Review

Thinking outside the F-box: novel ligands for novel receptors

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The importance of regulated proteolysis in the physiology and development of plants is highlighted by the large number of genes dedicated to proteasome-dependent protein degradation. Within the SCF class of E3 ubiquitin ligases are more than 700 F-box proteins that act as recognition modules to specifically target their dedicated substrates for ubiquitylation. This review focuses on very recent studies indicating that some F-box proteins function as phytohormone or light receptors, which directly perceive signals and facilitate specific target-protein degradation to regulate downstream pathways. If this new connection between ligand-regulated proteolysis and signaling proves to be more extensive, an entirely new way of understanding the control of signal transduction is in the offing.

Adding to the SCF complex(ity): ligand-binding F-box proteins

Post-transcriptional regulations, such as proteolysis, are important processes used by organisms to respond to environmental and intracellular signals by altering protein levels and activities. Many short-lived proteins are degraded through ubiquitin (Ub)–26S proteasome pathways rapidly after polyubiquitination. In *Arabidopsis* (*Arabidopsis thaliana*), more than 1300 genes are involved in the Ub–26S proteasome system. In this pathway, the reusable 76-amino-acid protein Ub is covalently attached to a specific target protein. The Ub conjugation to the target protein is achieved through the sequential action of three enzyme families, E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme) and E3 (Ub ligase). Ub is activated by E1 in an ATP-dependent manner and conjugated to E2. The Ub–E2 intermediate then transfers the Ub to a Lys residue of the substrate protein via an E3 recognition element. The ubiquitinated proteins are recognized and degraded by the 26S proteasome (Figure 1a) [1,2]. Substrates can be mono-ubiquitinated or polyubiquitinated through repeated Ub conjugation. Generally, monoubiquitination functions as a modifier that alters protein function, whereas polyubiquitinated proteins are targeted for degradation [3]. The *Arabidopsis* genome encodes two E1s, at least 45 E2s or E2-like proteins, and almost 1200 E3 components [1,4].

Of the different classes of E3 ligases, the SCF complex is the largest and best characterized [4,5]. The SCF ubiquitin ligase is composed of Cullin (CUL1), SUPPRESSOR OF KINETOCHORE PROTEIN1 (SKP1; in *Arabidopsis*, it is called *Arabidopsis* SKP1-like [ASK]), RING-BOX1 (RBX1) and an F-box protein. CUL1 functions as a scaffold protein, binding RBX1 at its carboxyl terminus and SKP1 at its N terminus. F-box proteins interact with SKP1 through the F-box motif at the N terminus of the protein (Figure 1a). The substrate specificity is conferred by a C-terminal protein-interaction motif, often comprised of kelch repeats, leucine-rich repeats (LRRs) or a WD40 domain [6]. With more than 700 F-box proteins in *Arabidopsis*, it is clear that SCF complexes are used to recognize a wide variety of substrate proteins. Recent studies have reported that F-box proteins participate in the regulation of many physiological phenomena, such as hormone responses, the circadian clock, flowering time and pathogen defense [4,5,7].

There are several excellent reviews on protein degradation, F-box proteins in general and hormone receptors [8–15]. Although gibberillic acid and ethylene signaling also involve F-box-protein-mediated processes [16–18], we focus here on recent findings demonstrating that certain types of F-box proteins can act directly as ligand receptors to modulate signaling pathways. This new paradigm implies that F-box proteins are not simply constitutive adaptors positioning a target for ubiquitination but are dynamically regulated by cellular components and the light environment.

StickLRRs for signaling: small-molecule ligands as 'molecular glue'

Our sense of what constitutes a plant hormone has expanded in recent years beyond the classical set of five: auxin, abscisic acid (ABA), cytokinin, ethylene and gibberellic acid. First in line were brassinosteroids, where genetic and molecular studies from more than ten years ago helped bring about their acceptance as an authentic sixth plant hormone [19]. Other small molecules, such as jasmonate and, very recently, strigolactones, have been shown to regulate plant development and physiology, to be effective at very low concentrations and to operate at a variety of sites in the plant. In these ways, they can be considered phytohormones in the traditional sense. The biosynthetic pathways and chemical structures of these eight growth regulators are diverse, but three of them are likely to share a common mechanism of action through the regulation of proteasome-dependent proteolysis (Figure 2).

