Supporting Information

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SI Materials and Methods

Bacteria and Plants, Infections, and Heat Stress Conditions. *Pseudomonas syringae* strains were described (1). Cytosolic *hsp70* mutants and overexpressing (line 8–7) Arabidopsis Columbia (Col) lines were from J. Parker (Max-Planck Institute, Cologne, Germany; refs. 2, 3) and Col *cphsp70* lines from H.M. Li (Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; ref. 4). PVX *hsp70-1* silencing and vector control constructs were from R. Terauchi (Iwate Biotechnology Research Center, Iwate, Japan) and VIGS was performed as described (5), with 3-week-old *Nicotiana benthamiana* infected with PVX transcripts and *P. syringae* infiltration 18 d later. HA-tagged $HopI1_{Pma}$ (JJ30) full-length and mutant versions and Agrobacterium-mediated plant transformation were described in refs. 1 and 6. Two Col and Nossen lines expressing Δrepeats (JJ196), QAA (JJ202), and ΔJ (JJ197) were used.

Escherichia coli and *P. syringae* pv. *maculicola* strain *Pma*ES4326 and its derivatives were grown as in ref. 7. *Arabidopsis thaliana*, mustard family plants, and pea (*Pisum sativum*) were grown in a 16-h light/8-h dark cycle at 20 °C. *N. benthamiana* and tomato "Money maker," "Bonnie Best," and "76R" were grown in a 16-h light/8-h dark cycle at 24 °C.

For heat treatments, Arabidopsis plants were grown at 20 °C and (*i*) kept at 30 °C after infection, (*ii*) incubated for 35 min at 50 °C, allowed 2-h recovery before infection and kept at 20 °C after infection, (*iii*) preincubated 1 d at 30 °C before infection and kept at 30 °C after infection, and (*iv*) control plants grown and infected at 20 °C.

Nineteen- to 21-day-old Arabidopsis and 4-week-old other plants were inoculated by blunt syringe infiltration at OD_{600} of 0.0003 or sprayed at OD_{600} of 0.005 (unless stated otherwise) with 0.02% silwet (8). Six to eight independent samples were averaged for each genotype for the *P. syringae* growth experiments. Unless stated otherwise, leaf disk samples were taken 3 days after infiltration and 5 days after spray inoculation, and ground and serial dilutions plated for growth analysis (8). All infection experiments were repeated at least twice with similar results and are shown with SE. Student's *t* test was used for statistical analysis (with Statview software; SAS Institute).

Chloroplast Isolation. Arabidopsis chloroplasts were isolated as in ref. 9 with modification. Leaves (2.5–3 g) of 19-d Arabidopsis were ground at 4 °C by polytron in grinding buffer (50 mM Hepes/KOH at pH 7.8, 0.33 M sorbitol, 10 mM EDTA, 10 mM $NaHCO_3$, 5 mM $MgCl_2$, and 25 mM Na-ascorbate), filtered through Miracloth (Calbiochem), centrifuged for 5 min at 1200 × *g*, the pellet was washed with 20 mL of the same buffer and resuspended in 1 mL of lysis buffer (50 mM phosphate buffer at pH 8.0, 150 mM NaCl, protease inhibitor). This method yields 70–80% intact chloroplasts with minimal contamination with other cellular compartments (≈1%; ref. 9). Different fractions were analyzed by by SDS/PAGE or blue-native PAGE and immunoblotting, or proteins were immunoprecipitated after adding igepal CA-630 to 0.2%.

Pea chloroplasts were isolated according to ref. 10.

