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Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance

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Plant diseases cause massive losses in agriculture. Increasing the natural defenses of plants may reduce the impact of phytopathogens on agricultural productivity. Pattern-recognition receptors (PRRs) detect microbes by recognizing conserved pathogen-associated molecular patterns (PAMPs)[1–3]. Although the overall importance of PAMP-triggered immunity for plant defense is established[2,3], it has not been used to confer disease resistance in crops. We report that activity of a PRR is retained after its transfer between two plant families. Expression of EFR (ref. 4), a PRR from the cruciferous plant *Arabidopsis thaliana*, confers responsiveness to bacterial elongation factor Tu in the solanaceous plants *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*), making them more resistant to a range of phytopathogenic bacteria from different genera. Our results in controlled laboratory conditions suggest that heterologous expression of PAMP recognition systems could be used to engineer broad-spectrum disease resistance to important bacterial pathogens, potentially enabling more durable and sustainable resistance in the field.

The ever-growing world population, the threat of climate change and the increasing interest in crop-derived biofuel production are some of the factors that threaten global food security. Microbial diseases and pests place major constraints on food production and agriculture. Agrochemical applications are the most common means of controlling these, but more sustainable methods are required[5]. One way to improve plant disease resistance is to enhance the capability of the plants' own innate immune system[6,7]. Although constitutive physical and chemical barriers contribute to their defense, like all higher eukaryotes, plants also depend for their survival on active recognition of microbial invaders. Plants can recognize potential pathogens via two perception systems[1]. One detects conserved microbial molecules, named pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs), through PRRs, leading to PAMP-triggered immunity (PTI). The other evolved to recognize microbial virulence effectors, usually through intracellular resistance proteins (R proteins), causing effector-triggered immunity (ETI).

ETI corresponds to what is classically referred to as gene-for-gene, vertical or race-specific resistance[1]. It generally occurs between cultivars of a given plant species bearing a particular *R* gene and a limited number of pathogenic strains carrying the matching virulence effector. *R* gene–mediated resistance is widely used in breeding programs to control plant diseases. However, this type of resistance rarely confers broad-spectrum disease resistance. Moreover, it is often rapidly overcome by evolving pathogens that lose or mutate the nonessential recognized effector or that produce new effectors to counteract ETI[1,8,9].

By definition, PAMPs are conserved across a wide range of microbes, which may or may not be pathogenic. Because these molecules are essential for viability or lifestyle, microbes are less likely to evade host immunity through mutation or deletion of PAMPs, compared with virulence effectors. PTI contributes to the plant innate immunity that is activated even during a susceptible (compatible) interaction. More notably, however, it probably constitutes an important aspect of non-host resistance, which accounts for why most plants are resistant to the majority of pathogens they encounter[10–17].

Relatively few plant PRRs have been identified. The leucine-rich repeat receptor kinases FLS2, EFR and XA21 recognize the bacterial PAMPs flagellin, elongation factor Tu (EF-Tu) and the type I–secreted sulfated protein 'activator of XA21-mediated immunity' (Ax21), respectively[2,3,18]. The transmembrane LysM domain–containing protein CeBiP binds the fungal PAMP chitin[2,3]. Perception of both chitin and unknown bacterial PAMP(s) require the LysM receptor kinase CERK1[2,3,19]. The receptor-like proteins EIX1 and EIX2 are PRRs for fungal xylanase, and the soluble protein GBP binds branched 1,6-1,3-β-glucans from the oomycete *Phytophthora sojae*[2,3].

Plant mutants in which PAMP recognition is affected are more susceptible to adapted pathogens (reflecting defects in basal resistance) and allow some degree of disease progression by non-adapted pathogens (reflecting defects in non-host resistance). For example, loss of flagellin recognition in *A. thaliana* and *N. benthamiana* enhances

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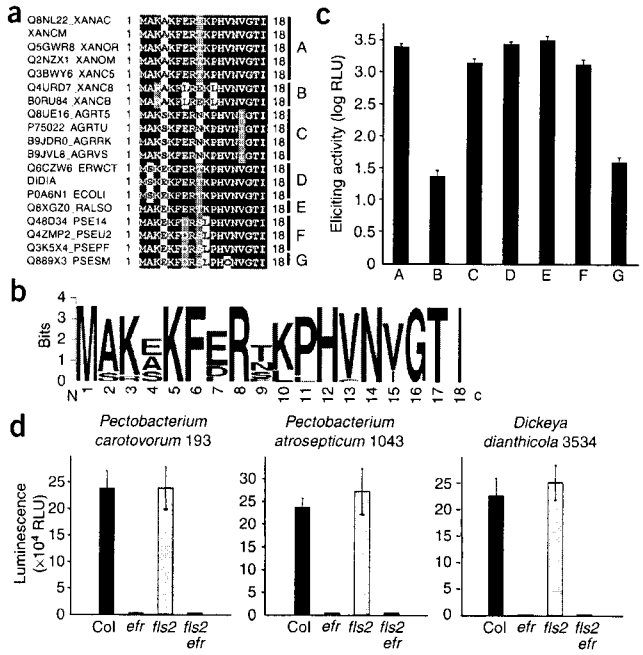

