Replicative Cycle of Ebola Virus

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ABSTRACT

Ebola virus responsible for hemorrhagic fever, belongs to filoviridae and fall in biosafety level-4 pathogen. The genome is linear, single stranded RNA, non-segmented and 19kb long. The genome encodes seven important structural proteins and all have essential role in virus replication. These structural proteins are nucleoprotein (NP), Large Protein (L Protein), Viral Protein 35 (VP35), Viral protein 40 (VP40), Glycoprotein (GP), Viral Protein 30 (VP30), and Viral Protein 24 (VP24). The replicative cycle of Ebola virus is crucial at the point of attachment and entry, while VP30 is involved in transcription. Once the virus enter into the cells, VP30 regulate the transcription and replication of viral genome. The phosphorylated VP30 block viral transcription because of the weakened interaction of promoter cofactor VP35. Several studies on recombinant EBOV and wild type EBOV have shown that VP30 containing serine 29 residue has major role in initiation of primary and secondary transcription. This difference is explained by alterations in the balance between the transcription and replication processes and appear to be associated with the state of VP30 phosphorylation. When replication have completed, newly synthesize genome and proteins are carried at site of budding where all these building blocks of virus come together to form virions and then release from the cell to infect other cells.

Keywords: Ebola virus, Structural Proteins, Micropinocytosis, Transcription, Niemann-Pick C1, Acylation, Nucleo-capsid, Nucleoprotein (NP), Large protein (L protein), Viral protein 35 (VP 35), Viral protein (VP 40), Viral protein 30 (VP 30), Viral protein 24 (VP 24), Glycoprotein (GP).

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1. INTRODUCTION

Ebola virus, the causative agent of Ebola hemorrhagic fever, is a well-known member of filoviridae family. Because the form of the virion similar to a twisted thread when examined through an electron microscope, the family name Filoviridae is derived from the Latin word "filum," meaning thread (Ascenzi et al. 2008; Feldman et al. 2013). The Filoviridae family, which belongs to the order Mononegavirales, comprises three genera. 1) Ebola virus 2) Marburg virus 3) Cueva virus (Jun et al. 2015; Burk et al. 2016). All of these have ssRNA, enveloped and a distinctive heterogeneous filamentous (filo; Thread) form (Slenczka 1999). The genome is uniformly 80 nm in diameter and ranges in length from 970nm to 1200 nm. A single molecule of linear, non-segmented, ssRNA, measuring about 19 kilobases (kb), is present in the virion core (Geisbert et al. 1995). Genus Ebola virus (EBOV) has five different distinct species: 1) Virus of Zaire (Zaire Ebola Virus); 2) Sudan's virus (Sudan Ebola Virus); 3) Bundibugyo virus (Bundibugyo Ebola virus); 4) Forest virus (Taï Forest Ebola Virus); 5) virus of Ruston (Reston Ebola virus) (Schieffelin et al. 2014). The first Ebola Virus Epidemic (EVD) reported in Zaire in 1976, which rebaptized to Democratic Republic of Congo, near the Ebola River (Nicastri et al. 2019). In 1976, epidemics of viral hemorrhagic fever (HF) were found to be caused by EBOV in the Congo and Sudan (Emond et al. 1977). EBOV caused disease known as EVD or Ebola Virus Disease, which is rare but deadly (with a mortality rate more than 90%) for mammals and non-human primates (Weingartl et al. 2013; Feldmann and Feldmann 2014).

The Ebola virus is categorized as a category A list pathogen and a biosafety level 4 pathogen. After an incubation period of 4 to 10 days, illness symptoms start to manifest. There are currently no authorized alternatives available for either postexposure prophylaxis or therapy. Virions are sensitive to lipid solvents, Photo induced alkylating probe 1,5 iodonaphthylazide, phenolic disinfectants, irradiations and formalin treatment (Mitchell et al. 1984; Warfield et al. 2007).

The major routes of transmission during an outbreak are nauseating humans or interaction with human bodies, although the natural reservoir of the virus is most likely fruit bats that are asymptomatic and infected with filoviruses, which have extremely high genetic diversity (Towner et al. 2009; Carroll et al. 2013). Other possible transmission methods of virus (present in saliva, stool, semen, body fluids) including direct touch, fomite, vaporizer and droplets (Bausch et al. 2007).

