

The Endomembrane System and the Problem of Protein Sorting

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"Yes there are two paths you can go by
But in the long run,
There's still time to change the road you're on."
Stairway to Heaven, Led Zeppelin

A 1976 review on the secretion of plant proteins and polysaccharides stated, "the only plant proteins which have been shown by autoradiography to be synthesized on rough ER are the storage proteins of *Vicia faba* cotyledons," and that they are transported to protein bodies by "... a process that has many analogies to secretion" (5). What was known about protein secretion in eukaryotes at that time? Key experiments of the late 1960s established the paradigm that secretory proteins are inserted into the endoplasmic reticulum (ER) by virtue of a transient signal peptide and then transported through the Golgi complex to their final destination. Not much more was known. In this short review we aim to summarize the enormous advances that have been made in the last 25 years in understanding the plant endomembrane system, and to show how studies of plant cells have yielded fundamental insights into eukaryotic cell biology.

THE UNIQUENESS AND DIVERSITY OF PLANT VACUOLES

In 1983 it was shown that the vacuolar storage proteins of legumes, when synthesized in *Xenopus* oocytes following RNA injection, did not accumulate in lysosomes, but were secreted into the incubation medium (2). This observation showed that plant vacuoles are not simply a variation of animal lysosomes. This study was followed by unsuccessful efforts to use yeast as an expression system. It eventually became clear, however, that plant expression systems should be used to search for plant vacuolar sorting signals. This approach resulted in the identification of the first signal in 1990—the C-terminal propeptide of barley lectin (3). Two months later the discovery of another vacuolar sorting signal, the N-terminal propeptide of sweet potato sporamin, was reported (22).

A few months before, a fundamental contribution to the establishment of the "bulk flow" model of eukaryotic protein traffic was made. It was observed that the insertion of bacterial proteins into the secretory pathway of plant cells by the addition of a signal peptide resulted in their secretion (9). This was the first demonstration of a bulk flow of proteins from the ER to the cell surface and extended previous results obtained using small peptides expressed in mammalian cells. The existence of bulk flow secretion also supported the hypothesis that vacuolar proteins need sorting signals. Following these pioneering studies, the study of vacuolar sorting exploded. About a dozen signals have currently been identified.

Important advances are being made concerning the mechanisms by which vacuolar proteins are sorted from secreted proteins. The first cloning of protein belonging to the sorting machinery of the plant endomembrane system has recently been reported. The transmembrane protein BP80/AtELP, which is thought to be the receptor that recognizes one of the signals (the so-called AsnProIle Arg motif), has been purified using affinity chromatography with peptides containing sorting signals and its cDNA cloned (17).

A second landmark in the field was the discovery in 1995 that in seeds, protein storage vacuoles are apparently not formed from the "normal" lytic vacuoles, but rather constitute a distinct compartment (13). This was followed in 1996 by the discovery that two types of vacuoles coexist in many young cells (24). This breakthrough was abetted by refinements in electron and confocal immunomicroscopy, and by the discovery of plant aquaporins (16), a family of proteins that has distinct members embedded in the tonoplasts of the various vacuole types. The circle was closed by the finding that protein traffic from the Golgi complex to lytic and storage vacuoles occurs by distinct routes that show different sensitivity to wortmannin, an inhibitor of phosphatidylinositol kinases and phospholipid biosynthesis (21). Clathrin-coated vesicles containing BP80/AtELP mediate traffic to lytic vacuoles via a prevacuolar compartment, which seems to perform functions similar to those of animal and yeast endosomes. In contrast, proteins destined for storage vacuoles are transported by "dense vesicles," structures that were actually discovered back in 1983 (6). The search for their receptor system is still

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ongoing. The hypothesis supporting the different ontogenies of lytic and storage vacuoles in developing seeds (13) contradicts a previous hypothesis claiming that the storage vacuoles are formed by subdivision of lytic vacuole during seed maturation (8). It is possible that more than one mechanism controls the biogenesis of storage vacuoles.

A third powerful and more recent approach takes advantage of the information in databases. As a result, many plant membrane (like the SNARE family) and cytosolic proteins controlling the specificity of vesicle formation and fusion between the ER, Golgi complex, and vacuoles have now been identified. These new molecular tools led to the discovery of the above mentioned prevacuolar compartment (26) and are enabling researchers to begin to understand the details of the protein-protein interactions involved in vesicle sorting (1). This approach is revealing a diversity of functions in the plant endomembrane system, many of which were unexpected based solely on sequence comparisons (1). Vacuolar sorting is now one of the most advanced and fascinating fields of research in eukaryotic cell biology.

