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Mini review

Induction and suppression of innate antiviral responses by picornaviruses

Qian Feng, Martijn A. Langereis, Frank J.M. van Kuppeveld *

Virology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, University of Utrecht, 3584CL Utrecht, The Netherlands

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ABSTRACT

The family *Picornaviridae* comprises of small, non-enveloped, positive-strand RNA viruses and contains many human and animal pathogens including enteroviruses (*e.g.* poliovirus, coxsackievirus, enterovirus 71 and rhinovirus), cardioviruses (*e.g.* encephalomyocarditis virus), hepatitis A virus and foot-and-mouth disease virus. Picornavirus infections activate a cytosolic RNA sensor, MDA5, which in turn, induces a type I interferon response, a crucial component of antiviral immunity. Moreover, picornaviruses activate the formation of stress granules (SGs), large aggregates of preassembled mRNPs (messenger ribonucleoprotein particles) to temporarily store these molecules upon cellular stress. Meanwhile, picornaviruses actively suppress these antiviral responses to ensure efficient replication. In this review we provide an overview of the induction and suppression of the MDA5-mediated IFN-α/β response and the cellular stress pathway by picornaviruses.

1. Introduction

On the front line of antiviral immunity is the production of type I interferons (IFN-α/β) and other antiviral cytokines at the site of infection. IFN-α/β can be produced by virtually all nucleated cell types and act in autocrine and paracrine manners (*i.e.* on both the infected cells and neighboring non-infected cells). IFN-α/β-receptor engagement induces the expression of large numbers of interferon-stimulated genes (ISGs), which together establish a so-called antiviral state in the recipient cells. To recognize the invading pathogens timely and correctly, cells employ specialized pattern recognition receptors (PRRs) to detect specific pathogen-associated molecular patterns (PAMPs). RIG-I-like receptors (RLRs) are a family of ubiquitously expressed PRRs that detect "non-self" viral RNAs in the cytoplasm. Two RLRs, RIG-I and MDA5, mediate IFN-α/β production upon infection of various RNA viruses [1,2]. While RIG-I is required for sensing, among others, paramyxoviruses, influenza virus, Japanese encephalitis virus and hepatitis C virus, MDA5 recognizes picornaviruses, mouse norovirus, mouse hepatitis virus and defective interfering particles of paramyxoviruses [1,2]. Another RLR, LGP2, lacks functional domains required for downstream signaling, and therefore has been long suspected to play regulatory roles on RIG-I and MDA5. Besides the IFN-α/β and inflammatory responses, cells also engage various other mechanisms to cope with undesirable conditions. One of such mechanisms is the formation of stress granules (SGs) in the presence of stress such as oxidative, heat, or nutrient stress, UV radiation and viral infections. Although the stress pathway was initially thought to function independently of classical innate antiviral responses such as IFN-α/β, it was recently suggested that the stress response may also directly or indirectly play an antiviral role [3].

During the long co-evolution with their hosts, viruses have acquired strategies to actively counteract host antiviral responses. Both the RLR-mediated IFN-α/β induction pathway as well as the stress pathway are targeted by various viruses [3,4]. This review summarizes our current knowledge on the recognition and suppression of host antiviral pathways by picornaviruses, a large family of human and animal pathogens. We focus on two important and well-studied genera of picornaviruses, namely *Enterovirus* and *Cardiovirus*, and discuss how their RNAs are recognized by RLRs, and how they antagonize the IFN-α/β induction pathway and SG formation in infected cells.

2. Picornaviruses

2.1. Classification and genetics of picornaviruses

Picornaviridae is a large and diverse virus family currently containing 26 genera. The *Enterovirus* genus contains hundreds of

* Corresponding author. Tel.: +31 30 253 4173; fax: +31 30 253 6723.
E-mail address: F.J.M.vanKuppeveld@uu.nl (Frank J.M. van Kuppeveld).

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(sero)types of important human pathogens [4–6]. Poliovirus (PV) is the causative agent of poliomyelitis. Various types of coxsackieviruses (CVs), echoviruses, and other enteroviruses (EVs) can cause viral meningitis, encephalitis, myocarditis and pancreatitis, and have also been implicated in the development of type I diabetes [5,7]. Enterovirus 71 (EV71) is an emerging virus that can cause severe neurological symptoms in young children, and have caused many outbreaks in the past decade, mostly in Southeast Asia [8]. Human rhinoviruses (HRVs) are responsible for approximately one third of common colds in adults, and are also associated with asthma exacerbations and chronic obstructive pulmonary disease (COPD) [9]. The *Cardiovirus* genus contains mostly animal pathogens such as encephalomyocarditis virus (EMCV) and Theiler's murine encephalomyelitis virus (TMEV). Both EMCV and TMEV are primarily rodent pathogens, but EMCV also causes severe, sometimes lethal, infections in other animals such as pigs, elephants, lions and primates, posing problems in zoos and national parks [10,11]. Recently, a new cardiovirus that infects humans has been discovered, namely the Saffold virus, which has been associated with gastroenteritis, and respiratory and neurological infections [12]. Other well-known genera are *Hepatovirus* (*e.g.* hepatitis A virus [HAV]), *Aphthovirus* (*e.g.* the foot-and-mouth disease virus [FMDV]), and *Parechovirus* (*e.g.* human parechovirus [HPeV]).

