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ISOLATION OF A DnaJ HOMOLOGOUS PROTEIN FROM *ARABIDOPSIS THALIANA* INTERACTING WITH NSm, THE TOMATO SPOTTED WILT TOSPOVIRUS MOVEMENT PROTEIN

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Introduction

For establishing a systemic host infection, plant viruses have to pass rigid cell walls and cross up to six different barriers (epidermis / mesophyll, mesophyll / mesophyll, mesophyll / bundle-sheath, bundle-sheath / phloem-parenchyma, phloem-parenchyma / companion-cell and companion-cell / sieve-element) before reaching the vascular system and finally phloem sieve elements. For a growing number of plant viruses it has been shown that at least for the movement between mesophyll cells the activity of virus encoded movement proteins (MPs) is one essential prerequisite to achieve cell-to-cell transport (CARRINGTON *et al.*, 1996). Plant infecting viruses have established at least two different mechanisms for cell-to-cell transport. Whereas the MPs of *Tobacco mosaic virus*, *Red clover necrotic mosaic virus* and *Cucumber mosaic virus* are able to increase the size-exclusion limit of plasmodesmata between mesophyll cells, *Cowpea mosaic virus*, *Cauliflower mosaic virus* and *Tomato spotted wilt virus* (TSWV) presumably move from cell to cell by inducing tubular structures (for review: LAZAROWITZ & BEACHY, 1999). Nevertheless, it is unclear by which appliances virus particles are guided and forced through such channels.

To shed some more light on the process of TSWV movement, we used the yeast two hybrid system to screen for host proteins interacting with NSm, the TSWV encoded MP. We report on an interaction between NSm and a DnaJ homologous protein from *Arabidopsis thaliana*, assuming that DnaJ like proteins could act as a co-factors during the transport of TSWV between mesophyll cells and/or in the vascular system.

Material and Methods

Plant inoculations

Arabidopsis thaliana (ecotype Columbia) plants were grown under short day conditions (8 hours light/16 hours darkness) in a growth chamber (Percival 35 LL) for 3-6 weeks, then transferred to a phytochamber which was set for long days (16 hours light/8 hours darkness). Two rosette leaves were mechanically inoculated with a fresh extract from infected leaves harvested from *Nicotiana rustica*. Two weeks after inoculation, 2 leaves were harvested every 2-3 days, frozen in liquid nitrogen and stored at –70° C for western blot analysis.

Recombinant DNA manipulations

DNA encoding the NSm protein of TSWV (L3, DSZM no. 0182; KELLMANN & SCHREIER, 1996) was cloned as a SmaI-XhoI fragment into the yeast/*E. coli* shuttle vector pAS2 (Clontech, USA) resulting in an in-frame fusion of the Gal4 DNA-binding domain and NSm. Expression of the chimaeric protein in yeast was confirmed by immunoblotting with an anti-Gal4 DNA binding domain antibody (Clontech, USA) as described in UHRIG *et al.* (1999). An oligo dT-primed cDNA library from *Arabidopsis thaliana* (ecotype Columbia) cell suspension culture was prepared in plasmid pACT2. Recombinant DNA work was performed using standard methods (SAMBROOK *et al.*, 1989).

Western blot analysis

Western blotting was performed by SDS/polyacrylamid gel electrophoresis of crude leaf extracts (dissolved in TBS-T (20mM Tris/Cl pH 7,4; 500 mM NaCl; 0,05% v/v Tween 20) subsequently blotted on PVDF membranes (Novex, USA). Presence of the TSWV N protein was determined by immunodetection using a polyclonal anti N antibody and goat anti-rabbit IgG conjugated with alkaline phosphatase as a secondary antibody. Staining of protein bands was performed by imbibing in NBT/BCIP solution (150 ug/ml nitroblue tetrazolium salt / 75 ug/ml 5-bromo-4-chloro-3-indolyl-phosphate).

Two hybrid assays

Yeast strain Y190 carrying the pAS2-NSm bait was transformed with 0,5 mg of DNA from the pACT2 cDNA library. The cells were plated on synthetic dropout medium (SD) lacking leucine, tryptophan and histidine supplemented with 25 mM 3-aminotriazole (DURFEE *et al.*, 1993). To verify their $lacZ^+$ phenotype as a second reporter for protein-protein interaction, the selected yeast colonies were lifted onto nylon membranes and

Transformed yeast was streaked onto synthetic dropout medium lacking leucine, tryptophane, histidine (LWH30) or lacking histidine (H30) supplemented with 30mM 3-aminotriazole to test for the his^+ interaction reporter. Subsequently, yeast colonies were filter-lifted and tested for $lacZ^+$ phenotype (second interaction reporter: blue staining of the respective sector after the addition of X-Gal).

