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O QUE SABEMOS SOBRE ANTIVIRAIS COM ATIVIDADE CONTRA O VÍRUS DO
MAYARO *IN VITRO*?

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FROM MOLECULES TO POTENTIAL VIRAL TARGETS”

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apresentado como requisito para
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AN UPDATE ON THE DEVELOPMENT OF ANTIVIRAL AGAINST MAYARO VIRUS: FROM MOLECULES TO POTENTIAL VIRAL TARGETS

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Abstract

Mayaro virus (MAYV) was first isolated in 1954 in Trinidad and Tobago islands, and it is the causative agent of Mayaro fever, a disease characterized by fever, rashes, headaches, myalgia, and arthralgia. The infection can progress to a chronic stage in over 50% of cases with persistent arthralgia, which can lead to disability of the infected individuals. MAYV is transmitted through the bite of the female *Haemagogus spp* mosquito genus. However, studies demonstrate that *Aedes aegypti* is also capable of transmitting it, contributing to the spread of MAYV beyond endemic areas, given the vast geographical distribution of the mosquito. Besides, the similarity of antigenic sites with other *Alphavirus* complicates the diagnoses of MAYV, contributing to underreporting of the disease. Nowadays, there are no antiviral drugs available to treat infected patients, being the clinical management only palliative based on analgesics and non-steroidal anti-inflammatory drugs. In this context, this review aims to summarize compounds that have demonstrated antiviral activity against MAYV *in vitro*, as well as discuss the potentiality of viral protein as targets for the development of drugs against MAYV. Finally, through rationalization of the data presented here in, we wish to encourage further research encompassing these compounds as potential anti-MAYV drug candidates.

INTRODUCTION

The Mayaro virus (MAYV) belongs to the *Togaviridae* family and *Alphavirus* genus, which is composed of a positive single-stranded RNA of 11,5 kb in length, covered by an icosahedral capsid and viral envelope (**Figure 1**)¹. It was first isolated in 1954 in Trinidad and Tobago islands from the blood of five febrile rural workers, and since then, sporadic outbreaks have been identified in countries with tropical forests, including Brazil, Bolivia, Suriname, Peru, Equator, Venezuela, and recently in Haiti². In addition, cases in North America³ and Europe⁴ were reported in citizens who traveled to South America.

MAYV belongs to the Semliki Complex, a subgroup of the *Alphavirus* genus that includes, for example, the Bebaru virus, Chikungunya virus (CHIKV), Getah virus, Una Forest virus (UNAV), O'nyong-nyong virus, among others. Viruses from this complex are classified into the same serological group, sharing common antigenic sites which can result in cross-reactivity in conventional serological tests, complicating the accurate diagnosis of MAYV infections and report of the disease incidence⁵.

MAYV infection causes the Mayaro Fever disease, which is characterized by acute fever, rashes, headache, retro-orbital pain, nausea, diarrhea⁵, myalgia, and arthralgia that, in more than 50% of cases, remain for months or years^{6,7}, culminating in physical disability⁸. In severe cases, Mayaro fever can cause intermittent fever, neurological complications, myocarditis, and ultimately, death⁵. The MAYV is transmitted through the bite of the *Haemagogus spp* mosquito, usually from infected non-human primates, birds, rodents, and small mammals to a susceptible human being⁹. *Aedes aegypti* was also been found to be able of transmitting the MAYV, and given the vast geographical distribution of the *Aedes* genus, it might contribute to its spread beyond endemic areas^{10,11}.

The MAYV replication cycle begins with virus entry in host cells via specific receptors on the cell surface, such as MXRA8, triggering a clathrin-mediated endocytosis^{12,13}. Through pH changes in the endosome, the interaction between the endosome membrane and the viral envelope leads to the viral RNA release into the cytoplasm. MAYV RNA is composed of two open reading frames (ORFs), in which the first is translated into the non-structural viral proteins (nsP) nsP1, nsP2, nsP3, and nsP4, in order to form the replicative complex or viral replicase. These proteins are responsible to produce a negative strand from the viral genome that serve as templates for new positive-sense strands, besides the sub-genomic 26S RNA¹. The other ORF is transcribed from the

negative strand and translated into a polyprotein which is processed into the structural proteins C, E1, E2, E3, and 6K, followed by the assembling of the viral components and virus release through exocytosis ⁵ (**Figure 2**).

Despite the high rates of chronification in infections caused by MAYV, there is no licensed vaccine or antiviral to treat Mayaro fever. The treatment of the disease consists in managing the symptoms with analgesics and/or non-steroidal anti-inflammatory drugs ¹⁴. However, the most effective therapy would be the use of antiviral with specific modes of action to inhibit the replication cycle. Therefore, research data have been developed to study the potential of natural and synthetic molecules with anti-MAYV activity. Here we summarize the natural and synthetic compounds previously described to possess activity against MAYV (**Table 1**). Thus, we critically compare molecules that could be further investigated *in vivo*, and also suggest protein targets to be further evaluated as anti-MAYV molecules. Finally, we aim to encourage further research encompassing these compounds as potential anti-MAYV drug candidates to treat infected patients.

INHIBITORS OF THE REPLICATIVE CYCLE OF MAYV

One of the first natural molecules tested for the potential to inhibit the MAYV replication *in vitro* was Prostaglandin A1 (PGA1) ¹⁵. This natural molecule possesses a hormone-like lipid structure and is produced by cells in the presence of injury and/or inflammation ¹⁶. Burlandy and Rebello demonstrated that 10 µg/mL of PGA1 reduced 99% of MAYV replication *in vitro* through a reduction plaque assay ¹⁵. To this, green monkey kidney cells (Vero cells) were infected with the virus at a multiplicity of infection (MOI) of 5 for 1h, and then, treated with non-cytotoxic concentrations of the compound. As a result, PGA1 inhibited viral replication in a dose-dependent manner with an EC₅₀ (50% effective concentration) of 3 µg/mL. Moreover, to suggest which stage of the replicative cycle was affected, a time of addition assay was performed. The results showed that PGA1 possesses the strongest effect when added to the cells simultaneously to the virus. The authors also identified a reduction in the synthesis of E1, E2, and C, and in the precursors of virus proteins, which suggests the effect in the early stages of viral replication. Additionally, an increase in the heat shock protein 70 (HSP70) was detected, and since this molecule is responsible for protein folding, disaggregation, and degradation to ensure proper cell function ¹⁷, it is possible to suggest a mode of action for PGA1 ¹⁵.

