Pathways for protein transport to seed storage vacuoles

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Abstract

Plant vacuoles have multiple functions: they can act both as digestive organelles and as receptacles for storage proteins. Different types of vacuoles can coexist in the same cell, which adds complexity to the process of targeting to these compartments. A fuller understanding of this process is of evident value when endeavouring to exploit the plant secretory pathway for heterologous protein production. Positive sorting signals are required in order to sort proteins to vacuoles, and these have been split into three groups: ctVSS (C-terminal VSS (vacuolar sorting signals)), ssVSS (sequence-specific VSS) and physical structure VSS. The current working model posits that soluble proteins are delivered from the Golgi apparatus to the lytic vacuoles in clathrin-coated vesicles by virtue of their ssVSS, or to the storage vacuole (PSV (protein-storage vacuole)) in dense vesicles in a manner dependent on ctVSS or physical structure VSS. Although targeting to LV appears to be receptor-mediated, no such receptor has been identified for the recruitment of proteins to the PSV. We have studied the vacuolar targeting of two castor bean (Ricinus communis L.) storage proteins, proricin and pro 2 S albumin, in their native endosperm and in the heterologous system of tobacco protoplasts. We have found that both these proteins contain bona fide ssVSS and bind to sorting receptors in vitro in a similarly sequence-specific manner. The apparent similarities to lytic VSS and possible implications with respect to the working model for transport to storage vacuoles are discussed.

Introduction

Vacuoles are multifunctional organelles that can be regarded as the defining feature of plant cells. In vegetative tissues, vacuoles can constitute up to 90% of the total cell volume. The principal function of vacuoles is the maintenance of cell turgour, but they are also involved in the turnover of macromolecules and the sequestration of toxic compounds and secondary metabolites [1]. Vacuoles participate in programmed cell death [2] and can accumulate proteins as an amino acid source for germination [3]. The fact that this array of seemingly contrasting functions can be performed by the same organelle can be explained with the previous finding that plant cells have different types of vacuoles [4], and these can coexist in a single cell [5,6]. Vacuoles can generally be divided into two main categories: (i) LVs (lytic vacuoles) are acidic compartments, rich in hydrolases and can be regarded as the equivalent of mammalian lysosomes; and (ii) PSVs (protein-storage vacuoles), on the other hand, are found in storage organs, mainly seeds, and can store large amounts of protein, which will be used as a source of nitrogen during seed germination [3].

Vacuolar sorting pathways

Topologically, both types of vacuoles, LVs and PSVs, are the intracellular endpoint of the plant secretory pathway. According to the current working model [3] for protein transport to vacuoles (Figure 1), proteins are initially translocated into the ER (endoplasmic reticulum). They then travel through the Golgi complex, where they can enter two possible pathways: (i) a route to LV via a PVC/MVB (prevacuolar compartment/multivesicular body) [7,8]. This is mediated by a sorting receptor, initially called BP-80 [9], which recruits clathrin coats by binding clathrin adaptor complexes [10,11]; (ii) a less well-characterized route to PSV, which may or may not use a receptor but employs non-coated ‘dense vesicles’ (DV) [12,13]. In vegetative cells, the two pathways coexist and the separate vacuoles ultimately merge to form the large central vacuole [14]. A third, alternative transport pathway has also been described in developing pumpkin cotyledons, where 2 S albumins leave the ER in large PAC (precursor-accumulating) vesicles. These bypass the Golgi complex and directly fuse with PSV [15].

VSS (vacuolar sorting signals)

The current model for vacuolar transport is based on the study of a limited number (∼30) of cargo proteins, most of which are soluble [3]. In general, vacuole-bound proteins must carry positive sorting information, in addition to the N-terminal signal peptide for translocation into the ER. VSS have been grouped into two main categories: (i) ssVSS
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Figure 1 | Working model for soluble protein sorting to vacuoles

All proteins are translocated into the ER lumen. Proteins destined to LV bind the sorting receptor BP-80, and enter CCVs. They are then released into the PVC/MVB, from where BP-80 is recycled, and are eventually delivered to LV. Most storage proteins are concentrated throughout the Golgi stack and packaged into DV, which eventually fuse with PSV. Some proteins can interact with BP-80 and be targeted to PSV, possibly via the multivesicular body. 2 S albumin in pumpkin cotyledon aggregates and buds off directly from the ER in PAC vesicles. These may receive glycosylated proteins from the Golgi.

