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Virus interactions with the actin cytoskeleton- what we know and do not know about SARS-CoV-2

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Abstract

Actin cytoskeleton and actin-dependent molecular and cellular events are responsible for the organization of the eukaryotic cells and their functions. All viruses, including SARS-CoV-2, depend on host cell organelles and molecular components for invasion and propagation. Thus, it is not surprising that they also interact, at many levels, with actin cytoskeleton of the host. There are many studies on how different viruses reconfigure and manipulate actin cytoskeleton of the host during successive steps of their life cycle. However, we know relatively little about the SARS-CoV-2 virus interactions with actin cytoskeleton. Here we describe the subversion of actin cytoskeleton strategies frequently used by different viruses during entry, assembly, and egress from the host cell. We emphasize what is known and unknown about SARS-CoV-2 in these subjects. This review should encourage investigation of SARS-Cov-2 functions in the uncharted territories and eventually help developing novel antiviral therapies or mitigate the severity of the COVID-19 disease.

Keywords: actin, viruses, SARS-Cov-2, COVID-19, endocytosis, Golgi, ER, cell junctions, syncytium, directional transmission

Introduction

In all eukaryotic cells, the actin cytoskeleton is indispensable for the anchoring and organization of the subcellular compartments and organelles. It regulates most cell functions, including cell-cell adhesion, cell movement, and cell division (1-3). The cortical actin participates in all events related to the expression and presentation of membrane/cell surface molecules, the formation and movement of endocytic/phagocytic vesicles, virus entry, exocytosis, and virus egress.

Additionally, the actin in the cell nucleus regulates DNA repair, chromatin remodeling and condensation status, and gene transcription (4, 5). Two main forms of actin in the eukaryotic cells are the monomeric globular G-actin and polymerized filamentous F-actin. Actin polymerization and G/F actin dynamics are regulated by the small GTPase RhoA and actin interacting proteins (2, 6-14). Because of the pleiotropy of actin function, it is not surprising that the life cycle of viruses, from the point of cell entry to the egress of the progeny, is intimately connected to the actin cytoskeleton of the host cell, and that viruses hijack various actin functions and modify or redirect them to enhance self-propagation. All studied viruses reconfigure and manipulate actin cytoskeleton of host to optimize the production of the progeny. It is known that RNA viruses can promote actin polymerization and reorganization (15). For example, the transmissible gastroenteritis virus (TGEV) and porcine hemagglutinating encephalomyelitis (PHEV) coronaviruses use Rac1/Cdc42 small GTPases of the RhoA family to induce actin polymerization (15-17). Additionally, the virus manipulates the cell adhesion complexes and modifies intercellular contacts, allowing a directional cell-to-cell spreading. The different strategies used by different viruses to manipulate actin cytoskeleton indicate that these strategies evolved independently (18, 19). Most of the studied actin-virus interactions describe viruses other than relatively novel SARS-CoV-2. Thus, here we describe the subversion of actin cytoskeleton strategies used by various viruses during entry, assembly, and egress from the host cells and point out what (if anything) we know about SARS-CoV-2 on those subjects. This review should encourage the exploration of SARS-Cov-2 behavior and functions in these uncharted areas and eventually help to develop novel anti-viral therapeutic or prevention strategies.

1. SARS-CoV-2 structure

The SARS-CoV-2 is a spherical structure of ~ 100nm diameter. In the center of the virus, there is a tightly packed positive-sense, single-strand, 29,811 nucleotide-long RNA, which is the so-called “translation-ready,” i.e., after the entry into the host cell translates (using the synthetic

