Endomembrane Distortions Induced by Viruses and Associated Proteins

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Formation of viral replication complex (VRC) is an essential step for genome replication of single-stranded positive RNA (ssRNA +) viruses infecting plants. They are composed of viral proteins as well as host proteins and generally localized to cellular endomembranes. Certain viral proteins are responsible for the recruitment of host proteins involved in endomembrane distortions to induce the formation of vesicles or small compartments.

Keywords: virus ; plant ; resistance

1. Plant Proteins Involved in Endomembrane distortions

Most of the proteins synthetized in the endoplasmic reticulum (ER) have a function in another localization and need to be exported from the compartment[1]. The coat protein complex II (COPII) proteins act on the ER to form backward vesicles allowing the exportation of proteins, mainly through the Golgi apparatus (GA), to allow protein maturation. Essential compounds required for the formation of vesicles on the ER are the GTPase SAR1, SEC12, the dimer SEC23/SEC24 and the heterodimer SEC13/SEC31[2][3].

The Golgi apparatus (GA) is a major component of protein maturation before they are sent to their functional environment[4]. Proteins in the GA are moved to one cisternae membrane disk to another, thanks to vesicles formed by the coat protein complex I (COPI). COPI is also involved in retrograde transport vesicle formation from the GA to the ER. Essential components of COPI are ADP-ribosylation factor 1 (ARF1), Golgi brefeldin resistance factor 1 (GBF1), a GTP guanidine-exchange factor, the heteroheptameric coat complex and the cytoplasmic tail of a receptor protein containing a retrieval signal[5][6][7].

The clathrin coat vesicles (CCVs) form the first protein complex involved in vesicle formation that has been observed. Nowadays, it remains the most fully understood coat complex in both plants and animals. CCVs can be formed at the trans-face of the GA, plasmatic membrane, vacuole and endosomes in order to target other organelles[8][9]. Essential components required to induce CCV formation are clathrin heavy chain (CHC) family proteins, chaperone HSC70, the adaptor Fer/Cip4 homology domain-only proteins 1 and 2 (FCHO1/2), the heterotetrameric adaptor protein (AP) complex, the receptor protein and a GTP-binding protein of the ARF family. Large numbers of co-factors are also essential to initiate coat complex recruitment, such as the DRP1 and DRP2 proteins[10][11][12].

Since vesicles are free in the cytosol, they have to fuse with the membrane of the targeted organelle. The N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins are involved in the regulation of the addressing and fusion of vesicles with the targeted organelle[13]. These proteins are localized to the membrane of the vesicle and to the membrane of the destination organelle. Usually, they are anchored to the membrane through a transmembrane domain located in the C-terminal region of the protein. SNARE proteins act in the donor and the receptor partner. v-SNARE proteins on the vesicle of the donor compartment interact with several t-SNARE proteins localized on the receptor organelle. Thus far, 56 members of the SNARE family have been identified in *Arabidopsis thaliana*[14][15][16].

The protein degradation pathway uses backward vesicles to transport proteins from one compartment or plasmatic membrane to the endosome, which is intended to fuse with the lysosome[17]. For their degradation, transmembrane proteins must also move inside the endosome and not remain on the membrane of their compartment. Therefore, an inward vesicle is formed inside the endosome. This structure, called the multivesicular body (MVB), will fuse with the lysosome or vacuole, releasing the vesicle into the lumen of the compartment in order to be degraded[18]. The endosomal sorting complex required for transport (ESCRT) is responsible for the formation of inward vesicles during MVB formation. Some proteins of ESCRT have also been involved in cytokinesis[19]. The ESCRT complex is divided into five small complexes: ESCRT-0, I, II and III and the Vacuolar protein sorting 4 (VPS4) complex. ESCRT-0 is composed of a

heterodimer of the proteins VPS27 and HSE1. ESCRT-I is composed of VPS23, VPS28, VPS37 and MVB1. In plants, homologues have been identified, except for MVB1. ESCRT-II is composed of VPS36, VPS22 and two VPS25. ESCRT-III is composed of VPS20, Sucrose non-fermenting 7 (SNF7), VPS24 and VPS2. Finally, the VPS4 complex is composed of several units of VPS4 and co-factors. Other co-factors such as BRO1 are essential in ESCRT initiation and recruitment, but their functions are not sufficiently described yet[20][21][22][23].

