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Review

Comparison of the peroxisomal matrix protein import system of different organisms. Exploration of possibilities for developing inhibitors of the import system of trypanosomatids for anti-parasite chemotherapy

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ABSTRACT

In recent decades, research on peroxisome biogenesis has been particularly boosted since the role of these organelles in metabolism became unraveled. Indeed in plants, yeasts and fungi, peroxisomes play an important role in the adaptation of metabolism during developmental processes and/or altered environmental conditions. In mammals their importance is illustrated by the fact that several severe human inherited diseases have been identified as peroxisome biogenesis disorders (PBD). Particularly interesting are the glycosomes – peroxisome-like organelles in trypanosomatids where the major part of the glycolytic pathway is sequestered – because it was demonstrated that proper compartmentalization of matrix proteins inside glycosomes is essential for the parasite.

Although the overall process of peroxisome biogenesis seems well conserved between species, careful study of the literature reveals nonetheless many differences at various steps. In this review, we present a comparison of the first two steps of peroxisome biogenesis – receptor loading and docking at the peroxisomal membrane – in yeasts, mammals, plants and trypanosomatids and highlight major differences in the import process between species despite the conservation of (some of) the proteins involved.

Some of the unique features of the process as it occurs in trypanosomatids will be discussed with regard to the possibilities for exploiting them for the development of compounds that could specifically disturb interactions between trypanosomatid peroxins. This strategy could eventually lead to the discovery of drugs against the diseases caused by these parasites.

1. Introduction

Peroxisomes, glycosomes and glyoxysomes are three members of the "peroxisome" family. These organelles, with a single membrane that envelopes a dense protein matrix, are widely distributed among eukaryotes and display a large variety of metabolic functions depending on organism and cell type.

The importance of the role played by peroxisomes in metabolism is illustrated by the fact that several human inherited diseases have been identified as peroxisome biogenesis disorders (PBD). Similarly, peroxisomes have been shown to play a crucial role in the metabolism of representative organisms of other major taxonomic groups such as plants, yeasts and fungi, and the adaptation of their metabolism during developmental processes and/or altered environmental conditions. The interest in glycosome biogenesis in the protist family Trypanosomatidae, which contains several species responsible for serious tropical diseases of humans, has been particularly boosted since it was demonstrated that proper compartmentalization of matrix proteins inside glycosomes is essential for survival of the parasite (Bakker et al., 2000; Blattner et al., 1998; Helfert et al., 2001; Moyersoen et al., 2004).

The biogenesis of peroxisomes involves a variety of processes such as the import of matrix proteins, the recruitment of lipids for membrane formation, the insertion of proteins in the organelle's membrane and fission of the organelle. So far, 31 proteins, called peroxins (acronym PEX) are known to mediate the distinct processes of the organelles' biogenesis. Most of these peroxins have been identified in *Saccharomyces cerevisiae* and orthologues of many of them have also been described for other yeasts and for fungi, mammalian cells, plants and protists.

The first step of matrix protein import involves the recognition of the proteins to be imported by either of two cytosolic

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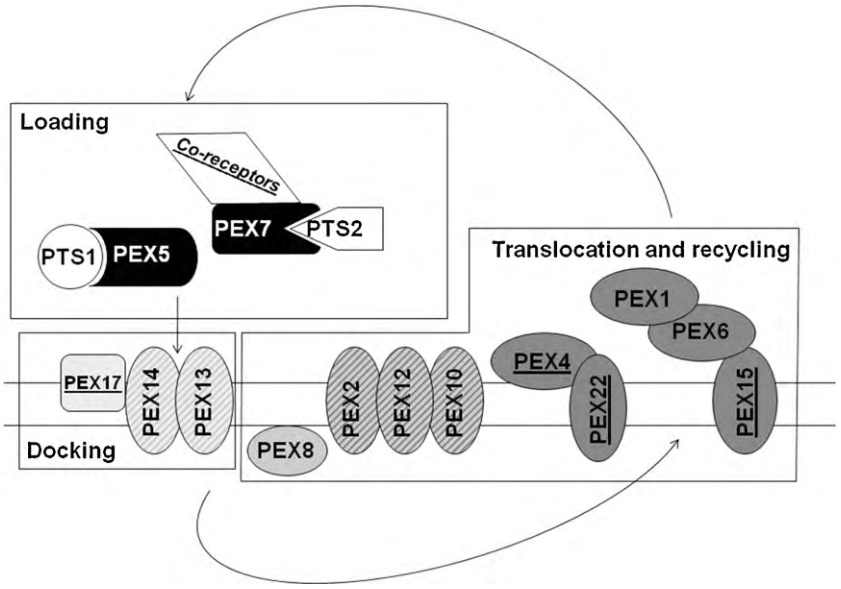

