Biochemical Aspects of Coronavirus Replication and Virus-Host Interaction

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nidovirus, transcription, translation

Abstract

Infection by different coronaviruses (CoVs) causes alterations in the transcriptional and translational patterns, cell cycle, cytoskeleton, and apoptosis pathways of the host cells. In addition, CoV infection may cause inflammation, alter immune and stress responses, and modify the coagulation pathways. The balance between the up- and downregulated genes could explain the pathogenesis caused by these viruses. We review specific aspects of CoV-host interactions. CoV genome replication takes place in the cytoplasm in a membrane-protected microenvironment and may control the cell machinery by locating some of their proteins in the host cell nucleus. CoVs initiate translation by cap-dependent and cap-independent mechanisms. CoV transcription involves a discontinuous RNA synthesis (template switching) during the extension of a negative copy of the subgenomic mRNAs. The requirement for base-pairing during transcription has been formally demonstrated in arteriviruses and CoVs. CoV N proteins have RNA chaperone activity that may help initiate template switching. Both viral and cellular proteins are required for replication and transcription, and the role of selected proteins is addressed.

Contents

MHV: mouse hepatitis virus

BCoV: bovine coronavirus

eIF: elongation initiation factor

INTRODUCTION

Extensive morphological and biochemical changes occur in coronavirus (CoV)-infected cells. Infection by different CoVs causes alterations in the transcription and translation patterns, cell cycle, cytoskeleton, and apoptosis pathways of the host cell. In addition, CoV infection may cause inflammation, alter immune and stress responses, and modify the coagulation pathways. A few selected changes are reviewed in this chapter, with a special focus on biochemical interactions during CoV replication and transcription.

NOVEL SIGNAL PATHWAY ALTERATIONS IN CoV INFECTION

Progress in the study of the effect of CoVs in the host has been made mostly with the mouse hepatitis virus (MHV) system and, more recently, with the severe and acute respiratory syndrome (SARS)-CoV. MHV and SARS-CoV infections induce mitogen-activated kinases (MAPKs), especially p38 MAPK (5, 82). In addition, activation of AP-1 and weak induction of Akt signaling pathways were found after SARS-CoV infection (80, 81). SARS-CoV N protein has been involved in the induction of these signaling pathways (45, 117). Special attention has been dedicated to the study of the cell signaling pathways altered after CoV infection and to the relationship between these alterations and the effects on the host leading to the pathology of disease.

Effect of CoV Infection on Host Cell Transcription and Translation

CoV infection affects both host cell transcription and translation. Interestingly, CoV gives rise to mRNAs that are structurally similar to those of their eukaryotic hosts, and this allows CoVs to parasitize the host machinery to translate the viral mRNA. The compartmentalization of CoV synthesis in virus factories could shield virus replication against the cell degradation induced by the virus. Alternatively, specific factors that interfere with host translation or transcription or other factors that enhance viral-specific synthesis must be responsible for the increase of virus-encoded molecules.

Infection by MHV leads to inhibition, but not a complete shutoff, of host protein translation (4, 120) that is accompanied by an increase of MHV protein synthesis (5, 62). The mechanism of selective CoV-specific protein synthesis, which occurs concomitantly with host protein inhibition in infected cells, is poorly characterized. Chimeric mRNAs containing bovine coronavirus (BCoV) or MHV leader sequences are translated more efficiently in CoV-infected cells (104, 120). As N protein binds to this sequence, it has been suggested that CoV mRNAs bind to N protein, forming a complex that may act as a strong translation initiation signal (121). Increased phosphorylation of elongation initiation factor (eIF)4E in CoV-infected cells (5, 82) has also been described

as a result of the activation of p38 MAPK. Phosphorylated eIF4E has a higher affinity for cap structures and eIF4G, and as a result, this usually leads to enhanced translation rates (41). Another possible mechanism of host cell protein synthesis shutoff in MHV-infected cells is the specific cleavage of 28S rRNA, an integral component of the 60S ribosome (4). Finally, specific host mRNAs are degraded in MHV-infected cells (62), and similar results were observed after infection with SARS-CoV, with downregulated genes involved in the host translational mechanism (68).

Researchers have monitored on a broad scale the effect of pathogens on host cell gene expression programs by using DNA microarrays, which clarify the effect of virus infection on cell transcriptosome. Alteration of the transcription pattern after CoV infection has been reported mainly for MHV and SARS-CoV infection. A comprehensive study (123) shows a higher perturbation of cellular gene transcription after SARS-CoV infection than after infection by human coronavirus (HCoV)-229E. In addition to the downregulation of genes involved in host cell translation and maintenance of cytoskeletal network, the upregulation of genes related to stress response, proapoptosis, proinflammation, and procoagulation were observed (68, 86, 123). The balance between the genes up- and downregulated after CoV infection could explain the pathogenesis and the differences in the severity of illness caused by these viruses (see below).

