COMMENTARY

Sorting out PtdIns(4,5)P₂ and clathrin-coated vesicles in plants

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Phosphoinositides and their binding proteins are regulators of many aspects of the vesicle-trafficking processes that underlie cellular physiology in animal cells. Relatively little is known, by comparison, of the contribution of phosphoinositides to membrane-trafficking phenomena in plants. A study in this issue of the Biochemical Journal by König et al. reports for the first time in this kingdom the association of PtdIns(4,5)P₂ with an endomembrane fraction enriched for clathrin. This work is discussed in the context of current evidence for constitutive and evoked endocytosis of membrane protein cargoes in plants.

Key words: Arabidopsis, clathrin, coated vesicle, endocytosis, phosphoinositide.

The trafficking of membrane vesicles and their cargoes underlies the maintenance of all cellular membranes and is central to the cellular homeostasis of all eukaryotes. Consequently, it is not surprising that the mechanisms by which vesicles bud from donor membranes, and by which vesicles fuse with acceptor membranes, are conserved across eukaryote kingdoms. Most of the evidence elaborating the molecular mechanisms of vesicle trafficking has come from the study of animal cells, and this is where we start the discussion.

The budding process is orchestrated by the assembly of coat proteins on a membrane surface with the concomitant capture of cargo molecules, concluding with the polymerization of coat proteins in a spherical protein cage enclosing a subducted membrane vesicle and its cargo. The recruitment of coat proteins from the cytosol to the membrane and their assembly into a macromolecular complex is a co-operative process involving accessory, or adaptor, proteins, membrane phospholipids and interactions between cargo and coat proteins. Included among these proteins are the soluble SNAREs [NSF (N-ethylmaleimide-sensitive factor)-attachment protein receptors], which provide the specificity for recognition of the target (acceptor) membrane. Once the vesicle has pinched off from the donor membrane, the coat complex disassociates and the subunits translocate to the cytosol, leaving a naked vesicle and its cargo.

The cargoes transported include membrane receptors, transporters and ion channels. Vesicles carrying cargoes move between plasma membrane and the ER (endoplasmic reticulum) and other membrane compartments, early and late endosomes, the Golgi and lysosomes, in accordance with the physiological requirements of the cell. Thus glucose transporters are recruited to and from the plasma membrane in insulin-responsive cells in response to changes in circulating blood glucose, whereas internalization of plasma membrane ion channels affords the cell the means of down-regulating receptor–ligand signalling at this barrier. Examples of ion-channel internalization can be found in plants and probably afford a mechanism for adaptive responses of the plant cell to changes in its environment.

Among cargo-selection processes, those mediated by COPI (coat protein complex 1), COPII and clathrin– adaptor complexes have received the most attention [1]. The different complexes associate with discrete populations of subcellular membranes. Export from the ER to the plasma membrane is mediated via COPII. Of its three cytosolic components, Sec23/Sec24, Sec13/Sec31 and Sar1, Sar1 is a small GTPase on which GDP/GTP exchange is catalysed by Sec12, an ER membrane protein. The COPII lattice is assembled from 24 Sec13/31 assembly units. Plants have orthologues of all four proteins. Proteins destined for export are recognized by ER-export motifs, one of which is the internal di-acidic Glu-Xaa-Asp motif. Not all Glu-Xaa-Asp motif proteins interact with Sec24. But, across kingdoms, such motifs are found in a variety of proteins, including the plant K⁺ channel KAT1.

COPI associates with the Golgi and, in a manner homologous with COPII-mediated processes, engages a small GTPase of the ARF (ADP-ribosylation factor) family. Cargo proteins include those containing KDEL motifs recognized for sorting by the KDEL receptor. Analysis of the Arabidopsis genome reveals that Arabidopsis has multiple members of the ARF family and multiple ARF GTPase-activating proteins.

The other major coat protein complex is that formed between clathrin and its multiple adaptor proteins. Clathrin–adaptor-mediated endocytosis is a major route for entry of material into the cytoplasm. The assembled clathrin network of the CCV (clathrin-coated vesicle) is built from trimers of the clathrin heavy chain (Mₐ ≈ 180), which are assembled with light chains (Mₐ ≈ 30) into a polyhedral lattice with interdigitated ‘legs’. Again, plants possess clathrin heavy and light chains. Interaction of clathrin with membranes requires the presence of adaptor proteins, identified first as heterotetrameric adaptor protein complexes. Different adaptor protein complexes associate with different membranes. Their association [and we will use the plasma-membrane association of AP-2 (adaptor protein 2) as an example] is driven in part by protein–protein interactions. Thus the μ2 subunit interacts with the transmembrane protein synaptotagmin, and is important in the selection of a subset of vesicle cargoes, including the transferrin receptor, whereas the α-subunit of the AP-2 heterotetramer interacts with the minor membrane lipid, PtdIns(4,5)P₂.

