Strigolactone biosynthesis and perception

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Strigolactones (SLs) are plant hormones that regulate shoot branching as well as known as root-derived signals for parasitic and symbiotic interactions. Since the first discovery of a naturally occurring SL, strigol, more than 40 years ago, the biosynthetic pathway has remained elusive. Recently, it was partially uncovered through the functional analysis of some biosynthetic components that were discovered from genetic studies using SL-deficient mutants. In addition, a perception component was also characterized through genetic and biochemical studies of a rice SL-insensitive mutant, *dwarf14*. In this review, we describe new findings on SL biosynthesis and focus on a recently identified SL precursor, carlactone. We also describe the perception mechanisms by an α/β -fold hydrolase family protein.

Introduction

Strigolactones (SLs) are a class of terpenoid lactones that were initially characterized as root-derived signals that induce the germination of parasitic plants such as *Striga* and *Orobanch*e species [1–3]. SLs were later identified as symbiotic signals for arbuscular mycorrhizal fungi, which facilitate the uptake of inorganic nutrients by plants [4,5]. In 2008, SLs were shown to act as endogenous hormones, or their precursors, that regulate shoot branching by using enhanced shoot-branching mutants of pea, *Arabidopsis* and rice [6,7]. The discovery of SLs as plant hormones has provided a link between genetically characterized shoot-branching mutants and chemically studied SLs, and strongly accelerated SL research. These enhanced shoot-branching mutants include *ramosus* (*rms*) of pea (*Pisum sativum*) [8–10], *more axillary growth* (*max*) of *Arabidopsis* [11–15], *decreased apical dominance* of petunia (*Petunia hybrida*) [16,17] and *dwarf* (*d*) or *high-tillering dwarf* (*htd*) of rice (*Oryza sativa*) [18–20]. In addition to the shoot branch-regulating function, SLs have been demonstrated to have various hormonal roles in plant development and adaptive responses [21–24]. Moreover, the discovery of SL-deficient and -insensitive mutants offered opportunities to study the SL biosynthetic pathway and SL

perception/signaling mechanisms. In the past few years, biochemical functional analysis of these genetically identified components led to the discovery of an important SL biosynthetic intermediate called carlactone (CL). Furthermore, an α/β -fold hydrolase family protein that was identified from studies using the rice SL-insensitive mutant, *d14*, was suggested to be involved in SL perception. In this review, we highlight recent discoveries regarding SL biosynthesis and perception.

SLs are biosynthesized from the carotenoid pathway via CL

In 2005, Matusova *et al.* [25] reported that SLs might be biosynthesized from a carotenoid pathway by using a carotenoid biosynthetic inhibitor, fluridone, and some carotenoid metabolism mutants. In 2008, two mutants of rice, *d17* and *d10*, defective in carotenoid cleavage dioxygenase 7 (CCD7) and CCD8, respectively, and the *ccd8/rms1* mutant of pea were shown to be SL-deficient mutants [6,7]. These results support the hypothesis that SLs are synthesized from carotenoid. In addition to these two CCDs, a cytochrome P450, MAX1, was shown to function in SL biosynthesis [26]. Furthermore, D27, a novel class of Fe-containing protein, was also found as a SL biosynthetic component [27].

Recently, CL, which has a SL-like carbon skeleton, was identified as a product of sequential reactions by three biosynthetic enzymes, D27, CCD7 and CCD8 using all-*trans*- β -carotene as a substrate *in vitro* [28^{••}]. In these sequential reactions, D27 was demonstrated to catalyze the reversible isomerization of all-*trans*- β -carotene at C-9 position to yield 9-*cis*- β -carotene. It was initially reported that CCD7 uses all-*trans*- β -carotene as a substrate and catalyzes a 9,10 oxidative cleavage to produce all-*trans*- β -10'-carotenal [29]. However, Alder *et al.* [28^{••}] demonstrated that CCD7 preferentially cleaves 9-*cis*- β -carotene to produce 9-*cis*- β -apo-10'-carotenal, which is then cleaved by CCD8 to give CL (Figure 1). In the final step catalyzed by CCD8, intramolecular rearrangement occurs to synthesize the D-ring part, the characteristic portion of SLs. Alder *et al.* [28^{••}] proposed the reaction