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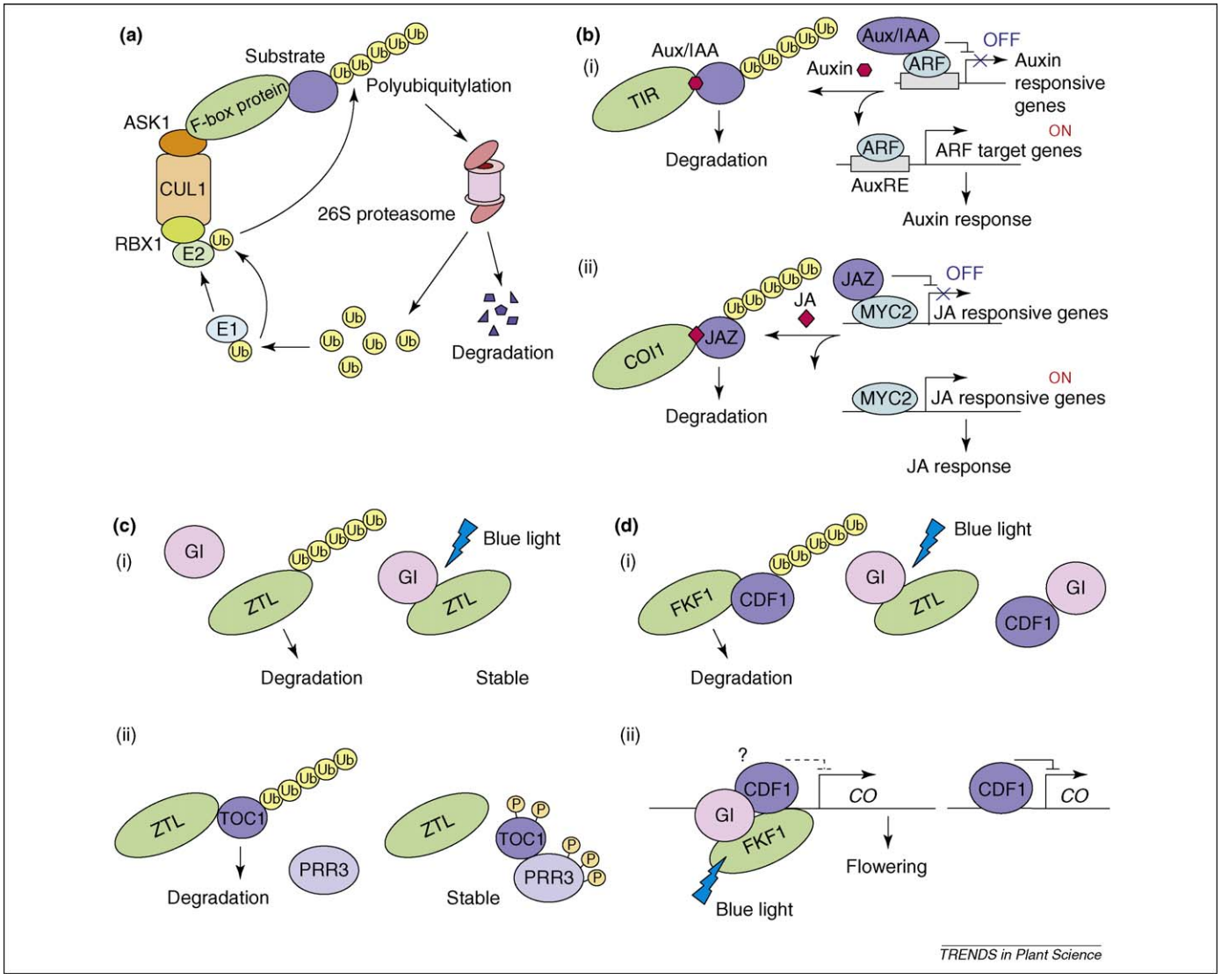

Figure 1. F-box-protein-dependent proteolysis. **(a)** The SCF complex is composed of CUL1, RBX1, ASK1, E2 and an F-box protein and facilitates the polyubiquitylation of a target substrate protein. The F-box protein serves as a substrate-specific link between the cullin-based complex and the target protein, which is polyubiquitylated and degraded via the 26S proteasome pathway. **(b)** Hormone mediated proteolysis. (i) Auxin-mediated proteolysis. At low levels of auxin, Aux/IAA and ARF form a dimer on an auxin response element (AuxRE) and repress the expression of auxin responsive genes. Auxin binding of TIR1 facilitates the association of TIR1 and Aux/IAA, leading to the polyubiquitylation and degradation of Aux/IAA. Degradation of Aux/IAA de-represses the expression of ARF target genes and auxin responses are switched on. (ii) Jasmonate-mediated proteolysis. The JA response involves a similar mechanism. A JAZ–MYC2 dimer represses JA-responsive gene expression. JA binding to COI1 facilitates the JAZ–COI1 interaction, and repression is released by JAZ degradation through SCF^{COI1}-dependent proteolysis. **(c)** Blue-light-mediated proteolysis – ZTL. (i) In the circadian clock, the F-box protein ZTL functions as a blue-light receptor. On its own, ZTL is degraded via the proteasome, but this process is inhibited upon absorption of blue light, which enhances the interaction with the stabilizing protein, GI. This allows ZTL protein to build to high levels near dusk. (ii) The SCF^{ZTL} targets the central clock components TOC1 and PRR5 (not depicted). TOC1 degradation is regulated by a competitive interaction with PRR3 and ZTL. When both TOC1 and PRR3 are highly phosphorylated, early in the night, they interact and inhibit ZTL–TOC1 binding, resulting in the stabilization and increase in TOC1 levels. When they are unphosphorylated, TOC1 is available for targeted degradation by ZTL. The phase-specific increase in ZTL levels near dusk helps sharpen the amplitude of TOC1 rhythm [64]. **(d)** Blue-light-mediated proteolysis – FKF1. (i) FKF1 functions as a blue-light receptor in the photoperiodic flowering pathway. FKF1 targets CDF1, a repressor of *CO* expression, for degradation. The GI and FKF1 interaction is blue-light-dependent, but GI and CDF1 also interact. (ii) GI, FKF1 and CDF1 control flowering through the regulation of *CO* transcription. GI interaction with CDF1 at the *CO* promoter might then recruit FKF1 to that site after blue absorbance by FKF1 enhances its affinity for GI. It is likely that they complex together at the *CO* promoter, where SCF^{FKF1} degrades CDF1, allowing *CO* transcription and flowering to proceed [65].

