European Journal of Cell Biology 95 (2016) 585–597

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Research paper

Pex17p-dependent assembly of Pex14p/Dyn2p-subcomplexes of the peroxisomal protein import machinery

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ARTICLE INFO

Article history:
Received 27 July 2016
Received in revised form
30 September 2016
Accepted 10 October 2016

Keywords:
Peroxisome biogenesis
Dyn2p
Protein import
PEX17

ABSTRACT

Peroxisomal matrix protein import is facilitated by cycling receptors that recognize their cargo proteins in the cytosol by peroxisomal targeting sequences (PTS). In the following, the assembled receptor-cargo complex is targeted to the peroxisomal membrane where it docks to the docking-complex as part of the peroxisomal translocation machinery. The docking-complex is composed of Pex13p, Pex14p and in yeast also Pex17p, whose function is still elusive. In order to characterize the function of Pex17p, we compared the composition and size of peroxisomal receptor-docking complexes from wild-type and *pex17Δ* cells. Our data demonstrate that the deficiency of Pex17p affects the stoichiometry of the constituents of an isolated 600 kDa complex and that *pex17Δ* cells lack a high molecular weight complex (>900 kDa) of unknown function. We identified the dynein light chain protein Dyn2p as an additional core component of the Pex14p/Pex17p-complex. Both, Pex14p and Pex17p interact directly with Dyn2p, but in vivo, Pex17p turned out to be prerequisite for an association of Dyn2p with Pex14p. Finally, like *pex17Δ* also *dyn2Δ* cells lack the high molecular weight complex. As *dyn2Δ* cells also display reduced peroxisomal function, our data indicate that Dyn2p-dependent formation of the high molecular weight Pex14p-complex is required to maintain peroxisomal function on wild-type level.

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1. Introduction

Peroxisomal matrix proteins are synthesized on cytosolic ribosomes and post-translationally targeted to the organelle matrix by peroxisomal import receptors. A remarkable difference between the peroxisomal protein import machinery compared to protein translocons from other organelles is the fact that the peroxisomal translocon imports proteins in a folded and even oligomerized state (Glover et al., 1994; McNew and Goodman, 1994). Moreover, the import receptors do not reside permanently at the membrane but cycle between cytosol and peroxisomes (Dodt and Gould, 1996; Marzioch et al., 1994). To target proteins to the peroxisomal matrix, the vast majority of these proteins possess the peroxisomal targeting signal type 1 (PTS1), a sequence frequently composed of the tripeptide SKL or a derivative thereof, which is located at the extreme C-terminus of the protein (Brocard and Hartig, 2006; Lanyon-Hogg et al., 2010). Some matrix proteins possess a PTS2, a nonapeptide located near the N-terminus (Lazarow, 2006). PTS1 and PTS2 are recognized and bound in the cytosol by the import receptors Pex5p and Pex7p, respectively (Rehling et al., 1996; van der Leij et al., 1993). Initial binding of the newly formed receptor-cargo complex to the peroxisomal membrane is performed by the docking complex, which consists of Pex13p, Pex14p and in yeast like *S. cerevisiae* also contains Pex17p (Albertini et al., 1997; Elgersma et al., 1996; Erdmann and Blobel, 1996; Gould et al., 1996; Huhse et al., 1998). The docking-complex assembles to a larger protein complex, the so called “importomer”, by association with the

Abbreviations: BN-PAGE, blue native polyacrylamide gel; LC–MS, liquid chromatography–mass spectrometry; PTS, peroxisomal targeting signal; RING, really interesting new gene; TPA, tobacco etch virus cleavage site-Protein A; SEC, size exclusion chromatography.

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http://dx.doi.org/10.1016/j.ejcb.2016.10.004
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Table 1
S. cerevisiae strains used in this study.

