Cellular events of strigolactone signalling and their crosstalk with auxin in roots

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Abstract

Strigolactones are a new group of plant hormones that suppress shoot branching. In roots, they regulate primary-root growth and lateral-root formation and increase root-hair elongation. Reception of strigolactones occurs via a specific cellular system which includes a D14-like/MAX2-like/SCF complex that, upon perception of strigolactone signalling, leads to certain degradation of receptors and to the release of downstream targets. This signalling pathway may eventually result in changes in actin-filament bundling, cellular trafficking, and PIN localization in the plasma membrane. As a result, auxin flux may be regulated in the shoot or root. Strigolactones are also involved with the response to phosphate conditions in roots, acting by both dampening auxin transport via depletion of PIN2 from the plasma membrane and inducing TIR1 transcription to increase auxin perception. In these instances and, possibly, others, strigolactones manipulate the auxin pathway, affecting its transport, perception or both. However, other mechanisms for strigolactone-regulated plant development and the involvement of other plant hormones are evident.

Key words: Actin, auxin, endocytosis, phosphate, PIN2, plasma membrane, polar transport, root, strigolactone, TIR1.

Introduction

Strigolactones are a new group of plant hormones. They were initially identified as plant hormones based on their inhibitory effect on the outgrowth of preformed axillary bud (Gomez-Roldan et al., 2008; Umehara et al., 2008). However, these compounds were actually first documented back in 1966, as highly active germination stimulants of parasitic plant seeds (Cook et al., 1966). The structure of strigol was firstly elucidated in 1972 (Cook et al., 1972) and, since then, a large number of studies have shown that strigolactones are produced and exuded from the roots as secondary metabolites which act as stimulators of the seed germination of parasitic plants, including Striga and Orobanche spp. (Cook et al., 1966, 1972; Yokota et al., 1998). Another role for strigolactones is in enhancing hyphal branching of the symbiotic arbuscular mycorrhizal fungi (Akiyama et al., 2005) and promoting the symbiotic interaction of plants with Rhizobium (Foo and Davies, 2011).

About 17 natural strigolactones have been isolated from different plant species. All share a common skeleton of four rings which consists of three ABC rings connected via an enol ether bridge to a D ring that belongs to butenolide. Different substitutions are found on the A and B rings (Tsuchiya and McCourt, 2009; Xie et al., 2010; Yoneyama et al., 2013; Zwanenburg and Pospíšil, 2013). Moreover, different activities of strigolactones were associated with the different molecular structures. For example, for the induction of seed germination of parasitic plants by strigolactones the α/β-unsaturated carbonyl moiety (of the C ring) needs to be connected to the D-ring via an enol ether unit. However, the presence of an extra methyl on the D-ring leads to a reduction in this activity (Zwanenburg et al., 2013).

The biosynthetic pathway of strigolactones is partially known. They are produced in a wide variety of plant species, including dicots, monocots, and primitive plants (Liang...
et al., 2010; Xie et al., 2010; Proust et al., 2011; Delaux et al., 2012), mainly from the roots. They are a result of cleavage of carotenoid precursors (Matusova et al., 2005) by the activity of several enzymes. These include two carotenoid cleavage dioxygenases (CCDs; Alder et al., 2012; Kohlen et al., 2012), a carotenoid isomerase (DWARF27; Lin et al., 2009, Waters et al., 2012a), and a class-III cytochrome P450 monoxygenase (Booker et al., 2005; Alder et al., 2012; Cardoso et al., 2014). Strigolactones are biosynthesized from the precursor carlactone, which is converted into the carboxylated metabolite carlactonic acid by MAX1 activity in Arabidopsis thaliana (Abe et al., 2014); however, the two homologues of MAX1 in rice (Oryza sativa) were shown to convert carlactone in Nicotiana benthamiana cells to ent-2’-epi-5-deoxystrigol and then to orobanchol, suggesting that MAX1 homologues can catalyse two distinct steps in strigolactone biosynthesis (Zhang et al., 2014).

The effects of strigolactones in plants are not just restricted to the repression of axillary bud outgrowth. They have been shown to act as positive regulators of secondary-shoot growth (Agusti et al., 2011) and as negative regulators of adventitious-root formation (Rasmussen et al., 2012). In roots, strigolactones increase cell numbers in the primary-root meristem (Ruyter-Spira et al., 2011; Koren et al., 2013) and regulate lateral-root formation. The latter is dependent on growth conditions—strigolactones suppress lateral-root formation under sufficient phosphate, but induce it under conditions of phosphate deficiency (Ruyter-Spira et al., 2011; Kapulnik et al., 2011a; De Cuyper et al., 2015). The addition of strigolactones to roots also increases root-hair elongation in the primary root (Kapulnik et al., 2011a).

