

UDC 578.233.4

CHARACTERIZATION OF THE YELLOW FEVER VIRUS REPLICATION COMPLEX USING AN NS1–GFP REPLICON

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ABSTRACT

Yellow fever virus is a mosquito-borne flavivirus responsible for severe human disease. Flaviviruses cause an estimated 400 million infections annually worldwide and are transmitted between animals and humans via arthropod vectors. Viral RNA replication is mediated by non-structural proteins that assemble into membrane-associated replication complexes (RC). Among these, the NS1 protein plays a critical role in replication complex formation and function. In this study, we investigated the replication complex of Yellow fever virus using sucrose density gradient ultracentrifugation. An NS1–GFP replicon system was constructed and employed to isolate and fractionate RC-associated components. This approach enables characterization of the molecular composition and organization of the replication complex and provides insights into the mechanisms underlying RNA replication in flaviviruses.

Keywords: Yellow fever virus, replication complex, flavivirus, ultracentrifugation.

INTRODUCTION

Yellow fever virus belongs to the family *Flaviviridae* and the genus *Flavivirus*, which includes several clinically important mosquito-borne viruses such as West Nile virus, Japanese encephalitis virus, and Dengue virus[1]. These viruses are responsible for a wide spectrum of severe human diseases. Despite their global impact, effective vaccines are unavailable for many flaviviruses, highlighting the importance of continued research in this field. In 2016, the World Health Organization declared Zika virus a Public Health Emergency of International Concern due to its rapid geographic spread and its association with neurological disorders and congenital abnormalities. The increasing incidence and geographic expansion of flavivirus infections present a growing public health challenge, driven in part by viral evolution and the emergence of genetically diverse strains[2].

The *Flaviviridae* family accounts for approximately 70 members, mostly grouped into several genera based on their genome, nature, and replication: tick-borne virus (the brightest representative of this genus is TBEV), mosquito-borne viruses (YFV, DENV, ZIKV, JEV), *Pestivirus* (BDV), and *Hepacivirus* (six genotypes of Hepatitis C virus)[3]. A meta-analysis of Yellow fever virus, Dengue virus, and Zika virus infections over the past decade estimated annual case numbers of approximately 86,000, 59,000, and 169,000, respectively, across more than 200 countries[4]. Dengue outbreaks, particularly in South Asia, have significantly impacted epidemiological and immunological research[5]. Flaviviruses are predominantly distributed in tropical and subtropical regions and are transmitted by arthropod vectors, primarily mosquitoes such as *Aedes aegypti* and *Aedes albopictus*, although some flaviviruses are tick-borne. Transmission occurs both vertically within vector populations

and horizontally between vectors and vertebrate hosts, including humans[6].

Flaviviruses are structurally and biologically similar positive-sense single-stranded RNA viruses with genomes of approximately 11,000 nucleotides, comprising a single open reading frame flanked by 5' and 3' untranslated regions[7]. After viral components penetrate the host organism's cytoplasm, the viral genome is released into the nucleocapsid, and RNA translation is initiated. Viral polyprotein is synthesized and further processed into the three structural and seven non-structural proteins. The structural proteins of flavivirus are capsid (C), pre-membrane (prM), and envelope (E) proteins. While non-structural proteins are high converted - NS1, NS3, NS5, and less converted proteins, namely, NS2a, NS2b, NS4a, NS4b (Figure 1)[8]. The capsid is essential for nucleocapsid formation; meanwhile, other structural proteins are responsible for viral entry and fusion. The viral RNA is subsequently used either for translation or for packaging into newly formed infectious virions. Besides that, it can be used for the synthesis of RNA strain[3,9].

