in the long run. However, migration rate in our experiment was density independent and migration was confined to the two nearest neighbors, whereas it is known that the dynamics of a metapopulation can vary depending on the exact form of density dependence (3J) and scheme of migration (11). Moreover, growth rates of Drosophila (and most insects, microbes, and fishes) are higher than those of mammals and birds, which are generally of greater concern for conservation. The intrinsic growth rates of subpopulations are also known to interact strongly with migration rate in producing observed metapopulation dynamics (12). Therefore, due caution should be exercised when extrapolating our results to natural populations.

References and Notes
8. J. Ripa, Oikos 89, 175 (2000).
20. In the experiment, the nine subpopulations (single-vial Drosophila cultures) were arranged on the periphery of a circle, with each vial exchanging migrants only with its two nearest neighbors (i.e., a one-dimensional array with periodic boundary conditions). In nature, such metapopulations might exist, for example, on the shorelines of lakes or along the edges of ecosystems. The simulations mimicked the experimental setup and focused on the behavior of nine coupled Ricker maps, with periodic boundary conditions. See supporting material on Science Online.
22. We define a population whose size fluctuates with higher amplitude across time to be less stable than one that has lower amplitude of fluctuation (‘‘constancy’’ attribute of stability (22)). We introduce a measure of stability, the fluctuation index (FI) of a series, given as

\[
FI = \frac{1}{N} \sum_{t=1}^{T} abs(N_{t+1} - N_{t})
\]

where \(N_t\) is the mean population size over \(T\) generations. FI thus reflects the mean one-step change in population size, scaled by average population size, over the study duration.
25. In the experiment, a subpopulation was deemed extinct when it did not contain at least one male and one female fly. When a CM subpopulation became extinct, it was restarted using four males and four females from a backup vial. The backup vials were maintained in parallel to the three treatments, under high larval crowding and yeast supplement to the adults. There were no restarts in LMMs or HMMs; extinct subpopulation vials remained empty until recognized by migrants from a neighboring subpopulation.
28. For example, in simulations with \(r = 2.8\), comparable to the estimated \(r\) in our CM subpopulations, the mean extinction rate was 0.75 out of 9 subpopulations per generation, versus 3.35 out of 9 for experimental QMs.
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A Plant miRNA Contributes to Antibacterial Resistance by Repressing Auxin Signaling

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Plants and animals activate defenses after perceiving pathogen-associated molecular patterns (PAMPs) such as bacterial flagellin. In Arabidopsis, perception of flagellin increases resistance to the bacterium Pseudomonas syringae, although the molecular mechanisms involved remain elusive. Here, we show that a flagellin-derived peptide induces a plant microRNA (miRNA) that negatively regulates messenger RNAs for the F-box auxin receptors TIR1, AFB2, and AFB3. Repression of auxin signaling restricts P. syringae growth, implicating auxin in disease susceptibility and miRNA-mediated suppression of auxin signaling in resistance.

Plants perceive a 22–amino acid peptide (flg22) from the N terminus of eubacterial flagellin (1). In Arabidopsis, flg22 triggers rapid changes in transcript levels, including down-regulation of a gene subset, potentially by posttranscriptional mechanisms (2). One posttranscriptional mechanism is RNA silencing, a sequence-specific mRNA degradation process mediated by 20- to 24-nucleotide (nt) RNAs known as short interfering RNAs (siRNAs) and microRNAs (miRNAs). Both are made from double-stranded RNA (dsRNA) by the ribonuclease III enzyme Dicer. Four paralogs (Dicer-like, or DCLs) are found in Arabidopsis. DCL2 produces viral-derived siRNAs (3) and siRNAs from antisense overlapping transcripts (4). DCL3 generates DNA repeat–associated siRNAs (3), whereas DCL4 synthesizes trans-acting siRNAs and mediates RNA interference (5–7). DCL1 excises miRNAs from intergenic stem-loop transcripts to promote cleavage of cellular transcripts carrying miRNA-complementary sequences (8).