2. Host Intracellular Membrane Association with Viral Replication Complexes

Cellular and molecular events leading to the appearance of symptoms in a plant infected by viruses are still imperfectly known. Viruses take control of host cell mechanisms to their advantage, which probably interferes with plant metabolism and development[24]. Thus, numerous studies have allowed improving the understanding of virus interactions with their hosts[25]. Replication of eukaryotic ssRNA(+) is associated with structural changes in intracellular membranes. They form vesicles and quasi-organelles that combine all the elements required for RNA accumulation and for the translation of viral replication proteins, called viral replication complexes (VRCs)[26][27]. VRCs are confined to defined structures, increasing the replication efficiency and preventing the activation of cellular defense mechanisms such as double-stranded RNA recognition during replication[28]. Membranes are an integral part of VRC formation. Their origins and their final destinations are different depending on the virus. For instance, carnation Italian ring spot virus (CIRV; genus *Tombusvirus*; family *Tombusviridae*) induces vesicles derived from the external membrane of mitochondria, and tobacco mosaic virus (TMV; genus *Tobamovirus*; family *Virgaviridae*) induces vesicles derived from the tonoplasts[29][30]. Studies seem to show that most cellular compartments can be a target for VRC formation but that each virus uses a defined host compartment[31].

The essential character of ssRNA(+) VRC anchoring in the membrane has been repeatedly shown in yeast by knock-out approaches of endomembrane trafficking mechanisms[32]. In plants, transient inhibition approaches of one gene of each of these mechanisms have shown the same reduction in the efficiency of viral accumulation[33][34]. Thanks to the development of membrane-based interaction assays in plant–virus protein interactions, several interactions between membrane-associated viral proteins and membrane trafficking proteins have been revealed in the last decade in plants. In **Table 1**, we regroup most of the data of interactions between a host membrane trafficking protein with viral proteins and approaches used to demonstrate the impact on viral accumulation in the host. In the following sections, we will detail these data and what we know about viral vesicles for the three major ssRNA(+) plant virus genera.

Table 1. Endomembrane trafficking proteins involved in virus replication. TBSV (tomato bushy stunt virus); CIRV (carnation Italian ring spot virus); RCNMV (red clover necrotic mosaic virus); TuMV (turnip mosaic virus); ZYMV (zucchini yellow mosaic virus); BMV (brome mosaic virus); CMV (cucumber mosaic virus).

Virus	Viral Protein	Host	Host Protein	Mechanisms	Relation between Pathogen and Host	Ref.
Tombusvirus						
TBSV	**p33**	***A. thaliana***	**VPS23, BRO1**	**ESCRT**	**Protein interaction**	[33]
TBSV	**p33**	**Yeast**	**VPS4, VPS24**	**ESCRT**	**Protein interaction**	[35]
TBSV	**p33**	**Yeast**	**VPS23**	**ESCRT**	**Protein interaction**	[36]
TBSV	**p33**	**Yeast**	**UFE1, USE1**	**SNARE**	**Protein interaction**	[37]
TBSV	**p33**	**Yeast**	**VPS34**	**ESCRT**	**Protein interaction**	[38]
TBSV	**-**	**Yeast**	**VPS15, VPS30, VPS34**	**ESCRT**	**KO reducing viral replication**	[38]
TBSV	**-**	**Yeast**	**VPS18, VPS32, VPS24, VPS29, VPS4, VPS41, DID2, VPS23, VPS28, VPS51, VPS61, VPS69**	**ESCRT, SNARE**	**KO reducing viral replication**	[35]

Virus	Viral Protein	Host	Host Protein	Mechanisms	Relation between Pathogen and Host	Ref.
TBSV, CIRV	-	*A. thaliana*	VPS4	ESCRT	Dominant-negative reducing viral replication	[34]
CIRV	p36	Yeast	VPS23	ESCRT	Protein interaction	[34]
TBSV	p33	Yeast	PEX19		Protein interaction	[39]
RCNMV	p27	In vitro	ARF1	COPI	Protein interaction	[40]
Potyvirus						
TuMV	6K2	In vitro	VTI11	SNARE	Protein interaction	[41]
TuMV	6K2	Yeast	VAP27	SNARE	Protein interaction	[42]
TuMV	6K2	Yeast	SEC24a	COPII	Protein interaction	[43]
TuMV	6K2, VPg, CP, CI	*A. thaliana*	DRP1/2	CCV	Protein interaction	[35] [44]
ZYMV	-	*Cucumis sativus*	VPS4	ESCRT	Substitution inducing resistance	[45]
Bromovirus* and *Cucumovirus						
BMV	1a	In vitro	SNF7	ESCRT	Protein interaction	[46]
BMV	1a	In vitro	RTN1p, RTN2p, YOP1p	RHP	KO reducing viral replication	[47]
BMV	1a	Yeast	VPS23, VPS20, SNF7, VPS24, VPS2, VPS4, DID2, VPS60	ESCRT	KO reducing viral replication	[46]
CMV		*Cucumis melo*	VPS41	SNARE	Substitution inducing resistance	[48]

