



# Ubiquitin ligases: cell-cycle control and cancer

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**Abstract** | A driving force of the cell cycle is the activation of cyclin-dependent kinases (CDKs), the activities of which are controlled by the ubiquitin-mediated proteolysis of key regulators such as cyclins and CDK inhibitors. Two ubiquitin ligases, the SKP1–CUL1–F-box-protein (SCF) complex and the anaphase-promoting complex/cyclosome (APC/C), are responsible for the specific ubiquitylation of many of these regulators. Deregulation of the proteolytic system might result in uncontrolled proliferation, genomic instability and cancer. Cumulative clinical evidence shows alterations in the ubiquitylation of cell-cycle regulators in the aetiology of many human malignancies. A better understanding of the ubiquitylation machinery will provide new insights into the regulatory biology of cell-cycle transitions and the development of anti-cancer drugs.

## Cyclin-dependent kinase

A protein kinase that controls cell-cycle progression in all eukaryotes and requires physical association with cyclins to achieve full enzymatic activity.

## Cyclin

The positive regulatory subunits of cyclin-dependent kinases, showing oscillating expression in the cell cycle.

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Deregulated cell-cycle control is a fundamental aspect of cancer. Normal cells only proliferate in response to developmental or other mitogenic signals that indicate a requirement for tissue growth, whereas the proliferation of cancer cells proceeds essentially unchecked. This indicates that cancer cells proliferate because of defects in internal and external proliferation-inhibitory signals. However, there must also be defects in the negative-feedback systems that should prevent such unregulated proliferation. We now have a detailed molecular picture of normal cell-cycle control, in which a series of kinases promote progression through each phase of the cell cycle. The phosphorylation of a variety of proteins by members of the cyclin-dependent kinase (CDK) family is crucial to this progression. Each CDK is dependent on a particular cyclin; therefore, the activity of each CDK can be controlled by the availability of its cyclin partner, and also the expression of a specific CDK inhibitor (CKI).

Levels of cyclin expression during cell division are periodic<sup>1</sup>. This is the result of a constant synthetic rate coupled with a defined window in the cycle of specific proteolysis, which is executed by the ubiquitin-proteasome system (UPS) (REF. 2). CKIs, negative-regulators of cyclin–CDK kinase complexes, are also targeted for degradation by the UPS. Therefore, the cell cycle is predominantly regulated by two types of post-translational protein modification — phosphorylation and ubiquitylation (BOX 1). The UPS comprises two discrete steps: the covalent attachment of multiple ubiquitin molecules to the protein substrate and degradation of the polyubiquitylated protein by the 26S proteasome complex<sup>3</sup> (FIG. 1).

The first step is mediated by at least three enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). An involvement of E1 in cancer has not been described, and only a few reports have linked E2 to cancer development<sup>4</sup>. By contrast, a large amount of evidence indicates that deregulation of E3 ligase, which is involved in many biological systems, often results in cancer development.

A direct molecular link between the deregulation of cell-cycle control and cancer was first discovered when it was shown that the genes that encode retinoblastoma protein (RB) and p53, both of which restrain cell-cycle progression, are frequently mutated or deleted in human cancers. Decreased expression of p27 CKI is also frequently found in various human cancers, and is a strong indicator of poor prognosis<sup>5</sup>. In contrast to RB and p53, mutations or deletions in p27 were found to be rare in cancer patients. Instead, a decrease in the protein stability of p27 contributes to its reduced levels in cancer cells<sup>6,7</sup>. These clinical findings were later explained by the overexpression of S-phase kinase-associated protein 2 (SKP2), a component of E3 ligase that targets p27 for degradation<sup>8</sup>. In contrast to the characterization of SKP2 as an oncogene, another component of E3 ligase, F-box and WD-40 domain protein 7 (FBW7), was biochemically and genetically shown to be a tumour suppressor<sup>8</sup>. Experimental and clinical data on SKP2 and FBW7, and also on other E3 ligases, indicates that the deregulation of the UPS in cell-cycle control is tightly linked to cancer development.

**Ubiquitin**

A 76-amino-acid polypeptide that is conjugated through an isopeptide linkage to other proteins. Such conjugates are most commonly targeted for degradation by the proteasome.

**Proteasome**

A large ~2.5-MDa multisubunit protein complex that binds to and subsequently degrades polyubiquitylated proteins in an ATP-dependent manner.

**Ubiquitin-conjugating enzyme (E2)**

An enzyme that accepts ubiquitin from a ubiquitin-activating enzyme (E1) and, together with a ubiquitin ligase (E3), transfers it to a substrate protein.

**Ubiquitin ligase (E3)**

A protein or protein complex that facilitates the transfer of ubiquitin from a ubiquitin-conjugating enzyme (E2) to a substrate. E3 enzymes provide platforms for binding E2 enzymes and specific substrates, thereby coordinating the ubiquitylation of the selected substrate.

**Cullins**

A family of proteins that are characterized by the presence of a distinct globular C-terminal domain (cullin homology domain) and a series of N-terminal repeats of a five helix bundle (cullin repeats).

**F-box protein**

A variable component of SCF E3 ligase that binds to SKP1 through the F-box domain. FBPs recognize specific substrates and, with the help of other subunits of the E3 ubiquitin ligase, deliver them to the E2 ubiquitin-conjugating enzyme.

**RING-finger proteins**

A family of proteins structurally defined by a particular folded protein domain that binds Zn<sup>2+</sup> through a four-point arrangement of cysteine and histidine amino acids. In most cases, a RING-finger protein interacts with an E2 and serves as an E3.

**At a glance**

- Two major classes of ubiquitin ligases, the SKP1–CUL1–F-box-protein (SCF) complex and the anaphase-promoting complex/cyclosome (APC/C), have a central role in cell-cycle regulation.
- The SCF complex and APC/C are structurally similar. Each is constituted of common subunits and a variable substrate-recognition subunit (F-box proteins for the SCF complex and activators for the APC/C). Three F-box proteins in the SCF complex — S-phase kinase-associated protein 2 (SKP2), F-box and WD-40 domain protein 7 (FBW7) and  $\beta$ -transducin repeat-containing protein ( $\beta$ -TRCP) — and two activators in the APC/C — cell division cycle 20 (CDC20) and CDH1 (also known as HCT1) — are the most important in cell-cycle regulation.
- SKP2 targets negative regulators of the cell cycle such as p27, p21 and p57 for degradation, and thereby promotes cell-cycle progression during S and G2 phases. SKP2 is upregulated in many human cancers.
- FBW7 induces the degradation of positive regulators of the cell cycle, such as MYC, JUN, cyclin E and Notch. FBW7 is often mutated in a subset of human cancers.
- $\beta$ -TRCP is a versatile F-box protein that recognizes several cell-cycle regulators — EMI1/2, WEE1A and CDC25A/B — in addition to its classical substrates,  $\beta$ -catenin and I $\kappa$ B. In some cancers,  $\beta$ -TRCP mutation or overexpression is found.
- CDC20 targets securin and mitotic cyclins for destruction, and thereby promotes sister-chromatid separation. CDC20 is the crucial mediator of the spindle checkpoint, which prevents aneuploidy and genomic instability. CDC20 is overexpressed in some cancers, although in others the CDC20 gene is mutated or deleted.
- CDH1 facilitates exit from M phase and maintains G1 phase by mediating the degradation of mitotic cyclins, non-CDK (cyclin-dependent kinase) mitotic kinases and some regulators of the formation of pre-replicative complexes. Deregulated expression or mutation of CDH1 as well as of most CDH1 targets have been described in human cancers.

