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Cyclophilins and cyclophilin inhibitors in nidovirus replication

Adriaan H. de Wilde, Uyen Pham, Clara C. Posthuma, Eric J. Snijder*

Molecular Virology Laboratory, Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT

Cyclophilins (Cyps) belong to the family of peptidyl-prolyl isomerases (PPIases). The PPIase activity of most Cyps is inhibited by the immunosuppressive drug cyclosporin A and several of its non-immunosuppressive analogs, which can also block the replication of nidoviruses (arteriviruses and coronaviruses). Cyclophilins have been reported to play an essential role in the replication of several other RNA viruses, including human immunodeficiency virus-1, hepatitis C virus, and influenza A virus. Likewise, the replication of various nidoviruses was reported to depend on Cyps or other PPIases. This review summarizes our current understanding of this class of nidovirus-host interactions, including the potential function of in particular CypA and the inhibitory effect of Cyp inhibitors. Also the involvement of the FK-506-binding proteins and parvulins is discussed. The nidovirus data are placed in a broader perspective by summarizing the most relevant data on Cyp interactions and Cyp inhibitors for other RNA viruses.

1. Nidoviruses, an introduction

The order *Nidovirales* currently comprises four families – the *Coronaviridae, Arteriviridae, Roniviridae,* and *Mesoniviridae* - that span across a wide range of hosts, including mammalian, avian, reptile, fish, and invertebrate species (https://talk.ictvonline.org/taxonomy/). Within this order, the coronaviruses and arteriviruses have been studied in most detail, due to the societal and economic impact of some family members, unusual features of their pathogenesis, and the complexity of their molecular biology. The latter includes having large to very large polycistronic positive-strand RNA genomes, with sizes ranging from 13 to 16 kb for arteriviruses, via ~ 20 kb for *meso*niviruses, to 26–34 kb for roni- and coronaviruses (Gorbalenya et al., 2006; Nga et al., 2011). The best-known members of the arterivirus family are porcine reproductive and respiratory syndrome virus (PRRSV) and equine arteritis virus (EAV). The coronaviruses (CoVs) are classified into two subfamilies: the *Torovirinae* and the *Coronavirinae*, the latter being subdivided into the genera *Alpha-*, *Beta-*, *Gamma-*, and *Deltacoronavirus*. Most mammalian CoVs are alpha- or betacoronaviruses and these genera include the four 'established' human coronaviruses (HCoVs 229E, OC43, NL63 and HKU1), the zoonotic coronaviruses causing severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (Vijay and Perlman, 2016), and related viruses from bats (Hu et al., 2017; Li et al., 2005) and camels (Sabir et al., 2016). Thus far, gamma- and deltacoronaviruses have been discovered mostly in avian species (Woo et al., 2012).

1.1. Societal and economic impact of nidoviruses

In the past 15 years, nidovirus research has been driven forward in particular by the emergence of two life-threatening CoVs in humans, the betacoronaviruses SARS-CoV and MERS-CoV, which most likely originate from bats and were introduced by zoonotic transfer from intermediate hosts, civet cats and dromedary camels for SARS-CoV and MERS-CoV, respectively (Ge et al., 2013; Hu et al., 2015; Menachery et al., 2015). Of note, the presence of neutralizing antibodies in camels suggests that MERS-CoV or related viruses may have been present in this reservoir for decades (Hu et al., 2015). The short-lived SARS-CoV outbreak in 2002–2003 resulted in 8098 reported cases leading to 774 deaths, while affecting 29 countries (http://www.who.int/csr/sars/en/). Between its emergence in 2012 and March 2018, MERS-CoV has caused > 2100 laboratory-confirmed human infections and at least 750 deaths (http://www.who.int/emergencies/mers-cov/en/). The clinical presentation of SARS-CoV and MERS-CoV ranges from asymptomatic or mild symptoms to acute respiratory disease, in the case of SARS originally described as an "atypical pneumonia" accompanied by fever and severe respiratory distress (Hui et al., 2014). The SARS-CoV and MERS-CoV outbreaks greatly augmented the interest in the CoV family, although human CoVs had already been known since the 1960's, when HCoV-OC43 and HCoV-229E were identified. These two viruses are known to cause mild respiratory disease and, after rhinoviruses, are a leading cause of common colds (10–15% of the cases; reviewed in Wat, 2004). More recently, two additional HCoVs were discovered, HCoV-

* Corresponding author.
E-mail address: E.J.Snijder@lumc.nl (E.J. Snijder).

