Virus Research 258 (2018) 64–67

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Sonchus yellow net virus core particles form on ring-like nuclear structure enriched in viral phosphoprotein

Tea Meulia[a], Lucy Stewart[b], Michael Goodin[c,*]

[a] Molecular and Cellular Imaging Center, Department of Plant Pathology, The Ohio State University, Wooster, OH, 44691, USA
[b] USDA-ARS Plant Pathology, 1680 Madison Ave, Wooster, OH, 44691, USA
[c] Department of Plant Pathology, University of Kentucky, Lexington, KY, 40546, USA

ARTICLE INFO

Keywords:
Rhabdovirus
GFP
Nicotiana benthamiana
Viroplasm
Inner nuclear membrane

ABSTRACT

The phosphoprotein (P) of the nucleorhabdovirus sonchus yellow net virus has been shown to accumulate in ring-shaped structures in virus-infected nuclei. Further examination by live-cell imaging, in combination with structural examination by transmission electron microscopy and immunolocalization demonstrated that P-rings do not form in association with nucleoli. Furthermore, viral cores were shown to condense on the nucleoplasm-contacting surface of the rings. The data presented here offer evidence for the site of nucleocapsid assembly in SYNV-infected nuclei.

Typical of viruses with negative-sense single-stranded RNA (NSS) genomes, the phosphoprotein (P) of the nucleorhabdovirus, sonchus yellow net virus (SYNV), is essential for virus replication (Amarasinghe et al., 2018; Wang et al., 2015; Ganesan et al., 2013). The roles of P in replication are several and these have largely been elucidated for SYNV and the animal rhabdovirus model vesicular stomatitis virus (VSV), most notably as a chaperone for the nucleocapsid (N) protein and co-factor with the large (L) protein to form the viral replicase/polymerase (Wang et al., 2015; Ganesan et al., 2013; Jackson and Li, 2016; Goodin et al., 2001; Whelan et al., 1995). For plant-adapted rhabdoviruses, functions of P proteins extend to include that of suppressor of RNA silencing (Mann et al., 2016). How these activities are coordinated in virus-infected plant cells remains equivocal. It is widely agreed that replication of plant-adapted NSS viruses takes place in viroplasm complexes composed largely of the N, P, and L proteins, in addition to viral RNAs (Deng et al., 2007; Martins et al., 1998). The earliest investigations of viroplasms utilized electron-microscopy, primarily at high magnification that featured virus particles and only a fraction of the surrounding nuclear contents. Later studies using live-cell fluorescence microscopy revealed the spatial relationship between sites of replication and morphogenesis (Goodin et al., 2007), which provided the intriguing discovery that the SYNV-P protein accumulated in ring-shaped structures in host cell nuclei when expressed in virus-infected cells (Goodin et al., 2007). Such rings did not form when P is expressed on its own, indicating that other virus-encoded factors are required for ring assembly. SYNV-N interacts with P and together their subnuclear localization is altered (Goodin et al., 2001), but co-expression of P with N outside the context of plant infection does not result in P-ring formation (Goodin et al., 2001; Deng et al., 2007).

In this study we re-examined P-ring formation and show that these sub-nuclear structures are not associated with nucleoli, and that nucleocapsid formation takes place on the outer surface of the rings. In order to localize P in SYNV-infected cells, the P open reading frame was expressed from pSITE-2C1, as a GFP fusion, in virus-infected transgenic *Nicotiana benthmiana* plants expressing the nucleolar marker Fibrillarin 1 fused to Red fluorescent protein (RFP:Fib1), as described previously (Fig. 1) (Goodin et al., 2007; Chakrabarty et al., 2007). Expression of recombinant proteins in leaf epidermal cells was achieved using agroinfiltration in plants at 14 days post inoculation, with examination by confocal microscopy two days thereafter. Microscopy for this study was conducted using an Olympus FV1000 laser-scanning confocal microscope as described previously (Goodin et al., 2007). As reported in previous studies, GFP:P formed rings in SYNV-infected plant cells (Fig. 1). The rings were not colocalized with the RFP:Fib1 marker, therefore we can rule out nucleoli as the site of ring formation.