A. Endomembrane Distortions during *Tombusviruses replication*

Tomato bushy stunt virus (TBSV), cucumber necrosis virus (CNV) and cymbidium ringspot virus (CymRSV; genus *Tombusvirus*; family *Tombusviridae*) induce membrane deformations at the peroxisome level in plants and yeasts [49][50]. They form spherule-like vesicles, similar to inward vesicles of MVBs, but maintaining a neck opening to the cytosol[51]. The modified peroxisomes contain host and viral replication proteins and viral RNA. These vesicles appear to relocate from peroxisomes to de novo peroxisomes derived from the endoplasmic reticulum (pERs)[52]. Mechanisms associated with TBSV movement from peroxisomes to pERs are not know. It has been observed that VRCs of TBSV are delocalized to the endoplasmic reticulum without affecting the efficiency of viral accumulation, when the peroxisome is absent in yeasts that do not express Peroxisomal biogenesis factor 3 or 19 (PEX3/19)[53]. Differently, the *Tombusvirus* carnation Italian ring spot virus (CIRV) induces the formation of MVB-like vesicles in the mitochondrial membrane[54]. The bipartite red clover necrotic mosaic virus (RCNMV; genus *Dianthovirus*; family *Tombusviridae*) induces membrane modifications and viral protein accumulation in the endoplasmic reticulum[55]. The kinetics of events during the formation of *Tombusvirus* VRCs are still unknown. However, the role of ESCRT in virus-induced distortion has been repeatedly shown. The SNARE mechanism also appears to be involved in the targeting of viral vesicles from peroxisomes to pERs[34][35].

The p33 protein of *Tombusvirus* is a transmembrane protein essential for the formation of VRCs. It is involved in a large number of interactions with other viral replication proteins and host proteins[56][57]. The interaction of the p33 protein with

the ESCRT proteins VPS23, VPS24, VPS20, VPS2 and VPS4 appears to be responsible for the formation of inward vesicles at peroxisomes, pERs and the ER[35][36]. An interaction of p33 with VPS34 and the Bro1 accessory protein has also been shown to be involved in the regulation of the ESCRT complex[38]. The p33 protein carries a targeting signal peptide responsible for the induction of COPI vesicle formation through interaction with the ARF1 protein. Inhibition of the protein interaction COPI–ARF1 inhibits the movement of viral vesicles from peroxisomes to pERs[52]. A direct interaction of TBSV p33 with SNARE UFE1 and USE1 has been shown. Their deletion delocalizes VRCs to the ER membrane and reduces TBSV replication in yeasts and plants. During CIRV replication, the protein responsible for the formation of VRCs has an additional domain. This protein, called p36, is responsible for VRC localization in mitochondria, in contrast with *Tombusviruses* encoding a p33[54][58]. Up to now, only the interaction of VPS23 with the p36 protein of CIRV has been shown. Loss of activity of most of these susceptibility factors by deletion of membrane trafficking proteins in yeasts or over-expression of dominant-negative mutants in plant leaves led to a significant reduction in RNA accumulation[32][34].

