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Hijacking of the host-cell response and translational control during influenza virus infection

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Abstract

Influenza virus is a major public health problem with annual deaths in the US of 36,000 with pandemic outbreaks, such as in 1918, resulting in deaths exceeding 20 million worldwide. Recently, there is much concern over the introduction of highly pathogenic avian influenza H5N1 viruses into the human population. Influenza virus has evolved complex translational control strategies that utilize cap-dependent translation initiation mechanisms and involve the recruitment of both viral and host-cell proteins to preferentially synthesize viral proteins and prevent activation of antiviral responses. Influenza virus is a member of the *Orthomyxoviridae* family of negative-stranded, segmented RNA viruses and represents a particularly attractive model system as viral replication strategies are closely intertwined with normal cellular processes including the host defense and stress pathways. In this chapter, we review the parallels between translational control in influenza virus infected cells and in stressed cells with a focus on selective translation of viral mRNAs and the antagonism of the dsRNA and host antiviral responses. Moreover, we will discuss how the use of genomic technologies such as DNA microarrays and high through-put proteomics can be used to gain new insights into the control of protein synthesis during viral infection and provide a near comprehensive view of virus-host interactions.

Keywords: Translation; RNA viruses; Influenza viruses; Functional genomics; Bioinformatics

1. Introduction

For more than a decade, we have been studying mechanisms of translational control using cells infected with influenza virus. The virus has developed translational control strategies that utilize cap-dependent translation initiation mechanisms and which involve the recruitment of host-cell proteins to preferentially synthesize viral proteins and prevent the activation of antiviral responses. Translational regulation is a critical component of the cellular response to a variety of stimuli, including growth-promoting and growth-repressing signals. Similarly, the cellular response to stress, such as viral infection, nutrient deprivation, accumulation of misfolded proteins and ER stress, and finally heat shock, involves translational control mechanisms that function to activate and to repress mRNA translation depending on environmental conditions. Moreover, there are interesting parallels between the translational regulation in influenza virus infected cells and in stressed cells. For example, during influenza virus infection there is a dramatic shutoff of cellular protein synthesis and the selective translation of viral mRNAs (Katze et al., 1986a,b, 1988; Katze and Krug, 1990; Garfinkel and Katze, 1992; Zurcher et al., 2000). In heat shocked or stressed cells, there is similarly a disruption of "normal" cellular protein synthesis and a subsequent redirection of translation to heat shock mRNAs (for recent reviews see Schroder and Kaufman, 2005; Holcik and Sonenberg, 2005; Clemens, 2005). Thus, influenza virus represents a particularly attractive model system, since viral replication strategies are closely intertwined with normal cellular processes, including host defense and stress pathways.

Influenza virus infection involves a series of steps, including attachment to sialylated glycoproteins via the viral hemagglutinin (HA), endocytosis of virus, and the pH-dependent fusion and release of viral genomic ribonuclearprotein (RNP) complexes (Fig. 1) for review see (Lamb and Krug, 2001). These complexes are then translocated to the nucleus where replication

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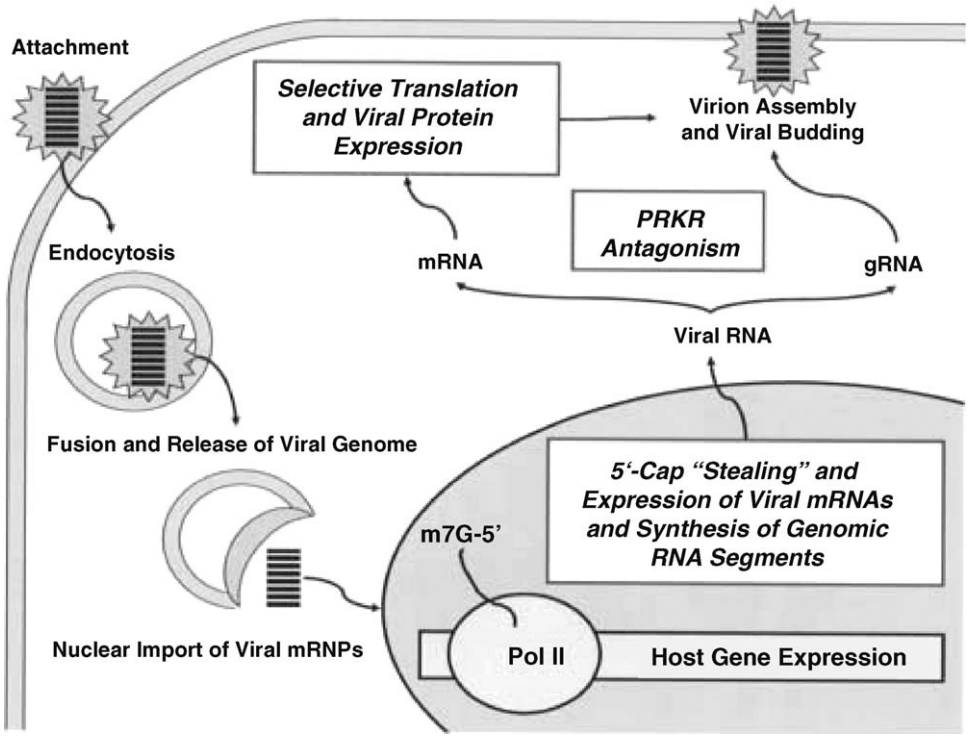

