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Assays for monitoring viral manipulation of host ARE-mRNA turnover

Jennifer A. Corcoran [a,b,1], Denys A. Khaperskyy [a,1], Craig McCormick [a,b,*]

[a] Department of Microbiology and Immunology, Dalhousie University, 5850 College Street, PO Box 15000, Halifax NS, Canada B3H 4R2
[b] Beatrice Hunter Cancer Research Institute, Dalhousie University, 5850 College Street, PO Box 15000, Halifax NS, Canada B3H 4R2

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ABSTRACT

Early host responses to viral infection rapidly induce an antiviral gene expression program that limits viral replication and recruits sentinel cells of the innate immune system. These responses are mediated by cytokines. The mRNAs that encode cytokines typically harbor destabilizing adenine- and uridine-rich elements (AREs) that direct their constitutive degradation in the cytoplasm. In response to a variety of signals, including viral infection, small pools of cytoplasmic ARE-mRNAs are rapidly stabilized and translated. Thus, mRNA stability plays a key role in antiviral gene expression. Intriguingly, recent studies have identified viral proteins that specifically target ARE-mRNAs for stabilization, suggesting that certain proteins encoded by ARE-mRNAs may be advantageous for infection. Here, we discuss the development of a suite of sensitive and complementary assays to monitor ARE-mRNA turnover. These include luciferase- and destabilized-GFP-based assays that can be adapted for high-throughput screening applications.

1. Introduction

In every infected cell, there is a pitched battle between virus and host to control gene expression. As obligate intracellular parasites, viruses require key components of the host transcription and translation machinery to express their genes. For this reason, viruses have evolved a variety of mechanisms to exploit cellular biosynthetic machinery for their own replication. Infection of host cells rapidly triggers countermeasures: antiviral cytokines that limit and alert the sentinel cells of the innate immune system. Thus, successful infection requires subversion of the host biosynthetic machinery and dampening of innate immune responses.

A variety of post-transcriptional mechanisms influence viral and host gene expression. Among these, the control of messenger RNA (mRNA) stability plays a central role. Cellular mRNAs bear a 5′-7-methylguanosine cap (5′-cap) and a 3′ poly(A) tail, which act together to protect from exonuclease digestion and facilitate translation. Most viral transcripts share these same structural features because they need to be recognized and translated by the same apparatus. Both host and viral mRNAs are ultimately subject to general mRNA turnover: destruction by host enzymes that direct deadenylation, decapping and exonucleolytic degradation [1]. In this way, the host mRNA decay machinery may represent a significant barrier to viral gene expression. In addition, some herpesviruses enforce 'host shutoff' to counter antiviral gene expression, promoting the degradation of host mRNAs. Host shutoff is mediated by viral nucleases and recent work has implicated selective interactions with the host mRNA turnover machinery [2]. Thus, the control of mRNA turnover represents an important battleground in controlling the outcome of infection.

Host mRNA decay machinery also prevents significant expression of mRNAs encoding antiviral gene products; thus, an important aspect of innate responses involves rapid stabilization of these labile mRNAs. Early host responses to viral infection depend on the stabilization of mRNAs encoding cytokines. Most cytokines are encoded by labile mRNAs that harbor adenine- and uridine-rich elements (AREs) in their 3′-untranslated regions (3′-UTRs), which direct constitutive degradation in the cytoplasm [3–6]. In this way, the levels of these mediators are normally kept low. Host ARE-binding proteins (ARE-BPs) receive inputs from a variety of signal transduction pathways and tightly regulate ARE-mRNA turnover. Perhaps the best characterized ARE-BP is tristetraprolin (TTP) is a CCCH-zinc finger protein that binds specifically to ARE-mRNAs via a nonamer sequence (UUAUUUAUU) and directs them to the exosome, an assembly of endo- and exonucleases that digest deadenylated mRNA from the 3′ end, and the P-body, a multiprotein complex involved in mRNA degradation and translation repression [7–9] (Fig. 1). The rapid degradation of ARE-mRNAs is subject to regulation by the p38 MAPK pathway. Stimulation of p38, long known to play a critical role in inflammation, results in significant increases in the half-lives of ARE-mRNAs and concomitant increases in translation. This blockade in ARE-mRNA degradation is mediated by the p38 target, MAPKAP-kinase-2 (MK2). The exact mechanism of MK2-mediated inhibition of ARE-mRNA decay is unknown, but is thought to involve phosphorylation of TTP on serines 52 and 178, which

