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The peroxisomal citrate synthase of *Saccharomyces cerevisiae contains* an *N*-terminal signal sequence for both peroxisomal and mitochondrial targeting

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Peroxisomal proteins are synthesized on free polyribosomes in the cytosol and transported into the organelle post-translationally. Most of the peroxisomal proteins have been reported to possess one of the three types of peroxisomal targeting signals (PTSs). PTS1 which is used by vast majority of peroxisomal proteins consists of the C-terminal tripeptide SKL (serine-lysine-leucine) or closely related variants, and is not cleaved off in the process of peroxisomal import. On the other hand, the second one, PTS2, is contained within the *N*-terminal part of the peroxisomal proteins, and the third one in the internal part of the proteins. We have previously found that *N*-terminal 15 amino acids of the peroxisomal citrate synthase (Cit2p) of *Saccharomyces cerevisiae*, is removed during its transport into peroxisomes notwithstanding the presence of PTS1. In the present study, we analyzed the function of the *N*-terminal sequence of Cit2p in protein sorting by using two *N*-terminal domain swapped citrate synthases, $Cit1p_{(1-85)}$::$Cit2p_{(69-460)}$ and $Cit2p_{(1-68)}$::$Cit1p_{(86-480)}$. Here, the former contains the 85-amino-acid *N*-terminal segment of the mitochondrial citrate synthase of *S. cerevisiae* (Cit1p) which harbors the *N*-terminal mitochondrial targeting signal (MTS) followed by the 392-amino-acid C-terminal segment of Cit2p and the latter harbors the 68-amino-acid N-terminal segment of Cit2p followed by the 395-amino-acid C-terminal segment of Cit1p. By cell fractionation followed by enzyme assay and western blotting, $Cit2p_{(1-68)}$::$Cit1p_{(86-480)}$ as well as $Cit1p_{(1-85)}$::$Cit2p_{(69-460)}$ was found to be transported into both mitochondria and peroxisomes, which suggested that the *N*-terminal region of Cit2p contains a signal sequence for targeting into both organelles. The localization of the fusion proteins containing the *N*-terminal sequence of Cit2p and a mutant green fluorescent protein (GFP2) was also analyzed by confocal microscopy, cell fractionation and western blotting. While $Cit2p_{(1-8)}$-GFP2 fusion protein containing the 8-amino-acid *N*-terminal segment of Cit2p remained in cytoplasm, $Cit2p_{(1-20)}$-GFP2 and $Cit2p_{(1-65)}$-GFP2 containing the 20- and 65-amino-acid *N*-terminal segment of Cit2p, respectively, were transported into both organelles. Consequently, the 20-amino-acid *N*-terminal segment of Cit2p was turned out to suffice for an ambidextrous signal sequence for protein import into both peroxisomes and mitochondria that is cleaved during the organelle targeting process. It would be interesting to figure out whether the ambidextrous *N*-terminal signal sequence of Cit2p found in this research requires a new peroxisomal import pathway by using the mutant strains of known peroxin genes and to search for a receptor specific for the *N*-terminal signal sequence of Cit2p. Furthermore, it is worth being revealed if the receptor is involved in mitochondrial import pathway as well as in peroxisomal one.