ARTICLES

A type III effector ADP-ribosylates RNA-binding proteins and quells plant immunity

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The bacterial plant pathogen *Pseudomonas syringae* injects effector proteins into host cells through a type III protein secretion system to cause disease. The enzymatic activities of most of *P. syringae* effectors and their targets remain obscure. Here we show that the type III effector HopU1 is a mono-ADP-ribosyltransferase (ADP-RT). HopU1 suppresses plant innate immunity in a manner dependent on its ADP-RT active site. The HopU1 substrates in *Arabidopsis thaliana* extracts were RNA-binding proteins that possess RNA-recognition motifs (RRMs). *A. thaliana* knockout lines defective in the glycine-rich RNA-binding protein GRP7 (also known as AtGRP7), a HopU1 substrate, were more susceptible than wild-type plants to *P. syringae*. The ADP-ribosylation of GRP7 by HopU1 required two arginines within the RRM, indicating that this modification may interfere with GRP7's ability to bind RNA. Our results suggest a pathogenic strategy where the ADP-ribosylation of RNA-binding proteins quells host immunity by affecting RNA metabolism and the plant defence transcriptome.

Many Gram-negative pathogens of plants and animals and other eukaryotic-associated bacteria use type III protein secretion systems[1,2]. Type III protein secretion systems are molecular syringes that inject bacterial proteins called effectors into eukaryotic host cells to modulate host physiology. In animal cells, their activities alter specific host cell functions, including phagocytosis, proinflammatory responses, apoptosis and intracellular trafficking[3]. Much less is understood about the activities and targets of type III effectors from plant pathogens. The emerging picture is that many type III effectors from plant pathogens suppress host immune responses[4–6]. Thus far, effectors that possess cysteine protease, tyrosine phosphatase and E3 ubiquitin ligase activities have been implicated in suppression of plant innate immunity[7–13]; however, the enzymatic activities for most plant pathogen type III effectors that suppress innate immunity remain unknown.

Genomic investigations of *Pseudomonas syringae* pv. tomato DC3000, a pathogen of *Arabidopsis thaliana* and tomato, have identified greater than 30 effector genes[14,15]. Among these, *hopO1-1*, *hopU1* and *hopO1-2* (formerly *hopPtoS1*, *hopPtoS2*, and *hopPtoS3*, respectively) are predicted to encode proteins that contain potential active sites of mono-ADP ribosyltransferases (ADP-RTs)[16,17] (Fig. 1a). ADP-RTs are well-characterized toxins in animal pathogens, including two that are type-III-injected[18,19], but they have not been demonstrated to be important in plant pathogenicity. Furthermore, genes that encode ADP-RTs have been found in eukaryotes, but have not been identified in plants[20].

hopU1 encodes a putative ADP-RT

To begin to characterize the DC3000 effector genes that potentially encode ADP-RTs we focused on *hopU1*, which encodes a product with similarity to known ADP-RTs (Fig. 1a). *hopU1* is downstream of an apparent type III promoter, of the *shcF* type III chaperone gene and of the *hopF2* effector gene in the DC3000 chromosome (Fig. 1b). Semi-quantitative reverse transcriptase polymerase chain reaction (RT–PCR) experiments indicated the *hopU1* gene is transcribed and its expression was elevated when DC3000 was grown in a medium that induces the expression of the type III protein secretion system (Fig. 1c) and HopU1 was shown to be type III-injected into plant cells on the basis of adenylate cyclase translocation assays (Supplementary Fig. 1a). A DC3000 Δ*hopU1* mutant was reduced sixfold in its ability to multiply in plant tissue and cause disease symptoms in *A. thaliana* ecotype Col-0 (Supplementary Fig. 1b).

HopU1 suppresses plant innate immunity

We earlier reported that DC3000 mutants defective in type III effectors that can suppress the hypersensitive response—a programmed cell death of plant cells associated with innate immunity—often show an enhanced ability to elicit a hypersensitive response[21]. To investigate whether the Δ*hopU1* mutant shared this phenotype we infiltrated wild-type DC3000 and the Δ*hopU1* mutant at different cell densities into *Nicotiana tabacum* cv. Xanthi (tobacco).We consistently found that the Δ*hopU1* mutant elicited a hypersensitive response in tobacco at cell densities below the threshold needed for wild-type DC3000 (Fig. 2a). When *hopU1* was expressed *in trans* in the Δ*hopU1* mutant it complemented this phenotype (Fig. 2a). We also assessed cell death by measuring the amount of ion leakage from plant cells and found that the Δ*hopU1* mutant caused more cell death than DC3000 and expression of *hopU1 in trans* reduced the amount of cell death to wild-type levels (Supplementary Fig. 2a). Taken together, these results provide genetic evidence that HopU1 acts as a suppressor of the non-host hypersensitive response.