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NL63 and HCoV-HKU1, which again are associated with respiratory disease (reviewed in Pyrc et al., 2007).

The potential impact of nidoviruses as veterinary pathogens is exemplified by the porcine epidemic diarrhea coronavirus (PEDV) and also by the arterivirus PRRSV, which both continue to cause major economic losses in the swine industry worldwide (Holtkamp et al., 2013; Lin et al., 2016). Likewise, ronivirus infections have done significant damage in the Asian shrimp farming industry (Flegel, 2012). Such outbreaks and the threat of additional emerging (zoonotic) nidoviruses, in combination with the lack of effective antiviral strategies, highlight the importance of advancing our knowledge of the replication of the members of this diverse virus order and their interactions with the host.

1.2. Nidovirus molecular biology

The conserved genome organization and expression strategy of nidoviruses includes the translation of two large replicase open reading frames (ORFs 1a and 1b) from the genomic RNA. This yields the replicase polyprotein (pp) 1a and, following a −1 ribosomal frameshift, the C-terminally extended pp1ab. The two polyproteins are proteolytically processed by multiple internal proteases to liberate (in the case of arteri- and coronaviruses) at least 13–16 nonstructural proteins (nsps). Among these nsps are subunits containing RNA-dependent RNA-polymerase and helicase functions, key players in the enzyme complex responsible for viral RNA synthesis. Together with recruited host cell proteins, nidovirus nsps form membrane-associated replication and transcription complexes (Gosert et al., 2002; Hagemeijer et al., 2012; Pedersen et al., 1999; van Hemert et al., 2008a, 2008b) that localize to a network of virus-induced structures, typically including double-membrane vesicles, in the perinuclear region of the infected cell (reviewed in de Wilde et al., 2017b; Romero-Brey and Bartenschlager, 2016; van der Hoeven et al., 2016). A nested set of subgenomic (sg) mRNAs is produced to express the structural and accessory proteins that are encoded downstream of the nidovirus replicase gene (Pasternak et al., 2006; Sawicki et al., 2007; Snijder et al., 2013; Sola et al., 2011). Despite these common features of viruses in the order *Nidovirales*, the various nidovirus taxa differ strikingly in the type, number, and size of their structural proteins, which also explains the observed variation in virion structure and morphology.

The recent outbreaks of emerging nidoviruses inspired extensive studies of their epidemiology and pathogenesis, and underlined the importance of developing prophylactic and therapeutic options, including vaccines and drugs targeting either viral functions or host factors recruited to support nidovirus replication. In this context, the inhibition of a range of RNA viruses by cyclophilin (Cyp) inhibitors (Hopkins and Gallay, 2015) prompted several research teams to investigate their impact on nidovirus replication, mainly for coronaviruses and - to a lesser extent – for arteriviruses. Below we will first describe the key features of members of the Cyp family and then summarize our current knowledge regarding their involvement in nidovirus replication. This includes the anti-nidoviral effect of cyclosporin A (CsA), the best known Cyp inhibitor (Borel et al., 1976; Handschumacher et al., 1984), and several of its non-immunosuppressive analogs. Finally, our current knowledge on the involvement of Cyps in the replication of other RNA viral pathogens is summarized, to illustrate the wide variety of mechanisms by which this common host factor can be involved in supporting viral replication.