Effect on Cell Cycle and Apoptosis

In general, viruses use host cell cycle regulation for their own replication advantage. Each virus promotes a different pattern of interference with the host cell cycle. MHV, infectious bronchitis virus (IBV), transmissible gastroenteritis virus (TGEV), and SARS-CoV infection lead to the accumulation of infected cells in the G0/G1 phase (20, 21, 143). Transcriptional profiling after SARS-CoV and HCoV-229E infection shows alteration in genes involved in cell cycle regulation, including upregulation of genes that can mediate growth arrest at the G1 phase (123). An accumulation of hypophosphorylated retinoblastoma protein has been involved in the arrest of the cell cycle progression in the G0/G1 phase after MHV infection. Four viral proteins, nonstructural protein 1 (nsp1) from MHV (21), SARS-CoV 3b and 7a proteins (143, 144), and N protein (23, 139), have been proposed as responsible for cell cycle arrest.

Many viruses encode proteins that can modulate apoptosis (7, 9, 97). Induction of apoptosis in infected cells can contribute directly to viral pathogenesis, whereas inhibition of apoptosis can prevent premature death of the infected cells, allowing the virus to replicate to a high titer or allowing the establishment of a persistent infection. Infection by CoVs, such as TGEV, MHV, and SARS-CoV, induces apoptosis in certain cells (3, 16, 37, 81, 136). Nevertheless, stimulation of both apoptotic and antiapoptotic molecules has been described (68, 80, 81, 86, 123). The delicate counterbalance of proapoptotic and antiapoptotic molecules during CoV infection should ensure cell survival during the early phase of infection to allow rapid multiplication of progeny virus before cell lysis occurs. Apoptosis induced by CoV infection is tissue specific, and this observation may explain the pathology of the infection and the effects on the host organism. For instance, the data obtained show that SARS-CoV infects epithelial cells of the enteric tract and induces an antiapoptotic response that may be important to inhibit or delay destruction of infected enterocytes, probably leading to an extension of virus production and shedding. These findings are consistent with clinical observations demonstrating a relatively normal endoscopic and microscopic appearance of the intestine in patients with SARS (69). On the other hand, CoV infection induces apoptosis in other tissues. SARS-CoV causes lymphopenia owing to the depletion of T lymphocytes by apoptosis

IBV: infectious bronchitis virus

TGEV: transmissible gastroenteritis virus

nsp: nonstructural protein

(140) or liver impairment owing to hepatocyte apoptosis (19).

Overexpression of several CoV proteins, such as MHV E protein, induces apoptosis (3). After SARS-CoV infection, T-cells undergo apoptosis likely owing to the E protein interacting directly with the antiapoptotic factor Bcl-xL (141). Protein 7a from SARS-CoV also induces apoptosis by a caspase-dependent pathway in several cell lines, including those derived from lung, kidney, and liver (122).

Effect of CoV Infection on Host Systems

CoV infection affects several host systems. Functional grouping of altered genes after CoV infection showed that more genes involved in inflammation, coagulation, and stress are upregulated in SARS-CoV than in HCoV-229E infection (123). Of the four CoVs known to infect human (HCoV-229E, HCoV-OC43, HCoV-NL63, and SARS-CoV), the first three are generally associated with mild upper respiratory tract infections such as the common cold in the immunocompetent host. In contrast, SARS-CoV causes respiratory failure in more than 60% of affected persons, with a mortality rate of 10%. The observed effect on host gene expression (123) may help to explain the pathologies caused by these CoVs.

CoV infection leads to the induction of inflammation. Several studies have shown that the proinflammatory response is increased after SARS-CoV infection (68, 86, 123, 138). For instance, the upregulation of IL-8 expression (26, 123), consistent with the increase of this chemokine in plasma of infected patients (138), may be of pathogenic importance, as the level of IL-8 has been positively correlated with disease severity in pulmonary infection by respiratory syncytial virus. In fact, a positive correlation was found between IL-8 levels in blood and alveolar spaces and the number of polymorphonuclear neutrophil in bronchoalveolar lavage of patients with pneumonia and acute respiratory distress syndrome (138). Other chemokines upregulated after SARS-CoV infection may mediate the chemotaxis of lymphocytes and neutrophils, contributing to the significant increase in neutrophil firing in the lung that may account for the localized nature of the response in SARS patients (86). Similar results were obtained with MHV, in which the neurovirulence of the virus correlated with the upregulation of proinflammatory cytokines. This is associated with the recruitment of lymphocytes and macrophages at the site of the infection, which may lead to encephalitis (72).