The recent approval of a proteasomal inhibitor, Bortezomib, by the US Food and Drugs Administration (FDA) for the treatment of relapsed and refractory multiple myeloma should further stimulate the search for specific E3 inhibitors. Targeting a specific E3 would selectively stabilize its target(s) and have an advantage over proteasomal inhibition by

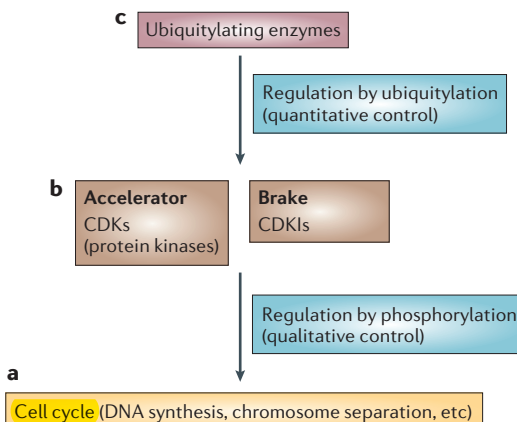
avoiding unwanted effects on other cellular proteins. Therefore, E3 ubiquitin ligases are expected to draw much attention as a promising class of new drug targets with a high level of specificity and less associated toxicity.

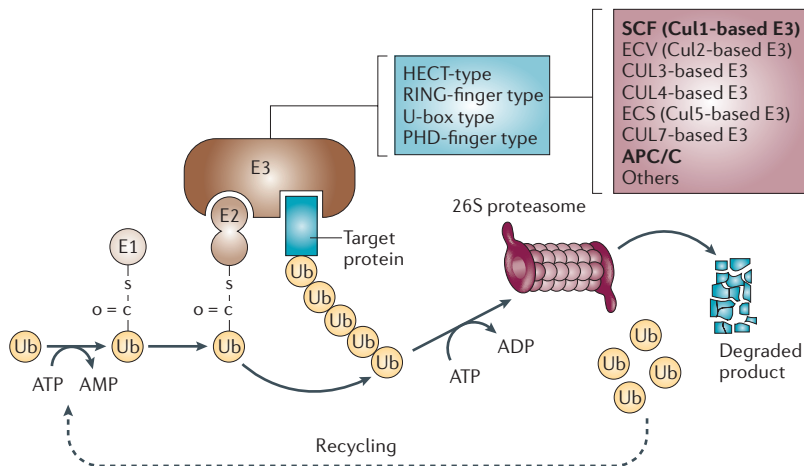
**SCF and APC/C: similar but distinct**

E3 components in the UPS are thought to be primarily responsible for the specific recognition of a large number of target proteins<sup>3</sup>. This requires both specificity and versatility, which are provided by the existence of 500–1,000 different E3 ligases. These are now categorized into four major classes on the basis of their specific structural motif: HECT-type, RING-finger-type, U-box-type or PHD-finger-type (FIG. 1). RING-finger-type E3s are thought to be the largest family and are further divided into subfamilies; one of these, the cullin-based E3 subfamily, is one of the largest single classes of E3. There are seven cullin-based E3s, including the SKP1–CUL1–F-box-protein (SCF) complex<sup>8</sup> and the anaphase-promoting complex/cyclosome (APC/C)<sup>9,10</sup>, both of which are involved in the proteolysis of core components of the cell-cycle machinery. The SCF complex consists of three invariable components — RBX1 (RING-finger protein), CUL1 (scaffold protein), and SKP1 (adaptor protein) — as well as one variable component, known as an F-box protein, that binds through its F-box motif to SKP1 and is responsible for substrate recognition (FIG. 2). Approximately 70 F-box proteins have been identified in humans, and they fall into three categories: those with WD40 repeats (FBXW), leucine-rich repeats (FBXL) or other domains (FBXO)<sup>11</sup>. Three F-box proteins — SKP2 (FBXL1), FBW7 (FBXW7) and  $\beta$ -transducin repeat-containing protein ( $\beta$ -TRCP) (FBXW1/11) — are thought to be involved in cell-cycle control (the functions of most other F-box proteins remain unknown).

**Box 1 | Three-layer regulation of the cell cycle**

Cell-cycle control can be described as a 3-layer process. The immediate phenomena of the cell cycle, including DNA synthesis and chromosome separation (layer a), are qualitatively controlled by phosphorylation. Movement through the cycle (layer b) depends on the activity of cyclin-dependent kinases (CDKs), which are promoted by accelerators — cyclins — and antagonized by brakes — CDK inhibitors (CKIs). The protein levels of cyclins, CKIs and many other cell-cycle-related regulators are quantitatively controlled by ubiquitylating enzymes (layer c). Accumulating clinical evidence shows various alterations in the ubiquitylation of cell-cycle regulators in the aetiology of many human malignancies.





**Figure 1 | Overview of the ubiquitin-proteasome pathway.** Ubiquitin (Ub) is a small 8-kDa protein, which is first transferred to the ubiquitin-activating enzyme, E1, in an ATP-dependent manner. This activated ubiquitin is then transferred to the ubiquitin-conjugating enzyme, E2. Finally, the ubiquitin is covalently attached to the target protein by E3 ubiquitin ligase, leading to the formation of a polyubiquitin chain. The polyubiquitinated protein is recognized by the 26S proteasome, and is destroyed in an ATP-dependent manner. There are many E3 ubiquitin ligases, which are categorized into four major classes: HECT-type, RING-finger-type, U-box-type and PHD-finger-type. RING-finger-type E3s are further divided into subfamilies, including cullin-based E3s, which constitute one of the largest classes of E3s. There are seven cullin-based E3s including the SKP1–CUL1–F-box-protein (SCF) complex and the anaphase-promoting complex/cyclosome (APC/C).

The APC/C is structurally similar to the SCF complex, and consists of invariable core components — APC11 (RBX1-related RING-finger protein), APC2 (CUL1-related scaffold protein) and at least 11 other components without a defined role — as well as a variable component known as an activator. There are two such variable components in mitotically cycling cells — cell division cycle 20 (CDC20) and CDH1 (also known as HCT1) — and they confer substrate specificity in the same way that F-box proteins do in the SCF complex<sup>9,10</sup>. Additional APC/C activators function during meiosis and in non-dividing cells (reviewed in REF. 12).

Despite the structural and biochemical similarities between the SCF complex and the APC/C, their cellular functions are different. This is highlighted by the timing of action of each E3 complex (FIG. 3a): the APC/C is active from mid-mitosis (anaphase) to the end of G1 phase, whereas the SCF complex, although originally thought to function mainly at the G1–S transition, is active from late G1 to early M phase. How are their roles clearly delineated? The most persuasive explanation is that they constitute a regulatory loop. Although the entire picture is not clear, several lines of evidence show that there is a tight interplay between the SCF complex and the APC/C<sup>13–16</sup> (FIG. 3b). The SCF complex and the APC/C also differ in their frequency of genetic alterations in **cancer**: far more alterations connected with **cancer** have been found for the SCF complex than for the APC/C.

**SKP2 is oncogenic**

SKP2 was originally discovered as a protein that associates with cyclin A–CDK2 in transformed cells<sup>17</sup>, and is

now known to be an F-box protein of the SCF complex. In conjunction with SKP2, the SCF complex targets p27 (REFS 18–20), p21 (REFS 21,22) and p57 (REF. 23) CKIs for degradation (FIG. 4). To date, it has also been reported that SKP2 targets p130, cyclin A, cyclin D1, free cyclin E, E2F1, ORC1, CDT1, CDK9, MYC, B-MYB, SMAD4, RAG2, UBP43, FOXO1 and papillomavirus E7 (reviewed in REF. 8). However, it is p27 that seems to be the primary target of SKP2, given that *Skp2*<sup>-/-</sup> mice show a marked accumulation of p27 (REF. 24), and that prominent cellular phenotypes apparent in *Skp2*<sup>-/-</sup> mice (see **Supplementary information S1** (table)) — including nuclear enlargement, polyploidy and an increased number of centrosomes that are probably caused by overreplication of chromosomes and centrosomes — disappear in *Skp2*<sup>-/-</sup> *p27*<sup>-/-</sup> double-mutant mice<sup>25,26</sup>.

