Reversible Methylation of N6-Methyladenosine in Plant Virus Infection

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N6-methyladenosine (m6A) is the most prevalent modification in the mRNAs of many eukaryotic species. The abundance and effects of m6A are determined by dynamic interactions between its methyltransferases (“writers”), demethylases (“erasers”), and binding proteins (“readers”). It has been indicated that there is a strong correlation between m6A and virus infection in mammals. In the case of plant virus infection, it appears that m6A plays a dual role. On the one hand, m6A acts as a plant immune response induced by virus infection, inhibiting viral replication or translation through methylation of viral genome RNAs. On the other hand, m6A acts as part of an infection strategy employed by plant viruses to overcome the host immune system by interacting with m6A-related proteins.

m6A methylation plant viruses defense mechanism

1. Plant Viruses May Act as an Inducer to Disrupt m6A Methylation

Significant changes in overall m6A levels after virus infection indicate that the viruses have induced m6A methylation. In mammals, SARS-CoV-2 infection triggered a global increase in host m6A methylome, exhibiting altered localization and motifs of m6A methylation in mRNAs [1]. In addition, the global cellular rate of m6A methylation increased upon HIV infection [2]. In plant viruses, it has been shown that m6A level is reduced upon *Tobacco mosaic virus* (TMV) infection in *Nicotiana tabacum* [3]. Meanwhile, the m6A methylation level of the plant endogenous mRNAs can be modified upon TMV infection. In addition, *alfalfa mosaic virus* (AMV) infection increases m6A levels in *Arabidopsis* [4]. These reports indicate that plant viruses may act as an inducer to disrupt m6A methylation.

However, how do plant viruses induce m6A methylation modification? One possibility is that the virus can interact with m6A-related proteins to negatively regulate their expression level such as METTL-like, demethylase homolog and ALKB-like, methylase homolog. These proteins remained unchanged or increased, resulting in reduced m6A methylation after virus infection [3]. For example, the gene expression level of m6A related protein (the potential demethylase XM_009801708, a protein partial homology among human AlkB Homolog 5 (ALKBH5) in *Arabidopsis* might be induced by TMV infection in *Nicotiana tabacum* at 14 and 21 days [3]. Another possibility is that plant m6A methylases make their major efforts to deal with the newly invading foreign viral RNA, which results in losing sufficient energy to perform the regular methylation of the plant endogenous gene.

2. Plant Viral RNA Can Be the Target of m6A Methylation

Methylation of m6A was shown to be conserved in the genomic RNAs of diverse mammalian viruses [5][6][7]. In plant viruses, the presence of m6A in the genomes of two members of the *Bromoviridae* family, AMV and *cucumber mosaic virus* (CMV), has been reported [4]. In the case of AMV, viral accumulation was reduced in inoculated leaves of a m6A demethylase (atALKBH9B) mutant in *Arabidopsis*. Whereas, higher m6A levels in AMV genomic RNAs were observed in these mutant plants. This suggests that m6A modification negatively affects viral infection. They found that the CMV genome also contains m6A but that it differs from AMV. The abundance of m6A methylation in viral RNA and virus infection were modified in atALKBH9B mutant plants, which might be due to the fact that the CMV coat protein (CP) did not interact with atALKBH9B in vivo [4]. This suggests a similar feature of m6A methylation in viruses that replicate in the cytoplasm of plant cells and mammalian. However, with these data, people know little about the status of m6A methylation in the AMV and CMV genome RNA.