Figure 1 Eliciting activities of elf18 peptides and EF-Tu from selected phytopathogenic bacteria in *A. thaliana*. (**a**) Alignment of elf18 regions from selected bacteria. Capital letters on the right indicate the subgroups of elf18 peptides. Accession numbers are from UniProtKB. XANAC, *X. axonopodis* pv. *citri* 306; XANCM, *X. campestris* pv. *musacearum* 4381; XANOR, *X. oryzae* pv. *oryzae* KXO85; XANOM, *X. oryzae* pv. *oryzae* MAFF 311018; XANC5, *X. campestris* pv. *vesicatoria* 85-10; XANC8, *X. campestris* pv. *campestris* 8004; XANCB, *X. campestris* pv. *campestris* B100; AGRT5, *A. tumefaciens* C58; AGRTU, *A. tumefaciens*; AGRRK, *Agrobacterium radiobacter* K84; AGRVS, *Agrobacterium vitis* S4; ERWCT, *E. carotovora* ssp. *atroseptica*/*P. atrosepticum* SCRI1043; DIDIA, *Dickeya dianthicola*/*E. chrysanthemi* SCRI3534; ECOLI, *E. coli* K12; RALSO, *R. solanacearum* GMI1000; PSE14, *P. syringae* pv. *phaseolicola* 1448A/Race 6; PSEU2, *Pss* B728a; PSEPF, *Pseudomonas fluorescens* Pf0-1; PSESM, *Pto* DC3000. (**b**) WebLogo representation of the elf18 consensus sequence. (**c**) Oxidative burst triggered by elf18 peptides from different subgroups as defined in **a**. We calculated the eliciting activity as the amount of relative light units (RLU) produced in response to 1 μM elf18 peptide minus the amount of ROS produced in response to water in wild-type (Col-0 ecotype) *A.* thaliana leaf discs. Results are averages ± s.e.m. (n = 12). (**d**) Oxidative burst triggered by 10 μl bacterial extracts from *P. carotovorum* 193, *P. atrosepticum* 1043 and *D. dianthicola* 3534 in *A. thaliana* leaf discs from wild-type (Col-0; black), *efr* (light gray), *fls2* (gray) and *fls2 efr* (white) plants, measured as RLU. Results are averages ± s.e.m. (n = 12). We repeated all experiments at least three times with similar results.

susceptibility to virulent, weakly virulent and non-adapted *Pseudomonas syringae* strains[10–13]. EFR contributes to resistance to *Agrobacterium tumefaciens* and weakly virulent strains of *P. syringae* pathovar *tomato* (*Pto*) DC3000 in *A. thaliana*[4,20]. The rice PRR XA21 confers resistance to *Xanthomonas oryzae* pv. *oryzae*[21].

Although some PAMPs are recognized by many plant species, others have a narrower range of recognition[3]. For example, flagellin is recognized by both monocotyledonous and dicotyledonous species, as reflected by the identification of FLS2 homologs in *A. thaliana*, tomato, *N. benthamiana* and rice[2]. In contrast, EF-Tu (or its eliciting epitope elf18), cold-shock protein (or its eliciting epitope csp22) and Ax21 are only known to be recognized naturally by members of the Brassicaceae, Solanaceae and some rice cultivars, respectively[3,18].

On the basis of this observation, we tested whether the transfer of new PAMP recognition capacities across plant families would confer broad-spectrum disease resistance. We hypothesized that pathogens that are adapted to a given host species might not have evolved virulence effectors targeting PRRs that are normally absent from the host species. We chose the Brassicaceae-specific PRR EFR (ref. 4) because the high level of conservation of EF-Tu protein sequences across bacteria[22] offers the possibility that EFR could confer resistance against a wide range of bacterial pathogens.

