Published in final edited form as:
Nat Rev Mol Cell Biol. 2013 June ; 14(6): . doi:10.1038/nrm3582.

Mechanisms and function of substrate recruitment by F-box proteins

Jeffrey R. Skaar[1], **Julia K. Pagan**[1], and **Michele Pagano**[2]

[1]Department of Pathology, New York University Cancer Institute, New York University School of Medicine, 522 First Avenue, SRB 1107, New York, New York 10016, USA

[2]Howard Hughes Medical Institute, Department of Pathology, New York University Cancer Institute, New York University School of Medicine, 522 First Avenue, SRB 1107, New York, New York 10016, USA

Abstract

S phase kinase-associated protein 1 (SKP1)–cullin 1 (CUL1)–F-box protein (SCF) ubiquitin ligase complexes use a family of F-box proteins as substrate adaptors to mediate the degradation of a large number of regulatory proteins involved in diverse processes. The dysregulation of SCF complexes and their substrates contributes to multiple pathologies. In the 14 years since the identification and annotation of the F-box protein family, the continued identification and characterization of novel substrates has greatly expanded our knowledge of the regulation of substrate targeting and the roles of F-box proteins in biological processes. Here, we focus on the evolution of our understanding of substrate recruitment by F-box proteins, the dysregulation of substrate recruitment in disease and potential avenues for F-box protein-directed disease therapies.

The irreversibility of ubiquitin-mediated proteolysis makes the ubiquitin–proteasome system the ultimate on–off switch of a cell. It controls many processes, particularly those that must proceed unidirectionally, such as the cell cycle or circadian oscillations. Covalently linked chains of the small protein ubiquitin are generated on substrates through an enzymatic cascade in which ubiquitin is activated by an E1 enzyme, transferred to an E2 ubiquitin-conjugating enzyme and then transferred to a substrate selected by an E3 ubiquitin ligase[1]. Ubiquitin chains of four or more moieties, linked through either Lys48 or Lys11 of each ubiquitin, direct substrates to the proteasome, which is a large complex of proteases that degrades ubiquitylated proteins[2]. Multiple monoubiquitins and other, non-Lys63-linked ubiquitin chains have also recently been implicated in protein degradation, and the study of alternative degradation signals is continuing[2,3]. It is estimated that >80% of proteins undergo ubiquitin-mediated degradation, so the selection of specific substrates by E3 ubiquitin ligases in response to specific stimuli is a crucial factor in cell regulation[4]. In humans, there are only two E1 enzymes and ~30 E2 enzymes, but there are hundreds of E3 enzymes. Many E3 ligases are modular, based on a core scaffold with interchangeable substrate-targeting subunits, which enable one piece of the core machinery to ubiquitylate many different substrates. The cullin–RING ligase (CRL) family complexes are the archetypes for these modular ubiquitin ligases (FIG. 1), and CRL1 ligases, better known as

*Correspondence to M.P. and J.R.S.*michele.pagano@nyumc.org; skaarlab@gmail.com.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Michele Pagano's homepage: http://pathology.med.nyu.edu/Pagano/The_Michele_Pagano_Laboratory.html

the S phase kinase-associated protein 1 (SKP1)– cullin 1 (CUL1)–F-box protein (SCF) complexes, are the best characterized[5].

SCF complexes are assembled using the scaffold protein CUL1. CUL1 bridges two essential biochemical functions of the SCF complex. The carboxyl terminus of CUL1 recruits the small RING protein RBX1, which directs the E2 enzyme to the E3 ligase, and the amino terminus of CUL1 binds SKP1 and a variable F-box protein that dictates substrate specificity[5]. As their name implies, F-box proteins contain the 40-amino-acid F-box domain (first identified in cyclin F (also known as FBXO1)), which binds SKP1 to create a link to CUL1. Sixty-nine human F-box proteins, each targeting multiple substrates, enable the core scaffold to select hundreds of proteins for degradation[6,7].

Although the generic SCF complex is considered well-characterized, many questions remain about the functions of specific SCF complexes. One glaring gap in our understanding of the SCF complexes is the number of orphan F-box proteins, for which no substrates are known. Our conceptions of the functions of SCF complexes are based on relatively few unique F-box protein– substrate pairs and the mechanisms controlling these pairings. Therefore, the field is rapidly evolving with the characterization of each orphan F-box protein. This Review highlights key recent advances in our mechanistic understanding of substrate recruitment by F-box proteins and our understanding of the overall biological roles of F-box proteins. An increased understanding of substrate targeting, coupled with knowledge of the roles of F-box proteins in disease, presents new targets for therapeutics, although this area of research remains in its infancy.

The F-box protein families

F-box proteins can be categorized on the basis of the presence of recognizable domains beyond the F-box domain, thereby generating three families[6,8,9]. In humans, the F-box and WD40 domain (FBXW) family is composed of 12 proteins, and the F-box and Leu-rich repeat (FBXL) family comprises 21 proteins. The remaining 36 F-box proteins were originally categorized as F-box only (FBXO) proteins, but contrary to their name, these F-box proteins often have conserved homology domains that were either not recognized or are not present in a large number of F-box proteins. Therefore, FBXO is more truly an abbreviation for F-box and other domains. At least 21 homology domains have been identified among the FBXO family members, and it is assumed that these homology domains mediate the interactions of F-box proteins with substrates[6]. So far, this hypothesis has received wide experimental support from studies of cyclin F, FBXO2, FBXO6 and FBXO11 (REFS 10–13).

Substrate recruitment to SCF complexes

SCF complexes are subject to general regulation through multiple mechanisms, including covalent modification with NEDD8 and the binding of various assembly factors (BOX 1). These general regulatory mechanisms are central to the biochemistry of the SCF complexes, but they remain poorly understood and/or controversial. In addition, as global regulators of SCF complexes, these mechanisms do not control specific substrates and biological functions. Overall, the ultimate regulation of the activity of specific SCF complexes occurs at the level of substrate recruitment. In response to stimuli, F-box proteins must rapidly and specifically bind their target proteins in the complex cellular milieu and recruit them to the core SCF scaffold, which exerts ubiquitin ligase activity. Conversely, binding between an F-box protein and its substrate can be perturbed in response to stimuli. Therefore, both F-box protein–substrate interfaces and the F-box proteins themselves are subject to tight regulation.

Canonical phosphodegrons

F-box proteins bind substrates in response to various stimuli, and in the canonical model, they bind short, defined degradation motifs (known as degrons)[14]. For many years, phosphorylation-dependent substrate recruitment was the paradigm for SCF complex function, and the best-characterized F-box proteins bind to phosphodegrons in their substrates. For example, β-transducin repeat-containing protein (βTrCP; which refers to F-box proteins encoded by both *FBXW1* and *FBXW11*) binds the consensus degron Asp-Ser-Gly- Xaa-Xaa-Ser (in which Xaa represents any amino acid and both Ser residues are phosphorylated), and FBXW7 binds the degron Thr-Pro-Pro-Xaa-Ser (in which the Thr and Ser residues are phosphorylated)[15,16]. Variation from the consensus degrons, such as substitution of Ser and Thr residues or the inclusion of phosphomimicking amino acids, is observed. Simple phosphodegrons can be phosphorylated by a single kinase. Alternatively, degrons with multiple phosphorylation sites can be targeted by multiple kinases and/or use priming phosphorylations, which adds increased complexity and stringency to substrate recognition by F-box proteins. For example, cyclin-dependent kinase 2 (CDK2) and glycogen synthase kinase 3 (GSK3) phosphorylate different residues of the cyclin E degron[17], and phosphorylation of the JUN degron by GSK3 requires prior phosphorylation of the degron by an additional kinase[18]. Degron phosphorylation can also require priming phosphorylations that are near, but not directly at, the F-box protein–substrate interface, such as the priming phosphorylations that are required for β-catenin regulation by βTrCP[19].

Finally, phosphodegrons can be used to fine-tune substrate recognition by setting a threshold of kinase activity that creates a direct linkage to a regulatory pathway. The yeast CDK inhibitor Sic1 is degraded to allow S phase entry, and recognition of Sic1 by the Cdc4 F-box protein requires the phosphorylation of at least six of nine specific amino acids of Sic1 by CDKs. These phosphorylations generate multiple suboptimal degrons, the binding affinity of which is sufficient to increase the local concentration of Sic1 associated with SCF^{Cdc4} (an SCF complex containing Cdc4), producing highly specific and efficient binding[20–22]. CDK activity increases during G1 phase of the cell cycle, eventually reaching a threshold level that is required for the recognition of Sic1 by SCF^{Cdc4}, resulting in Sic1 degradation and entry into S phase.

However, as the larger F-box protein family is explored, it is becoming clear that although direct recognition of phosphodegrons is a common mechanism for substrate recruitment, it is not the only mechanism (FIG. 2). In addition, multiple recruitment mechanisms can be combined, which enables more precise regulation of substrate selection by each SCF complex.

