Minireview

Structural and molecular biology of Sabiá virus

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Impact statement

We present the first comprehensive review of Brazilian mammarenavirus or Sabiá virus (SABV) biology, focusing on molecular and structural aspects of viral proteins. Literature on SABV is scarce, mostly due to the inexistent biosafety level 4 (BSL4) infrastructure necessary for SABV research, and usually focused on epidemiology or retrospective case studies of Brazilian hemorrhagic fever in humans. We used protein structure prediction and sequence comparisons to determine common and shared features of SABV proteins and their counterparts in New World (NW) and Old World (OW) arenaviruses. In addition to the state-of-the-art knowledge on SABV, we provide insights into SABV biology, transmission, interaction with the proposed cellular receptor, and viral replication inferred from protein biology. There are no vaccine candidates or specific treatments available against SABV infection or Brazilian hemorrhagic fever, for which we suggest viral protein targets that may be explored in search of effective countermeasures, including compounds, neutralizing antibodies, and vaccine platforms repositioned from their intended applications against related arenaviruses.

Abstract

Brazilian mammarenavirus, or Sabiá virus (SABV), is a New World (NW) arenavirus associated with fulminant hemorrhagic disease in humans and the sole biosafety level 4 microorganism ever isolated in Brazil. Since the isolation of SABV in the 1990s, studies on viral biology have been scarce, with no available countermeasures against SABV infection or disease. Here we provide a comprehensive review of SABV biology, including key aspects of SABV replication, and comparisons with related Old World and NW arenaviruses. SABV is most likely a rodent-borne virus, transmitted to humans, through exposure to urine and feces in peri-urban areas. Using protein structure prediction methods and alignments, we analyzed shared and unique features of SABV proteins (GPC, NP, Z, and L) that could be explored in search of therapeutic strategies, including repurposing intended application against arenaviruses. Highly conserved catalytic activities present in L protein could be targeted for broad-acting antiviral activity among arenaviruses, while protein-protein interactions, such as those between L and the matrix protein Z, have evolved in NW arenaviruses and should be specific to SABV. The nucleoprotein (NP) also shares targetable interaction interfaces with L and Z and exhibits exonuclease activity in the C-terminal domain, which may be involved in multiple aspects of SABV replication. Envelope glycoproteins GP1 and GP2 have been explored in the development of promising cross-reactive neutralizing antibodies and vaccines, some of which could be repurposed for SABV. GP1 remains a challenging target in SABV as evolutive pressures render it the most variable viral protein in terms of both sequence and structure, while antiviral strategies targeting the Z protein remain to be validated. In conclusion, the prediction and analysis of protein structures should revolutionize research on viruses such as SABV by facilitating the rational design of countermeasures while reducing dependence on sophisticated laboratory infrastructure for experimental validation.

Keywords: Sabiá virus, *Brazilian* mammarenavirus, New World arenavirus, hemorrhagic fever 25

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Introduction

The *Arenaviridae* are zoonotic viruses distributed worldwide and infecting different natural hosts, in which viruses within *Mammarenaviridae* have mammals as hosts.[1] Mammarenaviruses are divided into two independent groups: Old World (OW), endemic to central Africa, and New World (NW) which are endemic in the Americas.[1,2] Based on the established knowledge of NW and OW arenaviruses such as Machupo (MACV), Junin (JUNV), lymphocytic choriomeningitis virus (LCMV), and Lassa (LASV) virus, it is reasonable to assume that Brazilian mammarenavirus or Sabiá virus (SABV) shares key biological aspects, including virus structure, infection, replication, pathogenicity, and transmission. SABV is associated to a fulminant hemorrhagic disease in humans named Brazilian hemorrhagic fever (BHF).[3–5] Although reported SABV infections in humans are rare, SABV can be easily transmitted through aerosols and has a fatality rate of up to 50% (Figure 1(B)).[3] There are no specific treatments or vaccines against SABV. Diagnosis of BHF is difficult, with non-existent diagnostic methods besides sequencing/PCR testing restricted to reference or research laboratories, and usually performed retrospectively.[3] Thus, most SABV cases may go unnoticed, and the

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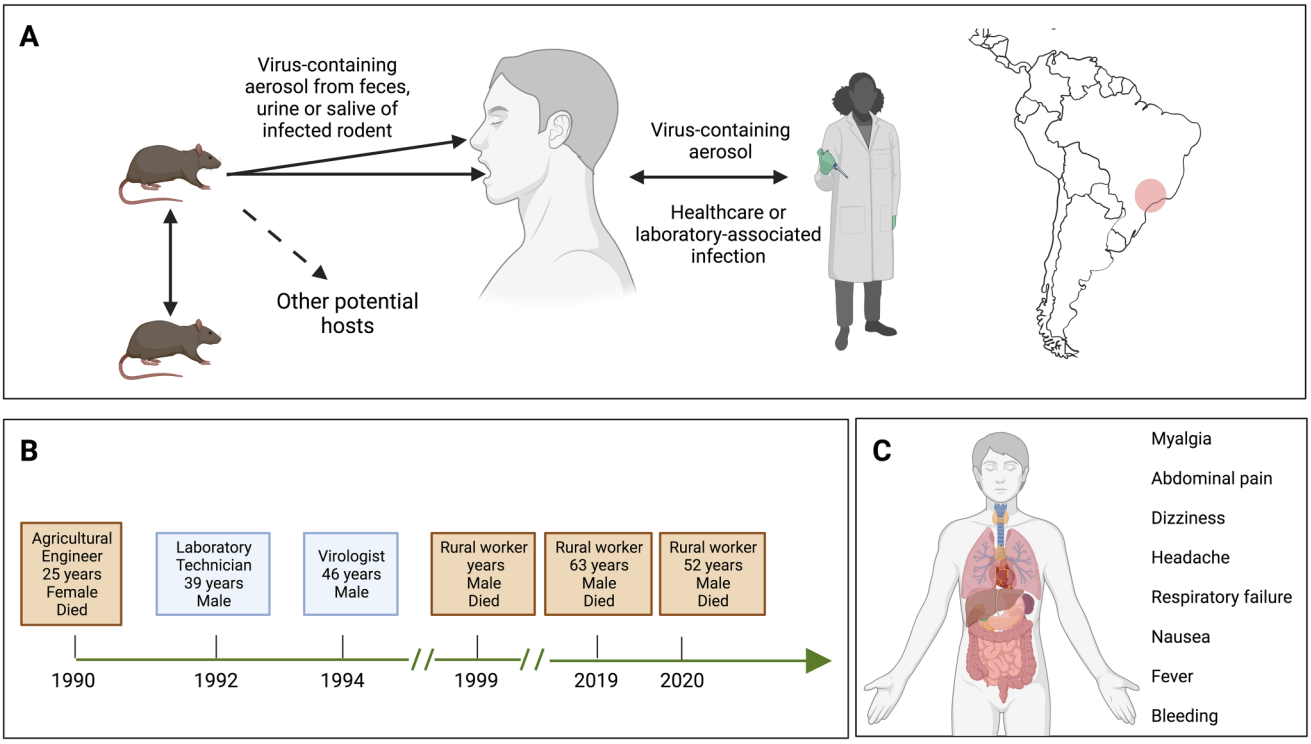