To confirm that EFR detects EF-Tu proteins from important phytopathogenic bacteria, we first assessed the variability and eliciting activity of elf18 peptides derived from a selection of phytopathogenic bacteria spanning several genera. The 'classical' elf18 peptide sequence (acetyl-MSKEKFERTKPHVNVGTI) is based on the EF-Tu from *Escherichia coli*[22]. Previous work based on alanine-scanning and deletion analyses has shown that a fully active minimal peptide requires the sequence acetyl-MxKxKFxRxxxxxxxxx (where x is any amino acid)[22]. Alignment of the 19 selected elf18 sequences revealed seven different groups, with a maximum of five residues differing from the *E. coli*–based elf18 (elf18^{Ecoli}; **Fig. 1a**). In agreement with the strong conservation of the consensus elf18 peptide (**Fig. 1b**), synthetic peptides from the seven groups all induced the production of reactive-oxygen species (ROS) in *A. thaliana* wild-type leaves (**Fig. 1c**). However, synthetic peptides from groups B and G (corresponding to strains of *Xanthomonas campestris* pv. *campestris* and *Pto* DC3000, respectively) showed only 0.8%–3.2% of the activity measured with peptides from the other groups (**Fig. 1c**). In the case of group B, this is probably due to the Lys→Arg substitution at position 3 of the elf18 peptide (**Fig. 1a**), a residue that is required for full eliciting activity[22]. Although elf18^{PtoDC3000} (group G) does not show any variability in key residues, it has five substitutions compared to elf18^{Ecoli} (group D; **Fig. 1a,b**), which probably explain its reduced activity in *A. thaliana* leaves (**Fig. 1c**)[22].

Sequence information necessary to predict elf18 peptides may not always be available for all bacterial strains, especially in the case of emerging pathogens. Nonetheless, EF-Tu elicitor activity can easily be detected by *A. thaliana* cells in heat-killed bacterial soluble extracts[22]. We therefore compared the ability of bacterial extracts to elicit the production of ROS in *A. thaliana* leaves. Extracts from *Pectobacterium carotovorum* 193 (formerly known as *Erwinia carotovora* 193), *Pectobacterium atrosepticum* SCRI1043 (formerly known as *E. carotovora* ssp. *atroseptica* SCRI1043) and *Dickeya dadantii* 3534 (formerly known as *Erwinia chrysanthemi* 3534) all elicited ROS production in wild-type or *fls2* mutant *A. thaliana* leaves (**Fig. 1d**). In contrast, this response was completely abolished in *efr* and *fls2 efr* mutant leaves, revealing that the major PAMP in these extracts recognized by *A. thaliana* is EF-Tu. In conclusion, we were able to demonstrate EF-Tu eliciting activities in all phytopathogenic bacteria tested.

Next, we tested whether stable transformation of two solanaceous species with *EFR* confers responsiveness to EF-Tu. We have previously reported that *Agrobacterium*-mediated transient expression of EFR in *N. benthamiana* is sufficient to confer binding and responses to elf18 (ref. 4). We generated transgenic *N. benthamiana* plants expressing *EFR* under the control of its native promoter and selected homozygous plants carrying the *EFR* transgene for detailed phenotypic analysis (**Fig. 2a**). Whereas wild-type *N. benthamiana* plants were insensitive to elf18, transgenic EFR plants produced ROS in response to elf18 (**Fig. 2b**). In addition to triggering an oxidative burst, elf18 also induces the expression of defense-marker genes and seedling growth inhibition in *A. thaliana*[22]. Indeed, the expression of the PAMP-inducible genes *CYP71D20*, *FLS2*, *ACRE132* and *WRKY22*

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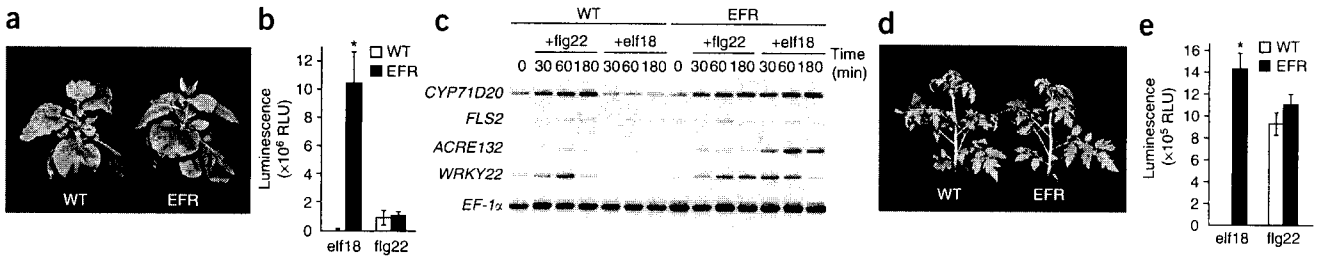