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To determine whether the predicted ADP-RT activity of HopU1 was required for suppression of the hypersensitive response, we ectopically expressed in the Δ*hopU1* mutant a HopU1 derivative ($HopU1_{DD}$) that had glutamic acid residues substituted with aspartic acids in the putative ADP-RT active site (Fig. 1a). This strain elicited an enhanced hypersensitive response similar to the Δ*hopU1* mutant control, suggesting that the suppression of the hypersensitive response required a functional ADP-RT active site (Fig. 2b). Ion leakage conductivity assays indicated that the Δ*hopU1* mutant expressing $HopU1_{DD}$ caused similar amounts of cell death to the Δ*hopU1* mutant control (Supplementary Fig. 2b). Thus, the hypersensitive response suppression activity of HopU1 requires a functional ADP-RT catalytic site.

We reasoned that HopU1 may be capable of suppressing other innate immune responses. To test this, we generated transgenic *A. thaliana* Col-0 plants that constitutively produced HopU1 fused to a haemagglutinin epitope (HopU1–HA). Bacterial flagellin often acts as a pathogen-associated molecular pattern, a conserved molecule from a microorganism recognized by animal and plant innate immune systems[22–24]. A conserved peptide from bacterial flagellin, flg22, has been shown to be effective at triggering callose (β-1-3 glucan) deposition[25]. The HopU1–HA-expressing transgenic plants treated with flg22 produced significantly reduced amounts of callose compared with wild-type plants (Fig. 2c, d). These plants also elicited a delayed atypical hypersensitive response in response to the type III effector AvrRpt2, which is recognized by the RPS2 resistance protein present in *A. thaliana* Col-0 (ref. 26; Supplementary Fig. 3). Together, these data indicate that HopU1 can suppress innate immune responses that are triggered by either a type III effector protein or a pathogen-associated molecular pattern.

HopU1 is an active ADP-RT

To explore whether HopU1 indeed possessed ADP-RT activity, we purified recombinant HopU1 and the catalytic site mutant ($HopU1_{DD}$), both fused to histidine affinity tags (Fig. 3a). The activity of recombinant HopU1–His was tested with poly-L-arginine, an artificial substrate for many ADP-RTs that can modify arginine residues. HopU1–His was capable of ADP-ribosylating poly-arginine in the presence of [^{32}P]-NAD, whereas the $HopU1_{DD}$–His mutant incorporated radioactivity in amounts similar to the BSA control (Fig. 3b). Therefore, HopU1 is an active ADP-RT that can modify arginine residues.

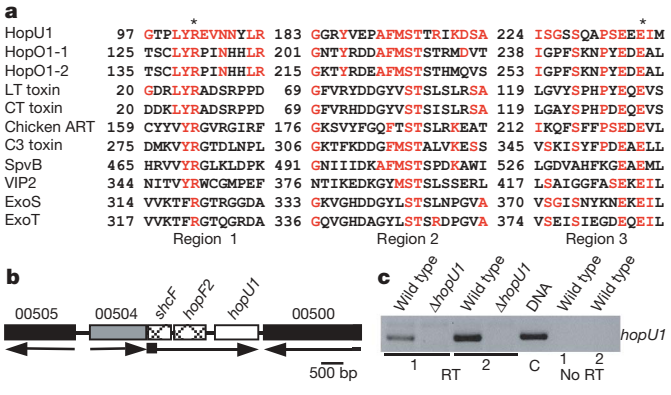

Figure 1 | HopU1 is a putative mono-ADP-ribosyltransferase that contributes to virulence. **a**, Alignment of the conserved regions of known mono-ADP-ribosyltransferases (ADP-RTs) with putative DC3000 ADP-RTs. Conserved residues are shown in red and the invariant amino acids of the cholera toxin group of ADP-RTs[42] are marked with asterisks. **b**, *hopU1* (white box) is downstream of a type-III-related promoter, the *shcF* type III chaperone gene and the *hopF2* effector gene (hatched boxes). **c**, RNA was isolated from DC3000 (wild type; WT) or the Δ*hopU1* mutant grown in either rich media (1) or a minimal medium that induces type-III-related genes (2) and used in RT–PCR reactions. A DNA control (C) and no reverse transcriptase controls (No RT) were included.

We next examined whether HopU1–His was capable of using plant proteins as substrates. Crude protein extracts from the leaves of *A. thaliana* ecotype Col-0 and tobacco were used in ADP-RT reactions. ADP-RT reactions were separated by SDS–polyacrylamide gel electrophoresis (PAGE), and subjected to autoradiography (Fig. 3c). At least two proteins in *A. thaliana* extracts and three in tobacco were ADP-ribosylated by HopU1–His. No labelled products were detected from reactions using the inactive $HopU1_{DD}$–His (Fig. 3c). Therefore, HopU1–His can use several *Arabidopsis* and tobacco proteins as substrates.

