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Adenovirus VA RNA: An essential pro-viral non-coding RNA

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ABSTRACT

Adenovirus (AdV) 'virus-associated' RNAs (VA RNAs) are exceptionally abundant (up to 10^8 copies/cell), heterogeneous, non-coding RNA transcripts (~150–200 nucleotides). The predominant species, VA RNA_I, is best recognized for its essential function in relieving the cellular anti-viral blockade of protein synthesis through inhibition of the double-stranded RNA-activated protein kinase (PKR). More recent evidence has revealed that VA RNAs also interfere with several other host cell processes, in part by virtue of the high level to which they accumulate. Following transcription by cellular RNA polymerase III, VA RNAs saturate the nuclear export protein Exportin 5 (Exp5) and the cellular endoribonculease Dicer, interfering with pre-micro (mi)RNA export and miRNA biogenesis, respectively. Dicer-processed VA RNA fragments are incorporated into the RNA-induced silencing complex (RISC) as 'mivaRNAs', where they may specifically target cellular genes. VA RNA_I also interacts with other innate immune proteins, including OAS1. While intact VA RNA_I has the paradoxical effect of activating OAS1, a non-natural VA RNA_I construct lacking the entire Terminal Stem has been reported to be a pseudoinhibitor of OAS1. Here, we show that a VA RNA_I construct corresponding to an authentic product of Dicer processing similarly fails to activate OAS1 but also retains only a modest level of inhibitory activity against PKR in contrast to the non-natural deletion construct. These findings underscore the complexity of the arms race between virus and host, and highlight the need for further exploration of the impact of VA RNA_I interactions with host defenses on the outcome of AdV infection beyond that of well-established PKR inhibition. Additional contributions of VA RNA_I heterogeneity resulting from variations in transcription initiation and termination to each of these functions remain open questions that are discussed here.

1. Introduction

Adenoviruses (AdVs) are naked, icosahedral, double-stranded DNA viruses that have been extensively studied for well over 60 years. Human AdVs include approximately 56 types divided into eight classes, A–G (Knipe and Howley, 2013). AdVs are pathogenic, and can cause a variety of different diseases including gastroenteritis, keratoconjunctivitis, hemorrhagic cystitis and acute respiratory disease. Class C AdVs, which include the most widely studied Ad2 and Ad5, are the most frequent cause of myocarditis in children under the age of one year and are a common cause of respiratory illness in children (Bowles et al., 2003; Edwards et al., 1985). However, in between 50% and 90% of cases, Class C AdV primary infections are asymptomatic (Edwards et al., 1985; Knipe and Howley, 2013). In addition to acute infection, AdVs are capable of both persistent (Fox et al., 1977) and true latent infection (Garnett et al., 2009; Neumann et al., 1987). In spite of their complex biology, our depth of knowledge of AdV coupled with its ease of genetic manipulation, and ability to infect a wide range of tissues, including both dividing and senescent cells, has lead to development of AdV as a promising candidate for gene and oncotherapy (Choi et al., 2012; Maekawa et al., 2013).

Abbreviations: AdV, Adenovirus (note, V is replaced by a number to denote a specific serotype); Ago-2, Argonaute-2; bp, base pair; DMS, dimethylsulfate; dsRNA, double-stranded RNA; eIF, eukaryotic translation initiation factor; Exp5, Exportin 5; FRT, flippase recognition target; miRNA, micro RNA; mivaRNA, VA RNA-derived miRNA; ncRNA, non-coding RNA; NF1, nuclear factor 1; nt(s), nucleotide(s); OAS, oligoadenylate synthetase; PAMP, pathogen-associated molecular pattern; PKR, dsRNA-activated protein kinase; RIG-I, retanoic acid-inducible gene 1; RISC, RNA-induced silencing complex; RNA Pol III, RNA polymerase III; SAXS, small angle X-ray scattering; SHAPE, selective 2′-hydroxyl acylation analyzed by primer extension; VA RNA, virus-associated RNA.

