The impact of RNA modifications on the biology of DNA virus infection

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Abstract

Approximately 170 RNA modifications have been identified and these are critical for determining the fate and function of cellular RNAs. Similar to human transcripts, viral RNAs possess an extensive RNA modification landscape. While initial efforts largely focused on investigating the RNA modification landscape in the context of RNA virus infection, a growing body of work has explored the impact of RNA modifications on DNA virus biology. These studies have revealed roles for RNA modifications in DNA virus infection, including gene regulation and viral pathogenesis. In this review, we will discuss the current knowledge on how RNA modifications impact DNA virus biology.

Keywords

RNA modifications; DNA virus; Innate immunity; Gene expression; Viral pathogenesis

1. Introduction

To date, nearly 170 post-transcriptional RNA modifications have been identified. RNA modifications are incredibly diverse and impart distinct biochemical and biophysical properties onto the modified nucleotide that are absent on the unmodified counterpart. As such, RNA modifications have been found to regulate multiple steps of RNA biogenesis, including transcription, pre-mRNA splicing, nuclear export, and translation (Roundtree et al., 2017a; Boccaletto et al., 2018). While initially believed to be static, emerging data over the last decade has determined that the installation as well as removal of RNA modifications can be modulated and is highly dependent on the cellular state.

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Viral infection results in drastic changes to cellular gene expression. While some of the changes are a result of the host responding to the infection, others are a result of viruses high-jacking the host cell machinery to drive or prevent specific gene expression programs that may affect their lifecycle (Bushell and Sarnow, 2002; Walsh et al., 2013). For example, several viruses are known to modulate cellular machineries to facilitate viral RNA export from the nucleus, increase viral RNA stability, enhance translation of specific messages, and facilitate packaging and release of viral particles (reviewed in (Davey et al., 2011; Thaker et al., 2019; Dai et al., 2020)). One mechanism by which the high-jacking of cellular machinery is achieved is through the manipulation of post-transcriptional RNA modifications (Cross et al., 2019).

RNA modifications, including N⁶-methyladenosine (m⁶A), 5-methylcytosine (m⁵C), Inosine, 2′-O-methylation, and pseudouridine (ψ) have been found on RNAs produced from both DNA and RNA viruses. Although the biological significance of viral RNA modifications has been more extensively investigated in the context of RNA virus infection, it is now appreciated that the lifecycle of both DNA and RNA viruses depend on post-transcriptional RNA modifications. In addition to affecting the viral lifecycle, RNA modifications also modulate the host immune response by serving as key chemical moieties detected during self vs nonself discrimination as well as altering the expression of antiviral molecules. Thus, RNA modifications play a critical role during both the progression and restriction of viral infection.

Unlike the RNA viruses, the genome of DNA viruses is not directly targeted by these RNA modifications, thus the functional importance of RNA modifications on DNA virus infection has been understudied. However, the last decade has witnessed an expansion of investigation into the biology of RNA modifications including their role in DNA virus biology. Here, we summarize significant discoveries made on the most studied modifications to date, m⁶A and inosine during DNA viral infection.

2. N⁶-Methyladenosine (m⁶A)

Methylation at the N⁶ position of adenosine (m⁶A) is the most prevalent internal RNA modification on mRNA. In addition to mRNAs, m⁶A is also present in several non-coding RNA species including circular RNA, long non-coding RNA (IncRNA), primary microRNA (pri-miRNA), ribosomal RNA (rRNA) and small nuclear RNA (snRNA) (Motorin and Helm, 2011; Alarcon et al., 2015; Pendleton et al., 2017; Yang et al., 2017; Zhou et al., 2017; Fazi and Fatica, 2019; Lei et al., 2020). An estimated 25% of all human transcripts are m⁶A modified (Dominissini et al., 2012; Meyer et al., 2012). m⁶A is found predominantly within the consensus motif DRACH (D = A, G or U; R = G or A; H = A, C or U), however, not all the DRACH motifs are modified and nearly 20% of m⁶A sites are not present within a DRACH motif (Linder et al., 2015).

The cellular m⁶A machinery consists of ‘writers’, proteins that deposit m⁶A, ‘erasers’, proteins that remove m⁶A, and ‘readers’, proteins that bind to m⁶A resulting in a biological consequence. The primary writer protein is METTL3 and is predominantly localized to the nucleus where it facilitates deposition of m⁶A co-transcriptionally on nascent RNAs (Liu et
al., 2014; Ke et al., 2017; Slobodin et al., 2017; Huang et al., 2019). The cellular proteins METTL14 and Wilms tumor associated protein (WTAP) form a complex with METTL3 and regulate its methyltransferase activity, modulating its stability, and regulating RNA binding (Wang et al., 2016). Additionally, VIRMA, Zinc Finger CCCH-Type Containing 3 (ZC3H3) and RNA binding motif protein 15 (RBM15/15B) also regulate the RNA targeting of the methyltransferase complex (Patil et al., 2016; Knuckles et al., 2018; Wen et al., 2018; Yue et al., 2018). In addition to the METTL3/14 complex, METTL16 has emerged as an additional m^6^A methyltransferase with activity on mRNA (Pendleton et al., 2017).

m^6^A can be reversed by the eraser proteins, fat mass and obesity-associated protein (FTO) and AlkB-homolog 5 (ALKBH5) (Jia et al., 2011; Zheng et al., 2013; Shen et al., 2015; Mauer et al., 2017; Wei et al., 2018). FTO demethylates both m^6^A and terminal N^6^,2'-O-dimethyladenosine(m^6^Am) while ALKBH5 exclusively demethylates m^6^A (Mauer et al., 2017; Wei et al., 2018). Because m^6^A is reversible, it has the potential to modulate viral replication and host immune responses during viral infection without major changes in transcription.

The best characterized group of cellular m^6^A readers are YTH domain containing proteins including YTHDC1, YTHDC2, YTHDF1, YTHDF2 and YTHDF3, which have an m^6^A binding pocket for recognition of modified RNAs (Li et al., 2014; Xu et al., 2014, 2015; Zhu et al., 2014). These proteins bind to m^6^A within RNAs and facilitate multiple m^6^A mediated downstream processes including RNA structure, translation, stability, alternative splicing, polyadenylation and localization (Fig. 1A) (Meyer and Jaffrey, 2014; Wang et al., 2014, 2015; Liu et al., 2015; Zhou et al., 2015; Xiao et al., 2016; Shi et al., 2017; Anders et al., 2018; Frye et al., 2018; Lesbirel et al., 2018; Yang et al., 2018; Park et al., 2019). Apart from YTHDC1, all other YTH reader proteins are localized to the cytoplasm. YTHDC1 regulates the splicing and nuclear export of m^6^A modified RNAs (Xiao et al., 2016; Roundtree et al., 2017b; Kasowitz et al., 2018). YTHDC2 is primarily expressed in testes where it plays a critical role in spermatogenesis and modulates the translation of m^6^A modified RNAs (Bailey et al., 2017; Hsu et al., 2017; Mao et al., 2019). In addition, YTHDC2 is found in complex with the cellular ribonuclease XRN-1 and has been shown to modulate ribonuclease mediated RNA decay during viral infection (Li et al., 2022; Macveigh-Fierro et al., 2022). YTHDF1 promotes cap-dependent translation by interacting with eukaryotic initiation factor 3 (eIF3), while YTHDF2 regulates the stability of RNA by recruiting the CCR4–NOT deadenylase complex (Wang et al., 2014, 2015; Du et al., 2016). YTHDF3 can regulate both translation and RNA decay as well as enhances the function of both YTHDF1 and YTHDF2 (Li et al., 2017a; Shi et al., 2017). In addition to the YTH domain containing proteins several additional cellular proteins are suggested to recognize m^6^A including heterogeneous nuclear ribonucleoproteins, hnRNPA2B1, hnRNPC and hnRNPG, eIF3D, FXR1, IGF2BP1, IGF2BP2, IGF2BP3 (Alarcon et al., 2015; Liu et al., 2015, 2017; Meyer et al., 2015; Edupuganti et al., 2017; Huang et al., 2018; Wu et al., 2018; Sun et al., 2019). How these readers impact the biology of m^6^A modified RNA is less well studied. In the next section, we discuss the current knowledge on m^6^A modification in DNA viral infection (Fig. 1B).
3. m6A in DNA virus infection