mechanism of CCD8; however, it has not yet been clarified. Intriguingly, CL suppressed the shoot branching of rice SL biosynthetic mutants such as *d10* and *d27*, as well as stimulated the germination of *Striga hermonthica* seeds, suggesting that CL is a biosynthetic precursor for SLs [28^{••}]. Before the discovery of CL, SLs were predicted to be biosynthesized by coupling of separately synthesized two portions, the ABC- and D-ring parts [25,30]. However, the biochemical analysis of these three enzymes revealed that one SL molecule is derived from one carotenoid molecule [28^{••}].

More recently, CL has been successfully identified in plant tissues using liquid chromatography-quadrupole/time-of-flight tandem mass spectrometry (LC-MS/MS), and its absolute stereochemistry has been determined [31[•]]. All SLs reported so far have the same stereochemistry at the C-2' position (*R* configuration); natural 5-deoxystrigol (5DS) is (+)-5DS or (-)-2'-*epi*-5DS [32,33] (Figure 1). Interestingly, the stereochemistry of endogenous CL was determined to be the same at the corresponding position (11*R* in CL; Figure 1). Furthermore, stable isotope-labeled CL ([¹³C]-CL) was converted into SLs, such as (-)-2'-*epi*-5DS and orobanchol, in rice plants. For these conversions, only (11*R*)-CL, but not (11*S*)-CL could be a precursor for SLs. These data conclusively demonstrate that (11*R*)-CL is an endogenous biosynthetic precursor for SLs in rice [31[•]].

In *Arabidopsis*, the detection of some SLs such as orobanchol and orobanchylacetate was reported [34]; however, Seto *et al.* [31[•]] could not detect these SLs using LC-MS/MS, possibly due to low abundance. Instead, another SL-like compound called SL-LIKE1 was found from *Arabidopsis* root extracts [31[•]]. Intriguingly, feeding experiments of [¹³C]-CL using *Arabidopsis* SL-deficient mutants such as *max4* and *max4 max1* double mutants demonstrated that CL is converted into SL-LIKE1 in a MAX1-dependent manner. These results demonstrate that MAX1 is involved in the metabolism pathway of CL. Grafting experiments carried out by Booker *et al.* [14], using *Arabidopsis max1* as a rootstock and *max3* or *max4* as a scion, resulted in a complementation of the shoot-branching phenotype

(Figure 2). These results are consistent with the idea that MAX1 is a downstream component of MAX3 and MAX4, and the feeding experiments strongly support this hypothesis. Moreover, the *max1* mutant accumulated an extremely high level of CL, suggesting that CL is a substrate for MAX1 [31•]. Although *max1* mutant plants accumulate high levels of CL, they show the branching phenotype as do *max3* and *max4* mutant plants, and SL treatment can restore the phenotype. These results indicate that CL is a biologically inactive precursor for SLs for shoot-branching inhibition. This finding was supported also by the observation that exogenously applied CL did not rescue the branching phenotype of the *max1* mutant but did rescue that of the *max4* mutant [35•].

CL should be further oxidized at least at two positions (C-19 and C-7) to be finally converted into the simplest SL, 5DS (Figure 1). MAX1 might be involved in these oxidation steps of CL (Figure 1). Grafting experiments using *max4* and *max1* also indicated that the biosynthetic intermediate in the pathway between MAX4 and MAX1 is a mobile precursor that can move from roots to shoots (Figure 2) [14]. If CL is a substrate for MAX1, it is possible that CL is the mobile intermediate in the SL biosynthetic pathway. In addition, there was a report on the presence of some known SLs such as orobanchol in the xylem sap of *Arabidopsis* and tomato, suggesting that root-derived SLs are transported to the aboveground part through the xylem to regulate shoot branching [34,36]. It would be interesting to examine whether CL is present in the xylem sap.

Although the SL biosynthetic pathway has not been fully clarified, the discovery of CL as an SL precursor is a breakthrough that significantly increased our knowledge on SL biosynthesis. We believe that the whole picture of the SL biosynthetic pathway, including the biochemical function of MAX1 and the chemical structure of SL-LIKE1, will be uncovered in the near future.