It has been widely accepted that p27 is a tumour suppressor, not only because of its activity as a CKI, but also because of evidence from mouse models<sup>27–29</sup> and the marked correlation between reduced p27 levels and poor prognosis found in clinical studies of patients with **cancer**<sup>5</sup>. Indeed, a reduction in the concentration of p27 is common in many types of human malignancies. However, in contrast with other tumour suppressors such as p53 or RB, mutation or deletion of the *p27* gene is an uncommon event in the development of human **cancers**, indicating that deregulation of p27 expression in human tumours is often due to post-transcriptional mechanisms. It has become evident that SKP2 expression is inversely correlated with levels of p27 in many **cancers**, and also with the grade of malignancy in certain human tumours (TABLE 1). In addition, frequent amplification and overexpression of the *SKP2* gene has been observed in **lung cancers**<sup>30,31</sup> and in cell lines expressing high-risk human papilloma virus<sup>32</sup>. Other components of the ubiquitylation machinery for p27, primarily CKS1, have oncogenic potential in patients with **colorectal carcinoma**<sup>33</sup>, and overexpression of SKP2 or CKS1 is strongly and independently associated with a loss of tumour differentiation and poor survival<sup>34</sup>. The oncogenic potential of SKP2 has also been shown in transgenic mouse models<sup>35,36</sup>. Furthermore, Ras signalling induces SKP2 expression through the binding of GA-binding protein, an Ets-family transcription factor, to the SKP2 promoter<sup>37</sup>. Such evidence supports the notion that SKP2 is a growth promoter and an oncoprotein.

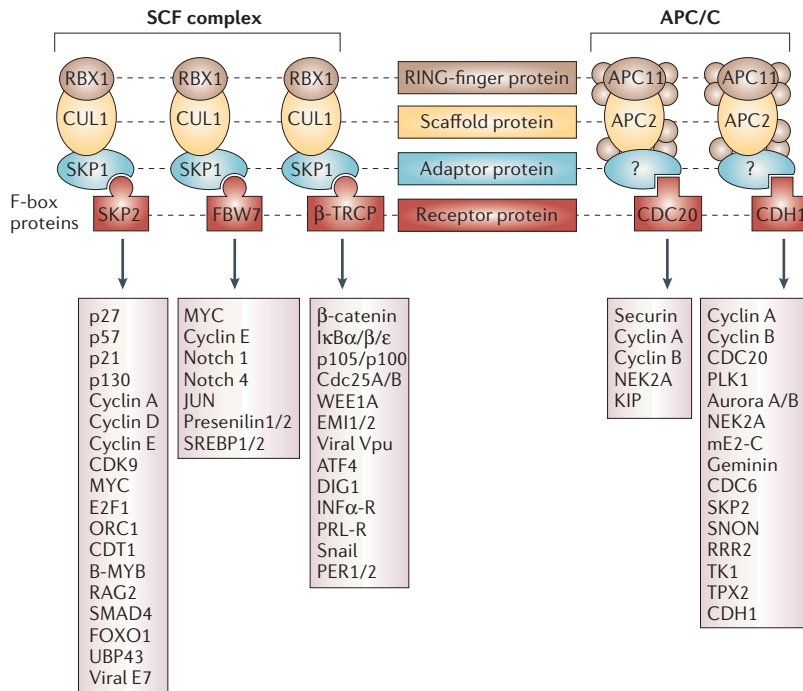
However, in the time since it became widely accepted that SKP2 mediates p27 degradation in G1 phase, several discrepancies have emerged. First, SKP2 is not expressed until early S phase, unequivocally later than the degradation of p27 apparent at G1 (REFS 17,38,39). Second, p27 is exported from the nucleus to the cytoplasm at G1 (REFS 39,40), whereas SKP2 is restricted to the nucleus<sup>41</sup>. Third, the downregulation of p27 at the G0–G1 transition occurs normally in *SKP2*<sup>-/-</sup> cells and is sensitive to proteasome inhibitors<sup>38</sup>. These temporal, spatial and genetic discrepancies indicate that p27 is degraded at G1 in the cytoplasm by a proteasome-dependent, but SKP2-independent, mechanism. We have recently purified an E3 enzyme, designated KIP1

**WD40 repeat**

A protein-interaction domain consisting of 40 amino-acid repeats that form a propeller-like structure, in which each repeat contributes a blade.

**Leucine-rich repeat**

A protein-sequence motif that contains regular occurrences of the amino acid leucine, which are present as tandem arrays in certain proteins. The back-to-back set of motifs was found to correspond to a small sub-domain structure in the protein that stacks next to adjacent repeats to form a parallel, β-sheet, arc-like structure.



**Figure 2 | The structural similarity of SCF and APC/C.** Both the SKP1–CUL1–F-box-protein (SCF) complex and the anaphase-promoting complex/cyclosome (APC/C) are constituted of common subunits and variable substrate-recognition subunits. The SCF complex consists of the invariable components RBX1 (RING-finger protein), CUL1 (scaffold protein) and SKP1 (adaptor protein) as well as a variable F-box-protein component, which is responsible for substrate recognition. Among more than 70 F-box proteins in humans, three proteins — S-phase kinase-associated protein 2 (SKP2), F-box and WD-40 domain protein 7 (FBW7) and β-transducin repeat-containing protein (β-TRCP) — are the main protagonists in cell-cycle control. The APC/C consists of the invariable core components APC1 (RBX1-related RING-finger protein), APC2 (CUL1-related scaffold protein) and at least 11 other components, the functions of which are not fully understood. In addition, there is the variable activator subunit, cell division cycle 20 (CDC20) or CDH1 (also known as HCT1), both of which confer substrate specificity in the same way as F-box proteins do in the SCF complex. Known substrates of each variable subunit in SCF and APC/C are shown<sup>8–10,97</sup>.

ubiquitylation-promoting complex (KPC), that interacts with, and ubiquitylates, p27 in G1 phase and is localized to the cytoplasm of mammalian cells<sup>42</sup>. KPC consists of two subunits, KPC1 and KPC2. KPC1 contains a RING-finger domain near its C terminus, and functions as the catalytic subunit<sup>43</sup>. KPC2 is a member of the UBL–UBA family of proteins. KPC2 stabilizes KPC1, recruits polyubiquitylated p27 and interacts with the 26S proteasome, thereby promoting the degradation of p27 (REF. 44). Inhibition of either KPC1 or KPC2 by RNA interference or with dominant-negative mutants delays p27 degradation at the G0–G1 transition<sup>42–44</sup>. These results indicate that the degradation of p27 is regulated by two distinct mechanisms: translocation-coupled cytoplasmic ubiquitylation by KPC at the G0–G1 transition and nuclear ubiquitylation by SKP2 during S and G2 phases (see **Supplementary information S2** (figure)). Given that, in a subset of breast cancers (32 out of 84 samples)<sup>45</sup>, p27 levels were low despite SKP2 not being expressed, it remains possible that the expression of KPC might be elevated in such cases.