In contrast, despite the upregulation of IL-8 in intestinal epithelial cells, biopsy specimens taken from the colon and terminal ileum of patients with SARS failed to demonstrate any inflammatory infiltrates (69). Neutrophil infiltration in the intestine of SARS patients may be limited despite neutrophilia due to changes of cytokine and chemokine levels in the intestinal environment. It has been observed that SARS-CoV infection inhibited production of IL-18 that is constitutively expressed in intestinal epithelial cells (26). Suppression of IL-18 levels reduced neutrophil accumulation in liver and lungs (31). The absence of T lymphocyte infiltration of the intestine in SARS patients may be a consequence of the profound decline of both $CD4^+$ and $CD8^+$ lymphocytes in the blood (30), possibly resulting from lymphocyte apoptosis. Although macrophage counts were increased in lungs, macrophage infiltration was absent from the gut of SARS patients. In agreement with this finding, in Caco-2 cells, SARS-CoV downregulates migration inhibition factor (MIF), which is a major factor produced by intestinal cells in response to microbial infection, regulation of macrophage emigration, inflammation, and cell metabolism (79).

Innate immunity probably is essential to control CoV infections in vivo. Cytokine profiling after SARS-CoV infection suggested an early activation of the innate immunity pathway (86). The same observations were made after MHV infection (95). IFN-γ is

critical in resolving MHV infection (91). IFNs have significant anti-SARS-CoV effects (25, 43, 78), and only immunocompromised transgenic mice with impaired IFN responses are infected with HCoV-229E (65) or SARS-CoV (51). CoVs have developed strategies to escape IFN responses. In fact, low levels of IFN have been found in SARS patients (86) probably owing to the interference of interferon regulatory factor 3 (IRF-3) activation (114).

Blood coagulation genes are also affected by SARS-CoV infection. The upregulation of several genes has been involved in the activation of this pathway (86, 123). The result leads to a procoagulation profile that mimics the pathological observations. In fact, at autopsy, many SARS patients have unusually disseminated small vessel thromboses in the lungs (40). Vascular damage in various tissues has also been reported (33). Activation of the procoagulation pathway in MHV infection resulting in confluent hepatocellular necrosis has also been described (32).

INFLUENCE OF VIRAL AND CELLULAR PROTEINS ON CoV REPLICATION

Cell Compartment Distribution of CoV Synthesis

CoV replication employs complex mechanisms that involve viral and cellular proteins. Similar to other positive-strand RNA viruses, CoV genome replication takes place in the cytoplasm in a membrane-protected microenvironment that contains all the protein functions required for viral RNA synthesis. Electron microscopy studies of MHV-infected cells have shown that these structures consist of double-membrane vesicles (DMVs) (42) that are generated possibly by using cellular autophagy-related processes (94). The cellular origin of MHV DMVs is under debate. While some data support that DMVs are derived from late endosomal membranes (131), other data suggest that DMVs have their origin in the endoplasmic reticulum (94, 108). Structures similar to DMVs have been found in SARS-CoV (110) and in the equine arteritis virus (EAV) infection (93), suggesting that CoV and arterivirus have a common replication strategy. The viral replication complex associated with these membranes apparently includes cellular proteins and up to 16 nsps, most of which are derived from the proteolytic processing of the replicase polyproteins pp1a and pp1ab (110).

Although CoV replication essentially takes place within the cytoplasm, CoVs may control the cell machinery by locating some of their proteins in the host cell nucleus. To date, two CoV proteins (N and 3b) (50, 139, 145) and another two from arteriviruses (N and nsp1) (98, 128) have been identified in the nucleolus of infected cells. The nucleolus has been implicated in many aspects of cell biology including functions such as ribosomal RNA synthesis and ribosome biogenesis, gene silencing, senescence, and cell cycle regulation (49). Viruses interact with nucleolar antigens, leading to their redistribution during infection (49). N protein from CoV genera α (TGEV), β (MHV), and γ (IBV) (50, 139) and also N protein from the arteriviruses porcine respiratory and reproductive syndrome virus (PRRSV) and EAV (98, 128) localize within the nucleolus. This may be a common feature among all nidovirus N proteins that influence host cell proliferation (139). However, the association of N protein with the nucleus may be cell dependent, as TGEV N protein has been identified in the nucleus of LLC-PK1 and Vero cells (139), but not in swine testis (ST) cells (17).

