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Oncogenic aberrations of cullin-dependent ubiquitin ligases

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Accumulating evidence points to a key role of the ubiquitin–proteasome pathway in oncogenesis. Aberrant proteolysis of substrates involved in cellular processes such as the cell division cycle, gene transcription, the DNA damage response and apoptosis has been reported to contribute significantly to neoplastic transformation. Cullin-dependent ubiquitin ligases (CDLs) form a class of structurally related multisubunit enzymes central to the ubiquitin-mediated proteolysis of many important biological substrates. In this review, we describe the role of CDLs in the ubiquitinylation of cancer-related substrates and discuss how altered ubiquitinylation by CDLs may contribute to tumor development.
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Introduction

The cellular abundance of many proto-oncoproteins and tumor suppressors is controlled by the ubiquitin–proteasome degradation pathway (Bashir and Pagano, 2003). Ubiquitin conjugation to a protein substrate requires a two-step reaction catalysed by sequential ubiquitin transferase enzymes. The first enzyme, E1, activates the entire pool of cellular ubiquitin proteins by forming high-energy, unstable thiolester bonds with ubiquitin (Hershko and Ciechanover, 1998). The second transferase enzyme is one of many ubiquitin conjugating enzymes (E2s or Ubcs), which covalently binds the ubiquitin moiety to the substrate. Multiple rounds of ubiquitin conjugations induce the polyubiquitinylation of the substrate, which is then targeted for degradation by the proteasome. A third class of enzymes, the ubiquitin ligases (E3s), determines the specificity of the reaction by simultaneously recognizing and directly associating with specific substrates and binding and positioning the E2 for ubiquitin conjugation to the substrate. Cullin-dependent ubiquitin ligases (CDLs) are one large class of E3s. These multisubunit ubiquitin ligases always contain two core components: a cullin subunit and a RING finger protein that stabilizes the E2–cullin interaction.

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Cullins

The name cullin derives from the fact that each member of this family 'culls' or sorts different substrates for ubiquitinylation (Kipreos *et al*., 1996). Budding yeast have three cullin proteins: CulA (also known as Cdc53 or Cul1), CulB (also known as Cul3) and CulC (also known as Cul8). Metazoans have at least five cullins (Cul1–5). Sequence homology extends across the whole length of Cul1–5, but it is greatest at the C-terminus that contains a so-called 'cullin domain'. Cullins derived from successive duplication events of an ancestral cullin gene that gave rise to two main branches: *CUL1/2/5* and *CUL3/4*. *Caenorhabditis elegans* cul6 (missing in vertebrates) appears to have arisen by a duplication event of the ancestral *CUL1* gene, while a later duplication separated mammalian *CUL4A* and *CUL4B*. An additional cullin member, Cul7, has been identified in humans (Dias *et al*., 2002; Arai *et al*., 2003); however, the homology is mostly in the cullin domain at the C-terminus. At least three additional cullin domain-containing proteins exist in mammals: KIAA0708 (Dias *et al*., 2002); Parc, which interacts with the tumor suppressor p53 controlling its subcellular localization (Nikolaev *et al*., 2003); and Apc2, a subunit of another E3 ubiquitin ligase – the anaphase promoting complex/cyclosome (APC/C) (Yu *et al*., 1998a). Most cullin members have been shown to associate with a RING finger protein (Figure 1). Cul1–7 interact with either Roc1 (also known as Rbx1) or the related Roc2 (Ohta *et al*., 1999), while Apc2 interacts with Apc11, another homolog of Roc1–2. The cullin–RING finger protein complex contains intrinsic ubiquitin ligase activity *in vitro* since purified recombinant cullin–Roc complexes are able to catalyse substrate-independent ubiquitin ligation by E2s, forming free ubiquitin chains. Similarly, Parc contains two RING domains that are likely responsible for its intrinsic substrate-independent ubiquitin ligation activity (Nikolaev *et al*., 2003).

CDLs are positively regulated by covalent conjugation of the Nedd8 ubiquitin-like protein to a specific lysine residue present in the cullin subunit (Hori *et al*., 1999). Conversely, deconjugation of Nedd8 from cullins by the isopeptidase activity of the COP9 signalosome inhibits their ubiquitin ligase activity (Schwechheimer and Deng, 2001). Despite the importance of the Nedd8 conjugation in modulating CDL functions, the signals that regulate cullin neddylation remain elusive. Rather, Nedd8 modification of cullins appears to be a constitu-

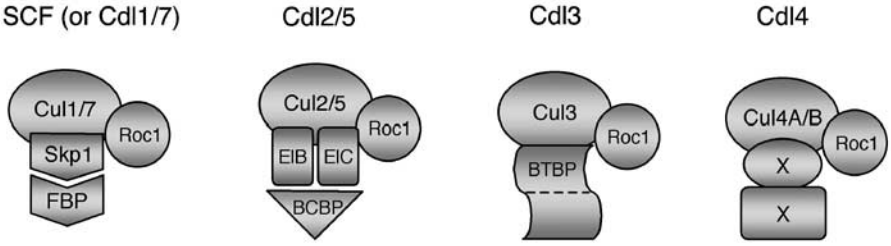

Figure 1 CDLs are composed of analogous modular proteins that assemble to form multiprotein complexes with ubiquitin ligase activity. See text for details

tive process that ensures the dynamic activity of CDLs by allowing cycles of assembly and disassembly of different substrate receptor subunits from CDLs.

The best-characterized mammalian cullin family member is Cul1, which is a component of a multiprotein ubiquitin ligase complex referred to as SCF (Skp1–Cul1–F box protein) or Cdl1 (Figure 1) (Feldman *et al*., 1997; Skowyra *et al*., 1997). In this complex, Cul1 acts as a molecular scaffold simultaneously interacting at the N-terminus with the adaptor subunit Skp1 and at the C-terminus with Roc1 or 2 and a specific ubiquitin conjugating enzyme (Ubc3, Ubc4 or Ubc5). Skp1, in turn, binds to one of many members of a family of F box proteins (FBPs) so named because they contain a 40-amino-acid motif called an 'F box' that is necessary to bind to Skp1 (Cenciarelli *et al*., 1999; Winston *et al*., 1999a; Kipreos and Pagano, 2000) (Figure 2). Each FBP can be matched with a discrete number of specific substrates through a protein–protein interaction domain (WD-40 domains, leucine-rich repeats, etc.). Thus, the substrate specificity of SCF complexes relies on the availability of a large number of FBPs (11 in yeast, 326 in worms, 29 in fly and approximately 70 in humans) each targeting specific substrates for degradation.

Complexes similar to SCF ligases are formed by analogous modular proteins that assemble to constitute different CDLs. Cul7, like Cul1, assembles an SCF-like E3 ubiquitin ligase (Cdl7) containing Roc1, Skp1 and the FBP Fbw6 (Dias *et al*., 2002). In contrast to Cul1, which can interact with Skp1 by itself, Cul7 is not capable of binding to Skp1 unless it is in complex with Fbw6, suggesting that Cdl7 cannot assemble with most FBPs. Also *C. elegans* Cul6 may function in SCF complexes since, like Cul1, it is able to interact with Skr3, an Skp1-related protein (Nayak *et al*., 2002). Interestingly, the fact that no Cul6 and Skr orthologs have been identified in mammals so far, together with the high number of FBPs present in *C. elegans*, suggests that the Skr, cullin and FBP families underwent a process of gene expansion specific for nematode development and physiology.

Both Cul2 and Cul5 are able to assemble in a complex (called Cdl2 and Cdl5, respectively) composed of a Roc protein, Elongin B, Elongin C, and one of many *S*OCS *b*ox *p*roteins (SBPs), where SOCS stands for suppressor of cytokine signaling (Kamura *et al*., 1998; Schoenfeld *et al*., 2000; Kamura *et al*., 2001) (Figure 1). Cdl2 and Cdl5 resemble the SCF complex, since sequence homologies have been identified between Skp1 and Elongin C, which are both adaptors for the substrate targeting subunit, and between the FBP and the SBP. SBPs, similarly to FBPs, constitute a large family of proteins that contains protein–protein interaction domains used to bind specific substrates (Hilton *et al*., 1998). Thus, as for the SCF complexes, a common core (either Elongin C–Elongin B–Cul2–Roc or Elongin C–Elongin B–Cul5–Roc) can be coupled to a large number of substrates through different and numerous SBPs. In addition, Cdl2 and Cdl5 can contain other substrate receptors that do not have a clear SOCS box but a more loose motif called a 'BC box' since it is necessary to bind to Elongin B and C (Kamura *et al*., 1998). However, it is likely that not all of the large number of BC box-containing proteins (BCBPs) identifiable in a computer analysis are indeed components of Cdl2 and Cdl5.