Figure 2 Transgenic expression of EFR in *N. benthamiana* and tomato confers elf18 responsiveness. (**a**) Four-week-old wild-type (left) and transgenic EFR (right) *N. benthamiana* plants. (**b**) Oxidative burst triggered by 100 nM elf18^{Ecoli} or flg22 in wild-type (white) or transgenic EFR (black) *N. benthamiana* leaf discs measured as RLU. Results are averages ± s.e.m. (n = 12). (**c**) Gene expression of marker genes determined by reverse transcriptase PCR. We treated wild-type and transgenic EFR *N. benthamiana* seedlings grown in axenic conditions with 100 nM elf18^{Ecoli} or flg22 for the times indicated. *EF-1α* is a housekeeping gene used a loading control. (**d**) Four-week-old wild-type (variety Moneymaker; left) and *EFR*-expressing transgenic tomato (right) plants. (**e**) Oxidative burst triggered by 100 nM elf18^{Ecoli} or flg22 in wild-type (variety Moneymaker; white) or transgenic EFR (black) tomato leaf discs measured as RLU. Results are averages ± s.e.m. (n = 12). *, $P < 0.05$ using Student's t-test. We repeated all experiments at least three times with similar results.

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was induced by elf18 in the transgenic plants expressing *EFR*, but not in wild-type plants (**Fig. 2c**). In addition, the growth of the transgenic seedlings was inhibited *in vitro* in the presence of elf18, whereas wild-type seedlings grew normally (**Supplementary Fig. 1**).

Similarly, homozygous transgenic tomato lines (*S. lycopersicum* variety Moneymaker) expressing *EFR* under the control of the constitutive promoter *35S* also gained elf18 responsiveness (**Fig. 2d,e**). Together, these results show that stable expression of *EFR* in *N. benthamiana* and tomato confers responsiveness to elf18.

The introduction of a receptor that is normally absent from a plant species could potentially affect the function of a preexisting receptor by, for example, competing for common signaling partners. To test whether the transfer of EFR to solanaceous plants could affect the function of the related endogenous PRR FLS2, we compared responsiveness to flg22 in wild-type and transgenic *N. benthamiana* plants expressing *EFR*. flg22 induced similar levels of ROS production and defense-marker gene expression in wild-type and transgenic plants expressing *EFR* (**Fig. 2b,c**). Therefore the presence of EFR has no detrimental effects on endogenous levels of FLS2.

Next, we tested whether EF-Tu responsiveness in the transgenic plants expressing *EFR* is associated with increased disease resistance to adapted virulent bacteria. Inoculation of *N. benthamiana* with *P. syringae* pv. *syringae* (*Pss*) B728a, the causal agent of bacterial brown spot of bean, caused severe disease symptoms and substantial bacterial growth[23] (**Fig. 3a**). However, transgenic *N. benthamiana* plants expressing *EFR* developed less severe disease symptoms and allowed only 1.25% of the bacterial growth observed in wild-type leaves 4 d post-inoculation (**Fig. 3a**).

To assess whether EFR confers resistance to other *P. syringae* pathovars, we next infected transgenic *N. benthamiana* plants expressing *EFR* with *P. syringae* pv. *tabaci* (*Pta*) 11528, the causal agent of tobacco wildfire (also known as angular leaf spot). Leaves of transgenic plants expressing *EFR* showed only minor disease symptoms compared with wild-type plants (**Fig. 3b**). This corresponded with very weak growth of *Pta* 11528 bacteria in leaves from transgenic plants expressing *EFR*, reaching only 0.02% of the bacterial growth observed in wild-type leaves 4 d post-inoculation (**Fig. 3b**).

We have previously reported that loss of EF-Tu recognition in *A. thaliana* leads to increased susceptibility to *A. tumefaciens*, the causal agent of crown gall disease. This demonstrates that EFR contributes to resistance to this phytopathogen in *A. thaliana*[4]. *N. benthamiana* is normally very amenable to *Agrobacterium*-mediated transient expression (**Supplementary Fig. 2**). In contrast,

