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Cell-to-cell transport in viral families: faster than usual

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Summary. – The most frequent way of virus dissemination is through the canonical receptor-mediated pathway. However, when unfavorable conditions, such as presence of antibodies appear, the viruses use more peculiar routes of transmission to protect themselves. Here we describe most of the routes, from syncytia formation, tunneling nanotubes and filopodia, through immunological and virological synapses to actin comets formation. We describe the cell-to-cell transport in different viral families to show that this way of virus distribution is present in almost all the mammalian virus families and is not as uncommon as it was thought. The knowledge of the ways of viral transport might lead us to exploit more successful therapeutic approaches and fight the most threatening diseases.

Keywords: cell-to-cell transmission; tunneling nanotubes; viral synapses; filopodia; actin comets

Introduction

Every action of the individual cell depends on communication with neighboring cells. Most of the communications are provided by different types of protrusions. Protrusions are present in different tissues from different organisms and are characteristic for distinct functional or structural properties. More efficient spread of the virus, than classical spread by receptor recognition, is direct cell-cell contact (Jolly *et al*., 2004; Mcdonald *et al*., 2003). Numerous studies have showed cellular protrusions mediated virus transfer for many viruses, including human T-cell leukemia virus type I (HTLV-1) (Van Prooyen *et al*., 2010), murine leukemia virus in living cells (Sherer *et al*., 2007), Marburg virus (Kolesnikova *et al*., 2007), African swine fever virus (Jouvenet *et al*., 2006) herpes viruses (Ady *et al*., 2016; Favoreel *et al*., 2005; Gill *et al*., 2008), influenza A viruses (Kumar *et al*., 2017; Roberts *et al*., 2015) and human immunodeficiency virus type 1 (HIV-1) (Sowinski *et al*., 2008). Cell-associated transmission is considered more rapid and efficient because it omits rate-limiting steps of cell-free spread: the release of virus particles, diffusion and entry occurs quickly at cell-cell contacts sites. What more, the viruses that utilize tight and adherence junctions for their transport are protected from the effect of neutralizing antibodies and other immune system components.

Filopodia as one of the earliest protrusions recognized (Gardel *et al*., 2010; Jacquemet *et al*., 2015), are actin-based protrusions observed at the leading edge of the migrating cells. First of all, filopodia "scan" the substrate followed by extension of lamellipodia toward the stabilized filopodia (Albrecht-Buehler, 1976; Trelstad *et al*., 1967). However, it was proved that the formation of filopodia did not always correlate with cell migration but instead correlated with intercellular signaling (Miller *et al*., 1995).

Cytonemes are defined as "filopodia of a special type" reaching up to 700 μm in length in Drosophila (Ramirez-Weber and Kornberg, 1999). Cytonemes connect cells sepa-

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Abbreviations: BoHV-1 = bovine herpesvirus 1; BVDV = bovine viral diarrhea virus; Env = envelope protein; gE = BoHV-1 glycoprotein gE; HCV = hepatitis C virus; HHV-1 = human alphaherpesvirus 1 (herpes simplex virus 1); HIV-1 = human immunodeficiency virus type 1; ICAM = intercellular adhesion molecule; JEV = Japanese encephalitis virus; K1 = keratin 1; LCMV = lymphocytic choriomeningitis virus; LFA-1 = lymphocyte function-associated antigen 1; MARV = Marburg virus; MV = mature virus; PRRSV = porcine reproductive and respiratory syndrome virus; SuHV-1 = suid alphaherpesvirus 1; TNT = tunneling nanotubes; Us3 = tegument protein Us3 kinase; VACV = vaccinia virus; VP26 = BoHV-1 structural protein VP26; VS = virological synapses; WV = wrapped virus

rated not only by distance but by other (non-participant) cells in the tissues, and are believed to deliver signaling molecules and their receptors between signal-sending and -receiving cells. Also cytonemes and tunneling nanotubes were found to form between cells in culture cells, allowing trafficking of vesicular organelles (Rustom *et al*., 2004). Cytonemes mediate the delivery of specific signaling ligands (morphogens) and their receptors to allow specific signal transduction between cell types that are far apart from each other, often separated by large numbers of intervening cells (Kornberg and Roy, 2014). As filopodia, cytonemes contain actin and need regulators of actin dynamics for their formation (Roy *et al*., 2014).

Tunneling nanotubes (TNT) were shown to form *de novo* between previously unconnected cells by extension of protrusions (Rustom *et al*., 2004) or by dislodgement of two previously-attached cells, which left TNTs behind (Sowinski *et al*., 2008). The name tunneling nanotube is taken from both their original discovery diameter size (50–200 nm), and also their tunneling ability in the extracellular matrix. They are thin tube structures protruding from one cell and connecting with the other to form a nanotubular network (You *et al*., 2014). These structures are filled with cytoskeletal filaments, like actin, microtubules and motor proteins. Usually, smaller (<100 μm) tubes of TNTs contain F-actin while thicker tubes (>100 μm) contain both F-actin and microtubules (Rustom *et al*., 2004; Sowinski *et al*., 2008; Wang *et al*., 2010). TNTs, by character of the connection can be divided into TNTs with open-end and closed-end. Open-ended TNTs support the continuity of cytoplasm between two cells allowing thus calcium signaling, free diffusion of membrane proteins or transport of large organelles such as mitochondria. Closed-ended TNTs do not propagate calcium signals nor free diffusion of membrane proteins (Gerdes and Carvalho, 2008). However, it was observed that the TNT tips of one cell adheres to the other surface of the other cell. These structures mostly contain actin and are found in cultured cells where they were observed to be loose in the media without attachment to the surface as well as attached (Naphade *et al*., 2015). However, the constituent cytoskeleton, actin or microtubule, present in TNTs determines the type of molecular motors (myosine, kinesins, dyneins), and thus specific cargoes, to be trafficked between cells.

