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Hitchhiking fads en route to peroxisomes

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A unique aspect of protein translocation across the peroxisomal membrane is that folded and oligomeric proteins get across this membrane (Purdue and Lazarow, 2001). The generality of this rule, its specific features, and its mechanism are not fully understood. A paper in this issue addresses, in a very thorough fashion, the assembly, cofactor binding, and import of an oligomeric protein, acyl-CoA oxidase (Aox), into the peroxisome matrix (Titorenko et al., 2002, this issue).

The import of folded oligomeric proteins into peroxisomes was known for proteins containing either one of the two major peroxisome targeting signals (PTSs),* PTS1 and PTS2 (Glover et al., 1994; McNew and Goodman, 1994). Although most peroxisomal matrix proteins use these PTSs for their targeting, a few proteins use neither (Purdue and Lazarow, 2001). One such protein lacking discernible PTS1 or PTS2 sequences is Aox from *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, and yet its import into peroxisomes is dependent on the PTS1 receptor, Pex5p (Purdue and Lazarow, 2001; Titorenko et al., 2002). Titorenko et al. (2002) show that the five acyl-CoA oxidase isoforms of *Y. lipolytica* are imported ($t_{1/2} \approx$ 4–5 min) into the peroxisome matrix as a heteropentameric cofactor-containing complex that is rapidly assembled ($t_{1/2} \approx$ 1 min) in the cytosol. The results demonstrate that even this pathway, which appears to be independent of the PTS1 and PTS2 sequences, allows folded and oligomerized proteins into the peroxisome matrix. Titorenko et al. (2002) reveal interesting features of oligomeric protein import that complement and extend previous results (Evers et al., 1994, 1996; Waterham et al., 1997; Stewart et al., 2001). Not too surprisingly, no rules apply generally to all oligomeric peroxisomal proteins.

The isoforms of Aox, designated Aox1p to Aox5p, assemble into a 443-kD heteropentameric complex. All isoforms have Aox activity but their specificities vary (Wang et al., 1999). Aox2p and Aox3p play a direct role in the assembly and perhaps indirectly in peroxisomal targeting of the Aox complex, but not in the binding of the cofactor, FAD (Titorenko et al., 2002). Unexpectedly, in the absence of one, two, or three of the Aox isoforms, the heteropentamer reduces in size with varying efficiencies to tetramers, trimers, or dimers, respectively, and all are imported at similar rates. The transport, but not the assembly, of the heteropentameric Aox complex is Pex5p dependent; but, because no PTS is known in any Aox isoform, it is unclear whether any subunit hitchhikes into peroxisomes in association with subunits that might have an undefined PTS. If this is the case, at least two subunits must have a PTS, or some affinity for Pex5p, because all five single Aox knockouts showed peroxisomal matrix locations for the other subunits (Titorenko et al., 2002). It would be interesting to know if Pex5p-binding sites are associated with Aox2p and Aox3p.

The types of oligomeric proteins imported into peroxisomes

Many peroxisomal proteins are oligomeric (Lazarow and Fujiki, 1985) and this is not the first analysis of either oligomerization or cofactor binding (Evers et al., 1994, 1996; Waterham et al., 1997; Brul et al., 1988; Stewart et al., 2001). Homotetrameric catalase (240 kD), which has heme as a cofactor, is preassembled in the cytosol and then imported into peroxisomes of human cells (Brul et al., 1988), but it has also been suggested that the tetramer may unfold (and disassemble) just before import and reassemble upon entry into peroxisomes (Middelkoop et al., 1993). The 443-kD heteropentameric Aox with 5 FADs is obviously bigger. However, the homooctameric alcohol oxidase (592 kD) that also binds FAD is imported as a monomer, the cofactor is bound inside the peroxisomes in *Hansenula polymorpha*, followed by assembly of the octamer (Evers et al., 1994, 1996). Is the oligomerization of alcohol oxidase inside peroxisomes an indication that the size limit of the translocation pore has been exceeded? Although it is a possibility, this answer is probably too simplistic, because not all oligomers below a certain size are assembled in the cytosol and imported as such (Table I). For example, monomeric isocitrate lyase is imported more efficiently than its homotetrameric (260 kD) form into plant peroxisomes (Crookes and Olsen, 1998), and rat liver catalase is reported to assemble into tetramers inside peroxisomes (Lazarow and Fujiki, 1985).

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*Abbreviations used in this paper: Aox, acyl-CoA oxidase; Cvt, cytoplasm-to-vacuole transport; PTS, peroxisome targeting signal.

Table I. **Import of oligomeric proteins into peroxisomes**

Oligomers	Proteins imported into peroxisomes	Cofactor involved
Homodimers	Thiolase (82 kDa), malate dehydrogenase (74 kDa), alanine:glyoxylate aminotransferase (86 kDa), *C. boidinii* dihydroxyacetone synthase (155 kDa)[a]	Thiamine pyrophosphate
Heterodimer	*S. cerevisiae* Eci1p/Dci1p (62 kDa)[a]	
Homotrimer	Chloroamphenicol acetyltransferase (72 kDa)[a]	
Homotetramer	*Y. lipolytica* isocitrate lyase (244 kDa)[a]	
Heteropentamer	5 isoforms of *Y. lipolytica* acyl-CoA oxidase (443 kDa)[a]	FAD
Homotetramer	Rat liver catalase (260 kDa)[b]	Heme
Homooctamer	Alcohol oxidase (~592 kDa) of methylotrophic yeasts[b]	FAD

Molecular masses of the multimers are shown in parentheses.
[a]Imported as an oligomer.
[b]Imported as a monomer.