Fig. 1. Influenza virus infection and the key events associated with translational control and viral replication. Diagram showing the steps in influenza virus infection from attachment and endocytosis of virus to the fusion of viral envelope with endosomal membrane and release and nuclear translocation of viral mRNP complexes. The key steps associated with regulation of host-cell translation are indicated and consist of the viral RNA replication mediated stealing of the 5′ cap structure of nacent host RNA polymerase II transcripts, suppression of PKR activation and selective translation of viral mRNAs and viral protein synthesis.

of viral RNAs occurs. All influenza virus mRNAs contain host-cell RNA sequences at the 5′ end that are obtained through a "cap snatching" mechanism. During this process, the influenza virus polymerase complex scavenges the 5′ end (10–13 nucleotides) of cellular polymerase II transcripts, which are subsequently used as primers for the transcription of viral mRNAs (Lamb and Krug, 2001). The influenza viral mRNA 5′ UTRs are typically 20–50 nucleotides (depending on the viral gene) with little apparent secondary structure and no upstream AUGs; however, there are conserved sequences within the viral 5′ UTRs. Despite its relative simplicity, we found that the influenza virus 5′ UTR is both necessary and sufficient to direct selective translation during infection (Garfinkel and Katze, 1992; Park and Katze, 1995; Park et al., 1999; Kash et al., 2002). In this chapter, we will review studies by our group and others that are defining the pathways and mechanisms by which influenza virus uses both viral and cellular proteins to control the efficient and selective translation of viral mRNAs. We will also discuss the recruitment of a host-cell mRNA binding protein recruited to stimulate cap-dependent protein synthesis via binding to conserved sequences in the viral mRNA 5′ UTRs. We will also review the antagonism of the dsRNA activated protein kinase PKR and suppression of the cellular antiviral response by the viral non-structural 1 (NS1) protein and the host-cell protein $P58^{IPK}$. We will then close with a discussion of the impact these various pathways make toward the high levels of viral protein synthesis and evasion of the host response to infection.

2. Selective translation of viral mRNAs

2.1. Recruitment of the host-cell mRNA-binding protein GRSF-1

We have identified several cellular proteins that bind to the influenza virus 5′ UTR, suggesting that both cellular and viral proteins have a role in directing selective translation. We began by using the yeast three-hybrid screen to identify and clone specific cellular proteins that bind viral mRNA (Park et al., 1999). We found that the cellular RNA-binding protein, GRSF-1 (G-Rich Sequence Factor-1), binds to specific conserved sequences within the influenza virus mRNA 5′ UTR. GRSF-1 is localized to the cytoplasm and is bound to poly(A) + mRNA (Qian and Wilusz, 1994). Western blot analysis suggests that there may be a family of related or alternatively spliced GRSF-1 proteins. Importantly, GRSF-1 is a member of the RRM (RNA Recognition Motifs) family of proteins and contains three potential RRMs and two auxiliary domains (Qian and Wilusz, 1994). RRMs have approximately 21 conserved amino acid residues spread along an 80–90 amino acid region, with the most conserved regions being the RNP-1 octamer sequence and RNP-2 hexamer sequence (Perez-Canadillas and Varani, 2001; Fierro-Monti and Mathews, 2000). RRM-containing proteins function in a variety of processes ranging from splicing to translational stimulation, raising the possibility that GRSF-1 plays additional roles in influenza virus replication.