HORIZON 2020

Labeling Flavivirus capsid protein to unravel its dynamics during infection and egress by correlative super-resolution fluorescence and cryo-electron microscopy

Informe

Información del proyecto

FLAVIC DYNAMICS

Identificador del acuerdo de subvención:
703519

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Periodic Reporting for period 1 - FLAVIC DYNAMICS (Labeling Flavivirus capsid protein to unravel its dynamics during infection and egress by correlative super-resolution fluorescence and cryo-electron microscopy)

Período documentado: 2017-09-01 hasta 2019-08-31

Resumen del contexto y de los objetivos generales del proyecto

Vector-borne diseases are major worldwide threats. Specifically, mosquito-borne diseases from the flavivirus genus such as West Nile, dengue, yellow fever and Zika viruses are raising major concerns on human health. There are few or no treatment/vaccine strategies to fight these viruses and the relatively poor knowledge of their life cycle (entry and egress) contributes to the deficit in treatment possibilities. A deeper knowledge of the virus cycle is required to unlock the development of antivirals. Understanding the dynamics of the viral proteins and their interactions in vivo strongly relies on quantitative and more recently super-resolution microscopies. These methodologies significantly depend on specific protein-labeling strategies. There are limited solutions for flavivirus labeling, a scenario that limits the results outcome. Significant advances in understanding flavivirus biology can be expected if such tools were available.
The major goal of this project was to understand the delivery of the flavivirus genome to its translation site at the cytoplasmic side of the ER membrane; the cellular components, the pH and machineries that intervene.
The focus of this project was not only to provide a labeling package of viral components that will foster flavivirus research. In addition, we explored alternative routes of Flaviviruses infection hypothesis such as cell-to-cell transmission, a phenomenon already described for HCV and HIV.
We have achieved successful YFV labelling in the envelop protein, lipid envelope and viral RNA, suitable to perform STORM.
YFV wt and vaccine strain viruses have their membrane fusion step in a different pH and endosome compartment.
ZIKV can employ alternative infection routes distinct from exo-endocytosis, to spread between cells.

Trabajo realizado desde el comienzo del proyecto hasta el final del período abarcado por el informe y los principales resultados hasta la fecha

Due to the early termination of the project as explained in the termination amendment, only one year of the total duration of 2 project years was concluded.
As such, and according to the project timeline and workpackage in the proposal, WP1, WP2, WP5 (with contingency plan) and WP7 were attained with successful deliverables.

WP1
We have succeeded to label YFV Envelop protein with two Alexa fluorescence dyes (A488 and A647). Moreover, we also successfully achieved proper labelling of the lipid envelope with DiD, DiO, DiI and Sm-BODIPY Fl lipid dyes. For this labelling we used highly pure/infectious YFV purified from a density gradient.
We had conducted our first 2D/3D STORM experiments having set the acquisition parameters and image reconstruction settings. We achieved the reconstruction of individual YFV viral particles,

averaging 50nm in diameter size (as expected).
An addition to the proposed WP was the achievement of specifically labelling the viral RNA.

WP2
Regarding FlAsH-C protein YFV we have selected the infectious clones containing YFV full genome to use for the insertion of the FlAsH binding sequence with Gibson cloning strategy. Selection and sequencing of clones was undergoing when project was interrupted.

WP3
To execute the CryoEM section of the project, I took several sessions of EM training at the Host Institution EM facility Ultrapole. Moreover, I took a practical workshop of CryoEM (Prato, Italy 9th-14th of April 2018). Due to biosafety level of the CryoEM facility at Institut Pasteur this workpackage, and al related to CryoEM of Flavivirus had to be solved based on a contingency plan. We were not authorized to use native Flavivirus, even if inactivated in the CryoEM instrument.
Based on this major drawback we had to develop a method to produce, purify and characterize Flavivirus Virus Like Particles so that these could be use as representative material of a native flavivirus viral particle. As such, I was responsible to establish a VLP USP/DSP platform for flavivirus using Expi293 suspension cell line. In conclusion I standardized all USP and DSP to produce high yields of flavivirus VLP. These could then be applied in CryoEM particle reconstruction steps of the project proposal.