CoV Translation

Genomes of positive-strand RNA viruses, such as members of the *Coronaviridae*, *Flaviviridae*, and *Togaviridae* families, contain an m7GpppN-cap structure at the 5′ end of the mRNA and are presumed to initiate translation in a cap-dependent manner. Coronavirus mRNAs have a polycistronic

DMV: double-membrane vesicle

EAV: equine arteritis virus

PRRSV: porcine respiratory and reproductive syndrome virus

configuration, except for the mRNA encoded by the most 3′ end of the genome, but usually only the 5′-terminal open reading frame (ORF) on each mRNA is abundantly translated by a cap-dependent mechanism (64). Nevertheless, there is evidence indicating that some of the coronavirus mRNAs are bicistronic (i.e., in SARS-CoV five mRNAs are functionally bicistronic) (125) or even tricistronic (77). These RNAs are translated by a variety of cap-independent mechanisms. (*a*) A common coronavirus mechanism of –1 ribosomal frameshifting generates two polypeptides of differing abundance from a mRNA (6, 12, 13, 47). This mechanism is also used by members of other virus families (*Retroviridae*, *Astroviridae*, and *Flaviviridae*) (34). (*b*) Internal ribosome entry onto IBV mRNA 3 (76, 77) and MHV mRNA 5 (67, 126) are mechanisms used to synthesize a second protein of lower abundance from a single transcript. (*c*) Scanning mechanisms synthesize less abundant proteins such as the I protein encoded by an ORF internal to the N gene that is expressed in a +1 frameshift in relation to the N ORF (39, 103, 105). (*d*) Downstream entry of ribosomes is an alternative translation mechanism described to express ORF 3b of some TGEV strains (87). By way of this mechanism the internal entry of ribosomes does not depend on an immediate upstream internal ribosomal entry structure (IRES). In this case, the ribosomes enter close to the ORF 3b start site by a mechanism that resembles shunting.

Translation can be regulated by viral or cellular factors acting in *trans* or by *cis*-acting elements within the 5′ UTR (104). It has been proposed that preferential translation of viral mRNA in MHV-infected cells is stimulated in part by the interaction of N protein with the tandemly repeated -UCYAA- sequence of the leader (120, 121), as described above. Protein synthesis is also dependent upon cellular translation factors such as the initiation factor eIF4F (35). CoV infection affects the phosphorylation of eIF4E, which might influence the synthesis of viral proteins (see Effect of CoV Infection on Host Cell Transcription and Translation, above).

Cellular mRNAs contain the cap structure at the 5′ end and a poly(A) tail at the 3′ end. Interaction between the 5′ and 3′ ends of these mRNAs increases translation efficiency (99). 5′-3′ linkage is mediated by the interaction of the 5′-end-bound eIF4G and the 3′-end-bound poly(A) binding protein (PABP) (88). CoVs also encode mRNAs with 5′ containing cap structures and 3′ poly(A) tails; therefore, they may use a communication between the 5′ and 3′ mRNA ends for a more efficient translation. Although PABP binding to CoV genome 3′-poly(A) affects CoV replication, no direct effect in translation has been reported (113).

CoV Genome Replication

CoV replication and transcription possibly require recognition of RNA genome 5′ and 3′ ends by viral and cellular proteins. Like all other positive-strand RNA viruses, CoV genome replication is mediated through the synthesis of a negative-strand RNA, which in turn is the template for the synthesis of progeny virus genomes. Mapping studies with MHV defective-interfering (DI) RNAs have indicated that 470 nt from the 5′ end and 436 nt from the 3′ end are required from DI RNA replication (60, 73). Both ends of the genome are necessary for positive-strand synthesis, whereas only the last 55 nt from the 3′ end and the poly(A) tail are required for the synthesis of negative-strand (74). Because during the synthesis of the positive-strand RNA the 3′ end of the genome is the last region reached by the viral polymerase, the replication signal at the 3′ end of the genome may interact with signals at the 5′ end to exert its effect on RNA synthesis. On the basis of this information, it has been postulated that the 5′ and 3′ ends of the genome interact during RNA replication (54, 63). Similar observations have been made for the regulation of MHV subgenomic (sg) mRNA transcription (75).

Although there is no apparent sequence complementarity between the 5′ and 3′ ends of the CoV genome, a direct interaction between both ends could be possible, as predicted for MHV and TGEV RNA genomes in protein-free media using computer programs (52, 106). However, there is experimental evidence for MHV (54) and TGEV (C. Galan, F. Almazan & L. Enjuanes, unpublished information) supporting the idea that the cross-talk between the 5′ and 3′ ends is mediated by proteins through RNA-protein and protein-protein interactions. Heterogeneous nuclear ribonucleoprotein (hnRNP) A1 has been identified as a major protein species binding to nt 90–170 (strongly) and to nt 260–350 (weakly) from the 3′ end of MHV RNA. These binding sites are complementary to the sites on the negative-strand RNA that bind another cellular protein, polypyrimidine tract-binding protein (PTB). Furthermore, hnRNP A1 and PTB also bind to the complementary strands at the 5′ end of MHV RNA and together mediate in vitro the formation of a ribonucleoprotein (RNP) complex involving the 5′ and 3′ ends of MHV RNA (54, 70, 71, 146). The functional relevance of hnRNP A1 in MHV replication has been established by experiments showing that overexpression of hnRNP A1 facilitates MHV replication, whereas dominant-negative mutants of hnRNP A1 reduce replication (107). Furthermore, mutations of the PTB and hnRNP A1 binding regions also impaired transcription of subgenomic RNA (53), suggesting a functional role in replication and transcription for the interaction between PTB and hnRNP A1.