machinery of the host) a set of viral proteins necessary for the RNA replication and formation of new copies of the virus (20-24). The RNA has 10-14 open reading frames (ORFs) and codes for 24-27 proteins (20, 25, 26). The viral transcription and replication also rely on intricate long-range RNA-RNA interactions with the host RNA (27). Viral RNA is surrounded by the protective coat, called the capsid, built of 1000 copies of RNA-binding nucleocapsid (N) protein (Fig. 1). The N protein is highly phosphorylated, has a molecular weight of 46.5 kDa, and through the binding to the genomic packaging signal facilitates the packaging of the viral genome within the capsid (28, 29). The capsid is surrounded by the membrane envelope, which derives from the membrane of the host cells but has inserted viral proteins: ~1000 copies of the membrane (M) protein, ~20 copies of the envelope (E) protein, and ~100 copies of the spike (S) protein (Fig. 1). The M protein has a molecular weight of 25-30 kDa, and it forms a dimer that shapes the viral membrane and promotes its binding to the capsid (29, 30). The E protein has a molecular weight of 8-12 kDa and an ion channel activity. It is crucial for virus assembly and its release from the host cell (29). The S proteins form the viral spikes. Each spike contains three copies of S protein and has a length of ~ 10nm (20, 29). The S protein has a molecular weight of 150 kDa, is N-glycosylated, and belongs to the class I fusion family of proteins, which facilitate binding to the receptors and allow membrane fusion (29, 31). The S protein has S1 and S2 subunits. The S1 has the receptor-binding domain, and the S2 mediates membrane fusion (32). The S protein-containing spikes have a high affinity to the angiotensin-converting enzyme 2 (ACE2) receptors on the host cells and facilitate entry of the virus (33-35). The binding of the virus to the host cells involves two steps, first is the docking of the virus to the ACE2, and second is the activation of the spike protein by the proteolytic cleavage at two sites by the host-encoded proteases (36, 37). The proteolytic cleavage is also a two-step process, the first cleavage occurs at the boundary of the S1 and S2 subunits, and the second exposes the S2 subunit. The first cleavage can occur before or after the binding to the receptor. The second cleavage occurs before or after endocytosis, through the activity of the serine protease TMPRSS2 or endosomal proteases, respectively. The second cleavage primes the S2 subunit for the fusion of the virus with the cell membrane. In the SARS-CoV-2 and MERS-CoV, the boundary between S1 and S2 subunits has several amino acids, which can also be cleaved by another serine protease, the furin. This stretch of amino acids is absent in the SARS-CoV (32, 36, 37). Additionally, the S protein has the sialic acid (Sia)-binding domain, which interacts with the sialic acid-containing molecules on the surface of the host cell and facilitates the virus entry (34). It seems that this dual feature of S protein is one of the reasons for the high transmissibility of the SARS-CoV-2 (33, 38). Recent studies indicate that the recruitment of the SARS-CoV-2 to the cell surface is

also facilitated by the heparan sulfate (HS) proteoglycan surface molecules. The HS interact with the Spike protein, increasing virus concentration and promoting engagement with the ACE2 receptors (39). Interestingly, the HCoV-OC43 and HCoV-HKU1 coronaviruses, which like SARS-CoV-2, belong to the beta-CoVs family (40), have an additional gene that expresses the hemagglutinin-esterase (HE) dimer protein (29). In these viruses, the S protein-containing spikes are interspersed with the shorter ~8nm stubby projections composed of HE protein. The HE protein has a dual function (41), it has a receptor-binding lectin domain that is specific for O-acetylated sialic acid (O-Ac-Sia) and promotes attachment of the virus to the sialic acid-containing molecules on the surface of a host cell, and a receptor destroying sialate-O-acetyl esterase that promotes virus release (33). It seems that the S and HE proteins are functionally interdependent and co-evolved to optimize the balance between virus attachment and release (42). The binding to the receptors and the virus entry into the cell (assessed for SARS-Cov-1) lasts ~10 min (20). The process of making new viruses within the cell lasts around 10 hours and ends with the release of ~ 1000 newly formed viruses (20). Below, we summarize what happens inside the infected cell, from the moment of virus attachment to the receptor till the release of the progeny, and how the actin cytoskeleton of the host is involved in these processes.

2. Actin-driven “surfing” of the virus

Some viruses, such as murine leukemia virus, human immunodeficiency virus, vesicular stomatitis virus, and human papilloma virus slide (surf) on the surface of host cell protrusions (filopodia), which contain actin filaments, toward the cell membrane territory with the highest endocytic activity (43) (Fig 2A). Although the virus can enter cells at the membrane located outside of filopodia, the filopodia-dependent entry, depending on the inherent ability of filopodia-region to bind and engulf various molecules, including viruses, is much more efficient and enhances virus entry and infection of the host (43). Confocal time-lapse microscopy of murine leukemia virus (MLV) infecting human embryonic kidney HEK 293 cells showed that the virus, after attaching to the tip of filopodia, moved downward toward the cell's body (43). Mechanism initiating the surfing is mainly unknown but certainly involves contact of the virus with its receptor(s) (43). The surfing itself is powered by the contraction (retrograde F-actin flow) of filopodium's actin filaments by the myosin II motors located within the cortical actin at the base of the filopodium. This creates the dragging force that moves virus/receptor complexes to the base of the filopodium. Cytochalasin D, which blocks the barbed ends of actin filaments, inhibits surfing (18, 43). Although there is no description of SARS-CoV-2 surfing, the high-resolution scanning microscopy showed that SARS-CoV-2 adheres to the surface of

filopodia-like projections of the African green monkey kidney Vero cells, which would suggest a possibility of surfing (44). Recently, Seyran et al. (34) described that prior the binding to the ACE2 receptor, the SARS-CoV-2 S protein interacts (through the flat sialic acid-binding domain at the N-terminal domain of the S1 subunit) with the sialic acid layer of the epithelium, allowing the virus to move on the epithelium surface to find and interact with the ACE2 receptor. It remains unknown if such movement of the virus has anything to do with, described above surfing perpetuated by the retrograde F-actin flow.

