

Japanese soil-borne wheat mosaic virus CP-RT protein associates with the endoplasmic reticulum network and mobile particles at the ER-Golgi interface during infection

Authors:

Nico Sprotte^{1,3}, Claudia J. Strauch^{1,4}, Petra Bauer¹, Emmanuel Boutant^{2,5}, Annette Niehl^{1*}

¹Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany

²UMR7021, CNRS, Laboratory of bio imaging and pathologies (LBP), University of Strasbourg, Faculty of Pharmacy, 74 route du Rhin - CS 60024, F-67400 Illkirch, France

³current address: Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Urban Green, Messeweg 11-12, 38104 Braunschweig, Germany

⁴current address: Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Biosafety in Plant Biotechnology, Erwin-Baur Straße 27, 06484 Quedlinburg, Germany

⁵current address: UMR7242, CNRS, Laboratory of Biotechnology and Cell Signaling (BSC), University of Strasbourg, ESBS, 300 Boulevard Sébastien Brant - CS 10413, F-67400 Illkirch, France

* Correspondence: Annette.niehl@julius-kuehn.de

Table S1 Primer sequences

Target	Name	Sequence 5'-3'	Purpose
JSBWMV CP-RT (pJS2)	CPRT_Seq_230_Rev1	TCACCGTCTTGAGTATGCGT	Sequencing pJS2
	CPRT_Seq1849_Rev1	CAAAGTTTCGCCCTACTGCA	
	CPRT_Seq590_For1	TGGTGTTGGCTGACGATTTG	
	CPRT_Seq_910_For1	CGACTTTCTTATCGTGCTTTATCG	
	CPRT_Seq_1204_For1	CGTCAGAGCAGTGAACAAGG	
	pJS2_Seq_3032_For1	GCCAACACTGCTCCTGTTTAC	
Entryvectors	M13uni (-21)	TGTAACACGACGGCCAGT	Sequencing Inserts of Entryvectors
	M13rev (-29)	CAGGAAACAGCTATGACC	
<i>H. vulgare</i> Elongation Factor 1- alpha	ELF1A_for1	CAGTGCTGGACTGCCACA	Testing cDNA
	ELF1A_rev1	CTCCACCACCATGGGCTT	