**Degron**

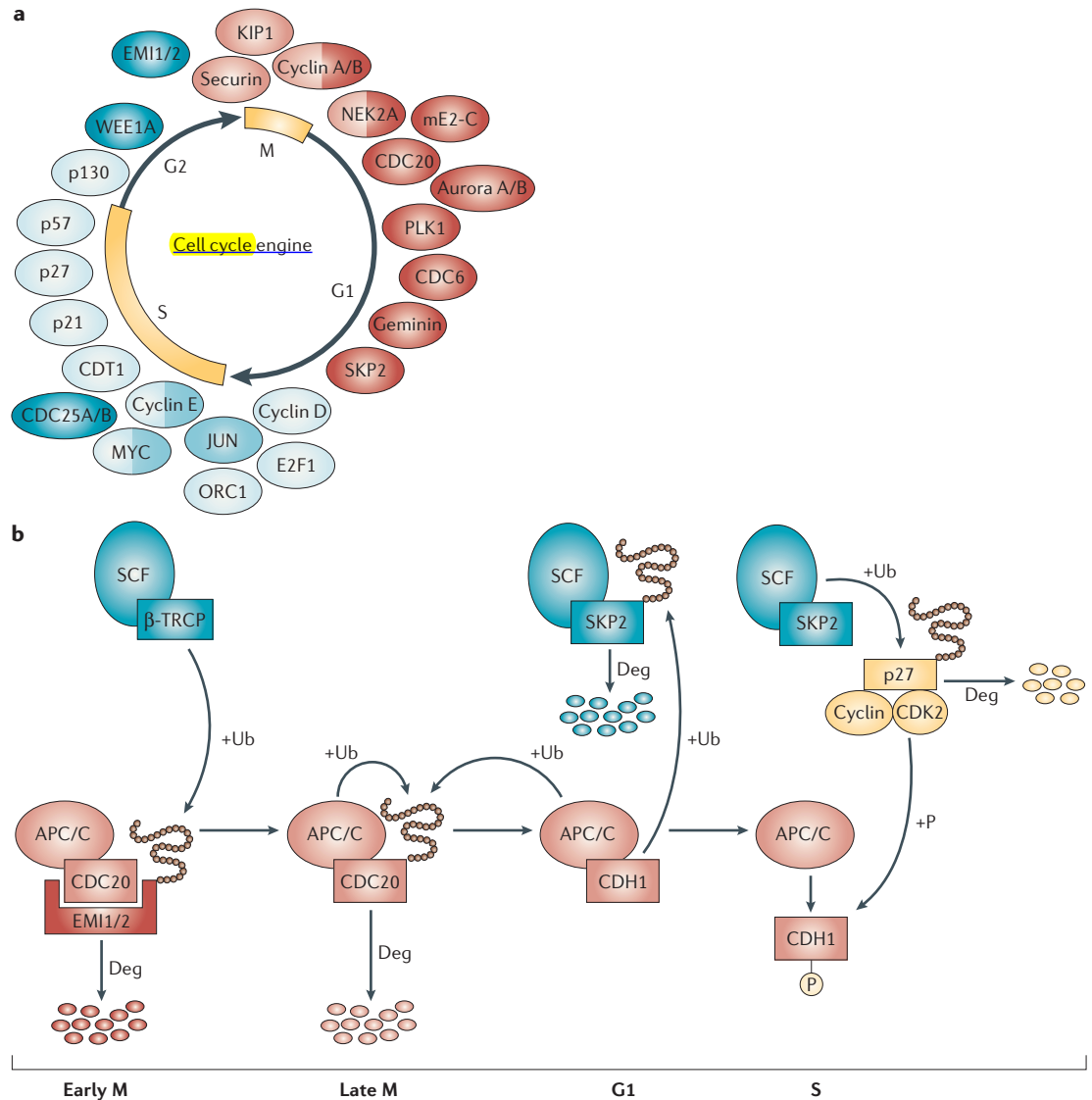
A portion of a protein that is necessary and sufficient to bring about its degradation by the ubiquitin–proteasome system.

**FBW7 as a tumour suppressor**

FBW7 is another F-box protein of the SCF complex that targets several oncoproteins, including cyclin E, MYC, JUN, Notch 1 and Notch 4 for degradation (reviewed in REF. 8) (FIG. 4). FBW7 was first discovered as a negative regulator of the lin-12 (Notch) signalling pathway in *Caenorhabditis elegans* by genetic screening<sup>46</sup>. We, and others, have generated mice that are deficient in FBW7 and found that the embryos die *in utero* at embryonic day 10.5, manifesting marked abnormalities in vascular development<sup>47,48</sup> (see **Supplementary information S1** (table)). Notch accumulates in *Fbw7*<sup>-/-</sup> embryos, resulting in an increased expression of HEY1, a transcriptional repressor that acts downstream of Notch and is implicated in vascular development<sup>47</sup>. So, FBW7 has a crucial role in mammalian vascular development by regulating Notch stability during embryogenesis.

Given that FBW7 is responsible for the degradation of the above-mentioned oncoproteins, FBW7 is thought to function as a tumour suppressor. Indeed, mutations in *FBW7* were found in ovarian cancer, breast cancer, lymphoma and colorectal cancer (TABLE 1). In animal models, *Fbw7*<sup>+/-</sup> mice have greater susceptibility to radiation-induced tumorigenesis, but most tumours retain and express the wild-type allele, indicating that FBW7 is a haplo-insufficient tumour-suppressor gene<sup>49</sup>. How does the decrease in FBW7 function result in cancer development? Deregulation of cyclin E has been considered to be a major factor in tumorigenesis because increased levels of cyclin E have been associated with various malignancies, and constitutive expression of cyclin E leads to genomic instability<sup>50</sup>. Furthermore, knockdown of cyclin E expression in the cancer lines in which FBW7 was also knocked out significantly reduced the extent of chromosomal instability<sup>51</sup>. However, recent studies do not always support this notion. Expression of cyclin E is not always increased in cancer cells in which FBW7 is mutated (REFS 49,52 and our unpublished observations). Furthermore, expression of cyclin E was unaffected in *Fbw7*<sup>-/-</sup> embryos<sup>47,49</sup>, and the concentration of cyclin E was increased only in the placenta<sup>48</sup>. Radiation-induced lymphomas are frequently observed in *Fbw7*<sup>+/-</sup> mice, but the level of cyclin E is not elevated<sup>49</sup>. It therefore seems likely that FBW7 contributes to cyclin E proteolysis in a context-dependent manner.

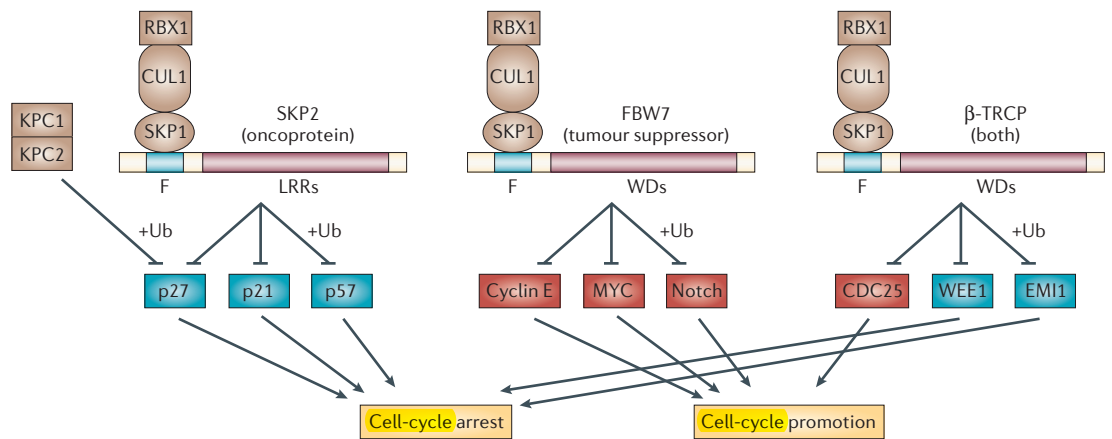
Another important substrate of FBW7 is MYC, the deregulation of which might be responsible for cancer development. Given that the expression level of MYC is increased in many malignant tumours, and many MYC mutations affect the stability of the encoded protein<sup>53</sup>, its turnover is thought to be a crucial determinant of carcinogenesis. MYC is ubiquitylated and degraded by the proteasome. The region of MYC that signals its ubiquitylation (the degron) overlaps with the transactivation domain, in which two highly conserved sequence elements — MYC box 1 (MB1) and MB2 — have been implicated in the proteolysis of MYC. In particular, phosphorylation of Thr58 and Ser62 in MB1 is an important determinant of MYC stability<sup>53</sup>. Growth factors control MYC stability primarily through the phosphorylation of these



