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SCF ubiquitin ligases in the maintenance of genome stability

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Abstract

In response to genotoxic stress, eukaryotic cells activate the DNA damage response (DDR), a series of pathways that coordinate cell cycle arrest and DNA repair to prevent deleterious mutations. In addition, cells possess checkpoint mechanisms that prevent aneuploidy by regulating the number of centrosomes and the spindle assembly. Among these mechanisms, ubiquitin-mediated degradation of key proteins has an important role in the regulation of the DDR, centrosome duplication and chromosome segregation. This review discusses the functions of a group of ubiquitin ligases, the SCF (SKP1-CUL1-F-box protein) family, in the maintenance of genome stability. Given that general proteasome inhibitors are currently used as anticancer agents, a better understanding of the ubiquitylation of specific targets by specific ubiquitin ligases may result in improved cancer therapeutics.

The DNA damage response and ubiquitylation

Deleterious mutations can occur in genomic DNA owing to chemical modifications originated from endogenous and exogenous sources such as oxidative metabolism, background and medical radiation, carcinogens, and cytotoxic chemotherapy. Although a background level of genetic variability may be pro-adaptive, mutations that interfere with replication, gene expression, and cell division can adversely affect multicellular organisms and result in premature aging, cancer, and organ degeneration [1-4]. Therefore, the maintenance of genome stability is essential for eukaryotic cell survival and allows the proper passage of genetic material to successive generations.

Genome stability maintenance involves many pathways that control the faithful licensing and replication of the genome, cell cycle regulation, centrosome duplication, and chromosome segregation. For example, the DNA damage response (DDR) detects lesions in the DNA, halts cell cycle progression, repairs damage, and re-initiates growth following DNA repair [1, 5]. Alternatively, if repair is impossible, the DDR can initiate senescence or

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apoptosis. The DDR is a remarkable and exquisite process that can be activated by a single abnormal DNA adduct on a millisecond timescale. By contrast, the consequences of the inability to accurately repair DNA damage can manifest years to decades later and even affect progeny. Several reviews summarize recent advances on the DDR and genome stability [5-9]. In addition to the DDR, cells possess systems to ensure proper chromosome segregation and, this way, prevent DNA damage and the generation of aneuploid cells (see Glossary) during cell division. For example, the spindle assembly checkpoint (SAC) ensures that all chromosomes are properly attached to the mitotic spindle before anaphase. Links between the SAC and ubiquitin ligases have been addressed previously [10-12]. Other structural components of the mitotic spindle apparatus are also important in ensuring that chromosomes are not broken or missegregated during mitosis. For example, the centrosome duplication cycle is tightly controlled to prevent formation of multipolar spindles [13, 14].

An additional system with a role in maintaining genome stability is the ubiquitin-proteasome system (UPS), which regulates a diverse array of processes, ranging from cell proliferation and death to circadian clock rhythms. The UPS consists of an ordered and regulated cascade involving three types of enzymes: E1 (ubiquitin activating enzyme or UBA), E2 (ubiquitin conjugating enzyme or UBC), and E3 (ubiquitin ligase) [15, 16]. In humans, there are two E1 proteins, ~30 E2 proteins and hundreds of E3 ligases; the E3 proteins provide substrate specificity to the UPS. RING domain-containing ligases represent the largest family of E3 proteins, with over six hundred members in humans [17].

