# Phytosterol metabolism in plant positive-strand RNA virus replication

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## *Abstract*

The genome of most plant viruses consists of a single positive-strand of RNA (+ssRNA). Successful replication of these viruses is fully dependent on the endomembrane system of the infected cells, which experiences a massive proliferation and a profound reshaping that enables assembly of the macromolecular complexes where virus genome replication occurs. Assembly of these viral replicase complexes (VRCs) requires a highly orchestrated interplay of multiple virus and co-opted host cell factors to create an optimal microenvironment for efficient assembly and functioning of the virus genome replication machinery. It is now widely accepted that VRC formation involves the recruitment of high levels of sterols, but the specific role of these essential components of cell membranes and the precise molecular mechanisms underlying sterol enrichment at VRCs are still poorly known. In this review we intend to summarize the most relevant knowledge on the role sterols in (+)ssRNA virus replication and discuss the potential of manipulating the plant sterol pathway to help plants fight these infectious agents.

## *Keywords*

Phytosterols, glycosylated sterols, RNA virus, plant biotic stress.

## *Declarations*

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## *Introduction*

The living nature of viruses has been a matter of debate since the first evidence of their existence was obtained in the late 19th century (Villarreal and Witzany, 2010; Artenstein, 2012). However, it is now widely accepted that viruses cannot be considered mere non-infectious disease-causing chemical agents, but pathogenic organisms responsible for causing a variety of diseases in many different hosts, including plants (Balloux and van Dorp, 2017; Fermin, 2018). In the past recent years, plant sterols, also known as phytosterols, have emerged as important players in the plant adaptive response to biotic stress induced by bacteria, fungi, nematodes and insects (Wang et al., 2012; Zhang et al., 2018; Castillo et al., 2019; Cabianca et al., 2021). However, virtually nothing is known about the role that these compounds might play, if any, in the plant defensive responses against virus infection, which cannot be entirely ruled out since depletion of sterol biosynthesis has been proposed as an antiviral defense strategy in algae and human cells (Blanc et al., 2011; Rosenwasser et al., 2014). This review is aimed at providing an overview of the role of phytosterols in the replication of single positive-strand of RNA (+ssRNA) plant viruses, and discusses the potential role of the phytosterol biosynthetic pathway as a new target to implement broad-spectrum plant virus disease control strategies.

## *Virus replication relies on infected host cell endomembranes*

Plant diseases caused by viruses represent a major threat to agriculture worldwide (Jones and Naidu, 2019). Nearly all plant viruses have a genome made of RNA, and most of these genomes consist of a single positive-strand of RNA (Carbonell et al., 2016). Upon viral infection, the primary metabolism of host cells experience a massive reprogramming to meet the increasing demand of energy needed to fuel cell defense responses against virus infection and sustain efficient virus multiplication. Rewiring of cellular lipid metabolism is an important part of this global metabolic response, not only because lipids serve as a source of energy and are involved in multiple defense signaling cascades, but also because they are the principal components of cell membranes (Llave, 2016; Zhang et al., 2019), which are critical for virus intracellular multiplication. Indeed, successful replication of (+)ssRNA viruses, including those infecting humans, animals and plants, is completely dependent on subcellular membranes (Strating and Kuppeveld, 2017). Host cells infected by a (+)ssRNA virus experience a massive proliferation and a profound rearrangement of the endomembrane system that result in the formation of novel organelle-like intracellular structures, referred to as replication compartments, that display different morphologies and may reach a size of 4 μm (Bassi et al., 1986; Cotton et al., 2009). These virus-induced membranous compartments have a unique composition of proteins and lipids (Jin et al., 2018a), and include a variable number of membrane invaginations or spherules (Figure 1), ranging between 30 to 200 nm in diameter, that are connected to the cytoplasm by narrow neck-like channels (Rubino et al., 2001; Schwartz