3. Virus entry into the cell

The coronaviruses can enter cells using various routes such as phagocytosis, micropinocytosis, or membrane fusion (19). However, recent studies showed that the preferable mode of entry of the SARS-CoV-2 virus is through the ACE2 receptor and clathrin-mediated endocytosis (45), which depends on a proper arrangement of cortical actin (Fig.3). Owczarek et al. (46) showed that both stabilization or depolymerization of actin interferes with endocytosis and virus entry of the common coronavirus OC43. During virus entry, the virus/receptor complex recruits clathrin, which coats the invaginating membrane pit. The initiation of membrane invagination is controlled by the multiprotein complex containing the membrane-associated protein intersectin - 1 that directs interactions between the endocytic vesicles and actin cytoskeleton, the epidermal growth factor receptor substrate 15 (Eps15) that is a scaffolding adaptor protein, and the membrane-sculpting F-BAR domain-containing Fer/Cip4 homology domain-only proteins 1 and 2 (FCHo1/2), (46). The FCHo1/2 recruits intersectin-1 and Eps15 and induces membrane invagination (46, 47). The Eps15 (48) recruits the adaptor protein 2 (AP2; (49), which recruits clathrin to form a lattice coating the membrane pit. The “neck” of the pit attracts amphiphysin protein (50), which in turn recruits the dynamin, which via binding and hydrolyzing GTP severs the “neck” of the pit from the cell membrane (51). The scission results in the formation of the clathrin-coated endosomal vesicle that contains the receptor and the virus (Fig. 3A); (46, 52).

4. The endosomal phase of the virus

The next step after the formation of the endosome containing SARS-CoV-2/receptor complex is the acidification of the endosome through the proton pump-dependent release of Ca^{2+} from the endosome via intracellular messenger nicotinic acid adenine dinucleotide phosphate (NAADP) sensitive two-pore channel (TPC2) (53). The acidification of the endosome occurs during the maturation of the early endosome, to the late endosome. In the late endosome, the complex of S protein and ACE2 receptor is cleaved by cathepsin-L, which promotes the fusion of viral and endosome membrane and the release of the viral RNA to the host-cell cytoplasm (Fig.3A); (54).

Studies showed that endosomal acidification inhibitors, such as chloroquine or bafilomycin A1 drastically reduce RNA release and replication of SARS-CoV-2 (55). The spatial arrangement of the endosomes and their trafficking within the cell, and the recycling of the endosomal membranes back to the cell surface, are supported by the actin filaments and microtubules and are powered by various motor proteins (56).

5. Virus replication and translation

Released viral RNA (often called the genomic RNA, gRNA) undergoes replication, transcription, and translation to produce viral proteins and progeny RNAs (22, 57); (Fig.3B). First, the two large open reading frames, ORF1a and ORF1b of viral RNA, are translated into the polyproteins pp1a and pp1ab, which after co-translational and post-translational modification are converted into the non-structural proteins (nsps), which participate in viral replication and transcription. (22, 57, 58). In the next step, viral gRNA generates (with the help of the nsps) the negative-sense RNA intermediates, which are the templates for synthesizing gRNAs of the next generation and subgenomic RNAs (sgRNAs). The sgRNAs will be translated into the viral structural proteins (N, E, M, and S), while the gRNAs will be assembled with the structural proteins to form the new progeny of the virus (Fig.3B). A recent analysis of the SARS-CoV-2 transcriptome showed that the viral RNA also has several ORFs of unknown functions (22).

In all positive-strand (+) RNA viruses, the replication of viral RNA and its transcription occurs within the virus-induced replication organelle, situated in the vicinity of the host cell nucleus (Fig. 3B). This replication compartment, which was recently found in the cells infected by the SARS-CoV (59), creates a protective niche for viral replication and transcription. It derives from the ER or other membranous structures of the host cell and consists of double-membrane vesicles (DMVs), double-membrane cups, called the spherules (DMSs), convoluted membranes (CMs), or/and tubules (57, 60, 61; 62). We do not know if actin participates in the establishment and maintenance of the replication organelle. However, the actin cytoskeleton involvement in the formation and structure of all membranous organelles in eukaryotic cells (2) would imply so. It is known that in the eukaryotic cells, actin regulates various transcription factors and that the binding to actin is necessary for the activity of all RNA polymerases (5). This implies that also the transcription of viral RNA should be actin-dependent. This assumption is supported by the older studies showing that actin cytoskeleton is involved in the replication and transcription of human parainfluenza virus type 3 (63). Moreover, the mutational analysis of respiratory syncytial virus (RSV) indicates that actin and profilin are necessary for viral transcription and that the divalent-cation-binding domain of actin is necessary for the binding and activation of viral RNA