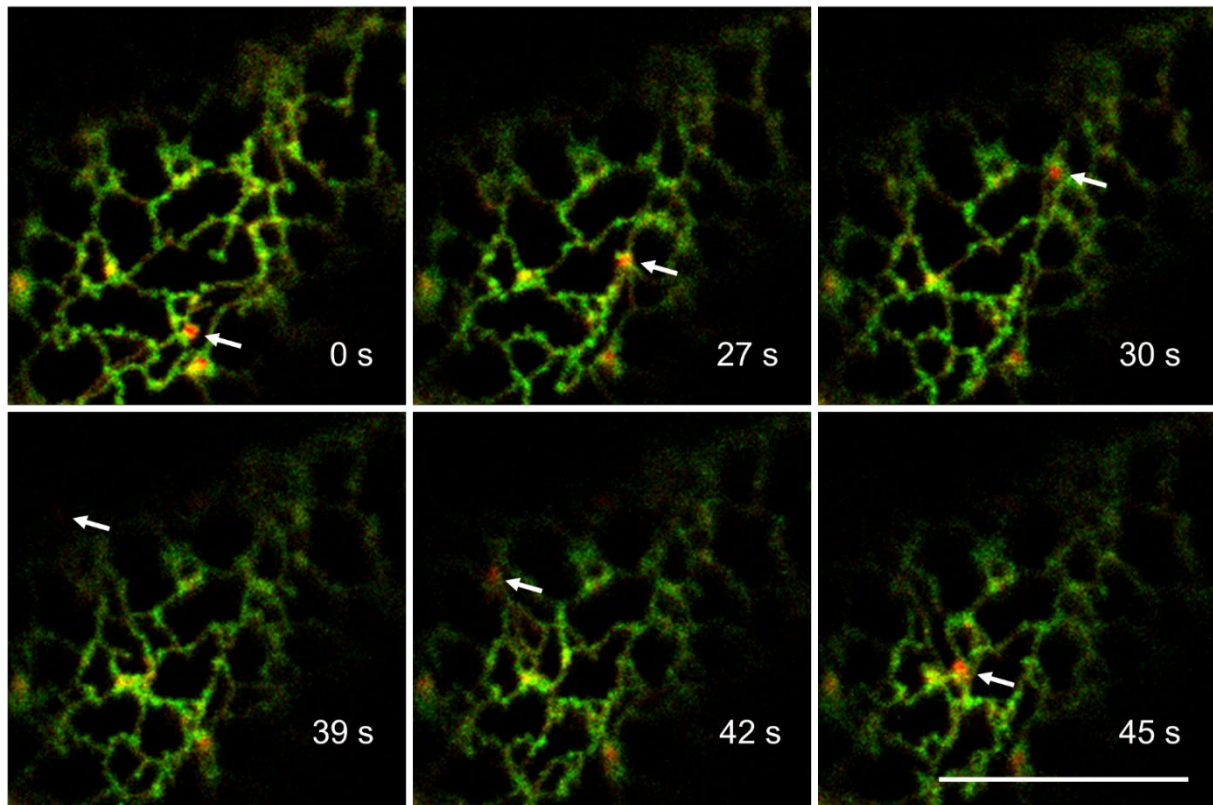


Figure S1 Images of a time series tracking the localization and movement of JSBWMV CP-RT:RFP in 16c plants

Ectopically expressed CP-RT:RFP localized to the ER network and to small dot-like particles moving in association with the ER network. Overlay images showing the CP-RT:RFP in red and ER in green are shown. Yellow fluorescence indicates co-localization. Arrows point to a moving CP-RT particle. Seconds of the time series are shown on the bottom. Scale bar, 10 μm .

