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Coat proteins, host factors and plant viral replication

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It was once believed that the sole biological function of viral coat protein (CP) is to encapsidate the viral genome, protecting it from degradation. The past several decades have witnessed a shift in this paradigm towards recognizing CPs as multifunctional proteins involved in almost every stage of the viral infection cycle. Such functional diversity is achieved via specific CP interactions with viral and host components in the infected cell. Different CP functions are tightly regulated both temporally and spatially through a variety of mechanisms including post-translational modifications and competing interactions. In the present review, we summarize the non-structural functions of plant viral CPs, placing special emphasis on their roles in viral genome replication and translation.

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Introduction

Successful viral infection depends on the careful execution of several consecutive and often overlapping stages of the viral life cycle. Viral genome uncoating, translation, replication, suppression of host defenses, movement and encapsidation all must be tightly coordinated to ensure a productive infection. Such coordination can be achieved either by binding of certain combinations of regulatory proteins to *cis*-acting and *trans*-acting viral regulatory sequences or by spatial and/or temporal separation of different viral functions. Complex interactions between viral non-structural proteins and host factors also play important regulatory roles during viral infection [1]. Surprisingly enough, many non-structural functions have been attributed in recent decades to viral coat proteins (CPs), which were once thought to only encapsidate the viral genome, protecting it from degradation. Although virion assembly and dissociation remain the canonical CP functions, we now know that CPs represent truly multifunctional proteins involved in practically every stage of the viral infection cycle. The CP functions include coordination of viral replication, translation and movement as well as modulation of host responses to viral infection and transmission of the virus to healthy plants. In the case of DNA viruses, CPs also facilitate nucleocytoplasmic shuttling of viral genomes. The non-structural functions of plant viral CPs are in the focus of this review.

Strategies to control CP expression in positive-sense RNA viruses

Proper timing and proper amount of CP synthesis are essential for successful viral infection. Tight control over CP gene expression is required for temporal separation of early viral genome replication from late virion assembly and movement. In many positive-sense RNA (ssRNA(+)) viruses, such temporal control over CP expression is achieved by the late production of CP from subgenomic RNAs (sgRNAs; Figure 1a). sgRNAs are produced efficiently only when the replication cycle is already established, thus preventing premature particle assembly at the early stages of infection. This allows viral RNA replication to proceed in the absence of competing CP-mediated processes such as encapsidation or movement. Other ssRNA(+) viruses employ an alternative genome expression strategy based on the synthesis of a single polyprotein (Figure 1b), which is proteolytically processed to yield smaller individual proteins. In such viruses, CP and replication proteins are produced in equimolar ratio, making temporal control over CP more complicated and therefore far less understood. One possibility is that, in these viruses, CP turnover is regulated by targeted proteosomal degradation [2••]. Another is that CP activity is regulated by post-translational modifications such as phosphorylation [3]. Yet another possibility is that virus-specific mechanisms boost viral RNA translation in the later stages of infection [4], bringing about large amount of CP required for virion assembly. Despite the different strategies used by different viruses to control CP production and availability, such control is universally required for CPs to properly perform their various functions.

Coordination of replication cycle in ssRNA(+) viruses

Infection with *Brome mosaic virus* (BMV; genera *Bromovirus*) offers a good example of how different amounts of CP present at different time points regulate the progression of viral infection cycle (reviewed in [5]). BMV CP is expressed from subgenomic RNA4 that is transcribed from the negative-strand of genomic RNA3 (Figure 1a). Early in viral infection, when the amount of CP is limited, CP enhances replication through binding to an RNA element named SLC within the 3′-tRNA-like

structure that contains the core promoter for genomic minus-strand RNA synthesis [6]. As CP levels rise towards the later stages of infection, the role of CP changes from one of replication enhancer to its inhibitor. Furthermore, at higher concentrations, CP also inhibits viral RNA translation [7••,8]. Such functional switching is achieved through concentration-dependent binding of CP to a second RNA element called B box located in the 5′UTR of BMV RNA1 and RNA2 [7••]. Thus, the carefully orchestrated changes in CP levels regulate the progression of BMV infection cycle from genome replication/translation to virion assembly. Interestingly, BMV virion assembly requires ongoing replication to increase CP specificity for packaging viral RNAs [9].