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The AdV genome is ~36 kilobases long with inverted terminal repeats. Genes are organized into early and late transcriptional units, and are transcribed from both strands of the DNA genome. All human AdV serotypes encode at least one highly structured, approximately 160 nucleotide (nt), non-coding "virus-associated RNA" (VA RNA) (Ma and Mathews, 1996b; Mathews and Shenk, 1991). Approximately 80% of all AdVs, including Ad2 and Ad5, encode two distinct VA RNA transcripts, VA RNA_I and VA RNA_{II} (Ma and Mathews, 1996b). VA RNAs from serotypes with only a single VA RNA gene most resemble the Class C VA RNA_I. VA RNA_I is essential for efficient virus replication: In Ad5, deletion of VA RNA_I led to an approximately 20-fold decrease in virus titer (Thimmappaya et al., 1982). While deletion of VA RNA_{II} caused no measurable decrease in AdV5 viability, deletion of both VA RNA_I and VA RNA_{II} decreased titers 60 fold (Bhat and Thimmappaya, 1984). This greater impact compared to the single VA RNA_I deletion suggests that while VA RNA_{II} can partly compensate for the absence of VA RNA_I, VA RNA_I plays the predominant pro-viral role. For this reason, the majority of research into the function of the AdV VA RNAs has focused on VA RNA_I and it is therefore the main focus of this review. Where the collective activities of both VA RNA_I and VA RNA_{II} are discussed, the term VA RNA will be used, while the individual VA RNAs will be referred to specifically as VA RNA_I and VA RNA_{II}.

During AdV infection, VA RNA_I and VA RNA_{II} accumulate in the cytoplasm beginning at ~18 h post infection, and by late in the lytic infection cycle (24 h), these RNAs have accumulated to extraordinarily high levels, 10^8 and 10^6 copies/cell, respectively (Mathews and Shenk, 1991). Like many viral RNAs, VA RNA_I interacts with multiple cellular proteins and likely has multiple functions during infection. The most established and best understood role of VA RNA_I is as an efficient inhibitor of the innate immune protein double-stranded (ds)RNA-activated kinase (PKR). Through this activity, VA RNA_I allows AdV to escape a key cellular response to dsRNA, a potent pathogen-associated molecular pattern (PAMP), thereby conferring AdV's observed resistance to interferon (Kitajewski et al., 1986; Mathews and Shenk, 1991). In addition to binding and inhibiting PKR, VA RNA_I interacts with the other innate immune system dsRNA-detecting proteins, RIG-I and OAS1 (Desai et al., 1995; Minamitani et al., 2011), as well as the host RNA interference (RNAi) machinery through the cellular endoribonuclease Dicer and Argonaute-2 (Ago-2) within the RNA-induced silencing complex (RISC) (Andersson et al., 2005; Xu et al., 2007). While most functions of VA RNA_I are associated with its accumulation late in infection, recent work has revealed a novel role for VA RNA_I early in infection as a repressor of Hepatoma-Derived Growth Factor (Kondo et al., 2014). Understanding the interactions of this RNA with host proteins during AdV infection will add to the growing body of knowledge of the multifaceted roles of non-coding RNAs (ncRNAs), improve our understanding of host-pathogen recognition, and promote effective development of AdV-based technologies. Of particular importance is building an understanding of how specific RNA features are critical for activation and inhibition of dsRNA-detecting innate immune proteins. Such proteins are responsible for the general detection of viruses and thus represent a potential target for influencing the outcome of infection. The ability to modulate and selectively activate these proteins is of particular interest for developing general antiviral therapies effective against viruses that rely upon manipulation of dsRNA-detecting innate immune proteins but for which there are no vaccines or treatments (Fei et al., 2011; Melchjorsen, 2013). For example, selective activation of PKR or the OAS/RNase L pathway in infected cells has already been demonstrated as a viable way to inhibit replication of a range of viruses including respiratory syncytial virus, encephalomyocarditis virus, and human parainfluenza virus 3 (Cirino et al., 1997; Rider et al., 2011; Thakur et al., 2007).