The EBOV structure plays vital act in peculiar infection. RNA based genome of Ebola virus encodes seven structural proteins from leader 3' to the trailer 5': 1) Nucleoprotein (NP); 2) Virion protein 35 (VP35); 3) VP40; 4) Glycoprotein (GP); 5) VP30; 6) VP24; 7) RNA- dependent RNA polymerase (L) (Hoenen et al. 2006; Martin et al. 2016). All the proteins have crucial role in virus replication and transcription as mention in Table 1.

Table 1: Role of structural genes in virus replication and pathogenesis

Proteins	Main Function
NP	Necessary for the development of nucleocapsid-like structures that encapsulates the viral genome, plays central role in virus replication and protects mRNA from destruction (Noda et al. 2005; Kirchdoerfer et al. 2015).
L	Participating in transcription, regulation of viral genome and mRNA editing (Volchov et al. 1999; Ayub et al. 2016).
VP35	Function as an innate immune antagonist; impairs dendritic cell maturation and inhibits antiviral effects by blocking protein kinase R (Basler et al. 2002).
VP40	Necessary for viral assembly at plasma membrane interlinked with viral and cellular component and budding from the host cell (Adu-Gyamfi et al. 2013).
GP $_{1,2}$	Virus attachment and entry; GP_1 make a link of cell surface receptors with viral particles; GP_2 consist of a fusion loop critical for membrane fusion (Volchkov et al. 1998).
VP30	Initiates transcription; involved in packaging of ssRNA and nucleocapsid assembly (Muhlberger et al. 1999).
VP24	Inhibit IFN- α/β and IFN-γ signaling through interaction with importins which is necessary for functional nucleocapsid (Han et al. 2003; Noda et al. 2007)

2. REPLICATIVE CYCLE

Similar to most negative stranded RNA viruses, the replication cycle of Ebola virus follows a basic similar pattern. A generalized sequence of the replicative stages is as follows:

3. ATTACHMENT AND ENTRY

The broad range of mammalian primary cells and cell lines that filoviruses can infect makes it challenging to pinpoint the specific cellular proteins that play a crucial role in viral attachment. After gaining entry parentally through the skin and mucous membrane, Ebola virus attaches to host surface though specific interaction among viral proteins and receptors present on the host's cell surface. The surface of Ebola virus is covered with glycoproteins (Chan et al. 2001) Earlier research has established that the interaction between the viral envelope GP1 protein and specific cell surface factors facilitates the attachment of the virus to its target cells (Chan et al. 2001). GP1 consists of three characteristic domains: 1. Receptor binding domain; 2. Glycan cap; 3. Heavily O-linked glycosylated mucin-like domain. In mature GP_1, receptor binding domain exists along with additional regions that engage with one or multiple receptors located on the surface cells (Kuhn et al. 2006). Although the EBOV mucin domain is not essential for virus entry (Yang et al. 2000; Jeffers et al. 2002), several roles have been proposed for this domain. The X-ray crystallography analysis revealed that the receptor-binding domain is encircled by the glycosylated glycan cap and MLD (membrane-proximal external region), forming a protective layer consisting of complex oligosaccharides (Beniac and Booth 2017). These include human folate receptors, β1 integrins, CLECs (C-type lectins) that specifically bind to glycans on the viral glycoprotein, and phosphatidylserine (PtdSer) receptors that cooperate with the viral envelope. These molecules play crucial roles in facilitating the entry and infection process of EBOV into host cells (Moller-Tank et al. 2013). The C-type lectin family consists of several important members, including DC-SIGN (dendritic cell specific intercellular adhesion molecule 3 grabbing non-integrin) and L-SIGN (liver/lymph node-specific ICAM-3 grabbing non-integrin), along with human macrophage galactose lectin. These lectins play crucial roles in various biological processes, such as cell adhesion and immune response regulation (Alvarez et al. 2002). Recently, a significant role has been attributed to cellular receptors that interact with PtdSer found in viral envelope. These receptors include TIM-1 and TIM-4, which relate to the T-cell immunoglobulin and mucin domain (TIM) family, as well as protein complexes comprising Gas6 or Protein S along with the TAM receptor family of tyrosine kinases (Tyro3, Axl, and Mer). It is well established that PtdSer is present in these interactions (Kondratowicz et al. 2011).