Among RING domain-containing E3 ligases, the largest group consists of cullin-RING ligases (CRLs), which are composed of a cullin protein scaffold that binds a RING protein in order to recruit an E2 [18]. CRL complexes recruit protein substrates through various adaptor proteins [19]. CRL1 complexes – also known as SKP1/CUL1/F-box protein (SCF) complexes – are the prototypical CRLs [20] (Fig. 1). They use cullin-1 (CUL1) to link the RING domain protein RBX1 (and its associated E2) to an F-box-containing protein via its association with the adaptor protein SKP1. There are 69 F-box proteins encoded in the human genome (allowing 69 distinct SCF complexes) that are divided into three subfamilies (FBXWs, FBXLs, and FBXOs) depending on the nature of domains other than the F-box domain, such as WD40 domains, leucine-rich repeats, or other domains, respectively [20, 21]. F-box proteins bring substrates to the core complex, resulting in ubiquitylation and consequent proteasome-mediated degradation of the substrates. The best-characterized F-box proteins often bind a distinct sequence in their substrates (known as “degron”), which typically needs to be phosphorylated by one or more signaling pathways for binding to occur, providing precise regulation of the degradation of SCF substrates.

In this review, we highlight some of the roles of the SCF family of E3 ligases in maintaining genome stability and discuss future research directions, with particular focus on the response to DNA damage, recovery from DNA damage, and centrosome duplication.

SCF and DNA damage-induced cell cycle arrest

The ability of mammalian cells to halt cell cycle progression following DNA damage is crucial to enable repair and maintain genome stability. SCF ligases are intimately linked with cell cycle regulation, and as a result, F-box proteins, such as β -transducin repeat-containing protein (β -TRCP), firmly link the control of cell cycle progression with the response to replication stresses and other sources of genotoxic stress.

β -TRCP in cell cycle checkpoints

β -TRCP is a component of the DDR that mediates the degradation of CDC25A, a key phosphatase in cell cycle control [22]. β -TRCP1 and its paralog β -TRCP2/FBXW11 are

biochemically indistinguishable and are jointly referred to as β -TRCP. Following initiation of DNA damage signaling, the CHK1 or CHK2 kinases phosphorylate CDC25A, one of three CDC25 family members that dephosphorylate and activate the cyclin-dependent kinases, CDK1 and CDK2. β -TRCP targets phosphorylated CDC25A for degradation in normally cycling cells during S phase and in response to genotoxic stress [22-25]. Inhibition of β -TRCP following DNA damage leads to a defective intra-S-phase checkpoint and mitotic catastrophe. β -TRCP-mediated degradation of CDC25A ensures attenuation of CDK1 activity and consequent cell cycle arrest. The regulation of CDC25A by SCF $^{\beta$ -TRCP (an SCF complex containing β -TRCP) demonstrates a theme of SCF biology; the degradation of SCF substrates often depends on regulated substrate modifications, such as phosphorylation. Thus, cell signaling pathways integrate multiple stimuli, leading to controlled substrate ubiquitylation and degradation.

Although it is clear that β -TRCP targets CDC25A for degradation, the precise mechanism regulating this event has remained somewhat opaque, most notably the regulation of the CDC25A degron by kinases. Unlike other β -TRCP substrates, which contain the consensus degron DSGxxS with both serines phosphorylated, CDC25A contains an alternate degron (76 SSESTDSG 83 , with serines 76, 79, and 82 phosphorylated) (Fig. 2A). How β -TRCP binds this degron has not been established by a crystal structure [23, 24]. Additionally, while both CHK1 and CHK2 were thought to mediate serine-76 phosphorylation, recent experiments in *CHK2*^{-/-} HCT116 cells suggest that CHK1, rather than CHK2, is primarily responsible for DNA damage-induced CDC25A destruction, although whether this represents a primary or compensatory mechanism is unknown [26]. Regardless, serine-76 phosphorylation is a priming phosphorylation, regulating the phosphorylation of the other degron serines by additional kinases, which directly control the binding of β -TRCP.