Auxin

Early work on the study of the molecular mechanism of auxin action identified the auxin and indole acetic acid (Aux/IAA) class of transcriptional regulators and the AUXIN RESPONSE FACTOR (ARF) family of transcription factors as essential players in the auxin-mediated control of gene expression. Subsequent work showed that these proteins interact *in vivo*, with Aux/IAA proteins inhibiting the positive transcriptional activity of the ARFs [8]. TRANSPORT INHIBITOR RESPONSE1 (TIR1) is an F-box protein first identified in a screen for auxin-transport inhibitors [20,21]. Soon after the cloning of TIR1, the Aux/IAA proteins were identified as physical interactors and substrates of an SCF^{TIR1} complex [22]. This result provided an explanation of TIR1 as a positive regulator of the auxin response through the targeted degradation of negative regulators, the Aux/IAA proteins (Figure 1bi).

How auxin facilitated the TIR1–Aux/IAA interactions became the next big question. Experiments using auxin-supplemented plant cell extracts in *in vitro* pull-down assays and separate work with extracts derived from auxin-treated plants made it clear that auxin was closely

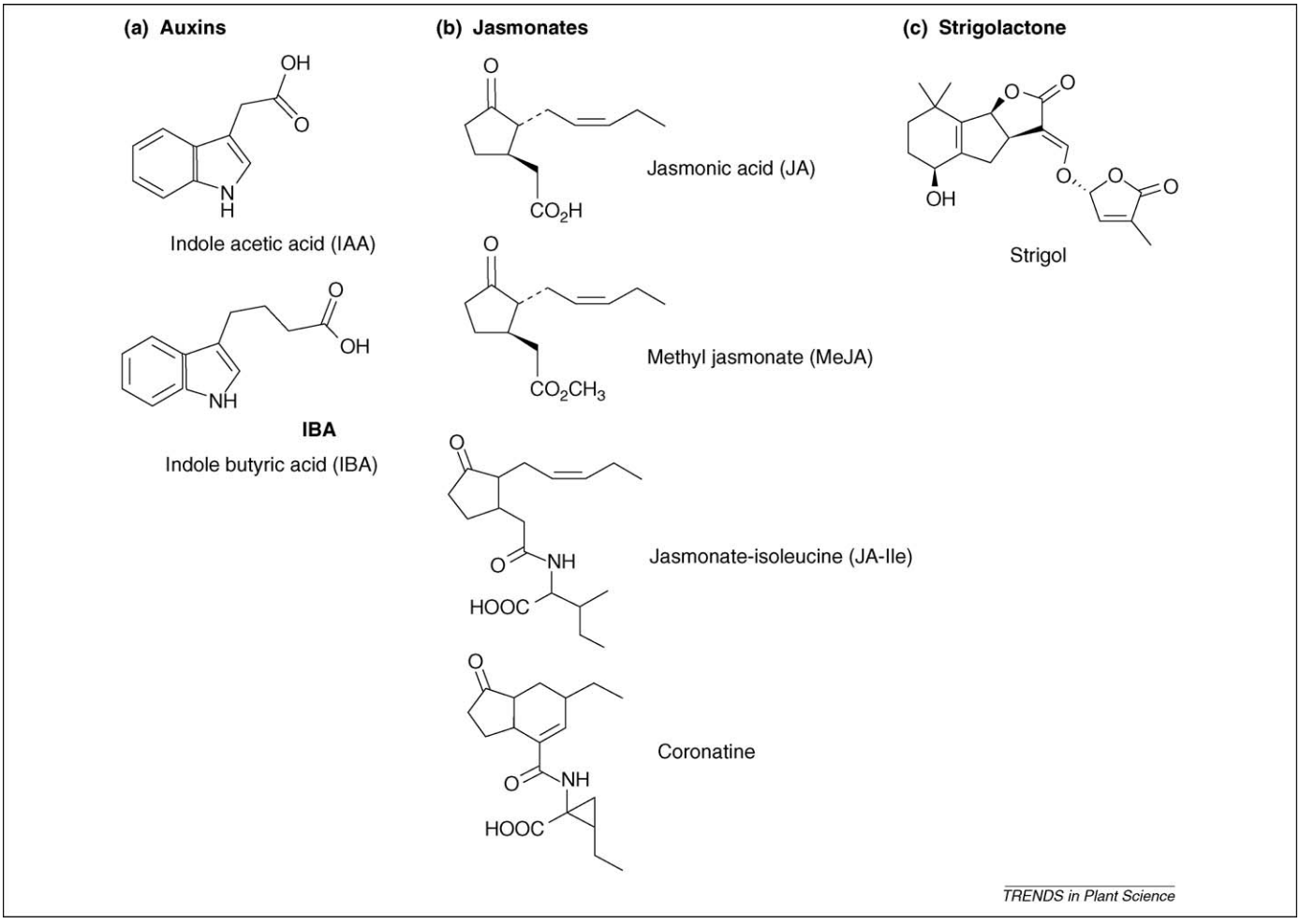

Figure 2. Small-molecule ligands. **(a)** Of these two bioactive auxins, only indole acetic acid (IAA) has been shown to bind to TIR1. **(b)** Although all three of these jasmonic acid (JA)-related compounds are bioactive, only JA-Ile was effective *in vitro* in facilitating a COI1–JAZ1 interaction [35]. Coronatine is a bacterically produced phytotoxin that mimics the action of MeJA in plants but has not been tested *in vitro*. **(c)** Strigol is one of many closely related compounds that are effective as root exudates in promoting germination of *Striga* spp. and other related parasitic plants [39].

associated with enhancing the binding between Aux/IAA proteins and SCF^{TIR1}, but the possibility remained of an unidentified third party auxin receptor [22,23]. The unprecedented finding that TIR1 binds auxin directly followed from a series of *in vitro* pull-downs using radiolabeled IAA and *Xenopus* and insect-cell heterologous expression systems [24,25]. When the crystal structure of TIR1–ASK1 was solved, the site of auxin binding was found near the upper surface of the horseshoe-shaped solenoid structure formed by the 18 LRRs of the protein [26]. The highly conserved domain