Cofactor-dependent substrate recognition

SKP2 (also known as FBXL1)-dependent degradation of the CDK inhibitor p27 presents one permutation of phosphorylation-directed substrate binding. Following the phosphorylation of Thr187 of p27 by a CDK, SKP2 binds p27 leading to its ubiquitylation and degradation. However, when this ubiquitylation reaction is reconstituted *in vitro*, SCF^{SKP2} by itself has poor ubiquitylation activity towards p27 (REFS 23,24). Full activity requires CKS1 (CDK regulatory subunit 1), an accessory protein that binds both SKP2 and p27 (REFS 25,26). This model seems to fit the canonical view of phosphorylation-dependent binding of F-box proteins, but the crystal structure of the SKP2–CKS1–p27 phosphopeptide complex indicates otherwise[27]. CKS1, but not SKP2, forms contacts with phosphorylated Thr187 of p27, so the accessory protein CKS1 is integral to substrate recognition by SKP2. It is currently unclear how many F-box proteins — and belonging to which families — might use accessory proteins and whether all accessory proteins control

substrate recognition. In this regard, FBXO4 has been reported to use the chaperone αβ-crystallin as a cofactor for phosphorylation-dependent substrate recognition, but it is not clear whether FBXO4 and/or αβ-crystallin directly recognize the phosphorylated substrate [28,29]. Ubiquitylation of the cofactor Met4 in yeast presents a variation on this theme. This transcription factor itself is inactivated by SCF^{Met30}-mediated ubiquitylation and, concomitantly, Met4 facilitates ubiquitylation of several of its cofactors, providing coordinate regulation[30].

Unmodified degrons

Degron phosphorylation facilitates the rapid and specific regulation of substrate selection by SCF complexes, but studies of cyclin F show that F-box proteins can also recognize unmodified degrons. Cyclin F does not bind CDKs, but its cyclin-homology domain recognizes an Arg-Xaa-(Ile, Leu) motif in its substrates in a manner similar to the way that other cyclins recognize their substrates for phosphorylation by CDKs[10,11]. Cyclin F uses this recognition mechanism for all known substrates, including the centrosomal protein CP110 and ribonucleotide reductase subunit M2 (RRM2), which provides evidence of the utility of unmodified degrons[10,11]. Because the Arg-Xaa-Leu degron is independent of any modification, substrates containing this motif would be degraded constitutively in the absence of additional regulatory mechanisms. Such mechanisms include the restriction of degron access and indirect regulation of substrate recognition through the control of cyclin F localization and stability.

Restricted degron access

Physical access of F-box proteins to substrate degrons can be controlled by the phosphorylation of motifs other than the degron, and such control can be combined with multiple degron types, including non-modified degrons and phosphodegrons. This regulation is exemplified by the ubiquitylation of RRM2 by cyclin F[10]. Ribonucleotide reductase generates deoxyribonucleotides for DNA synthesis, and in G2 phase of the cell cycle, the enzyme is inactivated by degradation of RRM2. To impart cell cycle-dependent regulation, access to the Arg-Xaa-Ile degron in RRM2 is controlled by CDK-mediated phosphorylation of RRM2. When CDK activity is low, the degron is obscured, but high CDK activity in G2 causes RRM2 phosphorylation at Thr33, which is not part of the degron, enabling recognition of the unmodified Arg-Xaa-Ile degron by cyclin F. Other SCF substrates might also use phosphorylation-regulated degron access, but in the case of phosphodegrons, it is difficult to determine whether these external phosphorylation events are priming events for the degron, control degron access, or both[31–33].

Indirect regulation of substrate recognition

$SCF^{cyclin\ F}$–mediated ubiquitylation of RRM2 also depends on the localization of both the substrate and SCF complex. RRM2 is a cytoplasmic protein, and cyclin F is a nuclear protein. Therefore, cyclin F-dependent RRM2 degradation requires nuclear import of RRM2 (REF. 10). Localization also affects the ubiquitylation of other cyclin F substrates, such as CP110. CP110 controls centrosome duplication, and its expression is closely linked to the cell cycle. CP110 localizes exclusively to the centrosome, and its degradation requires a subpopulation of cyclin F at the centrosome[11].

Subcellular localization also controls other F-box proteins. FBXL2 and FBXL20 contain Cys-Ala-Ala-Xaa (CAAX) motifs, which undergo isoprenylation, directing the proteins to membranes[34,35]. CAAX-dependent membrane localization of FBXL20 is required for the degradation of RAB3-interacting molecule 1 (RIM1), and membrane-associated FBXL2 is required for hepatitis C virus (HCV) replication (through unknown substrates).

The localization-dependent functions of cyclin F, FBXL2 and FBXL20 highlight the ability of SCF complexes to control substrates in both a spatial and temporal manner.

In fact, the temporal activation of cyclin F is regulated through tight control of its expression levels. Cyclin F levels are modulated throughout the cell cycle by transcription and by degradation involving an unknown ubiquitin ligase[10,36]. When cyclin F levels increase in G2 phase of the cell cycle, cyclin F substrates are degraded. Regulation of other F-box proteins by degradation has also been reported. The cell cycle-dependent oscillations of SKP2 levels are controlled by the APC/C^{CDH1} (that is, the anaphase-promoting complex (also known as the cyclosome) containing CDC20 homologue 1), and EMI1 (early mitotic inhibitor 1; also known as FBXO5) is regulated in a cell cycle-dependent manner by $SCF^{\beta TrCP}$ (REFS 37–40). F-box protein stability might also be controlled through autoubiquitylation mediated by the SCF scaffold, but the specificity of such regulation remains unclear[41].

Most examples of F-box protein degradation are linked to the cell cycle, but the regulation of FBXL5 is unique in this regard. FBXL5 mediates the degradation of iron regulatory protein 2 (IRP2) under conditions of high iron and/or oxygen levels, and in turn, IRP2 controls the translation and stability of iron-responsive mRNAs, including those encoding proteins related to iron metabolism and enzymes that are functionally dependent on iron[42–44]. The key feature of this regulatory system is a haemerythrin domain in FBXL5 that binds two iron ions. In iron-depleted conditions, the haemerythrin domain cannot bind iron, resulting in FBXL5 unfolding and degradation (through an unknown ubiquitin ligase)[45,46]. The properly folded haemerythrin domain confers stability to FBXL5 in the presence of iron, but it does not affect substrate binding, highlighting the direct regulation of F-box proteins in response to environmental cues. Iron availability might also affect FBXL5–IRP2 binding in a haemerythrin domain-independent manner, but this intriguing mechanism remains to be investigated[43]. The modification of F-box proteins, whether by iron coordination or covalent modifications, in response to stimuli to increase F-box protein stability and substrate degradation is an emerging theme that is also illustrated by ataxia-telangiectasia mutated (ATM)-mediated phosphorylation of FBXO31 in response to DNA damage[47]. Covalent modifications of F-box proteins are common, and it is possible that they could have a role beyond F-box protein stability, affecting either substrate recruitment or association with the SCF scaffold[48]. However, this hypothesis requires further investigation.

Inducible, non-covalent degrons

The regulation of FBXL5 by non-covalent binding of iron underscores that F-box proteins can respond to signals other than covalent posttranslational modifications. Studies of plant responses to the hormones `auxin` and `jasmonate` have shown that substrates can be recruited to F-box proteins through degrons that are a combination of protein sequence and non-peptide hormones or small molecules. The receptors for auxin and jasmonate (TIR1 (TRANSPORT INHIBITOR RESPONSE 1) and COI1 (CORONATINEINSENSITIVE 1), respectively) are F-box proteins that target the auxin–indoleacetic acid (AUX–IAA) and JA–ZIM domain (JAZ) families of transcriptional repressors, respectively, for degradation[49,50]. Binding of the hormones to their receptors fills the gaps between the substrates and the F-box proteins, extending the binding surface and functioning as ‘molecular glue’ to stabilize the interaction. The formation of this protein–hormone hybrid degron is essential for substrate degradation. In addition, both TIR1 and COI1 are similar to FBXL5 in that they incorporate a small molecule into their folded structure. TIR1 incorporates inositol hexakisphosphate, and COI1 incorporates inositol pentakisphosphate. Whether the incorporation of these small molecules has a regulatory role, similar to the incorporation of iron ions in FBXL5, remains to be determined. However, in the case of COI1, inositol

pentakisphosphate has a crucial role in degron recognition, helping to coordinate binding to a key carboxyl group in the hormone, and it is likely that inositol hexakisphosphate has a similar role in TIR1 (REF. 49).