Furthermore, Caldas and coworkers also assessed the effect of PGA1 on MAYV replication and the relevance of HSP70 in this process ¹⁶. Human cervix carcinoma cells (Hep-2 cells) were infected with MAYV with an MOI of 1 for 1h and treated with different concentrations of the compound. According to the authors, the PGA1 was not cytotoxic, presented an EC₅₀ of 1 µg/mL, and inhibited up to 95% of viral production at 6 µg/mL. The inhibition of MAYV replication was higher when the compound was added 1- or 2-hours post-infection (h.p.i.). In agreement with the previous study, PGA1 impaired p110, p62, E1/E2, and C synthesis, as well as a significantly increased HSP70 expression ¹⁶. The HSP70 increase seems to be related to the PGA1 antiviral effect since the inhibition of viral replication was only observed in PGA1 concentrations that induced the production of this HSP. In this context, the authors proposed that PGA1 may act in different stages of viral replication due to the different strategies presented by the molecule.

Alternatively, Carvalho and coworkers proposed the natural macromolecule Lactoferrin, a globular glycoprotein, as a source of treatment against MAYV ¹⁸. This molecule is produced by exocrine glands such as maternal milk and tears ¹⁹, was used in its apo form, apolactoferrin, found in bovine whey (bLf). To assess the effect of Lactoferrin against MAYV, Vero cells were infected with viral particles labeled with DiD (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine,4-chlorobenzenesulfonate salt), a lipophilic carbocyanine marker that expresses red fluorescence ²⁰, and then incubated with non-cytotoxic concentrations from 0.2 to 1.0 mg/mL of bLf during the pretreatment and viral infection. The assays demonstrated that bLf inhibited viral replication in a dose-dependent manner, with 1 mg/mL being able to inhibit 85% of viral infection. Furthermore, a time addition assay was also carried out to investigate which stage of the replication cycle was specifically affected ¹⁸. The bLf mainly acted in virus entry stage, suggesting that the molecule can interact with the sulfate groups of the glycosaminoglycans layer (GAGs) of the host cells, preventing the attachment of the virus. However, since the specific MAYV receptor at the time of the study was unknown ¹⁸, further studies involving the Mrxa8 are needed to corroborate its mechanism of action.

Another potential antiviral candidate against MAYV is the flavonoid proanthocyanidin [(-) - epicatechin- (4β → 8) - (-) - 4'-methylepigallocatechin] (PAC), a compound isolated from *Maytenus imbricata* roots ²¹. Ferraz and coworkers evaluated the viability of PAC employing Vero cells through an MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium Bromide)] which demonstrated no cytotoxic effect, with the CC₅₀ (50% cell cytotoxicity concentrations) over 1640 μ M. To assess the antiviral effect, cells were treated with PAC at different concentrations in the presence of MAYV, resulting in an EC₅₀ of 37.9 μ M and a SI (selectivity index) higher than 43²¹. The treatment at different stages of the virus cycle demonstrated that PAC's main effect is related to virucidal activity, which was confirmed by a dialysis membrane assay. The class of this compound was previously described with activity against other viruses with clinical importance such as Hepatitis B, C, and D, and Herpes Simplex 1 and 2^{22–25}. The authors proposed that the mechanism of action may be related to the interaction of proanthocyanins with the envelope glycoproteins, decreasing the infectivity of viral particles²¹.

Ginkgolic Acid (GA) is a compound isolated from *Ginkgo biloba* and is widely used in traditional Chinese medicine²⁶, since it possesses several described biological activities, such as anti-tumor^{27,28}, antibacterial²⁹, anti-parasitic³⁰, and antiviral³¹. In this context, Campos and coworkers evaluated the effect of this compound on the CHIKV, MAYV, and UNAV replication. Through a plaque reduction assay using epithelial cervical cancer cells (HeLa cells), it was found that GA at 10 μ M reduced the MAYV viral progeny in almost 2 log₁₀ at 16 h.p.i. To confirm these results, an immunofluorescence assay was performed, demonstrating that treated cells presented a decrease in viral antigens. Moreover, a time addition assay was performed adding GA to the cells hourly after adsorption, and supernatants were collected 24 h.p.i to measure viral progeny yields. As a result, the compound was able to mainly inhibit the initial stages of the viral cycle, which was confirmed by the suppression of E1 and E2 viral proteins in western blot²⁶. Despite the promising effects of GA against MAYV *in vitro*, the molecule was demonstrated to be toxic in other cell lines and animal studies^{32,33}. Therefore, the authors suggest that further investigations are needed to confirm the safety of the treatment, and the antiviral activity against MAYV.

Another promising inhibitor of MAYV is Silymarin, a compound extracted from *Silybum marianum*. This molecule possesses biological properties as antioxidant, anti-inflammatory, antifibrotic, and is widely used for the treatment of liver disorders³⁴. It was described to have antiviral activity against Hepatitis C virus³⁵ and CHIKV³⁶, presenting itself potential as a new therapeutic approach for MAYV infection. With this in mind, Camini and coworkers assessed the cytotoxic effect of Silymarin on

hepatocarcinoma cells (HepG2 cells), which resulted in a CC₅₀ of 103.5 µg/mL. The EC₅₀ of the compound was 3.58 µg/mL. Then, HepG2 cells were treated with 25 µg/mL of Silymarin and simultaneously infected with MAYV at an MOI of 5. Supernatants were collected 48 h.p.i. and used to infect Vero cells. The results demonstrated a reduction of up to two logs in MAYV replication when compared with non-treated cells, inhibiting almost 100% of the cytopathic effect. To confirm this result, the production of reactive species of oxygen (ROS) was measured employing infected HepG2 cells with a fluorogenic marker. The results confirmed that MAYV infection increased ROS generation in a time-dependent curve. In this context, the authors quantified the other oxidative stress biomarkers, such as malondialdehyde (MDA) and carbonyl protein, in the presence of MAYV and Silymarin, which culminated in a drop of those biomarkers, suggesting a protective effect on cells associated with the antiviral activity ³⁴.

