Microbial Pathogenesis 118 (2018) 146–153

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Role of Rab GTPases in HSV-1 infection: Molecular understanding of viral maturation and egress

Sohail Raza[a,*], Gualtiero Alvisi[b], Farzana Shahin[c,d], Urooj Husain[e], Masood Rabbani[a], Tahir Yaqub[a], Aftab Ahmad Anjum[a], Ali Ahmad Sheikh[a], Muhammad Nawaz[a], Muhammad Asad Ali[a]

[a] Department of Microbiology, University of Veterinary and Animal Sciences Lahore 54000, Pakistan
[b] Department of Molecular Medicine, University of Padua, 35121, Italy
[c] The State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China
[d] College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China
[e] Postgraduate Medical Institute Lahore 54000, Pakistan

ARTICLE INFO

Keywords:
Herpesvirus 1
Rab GTPases
Vesicular transport
Viral maturation
Egress

ABSTRACT

Most enveloped viruses exploit complex cellular pathways for assembly and egress from the host cell, and the large DNA virus Herpes simplex virus 1 (HSV-1) makes no exception, hijacking several cellular transport pathways for its glycoprotein trafficking and maturation, as well as for viral morphogenesis and egress according to the envelopment, de-envelopment and re-envelopment model. Importantly Rab GTPases, widely distributed master regulators of intracellular membrane trafficking pathways, have recently being tightly implicated in such process. Indeed, siRNA-mediated genetic ablation of specific Rab proteins differently affected HSV-1 production, suggesting a complex role of different Rab proteins in HSV-1 life cycle. In this review, we discuss how different Rabs can regulate HSV-1 assembly/egress and the potential therapeutic applications of such findings for the management of HSV-1 infections.

1. Introduction

Many enveloped viruses utilize cellular transport pathways during their assembly and egress from the host cell. For example, influenza virus and human immunodeficiency virus 1 (HIV-1) acquire their outer envelope by budding from the plasma membrane (PM) [1,2]. This process involves the interaction between envelope glycoproteins cytoplasmic tails and matrix proteins [3–5]. Moreover, hepatitis B and C viruses acquire their outer envelopes by budding from pre-Golgi membranes, before being released through the secretory pathway [6,7]. Larger enveloped viruses utilize more complex processes of envelopment and egress. For example, African swine fewer virus acquires its inner envelope from the endoplasmic reticulum (ER) and its outer envelope from the PM [8,9]. The triple membrane wrapped core of poxvirus and vaccinia virus are exposed by exocytosis and fusion of the outer membrane with the PM [10]. Moreover, the two outer membranes have been supposed to derive from the TGN or endosomes [11,12].

Herpes simplex virus 1 (HSV-1) is a large, double stranded (ds) DNA enveloped virus with diameter of nearly 200 nm. The mature virion consists of three definite structures found in all *Herpesviridae* members: nucleocapsid, tegument and envelope. The nucleocapsid contains the linear dsDNA surrounded by a proteinaceous icosahedral capsid [13]. HSV-1 tegument, composed by more than 20 virus encoded proteins [14], is believed to represent the link between the inner capsid and outer envelope [15]. The major envelope glycoproteins are gB, gC, and gD, while the minor envelope glycoproteins are gE, gG, gH, gI, gK, gL, gM, and gN. Such envelope glycoproteins are processed through the cellular exocytic pathway and play pivotal roles in virus replication through the regulation of virus attachment, entry, and cell to cell spread [16]. The morphogenesis of *Herpesviridae* members is a complex and dynamic process, that has been difficult to characterize at molecular level, and which is believed to follow the so-called envelopment-deenvelopment-reenvelopment model [17]. According to this model, HSV-1 genome replication and capsid assembly occur in the nucleus. Subsequently, viral capsids acquire a primary envelope at the inner nuclear membrane by budding in the perinuclear space (*envelopment*). The latter is subsequently lost by fusion with the outer nuclear membrane (*deenvelopment*), thus releasing naked capsids into the cytoplasm [18]. The site or source of final envelopment (reenvelopment) has been