synthesis (64, 65). Additionally, studies indicate that also the viruses replicating in the nucleus interact with nuclear actin for replication, assembly, and export of mRNA/ capsids (66). The fact that the interactions with actin are necessary for many viral functions suggests that the SARS-CoV-2 RNA transcription might also be actin-dependent despite that, to our knowledge, there is no direct information available on the subject.

6. Virus assembly and egress

The newly synthesized viral proteins and RNAs are transported to the ER/Golgi intermediate compartment (ERGIC, also referred to as the vesicular-tubular cluster (VTC; 67) where the SARS-CoV-2 RNA coated with N proteins are enveloped with the host-cell membrane containing viral M, E, and S proteins (Fig. 3C). From the ERGIC, the virus enters the trans-Golgi network (TGN)/Golgi complex for posttranslational modification of the proteins (68). Some time ago, a peculiar structure called “the juxtanuclear actin ring” was described in the coronavirus-infected cells (Fig. 3B). The ring, built of actin filament layers surrounding the cell nucleus and the adjacent ER and Golgi compartments facilitates viral genome replication and protein synthesis (19, 69). The sequestration of the molecules needed for virus replication, transcription, and translation by the actin ring may concentrate them in the vicinity of ER and Golgi for further processing. Another function of perinuclear actin ring was described in human adenovirus infection. In this case, the adenovirus early region 4 ORF4 protein (E4orf4) induces, via myosin II motor/ Src kinase/ Cdc42/N-Wasp/ RhoA kinase, and Rac1 pathways, actin polymerization around the nucleus. Subsequently, the actin ring, by recruiting endosomes, disrupting vesicular trafficking, and membrane dynamics, leads to the activation of an endo-lysosomal-based cell death pathway (70).

The egress of the newly assembled viruses from the cell occurs via exocytosis (54, 71-73), (Fig. 3C) Recent studies showed that SARS-CoV-2 and other β -Coronaviruses use a unique exit strategy. Instead of using the conventional secretory pathway used by other viruses, they use the deacidified lysosomes to egress from the cell (68). The exact mechanism of deacidification of the lysosomes, which deactivates the lytic enzymes is still unknown. Ghosh et al (68) suggest that the deacidification of lysosomes is either a direct consequence of too much cargo (viruses) and/or a dysfunctional proton pump, or that some of the viral proteins have a viroporin function that by the formation of hydrophilic pores modifies membrane permeability and Ca^{2+} homeostasis leading to lysosome deacidification. Also, it is still unknown how the viruses translocate from the TGN/Golgi to the lysosomes. Ghosh et al. (68) propose two possible routes. One route is the transfer from the Golgi to the multivesicular bodies (MVB), which mature into

the lysosomes via the activity of the Rab7 GTPase pathway (Fig. 3C). The fact that the inhibition of Rab7 reduces both the lysosome formation and the egress of the virus support this option (68). Another, more complicated option is the retrograde transport of the virus from the Golgi back to the ER/ERGIC and then to the lysosomes. The modification of the second option is the microphagy, when the fragments of ER containing the virus are directly engulfed by the lysosomes (68). Studies on the avian infectious bronchitis coronavirus showed that the budding of the virus from the cell membrane and the egress are actin filament-dependent and involve the interaction between actin and the amino acids A159 and K160 of the viral membrane M protein (19, 74). The scanning electron and atomic force microscopy showed that just before its egress the SARS-CoV induces the formation of pseudopodia and thickening of the cortical actin, which by bending the membrane expels the virus progeny from the host cell (75).

7. Actin comets

Many viruses, such as Vaccinia, Ebola, Marburg, and insect baculoviruses, use the actin “tails” or the so-called “actin comets” to be propelled within the cytoplasm to the cell periphery and/or to the outside of the infected cell (Fig. 2B). Viral proteins induce actin nucleation via the activity of the Arp2/3 complex. This is followed by the interaction with the myosin and kinesin motors and actin polymerization/depolymerization cycles, which propel the virus. Because the comet-associated viruses were noticed not only inside the cytoplasm but also in the vicinity of the cell-to-cell junctions, it has been proposed that the propelling force of the actin comet overcomes the actin reinforced cellular junctions allowing delivery of the virus to the adjacent cell (76-78). So far, there is no information if the coronaviruses and SARS-CoV-2 use the actin comets for the movement inside and out of the cell.

