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Review

The first minutes in the life of a peroxisomal matrix protein☆

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ABSTRACT

In the field of intracellular protein sorting, peroxisomes are most famous by their capacity to import oligomeric proteins. The data supporting this remarkable property are abundant and, understandably, have inspired a variety of hypothetical models on how newly synthesized (cytosolic) proteins reach the peroxisome matrix. However, there is also accumulating evidence suggesting that many peroxisomal oligomeric proteins actually arrive at the peroxisome still as monomers. In support of this idea, recent data suggest that PEX5, the shuttling receptor for peroxisomal matrix proteins, is also a chaperone/holdase, binding newly synthesized peroxisomal proteins in the cytosol and blocking their oligomerization. Here we review the data behind these two different perspectives and discuss their mechanistic implications on this protein sorting pathway. This article is part of a Special Issue entitled: Peroxisomes. Guest Editors: Ralf Erdmann.

"No part of the world can simply be read — it always must be interpreted, and those interpretations are subject to constant reevaluation."

Mark Ptashne [1].

1. Introduction

Peroxisomes are round-shaped organelles delimited by a single membrane. Their size, number and protein repertoire varies widely among organisms, cell types and even physiological conditions [2]. In mammals, peroxisomes have a relatively simple composition comprising about 100 different proteins [3,4]. Despite this simplicity, peroxisomes are involved in important metabolic pathways and, accordingly, mutations in genes encoding peroxisomal enzymes, peroxisomal membrane metabolite transporters, or proteins involved in peroxisome biogenesis cause devastating diseases in humans [5,6].

All peroxisomal matrix proteins are synthesized in the cytosol and post-translationally transported to the organelle [7]. Their specific sorting to this compartment is mediated by a complex machinery comprising a core of 10 evolutionary conserved peroxins (peroxins are proteins specifically involved in peroxisomal biogenesis) plus a set of additional proteins most of which are involved in ubiquitin conjugation and deconjugation ([8–13]; see also Table I in ref. [14], this issue).

In order to be sorted to the peroxisome matrix, a newly synthesized protein must have a peroxisomal targeting signal (PTS) in its polypeptide chain. There are two well-characterized types of PTSs: the PTS type 1 (PTS1), the most common, is a small peptide present at the C termini of proteins and frequently ends with the sequence S-K-L [15]; the PTS2 is a degenerated nonapeptide found at the N termini of a few peroxisomal proteins [16,17]. Unlike the PTS1, which is not processed upon import, the PTS2 is generally cleaved when the protein reaches the peroxisome matrix of higher eukaryotes [17].

In mammals, plants and many other organisms, sorting of both PTS1- and PTS2-containing proteins requires PEX5 [18–22], a monomeric protein of about 70 kDa possessing an intrinsically disordered N-terminal half and a globular C-terminal half comprising six tetratricopeptide repeat motifs (hereafter simply referred to as TPRs) [23–26]. Our knowledge on how PEX5 interacts with these two types of cargoes is still fragmented. Structural and protein-protein interaction studies have shown that the PTS1 signal interacts with the TPRs of PEX5 [25,27–29]. However, it is now clear that the PEX5-cargo protein interaction is not limited to this binding interface and that the N-terminal half of PEX5 and other regions of the PTS1 cargo proteins are also involved [30–34]. Much less is known on the PEX5-PTS2 cargo protein

Abbreviations: ACOX, acyl-CoA oxidase; AO, alcohol oxidase; CCS, copper chaperone of SOD1; DTM, docking/translocation machinery; Gpd1p, glycerol-3-phosphate dehydrogenase 1; LDHA/B/Bx, lactate dehydrogenase isoforms A, B and B readthrough-extended, respectively; PIM, peroxisomal import machinery; Pnc1p, nicotinamidase; PTS, protein targeting signal; SOD1, Cu/Zn superoxide dismutase; TPR, tetratricopeptide repeat; UOX, urate oxidase.

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interaction. Actually, until recently, it was frequently considered that the PEX5-PTS2 interaction might even not be direct but rather bridged by PEX7, a WD-repeat protein long-known to interact with both the PTS2 peptide and a small domain present in the N-terminal half of PEX5 [18–22,35]. However, recent structural data of a trimeric protein complex comprising yeast PEX7, an artificial PTS2 protein and a small fragment of PEX21 (the yeast orthologue of mammalian/plant PEX5 in the PTS2-mediated protein import pathway; [36,37]), revealed that PEX21 also interacts directly with the PTS2 peptide [38]. Given the functional and structural similarities between yeast PEX21 and the N-terminal half of mammalian/plant PEX5 [36,37], it is therefore likely that the same is valid for mammalian/plant PEX5. In agreement with this possibility, two recent studies have shown that the human PEX7.PTS2 interaction is drastically stabilized by PEX5 [39,40].

One of the most important properties of PEX5 and PEX7 regards their intracellular localization. Indeed, in contrast to all the other peroxins involved in this protein sorting pathway, which are peroxisomal proteins, pioneering studies on PEX5 and PEX7 revealed that both display a dual subcellular localization, cytosolic and peroxisomal [41,42]. This property, together with their capacity to bind PTS1 and PTS2 proteins, is at the basis of a central concept found in all mechanistic models published to date, namely, that newly synthesized peroxisomal matrix proteins are recognized in the cytosol and transported to the organelle by the shuttling receptors PEX5/PEX7; after delivering their cargoes into the organelle, the receptors return to the cytosol to promote additional rounds of protein transport [41,42].

