ELSEVIER

F-box proteins everywhere

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The ubiquitin proteasome system is a key regulator of many biological processes in all eukaryotes. This mechanism employs several types of enzymes, the most important of which are the ubiquitin E3 ligases that catalyse the attachment of polyubiquitin chains to target proteins for their subsequent degradation by the 26S proteasome. Among the E3 families, the SCF is the best understood; it consists of a multi-protein complex in which the F-box protein plays a crucial role by recruiting the target substrate. Strikingly, nearly 700 F-box proteins have been predicted in *Arabidopsis*, suggesting that plants have the capacity to assemble a multitude of SCF complexes, possibly controlling the stability of hundreds of substrates involved in a plethora of biological processes. Interestingly, viruses and even pathogenic bacteria have also found ways to hijack the plant SCF and to reprogram it for their own purposes.

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Introduction

Regulation of protein stability through the ubiquitin proteasome system (UPS) is an important mechanism that underlies numerous cellular and organismal processes [1]. Degradation via the UPS is a two-step process: the protein is first tagged by covalent attachment of ubiquitin and subsequently degraded by a multicatalytic protease complex called the 26S proteasome. The ubiquitin conjugation pathway involves several classes of enzymes, the most interesting being the ubiquitin protein ligases (or E3s) that are in charge of the substrate specificity. To date, several hundred different E3s have been predicted in sequenced metazoan and plant genomes, on the basis of commonly shared structural motifs. These E3s fall into different families, among which the SCF (SKP1-CUL1-F-box) is the largest and best characterised.

The SCF complex is composed of four major subunits: Cullin 1 (CUL1), SUPPRESSOR OF KINETOCHORE PROTEIN 1 (SKP1), RING-BOX 1 (RBX1)/REGULA-TOR OF CULLINS 1 (ROC1) and an F-box protein ([2•]; Figure 1). Structure–function studies in yeast and mammals have demonstrated that CUL1 functions as a scaffold in assembling the different subunits of the complex. Thus, CUL1 interacts at its carboxyl terminus with the RING-domain protein RBX1 (forming the core catalytic domain) and, at its amino terminus, with the adaptor protein SKP1, which links to one of the several F-box proteins. F-box proteins, in addition to the loosely conserved F-box motif that binds to SKP1, usually carry one of a variety of typical protein–protein interaction domains that confers substrate specificity to the SCF complexes. This review emphasizes important recent research on the function of F-box proteins in various aspects of plant biology (Table 1).

Dynamic assembly of a multiprotein complex

In plants, the so-called CUL1 (e.g. *Arabidopsis* AtCUL1) is phylogenetically distant from yeast or metazoan CUL1 members and falls into a separate phylogenetic clade [3]. Unlike vertebrates, but like *Caenorhabditis* and *Drosophila*, *Arabidopsis* also encodes a large family of *Arabidopsis* SKP1-LIKE (ASK) proteins [4]. Among the 21 members of this family, ASK1 and ASK2 seem to play prominent roles in plant SCF complexes. This is supported by the fact that they are the most conserved SKP1-related proteins with respect to yeast and human counterparts [5]. In addition, they interact with almost all of the *Arabidopsis* F-box proteins tested, which is not the case for other ASKs [5]. Finally, *ASK1* and *ASK2* are essential for embryogenesis [6], as is *AtCUL1* [3]. Nevertheless, the loss-of-function phenotype of *AtCUL1* is more dramatic than that of the *ask1 ask2* double mutant, and hence other ASKs might also contribute to SCF function during embryogenesis. Strikingly, the *Arabidopsis* genome encodes about 700 F-box proteins [7]. This number is significantly higher than that in other eukaryotes for which full genome sequences are available, and indicates that SCF-dependant ubiqui-tylation is a major route for selective protein degradation in plants.