Subcellular sites and chaperones for cofactor assembly

Interesting differences exist between Aox and other proteins regarding the site of cofactor addition and perhaps chaperone requirement. For Aox, the association of each subunit with FAD happens in the cytosol, before peroxisomal transport (Titorenko et al., 2002). However, monomers of *H. polymorpha* alcohol oxidase are transported into peroxisomes, and FAD is added inside peroxisomes (Evers et al., 1994, 1996; Waterham et al., 1997; Stewart et al., 2001). Specific proteins (chaperones) may be needed inside the peroxisomes for FAD binding to alcohol oxidase (Evers et al., 1996). No such chaperone requirement for FAD binding was defined for Aox, but the analysis was restricted to the five Aox isoforms (Titorenko et al., 2002).

Proteins that assist in the assembly of oligomeric complexes

The authors conclude that Aox2p and Aox3p play a key role in the rate and extent of formation of the pentameric complex in the cytosol, and they can substitute for one another (Titorenko et al., 2002). This was shown by the observation that, although single mutants affecting any of the five Aox genes caused the assembly and accumulation of tetrameric complexes in peroxisomes, in the *aox2KO aox3KO* double mutant, the other subunits were mostly monomeric and cytosolic. This result resembles the requirement of Pex20p for the assembly and dimerization of peroxisomal thiolase in the cytosol (Purdue and Lazarow, 2001). Aox2p and Aox3p favor the formation of the Aox complex by retarding its disassembly, but they are not needed for FAD acquisition by the subunits.

Oligomeric proteins are transported across membranes of prokaryotes and eukaryotes

Although most proteins are transported across cellular membranes in the unfolded state, the paradigm of transport of folded oligomeric proteins is more widespread than just across the peroxisomal membrane (Titorenko et al., 2002). In the cytoplasm-to-vacuole transport (Cvt) pathway, a dodecamer of a precursor to aminopeptidase I (prAPI) and a tetramer of α mannosidase (AMS1), are delivered across the vacuolar membrane (Abeliovich and Klionsky, 2001). The ΔpH-dependent translocation of proteins across the plant thylakoid membrane and the related twin-arginine translocation of proteins across the bacterial cytoplasmic membrane are other examples (Robinson and Bolhuis, 2001). One consequence of oligomeric protein transport is that subunits lacking a canonical targeting signal can enter piggyback into the organelle (Glover et al., 1994; McNew and Goodman, 1994; Lee et al., 1997; Rodrigue et al., 1999; Yang et al., 2001). Indeed, the twin-arginine translocation pathway allows the transport of a heterodimeric hydrogenase 2 complex in which the larger 60-kD subunit lacking known targeting signals crosses the bacterial periplasmic membrane in association with the smaller 30-kD subunit that has a signal sequence with the twin-arginine motif (Rodrigue et al., 1999). Furthermore, the large subunit is required for the membrane targeting and translocation of the small subunit, just as Aox2p and Aox3p are necessary for assembly and targeting of the Aox complex. The requirement of the small subunit for the acquisition of nickel by the large subunit is reminiscent of the need for a putative chaperone for FAD binding to alcohol oxidase (Evers et al., 1996). Thus, several features of the assembly of homo- and heterooligomers, such as the addition of cofactors, the requirement of specific proteins for complex assembly or cofactor addition, and the piggy-back transport of subunits lacking a targeting signal, are common to both prokaryotes and eukaryotes.

Mechanisms involved in the transport of oligomeric proteins across membranes

Three mechanisms can be envisioned for the delivery of multimeric proteins across biological membranes.

Delivery via a channel. This involves cargo translocation through a membrane-associated pore or channel that may exist in the membrane or be assembled there transiently in response to cargo delivery to the membrane. The other two modes bypass the need for a pore and even for direct translocation through the membrane in question.

Delivery of cargo bound by a single-membrane. The target membrane where the oligomeric cargo is bound invaginates and pinches off on the side of the target membrane where cargo is to be delivered, followed by degradation of the membrane around the cargo to deliver the contents. Although analogies have been drawn between this mechanism and endocytosis, better parallels may be multivesicular body formation from endosomes followed by vesicle delivery across the vacuolar membrane (Odorizzi et al., 1998), or the

transport of degradative cargo across the vacuolar membrane by microautophagy (Abeliovich and Klionsky, 2001).

Delivery of cargo wrapped in a double membrane by fusion to the target membrane. The first step of this process resembles the formation of Cvt vesicles or autophagosomes (Abeliovich and Klionsky, 2001). The outer membrane of the resulting vesicles fuses to the target membrane to deliver cargo, surrounded by a single membrane, to the other side of the target membrane. This would have to be followed by degradation of the membrane around the cargo to release the contents. Although the first of these mechanisms is favored, better definition and clarity regarding the translocon in the peroxisomal membrane (Purdue and Lazarow, 2001) are necessary to comprehend how folded oligomeric proteins get across.

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