β1 integrins are proteins responsible for attaching cells to the extracellular matrix. The Tyro3 protein kinase (TAM) family consists of Axl, Dtk, and Mer receptors, which are present on the cell's outer membrane in various cell types. When these receptors are activated, they promote cell migration, division, and viability, leading to enhanced cellular functions (Linger et al. 2008). Additionally, it has been shown that folate receptor serves as a coreceptor for Ebola virus and Marburg virus lycol protein making it easier for the viruses to connect to the cells that are expressing their glycoproteins and enter cells more quickly (Simmons et al. 2003; Sinn et al. 2003).

Upon binding to receptor, Ebola Virus move in the host cells through three mechanisms: (a) Macropinocytosis (Quinn et al. 2009), (b) Clathrin-mediated endocytosis (Bhattacharyya et al. 2011; Bhattacharyya et al. 2010) and (c) caveolin-mediated endocytosis. At present, micropinocytosis is supposed to be the chief endorsement process (Saeed et al. 2010; Nanbo et al. 2010; Mulherkar et al. 2011).

Macropinocytosis, observed in certain immune cells like dendritic cells and macrophages, is distinguished by actin-driven membrane ruffling (Jones 2007; Kerr and Teasdale 2009; Mercer and Helenius 2009). Macropinocytosis is linked to the activation of Rho GTPases, such as Rac1 and Cdc42, which trigger the development of membrane ruffles through actin polymerization. For instance, in Vero cells, the entry is mediated by T-cell immune globulin and mucin domain 1 (TIM 1) and involves the activation of the Phosphoinositide 3-kinase signaling pathway. On the other hand, SNB19 cells require TAM tyrosine kinase and phospholipase signaling for viral entry (Liu et al. 2020).

4. FUSION AND UNCOATING

After endocytosis, the subsequent stages involve viral membrane fusion and uncoating where the viral membrane merges with membrane bound vesicles to release viral genetic material in cytoplasm of host cell (Martin et al. 2016). Virion envelopes of enveloped viruses join with the cell's outer membrane during a process of attachment known as fusion (Levinson 2008). EBOV produced three discrete proteins from glycoprotein gene that are, glycoprotein, soluble glycoprotein, and small soluble glycoprotein. whose appearance is influenced, in part, by transcription excision at a specific site containing seven remains of uridine (Volchkov et al. 1995; Sanchez et al. 1996; Mehedi et al. 2011). Crucially, membrane fusion and receptor binding are accomplished by the same transmembrane GP. Within trans-Golgi network (TGN), host cell proteases, including furine, cleave EBOV GP to produce the two components glycoproteins that are GP1 and GP2 (Volchkov et al. 1998; Jeffers et al. 2002). A glycoprotein core, a receptor-binding domain, glycosyl capped, and a mucin-like domain are all components of the GP1 subunit. The GP2 subunit has a cytoplasmic tail, a transmembrane region, heptad repeats 1 and 2, and an internal fusion loop (Sanchez et al. 1996). GP1 plays a crucial role in attaching through receptor-binding site in the host cell (Kuhn et al. 2006). On the other hand, GP2 is responsible for facilitating host cell membrane and virus membrane fusion (Malashkevich et al. 1999). Additionally, the internal fusion loop of GP2 and glycan cap of GP1 may interact to limiting accessibility of fusion peptide and preventing from early fusion events (Weissenhorn et al. 1998). Low pH conditions are required for conformational alternation in the fusion loop that encourage fusion (Gregory et al. 2011).