AtCUL1 function is also regulated by the covalent linkage of a ubiquitin-like protein, called RELATED TO UBI-QUITIN1 (RUB1)/NEURAL PRECURSOR CELL EXPRESSED, DEVELOPMENTALLY DOWNRE-GULATED 8 (NEDD8) [8]. *Arabidopsis* encodes three RUB-like proteins, two of which (RUB1 and RUB2, are essential and regulate diverse processes throughout plant

Figure 1
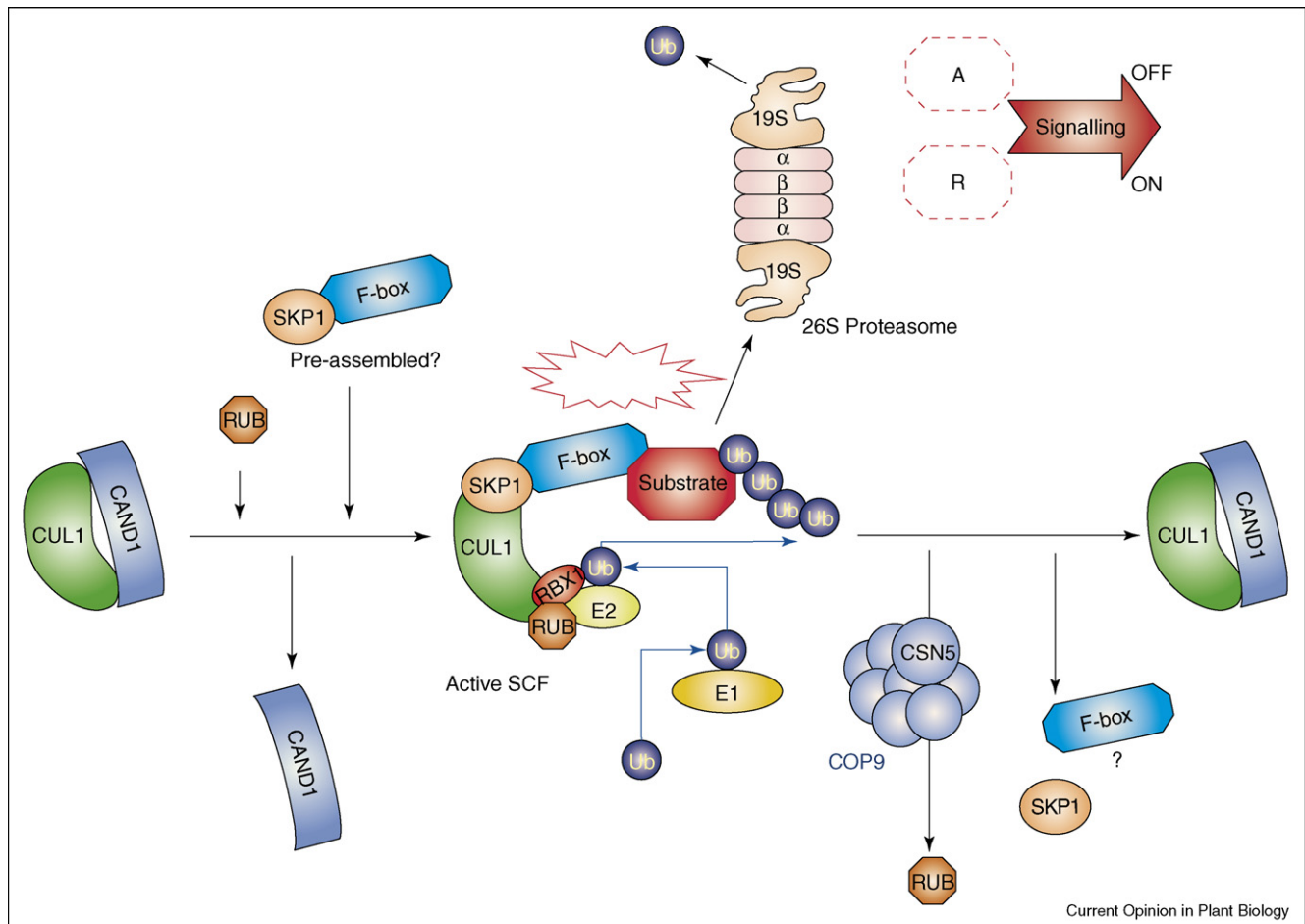
Model for SCF-dependent ubiquitylation and subsequent protein degradation. Free CUL1 interacts with CAND1. Upon RUB modification, CUL1 dissociates from CAND1. This allows the association of SKP1 (or ASK1/2 in *Arabidopsis*) and the F-box protein, which might already exist as an heterodimer before entering the complex. At this stage, the SCF is assembled. Additional important regulations (illustrated by a red star) are required for the SCF interact with its substrates. In most described cases, it is the substrate that is modified at the post-translational level, but additional regulations might operate; for example, the binding of auxin to the F-box protein in plants. Conjugation of ubiquitin to the protein target also requires two other enzymes: the ubiquitin-activating enzyme (E1) forms a high-energy thioester intermediate (E1-S~Ubi) that is then *trans*-esterified to one of the several ubiquitin-conjugating enzymes (E2). The transfer of ubiquitin from E2-S~Ubi to an ε-NH$_2$ group of an internal lysine residue in the target protein substrate is mediated by the SCF. A polyubiquitin chain is synthesized by successively adding ubiquitin moieties to the previously conjugated ubiquitin molecule. Multiubiquitylated proteins are then recognized by the 26S proteasome and proteolyzed into peptides, and ubiquitin is recycled through the action of de-ubiquitylating enzymes (not represented). As the protein target is usually either an activator (A) or a repressor (R) of a signalling pathway, its degradation switches the pathway either OFF or ON. What happens to the SCF after the substrate is polyubiquitylated is poorly understood. It is possible that RUB-deconjugation by the CSN5 subunit of the COP9 signalosome triggers the disassembly of the complex and thus resets the mechanism.