**Figure 3 | Cell-cycle regulation by the SCF complex and APC/C.** **a** | The SKP1–CUL1–F-box-protein (SCF) complex and the anaphase-promoting complex/cyclosome (APC/C) function at distinct stages in the cell cycle. SCF ubiquitylates substrates from late G1 to early M phase, whereas the APC/C is active from mid-M phase (anaphase) to the end of G1 phase. The approximate timing of the destruction of substrates mediated by the APC/C is shown in shades of red: light red denotes the presence of the cell division cycle 20 (CDC20) variable subunit, and dark red denotes the presence of CDH1 (also known as HCT1). Similarly, light green, mid green and dark green represent substrates ubiquitylated by the SCF complex in association with S-phase kinase-associated protein 2 (SKP2), F-box and WD-40 domain protein 7 (FBW7) and  $\beta$ -transducin repeat-containing protein ( $\beta$ -TRCP), respectively. The order of degradation is represented approximately in this figure. **b** | The boundaries between the activities of the SCF complex and the APC/C, and between the variable subunits, are governed by positive- and negative-feedback interactions between them. In early M phase, SCF $^{\beta$ -TRCP recognizes the phosphorylated APC/C $^{CDC20}$  inhibitors EMI1 and EMI2, causing their degradation and an increase in APC/C activity. In late M phase, CDC20 is ubiquitylated, and therefore degraded, by APC/C $^{CDH1}$  or by itself, leading to the transition from APC/C $^{CDC20}$  to APC/C $^{CDH1}$ . CDH1 recognizes the D box of SKP2 and induces its degradation, resulting in low SCF complex activity and the accumulation of cyclin-dependent kinase inhibitors (CKIs) such as p27. In turn, increased expression of SKP2 at the G1–S boundary promotes p27 degradation and activates S-cyclin/cyclin dependent kinase 2 (CDK2), which induces the dissociation of CDH1 from the core APC/C subunits by the phosphorylation of CDH1. So, APC/C activity declines at the G1–S boundary, allowing mitotic cyclins to accumulate gradually during S and G2 phases. P, phosphate; Ub, ubiquitin.

sites (see **Supplementary information S3** (figure)). Thr58 and Ser62 residues are frequently mutated in human tumours, which is consistent with the effect of phosphorylation on MYC stability<sup>53</sup>. We, and others,

have shown that FBW7 interacts with, and promotes the degradation of, MYC in a manner that is dependent on the phosphorylation of MB1 (REFS 54,55). Accumulation of MYC is also apparent in mouse



**Figure 4 | Functions of the SKP1–CUL1–F-box-protein (SCF) complex.** The variable components (F-box proteins) of the SCF complex — S-phase kinase-associated protein 2 (SKP2), F-box and WD-40 domain protein 7 (FBW7) and  $\beta$ -transducin repeat-containing protein ( $\beta$ -TRCP) — and their major targets are shown: red and green boxes indicate cell-cycle promoters and inhibitors, respectively. SKP2 promotes the cell cycle through the ubiquitylation of CDK inhibitors (CKIs), including p27, and therefore functions as an oncoprotein. The KIP1 ubiquitylation-promoting complex (KPC) also mediates p27 ubiquitylation. SKP2 is overexpressed in many cancers. By contrast, FBW7 targets cell-cycle promoters to inhibit the cell cycle, and serves as a tumour suppressor. Mutations or deletions in FBW7 are found in a subset of human cancers.  $\beta$ -TRCP is both a cell-cycle promoter and inhibitor. Both mutation and overexpression of  $\beta$ -TRCP are observed in human cancers. F, F-box domain; LRRs, leucine-rich repeats; Ub, ubiquitin; WDs, WD40 repeats.

*Fbw7<sup>-/-</sup>* cells<sup>54</sup>, as well as in lymphomas from *Fbw7<sup>+/-</sup>* mice<sup>49</sup>. Surprisingly, SKP2 also binds to MYC through its MB2 and helix-loop-helix–leucine-zipper (HLH-Zip) domains, and thereby mediates its ubiquitylation and degradation<sup>56,57</sup>. However, SKP2 unexpectedly increases the transactivation activity of MYC, indicating that SKP2 is a transcriptional cofactor. Consistent with this notion, MYC accumulates, but its transcriptional activity is reduced, in mouse *Skp2<sup>-/-</sup>* cells<sup>57</sup>. These observations indicate that two F-box proteins, FBW7 and SKP2, differentially regulate MYC stability by targeting MB1 and MB2 domains, respectively.

Increased expression of not only cyclin E and MYC but also other substrates of FBW7 might contribute to cancer development. Deregulation of wild-type Notch, Notch ligands or downstream targets has been detected in many human malignancies<sup>58</sup>. Truncated Notch proteins exhibit transforming activity both *in vitro* and in animal models<sup>59–62</sup>. Furthermore, Notch 1 was recently shown to be a downstream effector of oncogenic Ras, CDC20 and CDH1, and depletion of Notch 1 in Ras-transformed human cells was sufficient to abolish key elements of the neoplastic phenotype *in vitro* and *in vivo*<sup>63</sup>. *Notch4* was originally identified as *Int3*, a proto-oncogene that is a frequent target for the integration of mouse-mammary-tumour virus in mammary carcinomas<sup>64</sup>. The JUN oncoprotein is a major component of the transcription factor API1, the constitutive activation of which is apparent in various types of human tumour cells, indicating that API1 has an important role in human oncogenesis<sup>65</sup>. Mutation of the human *FBW7* gene might therefore result in the impaired degradation of these substrates and their subsequent accumulation, which might then contribute to carcinogenesis.

### $\beta$ -TRCP regulates many pathways

$\beta$ -TRCP is a versatile F-box protein of the SCF complex that targets various substrates for degradation, including crucial cell-cycle regulators such as EMI1/2 (REFS 13, 14, 66, 67), WEE1A (REF. 68) and CDC25A/B (REFS 69–71) (FIG. 4). The  $\beta$ -TRCP family is highly conserved and includes *Drosophila melanogaster* Slimb<sup>72</sup> and *Xenopus laevis*  $\beta$ -TrCP<sup>73</sup>, as well as mammalian  $\beta$ -TRCP1 (also termed FBW1a or FWD1) and  $\beta$ -TRCP2 (also known as FBW1b or HOS).  $\beta$ -TRCPs also target  $\beta$ -catenin and I $\kappa$ B for ubiquitylation and degradation<sup>8</sup>, and  *$\beta$ -TRCP1<sup>-/-</sup>* mice have a partial defect in their ability to eliminate  $\beta$ -catenin and I $\kappa$ B $\alpha$  in certain situations<sup>74</sup> (see Supplementary information S1 (table)). Other substrates that are degraded as a result of  $\beta$ -TRCP-dependent ubiquitylation include I $\kappa$ B $\beta$  and I $\kappa$ B $\epsilon$ , the p105 and p100 subunits of nuclear factor  $\kappa$ B (NF $\kappa$ B), ATF4 (CREB2), VPU protein of human immunodeficiency virus type 1, interferon receptor, prolactin receptor, mammalian circadian regulatory proteins PER1 and PER2, Snail and the discs large (hDLG) tumour suppressor (reviewed in REF. 8).  $\beta$ -TRCP recognizes the DSG(X)<sub>2+n</sub>S destruction motif, the serines of which are phosphorylated by specific kinases<sup>75</sup>.

There are many studies that show alterations of the genes that encode  $\beta$ -catenin and NF $\kappa$ B in various human cancers. Given that  $\beta$ -TRCP targets  $\beta$ -catenin and I $\kappa$ B for degradation, overexpression of  $\beta$ -TRCP is expected to have complex consequences: it inactivates the Wnt pathway, which stimulates cellular proliferation, but also activates the NF $\kappa$ B pathway, which antagonizes pro-apoptotic signals. Genetic alteration of  $\beta$ -TRCP genes in human cancers have been shown in several studies (TABLE 1). A nucleotide substitution in  $\beta$ -TRCP2 was identified in a gastric cancer cell line,

Table 1 | Alterations in the variable components of SCF and APC/C in human malignancies