After the virion has been internalized by micropinocytosis and has moved along the endocytic pathways, the receptor binding site is revealed by the host endosomal cysteine, and cathepsins proteases (low PH-dependent) such as L and B Proteases cleaves the GP1 and GP2 mucin-like domains and glycan capped (Gong et al. 2016). For the virus to connect with the Niemann-Pick C1 obligatory host receptor and transporter cholesterol, this type of proteolysis of EBOV GP1 is necessary (Carette et al. 2011). NPC1 is a thirteen-pass transmembrane protein that is found in delayed endosomes and is thought to be involved in the transport of lysosomal cholesterol. NPC1 is a crucial viral receptor and a host factor for the entry, infection, and pathogenesis of filoviruses (Miller et al. 2012). It has six small cytoplasmic loops, a cytoplasmic tail, 4 small and 3 large luminal loops, and 13 transmembrane domains. The sterol-sensing domain is housed within NPC1 transmembrane regions (Davies and Ioannou 2000). With the aid of a soluble NPC2 protein, it helps cholesterol exit late endosomes so that it can be redistributed to cellular membranes including the endoplasmic reticulum and plasma membrane (Sleat et al. 2004). The late endosome/early lysosome's NPC1 receptor and GP1,2 receptor binding site interact to cause conformational modification in GP1 and GP2, which guide the merging of the virion and endosomal membranes and releases viral genome in cytoplasm (Gong et al. 2016).

5. TRANSCRIPTION AND REPLICATION

The transcription of Ebola virus starts with the synthesis of viral mRNA genome from single stranded and negative sense RNA genome by formation of complementary sequence to existing negative sense sequence. Though the genome contains many nucleotides roughly estimated between 18,000 to 19,000 that encodes for many crucial proteins. Majorly Ebola virus has seven genes that code for many crucial proteins that play significant role in viral life cycle. Due to the diverse and complicated life cycle of Ebola virus, many factors including viral and host, help virus to evade immune system and to manipulate the immune response. The seven genes code for the proteins include nucleoproteins, viral protein 35, viral protein 40, viral protein 30, viral protein 24, glycoprotein and RNA dependent RNA polymerase(L) (Hoenen et al. 2006; Martin et al. 2016.). NP encloses a viral genome which proceed as a model for viral RNA transcription and replication (Ruigrok et al. 2011). Once the virus get entry into the cell, the replicative cycle begins within the host cell cytoplasm (Fig. 1). There also formed secondary sites, termed as inclusion bodies which are formed by the accumulation of NP and other vial proteins, serve as other site of transcription and replication of viral genome (Hoenen et al. 2012; Nanbo et al. 2013; Lier et al. 2017). NP and all its associated proteins play significant role in primary and secondary transcription of viral genome. Transcription starts at the promoter site of viral genome that leads to the transcription of gene from start (Weik et al. 2005). When ample number of proteins have been synthesized from newly made RNA transcript, it leads to the replication of filoviral genome and antigenome. The formation of more and more viral genome act as a template for the formation of more viral protein (referred as secondary transcription). The pre translational editing of EBOV GP gene result into three transcript, pre-sGP, pre-GP, and pre-ssGP, these transcripts respectively translated into pre-sGP pre-GP and pre-ssGP. sGP is encoded by the GP gene of all five species of Ebolavirus. It is initially synthesized as pre-sGP, a golgi-Specific precursor, which undergoes post-translational proteolytic cleavage at its C-terminus by cellular proteases, such as furin, to yield he mature form of the protein. The post translational editing involves cleavage of pre-sGP by furin into sGP and Δ-peptide (Delta peptides of filovirus are actually non-structural peptides and are termed as viroporins, major role in viral pathology) and cleaving Pre-GP forms GP post-translationally into GP1 and GP2 subunits (Sanchez et al. 1998; Volchkov et al. 1998; Jeffers et al. 2002). The mechanism of transcription and replication go side by side, but still the actual phenomenon of regulation of transcription and replication is unclear.

The protein named VP30 has major impact on transcription and replication of EBOV genome (Modrof et al. 2002; Martinez et al. 2008). Phosphorylation of VP30 results in the blockage of transcription and it is due to the weakened interaction of polymerase cofactor VP35. Non phosphorylated VP30 in association with the polymerase cofactor 35 and NP regulate the transcription. The VP30 phosphorylation occurs at six N- proximal serine residue (S29-S31, S42, S44, and S46) and at threonine 143 and 146 (Modrof et al. 2002.; Ilinykh et al. 2014). By checking the mutation at these residual points by alanine which shows up active transcription and aspartate with strong phosphorylated character shows up defective transcription (Elliott et al. 1985; Modrof et al. 2002; Martinez et al. 2008), it shows the actual behavior of phosphorylation towards viral protein transcription. The non-phosphorylated and weak phosphorylation of VP30 show up viral transcription along with replication. Current researches have shown that association in phosphorylated VP30 and polymerase complex ceases the transcription complex and favors the easy access of replicase complex to NP -RNA template (Martinez et al. 2011; Biedenkopf et al. 2013). Only dephosphorylated VP30 mediate viral transcription (referred as transcription activator). Several studies on recombinant EBOV and wild type EBOV have shown that VP30 containing serine 29 residue has major role in initiation of primary and secondary transcription (Elliott et al. 1985; Modrof et al. 2002; Modrof et al. 2003; Martinez et al. 2008; Biedenkopf et al. 2016).