RNA-binding proteins are HopU1 targets

To determine the identity of the proteins ADP-ribosylated by HopU1 in *A. thaliana* extracts, we separated ADP-RT reactions with two-dimensional (2D) PAGE followed by autoradiography (for representative examples, see Supplementary Fig. 4). One well-separated protein spot that stained with Coomassie blue had identical migration to a radiolabelled ADP-RT activity spot. It was analysed with tandem mass spectrometry and corresponded to chloroplast RNA-binding protein (CP-RBP) RBP31 (ref. 27; Supplementary Table 1). The other ADP-RT activity spots did not co-migrate with visible Coomassie-blue-stained protein spots, suggesting that these proteins

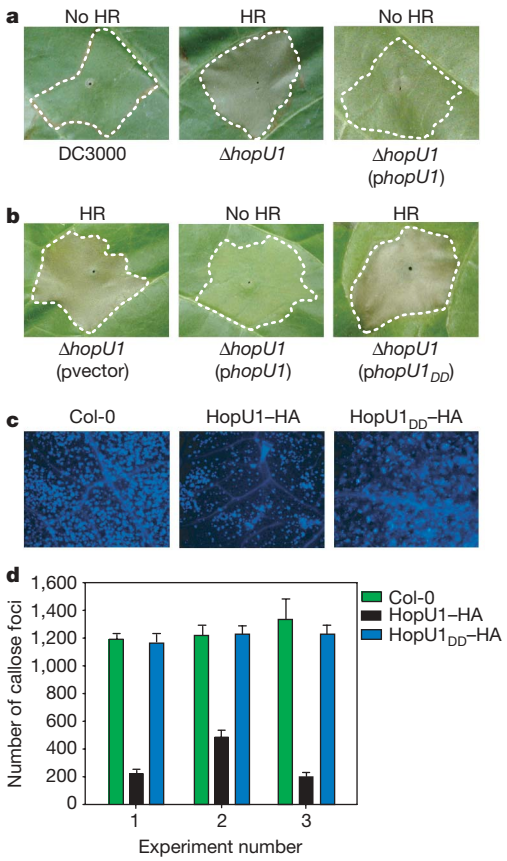

Figure 2 | HopU1 suppresses outputs of plant innate immunity. **a**, DC3000, Δ*hopU1* mutant and the Δ*hopU1* mutant expressing *hopU1* (p*hopU1*) were infiltrated into tobacco leaves at threshold cell densities (1×10^6 cells per ml). After 24 h the tissue was assessed for hypersensitive response (HR) production. **b**, The Δ*hopU1* mutant carrying a vector control (pvector), a *hopU1* construct (p*hopU1*) or a *hopU1* ADP-RT catalytic mutant construct (p$hopU1_{DD}$) were infiltrated into tobacco at 1×10^7 cells per ml and assessed for hypersensitive response production after 24 h. **c**, Callose deposition was visualized in *A. thaliana* plants expressing HopU1–HA or the $HopU1_{DD}$–HA mutant 16 h after treatment with flg22. **d**, Callose deposition was quantified by counting the number of callose foci per field of view. Twenty leaf regions (4 fields of view from 5 different leaves) were averaged and error bars (s.e.m.) are indicated. The experiment was repeated three times.

were in lower abundance. To enrich for less abundant substrates more concentrated *A. thaliana* extracts were made and fractionated using ion exchange chromatography. ADP-RT assays were performed on aliquots of each fraction (Supplementary Fig. 4b). Coomassie-blue-stained protein spots that had identical migration patterns to ADP-RT-modified protein spots visible on autoradiograms were analysed with tandem mass spectrometry. In total, we found three CP-RBPs and two glycine-rich RNA-binding proteins (GR-RBPs), GRP7 and GRP8 (ref. 28), to have been ADP-ribosylated by HopU1–His (Supplementary Table 1).

To confirm that the identified CP-RBPs and GR-RBPs were substrates for HopU1–His, we constructed glutathione *S*-transferase (GST) fusions. Substrate–GST fusions were partially purified with glutathione Sepharose or used as crude lysates in ADP-RT reactions. Each of the substrate–GST fusions could act as substrates for HopU1–His (Fig. 4a). Other plant protein–GST fusions corresponding to proteins neighbouring HopU1 substrates on 2D polyacrylamide gels were not ADP-ribosylated by HopU1–His. Thus, these CP-RBPs and GR-RBPs are indeed *in vitro* substrates for the HopU1 ADP-RT. We did not find additional HopU1 substrates in our extensive mass spectrometry analyses, suggesting that the *in vivo* targets of HopU1 are among these proteins.

