Cytoarchitecture of Zika virus infection in human neuroblastoma and Aedes albopictus cell lines

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ABSTRACT

The Zika virus (ZIKV) pandemic is a global concern due to its role in the development of congenital anomalies of the central nervous system. This mosquito-borne flavivirus alternates between mammalian and mosquito hosts, but information about the biogenesis of ZIKV is limited. Using a human neuroblastoma cell line (SK-N-SH) and an Aedes albopictus mosquito cell line (C6/36), we characterized ZIKV infection by immunofluorescence, transmission electron microscopy (TEM), and electron tomography (ET) to better understand infection in these disparate host cells. ZIKV replicated well in both cell lines, but infected SK-N-SH cells suffered a lytic crisis. Flaviviruses scavenge host cell membranes to serve as replication platforms and ZIKV showed the hallmarks of this process. Via TEM, we identified virus particles and 60–100 nm spherical vesicles. ET revealed these vesicular replication compartments contain smaller 20–30 nm spherular structures. Our studies indicate that SK-N-SH and C6/36 cells are relevant models for viral cytoarchitecture study.

1. Introduction

Originally discovered in 1947 in a sentinel Rhesus monkey during yellow fever studies in the Zika forest of Uganda and then isolated from mosquitoes in the same region in 1948 (Smithburn, 1951), ZIKV was regarded as an unimportant mosquito-borne flavivirus until its emergence in Latin America. Currently, multiple countries are reporting autochthonous outbreaks of Zika virus (ZIKV) with continuing mosquito-borne transmission since 2015 (World Health Organization, 2016), and other countries are experiencing significant cases of infection associated with non-vector mediated transmission. These include maternal-fetal transmission (Brasil et al., 2016), sexual transmission from and to both males and females (Foy et al., 2011; Centers for Disease Control and Prevention, 2016; Turmel et al., 2016; Musso et al., 2015), and blood transfusion (Musso et al., 2014). Previously considered a benign disease, ZIKV infection is now regarded as a serious disease.

ZIKV is a member of the Flaviviridae family, and is closely related to several other mosquito-borne flaviviruses (MBFV), such as, Dengue, West Nile virus and Yellow Fever (Miner et al., 2016). Flaviviruses are single-stranded, positive sense RNA viruses. After binding and entry into a cell, the 11 kb viral RNA genome is released into the cytoplasm and translated at the endoplasmic reticulum into a single polyprotein. Viral and host proteases cleave this polyprotein into 3 structural (capsid, membrane [M], and envelope [E]) and 7 nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins (Bartenschlager and Miller, 2008).

A remarkable feature of flavivirus biology is the interaction between virus replication and the cellular membranes. These viruses induce a marked proliferation and rearrangement of endoplasmic reticulum membranes and reconfigure them into a variety of vesicular structures that bulge into the ER lumen and serve as replication compartments. Within these compartments, NS5, the viral RNA-dependent RNA polymerase, in conjunction with other viral nonstructural proteins and host factors, synthesizes a double-stranded viral RNA, and forms a replication complex to produce viral progeny genomes. The progeny strands and the viral structural proteins associate on adjacent lamellae of the ER where immature particles are generated and intruded into

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the ER lumen. The particles are transported to and traverse the Golgi structures, acquiring glycosylation. Finally, in a post-Golgi vesicle, the covalent attachment between the preM and E structural proteins is cleaved by furin and the mature virion is formed and exits the cell (Roby et al., 2015).

A unique requirement of the viral biology of ZIKV and other MBFV is the need to replicate both in mammalian and mosquito cells. The ultrastructure of a number of vector-borne flavirviruses has been well-studied (Welsch et al., 2009; Gillespie et al., 2010; Romero-Brey and Bartenschlager, 2015; Offerdahl et al., 2012; Miorin et al., 2013; Junjhon et al., 2014; Whiteman et al., 2014; Bily et al., 2015). There have been recent reports examining ZIKV infection in human skin cells (Hamel et al., 2015) and African green monkey kidney cells (Ferreira Barreto-Vieira et al., 2016). In addition papers have depicted ZIKV replication in cells of neural origin (Qian et al., 2016; Hughes et al., 2016). However, the ultimate aim of our work is to characterize the cell biology and cytoarchitecture of ZIKV infection at extremely high resolution microscopy techniques, and for this purpose well-characterized cell lines of relevant origin are required. Therefore, in this study, we characterized the cytoarchitecture of ZIKV replication in human neuroblastoma and Aedes albopictus mosquito cells using a current South American ZIKV isolate. Additionally, we characterized the envelope protein’s localization to the endoplasmic reticulum and visualized the ultrastructural scaffolding of infection. We also employed electron tomography to provide a 3-dimensional comparison of ZIKV infection in both human and mosquito cell lines.