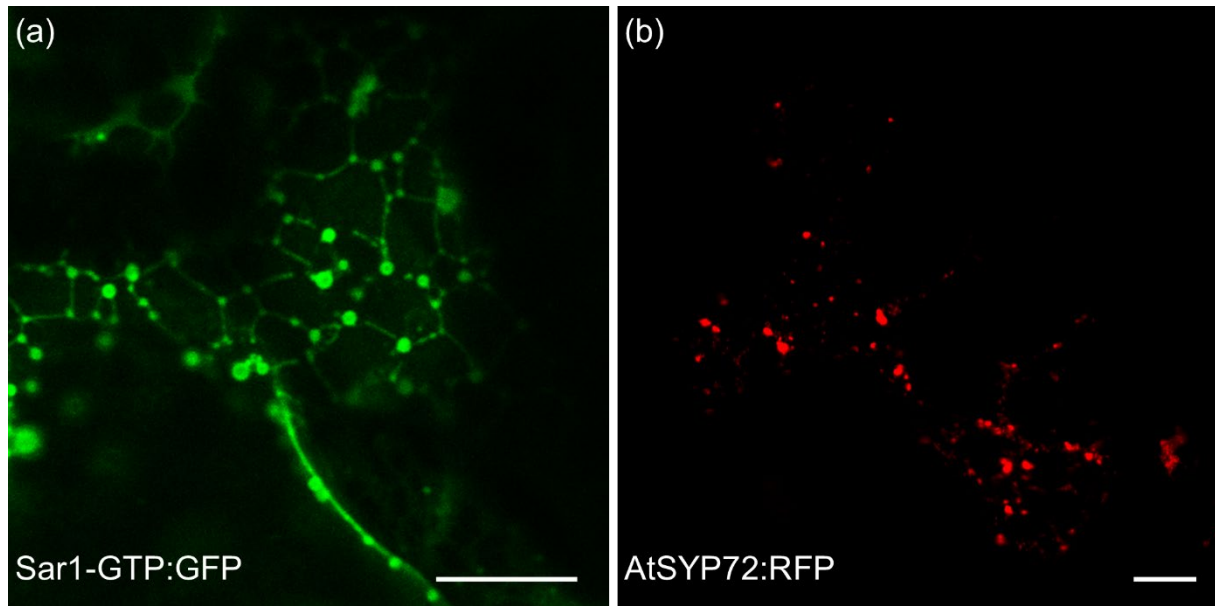


Figure S2 Sub-cellular localization of Sar1-GTP:GFP and AtSYP72:RFP

The dominant-negative Sar1-GTP:GFP interferes with ER to Golgi transport and localizes to specific dot-like ER sites and the ER network (daSilva et al. 2004; Osterrieder et al. 2010). AtSYP72:RFP is an ERIS marker (Lerich et al. 2012) and localizes to dot-like particles upon transient expression from *Agrobacteria*. Sar1-GTP:GFP and AtSYP72:RFP were agroinfiltrated into *N. benthamiana* leaves and imaged by CLSM two to three days post agroinfiltration. Scale bars, 10 µm.

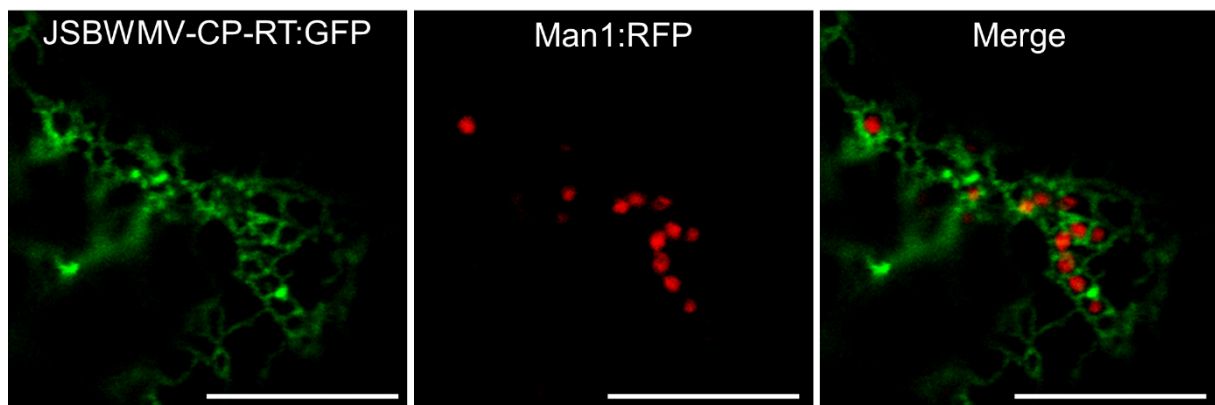


Figure S3 Visualization of Golgi in JSBWMV-CP-RT:GFP infection sites

The Golgi Marker Man1:RFP was ectopically expressed in JSBWMV-CP-RT:GFP infection sites by agroinfiltration. The picture was taken 8 days post agroinfiltration and 10 dpi. Left panel, GFP channel; middle panel, RFP channel; right panel, overlay image of GFP and RFP channels. Scale bars, 10 µm.