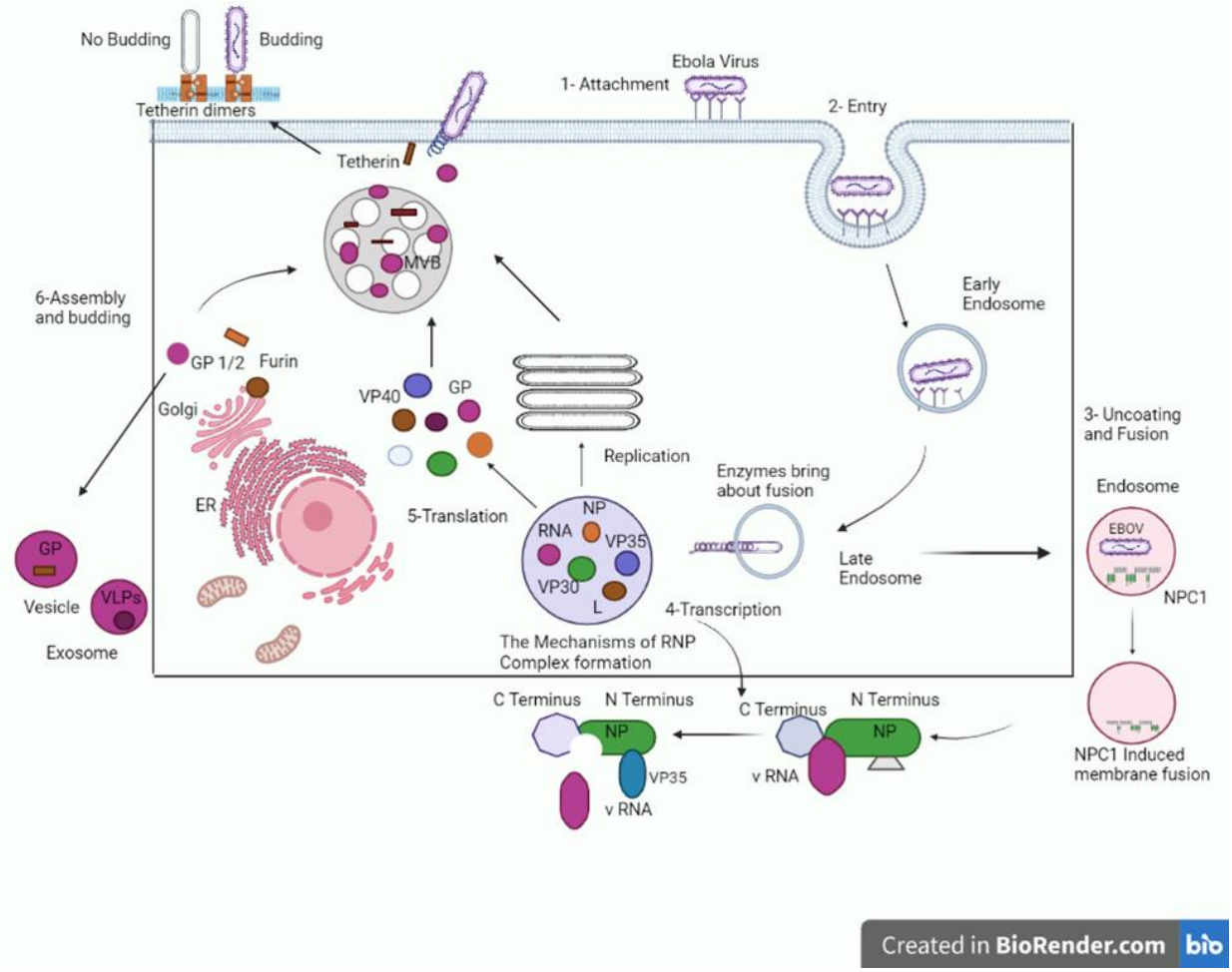

Fig. 1: Replicative cycle of Ebola Virus. Arrows represents the steps involved in Ebola virus replication. Virus attachment to cell surface receptors; Gains entry, Uncoat and fuse with membrane. Replication of genome and viral protein and after assembly and budding release a competent virus from cell.

