Molecular recognition mechanism of peroxisomal targeting signal–2, PTS2

In eukaryotic cells, peroxisomes are responsible for many important metabolic functions, such as catabolism of fatty acids. Dysfunctions of peroxisomes cause fatal developmental problems in humans and hinder proliferation of yeast cells. More than 20 conserved proteins are necessary to assemble functional peroxisomes and they are named peroxins (Pex) [1]. Peroxins target peroxisomal matrix proteins synthesized in cytosol to peroxisome and translocate them into the matrix. Those matrix proteins usually possess either of two types of peroxisomal targeting signals (PTS1 or PTS2) for appropriate targeting. While the structural basis of PTS1 recognition by peroxin 5 (Pex5p) has been revealed by the crystal structures, that of PTS2 remains to be determined [2]. We studied the recognition mechanism of PTS2 from *Saccharomyces cerevisiae* to reveal the general mechanism of PTS2 recognition [3].

PTS2 is a conserved nonapeptide motif (R-[L/V/I/Q]-XX-[L/V/I/H]-[L/S/G/A]-X-[H/Q]-[L/A]) at the N-terminal end of cargo proteins. In this motif, the residues at positions 1 and 8 showed strict limitations to only a few types of hydrophilic residues, whereas the residues at positions 2, 5 and 9 are limited to mainly hydrophobic residues [4]. Receptor peroxin Pex7p recognizes PTS2 only in the presence of a co-receptor peroxin [5]. In *S. cerevisiae*, either Pex18p or Pex21p functions as the co-receptor instead of Pex5pL, the longer isoform of Pex5p in mammals. By determining the crystal structure of a PTS2-recognition complex (Pex7p-Pex21p-PTS2), we endeavored to answer the following questions: why are the key residues of PTS2 limited to specific positions and specific types of residues, how does Pex7p form the binding site for PTS2, and how do the co-receptor peroxins support Pex7p for PTS2 recognition?

Well-diffracted crystals were obtained when we used individually purified Pex7p Δ257–265, the C-terminal 99 residues of Pex21p (Pex21pC), and Fox3pN-MBP to reconstitute the PTS2-recongnition complex (Fig. 1). Fox3pN-MBP is a fusion protein of the N-terminal 15 residues of *S. cerevisiae* Fox3p and the maltose binding protein from *Escherichia coli*. The best diffraction dataset was collected at beamline **BL41XU**. The crystal structure was determined by the molecular replacement method with the crystal structures of a WD40 protein and MBP as search models and refined to the resolution of 1.8 Å.

The crystal structure clearly defined a cooperative mechanism in which both Pex7p and Pex21pC are necessary for PTS2 recognition (Fig. 1). Pex7p forms a β-propeller structure that is similar to those of other WD40 proteins. Pex21pC has an extended shape with an α-helical region, a small β-sheet, and an extended C-terminal loop. Both the top surface of Pex7p and the helical region of Pex21pC share the rule to form a

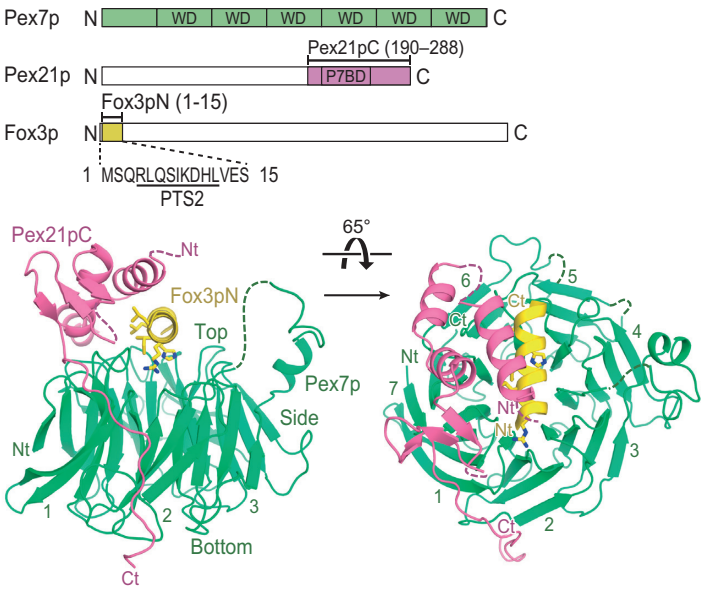

Fig. 1. Overall structure of the PTS2-recognition complex and sequence diagram of its components. Numbers in the lower panel indicate the number of blades of the β-propeller structure. The side chains of key residues of PTS2 in Fox3pN are shown as stick models. WD, WD40 motif; P7BD, Pex7p binding domain.

binding pocket for PTS2. These regions of Pex7p and Pex21pC are composed of well-conserved residues. Mammalian Pex5pL also contains a homologous region corresponding to the helical region of Pex21pC. This manner of pocket formation has not been reported in other targeting signal systems (e.g., PTS1, mitochondrial targeting signal, nuclear localization signal), in which binding pockets for targeting signals are formed by single receptor proteins.