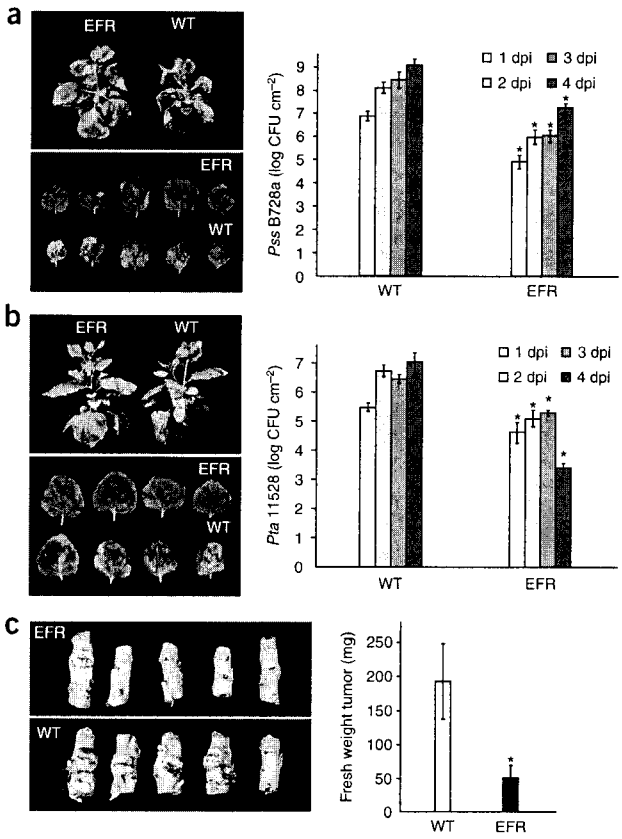

Figure 3 Transgenic expression of EFR in *N. benthamiana* confers broad-spectrum bacterial resistance. (**a,b**) Infection with *Pss* B728a (**a**) and *Pta* 11528 (**b**). We sprayed 4-week-old *N. benthamiana* plants with 10^8 colony-forming units (CFU) of bacteria per milliliter supplemented with 0.06% (vol/vol) Silwet-L77 and photographed them 6 days post-inoculation (dpi). Results are averages ± s.e.m. (n = 4). (**c**) Infection with *A. tumefaciens* A281. We stab-inoculated stems of 4-week-old *N. benthamiana* plants with bacteria that had been cultured on a plate for 2 d. We took pictures (left) and tumor fresh-weight measurements (right) at 3 weeks post-inoculation. Results are averages ± s.e.m. (n = 16). *, $P < 0.05$ using Student's t test. We repeated all experiments at least three times with similar results.

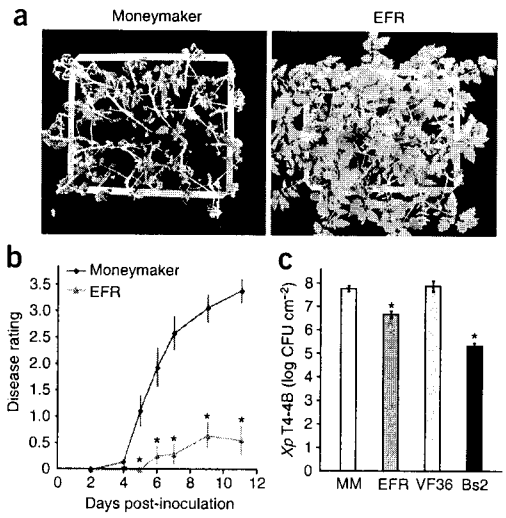

Figure 4 Transgenic expression of EFR in tomato confers broad-spectrum bacterial resistance. (**a**) Wild-type (variety Moneymaker; left) and transgenic EFR (right) tomato plants infected with *R. solanacearum* GMI1000. We drench-inoculated 4-week-old plants with 10^8 CFU ml^{-1} bacteria and photographed them 6 d after inoculation. (**b**) Disease scoring after infection with *R. solanacearum* GMI1000 in wild-type Moneymaker (blue) and transgenic EFR (red) tomato plants. Results are averages ± s.e.m. ($n = 24$). (**c**) Wild-type Moneymaker (MM) or VF36 and transgenic EFR- or Bs2-expressing tomato plants infected with *X. perforans* T4-4B. We dipped 6-week-old tomato plants in bacterial suspension (10^7 CFU ml^{-1} supplemented with 0.008% (vol/vol) Silwet-L77) and counted bacteria 14 d after inoculation. Results are averages ± s.e.m. ($n = 3$). *, $P < 0.05$ using Student's *t*-test. We repeated all experiments at least twice with similar results.

leaves from transgenic *N. benthamiana* plants expressing *EFR* were strongly impaired in the transient expression of a reporter transgene encoding the enzyme β-glucuronidase (GUS; **Supplementary Fig. 2**). Next, we tested whether EF-Tu perception restricts gall formation caused by a virulent tumorigenic *A. tumefaciens* strain. Notably, stab-inoculated stems of transgenic *N. benthamiana* plants expressing *EFR* developed tumors ~75% smaller than the ones present on wild-type stems (**Fig. 3c**).

