



# Native functionality and therapeutic targeting of arenaviral glycoproteins

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Surface glycoproteins direct cellular targeting, attachment, and membrane fusion of arenaviruses and are the primary target for neutralizing antibodies. Despite significant conservation of the glycoprotein architecture across the arenavirus family, there is considerable variation in the molecular recognition mechanisms used during host cell entry. We review recent progress in dissecting these infection events and describe how arenaviral glycoproteins can be targeted by small-molecule antivirals, the natural immune response, and immunoglobulin-based therapeutics. Arenaviral glycoprotein-mediated assembly and infection pathways present numerous opportunities and challenges for therapeutic intervention.

## Addresses

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## Introduction

Pathogenic arenaviruses from the *Arenaviridae* family can cause a range of human diseases, including severe hemorrhagic fever and fatal transplant-associated infection [1]. Whilst good medical practice [2] and community hygiene [3,4] can limit viral transmission, arenaviruses are under increasing scrutiny due to their categorization as biosecurity risks. Our increasing knowledge of arenavirus structure and pathobiology provides a detailed molecular picture of key events during viral zoonosis and immune resolution of infection. Here, we describe these recent advances and discuss the challenges and opportunities of targeting arenaviruses at that initial stage of host cell entry and viral biogenesis.

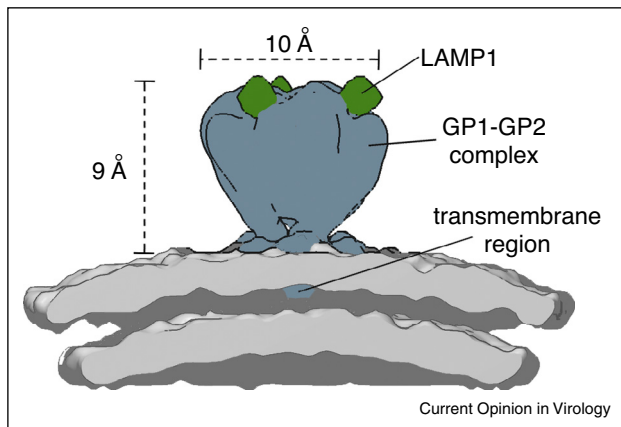
Mammalian arenaviruses are predominantly found in the Americas and Africa and are consequently commonly

classified as either New or Old World, respectively. While currently no clade distinctions are made amongst the Old World arenaviruses, New World arenaviruses are divided into four clades (A–D) [5]. Mammalian New and Old World arenaviruses reside in a range of rodent species and transmission of these viruses to humans is often associated with perturbation of natural habitats. For example, the causative agent for Bolivian hemorrhagic fever was termed Machupo virus (MACV) after an outbreak in the community around the Machupo river, where it is believed mice had been attracted by expanding agriculture. Although rodents are the most significant host reservoir, arenaviruses have also been identified in reptiles. Viruses belonging to the new genus *Reptarenavirus* within the family *Arenaviridae*, are believed to be the etiological agents of the fatal inclusion body disease in alethinophidian snakes [5,7•,8•,9•], but their role in human disease, if any, has not been established. Given the diverse nature of these animal reservoirs, it is likely that further host species will be identified.

The arenavirus genome is ambi-sense and consists of two separate segments of RNA, termed L and S. The L segment encodes the RNA-dependent polymerase (L protein) and zinc-binding matrix protein (Z protein) whereas the S segment encodes the nucleoprotein and the glycoprotein precursor (GPC). During the viral glycoprotein maturation, GPC is cleaved into three components; a stable signal peptide (SSP) of an unusual length that is retained as an essential component of the mature complex, an attachment glycoprotein (GP1) and fusion glycoprotein (GP2) [10]. Low resolution electron cryo-microscopy combined with sub-tomogram averaging has revealed that these three components associate to form a three-legged trimeric spike, measuring 9 nm in height and 10 nm in width (Figure 1), which undergoes pH-dependent conformational changes [11•].

## Structure of the arenaviral GP1 attachment glycoprotein

Crystal structures have been determined for the GP1 attachment glycoproteins of Old World Lassa virus (LASV) [12•] and the New World arenaviruses, MACV [13] and Junín virus (JUNV) [14•] (Figure 2). Despite low sequence conservation between these viral glycoproteins, all three structures display a highly similar compact  $\alpha/\beta$  fold. The domain consists of a twisted  $\beta$ -sheet, which is surrounded by an array of  $\alpha$ -helices. The N-linked

**Figure 1**

Cryo-EM analysis of LASV reveals the higher order architecture and receptor-binding properties of the Old World arenavirus SSP–GP1–GP2 spike complex. Schematic of the 14 Å-resolution structure of the LASV glycoprotein spike in complex with LAMP receptor at pH 5.0 (EMD accession code EMD-3293). Density corresponding to the GP1–GP2 complex is coloured blue, human LAMP1 is green, and the lipid bilayer is grey.