Our crystal structure revealed the precise interactions between the key residues of PTS2 and its receptor proteins. The 15 residues of Fox3pN, including the sequence of the PTS2, fold into an α-helix and fit tightly into the binding pocket (Fig. 2). The buried surface area of Fox3pN is large and accounts for 70% of its total surface area. By forming an α-helix, the key residues are separated into two groups: hydrophilic key residues (Fox3p Arg4 and His11) and hydrophobic key residues (Fox3p Leu5, Ile8, and Leu12). These two groups of key residues fit into the complementary grooves in the binding pocket of PTS2. The hydrophilic key residues form salt bridges and hydrogen bonds with the residues of the hydrophilic groove on the top surface of Pex7p (Pex7p Asp61, Glu106, Thr178, Glu222, and Glu304). The hydrophobic key residues are inserted into the hydrophobic groove between Pex7p and Pex21pC. Consequently, the hydrophobic residues from all three proteins form a hydrophobic core around the residue Pex21p Phe236 (Fig. 2).

The structure implies that this hydrophobic core is important in ternary complex formation. Therefore, we performed pull-down assays with purified proteins and *in vivo* PTS2-EGFP transport assays with *Δpex7* or *Δpex18Δpex21 S. cerevisiae* strains to analyze the effects of mutations (Pex7p L34D and Pex21p F236D) that were expected to destabilize the complex formation [3]. The results of pull-down assays clearly showed that wild-type Pex7p and Pex21pC were able to form stoichiometric ternary complexes, but Pex7p L34D and Pex21p F236D mutants could not. The expression of wild-type Pex7p in the *Δpex7* strain or wild-type Pex21pC in the *Δpex18Δpex21* strain was able to restore the PTS2-EGFP transport to peroxisomes, but the expression of Pex7p L34D and Pex21p F236D mutants could not. Thus, we confirmed that the cooperative hydrophobic core formation is necessary for strong binding of PTS2.

In summary, we reported, for the first time, the crystal structure of a PTS2-recognition complex and our findings revealed a cooperative mechanism for PTS2 recognition. That is, Pex7p and the co-receptor peroxin cooperatively form the binding pocket of PTS2. Residues of Pex7p and Pex21p forming the binding pocket of PTS2 are well conserved from yeast to humans, which suggests that it is likely the manner of PTS2 recognition in yeast is also used in higher eukaryotes. The specific assignment of the key residues in the PTS2 motif is to ensure that two types of key residues can be lined up in two directions upon forming the α-helix and can be recognized by two complementary grooves in the PTS2 binding pocket.

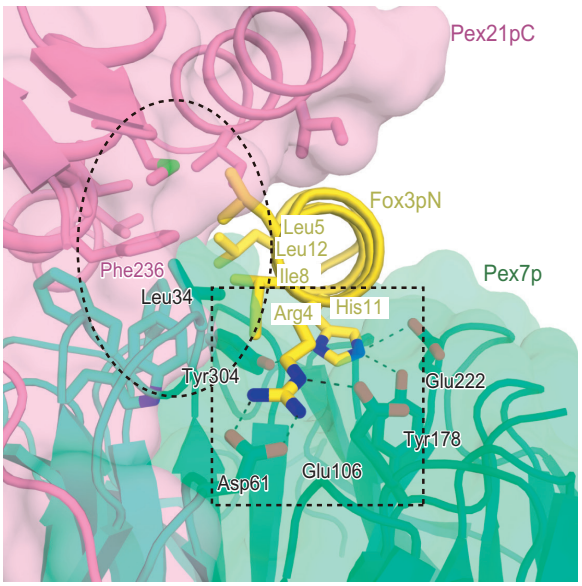

Fig. 2. Close-up view of the PTS2 binding pocket. The side chains of key residues of PTS2 and the residues of Pex7p and Pex21p that interact with PTS2 key residues are shown as stick models. Dashed ellipse indicates the position of hydrophobic core and dashed box indicates the hydrophilic groove.

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