HSP70 homolog functions in cell-to-cell movement of a plant virus

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Plant closteroviruses encode a homolog of the HSP70 (heat shock protein, 70 kDa) family of cellular proteins. To facilitate studies of the function of HSP70 homolog (HSP70h) in viral infection, the beet yellows closterovirus (BYV) was modified to express green fluorescent protein. This tagged virus was competent in cell-to-cell movement, producing multicellular infection foci similar to those formed by the wild-type BYV. Inactivation of the HSP70h gene by replacement of the start codon or by deletion of 493 codons resulted in complete arrest of BYV translocation from cell to cell. Identical movement-deficient phenotypes were observed in BYV variants possessing HSP70h that lacked the computer-predicted ATPase domain or the C-terminal domain, or that harbored point mutations in the putative catalytic site of the ATPase. These results demonstrate that the virus-specific member of the HSP70 family of molecular chaperones functions in intercellular translocation and represents an additional type of a plant viral-movement protein.

The molecular chaperones from the HSP70 family are ubiquitous proteins conserved among all types of cellular organisms (1). Different members of this family function in a variety of processes, such as cell recovery from stress, folding of nascent proteins, disassembly of oligomeric protein complexes, protein import into endoplasmic reticulum or mitochondria, transport of receptors, etc. (2–4). The HSP70 molecules possess two domains, an N-terminal ATPase domain, and a C-terminal domain harboring a peptide-binding pocket (5, 6). All characterized HSP70s act by ATP-dependent cycles of protein binding and release. Although the C-terminal domain plays a critical role in HSP70 interactions with target proteins, the ATPase domain also displays a protein-binding capacity that is an important determinant of the protein's functional specialization (7). In addition, it is thought that conformational changes associated with ATP hydrolysis may generate the mechanical force required to pull polypeptides into the lumen of the endoplasmic reticulum (8).

As a rule, viruses do not encode HSP70s, although many have been demonstrated to recruit cellular HSP70s to aid virion assembly or genome replication (see examples in refs. 9 and 10). Several animal and plant viruses specifically regulate the expression of cellular HSP70s (11, 12). Plant closteroviruses represent a unique family of large positive-strand RNA viruses that encode homologs of HSP70s (HSP70h; refs. 13–17). It seems likely that the HSP70h gene was acquired by a common ancestor of closteroviruses by recombination with a host mRNA coding for HSP70 (18). Computer-assisted analysis revealed that the structural elements identified in the ATPase domain of cellular HSP70s are conserved in closteroviral homologs (13, 19). Comparisons of the more variable C-terminal domains (1) showed limited similarity between cellular and viral HSP70s (ref. 13 and V.V.D., unpublished data).

To reveal the function of HSP70h in closterovirus infection, we have employed a reverse-genetic approach on a cDNA clone of a prototype closterovirus, beet yellows virus (BYV). Using transfection of the isolated plant protoplasts with the corresponding RNA transcripts, we have demonstrated that HSP70h is not involved in the processes of BYV RNA replication and transcription (20, 21). In particular, we have found that a point mutation abolishing HSP70h expression has no detectable effect on accumulation of the genomic or any of the subgenomic messenger RNAs encoding structural and nonstructural proteins of BYV (20). Moreover, a truncated BYV variant lacking the HSP70h gene and five other genes was fully competent in replication, producing elevated levels of the two remaining subgenomic RNAs (21). Similar results ruling out a role for HSP70h in accumulation of viral RNAs were obtained for two other closteroviruses distantly related to BYV (22, 23). In addition, there was no evidence of HSP70h involvement in RNA encapsidation (24).

One of the fundamental phases in the life cycles of plant viruses is a symplastic cell-to-cell movement via plasmodesmatal channels. Since the original discovery of the tobacco mosaic virus movement protein (MP) (25), proteins potentiating this process have been identified in diverse groups of plant viruses (for recent reviews see refs. 26–28). These MPs have been classified into several families (27, 28). Closteroviruses do not specify proteins that are closely related to previously identified MPs; it has been suggested that HSP70h functions in closteroviral transport analogously to the participation of cellular HSP70s in various translocation processes (13). The recent detection of HSP70h in the plasmodesmata of closterovirus-infected cells (24) and the reported ability of HSP70h to partially complement transport defects in unrelated viruses (29) provided indirect support for this hypothesis.