Ralstonia solanacearum and *Xanthomonas perforans* (previously known as *X. axonopodis* pv. *vesicatoria*) are major pathogens of solanaceous plants, causing bacterial wilt and spot diseases, respectively. Whereas soil drenching with *R. solanacearum* led to massive wilting of wild-type tomato plants, transgenic plants expressing *EFR* showed drastically reduced wilting symptoms (**Fig. 4a,b**). Similarly, transgenic tomato plants expressing *EFR* were more resistant to *X. perforans* than were wild-type strains of tomato plants (**Fig. 4c** and **Supplementary Fig. 3**). Transgenic *EFR* expression conferred resistance to *X. perforans*, albeit to a lesser extent than transgenic expression of the *Bs2* gene from pepper (*Capsicum annuum*) (**Fig. 4c** and **Supplementary Fig. 3**). The *Bs2* gene encodes an R protein that confers strong field resistance via recognition of avrBs2, an effector widely conserved in *X. perforans* isolates[24]. Notably, the resistance conferred by EFR appears less efficient against *X. perforans* than against other bacteria tested (**Figs. 3** and **4**). Overall, these results demonstrate that EF-Tu perception by EFR in transgenic solanaceous plants enhances resistance to adapted foliar and vascular phytopathogenic bacteria.

One problem often associated with the heterologous overexpression of defense-related genes in plants is constitutive activation of defense responses that can lead to necrosis and/or reduced growth[6,7]. Yet sterile and soil-grown transgenic *N. benthamiana* and tomato plants expressing *EFR* did not show any constitutive activation of ROS production (data not shown) or defense-gene expression (**Fig. 2c** and **Supplementary Fig. 4**) and did not show any developmental or growth defects when grown in nonsterile soil over several generations (**Fig. 2a,d**). Consistent with the specificity of EFR for bacterial EF-Tu and the absence of constitutive activation of defense reactions, transgenic plants expressing *EFR* were not more resistant to the fungal pathogen *Verticillium dahliae* (**Supplementary Fig. 5**), which, like *R. solanacearum*, is a vascular phytopathogen. Therefore, transgenic expression of *EFR* does not seem to constitutively activate defense responses under laboratory conditions.

Genetic engineering of new PAMP recognition specificity across plant families offers several advantages over the current alternatives to improving resistance to phytopathogens. Using the plant's own immune system to combat plant diseases should reduce agrochemical inputs and their associated financial, health and environmental costs. In comparison to classical breeding for *R* gene–mediated resistance, transgenic approaches will allow rapid transfer of new PRRs into several elite varieties, as well as into crops that are not amenable to classical breeding (e.g., banana). In contrast to R proteins, a given PRR has the potential to confer resistance to a wide range of pathogens that carry the recognized PAMP. Here, for example, EFR conferred resistance to bacteria belonging to genera as diverse as *Pseudomonas*, *Agrobacterium*, *Xanthomonas* and *Ralstonia*. More importantly, gene-for-gene resistance is often rapidly broken down by evolving pathogens. Given the conserved and essential nature of PAMPs, it may be less likely for pathogens to evolve to evade recognition by PRRs; PAMP mutations are more likely to penalize fitness.

It should nonetheless be noted that rare examples of allelic variation in PAMP genes have been reported. These reflect a virulence strategy used by a small number of successful pathogens to avoid recognition[2,3]. In addition, some virulence effectors from the phytopathogenic bacterium *Pto* DC3000 are known suppressors of PTI in *A. thaliana* and tomato[25]. For example, AvrPto and AvrPtoB directly target PRRs, including FLS2 and EFR[17,26–28]. However, these effectors are not able to fully suppress PTI during infection, as mutations in PRRs enhance susceptibility to virulent bacteria[10,17]. Notably, our results (**Figs. 3** and **4**) suggest that effectors secreted during infection by *Pss* B728a, *Pta* 11528, *A. tumefaciens* A281, *R. solanacearum* GMI1000 or *X. perforans* T4-4B cannot fully suppress the immunity conferred by the transgenic expression of EFR in tomato and *N. benthamiana*. Moreover, because plants and their pathogens engage each other in a constant evolutionary arms race, the possibility of eventual recognition escape or PTI suppression by existing, newly acquired or evolved effectors cannot be discounted. However, the reduced pathogen populations resulting from PTI activation will decrease the probability of new virulent pathogen isolates arising and thus decrease the likelihood that resistance might break down[29].