Several kinases can phosphorylate the CDC25A degron and affect β -TRCP-mediated turnover of CDC25A. For example, casein kinase I α (CKI α) phosphorylates the CDC25A degron on serines 79 and 82 to contribute to CDC25A turnover [27]. The serine-76 priming phosphorylation by either CHK1 or GSK3 β was required, further supporting the idea that an ordered series of events is required for CDC25A regulation. In fact, phosphorylation by PLK3 may prime CDC25A for GSK3 β phosphorylation [28, 29]. The identification of GSK3 β as a new Ser76 kinase suggests that CDC25A can receive inputs from the MAP kinase and PI3 kinase growth-control signaling pathways via GSK3 β and through cell cycle and DNA damaging pathways via CHK1. Notably, GSK3 β is most active in the G1 phase of the cell cycle, suggesting that the use of different kinases may be dictated by cell cycle phases. Additionally, the use of constitutively active kinases, such as CKI α , hints that combinatorial phosphorylation events are required to impart regulation to this system.

Indeed, CHK1-mediated degradation of CDC25A may also utilize additional degron kinases, such as NEK11 [30]. NEK11 phosphorylates CDC25A on Ser82 of the degron to regulate degradation of CDC25A in cells with and without DNA damage. NEK11-mediated phosphorylation of CDC25A required the priming phosphorylation of Ser76 by CHK1, and CHK1-dependent phosphorylation of NEK11 activated CDC25A degradation. In contrast to GSK3 β , NEK11 appeared most active in promoting CDC25A degradation in S and G2 phases. NEK11 expression increases during colon cancer progression, suggesting a role in tumorigenesis, and future research is required to confirm this link [31]. Overall, the identification of multiple degron kinases and sequential phosphorylations for CDC25A suggests regulation by multiple signaling pathways, although a consensus, unified model for the fine details of CDC25A degradation has not yet emerged. Finally, the importance of β -TRCP and CDC25 activity in additional checkpoints must be noted. In response to various non-genotoxic stresses, CDC25B is also degraded in a β -TRCP -dependent manner, but the

degradation depends on a different kinase (stress-activated MAP kinases) and two non-canonical degrons [32, 33].

Beyond CDC25A degradation, β -TRCP also affects the cell cycle in response to DNA damage through regulation of p53, a critical tumor suppressor that implements G1 arrest and maintains G2 arrest following DNA damage via the transcriptional induction of the cyclin-dependent kinase inhibitor p21. While investigating the effect of NF- κ B signaling on p53 stability, Xia *et al.* discovered that I κ B kinase 2 (IKK2/IKK β) phosphorylates p53 on Ser362 and Ser366 to stimulate SCF $^{\beta$ -TRCP-mediated p53 degradation [34]. Notably, this degradation is independent of MDM2, the primary ubiquitin ligase for p53, and β -TRCP can also target MDM2 for degradation. MDM2 lacks the classical DSGxxS degron, and multiple phosphodegrons, targeted by CKI, appear to be required for degradation via SCF $^{\beta$ -TRCP [35]. The interaction of CKI and MDM2 is enhanced following DNA damage, which would contribute to the increase in p53 levels following genotoxic stress. Accordingly, inactivation of β -TRCP or CKI leads to MDM2 accumulation, an increase in p53 activity, and resistance to DNA-damaging agents. However, a recent report suggests that the apparent degradation of MDM2 is an artifact due to MDM2 phosphorylation following DNA damage, which blocks the epitope for the most commonly used MDM2 western blotting antibodies [36].

Regulation of cyclin D1 by SCF complexes

Cyclin D1 is often overexpressed in tumors and is thought to be important in promoting tumorigenesis [37]. The levels of cyclin D1, as its name implies, oscillate during the cell division cycle due in part to ubiquitin-mediated proteolysis, but cyclin D1 is also degraded following DNA damage [38-40]. Several ubiquitin ligases, including FBXO4, FBXW8, FBXO31, SKP2, and β -TRCP, as well as the APC/C, have been reported to lead to cell cycle arrest following DNA damage through cyclin D1 degradation [22, 40-45], making the literature surrounding cyclin D1 degradation by SCF complexes controversial and confusing. Some of this confusion may result from the different stimuli and biochemical mechanisms proposed for degradation of cyclin D1 by SCF ubiquitin ligases.

