Experimental determination of organelle targeting-peptide cleavage sites using transient expression of green fluorescent protein translational fusions

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Résumé en anglais	The majority of nuclear-encoded organellar proteins contain a cleavable presequence, which is necessary for protein targeting and import into the correct cellular compartment. Knowledge about targeting-peptide cleavage sites is essential for the structural and functional characterization of the mature organellar proteins as well as for a deeper understanding of the import process. Because of the low consensus and high variability of presequences, bioinformatics of targeting-peptide cleavage fails to predict the length of the targeting peptide with high confidence. Therefore, we have developed a rapid and robust method to experimentally determine the cleavage site of the transit peptide for proteins imported into mitochondria or plastids. The protein precursor with green fluorescent protein (GFP) fused to its C-terminus is transiently expressed in cells (for animal proteins) or protoplasts (for plant proteins), allowing translocation into organelles and removal of the transit peptide. After lysis, the matured protein is immunopurified using an anti-GFP antibody coupled to magnetic beads. The N-terminal amino sequence is then determined by Edman microsequencing or mass spectrometry. The method has been validated using proteins with known targeting-peptide sequences and is suitable for animal and plant organelle-targeted proteins.
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