Genetic and biochemical approaches show that Cul3 is also a subunit of a modular ubiquitin ligase system (Cdl3) (Furukawa *et al*., 2003; Geyer *et al*., 2003; Pintard *et al*., 2003; Xu *et al*., 2003). In the attempt to identify the adaptor protein equivalent to Skp1 that plays a conserved function in the Cdl3 complex, *C. elegans* Cul3 was used as a bait in a yeast two-hybrid screen (Xu *et al*., 2003). In all, 11 Cul3-specific interactors were isolated. All have a common domain, previously termed *b*road-complex, *T*ramtrack and *B*ric-a-Brac (BTB), which is thought to play a role in protein–protein interaction (Zollman *et al*., 1994; Chen *et al*., 1995). BTB proteins (BTBPs) from different species can contain additional domains that are also known to mediate protein–protein interaction such as MATH domains, kelch repeats, WD40 repeats and Zn-finger repeats. Remarkably, the structure of Skp1 and the BTB domain of human promyelocytic leukemia zinc-finger (PLZF) protein revealed the presence of conserved structural features. Moreover, both Skp1 and BTBPs bind to a common interface in Cul1 and Cul3, respectively. These findings suggest that BTBPs might represent a common component of Cul3-dependent ubiquitin ligases (Figure 1). Each BTBP might act as the equivalent of the Skp1–FBP complex characteristic of the SCF. Indeed, BTBPs bind to Cul3 (as Skp1 to Cul1) and at the same time to a substrate (as the FBP). It is also possible that those BTBPs that lack other protein–protein interaction domains might associate

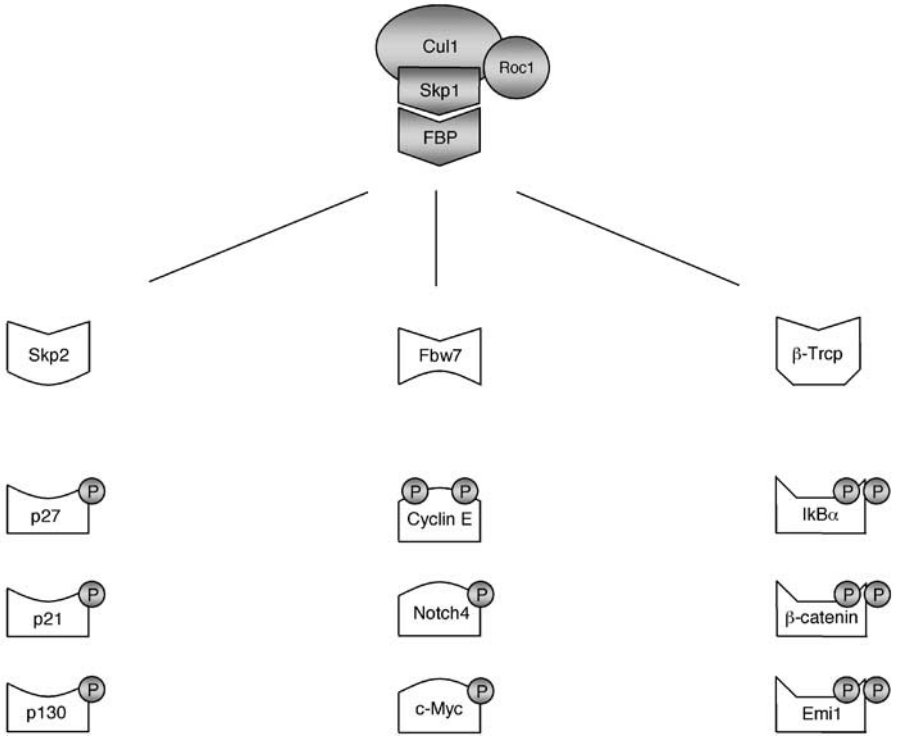

Figure 2 Three SCF complexes, SCF^{Skp2}, SCF^{Fbw7} and $SCF^{\beta\text{-}Trcp}$, contain a common core, composed of Cul1, Skp1 and Roc1, that associates with different FBPs. Each FBP acts as a substrate receptor subunit that is coupled to a discrete number of specific phosphorylated (indicated in the figure with a 'P') substrates through protein–protein interaction domains. The most extensively characterized substrates for these SCF ligases are shown

with additional adaptor proteins that in turn recognize specific targets.

Finally, Cul4 is also part of a complex with ubiquitin ligase activity (Chen *et al*., 2001; Nag *et al*., 2001). The finding that Cul4A associates with Roc1 and Damaged DNA-Binding proteins (DDB) to mediate the cellular response to DNA damage suggests that Cul4 is part of a multiprotein complex whose ubiquitin ligase activity plays an important part in tumorigenesis (Groisman *et al*., 2003) (Figure 1).

Since about 70 FBPs (Cenciarelli *et al*., 1999; Winston *et al*., 1999a; Kipreos and Pagano, 2000), 40 SBPs (Hilton *et al*., 1998; Kile *et al*., 2002) and 190 BTBPs (Zollman *et al*., 1994) have been identified in mammals, the cullin family appears to control the ubiquitinylation of a large number of cellular substrates. This review aims to summarize the current knowledge regarding oncogenic aberration of specific CDLs and some of their substrates, focusing particularly on Cul1–5.

Cdl1: the SCF complex

Cul1, the ortholog of yeast Cdc53, was the first member of the cullin family to be identified in metazoans (Kipreos *et al*., 1996). In *C. elegans*, loss-of-function mutations of *CUL1* lead to hyperplasia of all tissues with a shortened G1 phase of the cell cycle. Moreover, Cul1 is necessary for proper cell cycle exit. In mice, loss of *Cul1* results in early embryonic lethality (Dealy *et al*., 1999; Wang *et al*., 1999). *Cul1*-deficient embryos implant in the uterine wall but do not develop beyond embryonic day 5.5, before the onset of gastrulation. In addition, apoptosis is increased in the embryonic ectoderm. The early lethality in *Cul1*-deficient mice is likely due to the accumulation of a large number of substrates targeted by the SCF ubiquitin ligases. Since the FBP family has approximately 70 members, about 70 SCF complexes are expected to be present in mammalian cells, but only three of them have been extensively studied and matched with their correspondent substrates: SCF^{Skp2}, SCF^{Fbw7} and $SCF^{\beta\text{-}Trcp}$.

SCF^{Skp2}

Three groups have independently demonstrated a role for SCF^{Skp2} in the degradation of the cyclin-dependent kinase (CDK) inhibitor $p27^{Kip1}$ (Carrano *et al*., 1999; Marti *et al*., 1999; Tsvetkov *et al*., 1999). Skp2 interacts with p27, an association that requires phosphorylation of p27 on threonine 187 by CDKs. Ligation of phosphorylated p27 to ubiquitin can be reconstituted *in vitro* using purified components of the SCF^{Skp2} complex (Ganoth *et al*., 2001). In this context, it was demonstrated that SCF^{Skp2} requires an accessory protein, known as CDK subunit 1 (Cks1), for its ubiquitin ligase activity toward phosphorylated p27 (Ganoth *et al*., 2001; Spruck *et al*., 2001). *In vivo* expression of an F box-deleted Skp2 mutant, which acts as a dominant-negative mutant because it is unable to bind the Skp1–Cul1–Roc1 complex but is still capable of binding p27,

increases p27 stability. Accordingly, Skp2 knockdown by RNA interference or by using antisense oligonucleotides induces stabilization of p27. In addition, targeted inactivation of the mouse *Skp2* locus by homologous recombination results in accumulation of p27 (Nakayama *et al*., 2000). *Skp2*-deficient mice are viable, with no gross anatomic abnormalities, but their body weight is two-thirds of that of their littermate controls. The small size phenotype of the *Skp2* knockout mice is the opposite of the phenotype of the *p27−/−* mice, which are abnormally large. Moreover, *Skp2*-deficient fibroblasts show a reduced growth rate and centrosome overduplication. *Skp2−/−* hepatocytes have enlarged and polyploid nuclei. Similar abnormalities were found in the kidney, lung, testis and mouse embryonic fibroblasts (MEFs), whereas they are absent in all the other organs analysed. Remarkably, most of the cellular and histopathological defects observed in *Skp2*-deficient mice are suppressed in *Skp2−/−;p27−/−* mice, implicating p27 as a major substrate for Skp2 (K Nakayama, personal communication) (Figure 2).