3.1. Simian Virus 40 (SV40)

The polyomavirus Simian Virus 40 is a small DNA tumor virus. Its natural reservoir is the rhesus macaque in which the virus is maintained as a chronic infection primarily within the kidney epithelium. Although associations with human disease remain controversial, in laboratory animals SV40 can induce cancer including those of the brain and bone (Vilchez and Butel, 2004). SV40 is among the most extensively studied animal viruses and work on this virus has led to many fundamental discoveries in basic molecular biology including DNA replication and transcription (Garcea and Imperiale, 2003).

Early work on SV40 demonstrated the presence of m6A on both cytoplasmic and nuclear RNAs of infected cells. methyl-3H-methionine labeling of SV40 infected cells determined that SV40 RNAs are m6A modified, with higher levels of m6A detected in the cytoplasmic fraction compared to the nuclear fraction (Lavi and Shatkin, 1975). The location of the m6A sites were mapped in 1979 and it was determined that the late SV40 viral RNAs contain three m6A modified nucleotides on average and are located in a consensus sequence ‘Gpm6ApC’ (Canaani et al., 1979). To investigate the impact of m6A modification on SV40 viral RNAs, cycloleucine, a competitive inhibitor of methionine transferase was used to inhibit internal m6A formation (Finkel and Groner, 1983). Cycloleucine treated cells were reported to contain reduced levels of cytoplasmic human- and viral RNAs, while the levels of nuclear RNA was unchanged. Additionally, the levels of SV40 RNA transcription and the half-life of viral RNAs remained unchanged following cycloleucine treatment, suggesting a role for m6A in export of RNA from the nucleus. Despite the fact that m6A modifications on SV40 viral RNAs are identified in 1975 (Lavi and Shatkin, 1975), the functional relevance of this modification was not elucidated experimentally until 2018 (Tsai et al., 2018). The phenotypic effects of m6A modification in SV40 replication was assessed using overexpression of an m6A reader protein, YTHDF2 (Tsai et al., 2018). Increased viral replication and larger viral plaques were observed when YTHDF2 overexpressed, while the opposite effects were observed with mutational inactivation of either YTHDF2 or the methyl transferase METTL3. In addition, photocrosslinking assisted m6A-seq (PA-m6A seq) identified two and eleven m6A clusters within the early and late SV40 RNAs, respectively. Synonymous mutations that eliminate the majority of the m6A clusters within the late SV40 RNAs reduced viral replication indicating that m6A regulates viral replication. This study further showed that m6A modifications enhance the translation of late viral transcripts without modulating the alternative splicing and thereby regulate viral replication.

3.2. Adenovirus

Adenovirus (AdV) is a nuclear replicating DNA virus and consists of a family of more than 50 serotypes (Lynch and Kajon, 2016). AdV is the causative agent of febrile illness in young children as well as many upper respiratory tract syndromes (Lynch and Kajon, 2016). methyl-3H-methionine labeling of AdV infected cells identified that both nuclear and cytoplasmic AdV RNAs are m6A modified and quantitative analyses revealed the presence of four m6A modifications per viral transcript (Sommer et al., 1976). In addition, the internal m6A modifications are located within the 5’ two-thirds of the viral RNA.
Given that the m^6A sites were present in viral pre-mRNA as well as clustered in the 5’ region led to speculation that m^6A played a role in the splicing of viral RNAs. Indeed, recent work has demonstrated AdV m^6A modification impacts late viral RNA splicing (Price et al., 2020). Leveraging direct long-read RNA sequencing Price et al. mapped m^6A sites within the overlapping adenoviral transcripts at the nucleotide- and isoform level. This study determined that early and late viral transcripts are m^6A-modified and that the host methyltransferase METTL3 was responsible for their installation. Moreover, knockdown of METTL3 lead to reduced expression of viral late genes, while the expression of early genes remains unchanged at the RNA and protein levels. Reduced viral late gene expression upon METTL3 knockdown was not a result of altered transcription or viral RNA stability, instead however, METTL3 knockdown decreased the splicing efficiency of late transcripts. Interestingly, the absence of METTL3 did not affect viral genome replication, although it did lead to a significant decrease in viral particle production, indicating that m^6A is critical for the late stage of viral infection. How the functional specificity of m^6A in late viral transcripts is achieved needs to be further investigated. Growing evidence indicates that splicing and polyadenylation occur co-transcriptionally and affect each other (Li et al., 2017b). Given that early and late transcripts have a different number of polyadenylation sites (one in early and five in late transcripts (Donovan-Banfield et al., 2020)), one potential hypothesis is differential polyadenylation of viral transcripts is connected to differential m^6A functional specificity and downstream splicing in viral replication. Together, these studies have demonstrated a functional role for m^6A in AdV replication by regulating the splicing efficiency of viral late transcripts. However, future studies should be aimed at determining the mechanism of functional specificity m^6A and RNA processing in different stages of AdV replication.

3.3. Herpes Simplex Virus 1 (HSV-1)

Herpesviruses have a bipartite lifecycle with latent and lytic cycles. During latency, the viral genome expresses a minimum number of genes and persists within the infected host cell nucleus, while the full repertoire of viral genes is expressed during the lytic stage resulting in the production of infectious virions. Herpes Simplex virus 1 (HSV-1) infection is ubiquitous in the human population. HSV-1 establishes latency in the trigeminal ganglia and its reactivation can cause fever blisters at epithelial surfaces that are innervated by the infected ganglia. Experiments in 1977 demonstrated the presence of m^6A within HSV-1 RNAs, however, the biological relevance of these modifications remained unclear until recently (Moss et al., 1977). Methylated RNA immunoprecipitation sequencing (Me-RIP) using m^6A specific antibody in HSV-1 infected human rhabdomyosarcoma (RD) cells identified 12 m^6A peaks across the HSV-1 genome covering multiple viral transcripts (Feng et al., 2021).