Commonly, the TNTs are formed or modified by the influence of the stress, including H_2O_2, UV, virus infection, prion aggregation, serum starvation and high glucose concentration (Thayanithy *et al*., 2014b; Wang and Gerdes, 2015; Wang *et al*., 2011). Typically, stressed cells form TNTs to reach out to healthy cells, indicating that TNT formation may be a mechanism that helps protect cells from death. In the culture of rat hippocampal neurons and astrocytes, p53 and its downstream effectors (Egfr, Akt, phosphoinositide 3-kinase, and mTOR) are critical for TNT formation (Wang *et al*., 2011), although its requirement is likely cell type-specific (Andresen *et al*., 2013). These structures are critical requirement for development, and tissue homeostasis and regeneration. TNTs can also contribute to cellular differentiation and reprogramming by providing a highway to transfer cellular components from one cell to another cell (Takahashi *et al*., 2013). However, the TNTs also contribute to spread of the tumors (Thayanithy *et al*., 2014a), progression of neurodegenerative diseases and transfer of bacteria, viruses and prions (Dubey and Ben-Yehuda, 2011; Gousset *et al*., 2009; Roberts *et al*., 2015; Sowinski *et al*., 2008).

Microtubules-based nanotubes (MT-nanotubes) mainly contain microtubules and do not require regulators of the actin cytoskeleton for their formation. MT-nanotubes utilize components of cilia formation (e.g. intraflagellar transport (IFT) proteins) although they lack other characteristics of cilia (such as acetylated tubulin) (Inaba *et al*., 2015).

Synapses transmit cell-cell signal through the extracellular space, relying on mechanism of ligand-receptor signaling across tight cell-cell junctions. During formation of the immunological synapses between T-cells and antigen-presenting cells, filopodia precede the formation as a full synaptic cleft and might even persist as functional "probes" operating in the cell-cell interface (Williams *et al*., 2007). Crucial transition from a filopodial intermediate to a tighter cell-cell contact depends on the recruitment of additional adhesion proteins and intracellular adaptors. For example, clusters of E-cadherin are recruited to establish adherence junctions, Syn-CAM or neuroligin are needed for neurological synapses and ICAM-1, LFA1 for immunological synapses (Sherer and Mothes, 2008). In the absence of specialized epithelial or synaptic adhesion proteins, a filopodial intermediate is stabilized to form a prolonged and elongated filopodial bridge (Sherer and Mothes, 2008). Pseudorabies virus and herpes simplex virus spread throughout the nervous system by means of neuronal synapses. HIV-1 and HTLV-1 use "virological synapses" in infected lymphocytes so named in analogy to immunological synapses (Igakura *et al*., 2003).

The most specific virus cell-to-cell transport, actin comets or tails, are provided by *Poxviruses*. Thanks to this rapid cell-to-cell spread they replicate and spread extremely rapidly in the epidermis and endothelium. We will discuss this unique transport in detail further on in the *Poxviridae* chapter.

Also, syncytia formation can be classified as a special cell-to-cell transmission. Any viruses that are able to induce fusion machinery in the infected cells especially on basolateral surface of polarized epithelial cells and can

undergo pH-independent fusion at the plasma membrane are able to mediate cell-cell fusion and form syncytia. Some of the families causing formation of syncytia are *Herpesviridae*, *Paramyxoviridae* and *Coronaviridae* (Sattentau, 2008)

Cell-to-cell transport in viral families

Herpesviridae

Alphaherpesviruses are able to spread across the junctions between the membranes of cells, by fusion of adjacent cells or on long distances along neurons (Nobiron *et al*., 2003).

Bovine herpesvirus 1 (BoHV-1) is a widespread bovine pathogen, a member of the *Varicellovirus* genus of the subfamily *Alphaherpesvirinae*. BoHV-1 shares many features with human herpesviruses, such as herpes simplex virus 1 and varicella-zoster virus (Muylkens *et al*., 2007). Due to its strong immunomodulatory properties, BoHV-1 is an interesting model for studies of viral immune evasion and cell-to-cell spread (Tyborowska *et al*., 2000). BoHV-1 has a restricted host range with cattle as the natural host; it is propagated in cells of bovine origin, although it has recently been shown to also infect human tumor cells (Cuddington and Mossman, 2014).

It utilizes various types of intercellular projections. In the infected cells, quantification of intercellular extensions showed a significant increase in the number of projections. Different structural viral proteins (glycoprotein gE, tegument protein Us3 kinase, capsid structural protein VP26) individually or as parts of an assembled particle were present both inside and on the surface of nanotubes and also in bubble-like vesicles (gondolas) formed from nanotubes. Protein VP26 migrated mostly in compact structures, while gE and Us3 were mostly visible in gondolas (Merwaiss *et al*., 2019). Unenveloped capsids and glycoproteins were during axonal transport transported separately and assembled into virions at the destination (Thomas *et al*., 2009) (Fig. 1).

Suid alphaherpesvirus 1 (SuHV-1) or *Pseudorabies virus* (the subfamily *Alphaherpesvirinae*, the genus *Varicellovirus*) causes Aujeszky's disease or Pseudorabies. It is linear double-stranded DNA virus with wide host range infecting numerous species of domestic and wild animals, with pigs being the natural host and reservoir (Sun *et al*., 2016) In the SuHV-1 studies, it was for the first time shown that Us3 protein kinase, a highly conserved protein among the herpesviruses, stimulated the formation of long intercellular projections. The activity of Us3 was crucial for the formation of nanotubes. SuHV-1 infected cells formed nanotubes and the presence of intact virions was clearly visible inside nanotubes. The nanotubes formed by SuHV-1 infected cells were remarkably stable compared to most TNTs described (Favoreel *et al*., 2005; Jansens *et al*., 2017). The Us3 induced TNTs contained stabilized microtubules and the virus particles were individually transported in membrane-bound vesicles and released along the TNTs and at the contact area between a TNT and adjacent cell. Contact between Us3-induced TNTs and acceptor cells was very stable and rich for components of adherens junctions such as beta-catenin or E-cadherin at the contact area (Jansens *et al*., 2017) (Fig. 1).

Human alphaherpesvirus 1 or herpes simplex virus 1 (HHV-1) utilizes a specific cell-to-cell transmission system - formation of syncytia. Protein tyrosine phosphatase (PTP1B) is the first host factor identified to be specifically required for cell-to-cell spread, and it may be a therapeutic target for preventing HSV-1 diseases (Carmichael *et al*., 2018) (Fig. 1).