Flaviviruses are on average 40–50 nm in diameter. The replication complex (RC) of the Yellow fever virus is conserved among flaviviruses and is localized in the cytoplasm. The synthesis of a complementary negative-strand RNA intermediate initiates viral RNA replication. Flaviviruses, like many positive-sense RNA viruses, exploit host cell organelles to support replication, with the endoplasmic reticulum (ER) serving as the primary site[10,11]. RNA replication is associated with modified membranes of the perinuclear ER, where viral replication complexes are assembled. The ER plays a central role not only in RNA replication but also in protein synthesis, lipid metabolism, and virion assembly. Membrane rearrangements and the formation of specialized replication compartments are

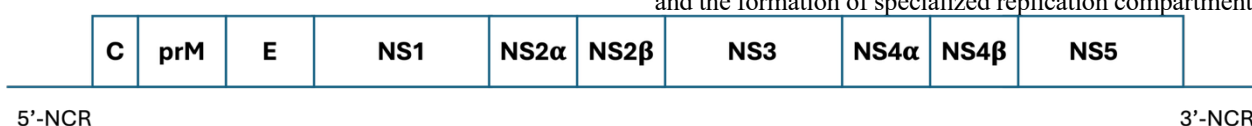


Figure 1. Structural organization of the Yellow fever virus.

essential for efficient viral replication. These processes are mediated by viral non-structural proteins, which coordinate the synthesis of the flaviviral genome[12,13].

As mentioned above, non-structural proteins are responsible for the efficiency of the flaviviruses' replication. Recent studies on this topic claimed that NS1 of flaviviruses plays a vital role in both viral genome synthesis and replication[14]. The evidence of these processes is the localization of the NS1 protein with double-stranded RNA and its association with intracellular membranes. NS1 protein is the initial non-structural protein that is translated, followed by envelopment, with a molecular weight of about 55 kDa[15,16]. Otherwise, NS2a participates in the replication process in the same way as NS1, but numerous research established that it can also be involved in the virion formation by viral RNA and structural proteins incorporation into the pathway[17,18]. The function of NS2b is associated with the mechanism of action of NS3. It's essential for the increase in NS3's protease activity[19]. Alternatively, it can be used as an engaging agent of NS3 into the endoplasmic reticulum. NS3 in complex with NS2b has an enzymatic activity and participates in RNA assembly and protein processing cycles[20]. NS4a is a compact non-structural protein that is critical for the rearrangement of membrane organelles and the membrane as a whole [21]. In another opinion, NS4a can be associated with NS1 and its function in the formation of replication organelles[22]. On the other hand, the significance of the NS4b protein is under discussion and could be related to the remodeling of the endoplasmic reticulum in flaviviruses[23]. NS5 is another protein that has protease activity. Like the NS3 protein, NS5 is a soluble protein that is located in the nucleus. The significance of the NS5 protein comes from translation and viral synthesis[24,25].

Methods for characterization of flavivirus replication complexes

Electron microscopy

Colloidal particles such as gold and silver are widely used to identify the localization of proteins and organelles[26]. Previous studies showed the localizations of most viral particles via the immunogold electron microscopy[27]. Structural localization of the NS1 protein of dengue virus, simultaneously with dsRNA loci, was found by Mackenzie et al[15]. This allowed us to build a hypothesis of the functions of the NS1 protein in the flavivirus's replication system. While observing the NS5 protein of yellow fever virus, performed by nuclear immunofluorescent (IF) staining[28]. A bunch of studies have shown the localization of structural and non-structural proteins in flaviviruses and identified that the envelope protein accumulates in the perinuclear region and cytoplasm, including previously described NS1-NS3 proteins[29]. The method is based on embedding-penetration, cryo-sectioning, and cell labelling with gold. Tissue or cell sections are fixed with paraformaldehyde, osmium tetroxide, or glutaraldehyde embedding with gelatin. Thin cryo-sections of fixed cells are commonly cut to 50-70 nm size and stored in sucrose and methylcellulose solutions. Pre-treated cells with antibodies against proteins on the cell surface must be incubated with colloidal particles conjugated with a secondary antibody[30].

Confocal microscopy

Use of the confocal microscopy technique for viral particle detection has started recently. Firstly, it is utilized for the identification of adeno-associated virus particles[31]. While authors reported using the same method for polyomavirus[32]. Nowadays, numerous studies have declared the advantages of confocal microscopy in the localization of viral particles and organelles, as well as protein dynamics in the cell. Crucial information about changes in a protein's localization can be caused by intracellular interactions of proteins that occur in cells[33]. Sample preparation procedures for confocal microscopy are similar for EM described before. Li et al. plated BHK-21 cell lines transfected with Japanese encephalitis virus (JEV) to detect cellular localization of DDX5. Unlike the control, where DDX5 localized in the nucleus and serum, in infected cells with JEV, DDX5 was found in the cytoplasm in colocalization with NS3 and NS5 proteins[34].