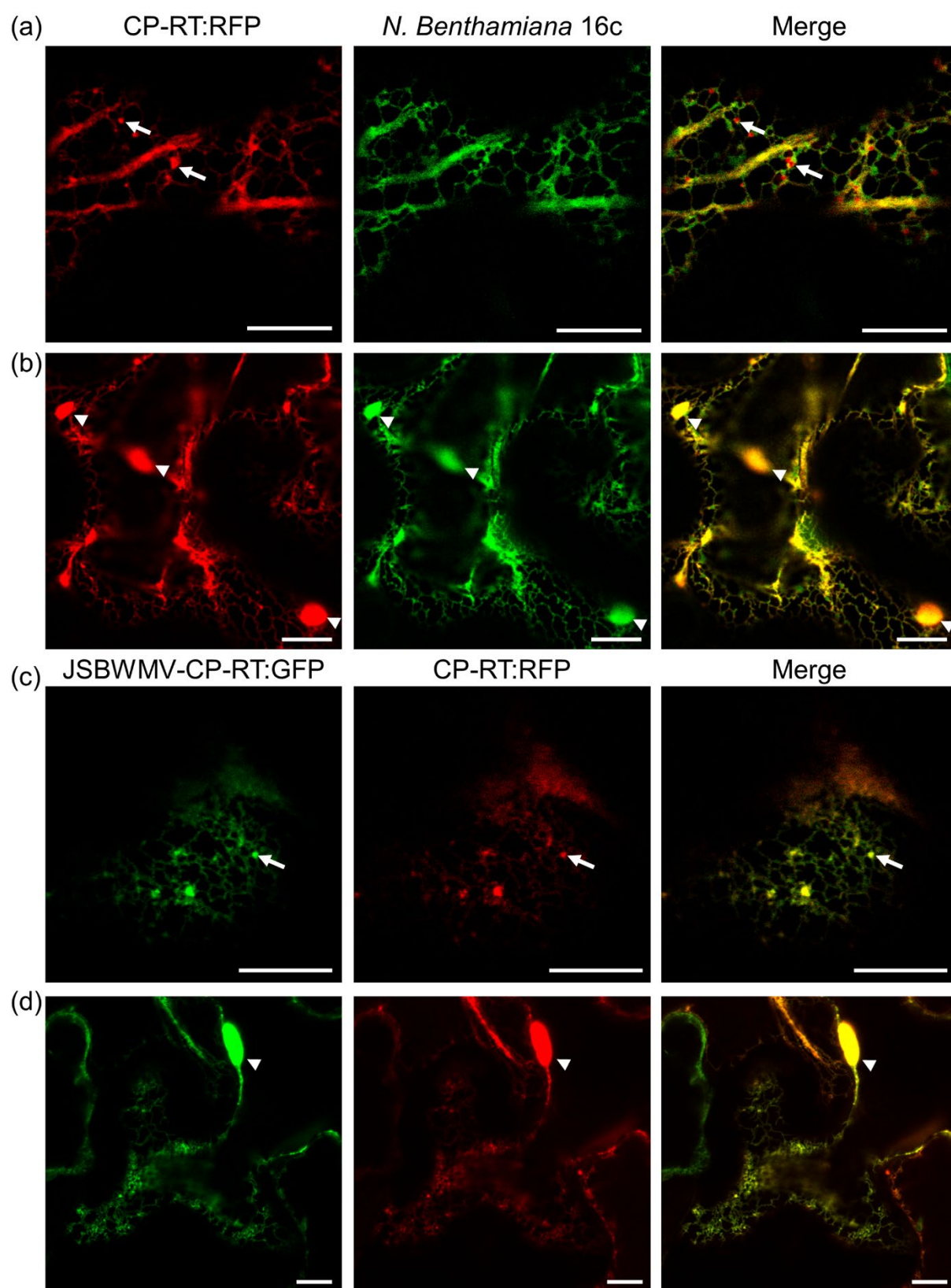


Figure S4 Ectopically expressed CP-RT localizes to the ER, forms dot-like particles and recruits virus-expressed CP-RT into aggregates

(a-b) Transient expression of CP-RT:RFP in a *N. benthamiana* 16c leaf epidermal cell. Arrows in (a) indicate dot-like particles formed by CP-RT:RFP. Arrowheads in (b) indicate aggregates formed by CP-

RT:RFP in cells with high expression levels of CP-RT:RFP. Pictures were taken four days post agroinfiltration. (c) Colocalization of JSBWMV-CP-RT:GFP and ectopically expressed CP-RT:RFP in a *N. benthamiana* leaf epidermal cell. The virus-expressed CP-RT:GFP protein colocalizes with the transiently expressed CP-RT:RFP. Arrow indicates dot-like structures formed by CP-RT:FP. Pictures were taken eight days after virus infection and 12 days post agroinfiltration. (d) Colocalization of JSBWMV-CP-RT:GFP and ectopically expressed CP-RT:RFP in a *N. benthamiana* leaf epidermal cell. Transient expression of CP-RT:RFP sometimes caused the formation of large aggregates in cells with high expression levels, which recruit the virus-expressed CP-RT:GFP into these aggregates. Arrowhead indicates CP-RT aggregate. Pictures were taken seven days after virus infection and 11 days post agroinfiltration. Left panels in (a) and (b), RFP channel, in (c) and (d), GFP channel; middle panels in (a) and (b), GFP channel, in (c) and (d), RFP channel; right panels, merge. All images were taken by confocal laser scanning microscopy. Scale bars = 10 μ m.