To elucidate the role of HSP70h in BYV cell-to-cell movement, a cDNA clone was further modified to accommodate an additional subgenomic promoter and a reporter ORF encoding the green fluorescent protein (GFP). Expression of GFP from the resulting ≈16.5-kb RNA genome of the tagged virus facilitated detection of virus movement and allowed us to demonstrate that the closteroviral HSP70h represents a distinct type of plant viral MP. The function of HSP70h in virus translocation from cell to cell adds yet another role to the list performed by the members of this multifaceted family of molecular chaperones.

Materials and Methods

Improvement of BYV cDNA Clone. The previously described BYV cDNA clone, pBYV-NA, was competent in replication, transcription, and assembly in isolated tobacco protoplasts (20, 21, 24). However, the corresponding RNA transcripts failed to

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Abbreviations: BYV, beet yellows closterovirus; GFP, green fluorescent protein; HSP70h, HSP70 homolog; MP, movement protein; GUS, β-glucuronidase; CP, major capsid protein; BYSV, beet yellow stunt closterovirus; d.p.i., day(s) postinoculation.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF190581).

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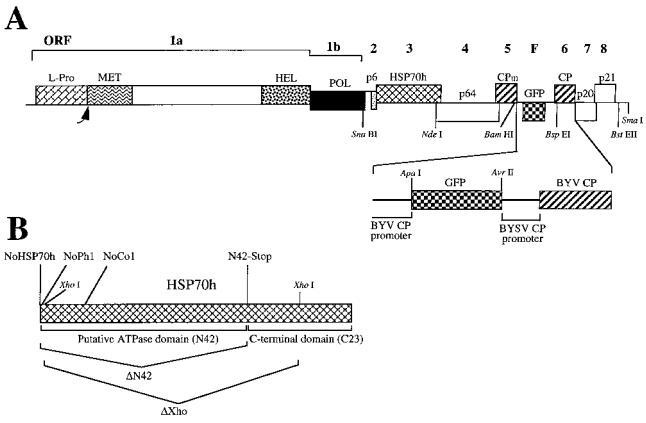

Fig. 1. (*A*) Diagram of the BYV genome with ORFs from 1a to 8, encoding leader proteinase (L-Pro), replication-associated proteins possessing putative methyltransferase (MET), RNA helicase (HEL), and RNA polymerase (POL) domains, 6-kDa protein (p6), HSP70 homolog (HSP70h), 64-kDa protein (p64), minor capsid protein (CPm), green fluorescent protein (GFP; ORF F, for foreign), major capsid protein (CP), 20-kDa protein (p20), and 21-kDa protein (p21). The bottom part of *A* is a close-up showing the reporter GFP ORF and CP ORF with adjacent promoter regions governing their expression. Selected restriction endonuclease sites are those mentioned in *Materials and Methods*. (*B*) Mutations introduced into the ORF encoding HSP70h (cross-hatched box). Point mutations and *Xho*I sites are shown above the box, whereas the location of the two major HSP70h domains and large deletion mutations are shown below the box.

induce local lesions characteristic of BYV infection on *Claytonia perfoliata* leaves (30). Furthermore, tagged pBYV-NA derivatives expressing β-glucuronidase (GUS) (21) were restricted to single cells in the systemic BYV host, *Nicotiana benthamiana* (V.V.P., Y.H., and V.V.D., unpublished results). We assumed that pBYV-NA and its tagged variants harbored defects in cell-to-cell movement, and we attempted to repair these defects by the generation and screening of additional cDNA clones derived from wild-type BYV RNA. Oligonucleotides 9,180 (5′-AAGTACGGGTACGAAAGTGGTG) and C15,453 (5′-GACTTCGCTAGGAGTGTGAAA) were used for reverse transcription–PCR amplification of the 3′-terminal region of BYV virion RNA from nucleotide 9,160 to the 3′-end as described (20). This region encodes nonreplicational proteins and, thus, seems likely to contain determinants of BYV transport. The resulting 6.3-kb PCR product was treated with *Sna*BI and *Bst*EII restriction endonucleases and cloned into appropriately digested pBYV-NA to replace the original *Sna*BI-*Bst*EII fragment (Fig. 1*A*).