FBXO4 and FBXW8 are proposed regulators of cyclin D1 cell cycle oscillation, but while they bind the same degron (using Thr286 of cyclin D1), their biochemical mechanisms seem to be different. Both FBXO4 and cyclin D1 are targets of GSK3 β signaling. GSK3 β and 14-3-3 ϵ activate FBXO4 by stimulating FBXO4 dimerization, a requirement for targeting cyclin D1 [41, 42, 46-48]. Therefore, FBXO4 is activated concurrent with the phosphorylation of Thr286 in cyclin D1 by GSK3 β [42]. The importance of FBXO4 dimerization in cyclin D1 degradation is further supported by dimerization-blocking mutations found in cancers that overexpress cyclin D1. Interestingly, FBXW8 also uses the phospho-Thr286 degron of cyclin D1, but FBXW8-mediated degradation of cyclin D1 uses the MEK-ERK pathway instead of GSK3 β signaling [43]. Regardless of the degron kinase, phosphorylated cyclin D1 moves from the nucleus to the cytoplasm, where it is ubiquitinated via cytoplasmic F-box proteins. FBXO4 also has nuclear targets, such as the telomeric protein TRF1, so it is unclear whether FBXO4 is exclusively cytoplasmic [49]. It is possible that FBXO4 and FBXW8 induce cyclin D1 degradation under different physiologic conditions, although the exact contribution from each remains a subject of investigation.

The relative contributions of FBXO4 and FBXW8 to cyclin D1 degradation is further clouded by the contrasting results obtained with mouse models. In one model, *Fbxo4* $-/-$ mice develop tumors (lymphomas and sarcomas) that express high levels of cyclin D1 [50], whereas *Fbxo4* $-/-$ mice from a second laboratory show no observable phenotype (K. Nakayama, personal communication). Moreover, the steady state levels and half-life of cyclin D1 in *Fbxo4* $-/-$, *Fbxw8* $-/-$ or *Fbxo4* $-/-$; *Fbxw8* $-/-$ mouse embryonic fibroblasts

(MEFs) are indistinguishable from those measured in wild type MEFs (K. Nakayama, personal communication). Finally, the silencing of both SKP2 and FBXO31 (see below) in *Fbxo4* $-/-$; *Fbxw8* $-/-$ MEFs does not affect cyclin D1 stability, making the contribution of the various SCF complexes to cyclin D1 degradation an open question.

Whereas multiple F-box proteins have been implicated in the cell cycle-dependent oscillation of cyclin D1, two F-box proteins have been linked to DNA damage-dependent cyclin D1 degradation: FBXO4 and FBXO31. Similar to its role in cell cycle-linked degradation of cyclin D1, FBXO4 also appears to control DNA damage-induced cyclin D1 degradation through GSK3 β -dependent phosphorylation of Thr286; however, the two processes require different upstream pathways, with the DNA damage-induced pathway displaying ATM dependence instead of RAS/RAF dependence [51]. Like FBXO4, FBXO31 binds cyclin D1 via phospho-Thr286, but only after DNA damage [45]. FBXO31-mediated cyclin D1 degradation may explain the rapid initiation of G1 arrest following damage versus the slow kinetics of the p53-dependent induction of p21 required for maintenance of arrest [40, 45]. FBXO31 is a direct target of ATM and is phosphorylated on Ser278, and the levels of FBXO31 increase after DNA damage, unlike FBXO4 or FBXW8 [45]. Therefore, FBXO31 may be physiologically significant only in the DNA damage response, not the cell cycle-dependent oscillations of unperturbed cells.

Notably, despite being active in different contexts, FBXO4, FBXW8, and FBXO31 use the same phosphodegron in cyclin D1. Further research is required to define the mechanisms governing the degron kinases after DNA damage and to understand why FBXW8 plays no role in DNA damage-induced cyclin D1 degradation despite using the same degron.