Figure 1. Basic biology of SABV. (A) Proposed mechanism of SABV transmission and geographic distribution. The virus is likely transmitted to humans through contact with rodent urine feces or saliva that contains the virus, particularly in areas where humans come into proximity with the rodents. Geographical distribution of SABV in Brazil (pink area) highlighting the Sao Paulo region of Brazil. (B) The most closely associated symptoms of infections caused by SABV. (C) Timeline of SABV infection. The natural infections are represented in orange, while the laboratory-associated accidental infections are shown in blue. Figures were created using BioRender.com.

possibility of SABV emergence constitutes a significant risk to human health.

Transmission of SABV to humans is most likely through ingestion or exposure to aerosols generated from feces, urine, and saliva containing virus in periurban areas where humans and wild rodents coexist (Figure 1(A)).[1,2,4,6] Rodents from the Muridae family are the reservoirs of most mammarenaviruses, and the determination of the geographic distribution of each arenavirus is contingent upon the range of its respective murine reservoir.[7] So far, SABV was detected only in Brazilian territory, and the natural reservoir has not been identified.[7–9] Rodent species from the *Calomys* genus may be a potential reservoir of SABV, as these rodents are broadly distributed across the Brazilian territory, particularly in regions where cases of SABV have been reported (Figure 1(A)). Moreover, *Calomys* species have been identified as carriers of JUNV,[10] MACV,[11] Latino (LATV),[12] and Pinhal[13] viruses.

The first documented case of SABV infection involved a 25-year-old agricultural engineer who succumbed to hemorrhagic fever in 1990[5] (Figure 1(B)). Subsequently, in 1992, a laboratory technician involved in characterizing the initial infection was hospitalized with influenza-like symptoms and later diagnosed with SABV infection.[14] Another case occurred in 1994, when a 46-year-old virologist working with SABV in a biosafety level 3 (BSL3) environment became infected and required hospitalization.[15] In 1999, a natural infection case was reported in a rural worker,[4] and in 2019, two individuals aged 63 and 52 years succumbed to natural SABV infection.[3] The initial symptoms of SABV infection can include fever, headache, malaise, and myalgia (Figure 1(C)). As the infection progresses, patients may experience more severe symptoms, such as nausea, vomiting, diarrhea, abdominal pain, and a rash.

Hemorrhagic symptoms, including bleeding from the nose, gums, and other mucous membranes, can also develop in severe cases. In some patients, SABV infection can progress rapidly, leading to multi-organ failure and shock. Neurological symptoms, such as confusion, seizures, and coma, have also been reported in some cases.[3–5,14,15]

Histopathological examinations revealed that lesions associated with SABV infection in humans were predominantly localized in the liver and the reticuloendothelial system. After infection, SABV can infect steatotic and apoptotic hepatocytes, Kupffer cells, and inflammatory portal cells.[3] The liver exhibited evidence of macrovesicular and microvesicular steatosis, hepatocyte apoptosis, and minimal inflammatory response. Transmission electron microscopy (TEM) analysis only detected a few virions. SABV induced a marked response from macrophages in the reticuloendothelial system, as evidenced by cellular phagocytosis in the liver by Kupffer cells, sinusoidal cells in the spleen, and macrophages in the bone marrow and lymph nodes.[3] Due to the lack of specific treatments, patients are hospitalized and

