Antiviral Research 101 (2014) 122–130

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

SARS-CoV ORF1b-encoded nonstructural proteins 12–16: Replicative enzymes as antiviral targets

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ARTICLE INFO

Article history:
Received 26 September 2013
Revised 22 October 2013
Accepted 11 November 2013
Available online 20 November 2013

Keywords:
Coronavirus
SARS-CoV
Replication
Transcription
Nonstructural proteins

ABSTRACT

The SARS (severe acute respiratory syndrome) pandemic caused ten years ago by the SARS-coronavirus (SARS-CoV) has stimulated a number of studies on the molecular biology of coronaviruses. This research has provided significant new insight into many mechanisms used by the coronavirus replication-transcription complex (RTC). The RTC directs and coordinates processes in order to replicate and transcribe the coronavirus genome, a single-stranded, positive-sense RNA of outstanding length (~27–32 kilobases). Here, we review the up-to-date knowledge on SARS-CoV replicative enzymes encoded in the ORF1b, *i.e.*, the main RNA-dependent RNA polymerase (nsp12), the helicase/triphosphatase (nsp13), two unusual ribonucleases (nsp14, nsp15) and RNA-cap methyltransferases (nsp14, nsp16). We also review how these enzymes co-operate with other viral co-factors (nsp7, nsp8, and nsp10) to regulate their activity. These last ten years of research on SARS-CoV have considerably contributed to unravel structural and functional details of one of the most fascinating replication/transcription machineries of the RNA virus world. This paper forms part of a series of invited articles in *Antiviral Research* on "From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses".

Contents

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0166-3542/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.antiviral.2013.11.006

1. Introduction

Coronaviruses are ubiquitous viruses infecting a large variety of hosts. In humans, they were mainly responsible for mild respiratory diseases until 2002/2003 when a novel coronavirus was identified as the cause of a severe acute respiratory syndrome (SARS). The SARS-coronavirus (SARS-CoV) infected ~8400 persons in the world, and differs from other human coronaviruses by its case/fatality rate (~10%) (Cheng et al., 2013). Its discovery greatly renewed interest in coronavirus research (Hilgenfeld and Peiris, 2013). SARS-CoV may have evolved from coronaviruses infecting bats (SARS-like CoVs) (Li et al., 2005). The virus became infectious in humans probably through an intermediate passage into civets and mutations at least in the structural protein S recognized by the host receptor (Wang and Eaton, 2007; Li, 2013).

Since April, 2012, a novel human pathogenic coronavirus of zoonotic origin, the Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged, causing severe pneumonia as well as kidney failure in some cases (de Groot et al., 2013; Zaki et al., 2012). As of November, 2013, more than 130 MERS-CoV-infected people have been identified, with a case fatality rate of ~50%. All are related in some way to travel to the Arabian Peninsula. MERS-CoV is a close relative of bat coronaviruses HKU4 and HKU5 (Drexler et al., 2013). The ongoing outbreak confirms that coronaviruses constitute a threat to public health at world level.

The *Coronavirinae* subfamily contains 4 genera (alpha, beta, gamma and deltacoronaviruses) and, together with the *Torovirinae* subfamily, form the *Coronaviridae* family. *Nidovirales* order is formed by *Coronaviridae*, *Roniviridae* (also named long genome nidoviruses), and the *Arteriviridae* (short genome nidoviruses) families. Recently, a new family of viruses infecting insects was created, in this viral order. It was named *Mesoniviridae* in reference to their middle-sized genomes between long and short genome nidoviruses (Nga et al., 2011).

Nidoviruses are positive-sense, single-stranded RNA viruses ((+) ssRNA), whose distinctive feature is to produce a set of subgenomic mRNAs (sg mRNAs) coding for structural and accessory proteins. Coronaviruses have a long genome that carries a cap structure at 5′ end and a polyA tail at the 3′ end. Genomic RNA can be directly translated into viral proteins after cell infection. The main characteristic that makes coronaviruses (and long-genome nidoviruses in general) of outstanding interest is the unusual length of their RNA genomes (27–32 kb). It is two to three time larger than that of other RNA viruses, but is thought to be associated with a higher genetic stability (Belshaw et al., 2008; Jenkins et al., 2002).