**Strigolactone reception**

Strigolactone reception occurs via a specific cellular system. This system has recently been described in other reviews (Bennett and Leyser, 2014; Koltai, 2014; Waldie et al., 2014; Waters et al., 2014) and is, therefore, only briefly treated here. One of the components of this reception system is an F-box protein, MAX2/D3/RMS4 (Stirnberg et al., 2002; Ishikawa et al., 2005; Johnson et al., 2006), which can link to an Skp, Cullin, F-box (SCF)-containing complex, catalysing the ubiquitination of proteins designated for proteasomal degradation (Moon et al., 2004). An additional component of strigolactone signalling is a protein of the α/β-hydrolase fold superfamily, designated D14 in rice (Arite et al., 2009). D14 and its orthologue in petunia (Petunia hybrida), DAD2, have been shown to bind and cleave the synthetic and biologically active strigolactone GR24 (Hamiaux et al., 2012; Kagiyama et al., 2013; Nakamura et al., 2013). Moreover, strigolactones induce the proteasomal degradation of D53 dependent on D14 and D3, whereas D53 can form a complex with D14 and D3. D53 is a class I Clp ATPase protein that acts as a repres- sor of strigolactone signalling. Its degradation by strigolactone is essential for strigolactone signaling in the rice shoot (Jiang et al., 2013; Zhou et al., 2013).

Together, these findings suggest that D14 acts to bind the mobile strigolactones. This binding induces the interaction of D14 with the SCF-D3 complex, resulting in tagging and then the degradation of proteins such as D53 via the ubiquitin—proteasome pathway (reviewed by Koltai, 2014; Waldie et al., 2014; Waters et al., 2014). Strigolactones induce, in a MAX2-dependent way, the proteasome-mediated degradation of D14. Hence, a negative feedback loop between D14 and strigolactones is suggested, which could effectively limit the duration and intensity of strigolactone signalling (Chevalier et al., 2014).

Another D14-like protein, KA12 (D14-LIKE), regulates seed germination and seedling growth in Arabidopsis in a MAX2-dependent pathway (Nelson et al., 2011; Waters et al., 2012b, 2014). However, KA12 is not a strigolactone receptor; rather, it is a receptor of the strigolactone-anal- ogous compound karrikin, originally found in forest-fire smoke and probably of an unknown plant-derived substrate (Flematti et al., 2004; Smith and Li, 2014). Involvement of other proteins with an α/β-hydrolase fold and degradation of other repressors may lead to the execution of different strigolactone-related processes, such as the formation of lateral or adventitious roots.

**Strigolactones regulate the plasma-membrane localization of PINs—the auxin-export proteins**

The activity of strigolactones as negative regulators of shoot branching was suggested to be derived from their ability to reduce the basipetal transport of auxin, thereby enhancing competition between branches (Crawford et al., 2010). The reduction of auxin transport by strigolactones could be derived from their regulation of the activity of PIN proteins, the auxin-efflux transporters. In tomato (Solanum lycopersi- cum) roots, functional involvement of GR24 with auxin efflux was suggested because exogenous supplementation of 2,4-D (a synthetic auxin that is not secreted by efflux carriers) led to reversion of the GR24-related root effect (Koltai et al., 2010). Indeed, both auxin-transport and shoot-branching phenotypes observed in various mutants were reproduced by a computational model in which strigolactone action was represented as an increase in the rate of PIN1 removal from the plasma membrane (Shinohara et al., 2013). Accordingly, experimental evidence supported the idea that strigolactone signalling triggers PIN1 depletion from the plasma membrane of xylem parenchyma cells in the stem (Shinohara et al., 2013). In roots, under conditions in which GR24 increases root-hair elongation, strigolactones increased, rather than decreased PIN2’s plasma-membrane localization and polarization in root-epidermis cells in a MAX2-dependent fashion (Pandya-Kumar et al., 2014).

The regulation by strigolactones on the plasma-membrane localization of PIN1 in the shoot was detected within minutes of strigolactone treatment and was dependent on clathrin-mediated membrane trafficking (Shinohara et al., 2013). Accordingly, in the root, the effect of strigolactones on the plasma-membrane localization of PIN2 was accompanied by increased PIN2 endocytosis, increased endosome trafficking in the epidermal cells, and changes in actin-filament architecture and dynamics (Pandya-Kumar et al., 2014). Since the polar position of the
PINs in the plasma membrane is an important factor determining the direction of auxin flux (Wiśniewska et al., 2006), all of these studies indicate that strigolactones regulate auxin flux by affecting PINs’ plasma-membrane localization.