Ultimately, our finding that PRRs can be transferred from one plant family to another provides a novel biotechnological approach to engineering disease resistance. We propose that combinations of several PRRs, as well as combinations of PRRs with R proteins recognizing widely distributed effectors (for example, Bs2) can be used to enable broad-spectrum disease resistance against multiple genera of plant pathogens with promising potential for durability under field conditions.

METHODS

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

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Note: Supplementary information is available on the Nature Biotechnology website.

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AUTHOR CONTRIBUTIONS
S.L., A.R.-C., E.S., N.P., D.D., H.P.E. and G.R. performed experiments and analyzed data. M.S. generated the transgenic plants. B.S. and B.P.H.J.T. contributed ideas, conceived experiments and analyzed data. J.D.G.J. initiated the project and contributed ideas. C.Z. initiated the project, conceived, designed and performed experiments, analyzed data, obtained funding, and wrote the manuscript. All authors commented on the manuscript prior to submission.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Plant growth. *A. thaliana* ecotype Columbia (Col-0) was the background for all mutant lines used in this study. We grew plants as one plant per pot at 20–21 °C with an 10-h photoperiod or on plates containing MS salts medium (Duchefa), 1% (wt/vol) sucrose and 1% (wt/vol) agar with a 16-h photoperiod. We grew *N. benthamiana* and tomato (*S. lycopersicum*) variety Moneymaker as one plant per pot at 22 °C with a 16-h photoperiod.

Generation of transgenic plants. We transferred the recombinant binary vectors *pGREENII-EFRp::EFR* and *pBIN19-35S::EFR* into *A. tumefaciens* strain Agl1 by electroporation. We generated transgenic *N. benthamiana* plants expressing *pGREENII-EFRp::EFR* as described[30], and transgenic tomato (*S. lycopersicum*) variety Moneymaker plants expressing *pBIN19-35S::EFR* essentially as described[31]. For *N. benthamiana*, we recovered 15 primary transformants after selection on phosphinothricin-containing plates. After transfer to soil, we collected leaf discs collected from individual primary transformant plants and tested them for gain of elf18 responsiveness in the luminal-based oxidative burst assay. Of 15 primary transformants, 9 gained elf18 responsiveness with similar levels. We randomly selected two of these lines and further selected homozygous progeny on the basis of segregation analyses on selection plates. The behavior of these lines was identical in terms of elf18 responsiveness as measured by oxidative burst and seedling growth inhibition. Therefore, we used only one line (no. 6-12-18) at the T4 stage for the final analysis reported in the manuscript.

For tomato, we recovered 20 primary transformants after selection on kanamycin-containing plant plates. After transfer to soil, we collected leaf discs from individual primary transformant plants and tested them for gain of elf18 responsiveness in the luminal-based oxidative burst assay. Of 20 primary transformants, 13 gained elf18 responsiveness with similar levels. In the next generations, we identified homozygous lines using segregation analysis on selection plates and quantitative real-time PCR[32]. We used two homozygous lines (no. P[1]-II7 and no. L[16]-I4) for physiological and pathological characterizations; these behaved similarly in all assays. The results presented in the manuscript are those of no. P[1]-II7.

Bioassays. We performed seedling growth inhibition and oxidative burst assays as previously described[25]. We ordered peptides from Peptron (http://www.peptron.com/).

To prepare bacterial extracts, we grew bacteria from a fresh culture on plates overnight in 50 ml of L medium (10 g l^{-1} bacto-peptone, 5 g l^{-1} yeast extract, 5 g l^{-1} sodium chloride, 1 g l^{-1} D-glucose, pH 7), pelleted them for 10 min, resuspended them in 2 ml sterile water, boiled them for 10 min at 95 °C, pelleted them for 10 min and collected the supernatant. We used 10 μl of bacterial extracts for the oxidative burst assays.

Sequence analysis. We retrieved EF-Tu sequences from UniProtKB (http://www.uniprot.org/help/uniprotkb) and generated the alignment using ClustalW2 (http://www.ebi.ac.uk/) and Boxshade (http://www.ch.embnet.org/) with default settings. We generated the consensus elf18 peptide using WebLogo (http://weblogo.berkeley.edu/).