Recombinant Proteins. $HopI1_{Psy}$ (JJ120) and control $HopX2_{Pma}$ (JJ128) were cloned into His-tag expression vector pMCSG7 according to ref. 11. HPD in $HopI1_{Psy}$ was mutagenized to QAA (JJ218) by PCR similarly as described for $HopI1_{Pma}$ (1). J domain of $HopI1_{Pma}$ (amino acids 334–432) was cloned into pET21, in frame with His-tag (JJ9). GST fusion clones of AtHsp70 cDNAs in pGEX-2T were from C. Guy (University of Florida, Gainesville, FL; ref. 12). GST vector was used as control. Purified Hlj1 (J domain-GST; ref. 13) and human mHsp70 were gifts from J. Brodsky (University of Pittsburgh, Pittsburgh). AtHsp70-1 used for ATPase activity was cloned into pGEX-2 from pENTRD clone provided by L. Noël (Centre National de la Recherche Scientifique-Commissariat à l'Énergie Atomique, St. Paul-lez-Durance, France). All proteins were expressed in *E. coli* Bl21 and purified on Ni-NTA agarose (Qiagen; His_6 fusions) or gluthatione agarose (Amersham; GST fusions) according to manufacturer instructions, using Tris·-NaCl buffers. Protein concentration was measured with Bio-Rad Bradford reagent and verified by SDS/PAGE.

ATPase Activity of Hsp70. Phosphate released by ATP hydrolysis was measured in colorimetric assay with molybdate and malachite green reagent following ref. 14. Reactions (25 μL) in 96-well plates contained 0.3 μM Hsp70 and 0.5 μM J protein (without GrpE), were started by adding ATP to final concentration 1 mM, and stopped after 1- or 2.5-h incubation at room temperature (14). Absorbance at 630 nm was measured in a plate reader. Phosphate standard curve was included in each experiment.

Pull-Down Assays, Immunoprecipitation, and Protein Analysis. For pull downs, recombinant proteins from *E. coli* lysates were immobilized on Ni-NTA or gluthatione resins (by following purification protocol until elution), incubated for 1 h at 4 °C with *E. coli* lysate containing interacting partner or with plant or chloroplast extract, eluted, and analyzed by immunoblotting.

IP with anti-HA matrix (Roche) followed manufacturer's IP protocol. Plant tissue (0.5 g) (leaves of 19- to 21-d Arabidopsis or *N. benthamiana* 2 d after Agroinfiltration) was ground in liquid nitrogen and suspended in 1 mL of lysis buffer (as in chloroplast isolation) with 0.2% igepal. Extract (0.5 mL) was incubated for 2 h at 4 °C with 75 μL of HA-matrix (Roche), and manufacturer's IP protocol was followed. For HopI1 mutants with lower expression (QAA, ΔJ), 1 mL of extract was used, whereas HopI1 and Δrepeats extracts were diluted twice and 1 mL of diluted extract was used. After IP proteins were separated by SDS/PAGE and analyzed either by Coomassie blue staining and LC-MS/MS or by immunoblotting. In all immunoblotting experiments, membranes were subsequently stained with Coomassie blue to control for loading by using standard procedure for gels. Most abundant protein bands (such as Rubisco) appear dark blue on a light blue background. Signal quantification was performed with ImageQuant (Molecular Dynamics).

Database Searching of Peptides Identified by LC-MS/MS. LC-MS/MS protein identification was performed at Chicago Biomedical Consortium and Stanford University. Proteins in gel bands were digested with trypsin, extracted, and peptides were identified on a Thermo LTQ after fractionation by using nano HPLC. MS/MS samples from immunoprecipitations from total extracts were searched against the IPI Arabadopsis database (v3.43) by using Mascot and SEQUEST search engines, and results were merged by using Scaffold. MS/MS Samples from immunoprecipitations from chloroplast extracts were analyzed by using Mascot, which was set up to search the NCBInr_080723 database (selected for Arabidopsis thaliana, unknown version, 51,937 entries).

Antibodies. Primary antibodies used were as follows: HA (Covance HA.11, monoclonal, 1:1,200); cytHsp70 (Stressgen SPA-817, monoclonal, 1:3,000 and SPA-757, rabbit polyclonal, 1:80,000, both specific for cytosolic Hsp70s; ref. 15); cpHsp70 [S78 from K. Keegstra (Michigan State University, East Lansing, MI; ref. 16), rabbit polyclonal, 1:3,000 for Arabidopsis, and from T. Leustek (Rutgers University, New Brunswick, NJ; ref. 17), guinea

pig polyclonal, 1:12,000 for peas], cFBP (Agrisera AS04043, rabbit polyclonal, 1:25,000). Secondary horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies (Thermo Scientific) were used at 1:20,000 dilution and anti-guinea pig (Sigma) at 1:100,000. Signal was detected with chemiluminescence (Thermo Scientific).