Screening of the RNA transcripts corresponding to 11 reassembled clones yielded 2 (designated as pBYV-1 and pBYV-4) that were capable of inducing multicellular local lesions indistinguishable from those produced by wild-type BYV. This result demonstrated that pBYV-1 and pBYV-4 possessed the copy of the BYV genome that was competent in cell-to-cell movement. The nucleotide sequencing revealed 21 single-nucleotide differences between pBYV-4 and pBYV-NA. Ten of these differences resulted in the amino acid substitutions affecting four BYV proteins. In particular, six amino acid substitutions were found in HSP70h. The complete nucleotide sequence of the pBYV-4 was deposited into the GenBank database under accession no. AF190581.

Engineering of GFP-Tagged BYV. The plasmid pBYV-CPGUS, in which a reporter ORF encoding GUS fused to a proteinase domain (Pro) derived from tobacco etch virus HC-Pro, was used to engineer a GFP-expressing BYV cassette (21). The GUS-Pro ORF was located between the first and second codons of the CP ORF. Briefly, the GUS ORF was replaced with the enhanced GFP ORF (CLONTECH) by using the *Apa*I and *Avr*II sites (Fig. 1*A*). Likewise, the Pro ORF located downstream from the GUS ORF in pBYV-CPGUS was replaced with the CP promoter derived from beet yellow stunt closterovirus (BYSV) (16). The borders of the 248-nucleotide-long promoter region were chosen arbitrarily from BYSV nucleotide 8,448 to the start site of the BYSV CP. As a result, the GFP ORF was placed under the control of the original BYV CP promoter, whereas BYV CP expression was driven by the heterologous BYSV promoter (Fig. 1*A*). This strategy of foreign gene expression is based on a modification of that designed by Donson *et al.* (31) for tobacco mosaic virus. The region harboring the GFP ORF and the BYSV promoter was cloned into pBYV-4 by using the *Bam*HI and *Bsp*EI sites. The resulting plasmid was designated pBYV-GFP (Fig. 1*A*).

Generation and Analysis of the Mutants. Site-directed mutagenesis (32) and a plasmid p65M-4, derived from pBYV-4 as described for p65M (20), were used to engineer six HSP70h mutants (Fig. 1*B*). The NoHSP70h mutant, in which the start codon in the HSP70h ORF was replaced with ATA, has been described (20). In the ΔXho mutant, the region between two *Xho*I sites was deleted in-frame to remove >80% of the HSP70h ORF (Fig. 1*B*). The mutant ΔN42 lacked the N-terminal 42-kDa ATPase domain of HSP70h (N42; amino acid residues 2–383) (Fig. 1*B*; refs. 13, 19). In the NoPh1 mutant, lysine was substituted for the conserved Asp^{7} located in the "phosphate 1" motif of BYV HSP70h, whereas in the NoCo1 mutant, the conserved Glu^{181} in the "connect 1" motif was replaced with Arg (Fig. 1*B*; ref. 19). A premature stop codon introduced after the ATPase domain in the N42-Stop mutant was designed to produce HSP70h lacking its C-terminal 23-kDa domain (C23 in Fig. 1*B*). In addition, revertants of the mutants NoHSP70h, NoPh1, and NoCo1 were generated by a second cycle of mutagenesis and designated YesHSP70h, YesPh1, and YesCo1, respectively. Each of these mutations was incorporated into the full-length clone, pBYV-GFP, at the *Sna*BI and *Nde*I sites (Fig. 1*A*).

