6 Abstract

Identification

In this study, a reverse genetic screen for peroxisomal proteins was used to analyze 78 protein bands of SDS-PAGE by mass spectrometry. 59 different proteins could be identified. 19 of the proteins proved to be peroxisomal, among them the 3 newly identified proteins Eci1p(enoyl-CoA isomerase), Dci1p (dienoyl-CoA isomerase) which play an accessory role in degradation of unsaturated fatty acids and Tes1p (thioesterase). 11 unknown proteins were suspected to be peroxisomal. In this study, an alternative approach for the analysis of the peroxisomal proteome was established, which combines protein separation during a 1-D SDS-PAGE with MALDI-MS and LC-ESI-MS. It was possible to identify 66 proteins, 25 peroxisomal and 17 proteins of not yet known localization

Functional analysis

The localization of newly identified proteins was confirmed by double-fluorescence microscopy using GFP fusion proteins in conjunction with a peroxisomal red fluorescent protein marker (PTS2DsRed) as well as by subcellular fractionation studies. To reveal the function of newly identified proteins, corresponding knockout strains were analyzed phenotypically for growth on various carbon sources.

It was shown, that **Yor084wp** functions as an oleic acid-inducible putative peroxisomal lipase/esterase. Import in the peroxisomal membrane is Pex5p-dependent. The observed phenotype of the analyzed mutant allows the conclusions that the corresponding gene has no effect of fatty acid degradation. The putative 2-hydroxyphytanoyl CoA lyase **Yel020cp** belongs to the peroxisomal, Pex5p-dependent imported matrix proteins. The protein possesses a thiamin-pyrophosphate-binding site. The gene seems to be not essential for peroxisomal biogenesis. **Ygl184cp** functions as a putative cystathionine β-lyase and in this study it was detected at the peroxisomal membrane. **Yir034cp** is a protein of the peroxisomal matrix that is imported in the organelle because of its PTS1-sequence. Its role in the biosynthesis of lysine leads to the hypothesis, that this kind of amino acid pathway is localized in yeast peroxisomes. For the acetyl-CoA hydrolase **Ybl015wp,** it was shown that it is imported in peroxisomes and in mitochondria because of the potential N-terminal peroxisomal (PTS2) as well as the mitochondrial targeting signal (MTS). **Ycr091wp** is also present in mitochondria and peroxisomes and functions presumably as a putative serine/threonine kinase in posttranslational modifications. In this study, the mitochondrial localization of the unknown protein **Yor228cp** was shown and three transmembrane domains were detected by *in silico* analysis, that led to the assumption that it is localized in the mitochondrial membrane. **Sfc1p/Yjr095wp** is a member of the mitochondrial carrier family

(MCF) and works as a succinate-fumarate transporter. A second, peroxisomal localization was not found. **Yhr199cp** was also found in mitochondria because of a mitochondria targeting signal, but its cellular role was not analyzed. The suspected peroxisomal localization of the **Yal054cp** could be disproved. The observed subcellular localization gave rise to the suspicion that it is localized in ER. **22** proteins were not peroxisomal, indicating that minor contaminations of the preparation were also detected due to the sensitivity of the method.

Pex11p

In this study, the putative function of Pex11p as a voltage-dependent channel in the peroxisomal membrane could not be demonstrated.

The expression of the yeast Pex11p as a TAP-fusion protein was successful and the protein can be purified by affinity columns and than used for further functional analysis.