Covalent, non-phosphorylation-based degron modifications

F-box proteins also recognize degrons with covalent modifications other than phosphorylation. FBXO2 and FBXO6 bind glycosylated substrates, such as pre-integrin β1 and T cell receptor α-chain, respectively, through F-box-associated (FBA) domains. Based on the presence of FBA domains, FBXO17, FBXO27 and FBXO44 are predicted to also bind glycosylated substrates[12,51,52]. The recognition of high-mannose oligosaccharides by FBXO2 and FBXO6 is in line with their function in endoplasmic reticulum (ER)-associated degradation (ERAD) of incorrectly folded proteins, as many proteins are glycosylated in the ER during the folding process, but clear functions for other sugarbinding F-box proteins remain unclear. In general, investigating the substrates and specificity of sugarbinding F-box proteins is an underdeveloped area of F-box protein biology, and no substrates have been identified for FBXO17, FBXO27 or FBXO44. However, these F-box proteins are likely to be functionally distinct based on non-overlapping affinities for different glycans and different expression profiles[12]. Finally, it is unclear whether binding to glycosylated degrons is mutually exclusive with other forms of substrate binding. FBXO6 targets `checkpoint kinase 1` (CHK1) for degradation, and although the nature of the CHK1 degron is currently not known, CHK1 has not been reported to be glycosylated[53].

Modifications blocking degron recognition

Although post-translational modifications of degrons often direct the binding of F-box proteins to substrates, they can also prevent substrate recognition by the F-box protein. FBXO11 recognizes an unmodified degron in `CDT2`, and phosphorylation of this degron by a CDK blocks FBXO11 binding[54,55]. Similarly, FBXL2 binding to p85β is also prevented by phosphorylation[56]. Although this mechanism has only been reported for two F-box protein–substrate pairs, it highlights an alternative model of degron recognition. Historically, the role of degron phosphorylation in substrate recognition has been viewed through kinase activity. However, phosphatases may facilitate degron recognition, as demonstrated by the regulation of the p85β degron by the protein Tyr phosphatase PTPL1 (REF. 56). Non-phosphorylation-based modifications could also block degron recognition.

Domain-based recognition

Finally, although most of the F-box protein field focuses on the concept of degrons as short stretches of amino acids, several findings indicate that not all F-box proteins recognize such short degrons. The short degron hypothesis has been challenged by reports of F-box protein–substrate interactions that are determined by conserved domain structures. FBXO4, which binds cyclin D1 in a phosphorylation-dependent manner, also binds the telomere protein TRF1 (telomeric repeat-binding factor 1) in a domain-dependent manner. In the FBXO4–TRF1 co-crystal structure, FBXO4 adopts a GTPase-like fold that binds to TRF1 independently of covalent modifications[57,58]. Only non-telomeric TRF1 is degraded, and this regulation is imparted by the binding of TIN2 (TRF1-interacting nuclear protein 2) to TRF1 at telomeres, which physically blocks the FBXO4-binding site on TRF1.

In addition, FBXL3 recognizes `cryptochrome 1 and cryptochrome 2` (CRY1 and CRY2) in a modification-independent manner through extended contacts between its Leu-rich repeats and the surface of the CRY proteins[59–62]. The C terminus of FBXL3 is also inserted into a conserved pocket in CRY that may be subject to competition with the flavine adenine dinucleotide (FAD) redox cofactor, which would also represent a new way to regulate substrate recruitment. This potentially novel mechanism requires further study.

CRY binding to FBXL3 is probably blocked by binding of the transcription factor period (PER), a core oscillatory component of the circadian clock (the levels of which are regulated by βTrCP) that couples CRY oscillations to PER. Other F-box protein–substrate pairs might also use domain or secondary structure recognition instead of degron recognition, but this form of regulation is difficult to prove without crystal structures.

Further diversification and regulation of substrate recruitment

As illustrated above, substrate recruitment by F-box proteins is regulated by multiple mechanisms, at the levels of both the substrate and F-box protein, including restriction of degron access, covalent degron modifications, regulated F-box protein stability and defined F-box protein localization. The diversity of targeting mechanisms for F-box protein substrates is continuing to increase. Methylation-dependent recognition of substrates has recently been reported for a CRL4 ubiquitin ligase through a DCAF (DDB1- and CUL4-associated factor) substrate adaptor[63], and the von Hippel–Lindau disease tumour suppressor (VHL), which is a substrate adaptor for CRL2, recognizes hydroxylated Pro residues in substrates[64], suggesting that F-box proteins could recognize alternative modifications. In addition, although hormone- and small molecule-regulated substrate binding have only been observed in plants, this mechanism can be functionally reconstituted in mammalian cells, which indicates that similar mechanisms for substrate recognition are possible in higher organisms[65]. Importantly, small molecule- induced binding of substrates is currently under investigation for the development of drugs that restore, activate or retarget E3 ubiquitin ligase activity in various diseases. The F-box protein–substrate interface is also a highly specific target for the development of therapeutic inhibitors (BOX 2).

Finally, the incorporation of F-box proteins into the SCF scaffold is an emerging area of research. SCF complexes can be disassembled in response to specific stimuli. This was shown by the removal of Met30 from SCF^{Met30} by Cdc48 in response to treatment of yeast with cadmium[66]. Dimerization of F-box proteins has also been observed, but the *in vivo* effect of F-box protein dimerization on substrate selection and ubiquitylation remains unclear[58,67–70]. Whereas dimerization of yeast Cdc4 contributes to substrate ubiquitylation, disrupting the dimerization of FBXW7, the human Cdc4 homologue, does not seem to affect substrate regulation[71]. The functional relevance of substrate adaptor dimerization is more firmly established for the CRL3 substrate adaptors Speckle-type POZ (SPOP) and Kelch-like ECH-associated protein (KEAP1). In each case, dimerization enables the recognition of two degrons in the substrate and could affect either the avidity of the substrate for the ligase or its positioning within the active ligase complex[71].

F-box proteins in disease

As discussed, F-box proteins regulate substrates in diverse biological pathways that control key dimensions of cellular life, including cell growth, cell division, development and differentiation, signalling responses, and cell survival and death (TABLE 1). Therefore, dysregulation of F-box protein-mediated ubiquitylation, which can occur via multiple distinct mechanisms (FIG. 3), has been implicated in many pathologies, including sleep disorders, mood disorders, diabetes, Parkinson's disease, bacterial infections and viral infections[35,72–80]. Our knowledge of the involvement of F-box proteins in many of these diseases (and others) will be expanded and cemented in the coming years. Notably, although F-box proteins function in diverse biological pathways, the evolution of the field is closely linked to studies of cell proliferation, so F-box protein function has been viewed largely in the context of cancer biology and the best-characterized F-box proteins, SKP2, FBXW7 and βTrCP (FIG. 4). These studies have provided insights into the ability of F-box proteins to function in a general or context-dependent manner, providing a valuable framework for the study of F-box proteins in other systems and diseases (FIG. 4).

SKP2, FBXW7 and βTrCP in cancer

The misregulated degradation of tumour suppressors or oncoproteins can drive tumorigenesis. Accordingly, F-box proteins can function as oncoproteins when overexpressed (if their substrates are tumour suppressors) or as tumour suppressors (if their substrates are oncoproteins). SKP2 is the archetypal oncogenic F-box protein. It promotes S phase entry through the ubiquitylation and degradation of the CDK inhibitor p27, and mouse models unequivocally confirm the role of the SKP2–p27 axis in tumorigenesis. In the vast majority of tumours, p27 is inactivated by SKP2-mediated degradation, not genetic deletion, and SKP2 overexpression correlates with high tumour grade and poor prognosis in a broad range of cancers[16]. Although SKP2 functions predominantly in the regulation of p27, it also targets other anti-proliferative substrates, such as p21 and p57, contributing to its function as an oncoprotein[7,16].

By contrast, loss-of-function mutations in FBXW7 have been identified in many cancers, which indicates that it might function as a tumour suppressor[81]. *FBXW7* is frequently deleted in tumours, and it is estimated that 6% of cancers have mutations in the *FBXW7* gene[82,83]. *FBXW7* mutations are detected most frequently in T cell acute lymphoblastic leukaemia (T-ALL; 31%), but mutations are also found in solid tumours, including cancers of the breast, intestine and bone. Mouse models have confirmed the tumour suppressor function of FBXW7; conditional deletion of *Fbxw7* leads to the development of haematological malignancies, such as thymic lymphoma and T-ALL[84,85]. FBXW7 substrates (including MYC, JUN, cyclin E and Notch) drive proliferation and tumorigenesis in conjunction with other mutations[81,86]. *Fbxw7* deletion causes premature loss of haematopoietic stem cells by promoting cell cycle entry and apoptosis in a p53-dependent manner. p53 loss leads to unchecked cell proliferation and cancer, supporting the idea that FBXW7 and p53 function synergistically to prevent tumorigenesis[86]. The loss of FBXW7 function also facilitates resistance to certain chemotherapy drugs, such as anti-tubulin therapies. This phenotype might be the result of increased levels of myeloid cell leukaemia sequence 1 (MCL1), an FBXW7 substrate and pro-survival factor that suppresses apoptosis[87,88].

