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Molecular Dissection of the Clathrin-Endocytosis Machinery in Plants

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Abstract In the last few years, the endocytic vesicular uptake in plant cells has gained increasing significance in several physiological processes. Therefore, an insight into plant clathrin endocytosis at the molecular level is essential. Plants do contain homologs to several key proteins of the mammalian clathrin-dependent endocytosis machinery, but so far only very few have been functionally characterized. Thus, this chapter deals first with the description of the molecular mechanism of clathrin-dependent endocytosis of non-plant organisms which is followed by the outline of similarities to plant endocytosis with an emphasis on its clathrin-dependency.

1 Molecular Mechanisms of Clathrin-Dependent Endocytosis in Non-Plant Organisms

1.1 Characterization of Clathrin-Coated Vesicle Coat and Accessory Proteins

Endocytosis, the uptake of nutrients and other macromolecules, starts at the outer border of a cell, the plasma membrane (PM), which invaginates at distinct sites in order to engulf the cargo molecules into endocytic clathrin-coated vesicles (CCV). After pinching off the PM the CCV shed off their coats thus enabling the naked vesicles to fuse with the first compartment, the early endosome, in order to release their content into the endocytic pathway (Gruenberg, 2001). At the molecular level, clathrin-mediated endocytosis (CME) can be subdivided into the distinct stages of cargo selection, recruitment of coat components, deformation of the PM, and finally budding/pinching of the CCV from the PM.

CCV are spherical vesicles surrounded by a lattice-like coat which was named after its main structure protein clathrin (kláthron in Greek meaning lattice) and the vesicles were therefore described to be clathrin-coated (Kanaseki and Kadota, 1969). Clathrin is a heterodimer consisting of one clathrin heavy chain (CHC) polypeptide of $\sim$ 190 kDa and one clathrin light chain (CLC) polypeptide of $\sim$ 25 kDa which trimerize into three-legged struc-

tures named triskelions, the subunits of the clathrin lattice (Kirchhausen, 2000a). The curvature of a closed polyhedral coat requires the constant number of 12 pentagons, while the number of hexagons may vary thus giving rise to different vesicle sizes (Crowther et al., 1976; Robinson and Depta, 1988; Mousavi et al., 2004). All non-plant organisms investigated so far contain a maximum of two CLC genes and a single gene for CHC, with the exception of some primates who in addition to the ubiquitously expressed and highly conserved CHC17 gene also contain the muscle-specific CHC22 gene (Liu et al., 2001). While the CHC gene from soybean was identified some years ago (Blackbourn and Jackson, 1996), the first plant CLC was identified only recently (Scheele and Holstein, 2002).

In addition to clathrin, the protein coat of endocytic CCV contains another main component, the heterotetrameric Adaptor (AP) complex AP-2 (Keen et al., 1979; Pearse and Robinson, 1984) which consists of the small σ2-adaptin ($\sim$ 20 kDa), the medium μ2-adaptin ($\sim$ 50 kDa) and two large subunits ($\sim$ 100 kDa), the β2- and α-adaptins (Kirchhausen, 1999). Thus, a cross-section through a CCV reveals a three-layered structure with the innermost vesicle membrane harbouring the transmembrane cargo molecules connected via the middle layer of adaptors to the outermost clathrin layer (Pearse et al., 2000).

Of the four mammalian AP complexes, the endocytic AP-2 complex is the best characterized at the molecular level (Collins et al., 2002). With the exception of the small σ2-adaptin, which is obviously a structural component, all other AP-2 adaptins have well-described functions assigned to their specific domains. Accordingly, the μ2-adaptin is the main receptor binding partner and its receptor-binding domain (RBD) serves as the interaction site for trans-membrane proteins, which in turn harbour the tyrosine-based internalization motif YXXϕ within their cytosolic tails (Bonifacino and Traub, 2003). Both large subunits of AP-2 have a tripartite structure consisting of an amino-terminal trunk portion that is connected via a flexible hinge region to the carboxy-terminal ear-domain. While β2-adaptin contains two clathrin interaction sites within its hinge-ear-region (Shih et al., 1995; Owen et al., 2000), the ear-domain of α-adaptin functions as a binding site for numerous accessory or network proteins which are crucial for vesicle budding (Owen et al., 1999; Conner and Schmid, 2002). Thus, α-adaptin performs a special regulatory role in the endocytic process and is considered as an endocytosis-specific protein, since unlike the σ-, μ- and β-adaptins it lacks isoforms in all other AP complexes. Based on their ability to interact with α-adaptin directly the network proteins are divided into two classes. The members of the primary network proteins, like the serine/threonin kinase α-adaptin-associated kinase (AAK1), epsin, EPS15, amphiphysin, AP180 and dynamin all contain the special α-adaptin binding motifs DPW/F and FXDXF that are present in variable numbers and positions (Owen et al., 1999), while the more extensive class of secondary network proteins are lacking these specific peptide motifs