We examined whether small RNAs—especially miRNAs—contribute to the rapid changes elicited by flg22. We analyzed transgenic Arabidopsis expressing the P1-He-Pro, P19, and P15 viral proteins that suppress miRNA- and siRNA-guided functions (9, 10), anticipating that transcripts repressed by flg22-stimulated small RNAs would be more abundant in these lines. Comparative transcript profiling of untreated

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transgenic seedlings and flg22-elicited wild-type seedlings identified a subset of mRNAs that fulfilled this criterion, including TIR1 (Transport Inhibitor Response 1) and two of its three functional paralogs, AFB2 and AFB3 (Auxin signaling F-Box proteins 2 and 3) (fig. S1, A and B). However, accumulation of AFB1, the third TIR1 paralog, was not discernibly altered in the suppressor lines (fig. S1B).

The F-box proteins TIR1, AFB1, AFB2, and AFB3 are receptors for the plant hormone auxin (11–13). Additionally, TIR1 and AFB transcripts are targets of miR393, a conserved miRNA (fig. S2) (14, 15). A modified rapid amplification of cDNA ends (RACE) assay confirmed that TIR1, AFB2, and AFB3 mRNAs are specifically cleaved by miR393 in a DCL1-dependent manner (Fig. 1A and fig. S3). However, polymerase chain reaction (PCR)-amplified cleavage products derived from AFB1 were hardly detectable and rarely cloned (Fig. 1A and fig. S3), confirming that AFB1 is partially resistant to miR393-directed cleavage. Presumably, this is due to a single mismatch in the miR393 complementary site found specifically in AFB1 mRNA (fig. S3).

Quantitative reverse transcription–polymerase chain reaction (RT-qPCR) analyses with primers flanking the miR393 target sites revealed a two- to threefold reduction in the levels of TIR1, AFB1, AFB2, and AFB3 30 min after flg22 elicitation of wild-type seedlings (Fig. 1B). Repression of TIR1, AFB2, and AFB3 was partially compromised in dcl1-9 seedlings (Fig. 1B), suggesting that a miRNA-directed pathway, probably involving miR393, contributes to TIR1, AFB2, and AFB3 down-regulation. However, flg22-triggered down-regulation of AFB1 was unaffected in dcl1-9, in agreement with its reduced sensitivity to miR393 (Fig. 1A and fig. S3). Therefore, repression of AFB1 is miRNA-independent and may exclusively occur at the translational level.

We analyzed miR393 levels in flg22-elicited Arabidopsis seedlings. Northern analysis showed a twofold increase in miR393 accumulation after 20 and 60 min, whereas levels of the unrelated miR171 remained unaltered (Fig. 2A). Similarly, miR393 levels were unaltered in seedlings treated with control flg22Aa-tum inactive peptide (Fig. 2A) (1). The Arabidopsis genome contains two miR393 precursors, on chromosomes 2 (At-miR393a) and 3 (At-miR393b) (16). We fused 1.5-kb DNA fragments upstream of At-miR393a and At-miR393b to the enhanced Green Fluorescence Protein (eGFP) and transformed them into Arabidopsis, producing transgenic lines AtmiR393a-p:eGFP and AtmiR393b-p:eGFP. Using RT-qPCR, we observed a twofold increase in eGFP mRNA level in three independent flg22-treated AtmiR393a-p:eGFP lines (Fig. 2B), consistent with the miR393 Northern analysis (Fig. 2A). However, eGFP levels were unaltered in three independent At-miR393b-p:eGFP-transgenic lines (Fig. 2B). These data suggest that up-regulation of miR393 by flg22 results from enhanced transcription of At-miR393a.

To assay TIR1 protein levels after flg22 treatment, we used Arabidopsis transformants expressing a Myc epitope–tagged TIR1 under the dexamethasone (Dex)-inducible promoter (Dex::TIR1-Myc) (17). Western blotting revealed a rapid reduction in TIR1-Myc levels upon flg22, but not flg22Aa-tum, treatment (Fig. 3A). Because TIR1 is part of the ubiquitin-ligase complex SCFTIR1 that interacts with Aux/IAA proteins to promote their degradation (17), we investigated whether Aux/IAA proteins became stabilized upon flg22 treatment. We used transgenic lines expressing a...
heat shock–inducible AXR3/IAA17 protein fused to the β-glucuronidase (GUS) reporter (HS::AXR3NT-GUS) (17). Seedlings were treated with either flg22 or flg22A.tum for 2 hours at 37°C and then stained for GUS. Flg22, but not flg22A.tum, triggered stabilization of AXR3NT-GUS (Fig. 3B), starting from 1.5 hours after flg22 elicitation (Fig. 3C), which coincided with the TIR1-Myc repression (Fig. 3A).