Point mutations that dysregulate the SCF-dependent turnover of oncogenic substrates usually affect the F-box protein–substrate interface. Most tumorigenic *FBXW7* point mutations are missense mutations in crucial residues of the substrate-binding region, and these mutations interfere with substrate recruitment[82,85]. Indeed, mutations of the two Arg residues in FBXW7 that directly contact the phosphodegron of the substrate account for 43% of cancer-associated *FBXW7* mutations. Other mutations inactivate FBXW7 by interfering with its localization, mutating the F-box domain or resulting in premature termination of translation. The F-box protein–substrate interface can also be affected by mutations in substrates. Degron mutations in oncogenic substrates, including FBXW7 substrates, are common in tumours. For example, the phosphodegron of MYC (Thr58; a GSK3 target site) is frequently mutated in patients with Burkitt's lymphoma, resulting in MYC stabilization[89,90]. In addition, mutations that disrupt Notch binding to FBXW7 are found in patients with T-ALL[83,91].

As demonstrated by mouse models, the roles of SKP2 and FBXW7 in tumorigenesis provide an idealized framework for the evaluation of F-box proteins and their substrates in disease. However, a closer examination of F-box proteins in cancer shows that their function can be more nuanced. The stimulus- and temporally-regulated control of substrate binding enables an F-box protein to regulate multiple substrates, including substrates with opposing biological roles. Therefore, some F-box proteins can have a marked cell type- and/or contextdependent function (FIG. 4). They can be tumour suppressors or oncoproteins

depending on which substrates are dysregulated and/or in which biological compartment they are dysregulated.

For example, although FBXW7 functions as a tumour suppressor in numerous cancers, it can also function in a pro-survival manner in multiple myelomas by mediating the degradation of p100 (also known as nuclear factor-κB2 (NF-κB2)), an inhibitor of NF-κB signalling[92]. Multiple myeloma cells are 'addicted' to NF-κB activity[93],[94], so degradation of p100 is required for their growth and proliferation. Intriguingly, this function seems to be B cell specific. Whereas FBXW7 mutations have been found in a wide variety of tumours, they have not been detected in tumours from the B cell lineage, including multiple myelomas, which might indicate a tissue-specific pro-survival role of FBXW7 (REF. 92). Notably, the proteasome inhibitor bortezomib is the frontline chemotherapy for multiple myelomas, and although the antitumour effects of bortezomib result from the stabilization of many substrates, p100 stabilization is likely to contribute to the effectiveness of this drug. In tissue culture, the pro-survival role of FBXW7 can also be countered by inhibitors of GSK3, the kinase that phosphorylates the p100 degron, suggesting a new avenue for therapy for B cell malignancies[92].

Another F-box protein with context-dependent functions is βTrCP, which has an important role in integrating both positive and negative growth signals throughout the cell cycle. βTrCP mediates the degradation of substrates that promote cell proliferation upon mitogenic stimulation in G1, but it also regulates both positive and negative components of the feedback loops that control the timing and progression of mitosis. βTrCP1 or βTrCP2 is overexpressed in multiple cancers, including colorectal cancer, pancreatic cancer, hepatoblastoma, breast cancer, melanoma and gastrointestinal cancers, which supports an oncogenic function for these proteins. In these settings, βTrCP may target various growth and survival inhibitors, such as BIMEL (an extra-long isoform of BIM), PDCD4 (programmed cell death 4) and IκB (inhibitor of κB) family members[16]. By contrast, βTrCP substrates with oncogenic properties, such as β-catenin, CDC25A and EMI1, are also overexpressed in cancers[95]–[97]. Notably, the contribution of loss of βTrCP function to the overexpression of these proteins is unclear, so although βTrCP regulates oncogenic substrates, it might not function as a bona fide tumour suppressor[16].

Context-dependent F-box protein function may also result from the context-dependent functions of substrates. For example, some βTrCP substrates, such as DEPTOR (DEP-domain containing mammalian target of rapamycin (mTOR)-interacting protein), seem to function as both tumour suppressors and oncoproteins. In general, DEPTOR functions as a tumour suppressor by blocking mTOR activity and inhibiting protein synthesis, cell proliferation and cell survival. Following mitogenic stimulation, DEPTOR is targeted for degradation by βTrCP, resulting in mTOR activation and cell proliferation[31]–[33]. Accordingly, DEPTOR levels are low in most cancers[98]. By contrast, DEPTOR levels are increased in many tumours with poor prognosis, which indicates that it might have oncogenic properties. Furthermore, DEPTOR is significantly overexpressed in a subset of multiple myelomas and is required for the survival of these cancer cells. Overexpressed DEPTOR maintains PI3K–AKT activation by disrupting the normal feedback regulation between mTOR complex 1 (mTORC1) and mTORC2. DEPTOR upregulation in multiple myelomas occurs largely as a result of increased transcription; the possibility of disrupted βTrCP-mediated DEPTOR degradation remains to be investigated.

FBXO11, a candidate tumour suppressor

SKP2, FBXW7 and βTrCP are valuable standards for the evaluation of the function of orphan F-box proteins as they are paired with substrates, and they highlight the perils of bestowing a generalized function on F-box proteins on the basis of a limited number of

substrates. Recently, FBXO11 was shown to mediate degradation of the oncoprotein BCL6 (B cell lymphoma 6), suggesting that FBXO11 is a tumour suppressor[13]. BCL6 functions as a transcription factor in B cell development, differentiation and activation, and increased BCL6 expression drives the development of diffuse large B cell lymphoma (DLBCL)[99]. BCL6 overexpression in DLBCL is caused by multiple mechanisms, including translocation to constitutively active promoters, promoter hypermutation or protein stabilization. Mutations in FBXO11 (deletions or inactivating missense mutations) are seen in ~20% of patients with DLBCL and DLBCL cell lines, corresponding with increased levels and stability of BCL6. These mutations are predominantly located in the CASH repeat domain of FBXO11, the presumptive substratebinding domain, and this is in agreement with the theory that substrate-binding domains are mutational hotspots. Reconstitution of FBXO11 expression in FBXO11-null DLBCL cell lines suppresses tumorigenicity in xenograft models, suggesting that downregulation of BCL6 levels by FBXO11 has an antitumour effect.

However, many questions regarding the FBXO11–BCL6 interaction remain. Similar to the mutation of FBXW7 substrates in cancer, it is possible that mutations in BCL6 that compromise FBXO11 binding could also contribute to high BCL6 levels in DLBCL, so tumour-derived point mutants of BCL6 require further investigation. In addition, the signalling pathways upstream of BCL6 degradation are unknown, although they seem to be separate from B cell receptor signalling and MAPK pathways[13]. FBXO11 might also have a role in normal B cell development. Notably, although naive B cells express *BCL6* mRNA, they do not express BCL6 protein, which indicates that BCL6 expression is post-translationally regulated. Finally, *FBXO11* mutations are also observed in cancers of the colon, lung, ovary, head and neck, as well as non-DLBCL lymphomas, which implies that FBXO11 functions as a tumour suppressor in multiple tissues[100–103]. FBXO11 also targets CDT2 for degradation, but the relevant targets in these other tissues remain unknown[54,55]. Mouse models investigating FBXO11 and its interactions with BCL6 and other substrates will undoubtedly shed light on its function in normal cells and tumour cells.

Emerging F-box proteins in cancer

Several other F-box proteins regulate the degradation of substrates that are involved in processes fundamentally related to tumorigenesis and tumour progression. For example, defects in centrosome copy number lead to aneuploidy and the loss of genome integrity, as seen in many types of cancer. Moreover, SCF complexes have an emerging role in controlling centrosome numbers, with several F-box proteins regulating the levels of centrosome proteins, including Polo-like kinase 4 (PLK4; which is regulated by βTrCP)[104,105], SAS6 (spindle assembly abnormal 6; which is regulated by FBXW5)[106] and CP110 (which is regulated by cyclin F)[11]. In addition to regulating the centrosome, cyclin F controls genome integrity through the degradation of RRM2 (REF. 10), and cyclin F downregulation has been reported in hepatocellular carcinoma[107]. Finally, FBXO9 mediates the degradation of mTORC1-associated `telomere length regulation protein 2` (TEL2) and `TELO2-interacting protein 1` (TTI1), removing feedback inhibition of mTORC2 and resulting in an increase in mTOR activity in a manner similar to DEPTOR overexpression[108–110]. Further characterization of these F-box proteins is required before their true roles in cancer are understood.