II, shared among the more than 20 Aux/IAA proteins, binds to TIR1 through a pocket formed by long loops contributed by three of the LRRs. Auxin lies at the bottom of this binding pocket but does not allosterically change TIR1 conformation. Further, a second small molecule, inositol hexakisphosphate ($InsP_6$), co-crystallizes with the complex and might act as a cofactor with auxin in the binding of TIR1 substrates. Together, they seem to act as a 'molecular glue', with auxin acting primarily to fill a cavity between TIR1 and the substrate polypeptide (domain II) by extending the protein interaction interface. IAA analogs (2,4-dichlorophenoxyacetic acid [2,4-D]; 1-naphthalene acetic acid [1-NAA]) also fit the pocket, but generally less well [26].

This exciting finding is only enhanced by knowing that TIR1 is part of a five-member family of auxin F-box (AFB) proteins, which display increasingly severe auxin response defects as multiple mutant combinations are assembled. This, together with their demonstrated auxin-binding properties, suggests overlapping functions among this group of proteins, and it is highly likely that they also bind auxins [27,28]. However, it is currently unclear (i) whether different auxins are bound; (ii) whether different auxin-binding affinities exist between family members; and (iii) whether their expression patterns differ to account for the subtly different roles they seem to play [28].

Jasmonate

Jasmonic acid (JA) has been well characterized as a mobile signal mediating biotic and abiotic stress, and it also regulates various aspects of plant growth and development [29]. As a chemical class, jasmonates are lipid derivatives (oxylipins), and their biological activity can be strongly affected by amino acid conjugation (e.g. JA-Ile) and methylation (MeJA). As in the case of auxin, it was a genetic screen that uncovered the role of an F-box protein in jasmonate signaling. The phytotoxin coronatine is structurally similar to MeJA and was used in a root-inhibition

screen to identify JA signaling elements [30,31]. Mutations in CORONATINE INSENSITIVE1 (COI1) cause insensitivity to coronatine, as well as to MeJA, and strong alleles are deficient in all aspects of the jasmonate response. COI1 is an LRR-containing F-box protein that is strongly related to TIR1 and that has been validated to participate in the formation of an SCF complex (SCF^{COI1}) [32,33].