SL perception by an α/β -fold hydrolase family protein

Possible perception or signaling components have been identified from the characterization of SL-insensitive mutants. *MAX2/D3/RMS4* encodes an F-box protein, which is known to be the substrate recognition subunit of SCF ubiquitin E3 ligase for proteasome-mediated proteolysis [18,37,38]. Some members of this protein family have been known to function as perception or signal transduction components of some plant hormones such as gibberellin (GA), jasmonate and auxin [39]. The rice *d14* mutant was also characterized as an SL-insensitive mutant [40]. Both the rice *d3* and *d14* mutants accumulated an excess amount of SL in plants [6,40]. These observations suggested that D3 or D14 family proteins are involved in SL perception.

Map-based cloning revealed that the *D14* gene encodes a protein that belongs to the α/β -fold hydrolase family [40]. Interestingly, the GA receptor GID1 also belongs to the same protein family [41], suggesting that D14 protein might act as an SL receptor. A difference between GID1 and D14 is that the catalytic triad (Ser, His, Asp), which is necessary for catalyzing the hydrolysis reaction, is conserved in D14, while His in the catalytic triad is replaced by Val in GID1 [41]. In agreement with the lack of the His residue, GID1 protein did not hydrolyze *p*-nitrophenyl acetate, which is often used as an artificial substrate for hydrolase family proteins [41]. In contrast, crystallographic analysis of D14 and its orthologs of petunia (DAD2) and *Arabidopsis* (AtD14) demonstrated that the catalytic triad is structurally conserved in these proteins [42[•]–44[•],45].

In fact, biochemical analysis of these recombinant proteins showed that they can catalyze the hydrolysis of an SL synthetic analog, GR24 (Figure 3) [42[•],43,45]. Furthermore, DAD2 was shown to interact with a petunia MAX2 ortholog, PhMAX2, in a GR24-dependent manner, as determined in yeast two-hybrid (Y2H) experiments [42[•]]. To evaluate the importance of hydrolyzing activity, the Ser residue in the catalytic triad was replaced by Ala (DAD2:S96A). The resulting DAD2:S96A protein had neither the GR24-hydrolyzing activity *in vitro* nor the shoot-branching inhibition function *in vivo*. Furthermore, DAD2:S96A did not interact with PhMAX2 in

the Y2H system. Moreover, the hydrolyzed products of GR24 by DAD2 did not restore the phenotypes of the petunia *dad2* mutant. From these results, the authors concluded that DAD2 is likely to be involved in SL perception, and that not the hydrolyzed products but the reaction itself is necessary to transduce the SL signal for shoot-branching inhibition.

Hamiaux *et al.* [42[•]] further examined the interaction between GR24 and DAD2 by differential scanning fluorimetry (DSF). DSF allows us to evaluate the interaction between protein and low-molecular weight compound by measuring the shift of protein melting temperature [46]. Usually, a receptor protein is stabilized and its melting point shifts to a higher temperature in the presence of a ligand molecule [46]. However, the melting point of DAD2 shifted to lower temperature with the addition of GR24, suggesting that GR24 induces destabilization of DAD2 protein [42[•]]. SL-dependent destabilization of D14 was also shown by Nakamura *et al.* [45] using a trypsin digestion assay. Although the mechanism for and the meaning of this destabilization have not been clarified, these results imply that GR24 can induce the conformational change in DAD2 and D14 protein structures. The melting temperature of DAD2:S96A protein did not shift with the addition of GR24, suggesting that the catalytic triad is important for GR24-induced DAD2 destabilization. It is not clear whether DAD2:S96A protein can still bind to GR24. Therefore, it has yet to be determined whether the hydrolysis reaction by DAD2 is necessary, or SL binding to DAD2 is sufficient, for transducing the hormonal signal. It is also important to use not only GR24, but also other SLs and SL analogs to determine the substrate specificity of DAD2.

