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REVIEW

Phosphorylation of the viral coat protein regulates RNA virus infection

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Haley S Hoover
C Cheng Kao

Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN, USA

Abstract: Coat proteins (CPs) are the most abundant protein produced during a viral infection. CPs have been shown to regulate the infection processes of RNA viruses, including RNA replication and gene expression. The numerous activities of the CP in infection are likely to require regulation, possibly through posttranslational modifications. Protein posttranslational modifications are involved in signal transduction, expanding and regulating protein function, and responding to changes in the environment. Accumulating evidence suggests that phosphorylation of viral CPs is involved in the regulation of the viral infection process from enabling virion disassembly to regulation of viral protein synthesis and replication. CP phosphorylation also affects viral trafficking and virion assembly. This review focuses on the regulatory roles that phosphorylation of CPs has in the life cycle of viruses with RNA genomes.

Keywords: viral capsid protein, posttranslational modification, phosphorylation, protein–RNA interaction

Introduction

Viral molecules must be able to sense and adapt to the environment. Mechanistically, this can be done by ligand recognition, protein conformational changes, or posttranslational modifications (PTMs).[1–5] PTMs are especially important in the molecular communication across cellular processes, including signal transduction and the host response to viral infection.[6–13] Viruses must utilize this form of cellular communication for their own nefarious purposes.

A well-understood form of PTM is the phosphorylation of specific amino acids in proteins. In 1954, casein was the first protein observed to be phosphorylated.[14] Protein phosphorylation has since been shown to alter protein structure and function in response to changes in the cellular environment.[15,16] Accumulating evidence suggests that all parts of the viral life cycle are regulated by phosphorylation. Phosphorylation of viral nonstructural proteins and the effects on viral infection have been reviewed previously.[17,18] This review focuses on the phosphorylation of viral coat protein(s) (CPs) in the life cycles of single-stranded (ss) RNA viruses. This review is not a comprehensive examination of all regulatory functions of phosphorylation of CPs, but provides specific examples and themes.

RNA viruses and CPs

RNA viruses make up a large number of plant, animal, and human viral pathogens, including over 80% of plant pathogens.[19,20] Over one-third of emerging and reemerging

Correspondence: Haley S Hoover
Department of Molecular and Cellular Biochemistry, Indiana University,
212 S Hawthorne Drive, Bloomington, IN 47401, USA
Tel +1 812 856 1467
Email haschoov@indiana.edu

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infections are caused by RNA viruses.[21,22] These viruses typically have small genomes that code for less than a dozen proteins, and the small size of their genomes does not eliminate the requirements for viral replication or evading host defenses. Indeed, it seems the majority, if not all, of the viral proteins have multiple functions. The CP, also known as the nucleoprotein (NP) and nucleocapsid (NC) protein or the Core, depending on whether the virion has an envelope, is often the most abundant viral protein produced. In addition to forming a protective shell around the viral genome, it is increasingly appreciated to be involved in encapsidation-independent activities, including the regulation of viral RNA synthesis, viral translation, and the modulation of the host innate immune response.[23]

Viruses typically encode one or two CPs with several common structural features (Figure 1).[23,24] Many CPs have highly flexible N- or C-terminal tails that contain positively charged residues that can interact with RNA. All viral CPs contain regions of higher order structure that form the shell-like domain around the encapsidated RNA in virions. Some viral CPs will contain a protruding domain following the shell domain that can form oligomeric structures and bind receptors.[25–27] Importantly, the CPs can associate with each other to form higher order structures similar to Lego blocks, which can expand the functions of the CPs by increasing the surface for interaction with cellular and viral molecules.

Virion structure, entry, and disassembly

An activity common among viral capsids is that they bind nucleic acids. Protein phosphorylation is known to affect protein binding to nucleic acids,[28,29] primarily through increasing the electrostatic repulsion between the negatively charged phosphate group and the negatively charged nucleic acid backbone. Phosphorylation tends to preferentially occur in less-structured regions of proteins.[30] A change in the charge and conformation of flexible regions in the viral CP, such as the N- or C-terminal tails, will alter the CP conformation to affect the interaction between the capsid and other viral and cellular molecules.[31]

Several plant RNA viruses use phosphorylation to regulate CP–RNA interactions and structure. Bamboo mosaic virus (BaMV) is a (+)-strand ssRNA virus and a member of the family Alphaflexiviridae. The BaMV CP can be phosphorylated in the C-terminal tail.[32–35] Mutations in the BaMV CP that prevented phosphorylation increased CP binding to the BaMV RNA, while replacement with glutamate, an amino acid that acts as a phosphomimetic, decreased CP interaction with RNA.[34] Weakened CP–RNA interaction was speculated to facilitate the release of RNA from the capsid.