2. Materials and methods

2.1. Cells and viruses

SK-N-SH human neuroblastoma cells (HTB-11, ATCC), obtained from a bone marrow sample from a 4-year-old female neuroblastoma patient, were maintained in Eagle’s minimal essential media (EMEM, ATCC) supplemented with 10% fetal calf serum (Life Technologies) (complete EMEM) at 37 °C in 5% CO₂. Aedes aegypti cells (CCL-125, ATCC) and Aedes albopictus cells were developed by Singh (1967) from pools of A. aegypti and A. albopictus larvae, respectively. Singh’s Aedes albopictus cells were further subcloned by Igarashi (1978) to create the C6/36 cell line (kindly provided by Dr. Stephen Whitehead, NIH/NIAID). Both mosquito cell lines were cultured in complete EMEM at 37 °C in 5% CO₂.

Zika virus Paraiba/2015 strain (ZIKV) isolated from a febrile female patient (kindly provided by Drs. Pedro F.C. Vasconcelos, Instituto Evandro Chagas, Brazil and Stephen Whitehead, NIH/NIAID) was propagated in African green monkey kidney cells (Vero, ATCC) at a multiplicity of infection (MOI) of 0.005. Viral titer was done by immunofocus assay as previously described (Offerdahl et al., 2012) using African green monkey kidney cells (Vero, ATCC), anti-flavivirus group antigen clone D1-4G2-4-15 (4G2, Millipore). Since antibodies against non-structural proteins were not available, staining for double-stranded RNA (dsRNA) was used as a surrogate marker for virus replication and stained by mouse monoclonal anti-dsRNA J2, IgG2α (English and Scientific Consulting). Secondary antibodies used were: Alexa Fluor 594 and 647-conjugated anti-mouse (Life Technologies). All antibodies were used at a dilution of 1:1000.

Cell volumes were determined by observation and measurement of nuclear (DAPI) and anti-E (4G2) staining of ZIKV infected cells using Zen 2012 SP1 software (Zeiss). Fifteen cells for each cell line were imaged and length, width, and height of both the 4G2 label and DAPI determined. Nuclear volumes for SK-N-SH and C6/36 cells were calculated assuming they were spheres. Due to length and width measurements, 4G2 staining volumes for SK-N-SH cells were calculated as rectangles, while C6/36 4G2 volumes were calculated assuming a spherical shape. The average nuclear and anti-E volumes of SK-N-SH cells were 2894 and 7913 µm³, respectively. Those for C6/36 cells were 354 and 645 µm³. Nuclear volumes were subtracted from the anti-E staining volumes to give the approximate volumes of virion/vesicle containing cellular areas.

2.2. Immunofluorescence microscopy

SK-N-SH and C6/36 cells were plated at 3×10⁴ cells/well in 8 well Labtek dishes (Nunc), infected with ZIKV Paraiba (MOI 10), and immunofluorescence assays were performed as previously described (Offerdahl et al., 2012). Mouse monoclonal Dylight 488-conjugated protein disulfide isomerase 1D3 (PDI, Enzo Life Sciences) was used as a cellular marker for the endoplasmic reticulum. The E protein of ZIKV was localized through the use of anti-flavivirus group antigen antibody, clone D1-4G2-4-15 (4G2, Millipore). Since antibodies against non-structural proteins were not available, staining for double-stranded RNA (dsRNA) was used as a surrogate marker for virus replication and stained by mouse monoclonal anti-dsRNA J2, IgG2α (English and Scientific Consulting). Secondary antibodies used were: Alexa Fluor 594 and 647-conjugated anti-mouse (Life Technologies). All antibodies were used at a dilution of 1:1000.

For transmission electron microscopy (TEM) and tomography, samples were prepared and processed essentially as described previously (Offerdahl et al., 2012), except that araldite embedding resin (SPI, Inc., West Chester, PA) was substituted for Spurr’s resin. Silver-colored sections were used for TEM. Sections for electron tomography (ET) were cut at a setting of 150 nm, and collected on Formvar/carbon-coated 2×1 mm copper or gold slot grids (Electron Microscopy Sciences, Hatfield, PA).