8. Actin and a direct cell-to-cell transmission of the virus

Cell-to-cell direct transmission that is faster and much more efficient than a cell-free spread, and allows circumventing immune response, especially antibody-mediated immunity of the host, is efficiently used by many viruses, including the SARS-CoV-2 (70, 78, 79, 80). Below we describe several possible routes for a direct transmission of the virus between cells. All these routes of transmission involve the actin cytoskeleton of the host cells.

a. Tunneling nanotubes (TNTs)

Tunneling nanotubes (TNTs) are long, thin (50-1500 nm in diameter), F-actin-based open-ended channels used by the neighboring cells to exchange large cargo, such as protein complexes, lipids, nucleic acids, vesicles, pathogens, and organelles (Golgi, ER, lysosomes, endosomes, and

mitochondria) at the distance of hundreds of micrometers (78, 79, 81- 85), (Fig.4) The ticker TNTs, which are used for the transport of very large cargo contain besides the F-actin also the microtubules. The traveling speed of the cargo through the TNTs is very fast, reaching ~ 7 nm/sec (85). Viruses can also “surf”, docking by the heparan sulfate (HS) receptors, on the surface of TNTs for long distances (Fig.4). The same receptors can also induce the fusion of the virus with the TNT membrane and induce endocytic internalization of the virus into the TNT lumen (85), (Fig. 4). Actin polymerization required for the formation of TNTs is regulated by the Rho GTPase signaling. including Rac1/Cdc42/ Pak1 pathways (86). Many viruses such as HIV-1, influenza, and coronaviruses induce the formation of TNTs to spread between cells (85, 86). Interestingly, the TNTs formed by *Mycobacterium tuberculosis* (known to enhance HIV-1 pathology) are used by the co-infecting HIV for the cell-to-cell transfer (86). We do not know if the SARS-Cov-2 uses TNTs to spread between cells. However, the observation that murine hepatitis (MHV) coronavirus and SARS-CoV-1 promote the formation of filopodia and membrane ruffling using Rac1/Cdc42/Pak1 pathways, and the ability of SARS-CoV-2 to bind to the surface HS, suggest that they can also induce the formation of TNTs. Certainly, this should be studied further in the context of SARS-CoV-2 as it may offer novel therapeutic targets for preventing or fighting COVID-19.

b. Virological synapse

Many viruses, including human T cell leukemia virus type 1 (HTLV-1), human immunodeficiency virus type 1 (HIV-1), and SIV retroviruses spread from cell to cell through the virally induced structure called the virological synapse (VS) or the infectious synapse, which shares similarities with the neurological, immunological, and plant synapses (87-90). During the synapse formation, the pre-synaptic and post-synaptic cells adhere to each other, but their membranes do not fuse. The virological synapse (VS) directly transmits viruses from the infected (pre-synaptic) to the uninfected (post-synaptic) cell (87). Also, the HIV-1, after being captured and internalized by the dendritic cells, is transferred through the immunological synapse to the interacting T cells. However, studies indicate that not only internalized virus is being transmitted, but also the virus attached to the surface of the dendritic cells. This type of viral transmission is called trans-infection (91).

The formation of any synapse, including the VS, involves the polarization of the F-actin filaments and the Rho GTPase signaling (92-95). The actin network at the synapse plays not only the structural function but is a scaffold for the signaling molecules and the clustering of the virus (92, 93). It seems that at least in the case of HIV-1, the synapse contains a local, F-actin-depleted zone that facilitates virus entry by removing the physical cortical actin barrier (96). Besides the

retroviruses, the coronaviruses also use the VS for a direct cell-to-cell transmission. Yang et al. (97) described the VS transmission of SARS-coronavirus (SARS-CoV) between dendritic cells (DC) and target cells. Recently, more and more studies suggest that the ability of the SARS-CoV2 to invade the neural system involves the transmission of the virus through the neurological synapses (98-100).