It has been proposed that SCF^{Skp2} controls the degradation of two other CDK inhibitors, $p21^{Cip1}$ and $p57^{Kip2}$, during the S phase of the cell cycle (Bornstein *et al*., 2003; Kamura *et al*., 2003). In fact, p21 and p57 are efficiently ubiquitinylated by the SCF^{Skp2} ubiquitin ligase complex *in vitro* and their rate of degradation is much slower in *Skp2*-deficient fibroblasts synchronized in S phase than in wild-type cells. Significantly, both p21 and p57 stabilization also occurs in *Skp2−/−;p27−/−* cells, ruling out the possibility that their accumulation could be caused indirectly by the increased percentage of cells in the G0/G1 phase of the cell cycle resulting from elevated levels of p27. The fact that both p21 and p57 are still degraded in G0/G1 even in the absence of Skp2 indicates that more than one system cooperates to regulate the turnover of these two CDK inhibitors.

SCF^{Skp2} also has a role in the degradation of the retinoblastoma-related 'pocket' protein p130 (Tedesco *et al*., 2002). The levels of p130 protein are regulated through the cell cycle, being maximal in G0/G1 and then decreasing as cells enter S phase despite the fact that the levels of p130 mRNA do not change significantly during the cell cycle. SCF^{Skp2} is responsible for this regulation by binding to p130 and mediating its ubiquitinylation. This interaction is phosphorylation dependent since phosphorylation of serine 672 is necessary for Skp2 binding and p130 destruction. Importantly, p130 accumulates in thymidine-arrested *Skp2−/−* MEFs confirming that p130 is a substrate of SCF^{Skp2} in S phase.

It has been reported that during the G1 to S transition of the cell cycle, Skp2 regulates the ubiquitinylation and stability of the transcription factor c-Myc, an oncoprotein that plays a major role in oncogenesis (Kim *et al*., 2003; von der Lehr *et al*., 2003). Surprisingly, Skp2 enhances c-Myc-induced S phase transition and activates c-Myc transcriptional activity. In fact, Skp2 overexpression was shown to induce the transcription of c-Myc target genes suggesting that Skp2 acts as a transcription coactivator of c-Myc. These results indicate that Skp2 can concurrently lead to degradation of tumor suppressors (p27, p57, p21 and p130) and to activation of an oncogene (c-Myc). While *Skp2*-deficient mice, which should have decreased c-Myc activity, can develop almost normally (Nakayama *et al*., 2000), *c-Myc* knockout mice die *in utero* between 9.5 and 10.5 days of gestation with severe abnormalities affecting the heart, pericardium and neural tube (Davis *et al*., 1993). One would naively expect that, *in vivo*, inactivation of Skp2 would compromise c-Myc function and result in a more severe phenotype. In addition, the mechanism by which ubiquitinylation can activate gene transcription and subsequently decrease protein stability is still unclear.

Other cancer-related substrates have been described to be degraded by SCF^{Skp2}. Li *et al*. (2003) found that the ubiquitinylation and degradation of the replication licensing factor Cdt1 is mediated by SCF^{Skp2}. Kiernan *et al*. (2001) proposed a mechanism in which Cyclin T1 recruits the cyclin-dependent kinase Cdk9 to SCF^{Skp2} leading to its ubiquitinylation and destruction. However, the role of SCF^{Skp2} in the regulation of Cdk9 is not yet well defined since a more recent study showed that Cdk9 is a stable protein that is not affected by Skp2 overexpression or downregulation, suggesting that Skp2/Cdk9 interaction has a physiological significance different from protein degradation (Garriga *et al*., 2003). Mendez *et al*. (2002) found that the origin replication factor Orc1 is ubiquitinylated and degraded on chromatin via Skp2. Charrasse *et al*. (2000) reported that Skp2 interacts with the oncoprotein B-Myb stimulating its degradation. Marti *et al*. (1999) reported that the transcription factor E2F1 is able to bind Skp2 and Cul1, and that mutations in the E2F1 N-terminal region that abolish binding to Skp2 decrease E2F1 ubiquitinylation and lead to its stabilization. Moreover, Skp2 seems to play a role in the ubiquitinylation and degradation of Cyclin D1 and Cyclin E. Cyclin D1, which is overexpressed in several human tumors, is ubiquitinylated and degraded by the proteasome, and some lines of evidence indicate that Skp2 might be implicated, at least in part, in this process (Yu *et al*., 1998b; Ganiatsas *et al*., 2001). Finally, Nakayama *et al*. (2000) found that Skp2 can bind to the CDK-unbound, inactive form of Cyclin E and mediate its ubiquitinylation. Skp2 interaction with Cyclin E seems to be independent of Cyclin E phosphorylation. Moreover, accumulation of free Cyclin E is not a secondary effect due to the elevated levels of p27 since it occurs also in *Skp2−/−;p27−/−* MEFs (K Nakayama, personal communication). All these studies implicate SCF^{Skp2} in the ubiquitinylation of numerous substrates. Notably, *Skp2−/−* MEFs display an accumulation of p27, p21, p130, p57 and unphosphorylated, inactive Cyclin E, while no stabilization of Cyclin D1 and E2F1 has been observed (Nakayama *et al*., 2000; Tedesco *et al*., 2002; Bornstein *et al*., 2003). The turnover of Orc1, Cdt1, B-Myb and c-Myc has not yet been investigated in *Skp2*-deficient cells.

The described role of Skp2 in inducing the ubiquitinylation and degradation of the tumor suppressor p27,

and more recently of p21, p57 and p130, indicates that Skp2 may be the product of a proto-oncogene. Several studies confirm that Skp2 has oncogenic properties. First, an inverse relationship between Skp2 and p27 protein levels was found in human lymphomas (Latres *et al*., 2001; Chiarle *et al*., 2002), breast carcinomas (Signoretti *et al*., 2002), epithelial dysplasias (Gstaiger *et al*., 2001), colorectal carcinomas (Hershko *et al*., 2001), oral squamous cell carcinomas (Kudo *et al*., 2001), small cell lung cancers (Yokoi *et al*., 2002), gastric carcinomas (Masuda *et al*., 2002) and prostate cancers (Ben-Izhak *et al*., 2003). In addition, Skp2 expression significantly and directly correlates with tumor malignancy and aggressiveness, and is associated with poor prognosis in human lymphomas (Latres *et al*., 2001; Chiarle *et al*., 2002), prostate cancers (Ben-Izhak *et al*., 2003) and ovarian adenocarcinomas (Shigemasa *et al*., 2003). Moreover, Skp2 protein expression increases during progression from epithelial dysplasia to invasive carcinoma (Gstaiger *et al*., 2001). Importantly, frequent amplification and overexpression of the *SKP2* gene has been observed in primary small cell lung cancers (Yokoi *et al*., 2002), and in cell lines expressing high-risk human papilloma virus (Dowen *et al*., 2003). Similarly, Cks1 message has been found highly expressed in a subgroup of breast cancers (ER-negative and Her-2-negative) (Signoretti *et al*., 2002), in hepatocellular carcinomas (Okabe *et al*., 2001), gastric adenocarcinomas (El-Rifai *et al*., 2001), epithelial ovarian cancers (Welsh *et al*., 2001) and non-small cell lung carcinomas (Inui *et al*., 2003). Accordingly, Cks1 protein levels are increased in colorectal carcinomas (D Hershko and M Pagano, unpublished) and prostate cancers (A Baron, M Loda and M Pagano, unpublished). It has been reported that Skp2 and H-RasG12V cooperatively transform primary rat fibroblasts as scored by colony formation in soft agar and tumor formation in nude mice (Gstaiger *et al*., 2001). Finally, ectopic expression of Skp2 in nonadherent fibroblasts leads to entry into S phase, p27 downregulation and cell division, indicating that anchorage-dependent growth, a characteristic of nontransformed cells, is abrogated by Skp2 overexpression (Carrano and Pagano, 2001). Two mouse models confirm the oncogenic properties of Skp2. In the first one, targeted Skp2 expression to the T-lymphoid lineage markedly cooperates with the activated oncogene N-Ras (Latres *et al*., 2001). Compared to transgenic animals expressing activated N-Ras alone, Skp2 coexpression causes an acceleration in tumor onset, increased penetrance of lymphomagenesis and decreased survival rate. Importantly, these tumors contain decreased levels of p27 compared with nontransformed cells. In the second mouse model, enforced expression of Skp2 in the prostate gland induces hyperplasia, dysplasia and low-grade carcinoma accompanied by increased proliferation, downregulation of p27 and high levels of mitotic markers such as Ki67 and Cyclin B1 (Shim *et al*., 2003). Thus, the mouse models are in agreement with the results in human tissues, confirming a major role for Skp2 in tumor development.