Identification of COI1 substrates came from transcript profiling studies examining the effects of JA on gene expression and from a genetic screen for jasmonate insensitivity (recovering *jai3*). From these studies, the JAZ (jasmonate ZIM domain) family of proteins was identified as a set of rapidly upregulated JA-responsive genes [34–36]. In a remarkable parallel to auxin signaling, JAZ proteins are negative regulators of JA-induced genes, which are controlled by the basic helix–loop–helix (bHLH) transcription factor MYC2 (Figure 1bii). Analogous to the Aux/IAA repressors, the 12 members of the JAZ protein family do not contain DNA-binding domains but bind to MYC2 through a conserved C-terminal Jas motif [13]. These similarities to auxin signaling inspired experiments to determine whether JAZ proteins are substrates of SCF^{COI1}. *In vivo* tests in *Arabdopsis* using JAZ–β-glucuronidase (GUS), JAI3–green fluorescent protein (GFP) or JAZ1–GFP showed JA-dependent degradation in wild-type plants, but JA had no effect in *coi1* mutants. Further support came from yeast (*Saccharomyces cerevisiae*) two-hybrid interaction tests, which showed that COI1 and JAZ1 binding depends on JA-Ile, and fails when MeJA or the JA precursor, 12-oxo-phytodienoic acid (OPDA), are used. *In vitro* pull-down tests using recombinant JAZ1-His incubated with COI1-myc from tomato (*Solanum lycopersicum*) extracts showed JA-Ile dose-dependent interactions [35,37].

Interestingly, mutation of key charged residues in the Jas motif severely abrogates the JAZ–COI1 interaction but does not affect the JAZ–MYC2 interaction. These mutations also diminish coronatine- and JA-Ile-dependent interactions between JAZ1 and COI1, implicating the jasmonate region as analogous to domain II of the Aux/IAA proteins [38]. If JA-Ile acts, like auxin, as a molecular glue to hold the Jas motif in place, then the interactions it effects seem to be fundamentally different from auxin's role in TIR1. The domain II peptide of Aux/IAA proteins binds to the auxin-bound TIR1 pocket largely through hydrophobic interactions [26]. By contrast, positively charged residues seem to be important for any JA-Ile-bound pocket in COI1 [38]. The final step, demonstration of JA-Ile bound to COI1, is all that remains to unequivocally demonstrate that COI1 is a jasmonate receptor [14].

Unlike the TIR1 family of AFB proteins, SCF^{COI1} seems the sole E3 ligase mediating JA responses [13]. Hence, the variation in the efficacy of different JA conjugates might result from some JA forms being differentially effective in facilitating the binding of some substrates over others (Figure 2). Alternatively, the temporal and spatial expression patterns of the JAZ substrates might be sufficiently varied to account for the wide range of JA-mediated effects.

Strigolactones

A third, very intriguing potential addition to the family of F-box-protein-based phytohormone receptors might come from recent reports that tie together plant parasitism, control of branching and senescence. Strigolactones connect these diverse reports, which came about, in part, as a result of a phenotype in search of a hormone. Strigol, one of the first of this family to be identified, has long been known to have strong stimulatory effects on seed germination in *Striga* spp. [39,40]. This genus of parasitic weeds takes a heavy toll on crop plants in Africa, and initiation of *Striga* seed growth is promoted by very low concentrations of strigol released from the roots of *Striga* hosts, such as maize (*Zea mays*), millet (*Sorghum vulgare*) and other cereal crops.

A completely separate line of research, initiated more than a decade ago, identified a novel mobile signal in pea (*Pisum sativum*) and *Petunia* responsible for the inhibition of shoot branching [41,42]. Grafting experiments in these species identified an acropetally mobile compound that inhibits lateral bud outgrowth. Subsequent work in these species, as well as in rice (*Oryza sativa*) and *Arabidopsis*, identified mutations in *MORE AXILLARY GROWTH3* (*MAX3*, also known as *RAMOSUS5* [*RMS5*], *HIGH-TILLERING DWARF1* [*HTD1*] and *DWARF17* [*D17*]) and *MAX4* (also known as *RMS1*, *D10* and *DECREASED APICAL DOMINANACE1* [*DAD1*]), which code for two different carotenoid cleavage dioxygenases (CCDs), *CCD7* and *CCD8*, respectively [43]. Mutations in these genes cause increased bushiness, or tillering, depending on the species. Two recent reports have linked the branching defects of these mutants with strigolactone deficiencies [44,45]. Strigolactone levels are strongly reduced in *ccd* mutants, and exogenous application of a synthetic strigol analog (GR24) rescues the branching defects. Thus, a firm link between shoot branching and strigolactones has defined a new class of terpenoid plant hormone (Figure 2). In this light, *Striga* species might have co-opted the natural production of strigolactones by host species to use them as a signal indicating that conditions for germination – and subsequent parasitism – are ripe for a successful next generation.