Cancer type	Observed alterations	References
<b>SKP2 (SCF)</b>		
Oro-pharyngo-laryngeal cancer	Correlation with poor prognosis; inverse correlation with p27 expression	127–131
Oesophageal cancer	Correlation with poor prognosis; inverse correlation with p27 expression	132
Gastric cancer	Correlation with poor prognosis; inverse correlation with p27 expression	133,134
Colon cancer	Correlation with poor prognosis; inverse correlation with p27 expression	34,135,136
Biliary tract cancer	Correlation with poor prognosis; no correlation with p27 expression	137
Lung cancer	Correlation with poor prognosis; inverse correlation with p27 expression; gene amplification; no correlation with p27 expression	30,31,138–141
Melanoma	Correlation with poor prognosis; inverse correlation with p27 expression	142,143
Glioma/Glioblastoma	Correlation with poor prognosis; inverse correlation with p27 expression; gene amplification	144,145
Breast cancer	Correlation with poor prognosis; inverse correlation with p27 expression	45
Renal cell cancer	Correlation with poor prognosis	146
Prostate cancer	Correlation with prognosis; inverse correlation with p27 expression	147–149
Transitional cell cancer	Correlation with poor prognosis	150
Cervix cancer	Correlation with poor prognosis; no correlation with p27 expression	151
Endometrial cancer	Correlation with poor prognosis	152
Ovarian cancer	Correlation with poor prognosis	153
Kaposi sarcoma	Correlation with poor prognosis; no correlation with p27 expression	154
Soft Tissue sarcoma	Correlation with poor prognosis; no correlation with p27 expression	155
Lymphoma and leukaemia	Correlation with poor prognosis; inverse expression of p27; no correlation with p27 expression	35,156–159
<b>FBW7(SCF)</b>		
Ovarian cancer	Mutation (only ~2%)	160
Breast cancer	Mutation and elevated level of cyclin E	161
	Loss of periodic expression of cyclin E	52
Endometrial cancer	Point mutation or deletion (>16%); inverse correlation with cyclin E expression; correlation with poor prognosis	162
	LOH (30%); no correlation with cyclin E expression	163
Colon cancer	Mutation	51
<b><math>\beta</math>-TRCP (SCF)</b>		
Gastric cancer	Mutation of $\beta$ -TRCP2 in cell line	76
Prostate cancer	Mutation (~10%)	77
Pancreatic cancer	Overexpression in cell line; correlation with NF $\kappa$ B activity and chemoresistance	81
Colon cancer	Elevation of mRNA & protein (56%); increase in $\beta$ -catenin and NF $\kappa$ B; correlation with poor prognosis	79
Hepatoblastoma	Elevation of mRNA; activation of Wnt signal	78
<b>CDC20 (APC/C)</b>		
Pancreatic cancer	Overexpression	164
Lung cancer	Upregulation in cDNA array	165
Gastric cancer	Upregulation in cDNA array	166
<b>CDH1 (APC/C)</b>		
Lymphoma (mouse)	Reduced expression	105

APC/C, anaphase-promoting complex/cyclosome;  $\beta$ -TRCP,  $\beta$ -transducin repeat-containing protein; CDC20, cell division cycle 20; FBW7, F-box and WD40-domain protein 7; LOH, loss of heterozygosity; NF $\kappa$ B, nuclear factor  $\kappa$ B; SCF, SKP1–CUL1–F-box-protein complex; SKP2, S-phase kinase-associated protein 2.

which led to an amino-acid substitution in the seventh WD40-repeat domain; this domain is conserved among  $\beta$ -TRCPs derived from human, mouse, *X. laevis*, and *D. melanogaster*<sup>76</sup>. In human prostate cancers, alterations

in  $\beta$ -TRCP1 were identified in 2 out of 22 samples<sup>77</sup>. Alterations in adenomatous polyposis coli (APC),  $\beta$ -TRCP1 and the  $\beta$ -catenin regulatory domain were not found together, consistent with their equivalent effects

on  $\beta$ -catenin stability. These data indicate that loss-of-function of  $\beta$ -TRCP results in the activation of the Wnt signalling pathway and cancer development.

Paradoxically,  $\beta$ -TRCP is highly expressed in some tumours. In hepatoblastoma (23 out of 23 cases), increased expression of  $\beta$ -TRCP1 was observed independent of the  $\beta$ -catenin mutational status<sup>78</sup>.  $\beta$ -TRCP1 overexpression was associated with the accumulation of intra-cytoplasmic and nuclear  $\beta$ -TRCP1 protein. Furthermore,  $\beta$ -TRCP1 mRNA and protein levels are increased compared with normal tissue in more than half of colorectal cancers (25 out of 45 cases)<sup>79</sup>. Increased  $\beta$ -TRCP1 levels were significantly associated with  $\beta$ -catenin activation and decreased apoptosis. Given that Wnt signalling increases the expression of  $\beta$ -TRCP mRNA and protein, probably by the stabilization of  $\beta$ -TRCP mRNA as a negative-feedback mechanism<sup>80</sup>, the overexpression of  $\beta$ -TRCP mRNA might not be the cause but the result of Wnt activation. Instead, activation of the NF $\kappa$ B pathway because of the overexpression of  $\beta$ -TRCP might be more important for cancer development. Chemoresistant pancreatic cancer cell lines exhibiting constitutive NF $\kappa$ B activity express significantly increased levels of  $\beta$ -TRCP1 (REF. 81). Suppression of  $\beta$ -TRCP1 by RNA interference reduces NF $\kappa$ B activation and chemoresistance. The effect of  $\beta$ -TRCP deregulation seems complex and needs to be analysed in more samples of human cancers.

Mice transgenic for  $\beta$ -TRCP1 in mammary-gland epithelium show increased ductal blanching and increased proliferation of the epithelial cells, correlating with increased NF $\kappa$ B activity, and finally develop tumours<sup>82</sup>. These findings indicate that aberrantly increased expression of  $\beta$ -TRCP induces cancer development. By contrast, no increased rate of cancer development was observed in  $\beta$ -TrCP1<sup>-/-</sup> mice<sup>13,74</sup>, although the possibility that  $\beta$ -TRCP2 compensates for the lack of functional  $\beta$ -TRCP1 still remains. A recent study of transgenic mice that specifically express either wild-type  $\beta$ -TrCP or a dominant-negative mutant of  $\beta$ -TrCP ( $\Delta$ F  $\beta$ -TrCP) showed that tumours develop in both types of transgenic mice<sup>83</sup>, indicating that deregulation of  $\beta$ -TrCP, either upregulation or downregulation, might result in cancer development.

$\beta$ -TRCP seems to participate in the regulation of cell division. *D. Melanogaster Slimb* mutants exhibit additional centrosomes and mitotic defects<sup>84</sup>. Defects in male fertility accompanied by testicular accumulation of spermatocytes in metaphase I were observed in  $\beta$ -TrCP1<sup>-/-</sup> mice<sup>13</sup>. Furthermore,  $\beta$ -TrCP1<sup>-/-</sup> fibroblasts manifest polyploidy, centrosome overduplication, impaired progression through mitosis and a reduced growth rate<sup>13,74</sup>. These abnormalities are attributable to the stabilization of EMI1, an inhibitor of the APC/C. EMI1 inhibits the APC/C in S and G2 phases of the cell cycle to ensure that the cyclin B–CDC20 complex does not prematurely activate the APC/C in early mitosis<sup>13,14</sup>. EMI2 (also called ERP1), an EMI1-related APC/C inhibitor, functions as a central mechanism of cytostatic factor in meiosis arrest (CSF, see below)<sup>66,67</sup>.

Other crucial substrates of  $\beta$ -TRCP in cell-cycle regulation are WEE1A and CDC25A, a kinase and a phosphatase that negatively and positively regulate CDK activity, respectively. WEE1A expression is decreased by ubiquitin-dependent degradation associated with its phosphorylation at the onset of M phase, and its degradation is required for the rapid activation of CDK1 (REF. 68). These findings suggest the existence of a feedback loop between CDK1 and WEE1A that ensures the rapid activation of CDK1 when cells are ready to divide. CDC25A is phosphorylated by the checkpoint kinases CHK1 and CHK2 in response to DNA damage or stalled DNA replication. Phosphorylation of CDC25A on Ser82 allows the protein to be recognized by  $\beta$ -TRCP and ubiquitinated<sup>69,70</sup>. These data indicate that  $\beta$ -TRCP has a prominent role in controlling the timing of entry into M phase, and in mediating the response to DNA damage. However, the effect of the deregulation of EMI1/2, WEE1A and CDC25A on cancer development has not been as extensively studied as Wnt or NF $\kappa$ B.