The function of the P rings was further examined by electron microscopy. Samples were collected from symptomatic leaves of SYNV-infected *Nicotiana benthamiana* plants 10 to 17 days-post-inoculation, and ultra-thin sections prepared as described previously (Redinbaugh et al., 2002; Fig. 2). SYNV was maintained by serial passages in N. benthamiana by rub-inoculation of three-leaf plants with carborundum as previously described (Senthil et al., 2005). Leaf samples were

* Corresponding author.
E-mail address: mgoodin@uky.edu (M. Goodin).

https://doi.org/10.1016/j.virusres.2018.10.005
Received 30 July 2018; Received in revised form 5 October 2018; Accepted 8 October 2018
Available online 09 October 2018
0168-1702/

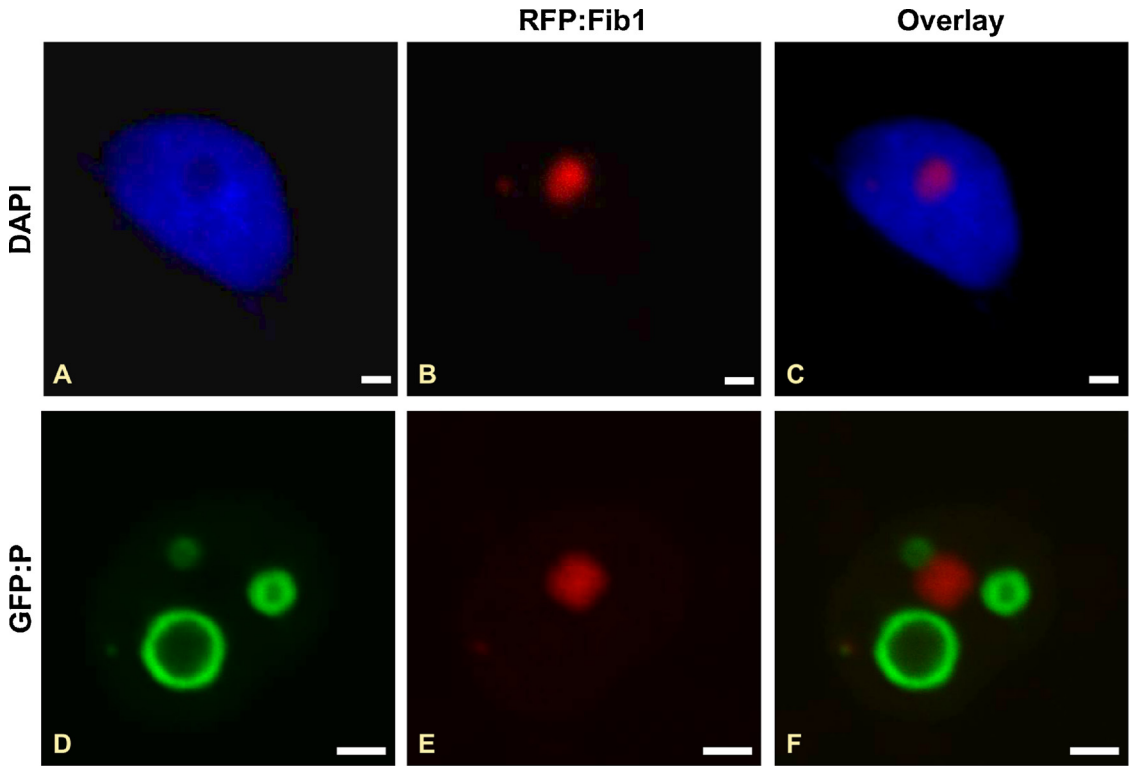

Fig. 1. GFP:P forms rings when expressed in SYNV-infected plant cells. Panels A–C. Localization of RFP:Fib1 in nuclei. **A**. DAPI counter-stained nucleus in leaf epidermal cells of transgenic *N. benthamiana* plants expressing nucleolar marker RFP:Fib1 (Nb-Fib1). **B**. RFP:Fib1 in nucleus shown in A. **C**. Overlay of panels A and B. Panels D–F. Localization of GFP:P in SYNV-infected Nb-Fib1 plants. **D**. GFP:P. **E**. RFP:Fib1 in cell shown in D. **F**. Overlay of D and E. Scale bar = 2 μm.

