central regulator of the plant immune system, largely uncharacterized in the root, directly influences root microbiome composition. Our results could open new avenues for modulating the root microbiome to enhance crop production and sustainability.

Note added in proof: Figure 1 was revised since this paper's original publication in *Science* Express.

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SUPPLEMENTARY MATERIALS

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PARASITIC PLANTS

Probing strigolactone receptors in *Striga hermonthica* with fluorescence

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Elucidating the signaling mechanism of strigolactones has been the key to controlling the devastating problem caused by the parasitic plant *Striga hermonthica*. To overcome the genetic intractability that has previously interfered with identification of the strigolactone receptor, we developed a fluorescence turn-on probe, Yoshimulactone Green (YLG), which activates strigolactone signaling and illuminates signal perception by the strigolactone receptors. Here we describe how strigolactones bind to and act via *ShHTL*s, the diverged family of α/β hydrolase-fold proteins in *Striga*. Live imaging using YLGs revealed that a dynamic wavelike propagation of strigolactone perception wakes up *Striga* seeds. We conclude that *ShHTL*s function as the strigolactone receptors mediating seed germination in *Striga*. Our findings enable access to strigolactone receptors and observation of the regulatory dynamics for strigolactone signal transduction in *Striga*.

Damages caused by the parasitic plant *Striga hermonthica* threaten food security in Africa. Infection of harvests by *Striga* leads to the loss of $10 billion U.S. dollars' worth of crops from the continent every year (*1*). Strigol and related strigolactones (*2*, *3*) derived from the host plants stimulate the germination of *Striga* by regulating the biosynthesis of plant hormones, including abscisic acid, gibberellins, and ethylene (*4*–*6*). So far, 17 strigolactones have been identified, which are unique according to the plant species (*7*–*9*). *Striga* recognizes host plants by sensing their particular strigolactone composition (*10*). However, the mechanism of how *Striga* senses minute amounts of structurally diverse strigolactones to identify their host targets remains unclear. Here we report the identification of the strigolactone receptor in *Striga*.

Strigolactones also function as plant hormones and as ecological signals for communicating with microbes (*11*–*13*). Genetic studies in model plants, including rice, *Arabidopsis*, and petunia, have led to identification of a group of α/β hydrolase-fold proteins as presumptive receptors for strigolactones (*14*–*17*). The unidentified strigolactone receptor in *Striga* may have a similar ligand selectivity to AtDWARF14 (AtD14), the strigolactone receptor in *Arabidopsis*, because AtD14 is also known to perceive natural and synthetic stimulants for *Striga* germination (*16*–*19*). However, *AtD14* regulates plant architecture, including shoot branching and root development, that has no obvious resemblance to *Striga* germination (*20*, *21*). In contrast, its homolog, *HYPOSENSITIVE TO LIGHT* (*AtHTL*)/*KARRIKIN INSENSITIVE2* (*KAI2*) is involved in seed germination stimulated by smoke-derived karrikins, a collection of imide-based agonists and non-natural stereoisomers of strigolactones in *Arabidopsis* (*16*, *22*, *23*). Therefore, the strigolactone receptors in *Striga* may have a comparable role to *AtHTL*, with ligand preferences similar to those of *AtD14* (fig. S1). On the other hand, the signaling processes of these homologs are highly related. Both AtD14 and AtHTL are considered to share an F-box protein, MORE AXILLARY GROWTH2 (AtMAX2), which directs their specific negative regulators to undergo ubiquitin-dependent proteasomal degradation (*24*, *25*). The ortholog of *AtMAX2* in *Striga* (*ShMAX2*) plays a role in regulating shoot branching and seed germination when expressed in *Arabidopsis*, thus suggesting that the signaling processes involving the F-box protein are conserved in *Striga* (*26*). Altogether, we hypothesized that *Striga* carries orthologs of either *AtD14* or *AtHTL* that have acquired new functions during the evolution of parasitism to respond to natural strigolactones and stimulate germination.