Samples were imaged for TEM and ET as described previously (Offerdahl et al., 2012), except as follows. Dual-axis tomographic tilt series of serial sections were collected using SerialEM software (University of Colorado, Boulder CO) (Mastronarde, 2005), and Ultrascan camera (Gatan, Inc., Pleasanton, CA). Volumetric reconstructions were created either manually or semi-automatically using the IMOD applications Etomo or Batchruntomo (University of Colorado) (Kremer et al., 1996), respectively. Counting of virions and virus-induced vesicles was performed using a modeling feature in IMOD to highlight spheres of a desired size and track them throughout the tomogram.

3. Results

3.1. Infection of human neuroblastoma and mosquito cells with Zika virus

In Latin America, the enzootic ZIKV is currently circulating in Aedes mosquitoes which then transmit the virus to humans causing disease. Both Aedes aegypti and Aedes albopictus mosquitoes have been implicated in ZIKV transmission. Therefore, we chose to evaluate the susceptibility of relevant cell lines to the 2015 ZIKV Paraiba, an isolate derived from a recent case in the current Brazilian outbreak. We
infected human neuroblastoma (SK-N-SH), *Aedes aegypti* (CCL-125), and *Aedes albopictus* (C6/36) cells with ZIKV at a multiplicity of infection (MOI) of 10. Serial samples were collected for determination of viral replication and evaluation of cytopathology. ZIKV replicated well in the SK-N-SH and C6/36 cell lines, reaching titers of $10^6$ focus forming units (ffu) per ml by 72 h post-ZIKV infection (hpi) in SK-N-SH cells and 96 hpi in C6/36 cells (Fig. 1). Conversely, CCL-125 cells showed negligible virus replication over the time course, with a maximum titer of $60 \text{ ffu/ml}$ at 72 hpi (data not shown).

The effects of virus replication on cellular morphology were observed via light microscopy and Giemsa staining for signs of cytopathic effect (CPE). By 48 hpi, CPE as manifest by rounding and cytoplasmic condensation of cells was evident in infected SK-N-SH cells (Fig. 2A). By 72 hpi, considerable CPE and sloughing of cells and syncytia formation were evident. After 96 hpi, an acute lytic crisis was apparent and few cells remained attached to the culture dish. On the other hand, C6/36 (Fig. 2B) and CCL-125 (data not shown) cells showed no overt signs of CPE over the course of evaluation (120 hpi).

Due to the failure of ZIKV to replicate in the CCL-125 cells, we continued our experiments with only the SK-N-SH and C6/36 cell lines.

3.2. Assessment of apoptosis induced by ZIKV in human neuroblastoma and *A. albopictus* cells

Widespread loss of the monolayer integrity in the neuroblastoma cells by 96–120 hpi suggested activation of cell death pathways. We, therefore, examined two parameters often associated with virus induced cell death: caspase activation and apoptosis. Immunofluorescence studies of infected SK-N-SH cells confirmed the presence of cleaved (active) caspase 3, an executioner caspase, indicating that the apoptosis cascade had been activated in these cells. (Fig. 3). Active caspase 3 staining was noted in some neuroblastoma cells as early as 24 hpi and was extensive by 72 hpi (Fig. 3B). The development of apoptosis was confirmed by the use of a terminal deoxynucleotidyltransferase-mediated TMR red-dUTP nick end labeling (TUNEL) assay (Fig. 4). TUNEL staining was first seen in SK-N-SH cells at 72 hpi but, presumably due to lysis and to cell sloughing, TUNEL positive cells were difficult to find at later time points. Thus, the lytic crisis observed in ZIKV-infected neuroblastoma cells was most likely effected by caspase-mediated apoptosis.

In infected C6/36 cells, some cleaved caspase 3 staining was seen at 48 hpi, became more common by 96 hpi and was widespread by 120 hpi. (Fig. 3B). Interestingly, when the C6/36 cells were subjected to TUNEL staining, only rare cells were TUNEL positive (Fig. 4). These results suggested that progression to apoptosis was being blocked even in the face of caspase activation.