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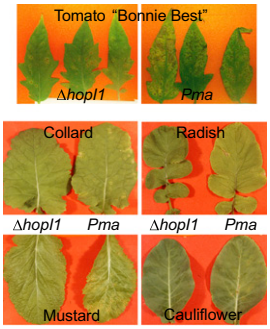

Fig. S1. HopI1$_{PmaES4326}$ is a virulence factor on many crops. Tomato and mustard family plants were sprayed with bacteria (OD_{600} = 0.005), and photos were taken 7 dpi. Deletion of *hopI1* resulted in reduced disease symptoms.

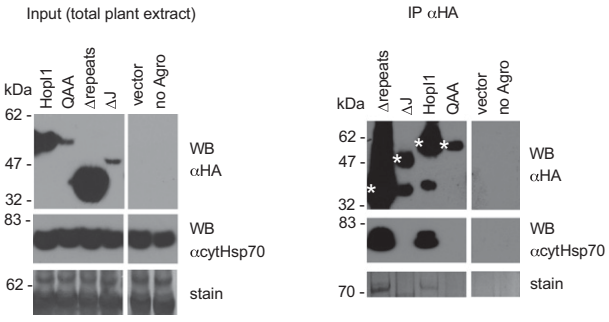

Fig. S2. J domain and HPD loop are necessary for HopI1 interaction with Hsp70. P/Q repeats of HopI1 are dispensable for this interaction. Proteins were immunoprecipitated with anti-HA matrix (*Right*) from *N. banthamiana* leaves transiently transformed with *hopI1*-HA variants or vector control, separated by SDS/PAGE and detected by HA antibody, Hsp70 antibody, or stained with Coomassie blue. All signals are from one exposure of one continuous membrane or gel. Cytosolic Hsp70 coprecipitated with HopI1 and HopI1Δrepeats. Proteins in total extracts (IP input) are shown in *Left* (one exposure of one continuous membrane). *HopI1-HA variants.