Aux/IAA proteins repress auxin signaling through heterodimerization with Auxin Response Factors (ARFs) (18). Those transcription factors ARFs bind to auxin-responsive elements (AuxREs) in promoters of primary auxin-response genes and activate (or repress) transcription (19). The flg22-induced stabilization of AXR3/IAA17 prompted us to determine whether flg22 inhibits auxin-response gene activation. Seedlings were treated for 1.5 hours with either flg22 or flg22A.tum, and transcripts of the primary auxin-response genes GH3-like, BDL/IAA12, and AXR3/IAA17 were monitored by RT-qPCR. This time point was chosen on the basis of the flg22-induced stabilization profile of AXR3/IAA17 (Fig. 3C). All three auxin-response genes were repressed at this time point (Fig. 3D). Thus, flg22 triggers events that contribute to rapid down-regulation of primary auxin-response genes.

Does repression of auxin signaling enhance bacterial disease resistance? We tested, in a tir1-1 mutant background, resistance in Arabidopsis transgenic lines that constitutively overexpress Myc epitope–tagged AFB1 (20). These lines contain higher levels of AFB1 mRNA as compared with tir1-1, and exhibit high AFB1-Myc protein accumulation (Fig. 4A). The partial resistance of AFB1 mRNA to miR393, together with its constitutive overexpression, suggested that both AFB1 mRNA and AFB1-Myc would remain unaffected by flg22 treatment, which was confirmed in semiquantitative RT-PCR and Western analysis (Fig. 4, B and C). We anticipated that constitutive overexpression of AFB1 would prevent the miR393-mediated suppression of auxin signaling, perhaps resulting in enhanced disease sensitivity.

**Fig. 3.** Flg22 triggers repression of TIR1 protein, stabilization of AXR3/IAA17, and repression of three primary auxin-response transcripts. (A) TIR1-Myc repression in response to flg22. (Upper panel) Western analysis using a Myc-specific antibody (anti-Myc). (Bottom panel) Ponceau staining of total proteins. Similar results were obtained in two independent experiments. (B) AXR3/IAA17 stabilization in leaves. HS::AXR3NT-GUS seedlings were transferred for 2 hours at 37°C with either 10 μM flg22 (right leaf) or 10 μM flg22A.tum (left leaf). Similar results were obtained in five independent experiments. (C) Kinetics of AXR3/IAA17 stabilization. HS::AXR3NT-GUS seedlings were treated as in (B). (Upper panel) Western analysis using anti-GUS. (Bottom panel) Ponceau staining of total proteins. Similar results were obtained in two independent experiments. (D) Flg22 reduces accumulation of three primary auxin-response genes. Col-0 seedlings were treated for 1.5 hours with either 10 μM flg22 (+) or 10 μM flg22A.tum (−). qRT-PCR were done to assess the relative mRNA level of GH3-like (At4g03400), BDL/IAA12 (At1g04550), and AXR3/IAA17 (At1g04250) upon flg22 as compared with flg22A.tum treatment. Error bars represent the standard deviation from four PCR results, and similar results were obtained in three independent experiments.

When AFB1-Myc–overexpressing plants were inoculated with virulent P. syringae pv. tomato (Pto) DC3000, they had bacterial titers that were about 20-fold higher than those of nontransformed or tir1-1 plants and they displayed enhanced disease symptoms (Fig. 4D, fig. S4A). Furthermore, no difference was observed with avirulent Pto DC3000 carrying AavrRpt2, which triggers race-specific resistance via resistance gene RPS2 (Fig. 4E) (21). Thus, suppression of auxin signaling, mediated at least partly by miR393, might specifically promote resistance to virulent Pto DC3000 but is not implicated in race-specific resistance.

