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Review

Mass spectrometry approaches to study plant endomembrane trafficking

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ABSTRACT

Intracellular proteins reside in highly controlled microenvironments in which they perform context specific functions. Trafficking pathways have evolved that enable proteins to be precisely delivered to the correct location but also to re-locate in response to environmental perturbation. Trafficking of membrane proteins to their correct endomembrane location is especially important to enable them to carry out their function. Although a considerable amount of knowledge about membrane protein trafficking in plants has been delivered by years of dedicated research, there are still significant gaps in our understanding of this process. Further knowledge of endomembrane trafficking is dependent on thorough characterization of the subcellular components that constitute the endomembrane system. Such studies are challenging for a number of reasons including the complexity of the plant endomembrane system, inability to purify individual constituents, discrimination protein cargo for full time residents of compartments, and the fact that many proteins function at more than one location.

In this review, we describe the components of the secretory pathway and focus on how mass spectrometry based proteomics methods have helped elucidation of this pathway. We demonstrate that the combination of targeted and untargeted approaches is allowing research into new areas of the secretory pathway investigation. Finally we describe new enabling technologies that will impact future studies in this area.

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1. Introduction

Intracellular proteins exist in controlled micro-environments where they fulfil different roles dependent on their local surroundings. It is therefore necessary for proteins to traffic within a cell to the correct location to enable them to carry out their function. This is particularly crucial for membrane proteins that must be trafficked through the endomembrane system to their functional location. This process is achieved by highly evolved trafficking pathways. Moreover, some membrane proteins may fulfill roles across multiple destinations, as many membrane proteins cycle between compartments as part of their function. Modulation of protein abundance at different locations is of paramount importance for responding to perturbations in the environment, such as biotic or abiotic stresses, as plants are sessile and cannot remove themselves from a suboptimal environment [1].

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Conventional protein trafficking within the endomembrane system is initiated at the endoplasmic reticulum (ER), from which newly synthesized membrane or soluble cargo proteins are transported through the Golgi apparatus, and on to the trans Golgi network (TGN) where they are sorted and further trafficked. Proteins, destined to be secreted, are transported to the plasma membrane (PM), whereas other proteins whose final destination is the plant vacuole, migrate through the multi vesicular body (MVB)/prevacuolar compartment (PVC)/late endosome (LE) as reviewed in [1]. The PM, apoplast and vacuole are the major destination regions for trafficked proteins, although PM proteins may also be endocytosed and recycled between the PM and TGN. If not recycled, proteins are delivered from the TGN to the vacuole via PVCs/MVBs/LEs, to the lytic vacuole where they are degraded [1]. There is increasing evidence that additional trafficking routes also exist in plant cells, but as this in has not yet been subjected to proteomic analysis, we will not describe such evidence in detail in this review (see future directions).

Our knowledge of membrane protein trafficking in plants has been brought about by many years of dedicated research, as reviewed in [2]. On-going research, however, continues to give unexpected insights into the complexities associated with plant membrane protein trafficking. For example, the chloroplast is also a destination organelle for the secretory pathway [3–5] via a TOC/TIC independent pathway of nuclear encoded chloroplast protein translocation, reinforcing that our understanding of even fundamental trafficking processes is far from complete.

To further our knowledge of endomembrane trafficking, it necessary to fully characterise the subcellular compartments that constitute this system. Methods to elucidate the system need to return accurate information about protein location, but must also be able to capture the dynamic nature of trafficking. Thorough characterisation of trafficking routes is non-trivial however, because the ER, Golgi and post-Golgi compartments contain both cargo and resident proteins, as well as transiently associating proteins. Accurate discrimination between these categories of proteins is required if robust models of protein trafficking are to be determined. Moreover, the Golgi, the hub of the secretory network, is made of a series of sub-compartments, or cisternae, through which resident proteins are thought to cycle, adding additional granularity to the system. As many proteins cycle between the compartments of the secretory pathway, it is perhaps more beneficial to think of proteins having a steady-state location within trafficking routes rather than having a single, fixed location. Although challenging, it is important to be able to map proteins with multiple locations and determine how the location of cargo and residents change in upon perturbation, if we are to understand plant responses at the systems level.