Bacteriophage SP6 RNA polymerase was used to generate capped RNA transcripts of the *Sma*I-linearized derivatives of pBYV-GFP as described (20). Eight-microliter aliquots of 50-μl reaction mixtures were applied manually to six carborundum-covered, mature leaves of *C. perfoliata* or *N. benthamiana*. Infection foci producing GFP-specific fluorescence were observed and photographed with a Leica (Deerfield, IL) DMRB microscope equipped with the GFP Plant Fluorescence filter set (excitation filter 470/40 nm and barrier filter 525/50 nm) and a Canon EOS Elan camera. Each inoculation experiment included the parental pBYV-GFP variant as a control and was reproduced at least twice. The Northern hybridization analysis was conducted as described (21). Statistical analysis of the data sets was performed by using Student's *t* test and Microsoft EXCEL 4 software package.

Results

Cell-to-Cell Movement of GFP-Tagged BYV. As illustrated in Fig. 1*A*, the GFP ORF was engineered downstream of the subgenomic promoter that normally directs the expression of the BYV CP. A heterologous promoter, derived from the distantly related closterovirus BYSV (16), was introduced to restore the production of BYV CP. This GFP-tagged BYV (BYV-GFP) was capable of replication in protoplasts, although the yield of the CP was reduced (data not shown), likely because of the increase in the genome size and the introduction of the additional promoter (21).

The competence of BYV-GFP for cell-to-cell movement was assessed by inoculation with the corresponding RNA transcripts onto leaves of *C. perfoliata.* This host responds to mechanical

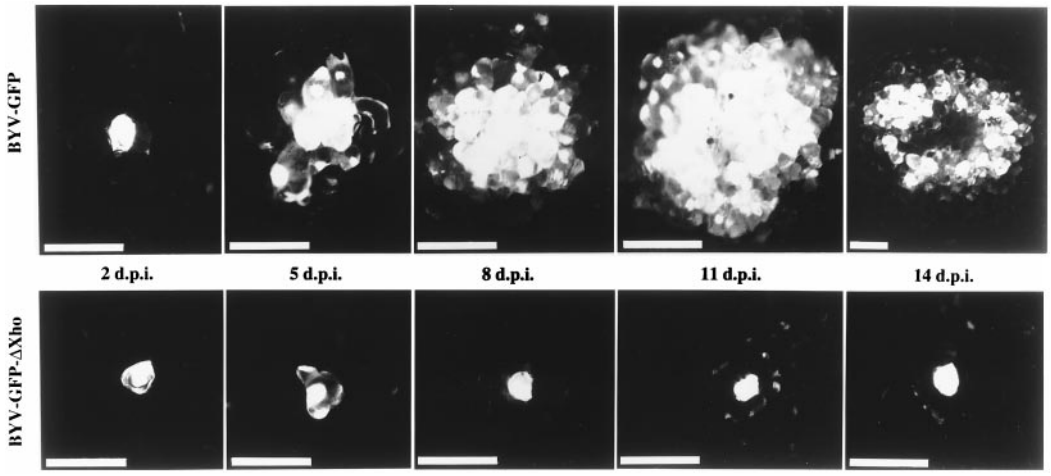

Fig. 2. Cell-to-cell movement of the GFP-expressing BYV (BYV-GFP) and its mutant variant lacking most of the HSP70h ORF (BYV-GFP-ΔXho) in mature leaves of *Claytonia perfoliata*. Photographs were taken with a fluorescent microscope and a GFP-specific filter. (Scale bars represent 25 μm.)

inoculation with wild-type BYV with the formation of characteristic red, local lesions (30). Single cells producing GFP-specific fluorescence were detected on the second day postinoculation (d.p.i.), and a gradual expansion of fluorescent infection foci continued until 11 d.p.i. (Fig. 2). At later times, necrotization of the foci resulted in fading fluorescence distinguishable only at the periphery (Fig. 2). Associated with this change in fluorescence was the appearance of visible red lesions identical to those formed after inoculation with wild-type BYV (data not shown).