The natural compounds may present limitations in terms of large-scale production and patentability, therefore, the production of synthetic and semi-synthetic compounds is an alternative and attractive approach ³⁷. Among the synthetic molecules, the Thienopyridine derivatives were described as antimicrobial agents against bacteria, protozoa, viruses ³⁸, and parasites ³⁹. In this context, Amorim and coworkers conducted a study based on the antiviral activity of thieno[2,3-b]pyridine derivatives in the MAYV cycle *in vitro* ⁴⁰. The cell viability and antiviral activity of 8 thieno[2,3-b]pyridine-5-carbonitrile derivatives (101 to 113) were investigated in Vero cells. Ribavirin was used as a positive control. As an outcome, the isomers showed less or similar toxicity to Ribavirin, with exception of molecules 112 and 113, which were more toxic to cells than the untreated control. Among the non-cytotoxic compounds, molecule 104 was the most viable compound, with a CC₅₀ of 2500 µM, an EC₅₀ of 20.0 µM (Ribavirin control EC₅₀ 62.5 µM), and SI of 125. To further assess the effect of molecule 104 on viral protein synthesis, Vero cells were infected at an MOI of 0.05 and treated with 100 µM of the compound in a time addition assay. Through the quantification of the plaque-forming assay, it was observed that compound 104 presented a higher effect when added before or simultaneously to the infection with the virus. Therefore, a virucidal assay was performed incubating the virus and the compound for 1h, then adding the inoculum to the cells, which confirmed that molecule 104 has a virucidal effect in concentrations higher than 25 µM. Besides that, the authors labeled the viral proteins with 35S-methionine to assess their synthesis after infection. The results showed a decrease in the protein synthesis in the presence of the

104. This effect was also confirmed by transmission electron microscopy, which demonstrated a reduction in the number of intracytoplasmic nucleocapsids and mature virus particles in treated cells.

Porphyrins are natural or synthetic organic molecules that can bear a metal ion in the center of their tetrapyrrolic structure ⁴¹. These molecules are capable of absorbing light and are widely used in photodynamic therapies. Additionally, the porphyrins have been shown to target the viral envelope, inactivating the viral particles of Dengue virus (DENV) and Yellow Fever virus (YFV) ⁴¹. Considering the activity of this class of compounds, Neris and coworkers evaluated the antiviral potential of the heme porphyrins Co-protoporphyrin IX (CoPPIX) and Sn-protoporphyrin IX (SnPPIX) in the presence or absence of light against CHIKV, Zika virus (ZIKV), MAYV, Vesicular Stomatitis virus (VSV), and Sindbis virus (SINV) in Baby Hamster Kidney Fibroblast Cells (BHK-21) or Vero cells ⁴². With no light stimulation, CoPPIX and SnPPIX presented a CC₅₀ of 762.3 μ M and 816 μ M, respectively, while in the presence of light the compounds demonstrated a CC₅₀ of 1018 μ M and 20.7 μ M, respectively. Later, 10⁷ PFU of viruses were pre-incubated with different concentrations of either compound in the absence of light for 1 hour at 37°C and then used to infect the cells with an MOI of 0.1. CoPPIX was able to completely inactivate all viruses tested at 300 μ M, with an EC₅₀ of 5.94 μ M, and SnPPIX with an EC₅₀ of 5.99 μ M. Alternatively, when incubated in the presence of light, SnPPIX had higher efficiency in reducing viral particles, resulting in the inactivation of viruses at 200 μ M with an EC₅₀ of 0.09 μ M, while CoPPIX demonstrated an EC₅₀ of 7.48 μ M. For this step, viruses and compounds were light stimulated for 10 minutes before incubation for 1 hour in the dark.

Additionally, to better investigate the CoPPIX and SnPPIX inhibition, MAYV was labeled with DiD, a marker that expresses red fluorescence and can be used to follow fusion between virus and endosomal membranes during endocytosis ⁴¹. After treatment with CoPPIX at 300 μ M, SnPPIX at 300 μ M, and SnPPIX at 10 μ M with light stimulation, MAYV was unable to fuse efficiently with the endosomal membrane. Thus, the authors imply that the porphyrins tested are hydrophobic molecules that might interact with viral envelope lipids, impairing the attachment and entry of the virus into the cells. Besides that, the photoactivation of some porphyrinic compounds, like SnPPIX, generates ROS, which can modify viral proteins in the envelope environment, producing an antiviral activity. The authors emphasize that a single intensity of light stimulation was performed,

and suggest that an increase in luminous intensity might increase SnPPIX virucidal efficiency ⁴¹.

Exploiting the host factors, the Src family of protein tyrosine kinases (SFK) are involved in intracellular signaling pathways related to viruses elimination ⁴³. However, during infection, the virus can interact with kinases favoring viral replication. Therefore, cell signaling inhibitors, including SFK inhibitors, have been tested for their antiviral activity against *Alphavirus* in human fibroblasts ⁴³. Since the knowledge of the signaling profile of SFK during *Alphavirus* is limited, the authors carried out a range of experiments using CHIKV infection, as a base to identify the kinase pathways involved in this genus infection. The SFK-phosphatidylinositol 3-kinase (PI3K)-Akt-mTOR signaling was demonstrated to be the main pathway during CHIKV infection. Then, the authors evaluate the effects of dasatinib and Torin, inhibitors of this pathway, on fibroblast infected with other viruses, such as MAYV. Through the viral titration of the supernatant collected from infected cells, it was shown that MAYV was significantly reduced by these inhibitors. The authors demonstrated that the SFK inhibitors blocked the subgenomic RNA translation, resulting in the impairment of *Alphaviruses* replication ⁴³.