We also transformed Arabidopsis with a construct with At-miR393a constitutively transcribed from the strong 35S promoter (Fig. 4F). Three independent T2 transgenic lines were selected for high miR393 accumulation (Fig. 4F). These lines exhibited lower TIR1 mRNA levels than controls (Fig. 4F), and in older plants, they displayed reduced apical dominance with multiple shoots, reminiscent of auxin signaling mutants. However, these developmental alterations were minor compared with those observed in tir1/afb multiple mutants (20). Four days after inoculation with virulent Pto DC3000, all three miR393-overexpressing lines, but not the controls, displayed fivefold lower bacterial titer, confirming that miR393 restricts Pto DC3000 growth (Fig. 4G). There was no difference in bacterial growth on transgenic lines overexpressing an artificial miRNA directed against GFP (22) (Fig. 4G).

We show here that a bacterial PAMP down-regulates auxin signaling in Arabidopsis by targeting auxin receptor transcripts. Augmenting auxin signaling through over-expressing a TIR1 paralog that is partially refractory to miR393 enhances susceptibility to virulent Pto DC3000, and, conversely, repressing auxin signaling through miR393 overexpression increases bacterial resistance. These results indicate that down-regulation of auxin signaling, resulting in ARF inactivation, is part of a plant-induced immune response (fig. S5). They also suggest that auxin promotes susceptibility to bacterial disease. This is consistent with other published findings. The tumorigenic P. syringae pv. savastanoi synthesizes high levels of indole-3-acetic acid (IAA) (23). Also, most P. syringae strains can produce IAA, and Pto DC3000 infection triggers increased IAA levels in Arabidopsis (24, 25). Consistent with these observations, exogenous application of the auxin analog 2,4-dichlorophenoxyacetic acid enhances Pto DC3000 disease symptoms (fig. S4B).

In addition to the contribution of miR393 in flg22-triggered repression of auxin signaling, our analysis also reveals a miR393-
miRNAs. on miR393 hold for other stress-induced involved in development, and it will previously been reported for plant miRNAs elicitation. This regulatory feature has not transcriptional repression provoked during tation and thus confer robustness to the TIR1 mRNAs present at the time of elicitation of miR393 by flg22 might deplete miRNA-producing cells (26). Rapid induction of miR393 by flg22 might deplete TIR1 mRNAs present at the time of elicitation and thus confer robustness to the transcriptional repression provoked during elicitation. This regulatory feature has not previously been reported for plant miRNAs involved in development, and it will be interesting to establish if these observations on miR393 hold for other stress-induced miRNAs.

References and Notes
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Erratum for the Report “A plant miRNA contributes to antibacterial resistance by repressing auxin signaling” by L. Navarro, P. Dunoyer, F. Jay, B. Arnold, N. Dharmasiri, M. Estelle, O. Voinnet, J. D. G. Jones

In the Report “A plant miRNA contributes to antibacterial resistance by repressing auxin signaling,” a loading control panel in Fig. 2A was incorrectly assembled. The authors have retrieved the original small RNA blots with their cognate rRNA loading controls. The original data show that the Report's biological conclusion (miR393 is induced in response to the active flagellin-derived peptide flg22) remains valid. Patrice Dunoyer, who assembled Fig. 2A, takes full responsibility for this mistake. The corrected figure panel and caption follow.

Northern analysis of miR393 (left panels) and miR171 (right panels) upon treatment with Flg22 (upper panels) or Flg22A.tum (bottom panels). Col-0 seedlings were treated with either 10 μM Flg22 or 10 μM Flg22A.tum. The same membrane was first probed with a miR393 probe (left panels) and subsequently stripped and reprobed with a miR171 probe (right panels). rRNA, ethidium bromide staining of ribosomal RNA; R, miRNA signal ratio between Flg22-treated versus Flg22A.tum-treated samples at each time point.
A Plant miRNA Contributes to Antibacterial Resistance by Repressing Auxin Signaling
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