Some previous work has found two cellular phosphatases i.e., PP1 and PP2A. PP1 and PP2A belong to the phosphoprotein phosphatase (PPP) superfamily. These phosphatases are important for dephosphorylation of VP30 which is mediated by NP (Modrof et al. 2002; Ilinykh et al. 2014; Lier et al. 2017). NP recruits the cellular phosphatase PP2A and VP30 in the viral inclusion bodies via some viral motifs. The degree of proximity between the VP30 and PP2A determines the efficient dephosphorylation of VP30 (Kirchdoerfer et al. 2016; Kruse et al. 2018).

6. ASSEMBLY AND BUDDING

When replication have completed, freshly created proteins and RNA of genome are carried at site of budding, where all these building blocks of virus come together to form virions (Harty et al. 2000; Martin-Serrano et al. 2001; Bavari et al. 2002; Timmins et al. 2003). Despite the few available reading frames for the Ebola virus, little is understood about viral assembly and the regulation of virus replication. According to some research, new formed bits of virus are gathered and budded at cell membrane, whereas viral duplication occurs in the cytoplasm (Feldmann et al. 1996, 1999).

The production of virus capsid with enclosed NA (cylinder shaped duct made up of associated NPNTDs with lumps), which amass in the area around the nucleus and transferred to the burgeoning sites at the cell membrane, is the first step in assembly of viral particles (Beniac et al. 2012; Bharat et al. 2012; Wan et al. 2017). Different functions in viral assembly and budding are played by Virus protein 40, Glycoprotein and NA complexes, and the slight grid protein (VP24) of the Ebola virus (Harty et al. 2000; Bavari et al. 2002; Han et al. 2003).

6.1. ROLE OF THE GLYCOPROTEIN

Glycoprotein produced by endoplasmic reticulum is translated at ER-bound ribosomes (Geisbert et al. 1995; Kolesnikova et al. 2000; Mittler et al. 2013), whereas all other virus-related proteins are decoded at open ribosomes in the cytosol. Acylation, oglycosylation, and ripening of N- linked glycans are all steps in processing of precursor GP before furin's proteolytic cleavage (IIto et al. 2001; Ji et al. 2005; Johnson et al. 2006). Another posttranslational alteration of viral GP, known as acylation, is essential for particle production, including virus assembly and budding. Following those procedures, VP40 and GP come across in the late endosome for assembly and budding (Neil et al. 2008). The Ebola virus is better able to emerge from these specialized microdomains when GP is localized to lipid raft domains (Bavari et al. 2002).

6.2. THE FUNCTION OF SLIGHT GRID VP24 PROTEIN

The function of VP24 has been hypothesized to involve assembly, budding, and, most recently, effective capsids with enclosed NA (nucleic acid) aggregation (Huang et al. 2002; Han et al. 2003). The number of released virions decreased when VP24 RNA was silenced, but viral transcription and duplication were unaffected (Huang et al. 2002).

6.3. ROLE OF THE NP PROTEIN

Nucleoproteins interactions with eachother and with RNA are carried by the hydrophobic amino group, end of the NP protein, whereas the hydrophilic Carboxyl end undergoes a change during the NP-VP40 collaboration (Mateo et al. 2010; Garcia-Dorival et al. 2016).

6.4. NP AND VP40 INTERACTION DURING THE FORMATION OF VP40-INDUCED VLPS

The sandwich structure of the Ebola virus protein VP40 consists of two structurally related realms (Dessen et al. 2000). The inhibition of viral transcription and replication through interactions between the matrix protein VP40 and NP may be accomplished by partial capsids with enclosed NA abridgment, while these interactions also promote NC envelopment at cell membrane and budding (Dolnik et al. 2010; Hoenen et al. 2010; Bharat et al. 2012; Kolesnikova et al. 2012; Wu et al. 2020). Before NP oligomerizes and the viral RNA is encapsidated concurrently with replication, a free amino group peptide of VP35 keeps nucleoprotein in a monomeric form (Kirchdoerfer et al.2015; Leung et al. 2015; Liu et al. 2017).