development [9]. RUB can be removed from CUL1 by the peptidase activity of the COP9-signalosome (CSN) [10]. Both the RUB conjugation and deconjugation pathways are important for optimal activity of the SCF, and it is thought that this modification controls the assembly and thus the activity of the complex (Figure 1). Indeed, it has been proposed that RUB1/NEDD8-modification of CUL1 dissociates CAND1 (CULLIN-ASSOCIATED AND NEDDYLATION-DISSOCIATED1), an inhibitor of the SCF, and consequently promotes the binding of SKP1 and an F-box protein to CUL1 [8]. *Arabidopsis* CAND1, which is encoded by a single gene, interacts preferentially with unmodified CUL1 and is also necessary for optimal SCF activity [11,12].

F-box proteins in plant hormone response pathways

Indole-3-acetic acid (IAA or auxin) is involved in many aspects of plant development and was the first phytohormone whose signalling pathway was shown to involve an SCF complex. The F-box protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1) is part of an SCF complex

Table 1

Overview of plant and microbial F-box proteins, their substrates and known biological functions.

F-box proteins	Motif	Demonstrated or putative substrates	Regulation	Biological process	References
TIR1 **AFB1-3**	LRR	Aux/IAA	Auxin binding	Auxin signalling	[13,17••,18••,19•,39]
COI1	LRR	Histone deacetylase?	?	JA signalling	[21,22,63]
SLY1 **SNE** **GID2**	–	DELLAs	Phosphorylation? Interaction with the GA-activated receptor?	GA signalling	[23–26,29,30,31••]
EBF1 and EBF2	LRR	EIN3	?	Ethylene signalling	[32–34]
TLP9	Tubby domains	?	?	ABA signalling?	[64]
EID1	Leucine-zipper	?	?	phyA signalling	[42,43]
AFR	Kelch repeats	?	?		[44]
ZTL	LOV/PAS domain and Kelch repeats	TOC1	?	Circadian clock	[45,47–49,50•,65]
FKF1		CDF1	?		
LKP2		?	?		
UFO **FIM**	–	?	?	Floral development	[35–37]
MAX2/ORE9	LRR	?	?	Shoot branching Leaf senescence	[38,66]
ARABIDILLO1 and ARABIDILLO2	Arm-repeats	?	?	Lateral root development	[40]
CEGENDUO	–	?	?		[41]
SFB/SLF	–	*S-RNAses*?	?	Self-incompatibility	[51,52••,53,55]
SKP2A	LRR	*E2Fc*?	Phosphorylation	Cell cycle	[67]
SON1	–	?	?	Defence response	[68]
CLINK	LxCxE motif	*pRB*?	?	Host DNA replication	[58]
P0	–	?	?	Host RNA silencing	[59•]
VirF	–	VIP1 and VirE2	?	T-DNA uncoating	[60,61•]

(–) indicates that the F-box protein does not contain a recognisable protein–protein interaction domain. Putative substrates, which have at least been shown (in yeast or *in vitro*) to physically interact with their respective F-box proteins, are indicated in italics, whereas demonstrated substrates are written in uppercase.