et al., 2002; Kopek et al., 2007; Cao et al., 2015; Wang et al., 2021). These invaginations serve as platforms for the assembly of the membrane-bound macromolecular complexes, the so-called viral replicase complexes (VRCs), where virus genome replication takes place to produce large amounts of (+)RNA genome copies. Moreover, there is increasing evidence suggesting that VRCs are also important for viral RNA translation, intracellular and possibly intercellular virus movement, and protection of the viral replication machinery from host defense mechanisms (Laliberté and Sanfaçon, 2010; Laliberté and Zheng, 2014; Nagy, 2016a; Jin et al., 2018a). The membranes of almost every organelle of infected plant cells, including the ER (Lee and Ahlquist, 2003), Golgi (Tilsner et al., 2012), peroxisomes (McCartney et al., 2005), mitochondria (Rubino et al., 2001), chloroplasts (Jin et al., 2018b), and the tonoplast (Wang et al., 2021), can be used as pre-assembly platforms for replication compartment formation and VRC assembly. Membrane origin as well as VRCs composition and organization varies depending on the type of virus, although different viruses exhibit some promiscuity in the selection of subcellular membranes for VRC assembly (Jonczyk et al., 2007; Laliberté and Zheng, 2014; Xu and Nagy, 2014), to the extent that plant +ssRNA virus replication has been demonstrated in isolated organelle membranes, yeast cells (*S. cerevisiae*), and yeast cell-free extracts. In fact, these alternative experimental systems have proven extremely helpful to unravel the specific roles of the different viral and co-opted host cell factors involved in viral multiplication (Nagy 2008; Xu et al., 2012; Nagy et al., 2016b). However, the reason why different viruses prefer specific subcellular membranes for replication and the underlying mechanisms still remain open questions.

## *Phytosterols are key players in virus genome replication*

The assembly of VRCs is a very complex process that requires a highly orchestrated interplay of multiple virus and host cell factors, including the viral (+)ssRNA, virus-encoded replication proteins, an increasingly growing number of host proteins playing a variety of housekeeping functions that may change when they are usurped by viruses, and membrane lipids such as sterols and phospholipids (Nagy, 2016; Jin et al., 2018a; Sasvari et al., 2018). The massive proliferation of intracellular membranes associated to VRC formation requires in turn an enhanced supply of their structural components to sustain the high rates of *de novo* membrane biogenesis (Carette et al., 2000; Lee and Ahlquist, 2003), in particular of sterols (Figure 1). Compelling evidence that plant virus replication is dependent on sterols was provided by Sharma and co-workers (2010) using yeast cells and *N. benthamiana* plants infected with *Tomato bushy stunt virus* (TBSV). This prototype virus of the Tombusvirus genus has been intensively studied and, together with other scientifically important viruses (Table 1), has greatly contributed to the current understanding of the principles of (+)ssRNA virus-plant host interactions (Yamamura and Scholthof, 2005; Nagy and Feng, 2021). Pharmacological inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) (Figure 2), the main regulatory enzyme of the sterol biosynthetic pathway (Hemmerlin et al., 2012), was shown to have a strong

inhibitory effect on TBSV RNA replication in yeast cells, and the same effect was observed when yeast sterol 4α-methyl oxidase (SMO) activity was depleted by means of chemical inhibition or by down-regulating the expression of the corresponding *ERG25* gene (Sharma et al., 2010). Yeast SMO catalyzes the removal of the two methyl groups at C4 position of the post-squalene sterol pathway intermediate 4,4-dimethyl zymosterol (Bard et al., 1996). Reduced virus genome replication concomitant to a delay in the appearance of infection symptoms was also observed in *N. benthamiana* upon both chemical inhibition of SMO activity and VIGS-mediated silencing of the corresponding *SMO1* and *SMO2* genes (Figure 2). Interestingly, the negative impact of sterol biosynthesis inhibition (SMO chemical inhibition) on virus replication in *N. benthamiana* protoplasts was reversed by exogenously added stigmasterol, and to a lesser extent by campesterol (Sharma et al., 2010). The differential complementation capacity of these two sterols might be due to the fact that stigmasterol is the most abundant sterol in tobacco cellular membranes (Cassim et al., 2019), and/or to the differential effects of these two sterols on membrane organization and biophysical properties (Grosjean et al., 2015). In contrast to other organisms, plants contain a mixture of sterols consisting of a variety of minor biosynthetic intermediates and three major $\Delta^5$-sterols, usually β-sitosterol, stigmasterol and campesterol (Moreau et al., 2002) (Figure 2), although cholesterol is also a major sterol in some members of the *Liliaceae*, *Solanaceae* and *Scrophulariaceae* families (Behrman and Gopalan, 2005) while $\Delta^5$-avenasterol is a major sterol in oats (Moreau et al., 2002) and $\Delta^7$-sterols are the predominant sterols in the *Cucurbitaceae* family (Akihisa et al., 1986; Fenner et al., 1989). Several studies have reported remarkable differences in the capacity of phytosterols to promote the formation of ordered domains in the membrane and organize their spatial distribution at the membrane surface. Campesterol has a strong ordering ability, which is in the same range of cholesterol and clearly higher than that of β-sitosterol, while stigmasterol has a much weaker ordering effect or may even lack this ability. Accordingly, cholesterol is also the most efficient sterol in modulating membrane stiffness/rigidity, thickness and molecular packing density, followed by campesterol, β-sitosterol, and stigmasterol. These differential effects on membrane properties have been attributed to the small variations in their structure (Shuler et al., 1991; Hodzic et al., 2008; Orädd et al., 2009; Grosjean, 2015; Fakih et al., 2018). Stigmasterol and β-sitosterol are 24-ethylsterols that differ only in a double bond at position C22 in the C17 alkyl side-chain of stigmasterol, while campesterol is a 24-methylsterol and cholesterol has no alkyl substituent at this position (Schaller, 2003) (Figure 2). Thus, subtle differences in the relative amounts of sterols can finely modulate plant membrane organization, biophysical properties, and biological function