However, of most interest here is the *MAX2* gene. Obtained from the same shoot-branching screen that recovered *MAX1* (a cytochrome P450) [46], MAX2 is an LRR-containing F-box protein that is related to TIR1 and COI1 and that defines an SCF^{MAX2} complex [47]. GR24 can rescue all of the *max* mutants (e.g. *max3* and *max4*) in the strigolactone biosynthetic pathway, except for *max2*. This and other genetic evidence put MAX2 downstream of the strigol hormone [47]. In view of the relationship of auxin and jasmonate to their respective LRR-F-box proteins, MAX2 is in strong contention as a strigolactone receptor.

Notably, *MAX2* had been recovered previously in a screen for senescence-delayed *Arabidopsis* mutants, and recently again in a screen for light signaling mutants. *max2* mutants are also affected in leaf shape and light-dependent germination [48–50]. The three separate isolations of the F-box protein known as ORESARA9 (ORE9), MAX2 and PLEIOTROPIC PHOTOSIGNALING (PPS) raise the intriguing question of how this protein is involved in these diverse processes. If strigalactones are indeed phytohormones, could specificity derive from altered substrate affinities caused by the binding of differently

modified versions of these compounds to MAX2? Several variations on the core structural unit of biologically active strigalactones have been isolated from different species, and all are able to stimulate seed germination in *Striga* and related species [39]. If stage- or tissue-specific forms of strigolactones exist within a plant, MAX2 could be recruited to switch to a different substrate under these conditions, greatly increasing the range of action of this single class of F-box protein. There is still much to be done to fully validate this scheme, but the possibilities are tantalizing.

Tangled up in blue: light as a ligand

Perhaps the most unusual example of a novel ligand-induced effector of an F-box protein is the role of blue light on the stability/activity of ZEITLUPE (ZTL) and FLAVIN BINDING KELCH-REPEAT, F-BOX1 (FKF1). Both proteins belong to a three-member family unique among all known F-box proteins for the presence of a LOV (light, oxygen and voltage sensing) domain N-terminal to the F-box. LOV domains are found in a wide variety of proteins, cutting across all the kingdoms of life. The known light-sensing LOV domains in plants and fungi (*Neurospora*) bind flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as the cofactor that confers blue-light-absorbing properties to these flavoproteins. In plants, the phototropins are protein kinases, dependent on two FMN-binding LOV moieties to enable blue-light mediated autophosphorylation, leading to phototropism [51]. In *Neurospora*, the LOV domain in WHITE COLLAR-1 (WC-1) binds FAD and confers blue-light-dependent DNA binding on this GATA-like transcription factor, which is central to the function of the circadian clock. In plants, FKF1 binds FMN and regulates the degradation of CYCLING OF DOF FACTOR1 (CDF1), and probably other CDF family members, which are repressors of the floral transition [52,53]. *FKF1* is transcriptionally controlled by the clock, but *fkf1* mutants are only late flowering and do not show circadian defects. ZTL also binds FMN and is very closely involved in the control of circadian period, targeting two related proteins, TIMING OF CAB EXPRESSION1 (TOC1) and PSEUDORESPONSE REGULATOR5 (PRR5), for proteasome-dependent degradation through SCF^{ZTL} [54–56]. Both TOC1 and PRR5 are nuclear-localized [55,57], but with no apparent DNA-binding motif, their specific molecular roles in the clock are still unclear [58,59].

The function of the ZTL LOV domain was equally uncertain until the discovery that GIGANTEA (GI) is essential to ZTL protein stability. GI, a novel protein found only in plants, is a key determinant of flowering time; *gi* mutants are very delayed in flowering under long days in *Arabidopsis*. GI also affects circadian period and hypocotyl elongation [60–62]. *ZTL* transcription is constitutive, and ZTL protein levels oscillate with a threefold amplitude under light/dark cycles and in constant light, indicating a circadian-clock-mediated post-translational control of protein abundance [63]. In *gi* mutants, ZTL levels are constitutively low, and ZTL is pegged at peak levels in GI overexpressers, implying that GI stabilizes this protein (Figure 1ci). GI and ZTL interact through the LOV domain, and in blue light the interaction is at least threefold stronger than in the dark or in red light [64]. Because *GI* transcription is clock-controlled, and GI protein levels closely follow GI mRNA rhythms, it is the cycling of GI levels and the blue-light-dependent GI–ZTL interaction that confers a circadian rhythm to ZTL abundance. The stabilization of ZTL by GI in turn affects the abundance of the ZTL substrates, TOC1 and PRR5. In the *gi* mutant, TOC1 protein, which normally cycles with high amplitude, is flattened and intermediate in expression, with higher than normal levels during the photoperiod.