S. cerevisiae strain	Description	Source or Ref.
UTL-7A (wild-type)	*MATα leu2-3, 112 ura3-52 trp1*	(Erdmann et al., 1989)
pex3Δ	*MATα leu2-3, 112 ura3-52 trp1, pex3:loxP*	(Rucktäschel et al., 2010)
pex17Δ	*MATα leu2-3, 112 ura3-52 trp1, pex17:loxP*	(Huhse et al., 1998)
dyn2Δ	*MATα leu2-3, 112 ura3-52 trp1, dyn2:loxP*	*this study*
UTL-7A-*Pex5p*^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, PEX5*^{TPA}*-kanMX6*	(Schäfer et al., 2004)
pex17Δ-Pex5p^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, pex17:loxP, PEX5*^{TPA}*-kanMX6*	*this study*
UTL-7A-*Pex13p*^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, PEX13*^{TPA}*-kanMX6*	*this study*
pex17Δ-Pex13p^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, pex17:loxP, PEX13*^{TPA}*-kanMX6*	*this study*
UTL-7A-*Pex14p*^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, PEX14*^{TPA}*-kanMX6*	(Agne et al., 2003)
pex17Δ-Pex14p^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, pex17:loxP, PEX14*^{TPA}*-kanMX6*	*this study*
UTL-7A-*Pex18p*^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, PEX18*^{TPA}*-kanMX6*	(Grunau et al., 2009)
pex17Δ-Pex18p^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, pex17:loxP, PEX18*^{TPA}*-kanMX6*	*this study*
dyn2Δ-Pex14p^{TPA}	*MATα leu2-3, 112 ura3-52 trp1, dyn2:loxP, PEX14*^{TPA}*-kanMX6*	*this study*

three really interesting new gene (RING)-finger proteins Pex2p, Pex10p and Pex12p, which takes place in a Pex8p-dependent manner (Agne et al., 2003). As demonstrated recently, upon docking, the import receptor Pex5p or the PTS2-co-receptor Pex18p becomes an integral constituent of a transient protein-conducting channel in the peroxisomal membrane (Meinecke et al., 2010; Montilla-Martinez et al., 2015). However, the detailed molecular mechanisms of pore formation and protein translocation remain elusive. Subsequent to cargo release into the peroxisomal matrix, the receptor is exported back to the cytosol. A prerequisite for this event is its mono- or polyubiquitination. Monoubiquitination primes the receptor for recycling, in particular, the ATP-dependent release from the peroxisome by the ATPase peroxins Pex1p and Pex6p (Miyata and Fujiki, 2005; Platta et al., 2005; Williams et al., 2007). Polyubiquitination leads to the degradation of the receptor by the ubiquitin-proteasome system (Francisco et al., 2014; Kiel et al., 2005; Platta et al., 2004).

Our knowledge on the function of Pex17p in peroxisome biogenesis is still scarce. Pex17p is a peroxin and its deficiency results in an import defect for PTS1- and PTS2-proteins (Huhse et al., 1998). Pex17p is a peripheral peroxisomal membrane protein that is targeted to peroxisomes via the Pex19p-dependent route for peroxisomal membrane proteins (Girzalsky et al., 2006). At the peroxisomal membrane, Pex17p interacts directly with Pex14p and as such is part of the peroxisomal receptor docking complex (Albertini et al., 1997; Girzalsky et al., 2006). However, its function in peroxisomal protein import is still unknown. To gain more insight into Pex17p-function, we compared isolated peroxisomal protein complexes obtained from wild-type and *pex17Δ* cells. Size exclusion chromatography and Blue-Native (BN)-gel electrophoresis revealed a significant decrease of a high-molecular weight subcomplex in strains lacking Pex17p. Protein profiling experiments using liquid chromatography-mass spectrometry (LC–MS) demonstrate that the absence of Pex17p is accompanied by the lack of the light chain of dynein, Dyn2p in this fraction. We show that Dyn2p is associated with Pex14p in a Pex17p-dependent manner. Chemical cross-linking experiments combined with MS (XL-MS) reveal binding sites between Dyn2p and both, Pex14p and Pex17p. Finally, deletion of either Pex17p or Dyn2p results in a growth defect on oleic acid medium and the absence of the high-molecular weight Pex14p-subcomplex.