B. Endomembrane Distortions during Potyviruses Replication

Potyviridae is the largest family of plant-infecting viruses[59]. This virus family cannot infect yeasts. Therefore, studies on proteins involved in the replication of these virusesmostly rely on transient inhibition of essential characters of plant membrane trafficking mechanisms and punctual mutation. Turnip mosaic virus (TuMV; genus Potyvirus; family Potyviridae) is the main model used to describe the vesicle formation in infected cells by Potyviruses. Viral replication of several Potyviruses has been localized to chloroplasts. Potyvirus membrane deformation in chloroplasts is characterized by the formation of a large compartment called the cytoplasmic inclusion (CI) body[60]. VRC assembly occurs in the ER compartment, and then VRCs are moved inside vesicles from the ER to chloroplasts[61][62][63]. The function of the CI body in chloroplasts is not clear. In contrast to Tombusviruses, the replication of TuMV occurs necessarily in chloroplasts. Vesicle translocation from the ER to chloroplasts is required for Potyviruses to succeed in replication[43][64]. For ssRNA(+) viruses which form endoplasmic reticulum VRCs and are addressed to another site, an interesting model was shown by H. Sanfaçon and J. Laliberté[31]. In this model, the authors suggest that a first budding event within the ER lumen is followed by a second budding event, allowing the formation of a second membrane, and upon detachment from the ER, this gives rise to a double-membrane vesicle in the cytoplasm[65]. Viral vesicles holding TuMV VRCs were also identified in theMVB compartment, which are released into the apoplast by fusion of the MVB to the plasmatic membrane[66]. The 6K2 protein of Potyviruses is a transmembrane protein responsible for ER-induced vesicles and for chloroplast targeting . The role of a second transmembrane protein encoded by the Potyvirus genome, called 6K1, is still poorly understood [67] [68]. The vesicle formation occurring in ER requires, at least, the recruitment by direct interaction of the COPII protein SEC24a by 6K2[43]. COPII vesicles are backward vesicles; thus, following the double-membrane vesicle model, another membrane trafficking mechanism should be involved in the first inward vesicle. For now, the only information supporting this model is a mutation in the ESCRT protein VPS4, involved in inward vesicles, which has been shown to induce resistance to the zucchini yellow mosaic virus (ZYMV, genus Potyvirus) in cucumber[45]. Then, the COPII vesicle, formed at the Golgi apparatus, is transported to the chloroplasts thanks to the interaction of 6K2 with Vesicle transport v-SNARE 11 (VTI11), an essential protein for TuMV replication[41]. A second SNARE protein, Syntaxin-71 (SYP71), is also involved in viral vesicle transport due to an indirect interaction between 6K2 and the SNARE co-factor Vesicle-associated protein 27 (VAP27)[42]. This interaction seems to be involved in MVB addressing of the viral vesicle. Involvement of clathrin coat vesicles (CCV) in ssRNA(+) virus replication has not been shown, except for TuMV. Recently, the interaction of the CCV DRP1 and DRP2 proteins with the 6K2, Viral protein genome-linked (VPg), Capsid protein (CP) and Cylindrical inclusion (CI) viral replication proteins was shown to be essential to TuMV replication[69]. However, the mechanisms associated with DRP1/2 recruitments are not well understood.

C. Endomembrane Distortions during Bromoviruses Replication

In plant and yeast cells, replicating Brome mosaic virus (BMV; genus Bromovirus; family Bromoviridae) RNAs occurs at the outer perinuclear endoplasmic reticulum (ER) membrane which is invaginated towards the lumen[70]. The BMV 1a protein serves as the primary organizer to form active replication compartments: it invaginates the outer ER membranes into the ER lumen to form spherules, recruits RNA templates into the interior of preformed spherules by recognizing the cis-element RE present only in viral genomic RNAs and also interacts with and recruits specific host factors to the site of viral replication[71][72]. Deletion of seven ESCRT proteins, VPS12, VPS20, SNF7, VPS24, VPS2, VPS4 and DID2 (VPS46), in yeasts induces a significant reduction in BMV RNA replication. Moreover, deletion of SNF7 leads to a total inhibition of RNA replication. Further investigation revealed a direct interaction of SNF7 with 1a[46]. BMV replication is also linked to the host reticulon homology domain protein (RHP) family. These proteins have been characterized in compartment shapes, which differ from a spherical shape-like ER[73]. Deletion of the three more expressed RHP protein reticulons 1 and 2 (RTN1/2) and YOP1 reduces BMV accumulation[47]. The mechanisms involved are not well understood. A genetic resistance to cucumber mosaic virus (CMV, genus Cucumovirus; family Bromoviridae) was shown to be based

on the SNARE protein VPS41. A single polymorphism led to a significant reduction in CMV accumulation in melon[74]. For now, the role of the SNARE complex in BMV replication is not known.

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