CP-RBPs and GR-RBPs belong to a group of RNA-binding proteins that all have in common an RNA-recognition motif (RRM)—a protein domain demonstrated to be necessary and sufficient for RNA binding[29,30]. Localization experiments found that HopU1–green fluorescent protein (GFP) (and HopU1–GUS) and the GR-RBP GRP7–GFP and GRP8–GFP fusion proteins were similarly localized to the cytoplasm and possibly to the nucleus, whereas the CP-RBP–GFP fusions were discretely localized to the chloroplast (Supplementary Fig. 5). Because of our localization experiments and the association of GR-RBPs with abiotic and biotic stress[31,32], we focused on the GR-RBPs as putative physiological targets of HopU1.

The GR-RBP GRP7 and GRP8 are homologous to each other, sharing 76.9% identity, and probably perform related functions. GRP7 has been shown to bind RNA and influence messenger RNA oscillations in response to circadian rhythms at the post-transcriptional level[33,34]. GRP7 contains 14 arginine residues that represent putative sites of ADP-ribosylation (Fig. 4b). We found that the RRM–GST fusion was ADP-ribosylated by HopU1–His, whereas the glycine-rich-domain–GST fusion was not (Fig. 4c). To determine the arginine residues required for ADP-ribosylation, each arginine of the RRM was individually mutated to lysine in full-length GRP7–GST fusions. When arginines in positions 47 or 49 of GRP7 were substituted with

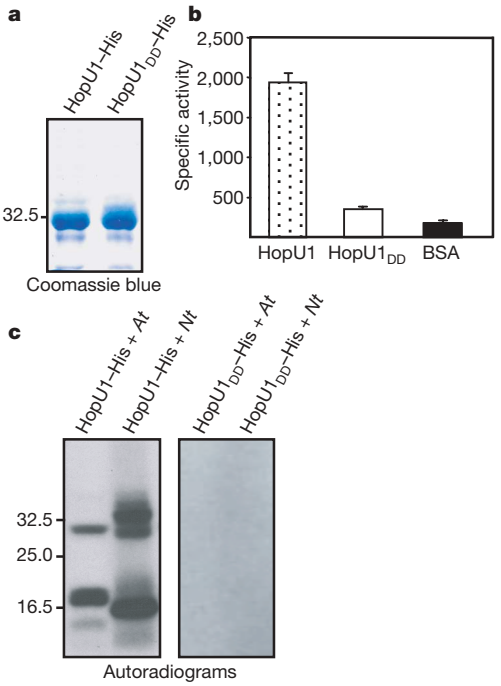

Figure 3 | HopU1–His ADP-ribosylates poly-L-arginine and proteins in *Arabidopsis* and tobacco. **a**, SDS–PAGE of partially purified HopU1–His and HopU1–His catalytic mutant ($HopU1_{DD}$–His) used in ADP-RT assays. **b**, HopU1–His (stippled bar), $HopU1_{DD}$–His (white bar), and a BSA control (black bar) were incubated with poly-L-arginine in the presence of [^{32}P]-NAD. ^{32}P-labelled products were quantified with liquid scintillation. The specific activity unit is μmol ^{32}P transferred per min $\times 10^{-10}$ per μmol of HopU1. The experiment was performed twice and the standard errors are indicated. **c**, Autoradiograms of ADP-RT assays with either *Arabidopsis* (*At*) or tobacco (*Nt*) extracts with HopU1–His or $HopU1_{DD}$–His. **a**, **c**, Molecular mass markers (kDa) are indicated.