2. Experimental procedures

Strains and Media – Saccharomyces cerevisiae strains used in this study are listed in Table 1. Genomic tagging of PEX5, PEX13, PEX14 or PEX18 with TEV-Protein A (TPA) was performed as described (Knop et al., 1999) using the primers listed in Table 2. Specifically, the following primers were used: Ku896/897 for PEX5, Ku1005/1006 for PEX13, Ku1043/1044 for PEX14 and Ku1176/1176 for PEX18. Deletion of DYN2 was performed by the 'short flanking homology' method as described before (Güldener et al., 1996), using the primer pair RE4256/RE4257. The PCR products were transformed in the corresponding strains according to (Schiestl and Gietz, 1989). Transformants were selected for geneticin resistance and proper integration was confirmed by PCR and immunoblotting.

Yeast complete (YPD) and minimal media (SD) have been described previously (Erdmann et al., 1989). YNO medium contained 0.1% (wt/vol) oleic acid, 0.05% (vol/vol) Tween 40, 0.1% (wt/vol) yeast extract, and 0.67% (wt/vol) yeast nitrogen base with amino acids (pH 6.0). YNO medium was supplemented with 0.1% (wt/vol) dextrose to obtain YNOD medium. Oleic acid plates were prepared as described previously and contain 0.1% (wt/vol) oleic acid, 0.5% (wt/vol) Tween 40, 0.1% (wt/vol) yeast extract, 0.67% (wt/vol) yeast nitrogen base, amino acids (pH 6.0), and 1% (wt/vol) agarose.

Yeast cell extracts- Yeast cells were grown on 0.3% SD medium to late exponential phase and subsequently for 15 h in YNOD. Cells were harvested and aliquots of 30 mg of cells were resuspended in 300 μl of potassium phosphate buffer (pH 7.4) containing 20% trichloroacetic acid. The samples were frozen at −80 °C for at least 30 min. Samples were sedimented, washed twice with ice-cold 80% acetone and resuspended in 80 μl of 10% (wt/vol) SDS/0.1 M NaOH

Table 2
Oligonucleotides used in this study.

Oligonucleotide	Sequence
Ku 896	5′ GAAGCCAGGCATGGACCTGAAAAGATTTAAAGGAGAATTTTCGTTTCGTACGCTGCAGGTCGAC 3′
Ku 897	5′ GAATTTGGGCAGTGATGCGAGAACATAAAATTGCGGAGAACCATAATCGATGAATTCGAGCTCG 3′
Ku1005	5′-GAAGAAAATTGAGCATGTTGATGATGAAACGCGTACACACCGTACGCTGCAGGTCGAC-3′
Ku1006	5′-TATATATATATGCGAATATATGTGTGCAAATATTGATGCAATCGATGAATTCGAGCTCG-3′
Ku 1043	5‘-AGTGTCCCTGACTGGCAAAATGGACAGGTCGAACACTCCATCCCACGTACGATGCAGGTCGAC-3‘
Ku 1044	5‘-AATTACAATTTCCGTTAAAAAACTAATTACTTACATAGAATTGCGATCGATGAATTCGAGCTCG-3‘
Ku1175	5‘-CTGAAATTCATGGTTTAAATTAAAGAAATTTCAAGGCCCGATCGATGAATTCGAGCT-3‘
Ku1176	5‘-GGCTGGTCTTGAGTTCCATGATGTTGAAGACAGAATTGCTCGTACGCTGCAGGTCGAC-3‘
RE4256	5‘-GAAACTGGGAAACGACATAAAGAAGAGCAAATTAAAACCAAACAGCTGAAGCTTCGTACGCT-3‘
RE4257	5‘-TAATAGTAATTGATTATCGATTAACTATTGAAGAAACACTAT ATAGGCCACTAGTGGATCTG-3‘

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