collected from symptomatic uninoculated leaves (above the inoculated leaves) at 10 dpi. Samples were placed in fixative containing 3% glutaraldehyde, 2% paraformaldehyde in 0.1 M potassium phosphate buffer pH 7.2 (PB), vacuum infiltrated and fixed for 2 h at room temperature and then overnight at 4 °C. After three washes with excess PB, samples were post-fixed with 1% osmium tetroxide, 1% uranyl acetate in PB for 1 h, and then dehydrated through a graded ethanol-propylene oxide series, and embedded in EM Bed812 resin (Electron Microscopy Sciences, Hartfield, PA). Ultrathin-sections were prepared using a Leica EM UC6 ultra-microtome. After staining with 2% aqueous uranyl acetate for 20 min, samples were incubated in Reynolds' lead citrate for 10 min.

Nuclei in virus-free cells were clearly defined, with the nucleoplasm exhibiting a granular appearance typical of plant nuclei (Martins et al., 1998; MacLeod et al., 1966) (Fig. 2A). In stark contrast, virus-infected nuclei contained well-defined rings that contrasted sharply with the surrounding nucleoplasm (Fig. 2B). SYNV particles were clearly identifiable in the perinuclear space of infected cells (Fig. 2B). Additionally, dark-staining protein aggregates were much more prevalent in virus-infected nuclei, particularly beneath the inner nuclear membrane. Magnification of the outer edge of the ring revealed nucleocapsids in various stages of assembly (Fig. 2C). The number of particles accumulating at the ring edge ranged from few to many, depending on the particular section (compare Fig. 2C with D). The thickness of the ring appeared to be related to the number of mature nucleocapsids in the nucleus, with increased thickness of the ring in nuclei where many particles were observed (compare Fig. 2B with E). Examination of micrographs taken at different stages of infection, suggests that nucleocapsids are transported to the nuclear envelope singly (Fig. 2E,F), but that numerous particles can accumulate in the same region of perinuclear space (Fig. 2B).

In order to link the observations shown in Figs. 1 and 2, it was necessary to establish that P is a major constituent of the observed rings. To achieve this, leaf samples taken from SYNV-infected plants were processed for electron microscopy immunolocalization following the protocol reported by Xu et al. (2007) with some modifications. As is standard for immunolabeling sample processing, osmium tetroxide and uranyl acetate post-fixation agents were omitted to preserve epitope integrity and access, and sections were stained only after the immunolocalizations, providing some contrast (see Fig. 3) but not as much as for structural study (Fig. 2). Quantification of the labeling was done by counting gold particles on the ring-like structure and other cell structures in five separate images. Ultra-thin sections were imaged on a Hitachi H-7500 transmission electron microscope with the SIA-L12C (Scientific Instruments and Applications, Duluth, GA) digital camera. Rabbit polyclonal antiserum against the P phosphoprotein used previously in an electron microscopy study (Martins et al., 1998) (Fig. 3) was used to localize this protein in ultra-thin sections to the dense material of the rings in SYNV-infected nuclei (Fig. 3A).

Our data support a model for SYNV virion assembly where core particles (e.g. nucleocapsids) assemble at P-protein foci or ring structures in the nucleus, and at least some particles bud out of the nucleus, acquiring an envelope, into the perinuclear space (see also [Jackson et al., 2005]). In the cells examined, very little cytoplasm was observed, and most viral particles observed were associated with large nuclei of infected cells. Thus, the route of cell-to-cell movement of virus (either in core nucleocapsids or enveloped virions) was not clear in this ultrastructural study. Most of the samples examined in the study contained mature particles accumulated in the perinuclear space. However, rare sections such as those shown in Fig. 2E suggest that P-rings are established well in advance of particle accumulation in the perinuclear space, and that expansion of the size of the P-ring may be proportional to accumulation of mature virions.

Taken together, we provide further evidence that the function of SYNV-P is spatially separated in infected nuclei from those of other virus-relevant activities such as association with nuclear membranes and movement (Goodin et al., 2007). We note that while rhabdovirus P proteins are expected to be abundant viral proteins due to the P gene