Another promising treatment is β -D-N4-hydroxycytidine (EIDD-1931), a ribonucleoside analog, which demonstrates antiviral activity against RNA viruses, such as SARS-COV-2, Equine Encephalitis Virus, and CHIKV ^{44,45} EIDD-1931 can rapidly reach plasma, be efficiently distributed in mice organs, and also present a high genetic barrier for the development of viral resistance ^{44,46}. A study conducted by Langendries and coworkers screened compounds with previously reported antiviral activity against other arboviruses, such as Favipiravir, Suramin, and also the EIDD-1931, against MAYV (strain TC625) ⁴⁵. For viability and antiviral assay, Vero cells were treated with serial dilutions of the compounds and infected by MAYV at an MOI of 0.01. As a result, the experiments demonstrated a CC₅₀ of > 100 μ M, > 2000 μ M, and 2837 μ M, and an EC₅₀ of 1.6 μ M, 124 μ M, and 79 μ M for EIDD-1931, Suramin, and Favipiravir, respectively. To evaluate which stage of the replicative cycle was affected by these compounds, a time addition assay was carried out. Vero cells were incubated with 300 μ M Favipiravir, 500 μ M Suramin, and 25 μ M EIDD-1931 2 hours before infection, and 0, 2, 4, 6, and 8 h.p.i. at an MOI of 1. Through end-point titration at 10 h.p.i and qRT-PCR quantification, the authors demonstrated that 3.1 μ M of EIDD-1931 and 75 μ M of Favipiravir still exhibited an inhibitory effect. It was also observed with Suramin at 500 μ M, producing a viral decrease

of $\sim 5 \log_{10}$. On the other hand, Favipiravir and EIDD-1931 demonstrated to inhibit 100% of viral replication ($\sim 8 \log_{10}$ reductions in infectious virus) at 300 and 12.5 μM , respectively. Later, EIDD-1931 impaired viral replication when added at 0, 2, and 4 h.p.i., with a moderated effect if added at 6 h.p.i. Differently, Suramin and Favipiravir reduced viral titers at 0 and 2 h.p.i., respectively. Throughout the results and previous data in the literature, the authors suggested that EIDD-1931 and Favipiravir impair viral RNA synthesis, resulting in non-functional copied viruses due to a large number of mutations in the viral ^{45,47} Additionally, it was also proposed that Suramin acted during the early steps of the MAYV life cycle, more specifically in the viral entry, in a similar mechanism demonstrated by studies with CHIKV ⁴⁸ and SARS-CoV-2 ⁴⁹.

Cyclic ketones are natural and synthetic compounds with antiviral ^{50,51}, antioxidant ⁵², and antifungal ⁵³ properties. The antiviral activity of 24 cyclic ketones was assessed by Fernandes and coworkers, by screening these compounds' antiviral activity in Vero cells through the MTT assay. Vero cells were infected with MAYV (MOI 1) and treated with each compound at concentrations ranging from 1 to 100 μM , and then incubated for 24 h. Among those, 8 compounds showed viability $\geq 50\%$ and were selected for viral inhibition assay. Using non-cytotoxic concentrations a plaque-forming assay was performed, and among these 8 compounds, compound 9-(5-(4-chlorophenyl)furan-2-yl)-3,6-dimethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2)-dione, a xanthenodione, inhibited the MAYV with EC_{50} of 21,5 μM and SI of 15,8. The authors also performed a time addition assay and found that the compound inhibits viral replication in the pre and post-treatment assays ⁵⁴.

MOLECULAR TARGET TO DRUG DEVELOPMENT AGAINST MAYV

The process to develop drugs might be a time-consumption process and sometimes results in unexpected outcomes ⁵⁵. Additionally, the lack of treatment against viral infections, mainly in neglected diseases, may jeopardize the control of the disease, as well as its consequences, such as the loss of human lives ⁵⁶. In this context, several strategies can be applied to accelerate the progress towards the development of novel molecules. Therefore, this section will focus on the MAYV proteins which could be exploited as a target for antiviral development, considering the following factors: i) previously described compounds with antiviral activity against MAYV and/or other *Alphaviruses*,

ii) resolved structure deposited in databanks, and iii) structural similarity and conservation among viruses.

The glycoprotein E1 possesses an ectodomain, subdivided into subdomains I, II, and III, and a transmembrane subdomain (TM). Similarly, the E2 ectodomain is composed of subdomains A, B, C, and D, and also a TM portion⁵⁷. The ectodomains of E1 and E2 interact with each other to form a portion of the spike structure outside the envelope (**Figure 3A-C**). On the other hand, the TM portion of E1 and E2 binds to the capsid protein above the lipid membrane, forming an E1-E2-capsid unit (**Figure 3D**). As observed for other *Alphaviruses*, MAYV possesses an asymmetric unit formed by the assembling of four E1-E2-capsid units resulting in 80 spikes⁵⁷. MAYV belongs to the arthritogenic viruses class, therefore, it is possible to suggest that Mxra8 is the main receptor related to the MAYV entry on host cells (**Figure 3E**)⁵⁸. In this context, considering that the glycoprotein protein complex interacts with Mxra8, mainly through E2, this contact might be impaired by active molecules through interference or destabilization of E2 binding sites^{13,57}. Molecules were described against other arthritogenic viruses, such as CHIKV, binding with glycoproteins and consequently inhibiting viral entry⁵⁹. Souza and coworkers described the activity of a coordinated organic compound that targets the E2 domain and plays a virucidal role in CHIKV replication. Here, we also described other compounds such as PAC, bLf, CoPPIX, and SnPPIX, which also presented a virucidal activity against MAYV infection. Even though, these molecules were not rationally designed to its specific targets, further studies could investigate whether their mode of action are related to these hint targets.

Another spot protein in MAYV replication is the non-structural protein Nsp4 (**Figure 4**). This protein acts as an RNA-dependent RNA polymerase (RdRp), being essential to RNA viruses replication⁶⁰, and therefore, it is attractive to antiviral research and development. To the best of our knowledge, the RdRp crystal structure of MAYV has not been determined and characterized yet, which might postpone drug design applied to this target. Regardless, the RdRp amino acid sequence is commonly conserved among viruses from the *Alphaviruses* genus⁶¹. Its structure is composed of fingers, the palm containing the GDD active site, and thumb domains⁶⁰. Therefore, it is possible to hypothesize that compounds with antiviral activity described in the RdRp of other viruses could act against MAYV infection⁶². Indeed, most of the described and approved drugs are nucleoside and non-nucleoside inhibitors that impair viral infections through RNA synthesis inhibition,

among them, Remdesivir, Azidothymidine (AZT), Sofosbuvir, and Lopinavir/Ritonavir, which are used to treat Ebola virus, Hepatitis C virus, and Human immunodeficiency virus infections ⁶³.