F-box proteins beyond cancer

Although F-box proteins have clear roles in cancer, their functions in other pathologies are gradually emerging as research into F-box proteins evolves beyond investigations of cell proliferation. This shift is exemplified by studies of the APC/C, a prominent cell cycle-associated ubiquitin ligase (FIG. 1) that also has roles in post-mitotic cells, such as

neurons[111]. In particular, the regulation of circadian rhythms by βTrCP and FBXL3 has been linked to sleep and mood disorders. βTrCP and FBXL3 control the degradation of PER1, PER2, CRY1 and CRY2, which negatively regulate the activity of the heterodimeric BMAL– CLOCK transcription factor at the core of the circadian system[59–61,112,113]. A mutation in PER2 has been found in individuals with `familial advanced sleep phase syndrome`, and this mutation causes increased nuclear export and decreased stability of PER2 (REFS 80,114,115). In addition, inhibitors of casein kinase 1 (CK1), the kinase that phosphorylates the βTrCP degron in PER proteins, have been used to alter circadian cycles in rodents[116,117]. The involvement of βTrCP in circadian regulation also highlights the context-dependent nature of this multifunctional F-box protein. FBXL3 has not yet been directly linked to a human disorder on the basis of mutation data. However, in addition to exhibiting circadian phenotypes, mice carrying a mutated form of FBXL3 show reduced anxiety- and depression-associated behaviours[118], and the FBXL3 substrates CRY1 and CRY2 have been linked to diabetes[75,76]. The FBXL3–CRY interface, particularly the interaction between the FAD-binding pocket of CRY proteins and the C terminus of FBXL3, is a promising site for pharmacological manipulation[62] (BOX 2). As CRY proteins are the only known substrates of FBXL3, it is currently unknown whether FBXL3 has a generalized function in circadian regulation.

Notably, FBXL21, an FBXL3 homologue, also binds CRY proteins, and the interplay of FBXL3 and FBXL21 will be an area of future investigation. It has been reported that FBXL21 is expressed predominantly in the suprachiasmatic nucleus, which is the region in the brain that functions as the master pacemaker in mammals[119], but more recent studies have proposed a more universal mechanism, in which cytoplasmic FBXL21 inhibits the degradation of CRY proteins in a manner mediated by nuclear FBXL3 (REFS 120,121). *Fbxl21*-mutant mice have circadian defects, and FBXL21 has been linked to schizophrenia[122]. Further research is required to fully integrate FBXL3 and FBXL21 into our understanding of circadian rhythm regulation and circadian-associated pathologies.

F-box proteins are also manipulated by multiple viruses, including HIV and HCV. The HIV viral protein U (Vpu) binds βTrCP and retargets the ubiquitylation activity of $SCF^{\beta TrCP}$ to CD4 and bone marrow stromal antigen 2 (BST2; also known as tetherin), facilitating the release and dispersion of the virus from the cell[72]. The hijacking of βTrCP by Vpu might also inhibit the normal functions of βTrCP, such as regulation of the NF-κB pathway[78,79]. Similarly, the HCV NS5A protein binds FBXL2, and FBXL2 function, including proper localization (see above), is required for HCV replication[35]. However, the effect of HCV on FBXL2 substrate selection is unclear, as are the substrates required for viral replication. Many other viruses also modulate the activity of host F-box proteins[123–125], and several viruses and bacteria encode their own F-box proteins[73,74], which indicates that the role of SCF complexes in microbial infections will expand in the future.

Finally, the association of F-box proteins with human disease highlights the importance of identifying more F-box protein substrates. For example, FBXO7 mutations have been identified in a subtype of Parkinson's disease, but there are few known FBXO7 substrates,with unknown relevance to Parkinson's disease[7, 77]. As the F-box field moves beyond cancer biology, the links between F-box proteins and human disease are likely to expand.

Concluding remarks

Fourteen years after the initial annotation of the F-box protein family, our understanding of the biochemical mechanisms of SCF ubiquitin ligases and their biological roles continues to evolve[8,9]. The old paradigm of phosphorylation-directed substrate recognition by F-box

proteins may still be dominant, but it is no longer absolute. The biochemical and biological functions of F-box proteins expand with each new substrate that is described, be it for an F-box protein with established substrates or an orphan F-box protein. The historical linkage between the SCF complex and the cell cycle has driven the investigation of this protein family in cancer, but it is likely that F-box proteins will have important roles in many other pathologies and pathways[35,75–77,118]. The identification of substrates for F-box proteins is of primary importance for determining the biological roles of each F-box family member, and as our knowledge of SCF biology expands, each SCF complex presents several avenues for the development of new therapies.

Acknowledgments

The authors apologize to their colleagues in the field for omitting their work owing to space constraints. J.R.S. is a Special Fellow of The Leukemia and Lymphoma Society. J.K.P. is supported by a fellowship from the Lymphoma Research Foundation. Work in the Pagano laboratory is supported by grants from the US National Institutes of Health (NIH) (R37CA076584 and R01GM057587). M.P. is an Investigator of the Howard Hughes Medical Institute.

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Glossary

Ubiquitin	A 76-amino-acid protein that can be covalently conjugated to Lys residues in other proteins to specify several protein fates. Polyubiquitin chains can be generated using seven internal Lys residues in ubiquitin. Lys11- or Lys48-linked chains target proteins to the proteasome, whereas other chains, such as Lys63-linked chains, have signalling roles. Monoubiquitylation also has a signalling role.
RING	Really interesting new gene (RING) proteins coordinate zinc using Cys and His residues in a cross-brace arrangement. RING proteins typically recruit E2 ubiquitin-conjugating enzymes.
NEDD8	A small ubiquitin-like protein that can be covalently conjugated to other proteins. Cullin proteins are the primary targets for neddylation, which activates the cullin–RING ligase.
Degrons	Small sections of a substrate that are recognized by a ubiquitin ligase and that are required for substrate degradation. Canonical degrons for F-box proteins are very short, conserved stretches of amino acids.
β-transducin repeat-containing protein (βTrCP)	Refers to two paralogous F-box proteins, βTrCP1 (FBXW1 (F-box and WD40 domain 1)) and βTrCP2 (FBXW11), that are biochemically indistinguishable. βTrCP recognizes phosphodegrons through WD40 repeats.

Cyclin-dependent kinase (CDK)	These are drivers of the cell cycle. The activity of these kinases is controlled by the availability of their cognate cyclins. The oscillation of cyclin levels during the cell cycle determines which CDKs are active.
αβ-crystallin	A chaperone protein that can be induced by heat, but unlike heat shock proteins, it does not re-fold proteins. Instead, αβ-crystallin forms protein aggregates.
Cyclin-homology domain	This domain normally determines the binding of cyclins to cyclin-dependent kinase substrates. The original cyclins were identified on the basis of their cyclic oscillations, but additional proteins contain a cyclin-homology domain, which has led to their designation as cyclins.
Ribonucleotide reductase subunit M2 (RRM2)	A subunit of the enzyme that converts ribonucleotides to deoxyribonucleotides for DNA replication. RRM2 is regulated by the cell cycle, whereas the RRM1 subunit is stable throughout the cell cycle.
Isoprenylation	The transfer of a farnesyl or geranyl–geranyl moiety to a carboxy-terminal Cys residue of a target protein. This modification facilitates protein recruitment to membranes.
RAB3-interacting molecule 1 (RIM1)	It is expressed near the active zone of the neuronal synapse and interacts with many presynaptic proteins, including RAB3. It controls calcium-evoked neurotransmitter release.
Auxin	A family of plant hormones required for growth signalling. Indole-3-acetic acid is the most potent auxin. They bind to TRANSPORT INHIBITOR RESPONSE 1, a plant F-box protein, promoting its interaction with auxin– indoleacetic acid transcriptional repressors.
Jasmonate	A lipid-based plant hormone that binds to CORONATINEINSENSITIVE 1, an F-box protein, and JA–ZIM domain (JAZ), a transcriptional regulator, promoting the degradation of JAZ.
Checkpoint kinase 1 (CHK1)	A Ser/Thr kinase that is required for checkpoint arrest and DNA repair following DNA damage.
CDT2	A DDB1- and CUL4-associated factor (DCAF) protein that mediates the degradation of SET domain-containing protein 8, p21 and CDT1, preventing re-replication in S phase.
Cryptochrome 1 and cryptochrome 2 (CRY1 and CRY2)	Mammalian CRY proteins function as transcriptional repressors within a negative feedback loop of the circadian cycle. CLOCK and BMAL1 activate the transcription of genes encoding period (PER) and CRY proteins, which then negatively regulate transcription by CLOCK–BMAL1. In lower organisms, CRY proteins function as photoreceptors, but this activity is not present in mammals.
p100	An inhibitor of nuclear factor-κB (NF-κB) signalling and the precursor for p52, which forms an active transcription factor in conjunction with REL family proteins. p100 is part of the non-

	canonical NF-κB signalling pathway, which largely responds to developmental signals.
Bortezomib	A proteasome inhibitor approved as frontline therapy for multiple myelomas. Originally, much of its effectiveness was ascribed to inhibition of canonical nuclear factor-κB signalling through stabilization of inhibitor of κB proteins.
DEPTOR	(DEP-domain containing mTOR-interacting protein). An inhibitor of the mammalian target of rapamycin (mTOR) kinase in the context of both mTOR complex 1 (mTORC1) and mTORC2.
Mammalian target of rapamycin	(mTOR). A PI3K that is an important regulator of cell growth and metabolism, particularly protein synthesis. mTOR forms two complexes, designated mTOR complex 1 (mTORC1) and mTORC2, which have multiple feedback links.
Telomere length regulation protein 2 and TELO2-interacting protein 1 (TEL2 and TTI1)	Evolutionarily conserved proteins that interact with all six mammalian PI3K-like protein kinases and control their abundance.
Familial advanced sleep phase syndrome	An inherited circadian and sleep disorder in which patients have altered circadian rhythms, with early sleep onset and early waking.