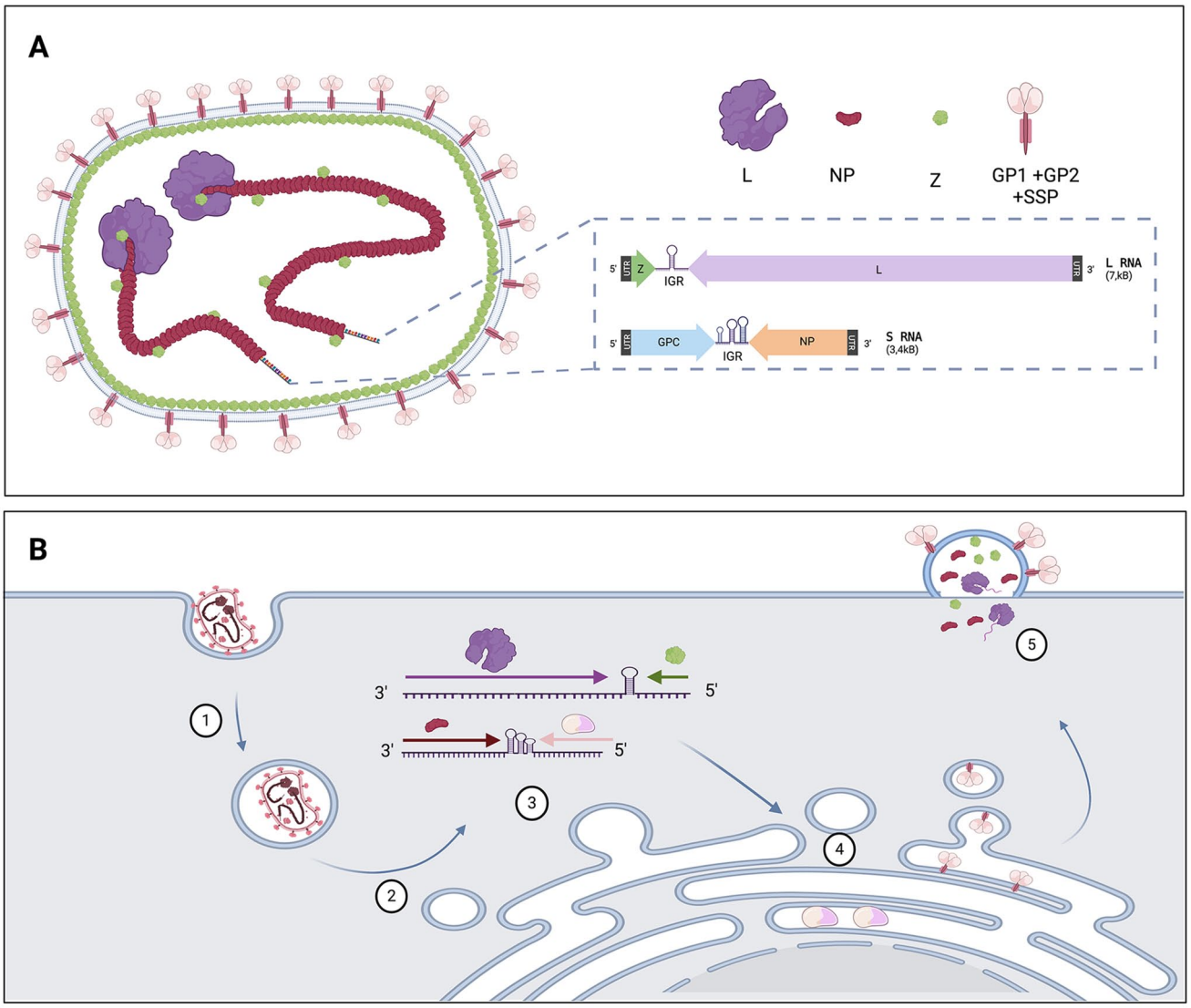

Figure 2. Schematic representation of the viral particle, genome, and life cycle of SABV. (A) The predicted structure of the assembled viral particle consists of the L and S segments, L polymerase protein, Z matrix protein, glycoprotein (GPC), nucleoprotein (NP), and ribosome. SABV genome is composed of an ambisense RNA genome consisting of two segments: L (large) and S (small). The L segment encodes the L and Z proteins, while the S segment encodes the GPC and NP. (B) SABV life cycle: The infection cycle of SABV is composed of (1) the virion attachment to the host cell and undergoes receptor-mediated endocytosis; (2) fusion with late endosome and acidification of endosome leading GP2 to envelope-mediated fusion, release of viral nucleocapsid-related proteins into the cytoplasm, and synthesis of antigenomic vRNA followed by the synthesis of genomic RNA; (3) early transcription of NP and L mRNA and translation followed by late GPC and Z mRNA transcription and translation; (4) GPC glycosylation and SSP cleavage in RE and cleavage of GP1 and GP2 into Golgi complex; (5) virus assembly and budding for virion release. Figures were created using BioRender.com.

submitted to supportive care, including intravenous fluids, electrolyte replacement, and blood products.

SABV is an enveloped RNA virus, pleiomorphic in shape, ranging in diameter from 30 to 400 nm, composed of all virus-encoded proteins (GP1, GP2, SSP, Z, L, and NP) and an ambisense segmented genome containing segments L (large) and S (small) (Figure 2(A)).[16] The single genomic segments of SABV contain two open reading frames, separated by an intergenic non-coding region (IGR).[16] The IGR in both the L and S segments form energetically stable stem-loop structures, with one and three hairpins, respectively. These structures may play a crucial role in mRNA synthesis termination and translational initiation of a second gene product. Notably, the S segment displays a unique predicted secondary structure, consisting of two large stem-loop structures and a smaller third structure.[16] This distinguishes SABV S RNA from the S RNAs of other arenaviruses, which are predicted to form just one (Lassa and LCMV), two (MACV), or three (JUNV and Sabia) stem-loop structures, as per the RNA fold webserver.[17,18] Arenaviruses may also pack one or more ribosomes from host cells into their virions, which are reminiscent of sand grains in electron microscope images, or "arena" in Latin.[19]

The L segment is approximately 7.2 kb in length and encodes for the L and Z protein.[20] L protein is a massive protein comprising all SABV-encoded enzymatic activity

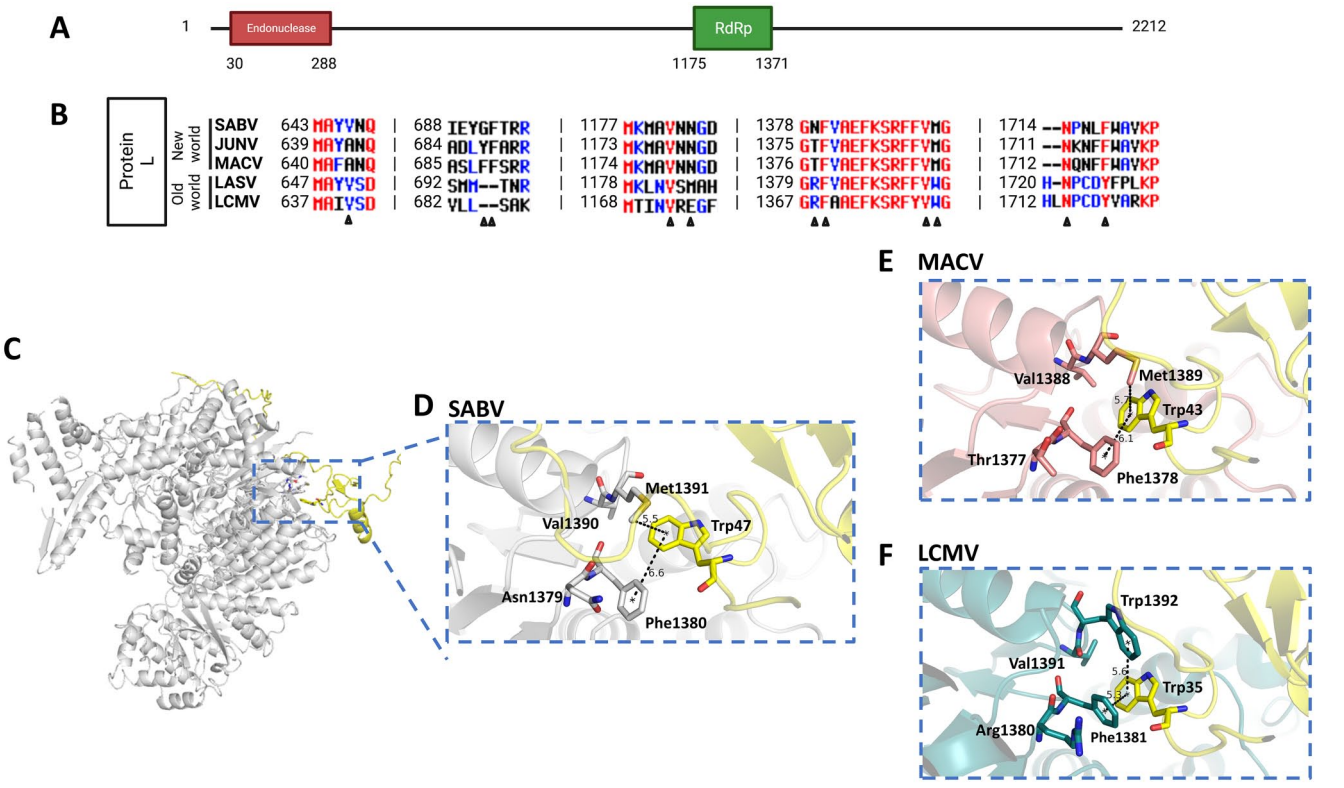

Figure 3. Model of arenavirus SABV polymerase. (A) Schematic representation of the linear organization of arenavirus L protein. (B) Sequence alignment comparison of New World and Old World arenavirus L protein. (C) Structural prediction model of SABV polimerase (Gray) and its interaction to Z protein (Yellow). Interaction interface inset to Z protein of SABV (D), MACV with pdb code 7CKM (E), and LCMV with pdb code 4O6I (F). Figure 3(A) and (B) were created using BioRender.com.

