PLOS | PATHOGENS

RESEARCH ARTICLE

The ER-Membrane Transport System Is Critical for Intercellular Trafficking of the NSm Movement Protein and Tomato Spotted Wilt Tospovirus

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Citation: Feng Z, Xue F, Xu M, Chen X, Zhao W, Garcia-Murria MJ, et al. (2016) The ER-Membrane Transport System Is Critical for Intercellular Trafficking of the NSm Movement Protein and Tomato Spotted Wilt Tospovirus. PLoS Pathog 12(2): e1005443. doi:10.1371/journal.ppat.1005443

Editor: Aiming Wang, Agriculture and Agri-Food Canada, CANADA

Received: September 11, 2015

Accepted: January 17, 2016

Published: February 10, 2016

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by the National Natural Science Foundation of China (31471746 and 31222045), the National Program on Key Basic Research Project of China (973 Program, 2014CB138400), the Special Fund for Agro-scientific Research in the Public Interest (201303028), the Fundamental Research Funds for the Central Universities (KYTZ201403), the Youth Talent Support Program of China and Distinguished Professor of Jiangsu Province to XT, and the Spanish Ministry of

Abstract

Plant viruses move through plasmodesmata to infect new cells. The plant endoplasmic reticulum (ER) is interconnected among cells via the ER desmotubule in the plasmodesma across the cell wall, forming a continuous ER network throughout the entire plant. This ER continuity is unique to plants and has been postulated to serve as a platform for the intercellular trafficking of macromolecules. In the present study, the contribution of the plant ER membrane transport system to the intercellular trafficking of the NSm movement protein and *Tomato spotted wilt tospovirus* (TSWV) is investigated. We showed that TSWV NSm is physically associated with the ER membrane in *Nicotiana benthamiana* plants. An NSm-GFP fusion protein transiently expressed in single leaf cells was trafficked into neighboring cells. Mutations in NSm that impaired its association with the ER or caused its mis-localization to other subcellular sites inhibited cell-to-cell trafficking. Pharmacological disruption of the ER network severely inhibited NSm-GFP trafficking but not GFP diffusion. In the *Arabidopsis thaliana* mutant *rhd3* with an impaired ER network, NSm-GFP trafficking was significantly reduced, whereas GFP diffusion was not affected. We also showed that the ER-to-Golgi secretion pathway and the cytoskeleton transport systems were not involved in the intercellular trafficking of TSWV NSm. Importantly, TSWV cell-to-cell spread was delayed in the ER-defective *rhd3* mutant, and this reduced viral infection was not due to reduced replication. On the basis of robust biochemical, cellular and genetic analysis, we established that the ER membrane transport system serves as an important direct route for intercellular trafficking of NSm and TSWV.

Economy and Competitiveness (BFU2012-39482 grant to IM). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Author Summary

Plant viruses may use different host cell transport machineries to move from one cell to another through plasmodesmata. The contribution of host cell transport systems to the intercellular movement of multipartite negative-strand RNA plant viruses including tospoviruses is poorly understood. We used *Tomato spotted wilt tospovirus* (TSWV) as a model to understand the mechanism of intercellular movement of tospoviruses. In this study, using *in vitro* and *in vivo* systems for characterizing membrane proteins, we identified that the TSWV NSm movement protein was physically associated with the ER membrane. NSm expressed in a single leaf cell was able to move into neighboring cells along the ER membrane network. The ER membrane in plants is a unique structure that runs between neighboring cells via the ER desmotubule of the plasmodesmata and forms a continuous network throughout the plant. Taking advantage of TSWV NSm being tightly associated with ER membrane and trafficked between cells through plasmodesmata, we demonstrated here by robust biochemical, cellullar and genetic evidence that the ER membrane transport system of plants serves as an important route for intercellular trafficking of the NSm movement protein and TSWV. Our findings have important new implications for mechanistic studies on intercellular trafficking of tospoviruses and other multipartite negative-strand RNA plant viruses.

Introduction

Plasmodesma-mediated macromolecular trafficking plays important roles in plant growth and development [1–3] and in plant–pathogen interactions [4–6]. Structurally, a plasmodesma is composed of the plasma membrane with a central, modified appressed endoplasmic reticulum (ER), the desmotubule [7]. Besides the long-established cell-to-cell transport of small molecules via plasmodesmata, macromolecules such as proteins and RNAs have been shown in the last two decades to traffic between cells through plasmodesmata (PD). Such macromolecular trafficking is crucial for viral infection [4–6], plant defense [8,9], and developmental regulation [1–3].

Plant viruses need to move within and between cells to establish systemic infection. To accomplish this task, the plant virus encodes a movement protein (MP) to facilitate intracellular trafficking of the viral genomes from the replication site to PD and to assist the spread of the viral replication complexes or viral particles between plant cells through PD [5,6,10–13]. Plant viruses not only utilize viral-encoded MPs or other viral components for viral intra- and intercellular movement, but also co-opt host cell transport machineries for their movement [13–17]. The cytoskeleton and membrane transport systems of cells are important for intracellular movement of vertebrate viruses (reviewed in [16]), essential for organellar trafficking within plant cells [18,19] and involved in the intercellular trafficking of macromolecules [20,21]. In the case of the best-studied plant virus, *Tobacco mosaic virus* (TMV), the ER membrane is important for its association with the viral replication complexes (VRC) and MP granules, whereas microtubules and microfilaments facilitated their movement on the ER (reviewed in [22]). The ER membrane also serves as an important platform for anchoring several other viral MPs, which are required for intracellular movement and viral spread [23–27]. The ER-to-Golgi secretory pathway is further involved in PD targeting and intercellular trafficking of several viruses [28–33]. Microfilaments and different myosin motors also participate in the intra- or intercellular movement of diverse MPs or viruses [28–30,34–40]. In addition, the endocytic pathway has also been shown to influence the movement of three viruses [41–43].