The MAYV nsP3 is another protein that might present itself as a target for antiviral development ⁶⁴. It is a modular protein with three domains: the N-terminal macrodomain, a central zinc-binding domain, and the C-terminal hypervariable domain (**Figure 4**) ⁶⁵. Each domain plays a different role in viral replication and interferes with the virulence among Old and New World Alphaviruses, being necessary for RNA synthesis ⁶¹. The MAYV macrodomain possesses four alpha-helices and six beta-strands ^{66,67}. On the other hand, the central zinc-binding domain plays an undefined, but essential role in minus-strand RNA synthesis, through an association with the macro domain, forming a ring-like structure to RNA binding ⁶⁸. Alternatively, the hypervariable domain is a large protein that upholds several insertions and deletions, and is responsible for interactions with multiple host proteins during viral replication, probably favoring virulence ^{69–71}. However, the macro domain is the most conserved site in the protein structure and could be exploited for drug development, particularly as a broad-spectrum antiviral. The macro domain site binds to ADP-ribose, dephosphorylate ADP-ribose-1'-phosphate, and possesses a de-ADP-ribose hydrolase activity, which is essential to viral replication ⁷². Several compounds were virtually screened employing the nsP3 macrodomain of CHIKV, Sin Nombre virus (SNV), and the Venezuelan equine encephalitis virus (VEEV) as targets ^{73–76}. Shimizu and collaborators, evaluated *in silico* and *in vitro* compounds designed specifically to the nsP3 macrodomain of CHIKV and identified the effect of two active compounds ⁷⁵. Therefore, it is possible to suggest the nsP3 macrodomain of MAYV as a relevant target for future antiviral discovery.

PERSPECTIVES

The MAYV infection can impact the quality of life of infected patients, due to the progressive arthralgia that results in a chronic condition and disabling disease. Besides that, the lack of approved antiviral treatment and vaccines against MAYV, associated with the presence of susceptible vectors, can lead to future outbreaks in tropical and subtropical countries.

In this context, this review summarized and discussed the literature concerning the compounds with anti-MAYV activity, as well as presented possible viral proteins that could be further exploited as a target for drug development. To the best of our knowledge, only the studies described here reported antiviral activity against MAYV infection, demonstrating that further researches on treatments against Mayaro fever are urgent. It is important to emphasize that the data presented here employed diverse methodologies, using different cell lines, MOI for infection assays, and design of the assays. However, these studies present relevant information on the antiviral activity of a range of molecules (natural and synthetic) against MAYV, and suggested possible mechanisms of action (MOA) related to each compound. The characterization of each MOA represents an advance for further studies since it clarifies the interactions among cells, viruses, and compounds ⁷⁷. Besides, the MOA against MAYV related to several compounds are still unknown ⁷⁸.

From the compounds described here, Lactoferrin, molecule 104 (thienopyridine derivative), and Silymarin presented significant antiviral activity against MAYV *in vitro*, with low or no cytotoxicity. EIDD-1931 is also a promising treatment since it was able to completely inhibit MAYV replication *in vitro*. However, these compounds need to be further investigated *in vivo* against MAYV. In addition, from the compounds cited in this review, Ginkgolic Acid (GA) was one of the most active compounds, and the only tested *in vivo*, demonstrating hepatotoxicity and nephrotoxicity effects, as well cytotoxicity in different cell lines. Qian and collaborators also observed that rats treated with either, high or low doses of GA, showed histopathological changes in the liver and kidneys. In addition, the serum levels of urea nitrogen and creatinine increased in the blood and total protein and albumin decreased, indicating kidney dysfunction and liver damage ³³. On the other hand, the genotoxicity evaluation of GA evidenced a low risk of genotoxicity *in vivo* ³². Therefore, more studies in human cells and animal models are necessary to analyze the safety of this compound.

Furthermore, we discussed MAYV proteins that could represent relevant targets for drug development. Here, we suggested the glycoprotein complex, the nsP4 (RdRp), and the nsP3 macrodomain as targets. The lack of resolved structures of several MAYV proteins, such as nsP1, nsP2, C, and E, invalidate them as targets for drug development. In addition, we only selected MAYV proteins as targets, acknowledging that focusing on host factors might produce cytotoxicity *in vitro*, and adverse effects *in vivo*.

CONCLUSIONS

The spread of MAYV infections in tropical and subtropical regions represents a threat to developing countries. Additionally, the lack of epidemiological data on Mayaro fever might underestimate the importance of this disease and its consequences. There is an urgent need for studies to understand the biology of the virus, not only of Mayaro but also for related viruses, thus creating paths and ammunition for future pandemics. In this context, the compounds described to possess anti-MAYV activity, and the possible targets discussed herein, may contribute to the future development of antiviral drugs against MAYV.

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Authors contribution

Literature research: M.P., M.S, V.R.G., and I.A.S.; Drafting of the manuscript: M.P., M.S.; Figures: V.R.G.; Design and Critical revision: I.A.S. and A.C.G.J. All authors reviewed the manuscript.

Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table 1: Compounds with antiviral activity against MAYV