Nonstructural proteins forming the replication/transcription complex (RTC) are coded by the genomic mRNA, where two overlapping ORFs are found. The ORF1a encodes for polyprotein 1a (pp1a) cleaved by viral proteases into 11 nonstructural protein (nsp1–nsp11). The second (ORF1b) produces 5 additional nonstructural proteins (nsp12–16) upon processing by the viral proteases contained into polyprotein 1ab (pp1ab) (Fig. 1). ORF1ab is translated upon a −1 ribosomal frameshift inside ORF1a, implying that nsp12–nsp16 are produced at significantly lower levels than ORF1a-encoded products. The nsp12–nsp16 proteins and their known co-factors/partners are the subject of this review. In an attempt to document their potential as drug targets, we report the present state of the art on the enzymatic machinery formed by the SARS-CoV ORF1b-encoded nonstructural proteins.

2. Nsp12 and nsp13, the presumed replication/transcription complex catalytic core

2.1. The RNA-dependent RNA polymerase nsp12

Nsp12 (102 kDa) is the most conserved protein in coronaviruses, and thus a central enzyme in the viral replication/transcription complex. It is an RNA-dependent RNA polymerase (RdRp) presenting all conserved motifs of canonical RdRps. The polymerase active site (Ser-Asp-Asp within motif C) is conserved in all nidoviruses. More intriguing is the presence of motif G (Gorbalenya et al., 2002), which is a signature of RdRps that initiate RNA synthesis in a primer-dependent manner (te Velthuis et al., 2010). Nsp12 of all coronaviruses also carries a ~42 kDa amino-terminal extension whose function is yet unknown. Even if bioinformatics studies did not reveal any specific signature sequence, this extension is necessary for RdRp activity (Cheng et al., 2005).

Although it plays a central role in viral replication, nsp12 RdRp is poorly characterized to date, certainly due to the difficulty to express and purify satisfactory amounts of protein. SARS-CoV nsp12 fused to a GST-tag exhibits weak polymerase activity *in vitro* using poly (rA)/oligo dT_{12-18} as a template (Cheng et al., 2005). Neither metal requirements nor the ability to initiate RNA synthesis with or without an RNA primer are clearly established, as reported by several authors which used full-length histidine-tagged nsp12 (Ahn et al., 2012; Cheng et al., 2005; te Velthuis et al., 2010). In any case, the *in vitro* nsp12 polymerase activity was weak, contrasting with the replication of ~30 kb-long coronavirus RNA genomes.

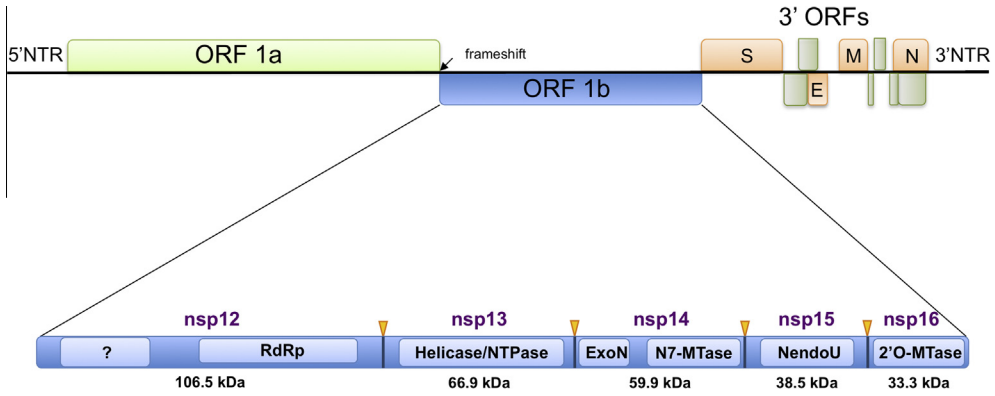

Fig. 1. Coronavirus genome organization and expression. Coronavirus genome is depicted and divided into five main segments: 5′NTR (nontranslated region), ORF1a (light green), ORF1b (blue), 3′ORFs, corresponding to all open reading frames coding for structural (orange) and accessory proteins (dark green), and 3′NTR. The ORF1b is zoomed and the derived nonstructural proteins are shown. The nsp5 protease cleavage sites (yellow arrow), the domains harbouring enzymatic activities (soft blue) and the size of each nsp are also depicted. ?: predicted domain of unknown function; RdRp, RNA-dependent RNA polymerase; NTPase, nucleoside triphosphatase, also capable of hydrolysizing 5′-triphosphate-RNA to 5′-diphosphate-RNA); ExoN, 3′ to 5′ exonuclease; N7-MTase, guanine-N7-methyltransferase; NendoU, endoribonuclease; 2′-*O*-MTase, 2′-*O*-methyltransferase. Protein size is indicated below each nsp.