Another important early discovery that has heavily influenced models on the mechanism of protein transport to the peroxisome matrix was the observation by several researchers that these organelles can acquire already oligomerized proteins from the cytosol (see Section 3.). Two main models were then proposed to explain this remarkable capacity of peroxisomes [43]. One, a translocation-based model, postulated the existence of large regulated channels/pores at the peroxisomal membrane; the other, proposed that large already-oligomerized cargo proteins might reach the peroxisomal matrix by an endocytosis-like mechanism. Data clearly favoring the translocation-based model came a few years later from the biochemical characterization of peroxisome-associated PEX5. Indeed, it was found that during its transient passage through the peroxisome, PEX5 acquires a transmembrane topology, exposing a small N-terminal domain into the cytosol whereas the bulky part of its polypeptide chain faces the organelle matrix [44,45]. Since the main cargo protein-binding domain of PEX5, the TPRs, resides at its C terminus and occupies about half of PEX5 polypeptide chain, this finding immediately suggested that cargo proteins are translocated across the organelle membrane by PEX5 itself when the receptor becomes inserted into a transmembrane protein complex of the peroxisome, the docking/translocation machinery (DTM) [44,46]. Subsequent characterization of the insertion of PEX5 into the DTM revealed that this step is a cargo-dependent but ATP-independent process, strongly suggesting that the driving force for protein translocation across the organelle membrane derives from strong protein-protein interactions involving PEX5 on one side and peroxins of the DTM on the other [46–48].

Although it is now generally accepted that peroxisomal proteins reach the organelle matrix using a translocation-based mechanism [8–10,49–51], there are still many questions (and disputes among researchers in the field) on the architecture and mechanism of the machinery that accomplishes this task. One of these questions regards precisely one of the most famous properties of peroxisomes, *i.e.*, their capacity to import already oligomerized proteins. Although the data supporting this property are abundant, several findings made over the years suggest that import of already oligomerized proteins may not be that frequent and that many of these proteins may actually arrive at the organelle still as monomers.

Here we summarize and discuss the main data behind these two different perspectives. As it will be apparent below, many of the points we raise argue against an oligomeric protein import model favoring instead a monomeric protein import model. This is not to say that the oligomeric protein import model is not valid at all. Actually, for a few components of the peroxisome, the oligomeric protein import model still provides the best explanation for their presence in the organelle (see Section 6.). Ultimately, our goal is to stimulate research on this topic so that the peroxisomal protein import machinery stops being one of the least understood protein import machineries of the eukaryotic cell.

2. Peroxisomal matrix proteins: the first events after synthesis

As stated in the previous section, peroxisomal matrix proteins are synthesized on soluble cytosolic ribosomes [7]. Thus, as with many other proteins that do not follow the secretory pathway, folding of their polypeptide chains is catalyzed by the cytosolic chaperone machinery and probably starts as soon as the first N-terminal amino acid residues emerge from the ribosomal polypeptide exit tunnel [52–58]. What happens to these proteins in the first seconds after folding has not been explored in detail. Nevertheless, it is reasonable to assume that peroxisomal matrix proteins that are monomers in their native state are simply recognized by cytosolic PEX5/PEX7 and transported to the organelle. However, for proteins that are homo-oligomers in their native conformation, the pathway may be different, as explained below.

An interesting property of several peroxisomal homo-oligomeric proteins regards the fact that they can be detected as soluble monomeric proteins immediately after synthesis, both *in vivo* [59–61] and *in vitro* [34,62,63]. This behavior suggests, on one hand, that these monomers are already at least partially folded and, on the other, that folding of monomers and their oligomerization to yield the native enzymes are not physically coupled events. The same conclusion is probably valid for monomer folding and monomer-PEX5 interaction because some active oligomeric peroxisomal proteins can be detected in the cytosol of cells lacking PEX5 [64–66] and the *in vitro* protein synthesis system used in the experiments referred to above has essentially no endogenous PEX5 [34,62,63]. Thus, it appears that newly synthesized proteins are released by the cytosolic chaperone machinery as soluble monomeric proteins independently of PEX5. An obvious implication of this reasoning is that all subsequent protein-protein interactions occurring in the cytosol are probably of stochastic nature. If so, one can consider two possible pathways for these proteins: 1) interaction with PEX5/PEX7 and/or 2) oligomerization. The first pathway leads us to a "monomeric protein import model" whereas the second is the basis of an "oligomeric protein import model" (see Fig. 1). Determining which pathway prevails is not a mechanistic detail of minor importance because, at the very least, it can provide us with valuable information on how the peroxisomal DTM functions.

3. Evidence for the oligomeric protein import model

The experimental evidence behind the concept that peroxisomal matrix proteins oligomerize in the cytosol before import into the organelle dates back to 1994 [43,67]. The experiments reported in those two studies consisted of expressing in the same cells two versions of a protein, which is homo-oligomeric in its native state. One version contained a PTS whereas the other lacked such a signal. Expression of the PTS-less protein alone resulted in its cytosolic localization, as expected. However, when this protein was co-expressed with the PTS-containing version, the two proteins were now found in the peroxisome. Apparently, the protein lacking the PTS was transported to the organelle piggy-backed with its PTS-containing partner. Similar findings were subsequently reported for other peroxisomal oligomeric proteins in several organisms/cell lines (see Table 1). There are three aspects of those experiments that deserve discussion. First, with only a few exceptions (see Section 6.), all these studies used experimental conditions that

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