Gene expression. We treated 2-week-old plants grown in liquid MS 1% (wt/vol) sucrose medium with 100 nM elf18 or flg22 for 0, 30, 60 or 180 min and froze them in liquid nitrogen. We extracted total RNA from seedlings with a RNeasy Plant Mini kit (Qiagen). We treated RNA samples with DNase Turbo DNA-free (Ambion), quantified with a Nanodrop spectrophotometer (Thermo Scientific) and reverse-transcribed 2 μg of total RNA into complementary DNA (cDNA) with SuperScript II reverse transcriptase (Invitrogen). We used 1 μl of cDNA in PCR under the following conditions: 95 °C, 2 min; (95 °C, 45 s; 58 °C, 45 s; 55 °C, 30 s; 72 °C, 1.5 min) × 25 cycles; 72 °C, 5 min.

We used the following primers: *CYP71D20*, 5′-AAGGTCCACCGCACCATGTCCTTAGAG-3′ and 5′-AAGAATTCCTTGCCCCTTGAGTACTTGC-3′; *FLS2*, 5′-AAGGATCCTGTGACTTGAAGCCTTCAA-3′ and 5′-AAGAATTCATTGGTAATTCATCAGCTCCTGTAA-3′; *ACRE132*, 5′-AAGGTCCAGCGAAGTCTCTGAGGGTGA-3′ and 5′- AAGAATTCCAATCCTAGCTCTGGCTCCTG-3′; *WRKY22*, 5′- AAGGTCCGGGATCTACATGCGGTGGT-3′ and 5′- AAGAATTCCGGGTCGGATCTATTTCG-3′; *PR1acidic*, 5′-TAGTCATGGGATTTGTTCTC-3′ and 5′-TCAGATCATACATCAAGCTG-3′; *EF-1α*, 5′-AAGGTCCAGTATGCCTGGGTGCTTGAC-3′ and 5′-AAGAATTCACAGGGACAGTTCCAATACCAC-3′.

We used the constitutively expressed *EF-1α* housekeeping gene as a loading control.

Disease assays. For infections with *Pss* B728a and *Pta* 11528, we sprayed four 4-week-old *N. benthamiana* plants with 10^8 CFU ml^{-1} bacteria in water supplemented with 0.06% (vol/vol) Silwet-L77. The bacterium inoculum was prepared from an overnight culture at 28 °C in King's B medium (20 g l^{-1} bacto-peptone, 1.5 g l^{-1} dipotassium hydrogen orthophosphate, 1% (vol/vol) glycerol, pH 7) started from a fresh culture on an agar plate. After spraying, we left plants uncovered for the duration of the experiment. We described bacterial populations by growth curve analysis as described[13].

For infections with *A. tumefaciens* A281, we stab-inoculated stems of 16 4-week-old *N. benthamiana* plants with a plastic tip that had been dipped into a fresh culture on an L-medium plate. We assessed tumor formation 3 weeks after inoculation by measuring the fresh weight of outgrowing tumors that were excised from the stem with a scalpel. We performed *Agrobacterium*-mediated transient expression with *pBIN19-35S::GUS:HA* as described[25].

For infections with *R. solanacearum* GMI1000, we transferred 4-week-old tomato plants into the inoculation facility (28 °C, 16-h photoperiod), grew bacteria in rich B medium (10 g l^{-1} bacto-peptone, 1 g l^{-1} yeast extract, 1 g l^{-1} casamino acids) and soil-drenched each tomato plant with 50 ml of 10^8 CFU ml^{-1}. We performed disease scoring daily using a visual index in which the numbers 1, 2, 3 and 4 correspond to 25%, 50%, 75% and 100% wilted leaves, respectively.

For infections with *X. perforans* T4-4B, we dipped 6-week-old tomato plants for 30 s into 10^7 CFU ml^{-1} bacteria in water supplemented with 0.008% (vol/vol) Silwett-L77. The bacterium inoculum was prepared from an overnight culture at 28 °C in NYGB medium (5 g l^{-1} bacto-peptone, 3 g l^{-1} yeast extract, 2% (vol/vol) glycerol, 1 mM magnesium chloride) started from a fresh culture on an agar plate. After dipping, we covered plants with plastic bags and returned them to the growth chamber for 3 d, then left them uncovered for the duration of the experiment. We described bacterial populations using growth curve analysis by grinding 1-cm^2 leaf samples in water and plating appropriate dilutions on NYGB supplemented with rifampicin 100 μg ml^{-1} to select for *X. perforans* T4-4B and cycloheximide 50 μg ml^{-1} to prevent fungal growth.

For infections with *V. dahliae*, we inoculated 10-day-old tomato plants or 4-week-old *N. benthamiana* plants with *V. dahliae* race JR2 (10^6 conidiospores per milliliter) by uprooting and subsequent root-dip inoculation, as described[33]. As a control, we mock-inoculated plants with water.

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