c. Virus interaction with the cellular junctions

A direct transfer of viruses between cells can also occur through intercellular junctions. Many viruses evolved various strategies to alter different types of cell junctions to allow viral penetration. There are three main categories of cell junctions. The tight (occluding) junctions support the exchange of small hydrophilic molecules. The anchoring junctions (adherens junctions, desmosomes, focal adhesions, and hemidesmosomes) attach cells to each other or the extracellular matrix. Adherens junctions and focal adhesions are connected to the bundles of actin filaments, while desmosomes and hemidesmosomes are connected to the intermediate filaments. The gap (communicating) junctions transfer small (below 1000Da) proteins, amino acids, nucleotides, small metabolites, ions, regulatory molecules, and secondary messengers (82, 101, 102). Studies showed that hepatitis C virus and retroviruses may enter through the tight junctions, human papilloma virus (HPV) through the adherens junctions, and HIV may modify gap junctions for the entry (103). Retroviruses modify the distribution of tight junction proteins occludin and claudin-1 and destroy tight junctions via RhoA/ROCK / MLC signaling pathways (103-105). Hepatitis C virus also affects occludin and claudin-1 and these proteins may also promote endocytosis of the virus (103, 106, 107). The HPV enters through the adherens junctions. It modifies the distribution of adherens junction proteins such as β -catenin, disrupting its association with E-cadherin and actin cytoskeleton (103, 108). The HIV hijacks the gap junctions to spread apoptosis-inducing factors to the uninfected cells and destroys, via mitogen-activated protein kinase (MAPK) pathway, the adherens and tight junctions (103, 109). Studies showed that the intermediate filament protein vimentin connected to the adherence junctions is essential for the viruses' life cycle, including entry, transport, replication, assembly, and egress (110). The intermediate filaments, actin, and microtubules are also crucial for the entry and propagation of dengue virus, Zika virus, and West Nile virus (111). Recent studies showed that the alterations of cell junctions induced by SARS-CoV-2 are highly damaging for the patients (112,113). Humans have over 100 proteins containing the PDZ (post-synaptic density protein) domains, some of which are the components of the cell junctions. The envelope (E) protein of SARS-CoV-2 and SARS-CoV-1 (called the SARS-CoV before the emergence of SARS-CoV-2)

has a PDZ-binding motif (PBM). The binding of viral PBM to the PALS1 (protein associated with Lin seven 1) present in human cell junctions relocates PALS1 from the junctions to the ERGIC compartment, where the virus is assembled. Envelope protein also binds and dislocates other PDZ-containing junctional proteins, including the adherens junction protein syntenin and the tight junction ZO-113 (Zonula Occludens-113) protein, which regulates the actin-relevant RhoA/ROCK pathway (114). Disruption of the junctional proteins in the inter-epithelial junctions of the lungs causes vascular leakage, damage of alveoli, and induces acute respiratory distress syndrome (ARDS) in COVID-19 patients (112, 115).

9. Virally induced syncytia

The pathological examination of the lungs of the patients with severe cases of COVID-19 infection shows the presence of SARS-CoV-2-infected, multinucleated syncytia of pneumocytes (116, 117). Additionally, these patients are often severely lymphopenic, i. e. they have a deficit of lymphocytes, which decreases their ability to fight the infection (118, 119). It is known that many viruses, including poxviruses, paramyxoviruses, herpes viruses, and retroviruses, induce the fusion of the infected cells into the multinuclear syncytia, which eventually die (120). Recent studies showed that SARS-CoV-2 infection causes the formation of the giant syncytia and the destruction of the tight junctions in the lung airway epithelium (113). There are two main types of syncytia: 1. homotypic syncytia that contain the same type of cells, and 2. heterotypic syncytia that result from the fusion of different types of cells. Recently, Zhang al. (119) described another type of syncytia in the lungs of COVID-19 patients. These are the syncytia of the lung cells containing the internalized lymphocytes (Fig.5). The cell fusion leading to the formation of the syncytia requires the rupture of the membranes of the fusing partners followed by the membrane rejoining. The whole process is facilitated by the fusogenic proteins and involves the restructuring of the supportive platform of the actin cytoskeleton underlying the cell membranes. The Spike protein of SARS-Cov-2 is a fusogen with homology to the mammalian syncytin-1, which is involved in the formation of the syncytial layers of the placenta (31). The model and the details of the fusion process are described in ref. 31 and 119. The virally induced syncytia not only enhance virus spreading but may also serve as a tool for the elimination of the immune cells, which fight the virus. Studies of COVID-19 patients showed that SARS-CoV-2 induces both homotypic syncytia of the pneumocytes, and the heterotypic cell structures, which contain fused lung cells with the engulfed lymphocytes inside (36, 119). These studies propose that the SARS-CoV-2 infected cells express the viral S (spike) protein on their surface. The interaction of the spike protein with the ACE2 receptor of the neighboring cell activates the calcium-activated

lipid scramblase TMEM16F (anoctamin 6) that exposes the phosphatidylserine on the cell surface and triggers the formation of syncytium, which, subsequently, engulfs the immune cells (116, 122). These studies suggest that syncytium-mediated lymphocyte internalization and elimination may be one of the causes of lymphopenia in COVID-19 patients (119-121). Recent studies indicate the antihelminthic drug niclosamide, suppress the activity of TMEM16F and inhibits the spike-induced syncytia formation in SARS-CoV-2 (123).