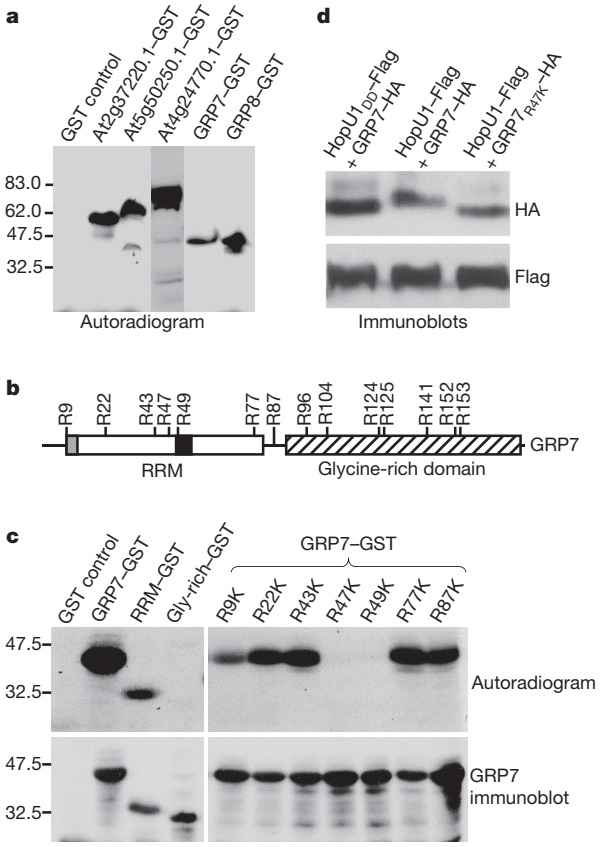

Figure 4 | HopU1–His ADP-ribosylates recombinant RNA-binding proteins *in vitro* and *in planta*. **a**, Recombinant CP-RBP and GR-RBP glutathione *S*-transferase (GST) fusions and a GST control were used in ADP-RT assays with HopU1–His. ADP-RT reactions were subjected to SDS–PAGE followed by autoradiography. The substrate fusions are listed with their corresponding *A. thaliana* locus or the protein name. **b**, Schematic representation of the RRM and glycine-rich domains within GRP7 (also known as AtGRP7) and the locations of the arginine residues (R) throughout the protein. Black and grey sections within the RRM domain depict RNP1 and RNP2, respectively, two conserved regions within RRM domains[29]. The locations of the arginine residues (R) are indicated. **c**, *In vitro* ADP-RT assays were done with HopU1–His and various GRP7–GST fusions. These included an RRM–GST fusion, a glycine-rich-domain–GST fusion, and GRP7–GST mutants that have each arginine within the RRM domain separately substituted with lysine. Autoradiograms (top panel) indicate the GRP7–GST derivatives that maintained the ability to be ADP-ribosylated and immunoblots (bottom panel) indicate the relative stability of each GRP7–GST derivative. **d**, GRP7 fused to an HA epitope (GRP7–HA), or a GRP7–HA derivative that is unable to be ADP-ribosylated ($GRP7_{R47K}$–HA) were transiently expressed in *N. benthamiana* with HopU1–Flag or the ADP-RT catalytic mutant $HopU1_{DD}$–Flag. After 40 h, leaf samples were subjected to immunoblot analysis using anti-HA and anti-Flag antibodies. **a**, **c**, Molecular mass markers (kDa) are indicated.

lysine, these GRP7–GST derivatives were no longer ADP-ribosylated, suggesting that one of these residues is the site of the ADP-ribose modification, whereas the other may be required for substrate recognition (Fig. 4c). Interestingly, on the basis of the RRM domain structure in other RNA-binding proteins, both of these residues would probably be solvent-exposed[35]. Moreover, the arginine in position 49 is within RNP1, the most conserved region of the RRM domain and one that has been directly implicated in RNA-binding[30].

To determine if GRP7 can be ADP-ribosylated by HopU1 *in planta*, we co-expressed HopU1–Flag or $HopU1_{DD}$–Flag and GRP7–HA or $GRP7_{R47K}$–HA (a GRP7 derivative that cannot be ADP-ribosylated) in *N. benthamiana* using *Agrobacterium* transient assays. After 40 h, plant extracts isolated from leaf tissue were separated on SDS–PAGE gels and analysed with immunoblots by using anti-Flag or anti-HA antibodies. We consistently observed an increase in the molecular mass of GRP7–HA when it was expressed *in planta* with HopU1–Flag, but not when expressed with $HopU1_{DD}$–Flag or when HopU1–Flag was expressed with $GRP7_{R47K}$–HA (Fig. 4d), suggesting that the increased molecular mass was due to ADP-ribosylation. Therefore, HopU1–Flag can ADP-ribosylate GRP7–HA inside the plant cell.

A *grp7* mutant is more susceptible to *P. syringae*

To determine the involvement of GRP7 in plant innate immunity, we identified an *A. thaliana* SALK homozygous T-DNA insertion line[36] in the *GRP7* locus, and confirmed that it did not produce *GRP7* mRNA and protein (Supplementary Fig. 6). To determine if this mutant, designated *grp7-1*, was altered in its responses to *P. syringae*, we infected wild-type *A. thaliana* Col-0 and the *grp7-1* mutant plants with DC3000 and a Δ*hrcC* mutant defective in the type III protein secretion system. DC3000 caused enhanced disease symptoms on *grp7-1* mutant plants compared with wild-type Col-0 (Fig. 5a). Each strain grew to higher levels in *grp7-1* plants compared with wild-type Col-0 plants (Fig. 5b), indicating that *grp7-1* plants were more susceptible to *P. syringae*. The growth difference was even more pronounced for the Δ*hrcC* mutant, which is probably due to the fact that this strain cannot inject any type III effectors, many of which suppress innate immunity. Importantly, we found that flg22-induced callose deposition was reduced in *grp7-1* plants compared with wild-type *A. thaliana* Col-0 (Fig. 5c), further supporting the idea that *grp7-1* mutant plants were impaired in their innate immune responses. Similar phenotypes were observed for an independent T-DNA mutant designated *grp7-2* (Supplementary Fig. 7).