## *Phytosterols enrichment at virus replication sites*

A more direct link between sterols and (+)ssRNA viral replication has been reported by Xu and Nagy (2017). The TBSV (+)ssRNA genome contains five open reading frames, two of which are translated directly from the viral genome yielding proteins p33 and p92

that are essential to RNA viral replication. TBSV p92 is the RNA dependent RNA polymerase itself whereas p33 is an RNA chaperone that interacts with viral (+)ssRNA and recruits it to the site of replication. Both are integral membrane proteins anchored in intracellular membranes to form the interior protein layer of VRCs (Gunawardene et al., 2017), where they play a primary role in building functional VRCs by establishing multiple interactions with a number of co-opted host factors. TBSV p33 and p92 have been found to interact with over 100 yeast proteins (Nagy et al., 2016a; Nagy and Feng, 2021) and both have also the ability to directly bind cholesterol *in vitro* through the three CARC- and CRAC-like sterol recognition/binding motifs (Fantini and Barrantes, 2013) identified within or near their two transmembrane domains (Xu and Nagy, 2017). It is thus logical to assume that direct binding of sterols to p33 and p92 is one of the mechanisms responsible for sterol recruitment to create the sterol-rich microdomains required for fully functional VRC formation in infected cell membranes. But for these proteins to have access to sterols, a complex network of interactions between virus-encoded and lipid-related host cell proteins has to be previously established. The p33 protein has also been shown to interact with the ER-localized Sac1 protein in yeast and *N. benthamiana* prior to the formation of the extensive replication compartments. Sac1 is a phosphatidylinositol phosphate (PI4P) phosphatase involved in the formation of membrane contact sites (MCS) between ER and other organelles that facilitates the vectorial transfer of sterols from the ER donor membrane to the closely apposed acceptor membranes (Wu et al., 2018). This interaction also helps p33 to hijack the ER-resident SNARE proteins Ufe1p and Use1p present in the ERAS (ER Arrival Site) subdomains in order to assemble initial small replication compartments at MCS between the ER and other subcellular organelles. These early replication complexes serve as pre-assembly hubs for the formation of large fully functional replication complexes, a process that involves membrane proliferation as well as the establishment and stabilization of additional protein interactions that promote further formation of MCS and mediate non-vesicular lipid transport between juxtaposed organellar membranes (Sasvari et al., 2018; Sasvari et al., 2020). Of particular relevance are the interactions of p33 with soluble oxysterol-binding (OSBP) related lipid transfer proteins (ORPs) like the yeast Osh3/5/6/7 and the ER-resident VAMP-associated proteins (VAPs) ScS2p and VAP27 of yeast and plants, respectively (Barajas et al., 2014). VAPs physically link the ER with other organelles forming MCS and have the capacity to interact with OSBPs and ORPs (Peretti et al., 2008). The high concentrations of Sac1p, ORPs and VAPs at MCS facilitates the redistribution and local enrichment of sterols at the sites of viral replication. The actin filament network appears to play an important role in recruiting host cell proteins and lipids for VRC assembly. The p33 replication protein has been found to interact and inhibit the actin depolymerization factor Cof1p (Nawaz-Ul-Rheman et al., 2016) involved in the rapid cycles of actin filament assembly-disassembly required for normal cell morphogenesis and motility (Lappalainen and Drubin, 1997; Kovar and Staiger, 2000). According to the model proposed by Nawaz-Ul-Rheman et al. (2016), transient blocking of the dynamic actin-filament network would hamper normal cellular distribution and

