Role of Luminal Domain on Intracellular Localization of Tobacco Membrane-Anchored Prolyl 4-Hydroxylase

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NtP4H1.1 is a Golgi-localizing type II integral membrane protein. Mutations in the cytoplasmic tail direct the protein to the endoplasmic reticulum (ER). We expressed a GFP fusion protein containing the mutant tail and the transmembrane region in tobacco BY-2 cells, and found that the protein localized in the Golgi. Therefore NtP4H1.1 contains multiple targeting information in different regions.

Key words: O-glycosylation; protein targeting determinant; higher plant; type II membrane protein; post-translational modification

The first step in the plant-specific O-glycosylation of proteins is the generation of Hyp residues in the proteins. Many Hyp residues are further glycosylated by arabinogalactan or oligo-arabinose glycan chains. The generation of Hyp residues in proteins is catalyzed by peptidyl-proline 4-dioxygenase, also called prolyl 4-hydroxylase (P4H). Plants have multiple P4Hs, divided into two classes. One of the classes, called type 1 P4H, contains an N-terminal type II signal anchor.1,2) Not only type 1 P4Hs but also many glycosyltransferases in the Golgi apparatus are type II membrane proteins. Many of them contain localization information in proteins, although the mechanism of Golgi localization varies across proteins.3) ER retention of a mutant of tobacco type 1 P4H (NtP4H1.1, formally tobacco PH1)3) suggests that this protein has multiple determinants of intracellular localization. We attempted to determine whether the luminal region of NtP4H1.1 contributes to intracellular localization.

NtP4H1.1 is composed of a short cytosolic tail of 12 amino acids, a transmembrane region of 22 amino acids, and the luminal region containing the catalytic domain. We have found that this protein and fusion with GFP localized predominantly in the cis-Golgi.2) We also found that replacement of the basic residues in the cytosolic tail with neutral amino acids prevented the export of GFP fusion from the ER.2) However, we occasionally observed that the mutant protein migrated to the ER and the Golgi apparatus, especially when expressed at high level. This suggests a possibility that even in the mutant protein information directing to the Golgi is present while other information to retain in the ER caused most of it to accumulate in the ER.

To assess this possibility, first we predicted the domain structure of NtP4H1.1. Homology modeling of it by SWISS-MODEL4) using Chramidomonas P4H5) as template suggested that its globular catalytic domain starts at the 75th proline residue and continues to the C-terminus. Next we assessed the possible higher-order structures of the N-terminal part of the luminal region comprised of 34th to 74th residues by Chou-Fasman prediction.5) Based on these analyses we predicted that NtP4H1.1 has the following structural units: An N-terminal cytosolic region, a transmembrane region, a flexible linker, an α-helical stalk, another flexible linker, and a globular catalytic domain (Fig. 1A).

We next addressed whether most of the luminal region of NtP4H1.1 has a role on intracellular localization by expressing a truncated version of NtP4H1.1 fused with GFP in tobacco BY-2 cells, and analyzed the localization. To detect GFP fluorescence, we used an IX81 fluorescence microscope (Olympus, Tokyo) equipped with IX2-DSU module (Olympus), which gives confocal images. Images were captured with an iXon DU-888E CCD camera (Andor, Belfast) and then analyzed by MetaMorph software (Molecular Devices, Sunnyvale).

GFP was fused just after the first linker sequence (Fig. 1B). Cells expressing this construct (NtP4H1.1Δcat-GFP) showed a punctate fluorescence pattern (Fig. 2, arrowheads) that was indistinguishable from that of full-length NtP4H1.1-GFP fusion (Fig. 2). The punctate pattern was clearly different from a perinuclear ring and intracellular strand-pattern of ER-localizing NtP4H1.1(QTT)-GFP, which lacks three basic amino acids in the cytosolic tail.6) When the same amino acid substitutions were introduced into a truncated construct [NtP4H1.1Δcat(QTT)-GFP], the fluorescence pattern was punctate and quite similar to that of the wild type (Fig. 2). This suggests that both of the truncated proteins migrate to the ER and Golgi apparatus, especially when expressed at high level. This suggests a possibility that even in the mutant protein information directing to the Golgi is present while other information to retain in the ER caused most of it to accumulate in the ER.

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proteins, regardless of basic residues in the cytosolic tail, localized in the Golgi apparatus.

To confirm that the localization sites of the truncated mutants were in the Golgi apparatus, we treated cells expressing corresponding GFP fusion proteins with brefeldin A (BFA) and analyzed the pattern of intracellular fluorescence. BFA treatment is known to redirect cis-Golgi localizing proteins to the endoplasmic reticulum.2,7) As shown in Fig. 3, BFA treatment directed both NtP4H1.1 cat-GFP and NtP4H1.1 cat(QTT)-GFP to the ER. This suggests that both NtP4H1.1 cat-GFP and NtP4H1.1 cat(QTT)-GFP localized to the cis-side of the Golgi apparatus, as in the case of NtP4H1.1 and its GFP fusion proteins.

As described above, we observed that deletion of most of the lumenal domain of tobacco prolyl hydroxylase NtP4H1.1 directed the GFP fusion protein to the Golgi apparatus regardless of the basic residues in the cytosolic tail, localized in the Golgi apparatus.

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References