PROTEIN ACCUMULATION WITHIN THE ER, A FINELY REGULATED PROCESS

Seed storage proteins are usually vacuolar, but several cereals utilize the ER to accumulate their storage proteins (prolamins) into large aggregates termed protein bodies. Cereals are the only known organisms that have a developmental program to store proteins in the ER. Prolamins cannot be extracted from seeds using simple aqueous buffers and were thought, therefore, to precipitate immediately after insertion into the ER lumen. The question of their inability to be transported along the secretory pathway seemed thus easily answered. The large variability between different prolamins also contributed to the notion that their structure may not be important to the accumulation process. However, recent studies suggest that despite their final deposition as very dense aggregates, the nascent storage proteins are folded as soluble monomers and then undergo specific assembly and deposition processes (27). In fact, the first protein-protein interactions detected in the plant secretory pathway were the association of the binding protein BiP, an ER-located member of the heat shock protein 70 family, to two storage proteins. More specifically, BiP was found to be associated with rice prolamins during their entire maturation into protein bodies (20), and with nascent monomers of phaseolin (a bean soluble vacuolar protein) until they assembled into trimers (30). These studies suggested that protein body formation is an assembly process that is similar to the oligomerization of soluble proteins. Prolamin accumulation within the ER may arise initially from extensive interactions with the chaperone machinery. Once the protein body is assembled, pro-

lamins may be physically unable to enter the small COP (coatamer protein) vesicles that leave the ER for the Golgi complex.

A widely held paradigm proposes that the biogenesis of endomembrane organelles is regulated solely by a special machinery, whereas the cargo proteins play no role in this process. Analyses of seed storage protein trafficking have refuted this paradigm, showing that biogenesis of organelles may also be determined by the cargo proteins. The storage proteins of barley generally accumulate in storage vacuoles. Yet elimination of one individual group of storage proteins, a consequence of a natural mutation, resulted in accumulation of the remaining storage proteins in ER-derived protein bodies (25). In a similar manner, expression of single type or a combination of two types of maize storage proteins in transgenic tobacco plants showed that individual proteins are transported to vacuoles, while the two types together accumulate in ER-associated compartments, similar to the situation in maize seeds (7). Such ER-associated compartments are not seen in wild-type tobacco cells and were apparently induced by the maize storage proteins.

DIRECT ROUTES FROM THE ER TO VACUOLES

The prevailing view of most eukaryotic biologists is that secretory proteins exit from the ER by means of COP vesicles and pass via the Golgi to their final destination (Fig. 1, route no.1). The discovery that wheat seeds possess an additional pathway for the ontogeny of storage vacuoles, which bypasses the Golgi, does not fit with this paradigm (19) (Fig. 1, route no. 2). Such a pathway in which proteins are directly delivered from the ER to the vacuole is mediated by very large vesicles that bud from the ER through an unknown mechanism and operates in parallel with the Golgi-mediated pathway. It took several years to discover that such a process is not unique to wheat endosperm and may represent a general mechanism of plant vacuolar ontogeny. Maize zeins expressed in transgenic tobacco seeds (7) as well as an endogenous vacuolar storage protein in pumpkin seeds (12) have been found to be delivered from the ER to vacuoles by similar processes (Fig. 1, route no. 4). Direct ER-to-vacuole transport is not limited to storage proteins: the intracellular traffic of at least one tonoplast aquaporin also seems to be Golgi independent (10). Another example is a thiol protease produced during mung bean seed germination. This enzyme leaves the ER in special, large vesicles termed KDEL vesicles (KV) vesicles that later fuse directly with the storage vacuoles (29) (Fig. 1, route no. 3).

It is interesting that this mung bean thiol protease, like its counterparts in other plants, possesses a C-terminal, ER-retrieval KDEL signal that is post-translationally removed (29). In all eukaryotes ER-