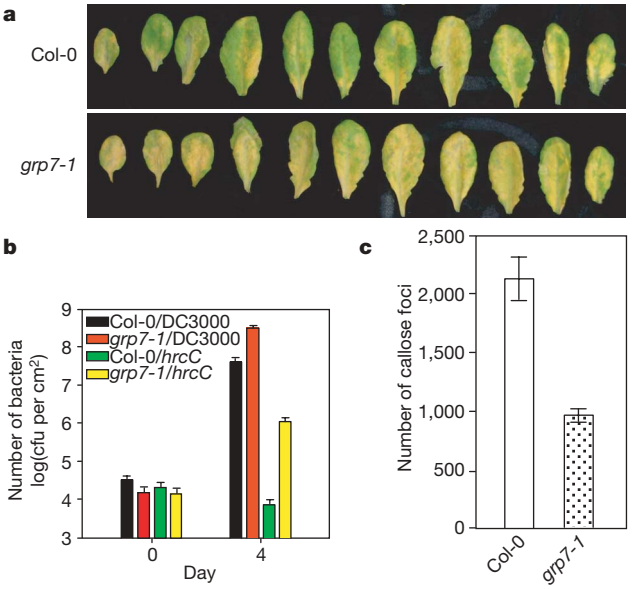

Figure 5 | Analyses of *A. thaliana grp7-1* mutant plants suggest GRP7 has a role in innate immunity. **a**, Disease symptoms in *grp7-1* plants and *A. thaliana* Col-0 plants after spray-inoculation with a cell density of 2×10^8 cells per ml. Pictures were taken after 5 days. The experiments in **a** and **b** were repeated five times with similar results. **b**, Bacterial growth assays of wild-type DC3000 and the Δ*hrcC* mutant spray-inoculated at a cell density of 2×10^8 cells per ml onto *grp7-1* and wild-type *A. thaliana* Col-0 plants. Results are shown as mean ± s.e.m. cfu, colony forming units. **c**, Callose deposition was determined in *A. thaliana* Col-0 and *grp7-1* mutant plants 16 h after treatment with flg22. The number of callose foci per field of view for 20 leaf regions (4 fields of view from 5 different leaves) were averaged ± s.e.m. The experiment was repeated three times with similar results.

Discussion

Several plant pathogen type III effectors are known to suppress outputs of innate immunity[4]; however, the enzymatic activity and substrates for these type III effectors remain poorly understood[14]. Indeed, there are only two cases for which both an enzymatic activity and a substrate for a plant pathogen type III effector are known[7–9]. The substrates of the HopU1 ADP-RT suggest an additional strategy used by bacterial pathogens to modulate plant innate immunity by indirectly affecting host RNA status. That is, GR-RBPs may act as key post-transcriptional regulators through either the trafficking, stabilization or processing of specific mRNAs in response to pathogen stress, and the ADP-ribosylation of the GR-RBPs by HopU1 may disrupt their activity (Supplementary Fig. 8). By disabling the function of GR-RBPs the pathogen may reduce the amount of immunity-related mRNAs available in the plant and tip the balance of the interaction in favour of the pathogen.

To our knowledge, this is the first demonstration of an active ADP-RT virulence protein in a plant pathogen. The DC3000 genome encodes at least two additional putative ADP-RTs, HopO1-1 and HopO1-2. These ADP-RTs possess low similarity with HopU1 and, therefore, probably target other plant proteins. ADP-RTs are well-characterized in animal pathogens; however, the ADP-RT substrates described here are new. Interestingly, IpaH9.8, a type III effector from the animal pathogen *Shigella flexneri*, was recently shown to bind the RRM-containing mammalian splicing factor U2AF[35], resulting in the suppression of pro-inflammatory cytokines[37]. The enzymatic activity of IpaH9.8 is presently unknown. The protein targets of HopU1 and IpaH9.8 indicate that animal and plant pathogens may alter RNA metabolism to quell the eukaryotic immune response.