Quantitative analysis of the 368 fluorescent foci observed at 12 d.p.i. revealed a broad size distribution. The result of this analysis, representing the complete data set, is shown as a histogram in Fig. 3*A*. Eighty percent of the foci were multicellular, ranging from two to nine cells in diameter. The prevalence

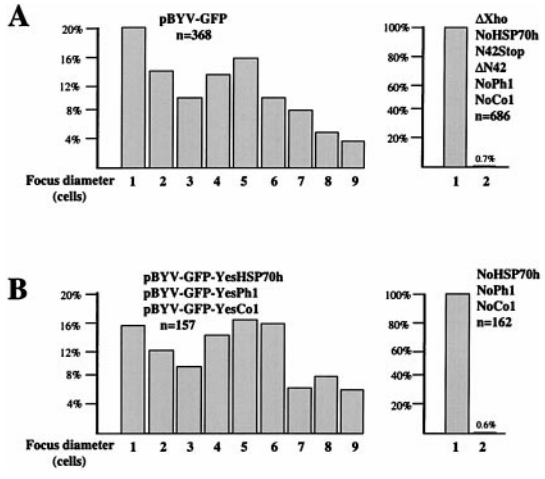

Fig. 3. Size distribution analysis of the fluorescent infection foci produced by RNA transcripts generated from pBYV-GFP and its variants, possessing mutations in the HSP70h ORF at 12 d.p.i. (*A*) Comparison between the parental BYV variant (BYV-GFP, derived from pBYV-GFP cDNA clone) and six mutant variants (see the text for further explanation). Because all the mutants exhibited identical movement-deficient phenotypes, the corresponding data were combined. (*B*) Comparison between the three revertant variants in which the original mutations were changed back to the wild-type (''Yes'') variants and parental mutant variants. Because all revertants exhibited identical movement-competent phenotypes, the corresponding data were combined. *n*, number of analyzed infection foci.

of multicellular foci and the induction of lesion formation by BYV-GFP indicated that the tagged virus provided an adequate model system for examining the local spread of BYV. The fact that 20% of the foci remained unicellular (Fig. 3*A*) suggested that the host plant possessed the capability to restrict BYV cell-to-cell movement at the early phase of infection.

We have also examined the cell-to-cell movement of BYV-GFP in a systemic host *N. benthamiana*, which does not restrict BYV spread via formation of the necrotic lesions (24). Fluorescent infection foci of up to 12 cells in diameter were observed at 12 d.p.i. on mature, inoculated leaves of *N. benthamiana* (data not shown). Likely because of the less restrictive nature of this BYV host, the proportion of unicellular foci was only 6% as compared with 20% for *C. perfoliata*. However, the susceptibility of *N. benthamiana* to mechanical inoculation with RNA transcripts was much lower than that of *C. perfoliata* (only 31 foci were found after analyses of 36 leaves), thus limiting the utility of *N. benthamiana* for analysis of the BYV local spread. The fact that the rate of increase in size of the infection foci in *C. perfoliata* was similar to that found in *N. benthamiana* (1.0 and 0.8 cell per day, respectively), further vindicated the use of the local lesion host for the study of BYV cell-to-cell movement.

HSP70h Is a Closteroviral MP. To test the possible role of HSP70h in closteroviral cell-to-cell movement, we generated the ΔXho mutant that lacked virtually all of the ATPase domain and half of the C-terminal domain due to the in-frame deletion (Fig. 1*B*). Time-course experiments using *C. perfoliata* leaves revealed complete restriction of the ΔXho mutant to single cells throughout the entire period of observation (Figs. 2 and 3*A*). An identical result was obtained with inoculation of the mutant transcripts into *N. benthamiana* leaves: All of the 33 foci found on the 36 inoculated leaves at 12 d.p.i. were unicellular. These results strongly suggested that HSP70h is essential for the movement of BYV from cell to cell.