Here we report the use of small-molecule tools to probe the function of strigolactone receptors. AtD14 hydrolyzes strigolactones into the ABC-ring and D-ring fragments during the signaling process (fig. S2) (*15*). We applied this reaction to

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develop a fluorogenic agonist for AtD14-type strigolactone receptors, Yoshimulactone Green (YLG). Fluorogenic substrates for hydrolyzing enzymes, such as protease, glycosidase, and phosphatase, have been widely used to track enzymatic activity (*27*). We designed YLG to be recognized by strigolactone receptors, with subsequent hydrolysis leading to the generation of fluorescent products (Fig. 1A and fig. S2). We proved this principle in several ways. Initially, we observed that recombinant AtD14 protein hydrolyzed YLG into fluorescein and the D-ring moiety in vitro. The increase in fluorescence with time and concentration showed a Michaelis constant (K_m^{YLG}) value at 0.63 μM and a catalytic cycle of 4.4 min per reaction (Fig. 1B and fig. S3). In contrast, YLG was poorly hydrolyzed by recombinant AtHTL protein, a characteristic shared with strigolactones, which selectively function through AtD14 (fig. S3) (*23*). YLG shares the binding pocket on AtD14 with physiologically active strigolactones, as indicated by the competition of hydrolysis of YLG alongside synthetic strigolactone, GR24, or natural strigolactones. Among the compounds investigated, (+)-5-deoxystrigol (5DS) displayed the strongest median inhibitory concentration (IC_{50}) value of 0.44 μM (Fig. 1C). This YLG competition assay reflects the binding specificity of the receptor, because the physiologically inactive analog, carba-GR24, failed to compete with YLG on hydrolysis by AtD14 (Fig. 1C) (*28*). We next found that treatment of YLG with wild-type *Arabidopsis* resulted in fluorescence in primary and lateral roots, where strigolactones have been reported to act on the growth or formation (Fig. 1D and fig. S4) (*21*); such fluorescence was absent in the *Atd14-1* loss-of-function mutant. As a third observation, we found that YLG restored shoot branching in the strigolactone biosynthetic mutant *max4-1* (Fig. 1E) (*29*). Together, these results led us to conclude that YLG works as an in vitro and in vivo fluorogenic agonist for *AtD14* in *Arabidopsis*.

YLG stimulated *Striga* germination, and subsequently 97% of germinating seeds emitted fluorescence (n = 229, Fig. 2A and fig. S5). Thus, YLG functions as a fluorogenic agonist in *Striga*, which cleaves the ligand as it is perceived. By searching a public *Striga* RNA-seq database, we