General regulation of SCF complexes

Several factors regulate the S phase kinase-associated protein 1 (SKP1)–cullin 1 (CUL1)–F-box protein (SCF) scaffold, and these factors often control all cullin–RING ligase (CRL) complexes. With the exception of CUL7, CRLs are regulated by neddylation, which is the covalent attachment of the small ubiquitin-like protein NEDD8 to the cullin subunit. This modification releases the RING finger subunit on a flexible tether, which positions the E2 enzyme closer to the substrate, thereby potentiating ubiquitylation. The neddylation status of cullins also determines their binding to cullin-associated and neddylation-dissociated 1 (CAND1), which can only bind non-neddylated cullins. CAND1 is an assembly factor that facilitates the rapid exchange of F-box proteins on the SCF scaffold[126–128] and other substrate adaptors on the additional CRL scaffolds. Like ubiquitylation, NEDD8 conjugation is accomplished through a cascade of E1, E2 and E3 enzymes. Cullins function as their own E3 ligases for NEDD8 by recruiting a NEDD8-specific E2 (either UBE2M (also known as UBC12) or UBE2F) to the complex. Neddylation is reversed through the action of the COP9 signalosome, a large, multisubunit complex that proteolytically cleaves NEDD8 from cullins. Combined, this system creates a cycle of neddylation and deneddylation that controls the assembly and activity of CRLs. More recently, glomulin was also shown to inhibit CRLs by blocking the access of the E2 enzymes to RING-box protein 1 (RBX1)[129]. Although the specific phenotypic effects of glomulin loss or inhibition manifest through an SCF complex that contains F-box and WD40 domain 7 (FBXW7), glomulin seems to affect the activity of most CRLs[130]. It is likely that other general regulators of CRLs will be discovered in the future.

F-box protein-targeted therapy

Rational design of inhibitors

Crystal structures of F-box protein–substrate complexes allow the rational design of inhibitors, as exemplified by the structure of S phase kinase-associated protein 1 (SKP1)–F-box and Leu-rich repeat 3 (FBXL3)–cryptochrome 2 (CRY2)[62]. Intriguingly, the tail of FBXL3 fits into a pocket in CRY2. In CRY proteins from other organisms, which (unlike mammalian CRY proteins) function as photoreceptors, this conserved pocket binds to flavin adenine dinucleotide (FAD)[131], and mammalian CRY proteins also have (extremely weak) binding to FAD. Therefore, FAD analogues could function as competitive inhibitors of FBXL3–CRY2 interactions. A compound that competes for FAD binding, inhibits CRY ubiquitylation and lengthens the circadian cycle was recently identified independently[132].

Virtual ligand screening (VLS) has also been used to identify inhibitory compounds *in silico* based on pockets in structures that are able to bind small molecules, such as the interface between SKP2, cyclin-dependent protein kinase regulatory subunit 1 (CKS1) and p27. This approach facilitated the discovery of compounds that competitively inhibit SKP2-mediated degradation of p27 (REF. 133).

High-throughput screening

High-throughput screens based on ubiquitin ligase activity are also being applied to SCF complexes and can identify inhibitors that target multiple steps in the ubiquitylation process, including kinase inhibitors, inhibitors of SCF complex assembly, allosteric inhibitors and degron inhibitors. One recent screen identified an allosteric inhibitor of yeast Cdc4 that distorts the WD40 domain and prevents substrate binding. This finding validates the concept of allosteric inhibition of F-box proteins[134]. Another compound disrupts binding between Skp1 and Met30, preventing SCF complex formation[135]. This compound is surprisingly selective for the Met30 F-box domain and does not inhibit the interaction between Skp1 and other F-box proteins.

De novo protein targeting

Two different strategies can be envisioned for restoring E3 ligase functions: stabilization of the interface between a mutated F-box protein and its substrates or the retargeting of substrates to a different, functional ligase. The first approach draws from the precedent established by auxin and TIR1 (TRANSPORT INHIBITOR RESPONSE 1)[50]. Provided that the mutated F-box protein is still expressed and only mildly unfolded, it may be possible to use a small molecule as a molecular glue or as an allosteric modifier of the structure of the F-box protein. However, this approach is technically daunting, specific for individual mutants and cannot correct large deletions.

Alternatively, protacs (proteolysis targeting chimaeras) are hetero-bivalent chimeric molecules that recruit an E3 ligase at one end (by mimicking a degron) and bind specific target proteins at the other end, which tethers the ligase to the substrate and results in target degradation[136]. Both peptide degron-based fusion protacs and small molecule-based protacs have been developed[137]. Protac technology has several limitations, including the inhibition of the endogenous ligase and nonspecific degradation of non-target proteins.

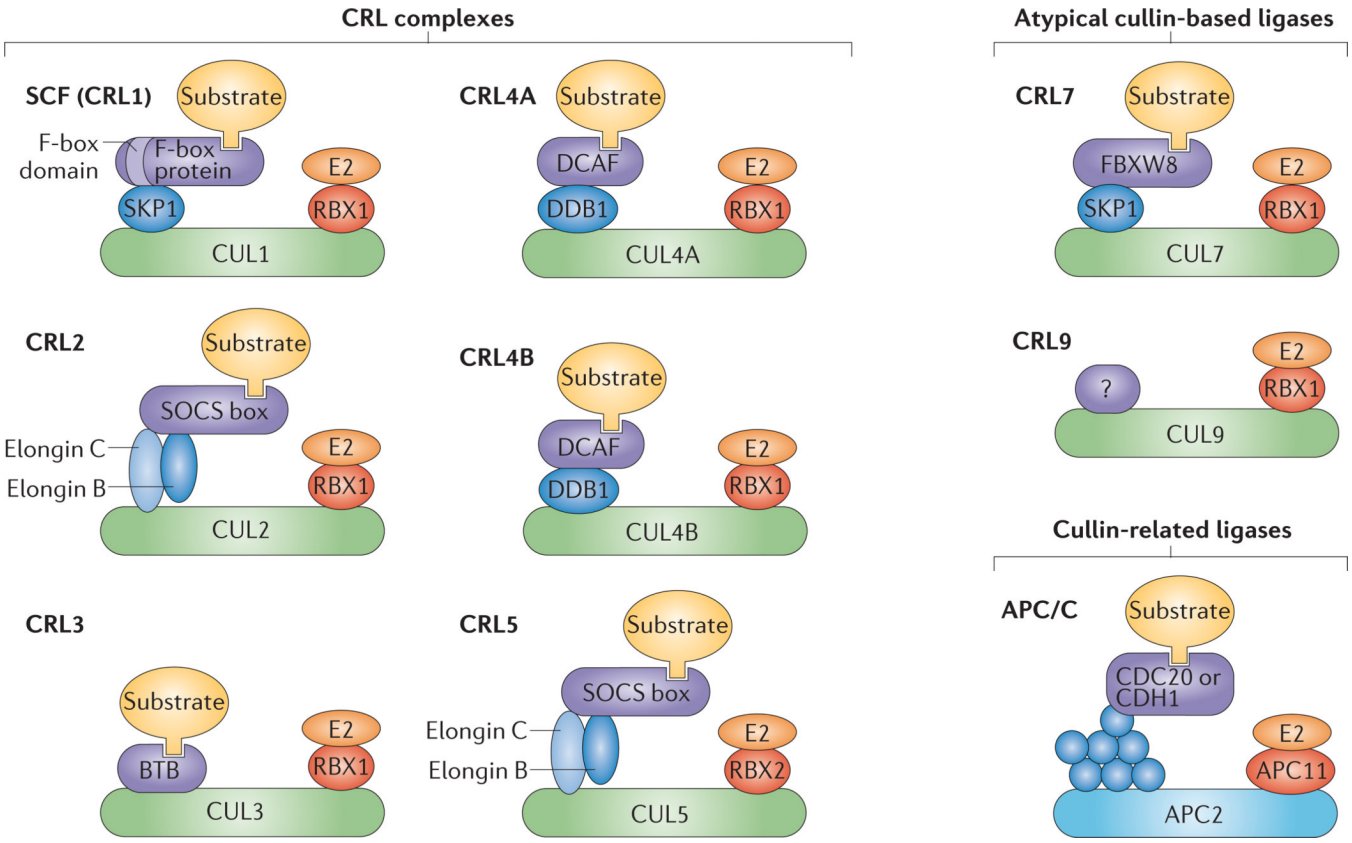

Figure 1. The cullin–RING ligase family

Cullin (CUL) proteins form the backbones of ubiquitin ligase complexes. CUL7 and CUL9 are atypical cullins owing to their large size and the incorporation of additional homology domains. APC2, the core of the APC/C (anaphase promoting complex; also known as the cyclosome), is distantly related to cullins. Each cullin–RING ligase (CRL) complex is modular, with variable substrate adaptors. CRL1 (better known as S phase kinase-associated protein 1 (SKP1)–CUL1–F-box protein (SCF) complex) uses SKP1 and F-box proteins as substrate adaptors, and CRL2 and CRL5 ligases use elongin B, elongin C and SOCS (suppressor of cytokine signalling) box proteins. The substrate adaptors for CRL3 are BTB (bric-a-brac-tramtrack-broad complex) proteins. CRL4A and CRL4B use DDB1 (DNA damage-binding protein 1) and DCAF (DDB1- and CUL4-associated factor) proteins. CRL7 uses SKP1 and a single F-box protein (F-box and WD40 domain 8 (FBXW8)). The substrate adaptors for CRL9 are not known. The full molecular architecture of the APC/C remains unclear, in part because it contains many more proteins (shown in blue) than the CRL complexes. APC/C uses either CDC20 or CDH1 (CDC20 homologue 1) as a substrate adaptor. The substrate for each complex is shown in yellow. RBX1, RING-box protein 1.