3.3. Localization of Zika virus E glycoprotein and site of virus replication in infected human neuroblastoma and *A. albopictus* cells

Previously studied vector-borne flaviviruses induce a pronounced
expansion and rearrangement of ER membranes in infected cells (Welsch et al., 2009; Gillespie et al., 2010; Offerdahl et al., 2012; Miorin et al., 2013; Junjhon et al., 2014; Whiteman et al., 2014; Bily et al., 2015). ZIKV infection of the human neuroblastoma cells led to a dramatic increase of ER as evidenced by a pronounced increase of staining for protein disulfide isomerase (PDI), an endoplasmic reticulum chaperone protein, compared to mock infected samples (Fig. 5). Signal for the ZIKV E glycoprotein was evident at 24 hpi (data not shown) and by 72 hpi, almost all cells in the culture were positive (Fig. 5). E glycoprotein was distinctly present in the cytoplasm and clearly overlapped with the PDI staining (Fig. 5), thus confirming that this viral structural protein was associated with the ER.

To identify sites of virus genome replication, we used an antibody for dsRNA, a surrogate marker for virus replication. A punctate pattern of dsRNA staining that overlapped with PDI staining for ER was clearly present in ZIKV infected neuroblastoma cells (Fig. 6). Mock infected cells did not stain for E protein (Fig. 5) or dsRNA (Fig. 6). Taken together, these results indicated that ZIKV replication was occurring in association with the ER in the neuroblastoma cells.

In C6/36 cells, ER expansion was apparent but slightly less obvious due to cell morphology (Fig. 5). The C6/36 cells are noticeably smaller than the SK-N-SH cells and more rounded in shape making differences in ER levels less striking than in the SK-N-SH. C6/36 cells showed a one day delay in reaching similar levels of ZIKV E protein staining at 48 hpi to those seen in SK-N-SH cells at 24 hpi (Fig. 5). Viral replication, as denoted by dsRNA staining, was similar in pattern and amount, but again delayed by 24 h compared with the neuroblastoma cells (Fig. 6). The delay in both ZIKV E protein and dsRNA staining corresponds with the viral titer seen in these two cell lines. In brief this data shows that, like other flaviviruses, ZIKV replication occurs within the confines of the ER in both mosquito and human cells.

3.4. The ultrastructure of ZIKV replication in human neuroblastoma and A. albopictus cells

After performing immunofluorescence assays to characterize ZIKV replication in both SK-N-SH and C6/36 cells by immunofluorescence and confocal microscopy, we turned to transmission electron microscopy (TEM) to visualize the cytoarchitecture of ZIKV infection in both human and mosquito cells at high resolution. In SK-N-SH cells, low magnification TEM images showed dramatic ER proliferation compared with mock-infected cells (Fig. 7A and B), encompassing nearly all of the cytoplasm (Fig. 7C). As has been reported for other flaviviruses (Welsch et al., 2009; Gillespie et al., 2010; Offerdahl et al., 2012; Miorin et al., 2013; Junjhon et al., 2014; Takasaki et al., 2001; Hase et al., 1987; Grief et al., 1997; Barth, 1992), ZIKV-induced vesicular replication compartments (or spherules), measuring 60–100 nm in diameter, were found within the rough-ER lumen, often in closely packed groups (Fig. 7E). Sometimes within the spherules, vesicles that appear to be a smaller sized (20–40 nm) were seen (Fig. 7E), however, we believe this to be due to the plane of section through the sample and not a difference in structure. Additionally, TEM showed virus particles (~30 nm in diameter), within membranes of the ER. The virions were often observed in small groups resembling “peas in a pod.” (Fig. 7G).

In the C6/36 cells, significant accumulations of ER expansion were also seen in ZIKV infected cells (Fig. 7D). With additional magnification, numerous vesicles (60–100 nm in diameter) were seen scattered within the ER scaffold (Fig. 7F and H). Virions were intermixed with these vesicles within the ER membranes, showing similar size (~30 nm) and distribution as seen with the neuroblastoma cells (Fig. 7F and H).

Like in other flavivirus studies (Welsch et al., 2009; Gillespie et al., 2010; Offerdahl et al., 2012; Junjhon et al., 2014), open necks or communicating pores between adjacent vesicles or connecting vesicles to the cytoplasm were seen in both cell lines. Previous works (Gillespie et al., 2010; Junjhon et al., 2014) have also noted structures within the
vesicles, which are believed to be the actual replication complexes. We observed these structures in both SK-N-SH and C6/36 cells; however, in some vesicles, the structures internal to the vesicle had a ring-like appearance and a diameter of 20–30 nm (Fig. 7H).