The BaMV CP is phosphorylated in plants by casein kinase 2 (CK2) of *Nicotiana benthamiana*.[34] CK2-alpha and the BaMV CP colocalized to the plasmodesmata that connect plant cells, suggesting that CP phosphorylation regulates cell-to-cell movement, possibly by facilitating the release of RNA from the ribonucleoprotein (RNP) complex after its passage through the plasmodesmata into a neighboring cell.

Potato virus A (PVA) is a (+)-strand ssRNA virus and a member of the family Potyviridae. In addition to RNA packaging, the PVA CP is involved in the spread of viral progeny to both neighboring cells and over long distances through

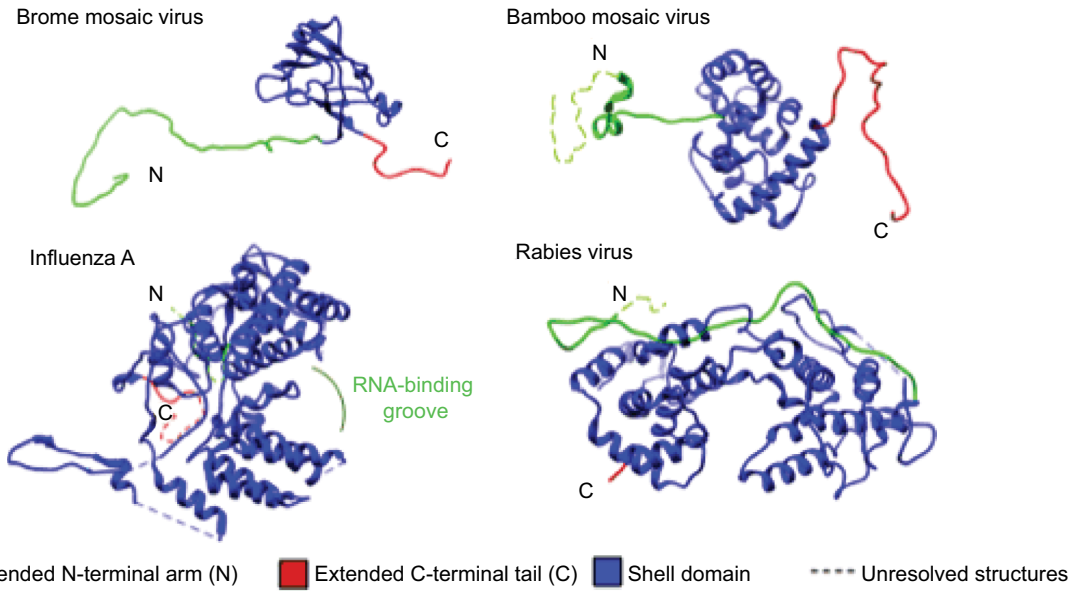

Figure 1 Examples of the structures of viral coat proteins and the features involved in RNA binding and formation of capsid oligomers.

the plant vasculature.[36] The PVA CP was phosphorylated by CK2 in the C-terminal region of the core domain that contacts RNA.[37,38] Phosphorylation of the CP reduced its affinity for RNA and was speculated to regulate the formation and stability of RNP complexes needed for viral trafficking. Furthermore, CPs with substitution that mimicked phosphorylated amino acids were defective in cell-to-cell and long-distance movement.[38]

Brome mosaic virus (BMV), a member of the family Bromoviridae that has a multipartite, (+)-strand, RNA genome, uses the CP to regulate replication and translation of the BMV genome. The CP does so through binding to regulatory motifs in the BMV RNA.[39] We had recently reported our findings that the BMV CP was heavily phosphorylated at the serine and threonine residues on the N-terminal arm that interacts with RNA.[5] Phosphorylation affected RNA accessibility in the virion and was able to alter CP binding to the encapsidated RNAs. Interestingly, BMV consists of three independent viral particles, each of which contains 180 subunits of the CP, and CP phosphorylation has a dramatic effect only on a subset of the particles. In the affected particles, the packing of the encapsidated RNAs is reorganized as a function of CP phosphorylation. This likely regulates the timing of BMV RNA release in the cell and the timing of BMV protein synthesis.[40] Notably, RNA sequences found to bind the CP include regulatory motifs in the RNAs that affect BMV translation and replication. Replacement of a threonine in the flexible N-terminal arm of the BMV CP with a phosphomimetic glutamate altered the ratios of encapsidated RNA, affected the kinetics of replication, and decreased the time needed for translation of the BMV CP. These results suggest that the degree of CP phosphorylation can regulate processes critical to BMV infection. An intriguing possibility is that these interactions may occur within the virion before the start of the infection process.