The strigolactone-signalling pathway affects auxin transport

As detailed above, cellular reception of strigolactone via a D14-like/MAX2-like/SCF module leads to the degradation of certain receptor(s) and to the release of downstream targets. The release of one of these targets may eventually result in the reduced bundling of actin filaments (Fig. 1). The polar localization of PINs is determined by the constitutive trafficking of PIN vesicles between the plasma membrane and endosomes (Geldner et al., 2001), and targeting of the PIN proteins to the plasma membrane is largely dependent on F-actin (Geldner et al., 2001; Nagawa et al., 2012). Hence, by affecting actin bundling, strigolactones probably affect cellular trafficking and PIN polar localization in the plasma membrane (Fig. 1; Koltai, 2014). Since the direction of auxin flux is determined largely by the polar position of the PINs in the plasma membrane (Wiśniewska et al., 2006), these strigolactone-induced changes in their plasma-membrane localization may eventually lead to the regulation of auxin flux in the shoot or root (Koltai, 2014; Waldie et al., 2014).

It could be that strigolactones and auxin are in a feedback loop (Koltai, 2014). This is because auxin stimulates its own transport by shaping actin filaments and PIN’s plasma-membrane localization (Waller et al., 2002; Nick et al., 2009). On the other hand, strigolactones may enhance auxin transport (in the root, under conditions of a GR24-positive effect on root-hair elongation), or dampen it (in the shoot; Crawford et al., 2010), and auxin is a positive regulator of strigolactone biosynthesis (Hayward et al., 2009). Hence, a signal from strigolactones to increase or decrease auxin transport may lead either to positive or to negative regulatory circuits between strigolactone and auxin involving the regulation of actin architecture and polar auxin transport (Koltai, 2014). However, strigolactone production in roots is also regulated by shoot-derived signals other than auxin (Yoneyama et al., 2014), and strigolactones were suggested to inhibit auxin biosynthesis associated with the attenuation of shoot gravitropism (Sang et al., 2014). Moreover, the rate of auxin transport and of strigolactones may feed back on PINs levels because auxin can act through TIR1/AFB to maintain PIN levels in the cell (particularly PIN2; Baster et al., 2013). On the other hand, under certain growth conditions, strigolactones may regulate auxin perception (described below). Thus, auxin–strigolactone cross-talk is likely to be more complicated.

Strigolactones affect the plant response to phosphate-stress conditions

In plants, strigolactones shape both shoot and root architecture in response to phosphate (Pi) conditions. Pi is the inorganic form of phosphorus (P) that is available to plants. P is an essential macronutrient and a limiting factor for plant growth and development (Bieleski, 1973; Maathuis, 2009). Plants modify their growth pattern and architecture to cope with Pi deprivation. Pi...
deprivation leads to an increase in strigolactone exudation across plant species (Yoneyama et al., 2007a, b, 2012). This boost in strigolactone levels was shown in both Arabidopsis and rice to be clearly correlated with a decrease in shoot branching under restricted-Pi growth conditions (Umehara et al., 2010; Kohlen et al., 2011). In addition, strigolactones were suggested to regulate leaf senescence in general (Snowden et al., 2005) and in the response to Pi deficiency in particular (Yamada et al., 2014). Significantly, strigolactone exudation from roots was reported to occur via the ABCG transporter PhPDR1, which is also up-regulated by low Pi conditions (Kretzschmar et al., 2012).

Root architecture is also altered in response to Pi deficiency (López-Bucio et al., 2003; Osmont et al., 2007). Changes in root architecture under these conditions include the inhibition of primary-root elongation (Sánchez-Calderón et al., 2005) and the promotion of lateral-root development (Nacry et al., 2005). The changes in root architecture under conditions of Pi deprivation are regulated by several plant hormones. For example, increased auxin sensitivity, mediated by an increase in the expression of the auxin receptor gene TIRI, is responsible for at least some of the changes in lateral-root formation in Arabidopsis under conditions of Pi deprivation (Pérez-Torres et al., 2008).

Strigolactones, via MAX2, are also involved in the regulation of root-system architecture in response to Pi deprivation. Although under conditions of sufficient Pi they negatively regulate lateral-root formation (Kapulnik et al., 2011a), strigolactones positively affect lateral-root formation when Pi is limited (Ruyter-Spira et al., 2011). The involvement of strigolactones and D3 in responses to Pi (and nitrate) has also been shown in rice for seminal-root length and lateral-root formation (Sun et al., 2014).