required for replication,[21] while the abundant matrix protein (Z)[22] plays a major role in viral assembly and budding from the infected cell.[20] The S segment is approximately 3.4 kb in length and encodes for the viral nucleoprotein (NP) and glycoprotein precursor (GPC).[20] NP binds to the viral RNA to form the ribonucleoprotein (RNP) complex and supports viral RNA replication and transcription.[23–25] GPC is cleaved into SSP, GP1, and GP2, which are involved in SABV tropism, attachment, and invasion of host cells.[26] Multiple interactions between the RNP complex, the envelope glycoproteins, multiple copies of Z, and the multifunctional L protein are necessary either for viral replication or formation of viable infectious SABV, which will be addressed in the following sections.

The life cycle of arenavirus initiates with the virus entering host cells via receptor-mediated endocytosis. Subsequently, pH-dependent membrane fusion occurs, leading to the release of viral nucleocapsid-related proteins (vNRPs) into the cytoplasm. Early and late transcription phases ensue, leading to genome replication, translation, and processing of the GPC. Finally, virion assembly and budding occur (Figure 2(B)). Arenavirus RNA synthesis occurs after delivery of two encapsidated S and L segments into the cytosol, each associated with the RNA-dependent RNA polymerase. The L protein initiates RNA synthesis from the 3′ end of the genomic template and leads to the production of genome-complementary (subgenomic), NP, and L mRNAs. These mRNAs are capped but not polyadenylated.[27] Transcription of GPC and Z mRNAs only occurs after one round of arenavirus replication, in which S and L antigenomes are synthesized. As a result, accumulation of NP mRNA and NP protein occurs earlier in replication when compared to GPC mRNA and the mature forms of SSP, GP1, and GP2 glycoproteins. Likewise, expression of L mRNA and protein precedes Z mRNA and protein expression.

L Protein

The RNA-directed RNA polymerase L or L protein is a 2212-amino-acid protein with approximately 253 kDa. L protein has two major domains, the RNA-dependent RNA polymerase and endonuclease domains (Figure 3(A)). During the replication cycle of SABV, L protein replicates the viral genome and participates in transcription of viral genes, and thus, is considered a major target for antiviral drug development.[28,29] An antiviral strategy targeting the L protein instead of other SABV proteins should be advantageous, as protein structure and catalytic activities present in L are highly conserved among arenaviruses (Figure 3(B)) and critical for viral replication, which suggests a reduced risk of emergence of drug-resistant variants and a potential for broad-spectrum antiviral activity. RNA-dependent RNA polymerase activity is not existent in vertebrate cells, and structural or functional similarities between host and arenaviral enzymes are little to none. Thus, off-target effects of candidate antiviral compounds targeting L protein are less likely, reducing the

risk of high toxicity when compared to antiviral compounds targeting host cell processes.[30]

The replication and transcription of the arenavirus genome depend on the coordinated activity of the Z and L proteins.[31] The Z protein binds as a staple to L protein, in the intersection between RdRp, PA-C-like, and PB2 N-like regions, preventing it from mediating viral RNA synthesis (Figure 3(C)). The structure of L protein complexed with Z in MACV (Figure 3(E)) and JUNV (Figure 3(F)) by Cryo-EM identified important sites of interaction with the Z protein.[29,32] Using a sequence alignment of L proteins from different arenaviruses, we mapped the interacting sites at positions A646, F691, F692, V1181, N1183, N1379, F1380, V1390, M1391, N1714, and F1718 in SABV (Figure 3(D)). A comparison of the amino acid sequences of Z and L of several NW and OW arenaviruses reveal some differences in these interaction sites. While amino acid residues V1181, F1380, V1390, and N1714 are highly conserved among arenaviruses, residues F692, N1183, M1391, and F1718 of the L protein are conserved only in NW arenaviruses, including SABV, most likely affecting function, interaction force, and protein activity. In LASV, the asparagine is replaced by a methionine, and in LCMV, by a glutamic acid (Figure 3(B)). Also, site F692 is conserved in NW arenaviruses but is absent in OW arenaviruses. The amino acids M1391 and F1718 found in NW arenaviruses are replaced by Tryptophan and Tyrosine, respectively, in OW arenaviruses (Figure 3(B)). Interestingly, we found that the site N1379 in SABV corresponds to a threonine residue in JUNV and MACV, but to an arginine residue in OW arenaviruses (Figure 3(B)). The N1379 mutation differentiates SABV from other NW arenaviruses and may be a product of selective pressure during SABV evolution, as this mutation may change interactions between Z and L proteins and modulate the activity of L. As the number of available sequences of SABV is limited, minor differences as N1379 mutation, as do other amino acid variations involved in the interaction between arenavirus Z and L proteins, must be experimentally validated. The molecular mechanisms underlying this interaction could be explored in the development of antiviral compounds that target viral protein-protein interactions instead of catalytic sites. Such an approach is also unlikely to cause toxicity due to off-target effects in mammalian cells but is posed to inhibit a specific arenavirus infection due to specificities as indicated in SABV.

Nucleoprotein

NP is the viral protein that is associated with viral RNA and the most abundant protein in arenavirus-infected cells.[23] NP organizes the viral genome by forming RNA-protein complexes named viral ribonucleoprotein (vRNP) particles, which participates in gene transcription and RNA replication.[24,25] The RNP complex locates at virus budding sites in association with the Z protein and host cell membranes that are subsequently recruited in developing virions. Studies using purified vRNPs showed that Z protein is complexed with vRNPs by binding NP.[33] Moreover, recent studies have elucidated the molecular basis for NP-Z interaction in different arenaviruses.[34]