However, we could not exclude the possibility that the deleted RNA sequence itself, rather than the encoded product, was required for viral transport. To test this possibility, we used the NoHSP70h mutant, in which the expression of HSP70h was abolished by a point mutation replacing the start codon in ORF 3 with ATA (Fig. 1*B*; refs. 20, 24). Inoculation of *C. perfoliata* revealed that the NoHSP70h variant was incapable of producing multicellular infection foci for at least 12 d.p.i. (Fig. 3*A*). Among 134 foci, only 1 was composed of two adjacent cells. The origin of this focus, as well as of occasional two-cell foci observed for

Table 1. Accumulation of the genomic and five subgenomic (sg) RNA species in BYV variants harboring mutations in the HSP70h ORF

BYV variant	% of level found for corresponding RNA species in parental BYV-GFP variant*					
	Genomic RNA	p6/HSP70h sgRNA†	p64 sgRNA	GFP sgRNA	CP sgRNA	p21 sgRNA
ΔXho	145 ± 26	149 ± 13	169 ± 7	160 ± 24	217 ± 65	163 ± 16
NoHSP70h	91 ± 14	89 ± 15	96 ± 14	103 ± 12	113 ± 29	102 ± 14
ΔN42	119 ± 26	115 ± 25	128 ± 27	131 ± 28	169 ± 63	124 ± 21
N42 Stop	106 ± 26	105 ± 28	115 ± 28	122 ± 27	142 ± 55	121 ± 19
NoPh1	96 ± 20	96 ± 16	108 ± 15	115 ± 12	137 ± 33	118 ± 15
NoCo1	93 ± 22	91 ± 27	100 ± 26	108 ± 23	123 ± 46	107 ± 14

*The levels of RNA accumulation were determined in tobacco protoplasts at 4 days posttransfection with corresponding RNA transcripts. Each sgRNA is designated according to its encoded product.

†Because of the small size of the ORF encoding p6, it is not fully established whether p6 and HSP70h are translated from the same or separate sgRNAs.

other mutants (Fig. 3), may depend on simultaneous inoculation of two adjacent cells rather than on virus translocation from one cell to another.

To ensure that the mutant phenotype depended on the replaced start codon rather than on the incidental second-site mutation, we reverted the ATA to the wild-type ATG start codon. The corresponding revertant, designated YesHSP70h, was fully competent in cell-to-cell movement (Fig. 3*B*). Statistical analysis revealed that the YesHSP70h variant was not significantly different from the parental BYV-GFP ($P > 0.1$). In contrast, NoHSP70h mutant was significantly different from both the revertant and parental variants ($P < 0.001$). On the basis of these data, we concluded that HSP70h is indispensable for virus translocation from cell to cell.

Structural Requirements for the Movement Activity of HSP70h. All cellular HSP70s possess two distinct domains, the N-terminal ATPase domain and the C-terminal domain primarily involved in protein–protein interactions. To examine the role of a computer-predicted ATPase domain (13) and a C-terminal domain in BYV transport, we generated two mutants, each expressing only one of these domains. The ΔN42 mutant expressed only the C-terminal domain because of a deletion of the upstream part of the HSP70h ORF, except for the start codon; whereas in the N42-Stop mutant, a premature termination codon was introduced after the putative ATPase domain (Fig. 1*B*). Complete arrest of the movement in these two mutant BYV variants in *C. perfoliata* demonstrated that each of two major HSP70h domains is essential for the protein function (Fig. 3*A*).