Using transgenic animal models it has been shown that interference with Skp1 and Cul1 functions *in vivo* leads to reduced cell proliferation and, after a period of latency, to genetic instability and neoplastic transformation (Piva *et al*., 2002). Thus, the idea of targeting Cul1 or the Nedd8 pathway does not represent a valid approach for the therapy of human diseases since it will result in decreased cell proliferation, likely due to the accumulation of substrates such as p27 and p21, but also in devastating unwanted effects (i.e., malignant transformation). In contrast, inhibition of Skp2 activity results in a decreased malignant potential of cancer cells since they show a reduced ability to grow in the absence of cell adhesion (Signoretti *et al*., 2002) and no longer proliferate when injected with an anti-Skp2 antibody (Zhang *et al*., 1995) or when transfected with an Skp2 siRNA duplex (Bashir *et al*., 2004). In conclusion, Skp2 inhibitors might represent a specific and valid therapeutic option in epithelial tumors and lymphomas.

SCF^{Fbw7}

Three groups have reported that Cyclin E ubiquitinylation is mediated by the ubiquitin ligase SCF^{Fbw7} (Koepp *et al*., 2001; Moberg *et al*., 2001; Strohmaier *et al*., 2001). In a genetic screen to identify genes that restrain cell proliferation, Hariharan's group identified *archipelago*, a gene encoding the *Drosophila* ortholog of the *C. elegans* F box protein Sel-10 and human Fbw7 (Moberg *et al*., 2001). Importantly, *archipelago* mutant cells express higher levels of Cyclin E protein, but not mRNA, compared with wild-type control cells. These results, together with the direct physical interaction between Archipelago and Cyclin E, suggested a role for Archipelago in the degradation of Cyclin E. A different genetic approach was used by Koepp *et al*. (2001) and Strohmayer *et al*. (2001) to identify the specific SCF ubiquitin ligase responsible for Cyclin E ubiquitinylation and degradation. In a stability assay in yeast, it was found that Cyclin E is stabilized in CulA, Skp1 and Cdc4 mutants. No difference in Cyclin E turnover was detected in mutant strains for other yeast FBPs. Moreover, Fbw7 (also called hCdc4) can partially rescue the *CDC4* mutation in yeast. To confirm this specificity, the binding of Cyclin E with different mammalian FBPs was tested. Of these, only Fbw7 is able to associate physically with Cyclin E. The interaction between Fbw7 and Cyclin E is phosphorylation dependent since mutation on two residues phosphorylated by Cdk2 (threonine 62 and 380) impairs Cyclin E ability to bind Fbw7. Furthermore, SCF^{Fbw7} is able to ubiquitinylate Cyclin E in a phosphorylation-dependent manner *in vitro*. Thus, whereas the degradation of free, inactive Cyclin E, via Skp2, occurs in a phosphorylation-independent fashion, the degradation of active, Cdk2-bound Cyclin E, via Fbw7, requires phosphorylation. Finally, overexpression of Fbw7 leads to decreased levels of Cyclin E, and, conversely, inhibition of Fbw7 by RNA interference causes accumulation of Cyclin E. Taken together, these results demonstrate that SCF^{Fbw7}

controls the stability of Cyclin E in mammalian cells and that this role is well conserved through evolution. Remarkably, one of the three alternative transcripts of the *FBW7* gene is induced by the tumor suppressor p53 in response to genotoxic stresses caused by UV irradiation and adriamycin treatment (Kimura *et al*., 2003), suggesting that to arrest the cell cycle p53 might induce Cyclin E degradation via upregulation of Fbw7.

Sel-10 (the worm name for Fbw7) was originally identified as a negative regulator of the Notch pathway in *C. elegans*. In addition, mammalian Fbw7 was shown to target Notch1 and Notch4 for ubiquitin-dependent degradation (Gupta-Rossi *et al*., 2001; Oberg *et al*., 2001; Wu *et al*., 2001). The involvement of Fbw7 in the control of the Notch pathway is confirmed by the finding that Notch4 accumulates in *Fbw7*-deficient embryos, which die *in utero* at embryonic day 10.5 and manifest abnormal vascular development (K Nakayama, personal communication). In addition, Notch4 degradation is impaired in *Fbw7−/−* cells. In contrast, the expression of Notch1, Notch2 and Notch3 is comparable among different genotypes. The finding that *Fbw7−/−* embryos exhibit severe defects in vascular development is likely due to the upregulation of the transcriptional repressor Hey-1, a downstream effector of Notch signaling.

Fbw7 is also responsible for the degradation of the oncoprotein c-Myc (B Clurman, I Hariharan and K Nakayama, personal communication). This degradation is phosphorylation dependent since phosphorylation of threonine 58 is necessary for c-Myc degradation.

The role of Fbw7 in the degradation of Cyclin E, Notch and c-Myc suggests that it might function as a tumor suppressor. Levels of Cyclin E are often increased in ovarian and breast tumors (Keyomarsi and Herliczek, 1997). Importantly, mutations of *FBW7* were found in three out of ten ovarian cancer cell lines analysed, indicating that mutations of *FBW7* might be responsible for elevated levels of Cyclin E in this tumor (Moberg *et al*., 2001). In addition, one breast cancer cell line, SUM149PT, that displays elevated levels of Cyclin E expresses an Fbw7 mutant form encoding a protein lacking the last four WD40 domains that are responsible for binding to the substrates (Strohmaier *et al*., 2001). Moreover, the human *FBW7* gene is mutated in 16% of primary endometrial adenocarcinomas, and loss of heterozygosity (LOH) was detected in most of these tumors (Spruck *et al*., 2002). It would be interesting to analyse the levels c-Myc and Notch in those human primary tumors where mutations of *FBW7* have been found. Preliminary studies indicate that *FBW7* mutations are often associated with high tumor aggressiveness. *FBW7* might be mutated in other tumor types since chromosome region 4q32, where *FBW7* has been mapped, is deleted in 67% of lung cancers, 63% of head and neck cancers, 41% of testicular cancers and 27% of breast cancers.

$SCF^{\beta\text{-}Trcp}$

The mammalian $SCF^{\beta\text{-}Trcp}$ (β-transducin repeat-containing protein) has been implicated in the regulation of at least two different signal transduction pathways, Wnt/Wingless and NF-κB, by mediating the ubiquitinylation and degradation of the transcriptional coactivator β-catenin and the NF-κB inhibitor IκB, respectively (Maniatis, 1999). Furthermore, β-Trcp controls cell cycle progression by stimulating the degradation of Emi1 (Early mitotic inhibitor 1) (Guardavaccaro *et al*., 2003; Margottin-Goguet *et al*., 2003; Peters, 2003) and Cdc25A (Busino *et al*., 2003). It has been shown that additional substrates are degraded via β-Trcp-dependent ubiquitinylation like the transcription factor ATF4/CREB2 (Lassot *et al*., 2001), NF-κB/p105 (Orian *et al*., 2000), NF-κB/p100 (Fong and Sun, 2002) and the discs large (hDlg) tumor suppressor (Mantovani and Banks, 2003). All these substrates share a common motif, DSGxx(x)S, and they can be recognized by β-Trcp once the two serine residues of this element are phosphorylated. However, at least in non-mammalian organisms, β-Trcp substrates that lack this motif have been identified. In human and mouse cells, two closely related paralogous β-Trcp gene products are present, known as β-Trcp1 (or Fbw1A) and β-Trcp2 (also called Fbw1B or HOS). β-Trcp1 and β-Trcp2 are ubiquitously expressed in both human and mouse tissues (Cenciarelli *et al*., 1999; Koike *et al*., 2000; Maruyama *et al*., 2001) and have similar biochemical properties in their capability to mediate the ubiquitinylation of their specific substrates. The function of β-Trcp in the degradation of β-catenin and IκBα is conserved through evolution since loss-of-function mutations of *slimb*, the *Drosophila* ortholog of β-Trcp, result in the accumulation of Armadillo, the *Drosophila* ortholog of β-catenin (Jiang and Struhl, 1998), and reduced expression of twist and snail, two downstream targets of dorsal/NF-κB (Spencer *et al*., 1999). *Slimb* mutations cause also a defective proteolytic processing of Cubitus interruptus (Ci) and ectopic expression of Hedgehog-responsive genes (Jiang and Struhl, 1998), suggesting that Ci might be a substrate of Slimb. In addition, it has been reported that Slimb is implicated in the degradation of the circadian clock protein Period (Ko *et al*., 2002). Finally, the *C. elegans* β-Trcp ortholog lin-23 functions to regulate negatively cell cycle progression, since *lin-23* null mutant cells undergo extra divisions, generating supernumerary cells (Kipreos *et al*., 2000).