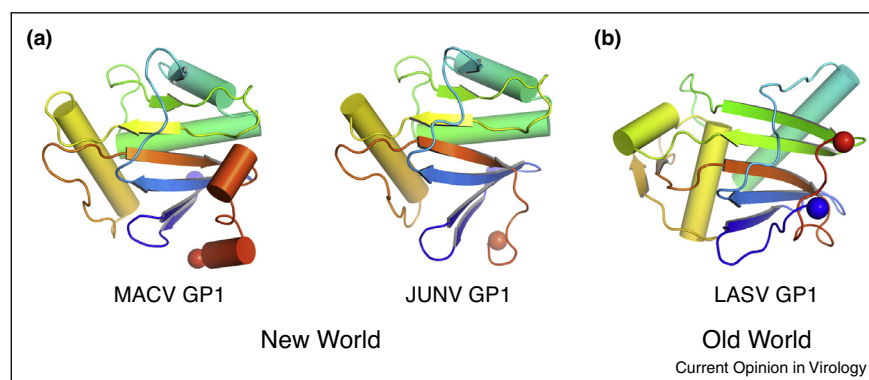
glycosylation sites cluster around the perimeter of the fold, although bioinformatics analysis revealed that they display only limited conservation in absolute position across the family [13]. The most significant conformational difference between the New World and LASV GP1 structures is the re-positioning of the termini [12<sup>\*</sup>]. This has been attributed to a pH-induced conformational change, reflecting the different pH conditions used for crystallization. This hypothesis is consistent with the conformational change that LASV GP1 has been proposed to undergo upon entry into endosomal compartments of the host cell [15<sup>••</sup>]. Nevertheless, this remains to

be systematically tested to exclude the effect of primary sequence divergence between the glycoproteins and other experimental factors such as crystal lattice effects.

### Attachment of New World arenaviruses at the host-cell surface

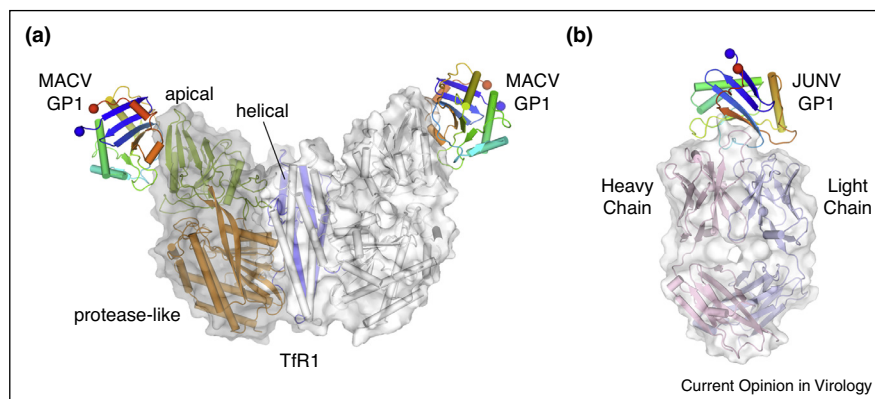
New and Old World arenaviruses utilize different receptors. The hemorrhagic New World clade B JUNV, MACV, Guanarito (GTOV), Sabia (SABV) and Chapare (CHPV) viruses, as well as the apathogenic arenaviruses of the same clade, use transferrin receptor 1 (TfR1) for cell entry into their natural rodent hosts [16,17]. Pathogenic arenaviruses display protein recognition modes by GP1 that lead to cross-reactivity with human TfR1, providing a rationale for viral zoonosis into human populations. This phenomenon is also observed in North American clade D (formerly known as clade A/B or A/rec) [5] viruses. These viruses also utilize TfR1 orthologs of their respective natural rodent hosts, with one member of the Whitewater Arroyo species complex showing additional ability to utilize human TfR1 [18]. Furthermore, although North American arenaviruses are generally considered non-pathogenic, Whitewater Arroyo virus has been tentatively implicated in the death of three people in California [19].