Interestingly, it was observed that the expression of the m^6A modification machinery is modulated early in HSV infection, with lower expression of erasers and higher expression of writers and readers leading to increased HSV replication. Along this line, inhibition of m^6A deposition using the methyltransferase inhibitor 3-deazaadenosine (DAA) significantly reduced viral genome replication and virion production. In addition, a recent study investigating HSV-1 infection of normal human dermal fibroblasts (NHDFs) observed
the cytoplasmic relocalization of the m^6A machinery, including METTL3 and METTL14 (Srinivas et al., 2021). Cytoplasmic redistribution increased as the lytic cycle progressed and was dependent on the immediate early viral protein ICP27, which is known to regulate RNA biogenesis (Rutkowski et al., 2015) and alternative splicing. Accordingly, while knockdown of METTL3 and METTL14 significantly decreases the viral gene expression early in infection, the effect of knockdown on viral gene expression is minimal at later time points.

3.4. Kaposi’s sarcoma-associated herpesvirus (KSHV)

Kaposi’s sarcoma-associated herpesvirus is the etiological agent of several human malignancies including primary effusion lymphoma (PEL), Kaposi’s sarcoma (KS) and Multicentric Castleman’s disease (MCD) (Cesarman et al., 2019). While lymphotrophic, KSHV can establish a latent infection in cell types of diverse origin, and along this line, KS is an endothelial cell derived lesion.

In 2017, Ye et al. utilized Me-RIP to map m^6A in the PEL cell line BCBL1 and determined that both latent and lytic viral transcripts are marked with m^6A, with increased levels of methylation during lytic replication (Ye et al., 2017). Demonstrating a role for m^6A during infection, siRNA depletion of METTL3 decreased viral lytic gene expression and virion production, while knockdown of the eraser, FTO, had opposite effects. The viral replication phenotype was ultimately ascribed to the presence of m^6A in the RNA encoding the master viral lytic transactivator, replication and transcription activator (RTA). m^6A within the RTA pre-mRNA are bound by the m^6A reader protein YTHDC1 which facilitates pre-mRNA splicing thus enhancing RTA protein expression.

Additional work from Hesser et al. identified m^6A sites in two different KSHV infected cell lines, the renal carcinoma cell line, iSLK.219, which is infected with the recombinant KSHV.219 virus as well as in TREx-BCBL1 cells, which are similar to BCBL1 cells except that a doxycycline-inducible copy of the viral transactivator, RTA, is integrated into the host genome (Nakamura et al., 2003; Hesser et al., 2018). Consistent with the work of (Ye et al., 2017), m^6A was identified in several viral transcripts, including RTA, and m^6A levels were demonstrated to increase during lytic reactivation. Interestingly, while knockdown of METTL3 or YTHDF2 lead to reduced RTA expression and virion production in iSLK-219 cells, their depletion in TREx-BCBL1 cells led to increased RTA expression. However, the levels of virions produced were not affected relative to the control. The virion production phenotype is contradictory to the previously reported results in BCBL1 cells (Ye et al., 2017).

Expanding the systems in which KSHV m^6A modification were investigated, Tan et al. mapped the epitranscriptomes of five different KSHV-infected cell lines and observed conserved as well as cell-type specific m^6A sites within viral RNAs (Tan et al., 2018). Consistent with previous studies, both latent and lytic viral RNAs were m^6A modified. However, in contrast to the study by Hesser et al., YTHDF2 depletion was found to enhance KSHV lytic reactivation in iSLK.219 cells. Moreover, Tan et al., observed that knockdown of YTHDF2 led to increased expression of viral lytic transcripts as a result of increased viral RNA stability, suggesting YTHDF2 may inhibit viral replication through
the modulation of viral RNA stability. Overall, three independent studies reported that the KSHV transcriptome is m\(^6\)A modified, and that modification affects viral replication in a cell-type specific manner by modulating viral RNA splicing and stability.

The observed phenotypic differences upon depleting m\(^6\)A writers or readers indicate there are cell-type and context-dependent dependent processes that are regulated by m\(^6\)A. It is possible that the location of m\(^6\)A within specific RNAs varies between different cell types and that this alters how m\(^6\)A on viral RNAs are interpreted. Along this line, the cellular m\(^6\)A profiles from iSLK.219 cells reported in Hesser et al. and Tan et al. should be compared to determine whether the m\(^6\)A are similarly localized. Similarly, the virion production phenotype is different between BCBL1 cells from Ye et al. and TREx-BCBL1 cells from Hesser et al. These differences may also result from distinct m\(^6\)A profiles leading to differential interpretation of the viral m\(^6\)A profiles.

Investigations into how m\(^6\)A regulates the KSHV lifecycle also resulted in the identification of additional m\(^6\)A reader proteins (Baquero-Perez et al., 2019). Using an in vitro RNA-protein pulldown assay in which the bait RNA was a methylated hairpin derived from RTA, eight members of the ‘Tudor family’, including Staphylococcus nuclease domain containing protein 1 (SND1) were identified by mass spectrometry as potential m\(^6\)A binding proteins. In vitro binding assays suggest that SND1 exhibits the greatest selectivity in binding to m\(^6\)A modified RNA. Furthermore, knockdown of SND1 reduced the stability of RTA pre-mRNA, resulting in reduced RTA protein and a reduction in viral lytic gene expression and replication. This data indicate that a novel m\(^6\)A reader, SND1 acts as a critical regulator of viral gene expression and replication.

The KSHV endoribonuclease SOX has been shown to cleave nearly 70% of host transcripts. The remaining 30% of the transcripts that escape SOX-mediated decay are hypothesized to carry SOX resistance elements (SREs) (Glaunsinger and Ganem, 2004; Lee et al., 2017). Interestingly, in the case of identified SREs, some have been found to associate with m\(^6\)A readers, suggesting a role for m\(^6\)A in escape from SOX mediated decay (Hutin et al., 2013; Muller et al., 2015; Muller and Glaunsinger, 2017). Along this line, a recent study has discovered that the Interleukin-6 (IL-6) mRNA, a well-known SOX resistant transcript, is m\(^6\)A modified within its SRE and is necessary to facilitate recruitment of YTHDC2 and promote escape from SOX (Macveigh-Fierro et al., 2022). Together these data indicate m\(^6\)A plays an important role in regulating the expression of viral- and host transcripts in KSHV-infected cells.