Several groups have demonstrated a role for mammalian β-Trcp1 and β-Trcp2 in the ubiquitinylation of β-catenin *in vitro* and in cultured cells (Hart *et al*., 1999; Kitagawa *et al*., 1999; Latres *et al*., 1999; Winston *et al*., 1999b). Under normal conditions, β-catenin associates with the adenomatous polyposis coli (APC) tumor suppressor, axin, and Gsk3β. In this complex, β-catenin is constitutively phosphorylated by Gsk3β on the two serine residues of the β-Trcp binding motif allowing the binding of β-catenin to $SCF^{\beta\text{-}Trcp}$ and its subsequent ubiquitinylation.

CTNNB1 (the gene encoding β-catenin) and other regulatory genes in this pathway are often mutated in primary human cancers such as colorectal, hepatocellular, desmoid, ovarian, endometrial, thyroid, prostate tumors and malignant melanomas (Polakis, 1999, 2000). In all the cases, the common consequence of these mutations is β-catenin accumulation, its translocation into the nucleus and transcriptional activation of target genes such as c-Myc and Cyclin D. Several mutations in *CTNNB1* found in human tumors affect the specific serine residues or the amino acids adjacent to them that mediate the phosphorylation-dependent interaction of β-catenin with β-Trcp (Polakis, 2000). β-catenin can be oncogenically activated not only by direct mutation but also by inactivation of APC. Alterations of the *APC* gene occur in 80% of the human colon cancers. In the absence of APC, β-catenin cannot be phosphorylated by Gsk3β, and it consequently accumulates and translocates into the nucleus (Polakis, 2000).

Degradation of β-catenin by β-Trcp suggests a role for this FBP as a tumor suppressor gene, the alteration of which may be found in human tumors. However, no mutations in the *BTRC* gene (encoding β-Trcp1) or *BTRC2* (encoding β-Trcp2) have been found in colon cancers wild type for *CTNNB1* and *APC*. The only reports showing genetic alterations of the *BTRC* genes in human tumors are those by Saitoh and Katoh (2001) and Gerstein *et al*. (2002). The first group searched for genetic alterations of *BTRC2* in gastric cancer. One nucleotide substitution was identified in OKAJIMA cells, which leads to an amino-acid substitution in the seventh WD40 repeat domain. In the second study, 22 samples including prostate tumor cell lines, xenographs and primary prostate tumors were analysed. Two alterations in *BTRC* were found, which are expected to render the protein deficient in β-catenin binding. In this context, it is worth noting that disrupting the *Btrc* locus in mice or silencing of either β-Trcp1 or β-Trcp2 alone in human cells is not sufficient to induce significant defects in β-catenin degradation (Guardavaccaro *et al*., 2003). Stabilization of β-catenin requires instead the inactivation of both β-Trcp1 and β-Trcp2, indicating that mutation of either *BTRC* or *BTRC2* alone might not provide a substantial cell growth advantage. Furthermore, given the role of β-Trcp in the degradation of IκB proteins, it is possible that inactivating mutations in *BTRC* are incompatible with transformation because of an increase of apoptosis as a result of NF-κB inhibition.

NF-κB is a transcription factor that is sequestered in the cytoplasm by association with a member of a class of NF-κB inhibitors called IκB proteins (Karin and Ben-Neriah, 2000). In response to a variety of stimuli such as extracellular signals, virus infection and ionizing radiation, IκB proteins are phosphorylated by the IκB kinase complex and degraded by the ubiquitin–proteasome pathway, releasing NF-κB that, in turn, translocates into the nucleus and activates the transcription of a large number of target genes. In 1998 Ben-Neriah's group, using biochemical methods, identified β-Trcp1 as the receptor component of the IκBα–ubiquitin ligase (Yaron *et al*., 1998). Subsequently, other groups (Hatakeyama *et al*., 1999; Hattori *et al*., 1999; Kroll *et al*., 1999; Shirane *et al*., 1999; Spencer *et al*., 1999; Winston *et al*., 1999b; Wu and Ghosh, 1999) provided additional strong evidences that IκBα phosphorylation on the two serine residues of the β-Trcp binding domain leads to IκBα association with $SCF^{\beta\text{-Trcp1/2}}$. The NF-κB transcription factors are regulators of the immune response. They are also implicated in the positive regulation of cell proliferation and inhibition of apoptosis, and accumulating evidence points to a role for NF-κB in neoplastic transformation (Luque and Gelinas, 1997; Rayet and Gelinas, 1999). Indeed, chromosomal aberrations and various mutations in genes encoding NF-κB transcription factors have been found in many different human tumors such as lung, colon, breast, and prostate cancers, Hodgkin's lymphomas, Ewing sarcomas, pancreatic carcinomas, familial medullary thyroid carcinomas and nasopharyngeal carcinomas. Alterations decreasing the expression and the function of IκB proteins have also been observed in many cancers (Rayet and Gelinas, 1999). All these different aberrations lead to hyperactivation of the NF-κB signaling pathway. Since many studies have reported that NF-κB proteins can inhibit apoptosis in response to a variety of stimuli, it is thought that constitutive NF-κB activity contributes to tumor development by promoting cell survival. The role of β-Trcp in the ubiquitinylation of IκB proteins indicates that β-Trcp could control the NF-κB-dependent apoptotic pathway. Interestingly, it has been found that inhibition of β-Trcp by overexpressing a dominant-negative β-Trcp2 mutant sensitizes human melanoma cell lines to apoptosis induced by cytokines, cisplatin, ionizing radiation and UV irradiation (Soldatenkov *et al*., 1999). Inhibition of β-Trcp renders cells more susceptible to apoptotic cell death because of IκB accumulation but likely also because of the stabilization of other substrates, such as β-catenin, Emi1 and Cdc25A, which could potentially push cells out of quiescence, a state in which cells are more resistance to apoptosis. Bhatia *et al*. (2002) found that the β-Trcp2 transcript is upregulated in chemically induced mouse papillomas and squamous cell carcinomas and this overexpression is associated with accelerated degradation of IκBα and constitutive activation of NF-κB. Moreover, β-Trcp1 overexpression in mouse mammary gland epithelium leads to increased ductal branching and elevated proliferation of the epithelial cells correlating with enhanced NF-κB activity (Y Kudo, D Guardavaccaro, P Gonzalez and M Pagano, unpublished results). All together, these results raise the possibility that inhibition of β-Trcp may augment the therapeutic response of tumor cell death-inducing protocols.

Recently, genetic and biochemical approaches have revealed an unexpected role for $SCF^{\beta\text{-Trcp1}}$ in the regulation of both meiosis and mitosis (Guardavaccaro *et al*., 2003). Inactivation of the *Btrc* gene in mice causes a defective spermatogenesis with accumulation of primary spermatocytes in metaphase and the appearance of multinucleated spermatids. Moreover, a subset

of *Btrc*-deficient spermatocytes displays spindle abnormalities and misalignment of chromosomes. These meiotic defects correspond to mitotic defects in somatic cells. First, *Btrc* knockout mice develop tumors at low incidence (unpublished results). In addition, MEFs isolated from *Btrc*−/− embryos progress more slowly through mitosis if compared with wild-type cells. *Btrc*-deficient MEFs display centrosome overduplication, multipolar metaphase spindles and misalignment of chromosomes. Moreover, stabilization of Emi1 was found both in *Btrc*−/− MEFs and in HeLa cells in which β-Trcp1 expression was silenced by RNA interference. It has been reported that Emi1 inhibits APC/C activity thereby preventing destruction of mitotic cyclins and other APC/C substrates like Securin, Aurora-A and Nek2 (Peters, 2002). Emi1 contains a canonical DSGxxS β-Trcp1 binding domain suggesting that it could be a direct substrate of β-Trcp1. Several results validate this hypothesis. Emi1 is able to bind β-Trcp1 both *in vitro* and *in vivo*, and this binding depends on the presence of the two serine residues of the β-Trcp binding domain. Wild-type Emi1 is stabilized when overexpressed in mitotic *Btrc*−/− MEFs, and a mutant form of Emi1, in which both serines have been mutated into alanine, is stable in MEFs of both genotypes. In a cell-free assay for Emi1 ubiquitinylation, Emi1-ubiquitin ligation activity was found to be higher in the extract from wild-type prometaphase MEFs than from *Btrc*−/− prometaphase MEFs. Mutant Emi1 is not ubiquitinylated by either extracts. Remarkably, the addition of purified recombinant β-Trcp1 to the extract from *Btrc*−/− MEFs in prometaphase is able to rescue the defective Emi1 ubiquitinylation. The levels of Emi1 transcript and protein are upregulated in many human tumors, in particular breast, lung, colon, uterus and ovary (Hsu *et al*., 2002; van't Veer *et al*., 2002). Emi1 degradation by β-Trcp in early mitosis suggests a mechanism for how Emi1 upregulation may contribute to genomic instability. In fact, Emi1 accumulation leads to inhibition of APC/C and subsequent overexpression of APC/C substrates such as Cyclin A, Aurora-A and Securin causing an error-prone mitosis (Reimann *et al*., 2001).