Recovery from genotoxic stress

The timing of cell cycle re-entry following DNA damage repair is critical to genome stability. Several examples of the role of SCF ubiquitin ligases in damage recovery have been discovered to date, most notably involving β -TRCP and FBXO6.

Claspin and WEE1 degradation

PLK1 is a protein kinase that is required for entry into mitosis. In response to DNA damage, PLK1 activity is eliminated both by dephosphorylation and APC/C^{Cdh1}-mediated degradation [52, 53]. Conversely, PLK1 activity plays a fundamental role in the recovery from genotoxic stress. During recovery, PLK1 levels increase, and PLK1 is activated by the action of Bora and Aurora A, which promote PLK1 phosphorylation [52, 54]. One key function of PLK1 activation is to facilitate substrate targeting by SCF ubiquitin ligases. PLK1 and CDK1 phosphorylate WEE1, a CDK1 inhibitory kinase that is downregulated at the onset of mitosis, leading to β -TRCP-mediated degradation of WEE1 (Fig. 2B) [55]. In addition to removing direct blocks to cell cycle progression, PLK1 activity and β -TRCP also regulate the cessation of CHK1 signaling during recovery from DNA damage. Claspin mediates CHK1 activation by ATR in response to genotoxic stress, and PLK1-dependent phosphorylation of Claspin leads to β -TRCP-mediated degradation and termination of the DNA replication checkpoint, enabling checkpoint recovery [56-58].

CHK1 degradation

Claspin degradation blocks further CHK1 activation during recovery from DNA damage, but phosphorylated (active) CHK1 is inactivated by CUL1-dependent degradation [59]. An siRNA-based approach identified FBXO6 as the F-box protein responsible for CHK1 degradation [60], and the levels of FBXO6 and CHK1 show an inverse correlation in cultured cells and breast tumor tissues, supporting the notion that FBXO6 regulates CHK1. Notably, inhibition of CHK1 degradation due to low levels of FBXO6 is associated with

resistance to the DNA damaging chemotherapeutic camptothecin [60, 61], implying that the lack of degradation of CHK1 leads to increased or persistent DNA damage signaling.

F-box proteins in chromosome segregation

Centrosome homeostasis, the duplication of centrioles, chromosome segregation, and proper cell division are essential components of genome stability [13, 14]. Earlier work demonstrated that the UPS, and SCF ubiquitin ligases in particular, play a role in centrosome biology. Proteasome inhibitors block both centriole separation *in vitro* and centrosome duplication in *Xenopus* egg extracts, and both CUL1 and SKP1 localize to interphase and mitotic centrosomes [62]. Furthermore, transgenic mice that express a dominant negative CUL1 mutant show centrosome and mitotic spindle abnormalities that lead to impaired chromosome segregation and multinucleated cells [63]. More recently, individual F-box proteins and their substrates have been implicated in centrosome homeostasis, giving SCF complexes specific roles in centrosome replication. Centrosome replication involves both centriole duplication and disengagement, and cyclin F (also known as FBXO1) and FBXW5 appear to play key roles in these processes.

SCF targets control centrosome duplication

CP110, a positive regulator of centrosome duplication, was identified as a substrate of SCF^{Cyclin F} [64] (Fig. 3A). Unlike other cyclins, cyclin F does not bind to or activate cyclin-dependent kinases, and unlike other F-box proteins, cyclin F binds CP110 through a mechanism that does not involve phosphorylation of the substrate degron. Instead, a cyclin-binding RxL motif controls the cyclin F-CP110 interaction, and CP110 degradation may also be regulated by changes in cyclin F localization and expression. Cyclin F-mediated degradation of CP110 ensures the duplication of centrosomes only once per cell cycle, and uncoupling CP110 from cyclin F-mediated regulation promotes centrosome overduplication, with consequent induction of abnormal mitotic spindles, chromosome abnormalities, and micronuclei [64]. These phenotypes promote genome instability and aneuploidy, two hallmarks of cancer, and suggest that cyclin F may function in other genome integrity pathways.