Immunofluorescent staining

The possibility of staining RNA *in situ* was reported previously, especially for heterogeneous RNA[35]. The visualization of RNA could be performed without isolation methods. There are antibodies specific for the target dsRNA to stain it *in situ* or in extracted form[36]. The technique focused on the recognition of target RNA structure by primary antibodies and staining them. While the authors declared the different staining reactions of cell lines. Stollar et al. reported staining of nucleoplasm and leaving the nucleoli unstained in Vero cell and *Aedes albopictus* cell lines, while the nuclei of BHK-21 and HeLa cell lines failed[37]. Son et al. observed dsRNA's localization visually by immunofluorescence staining of fixed, infected Vero B6 and DBT (astrocytoma-derived) cell lines[38]. Besides the nuclei and nucleoplasm detection, the cytoplasm visualization was characterized by using monoclonal antibodies to recognize TBEV's dsRNA and co-recognized the replication sites[37]. Although this technique has incredible potential in the detection of cells' RNA and its size, shape, and localization, it also has limitations in various cell lines. The main hypothesis of different reaction is the ratio of RNA synthesis and processing[39].

Dynamic light scattering spectroscopy (DLS)

Dynamic light scattering spectroscopy is a method using static light to measure intensity fluctuations caused by the Brownian motion of particles without destruction of the sample. The technique is based on collecting and measuring the lights at a particular angle by a detector. The angle less than 30° compatible with detecting larger particles, while the angle greater than 90° sensitive for small particles. Therefore, the right angle is the vital criterion for accurate measurements. Due to the Brownian motion, small particles move faster while the motion of larger particles takes a lot of time, and the fluctuation of lights is the basis of size measuring and can be calculated by the Stokes-Einstein equation[40]. This technique allows to detect size and structure by DLS spectroscopy and can be applicable for a diverse type of biological objects such as proteins, viruses, and liposomes[41]. DLS coupled with microscopy allow to measure cell dynamics of organelles, components of the cytoskeleton, and others[42]. Dynamics of the cell nucleus in the G1 phase of division were described by using dynamic light scattering[43]. Other studies reported the localization of intracellular dynamics in cancer cells[44].

Chromatography

Chromatography is a great option for the isolation and purification of biological agents as well as particles. The separation process is based on their characteristics such as size, shape, polarity, and affinity. Crooks et al. separated infected cells with tick-borne encephalitis (TBE) virus cells on a column of Sephacryl chromatography, and eluate fractions were then purified[45]. Coupled with electron microscopy and centrifugation column chromatography allowed authors to separate and purify nonstructural proteins and E polypeptide of TBE with high yields. The Skalica strain of tick-borne encephalitis virus was purified via affinity chromatography. Monoclonal antibodies against to TBE virus were used to bind to the affinity chromatography, and this allowed to separate and fractionate it on the column[46]. Size exclusion chromatography was successfully applied for the purification of extracellular vesicles extracted from pathogens. The latest research describes the application of a chromatography system for the separation, fractionation, and purification of viral particles due to its uncomplicated principles[47].

Density gradient centrifugation

Classic purification methods in virology are based on virus membrane degradation. In order to avoid the decomposition of biological and non-biological agents, a fractionation technique is applied, which is based on density formation during the centrifugation process. The fractionation method of viral particles allows researchers to divide and separate organelles and cell compartments grounded in their biochemical and physical features, especially on buoyant density of particles as well as size, shape, and mass[48]. This method commonly uses sucrose, iodixanol, and cesium chloride as a dense medium. Besides those reagents, also as a medium can be used glycerol, cesium sulfate, nycodenz, and Percoll can also be used as a medium[49]. The choice of materials depends on what purpose it will use. Choosing iodixanol as a density formation material is attributable to the speed of the protocol, while cesium chloride provides better resolution of viral components but usually consumes more time[50]. As a result, the components and cell compartments with large mass precipitate on the bottom side of the gradient, and the lighter particles collect on the top of the tube.