Another recorded change in roots in response to Pi deprivation is an increase in root-hair length and density. This probably occurs to expand the root surface area, thereby enhancing nutrient acquisition (Péret et al., 2011). Strigolactones are necessary for the increase in root-hair density under conditions of Pi deficiency in Arabidopsis seedlings shortly after germination (Mayzlish-Gati et al., 2012). Moreover, the strigolactone-response mutant max2, under conditions of Pi deprivation, showed a lack of a proper response to low Pi conditions and a reduction, rather than an induction, of TIRI expression (Mayzlish-Gati et al., 2012). This is in accordance with the need for an increase in TIRI expression for the response to low Pi levels in the wild type (Pérez-Torres et al., 2008).

**Root response to Pi deprivation involves a reduction in plasma-membrane localization of PIN2, dependent on strigolactone/MAX2 signalling**

The plant’s response to low-Pi conditions is an active process that involves an increase in actin-filament bundling in root cells early in seedling development. As a result, endosome movement and PIN2 trafficking and polarization in the plasma membrane are reduced (Kumar et al., 2015).

Furthermore, as indicated above, the seedlings’ response to conditions of Pi deficiency by increased root-hair density is strigolactone/MAX2-dependent (Mayzlish-Gati et al., 2012). This strigolactone/MAX2-dependent response to Pi deprivation was found to involve depletion of PIN2 proteins from the plasma membrane of epidermal root cells, and a strigolactone/MAX2-dependent increase in actin bundling under these conditions (Fig. 1; Kumar et al., 2015). Thus, functional strigolactone/MAX2 signalling may be needed for these cellular events in response to low Pi during the early stages of seedling development.

As already noted, these changes in PIN2 localization to the plasma membrane and polarization (Gonzalez-Mendoza et al., 2013; Kumar et al., 2015) may lead to disturbances in auxin flux. Accordingly, the involvement of strigolactones and D3 in the responses to Pi in rice for seminal-root length and lateral-root formation was suggested to involve changes in auxin transport from the shoots to the roots (Sun et al., 2014).

**Concluding remarks**

Strigolactones regulate plant development and growth via close crosstalk with auxin. As discussed in this review, shoot branching may largely be a result of strigolactone depletion of PIN1 from the plasma membrane, which may lead to dampening of polar auxin transport (Shinohara et al., 2013). In the case of the root response to conditions of Pi deficiency, strigolactones are likely to act in more than one way to manipulate the auxin pathway (Fig. 1). One is by dampening auxin transport via depletion of PIN2 from the plasma membrane (Kumar et al., 2015). Another involves the induction of TIRI transcription and thus a probable increase in auxin perception (Mayzlish-Gati et al., 2012). Together, the suggested strigolactone/MAX2-dependent reduction of auxin efflux and the induction of auxin perception probably lead to an enhanced response to auxin, resulting in, for example, increased density and length of root hairs, which are typical root responses to conditions of Pi deficiency (Peret et al., 2011).

In these instances, and possibly others, strigolactones manipulate auxin transport, perception or both. However, these mechanisms of strigolactone–auxin cross-talk may not underlie all strigolactone-regulated plant development. For example, strong evidence suggests that strigolactones induce expression of the bud-specific target gene BRANCHED1 (BRC1), which encodes a transcription factor repressing bud outgrowth (Braun et al., 2012; Dun et al., 2012). Here, strigolactones act as an auxin-promoted secondary messenger acting antagonistically to cytokinin, as cytokinin promotes bud outgrowth by repressing BRC1 expression (Aguilar-Martinez et al., 2007). Interestingly, overexpression of BRC1 facilitates a fast and generalized growth arrest in the root and shoot apical meristems and in leaf primordia. This and additional evidence, including the BRC1 expression profile, suggest that BRC1 has a role in bud arrest triggered by shade, an important adaptive plant response (González-Grandío et al., 2013). Also, auxin and cytokinin have been suggested to be dominant regulators in decapitation-induced branching; however, in intact
plants, strigolactones are more important for the regulation of shoot branching (Young et al., 2014).

Additional evidence suggests that the strigolactone-signalling pathway is integrated with additional hormone-signalling pathways (Kapulnik et al., 2011b; Wang et al., 2013). Together, strigolactones, auxin, and other plant hormones are likely to form a carefully co-ordinated hormonal network for the regulation of proper plant growth and development. At least part of this co-ordinated network may rely on changes in trafficking of plasma membrane proteins, such as PINs, which are derived from changes in actin architecture.

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Strigolactone crosstalk with auxin | 4861