Other proteins involved in centrosome duplication are also targeted by SCF ubiquitin ligases (Fig. 3A). FBXW5 was identified as a centrosome regulator by siRNA screening, and it targets the centriole assembly factor SAS-6 for degradation (Fig. 2)[65]. This degradation event is presumed to occur in G2 phase, and alterations in FBXW5 levels lead to reciprocal changes in SAS-6 levels and in centriole numbers. The absence of a known degron in SAS-6 prevents the identification of potential regulatory factors for the FBXW5-SAS-6 interaction, but FBXW5 is highly regulated. FBXW5 is degraded via the APC/C in G1, and PLK4-mediated phosphorylation of FBXW5 likely blocks the ability of FBXW5 to degrade SAS-6 in S phase. Although these results suggest that FBXW5 plays a key role in centrosome duplication, FBXW5 expression only partially rescues the effects of PLK4 depletion on centriole number, suggesting that other, unknown mechanisms control centrosome duplication in parallel.

Cyclin F and FBXW5 regulate the replication of disengaged centrosomes, but SCF complexes also regulate centrosome duplication events in the absence of disengagement (Fig. 3A). PLK4 levels directly correlate with centriole number, and changes in PLK4, by either depletion or ectopic expression, lead to altered centriole number [66]. Therefore, PLK4 levels are tightly controlled. CUL1 was identified as a suppressor of centriole multiplication through regulation of PLK4 levels [67], and later, the F-box protein required for this regulation was identified in both flies and humans. SCF^{Slimb}, the *Drosophila* equivalent of SCF ^{β -TRCP}, regulates PLK4 levels [68, 69], and in mammalian cells, β -TRCP

targets PLK4 for degradation to block centriole re-duplication [70], supporting the previously described centrosome overduplication in β -*Trcp1*^{-/-} MEFs [71].

Regulation of Bora and REST by β -TRCP

Although SCF complexes affect chromosome segregation through centrosome regulation, they can also regulate other components of the mitotic spindle. β -TRCP controls multiple aspects of the mitotic spindle, both directly and indirectly, through degradation of Bora and the transcription factor REST (repressor element 1 silencing transcription factor; also known as NRSF) [54, 72]. Changes in Bora levels affect Aurora A localization and function at spindle poles, and expression of a stable Bora variant that is resistant to β -TRCP-mediated degradation induces formation of monopolar spindles and delays the metaphase-anaphase transition. Notably, this network is intimately linked with the PLK1-dependent pathways that control the recovery from genotoxic stress (Fig. 2B). PLK1 triggers the β -TRCP-dependent degradation of Bora in M-phase, while Bora and Aurora A activate PLK1 during checkpoint recovery [52]. This feedback loop links and controls physiologic cell cycle progression, centrosome duplication, and recovery from the G2 DNA damage checkpoint (Fig. 2B) and suggests that β -TRCP is a biological hub, functioning at the interface of multiple processes.

β -TRCP also regulates the mitotic spindle indirectly through the proteolysis of REST (Fig. 3B). In addition to controlling neurogenesis [73], the regulation of REST by β -TRCP plays a role in cell proliferation through transcriptional repression of MAD2 [72], an essential mitotic spindle checkpoint component, which prevents sister chromatid separation until the microtubules radiating from the spindle poles are correctly attached to the kinetochores. β -TRCP-dependent degradation of REST during G2 permits optimal activation of the spindle checkpoint, but high levels of REST or truncated REST variants (lacking the β -TRCP degron) found in human tumors may contribute to oncogenesis by inhibiting the spindle checkpoint, promoting chromosome instability [72].