Poxviridae

Vaccinia virus (VACV) as a member of the *Poxviridae* family has a unique way of transmission, forming of actin comets. This is a very complex mechanism, that includes great regulation and is mediated and fine-tuned by a multitude of host factors (Newsome and Marzook, 2015).

Mature virus (MV) particles are formed in the perinuclear replication center called virus factory. These particles are formed by single membrane derived from the endoplasmatic reticulum and over 100 viral proteins (Resch *et al*., 2007). In early endosomes and trans-Golgi network compartments, a subset of MV acquires two additional membrane layers to form wrapped virus (WV). WV is the only viral form that is able to promote actin nucleation. By adding additional membranes WV acquires additional membrane associated proteins (WV-specific proteins), such as A36, F12 and E2 functioning in recruitment and stabilization of microtubule motor complex kinesin-1. This interaction drives the viral particle from the virus factory to the cell periphery (Carpentier *et al*., 2015; Dodding *et al*., 2011). By cooperation of proteins carried by the virus and cellular proteins, the virus is transported on to extracellular side of the membrane. Actin nucleation by extracellular WV is executed by the cellular actin nucleator, the Arp2/3 complex to promote *de novo* seeding of actin filaments at 70° branch points on existing actin filaments. Following nucleation, actin polymerization is in a constant state of movement; as rapidly as actin is nucleated at the cytoplasmic/virus interface and filaments extend, actin polymers are disassembled, giving rise to a characteristic comet morphology (also referred to as actin „tails"). Usually, cells infected with VACV show 5–50 virus-associated actin comets of about 3.5 μm in length,

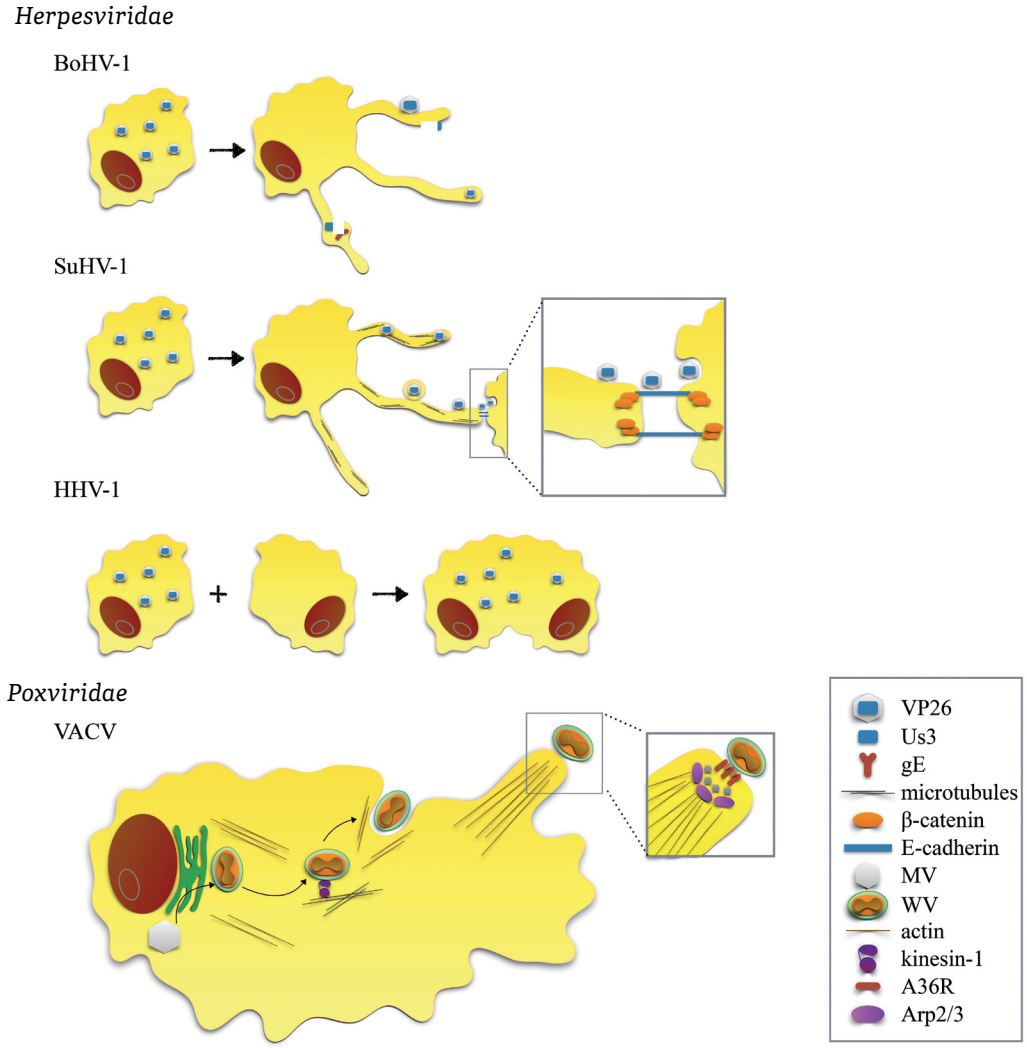

Fig. 1

Different types of cell-to-cell transports in DNA viruses

Herpesviridae: In BoHV-1 infected cells the significant increase in number of projections is induced. Capsid structural protein VP26 migrates in compact structures, while glycoprotein gE and tegument protein Us3 kinase is mostly present in gondolas (bubble-like vesicles). They utilize different types of intercellular projections. SuHV-1 stimulates the formation of long intercellular projections. Virions are present inside the nanotubes. TNTs contain stabilized microtubules. Virus particles are individually transported in membrane bound vesicles and released along TNTs and at contact with adjacent cell. Contacts are very stable and rich for components of adherens junctions such as beta-catenin or E-cadherin at the contact area. HHV-1 utilizes for its transmission the formation of syncytia. *Poxviridae:* VACV utilizes the formation of actin comets. Mature virions (MV) are formed in perinuclear replication center. In trans-Golgi network, MVs acquire additional membrane layers to form wrapped virus (WV). WVs associated with kinesin-1 motor are moved to the cell periphery and virus is transported on to extracellular side of the membrane. Actin nucleation is executed by Arp2/3 complex to form actin "tails" that propel WV to the neighboring cell.

although there is great variation between cell types (Dodding *et al.*, 2011). At any point in time, 20–30% of cell-associated extracellular virus will be adjacent to an actin comet (Humphries *et al.*, 2012). Actin comets on the apical surface of isolated VACV-infected cells manifest as long virus tipped cytoplasmic extensions to promote delivery of WV to adjacent cells, perhaps by overcoming the cortical actin-reinforced plasma membrane of adjacent cells. Actin-based motility is concentrated at the cell-to-cell junctions (Rietdorf *et al.*, 2001) (Fig. 1).

