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SARS coronavirus replicase proteins in pathogenesis

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Abstract

Much progress has been made in understanding the role of structural and accessory proteins in the pathogenesis of severe acute respiratory syndrome coronavirus (SARS-CoV) infections. The SARS epidemic also brought new attention to the proteins translated from ORF1a and ORF1b of the input genome RNA, also known as the replicase/transcriptase gene. Evidence for change within the ORF1ab coding sequence during the SARS epidemic, as well as evidence from studies with other coronaviruses, indicates that it is likely that the ORF1ab proteins play roles in virus pathogenesis distinct from or in addition to functions directly involved in viral replication. Recent reverse genetic studies have confirmed that proteins of ORF1ab may be involved in cellular signaling and modification of cellular gene expression, as well as virulence by mechanisms yet to be determined. Thus, the evolution of the ORF1ab proteins may be determined as much by issues of host range and virulence as they are by specific requirements for intracellular replication.
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1. Introduction

The coronaviruses express the largest and most complex polyproteins of any RNA viruses. The polyproteins are translated from the genome RNA open reading frames 1a and 1b, and are known as replicase, replicase/transcriptase, or polymerase polyproteins, in recognition of the predicted and demonstrated roles in viral RNA synthesis. However, the appellation of “replicase/transcriptase”, while appropriate, is an incomplete description of all probable ORF1ab protein functions. It has been predicted, as well as demonstrated in some cases, that the mature proteins in the polyprotein may serve roles distinct from or in addition to roles in viral RNA synthesis. More specifically, it is becoming clear that proteins or protein domains encoded in ORF1ab may serve specific roles in virulence, virus–cell interactions and/or alterations of virus–host response.

Two events in the history of coronavirus biology have dramatically accelerated the studies and discoveries in protein functions: the SARS epidemic and the development of reverse genetic strategies for the study of coronavirus replication. The rapid identification and sequencing of SARS-CoV isolates led to bioinformatics analyses highlighting both conserved and divergent regions of the replicase genes, particularly in relationship with known group 2 coronaviruses such as mouse hepatitis virus (MHV). In addition, the detailed analysis of animal and human isolates of SARS-CoV during the course of the epidemic revealed evidence of adaptive mutations in the replicase to an extent that matched or exceeded that in the structural proteins.

Concurrently, the rapid establishment of a reverse genetic system for SARS-CoV, as well as the development of reverse genetic systems for other group 2 coronaviruses (Yount et al., 2003, 2002), allowed direct studies of conserved and divergent domains of the replicase in replication. Subsequently, it has become clear that the replicase gene proteins will likely demonstrate multiple functions, many of them novel, in viral pathogenesis. This review will (1) summarize the organization, expression, processing, and putative replication functions of the nonstructural proteins (nsps 1–16) of SARS-CoV; (2) describe studies of ORF1b nsps demonstrating interactions with host cells or host immune response; (3) describe studies of SARS-CoV

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nsps that support functions for the proteins in pathogenesis and adaptation.

1.1. Coronavirus life cycle

Attachment of the virion to the cell surface via a receptor constitutes the first step in the coronavirus life cycle and is perhaps the most important tropism determinant, since coronavirus genomes can replicate in many different cell types when transfected. Receptors vary widely across coronaviruses: for SARS-CoV, human angiotensin converting enzyme 2 (hACE2) can serve as a receptor (Li et al., 2003). Following attachment, the genome enters the cell via a cathepsin L-dependent mechanism (Huang et al., 2006; Simmons et al., 2005). Once inside the cell cytoplasm, the genome serves as an mRNA for the first open reading frame (ORF1), from which the viral replication proteins are translated and processed. These proteins induce and assemble on double-membrane vesicles and become the sites for viral RNA synthesis, and thus comprise the replication complexes. These replication complexes are likely responsible for all viral RNA synthetic activities in the viral life cycle.

Viral RNA synthesis involves two stages: in one stage, input genome RNA is replicated through transcription of a minus-strand template. This is referred to as genome replication. In the other stage, subgenomic mRNAs are transcribed and subsequently used for translation of structural and accessory proteins from downstream ORFs (ORFs 2–9 for SARS-CoV). This stage is referred to as subgenomic RNA transcription and, as it leads to translation of structural proteins, is essential for virion formation and the completion of a productive viral replication cycle (Sawicki and Sawicki, 2005; Sawicki et al., 2007).