METHODS SUMMARY

Bacterial strains (and plasmids) and primers used in this work are listed in Supplementary Table 2 and 3, respectively. A Δ*hopU1* mutant was made by homologous recombination. Expression of *hopU1* was assessed with RT–PCR[38]. Construction of *A. thaliana* Col-0 transgenic plants was done using the *Agrobacterium*-mediated floral dip method[39]. DC3000 strains were tested for their ability to elicit an hypersensitive response on *Nicotiana tabacum* cv. Xanthi by infiltrating strains resuspended in 5 mM MES (pH 5.6) at a cell density of 1×10^6 or 1×10^7 cells per ml. Pathogenicity assays with *A. thaliana* plants were performed by spray-inoculation with bacterial suspensions[12]. Seeds of the *Arabidopsis* SALK_039556.21.25.x and SALK_051743.42.85.x lines, corresponding to the *grp7-1* and *grp7-2* mutants, respectively, were obtained from the *Arabidopsis* Biological Resource Center at Ohio State University. *A. thaliana* plants were infiltrated with 1 μM of flg22 and stained with aniline blue for callose as described[25]. Leaves were examined with a Zeiss AxioPlan2 Imaging System microscope with fluorescence. The poly-L-arginine assay was done as previously described[40]. The ADP-RT assay was adapted from a described protocol[41]. GRP7–GST site-directed mutant constructs were made using the QuikChange Site-Directed Mutagenesis kit (Stratagene). *In planta* HopU1 ADP-RT assays with GRP7 were done using *Agrobacterium* delivery as described[21] and assessed with immunoblots.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions Z.Q.F. constructed the DC3000 Δ*hopU1* mutant, made the transgenic HopU1–HA-expressing *A. thaliana* plants, and performed the experiments in Figs 1a, b; 2a, b; 3; 4a, d and Supplementary Figs 1, 2, 4, 5b–d and 6d; M.G. identified the homozygous *A. thaliana grp7* mutant plants, cloned the HopU1–His substrate complementary DNAs, and performed the experiments in Figs 2c, d and 5 and Supplementary Figs 3, 4, 6a–c and 7; B.-r.J. performed the experiments in Figs 1c and 4c and Supplementary Fig. 5a, d; and F.T. provided technical support for several experiments. T.E.E. helped direct the identification of the HopU1–His substrates; R.L.C. performed the mass spectrometry and peptide database searches; D.S. provided the anti-GRP antibody, plasmids pGRP7-Gly and pGRP7-RRM, and insights on RNA-binding proteins; J.R.A. helped design the experimental plan, designed Supplementary Fig. 8, and was the primary writer of the paper. All of the authors discussed the results and commented on the paper.

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METHODS

Bacterial strains and nucleotide primers. Bacterial strains and plasmids used in this work are listed in Supplementary Table 2. The primers used are listed in Supplementary Table 3.

Construction of the DC3000 Δ*hopU1* mutant. A DC3000 Δ*hopU1* mutant was made by amplifying a 2 kb region upstream and downstream of *hopU1* using PCR with primer sets P1058/P1059 and P1078/P1079. The DNA fragment upstream of *hopU1* was ligated into pHP45Ω using *Bam*HI and *Hind*III sites. The DNA fragment downstream of *hopU1* was ligated into the pHP45Ω derivative containing the upstream fragment using *Xba*I and *Sac*I restriction enzymes such that the *hopU1* flanking regions were on either side of an Ω fragment in the same orientation. This cassette was ligated into the broad-host-range vector pRK415 using *Bam*HI and *Sac*I restriction enzymes. The resulting construct was electroporated into DC3000 and homologous recombination was selected for by selecting for retention of the antibiotic marker linked to the mutation, and loss of the plasmid marker. The resulting mutant, UNL141, was confirmed with PCR, using primers that annealed to the flanking regions.

RT–PCR to determine *hopU1* expression. The isolation of DC3000 RNA and reverse transcriptase PCR were performed as previously described[38].

Construction of *A. thaliana* Col-0 transgenic plants expressing HopU1–HA and HopU1$_{DD}$–HA. *hopU1* was amplified with PCR and recombined into the Gateway entry vector pENTR/D-TOPO. Site-directed mutagenesis was carried out on this entry construct to change the two glutamic acids in the ADP-RT active site to aspartic acids using the QuikChange Site-Directed Mutagenesis kit (Stratagene). The resulting constructs were recombined into binary Gateway destination vectors such that the *hopU1* derivatives were downstream of a *CaMV 35S* promoter; these were then electroporated into *Agrobacterium tumefaciens* C58C1 and transformed into *A. thaliana* Col-0 using the *Agrobacterium*-mediated floral dip method[39]. Plants were propagated through four generations and confirmed to constitutively produce HopU1–HA or HopU1$_{DD}$–HA, with immunoblots using anti-HA antibodies.

Plant materials and bioassays. *P. syringae* pv. tomato DC3000 strains were tested for their ability to elicit an hypersensitive response on *Nicotiana tabacum* cv. Xanthi by infiltrating strains resuspended in 5 mM MES (pH 5.6) at a cell density of 1×10^6 or 1×10^7 cells per ml with a needleless syringe. The Δ*hopU1* mutant UNL141 strain carrying plasmids pLN1981 (encoding HopU1), pLN1982 (encoding HopU1$_{DD}$), or pML123 (empty vector) were used in hypersensitive response suppression experiments. Pathogenicity assays with *A. thaliana* Col-0 and *grp7* mutant plants were performed by spray-inoculation with bacterial suspensions that were adjusted to an A_{600} of 0.2, as described[12]. Bacteria were enumerated from leaf tissue by plating dilutions on KB plates with the appropriate antibiotics. Seeds of *Arabidopsis* SALK lines SALK_039556.21.25.x and SALK_051743.42.85.x, which were used to identify *grp7-1* and *grp7-2* mutants, respectively, were obtained from the Ohio State University Biological Resource Center. Further description of methods used to confirm the homozygosity of the T-DNA inserts and the absence of *GRP7* RNA and GRP7 protein is in the Supplementary Methods.