Therefore, SCF ubiquitin ligases control the fidelity of chromosome segregation at multiple levels, from centrosome duplication to mitotic spindle regulation to transcriptional regulation of the spindle assembly checkpoint. The complex nature and integration of these networks suggests that SCF ubiquitin ligases may be involved in other, unexplored aspects of these pathways.

Concluding remarks

The role of SCF complexes and proteasome-mediated degradation in cell cycle checkpoints and other processes involved in the maintenance of genome integrity is well established, but it remains an extremely active field of research. Remarkably, relatively few of the 69 F-box proteins have known substrates, and even fewer have known substrates involved in maintaining genome stability. However, even the small number of F-box protein-substrate pairs that are known to be involved in the DDR and genome stability has established SCF complexes as key players in the response to DNA damage, and it is likely that the role of SCF complexes in genome stability will continue to expand with further investigation.

One key area that is likely to feature SCF complex function is the “resetting” of the DNA damage response following repair. Similar to the regulation of cell cycle progression, the proteolytic regulation of components of the DDR could efficiently restrict DDR signaling. Additionally, DNA repair uses nucleases, and overexpression or activation of these nucleases might be toxic; degradation of these nucleases would remove this threat to genome stability [7, 16]. Finally, although K63-based ubiquitylation (which does not lead to degradation, as reviewed in [74]) has a clear role in propagating the DDR signal, no SCF

ligases have been implicated in the activation or amplification of DDR signaling, but degradation-associated ubiquitylation will likely be linked to DDR signaling at some point in the future. In this respect, several F-box proteins have been identified in siRNA screens for DNA damage-associated proteins, but their biological functions remain unknown [75-77].

At a more mechanistic level, many questions remain about the biochemical aspects of SCF complex function in the maintenance of genome stability. For example, the roles of SCF ligases in regulating centrosomes and chromosome segregation also suggest the importance of understanding the subcellular localization – and localized function - of ubiquitin ligases. The varying functions of the isoforms of FBXW7 which occupy discrete subcellular compartments illustrates this principle [78]. These examples support the possibility that the role of F-box proteins in the DNA damage response will be controlled by the localization or redistribution of F-box proteins to sites of DNA damage. Finally, the ability of multiple F-box complexes to regulate the same substrate in a spatial- and context-dependent manner is an underexplored area of SCF biology that could reveal important links between DNA damage responses and normal physiologic processes.

Genome instability is a hallmark of cancer, and several F-box proteins are known oncoproteins or tumor suppressors. Given the crucial function of SCF complexes in genome stability maintenance, deregulation of SCF complexes might allow cancer cells to bypass checkpoints, generate growth-promoting mutations, or become insensitive to DNA damaging therapies. The targeting of either the entire UPS by proteasome inhibitors or the CRL family by NEDDylation inhibitors shows promise as an anticancer strategy [79-81]. However, these approaches are fairly non-specific, and the inhibition of an individual SCF complex could be equally efficacious, with fewer secondary effects. The targeting of SCF complexes with small molecule inhibitors is feasible, both in concept and in practice [82-84], so the translation of recent discoveries in SCF biology to the clinic is the next frontier in this field of investigation.

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Glossary

Aneuploid cells	cells that have an abnormal number of chromosomes.
APC^{CDH1}	The anaphase promoting complex (APC) can use two different adaptor proteins, CDC20 and CDH1. The adaptor protein for any one complex is denoted in superscript.
ATM	ataxia telangiectasia mutated protein, which is a protein kinase that is activated after DNA damage.
Aurora A	protein kinase that is required for proper mitosis.
Bora	protein that binds and activates the Aurora A kinase. It is required for multiple aspects of mitosis.
BRAF	a Raf family protein (see Raf) that is required for RAS pathway signal transduction.