Retroviridae

In retroviruses, the induction of synapse-like structures facilitating cell-to-cell spread is triggered by the interaction of the viral glycoprotein (Env) with specific host receptors. Blocking the Env–cell receptor interaction abolishes cell-to-cell transmission (Jolly and Sattentau, 2004; Sherer *et al*., 2007).

Human immunodeficiency virus 1 (HIV-1) is the etiological agent of acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi *et al*., 1983). It infects cells by multiple mechanisms, either as cell-free or cell associated particles (Casartelli, 2016). HIV-1 infection is more efficient when the virus is transmitted through direct cell contacts (Bracq *et al*., 2018).

Viral synapses (VS) are direct adhesive cell-cell contact structures that can be formed between HIV-1 infected and uninfected $CD4^+$ T cells. The T cell VS is characterized as an actin-dependent polarization of viral proteins Env and Gag on the infected cells and CD4 receptor on the uninfected cell, forming cell-to-cell contact region (Jolly *et al*., 2004). Other molecules, such as intercellular adhesion molecules (ICAM) 1 and 3 and LFA-1 may further stabilize the VS (Jolly *et al*., 2007; Jolly and Sattentau, 2004). Cell-to-cell infection, at least *in vitro* is more efficient (Chen *et al*., 2007; Sourisseau *et al*., 2007), enables the resistance of the virus to certain classes of antiviral drugs (Sigal *et al*., 2011; Titanji *et al*., 2013) and to broadly neutralizing antibodies (Li *et al*., 2017; Reh *et al*., 2015). What more, cell-to-cell transmission of HIV-1 is an important route that leads to the establishment of latent infection (Pedro *et al*., 2019). VS can also take place between adjacent cells or on relatively long distances such as in the case of filopodia (Sherer *et al*., 2007; Sowinski *et al*., 2008).

In several studies a model of cell-to-cell transmission of HIV-1 initially transferred across the VS in a co-receptor independent manner into trypsin-resistant endocytic compartments within the HIV-1 uninfected target $CD41^+$ T cells is supported (Chen *et al*., 2007; Dale *et al*., 2011; Sloan *et al*., 2013). Time-lapse imaging studies showed interaction between Env and CD4 prior to the recruitment of Gag to the cell-cell contact region (Hubner *et al*., 2009), indicating that Env initially functions as an adhesion molecule already during formation of VS (Chen, 2012).

In HIV-1 also another type of cell-to-cell type infection can occur. This mechanism is called trans-infection and it is provided by infectious synapse. The infectious synapse forms when the virus is captured by a cell without itself becoming infected and directs the intact particle to the target cell through the cell-cell contact (Kijewski and Gummuluru, 2015). Trans-infection is usually associated with transmission from myeloid antigen presenting cells to $CD4^+$ T cells, such as macrophages and dendritic cells, but can occur between different other cell types (Pedro *et al*., 2019). Usually, HIV-1 is captured by surface molecules of APC, such as C-type lectin SIGLEC-1 and stored in non-lysosomal compartments where it avoids degradation and antibody neutralization (Gummuluru *et al*., 2014). After this, interaction between two cells mediated by ICAM-1 and LFA-1 are arranged to form infectious synapse (Rodriguez-Plata *et al*., 2013). Meanwhile, complimentary processes accompanied by the cytoskeleton reorganization, are occurring in the $CD4^+$ T cell. Variety of molecules are directed to the site of contact including CD4, CXCR4 and CCR5 and the receptors required by HIV-1 entry (Pedro *et al*., 2019).

Also synapse independent cell-cell transmissions are utilized by HIV-1, such as phagocytosis, where macrophages phagocytosing dying HIV-1-infected $CD4^+$ T cells subsequently become infected (Baxter *et al*., 2014); syncytium formation, which occurs as a consequence of HIV-1-gp120 on infected cells interacting with CD4 on uninfected cells resulting in the fusion of the two cell membranes (Bracq *et al*., 2018); tunneling nanotubes, where the virus usually transfers through the nanotubes inside the endosomes (Kadiu and Gendelman, 2011a,b); and transcytosis (Pedro *et al*., 2019).

The Nef (negative regulatory factor of HIV) HIV-1 accessory protein is critical for HIV-1 pathogenesis and can self-disseminate in culture via TNTs. Nef can regulate Myosin-X (Myo10), a TNT inducer in neuronal cells, expression, thereby inducing TNT formation, resulting in its own transfer from macrophages to T cells (Uhl *et al*., 2019).

Transcytosis is a process where cells, usually mucosal epithelial cells, capable of internalizing viral particles into vesicles at the apical surface, transport the vesicles to the basal layer and transmit them to $CD4^+$ T cells (Anderson, 2014; Kinlock *et al*., 2014).

During HIV-1 and tuberculosis co-infection, the tuberculosis-associated microenvironment triggers IL-10/STAT3-dependent tunneling nanotube formation in macrophages and promotes thus HIV-1 dissemination (Souriant *et al*., 2019) (Fig. 2).