* Corresponding author. Department of Microbiology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan.
E-mail address: sohail.raza@uvas.edu.pk (S. Raza).

https://doi.org/10.1016/j.micpath.2018.03.028
Received 16 October 2017; Received in revised form 14 March 2018; Accepted 15 March 2018
Available online 16 March 2018
0882-4010/

subject of intense debate, with new players being constantly added to the process, which now is believed to involve, in addition to multivesicular bodies, also endocytic tublues derived from the PM [19,20]. In addition, recent experimental evidences support a pivotal role of the TGN in HSV-1 secondary envelopment [21]. The TGN comprises three cisternae on the trans side of the Golgi apparatus, all cisternae are able to bud and extend tubules [22]. A recombinant HSV-1 had been constructed that expresses the outer capsid protein VP26, the tegument protein VP22 and the envelope glyocoprotein gB as fusion proteins with three different fluorescent proteins. The fluorescently tagged viral capsids mainly localized at membranes positive for TGN46, the major TGN marker, interestingly gB was clearly found at the TGN. VP22 also found to be facilitated by TGN localization and optimal virion incorporation. This is providing the evidence that wrapping could take place on TGN membranes. Such hypothesis is further strengthened by the fact that most of the viral glycoproteins also colocalized with TGN46 [23,24]. The localization of HSV-1 glyco- and tegument proteins at TGN-derived membranes is very important for secondary envelopment of virions. The standard route for most of the membrane proteins is to PM [25].

Otherwise, the cargo will contain specific motifs that interact with clathrin adaptor proteins which help in vesicle formation. There are three classes of potential membrane trafficking motifs: acidic cluster motif, tyrosine-based motifs and dileucine-based motif. All of these motifs are recognized by components of protein coats with association of the cytosolic domain of membranes [25]. These motifs mediate important roles in vesicles transport. HSV-1 gB and gE glycoproteins, contain tyrosine and dileucine motifs respectively. In addition, HSV-1 gM, gK and pUL20 proteins have tyrosine motifs thatpromote endocytosis through signals from endosome to TGN transport [26]. Further studies showed that gE and TGN46 relocalize to the lateral membranes upon HSV-1 infection of polarized cells [27]. However, in HSV-1 infected human fetal DRGs axon cells (DRG neurons in vitro have a highly polarized structure), TGN46 was detected around viral capsids by using immunogold labeling [28]. Along with specific motifs, several cellular proteins regulate the trafficking events. Key regulators in particular trafficking events are Rab GTPases [29]. Recent studies found that members of Rab GTPases proteins play a key role in glycoprotein trafficking and secondary envelopment of HSV-1 [30].

Since Rab GTPases are becoming attractive therapeutic targets because of their involvement in a number of human diseases, including cancer and viral infections, such findings might open the door to the development of new, highly-needed therapeutics to combat *Herpesviridae* infections.

In this review, we describe how individual Rab GTPases differentially regulate HSV-1 glycoprotein trafficking, secondary envelopment and life cycle and discuss the therapeutic potential implication of such findings for the control of HSV-1 infection.