Compound	Inhibition	Mechanism	EC ₅₀	CC ₅₀	Cell lineage
Prostaglandin A1 (PGA1)	Post-entry	Reduction in the synthesis of E1, E2, C, and in the precursors of virus proteins	1 µg/mL	ND	Hep-2
			3 µg/mL	ND	Vero
Lactoferrin	Viral Entry	Prevents the viral entrance	ND	ND	Vero
Proanthocyanidin (PAC)	Viral Entry	Interacts with proanthocyanins on the envelope glycoproteins, preventing viral entry	37.9 µM	> 1640 µM	Vero
Ginkgolic Acid (GA)	Post-entry	Inhibits the initial stages of the viral cycle, and suppresses the expression of E1 and E2 viral proteins	ND	ND	Vero, HeLa
Silymarin	Host cells	Decreases virus-induced oxidative stress on host cells associated with the antiviral activity	3.58 µg/ml	103.5 µg/mL	HepG2
Thienopyridine Derivatives	Post-entry	Decreases viral protein synthesis	20.0 µM	2500 µM	Vero
Co-protoporphyrin IX (CoPPIX)	Viral Entry	Impairs viral entry and adsorption	5.94 µM - without light 7.48 µM - with light	762.3 µM - without light 1018 µM - with light	BHK-21, Vero
Sn-protoporphyrin IX (SnPPIX)	Viral Entry	Impairs viral entry and adsorption, generates ROS	5.99 µM - without light 0.09 µM - with light	816 µM - without light 20.7 µM - with light	BHK-21, Vero
Kinases enzyme (SFK) inhibitors	Post-entry	Blocks the subgenomic RNA translation, impairing viral replication	ND	ND	ND
β-D-N4-hydroxycytidine (EIDD-1931)	Post-entry	Impairs viral RNA synthesis, resulting in non-functional copied viruses	1.6 µM	> 100 µM	Vero
Favipiravir	Post-entry	Impairs viral RNA synthesis, resulting in non-functional copied viruses	79 µM	2837 µM	Vero
Suramin	Viral Entry	Inhibits viral entry	124 µM	> 2000 µM	Vero
9-(5-(4-chlorophenyl)furan-2-yl)-3,6-dimethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2))-dione	ND	Inhibits viral in pretreatment and post-entry assay.	21,5 µM	338.8 µM	Vero

ND: not described; EC₅₀: effective concentration of 50%; CC₅₀: cytotoxic concentration of 50%.

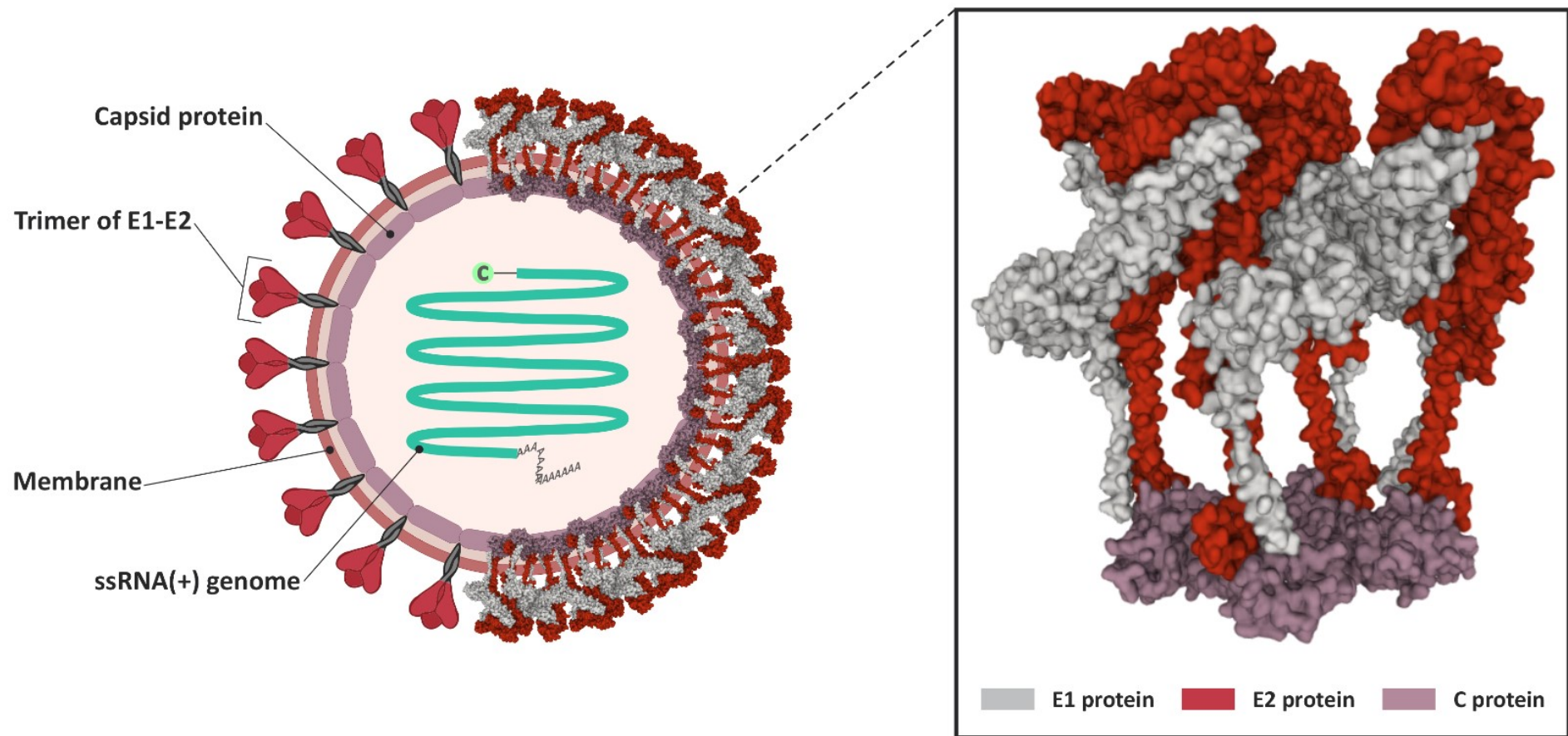


Figure 1: Schematic structure of MAYV virion. The virion is composed of a positive single-stranded RNA with an icosahedral capsid formed by the capsid protein (C), covered by an envelope membrane inserted of the E1, E2, and E3 glycoproteins. The E3 protein is not shown, since its structure is not available in the current database.