Flaviviridae

Hepatitis C virus (HCV), is a hepatotropic virus, resulting in acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) in humans. It contains a single-stranded and positive-sense RNA genome and a cellular membrane-derived envelope. The viral polyprotein is cleaved into structural (core, E1, E2, and p7) and nonstructural (NS) viral proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Lindenbach and Rice, 2005). HCV infection occurs in two different forms, cell-free and cell-to-cell transmission. Cell-free transmission is the major route

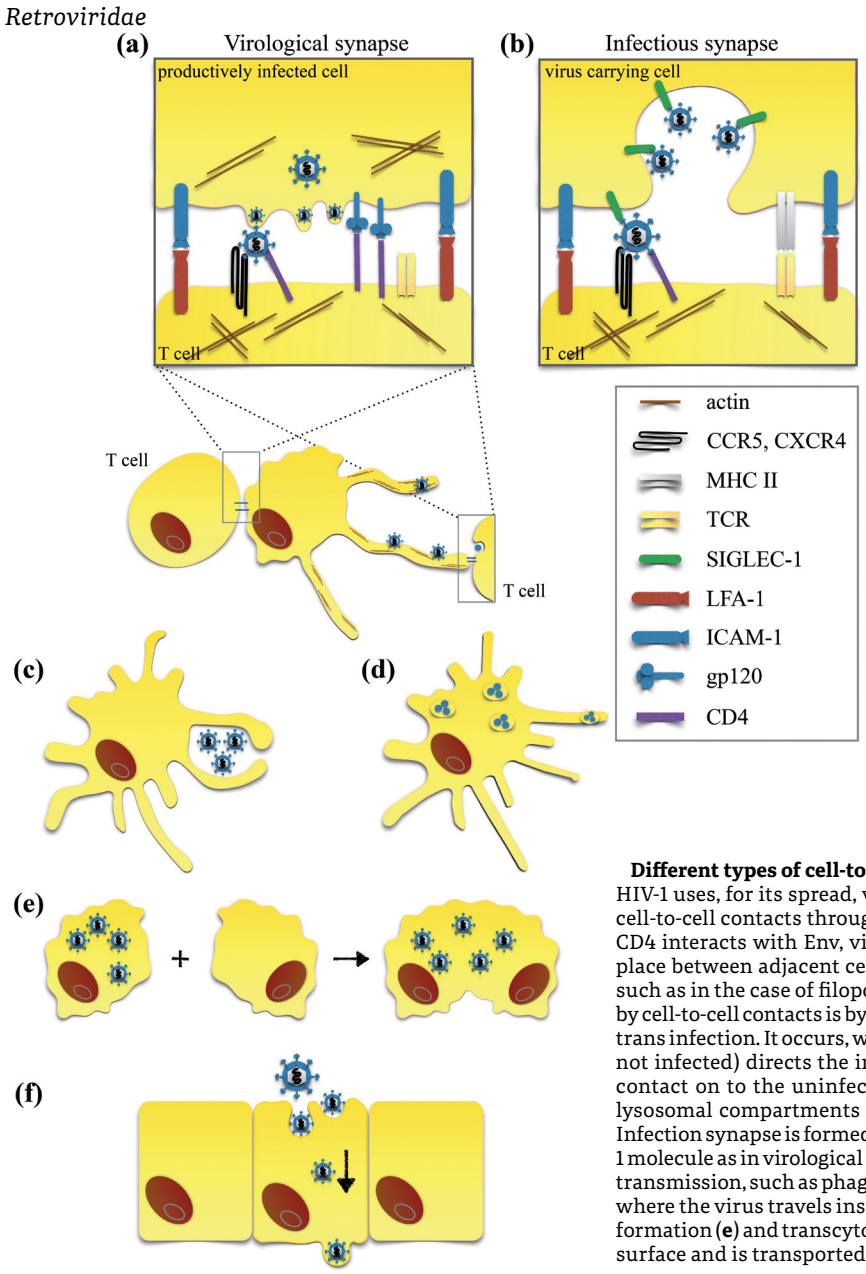

Fig. 2

Different types of cell-to-cell transports in Retroviruses
HIV-1 uses, for its spread, virological synapses (**a**) that form cell-to-cell contacts through ICAM 1, 3 and LFA-1 molecules. CD4 interacts with Env, viral envelope protein. VS can take place between adjacent cells or on relatively long distances such as in the case of filopodia. The second type of infection by cell-to-cell contacts is by infectious synapse (**b**) also named trans infection. It occurs, when cell with captured virion (but not infected) directs the intact particle though the cell-cell contact on to the uninfected cell. Virion is stored in non-lysosomal compartments captured by SIGLEC-1 molecules. Infection synapse is formed by interaction of ICAM-1 and LFA-1 molecule as in virological synapse. Other types of cell-to-cell transmission, such as phagocytosis (**c**), tunneling nanotubes, where the virus travels inside the endosomes (**d**), syncytium formation (**e**) and transcytosis, where the virion enters apical surface and is transported to the basal layer (**f**), are utilized.

(>90%) of HCV infection, which can be blocked by E1/E2-specific monoclonal antibodies. Cell-cell transmission is responsible for the spread of HCV between neighboring cells and is not affected by HCV-neutralizing antibodies (Brimacombe *et al.*, 2011; Timpe *et al.*, 2008). Thus, it is thought that cell-to-cell transmission may contribute to the escape of the host immune response against HCV, resulting in persistent infection. HCV utilizes virological synapses or membrane nanotubes as a way of cell-to-cell transmission (Carloni *et al.*, 2012).

The cell-to-cell transmission of HCV depends on the expression of two cellular proteins, also functioning as post-attachment receptors for the entry of free virus, claudin-1 and occludin present in the junction cell-cell contacts (Brimacombe *et al.*, 2011; Carloni *et al.*, 2012; Timpe *et al.*, 2008; Witteveldt *et al.*, 2009). The host cell molecule CD81 and the tight junction protein claudin 1 (CLDN1) are critical factors for HCV entry. The presence of soluble CD81 and anti-CD81 abrogated cell-free infection of Huh-7.5 cells and partially inhibited cell-cell transfer

of infection. CD81-negative HepG2 hepatoma cells were resistant to cell-free virus infection, however they could be infected after co-culturing with cells infected with a different strain in the presence of neutralizing antibodies, confirming that CD81-independent routes of cell-cell transmission exist. Further experiments suggest that cell-cell transmission is dependent on CLDN1 expression. However, it is suggested that CLDN1 is required but that CD81-dependent and independent routes exist (Timpe *et al*., 2008; Witteveldt *et al*., 2009) (Fig. 3).