According to the prediction and identification of m6A methylation in viral RNA, it is mainly concentrated in the CDS region, while m6A methylation of plant endogenous gene mRNA is mostly concentrated in the 3'UTR of TMV [3]. This indicates that before the plant was successfully infected, the 5' and 3'UTRs of the virus had been modified by the m6A methylation mechanism of the plant, resulting in unsuccessful viral replication and/or translation. In this case, m6A methylation in the CDS region has been detected in viral RNA as well, indicating that these methylations may not influence virus replication and proliferation but act as a protective mechanism for viral RNA and protect them from being degraded by RNase. The other question that remains unclear is whether the m6A methylations affect the translation of viral RNA.

m6A methylation occurs not only on RNA viruses but also on DNA viruses [8]. A novel function of m6A RNA methylation regulates the UV-induced DNA damage response, supporting a model whereby m6A RNA serves as a beacon to facilitate repair and cell survival [9]. This is also agreed with the evidence that the DNA viruses are also subjected to be modified by m6A methylation in mammals, such as Simian virus 40 (SV40) [10][11][12] and Kaposi's sarcoma-associated herpesvirus (KSHV) [13]. The m6A methylation in plant DNA viruses remains unknown.

3. m6A Methylation Is One of the Defense Mechanisms against Plant Viral Infection

In mammals, RNA m6A methylation is catalyzed by a polyprotein complex composed of METTL3, METTL14, Wilms' tumor 1-associating protein (WTAP), the human homolog of *Drosophila* Virilizer (KIAA1429) [14], and several cofactors not yet identified [15][16].

In some cases, viral infection are positively (in the case of hepatitis C virus, HCV) and negatively (in the case of Zika virus, ZIKV) regulated by knockdown of METTL3/14 and ALKBH5, or FTO (fat mass and obesity-associated protein), respectively [6][17]. Although m6A has long been known to exist in plant mRNAs, the proteins involved in m6A methylation have only recently been detected through mutant analysis, homology search, and mRNA interactome capture in *Arabidopsis thaliana* [6][17]. The review by Marlene Reichel et al., showed that the

orthologues of several methylosome subunits have been identified and were shown to interact with each other. METHYLTRANSFERASE A (MTA), a METTL3 homolog that plays a critical role in plant development in *Arabidopsis*, has been identified [18][19]. The *Arabidopsis* FIP37 protein, a plant homolog of WTAP interacting with MTA both in vitro and in vivo, is essential for mediating m6A mRNA modification of key shoot meristem genes [18][20]. In the study of a protein with homology to VIRMA/KIAA1429 involved in m6A formation in mammals [14], m6A levels in the vir-1 mutant were reduced to approximately 10%, and the mutant showed aberrant formation of lateral roots and root caps as well as aberrant cotyledon development [21]. METHYLTRANSFERASE B (MTB), an orthologue of human METTL14 [21] may display enzymatic activity in *Arabidopsis* [22]. m6A levels were reduced to 50% in an inducible MTB RNAi line [21]. An additional component of the *Arabidopsis* writer complex was HAKAI, the orthologue of an E3 ubiquitin ligase. The m6A levels are reduced to 35% in hakai mutant lines without obvious phenotypes [21]. The *Arabidopsis* homolog of RBM15 is FPA, which regulates the flowering time by RNA-mediated chromatin silencing of the floral repressor FLOWERING LOCUS C (FLC) [23][24].

YT521-B homology (YTH) domain proteins are important m6A readers with established functions in animals. Plants contain 13 previously identified *Arabidopsis* YTH domain-containing proteins [25]. Two labs established the relevance of a cytoplasmic m6A-YTH regulatory module in the timing and execution of plant organogenesis [26][27]. The cytoplasmic *Arabidopsis thaliana* YTH domain proteins, EVOLUTIONARILY CONSERVED C-TERMINAL REGION2/3 (ECT2/3) are required for the correct timing of leaf formation and normal leaf morphology [27]. ECT2 has been proposed to promote m6A-dependent stability by binding the 3′untranslated regions (3′UTRs) of target genes [26] both in the nucleus and the cytoplasm.

Some researchers also demonstrated that m6A methylation is crosslinked with the stress response to heat shock in plants. Upon heat stress, YTHDF2 relocates to the nucleus where it binds to m6A sites in the 5′UTR of stress-induced transcripts, including HSP70, thereby preventing FTO from demethylation and promoting translation [28].