Hence, unlike with TIR1 and COI1, the ligand-binding (blue-light absorption) of ZTL does not directly affect its affinity for TOC1 (or PRR5). Rather, TOC1 and PRR5 levels are regulated indirectly through GI-mediated ZTL stabilization. The dynamics of the GI–ZTL–TOC1 interaction are still unclear, but it is likely that a GI–ZTL interaction excludes TOC1, helping to boost TOC1 amplitude during the diurnal cycle [55]. The strength of the ZTL–TOC1 interaction seems to be independent of light [64], but phosphorylation-dependent interactions with other PRR proteins (e.g. PRR3) might help sequester TOC1 away from ZTL early in the night to help boost TOC1 amplitude [55] (Figure 1cii).

Interaction between FKF1 and GI is also blue-light-dependent, but the effect on FKF1 protein abundance is unclear. What seems likely is that some form of a FKF1–GI–CDF1 complex occupies the promoter of the key flowering-time gene *CONSTANS* (*CO*) late in the photoperiod. One model posits GI first binding to *CO*-promoter-bound CDF1 and then recruiting FKF1 to the site through a blue-light-mediated GI–FKF1 interaction (Figure 1d). Repression of *CO* transcription is then released when SCF^{FKF1}-mediated ubiquitination degrades CDF1 [65]. This view has GI acting as a site-specific (*CO* chromatin) intermediary between FKF1 and CDF1. Still unresolved is the specific nature of the molecular relationship between the three proteins because the same N-terminal portion of GI interacts with both FKF1 and CDF1. Does blue-light absorption by FKF1 effectively outcompete CDF1 for GI binding? How well does FKF1 interact with unbound CDF1, if at all? It will also be interesting to determine whether all the components of SCF^{FKF1} are present at the *CO* promoter, indicating a chromatin-localization of this class of E3 ligase, similar to findings in mammalian and yeast systems [66].

In these two examples, blue light has different roles. For ZTL, the GI–ZTL interaction stabilizes ZTL and might even inhibit formation of SCF^{ZTL}–TOC1 and SCF^{ZTL}–PRR5 complexes. For FKF1, the GI–FKF1 interaction might be important to facilitate subsequent FKF1–CDF1 binding, and GI might act as a type of scaffold protein. In both cases, F-box protein stability and/or activity is greatly modified through the absorption of a light ligand. The ZTL family comprises a novel type of photoreceptor, where light does not initiate a classic signal amplification cascade but simply alters the proteolytic potential of this unique class of F-box proteins.

Perspectives

Whereas the ZTL-type F-box proteins are in a small class of their own, there are more than 42 LRR-type F-box proteins