function of pro-viral host factors, which might then become more accessible for redirection to the sites of virus replication and VRC formation.

Sterol enrichment at virus replication sites can be further promoted by the interaction of Ufe1p with sterol biosynthetic enzymes, like the ER-resident squalene synthase (Busquets et al., 2008), which was localized near the replication compartments in both yeast and plant cells infected with TBSV (Sasvari et al., 2018). Whether other ER-bound sterol biosynthetic enzymes are recruited close to viral replication sites requires further investigation, as it is also the case of the origin of sterols that accumulate at virus replication sites. Virus replication inhibition time-course experiments provided evidence that TBSV replication in yeast and plants is more dependent on newly formed sterols than on the preexisting ones, particularly at early stages of infection (Sharma et al., 2010), and more recently it has been shown that wheat plants infected with *Barley yellow dwarf virus* (BYDV) show significantly increased levels of total sterols (Porras et al., 2018). The proposed involvement of Sac1, ORPs and VAPs in selective enrichment of sterols at replication compartments and the recruitment of sterol biosynthetic enzymes nearby these structures suggests that sterols required for the assembly and functioning of virus replication machinery are indeed synthesized *de novo* in the ER. It is generally accepted that sterols are synthesized at the ER and then rapidly exported to the PM where they primarily accumulate (Hartmann, 1998; Benveniste, 2004; Hartmann, 2004), although the possibility that some steps of the post-squalene segment of the pathway (Figure 2) localize into the PM cannot be completely ruled out (Silvestro et al., 2013). However, a more recent report showed that sterol levels in both total cellular membranes and the detergent resistant membrane (DRM) fractions of yeast and plant cells infected with TBSV, remain essentially unchanged, thus suggesting that virus-induced recruitment of sterols at sites of replication involves rapid transport from existing cellular pools of sterols rather than induction of *de novo* biosynthesis (Xu and Nagy, 2017). This observation is somewhat surprising since active membrane biogenesis would be expected to require an extra supply of their primary structural components. In fact, phospholipid biosynthesis in TBSV-infected yeast and plant cells is clearly enhanced (Sharma et al., 2010; Xu and Nagy, 2017). Thus, it is reasonable to speculate that *de novo* sterol biosynthesis in the ER acts in close coordination with internal redistribution of pre-existing sterol pools, such as PM sterols (Lin et al., 2021) or even the esterified sterols accumulated in the cytoplasmic lipid bodies (Bouvier-Navé et al., 2010; Lara et al., 2018) (Figure 2), in order to meet the enhanced demand of these compounds triggered by virus replication. To establish the origin of these sterols is certainly an interesting subject for further studies, but either case it is clear that efficient virus replication in infected cells requires an active intracellular trafficking of these compounds.

## *Role of phytosterols in virus replication compartments*

The precise role of sterol accumulation at virus-induced replication compartments remains unclear, but several possible functions have been suggested (Figure 1). Sterols