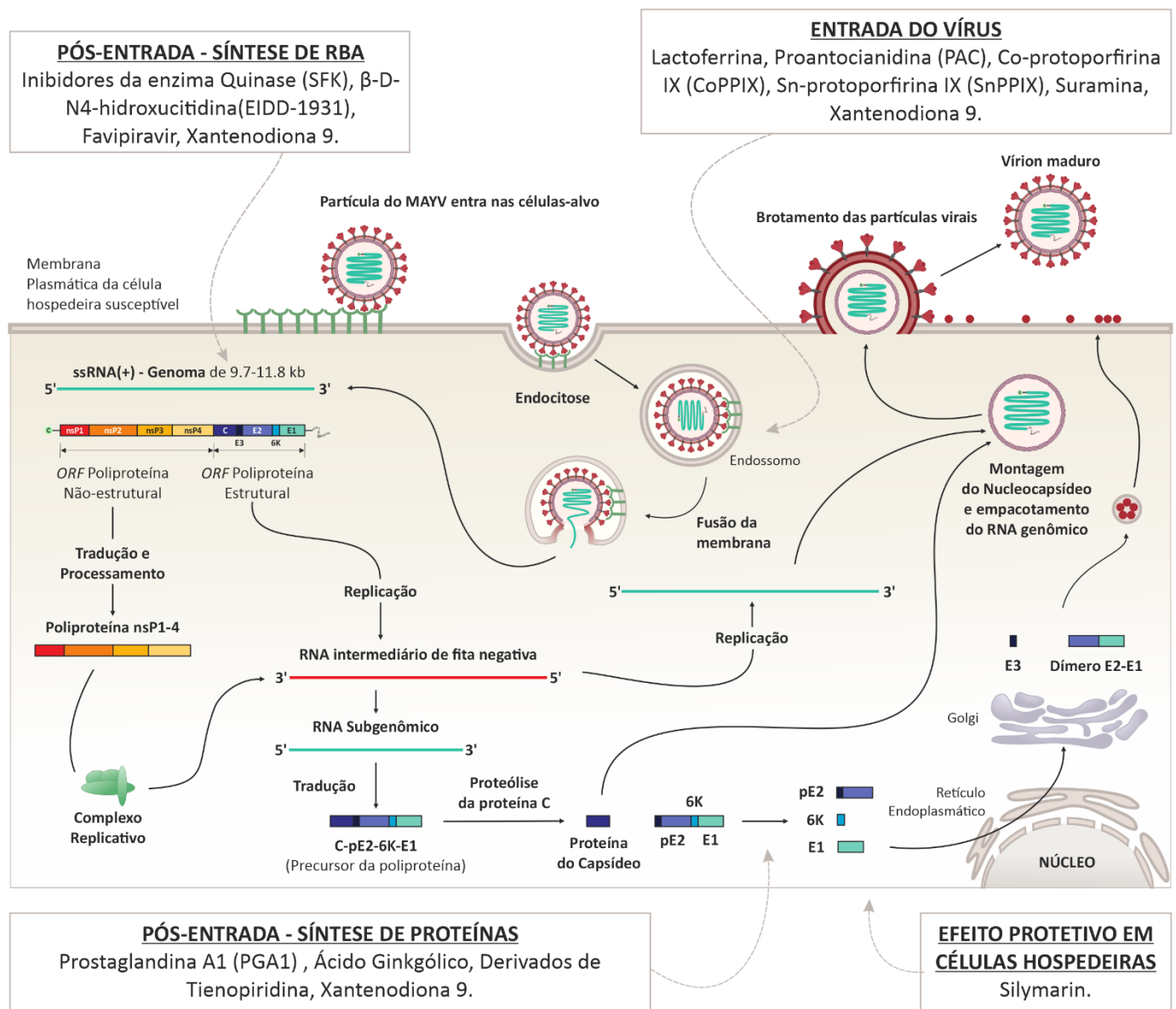


Figure 2: Schematic representation of MAYV replicative cycle. MAYV attaches to the host cells by the interaction between the host receptors and glycoproteins. After entry, the viral uncoating process results in the release of the viral genome, and the replication stage occurs (translation and transcription). Structural proteins are produced through an intermediate subgenomic RNA and forwarded to assembly, packaging, and virus release. Compounds with antiviral activity against MAYV are indicated in each step of the virus replication cycle.