(sequence-specific sorting signals). ssVSS reside within cleavable propeptides that can be found at any position within the precursor proteins [3]. The sorting motif is centred around a critical Ile/Leu residue within the tetrapeptide [N/L][P/I/L][I/P][R/N/S]. Mutation of the Ile/Leu residue completely abolishes vacuolar targeting and results in secretion of the protein [3,16]; (ii) ctVSS comprise a number of C-terminal propeptides rich in hydrophobic residues, but very variable in sequence. A consensus motif has so far proven difficult to identify, due to sequence heterogeneity in the limited number of proteins studied so far. It seems that the major requirement is for the C-terminus to be exposed: addition of two glycine residues to several ctVSS can result in protein secretion [3,16], suggestive of masking of the VSS.

ssVSS have been shown to interact in vitro with the BP-80 family of receptors [9,17,18] and, for this reason, proteins carrying ssVSS have been postulated to travel to the lytic vacuoles. In contrast, proteins carrying ctVSS are thought to be targeted to PSVs in DV or PAC vesicles [3] via aggregation-based sorting. Proteins of the 11 S globulin family are known to form insoluble, membrane-bound complexes as early as the Golgi apparatus [23,24]. For the bean 7 S storage protein phaseolin, membrane association has been linked to the presence of its hydrophobic ctVSS, the tetrapeptide AFVY [25–27]. A sorting mechanism similar to regulated protein secretion in mammalian endocrine and exocrine gland cells [28,29] has been envisaged, by which proteins can aggregate around membrane-bound nucleation factors and strongly interact with the Golgi membrane, triggering DV budding. Non-storage material trapped in the vesicles would then be sorted out of DV by means of CCVs (clathrin-coated vesicles) [13]. A potential candidate for the nucleation factor is the plant-specific RMR protein [30]. This protein travels through, but is not recycled to, the Golgi complex, and has been shown to bind the storage protein ctVSS in vitro. Again, very little is known about the machinery responsible for this sorting mechanism.

Recently, the ‘one vacuole, one pathway’ model has been called into question by the finding that some storage proteins, such as ricin and castor bean 2 S albumin, carry ssVSS and can interact with BP-80 in vitro [19–21]. Also, it has recently been shown that insertional inactivation of the seed-specific isoform of the receptor BP-80 in Arabidopsis results in the partial secretion of all major classes of storage proteins [22]. It is therefore possible that some storage proteins may interact with BP-80 and be recruited into CCVs. In this case, there must exist a mechanism to sort this cargo away from the LV, assuming that LVs are present in the cell. It is possible that this sorting occurs within the multivesicular body, although nothing is known about this process [3].

It is clear that there are several, major outstanding questions in the study of vacuolar transport pathways: is BP-80 a receptor for storage proteins? Is RMR a receptor or a nucleation factor? What is the sorting signal for one of the major classes of storage proteins, the 11 S globulins? What is the role of the multivesicular body? What factors determine...
the identity and function of different vacuoles in different tissues? How does aggregation play a role in sorting?

As stated previously, the paucity of data available on the signals and routes of vacuolar proteins is a serious limiting factor in our understanding of the vacuolar sorting process. In the case of the model plant Arabidopsis thaliana, this is even more striking. Only one protein (aluerain) carrying ssVSS has been characterized to date [10], and no endogenous proteins carrying ctVSS have been studied. A very recent, comprehensive study of the vacuolar proteome of Arabidopsis has now identified a relevant number of bona fide vacuolar proteins [31]. Remarkably, analysis of their precursors’ primary structures only identified ctVSS or ssVSS in a small proportion of vacuolar residents [31]. This clearly indicates that there may be many alternative mechanisms for sorting and that our current model for vacuolar sorting may be drastically revised in the future. A multidisciplinary approach is clearly required to tackle this complex but exciting biological question.

References