Cesium chloride density gradient

Cesium chloride-mediated precipitation of viral compartments has been widely used for the purification of numerous viruses, including adenoviruses and polyomaviruses. Typically, this approach requires ultracentrifugation at high speeds ($\sim 100,000 \times g$) to achieve efficient separation of viral fractions. However, Nasukawa et al. described an alternative strategy employing lower centrifugal force ($\sim 40,000 \times g$) applied

over an extended duration[51]. Following centrifugation, the sample is resolved into multiple fractions (e.g., cytoplasmic, membrane, and nuclear) based on differences in buoyant density within the cesium chloride gradient. In most cases, the upper layer contains low-density cellular components.

Iodixanol density gradient

Iodixanol, another reagent, can potentially be used for virus purification based on the density of different concentrations of iodixanol. Harvested cells were used for separation, followed by sedimentation by ultracentrifuge and flotation[52]. This technique was used for the extraction and replication complex of alphavirus by iodixanol density gradient fractionation. By gradient fractionation, they separated samples for several bands after SDS-electrophoresis, where each fraction contains cellular compartments. Predominantly, proteins are found at the top layer, while lipids can be found in membrane fractions and pellets[53].

Sucrose density gradient

Sucrose gradient fractionation is a commonly used technique for the purification of viral replication complexes as well as nucleic acids. Most generic protocols used for the flavivirus family have been explained by Uchil et al. They suggested using different densities of sucrose to perform a gradient, which allowed the separation of the fractions of viral compartments[54]. Tseng et al. described the application of sucrose dense gradient fractionation to investigate the association of non-structural protein 5 with the virus membrane[55]. Another study on the KUN virus proved the localization of viral products solely in heavy fractions 4 and 5[13].

MATERIALS AND METHODS

Cell culturing

BHK-21 (baby hamster kidney) cell lines were cultured on Dulbecco's Modified Eagle medium supplemented with 5% fetal bovine serum, 10 ug/ml penicillin and streptomycin antibiotics, non-essential amino acid, L-glutamine at 37 °C with provided 5% CO₂ in P100 Petri dishes due to 90-95% confluency. Cells were reseeded and passaged several times in P150 dishes. The condition of naive BHK-21 was visually controlled.

Transfection

Naive BHK-21 cells were transfected with the Laura-17 plasmid construction on Bio-Rad Gene Pulser Xcell following the exponential protocol (Figure 2). Before the procedure, cells were washed with 3 mL of 1x PBS and detached with 1.5 mL of trypsin solution that covered the surface of P150 dishes. Cells were incubated at 37 °C for 5 min in a CO₂ incubator. Cells were mixed with 7 mL of medium to inactivate

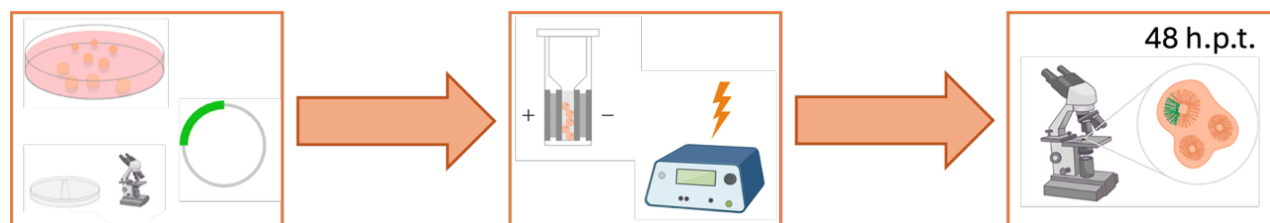


Figure 2. Schematics of the transfection via the electroporation of the mammalian cell lines.

trypsin activity and broken up the cell clumps by pipetting up and down several times. Cells in medium were collected and transferred into a 15 mL Falcon for centrifugation at 1000 x g for 5 min at 4 °C. Medium was discarded, and 7 mL of cold 1x PBS was added. The cell pellet was pipetted 10 times and centrifuged again. Pellet was resuspended in 450 uL of PBS and transferred to a tube. 5 ug of plasmid was added directly into the cells and transferred into the transfection cuvette. For the electroporation transfection process, we used an exponential protocol. The pulse was given twice: at 25 uF, 1500 V, 0.5s consistency, and the cuvettes were incubated at room temperature for 10 min.