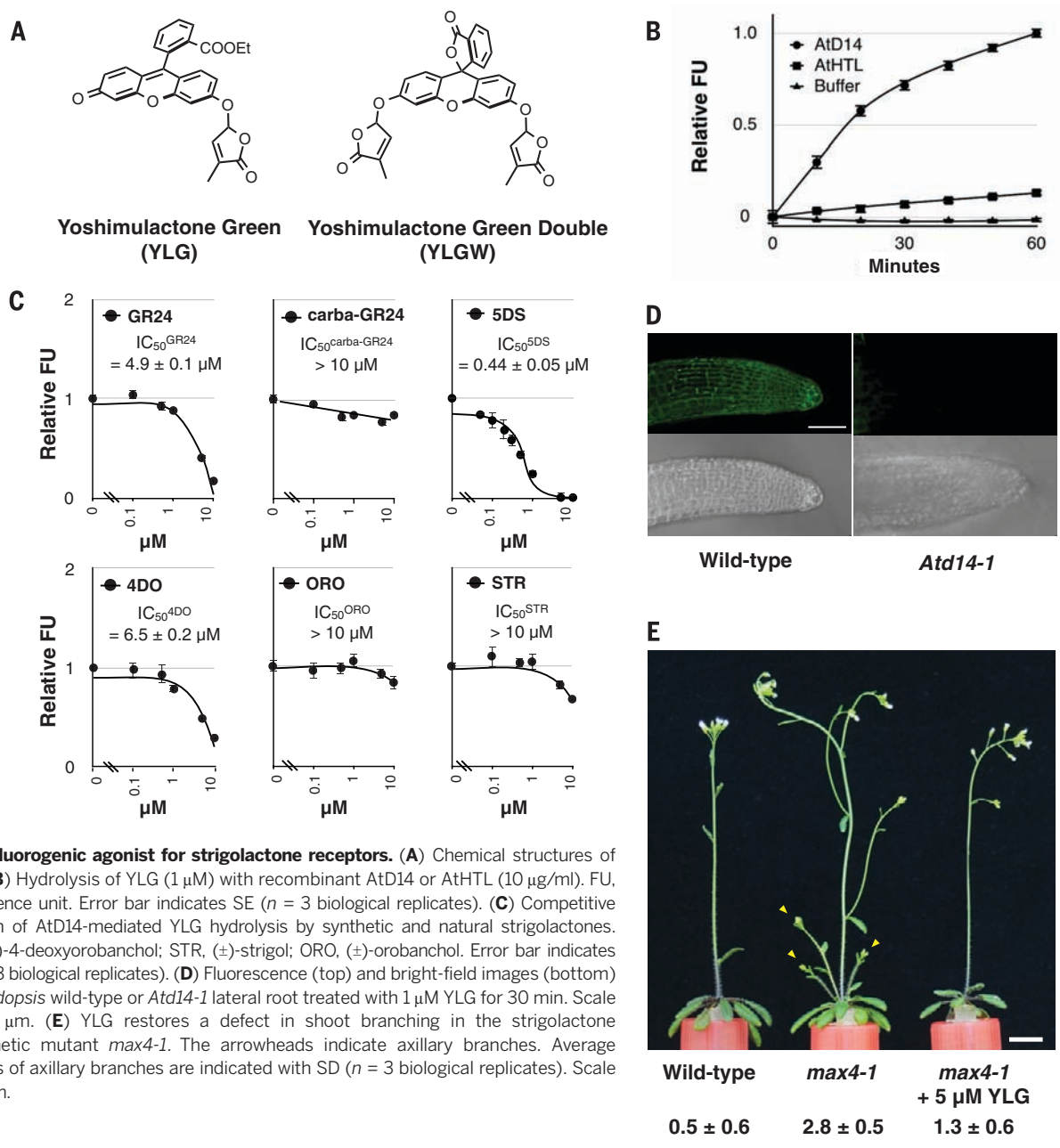

Fig. 1. Fluorogenic agonist for strigolactone receptors. (**A**) Chemical structures of YLGs. (**B**) Hydrolysis of YLG (1 μM) with recombinant AtD14 or AtHTL (10 μg/ml). FU, fluorescence unit. Error bar indicates SE (n = 3 biological replicates). (**C**) Competitive inhibition of AtD14-mediated YLG hydrolysis by synthetic and natural strigolactones. 4DO, (+)-4-deoxyorobanchol; STR, (±)-strigol; ORO, (±)-orobanchol. Error bar indicates SE (n = 3 biological replicates). (**D**) Fluorescence (top) and bright-field images (bottom) of *Arabidopsis* wild-type or *Atd14-1* lateral root treated with 1 μM YLG for 30 min. Scale bar, 50 μm. (**E**) YLG restores a defect in shoot branching in the strigolactone biosynthetic mutant *max4-1*. The arrowheads indicate axillary branches. Average numbers of axillary branches are indicated with SD (n = 3 biological replicates). Scale bar, 1 cm.

identified 12 genes as candidate receptors with sequences related to *AtD14* and *AtHTL: ShD14* and *ShHTL1* to *ShHTL11* (Fig. 2B and fig. S5). All 12 proteins have a conserved catalytic triad in α/β hydrolase, although only 10 recombinant proteins (*ShHTL2* to *-11*) hydrolyzed YLG and GR24 (Fig. 2C and figs. S5 and S6). To test the function of YLG in seed germination, we expressed *ShHTL7* in the *Arabidopsis Athtl-3* mutant, which is defective in germination (*16*). YLG and GR24 stimulated germination in the transgenic lines but not in the parental *Athtl-3*, indicating that *ShHTL7* is a functional strigolactone receptor that supports germination in *Arabidopsis* (Fig. 2D).