CAAX domain	canonical CAAX domains are a short stretch of amino acids (Cys-Ala-Ala-any amino acid) and dictate the lipidation (addition of hydrophobic molecules) of proteins. Lipidation directs the protein to membranes.
CDH1	CDH1 is one of two adaptors for the APC.
CDC20	CDC20 is one of two adaptors for the APC.
Claspin	a protein that monitors DNA replication, and upon replication stress or DNA damage, it mediates the activation of CHK1 by ATR.
Cyclin D1	the activating subunit for cyclin-dependent kinase complexes using CDK4 or CDK6. Like other cyclins, the levels of cyclin D1 oscillate during the cell cycle, with cyclin D1 levels being highest near the G1-S transition.
F-box proteins	proteins that contain a short, 40 amino acid domain that is homologous to a region in cyclin F. This domain mediates the binding of F-box proteins to SKP1, allowing their incorporation into SCF complexes.
Mitotic spindle	a bipolar network of tubulin filaments that forms during mitosis and facilitates the segregation of chromosomes into daughter cells.
NEDD8	a small ubiquitin-like protein that can be covalently attached to other proteins to modify their functions. NEDD8 is best known for the modification of the cullin family of proteins, which require NEDDylation for their activity.
RAF	a MAP kinase kinase kinase (MAP3K) that functions downstream of RAS proteins in signal transduction cascades.
RAS	the RAS protein family (H- RAS, K- RAS, and N- RAS) of small GTPase proteins functions in many signal transduction pathways and has a well-defined role in growth control pathways and tumorigenesis.
14-3-3ε	a phosphoserine.binding protein involved in multiple signal transduction cascades.

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Figure 1. The SCF complex

The SCF (SKP1/CUL1/F-box protein) complexes are assembled using a CUL1 scaffold. CUL1 recruits an E2 ubiquitin conjugating enzyme through RBX1. Substrates are recruited to the complex by SKP1 and a variable F-box protein that determines substrate specificity.

Figure 2. β -TRCP and the DNA damage response

(a) β -TRCP typically recognizes substrate degrons containing DSGxxS motifs, in which certain serines or threonines are phosphorylated (bold). Most substrates (such as β -catenin, Ikb α , and claspin) contain the consensus motif, whereas other substrates (such as Bora and WEE1) have variant motifs. CDC25A is recognized through an alternate phospho-degron. The three initial residues shown, SSE, represent the priming kinase site for CDC25A and are not part of the true degron that dictates binding to β -TRCP. **(b)** The F-box protein β -TRCP controls DNA damage-induced cell cycle arrest, cessation of DNA damage signaling, and recovery from cell cycle arrest. β -TRCP leads to the degradation of CDC25A (a CDK1 activator) in a CHK1-dependent manner. Upon removal of genotoxic stress, Claspin (a co-activator for ATR-mediated phosphorylation of CHK1) and WEE1 are degraded in a β -TRCP- and PLK1-dependent manner to stop DNA damage signaling and allow exit from cell cycle arrest. Thus, β -TRCP is involved in DNA damage-induced cell cycle arrest (through CDC25 degradation), the cessation of DNA damage signaling (through CHK1 inhibition by Claspin degradation), and the recovery from DNA damage (via degradation of WEE1) through a feedback loop involving the regulation of multiple kinases. Notably, the key kinases for β -TRCP substrate degradation, CHK1 and PLK1, are also regulated by β -TRCP through the degradation of their activators (Claspin and Bora, respectively).

Figure 3. SCF ubiquitin ligases in centriole duplication and chromosome segregation
(a) PLK4, SAS6, and CP110 are SCF substrates that are required for centrosome duplication. PLK4 is targeted by β -TRCP; SAS6 is targeted by FBXW5; and CP110 is targeted by cyclin F. Further regulation of FBXW5 is provided by APC/C-mediated degradation. PLK4 has additional substrates, including FBXW5, which may also affect centrosome duplication. **(b)** REST, which controls the spindle assembly checkpoint (SAC) through transcriptional regulation of MAD2, is also targeted by β -TRCP.