#### CDC20: target of the spindle checkpoint and CSF

CDC20 and CDH1 are believed to be primarily responsible for substrate recognition by the APC/C<sup>85</sup>. CDC20 and CDH1 recognize proteins that have a destruction box (D box) with the consensus sequence RxxLxxxxN (REF. 2) or a KEN box with the consensus sequence KENxxxxN (REFS 86,87). The windows of activity of APC/C<sup>CDC20</sup> and APC/C<sup>CDH1</sup> are clearly different (FIG. 5): the former is active from mid-mitosis (anaphase) to late mitosis, whereas the latter is activated at late mitosis, remains active through G1 phase and is extinguished at the G1–S boundary<sup>9,10</sup>. APC/C<sup>CDC20</sup> contributes to the proteolysis of securin, and thereby triggers chromosomal separation at anaphase. After replication, sister chromatids are accompanied until anaphase by the multiprotein complex cohesin. Separase cleaves cohesin, but its activity is suppressed by securin. The degradation of securin by APC/C<sup>CDC20</sup> activates separase, resulting in the cleavage of cohesin and separation of sister chromatids<sup>88–90</sup>. Although CDC20 is expressed in G2 phase before the entry to M phase, the activity of APC/C<sup>CDC20</sup> is suppressed until spindle attachment at kinetochores is completed in mitosis. This surveillance system, known as the spindle checkpoint, precludes precocious segregation of chromosomes, which would result in abnormal chromosome number (aneuploidy), a prevalent form of genetic instability in human cancers<sup>91</sup>. The main mediators of this system are mitotic arrest deficient (Mad) and budding uninhibited by benzimidazole (Bub) proteins. Among them, MAD2, BUBR1 and BUB3 form the mitotic checkpoint complex (MCC), which binds to, and inhibits, the function of CDC20 (REF. 91). Once all kinetochores are attached to spindles at the metaphase–anaphase transition the MCC is dissociated from CDC20, resulting in the activation of APC/C<sup>CDC20</sup> and chromosome segregation<sup>9,10</sup>.

Given that the major targets of CDC20 are securin and mitotic cyclins, it is expected that deregulation of CDC20-dependent proteolysis might result in aneuploidy and, finally, cause cancer. Securin is frequently overexpressed in human neoplasms<sup>92</sup>. In addition,

#### Spindle

A highly dynamic, bipolar array of microtubules that forms during mitosis or meiosis and serves to move the duplicated chromosomes apart.

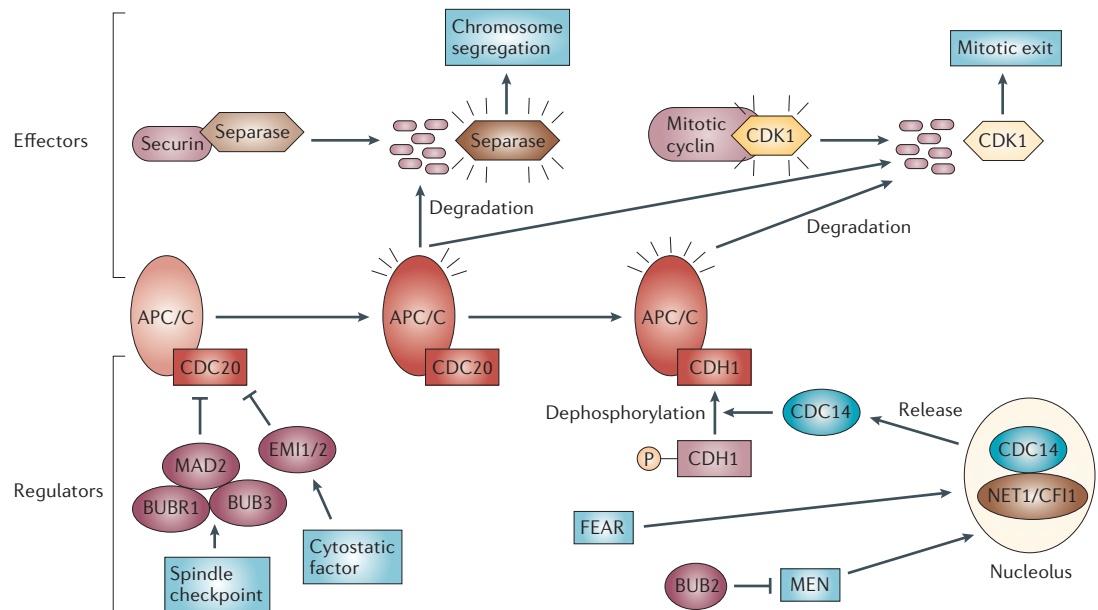
#### Kinetochores

The complicated protein assembly that links the specialized areas of condensed chromosomes, known as centromeres, to the microtubule-based mitotic spindle.

#### Aneuploidy

The ploidy of a cell refers to the number of sets of chromosomes that it contains. Aneuploid karyotypes are those of which chromosome complements are not a simple multiple of the haploid set.





**Figure 5 | Regulation and function of the anaphase-promoting complex/cyclosome (APC/C).** Until all spindles have attached to the kinetochores at mitosis metaphase, cell division cycle 20 (CDC20) is inhibited by binding to MAD2, BUBR1 and BUB3; this system is called spindle checkpoint. In meiosis, eggs are arrested at metaphase by cytostatic factor (CSF), in which EMI2 is essential for the inhibition of CDC20-dependent proteolysis. Release from spindle checkpoint or CSF-arrest activates APC/C<sup>CDC20</sup> and promotes the proteolysis of securin to allow separate to cleave cohesin, resulting in chromosome separation. APC/C<sup>CDC20</sup> also targets mitotic cyclins for degradation. APC/C switches its activator from CDC20 to CDH1 (also known as HCT1) at late M phase. CDH1 associates with the core APC/C subunits when dephosphorylated by CDC14 phosphatase. CDC14 is sequestered in the nucleolus during most of the cell cycle by its inhibitor NET1/CFI1. The release of CDC14 from its inhibitor is mediated by the mitotic exit network (MEN) and the CDC fourteen early anaphase release (FEAR) network.

blocked proteolysis of securin by APC/C<sup>CDC20</sup> leads to genomic instability in cultured cells<sup>93</sup>. On the other hand, cells that lack securin lose chromosomes at a high frequency because of abnormal anaphases, during which cells make repeated unsuccessful attempts to segregate their chromosomes<sup>94</sup>. This apparent discrepancy might be explained by the fact that securin is required to fully activate separate<sup>94</sup>; therefore, both overexpression and loss of securin lead to inactive separate, causing problems in sister chromatid separation and the loss of chromosomes. The deregulation of CDC20-dependent proteolysis is important and is likely to be involved in tumorigenesis. CDC20 is overexpressed in some human cancers, although there are also some reports that *CDC20* is mutated or deleted (TABLE 1). However, our knowledge of the mechanisms that regulate APC/C is developing rapidly, and more is likely to emerge about the role of these mechanisms in the development of cancer.

CDC20 is a target of APC/C inhibition by the spindle checkpoint not only in mitosis but also in meiosis. Vertebrate eggs awaiting fertilization are arrested at the metaphase stage of meiosis II by cytostatic factor (CSF). This inhibits APC/C<sup>CDC20</sup>, triggering anaphase onset and mitotic/meiotic exit by targeting securin for destruction. On fertilization, a transient rise in free intracellular calcium releases the egg from CSF arrest and activates APC/C. Recent studies<sup>95,96</sup> have uncovered the mechanism of release from CSF arrest: phosphorylation of

EMI2, an inhibitor of CDC20, by calcium-activated CaMKII leads to the recruitment of polo-like kinase 1 (PLK1). The recruitment of PLK1 then triggers the destruction of EMI2 by phosphorylating a site that is known to serve as a phosphorylation-dependent degradation signal (DSGX<sub>3</sub>S) for SCF<sup>β-TRCP</sup> (FIG. 3b). Mad/Bub and EMI1/2 inhibitors have a crucial role in preventing the premature activation of APC/C<sup>CDC20</sup> in response to spindle checkpoint and developmental signals.