3.5. Three-dimensional electron tomography of ZIKV replication in human neuroblastoma and A. albopictus cells

The use of dual-axis electron tomography (ET) provided significant additional information to the 2-D images described in the previous section. The 3-D imagery clearly demonstrated that spherical replication compartments intruded into ER cisternae and were attached to the lamella of the ER in both the human neuroblastoma (Fig. 8A and Movie S1) and A. albopictus (Fig. 8B and Movie S2) cells. A small number of the vesicular replication compartments were obviously tubular rather than spherical in form, as has been reported in other flavivirus models (Welsch et al., 2009; Offerdahl et al., 2012; Junjhon et al., 2014; Takasaki et al., 2001; Hase et al., 1987; Grief et al., 1997; Barth, 1992).

The dual-tilt tomography also revealed that the circular structures noted within the vesicles were, in fact, “hollow” spherical structures, approximately 20–30 nm in diameter, an observation not evident by standard TEM (Movie S1 and S2). Interestingly, these smaller spheres sometimes appeared attached to the wall of the vesicle where the vesicles were contiguous with the ER lamellae (Fig. 8A and B, insets). Tomography confirmed that the virions were in membrane bound profiles of ER, and in some cases, they were adjacent to the replication compartments (Fig. 8A and B). We did not observe any obvious structures suggestive of nascent virions budding into the ER.

In an effort to estimate the number of vesicles and virions within a single cell, we utilized a software modeling function of the IMOD suite, described in the materials and methods, to select and enumerate these structures within the chosen imaging areas in both cell lines. In the SK-N-SH cells, 598 virus particles and 267 virus-induced vesicles were counted per cubic micrometer. Fewer virions and vesicles were seen in the C6/36 cells, 212 and 188 per cubic micrometer, respectively. These values, although accurate for the sections imaged, are almost certainly not representative of the entire infected cell volume, as areas enriched for vesicles, virions, and proliferated ER were preferentially chosen for ET imaging. However, in an effort to estimate the amount of virions and vesicles present per cell, we determined, via confocal microscopy (described in material and methods), the approximate volume of 4G2 antibody staining material for each cell line to allow extrapolation of the particle and vesicles counts established by our ET experiments. For SK-N-SH cells, this calculated to 3×10⁶ virions and 1×10⁶ vesicles per cell while the smaller C6/36 cells were estimated to have 2×10⁵ virions and 2×10⁵ vesicles per cell. Virus particle and vesicles counts for both cells lines were performed at the 72hpi time point. Interestingly, the one log difference in virion and

![Fig. 4. TUNEL staining during ZIKV Paraiba infection in SK-N-SH and C6/36 cells. Cells were mock-infected (A) or infected with ZIKV Paraiba at a MOI of 10 (B), fixed, and TUNEL stained to visualize single-stranded DNA breaks (red) and nuclei were counterstained with DAPI (blue). DNase I treated cells were used as a positive control and the TUNEL enzyme was omitted from the negative control samples (A). 63× magnification.](image-url)
vesicle counts between SK-N-SH and C6/36 cells parallels the one log difference seen in virus titer (Fig. 1) at this time point.

4. Discussion

The recent explosion of ZIKV infections in Latin America has led to a surge of interest in this emerging virus. Amid global travel concerns and a paucity of information on the effects of ZIKV on humans, ZIKV research has quickly expanded. Driving much of the concern is the pronounced neurotropism of this virus as well as the expanding catalog of structural and functional neurological problems patent in infected human fetuses and newborns (Anderson et al., 2016).

In order to model and study the cytoarchitecture and cell biology of ZIKV, the human neuroblastoma cell line, SK-N-SH, offers a convenient in vitro model system. We have shown these cells to be permissive for ZIKV infection in their undifferentiated state supporting virus production of up to $10^7$ ffu/ml. ZIKV replication in these cells, illuminated by immunofluorescence, was characterized by abundant staining for the E glycoprotein, increasing quantities of dsRNA, and expansion of the ER as infection progressed. Furthermore, clear evidence of caspase mediated programmed cell death was apparent in the neuroblastoma cell line, leading to an acute lytic crisis. This is very similar to observations from our lab with a tick-borne flavivirus (Mlera et al., 2016; Mlera et al., 2015), where the lytic crisis is followed by necrosis.