We next evaluated the relevance of hydrolysis in the evolution of parasitism (Fig. 2 and fig. S5). Phylogenetic analysis showed that the 10 genes in the YLG hydrolysis trait (*ShHTL2* to *-11*) coincide with a *Striga*-characteristic gene cluster extended from *AtHTL,* which suggests that these genes function in seed germination and subsequently acquire YLG hydrolytic activity (Fig. 2B). Figure 2C shows that a subgroup including *ShHTL4* to *-11* binds to natural strigolactones with moderate to high affinity. Moreover, the expression of this subgroup was induced by the potentiating treatment of seed conditioning, which may coordinate receptors to perceive strigolactones for germination (fig. S5). Of this subgroup, *ShHTL6* and *ShHTL7* showed indiscriminately high affinity to all of the strigolactones tested, whereas others favor particular strigolactones (Fig. 2C). Thus, from a few promiscuous receptors, multiple specialized receptors seem to have evolved to detect structurally diverse strigolactones more efficiently. This would have led to each different strigolactone being perceived by a different combination of receptors. Among the strigolactones that we tested, 5DS showed high-affinity binding (IC50 < 1 μM) to most of the receptors, which is consistent with its potency in stimulating the germination of *Striga* (fig. S7). This result may explain why 5DS-producing cultivars of some economically important crops such as sorghum, maize, and millet are susceptible to *Striga* (*10*, *30*). It is noteworthy that the strain we used was harvested from *Striga* growing on sorghum. It is possible that other strigolactones than 5DS may act as high-affinity ligands for the receptors in the locally propagated *Striga* population, which have adapted to different hosts.

The catalytically activated fluorescence in YLG allows us to track signal perception by the strigolactone receptors in intact *Striga* seeds.