The majority of NP is organized in two structured domains, named amino and carboxy terminal domains (NTD, CTD), according to structural investigations and functional analyses performed with different arenaviruses.[35–38] SABV NP comprise 62.8 KDa with 562 amino acid residues, with prediction models showing an overall organization similar to that of related arenaviruses, with ordered NTD and CTD united by a short and disordered linker (Figure 4). The NTD (SABV 9, 338 aa) is an alpha-helical motif with an RNA-binding site described as a pocket with positively charged residues to anchor phosphorylated ribonucleotides (K249, R296, K305, and R319 for SABV) and a hydrophobic cavity for cap-binding activity (W160, L168, and F172 for SABV).[35] RNA binding is regulated through a gating mechanism, where the apo-form conformation presents an alpha helix 5 extension, with the alpha helix 6 orientated over the RNA binding site, establishing a trimer-ring with threefold symmetry in closed conformation. Upon the RNA/NP complex formation, the cleft hidden by alpha helix 5-6 is opened, and the interaction with viral RNA drives oligomerization. Such conformation is characterized by a more linear head-to-tail alignment, in which the oligomer binds viral RNA, protecting from host RNAse activity and facilitating access by the L protein RNA polymerase.[38] NP also presents a 3′–5′ exoribonuclease activity described in LASV[39] located in the CTD (SABV 357; 556 aa), with participation of residues D381, E383, D458, D517, and H522 that are located adjacent to the beta-sheet and found to be conserved in arenaviruses.[35,39] This positively charged catalytic site depends on divalent cations for activity, on pocket size, and the location of amino acid residues, as shown for JUNV NP, which has D457 placed further from the site and presents no exonuclease activity *in vitro*.[36] The roles of CTD exonuclease activity in NP includes the suppression of IFN production, by depleting viral RNAs that would serve as pathogen-associated molecular patterns (PAMPs)[40] and trigger innate immunity in infected cells.[35]

Glycoprotein precursor

SABV GPC is a 70–80 kDa polyprotein precursor to the stable signal peptide (SSP), glycoproteins GP1 and GP2, as in other arenaviruses[41] (Figure 5(A)). Once expressed in infected cells, GPC localizes to cellular membranes, which are eventually subverted into the SABV lipid envelope. GPC is cleaved by the host protease SKI-1/S1 P and later by the host signal peptidase, generating mature and functional viral proteins that form a tripartite (SSP-GP1-GP2) complex that is finally organized in the trimeric viral spike.[26]

SSP is a small myristoylated transmembrane protein of approximately 6 kDa that directs the nascent GPC polyprotein to the cellular membrane. SSP remains on the surface of SABV virions and participates in maturation and pH-dependent membrane fusion activity of the GP complex,

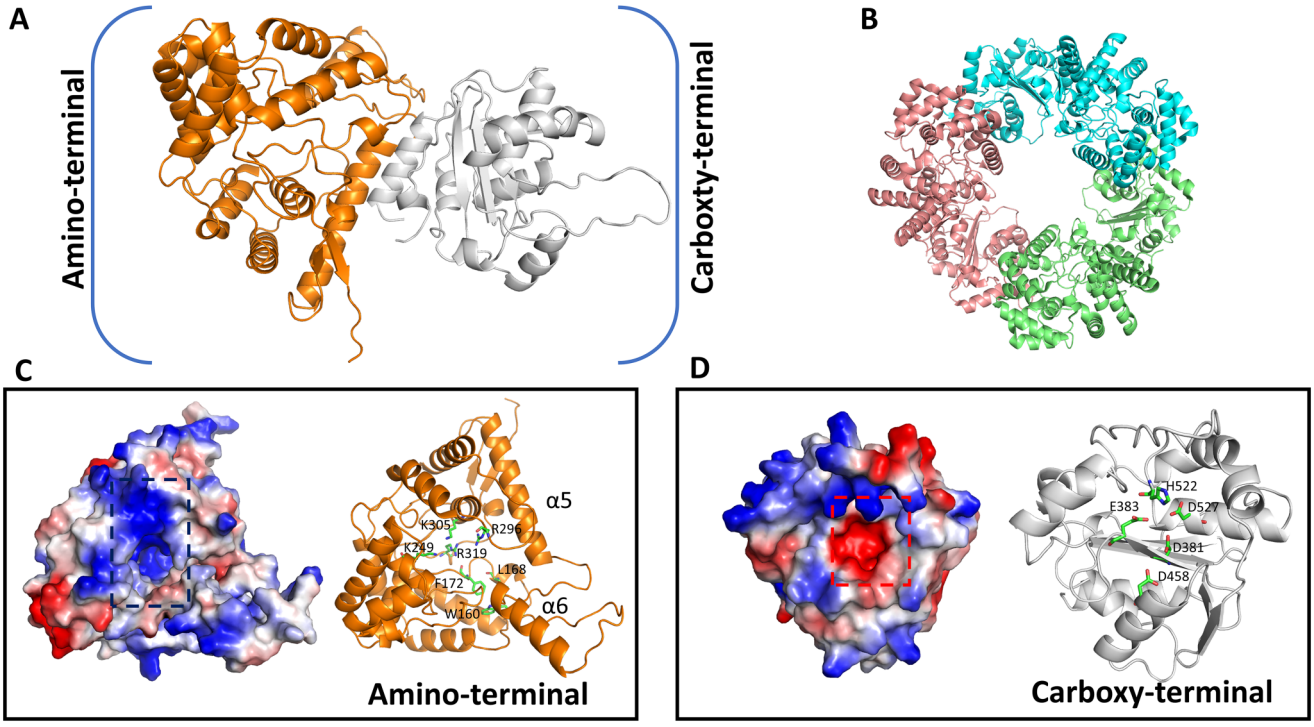

Figure 4. General prediction architecture of an SABV nucleocapsid structure. (A) Prediction cartoon diagram of SABV NP protomer, with its amino-terminal (orange) and carboxy-terminal (gray) domains. (B) Homotrimer described for LASV NP in APO configuration (PDB code 3MWP). (C) SABV N-terminal domain electrostatic surface potential representation (left) showing the positively charged RNA binding site (blue dash line square) and cartoon representation (right) with the residues that compose the site in green sticks. (D) SABV C-terminal domain electrostatic surface potential representation (left) showing in red the negatively charged exoribonuclease cavity (red dash line square) and cartoon representation (right) with the residues that compose the site in green sticks.

which is an uncommon feature of viral signal peptides.[42] GP1 and GP2 are essential viral factors involved in arenavirus entry into the host cells. GP1 is a heavily glycosylated roughly globular protein of 40–46 kDa located at the top of the viral spike and non-covalently associated with GP2, a 35-kDa transmembrane protein anchoring the complex to the viral lipid envelope.[43,44] GP1 binds the cellular receptor in mammalian hosts, which in humans is the transferrin receptor 1 (TfR1), mediating the attachment of SABV to target cells. As iron is an essential nutrient, TfR1 is expressed in almost every cell type and in high levels in tissues with increased proliferative capacity, such as bone marrow and placenta, indicating that SABV may be able to infect multiple host tissues.[45] Available evidence supports arenavirus infection of endothelial cells, leukocytes, and erythrocytes,[46] which reportedly expresses TfR1. GP2 is a class I fusion protein that has an ectodomain containing the fusion peptides and heptad repeats, a transmembrane domain, and a short cytoplasmic tail with two zinc-binding clusters that are involved in SSP interaction.[20,47] Exposure to acidic pH in endosomes leads to conformational changes in receptor-bound GP1 resulting in its release from GP2. Subsequently, N-terminal hydrophobic fusion peptides in GP2 are exposed and directed against the endosome membrane,[48] forming a metastable pre-hairpin structure which spontaneously folds back on itself, forming a six-helix bundle. These conformational changes bring the arenavirus envelope and endosomal membranes in proximity, leading to membrane fusion and delivery of the viral genome into the host cell cytoplasm.[49]

