Defining the membrane topologies of PEX13 and PEX14

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Abstract

PEX13 and PEX14 are two key components of the transmembrane hydrophilic channel through which newly synthesized peroxisomal proteins are translocated into the matrix of the organelle. The architecture of this hydrophilic channel remains an enigma, so much so, that even though the functional domains of PEX13 and PEX14 and their protein-protein interactions are relatively well characterized, the membrane topologies of these two proteins are still poorly defined. In this work, using protease-protection assays performed with both proteoliposomes and rat liver peroxisomes, followed by mass spectrometry, Edman degradation and western blotting techniques, the actual topologies of these proteins at the peroxisomal membrane were determined. Our results indicate that PEX14 is indeed a transmembrane protein with a N_{in}-C_{out} topology and that PEX13, contrarily to previous predictions, exposes its C-terminal SH3 domain to the matrix while its N terminus faces the cytosol. These results provide new insights over the organization of the peroxisomal protein import machinery.

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