The hydrolysis reaction of D14/AtD14/DAD2 was reported to be very slow [42[•],43]. In agreement with this, the interaction with GR24 could also be detected as binding activity. Direct binding ability of AtD14 to GR24 was examined by the isothermal titration calorimetry (ITC) method, showing that AtD14 can bind to GR24 with a K_d value at sub-micromolar range [44[•]]. However, so far, there have been no reports on the D14–GR24 (or other SLs)

complex structure. On the other hand, Nakamura *et al.* [45] reported the complex structure of D14 with a hydrolytic product, D-ring (hydroxymethylbutenolide; HMB, Figure 3), as was obtained by soaking crystallized D14 with another SL analog, (-)-2'-*epi*-GR7. The authors also found that application of a high concentration of HMB weakly decreased the tiller length of the rice *d27* mutant. They proposed that HMB produced by D14 protein acts as an active form for shoot-branching inhibition. However, there is no evidence that the D14–HMB complex is involved in transducing the hormonal signal in the shoot-branching inhibition pathway.

More recently, the characterization of a dominant SL-insensitive rice mutant *dwarf53* (*d53*) was reported [47^{••},48^{••}]. D53 protein was shown to interact with D14 in a GR24-dependent manner. GR24 treatment caused D53 degradation through the proteasome pathway in a manner that requires D14 and the SCF^{D3} ubiquitin ligase. These results suggest that D53 acts as a repressor in the SL signaling pathway. More details about D53 protein are discussed in another review in this issue (Smith and Li). To better understand SL perception and signaling, we should learn the mechanisms by which D14 interacts with D53 and what kind of SLs can induce this interaction. The results from DSF analysis implied that GR24 induces a conformational change of DAD2 protein and causes protein destabilization. This conformational change might be important for the interaction with D53 protein. Thus, it is important to understand the mechanism for this protein destabilization in detail.

Together the reported data suggest that D14 family proteins are involved in SL perception. However, more detailed analysis would be needed to understand how D14 recognizes SLs and transduces the hormonal signal to regulate shoot branching. In particular, the meaning of hydrolytic degradation of SLs has not been fully understood as discussed above. Natural SLs with diverse chemical structures have been identified [49–51]. However, the active form as a shoot branch-inhibiting hormone has not yet been demonstrated. To address these remaining questions, it would be

informative to evaluate the interaction between D14 and many known SLs including natural ones.

Conclusions

Over the past few years, significant progress has been made in our understanding of SL biosynthesis and perception. In both cases, key components have been found from genetic studies using shoot-branching mutants. Thus, as has been the case with other hormone biosynthesis and signaling pathways, genetic studies in combination with biochemistry have been powerful in increasing understanding of the SL biosynthesis pathway and SL perception mechanisms.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Cook CE, Whichard LP, Turner B, Wall ME, Egley GH: **Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant.** *Science* 1966, **154**:1189-1190.
2. Humphrey AJ, Beale MH: **Strigol: biogenesis and physiological activity.** *Phytochemistry* 2006, **67**:636-640.
3. Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH: **Secondary metabolite signalling in host-parasitic plant interactions.** *Curr Opin Plant Biol* 2003, **6**:358-364.
4. Akiyama K, Matsuzaki K, Hayashi H: **Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi.** *Nature* 2005, **435**:824-827.
5. Akiyama K, Ogasawara S, Ito S, Hayashi H: **Structural requirements of strigolactones for**