At the protein level, SABV SSP and GP2 share remarkable similarities with homologous proteins in both NW and OW arenaviruses (Figure 5(B)), which is in accordance with the fact both SSP and GP2 are highly conserved in arenaviruses. Such degree of conservation is reflected by the roles of both SSP and GP2 in viral fusion and in other steps of viral replication,[48] which are critical in viral biology and nearly identical in all arenaviruses studied to date. We also observed that several asparagine residues in SABV GP2 were conserved in OW and NW arenaviruses, which indicates that N-glycosylations are likely to be conserved, in line with critical roles played by glycosylations, such as in interaction of arenaviruses with cellular receptors, supporting viral fusion and in immune evasion/recognition.[41,49] Recent evidence indicates that neutralizing antibodies generated against GP2 were found to cross-react against multiple NW and OW arenaviruses but could not neutralize viruses in functional and validation assays, indicating that our understanding of the roles played by GP2 in arenaviruses remain incomplete.[50]

Conversely, SABV GP1 differs from every other NW or OW GP1, and GP1 is not conserved at levels of protein sequence and structure in NW or OW arenaviruses.[45] Little identity shared by arenavirus GP1 proteins has been attributed to arenavirus speciation, as each viral species has become adapted to a specific rodent reservoir.[7] Thus, GP1 in each arenavirus, including SABV, would have evolved to bind cellular receptors from the respective reservoir, resulting in the significant diversity observed besides the fact that GP1 play a role as a host attachment factor in every arenavirus. Although SABV GP1 is known to bind with human TfR1

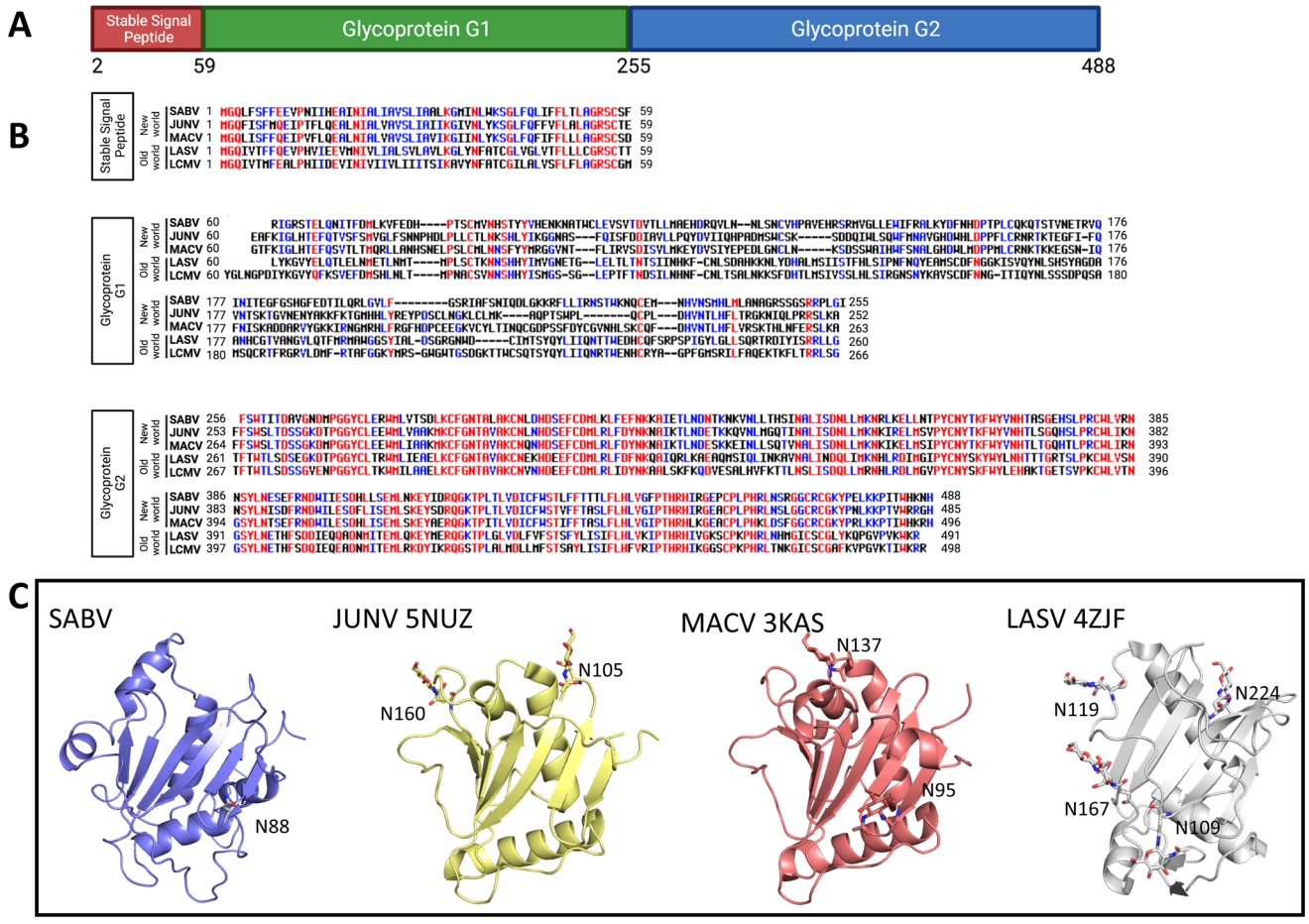

Figure 5. Domain organization of SABV glycoprotein. (A) Linear representation of GPC domains. (B) Sequence alignment of SABV SSP, GP1, and GP2 domains with NW and OW arenaviruses GP proteins. (C) Ribbon representation of GP1 domains of SABV prediction model (blue), JUNV (yellow, PDB code 5NUZ), MACV (magenta, PDB code 3KAS), and LASV (gray, PDB code 4ZJF). The first N-acetylglucosamine from N-glycosylations and asparagines are shown in sticks in respective structures. Figures 5(A) and (B) were created using BioRender.com.

and to cause severe disease in humans,[3] a feature associated with TfR1 binding in pathogenic NW arenaviruses,[51] there is substantial evidence indicating that SABV may use other cellular receptors in mammalian hosts.[41,45,49] Comparison of the predicted SABV GP1 structure with crystallographic structures of related arenaviruses (Figure 5(C)) supports the structural diversity inferred from protein sequence. We observed that SABV GP1 N89 is the single asparagine residue conserved in OW and NW arenaviruses, located in the GP1 N-terminal end (Figure 5(B)). Besides little sequence identity, SABV GP1 N89 is predicted as an N-glycosylation site positioned in similar regions in MACV and LASV GP1. The biological significance of such post-translational modification to be conserved in SABV, MACV, and LASV, but not in JUNV, is yet to be uncovered. We speculate these variations may reflect specificities in NW arenavirus maturation during the replicative cycle[44] or evolution toward different vertebrate host cellular receptors.

