to the peroxisomal membrane by PEX26. Hydrolysis of ATP by PEX1 and PEX6 appears to cause a conformational change in PEX1:PEX6:PEX26 that removes Ub:PEX5L from the peroxisomal membrane and into the cytosol (reviewed in Saffert et al. 2017). ZFAND6 probably binds ubiquitinated PEX5 and PEX6 and acts as an export factor (Miyata et al. 2012). Export of PEX7 back to the cytosol requires export of PEX5L but PEX7 and PEX5L appear to be exported separately (Rodrigues et al. 2014).


Followed by: USP9X binds Ub:PEX5L

Literature references


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
The deubiquitinating enzyme USP9X binds ubiquitinated PEX5L (ubiquitin conjugated to the large isoform of PEX5, Ub:PEX5L) and then hydrolyzes the thioester bond between the carboxyl terminus of ubiquitin and cysteine-11 of PEX5L (Grou et al. 2012).


**Followed by:** USP9X hydrolyzes Ub:PEX5L yielding PEX5L and Ubiquitin

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, PP., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, JE.</td>
</tr>
</tbody>
</table>
USP9X hydrolyzes Ub:PEX5L yielding PEX5L and Ubiquitin

**Location:** Peroxisomal protein import

**Stable identifier:** R-HSA-9033491

**Type:** transition

**Compartments:** cytosol

The deubiquitinating enzyme USP9X hydrolyzes the thioester bond between the carboxyl terminus of ubiquitin and cysteine-11 of PEX5L (Grou et al. 2012). The thioester bond is unstable and appears to be also spontaneously (non-enzymatically) disrupted by nucleophilic attack of small metabolites such as reduced glutathione (Grou et al. 2009).

**Preceded by:** USP9X binds Ub:PEX5L

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Author/Editor</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-20</td>
<td>Authored, Edited</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
</tr>
</tbody>
</table>

Van Veldhoven, PP., Fransen, M.

Azevedo, JE.
After proteins are imported into the peroxisome a subset of proteins are cleaved by the protease TYSND1 (Okumoto et al. 2011). Based on mutagenesis of human TYSND1 (Okumoto et al. 2011) and the homolog in Arabidopsis (Schuhmann et al. 2008), TYSND1 appears to be a trypsin-like serine protease containing a conserved histidine aspartate serine triad essential for catalysis. Mice lacking Tysnd1 have reduced peroxisomal localization of some peroxisomal enzymes and exhibit reduced beta-oxidation of fatty acids and metabolism of phytanic acid (Mizuno et al. 2013). Male mice lacking Tysnd1 are sterile due to sperm that lack acrosomal caps.

**Literature references**


<table>
<thead>
<tr>
<th>Editions</th>
<th>Authored, Edited</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017-12-22</td>
<td>Authored, Edited</td>
<td>May, B.</td>
</tr>
<tr>
<td>2018-02-13</td>
<td>Reviewed</td>
<td>Van Veldhoven, P.P., Fransen, M.</td>
</tr>
<tr>
<td>2018-03-12</td>
<td>Reviewed</td>
<td>Azevedo, J.E.</td>
</tr>
</tbody>
</table>
**Table of Contents**

- **Introduction**
- Peroxisomal protein import
  - PEX5S,L binds cargo proteins containing PTS1
  - Cargo of PEX5S,L translocates from the cytosol to the peroxisomal matrix
  - PEX2:PEX10:PEX12 binds PEX5S,L (in PEX5S:PEX13:PEX14 and Ub:UBE2D1,2,3)
  - PEX2:PEX10:PEX12 monoubiquitinates PEX5S,L at cysteine-11
  - USP9X binds Ub:PEX5S
  - USP9X hydrolyzes Ub:PEX5S yielding PEX5S and Ubiquitin
  - PEX7 binds cargo proteins containing PTS2
  - PEX5L binds PEX7:Cargo protein
  - PEX5L:PEX7:Cargo binds PEX13:PEX14:PEX2:PEX10:PEX12 (Docking and Translocation Module)
  - Cargo of PEX5L:PEX7 translocates from the cytosol to the peroxisomal matrix
  - PEX2:PEX10:PEX12 binds PEX5L (in PEX5L:PEX7:PEX13:PEX14:PEX2:PEX10:PEX12 and Ub:UBE2D1,2,3)
  - PEX2:PEX10:PEX12 monoubiquitinates PEX5L at cysteine-11
  - PEX1:PEX6:PEX26:ZFAND6:Ub:PEX5L and PEX7 from PEX14:PEX13:PEX2:PEX10:PEX12 and translocates PEX5L and PEX7 from the peroxisomal membrane to the cytosol
  - USP9X binds Ub:PEX5L
  - USP9X hydrolyzes Ub:PEX5L yielding PEX5L and Ubiquitin
  - TYSND1 cleaves peroxisomal proteins

---

[https://reactome.org](https://reactome.org)