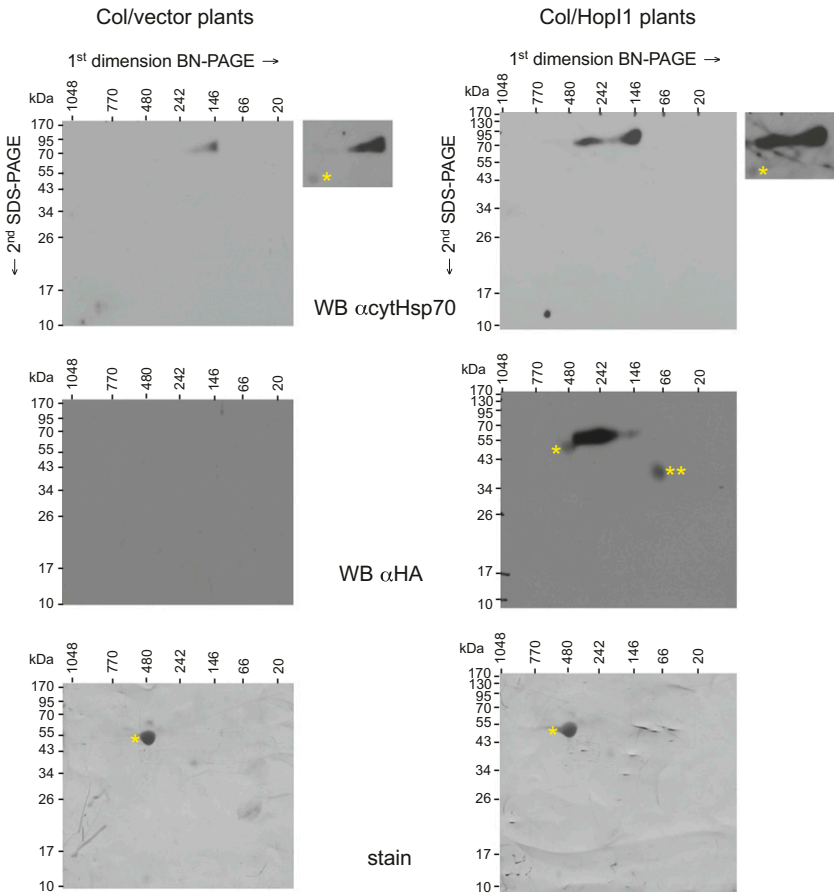

Fig. S3. HopI1-HA and cytHsp70 form similar-sized complexes in HopI1-expressing Arabidopsis. Proteins from Col/vector (*Left*) and Col/HopI1 (*Right*) plants were separated on 2D gels (first dimension, blue native gel; second dimension, SDS/PAGE) and the same membrane was probed with cytHsp70 antibody, HA antibody, and stained with Coomassie blue. Second dimension (SDS/PAGE) verified that signals in BN gel were from proteins of correct size (HopI1-HA and cytHsp70). Large cytHsp70 complexes were not detected in WT plants even with very long exposure (*Insets*). Same exposure is shown for Col/vector and Col/HopI1. *Rubisco; **HopI1 degradation product.

