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C-Terminal Propeptides and Vacuolar Sorting by BP-80–Type Proteins: Not All C-Terminal Propeptides Are Equal

A recent discussion in the pages of THE PLANT CELL concerning the C-terminal vacuolar targeting signal of Na-PI and the interaction of this protein with a BP-80 ortholog (Smith, 1999; Jiang and Rogers, 1999; Miller and Anderson, 1999) perhaps espouses the over-simplified view that all C-terminal propeptide signals mediate vacuolar delivery by a common mechanism.

Historically, vacuolar sorting signals have been classified as N-terminal propeptides (NTPPs), C-terminal propeptides (CTPPs), and internal signals (Matsuoka and Neuhaus, 1999). The vacuolar targeting mediated by the CTPPs of barley lectin and tobacco chitinase precursors is sensitive to both high concentration of wortmannin in logarithmically grown tobacco BY-2 cells (Matsuoka et al., 1995) and to the addition of Gly residues at the C terminus (Dombrowski et al., 1993; Koide et al., 1999). Thus, these propeptides form a distinct class of signal, known as the C-terminal vacuolar sorting signal (ctVSS; Matsuoka and Neuhaus, 1999).

In contrast, vacuolar sorting mediated by the NTPP of sporamin is relatively resistant to wortmannin treatment in tobacco BY-2 cells, suggesting a distinct cellular mechanism (Matsuoka et al., 1995). The N-terminal signals of sporamin and aleurain precursors contain a conserved NPIR tetrapeptide sequence that facilitates binding to the vacuolar sorting receptor, BP-80 (Neuhaus and Rogers, 1998). Similarly, the NTPP of PT20, a 20-kD potato proteinase inhibitor (unrelated to Na-PI), contains an NPIXL motif that functions as a vacuolar sorting signal (Koide et al., 1999). Interestingly, recent observations indicate that the vacuolar sorting signal in the NTPP of the sporamin precursor can function at the C terminus of the protein to render vacuolar sorting resistant to either wortmannin or the addition of Gly residues (Koide et al., 1997, 1999). This observation is consistent with the finding that the C-terminal vacuolar targeting determinant of Brazil nut 2S albumin can bind to BP-80 in vitro (Kirsch et al., 1996) and that the pumpkin 2S albumin CTPP can bind a BP-80 ortholog in pumpkin via an NLPS sequence that functionally resembles NPIR sequences (Shimada et al., 1997). Thus, these targeting signals are currently classified as sequence-specific vacuolar sorting signals (ssVSS), regardless of their location (Matsuoka and Neuhaus, 1999).

Whereas the NTPPs of sporamin and aleurain share an NPIR motif that promotes pH-dependent binding of BP-80 in vitro, recent mutation analysis of the ssVSS of the sporamin precursor indicates that the NPIR motif is not the consensus sequence that functions as a vacuolar targeting determinant. Rather, the amino acid requirement to function as a sorting signal is $\mathbf{X_1\text{-}X_2\text{-}I/L\text{-}X_3\text{-}X_4}$, where X_1 lacks a small hydrophobic side chain (Asn is the preferred amino acid), X_2 may not be an acidic amino acid, X_3 is any amino acid, and X_4 is a large and preferably hydrophobic residue. We propose that hydrophobic interactions between the side chain of Ile/Leu and a receptor would in fact play a pivotal role in sorting (Matsuoka and Nakamura, 1999). The NPIR sequence of the aleurain propeptide does not fully conform to these requirements, because proline, which is a small side chain imino acid, lies immediately downstream from the NPIR. Indeed, the aleurain NTPP contains information additional to the NPIR region that is required for efficient sorting to the vacuole (Holwerda et al., 1992), and these non-NPIR regions are recognized by BP-80 in a mechanism that is independent of the NPIR-recognition domain (J.C. Rogers, personal communication).

In the CTPP of the Na-PI precursor, there is an NDLQK sequence that

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partially matches the amino acid requirement of the sporamin ssVSS. Therefore, this NDLQK sequence may be the sorting determinant recognized by a *Nicotiana alata* BP-80 ortholog. It is probable that the NDLQK sequence can function at least as a partial ssVSS as does the NPIR region of the aleurain precursor. Conceivably, this NDLQK region could combine with other region(s) of the Na-PI precursor to contribute to the association of Na-PI with an *N. alata* BP-80 ortholog (Miller et al., 1999). However, the possibility remains that the Na-PI CTPP could function as both a ctVSS and an ssVSS. For example, the β-1,3-glucanase precursor, which is sorted to the vacuole by its CTPP, shows an intermediate sensitivity to wortmannin (Matsuoka et al., 1995). Furthermore, the NTPP sequence of the sporamin precursor, when attached to the C terminus of sporamin, confers resistance to wortmannin and modification by Gly, although the disruption of the NPIRL determinant designates this propeptide as a ctVSS (Koide et al., 1997, 1999). Thus, the cellular mechanisms that recognize vacuolar sorting determinants are very complex and may be partially redundant. The presence of a ctVSS in the Na-PI CTPP could be tested by the addition of Gly residues at its C terminus or by assessing sensitivity to wortmannin.

A remaining question is whether the cross-linking of Na-PI and BP-80 ortholog observed with isolated PVCs (Miller et al., 1999) represents the in vivo situation of sorting. A current model of vacuolar sorting is that a receptor–ligand complex is formed at the *trans* side of the Golgi apparatus and that this complex dissociates upon exposure to the lower pH in the PVC (Neuhaus and Rogers, 1998). If this model is correct, the Na-PI precursor may not be associated with a sorting receptor even though these two proteins are present in the same PVC. Thus, the observed association (Miller et al., 1999) could be attributed to the possible artifactual neutralization of the PVC internal milieu during isolation. Indeed, cross-linking reactions of the type used by Miller et al. (1999) generally require neutral or alkaline conditions, so that the cross-linking of the Na-PI precursor to the BP-80 ortholog would be unlikely within the acidic PVC. The cross-linking data of Miller et al. (1999) should therefore be interpreted with caution. Further in vivo experimental evidence will be required to reach the conclusion that BP-80 and its orthologs, which function as ssVSS binding proteins in vitro, are the receptors that sort ssVSS-containing proteins to the (lytic) vacuole.

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