* Corresponding author at: Department of Microbiology and Immunology, Dalhousie University, 5850 College Street, PO Box 15000, Halifax NS, Canada B3H 4R2.
E-mail address: craig.mccormick@dal.ca (C. McCormick).
[1] These authors contributed equally to this work.

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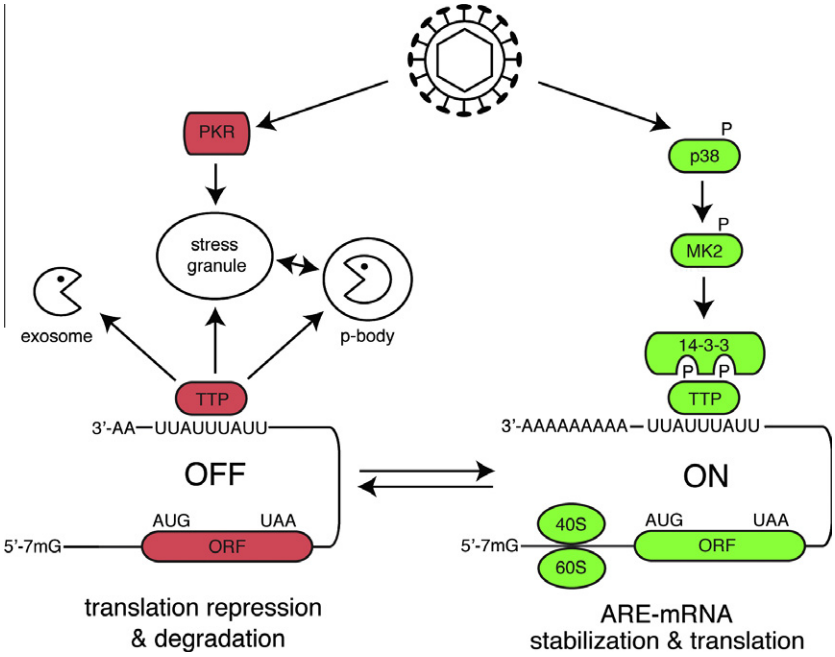

Fig. 1. Regulation of AU-rich element (ARE)-mediated mRNA decay in virus-infected cells. Recognition of viral dsRNA triggers dimerization and autophosphorylation of PKR, resulting in kinase activation [12]. Active PKR then phosphorylates eukaryotic initiation factor 2 alpha (eIF2α), and promotes the assembly of a translationally incompetent initiation complexes that nucleate cytoplasmic stress granules (SGs) [13–15]. SGs serve as triage sites, where translationally stalled mRNAs await reinitiation or degradation. TTP localizes to both SGs and associated P-bodies, and is thought to ferry ARE-mRNA cargo from SGs to P-bodies for translation repression and degradation [16]. All of these mRNA trafficking events are reversible, and there is evidence that mRNAs can exit P-bodies to re-enter translation [51]. Many viruses also trigger activation of p38 and the downstream kinase MK2 [17–21]. MK2-mediated phosphorylation of TTP, and consequent association with 14-3-3 scaffolding proteins, prevents SG localization, thus protecting bound ARE-mRNAs from translational stalling and degradation [22].

generates consensus binding sites for cytoplasmic 14-3-3 scaffolding proteins, perhaps preventing the recruitment of the exosome [10,11].