After infecting BHK-21 cells with Laura-17 was cultured with 5% fetal bovine serum containing complete DMEM. At 48 h.p.i. GFP production was seen by visualizing on the Zeiss Axiocam Microscope. GFP-producing infected BHK-21 cells were passaged 10 times following the protocol due to the 95% confluency. BHK-21 cells didn't accumulate a mutation during the passaging.

YFV replication complex extraction

Infected BHK-21 cells were washed once with 1x PBS and detached from the Petri dish surface with trypsin and incubated for 5 min at 37 °C with 5% of CO₂. Infected cells were pelleted through centrifugation at 2500 x g for 5 min. Collected cells were used for the endoplasmic reticulum extraction. For the flavivirus's replicative complex extraction, we used the protocol described by Uchil et al[54]. The extraction process had been done according to the protocol with minor modifications. Pellet was incubated in 1% Triton X-100 on ice for 1 h for cellular plasmatic membrane disruption and centrifuged at 16 000 x g for 15 min at 4 °C. The pellet contains a heavy membrane fraction (P16), which was resuspended in 0.5 mL of TNMg buffer (10 mM Tris (pH 8.8), 10 mM sodium acetate, and 1.5 mM magnesium chloride). Sucrose dense gradient was prepared in polyallomer centrifugation tubes for 12,5 mL compatible with the Beckman Coulter SW-41 rotor. The bottom of the tube contained 0.5 mL of 75% sucrose (w/v), then 0.5 mL of the sample in TNMg mixed with 4 mL of 75% sucrose (w/v), the upper phase contains 4 mL of 55% sucrose (w/v) and 0.5 mL of 5% sucrose solution (w/v) above it. The heavy membrane fraction P16 fraction was separated via ultracentrifugation at 35 000 x g, for 18 hours at 4 °C.

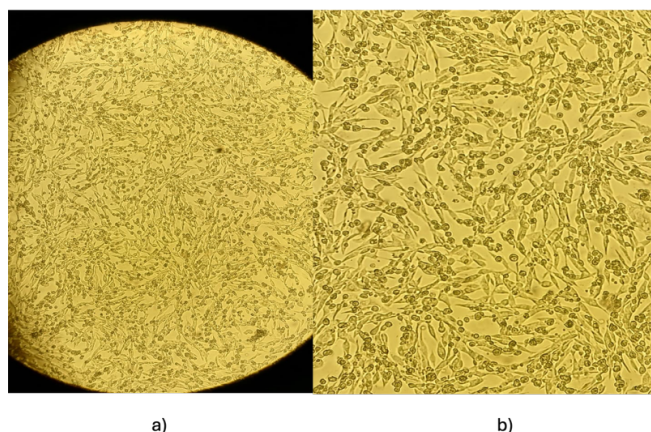


Figure 3. a) Naive BHK-21 cells cultured in the DMEM medium, 5x optical zoom; b) BHK-21 cells in 10x dimension zoom in a Zeiss Axiocam Microscope

Thirteen fractions of sucrose dense gradient were collected by 1 mL volume in Eppendorf tubes. Sucrose from fractions was washed through the resuspension of each fraction in a 1x RNase-free cold phosphatase saline solution (PBS). Then fractions were centrifuged at 30 000x g for 30 min at 4 °C.

Western blot analysis

The replication complex in gradient fractions was checked by performing western blotting. For the western blotting technique, gradient fractions were electrophoresed on the 12% SDS-polyacrylamide gel. The bands were transferred onto the PVDF membrane at 30V for 1h. Samples were fixed in the membrane by soaking for 5 min in ponceau solution and blocked in 5% dry milk solution. As a primary antibody, we treated the membrane with the combination of anti-GFP and anti-NS1 antibodies (Invitrogen, Thermo Fisher Scientific) in the concentration 1:1500 and 1:500, respectively. Membrane was washed and incubated with DyLight 680 secondary antibody-conjugate (Cell Signaling Technology) in a ratio of 1:10000. Visualization of signals occurred in the Odyssey DLx Infrared Imager.

RESULTS

Cell culturing and transfection

Naive BHK-21 cells at 90–95% confluency (Figure 3) were transfected with the GFP–YFV/NS1 plasmid construct by electroporation.