- hyphal branching in AM fungi.** *Plant Cell Physiol* 2010, **51**:1104-1117.
6. Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K *et al.*: **Inhibition of shoot branching by new terpenoid plant hormones.** *Nature* 2008, **455**:195-200.
 7. Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC *et al.*: **Strigolactone inhibition of shoot branching.** *Nature* 2008, **455**:189-194.
 8. Beveridge CA, Ross JJ, Murfet IC: **Branching mutant rms-2 in *Pisum sativum* (grafting studies and endogenous indole-3-acetic acid levels).** *Plant Physiol* 1994, **104**:953-959.
 9. Beveridge CA, Ross JJ, Murfet IC: **Branching in pea (action of genes Rms3 and Rms4).** *Plant Physiol* 1996, **110**:859-865.
 10. Beveridge CA, Symons GM, Turnbull CG: **Auxin inhibition of decapitation-induced branching is dependent on graft-transmissible signals regulated by genes Rms1 and Rms2.** *Plant Physiol* 2000, **123**:689-698.
 11. Stirnberg P, van De Sande K, Leyser HM: **MAX1 and MAX2 control shoot lateral branching in Arabidopsis.** *Development* 2002, **129**:1131-1141.
 12. Sorefan K, Booker J, Haurogne K, Goussot M, Bainbridge K, Foo E, Chatfield S, Ward S, Beveridge C, Rameau C *et al.*: **MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea.** *Genes Dev* 2003, **17**:1469-1474.
 13. Booker J, Auldridge M, Wills S, McCarty D, Klee H, Leyser O: **MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule.** *Curr Biol* 2004, **14**:1232-1238.
 14. Booker J, Sieberer T, Wright W, Williamson L, Willett B, Stirnberg P, Turnbull C, Srinivasan M, Goddard P, Leyser O: **MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone.** *Developmental Cell* 2005, **8**:443-449.
 15. Turnbull CG, Booker JP, Leyser HM: **Micrografting techniques for testing long-distance signalling in Arabidopsis.** *Plant J* 2002, **32**:255-262.
 16. Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, Simons JL, Karunairetnam S, Gleave AP, Clark DG, Klee HJ: **The Decreased apical dominance1/Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development.** *Plant Cell* 2005, **17**:746-759.
 17. Simons JL, Napoli CA, Janssen BJ, Plummer KM, Snowden KC: **Analysis of the DECREASED APICAL DOMINANCE genes of petunia in the control of axillary**

- branching.** *Plant Physiol* 2007, **143**:697-706.
18. Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyoizuka J: **Suppression of tiller bud activity in tillering dwarf mutants of rice.** *Plant Cell Physiol* 2005, **46**:79-86.
 19. Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, Zhu L: **The rice HIGH-TILLERING DWARF1 encoding an ortholog of Arabidopsis MAX3 is required for negative regulation of the outgrowth of axillary buds.** *Plant J* 2006, **48**:687-698.
 20. Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyoizuka J: **DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice.** *Plant J* 2007, **51**:1019-1029.
 21. Seto Y, Kameoka H, Yamaguchi S, Kyoizuka J: **Recent advances in strigolactone research: chemical and biological aspects.** *Plant Cell Physiol* 2012, **53**:1843-1853.
 22. Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H: **The biology of strigolactones.** *Trends Plant Sci* 2013, **18**:72-83.
 23. Brewer PB, Koltai H, Beveridge CA: **Diverse roles of strigolactones in plant development.** *Mol Plant* 2013, **6**:18-28.
 24. de Saint Germain A, Bonhomme S, Boyer FD, Rameau C: **Novel insights into strigolactone distribution and signalling.** *Curr Opin Plant Biol* 2013, **16**:583-589.
 25. Matusova R, Rani K, Verstappen FW, Franssen MC, Beale MH, Bouwmeester HJ: **The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanch* spp. are derived from the carotenoid pathway.** *Plant Physiol* 2005, **139**:920-934.
 26. Crawford S, Shinohara N, Sieberer T, Williamson L, George G, Hepworth J, Muller D, Domagalska MA, Leyser O: **Strigolactones enhance competition between shoot branches by dampening auxin transport.** *Development* 2010, **137**:2905-2913.
 27. Lin H, Wang R, Qian Q, Yan M, Meng X, Fu Z, Yan C, Jiang B, Su Z, Li J *et al.*: **DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth.** *Plant Cell* 2009, **21**:1512-1525.
 28. Alder A, Jamil M, Marzorati M, Bruno M, Vermathen M, Bigler P, Ghisla S, Bouwmeester H, Beyer P, Al-Babili S: **The path from beta-carotene to carlactone, a strigolactone-like plant hormone.** *Science* 2012, **335**:1348-1351.
- The authors identified CL as a product of sequential reactions of recombinant D27, CCD7 and CCD8 *in vitro*, and proposed a new biosynthetic pathway of SLs. In this paper, D27 was found to be a β -carotene isomerase, and the true substrates for CCD7 and CCD8 were found to be 9-*cis*- β -carotene and 9-*cis*-10-apo-carotenal, respectively.
29. Schwartz SH, Qin X, Loewen MC: **The biochemical characterization of two carotenoid cleavage enzymes from Arabidopsis indicates that a carotenoid-derived compound**