Structural analysis of MACV GP1 in complex with human TfR1 provides a molecular rationale for observed spill over of New World arenaviruses into human populations [20–22] (Figure 3a). MACV GP1 binds to the apical domain of TfR1, at a site not used by either of the physiological ligands transferrin and hereditary hemochromatosis associated protein. Interestingly, it has been suggested antibodies that target TfR1 apical domain may have antiviral potential [23]. However, it is important to note that parameters beyond receptor specificity alone may dictate effective zoonosis. In one extreme example, a

**Figure 2**

Comparison of New and Old World arenaviral GP1 attachment glycoprotein folds. **(a)** Crystal structures of New World MACV (PDB accession code 2WFO, left) and JUNV (PDB accession code 5EN2, right) GP1 glycoproteins. **(b)** Crystal structure of Old World LASV GP1 glycoprotein (PDB accession code 4ZJF). Structures are shown in cartoon representation and ramped from blue at the N-terminus to red at the C-terminus. The N-terminus and C-terminus are shown as blue and red spheres, respectively.

Figure 3



Crystal structures of receptor-bound and antibody-bound New World arenavirus GP1 complexes. **(a)** Crystal structure of MACV GP1 bound to the Tfr1 ectodomain (PDB accession code 3KAS). The Tfr1 homodimer is shown as a cartoon with a transparent van der Waals surface. One protomer of the dimer is coloured according to domain (apical domain coloured green, protease-like domain coloured orange, and helical domain coloured blue) and the other white. MACV GP1 is coloured as in Figure 2. **(b)** Crystal structure of JUNV GP1 bound to the Fab of a neutralizing monoclonal antibody derived from a mouse (PDB accession code 5EN2). The Fab heterodimer is shown as a cartoon with a surrounding van der Waals surface. The heavy chain of the Fab is coloured pink and the light chain coloured blue. JUNV GP1 is coloured as in Figure 2.

recent study on reptarenavirus propagation in cell culture demonstrated ready infection of University of Helsinki virus in human cells although this was only efficient at 30 °C and was significantly impeded at mammalian body temperature [24]. Another example is the finding that all known human-pathogenic arenaviruses, but not non-pathogenic arenaviruses, share a Z-protein-mediated innate immune suppression mechanism that blunts the interferon-mediated antiviral responses [25]. These observations underscore that whilst arenavirus–cell surface interactions are necessary for infection, these conditions alone may not be sufficient to establish infection.

### Old World arenavirus attachment factors

No Old World arenaviruses are known to utilize the Tfr1-mediated host-cell entry pathway. Instead, Old World arenaviruses such as LASV, lymphocytic choriomeningitis virus (LCMV), Mobala, as well as clade C New World viruses Oliveros and Latino, have been shown to functionally recognize the  $\alpha$  subunit of dystroglycan ( $\alpha$ -dystroglycan) [26–31]. Dystroglycan is widely expressed and is integral in linking the extracellular matrix to the cytoskeleton [32,33]. Interestingly, LASV recognition is dependent on the structure of the conjugated O-glycans displayed on  $\alpha$ -dystroglycan. This leads to a dependency for O-glycan biosynthesis factors such as the transferase LARGE, which is important in elaborating O-mannosyl initiated glycans on  $\alpha$ -dystroglycan [30,34,35]. In addition to these entry mechanisms, lectin-mediated and other protein-mediated pathways may contribute to LASV infection of certain cell types [36].

During endocytosis and exposure to the acidic interior of late endosomes (pH 5–5.5), LASV binds to lysosomal associated membrane protein 1 (LAMP-1) [15\*\*] at the tip of the viral spike (Figure 1) [11\*\*]. This pH-dependent interaction [15\*\*] is controlled by a histidine-switch at the putative binding face of LASV GP1 [12\*] and is dependent on both  $\alpha$ 2,3-linked sialylation and the presence of a single N-linked glycan on LAMP-1 [15\*\*]. Although the molecular bases for LASV GP1–dystroglycan and GP1–LAMP-1 interactions are yet to be elucidated, the shared dependency on glycans for both complexes is suggestive of related modes of interaction. Immunologically, Jae *et al.* make the interesting observation that the upper and lower human respiratory tract is dominated by  $\alpha$ 2,6-linked and  $\alpha$ 2,3-linked sialylated glycans, respectively, which may impede LASV infection [15\*\*].

LAMP-1 dependency does not appear to be shared across Old World arenaviruses and, for example, is not required for LCMV infection [15\*\*]. The variable dependency on the lysosomal factors would seem to suggest that we have an incomplete picture of this critical stage of the infection cycle.