Beet black scorch virus (BBSV) is a (+)-strand ssRNA virus of the family Tombusviridae. The stability of the virions is essential for systemic infection of *N. benthamiana*.[41] The BBSV CP was phosphorylated at four residues, one of which, T41, was located in a loop of a hinge structure that connected the flexible sequence and the structured domain.[42,43] The hinge is C-terminal to the RNA-binding domain, and phosphorylation of T41 is not expected to directly affect RNA binding. Mutations to T41 prevented replication and gene expression, resulting in the formation of small, irregularly shaped virions that are unstable and defective in encapsidating viral RNA, and abolished viral systemic movement in *N. benthamiana*.[43] This suggests that T41 phosphorylation may impact the secondary and tertiary precapsid changes that occur during virus particle morphogenesis. Protein kinase A was shown to phosphorylate the BBSV CP. Further studies are underway to determine if T41 phosphorylation/dephosphorylation cycles provide molecular switches for virion assembly, by examining the viral assembly intermediates.

Potato virus X (PVX) is a (+)-strand ssRNA virus and a member of the family Alphaflexiviridae. The PVX CP is required for cell-to-cell movement. The PVX CP was phosphorylated, with major phosphorylation sites(s) in the N-terminal arm.[44] Interestingly, phosphorylation of the PVX CP enabled the translation of the encapsidated RNA. How this process occurs is unknown, but Atabekov et al speculate that conformational changes that occur due to CP phosphorylation allow ribosomes to access the encapsidated RNA.[44]

Significant insights into how phosphorylation affects CP–RNA interactions have been revealed by animal RNA virus studies. Infectious bronchitis virus (IBV) is a (+)-strand ssRNA enveloped virus that belongs to the family Coronaviridae. The IBV NC protein is required for efficient transcription of viral RNAs.[45,46] The NC protein was phosphorylated proximal to RNA-binding sites in the globular region and the C-terminus of the CP.[47,48] IBV NC protein phosphorylation at the C-terminus proved to be involved in the regulation of NC protein–RNA binding and in the discrimination between viral and cellular RNAs.[48,49]

Rubella virus (RuV) is an enveloped (+)-strand ssRNA virus and a member of the family Togaviridae. The RuV CP is involved in RNA packaging, a regulated event that occurs on the Golgi complex membrane. It interacts with multiple host cell proteins and appears to be proapoptoic in several cell lines.[50–53] The RuV CP was phosphorylated at multiple residues, with the major site at S46 in the RNA-binding domain.[54–58] Hypophosphorylated mutants at S46 bind RNA more efficiently than the CPs retaining wild type levels of phosphorylation. The protein phosphatase PP1A is known to dephosphorylate RuV in vitro,[58] but the kinase(s) responsible for RuV CP phosphorylation remains to be identified.

West Nile virus (WNV) is an enveloped (+)-strand RNA virus and a member of the family Flaviviridae. In addition to its role in RNA encapsidation, the WNV CP interacts with host proteins, including importin-alpha, and is involved in initiating apoptosis.[59,60] The WNV CP is involved in initiating apoptosis through activation of caspases and disruption of mitochondrial functions.[60] The CP has been shown to enter the cell nucleus by interacting with importin-alpha through a nuclear localization sequence.[59] The initiation of apoptosis was revealed to be due to the sequestration of HDM2 by the

CP into the nucleolus, inducing p53-mediated apoptosis.[61] The WNV CP is phosphorylated by PKC at the carboxy termini.[62] The presence of a PKC inhibitor decreased the association of CP with importin-alpha, resulting in increased accumulation of the CP in the cytoplasm. Nuclear localization sequence-dependent nuclear import has been shown to be regulated by phosphorylation.[63–66] Phosphorylation of WNV CP enhances its binding to HDM2.[62] This stabilizes the p53 protein by preventing HDM2–p53 complex formation, causing accumulation of p53 and its downstream target protein Bax, and initiating apoptotic events.

The recombinant WNV CP does not bind to RNA unless the phosphates in the CP have been removed.[67] Phosphorylation could prevent premature encapsidation of the RNA. Since the virus assembly occurs in the cytoplasm, dephosphorylation of the CP reduces interactions with importin-alpha to increase cytoplasmic localization of the CP and the virion assembly. This is consistent with the observation of the CP trafficking from the nucleus to the cytoplasm late in infection, and a relative reduction in CP phosphorylation. Furthermore, phosphorylation of the CP modulates the rate of oligomerization, with higher-order oligomers forming more rapidly with hypophosphorylated mutants than with the wild-type CP.