that mediates auxin-dependant transcriptional control by targeting certain AUX/IAA proteins for ubiquitin-dependant degradation [13]. AUX/IAA proteins serve as repressors of auxin action by binding to and blocking the AUXIN RESPONSE FACTOR (ARF) transcription factors, which activate auxin-inducible genes [14]. Although auxin is known to stimulate the binding of Aux/IAA proteins by the SCF^TIR1 complex [13,15], the molecular details of this mechanism were unknown until recently. Pharmacological and biochemical studies showed that post-translational modifications of the Aux/IAA proteins, such as phosphorylation (a modification occurring on many SCF substrates), are not involved in this mechanism [15,16]. The major breakthrough was achieved, however, when two different laboratories demonstrated that auxin binds to TIR1 and, as a consequence, promotes the interaction of SCF^TIR1 with the Aux/IAA proteins [17••,18••]. This finding is very important because it establishes TIR1 as an auxin receptor. Furthermore, it suggests that F-box proteins have the capacity to bind directly to small signalling molecules and that this binding can modify SCF activity.

Nevertheless, we still do not know which protein domain of TIR1 binds to auxin and how this binding promotes SCF^TIR1 interaction with the Aux/IAA proteins.

TIR1 loss-of-function mutants exhibit only a weak auxin-resistance phenotype, and so it is likely that other similar auxin receptors exist. Indeed, three additional TIR1-related F-box proteins, called AUXIN SIGNALLING F-BOX PROTEIN 1–3 (AFB1–3), that also interact with Aux/IAA proteins in an auxin-dependant manner have been identified [19•]. Genetic evidence indicates that all four F-box proteins act redundantly to mediate auxin responses during embryogenesis and throughout plant development. Interestingly, recent work has also shown that expression of these F-box proteins is repressed by bacterial flagellin through a mechanism that involves a microRNA (miRNA) [20•]. This evidence indicates that downregulation of auxin signalling is part of a pathogen-induced immune response [20•].

SCF complexes also regulate other phytohormones signalling pathways, including the jasmonate, gibberellin

and ethylene pathways. Regulation of jasmonate signalling involves the *Arabidopsis* F-box protein CORONATINE INSENSITIVE1 (COI1), which is part of an SCF complex [21,22]. At present, COI1's protein target(s) remain(s) unknown. Knowledge is more advanced for the gibberellin (GA) signalling pathway, which is regulated in *Arabidopsis* by the F-box proteins SLEEPY1 (SLY1) and SNEEZY (SNE) [23–25] and in rice by the F-box protein GID2 [26]. Like TIR1, these F-box proteins are involved in the degradation of negative regulators of phytohormones responses. In the GA response, these negative regulators are the DELLA proteins, which belong to the GRAS superfamily of putative transcriptional regulators that directly or indirectly repress the expression of GA-induced genes [27]. DELLAs seem to modulate plant growth in response to diverse environmental signals and, in particular, can restrain plant growth in adverse conditions [28•]. Conversely, GA stimulates plant growth by promoting the destruction of DELLAs. In contrast to auxin signalling, the degradation of DELLA proteins appears to be regulated by phosphorylation [26,29,30]. Bioactive GAs do not bind to the F-box proteins directly but rather bind to a recently identified receptor, GIBBERELLIN INSENSITIVE DWARF1 (GID1) [31••]. Interestingly, GID1 interacts with the rice DELLA-related protein SLENDER RICE1 (SLR1) in a GA-dependent manner and renders SLR1 degradable by the SCF^{GID2} proteasome pathway. However, the molecular details of these interactions are not known and the role of DELLA phosphorylation in this model is unclear.

In the signalling pathway for the gaseous plant hormone ethylene, two *Arabidopsis* F-box proteins, EIN3 BINDING F-BOX PROTEIN 1 (EBF1) and EBF2, target the transcriptional activator ETHYLENE INSENSITIVE3 (EIN3) for degradation [32–34]. EIN3 is expressed constitutively but is unable to accumulate because it is subjected to permanent proteolysis mediated by EBF1 and EBF2. EIN3 becomes stabilised and acts on its target promoters only upon perception of ethylene. It is worth noting that SCF-dependent proteolysis in ethylene signalling differs significantly from that in the responses to auxin and GA in that a transcription activator (EIN3) instead of repressors (Aux/IAA and DELLA proteins, respectively) is degraded. Moreover, proteolysis is switched off after ethylene perception whereas it is activated in response to auxin and GA, probably by the binding of the hormones to their receptors.