### 3.5. Epstein Barr virus (EBV)

Epstein Barr virus is a human gammaherpesvirus that is ubiquitous in the human population. Infection is associated with ~1.5% of all cancers worldwide, including B-cell lymphomas, epithelial cell carcinomas, and gastric carcinoma (Farrell, 2019). The expression of m\(^6\)A machinery including METTL14, ALKBH5 and YTHDF2 is modulated in EBV positive lymphoblastoid (LCLs) and Burkitt’s lymphoma cell lines (Akarta) (Lang et al., 2019). Specifically, METTL14 expression is increased in EBV positive cells at the RNA and protein levels. Interestingly, upon entering the lytic cycle METTL14 expression is decreased.
Recent studies leveraging Me-RIP seq have discovered m⁶A modifications within EBV RNAs and determined their role in the EBV lifecycle and tumorigenesis (Lang et al., 2019). While both latent and lytic EBV transcripts are modified, the level of m⁶A modification decreases during lytic reactivation compared to latency. The decrease in modification correlates well with the timing of the decreased METTL14 expression. The EBV-encoded latent antigen, EBNA3C, physically interacts with METTL14 and stabilizes it during latent infection. The interaction between EBNA3C and METTL14 is mediated by amino acids 100–200 within EBNA3C. METTL14 positively regulates the growth and proliferation of EBV-infected cells along with EBNA3C and knockdown of both METTL14 and EBNA3C resulted in significantly reduced tumor formation in xenograft models (Lang et al., 2019).

m⁶A has been detected in EBV RNA from various EBV-infected cell lines, human nasopharyngeal carcinoma (NPC) biopsies and patient derived xenograft samples (Xia et al., 2021), and are primarily localized within the coding region of EBV transcripts. YTHDF1 was found to interact with EBV mRNAs encoding BZLF1 and BRLF1, the main viral transcription activators, and promote their decay through the recruitment of the cellular RNA degradation proteins ZAP, DDX17 and DCP2. Accordingly, knockdown of YTHDF1 stabilizes BZLF1 and BRLF1 mRNAs and promotes EBV reactivation (Xia et al., 2021).

Epstein-Barr nuclear antigen 2 (EBNA2) is one of the six viral nuclear proteins expressed in latently infected B lymphocytes and its mRNA has also been observed to be m⁶A modified in EBV-infected BJAB cells (a normally EBV-negative B cell lymphoma cell line) (Zheng et al., 2021). Knockdown of METTL3 decreases the expression of EBNA2 at the RNA and protein levels, while knockdown of FTO exerts opposite effects. Interestingly, the YTHDF1–3 cytoplasmic readers are all capable of binding to EBNA2, however their knockdown differentially affects EBNA2 RNA expression. For example, knockdown of YTHDF1 decreases the expression of EBNA2, while knockdown of YTHDF2 and YTHDF3 enhances EBNA2 expression.

In summary, m⁶A marks are identified within EBV transcripts in both latent and lytic infected cells and m⁶A modification modulates the expression of several EBV RNAs that are critical for viral lifecycle and pathogenesis.

3.6. Human cytomegalovirus (HCMV)

HCMV is a human beta-herpesvirus for which the majority of the human population is seropositive. In healthy individuals infection is mostly asymptomatic, however, in solid organ and stem cell transplant recipients it is among the leading causes for morbidity (Ljungman et al., 2010; Razonable et al., 2013). Moreover, in utero infection is the leading cause of developmental delay and deafness worldwide (Pass, 2005; Hart et al., 2012).

Studies using primary human fibroblasts have demonstrated that HCMV infection increases the expression of m⁶A writers and readers and 21 viral transcripts have been identified that are m⁶A-modified (Rubio et al., 2018; Winkler et al., 2019). Interestingly, however, while depletion of METTL3 resulted in a reduction in viral replication it did not significantly affect the expression of viral m⁶A-modified RNAs (Winkler et al., 2019). In contrast, significant changes were observed in the expression of cellular interferon stimulated genes.
(ISGs). Furthermore, the mRNA encoding interferon beta (IFNβ) was found to be m^6A modified and was stabilized by METTL3 or YTHDF2 depletion. The m^6A-mediated inhibition of HCMV growth was driven by the enhanced type 1 interferon (IFN) response as small-molecule mediated-blocking of the IFN response rescued HCMV replication. Together, the functional role of m^6A modification on viral transcripts is still unclear, however these findings describe m^6A acts as negative regulator of the IFN response by dictating increased turnover of IFN mRNAs and consequently facilitating viral replication.

3.7. Hepatitis B virus (HBV)

HBV is a Hepadnaviridus and infection of humans can result in chronic hepatitis resulting in an increased risk for developing cirrhosis and hepatocellular carcinoma (Seeger and Mason, 2015). Although a DNA virus, HBV replication is unique and goes through an RNA intermediate called pregenomic RNA (pgRNA) that is reverse transcribed to produced viral DNA (Seeger and Mason, 2000).

Me-RIP seq identified modified sites within HBV RNAs isolated from cell lines expressing HBV genome as well as liver tissues from HBV-infected patients. The sites of m^6A were located within an RNA structural element termed the epsilon loop of HBV RNAs (Imam et al., 2018). All HBV RNAs contain the epsilon loop at their 3’ end, while within pgRNA the epsilon loop is located at both 3’and 5’ termini.

Knockdown of the m^6A modification machinery revealed a role for m^6A in the HBV lifecycle. For example, in the absence of METTL3 and METTL14, HBV RNA stability and protein abundance is increased, while the reverse transcription of pgRNA is decreased. In contrast, in the absence of FTO and ALKBH5 viral RNA and protein is decreased and reverse transcription of pgRNA is increased (Imam et al., 2018).

RNAs with m^6A have been shown to modulate the activity of the cellular double-stranded RNA (dsRNA) sensor retinoic acid inducible gene-I (RIG-I). The assembly of RIG-I filaments and subsequent activation of immune signaling is inhibited by the presence of m^6A within dsRNAs (Kariko et al., 2005; Peisley et al., 2013; Durbin et al., 2016). Consistent with the previous findings, studies have demonstrated a role for HBV RNA m^6A modification in modulation of the immune response (Imam et al., 2020; Kim et al., 2020). The epsilon loop within HBV RNAs is recognized by RIG-I to induce host immune responses (Sato et al., 2015), and m^6A modification within the loop negatively affects the recognition of viral RNAs by RIG-I (Kim et al., 2020). Mechanistically, it was demonstrated that YTHDF2 binds to the m^6A modified viral RNAs thus preventing RIG-I sensing of the epsilon loop and subsequent induction of interferon signaling. Interestingly, however, although YTHDF2 binding prevents interferon induction, the interaction has been proposed to enhance HBV RNA degradation by the cellular interferon stimulatory gene 20 (ISG20). Co-immunoprecipitation assays demonstrated that ISG20 and YTHDF2 interact with each other independent of the m^6A site. YTHDF2 recruits ISG20 to the m^6A modified HBV RNAs. Upon recruitment ISG20 executes an exonuclease activity to degrade the methylated viral RNAs. This YTHDF2/ISG20 mediated RNA decay is abolished in methyltransferase depleted cells indicating m^6A regulates RNA decay in HBV infection. In summary, these
two studies reported a new role for m\textsuperscript{6}A in modulating the host immune responses upon HBV infection.

In addition to modulating antiviral response, m\textsuperscript{6}A modification impacts nuclear export of modified viral RNAs (Kim et al., 2021). Leveraging MeRIP qRT-PCR using primers that recognize a shared 3′-UTR sequence present in all HBV transcripts, including pgRNA, Kim et al. proposed a role for m\textsuperscript{6}A in facilitating nuclear export of methylated RNAs. Increased nuclear accumulation of HBV transcripts are identified in HBV mutants that lack m\textsuperscript{6}A modifications, indicating that m\textsuperscript{6}A regulates the subcellular distribution of HBV RNAs. Cellular m\textsuperscript{6}A readers, YTHDC1 and fragile X mental retardation protein (FMRP) bind to the m\textsuperscript{6}A modified viral RNAs and facilitate their nuclear export (Kim et al., 2021).