The NC of the SARS coronavirus (SARS-CoV), an enveloped (+)-strand ssRNA virus, is active in many regulatory processes including viral RNA replication,[68] subgenomic RNA synthesis,[45,69] deregulation of the host cell cycle,[70–72] inhibition of interferon production,[73–75] and upregulation of cytochrome oxidase 2 production to result in oxidative stress.[76] Perhaps, the multitude of activities is regulated by PTMs of the NC. SARS-CoV NC protein was phosphorylated at several serine residues in a serine–arginine rich motif in the central domain required for oligomerization of the NC protein and translocation of the NC to the nucleus.[72,77–79] Phosphorylation affects the interaction between NC subunits and regulates the ability of NC to suppress translation.[78] Glycogen synthase kinase 3, one of the kinases that phosphorylate NC protein, can be inhibited to suppress the replication of SARS-CoV.[80]

Rabies virus (RV) is a (−)-strand ssRNA enveloped virus and a member of the family Rhabdoviridae. The NP of (−)-strand RNA viruses regulates the transition from viral RNA transcription to replication,[81] and the RV NP is no exception. The RV NP is phosphorylated proximal to the putative RNA-binding domain in the C-terminal region of the NP.[82,83] The unphosphorylated form of the NP encapsidated RNA more efficiently.[84] Cellular kinases and phosphatase(s) responsible for the phosphorylation of RV remain to be identified.

The unphosphorylated form of the RV NP encapsidates RNA efficiently, but early encapsidation can decrease the level of viral RNA transcription and replication.[84,85] As with all (−)-strand ssRNA viruses, in RV, the genomic RNA is not infectious unless complexed to the NC.[86] Wu et al proposed that interaction with genomic RNA causes a conformational change in NP to allow phosphorylation of the NP, which results in weakened interaction with the RNA.[85] Reduced NP–RNA interaction is proposed to allow access by the RNA polymerase complex and viral transcription and replication. How this process occurs needs to be addressed, but the structure of the amino acid and the net negative charge of the phosphate are important for viral transcription and replication.[85]

The measles virus (MeV) is an enveloped (−)-strand ssRNA virus that belongs to the family Paramyxoviridae. The NP encapsidates RNA, supports replication and transcription of viral RNA,[87] and is involved in suppression of immune response.[88–92] The NP was phosphorylated in the disordered C-terminal region, impacting genomic RNA stability.[93–96] Early in infection, this downregulates MeV gene expression. Transcription of the NP gene has been shown to induce cytokines in both a time- and dose-dependent manner.[97] A decrease in MeV gene expression has been shown to decrease the production of proinflammatory cytokines, likely due to decreased recognition by innate immune receptors.[96]

Influenza A virus is an enveloped (−)-strand ssRNA virus and a member of the family Orthomyoxoviridae. The influenza A NP is a component of the RNP complex and its oligomerization is required for the transcription and replication of full-length RNA genome segments,[98–101] but it needs to remain in a monomeric state before the assembly of the RNP complex.[102,103] The NP was phosphorylated at multiple sites, and reversible phosphorylation of S165, located in the "groove" of the NP which interacts with the "tail loop" of another NP, meditates NP oligomerization.[100,104] Substitution of S165 to phosphomimetics resulted in primarily monomeric NP, while a change to an alanine resulted in oligomers of the NP.[98,102,105] Viral infection was significantly reduced with alanine and aspartate substitutions, while substitution with glutamate failed to generate infectious virus.[105] RNP complexes made with mutant NP had decreased accumulation of viral mRNAs.

Concluding remarks

Identifying phosphorylated proteins remains a challenge due to the stoichiometric and labile nature of phosphorylation, but substantial contributions have shown that phosphorylation of CP from RNA viruses has an important impact on viral infection from entry to viral gene expression to virion assembly. Phosphorylation of the CP plays a significant role by modulating CP–RNA interaction, the structure and stability of viral particles, and the intracellular locations of viral molecules. Protein kinases regulate essentially every cellular process and often respond to the conditions in the cell. Therefore, it makes sense that viruses will borrow or steal these enzymes for their own purposes. Protein phosphatases are also involved in signal transduction.[10–13] Phosphatases such as Shp1 and Shp2 regulate DNA virus infection as well as signal transduction pathways that can affect innate immune responses.[106–108] It is likely that they are also involved in RNA virus infection. It is highly likely that cellular kinases and phosphatases will act on viral CPs as a way to prevent the viral infection process. With adeno-associated virus, phosphorylation led to degradation of the capsids.[109] Furthermore, kinase and phosphatase activities will change with environmental input experienced by the host cell. Thus, they will affect viral infection as a function of the environment.

Although this review focuses on phosphorylation of the CP in RNA viruses, other PTMs are likely to play a role in the viral infection process. Further evaluation of the role of CP phosphorylation, including identification of kinases and phosphatases, could lead to potential therapeutics.

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Author contributions

HH and CK contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

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