The rate of infection is checked by the presence of fluorescence at 72 hours post-transfection (h.p.t.). Visible GFP production in the transfected BHK-21 cell lines was successful evidence of the infectious process. It demonstrated genetic stability from passage to passage. According to the latest studies, the production of GFP accumulates in a perinuclear area of cells. This was proved by the visual screening of GFP production in the BHK-21 cells and staining of fixed cells with DAPI-containing reagent (Figure 4).

The re-seeding frequency reached over 10 passages before the extraction process was initiated. For the replication complex, at least 2×10^7 cells/mL of the monolayer of infected cells.

YFV replication complex extraction

Based on GFP-NS1 replicon construction, the replication complex's localization site was uncovered. Infected with GFP-YFV/NS1 plasmid, BHK-21 cells that reached confluency rates were used for the extraction process. The cells were lysed in a detergent-containing solution that directly affects the plasma membrane as a degradation factor. Centrifugation of the lysed cells allowed the separation of the heavy membrane fraction, which accumulates in the pellet (P16K). Supernatant is the post-mitochondrial fraction, which contains subcellular components. Post-mitochondrial S16K fraction was saved for further comparative analysis of western blotting.

Sucrose dense gradient was chosen as a fractionation method due to the creation of a stable, high-density medium for sedimentation. This technique is applied for separating the subcellular compartments according to their buoyant density grades. More buoyant cellular components gather at the top of the gradient, while denser compartments localize at the bottom of the sucrose gradient, as reflected in their density. After ultracentrifugation, two cloud-like visible bands were seen on the gradient (fraction 4-5). The fractionation process demonstrated these bands as an accumulation of the fusion proteins

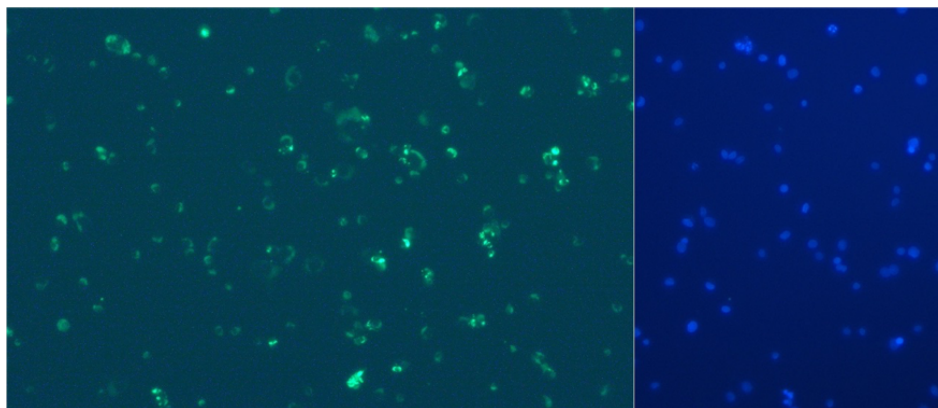


Figure 4. Infected with YFV/NS1-GFP construction BHK-21 cell lines at 48 h.p.i. a. GFP producing infected BHK-21 cell line, 10x zoom; b. Infected BHK-21 cells' nuclei stained with DAPI-containing reagent, 10x zoom.

of rival replication complexes. Twelve fractions and a pellet were used for further Western blotting analysis.

SDS-PAGE and Western blot analysis

All fractions after the sedimentation process were used for the western blotting. Post-mitochondrial supernatant and pellet fractions were analyzed in 10% SDS-polyacrylamide gel electrophoresis prepared in TGE buffer, as shown in Figure 5.

As a preliminary analysis, the gel was stained in Coomassie Brilliant R-250 and then incubated in a destaining solution. As a positive control, an infected BHK-21 cellular suspension in 1x phosphatase solution. All bands of replication complexes matched the molecular weight of the YFV/NS1-GFP construction. Analysis of visible bands identified fusion proteins in 74kDa. After the extraction of replication complexes, the gradient fraction was analyzed by western blotting, which follows gel electrophoresis. Twelve fractions and a pellet were loaded into an SDS gel and separated according to the protocol. Afterward, the gel was used for transferring to blot paper. After the antibody treatments, the signals were detected by the channels red 700 nm and green 800 nm on the Odyssey Imager. Treated with a mix of monoclonal anti-NS1 and anti-GFP antibodies, membranes showed the localization of the fusion proteins as a double band in the fractions 4-5 by using engineered replicon construction (Figure 6).