HBV-encoded proteins have also been shown to be important for maintaining m\textsuperscript{6}A levels within viral RNAs. Loss of HBV protein x (HBx) results in reduced m\textsuperscript{6}A modification within viral RNAs suggesting that viral HBx protein is critical for their modification (Kim and Siddiqui, 2021). Chromatin immunoprecipitation (CHIP) assays indicate that cellular methyltransferases and HBx occupy the same genomic locus on an HBV mini chromosome. In addition, HBx interacts with METTL3 and METTL14 to facilitate their nuclear import and truncation studies have determined that amino acids 50–100 of HBx are responsible for interacting with cellular methyltransferases and modulating m\textsuperscript{6}A modification of viral RNAs.

Recent studies have also demonstrated that HBx expression is regulated by m\textsuperscript{6}A within its own mRNA (Kim and Siddiqui, 2022). The site of modification is located within the coding region at nucleotide 1616. Depleting m\textsuperscript{6}A at 1616 site either by silencing methyltransferases or mutating the methylation site leads to increased HBx levels, suggesting that m\textsuperscript{6}A helps to stabilize the HBx mRNA. In sum, m\textsuperscript{6}A regulates the expression of HBx which in turn modulates m\textsuperscript{6}A of other viral transcripts in HBV lifecycle (Kim and Siddiqui, 2021, 2022).

m\textsuperscript{6}A has also been recently demonstrated to play a role in HBV-related liver fibrosis by modulating immune cell infiltration (Zhao et al., 2022). This study generated a m\textsuperscript{6}A-Score (m\textsuperscript{6}A-S) to represent the levels of m\textsuperscript{6}A modifications in chronically infected HBV individuals and the results suggests m\textsuperscript{6}A-S is positively correlated with immune cell infiltration and the progression of liver fibrosis. Collectively, m\textsuperscript{6}A plays a critical role in not only regulating the HBV lifecycle through modulating RNA stability and viral DNA synthesis, but also modulating the host immune responses to HBV infection.

4. Adenosine-to-Inosine editing (A-to-I editing)

RNA editing alters genomic encoded information to generate transcriptome diversity, which is important for many biological processes including metabolism and immunity. In the animal kingdom, A-to-I RNA editing is the most common and well-studied type of RNA editing (Fig. 2 A). Recent studies estimate over 100 million A-to-I editing sites in the human transcriptome (Bazak et al., 2014; Ulbricht and Emeson, 2014). Since inosine is recognized as guanosine by cellular machinery, A-to-I editing can change codon specificity and give rise to different protein isoforms. However, to date, most edited sites are located within
non-coding regions including introns and UTRs (Yang et al., 2013; Ramaswami and Li, 2014). A-to-I editing has also been observed in various noncoding RNAs including miRNA precursors where it can influence miRNA biogenesis and target selection (Mingardi et al., 2018). Proper levels of A-to-I editing is important for neuronal development and growth and aberrant editing levels are associated with many human cancers, neurological disorders, autoimmune disorders and viral infections (Slotkin and Nishikura, 2013; Zipeto et al., 2015).

A-to-I editing is carried out by a family of proteins called adenosine deaminases acting on RNAs (ADARs) that catalyze the deamination at position C6 of adenosine (Bass et al., 1997). ADARs were originally identified as dsRNA unwinding activity in Xenopus laevis oocytes as A-to-I editing generates I:U mismatches within the dsRNAs (Bass and Weintraub, 1987; Rebagliati and Melton, 1987). ADARs regulates cellular processes, including immune responses by altering the RNA sequence (A-to-I) and RNA structure (destabilizing dsRNAs). In mammals there are three different members in the ADAR family: ADAR1, ADAR2 and ADAR3 (Fig. 2B).

The expression of ADAR1 is driven by four alternative promoters, one interferon inducible and three constitutively active (George and Samuel, 1999b, 1999a; Kawakubo and Samuel, 2000). As a result of the alternative promoters ADAR1 is expressed in two isoforms, p110 and p150. ADAR1 p110 is generated from the constitutively active promoters and predominantly localizes to the nucleus (George and Samuel, 1999b, 1999a), while ADAR1 p150 is generated from the interferon inducible promoter and shuttles between the nucleus and cytoplasm (George and Samuel, 1999b, 1999a). ADAR1 p110 and p150 have three dsRNA binding domains (dsRBDs) that recognize dsRNA and a C-terminal catalytic domain that mediates the hydrolytic deamination of the adenosine residues. Mice lacking ADAR1 are embryonically lethal at stage E11.5 to E13.5 (Hartner et al., 2004; Wang et al., 2004) and subsequent studies reported deregulation of interferon signaling in the absence of ADAR1 (Hartner et al., 2009). ADAR1 prevents the activation of interferon signaling by cellular dsRNA sensor melanoma differentiation-associated protein 5 (MDA5) (Mannion et al., 2014; Liddicoat et al., 2015; Pestal et al., 2015). Moreover, the endoribonuclease RNase L is activated upon ADAR depletion and mediates significant cellular lethality (Li et al., 2017c). Thus, ADAR1 plays a critical role in innate immune signaling in mammals.

ADAR2 is constitutively expressed and exclusively localized to the nucleus (Desterro et al., 2003; George et al., 2011). ADAR2 is primarily responsible for site-specific editing, such as the sequence-specific editing that leads to translational recoding events (Tan et al., 2017). The deaminase activity of ADAR2 is regulated by post-translational modifications and alternative splicing (Gerber et al., 1997; Lai et al., 1997; Marcucci et al., 2011; Li et al., 2015). In addition, ADAR2 edits its own mRNA to regulate the deaminase activity within the cells (Rueter et al., 1999). Self-editing of ADAR2 leads to a frameshift within the coding sequence leading to synthesis of a truncated protein with decreased deaminase activity. In contrast to adar1 deficient mice, mice lacking adar2 are viable. However, adar2 null mice develop epileptic seizures and die at postnatal day 20 (Higuchi et al., 2000). The postnatal seizures in adar2 deficient mice is rescued by introducing A-to-I edited glutamate receptor subunit B (Glu-RB) at the Q/R site (Higuchi et al., 2000), suggesting editing at the Glu-RB Q/R site is critical for normal development in mammals.
Expression of the third member of the ADAR family, ADAR3, is restricted to the mammalian nervous system (Melcher et al., 1996; Chen et al., 2000). Similar to ADAR1 and ADAR2, ADAR3 also has a C-terminal deaminase domain, however ADAR3 has not been observed to exhibit deamination activity in vivo (Melcher et al., 1996; Chen et al., 2000). In contrast, ADAR3 has been shown to inhibit the deamination activity of the active mammalian ADARs in vitro, suggesting a regulatory role of ADAR3 (Chen et al., 2000). Mice lacking the full length ADAR3 (deleted dsRBDS) exhibited increased anxiety and hippocampus dependent short- and long-term memory formation (Mladenova et al., 2018). In addition, genes linked to synaptic function were misregulated in ADAR3 deficient mice suggesting ADAR3 plays an important role in regulating brain function. In the following section, we discuss the current knowledge on A-to-I editing in DNA viral infection (Fig. 2C).