PtdIns(4,5)P₂ is one of a family of seven phosphoinositides, sn-1,2-glycerophospholipids with a myo-inositol headgroup to which monoester phosphates are esterified in different positions. The last phosphoinositides to be identified, in 1997, were

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PtdIns5P and PtdIns(3,5)P_2, and all phosphoinositides, with the exception of PtdIns(3,4,5)P_3, are present in plants. The intervening decade has witnessed the identification, and the structural and functional characterization, of a plethora of phosphoinositide-binding proteins [2]. Among phosphoinositide-binding proteins, the PH (pleckstrin homology) domain of HsPLCδ1 (Homo sapiens phospholipase Cδ1) has found widespread use, fused to GFP (green fluorescent protein) as a reporter of PtdIns(4,5)P_2 dynamics in diverse cellular contexts. The variety of phosphoinositide-binding proteins identified in animals include a number of proteins which are monomeric adaptors of clathrin, and also AP-2 interactors. Some of these adaptors have been shown to be responsible for cargo selection. β-Arrestin, which recruits ligand activated G-protein-coupled receptors for internalization, and epsin are AP-2-interacting proteins which also bind PtdIns(4,5)P_2. In the case of epsin, PtdIns(4,5)P_2 binding is conferred by an ENTH (epsin N-terminal homology) domain.

Of the variety of phosphoinositide-binding domains present in the Arabidopsis genome, a number of proteins with ENTH domains and variants more similar to the ENTH domain of the neuronal protein AP180 have been identified. Some of these have been shown to interact with subunits of plant adaptor protein complexes. These complexes are not exclusively plasma-membrane-located, and particularly strong evidence implicates a role for AtEPSINR1 (Arabidopsis thaliana epsinR1) in the targeting of cargoes to the vacuole (for a review, see [3]).

It is clear from the above that phosphoinositide binding, especially of PtdIns(4,5)P_2, is a significant factor in initiation of CCV formation at the plasma membrane of mammalian cells. Experiments in model systems and transfected cells indicate that AP-2 recruitment is dependent upon the activity of a PtdIns4P 5-kinase, also known as a type 1 ‘PIP kinase’, an enzyme that phosphorylates PtdIns4P on the 5-position to make PtdIns(4,5)P_2.

Work by König et al. [4] in this issue of the Biochemical Journal directly addresses, for the first time in plants, the association of phosphoinositide metabolism, particularly that of PtdIns(4,5)P_2, with the formation of clathrin-enriched vesicles. The authors treated Arabidopsis plants grown in hypertonic conditions with hypertonic solutions of NaCl. They harvested rosette leaves at intervals thereafter, and undertook a subcellular fractionation approach to obtain membrane/organelle fractions. Work from a number of groups had previously shown that Arabidopsis accumulates PtdIns(4,5)P_2 following salt stress. Suspension of plant cells in hyperosmotic media causes plasmolysis, the shrinking of the plasma membrane away from the cell wall as a consequence of the decrease in turgor pressure consequent on the efflux of water. By measuring the mass levels of membrane phospholipids after TLC separation and GC determination of fatty acids, the present study showed an accumulation of PtdIns(4,5)P_2 in a plasma-membrane-enriched membrane fraction within 15 min of salt stress. The fatty acid profiles of both PtdIns(4,5)P_2 and PtdIns4P were proportionately more saturated and monounsaturated than ‘structural’ phospholipids. At later time points, the increase in PtdIns(4,5)P_2 was no longer associated with plasma-membrane-enriched fractions, but resided with endomembrane fraction that was depleted of ER, plastid, nuclear-enzyme sequences and also of plastid phospholipids. Analysis of the protein content of these PtdIns(4,5)P_2-enriched membranes identified this fraction to be enriched for clathrin, and showed further an increase in clathrin after 60 min of salt stress.Transient co-expression of a PLCδ1θ reporter of PtdIns(4,5)P_2 with eYFP (enhanced yellow fluorescent protein)-tagged clathrin revealed plasma membrane and cytosolic locations respectively for PtdIns(4,5)P_2 and clathrin in onion epidermal cells. Salt-stress induced a relocalization of clathrin reporter to coincide with that of the PtdIns(4,5)P_2 reporter at the plasma membrane within 2 min, but analysis was not reported beyond this time point. What the paper does not do is identify the morphology of vesicles endocytosed from the plasma membrane. This may be very important as the architecture, size, coat thickness and cargo content are all determinants of the ultrastructural definition of vesicles as CCVs [5].