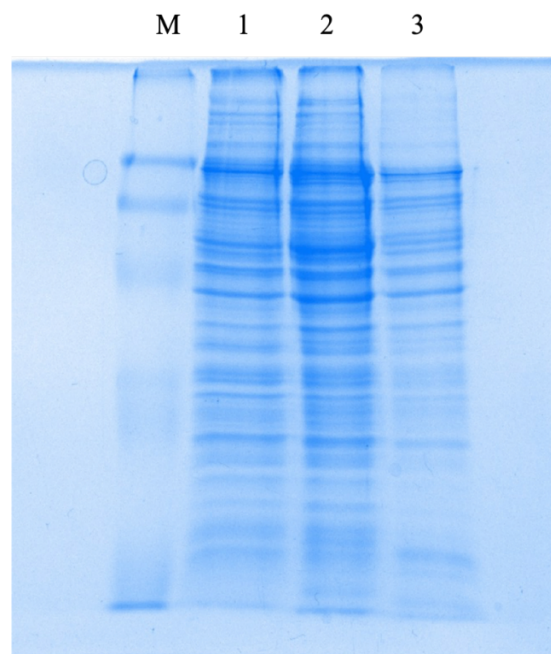


Figure 5. 10% SDS-Polyacrylamide gel electrophoresis. M. ColorBurst Electrophoresis Maker, mw 8-220 kDa; 1. Post mitochondrial S16K fraction; 2. Heavy membrane fraction; 3. Infected BHK-21 cell suspension in 1x PBS.

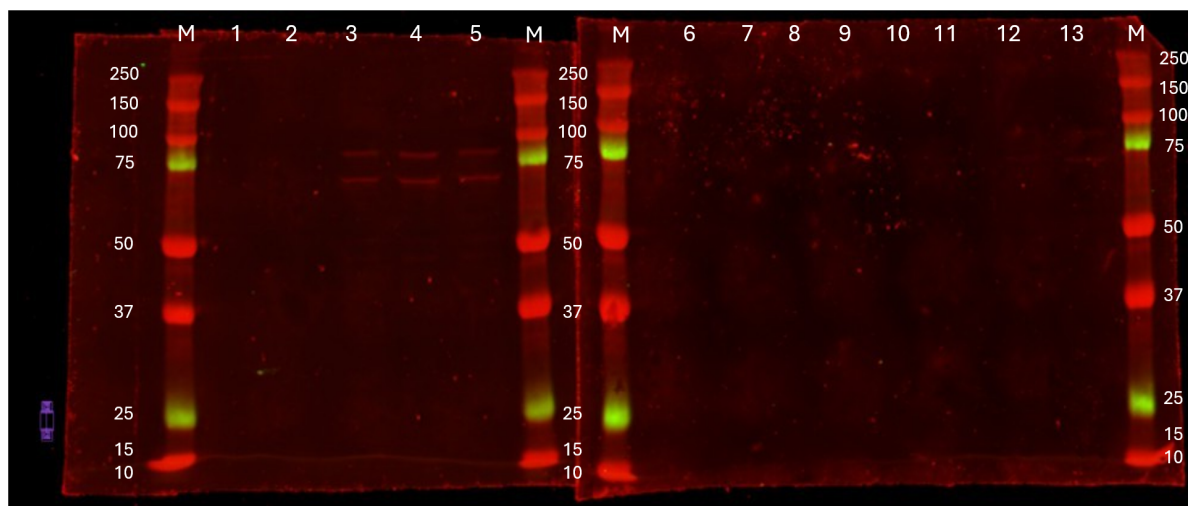


Figure 6. Western-Blot analysis of the gradient fractions treated with anti-NS1 and anti-GFP monoclonal antibodies. Lane M – Precision Plus Protein Kaleidoscope marker, 1-12 – sucrose gradient fractions, 13 – pellet of gradient, resuspended in 1x PBS.