Finally, it has been recently found that β-Trcp targets Cdc25A, a Cdk1 and Cdk2 activating phosphatase, for degradation during S phase and in response to ionizing radiation-induced DNA damage (Busino *et al*., 2003). Cdc25A is able to interact with $SCF^{\beta\text{-Trcp1/2}}$ through a β-Trcp binding domain (DSGxxxxS) that is required for Cdc25A ubiquitinylation and degradation. Silencing of β-Trcp by RNA interference causes accumulation of Cdc25A in the S phase of the cell cycle and Cdc25A defective destruction in response to ionizing radiation, indicating that β-Trcp has a role in the S phase checkpoint.

Cul1 and Skp1 are localized on the centrosome and play a key role in centriole splitting (Freed *et al*., 1999; Gstaiger *et al*., 1999). Cul1 and Skp1 also control later steps of the centrosome cycle as shown by the fact that enforced expression of a Cul1 dominant-negative mutant induces multiple centrosome abnormalities, not only a failure of the centrioles to separate (Piva *et al*., 2002). The FBP interacting with Cul1 and Skp1 to form a centrosomal SCF complex is not known, and it is actually possible that multiple SCF E3s, each containing a different FBP, are involved in different phases of the centrosomal cycle. So far, two genes encoding FBPs (Skp2 and β-Trcp1) have been inactivated in mice and both show overduplication of centrosomes (Nakayama *et al*., 2000; Guardavaccaro *et al*., 2003). Skp2 deficiency induces endoreduplication and inhibits the entry into mitosis via a mechanism that is not yet understood. Thus, centrosomal overduplication in Skp2−/− cells might be the result of a prolonged period spent in S phase. In fact, the centrosome cycle is dissociated from the cell division cycle since an arrest either at G1/S or in mitosis does not block centrosomal duplication, hence generating multiple centrosomes per cell (Gard *et al*., 1990; Balczon *et al*., 1995). β-Trcp1 deficiency might induce centrosomal overduplication by its ability to delay mitosis progression by increasing Emi1 levels and consequently inducing an inhibition of APC/C^{Cdc20}. In agreement with this hypothesis, overexpression of Emi1 causes centrosomal overduplication. APC/C substrates, such as Cyclin A, Aurora-A, Plk1, Cdc25A and Nek2, stabilized as a result of APC/C inhibition by Emi1, could play a role in the amplification and separation of centrosomes in the absence of β-Trcp1 function. Despite the role of Skp2 in regulating the centrosomal cycle, evidence that this protein is stably associated with the centrosomes is lacking. In contrast, β-Trcp1 has been recently found localized on the centrosomes by a mass spectrometry-based proteomic analysis (Andersen *et al*., 2003).

Cdl2 and Cdl5

In *C. elegans*, Cul2 functions as a positive cell cycle regulator. It is expressed mainly in proliferating cells, and disruption of Cul2 expression induces G1 arrest of germ cells (Feng *et al*., 1999). This block correlates with the accumulation of the CDK inhibitor Cki-1. Cul2 is also necessary for proper movement of the cytoskeleton and mitotic chromosome condensation. As with Cul1, Cul2 is expected to bind a large number of substrate receptor subunits given that a large number of SBPs and BCBPs have been identified (Hilton *et al*., 1998; Kile *et al*., 2002).

It has been shown that the product of the von Hippel–Lindau (VHL) tumor suppressor gene interacts, via its BC box, with Cul2, Elongin B, Elongin C and Roc1 to form a Cdl2, also known as VBC complex (Kaelin, 2002). Various studies revealed that, under normoxic conditions, $Cdl2^{VHL}$ targets the hypoxia-inducible factor subunits HIF-1α and HIF-2α for ubiquitinylation and degradation, shedding light on the cancer pathogenesis of the VHL syndrome (Kondo *et al*., 2002; Maranchie *et al*., 2002). The VHL disease is caused by germline mutations of the *VHL* gene (Pugh and Ratcliffe, 2003). Patients with VHL are predisposed to a variety of tumors, including renal carcinomas, pheochromocytomas, central nervous system hemangiosarcomas and retinal angiomas. These tumors develop when the

remaining wild-type allele is inactivated by deletions, mutations or hypermethylation. Moreover, the majority of sporadic renal clear cell carcinomas also display loss of both *VHL* alleles. A hallmark of VHL-associated neoplasms is their hypervascular nature due to the constitutive expression of hypoxia-inducible genes, like those encoding erythropoietin, vascular endothelial growth factor and glucose transporter. In this context, the identification of the HIF-α complex as a proteolytic target of the $Cdl2^{VHL}$ E3 ubiquitin ligase provides a link between the ubiquitin–proteasome system and the tumor suppressor activity of VHL. Importantly, VHL mutants in *C. elegans* appear indistinguishable from wild-type worms at normal oxygen levels (ET Kipreos, personal communication) indicating that the cell cycle phenotype observed in the *cul2* knockout is not due to the loss of VHL function. These results suggest the presence of a different substrate receptor in Cdl2 that is responsible for its cell cycle functions.

Another BCBP, known as mediator subunit Med8, has been shown to assemble with Cul2, Elongin B and C, and Roc1, via its BC box to form a complex recruiting ubiquitin ligase activity to the RNA polymerase transcriptional machinery (Brower *et al*., 2002).

The SOCS box domain is contained in more than 40 proteins belonging to different families, the canonical SOCS family comprising Socs1–7 and the cytokine-inducible SH2-containing proteins (CISs), the ankyrin repeat proteins with a SOCS box (ASBs), the SPRY domain proteins with a SOCS box (SSBs), the WD40 repeat proteins with a SOCS box (WSBs), the tubby domain-containing proteins with a SOCS box (TSBs), the Rar proteins and the Neuralized family of proteins (Hilton *et al*., 1998). All these polypeptides contain, besides the SOCS box, additional domains involved in protein–protein interactions. Many of these, such as Socs1, Socs3, Wsb1, Asb2 and Rar, have been shown to associate with Elongin B and Elongin C (Kamura *et al*., 1998), but demonstrations that these complexes interact with Cullin2 and Roc1 and have ubiquitin ligase activity are still missing for most of them.

Socs1, an *S*rc *h*omology *2* (SH2)-containing protein that negatively controls cytokine and growth factor signaling, interacts with Cul2, Elongin B and Elongin C (De Sepulveda *et al*., 2000; Kamizono *et al*., 2001). This complex has been found to function as a ubiquitin ligase and promote ubiquitin-dependent degradation of Tel-Jak2, a gene product found in human leukemia derived from the fusion of the *TEL* gene to the *JAK2* tyrosine kinase gene. Moreover, overexpression of Socs1 can efficiently suppress the transforming potential of Tel-Jak2 (Kamizono *et al*., 2001). $Cdl2^{Socs1}$ can also mediate the ubiquitinylation and subsequent degradation of the hematopoiesis-specific guanine nucleotide exchange factor Vav, a human oncoprotein expressed in hematopoietic cells (De Sepulveda *et al*., 2000). Finally, Socs1 promotes the ubiquitinylation and degradation of the insulin receptor substrates IRS1 and IRS2 (Rui *et al*., 2002).

It has been reported that besides Cul2, Cul5 is also able to assemble with Roc1, Elongin B, Elongin C and a BCBP to form a similar multiprotein complex with ubiquitin ligase activity (Kamura *et al*., 2001). Several SBPs and BCBPs have been shown to associate with Cul5–Roc1–Elongin B–Elongin C: Muf-1, a leucine-rich repeat containing protein, Socs1, Elongin A and WSB-1 (Kamura *et al*., 2001). The Cul5–Elongin B–Elongin C–Roc1 complex can also interact with the two adenovirus proteins E4orf6 and E1B55k (Querido *et al*., 2001a). It was previously shown that these two proteins are able to bind the tumor suppressor p53, inhibit its transcriptional activity and induce its efficient degradation (Querido *et al*., 2001b). E4orf6 and E1B55k, bound to the Cul5–Elongin B–Elongin C–Roc1 complex, are able to induce p53 ubiquitinylation *in vitro* in the presence of E1 and an E2. E4orf6/E1B55k-mediated degradation of p53 is analogous to that induced by the human papilloma virus (HPV) E6 oncoprotein, which recruits the cellular ubiquitin ligase, E6-associated protein, to p53, thereby targeting it for degradation (Pagano and Benmaamar, 2003). Hence, in both cases, a viral oncoprotein can associate with a cellular ubiquitin ligase enzyme and use it to eliminate the tumor suppressor p53.