The present study is illuminated against a backdrop of recent reports which identify CCV formation as a route for constitutive endocytic internalization of a number of plant proteins. Significantly, overexpression of the C-terminal part of the clathrin heavy chain disrupted coat formation and blocked endocytosis of putative auxin transporters in plant protoplasts. Moreover, tyrophostin A23, which in animal cells inhibits phosphorylation of tyrosine-containing motifs such as those displayed by the CCV cargo transferrin receptor, and which are recognized by the μ-subunit of the AP-2 complex, also blocked internalization of transferrin receptor heterologously expressed in plant cells [6].

Whether CCVs prove to be the principal route for activated or evoked endocytosis of plasma membrane protein cargoes in plants remains to be established convincingly. The field is relatively in its infancy in plants, but nevertheless ligand-induced internalization has been reported for FLS2, a pattern-recognition receptor of Arabidopsis, whereas quite a substantial body of work from the groups of Michael Blatt, Gerhard Thiel and Ulrike Homann has described trafficking of the plasma-membrane K⁺ channel KAT1. These groups have variously described endocytosis of KAT1 against high turgor in guard cells, association of KAT1 with Sec24 of COPII via a di-acidic ER export motif in KAT1, and in the present context ABA (abscisic acid)-evoked endocytosis of this channel [7]. The latter study has revealed that internalization is selective for KAT1, occurs with a half-time of 11 min, and that KAT1 is internalized to an as-yet-unidentified endomembrane location.

At present, there has been no direct test of the involvement of PtdIns(4,5)P_2 or PtdIns(4,5)P_2-binding cargo-specific adaptor proteins in the plant phenomena described above. In the context of the present study, it is not clear what the physiological significance is of the excursion in membrane dynamics induced on supplementation of media to >800–1600 mosmol/kg. Whether the changes in PtdIns(4,5)P_2 reported are a consequence of clathrin assembly, a priming factor thereof, or merely coincidental remain to be established. In this regard, the application of approaches that selectively manipulate plasma membrane PtdIns(4,5)P_2 levels, such as chemically induced translocation of PtdIns(4,5)P_2-specific enzymes [8], could prove particularly powerful.

It is also worth remembering that much of our understanding of Ca²⁺-activated exocytosis, and the priming thereof by PtdIns(4,5)P_2, was elaborated in permeabilized cell systems which afforded manipulation and identification of cytosolic components, including a PtdIns4P 5-kinase critical to the exocytotic process [9]. The PtdIns4P 5-kinase-dependent assembly of coated vesicles has similarly been demonstrated with model membrane systems.

Considering PtdIns4P 5-kinases, the Arabidopsis genome harbours a family of 11 genes with similarity to their mammalian counterparts. These 11 can be divided into two families represented by AtPIP5K (Arabidopsis thaliana PtdIns4P 5-kinase)1-9 and AtPIP5K10-11. The former differ from the latter and from other eukaryote PtdIns4P 5-kinases by the presence of N-terminal multiple tandem repeats of a MORN (membrane occupation and recognition) domain that has variously been reported either to
regulate enzyme activity in the case of AtPIP5K1, or to have no such effect in the case of AtPIP5K3. Although plant MORN domains have been reported to bind PtdIns(4,5)P₂, evidence is accumulating from other organisms that MORN domains probably constitute protein–protein interaction domains [10]. It may be extravagant to suggest that plant PtdIns4P 5-kinases regulate clathrin–adaptor-mediated endocytosis of particular membrane protein cargoes at the moment. However, if experimental manipulation of PtdIns(4,5)P₂ proves that PtdIns(4,5)P₂ is involved either in sorting of cargo for endocytosis and/or in clathrin assembly, this commentator is tempted to speculate that the protein–protein interactions of the MORN domain-containing PtdIns4P 5-kinases will have a key role in regulation of the macromolecular machinery of plant endocytosis.

REFERENCES


Received 11 September 2008; accepted 18 September 2008
Published on the Internet 15 October 2008, doi:10.1042/BJ20081830