A.) Interaction of NSm and the protein *encoded on* pAD-A39. SNF1/SNF4: Interaction of yeast proteinkinase SNF1 and the activator protein SNF4 (FIELDS & SONG, 1989; positive control), AD/BD-NSm: Gal4 activation domain (AD) plus Gal4 DNA-binding domain (BD)-NSm fusion protein, AD-A39/BD-NSm: pAD-A39 encoded protein plus NSm linked to Gal4 BD, empty vectors: Gal4 BD plus Gal4 AD (negative control).

B.) Specific interaction between the pAD-A39 encoded protein and NSm. SNF1/SNF4: positive control, AD-A39/-: single transformation of pAD-A39 encoded protein, sectors 3 to 8: pAD-A39 encoded protein co-transformed with: Gal4 DNA-binding domain in fusion with NSm (BD-NSm), TSWV nucleocapsid (N) protein (BD-N), TSWV non structural protein NSs (BD-NSs), WD-protein encoded by the pleiotropic regulatory locus 1 from Arabidopsis (BD-PRL), replicator protein of wheat dwarf geminivirus (BD-Rep), yeast SNF1 proteinkinase (BD-SNF1).

A39 is homologous to DnaJ like chaperones

The *Arabidopsis thaliana* cDNA insert of library plasmid pAD-A39 was sequenced and compared with the EMBL databank. The homology BLAST search revealed that the cDNA encodes a DnaJ like protein. The complete gene has been sequenced during the *Arabidopsis* sequencing project. The full length protein is composed of 348 amino acids, 314 amino acids of the C-terminus are present in pAD-A39.

Discussion

Plant viruses systemically infect their hosts by expressing MPs, which mediate virus cell-to-cell transport. Recently it was shown that the movement mechanism mediated by the RCNMV encoded MP resembles RNA- and protein transport mechanisms at least in the vascular system of plants (XOCONOSTLE-CÁZARES *et al.*, 1999). Accordingly, it can be assumed that viral MPs alter the hosts transport functions to establish spreading of the virus throughout the plant by interaction with host proteins. One example for a host transport function, which also seems to be utilized by viral MPs, was given by the maize homeobox protein KN1, which was thought to use a pathway through plasmodesmata involving a plasmodesmal receptor and/or cytoplasmic chaperone activity (KRAGLER *et al.*, 1998).

From several examinations it is well accepted that NSm represents the movement protein of TSWV, however, the mode of action of NSm is still unclear. Electronmicrographs showed that NSm is located at plasmodesmata which connect mesophyll cells and therefore conclusively is associated with symplastic transport mechanisms (KORMELINK *et al.*, 1994). NSm is able to induce tubular structures in mesophyll cells, which also can be observed in TSWV transfected or NSm overexpressing tobacco protoplasts (STORMS et al, 1995). The entire path of spreading of TSWV from the site of infection into the vasculature, however, has not yet been enlighted. We started a two hybrid screen using the NSm protein as a bait. An activation domain tagged cDNA library from *Arabidopsis thaliana* was examined since this species was shown to be susceptible for TSWV (GERMAN *et al.*, 1995; Figure 1). We isolated a clone designated A39, which specifically interacts with NSm (Figure 2) and shows homology to DnaJ like chaperones.

The homology of A39 to DnaJ like chaperones allows the deduction of possible functions. To date, all members of the Hsp70 family (DnaK, DnaJ and GrpE) are known to be involved in a wide variety of different cellular processes (KELLEY, 1998). A typical role of DnaJ in protecting proteins during stress in procaryotes is to bind a substrate protein and to interact subsequently with DnaK, where ATP hydrolysis takes place leading to a lock-in of the substrate protein into DnaK (BUCKAU & HORWICH, 1998). Therefore, one possible function of the DnaJ homologous chaperone in plants is related to stress conditions, e.g. virus infection. ZIMMERMANN (1998) presented a model for putative roles of Hsp70 and DnaJ (Hsp40) in post- as well as cotranslational protein transport into the mammalian endoplasmatic reticulum (ER). The transport function of the mammalian DnaJ protein at the ER correlates with the presence of appressed ER (desmotubuli) present in plant plasmodesmata.

Other experiments demonstrate a relationship between DnaJ like proteins and the cytoskeleton, e.g. loss of the activity of the DnaJ homologous protein YDJ1 in yeast caused an aberrant microtubule formation, and interaction between YDJ1 with the gamma tubulin TUB4 supported the idea that YDJ1 plays an important role in the regulation of microtubule formation (OKA *et al.*, 1998). Based on the strong homology of A39 with DnaJ like proteins it is conceivable that the NSm-A39 interaction starts deviating microtubule penetration through plasmodesmata in plant tissues. Similarly, the interaction of the MP of TMV with the cytoskeleton allowed viral RNA or virus particles to move along the microtubules to plasmodesmata (HEINLEIN *et al.*, 1995;

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