CoV N protein forms a RNP complex with genomic RNA. In addition to its role in virus assembly (38, 96), N protein likely has a prominent role in CoV replication and transcription, as it influences many viral and cellular processes. In fact, at early times after infection, CoV N protein colocalizes with the replication complex in DMVs, the site of CoV RNA synthesis (11). Furthermore, the presence of N protein enhances the rescue of infectious virus from cDNA clones generated from different CoVs, such as IBV (18), HCoV-229E (101), and TGEV (142), using RNA in vitro transcripts.

The requirement of N protein for virus replication and transcription has been under debate. Certain researchers suggest that N protein plays a role in CoV RNA synthesis (8, 27, 61, 66, 85, 115), whereas others using either CoV (124) or arterivirus systems (84) claim that N protein is not essential. Using TGEV- and HCoV-229E-derived replicons, two groups have shown that only background levels of CoV RNA synthesis are produced in the absence of N protein (1, 101). In these systems, the presence of N protein either in *cis* or in *trans* is required for efficient CoV RNA synthesis (1). A quantitative analysis of TGEV replicon activity showed an increase of more than 100-fold when N protein was provided in *cis*, and an increase of more than 1000-fold when N protein was provided in *trans* (1). Whether the effect of N protein is at the level of replication, transcription, or both remains to be determined.

Despite the variable size of N protein from different CoVs, it presents a conserved pattern of secondary structural elements with a three-domain organization (90) (**Figure 1**). N protein domains I and III are the most unstructured and divergent between CoVs, while domain II is more conserved and includes highly conserved alpha helices and a serine-rich domain (14, 17). Several active N protein domains have been mapped, such as a RNA binding domain (66, 85), the oligomerization domain (44, 118), and the M protein binding domain (46, 57), also involved in N protein oligomerization. N protein activity should be a result of its self-interaction and interaction with other viral and cellular proteins and with virus and host cell nucleic acids. The phosphorylation state has been proposed to regulate N protein functions and to cause conformational changes in N protein structure (22, 83). TGEV (17) and IBV (22) phosphoserine residues have been mapped within

hnRNP A1: heterogeneous nuclear ribonucleoprotein A1

PTB: polypyrimidine tract-binding protein

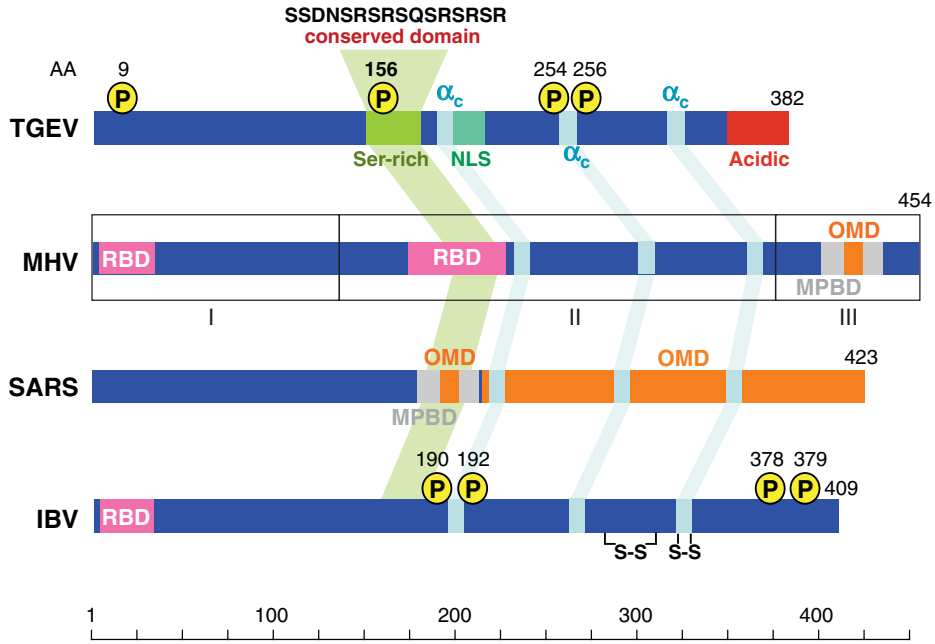

Figure 1

Scheme of N protein from different CoVs. The organization of N protein from four representative CoVs of genera α (TGEV), β (MHV and SARS-CoV), and γ (IBV) is indicated. Conserved predicted structural elements are joined by blue shadowing zones. The three-domain organization proposed for MHV N protein by P. Masters' group (90) is indicated as open boxes over MHV N protein (I, II, and III). P, phosphorylation sites; α_c, protein domains with highly conserved alpha structure; AA, amino acid; NLS, nuclear localization signal; RBD, RNA binding domain; OMD, oligomerization domain; MPBD, M protein binding domain; S-S, disulfide bridge. The scale at the bottom indicates the approximate amino acid number.

the CoV N protein primary and secondary structures. SARS-CoV N protein phosphorylation has also been demonstrated, but the precise amino acid residues involved have not been mapped (116). N protein phosphorylation could affect its secondary structure with the introduction of negative charges into a basic environment, affecting N protein RNA binding activity.