### CDH1 maintains G1 phase

Whereas CDC20 targets securin and mitotic cyclins during anaphase, CDH1 recognizes various proteins in late M and G1 phases, such as mitotic cyclins, CDC20, CDH1, Aurora A, Aurora B, PLK1, NEK2A, geminin, CDC6, mE2-C, SNON, ribonucleotide reductase R2, thymidine kinase 1, TPX2 and SKP2 (reviewed in REFS 10,97). Unlike CDC20, CDH1 levels are relatively constant throughout the cell cycle<sup>98–100</sup>. CDH1 activity is regulated by cell-cycle-dependent phosphorylation and dephosphorylation, being unphosphorylated in late M and G1 phases, and then phosphorylated during S, G2 and early M phases<sup>99,100</sup>. The phosphorylation of CDH1 by cyclin-CDK inhibits APC/C activation by preventing CDH1 from binding to the core APC/C subunits, whereas dephosphorylation of CDH1 by CDC14 phosphatase induces APC/C activation by allowing CDH1 to access the core APC/C subunits<sup>100,101</sup>. CDC14 activity is

tightly regulated by its localization (FIG. 5). It is bound to an inhibitor, NET1/CFI1, which keeps it inactive in the nucleolus for most of the cell cycle. However, from early anaphase until telophase, the interaction between the two proteins is lost and CDC14 becomes active, resulting in the dephosphorylation of CDH1 and, in turn, the formation of active APC/C<sup>CDH1</sup>. The release of CDC14 from its inhibitor is mediated by two pathways: the mitotic exit network (MEN), a group of proteins forming a Ras-like signalling cascade<sup>102–104</sup>, and the CDC fourteen early anaphase release (FEAR) network<sup>104,105</sup>.

The deregulation of APC/C activity might result in the uncontrolled expression of its substrates that are involved in cell proliferation and genomic stability. Reduced expression of CDH1 has been described during the malignant progression of a B-lymphoma cell line<sup>106</sup>, and recently it has been shown that two essential subunits of the APC/C, APC6 (also known as CDC16) and APC8 (also known as CDC23), often have inactivating mutations in human colon cancer cells<sup>107</sup>. Moreover, overexpression of a mutated APC8 gene in a colon cancer cell line leads to the deregulation of cyclin B and cell-cycle progression<sup>107</sup>. Indeed, overexpression of mitotic cyclins results in genomic instability and is found in various cancers<sup>107,108</sup>.

Precise control of DNA replication and prevention of re-replication by licensing origin firing are essential for the maintenance of genomic integrity. The formation of pre-replicative complexes (pre-RCs) at origins of DNA replication is antagonized by CDK-dependent phosphorylation of the components of pre-RCs. G1 is the only cell-cycle phase with low CDK activity, which is essential for the loading of pre-RCs at origins of DNA replication. One of the major roles of APC/C<sup>CDH1</sup> seems to be to ensure this low CDK activity. First, APC/C<sup>CDH1</sup> mediates constitutive proteolysis of mitotic cyclins throughout the G1 phase<sup>99,109</sup>. Second, APC/C<sup>CDH1</sup> recognizes SKP2 and mediates its proteolysis<sup>15,16</sup>, resulting in the accumulation of CKIs p27, p21 and p57, suppressing CDK activity<sup>8</sup>. Third, APC/C<sup>CDH1</sup> promotes the degradation of geminin<sup>110</sup>, an inhibitor of the DNA replication factor CDT1, which is involved in the formation of pre-RCs. The downregulation of geminin by APC/C<sup>CDH1</sup> allows CDT1 to access the origins. Therefore, APC/C<sup>CDH1</sup> promotes the formation of pre-RCs at origins in G1 phase. Deregulated expression of CDT1 and its inhibitor geminin have been described in many types of human cancers<sup>11</sup>, indicating that deregulated control of pre-RC loading contributes to the development of genetically unstable cancer cells.

Not only cyclin-CDK, but also mitotic non-CDK kinases such as Aurora A, PLK1 and NEK2A are down-regulated by APC/C<sup>CDH1</sup> during G1 phase<sup>9,10</sup>. Aurora A accumulates in G2 and M phase, contributing to centrosome duplication and separation, and to spindle assembly<sup>112,113</sup>. Increased levels of Aurora A have been described in many human cancers<sup>14–119</sup>, and lead to cells passing through mitosis without cytokinesis, producing tetraploid progeny, which can give rise to aneuploid cells in subsequent cell division, particularly in the absence of the tumour suppressor p53 (REF. 120).

Plks are key enzymes that control the mitotic entry of proliferating cells and regulate many aspects of mitosis necessary for successful cytokinesis<sup>121</sup>. PLK1 is the best characterized of the four Plks and is overexpressed in many tumour types, with overexpression frequently an indicator of poor prognosis. NEK2A is a member of the NIMA-related serine/threonine kinase family, the activity of which is cell-cycle-regulated, peaking in S and G2 phase<sup>122</sup>. NEK2 is a core component of the human centrosome throughout the cell cycle, and might trigger centrosome separation at the onset of mitosis through the phosphorylation of multiple linker components. Data are emerging that NEK2 is abnormally expressed in various human malignancies such as breast, cervical and prostate carcinomas, Ewing sarcoma and lymphomas<sup>123</sup>. Therefore, the dysfunction of APC/C<sup>CDH1</sup> might result in the abnormal accumulation of both mitotic CDK and non-CDK kinases, leading to cancer development.

### Conclusions and perspectives

Together, biochemistry, cell biology, animal genetics and clinical science have produced a large amount of evidence that SCF and APC/C E3 ligases play an integral part in the highly ordered progression of the cell cycle, and that their deregulation contributes to tumorigenesis. The main goal of studying E3-ligase control of the cell cycle is to develop a drug that specifically targets these enzymes for cancer therapy. However, attaining this goal is expected to be difficult, although not impossible, given that they do not contain a canonical active site and their mode of action involves protein–protein interactions. For example, a small compound named Nutlins was shown to inhibit MDM2 (the ligase for p53) by binding to its p53-binding pocket, thereby blocking access and resulting in p53 accumulation<sup>124</sup>. This indicates that specific inhibitors that interfere with the interaction between the SCF complex or the APC/C and their substrates could be developed.

On the other hand, attempts to develop proteasome inhibitors for cancer therapy have been questioned because suppression of the proteasome is likely to be highly toxic to normal cells. However, cancer cells with uncontrolled proteolysis unexpectedly turned out to be more sensitive to such drugs than normal cells, making lower doses more effective and therefore limiting the side effects of these inhibitors<sup>125</sup>. The recent success in developing the proteasome inhibitor Bortezomib for the treatment of relapsed multiple myeloma is encouraging, even though the molecular mechanism of action has not been fully uncovered. Treatment with Bortezomib effectively blocks NFκB activation, which is a prominent instigator of chemoresistance to anti-apoptotic molecules<sup>125</sup>. However, several lines of evidence indicate that, while important, the downregulation of NFκB activity is not solely responsible for the activity of proteasome inhibitors. Proteasome inhibitors also induce upregulation of p27 and MYC, presenting the cell with contradictory signals, and this conflict leads to the initiation of apoptotic pathways<sup>126</sup>.

Two opposing strategies, the development of broad or specific inhibitors of the UPS, are being adopted in parallel in the hope of producing anti-tumour drugs. The successful development of the proteasome inhibitor Bortezomib for cancer therapy allows us to reconsider whether developing drugs that are highly specific is indeed required and worth the huge effort and cost. Broad inhibitors might attack cancer cells through multiple pathways such as cell cycle, apoptosis and signal transduction, and might be more

effective than specific inhibitors. However, the broad nature of the drugs inevitably involves the risk of side effects because it also damages normal cells, and the administered dose needs to be carefully monitored. Specific inhibitors might overcome this problem. It is impossible to predict at present which strategy will be more successful, but for either strategy to succeed it is important to discover the molecular mechanisms that are responsible for cell-cycle regulation by ubiquitin ligases.

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#### Competing Interests

The authors declare no competing financial interests.

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