5. A-to-I editing during DNA virus infection

5.1. Mouse polyoma virus (MPyV)

MPyV is a dsDNA virus belonging to the polyomaviridae family and is an often cause of tumors in rodents. The MPyV lifecycle consists of early and late phases, with the early phase occurring prior to DNA replication and the late phase post-DNA replication. Early phase and late phase transcripts are expressed on opposites strands in opposing orientations and several studies have observed that their expression is anti-correlated (Liu et al., 1994; Carmichael, 2016). Studies have demonstrated that antisense late RNAs base-pair with overlapping early RNAs and lead to robust A-to-I editing of the hybrid sequences (Kumar and Carmichael, 1997). In fact, roughly half of the early RNAs detected during the late phase of MPyV replication are edited by ADARs, with ~50% of the adenosines being converted into inosines. Moreover, consistent with human ADAR1 nucleotide preferences (Lehmann and Bass, 2000), edited adenosines are enriched for a 5′ neighbor nucleotide with U > A > C =G, while with 3′ sequence preference was not observed. Interestingly, nuclear cytoplasmic fractionation demonstrated extensively edited early viral RNAs were retained in the nucleus and excluded from being translated by the ribosomes (Kumar and Carmichael, 1997).

A-to-I editing has also been implicated in regulating the early-to-late switch (Gu et al., 2009). Although both early and late RNAs are transcribed during the early phase of infection late RNAs are highly unstable resulting in reduced abundance. Reduced late transcript stability was speculated to be a result of A-to-I editing as it was observed in both early and late RNAs within the overlapping region of their polyadenylation signals. Indeed, knockdown of ADAR1 led to reduced viral replication and a nearly 20-fold reduced late-to-early RNA ratio.

Furthermore, (George and Samuel, 2011) reported the kinetics and growth yields of MPyV in adar1 and adar2 deficient mouse embryo fibroblasts (MEFs). The absence of either ADAR1 or ADAR2 did not affect viral growth. However, adar1 deficient cells exhibited increased cytopathic effect compared to wild type and adar2 deficient cells. The expression of ADAR1 p110 rescued this cytopathic effect suggesting that ADAR1 is modulating the host cell survival in MPyV infection. In addition, the expression of early viral proteins was
higher in adar1 deficient cells compared to wild type and adar2 deficient cells, while the expression of late viral proteins was higher in both adar1 and adar2 deficient cells compared to wild type cells. Together, ADARs are involved in regulating the early-to-late switch of MPyV life cycle and modulating the host cell survival in MPyV infection.

5.2. Adenovirus

Two small regulatory RNAs called Virus associated (VA) RNAs are encoded by Adenovirus. VA RNAs are highly structured, contain two imperfectly base-paired stem regions, one terminal stem region and an apical stem loop (Ma and Mathews, 1996b, 1996a). VA RNAs accumulate in the cytoplasm and are required for efficient protein synthesis in adenovirus infected cells through the antagonism of the cellular dsRNA sensor PKR (Soderlund et al., 1976; Schneider et al., 1984; Kitajewski et al., 1986). In addition to PKR, VA also inhibits the deaminase activity of ADAR1 (Lei et al., 1998). Cytoplasmic extracts prepared from IFN treated and untreated cells exhibited reduced deamination activity upon the addition of VA RNA (Lei et al., 1998). However, A-to-I editing was not detected within the VA RNAs. Mutational analysis of VA RNA suggests that the central domain of the RNA is essential for repressing ADAR1 activity. The central domain is not a perfectly base-paired region, and this could account for the lack of editing by ADAR1 (Lei et al., 1998). The molecular mechanism of how VA RNA inhibits ADAR1 deaminase activity is not known although it is proposed that VA RNAs may compete with other RNAs for binding to ADAR1 or result in a conformational change of ADAR1 resulting in reduced editing activity.

5.3. KSHV

Kaposin (K12) is one of the few abundantly expressed latent KSHV transcripts. Two alternate transcripts lead to the production of the three Kaposin isoforms A, B and C (Sadler et al., 1999). Early efforts to catalog viral miRNAs resulted in the identification of 10 pre-miRNAs encoded by the KSHV genome and interestingly all were confined to the 5-kb region of the viral genome that also encodes K12 (Pfeffer et al., 2005). Two sequence variants of KSHV-miRNA-10 were detected, one A-containing and the other G-containing at position 2. PCR-amplification of the viral genome and sequencing was only able to recover miRNA-10 sequences harboring an A, suggesting that the pre-miRNA is edited. Interestingly, all but one, miRNA-10, is located within the intronic region of the Kaposin transcript. miRNA-10 is within the ORF of Kaposin A and C, and thus A-to-I editing of the Kaposin transcript could potentially expand the viral proteome. Indeed, sequencing of poly-A selected cDNA clones resulted in the identification of edited polyA+ RNAs. Editing at genomic position 117,990 within the Kaposin ORF affects the amino acid sequence of Kaposin A and C (Glycine to Serine at amino acid 38) (Gandy et al., 2007).

Kaposin A exhibits transforming activity in cultured cells and can induce tumor formation in mice (Muralidhar et al., 1998). Gandy at al. investigated the effects of A-to-I editing within Kaposin A and its ability to transform cells. Expression constructs with Kaposin A harboring either an A or G at position 117,990 were transfected into Rat3 cells and the formation of morphologically distinct highly refractile loci was quantified. Compared to non-transfected controls, Kaposin A with an A at position 117,990 exhibited an increased
number of foci in transfected Rat3 cells, while Kaposin A with G at the same position did not induce foci formation (Gandy et al., 2007). In addition, mice injected with Rat3 cells stably expressing non-edited Kaposin A (an A position 117,990) developed tumors as early as two weeks post-injection. In contrast mice injected with Rat3 cells expressing G containing Kaposin A did not develop tumors post-injection. Thus, A-to-I editing of Kaposin A affects its transforming properties (Gandy et al., 2007).

In addition to impacting the oncogenic properties of KSHV proteins a recent study reported that ADAR1 is required for optimal KSHV lytic reactivation (Zhang et al., 2020). Knockdown of ADAR1 in iSLK.219 and PEL cells reduced viral gene expression and viral replication. Additionally, the absence of ADAR1 led to an increase in the expression of type I interferons and an array of antiviral response genes in a RIG-I-like receptor (RLR) pathway dependent manner. siRNA-mediated depletion of RIG-I, MDA5 and mitochondrial antiviral-signaling protein (MAVS) reduced interferon production and reduced viral gene expression in ADAR1 deficient cells. Collectively, A-to-I editing has the potential to regulate the transforming properties of a KSHV protein and is necessary for optimal lytic reactivation.