Matrix protein Z

Protein Z is the smallest protein transcribed by arenaviruses, with approximately 100 amino acid residues, and SABV protein Z has 11 KDa and comprises three main regions. Z presents intrinsically disordered and unstructured regions in both ends: N-terminal (NTD) and C-terminal (CTD) domains, with a central structured portion. The Z NTD from SABV comprises the first 36 residues, which share little identity with NTDs from other arenaviruses, but presenting a conserved myristoylation (Figure 6(A)) modifying the initial glycine residue in Z. The Z CTD presents some conserved regions involved in protein-protein interaction described as late domains (variable). The central structured domain of Z protein harbors the zinc finger domain with the RING motif, which comprises 50 residues in SABV (37–87 aa) (Figure 6(E)). The RING motif contains an alpha helix, two antiparallel beta strands, and two zinc-binding sites.

Protein N-myristoylation refers to an irreversible covalent attachment of myristate, an attachment of a 14-carbon saturated fatty acid, to the very N-terminal glycine of viral proteins required to a myriad of cell destination and functions, and after a methionine aminopeptidase removes the starter methionine from this process, the host cell enzyme N-myristoyl transferase (NMT) uses myristoyl-CoA as a substrate and catalyzes the reaction.[52] The matrix Z protein plays an important driving force in the budding process of arenaviruses, in which N-myristoylation plays a major role.[53] The initial 10 amino acid residues of SABV Z NTD are mostly

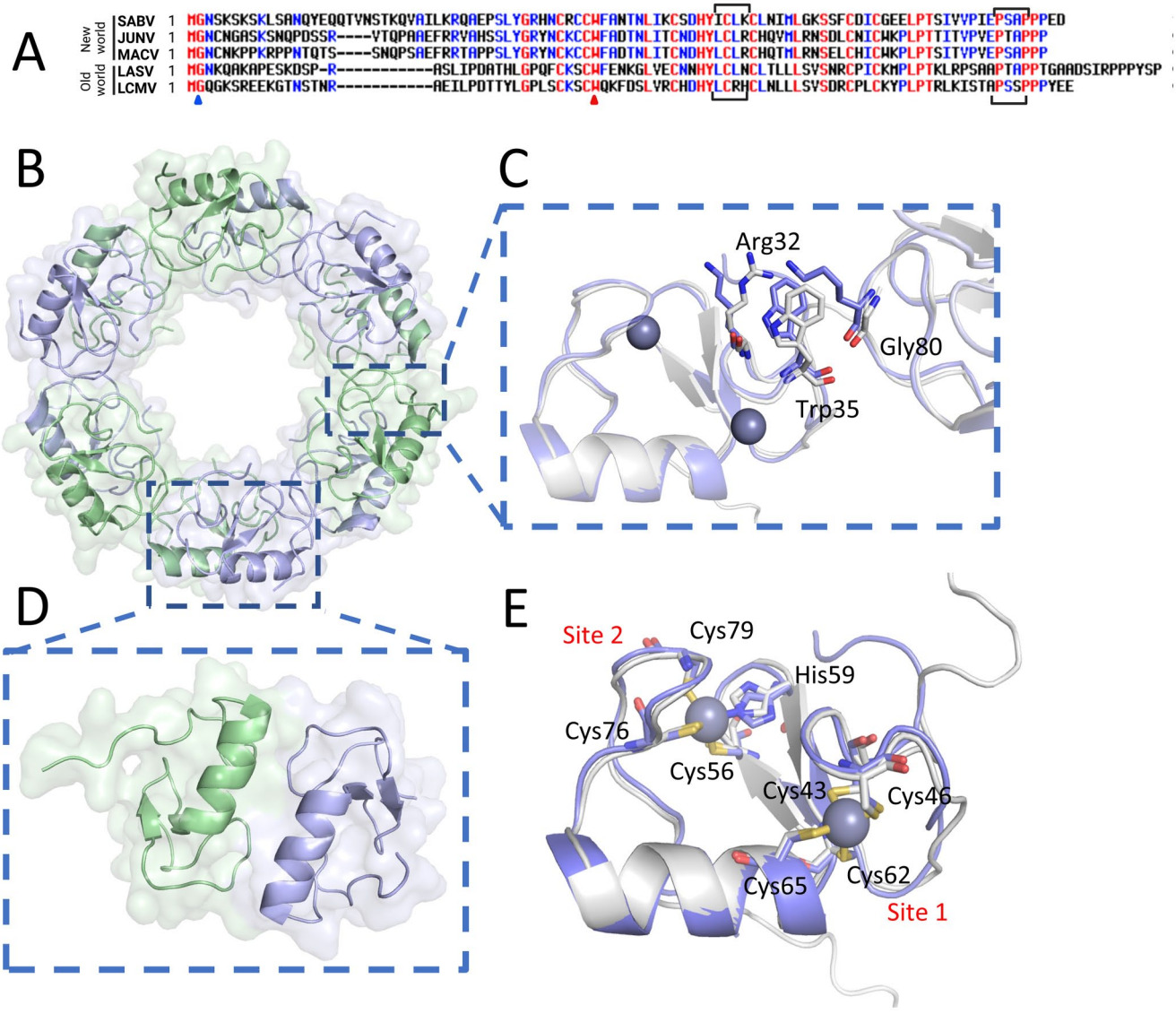

Figure 6. Structural configuration of Z protein. (A) Schematic alignment representation of Z protein from different arenaviruses, showing a conserved N-terminal glicine myristoylation site (blue arrow) and W47 shown to interface interaction to L protein (red arrow). Moreover, the conserved late domains are demonstrated in black brackets. (B) The prediction model of the dodecamer oligomerization of Z protein in cartoon diagram. (C) Interaction interface Z protein protomers and the residues involved in gray sticks for SABV and blue sticks for LASV. (D) Dimer configuration for the predicted model of SABV. (E) The monomeric model of SABV Z (gray) superimposed to LASV Z protein (blue) (PDB code 5I72), with both zinc-binding sites (zinc as blue spheres), presenting the component residues from the sites in the similar coordination. Figures 6(A) was created using BioRender.com.