might help stabilize these organelle-like intracellular structures during all the time needed for efficient viral replication; for example, by limiting spontaneous lipid and protein diffusion in the membrane bilayer due to its rigidifying effect on cell membranes. Sterols have the capacity to interact with other membrane lipids containing saturated or relatively saturated acyl chains, such as sphingolipids and to a lesser extend phospholipids, thus promoting tighter lipid packing in membranes, which under extreme conditions may lead to liquid-ordered phase formation (Bigay and Antonny, 2012). In addition to membrane fluidity, sterols might also play a role in modulating membrane curvature, since the formation of the membranous compartments sustaining viral replication requires profound bending and deformation of subcellular membranes (Figure 1). The elastic properties of lipid bilayers forming eukaryotic cell membranes make them resistant to spontaneous deformation, so that active mechanisms are required to re-shape them. Even though sterols do not favor membrane curvature by themselves, they can modulate it by facilitating the local enrichment of other host lipids and proteins that contain domains or motifs that are specialized in sensing, generating or stabilizing membrane curvature, at the expense of other host components that are excluded from replication compartments. In fact, there are proteins that act directly by changing lipids, whereas others provide scaffolding and forces that impose tension on membranes (McMahon and Gallop, 2005; McMahon and Boucrot, 2015). Sterols in sterol-rich subdomains may also affect the topology, structure, oligomerization state and stability of the proteins embedded in the membranes with which they interact (Xu and Nagy 2017) with the consequent functional implications. In fact, sterols have been reported to be important for stabilization of TBSV p92 polymerase in yeast cells (Sharma et al., 2010), and changes in the relative proportions of membrane sterols are known to have an important effect on proper positioning and activity of membrane bound proteins (Carruthers and Melchior, 1986; Cooke and Burden, 1990; Grandmougin-Ferjani et al., 1997; Men et al., 2008; Mlayeh et al., 2010). The multiplicity of mechanisms through which sterol enrichment in VRCs may change membrane structure, dynamics and function strongly suggests a multifactorial contribution of sterols to create an optimal membrane environment for virus replication.

Despite the growing evidence supporting a critical role of sterols in (+)ssRNA virus genome replication, almost nothing is known about the function that individual sterol species and/or specific fractions might play in this process. More than 250 different phytosterols have been described (Nes, 2011), with each plant species having a characteristic composition of sterols that may also vary depending on the organs and tissues, and their developmental stages (Grunwald, 1978; Fenner et al., 1989; Schrick et al., 2011). Furthermore, sterols can be present in free form (FS) and conjugated as steryl glycosides (SG), acyl steryl glycosides (ASG), and steryl esters (SE) (Figure 2). In general, the sterol species in the conjugated forms are the same as those present in free form although their relative proportions may be different (Duperon et al., 1984; Münger et al., 2015). SE accumulate in cytoplasmic lipid droplets and serve as an FS storage form, while SG and ASG localize together with FS in cell membranes and are enriched in DRMs along with FS, sphingolipids and proteins (Ferrer et al., 2017). Thus, the emerging

picture is that SG and ASG are also important players in determining membrane organization and functionality. In fact, SG and ASG have the same capacity to promote order in the lipid bilayer than FS, and effect that is enhanced when these different forms of sterols are combined in the bilayer (Moreau et al., 2002; Grosjean et al., 2015; Cassim et al., 2019). This is of particular importace in those plant species with high levels of glycosylated sterols. In fact, the relative proportions of free and conjugated sterols fractions may vary greatly depending on the species. In most plants, FS are by far the predominant form of sterols while SG and ASG are relatively minor components of the total sterol fraction ranging from 10 to 30% of the total sterol content. However, in plants of the Solanaceae family, SG and ASG are the predominant forms of membrane sterols. For instance, in leaves of tomato, potato and eggplant, glycosylated sterols may represent up to 80% of total sterols (Duperon et al., 1984; Moreau et al., 2002; Furt et al., 2010; Nyström et al., 2012). It is important to bear in mind that the Solanaceae family includes widely cultivated crops of great economic importance, such as potato (*S. tuberosum*), tomato (*S. lycopersicum*), eggplant (*S. melongena*), pepper (*C. annuum*) or tobacco (*N. tabacum*), that can be infected by many (+)ssRNA viruses (Hančinský et al., 2020).