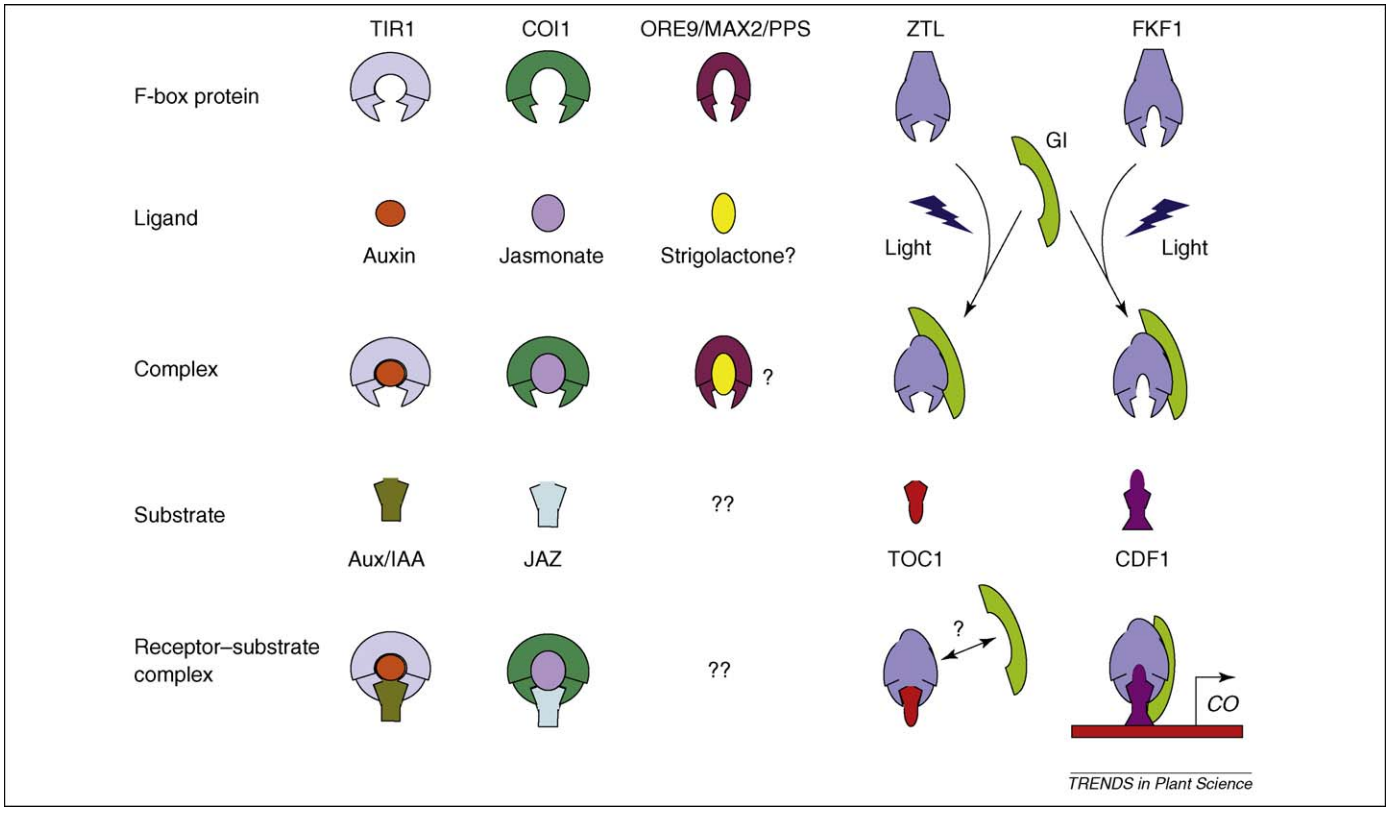

Figure 3. A comparison of receptor F-box proteins. Three examples of LRR-type F-box proteins are aligned based on relationships between known and possible small-molecule ligands and known proteolytic substrates. TIR1 is the prototype of this class of F-box protein, with the complexing of Aux/IAA substrates to TIR1 dependent on auxin bound to the LRR. Not shown is inositol hexakisphopshate ($InsP_6$), which is known to co-purify with TIR1. A COI1-jasmonate-dependent binding of JAZ substrates is also supported by recent reports (see text), and the ligand–F-box relationship here is highly analogous to the TIR–auxin interaction. More speculative is the notion that strigalactones form a third class of small-molecule ligands that bind to the ORE9/MAX2/PPS F-box protein. Currently, there is only genetic and other circumstantial data to support this view, but once substrates are identified there will be rapid progress in testing this hypothesis. When light is viewed as a 'ligand', the ZTL family of proteins fit well into the same scheme. Here, blue light is directly absorbed by the LOV domains of ZTL and FKF1, which greatly enhances GI binding to these proteins (not shown is the third family member, LOV KELCH PROTEIN2 [LKP2], which might have a role in the circadian clock, but it has not been well defined). This interaction stabilizes ZTL and its levels rise in phase with GI, which is transcriptionally controlled by the clock with a peak expression near dusk. In this way, a circadian rhythm is conferred on ZTL post-translationally. TOC1 interaction with ZTL does not require GI binding and might even be sterically inhibited. But the higher ZTL levels help to establish high amplitude cycling of TOC1. By contrast, a light-mediated GI–FKF1 interaction seems to be essential for the recruitment of FKF1 to the *CO* promoter, where the repressor CDF1 is degraded by SCF^{FKF1}. Both GI–CDF1 and GI–FKF1 interactions are key to this de-repression, and it is possible that a three-protein complex exists at the chromatin. In this model, GI has a more direct role in the proteolytic process than with ZTL, because it interacts with both FKF1 and CDF1 through the same N-terminal region.

in plants and another 160 plant-specific LRR derivatives [6]. Given this diversity, it is especially intriguing to consider the extensive possibilities for small-ligand-based signal perception mediated by SCF E3 ligases (Figure 3). Most of the classical hormones are products of secondary metabolic pathways. These pathways are also the source of the immense complexity of plant biochemicals, meaning that a host of additional 'growth regulators' might lie undiscovered [67]. For example, flavonoids have been implicated in the modulation of developmental processes as diverse as auxin transport, pollen germination, root hair growth and allelopathic responses [68], and in systemic acquired resistance (SAR), some signaling-associated small molecules, such as methyl salicylate and an unknown lipid-derived compound, lack known receptors [69]. These are only three examples of potential candidate ligands for still-unidentified F-box proteins that might be involved in plant development and defense.

If substrates are known, clever high-throughput screens using libraries of known or suspected naturally occurring small molecules might be devised to identify ligands that enhance or diminish interactions. Techniques and methods from the field of chemical genomics have already been used to identify several synthetic agonists and antagonists of TIR1 function and auxin responsiveness [70,71]. Newly identified synthetic ligands of known F-box proteins might be exploited to probe the biophysical parameters of the ligand–receptor interaction. They might also be employed to modulate SCF activity through a poisoning or superactivation of the F-box protein, depending on the compound applied. But the greatest challenge will be to determine which of the many remaining F-box proteins of unknown function might also require a type of 'molecular glue' to adhere receptor to substrate.

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