Microscopic detection of callose deposition. *A. thaliana* Col-0 wild-type, Col-0 *grp7* mutant plants and *A. thaliana* expressing HopU1–HA or HopU1$_{DD}$–HA were infiltrated with 1 μM of flg22. After 16 h, the leaves were excised, cleared, and stained with aniline blue for callose, as described[25]. Leaves were examined with a Zeiss AxioPlan2 Imaging System microscope with fluorescence. The number of callose depositions was determined with Quantity One software (Bio-Rad). Twenty fields of view (each 0.56 mm^2) were analysed and averaged. The average and standard errors of three independent assays for each treatment were recorded.

Purification of HopU1–His and HopU1$_{DD}$–His mutation derivative. HopU1-His and HopU1$_{DD}$-His mutation derivative were affinity-purified from *Escherichia coli* BL21 (DE3) using Ni-NTA resin following the manufacturer's instructions (Qiagen). Purified HopU1–His and HopU1$_{DD}$–His were stored in aliquots at −80 °C or on ice. HopU1–His and HopU1$_{DD}$–His protein concentrations were measured with the BioRad Protein Assay Kit and the purity of the proteins was examined by SDS–polyacrylamide gel electrophoresis (PAGE).

Poly-L-Arginine ADP-RT assay. To determine if HopU1 ADP-ribosylated the artificial substrate poly-L-arginine, we used a previously described protocol[40]. Briefly, homo-poly-L-arginine (80 μl of 10 mg ml^{-1} in 0.1 M dimethyl glutaric acid buffer pH 7.0), 1 μM [^{32}P]-NAD (GE Healthcare) radiolabelled on the ADP-ribose moiety and 1 μM HopU1–His or HopU1$_{DD}$–His were incubated at room temperature for 1 h, suspended in 0.1 M phosphate buffer, centrifuged, and resuspended in 250 μl of 0.1 M HCl and 500 μl of 0.1 M dimethyl glutaric acid buffer (pH 7.0). Incorporated radioactivity was determined using liquid scintillation. BSA (1 μM) was used as a control.

ADP-RT assays with plant extracts and recombinant substrates. The ADP-RT assay was adapted from a described protocol[41]. Briefly, approximately 10 ng of partially-purified HopU1–His was incubated with 5 μCi of [^{32}P]-NAD (1 Ci μmole^{-1}) and plant extracts (*A. thaliana* Col-0 or tobacco), *E. coli* extracts, or 1–2 μg of partially purified recombinant substrates. The reaction was stopped by adding SDS sample buffer, after incubation at room temperature for 1 h. Samples were subjected to one-dimensional or two-dimensional SDS–PAGE followed by autoradiography of dried gels.

***GRP7–GST* plasmids and site-directed mutagenesis of *GRP7–GST*.** The nucleotides corresponding to the RRM domain and the glycine-rich domain of GRP7 were separately PCR-amplified using primer sets P2578/P2579 and P2580/P2581, respectively, and ligated into the GST vector pGEX-5X-1. The resulting plasmids encoded an RRM–GST fusion (pGRP7-RRM) and glycine-rich-domain–GST fusion (pGRP7-Gly). Several constructs encoding full-length GRP7–GST fusions in which individual arginine residues in the RRM domain were substituted with lysines were made using the QuikChange Site-Directed Mutagenesis (Stratagene). The primers used to introduce these mutations and the resulting plasmids are listed in Supplementary Tables 2 and 3. These constructs were separately expressed in *E. coli* to make lysates that contained GRP7–GST derivatives, which were used in ADP-RT assays.

***In planta* HopU1 ADP-RT assays with GRP7, using *Agrobacterium* delivery.** *Agrobacterium* transient co-delivery of *GRP7–HA* and *hopU1–Flag* or *GRP7–HA* and *hopU1$_{DD}$–Flag* DNA were done as described[21]. After 40 h, plant tissue was harvested with a No. 8 cork borer (12-mm diameter), ground in liquid nitrogen, and resuspended in SDS sample buffer. Samples were subjected to SDS–PAGE and immunoblot analysis using anti-Flag or anti-HA antibodies to determine the molecular mass of GRP7 in the presence or absence of active HopU1–Flag.