### Viral membrane fusion with the host

Following arenaviral endocytosis, the GP1 is shed and membrane fusion is mediated by GP2. It is suggested that GP1 may act as a molecular shield preventing premature conformational changes [11\*\*]. Whilst pre-fusion crystal structures of the GP2 have yet to be determined, the structures of LCMV and GTOV GP2 in their post-fusion conformation reveal a well-conserved, class I fusion protein architecture common to a number of enveloped

viruses such as the *Orthomyxoviridae*, *Filoviridae*, and *Paramyxoviridae* [37,38]. Class I fusion glycoproteins exhibit an  $\alpha$ -helical architecture and form an extended coiled-coil structure upon fusion. Upon cleavage of the GPC, the GP2 is rendered metastable and exposure to endosomal pH drives consequent rearrangements and membrane merger [10]. Interestingly, small molecule inhibitors of arenavirus cell entry are believed to target the pH-sensitive molecular interface between SSP and GP2, resulting in inhibition of pH-induced fusion [10,39].

### Arenaviral glycoproteins are targeted by neutralizing antibodies

The high mortality rate arising from infection by some arenaviruses, such as 15–30% mortality in JUNV infection, indicates that the human immune system can fail to control infection [40]. However, passive transfer of immunoglobulins from convalescent patients is an effective treatment for acute human JUNV infections [41–43]. The molecular basis for viral neutralization has elegantly been revealed through the elucidation of the crystal structure of the JUNV GP1 in complex with a neutralizing IgG2a Fab [14<sup>\*\*</sup>] (Figure 3b). The originating antibody was raised in mice by immunization with inactivated JUNV [44]. The antibody achieves neutralization through the recognition of the Tfr1 binding interface. The interaction mimics a central GP1-receptor contact by inserting a tyrosine residue into a pocket on the GP1. Thus, neutralization is most probably achieved by interfering with binding to the host cell receptor. Consistent with this hypothesis, Mahmutovic *et al.* demonstrate that the receptor binding site is also a targeted by antibodies contained in the polyclonal immunoglobulin pool of JUNV survivors [14<sup>\*\*</sup>]. Given the practical limitations of convalescent serum therapy [41], the GP1-Tfr1 interface provides a promising target for rational development of alternative antibody and small-molecule antivirals [45]. An example of promising antibody therapy is a glycoprotein-specific IgG cocktail, produced by DNA vaccination, that has been demonstrated to protect experimental animals against lethal JUNV and GTOV challenge [46]. Encouragingly, some, albeit limited, cross-neutralization was observed between the JUNV and MACV glycoprotein-specific sera [46].

### Prospects for arenaviral vaccines

Although cell-mediated immunity is likely to play an important role, the ready neutralization of JUNV by antibodies is consistent with the development of the only currently available arenavirus vaccine Candid#1, which is composed of live-attenuated JUNV and is licensed in Argentina for the vaccination of populations within high-risk areas [47]. The successful development of a JUNV vaccine has stimulated considerable interest in the development of vaccines to other arenaviruses and potentially cross-protective vaccines [46,48–51].

The success in vaccine development and the efficacy of survivor plasma transfusion in the treatment of JUNV-mediated hemorrhagic fever has, however, not been mirrored in LASV vaccinology [52,53]. Delayed and weak neutralizing antibody responses have been observed both in natural infection [54] and in candidate vaccine trials [55,56]. Interestingly, the higher glycan density on the LASV GP1 compared to JUNV GP1 does seem to shield the LASV envelope against efficient antibody-mediated neutralization [57]. Despite these observations, the discovery of antibodies that can bind to and penetrate the glycan shield of the attachment glycoprotein of the human immunodeficiency virus gives hope that these glycan effects are not insurmountable [58–60]. Nevertheless, it is currently uncertain whether successful arenaviral vaccines will be dependent on potent antibodies or whether they will rely predominantly on a strong cellular component.

### Antivirals targeting glycoprotein folding

Although ribavirin has been found somewhat effective for the treatment of hemorrhagic fevers caused by arenaviruses such as LASV and JUNV [61], there are currently no virus-specific small-molecule antiviral agents approved for the treatment of arenaviruses. However, arenaviruses have been shown to be vulnerable to drugs that target viral glycoprotein folding by inhibiting host cell ER  $\alpha$ -glucosidases, such as iminosugars. These small molecule drugs have demonstrated *in vitro* efficacy against Pichinde virus, JUNV, and Tacaribe virus [62<sup>\*</sup>]. The initial success of these compounds highlights the differential dependency of the glycan-mediated folding pathway between self and viral glycoprotein biosynthesis and suggests a promising window for therapeutic control of infection.

### Perspectives

Recent structural analyses of arenavirus glycoproteins, alone and in complex with receptors and antibodies, have revealed that these surface-exposed molecules are both key determinants of species tropism and clear targets for therapeutic intervention. We anticipate that further structural and mechanistic studies will further help to define features of the arenaviral life cycle that can be targeted in next generation cross-protective vaccines and therapies.

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