Bovine viral diarrhea virus (BVDV) infects cattle and causes major economic losses to the livestock industry. The viral particle is formed by lipid bilayer with protruding envelope glycoproteins E^{rns}, E1 and E2, capsid protein C and the RNA genome (Callens *et al*., 2016; Thiel *et al*., 1991). E2 determines the cellular tropism and interacts with cellular receptors (Liang *et al*., 2003; Maurer *et al*., 2004).

In BVDV infected cells the main mechanism of propagation was antibody resistant spread (Merwaiss *et al*., 2019), referring to the cell-to-cell spread without leaving the cell. The presence of the CD46 receptor is necessary for both cell-free and cell-to-cell transmission (Brimacombe *et al*., 2011; Carloni *et al*., 2012; Timpe *et al*., 2008; Witteveldt *et al*., 2009). Also, the envelope protein E2 is required for cell-associated spread, what was proved by blocking the cell-to-cell transmission by soluble E2. A proposed mechanism of BVDV cell-to-cell transmission involves the egress of complete virus particles in exocytic vesicles and accumulation in the extracellular space at sites of cell-cell contact (Schmeiser *et al*., 2014). Envelope protein E2 attachment to the plasma membrane engages a cellular co-receptor on the target cell which mediates internalization of spreading virions by clathrin dependent endocytosis (Merwaiss *et al*., 2019) (Fig. 3).

The neurotropic *Japanese encephalitis virus* (JEV) causes Japanese encephalitis, an uncontrolled inflammatory disease of the central nervous system. It is a single stranded positive sense RNA virus encoding 3 structural proteins, capsid (C), precursor of membrane protein and envelope protein (E) and 7 non-structural proteins (Misra and Kalita, 2010). The absence of E protein in JEV-infected microglia cells makes it impossible to form the virus particles. However, detection of intracellular dsRNA in microglia demonstrates a replicative form of JEV viral RNA. Viral RNA may be sufficient for cell-to-cell transmission and the recovery of infectious virus (Boyce and Roy, 2007; Yun *et al*., 2007). A cell-to-cell transmission of viral RNA is alternative mechanism enabling the generation of new virus particles (Zhong *et al*., 2013). Human microglia incapable to generate infectious virus particles and production of microglia-associated JEV by target cells depends on cell contact (Lannes *et al*., 2017). Microglia may use virological synapses or membrane protrusions such as nanotubes and/or filopodia structures. Virological synapses require the co-operation of adhesion molecules together with microtubules and actin cytoskeleton stabilization (Bracq *et al*., 2018). The transmission of JEV from microglia to target cells involves CX_3CR1-CX_3CL1 interaction. However, additional or alternative factors may be involved in transmission, since the antagonist of CX_3CR1 did not completely abrogated the JEV transmission (Lannes *et al*., 2019). For instance, DC-SIGN expressed in human microglia (Lambert *et al*., 2008), promotes dendritic cell-to-T cell transmission of JEV (Wang *et al*., 2017) and mediates cellular modifications such as cytoskeleton remodeling promoting filopodia extension (Nikolic *et al*., 2011) (Fig. 3).

Togaviridae

Some members of the *Alphavirus* genus are able to induce the formation of TNTs in several cell types. This induction of TNTs is dependent on both the E2 envelope glycoprotein and the Cp capsid protein, but the cellular pathways through which they act are still unknown (Martinez and Kielian, 2016). The intercellular extensions were long (>10 μm), contained actin and tubulin, and formed flattened contacts with adjacent cells, however they did not mediate membrane or cytoplasmatic continuity between cells. The formation of TNTs did not require the virus receptor, or active particle budding, however it required the E2-capsid protein interaction and TNTs also formed in presence of structural proteins only (Martinez and Kielian, 2016) (Fig. 3).

Arteriviridae

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped positive stranded RNA virus. In infected pigs it mainly infects subsets of swine macrophages present in lungs and lymphoid organs (Labarque *et al*., 2000). A novel mechanism of antiapoptosis/necrosis in PRRSV infected cells was discovered. PRRSV infection induces increased formation of intercellular nanotube connections, and TNTs are determined to be involved in mitochondria transfer between infected and non-infected cells. More importantly, transfer of functional mitochondria through nanotubes rescued the host cell from apoptosis/necrosis in the early stage of infection by PRRSV. On the other hand, mitochondria were observed as a potential transporter of viral infectious materials for cell-to-cell spreading of the infection (Guo *et al*., 2018) (Fig. 3).

Filoviridae

Marburg virus (MARV) causes fulminant hemorrhagic disease in humans and non-human primates with high