The complexity of the plant secretory pathway, compared to its animal counterpart, means that a variety of techniques are required for its thorough investigation. Given that the endomembrane systems pervades the entire cell, they must be understood in a whole cell context employing approaches that capture information at the whole cell level. Conversely, the transient cargo content of different trafficking vesicles, and the temporary association trafficking regulators with vesicles, might not be visible against a whole-cell background. Isolation and out-of-context analysis of these compartments can therefore play an equally important role.

Methods applied to determine the molecular machinery of the endomembrane system fall into two broad categories; hypothesis testing, hypothesis generating. The former set out to prove the location(s) of a set of pre-conceived proteins within the secretary pathway, typically by observation of a fluorophore-tagged protein or an antibody raised against the protein of interest [2,6–8].

The latter category centres round either isolating or enriching a sub-cellular compartment of interest and then using protein identification methods to get a 'parts-list' of proteins present. In effect, these approaches do require some prior knowledge, as 'marker proteins' from compartments of interest are useful for monitoring compartment enrichment strategies and in data analysis.

Within the latter category of methods, isolation and enrichment approaches have been used to great effect to determine proteins within the secretory pathway. However, each has its strengths and limitations, whether in distinguishing full-time residents of compartments, identifying cargo contents or determining which proteins are likely to function by shuttling between secretory compartments. These methods have been further supplemented with studies that use chemical approaches to determine the behaviour of sets of proteins within endomembrane compartments [9,10].

In this review, we discuss the constituent parts of the secretory pathway and describe how mass spectrometry based proteomics methods have aided our understanding of the secretory pathway. We show how combining targeted and untargeted approaches can be an effective way of opening up new areas of the secretory pathway for investigation. Finally we discuss new enabling technologies that will impact future studies, by mapping trafficking pathways at unprecedented resolution, and investigating the heterogeneous distribution of proteins in membranes.

2. ER and golgi proteomes

Typically in cellular biology, organelle isolation has been a prerequisite for their functional, metabolic and proteomic characterization. Confident assignment of proteins to an organelle requires isolation at high purity levels. A number of techniques have been developed that exploit the density, size or surface properties of different organelles, the most popular of which is density centrifugation [11]. Although the intact ER and Golgi are visually distinct, in plants they are remarkably similar in density, and thus separation to the required purity levels for meaningful proteomic analysis is not possible. For this reason, the concerted efforts to study major Arabidopsis organelles of the early 2000 s by proteomic analysis did not include the ER and Golgi. Nevertheless, the first substantial description of the plant ER and Golgi proteomes was achieved using density centrifugation during this period.

The LOPIT (localisation of organelle proteins using isotope tagging) technique was first conceived as a means of investigating the Arabidopsis Golgi proteome without resorting to organelle isolation [12–14]. LOPIT is a quantitative proteomics method for the high-throughput and simultaneous assignment of multiple subcellular compartments. It does not rely on the purification of sub-cellular compartments of interest. It combines biochemical fractionation by differential or equilibrium density-gradient centrifugation, and multiplexing quantitative proteomics methods applied to fractionated proteins (see Fig. 1 for LOPIT protocol). Cells are lysed under detergent-free conditions ensuring minimal disruption to organelle integrity. Membrane bound organelles and large protein complexes are then separated based on their various physical properties such as their characteristic buoyant densities by equilibrium ultracentrifugation or size and density by differential centrifugation. None of these approaches deliver discrete purified fractions, but different sub-cellular compartments display distinct enrichment patterns and correlation profiles, that can be characterised by quantitative methods. In the case of LOPIT, fractions representing peak enrichment for organelles of interest are selected for shotgun proteomics approaches involving proteolytic digestion of proteins extracted from fractions to peptides. The resulting peptides are differentially labelled with amine-reactive Tandem Mass Tag (TMT) reagents [15], which enables peptides derived from each fraction to be distinguished by tandem mass spectrometry (MS) workflows. For each peptide analysed, tag specific reporter ions are liberated during peptide fragmentation during tandem MS and

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