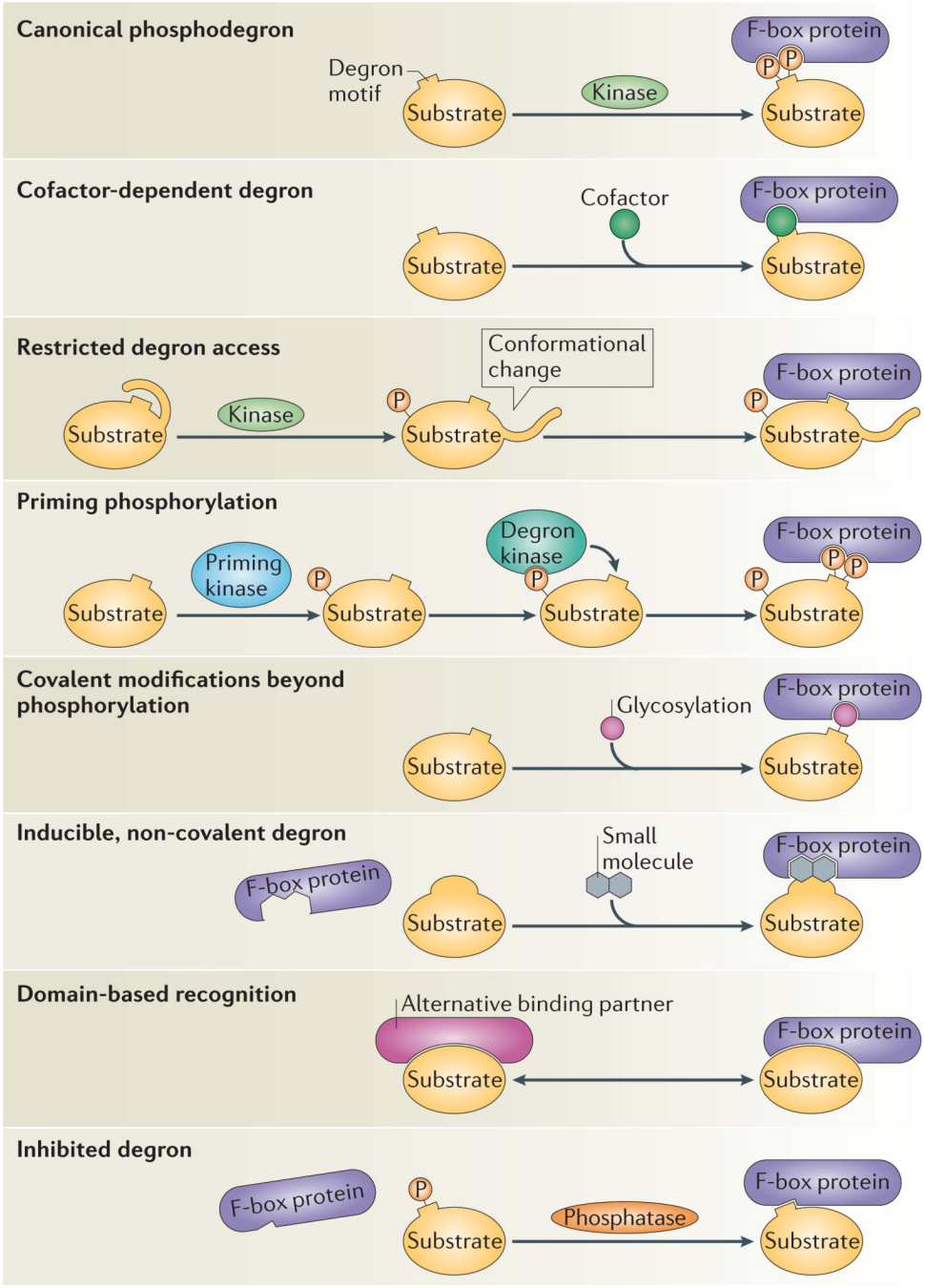

Figure 2. Recognition of substrates by F-box proteins

F-box proteins recognize their substrates in multiple ways. Often, various recognition mechanisms are combined to impart precise regulation of substrate degradation. Eight different modes of recognition and regulation are shown, as discussed in the main text. These simple concepts of regulation and recognition are often combined in substrates. For example, phosphodegron recognition can be combined with priming phosphorylations, or the recognition of non-modified degrons can be paired with restricted degron access.

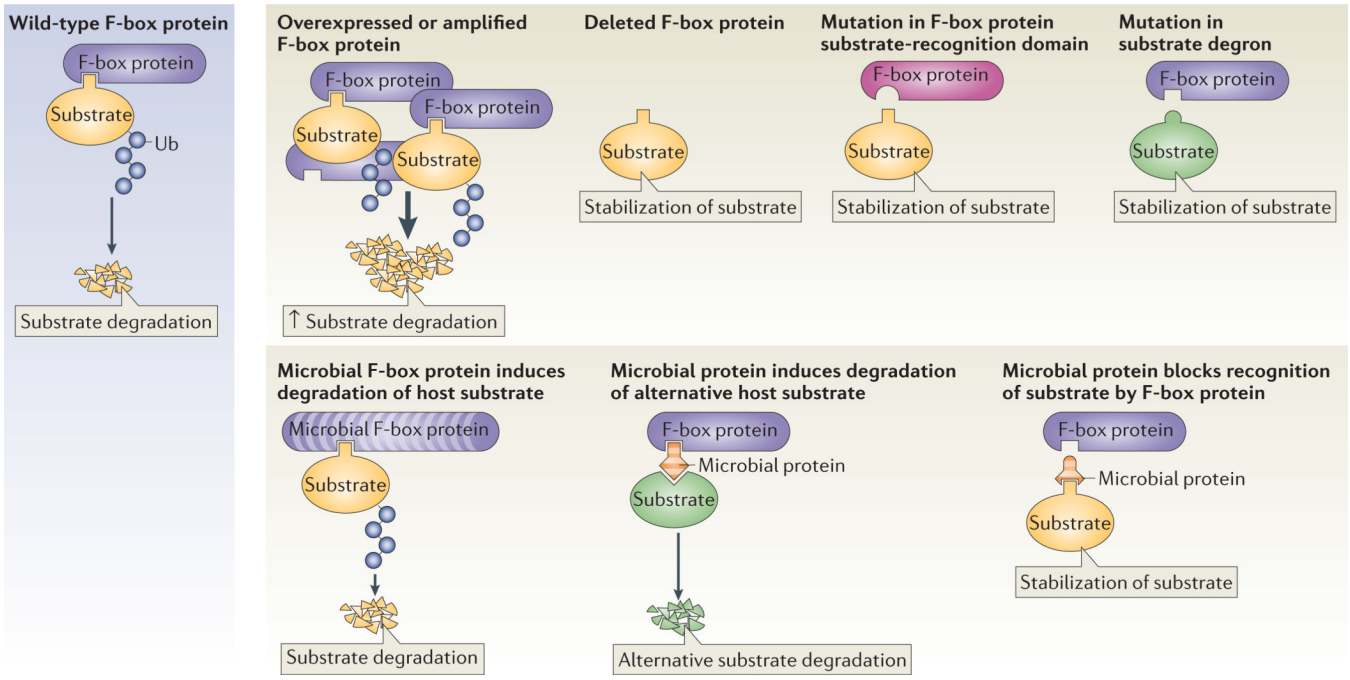

Figure 3. Dysregulation of F-box protein-mediated degradation in disease

Dysregulation of F-box protein-mediated degradation can occur by the overexpression of an F-box protein, deletion of an F-box protein, point mutation of an F-box protein or mutation of substrate degrons. In addition, the expression of microbial proteins can increase or decrease substrate degradation, or lead to the degradation of alternative substrates. Ub, ubiquitylation.