Another well-studied example of a virus requiring CP for efficient viral RNA translation/replication is *Alfalfa mosaic virus* (AMV; genus *Alfamovirus*). In a phenomenon termed 'genome activation', AMV genomic RNAs require the presence of a few 'activating' CP molecules for infectivity. One explanation of this phenomenon is that AMV CP enhances viral RNA translation through simultaneous binding to secondary structures at the 3′ ends of AMV RNAs and 5′-cap-bound translation initiation factors, thus forming a closed-loop RNA structure [10•,11,12•,13•]. In this manner, AMV CP may enhance viral RNA translation by mimicking the function of the poly(A) binding protein (PABP) in translation of cellular mRNAs. In an alternative model, interaction with AMV CP allows the 3′-UTR of viral RNA to assume a highly ordered tRNA-like conformation [14••]. This conformation is then recognized by viral RNA-dependent RNA polymerase (RdRP) as an initiation site for minus-strand RNA synthesis. The formation of the RdRP-RNA complex is significantly enhanced in the presence of CP [12•], suggesting that CP plays an active role during initiation of viral replication. Because both models are supported by independent experimental evidence, they may not be mutually exclusive. That is, AMV CP may enhance both viral RNA translation and replication, depending on the stage of infection. To determine whether this is indeed the case, new experimental approaches will be needed to separately assess the AMV CP involvement in viral translation and replication *in vivo* in the context of AMV infection.

Assembly of viral replication complexes

Many RNA viruses assemble their viral replication complexes (VRCs) on intracellular membranes. This process is associated with rearrangement of, for example, endoplasmic reticulum (ER) and formation of membrane vesicles, which represent organelle-like compartments for viral replication [15,16]. These vesicles contain viral replication proteins and RNA templates shielded from host defenses and sequestered from competing processes. A new and surprising twist to this concept is that BMV CP itself has the intrinsic property to induce ER membrane rearrangements and formation of vesicles similar to those present in infected cells [17••]. Thus, CP may play an important and previously unsuspected role in organizing local membrane environment for efficient viral replication. Furthermore, BMV CP co-localizes with sites of viral replication in infected cells, suggesting that replication and virion assembly are spatially coupled. Such coupling is beneficial for the virus because it allows selective encapsidation of newly synthesized viral RNA and its protection from nuclease degradation immediately after synthesis.

In some viruses, CP is dispensable for replication. For example, replication of *Tobacco mosaic virus* (TMV; genus *Tobamovirus*) does not require CP, but its expression leads to a more rapid appearance of VRCs and increase in their size [18•]. Therefore, TMV CP may play an accessory role in the formation of VRCs, possibly through regulation of sgRNA production.

Chaperone-mediated regulation of CP functions during replication

Infection with many plant viruses induces the expression of host *HSP70* genes, encoding molecular chaperones [19]. In potyviruses, members of the heat shock protein 70 family interact with viral RdRP and appear to be putative VRC components [2••,20]. Moreover, a CP-mediated defect in the replication of *Potato virus A* (PVA; genus *Potyvirus*) is observed in *HSP70*-silenced plants [2••]. J-domain proteins CPIPs, co-chaperones of HSP70, interact with potyviral CPs and represent important susceptibility factors for potyvirus infection [2••,21•]. Interestingly, ectopic expression of PVA CP inhibits viral gene expression, but CPIP is able to counteract the inhibition in association with HSP70 [2••]. Thus, the HSP70/CPIP-dependent mechanism may prevent premature particle assembly at the early stages of infection, allowing for efficient viral RNA replication and translation.

Another J-domain protein, designated NbDnaJ, binds to the CP of *Potato virus X* (PVX; genus *Potexvirus*) in *Nicotiana benthamiana* [22••]. The protein specifically recognizes a structure in the 5′ untranslated region of PVX RNA called stem-loop 1 (SL1), which interacts with CP and is essential for viral replication and movement [23]. Experiments in *NbDnaJ*-silenced plants or in plants overexpressing *NbDnaJ* suggest that NbDnaJ is a negative regulator of PVX replication and movement at the early stages of infection, acting through interaction with CP and SL1 RNA [22••]. An emerging picture is that J-domain co-chaperones are often associated with plant viral replication, but they do not necessarily exert their effects through CPs. For example, a J-domian protein of yeast, Ydj1p, is involved in the formation of BMV VRCs and (−)-strand synthesis, but it functions through viral replication proteins [24].