All coronaviruses possess four well-characterized structural proteins: S (spike), E (envelope), M (membrane protein), and N (nucleocapsid). These proteins are translated from subgenomic RNAs 2, 4, 5, and 9a, respectively, in SARS-CoV. MHV studies have shown that S, E, and M undergo modification in the Golgi prior to virus assembly (Bost et al., 2000, 2001; Klumperman et al., 1994; Krijnse-Locker et al., 1994). The viral N protein has been shown in MHV to colocalize with viral replicase proteins and at sites of virus assembly at late times post-infection (Bost et al., 2000; Denison et al., 1999; Sims et al., 2000; van der Meer et al., 1999). Viral assembly is precipitated by E and M, which induce a curvature of the budding membrane (Raamsman et al., 2000). Interactions between M, N, and viral RNA lead to S recruitment and RNA packaging into these structures, which form the budding virion (Narayanan et al., 2000; Opstelten et al., 1993, 1995). The viral E protein then aids in the final pinching off of the virion (Fischer et al., 1998). Coronavirus virions are shuttled to the cell surface in large exocytic vesicles, and the virions are released from the cell in a process that does not require cell lysis.

1.2. Coronavirus genome organization and ORF1ab expression and processing

The coronavirus input genome RNA is positive-stranded and functions as an mRNA, from which gene 1 is translated by host–cell ribosomes from two overlapping open reading frames, ORF1a and 1b (Fig. 1). Translation of ORF1a results in a theoretical polyprotein of ∼500 kDa, while translation of ORF1ab results an ∼800 kDa polyprotein (Baranov et al., 2005; Dos Ramos et al., 2004). The ORF 1a and 1ab polyproteins are not detected during infection, since they are most likely processed co- and post-translationally into intermediate and mature proteins by proteinase activities in the nascent polyproteins (Harcourt et al., 2004). The number of proteinases varies by species. All coronaviruses encode a cysteine proteinase in nsp 5 that is referred to as the 3C-like proteinase (3CLpro), or more recently as Mpro (Anand et al., 2003; Tan et al., 2005). The nsp 5 proteinase is responsible for processing the C-terminus of nsp 4 through nsp 16 for all coronaviruses (Anand et al., 2003; Bost et al., 2000; Denison et al., 1998, 1999; Lu et al., 1998; Tan et al., 2005). In contrast, nsps 1–3 are cleaved by either one or two papain-like proteinase activities (PLP) within nsp 3 (Anand et al., 2003; Bonilla et al., 1995, 1997; Dong and Baker, 1994; Harcourt et al., 2004; Kanjanahaluethai and Baker, 2001; Snijder et al., 2003; Tan et al., 2005; Teng et al., 1999; Ziebuhr et al., 2000). Group 1 (HCoV-229E) and group 2a (MHV) coronaviruses each encode PLP1 and PLP2, while group 2b (SARS-CoV) and group 3 (Avian infectious bronchitis virus—IBV) coronaviruses each encode only one PLP in the position of PLP2. IBV possesses an inactive remnant of PLP1, while SARS-CoV PLP1 appears to have been completely lost (Snijder et al., 2003) (Fig. 2). Regardless of the number of PLPs present, nsps 1–3 are processed by papain-like proteinase(s) for all coronaviruses. These distinct processing networks may serve to regulate the liberation of intermediate and mature protein species that perform conserved functions across coronavirus groups.

1.3. Evolution and adaptation

Prior to the SARS epidemic, studies of coronavirus evolution were limited to established laboratory virus strains, and focused exposure to chemical mutagens and subsequent analysis of adapted strains. The most elegant work came from studies of gradual adaptation of MHV to Syrian baby hamster kidney (BHK) cells. The resulting passaged virus grew efficiently in murine, hamster, human, and primate cells, clearly demonstrating the ability of coronaviruses to cross species barriers (Baric et al., 1997). This report was remarkably prescient, coming less than 10 years before the natural experiment of SARS-CoV transmission from animals into humans. The SARS epidemic created a unique opportunity to study the changes in an animal virus as it encountered a previously unexposed human population, both geographically and temporally. A study of 63 isolates of SARS-CoV, including animal and human strains was performed by the Chinese SARS consortium to determine rates of mutation along the course of the epidemic (Chinese, 2004). Complete sequencing of the isolates allowed detailed direct and statistical analysis of adaptation in SARS-CoV, and it led to conclusions about the most variable regions of the genome during the course of the epidemic. As expected, the S coding region demonstrated the most mutations resulting in non-synonymous amino acid

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