Because the ATPase domain of at least some cellular HSP70s has been demonstrated to provide specialized functions distinct from its ATPase activity (7), we were interested in testing whether this activity is required for BYV transport. To this end, two point mutations targeting key residues in the putative active center of the ATPase domain were introduced into HSP70h. The invariant, negatively charged residues Asp^{7} and Glu^{181} that belong to phosphate 1 and connect 1 motifs, respectively, and which in cellular HSP70s are critical for ATP binding through interaction with Mg^{2+} ions (19), were replaced with positively charged amino acid residues in the NoPh1 and NoCo1 mutants. These mutations are presumed to interfere specifically with the ability of HSP70h to bind and hydrolyze ATP. As seen in Fig. 3*A*, neither the NoPh1 nor the NoCo1 mutant was capable of intercellular translocation. To exclude the possible involvement of the spontaneous second-site mutations, each of the NoPh1 and NoCo1 mutations was reverted to the wild type, resulting in YesPh1 and YesCo1 variants. Inoculation experiments revealed the complete restoration of cell-to-cell movement in these revertants (Fig. 3*B*). Statistical analyses of the data indicated that the extent of cell-to-cell spread of each revertant was not significantly different from BYV-GFP ($P > 0.1$), and that the NoPh1 and NoCo1 mutants were different from corresponding revertants and a parental BYV-GFP variant ($P < 0.001$). These results strongly suggest that each of the domains of HSP70h as well as the putative ATPase activity are essential for protein function in closterovirus movement from cell to cell.

Replication of HSP70h Mutants in Tobacco Protoplasts. Although it was demonstrated previously that HSP70h is not required for accumulation of the genomic and subgenomic RNAs of BYV (20, 21), we analyzed each of the HSP70h mutants described in this work by using transfection of corresponding RNA transcripts into isolated tobacco protoplasts and Northern hybridization. The levels of the genomic RNA detected at 4 days posttransfection for each of the HSP70h mutants, with the exception of ΔXho, were not significantly different from each other and from that of the parental BYV-GFP variant (Table 1; $P > 0.1$). The ΔXho mutant exhibited marginally significant ($P = 0.05$), ≈50% elevation in RNA level, likely due to improved replication of the truncated genome.

We have also analyzed accumulation of the five subgenomic RNAs encoding p6/HSP70h, p64, GFP, CP, and p21. The subgenomic RNA species encoding the CPm and p20 were not quantified because of their relatively low abundance and a background resulting from ribosomal RNAs (21). The results of this analysis (shown in Table 1) demonstrated that the mutations introduced into HSP70h did not have any negative effect on the steady-state levels of subgenomic RNAs. The ΔXho mutant accumulated higher levels of the subgenomic RNAs than those levels found in the parental variant BYV-GFP. These results reconfirmed the fact that HSP70h is not involved in RNA replication, transcription, or regulation of RNA stability.

Discussion

Molecular chaperones from the HSP70 family are conserved among all living cells. The closteroviruses represent a unique family of plant viruses that has acquired an HSP70 gene in the course of its evolution (18). Other viruses that rely on host HSP70s include bacteriophage λ, which requires DnaK (bacterial HSP70) to assemble its replication complex (2), and several viruses that utilize HSP70s in the virion assembly process (see examples in ref. 9). What is the function commissioned to HSP70 by a closterovirus? It was shown earlier (20, 21) and confirmed in this study that unlike bacteriophage λ, BYV does not require HSP70h for genome replication or transcription. Furthermore, HSP70h seems to be dispensable for the encapsidation of BYV RNA (24).

In this paper, we describe the construction of a GFP-producing BYV variant and demonstrate that GFP detection provides an adequate tool to observe cell-to-cell movement of BYV. Time-course experiments using a local lesion host revealed GFP fluorescence in initially inoculated cells early in infection. The spread of GFP from cell to cell at later times was eventually limited to localized infection foci indistinguishable from those formed by wild-type BYV. Necrotization of these foci at the terminal phase of tissue lysis resulted in a gradual, center-to-periphery disappearance of the GFP fluorescence. As expected, the BYV-GFP movement in a systemic host also produced similar multicellular infection foci, but did not induce tissue necrotization.

The BYV-GFP system was utilized to address the possible role of HSP70h in viral cell-to-cell movement. To this end, we generated three types of HSP70h mutants. In one type, HSP70h expression was abolished because of start codon replacement or deletion of most of the HSP70h ORF. Complete restriction of these BYV variants to single, initially inoculated cells in local lesion and systemic hosts of BYV unequivocally demonstrated that HSP70h is essential for BYV cell-to-cell movement.