F-box proteins in lateral root formation
Several F-box proteins have been implicated in organ formation and development. These proteins include UNUSUAL FLORAL ORGANS (UFO) and FIMBRIATA (FIM), which control multiple aspects of floral development [35–37], and MAX2, which represses shoot lateral branching [38]. As auxin plays a pivotal role in almost every aspect of plant development, it is perhaps not surprising that a mutant that has a defect in the *Arabidopsis* F-box protein TIR1 is deficient in lateral root formation [39]. Recently, other classes of *Arabidopsis* F-box proteins were also shown to be involved in lateral root formation. Thus, two Armadillo-related F-box proteins (called ARABIDILLO-1 and ARABIDILLO-2) promote root branching by a mechanism that does not seem to involve modulation of auxin perception or response [40]. Finally, whereas loss of *TIR1* or *ARABIDILLO-1* and *ARABIDILLO-2* function reduces lateral root formation, a mutation in another F-box gene, *CEGENDUO*, leads to an increase in lateral root production [41], suggesting a complex interplay of degradation events in lateral root development.

F-box proteins in light signalling and clock control
F-box proteins have been implicated in phytochrome A (phyA)-dependant light signalling. Mutants that have defects in the F-box-protein encoding gene *EMPFINDLICHER IM DUNKELROTEN LICHT* (*EID1*) exhibit increased far-red light sensitivity and, thus, it has been proposed that an SCF^{EDI1} E3 targets positive phyA signal transducers(s) for proteolysis [42]. Moreover, *EID1* modulates phyA-dependant light responses during all stages of plant development [43]. ATTENUATED FAR-RED RESPONSE (AFR) is another F-box protein that is involved in phyA-dependant signalling [44]. Mutations in *EID1* lead to hypersensitivity to far-red light whereas, conversely, reduction of AFR protein by RNA interference leads to far-red light hyposensitivity. Thus, the AFR protein might mediate the degradation of a repressor of phyA signalling. The identification of protein targets of both EID1 and AFR will certainly help to unravel their complex interplay in light responses.

The role of SCF-dependant protein degradation is better understood in the control of the photoperiod. The circadian clock allows plants to measure day length and thus to control various physiological and developmental processes, such as flowering time. The F-box protein ZEITLUPE (ZTL) was the first UPS component to be implicated in the plant circadian system [45] and was shown to assemble into an SCF complex *in vivo* [46]. ZTL is involved in the dark-dependant degradation of TIMING OF CAB EXPRESSION 1 (TOC1) [47], a component of the oscillator of the circadian clock. TOC1 promotes transcription of CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), two other core components of the central oscillator of the circadian clock. To explain ZTL's function in periodicity control, however, it seems likely that ZTL has substrates in addition to TOC1 [48]. ZTL belongs to a small family of three genes, which also includes the FLAVIN-BINDING, KELCH-REPEAT,

F-box 1 (FKF1) and the LOV KELCH PROTEIN2 (LKP2). All three proteins contain an amino-terminal LIGHT, OXYGEN OR VOLTAGE (LOV) domain, a central F-box, and Kelch repeats in the carboxy-terminal domain. Interestingly, the LOV domain of FKF1 binds the chromophore flavine mononucleotide, and it has been suggested that FKF1 might function as a periodic blue-light photoreceptor [49]. Moreover, FKF1 was found to control the daytime expression of CONSTANS (CO), which is crucial for photoperiod-dependant flowering [49]. However, the mechanism by which the temporal expression of CO is controlled by FKF1 remained unknown until recently. New data now indicate that FKF1 targets CYCLING DOF FACTOR 1 (CDF1), a Dof (DNA binding with one finger) transcriptional repressor of *CO* [50•].