- inhibits lateral branching.** *J Biol Chem* 2004, **279**:46940-46945.
30. Rani K, Zwanenburg B, Sugimoto Y, Yoneyama K, Bouwmeester HJ: **Biosynthetic considerations could assist the structure elucidation of host plant produced rhizosphere signalling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants.** *Plant Physiol Biochem* 2008, **46**:617-626.
 31. Seto Y, Sado A, Asami K, Hanada A, Umehara M, Akiyama K, Yamaguchi S: **Carlactone is an endogenous biosynthetic precursor for strigolactones.** *Proc Natl Acad Sci U S A* 2014, **111**:1640-1645.
- The authors firstly identified an endogenous CL using LC-MS/MS. They also found that CL is a precursor for SLs by feeding experiments using [¹³C]-CL.
32. Xie X, Yoneyama K, Kisugi T, Uchida K, Ito S, Akiyama K, Hayashi H, Yokota T, Nomura T: **Confirming stereochemical structures of strigolactones produced by rice and tobacco.** *Mol Plant* 2013, **6**:153-163.
 33. Ueno K, Nomura S, Muranaka S, Mizutani M, Takikawa H, Sugimoto Y: **Ent-2'-epi-orobanchol and its acetate, as germination stimulants for *Striga gesnerioides* seeds isolated from cowpea and red clover.** *J Agric Food Chem* 2011, **59**:10485-10490.
 34. Kohlen W, Charnikhova T, Liu Q, Bours R, Domagalska MA, Beguerie S, Verstappen F, Leyser O, Bouwmeester H, Ruyter-Spira C: **Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in nonarbuscular mycorrhizal host *Arabidopsis*.** *Plant Physiol* 2011, **155**:974-987.
 35. Scaffidi A, Waters MT, Ghisalberti EL, Dixon KW, Flematti GR, Smith SM: **Carlactone-independent seedling morphogenesis in *Arabidopsis*.** *Plant J* 2013, **76**:1-9.
- The authors reported the first chemical synthesis of CL. They found that CL suppressed *Arabidopsis* shoot branching in a manner dependent on MAX1, while the activity on hypocotyl elongation was independent of MAX1.
36. Kohlen W, Charnikhova T, Lammers M, Pollina T, Toth P, Haider I, Pozo MJ, de Maagd RA, Ruyter-Spira C, Bouwmeester HJ *et al*: **The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SICCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis.** *New Phytol* 2012, **196**:535-547.
 37. Stirnberg P, van de Sande K, Leyser HMO: **MAX1 and MAX2 control shoot lateral**

- branching in Arabidopsis.** *Development* 2002, **129**:1131-1141.
38. Johnson X, Breich T, Dun EA, Goussot M, Haurogne K, Beveridge CA, Rameau C: **Branching genes are conserved across species. Genes controlling a novel signal in pea are coregulated by other long-distance signals.** *Plant Physiol* 2006, **142**:1014-1026.
 39. Lechner E, Achard P, Vansiri A, Potuschak T, Genschik P: **F-box proteins everywhere.** *Curr Opin Plant Biol* 2006, **9**:631-638.
 40. Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyoizuka J: **d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers.** *Plant Cell Physiol* 2009, **50**:1416-1424.
 41. Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I *et al*: **GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin.** *Nature* 2005, **437**:693-698.
 42. Hamiaux C, Drummond RS, Janssen BJ, Ledger SE, Cooney JM, Newcomb RD, Snowden KC: **DAD2 is an α/β hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone.** *Curr Biol* 2012, **22**:2032-2036.
- The authors demonstrated that DAD2, a D14 ortholog in petunia, is likely to be involved in SL perception. This is the first report on the crystal structure of a D14 family protein, and the hydrolase activity of DAD2 was found using GR24 as a substrate.
43. Zhao LH, Zhou XE, Wu ZS, Yi W, Xu Y, Li S, Xu TH, Liu Y, Chen RZ, Kovach A *et al*: **Crystal structures of two phytohormone signal-transducing α/β hydrolases: karrikin-signaling KAI2 and strigolactone-signaling DWARF14.** *Cell Res* 2013, **23**:436-439.
 44. Kagiya M, Hirano Y, Mori T, Kim SY, Kyoizuka J, Seto Y, Yamaguchi S, Hakoshima T: **Structures of D14 and D14L in the strigolactone and karrikin signaling pathways.** *Genes Cells* 2013, **18**:147-160.
- The authors showed that D14 protein can bind to GR24 by ITC. They also demonstrated the crystal structures of D14 and its closely related homolog D14LIKE. D14LIKE protein is predicted to be the receptor of karrikin, a smoke-derived germination-inducing substance. They firstly demonstrated the binding ability of D14LIKE to karrikin by ITC.
45. Nakamura H, Xue YL, Miyakawa T, Hou F, Qin HM, Fukui K, Shi X, Ito E, Ito S, Park SH *et al*: **Molecular mechanism of strigolactone perception by DWARF14.** *Nat Commun* 2013, **4**:2613.
 46. Niesen FH, Berglund H, Vedadi M: **The use of differential scanning fluorimetry to detect**