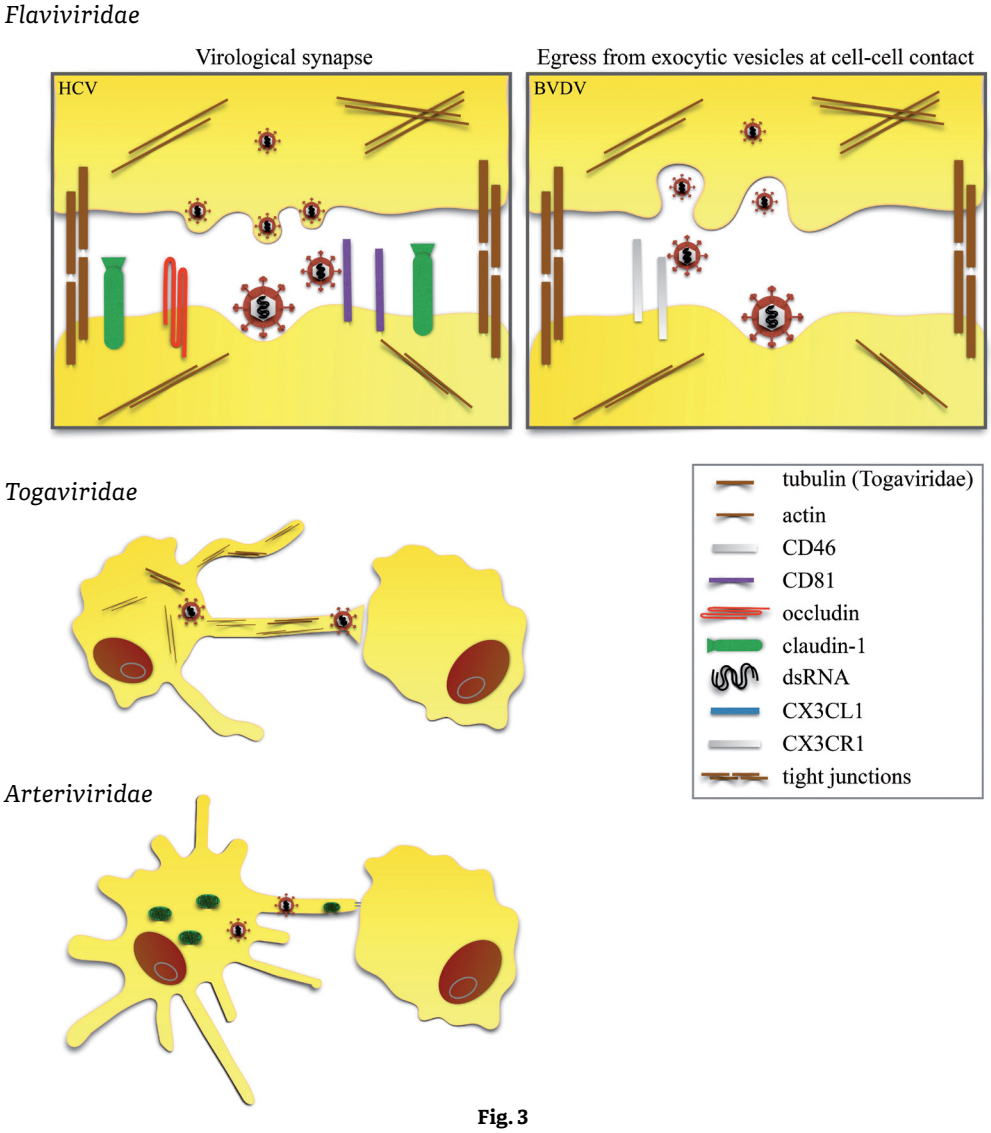

Fig. 3

Different types of cell-to-cell transports in RNA viruses with positive polarity

Flaviviridae: In HCV, cell-to-cell transmission depends on the post-attachment receptors claudin-1 and occluding, present in tight junctions cell-cell contacts, and host cell molecule CD81 that binds envelope protein E2. BVDV infection is mediated by egress of complete virus particles in exocytic vesicles and accumulation in the extracellular space at sites of cell-cell contact. Presence of CD46 is necessary for cell-to-cell spread. JEV uses virological synapses or membrane protrusions such as nanotubes and/or filopodia structures. Virological synapses require the co-operation of adhesion molecules together with microtubules and actin cytoskeleton stabilization and CX_3CR1-CX_3CL1 interaction. Viral RNA may be sufficient for cell-to-cell transmission. *Togaviridae*: The intercellular extensions contain actin and tubulin, and form flattened contacts with adjacent cells. There is no mediate membrane or cytoplasmatic continuity between cells. *Arteriviridae:* PRRSV infection induces increased formation of intercellular nanotube connections. TNTs are involved in the mitochondria transfer between cells.

mortality rates (Peters, 2005). It is a single-stranded negative-sense RNA virus encoding seven structural proteins, viral polymerase; VP35 and VP30 associated with nucleoprotein; glycoprotein present in viral envelope and mediating the viral entry and matrix proteins VP40 and VP24 playing a key role in virion assembly (Kolesnikova *et al.*, 2002).

It is known that MARV is strongly associated with the actin cytoskeleton, which is essential for MARV release from the cell and the viral particles in the process of budding are associated with filopodia (Kolesnikova *et al.*, 2007). VP40 transported in the multivesicular bodies to the place of virion completion is closely associated with filopodia. Filopodia thus can play a strategic role in the