2. Vesicular transport

In eukaryotic cells the membranous compartments play a major role in cell functions by interacting with the outer environment. The communication between different cellular compartments is achieved by vesicular transport, which enables cargoes shuttling through the exocytic and the endocytic pathways. So far, three main kinds of transport vesicles have been characterized: clathrin coated, coat protein complex I (COPI) and coat protein complex II (COPII) vesicles. Clathrin coated vesicles mediate the vesicle trafficking within the endosomal membrane system [31]. COPI vesicles play a role in intra Golgi transport as well as in the initial phase of ER to Golgi anterograde transport and in Golgi to ER retrograded transport, whereas ER-generated COPII vesicles are involved in ER to the Golgi anterograde transport [32]. COPII vesicles originate from the ER and are involved in transport of secretory proteins to the Golgi [33]. Cargo proteins from ER communicate with COPI coat through adapter transmembrane proteins. The COPI vesicles ensure proper distribution of proteins within the Golgi stacks and are involved in the anterograde transport between the Golgi cisternae, thus playing an important role in cisternal maturation. The traffic using COPI vesicles is also important for ER quality control. The latter mechanism ensures the correct assembly of some multisubunit membrane proteins. These protein assemblies contain diarginine signals responsible for ER retrieval/retention after interaction with coatomer. Such retention signals are masked upon subunit assembly, thus enabling the complexes to exit the ER. Importantly, the first phase of cargo exit from the ER is mediated by COPII coated vesicles. The transition from COPII to COPI vesicles is mediated by the activation of Arf1. Indeed, inhibition of Arf1 activation blocks ER to Golgi transport [34]. The transport of secretory proteins starts from the ER, where they are translated and translocated for folding and post-translational modifications. The correctly assembled and folded proteins, are subsequently exported to the ER-Golgi intermediated compartment (ERGIC) or the Golgi complex in COPII transport vesicles [35]. In most of the eukaryotes, COPII vesicle mediated protein export from specialized regions of the ER named as transitional ER (tER). In these sites, cytoplasmic proteins, collectively called as COPII coat, generate COPII vesicles. The formation of COPII coat is initiated through the conversion of small Ras-like GTPase Sar1-GDP to Sar1-GTP. This conversion is mediated by a guanine nucleotide exchange factor (GEF). Several studies indicated the roles of other additional factors like Sec16 and Sed4 in COPII vesicle formation. There are several different types of export signals found on transmembrane cargo proteins for efficient transport from ER to the Golgi route [36]. For example, mammalian Kir2.1 potassium channel protein has cytoplasmically exposed di-acidic motifs that are necessary for efficient transport from the ER [37]. In addition, specific transmembrane cargo receptors are required for the export of soluble luminal cargoes. These receptors mediated the interaction of cargo with the COPII subunits. For example, ERGIC-53, a transmembrane lectin, is essential for the transport of certain glycoproteins. Like soluble cargoes, GPI-anchored proteins also require a transmembrane for efficient exit from ER [36]. As an example, yeast p24 protein, functions as an adapter protein for efficient ER release of GPI-anchored cargo Gas1 [38]. The Sec23/24, Sec/31 and GTP-locked Sar1 with a non-hydrolysable GTP analog are the components required for the generation of COPII vesicles. Therefore, GTP hydrolysis by Sar1 is important for COPII vesicle formation. Membrane-bound Sar1-GTP form Sec23/24-Sar1 complex, which selects cargo to form a pre-budding complex. Subsequently, the Sec13-Sec31 heterotetramer is likely to cross-link the pre-budding complex which provide the outer layer of the coat and drive membrane deformation to generate COPII vesicles. The COPII vesicles shed their coats before fusion with Golgi and this uncoating is achieved by GTP hydrolysis mediated by Sar1. Sar1 not only recruits the COPII components but also serves as mediator of membrane deformation and vesicle fission. The ability of Sar1 to deform liposomes into tubules correlates with its ability to form vesicles. COPII components are also important for the transfer of the large cargoes within conventional COPII vesicles. However, it is still not clear whether COPII coat cages the membrane encircling large macromolecular complexes or have an indirect function in transport carrier biogenesis [36]. In many high eukaryotes, clathrin coated endocytosis is essential for several PM processes. These processes include signal transduction, neurotransmission and other PM activities. Clathrin mediated endocytosis is the uptake of the material from the cell surface using clathrin coated vesicles. There are several adapter proteins that help for the packaging of different cargoes [39]. There are five different steps of clathrin coated vesicle formation. These steps named initiation, cargo selection, coat assembly, scission and uncoating. Clathrin does not attach directly to the cargo receptors. With the help of adaptor proteins, complexes and accessory proteins, clathrin is recruited to the PM. All the accessory proteins are actually cytoplasmic proteins that are recruited to the site of budding and subsequently recycled back to the cytoplasm where they can be engaged in another cycle of endocytosis