- ligand interactions that promote protein stability.** *Nat Protoc* 2007, **2**:2212-2221.
47. Jiang L, Liu X, Xiong G, Liu H, Chen F, Wang L, Meng X, Liu G, Yu H, Yuan Y *et al.*: **DWARF 53 acts as a repressor of strigolactone signalling in rice.** *Nature* 2013, **504**:401-405.
- The authors characterized a dominant SL-insensitive rice mutant *d53* and found that D53 is a key repressor in the SL signaling pathway. They showed that GR24 induces D53 degradation by the proteasome in a D14⁻ and D3-dependent manner.
48. Zhou F, Lin Q, Zhu L, Ren Y, Zhou K, Shabek N, Wu F, Mao H, Dong W, Gan L *et al.*: **D14-SCFD3-dependent degradation of D53 regulates strigolactone signalling.** *Nature* 2013, **504**:406-410.
- Like Jiang *et al.* [47●●], the authors characterized the rice *dwarf53* (*d53*) mutant and found that D53 is a critical repressor in the SL signaling pathway. They showed that GR24 induces D53 degradation by the proteasome in a D14⁻ and D3-dependent manner.
49. Yoneyama K, Xie X, Takeuchi Y: **Strigolactones: structures and biological activities.** *Pest Manag Sci* 2009, **65**:467-470.
50. Yoneyama K, Awad AA, Xie X, Takeuchi Y: **Strigolactones as germination stimulants for root parasitic plants.** *Plant Cell Physiol* 2010, **51**:1095-1103.
51. Xie X, Yoneyama K: **The strigolactone story.** *Annu Rev Phytopathol* 2010, **48**:93-117.

Figure Captions

Figure 1

The SL biosynthetic pathway. CL is produced from all-*trans*- β -carotene by three enzymes, D27, CCD7 and CCD8. D27 catalyzes the isomerization reaction of all-*trans*- β -carotene at the C-9 position. CL would be oxidized by oxidation enzyme(s) such as the cytochrome P450 monooxygenase MAX1 and then converted to (+)-5DS or (-)-2'-*epi*-5DS, which are naturally occurring isomers of 5DS. 5DS might be converted into some other SLs. Blue, red, green and orange characters indicate genes of rice, *Arabidopsis*, pea and petunia, respectively. Single arrows show conversions catalyzed by a single enzyme. Double arrows denote conversions possibly catalyzed by one or more enzymes.

Figure 2

The plausible biosynthetic pathway of SL in *Arabidopsis* and a graphical summary of grafting experiments using *Arabidopsis max* mutants. The shoot-branching phenotype of the *max4* mutant was complemented by grafting onto the *max1* rootstock [14]. An extremely high level of CL that accumulated in the *max1* mutant supports the idea that CL is a substrate for MAX1 [31[●]]. Taken together, CL is a good candidate for the mobile signal which can move from roots to shoots in the *max4/max1* (shoot/root) graft.

Figure 3

Hydrolysis of GR24 by D14/DAD2/AtD14 proteins. Red, blue and orange characters indicate genes of rice, *Arabidopsis* and petunia, respectively.

Figure 1

Figure 2

Figure 3