Up to now, no anti-viral activities of those identified m6A related proteins have been described in plants at present. Transcriptome-wide m6A profiling found a significant variation in the m6A modification patterns between the resistant and susceptible wheat varieties. Different m6A RNA modifications in the two varieties demonstrated regulation of gene expression and pathogen–plant interaction-related pathways [29]. The m6A demethylase activity of atALKBH9B modulates AMV infection and the m6A abundance in its genomic RNAs, indicating that plant m6A methylation might be involved in viral infection [4].

4. The Virus Encodes AlkB Protein to Promote Virus Infection

Escherichia coli AlkB proteins (members of the 2-oxoglutarate (2OG)- and Fe(II)-dependent oxygenase superfamily) are involved in DNA and RNA repair [30][31][32]. Eukaryotes usually have several proteins encoding ALKB-like genes. Nine ALKB homologs have been identified in mammals: ALKBH1-8 and FTO. Among them, ALKB5 is well studied. In HCV and ZIKV, viral titer was negatively affected when m6A modification of their genomic RNAs were regulated by the knockdown of ALKBH5 and FTO. m6A abundance in the ZIKV genome negatively

affected the viral titer [6]. The production of infectious virus decreased when subjected by the depletion of FTO but not ALKBH5 [17]. The *Arabidopsis* genome contains 13 homologs (atALKBH1-10B) of *E. coli* AlkB [33]. According to the subcellular localization assay, all these proteins display a nucleocytoplasmic localization pattern except for atALKBH1D, which localizes to the chloroplast as well, and atALKBH9B, which is exclusively cytoplasmic [33]. The function of most proteins remains unknown. It has been demonstrated that the demethylase activity of atALKBH9B modulates viral infection of AMV but not of CMV. Whereas, it appears that atALKBH10B is involved in the regulatory network of floral transition in *Arabidopsis* [34][35]. This indicates that the host RNA methyltransferase machinery may represent an additional host regulatory mechanism to counter infection by viruses.

Several plant viruses have been found to contain ALKB protein homologs or domains, suggesting a counter-defense mechanism exerted by these viruses. In 2005, plant virologists found ALKB-like domains in 22 different single-stranded RNA positive-stranded plant viruses based on protein library sequence alignments.

Table 1. Plant viruses involved in m6A methylation or contained ALKB domains.

Virus.	M6A-Related Proteins	Summary of Knowledge	References
Aalfalfa mosaic virus (AMV)	ALKBH9B (At2g17970)	The demethylation activity of atALKBH9B affected the infectivity of AMV by interacting with CP of AMV. Suppression of atALKBH9B increased the relative abundance of m6A in the AMVgenome, impairing the systemic invasion of the plant.	Martinez-Perez et al., 2017
Cucumber mosaic virus (CMV)	ALKBH9B (At2g17970)	atALKBH9B does not have any effect on CMV infection. atALKBH9B does not interact with CP of CMV.	Martinez-Perez et al., 2017
Tobacco mosaic virus (TMV)	The potential demethylase XM_009801708 in *Nicotiana tabacum.*	The overall level of m6A decreases after (TMV) infection in *Nicotiana tabacum*. The expression level of XM_009801708 is increased upon TMV infection.	Zhurui et al., 2018
Grapevine virus A (GVA)	Containing ALKB domain in viral genome	Maintaining the integrity of the viral RNA genome through removal of deleterious RNA damage.	Van den Born et al., 2008
Blueberry scorch virus (BlScV)	Containing ALKB domain in viral genome	Maintaining the integrity of the viral RNA genome through removal of deleterious RNA damage.	Van den Born et al., 2008
Blackberry virusY (BVY)	Containing ALKB domain in viral genome	Maintaining the integrity of the viral RNA genome through removal of deleterious RNA damage.	Van den Born et al., 2008
Little cherry virus (LChV-2)	Containing ALKB domain in viral	Not tested.	Van den Born et al.,