6.5. MICROTUBULES ARE REQUIRED FOR VLP BUDDING

EBV uses processes based on microtubules to mediate within cell conveyance of NCs to cell membrane and their integration into virions (Greber and Way 2006). VP40 facilitates the association of the Ebola

virus with microtubules. NP comes together to create helical tubes and then joins forces with VP35 and VP24 to form a nucleocapsid-like structure, after forming NC like structure, they are transferred at cell membrane by means of tubulin polymers and interrelates with VP40 to be integrated into virions (Noda et al. 2006; Baker et al. 2016).

6.6. VIRAL PROTEIN 40 IS ESSENTIAL FOR VIRION AMALGAMATION AND THE CONVEYANCE OF NC-LIKE STRUCTURES

The Ebola virus's most prevalent virion protein, VP40, is found underneath the envelop and is important for competent virus release (Harty et al. 2000; Jasenosky et al. 2001; Timmins et al. 2001). The free amino group and carboxyl group realms of VP40 have a distinct folding pattern (Bornholdt et al. 2013), while the Carboxyl side realm of VP 40 is required for membrane contact, the amino group region is sufficient for oligomerization (Ruigrok et al. 2000). The presence of late realm sequences at the amino group side of VP40 including Tsg101 and Vps4 have been found to interact with the components of cells and supports the involvement of VP40 in budding (Harty et al. 2000; Martin-Serrano et al. 2001; Licata et al.2003; Timmins et al. 2003; Yasuda et al. 2003). For a complete virus to be released, cell collaboration between VP40 and inner leaflet also happens as VP40 electrostatic and hydrophobic components are linked to plasma membrane PS which controls the location and oligomerization of VP40 on inner leaflet of plasma membrane (Moller-Tank et al. 2013; Moller-Tank et al. 2014) (Fig. 1).

Another important mechanism in Fledgling is the interaction between GP2 and small glycosylated membrane protein, which can cause an entire virus particle preservation on the cell membrane and is triggered by IFN-α (Neil et al. 2008; Lopez et al. 2010; NH Vande Burgt et al. 2015). A hydrophobic membrane spanning realm and glycosyl cap found in GP2 are thought to contribute significantly to tetherin antagonism (Han et al. 2003; Gnirss et al. 2014).

Since interactions with host cell components are necessary to facilitate the long filovirus NC's movement, it cannot get to the budding site by diffusion alone. An actin cytoskeleton drives the trafficking of filovirus NCs (Licata et al. 2004; Schudt et al. 2013, 2015; Takamatsu et al. 2018). Actin comet tails on one side of moving NCs and NCLS indicate a transport mechanism based on the polymerization of branching actin filaments (Welch et al. 2013; Mueller et al. 2014). Inside IBs, transport-capable NCs made up of all the NC proteins are produced. Actin appendages are created at 1 side of the NC in the cytosol, that propels their movement outside the IBs. Maturing of viruses occurs mostly in long, slender cellular protrusions after reaching the cell membrane, where myosin 10 may facilitate the movement of capsids with enclosed NA along parallel microfilaments. The favored budding sites for filoviruses are enriched filopodia (Kolesnikova et al. 2007; Schudt et al. 2013. 2015; Dolnik et al. 2014). Strongly enhancing NCLS recruitment into filopodia is EBOV VP40 (Takamatsu et al. 2018). Long, thin cellular protrusions known as filopodia are distinctive parallel microfilaments which are cross-linked by fascin (Bornholdt et al. 2013).

In addition to the cell membrane, the internal membranes of MVBs and late endosomes have also been found to host filoviral maturing (Silvestri et al. 2007). Any of two alternative ways of viral budding can occur in virus-infected cells that had numerous virions on their surface. Although numerous virions can emerge horizontally through the cell membrane, filamentous virions are discharged vertically from the cell surface (Roberts and Compans 1998; Brown et al. 2002; Simpson-Holley et al. 2002).

7. CONCLUSION

In conclusion, tremendous advancements have been achieved in the process of Ebola Virus replicative cycles, but still numerous areas that need more clarification.

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