2.2. Life cycle of picornaviruses

Members of the *Picornaviridae* family are small, non-enveloped, positive-strand RNA viruses. The viral genome, a single-stranded (ss) RNA molecule of 7.5–8.5 kb, encodes a single open reading frame (ORF), an untranslated region (UTR) at either terminus, and a poly(A) tail at the extreme 3′ end. The 5′ terminus of the viral RNA is coupled to a small viral peptide VPg (also known as 3B) *via* a phosphodiester bond as a result of VPg-primed viral RNA replication. The genomic RNA also contains several structured RNA elements that are crucial for virus replication. The internal ribosomal entry site (IRES) in the 5′ UTR contains several stem–loop structures and drives viral cap-independent translation. Stem–loop structures in the UTRs and a *cis*-acting RNA element (CRE) – the position of which varies across different genera – are crucial for viral RNA replication [13].

Picornaviruses share a similar life cycle (Fig. 1), with some details varying across genera [13]. Infection is initiated *via* receptor-mediated endocytosis, followed by uncoating to release the genomic RNA in the cytoplasm. A cellular enzyme then releases the VPg peptide from the genomic RNA [14], for reasons not yet understood, generating a single-stranded viral RNA carrying a 5′ monophosphate group. The viral genome is then immediately translated by the host translation machinery to generate a large polyprotein, which undergoes proteolytic processing by the virally encoded proteinases. All picornaviruses carry a $3C^{pro}$, which mediates most of the proteolytic processing, and members of some genera carry an additional proteinase that also participates (*e.g.* $2A^{pro}$ for enteroviruses and L^{pro} for aphthoviruses). Additionally, these viral proteinases also cleave host factors to aid virus RNA replication and/or to evade host antiviral responses. Next, several viral non-structural proteins hijack regulatory mechanisms of host membrane metabolism to induce extensive remodeling of the intracellular membranous structures to form the so-called replication organelles (ROs) where viral RNA replication takes place. The process of RNA replication is carried out by the virally encoded RNA-dependent RNA polymerase $3D^{pol}$ (Fig. 2). First, $3D^{pol}$ uridylylyates VPg, and uses the resulting VPg-pU-pU as a primer to transcribe the positive-strand RNA into a complementary, negative-strand RNA molecule. During this process, a long dsRNA intermediate product is produced, which is referred to as the replicative form (RF). Next, $3D^{pol}$ uses the negative-strand RNA as a template, and again VPg-pU-pU as a primer, to produce a large number of nascent positive strands. This step leads to the production of another intermediate product, namely the replicative intermediate (RI), which comprises of a single negative-strand RNA and multiple incomplete positive-strand RNAs that are undergoing active transcription. The completed nascent positive-strand RNAs either enter a new round of translation and RNA replication, or are encapsidated to form new virions. At the end of the replication cycle, progeny virus particles are released by cell lysis [13].

2A and L are the two most divergent picornaviral proteins. Both can act as proteinases in some genera but not in others [13]. Because of their activities in inducing host shutoff, modulating cell death and counter-acting immune responses, these proteins have been classified as viral security proteins [15]. In fact, the L proteins

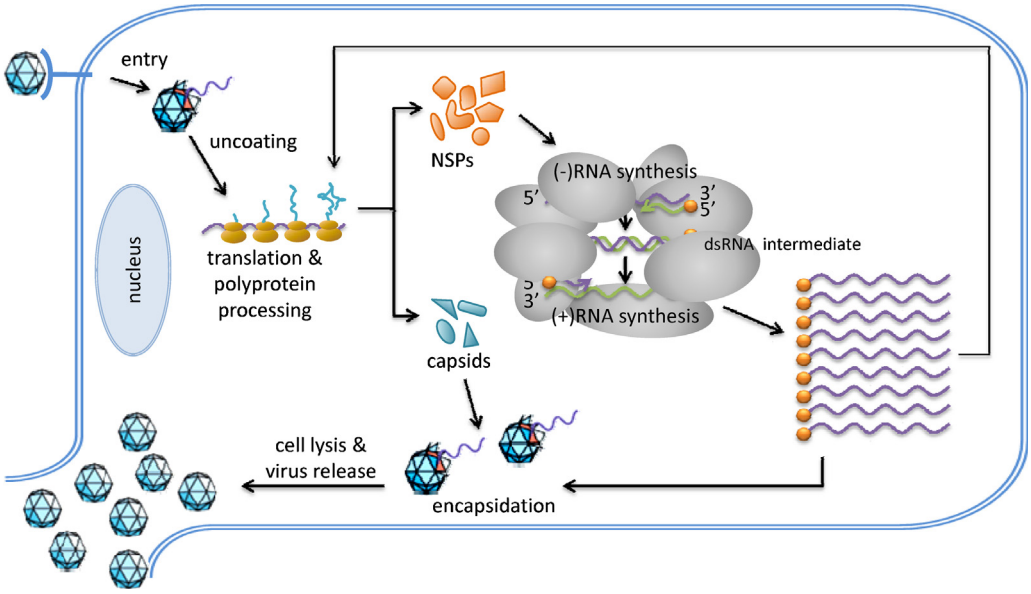

Fig. 1. Life cycle of picornaviruses. NSPs, non-structural proteins. Purple line, viral positive-strand (+)RNAs. Green line, viral negative-strand (−)RNAs. Orange circle, VPg/3B.