Transcription-regulating sequences (TRSs): highly conserved sequences preceding each *Nidovirales* gene controlling the production of subgenomic mRNAs

CoV Transcription

CoV transcription, and in general transcription in the order *Nidovirales*, is an RNA-dependent RNA synthesis that includes a discontinuous step during the production of sg mRNAs (64, 100). This transcription process ultimately generates a nested set of sg mRNAs that are 5′- and 3′-coterminal with the virus genome. The common 5′-terminal leader sequence, which in TGEV has 93 nt, is fused to the 5′ end of the mRNA coding sequence (body) by a discontinuous transcription mechanism. Sequences preceding each gene represent signals for discontinuous transcription of sg mRNAs. These transcription-regulating sequences (TRSs) include a conserved core sequence (CS), which in TGEV is (5′-CUAAAC-3′) identical for all gene CSs of the mRNA body (CS-B), and the 5′ and 3′ flanking sequences (5′ TRS and 3′ TRS, respectively) that regulate transcription (2, 112). As this CS sequence is also found at the 3′ end of the leader sequence

(CS-L), it could base-pair with the nascent minus strand complementary to each CS-B (cCS-B). In fact, the requirement for base-pairing during transcription has been formally demonstrated in arteriviruses (92, 134) and CoVs (147) by experiments in which base-pairing between CS-L and the complement of CS-B was engineered in infectious genomic cDNAs. The data obtained are compatible with a transcription model that includes three steps (**Figure 2**): (*a*) formation of transcription initiation 5′-3′ complexes in the genomic RNA, (*b*) basepair scanning of the nascent minus-strand RNA by the TRS-L, and (*c*) template switching during synthesis of the negative strand to complete the minus-strand sgRNA. Template switching takes place after copying the CS sequence and can be predicted in silico on the basis of the high base-pairing score between the nascent minus-strand RNA and the TRS-L and minimum free energy (ΔG) of the duplex formation (112, 147). The synthesis of sg mRNAs proceeds only when the CS is located in an optimal sequence