WP4
NA

WP5
By using Envelop protein and lipid envelope labelled YFV (wt and 17D) I have studied the fusion steps of YFV during cellular entry. The aim of this study was to assess if the two viruses would have a different pH threshold for fusion with the host cell membrane at the endosomal membranes.
The attenuated vaccine strain (YF17D) contains 12 out of the 32 aa mutations in the E protein. These mutations may have an effect that, during entry, the two viruses may fuse in different endocytic compartments. We showed that the majority of YF17D fusogenic particles are localized in Rab7/Rab9 late endosomes, whereas YFwt fusion events are localized in early EEA-1/Rab5 endosomes. We have also measured, using a pH sensitive probe (pHrodo), the actual pH of the endosome vesicles where viral fusion was detected. Our results show that YF17D membrane fusion occurs at a more acidic pH than for YFwt further supporting that the two viruses fuse in different compartments.
Since viral fusion marks the checkpoint infection detection and subsequent host response, such finding may provide a novel insight into the molecular mechanisms leading to YF17D protective immune response that could be translated to other flavivirus.

WP6
NA

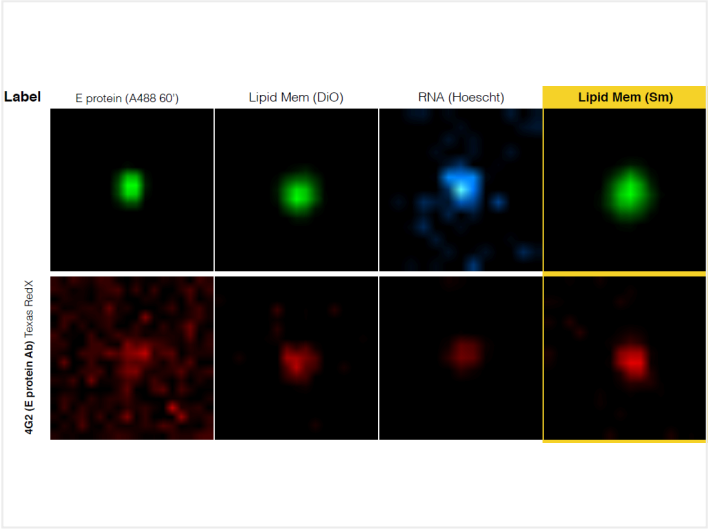
WP7
This exploratory WP served as a gateway to foster new collaborations. A joint effort between different

fields of research (cell biology and Virology) that aimed for breakthrough findings in flavivirus state-of-the-art. Together with Chiara Zurzolo research group we have studied the possibility of new flavivirus (Zika in particular) cell-to-cell transmission routes.

Results gathered in WP5 and WP7 are currently being drafted as manuscripts for publishing. The outcome of WP1 will set the fundamental for particle averaging reconstruction of virus particles based on STORM data and it will be published as a short communication.

Avances que van más allá del estado de la técnica e impacto potencial esperado (incluida la repercusión socioeconómica y las implicaciones sociales más amplias del proyecto hasta la fecha)

WP5 and WP7 have major impact on the current state-of-the-art of flavivirus virus biology.
On one side we have found that wt and vaccine strain YFV fuse in distinct endosome compartments with different pH. This finding may help to understand why the vaccine strain is such a great immunogen whereas the wt is not. Such information could be translated to other flavivirus helping to develop better vaccine strategies.
On ZIKA virus infection, the finding that such viruses use cell-to-cell routes to spread infection is a major breakthrough. This alternative ZIKV cellular infection route to the “classical” exo-endocytosis mechanism, using cell-to-cell contact dependent manner connections. Such infection route is unreachable to circulating neutralizing antibodies or other types of antiviral drugs that may be used to block circulating virus infection. It would be an advantage and a major acquired virus fitness that confers protection against the immune system. For the future, immunotherapeutic approaches based solely on neutralizing antibodies will need to take in account the ZIKV cell-to-cell spreading described here and the search for a TNT inhibitor that does not interfere with the normal cellular function will be of major interest, as it may serve as a complimentary treatment for controlling the viral infection. In addition, the finding that ZIKV can use TNT-mediated transmission to infect different cell types and tissues (epithelial to neuronal cells) suggests how ZIKV may increase its tropism, pathogenicity and infectivity to non-permissive cells.



yfv-labelling.png

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