## *Phytosterol metabolism and virus management strategies*

The strong sterol dependence of (+)ssRNA virus replication in infected host cells makes the biosynthetic pathway of these compounds (Figure 2) a promising new target to develop broad-spectrum plant virus disease control strategies based on the modification of sterol profiles. In fact, this kind of plant disease management strategy is already being explored as a new way of insect pest control, since herbivore insects survival depends on diet-acquired plant sterols to synthesize their own preferred sterols (Jing and Behmer 2020). A recent study has shown that altered sterols profiles in Arabidopsis plants may have a negative effect on aphid growth and reproduction (Chen et al., 2020). Interestingly, viruses appear to take advantage of host sterols not only for replication purposes but also for their subsequent dissemination. The increase of sterol content in wheat plants infected with BYDV has been correlated with enhanced feeding rates and fecundity of specific aphid vectors involved in its transmission, thus increasing the probabilities of virus acquisition and spread (Porras et al., 2018). The possibility to hamper or even block virus replication by completely removing plant cell sterols is not realistic because of the essential structural and functional roles of these primary metabolites. What does seem more plausible is the possibility to introduce relatively minor changes in the quantitative and/or qualitative profiles of free and conjugated sterols that might compromise to a greater or lesser extent virus replication without affecting the capability of sterols to continue fulfilling their essential biological roles, similarly to what has been done in the case of phospholipid metabolism (He et al., 2019). The key point for implementing this sort of approaches is to find the best trade-off between impaired virus replication and normal plant cell viability and performance, which necessarily requires a more detailed knowledge of the specific quantitative and qualitative requirements of sterols for efficient

virus replication. This approach, in combination with other prophylactic anti-viral measures, might contribute to enhance plant resistance against viral pathogens in an environmentally friendly way.

## *Conclusions and future directions*

Over the past recent years our knowledge on the molecular events underlying plant (+)ssRNA virus replication has progressed impressively. It is now well established that building up the molecular machinery required for efficient virus genome replication relies on a highly complex and dynamic interplay between virus and plant host cell factors, including proteins and lipids, in which sterols appear to play a primary role in creating an optimal environment for virus genome replication. However, much work is still needed in order to establish the precise molecular and cellular mechanisms underlying the recruitment and the role of plant cell host sterols at virus replication sites. For instance, it is not yet clear whether these sterols are synthesized *de novo* or transferred to the virus replication sites from a preexisting pool of sterols, or even if both sterol sources are used in a timely regulated manner during virus genome replication. Minor changes in sterol structure may have a very different impact on membrane biophysical properties, which raises the question as to whether there is a preference for sterols with specific structural features or, on the contrary, sterol enrichment at virus replication sites is simply a matter of quantity. The role of glycosylated sterols in these sites is another aspect that has yet to be addressed, particularly in those plant species where these conjugated forms are the predominant membrane sterols, such as the Solanaceae. Answering these and other still open questions will further enhance our understanding of the role of sterols in (+)ssRNA virus genome replication, thus providing a knowledge base that can also be applied to the rational design of broad-spectrum virus management strategies based on altering the qualitative and/or quantitative profile of sterols to hinder or even block the virus genome replication process.

## *Figure captions*

**Fig. 1** Formation of virus replication compartments in infected plant cells involves a massive proliferation and a profound rearrangement of the targeted endomembrane system that results in the formation of novel organelle-like intracellular structures enriched in membrane sterols. These key components of cell membranes help to create an optimal environment for virus replication machinery, most likely acting at different levels. ER, endoplasmic reticulum; PM, plasma membrane; LB, cytoplasmic lipid bodies.

**Fig. 2** Schematic representation of the metabolic pathway leading to the synthesis of free and conjugated phytosterols. Solid arrows indicate single enzymatic steps whereas dashed arrows denote multiple steps. HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; SQS, squalene synthase; SMO1/2, sterol 4α-methyl oxidase 1 and 2; C22-des, sterol C22

desaturase; SGT, sterol glycosyltransferase; SGAT, sterol glycoside acyltransferase: ASAT, acyl-CoA:sterol acyltransferase; PSAT, phospholipid:sterol acyltransferase. Cholesterol has no alkyl substituent at position C24 , which is highlighted with a red dot. Campesterol is a 24-methyl sterol while β-sitosterol and stigmasterol are 24-ethyl sterols.