5.4. EBV

Primary transcripts encoding miRNAs consist of a short dsRNA stem region and these transcripts can act as substrates for ADARs (Bass, 2000). Similar to mammalian pri-miRNAs (Blow et al., 2006; Peng et al., 2012), primary transcripts of many EBV miRNAs were identified with A-to-I changes in RNA-seq data (Iizasa et al., 2010; Lei et al., 2013a). Initial studies identified editing within four pri-miRNAs (pri-miR-BHRF1, pri-miR-BART6, pri-miR-BART8 and pri-miR-BART16) in three latently infected EBV positive human cells including Burkitt lymphoma, nasopharyngeal cancer and lymphoblastoid cells (Iizasa et al., 2010). The level of editing is relatively low at sites within pri-miR-BHRF1, pri-miR-BART8 and pri-miR-BART16. In contrast, pri-miR-BART6 exhibits higher levels of editing (50–70%) at + 20 site and the biological function of editing at the + 20 site has been investigated. In vitro pri-miRNA processing assays suggests that editing at + 20 within pri-miR-BART6 reduces cleavage by Drosha in Burkitt lymphoma and nasopharyngeal cancer cells while no effect on Drosha cleavage was detected in lymphoblastoid cells. Furthermore, Dicer was identified as the major transcript targeted by one of the mature miRNAs produced from pri-miR-BART6 (miR-BART6-5p). The level of Dicer repression by unedited miR-BART6-5p is higher than the repression levels by the edited miR-BART6-5p due to reduced efficiency of loading the miRNAs into the miRNA induced silencing complex (miRISC). Together this study reported the first evidence of A-to-I editing in regulating the miRNA loading into miRISCs and modulation of mature miRNA function.

In addition to the previously identified EBV pri-miRNAs, (Lei et al., 2013a) identified editing within another primary miRNA transcript, pri-miR-BART3 (Lei et al., 2013a). Pri-miR-BART3 is edited at four different sites located at the Drosha cleavage site and within the seed region. Previous studies identified the Dice1 tumor suppressor as a cellular target of miR-BART3-5p (Lei et al., 2013b). A-to-I editing at the second site within the seed region of miR-BART3-5p resulted in reduced repression of Dice1 compared to the
unedited miR-BART3-5p. In addition to target selection, editing within the pri-miR-BART3 also reduced miRNA biogenesis and led to decreased levels of mature miRNAs. In sum, these data suggest a functional role of EBV primary miRNA editing in regulating miRNA biogenesis and function. However, how the global cellular and viral transcriptome are affected by these edited EBV miRNAs has not been determined.

A-to-I editing was also observed within noncoding regions of transcripts originating from bidirectional transcription within the EBV origin of replication P (oriP) (Cao et al., 2015). Both the leftward transcript oriPtLs and the rightward transcript oriPtRs are localized in the nucleus. RNA-seq studies on EBV positive Akata cells identified a high density of A-to-I changes within the Family of Repeats (FR) region of oriPtLs and oriPtRs. In fact, nearly 73% and 66% of all adenosines within the FR regions were edited, and the levels of editing were as high as 68% and 74% at individual edited adenosines within oriPtLs and oriPtRs, respectively. In addition, RNA immunoprecipitation assays suggest that ADAR1 and the paraspeckle complex assembly factor NONO can bind oriPt transcripts. Hyper-edited RNAs are sequestered in paraspeckles (Hundley and Bass, 2010). Editing within oriPt transcripts and the subsequent sequestration in paraspeckles could be involved in the antiviral/stress response, however further studies are required to dissect this.

5.5. HBV

ADAR1 regulates HBV replication via modulating the miRNA biogenesis pathway in infected hepatocytes (Liu et al., 2019). Human hepatocyte cells (Huh7) infected with HBV were treated with different doses of interferon alpha (IFN-α) to determine the effects of interferon treatment on viral replication. IFN-α treated cells exhibited increased expression of ADAR1 and decreased expression of HBV RNA in an IFN-α dose dependent manner. Knockdown of ADAR1 in this context led to increased expression of HBV RNA suggesting ADAR1 negatively regulates HBV transcript levels. A predominantly expressed miRNA in hepatocytes, miR-122, was upregulated by ADAR1 expression, whereas ADAR2 had no effect in regulating miR-122 expression. Transfection of miR-122 mimics and miR-122 inhibitors resulted in decreased and increased expression of HBV RNAs, respectively, suggesting miR-122 negatively regulates the expression of HBV RNA. Additionally, ADAR1 regulates the expression of miR-122 via the previously known miR-122 targets cyclin G1 and p53 (Gramantieri et al., 2007; Wang et al., 2012). Like wild-type ADAR1, ADAR1 catalytic mutant (E912Q), also regulates miR-122 and HBV RNA levels, suggesting the deamination activity of ADAR1 is not required for regulating either miR-122 or HBV RNA and that ADAR1 regulates HBV RNA expression via miRNA biogenesis.

In contrast to Liu et al. two subsequent studies reported that ADAR1 promotes HBV replication (Yuan et al., 2020; Wang et al., 2021). Knockdown of ADAR1 reduced the levels of HBV replicative DNA (HBV DNA), while over expression of ADAR1 enhances HBV DNA in HepG2.2.1.5 cells independent of the HBV genotypes (Yuan et al., 2020). ADAR1 mutants harboring mutations within the deaminase domain led to decreased HBV DNA replication compared to the wild type ADAR1, suggesting that the demainase activity of ADAR1 is critical for promoting HBV replication.
Previous studies had suggested that HBV counteracts host innate immune responses mediated by pattern recognition receptors (PRRs). Recent studies by Wang et al. reported an ADAR1-mediated molecular mechanism by which HBV manipulates the host immune responses. Human hepatocytes are defective in sensing DNA, however RNA sensing pathways still function in these cells. ADAR1 RIP assays suggested that HBV RNAs are bound by ADAR1 and the presence of multiple ADAR1 binding sites apart from the 5’ and 3’ epsilon loops (Wang et al., 2021). Mutations within one dsRBD did not affect ADAR1 binding to HBV RNA. However, simultaneous mutations within any two of the dsRBDs led to significantly reduced interaction with HBV RNAs, suggesting at least two dsRBDs are required for ADAR1 to bind HBV RNAs. Furthermore, RNA-seq identified 11 A-to-I editing sites within HBV RNA. HepG2 cells transfected with HBV RNA with all 11 edited adenosines mutated into guanosines led to reduced levels of interferon production compared to HBV RNA with adenosines. Along this line, HBV RNAs are highly enriched in RIG-I and MDA5 RIPs in the absence of ADAR1. In sum, ADAR1 promotes HBV replication by editing the HBV RNAs to escape from host immune recognition.

ADAR1 genetic variants are associated with differential clinical outcomes in patients with chronic hepatitis B. For example, single nucleotide polymorphism (SNP) rs4845384 located within ADAR1 affects the efficiency of IFN-α therapy (Wu et al., 2012). The AA carriers at rs4845384 expressed lower levels of ADAR1 compared to GG carriers. Moreover, the frequency of A at rs4845384 is lower in individuals who had spontaneously clear HBV compared to individuals with chronic infection (Wu et al., 2014). Taken together, these findings suggests ADAR1 has both antiviral and proviral roles during HBV infection.