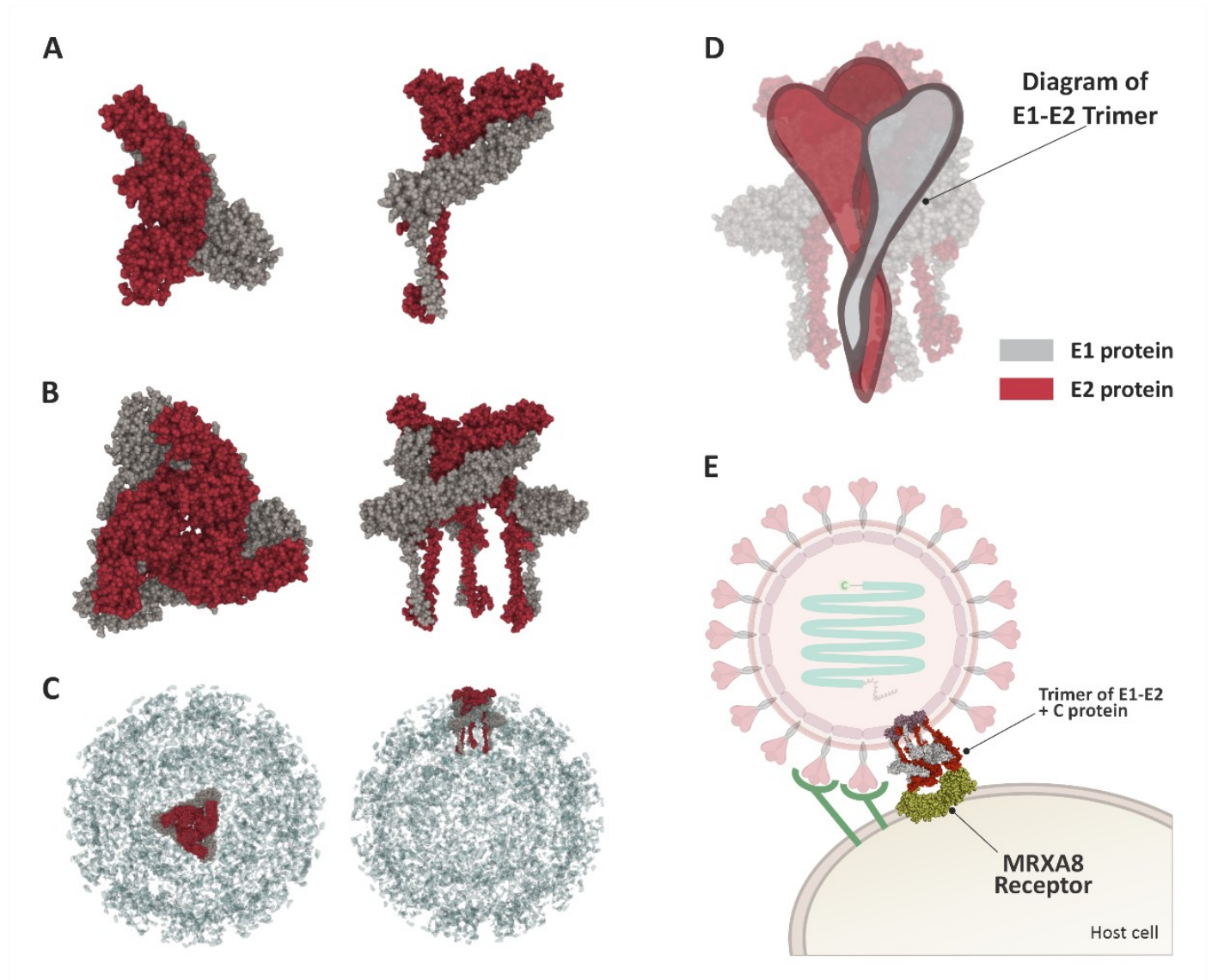


Figure 3: Schematic structure of MAYV glycoprotein. The glycoproteins E1 and E2 interact forming a dimeric unit (A), that is further associated to form a trimer (B). The localization of the trimers is demonstrated in (C), and a diagram of the E1-E2 trimer is shown in (D). The interaction between the glycoproteins and the MRXA8 receptor in host cells is demonstrated in (E).

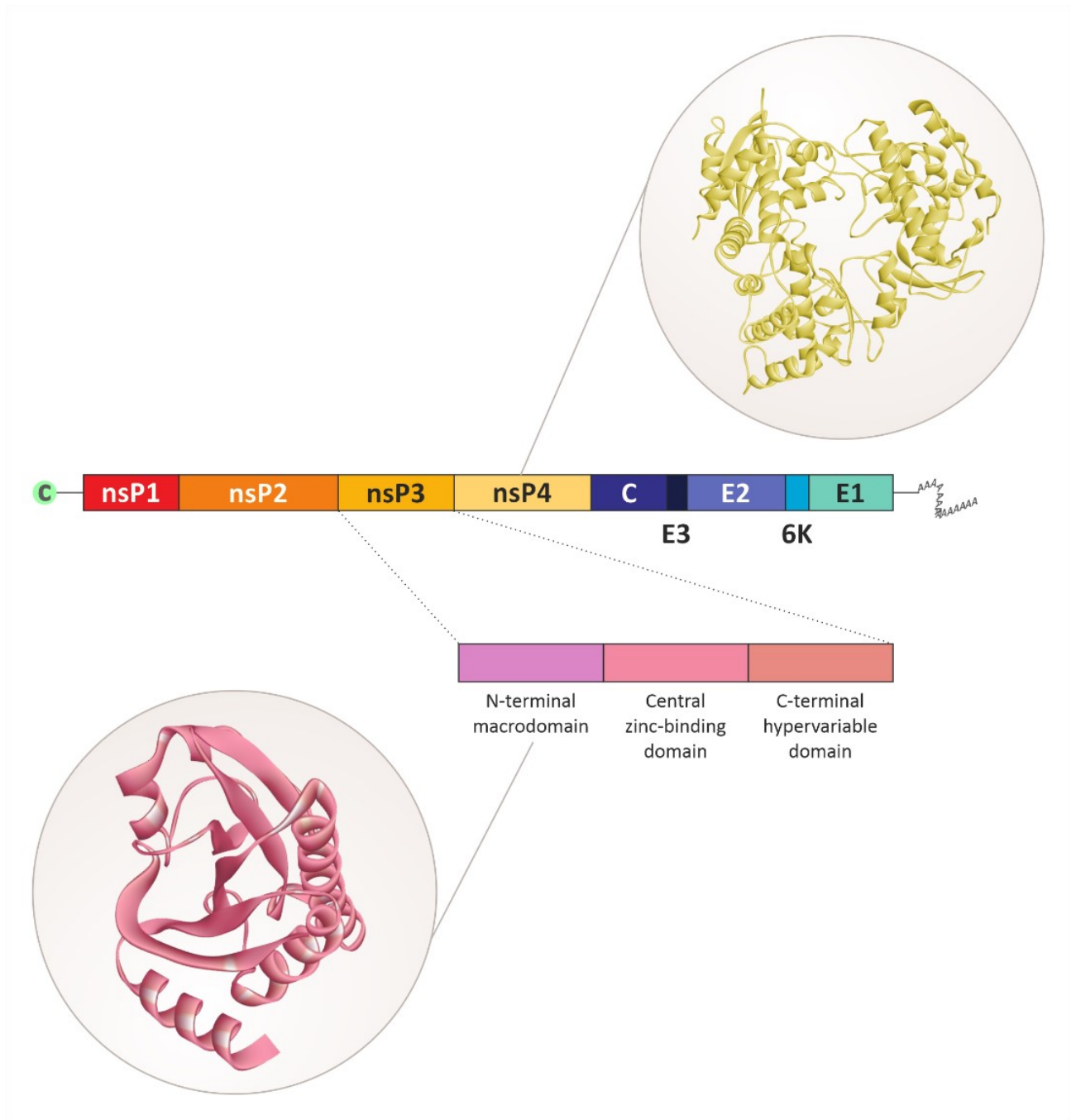


Figure 4: Schematic structure of MAYV genome and the crystal structure of nsP3 macrodomain and nsP4. Nsp3 is a modular protein with three domains: the N-terminal macrodomain, a central zinc-binding domain, and the C-terminal hypervariable domain, being the macrodomain constituted by four alpha-helices and six beta-strands. The nsP4 protein acts as an RNA-dependent RNA polymerase (RdRp), and its structure is composed of fingers, the palm containing the GDD active site, and thumb domains.