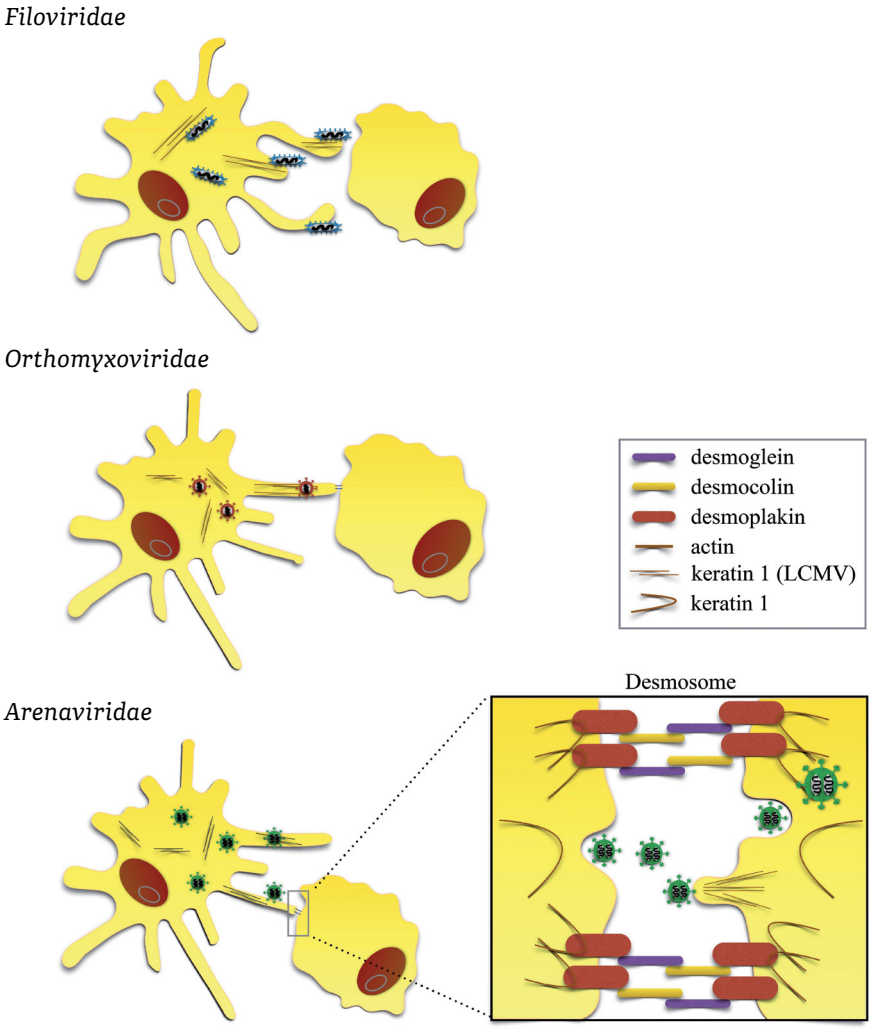

Fig. 4

Different types of cell-to-cell transports in RNA viruses with negative polarity

Filoviridae: MARV is strongly associated with actin. Virion completion is closely associated with filopodia. *Orthomyxoviridae*: IAV increases the frequency of nanotube formation. Viral components are present inside the nanotubes. *Arenaviridae*: LCMV nucleoprotein interacts with keratin 1 (K1), which facilitates its transfer preferentially to the desmosomes, tight junctions consisting of desmoplakin, desmocolin and desmoglein strongly associated with keratin 1. Here the virus crosses the intercellular space. LCMV virion components can also be transported along the nanotubes – inside and also in the extracellular space. LCMV might also utilize actin "tails".

spread of MARV in infected tissue because they can guide viral particles directly to new target cells (Kolesnikova *et al.*, 2007). However, the direct cell-to-cell dissemination has not been proved yet (Fig. 4).

Orthomyxoviridae

Influenza A virus, contains a negative-stranded segmented RNA genome. In humans, it causes respiratory disease with epidemic to pandemic potential. Despite the presence of circulating protective levels of hemagglutination-inhibiting antibodies, influenza viruses can still spread to cause disease. In the experiments with PR8 influenza A virus strain, the infection increased the frequency of nanotube formation, suggesting that the virus may utilize these cellular protrusions to its dissemination. Also, after the blocking the spread of the cell-free virus by Oseltamivir or neutralizing antibodies in the co-cultured infected and uninfected cells, the uninfected cell became infected. The nanotubes were observed to form between the infected and uninfected cells and the transfer of the viral components could be seen in these nanotubes. After

Table. 1 Cell-to-cell transport in different viral families

Family	Example species		Type of transport
	Name	Abbreviation	
Herpesviridae	Bovine herpesvirus-1	BoHV-1	tunneling nanotubes
	Suid alphaherpesvirus 1, pseudorabies virus	SuHV-1	tunneling nanotubes
	Human alphaherpesvirus 1, herpes simplex virus 1	HHV-1	syncytia
Poxviridae	Vaccinia virus	VACV	actin comets
Retroviridae	Human immunodeficiency virus	HIV	viral synapses infection synapses syncytia tunneling nanotubes transcytosis
Flaviviridae	Hepatitis C virus	HCV	viral synapses tunneling nanotubes
	Bovine viral diarrhea virus	BVDV	exocytosis vesicles
	Japanese encephalitis virus	JEV	viral synapses tunneling nanotubes filopodia
Togaviridae			tunneling nanotubes
Coronaviridae			syncytia
Arteriviridae	Porcine reproductive and respiratory syndrome virus	PRRSV	tunneling nanotubes
Paramyxoviridae			syncytia
Filoviridae	Marburg virus	MARV	filopodia ???
Orthomyxoviridae	Influenza A virus	IAV	tunneling nanotubes
Arenaviridae	Lymphocytic choriomeningitis virus	LCMV	tunneling nanotubes actin comets

the inhibition of actin polymerization, the TNT formation and transfer of viral genome was attenuated, proving the possible strategy of virus dissemination by TNTs (Kumar *et al*., 2017) (Fig. 4).

Arenaviridae

Lymphocytic choriomeningitis virus (LCMV) is an RNA virus causing rodent-transmitted persistent infections. It is an important experimental model system to study acute and persistent viral infections, and also a neglected human pathogen of clinical significance (Laposova *et al*., 2013). Specific strain MX establishes persistent infection without the yielding of infectious virus, using thus cell-to-cell contacts for dissemination. Earlier, we have proved that the viral nucleoprotein interacts with keratin 1 (K1), which facilitates its transfer preferentially to the desmosomes, tight junctions connecting two adjacent cells. Here the virus can cross the intercellular space to the neighboring cell, protected from the immune system. Also, the presence of the MX strain induced the expression of K1 and desmosomes (Labudova *et al*., 2009). K1 is essential for the persistent infection of LCMV strain MX and its absence effectively slowed down the course of infection. The presence of the virus enhanced the K1 expression, while the presence of K1 protein potentiated the viral cell-to-cell spread in persistently infected cells (Labudova *et al*., 2019).

We have also proposed the possibility that the LCMV strain utilizes other types of cell-to-cell transmission. Firstly, the utilization of actin, similarly to the vaccinia virus, as the viral nucleoprotein could be seen on the ends of the actin filaments and the infected cells showed richer actin network. Secondly, inside the TNTs, as we could see the particles travelling along the nanotubes and thirdly, traveling along the protrusions associated with the membrane (Labudova *et al*., 2018).

During persistent infection, the virus exploits the host cell without disturbing its vital functions. However, microenvironmental hypoxia can disrupt this delicate balance and escalate virus pathogenesis. It was demonstrated that exposure of cells persistently infected with LCMV MX to chronic hypoxia resulted in increased expression of all virus genes in hypoxia inducible factor-dependent manner (Tomaskova *et al*., 2011). After the exposure to hypoxia the infectious virions begun to form and a traditional receptor-mediated transmission took place instead (Fig. 4).

Conclusion

From many studies it is clearly seen that the viruses don't depend only on the canonic receptor-mediated transmission. They can use many different ways to endure in the cell population even if the most common transmission fails. The viruses from most of the families were able to establish a different path to infect the neighboring cells to survive. They have found the way how to evade the neutralizing antibodies and found a faster way to disseminate. The knowledge about different paths of transmission gives us the possibilities to fight the viruses on both fronts - the classical receptor-mediated and cell-to-cell mediated - and may help us to eliminate some of the threatening diseases.

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