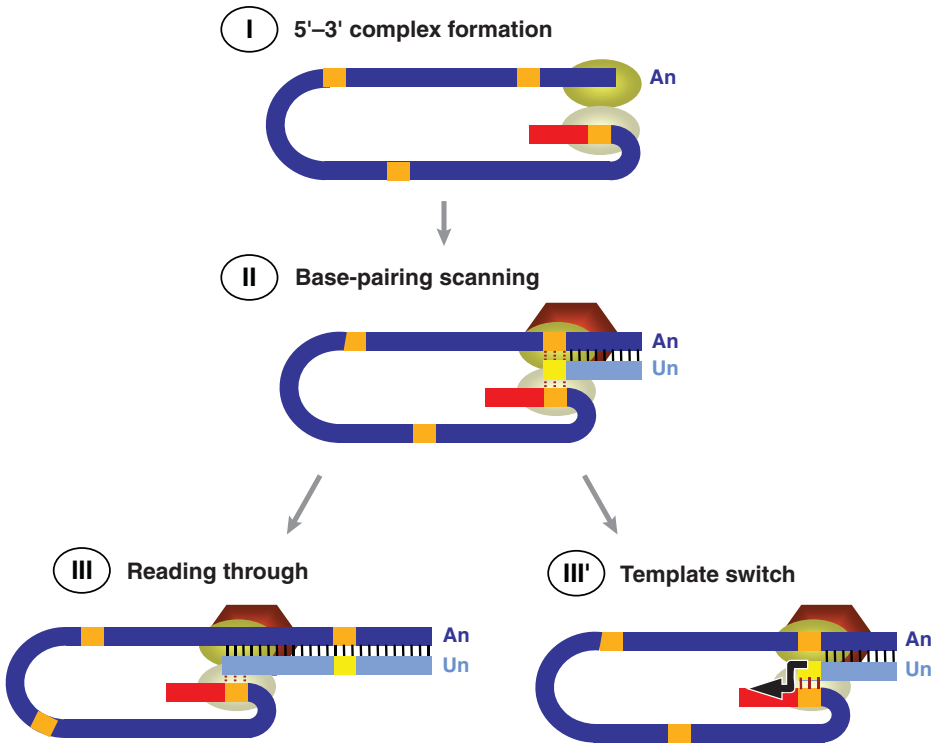

Figure 2

Three-step working model of CoV transcription. (I) 5′-3′ complex formation step. Proteins binding the 5′ and 3′ end TGEV sequences are represented by ellipsoids. Leader sequence is indicated with a red bar, CS sequences are indicated with an orange or yellow bar. An, poly(A) tail. (II) Basepair scanning step. Minus-strand RNA is light blue, and positive-strand RNA is dark blue. The transcription complex is represented by the hexagon. Vertical dotted bars represent the base-pairing scanning by the TRS-L sequence in the transcription process. Vertical solid bars indicate complementarity between the genomic RNA (gRNA) and the nascent minus strand. Un, poly(U) tail. (III) The synthesis of the negative strand can continue to make a longer sgRNA (III), or a template switching step can take place (III′) as indicated in the text. The thick arrow indicates the switch in the template made by the transcription complex to complete the synthesis of minus-strand sgRNA.

Hel: helicase

RNA chaperones: proteins that bind RNA with broad specificity and decrease the activation energy required for a transition between two states

context (2, 89, 112, 147). Base-pairing between leader and body beyond the CS is a determinant regulation factor in CoV transcription (112). In TRS mutants with increasing complementarity between TRS-L and cTRS-B, a tendency to reach a plateau in ΔG values was identified. This observation suggests that a more precise definition of the TRS limits, consisting of the central CS and around four nucleotides flanking the 5′ and 3′ ends of the CS (112), might be proposed.

According to the working model of transcription proposed by our laboratory (**Figure 2**), the first step would be the formation of a complex in which the leader TRS interacts with a transcription complex formed by viral and cellular proteins and with the 3′ end of the genome. Candidate proteins potentially forming the transcription complex have been reported by several laboratories. On the viral side, essential proteins in transcription should be the RNA-dependent RNA polymerase (RdRp), the helicase (Hel), and possibly N protein (see above). Other viral replicase proteins probably contribute to the regulation of transcription and are most likely involved in the formation of this complex. MHV RdRp (nsp12) interacts with other replicase-encoded products such as nsp4 (3CLpro), nsp8 (p22), and nsp9 (p12) (15). Biochemical studies have shown that SARS-CoV nsp9 is a single-stranded RNA binding protein (36, 119). In addition, MHV nsp10 has been implicated in minus-strand RNA synthesis (109).

A set of three conserved domains (nsp14, nsp15, and nsp16) has been identified in the C-terminal region of pp1ab by Sacha Gorbalenya bioinformatics analysis. The predicted activities of these domains are 3′-to-5′ exonuclease, uridylate-specific endoribonuclease (NendoU), and *S*-adenosylmethionine-dependent ribose 2′-*O*-methyltransferase (2′-*O*-MT) (111, 135). These domains are conserved in CoVs, toroviruses, and roniviruses, but only the NendoU domain is also conserved in arteriviruses. NendoU may play a role in CoV transcription by specifically cutting double-stranded RNA (transcriptive intermediates) generated during the synthesis of the nascent RNA of negative polarity. NendoU nuclease has a strong preference for cleavage at GU(U) sequences in double-stranded RNA substrates (58). The GU(U) sequence at the 3′ terminus of nascent minus-strand RNAs, which corresponds to conserved AAC nucleotides in the core of the CoV gene TRS elements, might be substrate of this activity. Therefore, NendoU activity might be involved in the transcription of sg mRNAs. Data from our laboratory, in which we analyzed approximately 90 different sgRNAs generated during the mutagenesis of a TGEV CS (147), and from another laboratory (101) support the functional relevance of the AAC sequence in transcription, but further studies are required to provide a direct link to transcription of enzymes such as the uridylate-specific endoribonuclease.

In addition, viral and cell proteins most likely play a role in arterivirus and CoV transcription regulation. EAV nsp1 has been involved in arterivirus transcription (129, 132) and interacts with cellular p100 (127). This interaction might be important for viral sgRNA synthesis, either directly or by recruiting a p100 binding protein to the viral RdRp complex. Both nsp1 and Hel contain zinc binding domains that could mediate RNA-protein interactions (129, 133). In fact, mutagenesis in the nsp1 zinc binding domain affects transcription regulation.