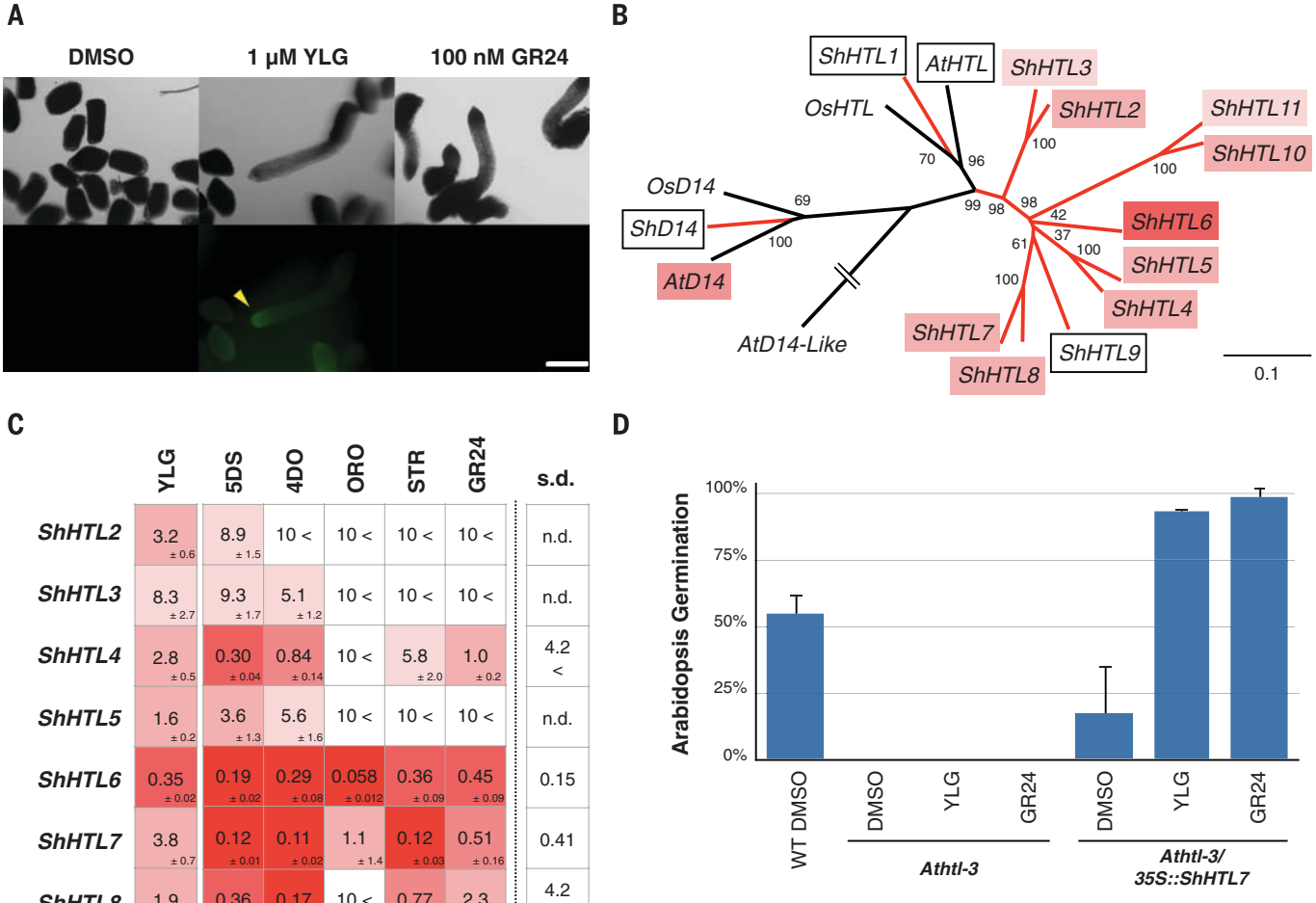

Fig. 2. Identification of strigolactone receptors in *Striga*. (**A**) YLG stimulates *Striga* seed germination with simultaneous release of the fluorophore. Top, bright-field image; bottom, fluorescence image. The arrowhead indicates the root tip. Scale bar, 300 μm. (**B**) Phylogenetic tree for strigolactone receptors in *Striga* (red line) as well as *Arabidopsis* and rice (black lines). The colors correspond to the heat map for the K_m^{YLG} values in (C). Bootstrap values of 1000 replicates are indicated by each node. (**C**) Biochemical parameters for the interactions of YLG, GR24, and natural strigolactones with *ShHTL*s. K_m^{YLG} and IC_{50} values for strigolactones are presented as a heat map with SD (n = 3 biological replicates). The variations among the IC_{50} values of strigolactones on each *ShHTL* are indicated in the right column as SD. (**D**) YLG and GR24 stimulate germination in *Arabidopsis Athtl-3* expressing *ShHTL7* under a 35S promoter. The error bar indicates SD (n = 3 independent lines).