The latent double stranded RNA (dsRNA)-dependent protein kinase PKR also plays an important role in antiviral defense. Recognition of viral dsRNA triggers dimerization and autophosphorylation of PKR that is mandatory for kinase activation [12]. Active PKR then phosphorylates eukaryotic initiation factor 2 alpha (eIF2α), and promotes the assembly of a translationally incompetent initiation complex that accumulates, together with a substantial fraction of polyadenylated mRNA, at discrete cytoplasmic foci known as stress granules (SGs) [13–15] (Fig. 1). SGs serve as triage sites, where translationally stalled mRNAs await reinitiation or degradation. TTP localizes to both SGs and associated P-bodies, and is thought to ferry ARE-mRNA cargo from SGs to P-bodies for degradation [16]. In this way, TTP should facilitate rapid ARE-mRNA degradation in virus-infected cells. However, many viruses also trigger activation of p38 and the downstream kinase MK2 [17–21]. MK2-mediated phosphorylation of TTP, and consequent association with 14-3-3 scaffolding proteins, prevents SG localization, thus protecting bound ARE-mRNAs from translational stalling and degradation [22]. Thus, in virus-infected cells with elevated MK2 activity, ARE-mRNAs likely represent a 'privileged' class of transcripts that should be preferentially stabilized and translated (Fig. 1).

Considering that host pro-inflammatory cytokine responses are designed to alert the immune system and limit viral infection, it seems deeply paradoxical that a virus would encode proteins that trigger the stabilization of ARE-mRNAs. What advantage could a virus obtain from ARE-mRNA stabilization? Important clues have been revealed by the study of the Kaposi's sarcoma-associated herpesvirus (KSHV, a.k.a. human herpesvirus-8), the infectious cause of the AIDS-associated malignancy Kaposi's sarcoma (KS). In KS, the predominant tumor cell is the KSHV-infected, proliferating 'spindle cell', thought to be of endothelial origin. Spindle cells secrete a variety of pro-inflammatory and pro-angiogenic cytokines, chemokines and growth factors that can promote tumorigenesis [23]. Importantly, these responses can be recapitulated by de novo KSHV infection of primary endothelial cells, indicating that KSHV plays an active role in reprogramming endothelial gene expression [24–26]. This reprogramming is not limited to effects on transcription; the latent kaposin B protein potently stabilizes ARE-mRNAs by binding and activating MK2 [17]. Beyond playing an important role in tumorigenesis, KSHV-mediated stabilization of ARE-mRNAs may allow for evasion of immune surveillance. Enhanced secretion of pro-inflammatory mediators by KSHV-infected cells would be expected to promote the recruitment of mononuclear leukocytes (lymphocytes, monocytes and neutrophils) to the tumor site. However, recent work has shown that KSHV-induced interleukin-6 (IL6) selectively limits the recruitment of neutrophils [27], a cell type with anti-viral and anti-tumor activities. This confirms the observation that neutrophils are sparse within KS lesions, where the inflammatory infiltrate is composed mainly of lymphocytic cells [28]. Together, these studies suggest that KSHV-mediated increases in IL6 transcription and ARE-mRNA stability may allow simultaneous viral evasion of immune surveillance and promotion of tumorigenesis.

The ability to measure kinetics of mRNA decay directly is essential for the characterization of viral regulators of ARE-mRNA turnover. Changes in abundance of certain mRNA species over time are measured by northern blotting, reverse-transcription real-time quantitative PCR (RT-QPCR), and cDNA microarray technology. These methods are often employed individually or in combinations for analyses of endogenous ARE-mRNA transcripts. Microarray analyses allow comprehensive survey of many transcripts simultaneously and are most applicable for identification of subsets of ARE-mRNA transcripts whose turnover is affected by a particular regulatory factor or condition [29,30]. RT-QPCR is rapid and less expensive than microarray analysis, has higher sensitivity and is