2. Cyclophilins and cyclophilin inhibitors

The peptidyl/prolyl isomerases (PPIases) comprise the immunophilin superfamily, to which the Cyps and the FK506-binding proteins (FKBPs) families belong, and the parvulin protein family (Schiene-Fischer, 2006). Cyps and FKBPs are ubiquitous in both eukaryotes and prokaryotes, and both protein families were initially identified on the basis of their ability to bind the immunosuppressive drugs CsA (Handschumacher et al., 1984), and FK506 or rapamycin (Lane et al., 1991), respectively. The parvulins were initially discovered in the cytoplasm of *E. coli* (Rahfeld et al., 1994) and are the smallest proteins known to have PPIase activity. This activity is essential for catalyzing the *cis-trans* isomerization of the peptide bond upstream of proline residues, which is a rate-limiting step in protein folding (Lang et al., 1987; Schmid, 1993). The identification of the first protein with PPIase activity (Fischer et al., 1984) coincided with the purification from bovine thymocytes of a cellular protein with high affinity for the immunosuppressant CsA: cyclosporin-binding protein A (CypA) (Handschumacher et al., 1984). Five years later, it was discovered that both proteins were one and the same (Fischer et al., 1989; Takahashi et al., 1989). Cyps are involved in a wide range of cellular processes, including protein folding, protein trafficking, and cell signaling (Naoumov, 2014). Despite the fact that all members of the PPIase superfamily share the same enzymatic activity, protein sequences and structures differ enormously between the three families (Barik, 2006; Davis et al., 2010; Hanes, 2015). The human genome is currently believed to encode 19 cyclophilins, 18 FKBPs, and three parvulins (Pin1, Par14, and Par17) (Gray et al., 2015).

Cyclophilins have been identified in a range of organisms, including mammals, plants, insects, fungi, and bacteria (Barik, 2006; Wang and Heitman, 2005). Not all Cyps catalyze the *cis-trans* isomerization of proline-preceding peptide bonds; in fact in vitro PPIase activity has been demonstrated for only seven of the human Cyps (Davis et al., 2010). Some Cyps, like CypA, consist of solely a PPIase domain, while in other Cyps this domain is flanked by additional sequences or modular domains, which control their subcellular localization and/or are thought to be specific for cellular functions (Barik, 2006; Schiene-Fischer, 2015). Despite the fact that Cyps have been implicated in a range of cellular processes, the function of many Cyps is unknown. Also, it has proven to be difficult to identify the natural substrates of the PPIase activity (reviewed in Hopkins and Gallay, 2015). Besides their role in specific cellular functions (reviewed in Naoumov, 2014), CypA, CypB, and CypD have been shown to also function in the replication of certain groups of RNA viruses. Below we will briefly summarize the cellular function of these Cyps.

2.1. Cyclophilin A

The 18-kDa cytosolic CypA is also referred to as peptidyl-prolyl isomerase A (PPIA) or Cyp18. It is one of the most abundant proteins in the cytoplasm (0.1–0.4% of total protein content) and is expressed in all tissues (Harding et al., 1986; Ryffel et al., 1991). In the cytosol, CypA plays a role in a broad range of cellular functions, like facilitating protein folding, protein trafficking, T-cell activation, and cell signaling (reviewed in Naoumov, 2014; Nigro et al., 2013). Although CypA normally is an intracellular protein, inflammatory stimuli like infections, hypoxia, or oxidative stress can elicit CypA secretion via a vesicular transport mechanism that depends on Rho kinase activation (reviewed in Bukrinsky, 2015). CypA proved to be non-essential for cell growth as depletion of CypA in cells or in $PPIA^{-/-}$ knockout mice did not affect survival and/or growth kinetics (Chatterji et al., 2009; Colgan et al., 2005; de Wilde et al., 2017c).

2.2. Cyclophilin B

The 22-kDa CypB essentially consists of a PPIase domain that is equipped with a cleavable N-terminal signal sequence to target the protein to the lumen of the endoplasmic reticulum (ER) (Price et al., 1991; Spik et al., 1991). N-terminally truncated CypB is secreted in response to inflammatory stimuli, although the mechanism by which CypB is cleaved is currently unclear (reviewed in Bukrinsky, 2015). In addition, binding of CsA to the CsA-binding site in CypB induces the release of CypB from the ER via the secretory pathway. It has been