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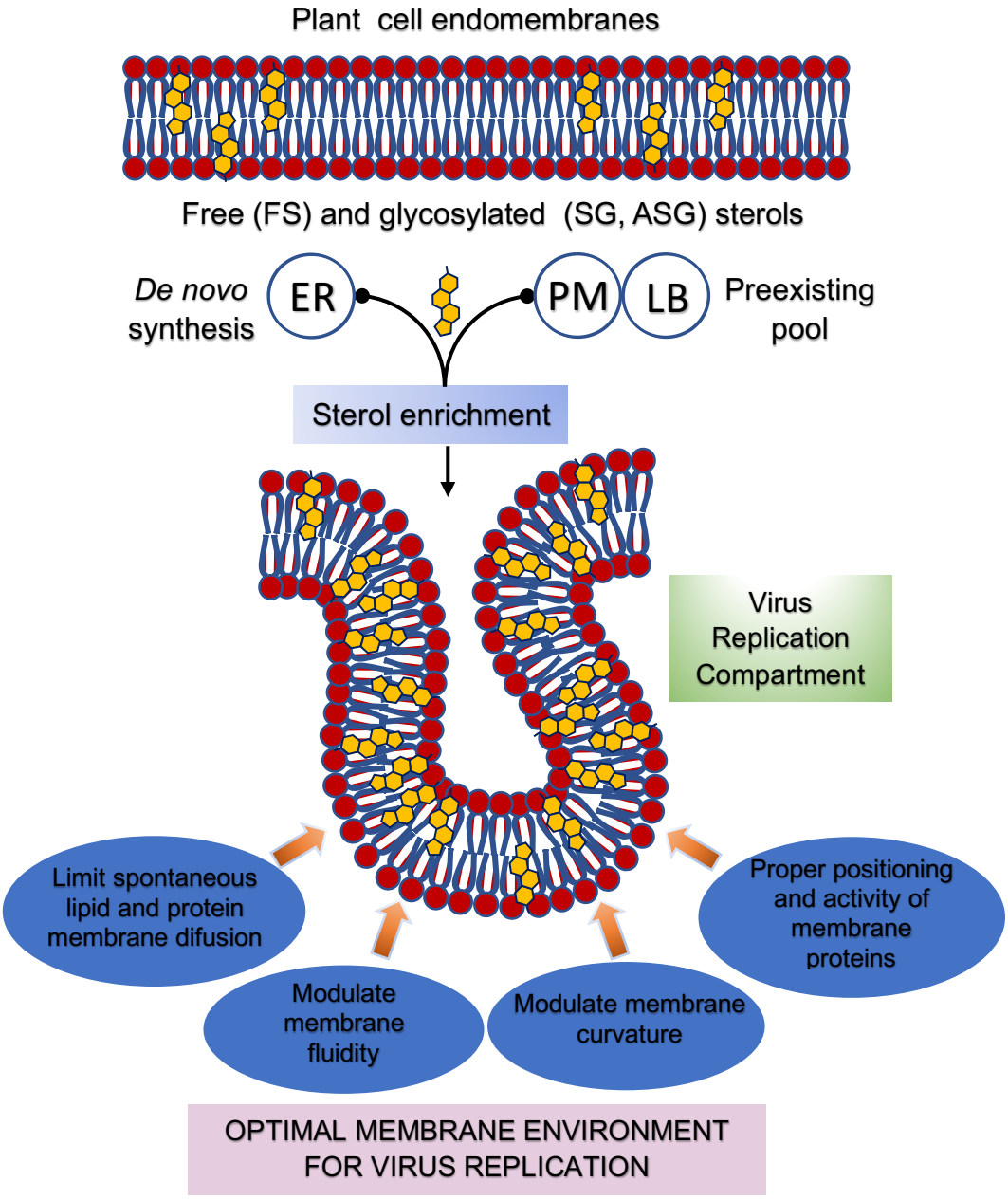
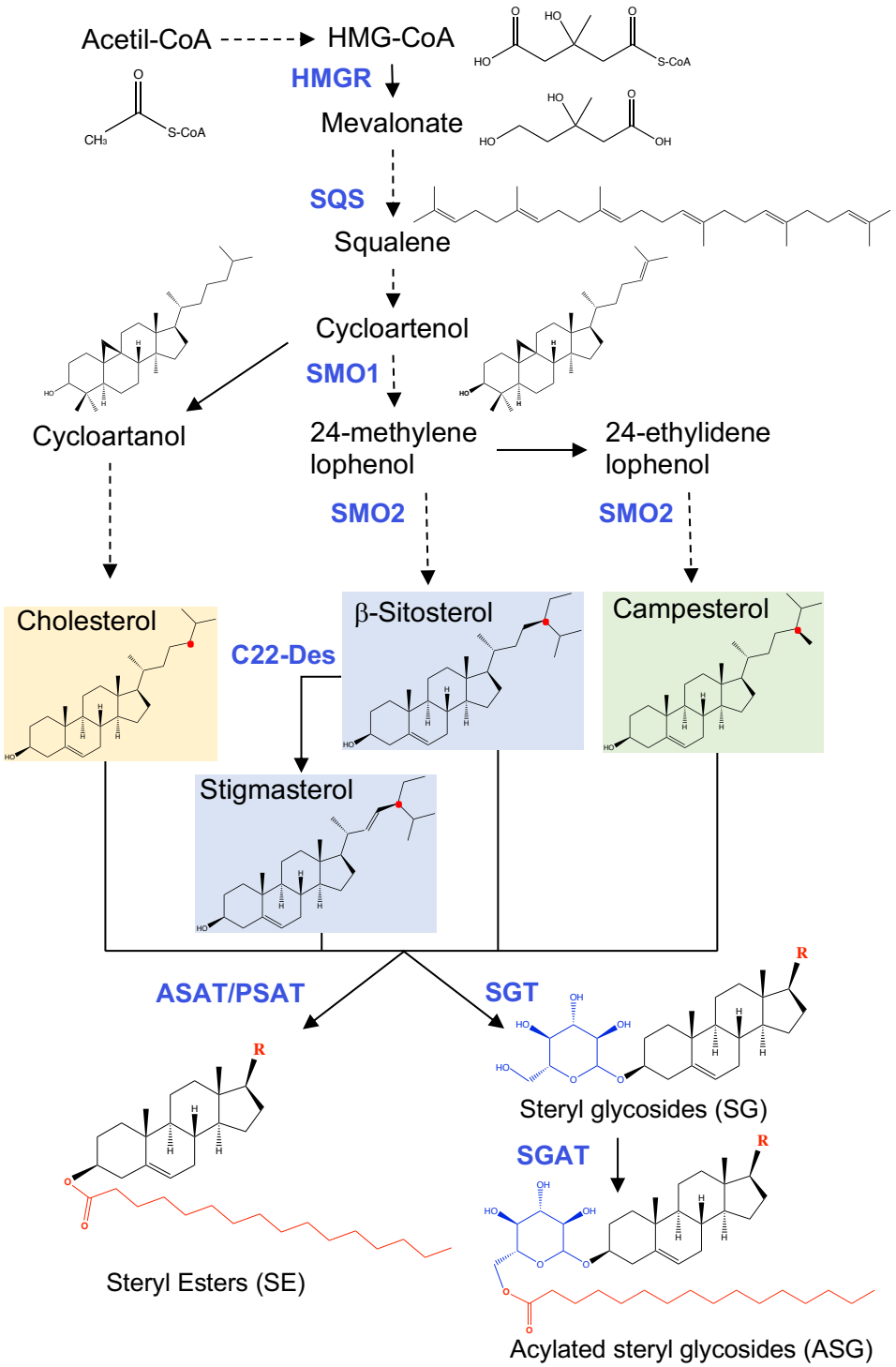
# Figure 1


Plant cell endomembranes
Free (FS) and glycosylated (SG, ASG) sterols
De novo synthesis
ER
PM
LB
Preexisting pool
Sterol enrichment
Virus Replication Compartment
Limit spontaneous lipid and protein membrane difusion
Modulate membrane fluidity
Modulate membrane curvature
Proper positioning and activity of membrane proteins
OPTIMAL MEMBRANE ENVIRONMENT FOR VIRUS REPLICATION

# Figure 2


Acetil-CoA
HMG-CoA
HMGR
Mevalonate
SQS
Squalene
Cycloartenol
SMO1
Cycloartanol
24-methylene lophenol
24-ethylidene lophenol
SMO2
SMO2
Cholesterol
C22-Des
β-Sitosterol
Campesterol
Stigmasterol
ASAT/PSAT
SGT
Steryl glycosides (SG)
SGAT
Steryl Esters (SE)
Acylated steryl glycosides (ASG)