Fig. 1. Schematic representation of peroxisome biogenesis as described for yeast. For the translocation step PEX14 and PEX5 are sufficient to form a pore in the peroxisomal membrane, which opens upon binding of cytosolic PEX5 with bound PTS1 proteins and so allows the import of PTS proteins into the matrix (Meinecke et al., 2010). The peroxins with underlined names are not present in mammals (see the text for details). Recent data showed unambiguously that the RING-finger peroxin complex (PEX2, PEX10, PEX12) is involved in the ubiquitin-dependent recycling of the (co)receptors. Whether they are also involved in the receptor-cargo translocation step, as suggested by older experiments is a matter of debate (see text).

receptors, PEX5 and PEX7, that are implicated in two convergent pathways (Fig. 1). One receptor, PEX5, is responsible for the interaction with proteins containing a type 1 peroxisomal-targeting signal (PTS1) and the other pathway starts with receptor PEX7 that recognizes an entirely different, type 2 signal (PTS2). Following this recognition, the cargo-loaded receptors interact with the peroxisomal membrane through a docking complex composed, in yeast, of PEX13, PEX14 and PEX17. However, PEX14 and the PTS-receptor are probably sufficient to form the translocon (Ma et al., 2009). Indeed, *in vitro* confirmation was obtained recently for the hypothesis that a pore composed of PEX14 and PEX5 forms, that opens when it encounters soluble PEX5 loaded with a PTS1 (Meinecke et al., 2010). Subsequently, the cargo-receptor complex is translocated across the membrane by an as yet poorly understood process that may involve PEX8 and the RING-finger complex composed of PEX2, PEX10 and PEX12. After releasing their cargo into the matrix, the receptors are cycled back to the cytosol. In the case of PEX5 recycling involves the action of two predominantly cytosolic ATPases of the AAA+-protein family (ATPases associated with various cellular activities), PEX1 and PEX6, which are also associated with the peroxisomal membrane via PEX15 in yeast or PEX26 in mammalian cells. The RING-complex (PEX2, PEX10 and PEX12) and PEX4 (in yeast) or UbcH5a/b/c (in mammalian cells) are involved in ubiquitination of the receptor (Kiel et al., 2005; Kragt et al., 2005), providing the signal for its retrieval (from the matrix or membrane) and delivery to the peroxin complex responsible for its ATP-dependent translocation back to the cytosol (Miyata and Fujiki, 2005; Platta et al., 2009). In the case of PEX7, despite strong evidence that the cargo-loaded receptor enters the lumen of the peroxisome, followed by its recycling to the cytosol, information about the details of this recycling mechanism is still almost entirely lacking. However, ubiquitination of PEX20, a cofactor of PEX7 in *Pichia pastoris* that translocates into and out of peroxisomes, was shown to be essential for its recycling to the cytosol, also suggesting a role of ubiquitination in the PTS2 import pathway (Leon et al., 2006).

PTS1 and PTS2 sequences are largely conserved between species and each class of targeting signals is recognized by homologous proteins (Brocard and Hartig, 2006; Petriv et al., 2004). However, the way by which the receptors interact with the PTS-containing proteins, the interactions between the two cytosolic receptors, and between the receptors and the docking complex, show considerable variations. In this review, we intend to highlight this large variation, as well as presenting the common aspects, in the biogenesis of peroxisomes in different organisms. To that end, we will present a comparison of the first two steps of matrix protein import of peroxisomes from mammals, yeasts, plants and trypanosomes: the binding of the protein to be imported to a receptor (PEX5 or PEX7) and the docking of the charged receptor onto a protein complex at the peroxisomal membrane (PEX13 and PEX14). Then, by focusing on some of the unique characteristics of the process as it occurs in trypanosomatids, we will explore the possibilities for exploiting these features for developing compounds that will specifically interfere with the function of the peroxins of the parasites and thus may serve as lead compounds for drugs against parasitic diseases. Indeed, evidence will be presented that any interference with the import process of the parasite that will lead to mislocalization of matrix proteins in the cytosol and thus to parasite death, may be exploited in the development of drugs for curing tropical trypanosomatid parasite-borne diseases. These diseases comprise sleeping sickness or human African trypanosomiasis in Africa, caused by different subspecies of *Trypanosoma brucei*, Chagas' disease in Latin America inflicted by *Trypanosoma cruzi* and the various manifestations of leishmaniasis that occur in tropical and subtropical zones world-wide and result from infection by one of about 20 human infective *Leishmania* species. No adequate drugs are currently available for these so-called neglected diseases that will often be fatal without treatment and especially afflict people in economically-deprived societies. At present, treatment involves the use of toxic drugs for many of which resistance is spreading (Delespaux and de Koning, 2007; Matovu et al., 2001; Nwaka and Hudson, 2006). Therefore, there is a large need for the identification and validation of new drug targets in these parasites and the subsequent development of efficacious, non-toxic compounds interfering with the functioning of these targets.