Conclusions

The information presented here indicates that the interactions of the viruses, including SARS-CoV-2, with the actin cytoskeleton and the actin-related cell functions and signaling pathways, are of crucial importance for virus functions, infectivity, and pathogenicity. It is also clear that the knowledge on the SARS-CoV-2 interactions with the actin and other components of the cytoskeleton is still very limited and requires further studies. A better understanding of these interactions, processes, and the participating viral and cellular components can help design novel antiviral therapies and/or mitigate the severity of COVID-19 symptoms and damaging, long-term effects. The conventional approaches to fight viral infections include vaccinations, antiviral drugs, and preventing the overactivation of the immune response. The latter is especially important in COVID-19 disease, in which the overproduction of inflammatory factors causes cytokine storm in the patients' lungs (124). The recent analysis of gene expression profile in the SARS-CoV/SARS-CoV-2 infection using the Through Gene Ontology (GO) and MetaCore showed that the upregulation of actin/cytoskeleton remodeling via the RhoA pathway is crucial for the infection (125). Thus, we propose that the interference with the actin-dependent function of virus and/or host immune cells using RhoA pathway inhibitors, such as the multiple sclerosis drugs Fingolimod and Syponimod, might be a novel therapeutical approach in fighting viral diseases (126).

Conflict of interests

None of the authors has a conflict of interests

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Figure Legends

Figure 1. SARS-CoV-2 structure

The SARS-CoV-2 consists of the nucleocapsid and the envelope. The nucleocapsid contains ss(+)RNA surrounded by the RNA-binding nucleocapsid (N) proteins. The nucleocapsid is surrounded by the lipid bilayer membrane, which derives from the cell membrane of the host cell but has inserted viral proteins: membrane (M) proteins (M), envelope (E) proteins, and the spike (S) proteins.

Figure 2. Hypothetical SARS-CoV-2 surfing and actin comets.

A) Filopodia contain the bundles of actin filaments. The virus bound to its receptor surfs on the surface of the cell membrane, from the tip to the bottom of the filopodia, and to the membrane region with the highest endocytic activity for a subsequent internalization. The motor proteins located at the bottom of the filopodium cause actin filament contraction and retrograde movement, which propels the receptor-bound virus. **B)** Viruses can also induce actin polymerization and the formation of the actin filament comets (tails), which propel the virus within the cell toward the cell surface or expel it from the cell. Viral proteins induce actin nucleation via the activity of the Arp2/3 complex, and the myosin and kinesin motor-dependent actin polymerization/depolymerization cycles propel the virus. Although these types of movement are used by many different viruses, so far, we do not know if SARS-CoV-2 may surf or use the actin comets for the movement.

Figure 3. SARS-CoV-2 life cycle

A) Diagram summarizes virus endocytosis. The preferable mode of SARS-CoV-2 entry to the host cell is the clathrin-mediated endocytosis. The binding of the virus to the ACE2 receptor induces, through the action of membrane sculpting complex (FCHo1/2, interscetin-1 and Eps15), the rearrangement of the cortical actin, membrane bending, and recruitment of clathrin to the surface of the membrane pit. Subsequently, the “neck” of the pit recruits the dynamin, which cuts off the “neck” of the pit from the cell membrane. The resulting clathrin-coated early endosome contains the virus and the receptor. The early endosome matures through the acidification into the late endosome. In the late endosome, the virus is cleaved off the receptor by cathepsin-L, which also promotes viral and endosomal membrane fusion. The receptor recycles back to the cell membrane, and the viral genomic RNA (gRNA) is released into the cytoplasm. The movement of endocytic vesicles and their spatial distribution within cell cytoplasm are actin/motor protein dependent. **B)** Summary of the post-endocytic phase of the virus life cycle. Released viral RNA (gRNA) is translated into the non-structural proteins (nsps), which will be

used for subsequent viral replication and transcription. First, viral gRNA makes (with the help of the nsps, RNA polymerases, and involvement of the globular (G) actin) the negative-sense RNA intermediates (not shown here), for the synthesis of gRNAs of the next generation, and subgenomic RNAs (sgRNAs). The sgRNAs are translated into the viral structural proteins (N, E, M, E, and S), while the gRNAs are assembled with the structural proteins to form the new viruses. Viral replication and transcription occur within the specialized virally induced, ER-derived, vesicular/membrane compartment called the replication organelle. The newly synthesized viral proteins and RNAs move to Golgi where the SARS-CoV-2 RNA coated with N proteins are enclosed by the host-cell membrane containing viral M, E, and S proteins. The maintenance and positioning of ER and Golgi complex depend on the actin filament. Also, the ER/replication organelle and Golgi area are surrounded by the ring of actin filaments, which probably helps to concentrate the factors involved in the synthesis of the new virus within the ER/Golgi complex. Finally, the newly formed viruses are released from the cell through exocytosis. C) Summary of the virus exocytosis and egress. SARS-CoV-2 containing vesicles translocate through the multivesicular bodies, which probably mature into lysosomes, which had been deacidified to deactivate their lytic enzymes. The virus-containing lysosomes move to and fuse with the cell membrane. The virally induced rearrangements of cortical actin and membrane bending, expel the virus from the cell.

