

STRUCTURAL ANALYSIS OF VIRAL REPLICATION AND MORPHOGENESIS INSIDE VIRAL FACTORIES Juan Fontana (1), Reyes R. Novoa (1), Gloria Calderita (1), Pilar Cabezas (1), José J. Fernández (2), Teryl K. Frey (3) and Cristina Risco (1). (1) Centro Nacional de Biotecnología, CSIC, Campus Universidad Autónoma de Madrid, Darwin 3, Cantoblanco, 28049 Madrid, Spain; (2) Departamento de Arquitectura de Computadores, Universidad de Almería, 04120 Almería, Spain; (3) Department of Biology, Georgia State University, Atlanta, GA, USA. E-mail: crisco@cnb.uam.es

Viral factories are associations of cell organelles that build a unique structure where viruses replicate and assemble [1]. Non-related viruses may use similar signalling pathways to recruit and modify cell organelles. How genome replication, transport to assembly sites and maturation of viral intermediates take place inside the factory in a coordinate way is largely unknown. We are studying the architecture and dynamics of these complex structures using two viral systems: Rubella virus and Bunyaviruses. Rubella virus replicates inside modified lysosomes and assemble in Golgi stacks [2, 3]. Modified lysosomes and Golgi elements together with mitochondria and rough endoplasmic reticulum cisternae build the viral factory. Using cells expressing replicons in the presence or absence of viral capsid protein [4, 5] we have localized the viral replicase and the capsid protein in membranes associated to lysosomes by confocal and immunoelectron microscopy. 3D reconstruction from serial sections show that these membranes are rigid sheets where viral factors could make 2D arrays for a more efficient replication process. Viral morphogenesis starts in Golgi membranes that surround the replication complexes. Viral immature precursors change their structure and mature inside the Golgi stack [6]. We have identified another process of viral maturation inside the Golgi of Bunyavirus-infected cells, where viral precursors change their structure in a *trans*-Golgi dependent manner [7, 8]. Factories of Bunyamwera virus are large perinuclear structures where mitochondria and Golgi membranes are recruited [8]. We have localized the viral polymerase and double-stranded RNA in tubular structures inserted in Golgi stacks. These tubes are the replication complexes of the virus and seem to participate in the recruitment of mitochondria to the viral factory. We are obtaining 3D maps of these viral factories to understand how genome replication and viral assembly are spatially connected. Three-dimensional

reconstruction from serial sections and 3D images from tantalum metal replicas of intracellular structures [9] will help us to study higher resolution 3D maps of these complex intracellular regions, to be obtained by electron tomography [10, 11].

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