2. Expression, localization, and structure of AdV VA RNAs

2.1. VA RNA gene structure and transcription

The VA RNA genes encode transcripts of ~150–200 nt in length. Accumulation of VA RNA_I was first observed late in Ad2 infection (Reich et al., 1966), and a second RNA species, VA RNA_{II}, was later found to be present in lower amounts, at approximately a 1:40 ratio (Soderlund et al., 1976). Both VA RNAs are transcribed by the cellular RNA Polymerase III (RNA Pol III) (Soderlund et al., 1976; Weinmann et al., 1974). RNA Pol III requires two intragenic

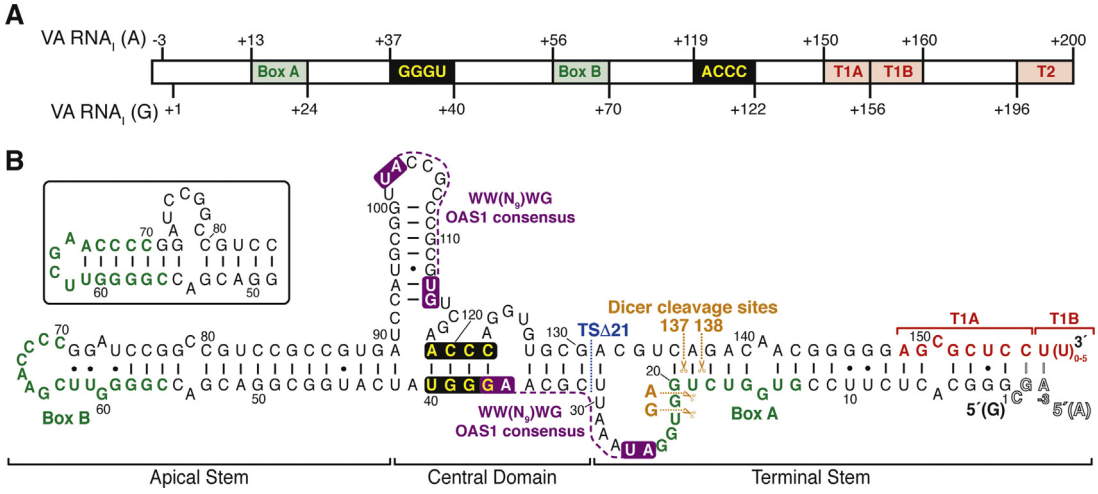

Fig. 1. VA RNA_I gene organization and transcript secondary structure. (**A**) AdV VA RNA_I is transcribed from two distinct start sites: A (−3) and G (+1) producing VA RNA_I(A) and VA RNA_I(G) transcripts, respectively. Locations of the RNA Pol III Box A and B promoter regions (green), internal terminator sequence (T1A, red), first d(A_4) terminator sequence (T1B, red), backup terminator sequence (T2, red), and pair of universally conserved complementary tetranucleotide sequences (yellow) are indicated. (**B**) Sequence and secondary structure of Ad2 VA RNA_I, which is comprised of three conserved structural domains: Terminal Stem, Central Domain, and Apical Stem. The RNA Pol III promoter sequences, conserved tetranucleotides, and T1A/T1B terminator sequences are colored as in *panel A*. Two WW(N$_9$)WG OAS1 consensus activation sequences (purple), sites of Dicer cleavage (orange dotted lines), and the site of TSΔ21 RNA truncation (blue dotted line) are mapped on the RNA secondary structure. 5′-End nucleotides arising from alternative transcription start site in VA RNA_I (A) are shown in outline font. The VA RNA_I Apical Stem can adopt two functionally non-equivalent structures; the alternate folding with base pairing between G57–G60 and C67–C70 which results in the formation of a loop within the helix is shown (boxed). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)