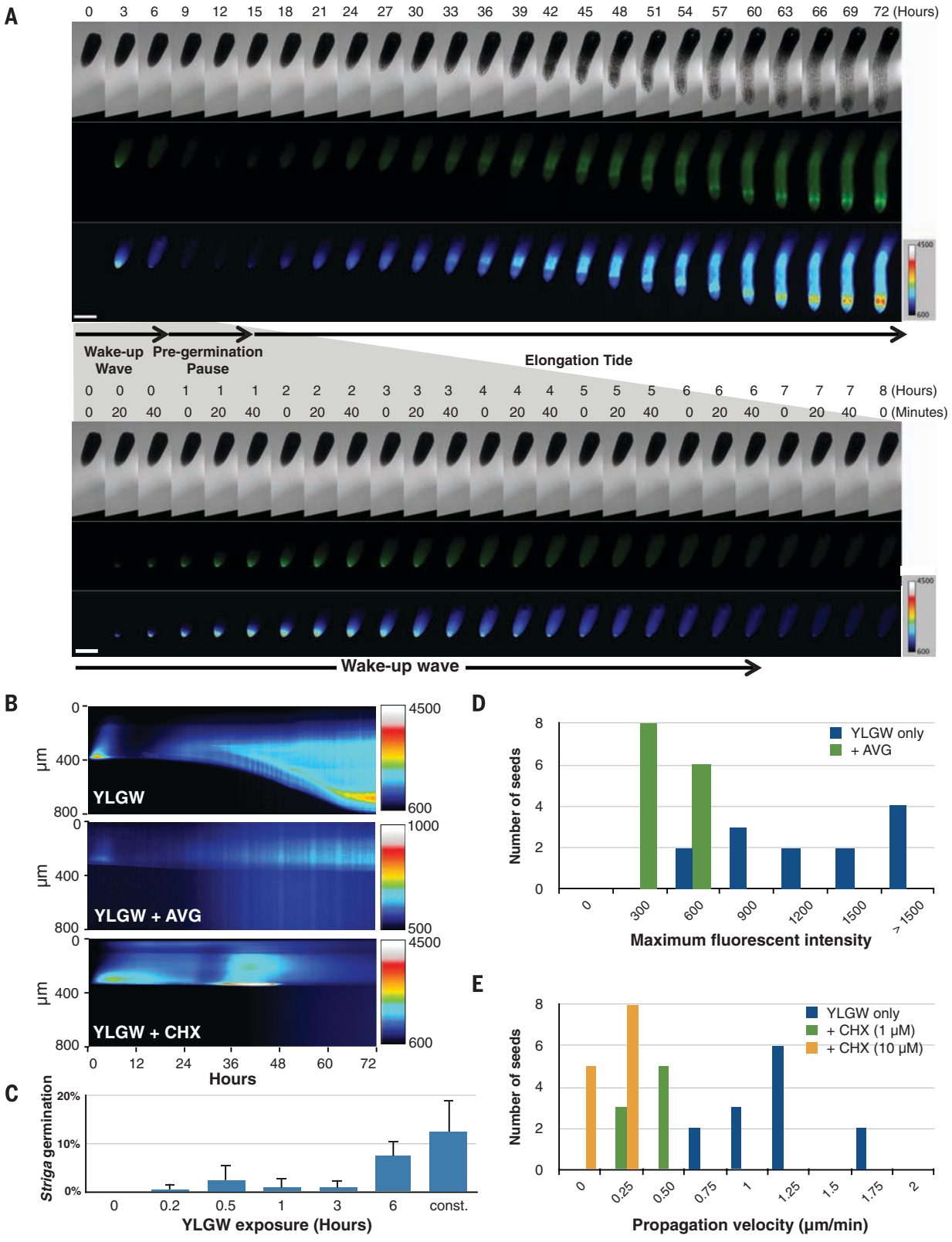

Fig. 3. Perception dynamics of strigolactone receptors during seed germination in *Striga*. (**A**) Time series of fluorescence images on the germinating *Striga* seed treated with YLGW (200 pmol) every 3 hours up to 72 hours or every 20 min up to 8 hours. Scale bar, 200 µm. (**B**) Kymographic analysis of YLGW-induced *Striga* germination in the presence of inhibitors. The detail of the kymograph is explained in the supplementary information. (**C**) Required period of YLGW exposure for *Striga* germination. *Striga* seeds were treated with YLGW for the indicated period (hours). Const., constantly exposed to YLGW. The error bar indicates SD (n = 3 biological replicates). (**D**) Statistical analysis for the effect of AVG on maximum fluorescent intensity (gray value) during germination. (**E**) Statistical analysis for the effect of CHX on the first wave. CHX slowed the propagation of the wake-up wave.

For this purpose, we improved the on/off ratio of YLG by attaching another D-ring to fluorescein (YLGW, Fig. 1A). This modification reduced the potency and selectivity toward strigolactone receptors but improved signal resolution and stability. We used YLGW to visualize their response at 5-min intervals over 3 days (Fig. 3A, movie S1, and figs. S8 and S9). Within 20 min of YLGW application, fluorescence appeared at the root tip of the *Striga* embryo. The fluorescence diffused toward the cotyledon over 6 hours (the wake-up wave) and then disappeared (the pregermination pause). The loss of fluorescence may arise from leakage of the fluorescent dye produced, and it indicates a reduction of hydrolytic activity within the cell. Morphological signs of germination (root elongation) accompanied the second fluorescence wave from the root tip (the elongation tide). All germinating embryos (n = 13) followed these three stages, although with varying kinetics (fig. S9). The fluorescence dynamics depended on the hydrolysis of YLGW, because GR24 treatment alone did not generate fluorescence (movie S2 and fig. S9). The dynamics are also linked to germination, because the nonconditioned embryo showed nonspecific fluorescence over the entire embryo (movie S3 and fig. S9). Neither *Arabidopsis* (nonparasitic) nor *Phtheirospermum japonicum* (hemiparasitic), which are known to germinate independently of strigolactone, showed wavelike propagation of fluorescence (movies S4 and S5). These data suggest that the perception dynamics are related to strigolactone-dependent germination. Pulse-feeding experiments showed that *Striga* seeds require at least 6 hours of exposure to YLGW for efficient germination, corresponding to the completion of the wake-up wave (Fig. 3C). This observation indicates that the wake-up wave is necessary for efficient germination. RT-PCR analysis after GR24 treatment showed only mild induction in several *ShHTL*s, which suggests that transcriptional regulation of these genes is of limited importance in the perception dynamics (fig. S5).