**Fig. 5.** Localization of ZIKV E to endoplasmic reticulum (PDI) in SK-N-SH and C6/36 cells. Cells were mock-infected (A) or infected at a MOI of 10 with ZIKV Paraiba (B), fixed, and stained for ZIKV E protein (4G2, red) and protein disulfide isomerase (PDI, green). Areas of colocalization between these two proteins appear yellow. Nuclei were counterstained with DAPI (blue). 72 h post infection, 63× magnification.

**Fig. 6.** Localization of dsRNA to endoplasmic reticulum (PDI) in SK-N-SH and C6/36 cells. Cells were mock-infected (A) or infected with ZIKV Paraiba at a MOI of 10 (B), fixed, and stained for double-stranded RNA (J2, red) and protein disulfide isomerase (PDI, green). Areas of colocalization between these two proteins appear yellow. Nuclei were counterstained with DAPI (blue). 72 h post infection, 63× magnification.
by the initiation and long-term maintenance of a persistent infection. In the light of long-term presence of ZIKV in human semen (Turmel et al., 2016), it will be important to determine if ZIKV persistence can also be established in SK-N-SH cells and to characterize that state. Furthermore, the SK-N-SH neuroblastoma cells undergo differentiation and neurite generation when treated with retinoic acid (Kraveka et al., 2003). Recent work by others (Hughes et al., 2016) has found that differentiation of some neuroblastoma cell lines can confer a resistance to ZIKV infection. It would be interesting to determine if this occurs with the SK-N-SH cells, to examine terminally differentiated neuroblastoma cells for ultrastructural changes that may not be apparent via immunofluorescence, and finally to determine the mechanism for the resistance.

As an arbovirus virus, ZIKV is cycled between Aedes mosquitoes...
and vertebrate hosts. Therefore, in addition to looking at neuroblastoma cells, we wanted to characterize the cytoarchitecture of ZIKV replication in mosquito cells as well. The primary vector for ZIKV is *Aedes aegypti* larvae (Singh, 1967). Our attempts to infect CCL-125 with ZIKV revealed very minimal virus production, and the cells showed no signs of morphological changes 120 h after ZIKV infection. Early studies reported a lack of susceptibility of CCL-125 to some mosquito-borne flaviviruses (Japanese encephalitis and Dengue 1–4), despite a permissiveness for West Nile virus (Singh and Paul, 1968). However, a more recent report showed that Dengue-2 did replicate in the CCL-125 cells (Wikan et al., 2009). Thus, the resistance of CCL-125 to ZIKV may not be entirely unexpected.

Since *Aedes albopictus* mosquitoes have also been implicated in ZIKV transmission, we examined the C6/36 cells derived from this species. Although C6/36 cells showed no morphological changes via light microscopy with ZIKV infection, they were clearly permissive for ZIKV. Upon closer examination by fluorescence staining patterns that were similar to the human neuroblastoma cell lines. Both cell types exhibited the hallmark membrane proliferation and rearrangements seen with many positive-strand RNA viruses (Romero-Brey and Bartenschlager, 2015). The 60–100 nm spherical virus-induced replication compartments were abundant and a clear apposition to the ER lamella was evident. The structures within the replication compartments were revealed by ET to be 20–30 nm “hollow” spherical structures that abutted the connection between the replication compartment and the adjacent cytoplasm. Some of the replication compartments were clearly tubular in profile, but still had the smaller internal structures. Others have shown that these smaller structures within the replication compartments can be disrupted by RNase treatment under condition specific for dsRNA digestion (Gillespie et al., 2010). Future experiments are planned to better characterize the biogenesis of the replication compartments as well as the full complement of viral and cellular components contained therein.

Virus particles were evident in the ER cisternae and in some instances were immediately adjacent to replication compartments. The virions we observed were ~30 nm in diameter, in contrast to the 50 nm diameter reported for purified ZIKV particles (Kostyuchenko et al., 2016; Sirohi et al., 2016). We believe this difference to be related to differences in virus particle maturity between purified virus particles and particles we observed that are likely nucleocapsids located within the lumen of the ER (Hase et al., 1987; Mackenzie and Westaway, 2001). We did not identify any structures consistent with immature virions budding into the ER in either cell line. It is likely that higher resolution microscopy will be required to identify immature budding virions. In summary, we have performed initial characterization of ZIKV in two very relevant cell types. This work will provide a solid framework for the detailed study of viral biogenesis and assembly.

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References

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