Virus.	M6A-Related Proteins	Summary of Knowledge	References
	genome		2008
Citric leave blotch virus (CLBV)	Containing ALKB domain in viral genome	Not tested.	Van den Born et al., 2008
Chrysanthemum virus B (CVB)	Containing ALKB domain in viral genome	Not tested.	Van den Born et al., 2008
Lily symptomless virus (LSV)	Containing ALKB domain in viral genome	Not tested.	Van den Born et al., 2008
Apple stem pitting virus (ASPV)	Containing ALKB domain in viral genome	Not tested.	Van den Born et al., 2008
Garlic latent virus (GLV)	Containing ALKB domain in viral genome	Not tested.	Van den Born et al., 2008
Zygocactus virusX (ZVX)	Containing ALKB domain in viral genome	Not tested.	Van den Born et al., 2008
Burdock mottle virus (BdMoV)	Containing ALKB domain in viral genome	Not tested.	Kondo et al., 2013
Black raspberry necrosis virus (BRNV)	Containing ALKB domain in viral genome	Not tested.	McGavin et al., 2010

5. The Interplay between m6A Methylation and Viruses in Plant

A model of interplay between the m6A methylation and plant viruses (**Figure 1**) was proposed. On the one hand, the m6A methylation system can be used as a defense mechanism for the host to resist foreign invasion upon viral invasion. The viral RNA could be methylated by METTL-like proteins, together with YTH domain proteins, affecting the stability, translation, or viral particle packaging during viral infection. Alternatively, the positions of m6A methylation on the RNA can directly inhibit the replication of viral genome to repress viral replication. On the other hand, viral RNA may also induce an imbalance in the m6A methylation and demethylation status of the host endogenous genes to regulate its expression. This can, in turn, indirectly influence the viral infection. However, several mysteries remain to be clarified, such as (i) how plants utilize the m6A methylation

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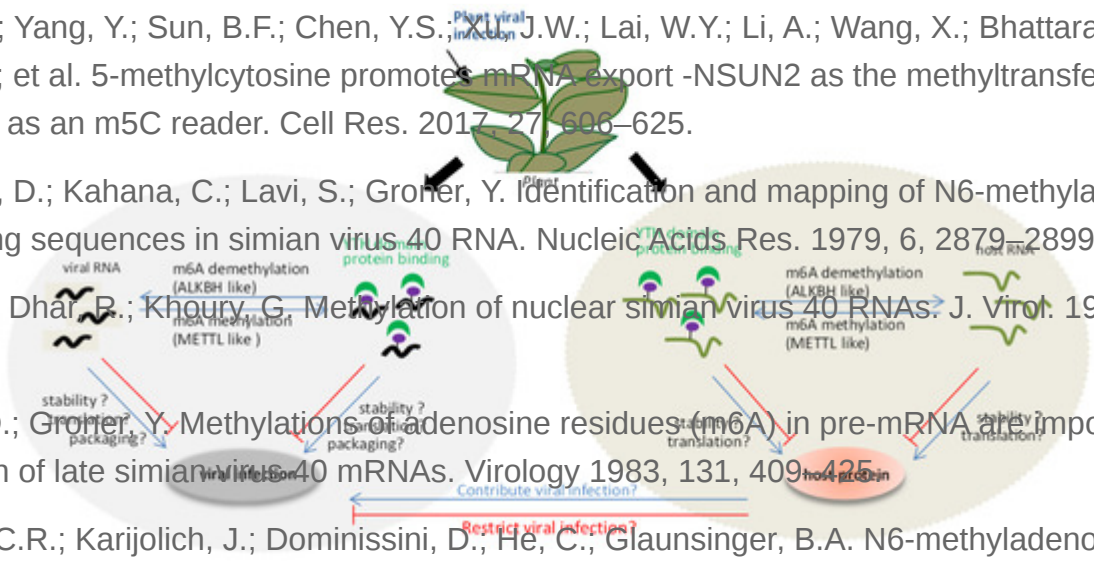

Figure 1. Schematic overview of virus-induced regulation of m6A methylation. When viral RNAs invade into plant cells, the m6A RNA methylase, demethylase and YTH domain proteins will modify the viral RNA. This might directly affect viral RNA stability, translation, and viral particle packaging. Alternatively, viral infection indirectly influences the expression level of host antiviral proteins, which could contribute or restrict viral infection.

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