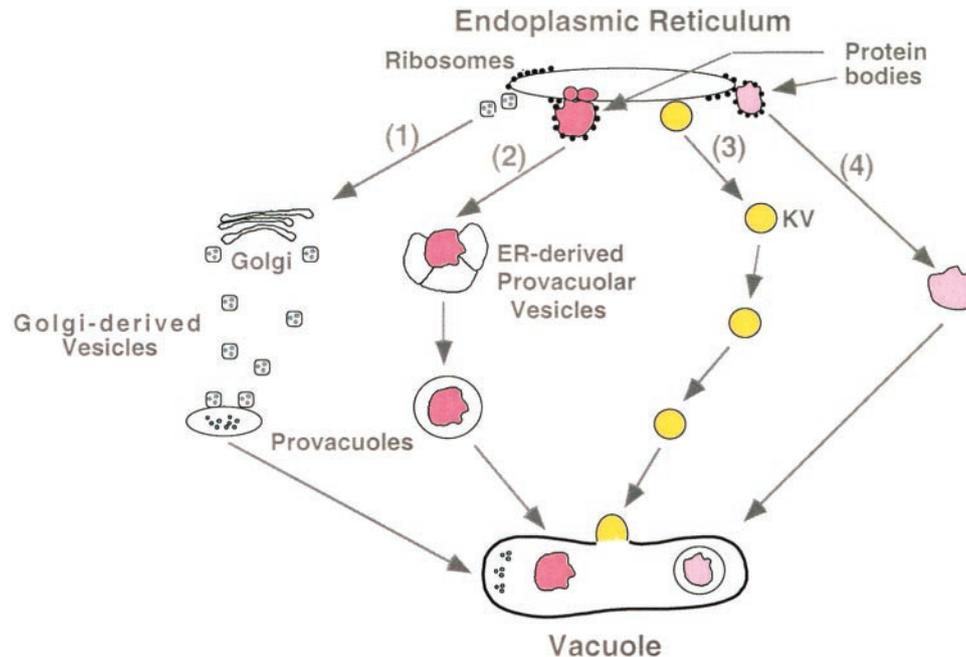


Figure 1. Schematic representation of direct routes of proteins from the ER to vacuoles. 1, The classical vesicular trafficking route via the Golgi and provacuoles, depicted for comparison; 2, a direct route from the ER to vacuoles that transports storage proteins in wheat endosperm; 3, budding of KV vesicles containing a mung bean thiol protease from the ER and their subsequent direct fusion with the vacuole; 4, a direct route from the ER to vacuoles found in seeds of transgenic tobacco plants expressing maize zein storage proteins. The pathway generating pumpkin precursor accumulating (PAC) vesicles from the ER is similar to route number 4, whereas their subsequent internalization into vacuoles appears morphologically similar to route number 2. The routes illustrated in pathways 2 and 4 appear morphologically similar to autophagy. In route number 2 the ER-derived protein bodies are wrapped by provacuolar vesicles in a morphologically similar manner to the formation of autophagosomes. In route number 4 the ER-derived protein bodies are internalized directly into vacuoles.

retrieval (K/H) DEL signals are the major means by which soluble residents of the ER accumulate in this compartment: a receptor located in the Golgi complex retrieves (K/H) DEL-containing proteins back into the ER. However, the KDEL tetrapeptide does not prevent (or may even promote) accumulation into special large vesicles, like those containing the mung bean protease. The contribution of plant biology to the elucidation of (K/H) DEL function does not end at this point. Despite having a permanent KDEL signal, the auxin-binding protein (ABP1) is present in low amounts at the cell surface (14) where it binds auxin. This indicates that ABP1 uses the KDEL signal to regulate its subcellular location rather than simply as a means to be retained in the ER.

NOVEL INSIGHTS INTO THE PLANT GOLGI COMPLEX

The Golgi complex is a factory for the production of complex carbohydrates and a crossroad for protein traffic. The end of the 20th century brought a better understanding of the known roles of the plant Golgi complex, as well as novel insights concerning the integration of this compartment into the plant endomembrane system. A peculiar feature of the plant Golgi complex is its well-known involvement in the

formation of the cell plate during cell division. The cell plate is a unique plant structure formed when Golgi-derived vesicles accumulate in the phragmoplast and begin to fuse, first into tubules, and then into sheets that enlarge toward the cell periphery. Using two different approaches, the first two specific proteins involved in cell plate formation were identified in 1996 and 1997. Phragmoplastin was cloned and found to be a homolog of yeast and mammalian dynamins, which are GTPases involved in various steps of the secretory pathway (11). Screening of *Arabidopsis* mutants impaired in embryogenesis identified a novel t-SNARE (KNOLLE gene product) that is localized in the cell plate (18), and which is expressed only during cell division.

Unlike mammalian cells where the Golgi complex is condensed in a limited perinuclear region, plant cells contain a large number of Golgi stacks distributed in the cytoplasm. Green fluorescent protein fusions recently allowed the visualization of Golgi stacks *in vivo* and revealed unsuspected dynamic relationships with the ER, mediated by actin filaments (4, 23). Based on these observations, new models for the role of Golgi movement in intracellular traffic have been formulated, which have changed our static view of the endomembrane compartments (4, 23).

EPILOGUE

The last quarter of the 20th century was characterized by explosive research and major discoveries concerning the plant endomembrane system. Among the major challenges for future research are: (i) the identification of the trafficking and sorting machineries that regulate Golgi-mediated and Golgi-independent protein transport to storage vacuoles, and (ii) the elucidation of the role of the secretory pathway in plant specific processes. In this latter respect we have already mentioned cell plate formation. Recent discoveries, however, suggest that secretory pathways may also be important for understanding plant-pathogen interactions and auxin transport (which in turn regulates a variety of developmental processes). For example, it has been found that the synthesis of ER-resident molecular chaperones in plants is regulated not only by the unfolded protein response, but also by a novel, pathogen-activated signal transduction pathway (15). In addition, the cloning of the GNOM gene has revealed that it encodes for an ADP-ribosylation factor/guanine nucleotide exchanger that mediates specific vesicle coating and budding to control polarized transport of the auxin efflux carrier (28).

In the late 1970s, cell biology studies involved mainly microscopical and biochemical analysis of trafficking routes. Gene cloning was a novelty and transgenic plants were a dream. We are now entering "the post-genomic era" of plant biology. Site-directed mutagenesis, transgenic plants, large scale sequencing, and reverse genetics, as well as the development of confocal microscopy, the use of green fluorescent protein, and refinement of immunoelectron microscopy have already and will continue to have a tremendous impact on the study of the endomembrane system. Dominant negative mutants, the new tools of biochemistry (in vitro vesicle formation systems and large-scale maps of protein-protein interactions), and functional genomics will also play major roles.

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