Regulatory regions of CoV genomic RNA interact with at least three proteins: hnRNP A1 (71, 107), PTB (53, 70), and viral N protein (8, 115). These proteins may act as mediators between the leader TRS and the transcription complex at the body TRS. In fact, binding between hnRNPA1 and PTB (54), hnRNP A1 and N protein (137), and PTB and N protein (24) has been documented. These interactions may be involved in the formation of RNP complexes that function in CoV RNA

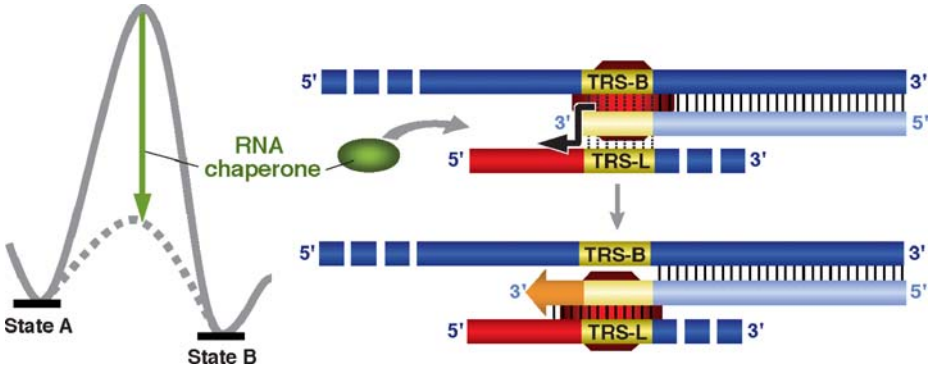

Figure 3

RNA chaperone involvement in template switching during CoV transcription. Left panel: Scheme of RNA chaperone activity. Right panel: Template switching step and elements involved. RNA chaperone is represented by a green ellipsoid.

synthesis. The N protein of MHV binds the leader RNA and is thought to be involved in MHV RNA transcription (8, 115). It has been proposed that the interaction between hnRNP A1 and N proteins brings the leader RNA to the CS sequence of the negative-strand RNA during sg mRNA transcription. In fact, N protein interacts with hnRNP A1 both in vitro and in vivo (137). Furthermore, there are data directly involving hnRNP A1 and PTB in CoV transcription. The extent of hnRNP A1 binding to the complement of TRS sequences correlated to the transcription efficiency from those TRSs (146) in the MHV model. Similarly, binding of PTB to MHV RNA leader or to sequences complementary to 3′-UTR regions was related to transcription efficiency (53, 70). This finding suggests that PTB plays a role in regulating viral RNA synthesis, and thus the interaction of N protein with PTB could modulate transcription (24).

Template switching during transcription represents a displacement of one sequence in the genome acting as a template RNA by another template, the leader sequence. RNA chaperones may help to overcome the energy barrier threshold associated with these types of processes. RNA chaperones are proteins that bind RNA with broad specificity and decrease the activation energy required for a transition between two states (28, 48, 102). Template switching during CoV transcription could be interpreted as a transition between two states. In the first state, a duplex between the nascent minus-strand RNA and the genomic positive-strand RNA used as template is formed; in the second state, the nascent minus-strand RNA is paired with the TRS of the leader sequence (**Figure 3**). RNA chaperones could be involved in template switching by decreasing the energy required for the transition from the first to the second duplex. RNA chaperones are proteins with the highest frequency of long intrinsically disordered regions (59). CoV N proteins also are highly disordered proteins, and we have shown that TGEV and SARS-CoV N proteins have RNA chaperone activity in vitro (148). To date, four viral RNA chaperones have been described and all of them are the nucleocapsid protein from RNA viruses: retrovirus (10, 130), hepatitis delta virus (55, 56), hepatitis C virus (29), and CoVs. The identification of chaperone activity in CoV, and its potential involvement in template switching during CoV transcription, links thermodynamic and molecular aspects of CoV transcription.

SUMMARY POINTS

1. Infection by different CoVs causes alterations in the transcription and translation patterns, cell cycle, cytoskeleton, and apoptosis and coagulation pathways of the host cell. CoV infection may also cause inflammation and affect immune and stress responses. Alteration of gene expression after CoV infection may explain the disease caused by these viruses.
2. CoV infection is initiated by genome translation, a process that takes place by cap-dependent and cap-independent mechanisms.
3. CoV replication involves both viral and cellular proteins and takes place in the cytoplasm in a membrane-protected microenvironment. CoVs may control the cell machinery by locating some of their proteins in the host cell nucleus.
4. CoV replication and transcription possibly require cross-talk between the 5′ and 3′ ends, and CoV transcription involves a discontinuous RNA synthesis during the extension of a negative copy of the sg mRNAs. This process is regulated by TRSs preceding each gene.
5. A model for CoV transcription involving three steps has been proposed: (*a*) formation of 5′-3′ complexes in the genomic RNA, (*b*) base-pairing scanning of the nascent minus-strand RNA by the leader TRS, and (*c*) template switching during synthesis of the negative strand to complete the minus-strand sgRNA.
6. The requirement for base-pairing during transcription has been formally demonstrated in arteriviruses and CoVs. CoV N proteins have RNA chaperone activity that may help initiate template switching.

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