In addition, these data confirmed the fact that the spread of GFP fluorescence was mediated by viral spread rather than by independent translocation of GFP. This proof is important in light of the recent finding that GFP has the ability to traffic from phloem companion cells into sieve elements and to be transported symplastically into sink tissues, including leaf mesophyll cells (33). Moreover, in the sink leaves of *Nicotiana* spp., free GFP is able to traffic between the mesophyll cells (34). Our data, along with extensive previous research (see examples in refs. 34–38), indicate that GFP remains to be a reliable reporter of virus cell-to-cell movement, provided that the experiments are conducted with mature source leaves rather than young sink leaves.

Analysis of the second type of HSP70h mutants indicated that each of the two HSP70h domains, the putative N-terminal ATPase domain and the C-terminal domain, is required for protein function. Furthermore, point mutations designed to inactivate the computer-predicted catalytic center of the ATPase domain in the third type of the mutants also abolished HSP70h function, suggesting that ATPase activity is indispensable for BYV transport. It should be stressed that each of the six mutants used in this study exhibited full competence in RNA replication and transcription. Collectively, these results establish HSP70h as a distinct type of viral MP and show that HSP70 can function in intercellular communications.

Although closteroviral HSP70h is structurally unrelated to the MPs of other plant viruses, it shares several features with diverse MP families. Similar to the p30 MP of tobacco mosaic virus, BYV HSP70h is expressed early in infection (cf. refs. 39 and 21) and is found in plasmodesmata, the structures that interconnect adjacent cells and provide a route for viral trafficking (cf. refs. 37 and 24). Moreover, HSP70h seems to possess *in vitro* microtubule-binding ability; it has been demonstrated that tobacco mosaic virus MP binds microtubules *in vivo* (cf. refs. 36, 40, and 41).

There are at least two types of movement-associated proteins that, similar to HSP70h, seem to possess an ATPase activity. One is the CI protein from potyviruses that is involved in RNA replication and in cell-to-cell movement (38, 42). Another is a member of the ''triple gene block'' MPs found in several groups of plant viruses. An example of such a protein is provided by a 58-kDa βb protein of a hordeivirus (43, 44). Our data implicating the ATPase activity in HSP70h function raise the possibility that ATP hydrolysis may generate a force required for viral translocation. An analogous idea was discussed in relation to the role of cellular HSP70s in pulling proteins across organellar membranes (8).

Similar to plant viruses that encode triple gene block proteins, closteroviruses possess at least three MPs. In recent studies we have shown that, in addition to HSP70h, a 6-kDa hydrophobic protein and a 64-kDa protein are necessary but not sufficient for virus translocation (D. V. Alzhanova, Y.H., V.V.P., and V.V.D., unpublished data). These studies suggest that, like the cellular HSP70s, viral HSP70h acts in association with several protein partners. It is not yet known whether HSP70h possesses an ability to bind genomic nucleic acid, as in the case of MPs from several diverse virus families (see examples in refs. 45–47). However, there are three lines of evidence suggesting that HSP70h may act by means of interaction with virions. First, ImmunoGold-electron microscopy analysis of BYV-infected cells revealed colocalization of HSP70h with virion masses (24). Second, it was demonstrated for a lettuce infectious yellows virus, which also belongs to the family *Closteroviridae*, that HSP70h copurifies with the virions (48). Third, our recent finding that each of the BYV capsid proteins is essential for virus translocation indicated that assembled virions may be required for this process (D. V. Alzhanova, Y.H., V.V.P., and V.V.D., unpublished data).

Although the mechanism of HSP70h action is not known, apparent association of HSP70h with the virions and plasmodesmata (24) suggests that it may bind virions, chaperone them toward the plasmodesmata, and pull them into plasmodesmatal channels. Whatever the mechanism of HSP70h action, it will be intriguing to see whether cellular HSP70s are also involved in intercellular communications.

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