The addition of ethylene results in the strigolactone-independent germination of *Striga* seeds, and this approach has been used to extirpate *Striga* seeds from farmers' fields (*1*). To further explore the relationship of strigolactone and ethylene with germination, we inhibited germination using either the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) or the protein translation inhibitor cycloheximide (CHX) (*5*). Both compounds inhibited *Striga* germination induced by GR24 or YLGW in a dose-dependent manner (fig. S10). However, the response differed for YLGW-dependent fluorescence (Fig. 3, B, D, and E, and movies S6 to S8). AVG caused a loss of fluorescence intensity, whereas CHX delayed the arrival of the wake-up wave. Thus, protein translation is required to produce the factors that wake up the entire embryo by spreading competence to respond to strigolactones from the root tip.

Ethylene, the biosynthesis of which is induced by strigolactone signaling, enhances strigolactone perception and thus forms an amplification loop (*5*). This signal amplification may explain how *Striga* recognizes minute amounts of strigolactones in the soil.

We envisage that the identification of strigolactone receptors and the establishment of a small-molecule reporter system will accelerate research to combat *Striga*.

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SUPPLEMENTARY MATERIALS

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Movies S1 to S8

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RNA SPLICING

An alternative splicing event amplifies evolutionary differences between vertebrates

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Alternative splicing (AS) generates extensive transcriptomic and proteomic complexity. However, the functions of species- and lineage-specific splice variants are largely unknown. Here we show that mammalian-specific skipping of polypyrimidine tract–binding protein 1 (PTBP1) exon 9 alters the splicing regulatory activities of PTBP1 and affects the inclusion levels of numerous exons. During neurogenesis, skipping of exon 9 reduces PTBP1 repressive activity so as to facilitate activation of a brain-specific AS program. Engineered skipping of the orthologous exon in chicken cells induces a large number of mammalian-like AS changes in PTBP1 target exons. These results thus reveal that a single exon-skipping event in an RNA binding regulator directs numerous AS changes between species. Our results further suggest that these changes contributed to evolutionary differences in the formation of vertebrate nervous systems.

A major challenge in evolutionary biology is to determine which gene regulatory changes contributed to species-specific phenotypes (*1*–*3*). Comparative transcriptomic analyses revealed that vertebrate organ alternative splicing (AS) patterns diverged more rapidly than gene expression differences (*4*–*6*). These AS differences were largely attributed to changes in the use of conserved cis-regulatory elements (*4*, *5*). However, a small number of lineage- and species-

Science

Probing strigolactone receptors in *Striga hermonthica* with fluorescence

Yuichiro Tsuchiya, Masahiko Yoshimura, Yoshikatsu Sato, Keiko Kuwata, Shigeo Toh, Duncan Holbrook-Smith, Hua Zhang, Peter McCourt, Kenichiro Itami, Toshinori Kinoshita and Shinya Hagihara

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Germination signals illuminated

The noxious weed *Striga* can take down an entire crop. Fields in Africa are particularly susceptible to the devastation it can cause. *Striga* seeds germinate in response to faint traces of the hormone strigolactone released by its targets. Tsuchiya *et al.* designed a mimic of strigolactone that, when cleaved by the Striga receptor, generates a fluorescent end product. This photogenic mimic lit up *Striga* seeds upon germination and led to the identification of its strigolactone receptor. Abolishing the activity of this receptor could be an effective defensive strategy.

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