Cdl3

In *C. elegans,* depletion of Cul3 by RNA interference causes defects in early embryogenesis resulting in abnormal microfilament and microtubule organization (Kurz *et al*., 2002). Cul3 is also required for proper development in mice since inactivation of the *Cul3* locus by homologous recombination results in embryonic lethality prior to 7.5 days of gestation with defects both in embryonic and extraembryonic compartments (Singer *et al*., 1999). The extraembryonic tissues are completely disorganized and the trophectoderm develops abnormally. Gastrulation is also abnormal in *Cul3*-deficient embryos. In addition, increased levels of free (non-Cdk2-associated) Cyclin E protein were observed in the ectoplacental cone and extraembryonic ectoderm of *Cul3*$-/-$ embryos (Singer *et al*., 1999). However, it is unlikely that the early embryonic lethality of Cul3-deficient mice could be solely caused by a defective degradation of a subpopulation of Cyclin E, and the recent discovery that in *C. elegans* Cul3 interacts with several BTBPs likely targeting a high number of different substrates (see Introduction) is in favor of this scenario. One of these BTBPs, Mel-26, binds to the microtubule-severing protein Mei-1/katanin. Mei-1 is an essential component of the *C. elegans* meiotic spindle. Although the precise role of Mei-1 protein in the meiotic spindle is unknown, it has been found that Mei-1 is degraded at the meiosis to mitosis transition and that Cul3 is required for its degradation. Moreover, loss-of-function mutations of *mel-26* result in Mei-1 protein ectopically localized in mitotic spindles and centrosomes leading to small and misoriented cleavage spindles and arrest at the single-cell embryo stage (Dow and Mains, 1998). Moreover, loss-of-function mutations in *mei-1*

fully suppress these defects (Kurz *et al*., 2002). Cul3 silencing by RNA interference results in abortive cytokinesis and analogous spindle orientation defects with misoriented spindles that become displaced toward one pole (Kurz *et al*., 2002). These genetic results, together with the finding that Mel-26 physically interacts with Mei-1 (Furukawa *et al*., 2003; Geyer *et al*., 2003; Pintard *et al*., 2003; Xu *et al*., 2003), strongly indicate that a Cul3-dependent ubiquitin ligase, $Cdl3^{Mel-26}$, might target Mei-1 for degradation. However, a direct biochemical assay demonstrating Mei-1 ubiquitinylation by $Cdl3^{Mel-26}$ is still missing. The discovery that Cul3 is capable of associating with many adaptor proteins that in turn recognize specific targets, together with the role in oncogenesis played by other cullins, leads to the speculation that Cdl3 might control the ubiquitinylation and degradation of cancer-related proteins. In support of this hypothesis, in the developing eye disc of *D. melanogaster*, Cul3 controls the stability of Cubitus interruptus (Ou *et al*., 2002), a major player of the Hedgehog signaling pathway. This latter has been found to be involved in the genesis of many different human tumors, such as basal cell carcinomas, medulloblastomas (Matise and Joyner, 1999), pancreatic adenocarcinomas (Thayer *et al*., 2003) and in various tumors of the digestive tract (Berman *et al*., 2003).

Finally, *S. pombe* Cul3 interacts with the BTBP Btb3p (Geyer *et al*., 2003), the fission yeast ortholog of human Bpoz2, a protein that is thought to be implicated in the growth arrest mediated by the tumor suppressor PTEN (Unoki and Nakamura, 2001).

Cdl4

The gene encoding Cul4A is amplified in 16% of primary breast cancers, and RNA *in situ* hybridization analysis indicates that 47% of primary breast cancers overexpress Cul4A (Chen *et al*., 1998). Moreover, 13q34, a chromosome region where *CUL4A* is mapped, is frequently amplified in primary hepatocellular carcinomas (HCC) and in several other tumors including primary esophageal squamous carcinomas (Yasui *et al*., 2002). Accordingly, *CUL4A* shows amplification and consequent overexpression in certain primary HCC. These findings suggest that Cul4A plays an important role in tumorigenesis. Interestingly, Groisman *et al*. (2003) have shown that Cul4A is involved in nucleotide excision repair (NER), an essential cellular defense mechanism against the oncogenic consequence of ultraviolet light. They reported that two NER proteins, known as DDB2 and CSA, are associated with identical complexes containing Cul4A, Roc1, DDB1 and the COP9 signalosome. These complexes display ubiquitin activity that is regulated in response to UV irradiation. Inhibition of the COP9 signalosome causes defects in the NER process. However, it is still unclear if the DDB proteins act as substrate recognition subunits or are direct targets of Cdl4, since two groups found that Cul4A induces ubiquitinylation of DDB2 and increases its degradation (Chen *et al*., 2001; Nag *et al*., 2001). Interestingly, DDB2 mutants have been identified from patients affected by xeroderma pigmentosum, a disease characterized by defective NER and predisposition to skin cancer (Nag *et al*., 2001). DDB2 mutants are characterized by a defect in the repair of UV-damaged DNA, suggesting that abnormal expression of Cul4A results in reduced DDB levels, thus impairing the ability of DDB in repairing the DNA of tumor cells.

It has been shown that Cul4 has a role in maintaining genomic stability by temporally restricting DNA replication licensing in *C. elegans* (Zhong *et al*., 2003). Silencing of Cul4 by RNA interference leads to massive DNA re-replication and S phase accumulation of the replication-licensing factor Cdt1, which, at least in mammals, could also be a target of Cul1 (see above). It has been proposed that Cul4 functions in S phase to degrade Cdt1 in order to prevent DNA re-replication.

All together, these results indicate that Cul4 plays an important role in regulating DNA replication and repair, and suggest a mechanism by which overexpression of Cul4A can contribute to tumor development.

Conclusions

Aberrant activities of many CDLs in human tumors have been described. Undoubtedly, the coming years promise a plethora of studies that will address how defective or overactive protein degradation contributes to tumor development. Furthermore, many crucial protein substrates that play a major role in tumorigenesis are not yet linked to their specific ubiquitin ligases, the identification of which will provide valuable knowledge for developing new therapeutic agents for cancer treatment.

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References

Andersen JS, Wilkinson CJ, Mayor T, Mortensen P, Nigg EA and Mann M. (2003). *Nature*, **426,** 570–574.

Arai T, Kasper J, Skaar J, Ali S, Takahashi C and Decaprio J. (2003). *Proc. Natl. Acad. Sci. USA*, **100,** 9855–9860.

Balczon R, Bao L, Zimmer WE, Brown K, Zinkowski RP and Brinkley BR. (1995). *J. Cell Biol.*, **130,** 105–115.

Bashir T and Pagano M. (2003). *Adv. Cancer Res.*, **88,** 101–144.