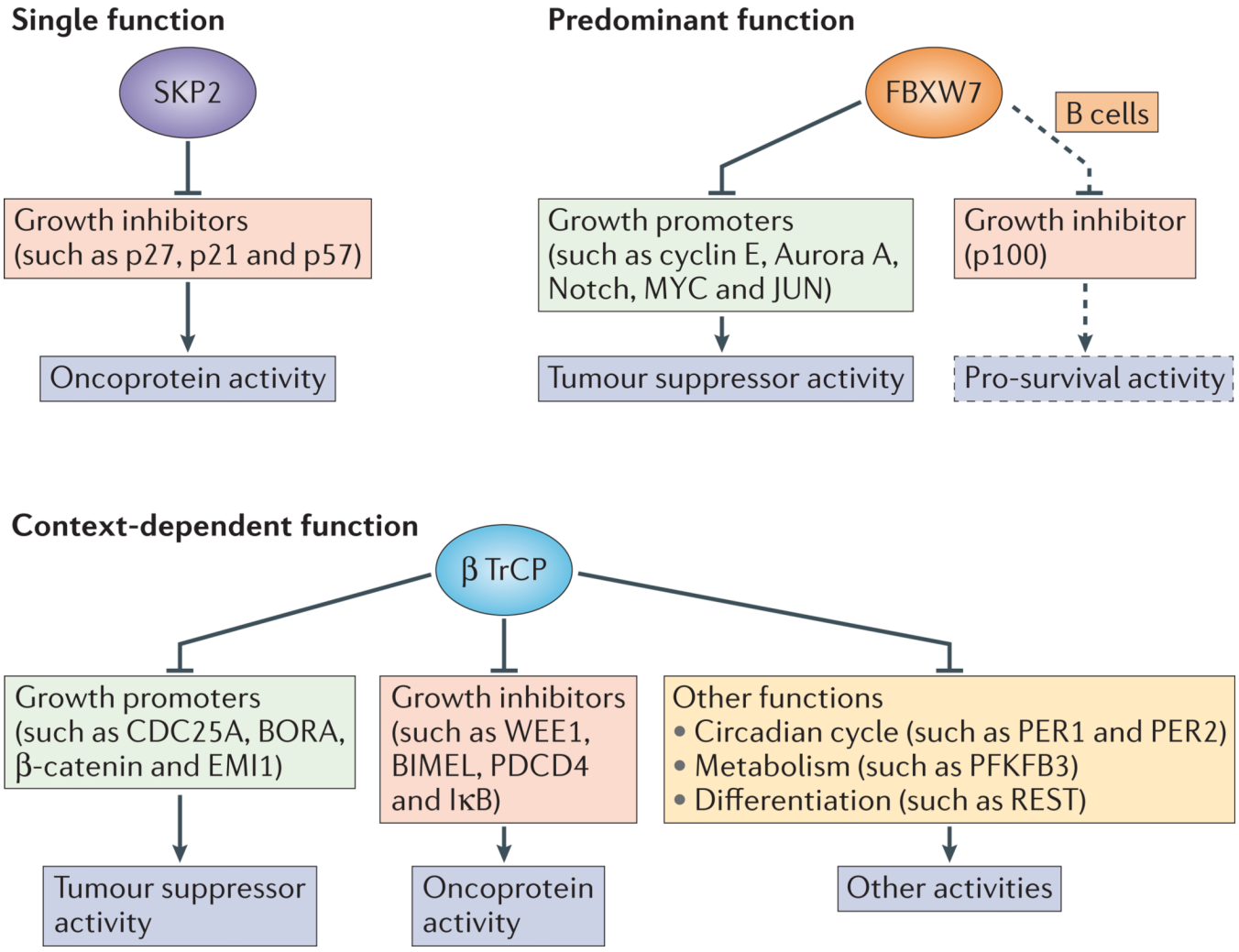

Figure 4. Generalized and context-dependent functions of F-box proteins

Studies of S phase kinase-associated protein 2 (SKP2), F-box and WD40 domain 7 (FBXW7) and β-transducin repeat-containing protein (βTrCP) show that F-box proteins can have generalized and/or context-dependent functions. As shown in mouse models, SKP2 functions as an oncoprotein that ubiquitylates and hence degrades growth suppressive substrates. Moreover, in mouse models, FBXW7 functions as a tumour suppressor by ubiquitylating growth-promoting substrates, but this role is cell type specific. In B cell lineages, FBXW7 actually has a pro-survival role by mediating the degradation of p100, an inhibitor of nuclear factor-κB (NF-κB) signalling. βTrCP function is highly stimulus and cell type specific, and it has a role in many disparate pathways, including pathways beyond cell growth and proliferation. BORA, aurora borealis; BIMEL, BCL-2-interacting mediator of cell death extra long; EMI1, early mitotic inhibitor 1; IκB, inhibitor of κB; PDCD4, programmed cell death 4; PER, period; PFKFB3, 6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 3; REST, RE1-silencing transcription factor.

Table 1

Control of key dimensions of cellular life by F-box proteins

F-box protein	Substrate	Function of substrate	Mechanism of degron regulation
Differentiation and development			
βTrCP	β-catenin	WNT signalling	Phosphodegron, priming phosphorylation
βTrCP	REST	Neural differentiation, cell cycle	Phosphodegron
βTrCP	SNAIL	Epithelial–mesenchymal transition	Phosphodegron, priming phosphorylation
FBXL14	SNAIL	Epithelial–mesenchymal transition	Unknown
FBXO11	BCL6	B cell development	Unknown
FBXO11	CDT2	DNA replication, seam cells (in worms)	Blocked by phosphorylation
FBXO32	MyoD	Muscle cell differentiation	Unknown
FBXW7	CEBPα	Adipocyte differentiation	Phosphodegron
FBXW7	SREBP1	Adipocyte differentiation	Phosphodegron
Cell division			
βTrCP	BORA	Mitotic signalling	Phosphodegron
βTrCP	EMI1	APC/C inhibitor	Phosphodegron
βTrCP	PLK4	Centriole duplication	Phosphodegron
βTrCP	WEE1	Kinase, CDK inhibitor	Phosphodegron
Cyclin F	CP110	Centriole duplication	Unmodified, localization dependent
Cyclin F	RRM2	dNTP production	Restricted access
FBXW5	EPS8	Actin remodelling	Unknown
FBXW7	Cyclin E	Cell cycle	Phosphodegron
SKP2	p27	CDK inhibitor	Cofactor-dependent
Cell death and survival			
βTrCP	BIMEL	Apoptosis	Phosphodegron
βTrCP	IκB	Inhibitor of NF-κB signalling	Phosphodegron
FBXO7	cIAP1	Apoptosis inhibitor	Unknown
FBXW7	MCL1	Survival factor	Phosphodegron
FBXW7	p100	Non-canonical NF-κB signalling	Phosphodegron
Cell growth			
βTrCP	DEPTOR	Inhibitor of mTOR	Phosphodegron, priming phosphorylation
βTrCP	PDCD4	Inhibits protein synthesis	Phosphodegron
FBXO9	TEL2 and TTI1	mTOR signalling	Phosphodegron
FBXW7	JUN	Mitogenic signalling	Phosphodegron
FBXW7	MYC	Cell proliferation	Phosphodegron
Signalling			
βTrCP	CDC25A	Phosphatase, CDK activator	Phosphodegron
βTrCP	PER1 and PER2	Circadian rhythm	Phosphodegron
FBXL2	p85β	PI3K signalling	Blocked by phosphorylation

F-box protein	Substrate	Function of substrate	Mechanism of degron regulation
FBXL3	CRY1 and CRY2	Circadian rhythm	Domain recognition, possibly ligand
FBXL5	IRP2	Iron homeostasis	Unknown

APC/C, anaphase-promoting complex (also known as the cyclosome); βTrCP, β-transducin repeat-containing protein; BCL6, B cell lymphoma 6; BIMEL, BCL-2-interacting mediator of cell death extra long; BORA, aurora borealis; CDK, cyclin-dependent kinase; CEBPα, CCAAT-enhancer-binding protein-α; cIAP1, cellular inhibitor of apoptosis 1; CP110, centrosomal protein 110; CRY, cryptochrome; DEPTOR, DEP-domain containing mTOR-interacting protein; EMI1, early mitotic inhibitor 1; EPS8, EGFR kinase substrate 8; FBXL, F-box and Leu-rich repeat; FBXO, F-box only; FBXW, F-box and WD40 domain; IκB, inhibitor of κB; IRP2, iron-regulatory protein 2; MCL1, myeloid cell leukaemia sequence 1; mTOR, mammalian target of rapamycin; MyoD, myoblast determination; NF-κB, nuclear factor-κB; PDCD4, programmed cell death 4; PER, period; PLK4, Polo-like kinase 4; REST, RE1-silencing transcription factor; RRM2, ribonucleotide reductase subunit M2; SREBP1, sterol regulatory element-binding protein 1; SKP2, S phase kinase-associated protein 2; TEL2, telomere length regulation protein 2; TTI1, TELO2-interacting protein 1.