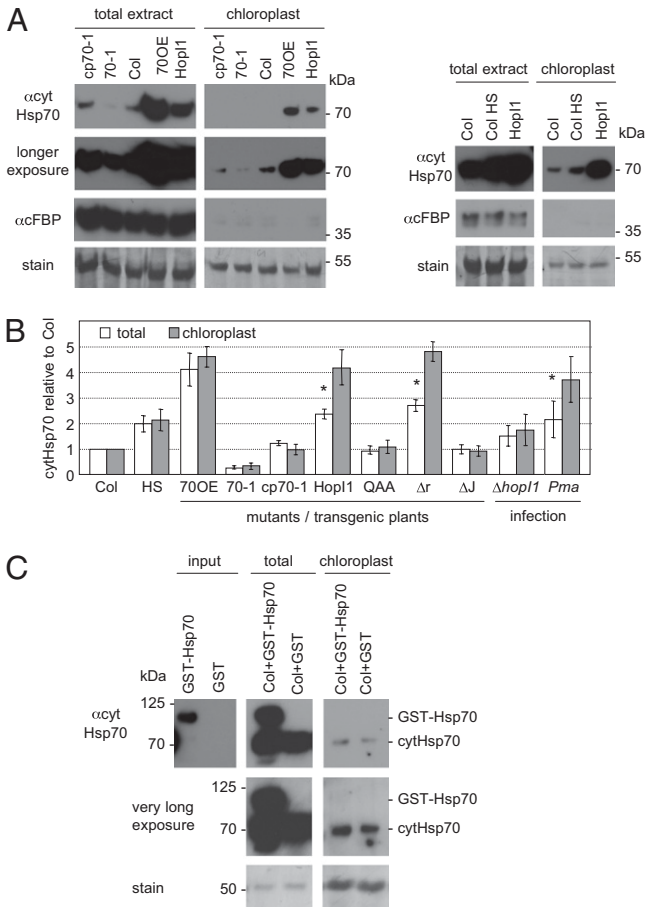

Fig. S4. HopI1 induces cytHsp70 level and recruits it to chloroplasts. All plants with elevated total cytHsp70 amount also had higher amount of cytHsp70 associated with chloroplasts. In Arabidopsis-overexpressing Hsp70-1 (70OE) or WT Col after 35 min of heat shock at 50 °C (HS), the increase in chloroplast fraction was proportional to increase in total extracts. However, plants expressing HopI1, Δrepeats (Δr), or infected with *Pma* had an almost twice higher cytHsp70 ratio (relative to Col or vector-transformed plants) in chloroplast-enriched than in total extract (4 times more than Col in chloroplast-enriched fraction and 2 times more than Col in total extract). (*A*) Cytosolic Hsp70 in total and chloroplast-enriched extracts was detected with cytHsp70 antibody (signals from all samples in both extracts are from one exposure of one continuous membrane). Later, the same membrane was incubated with cytosol-specific cFBP (cytosolic fructose-1,6-bisphosphatase) antibody for quality control of chloroplast-enriched fraction and stained with Coomassie blue for loading control. (*B*) Relative amounts of cytHsp70 in total (white) and chloroplast-enriched (gray) extracts were measured from multiple samples by using ImageQuant. Western blot signals from plants in *A* and Fig. 3 were quantified from different exposures for chloroplast and total extracts (shortest exposure where the weakest signal could be detected), corrected for amount of Rubisco, and presented relative to Col or Col/vector (therefore cytHsp70 in total and chloroplast Col extracts are taken as 1). Mean with SE of eight independent samples for HopI1 plants and heat shock treatment, at least six samples for infected plants, five for 70OE, at least three for HopI1 mutants, and two for *hsp*70-1 (70-1) and cp*hsp*70-1 (cp70-1) mutants is shown. All experiments and gels contained Col (for *hsp70* mutants, heat shock and infection) or Col/vector control (for transgenic plants). *Increase of cytHsp70 in chloroplast-enriched fraction was higher than in total extract in plants expressing HopI1 and Δrepeats (Δr), and plants infected with *Pma* ($P < 0.05$). (*C*) Cytosolic Hsp70 is not a contaminant in chloroplast-enriched fraction. Recombinant GST-AtHsp70-1 (cytoslic) or control GST was added to Arabidopsis extract before chloroplast isolation in amount similar to endogenous cytosolic Hsp70 (5 μg of GST-Hsp70 was added to 1 g of tissue ground in 10 mL of buffer). GST-Hsp70 was not detected in chloroplast-enriched fraction, whereas endogenous cytHsp70 was detected. Signals in total and chloroplast-enriched extracts are from one exposure of one continuous membrane.

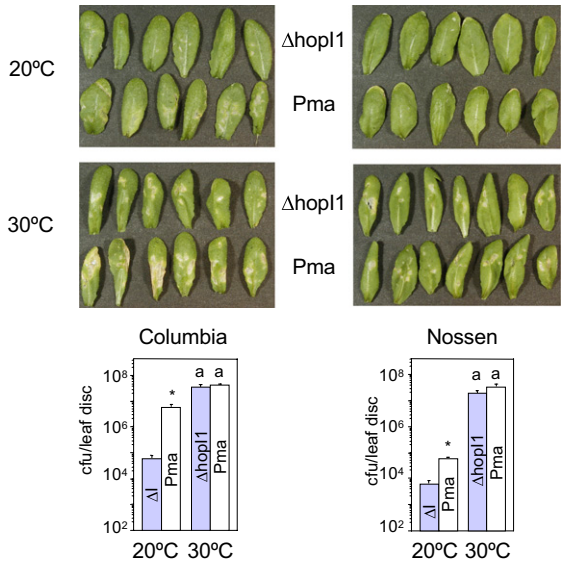

Fig. S5. Plants are more susceptible to infection at 30 °C. HopI1 is not needed for *Pma* virulence at 30 °C. Disease symptoms 5 d after spray infection (OD_{600} = 0.01) and bacterial growth 3 dpi are shown. Blue bars, Δ*hopI1;* white bars, wild-type *P. syringae* growth. At 20 °C, ΔhopI1 (blue bars) grew less than Pma (white bars; $*P <$ 0.01) in Col and Nossen Arabidopsis. At 30 °C, growth of both strains was the same and it was higher than at 20 °C ($^{a}P < 0.01$) in Nossen (in both experiments) and sometimes in Columbia (in three of five experiments).

Table S1. Hsp70 peptides identified by LC/MS/MS in three of the most abundant Hsp70 isoforms coimmunoprecipitated with HopI1-HA from Arabidopsis leaf and chloroplast-enriched extracts

	Total leaf extract			Chloroplast extract		
Isoforms	Unique peptides*	Specific peptides†	% coverage	Unique peptides*	Specific peptides†	% coverage
Hsp70-1	19	6	32	30	11	44
Hsp70-3	15	6	26	27	10	43
Hsp70-4	11	2	22	19	6	28

One specific peptide each from Hsp70-2 and chloroplast cpHsp70-1 (Hsp70-6) was also identified.
*Number of different peptides that match the isoform shown, but may also match one or more other isoforms.
†Peptides specific to one Hsp70 isoform.