CONCLUSION

This study establishes a density-based framework for characterizing viral replication complexes and demonstrates its application to the Yellow fever virus. The results confirm that the flaviviral replication complex is a conserved, membrane-associated structure that functions as a platform for RNA replication. The NS1–GFP replicon enabled targeted visualization and tracking of replication complex components, revealing their association with endoplasmic reticulum–derived membranes. Sucrose density gradient centrifugation effectively resolved cellular compartments, with replication complex–enriched fractions consistently localized to fractions 4–5 of the post-mitochondrial gradient. These findings were supported by Western blot analysis, which demonstrated specific detection of NS1–GFP in the corresponding fractions. Together, the data validate the combined use of gradient fractionation and immunodetection as a reliable approach for replication complex analysis. Further characterization by LC–MS/MS will provide a comprehensive definition of the molecular composition and functional organization of the replication complex.

FUNDING

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan under the Grant No. AP23488393 “Study of the intracellular interactome of viral replication complex proteins”.

AUTHOR CONTRIBUTIONS

Conceptualization: A.S., P.T.; methodology: D.A., B.I.; statistical analysis: S.B.; study design: A.S., P.T.; data interpretation: D.A., A.S.; original draft preparation: D.A.; review and editing: S.A., A.S.; supervision: P.T. All authors have read and agreed to the published version of the manuscript.

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ХАРАКТЕРИЗАЦИЯ РЕПЛИКАТИВНОГО КОМПЛЕКСА ВИРУСА ЖЁЛТОЙ ЛИХОРАДКИ С ИСПОЛЬЗОВАНИЕМ РЕПЛИКОНА NS1–GFP

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АБСТРАКТ

Вирус жёлтой лихорадки – флавивирус, который является возбудителем множеств тяжелых заболеваний человека, переносчиком которого является комары. По предположительным оценкам, около 400 миллион случаев заражения и передачи от животных к людям через членистоногих переносчиков ежегодно регистрируются в мире. Репликация вирусной РНК опосредуется неструктурными белками, которые собираются в связанные с мембраной репликативных комплексов. Среди прочих неструктурных белков NS1 имеет важную роль в формировании и функционировании репликативных комплексов. В данном исследовании мы исследовали комплекс репликативного комплекса вируса жёлтой лихорадки с помощью ультрацентрифугирование на градиенте плотности сахарозы. Была сконструирована и использована система репликона NS1–GFP для выделения и фракционирования, ассоциированных с репликативным комплексом, компонентов. Данный подход позволяет охарактеризовать молекулярный состав и организацию репликативного комплекса и свидетельствует о механизмах, лежащих в основе репликации РНК у флавивирусов.

Ключевые слова: Вирус жёлтой лихорадки, репликационный комплекс, флавивирус, ультрацентрифугирование

NS1–GFP РЕПЛИКОН НЕГІЗІНДЕ САРЫ ҚЫЗБА ВИРУСЫНЫҢ РЕПЛИКАТИВТІК КЕШЕНІН СІПАТТАУ

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ТҮЙІН

Сары қызба вирусы – тасымалдаушысы масалар болып табылатын флавивирус, көптеген аурулардың қоздырушысы. Статистикалық болжамдарға сәйкес, әлем бойынша жылына шамамен 400 миллионға жуық жануарлардан адамға жұқтыру жағдайлары тіркеледі. Вирустың РНК репликациясы мембранамен байланысқан репликативтік кешенде жиналатын құрылымдық емес ақуыздардың көмегімен жүзеге асырылады. Өзге құрылымдық емес ақуыздардың арасында NS1 ақуызы репликативтік кешеннің қалыптасуы мен қызметінде маңызды рөл атқарады. Осы зерттеу жұмысында біз сары қызба вирусының репликативтік кешенінің сахарозаның тығыздық градиентінде ультрацентрифугалау көмегімен зерттедік. Репликативтік кешенмен байланысты компоненттерді бөліп алу мен фракциялау мақсатында NS1–GFP репликон жүйесі жасалып, қолданылды. Аталған әдіс репликативтік кешеннің молекулалық құрамы мен оның ұйымдастырылу жүйесін сипаттауға ықпал етіп, флавивирустардың РНК репликациясының негізінде жатқан механизмдерді анықтап, зерттеуге мүмкіндік берді.

Кілтті сөздер: Сары қызба вирусы, репликативті кешен, флавивирус, ультрацентрифугалау