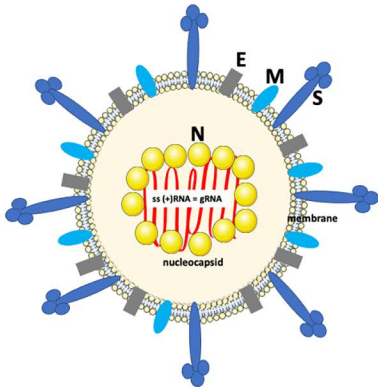
Figure 4. Hypothetical TNT- dependent transfer of the SARS-CoV-2.

The binding of the S protein of the SARS-CoV-2 to the heparan sulfate (HS) proteoglycan molecule (which acts as a co-receptor for the ACE2 binding) present on the cell surface, induces the formation of a tunneling nanotube (TNT) between the infected cell and the neighboring noninfected cell. The virus is transferred within the lumen of the TNT. The TNT contains actin filaments. The Rho GTPase signaling including Rac1/Cdc42/ Pak1 pathways regulates actin polymerization, which is required for the formation of TNT. The virus can also, after binding to the HS, surf on the surface of TNT. The virus on the surface of TNT can also internalize into the TNT interior. Although there is no data that the SARS-CoV-2 spreads through the TNTs, the fact that its S protein binds to the HS, and that a closely related SARS-CoV-1 can induce various types of cytoplasmic extensions, suggests so.

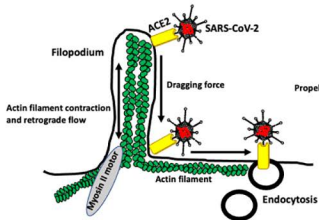
Figure 5. SARS-CoV-2 induced syncytia

After the cell infection and release of the viral RNA, the infected cell synthesizes viral Spike (S) protein. The S protein expressed on the surface of the infected cell interacts with the ACE2 receptor of the uninfected cell. Because the viral S protein has fusogenic properties, this

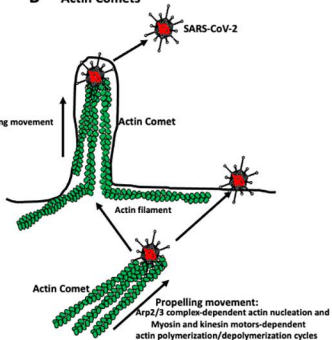
interaction initiates a cascade of events, which eventually lead to the apposition of neighboring cell membranes, membrane scission, and cell fusion. The interaction of the spike protein with the ACE2 receptor of the neighboring cell activates the calcium-activated lipid scramblase TMEM16F (anoctamin 6) that exposes phosphatidylserine on the cell surface and triggers the formation of the syncytium. The SARS-CoV-2 induces not only the formation of the pneumocyte syncytium but also the formation of heterotypic syncytia when the pneumocyte-derived syncytia engulf the lymphocytes. The internalized lymphocytes undergo apoptosis and die. SARS-CoV-2 uses this method to eliminate from the lungs the virus-fighting lymphocytes.

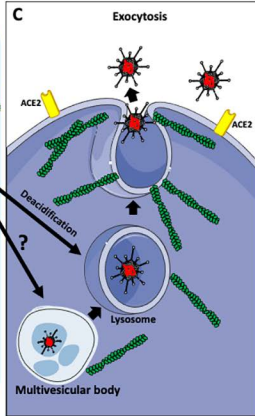
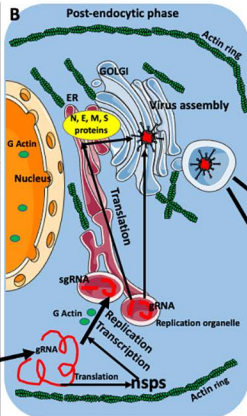
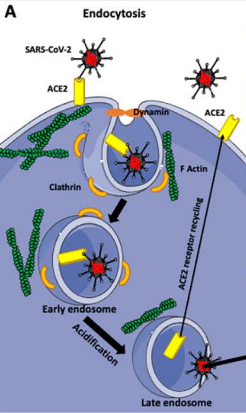


A Virus Surfing



B Actin Comets





TNT- dependent transfer of the virus