5.6. HCMV

A-to-I editing within HCMV RNAs and the biological significance of RNA editing in pathogenesis is largely unknown. However, ADARs are involved in modulating the host immune response upon HCMV infection (Nachmani et al., 2014). The interferon inducible ADAR1 p150 is induced by many DNA and RNA viral infections (Samuel, 2012). However, HCMV infected human foreskin fibroblasts (HFFs) showed induced expression of the constitutively expressed ADAR1 isoform p110, while the expression of ADAR1 p150 did not significantly changed (Nachmani et al., 2014). ADAR1 p110 affects the editing of one of the most abundantly edited miRNAs, miR-376a (Kawahara et al., 2007). Knockdown and overexpression of ADAR1 p110 in HCMV-infected HFFs led to reduced and increased editing of miR-376a, respectively. Editing within the seed region of miR-376a altered the target specificity within the host cell; edited miR-376a lost its ability to repress a previously known target, MICB, while gained ability to repress a new target, HLA-E, a natural killer (NK) cell inhibitory ligand (Nachmani et al., 2010). HLA-E was predicted to have two binding sites for edited miR-376a within the 3’-UTR and mutations within these predicted binding sites abolished repression, suggesting that the edited miR-376a directly binds to HLA-E to regulate the expression. Furthermore, transfection of anti-miR-376a sponges specific to edited miR-376a during HCMV infection resulted in increased expression of HLA-E compared to HCMV-infected HFFs with control sponges. In addition, HCMV-infected HFFs expressing anti-miR-376a sponges and control sponges were subjected to cell killing by NK cells isolated from donors. The expression of anti-miR-376a sponges that
sequester edited miR-376a resulted in increased expression of HLA-E, reduced inhibition by NK cells as well as killing. In sum, ADAR1 p110 mediated editing of miR-376a leads to immune modulation of HCMV-infected cells and provides an advantage to HCMV in infected cells (Nachmani et al., 2010).

5.7. Vaccinia (VACV)

The Pox virus VACV is widely used in live vaccines to immunize individuals against smallpox (Smith et al., 2013). Unlike other DNA viruses, VACV replicates in the cytoplasm. A-to-I editing within VACV RNAs and their role in viral pathogenesis are largely unknown. VACV has evolved multiple mechanisms to antagonize host immune responses including inhibiting one of the major antiviral proteins, PKR (Davies et al., 1993; Langland and Jacobs, 2002). A viral encoded dsRNA binding protein E3L inhibits PKR activation by sequestering dsRNA and interfering with the RNA binding ability of PKR (Davies et al., 1993; Langland and Jacobs, 2002). Similar to PKR, E3L acts as a potent inhibitor of host ADAR1 (Liu et al., 2001). In vitro deamination assays suggest that E3L efficiently suppresses the activity of ADAR1. Both ADAR1 isoforms p110 and p150 were inhibited by E3L and this inhibition was independent of the dsRNA binding activity of ADAR1. In contrast, E3L dsRNA binding mutant and an E3L N44A/W66L Z-DNA binding domain mutant exhibited significantly reduced inhibitory effect on ADAR1 deaminase activity compared to wild type E3L. Furthermore, E3L was able to inhibit the site-selective editing of natural substrates including the glutamate receptor subunit B, R/G site. Together, these studies indicate that the VACV protein E3L inhibits ADAR1, however future studies should be aimed at determining the molecular mechanisms of ADAR1 inhibition by E3L and its role in viral replication and pathogenesis.

5.8. Human papillomavirus (HPV)

*Human papillomavirus* infects keratinocytes. While HPV infections in most individuals clear up spontaneously, persistent HPV infections are associated with multiple cancers including cervical and anal (Braaten and Laufer, 2008). HPV has evolved mechanisms to evade the host immune responses by altering the expression of pattern recognition receptors and suppression of interferon responses (Bordignon et al., 2017). Pujantell et al. (2019), investigated the role of ADAR1 in innate immune signaling and antiviral responses upon HPV infection. Knockdown of ADAR1 in HPV16 genome integrated keratinocytes (SiHa) led to increased expression of RIG-I and MDA5, as well as IFNB, CXCL10 and transcriptional factor IRF7. In addition, phosphorylation of IRF7 and STAT1 was detected in the absence of ADAR1. ADAR1 deficiency in SiHa cells led to increased expression of cytokines suggesting the absence of ADAR1 stimulates a proinflammatory environment in HPV infected cells. Furthermore, depletion of ADAR1 increased the expression of viral proteins E1 and E7 in SiHa cells. A-to-I editing within HPV RNAs has not been identified, and thus the regulation of viral gene expression by ADAR1 is independent of deamination of viral transcripts (Pujantell et al., 2019). Collectively this work suggests that ADAR1 regulates innate immune responses to modulate viral gene expression in HPV infection.
6. Conclusions

RNA modifications affect RNA structure and function. Despite RNA modifications being discovered in viral genomes nearly half a century ago it has really been the last decade that a functional role for RNA modifications during viral infection has emerged. To date, studies have clearly established roles for the host- and viral epitranscriptomes in viral pathogenesis and host immune responses. Studies on RNA modifications in DNA viral infections are limited; m\(^6\)A and inosine are the two most studied RNA modifications in DNA viruses to date. Overall, m\(^6\)A and inosine modifications are neither proviral or antiviral, but instead modulate aspects of DNA viral replication and pathogenesis. Future studies should be aimed at investigating other less studied modifications including m\(^1\)A, m\(^5\)C, and pseudouridine, and their functional roles in DNA viral replication. Further, the dysregulated cellular pathways caused by altered RNA modifications need to be determined to identify the potential novel therapeutic RNAs to target in DNA viral infections.

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Data availability

No data was used for the research described in the article.

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Fig. 1.
The m\(^6\)A modification and its effects on dsDNA viral replication and pathogenesis. (A) m\(^6\)A is deposited by cellular methyltransferase complex METTL3/METTL14 and reversed by FTO and ALKBH5. m\(^6\)A can be recognized by reader proteins and lead to regulation of many steps in RNA biogenesis. (B) Like m\(^6\)A modifications in RNA viruses, m\(^6\)A modifications differentially impacts the steps of viral replication depending on different dsDNA viruses.
Fig. 2.
The A-to-I editing and its effects on dsDNA viral replication and pathogenesis. (A) A-to-I editing is catalyzed by ADARs via a hydrolytic deamination reaction. Inosines are recognized as guanosines by cellular machineries, thus A-to-I editing impacts recoding, splicing and small RNA mediated gene regulation of cellular RNAs. (B) Schematic representation of ADAR family proteins in humans. All of them have two or more dsRBDs and a deaminase domain. In addition, ADAR1 contains a Z-domain and ADAR3 contains a...
R domain at the N-terminus. (C) A-to-I editing within dsDNA viral transcripts differentially impacts steps of viral replication and pathogenesis depending on different viruses.