F-box proteins in pollen recognition and rejection

Self-incompatibility interactions in Solanaceae, Scrophulariaceae and Rosaceae, which prevent inbreeding, are controlled by pistil-expressed S-RNases that act as cytotoxins to inhibit the growth of pollen that has a matching *S*-allele [51]. Strikingly, clusters of F-box genes known as *SFB* or *SLF* (*S-linked F-box* genes) have recently been found close to the S-RNase genes in *Petunia*, and these genes have been proposed to control specificity on the pollen side [52••]. A role for an F-box protein, AhSLF-S2, in self-incompatibility has also been demonstrated in *Antirrhinum* [53]. AhSLF-S2 is able to interact not only with an *Antirrhinum* pollen-specific ASK1-like protein [54] but also directly with its putative substrates: the S-RNases [55]. A current model therefore proposes that the F-box proteins specifically inhibit non-self S-RNases by targeting them for ubiquitin-dependant degradation. However, this model is not consistent with the fact that the *Antirrhinum* AhSLF-S2 protein also binds self-S-RNase, at least *in vitro* [55], or with the finding that an *slf* loss-of-function mutant in *Prunus avium* is self-compatible [56]. According to the model, the absence of the F-box should lead to universally incompatible pollen. Thus, additional research is needed to elucidate the molecular details of this system.

F-box proteins encoded by plant pathogenic microbes

It is well established that animal viruses manipulate the UPS to favour their infection [57]. In some cases, viruses directly encode E3 components, whereas in others, host E3s are redirected to serve viral purposes. Interestingly, two plant viruses have been found to encode F-box proteins. The Faba bean necrotic yellow virus protein CELL CYCLE LINK (CLINK) contains an F-box motif and binds to MsSKP1, an alfalfa SKP1 homologue [58]. The function of CLINK has not yet been established but it is suspected to trigger host DNA replication by targeting a RETINOBLASTOMA RELATED PROTEIN

(RBR) protein. Interestingly, an F-box motif was also recently found in the polerovirus P0 protein, a suppressor of gene silencing [59•]. Mutations in the F-box abolish P0's interaction with the SKP1-related ASK1/2 and reduce its silencing suppressor activity, thus diminishing virus pathogenicity. Consistently, SKP1 knockdown in *Nicotiana benthamiana* conferred higher plant resistance to polerovirus infection. An interesting hypothesis is that P0 is part of an SCF complex that targets a component of the host posttranscriptional gene-silencing machinery.

No less smart than viruses, pathogenic bacteria have also found ways to re-design SCF complexes. The first example came from *Agrobacterium tumefaciens*, which leads to the formation of crown gall tumors. This bacterium encodes an F-box protein called VirF that functions within the plant cell and where it interacts with plant SKP1-related ASK1/2 proteins [60]. Although VirF is specifically required during the infection process, its mechanism of action has remained uncharacterised. However, a recent report provides strong evidence that VirF is involved in turnover of both the host protein VIP1 and the bacteria-encoded protein VirE2, and thus might contribute to the uncoating of the T-DNA before its integration into the plant genome [61•]. The fact that VirF is required for some but not all plant species is still intriguing. VirF will very likely not be the sole example of this kind, as a glimpse into other bacterial genomes reveals additional putative F-box proteins. One example is the soil pathogenic bacterium *Ralstonia solanacearum*, which encodes several F-box proteins, some of which are translocated into the plant cell through the type III secretion system (S Genin, N Peeters, pers. comm.).

Conclusions and perspectives

If the nearly 700 predicted *Arabidopsis* F-box proteins [7] all form SCF complexes, it is evident that we are still very far from having an integrated picture of their functional repertory. Conditional mutants that affect core components of the SCF, such as the recently described *auxin response 6-3* (*axr6-3*) allele of *AtCUL1* provide further evidence that novel pathways that are regulated by SCFs remain to be characterized [62]. Elucidation of these pathways, at the molecular level, will certainly keep more than one laboratory busy in the coming years. Such a goal will be challenging, however, for at least two reasons. First, many F-box proteins are encoded by large gene families, which makes genetic approaches difficult because of functional redundancy. Second, in many cases, the protein target of an SCF requires post-translational modification(s) (often phosphorylation) in order to be recognised. Even worse, as reported for TIR1, the SCF itself might bind directly to different signalling molecules in order to interact with its substrate(s). But never fear, our guess is that we will learn much more in the near future about novel F-box protein targets and novel signalling pathways in which they are involved, as well as

about SCF regulation by docking proteins and even small metabolic compounds.

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