basic (MGNSKSKSK) and cluster, with the participation of the N-myristoyl, to stabilize membrane lipids[52] and guide the assembly of Z in the inner part of the infected cell membrane, as demonstrated in LCMV and LASV infections.[54]

The Z protein RING domain is associated to a multitude of functions in arenaviruses including viral particle formation, viral RNA synthesis, and inhibition of IFN production. The Z protein RING domain interacts with the RNA helicase RIG-I in infected cells and inhibits subsequent activation of innate immune responses,[55–57] and the SABV RING domain (Figure 6(E)) shares conserved motifs and interaction sites described in other arenaviruses, most notably where the two zinc cations are located. The zinc ions are coordinated by four cysteines (first site) and three cysteines with one histidine (second site) which are presented in a typical cross-brace fashion; with C43, C46, C62, and C65 for the first site and C56, H59, C76, and C79 for the second. Both sites are disposed in opposing directions of the protomer in solved structures of Lassa virus (PDB code: 2M1 S and 5I72),[58,59] with site 1 being described as an inhibitor of eukaryotic translation initiation factor eIF4E.[58]

The RING domain of Z is also involved in the interaction between Z and the L protein,[60] in which Z inhibits viral RNA replication and transcription. In SABV, the interaction between L and Z[61] involves residues of R40, N42, W47, L53, and Y60, adjacent to the zinc-binding site 1, with W47 being critical for interaction.[62] The LACV protein Z structure, solved using x-ray crystallography, presented evidence of self-association in the asymmetric unit, a dodecameric oligomerization of six dimers[59] (Figure 6(B)). The dimer is stabilized by

a hydrophobic core formed by the alpha-helical interface (Figure 6(D)) and the oligomer interface by a cation-pi interaction of W35 packing with Y32 and Y68 (SABV homologous R44, W47, and G80) (Figure 6(C)); however, mutants W35A prove to revert the dodecamer to its monomeric state.

The SABV Z protein contains late domains that participate in the interactions with multiple protein-binding partners. These late domains are conserved in OW and NW arenaviruses and present in the matrix proteins of other enveloped RNA viruses.[63] The SABV C49 residue within the zinc-biding site 1 also constitutes the first YICL tetrapeptide late domains. In addition, the C-terminal regions of all arenavirus Z proteins contain late domains of the P[T/S]AP- and PPPY-type. These motifs are important,[64] to recruit NP during the budding process, and were assessed using virus-like particles.[60,65] Moreover, the C-terminal PTAP motif was shown to interact with Tsg101 in the endosomal sorting complexes requires for transport (ESCRT) machinery in host cells and found to be conserved in SABV.

Conclusions and perspectives

The severity of the disease caused by SABV, our limited understanding of SABV biology, and the potential of SABV to emerge indicate that this virus constitutes a significant risk to human health. Such risk is recognized by the World Health Organization and several interested parties and funding bodies worldwide. Further research is needed to identify the natural reservoirs of SABV so that strategies to prevent and control SABV transmission to humans may be devised, based on the virus ecology.

A BSL4 laboratory infrastructure is a requirement for the manipulation of and research on infectious SABV, and the lack thereof in Brazil, where the virus was discovered and determined as pathogenic to humans, may have impaired a more significant advance on our current understanding of the virus and associated disease. Consequently, the necessity to implement infrastructure for research on maximum containment pathogens becomes imperative, following a worldwide trend to increase access to such facilities. Difficulties to perform a differential diagnosis of hemorrhagic fevers is a critical issue in Brazil, which is endemic for yellow fever and dengue, and in Latin America, where other pathogenic NW arenaviruses are endemic, among other possible causative agents. The use of recombinant SABV proteins does not require high or maximum containment facilities and could be readily used to develop diagnostic tools, such as enzyme-linked immunosorbent assay (ELISA) tests to detect anti-SABV IgM or IgG.[66] SABV GPC is the most likely candidate antigen for such ELISA assays although NPs have been used for the diagnosis[67] of OW and NW arenaviruses infections.[66]

Several target-based strategies and drug repurposing have been explored in the development of specific treatments against severe diseases caused by arenaviruses. Broad-acting antiviral agents against SABV and others NW arenaviruses are available.[4,48,50,68] A few examples include nucleoside analogs such as ribavirin and favipiravir, expected to target the RNA polymerase activity of L protein. Favipiravir was protective against LASV and JUNV in animal models, but its efficacy in treating arenavirus hemorrhagic fever patients remains to be confirmed.[48] Likewise, inhibition of SKI-1/S1 P activity impairs GPC processing and release of infectious virions, leading to the use of SKI-1/S1 P inhibitors as antiviral drugs against OW and NW arenaviruses.[50] The antiviral activity of isavuconazole against LASV, which targets a conserved interaction between SSP and GP2, was also shown to be effective against NW arenaviruses *in vitro*.[68] Moreover these candidate treatments prove effective in human patients, and we would expect similar efficacy when treating SABV infections, as these targets or processes are highly conserved in arenavirus biology. In contrast, therapeutic strategies targeting GP1 are unlikely to be successfully repurposed against SABV, including neutralizing antibodies[41] or compounds,[69] due to the lack of conservation. We cannot predict how effective TfR1-targeted strategies would be against SABV *in vivo*, as evidence indicates that SABV may use other cellular receptors yet to be discovered.

Recently, the vaccine platform MOPEVAC, consisting a hyperattenuated Mopeia virus (MOPV) expressing pathogenic arenavirus GPC, was successfully employed against MACV infection in non-human primates, while inducing varied levels of cross-protection against other NW arenaviruses including SABV.[70] Vaccination against pathogenic arenaviruses, as exemplified by the live attenuated Candid#1 vaccine against JUNV, significantly reduces the incidence of disease, which in cases of Argentinian hemorrhagic fever may reach mortality rates of up to 30%.[71,72] Therefore, first- or second-generation live attenuated vaccines should be suitable platforms for the development of SABV vaccine, in addition to vaccine platforms recently used to mitigate the effects of the COVID-19 pandemic.

Finally, the use of technologies enabling the prediction of protein structure and function, as used in this article to substantiate discussion, analysis, and design of hypotheses, is undoubtedly effective for research on NW arenaviruses such as SABV.

AUTHORS' CONTRIBUTIONS

EHSB generated protein structure models. TDMH prepared figures and performed sequence alignments. REM conceived the paper. All authors wrote and revised the manuscript.

AUTHORS' NOTE

SABV models were generated using Phyre2[73] and Alphafold.[74]

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