Bashir T, Dorrello NV, Amador V, Guardavaccaro D and Pagano M. (2004). *Nature*, in press.
Ben-Izhak O, Lahav-Baratz S, Meretyk S, Ben-Eliezer S, Sabo E, Dirnfeld M, Cohen S and Ciechanover A. (2003). *J. Urol.*, **170,** 241–245.
Berman DM, Karhadkar SS, Maitra A, Montes de Oca R, Gerstenblith MR, Briggs K, ARParker AR, Shimada Y, Eshleman JR, Watkins DN and Beachy PA. (2003). *Nature*, **425,** 846–851.
Bhatia N, Herter JR, Slaga TJ, Fuchs SY and Spiegelman VS. (2002). *Oncogene*, **21,** 1501–1509.
Bornstein G, Bloom J, Sitry-Shevah D, Nakayama K, Pagano M and Hershko A. (2003). *J. Biol. Chem.*, **278,** 25752–25757.
Brower CS, Sato S, Tomomori-Sato C, Kamura T, Pause A, Stearman R, Klausner RD, Malik S, Lane WS, Sorokina I, Roeder RG, Conaway JW and Conaway RC. (2002). *Proc. Natl. Acad. Sci. USA*, **99,** 10353–10358.
Busino L, Donzelli M, Chiesa M, Guardavaccaro D, Ganoth D, Dorrello NV, Hershko A, Pagano M and Draetta GF. (2003). *Nature*, **426,** 87–91.
Carrano AC, Eytan E, Hershko A and Pagano M. (1999). *Nat. Cell Biol.*, **1,** 193–199.
Carrano AC and Pagano M. (2001). *J. Cell Biol.*, **153,** 1381–1389.
Cenciarelli C, Chiaur DS, Guardavaccaro D, Parks W, Vidal M and Pagano M. (1999). *Curr. Biol.*, **9,** 1177–1179.
Charrasse S, Carena I, Brondani V, Klempnauer KH and Ferrari S. (2000). *Oncogene*, **19,** 2986–2995.
Chen LC, Manjeshwar S, Lu Y, Moore D, Ljung BM, Kuo WL, Dairkee SH, Wernick M, Collins C and Smith HS. (1998). *Cancer Res.*, **58,** 3677–3683.
Chen W, Zollman S, Couderc JL and Laski FA. (1995). *Mol. Cell. Biol.*, **15,** 3424–3429.
Chen X, Zhang Y, Douglas L and Zhou P. (2001). *J. Biol. Chem.*, **276,** 48175–48182.
Chiarle R, Yan P, Piva R, Boggino H, Skolnik J, Novero D, Palestro G, DeWolf C, Chilosi M, Pagano M and Inghirami G. (2002). *Am. J. Pathol.*, **160,** 1457–1466.
Davis AC, Wims M, Spotts GD, Hann SR and Bradley A. (1993). *Genes Dev.*, **7,** 671–682.
De Sepulveda P, Ilangumaran S and Rottapel R. (2000). *J. Biol. Chem.*, **275,** 14005–14008.
Dealy MJ, Nguyen KV, Lo J, Gstaiger M, Krek W, Elson D, Arbeit J, Kipreos ET and Johnson RS. (1999). *Nat. Genet.*, **23,** 245–248.
Dias DC, Dolios G, Wang R and Pan ZQ. (2002). *Proc. Natl. Acad. Sci. USA*, **99,** 16601–16606.
Dow MR and Mains PE. (1998). *Genetics*, **150,** 119–128.
Dowen SE, Neutze DM, Pett MR, Cottage A, Stern P, Coleman N and Stanley MA. (2003). *Oncogene*, **22,** 2531–2540.
El-Rifai W, Frierson Jr HF, Harper JC, Powell SM and Knuutila S. (2001). *Int. J. Cancer*, **92,** 832–838.
Feldman RM, Correll CC, Kaplan KB and Deshaies RJ. (1997). *Cell*, **91,** 221–230.
Feng H, Zhong W, Punkosdy G, Gu S, Zhou L, Seabolt EK and Kipreos ET. (1999). *Nat. Cell Biol.*, **1,** 486–492.
Fong A and Sun SC. (2002). *J. Biol. Chem.*, **277,** 22111–22114.
Freed E, Lacey KR, Huie P, Lyapina SA, Deshaies RJ, Stearns T and Jackson PK. (1999). *Genes Dev.*, **13,** 2242–2257.
Furukawa M, He YJ, Borchers C and Xiong Y. (2003). *Nat. Cell Biol.*, **5,** 1001–1007.
Ganiatsas S, Dow R, Thompson A, Schulman B and Germain D. (2001). *Oncogene*, **20,** 3641–3650.
Ganoth D, Bornstein G, Ko T, Larsen B, Tyers M, Pagano M and Hershko A. (2001). *Nat. Cell Biol.*, **3,** 321–324.
Gard DL, Hafezi S, Zhang T and Doxsey SJ. (1990). *J. Cell Biol.*, **110,** 2033–2042.
Garriga J, Bhattacharya S, Calbo J, Marshall RM, Truongcao M, Haines DS and Grana X. (2003). *Mol. Cell. Biol.*, **23,** 5165–5173.
Gerstein AV, Almeida TA, Zhao G, Chess E, Shih Ie M, Buhler K, Pienta K, Rubin MA, Vessella R and Papadopoulos N. (2002). *Genes Chromosomes Cancer*, **34,** 9–16.
Geyer R, Wee S, Anderson S, Yates J and Wolf DA. (2003). *Mol. Cell*, **12,** 783–790.
Groisman R, Polanowska J, Kuraoka I, Sawada J, Saijo M, Drapkin R, Kisselev AF, Tanaka K and Nakatani Y. (2003). *Cell*, **113,** 357–367.
Gstaiger M, Jordan R, Lim M, Catzavelos C, Mestan J, Slingerland J and Krek W. (2001). *Proc. Natl. Acad. Sci. USA*, **98,** 5043–5048.
Gstaiger M, Marti A and Krek W. (1999). *Exp. Cell Res.*, **247,** 554–562.
Guardavaccaro D, Kudo Y, Boulaire J, Barchi M, Busino L, Donzelli M, Margottin F, Jackson PK, Yamasaki L and Pagano M. (2003). *Dev. Cell*, **4,** 799–812.
Gupta-Rossi N, Le Bail O, Gonen H, Brou C, Logeat F, Six E, Ciechanover A and Israel A. (2001). *J. Biol. Chem.*, **276,** 34371–34378.
Hart M, Concordet JP, Lassot I, Albert I, del los Santos R, Durand H, Perret C, Rubinfeld B, Margottin F, Benarous R and Polakis P. (1999). *Curr. Biol.*, **9,** 207–210.
Hatakeyama S, Kitagawa M, Nakayama K, Shirane M, Matsumoto M, Hattori K, Higashi H, Nakano H, Okumura K, Onoe K, Good RA and Nakayama KI. (1999). *Proc. Natl. Acad. Sci. USA*, **96,** 3859–3863.
Hattori K, Hatakeyama S, Shirane M, Matsumoto M and Nakayama K. (1999). *J. Biol. Chem.*, **274,** 29641–29647.
Hershko A and Ciechanover A. (1998). *Annu. Rev. Biochem.*, **67,** 425–479.
Hershko D, Bornstein G, Ben-Izhak O, Carrano A, Pagano M, Krausz M and Hershko A. (2001). *Cancer*, **91,** 1745–1751.
Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcalf D and Nicola NA. (1998). *Proc. Natl. Acad. Sci. USA*, **95,** 114–119.
Hori T, Osaka F, Chiba T, Miyamoto C, Okabayashi K, Shimbara N, Kato S and Tanaka K. (1999). *Oncogene*, **18,** 6829–6834.
Hsu JY, Reimann JD, Sorensen CS, Lukas J and Jackson PK. (2002). *Nat. Cell Biol.*, **4,** 358–366.
Inui N, Kitagawa K, Miwa S, Hattori T, Chida K, Nakamura H and Kitagawa M. (2003). *Biochem. Biophys. Res. Commun.*, **303,** 978–984.
Jiang J and Struhl G. (1998). *Nature*, **391,** 493–496.
Kaelin Jr WG. (2002). *Nat. Rev. Cancer*, **2,** 673–682.
Kamizono S, Hanada T, Yasukawa H, Minoguchi S, Kato R, Minoguchi M, Hattori K, Hatakeyama S, Yada M, Morita S, Kitamura T, Kato H, Nakayama K and Yoshimura A. (2001). *J. Biol. Chem.*, **276,** 12530–12538.
Kamura T, Burian D, Yan Q, Schmidt SL, Lane WS, Querido E, Branton PE, Shilatifard A, Conaway RC and Conaway JW. (2001). *J. Biol. Chem.*, **276,** 29748–29753.
Kamura T, Hara T, Kotoshiba S, Yada M, Ishida N, Imaki H, Hatakeyama S, Nakayama K and Nakayama Ki. (2003). *Proc. Natl. Acad. Sci. USA*, **100,** 10231–10236.
Kamura T, Sato S, Haque D, Liu L, Kaelin Jr WG, Conaway RC and Conaway JW. (1998). *Genes Dev.*, **12,** 3872–3881.
Karin M and Ben-Neriah Y. (2000). *Annu. Rev. Immunol.*, **18,** 621–663.

Keyomarsi K and Herliczek TW. (1997). *Prog. Cell Cycle Res.*, **3,** 171–191.
Kiernan RE, Emiliani S, Nakayama K, Castro A, Labbe JC, Lorca T, Nakayama KI and Benkirane M. (2001). *Mol. Cell. Biol.*, **21,** 7956–7970.
Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM and Hilton DJ. (2002). *Trends Biochem. Sci.*, **27,** 235–241.
Kim SY, Herbst A, Tworkowski KA, Salghetti SE and Tansey WP. (2003). *Mol. Cell*, **11,** 1177–1188.
Kimura T, Gotoh M, Nakamura Y and Arakawa H. (2003). *Cancer Sci.*, **94,** 431–436.
Kipreos E and Pagano M. (2000). *Genome Biol.,* 1 REVIEWS3002.
Kipreos ET, Gohel SP and Hedgecock EM. (2000). *Development*, **127,** 5071–5082.
Kipreos ET, Lander L, Wing J, He W and Hedgecock E. (1996). *Cell*, **85,** 829–839.
Kitagawa M, Hatakeyama S, Shirane M, Matsumoto M, Ishida N, Hattori K, Nakamichi I, Kikuchi A, Nakayama KI and Nakayama K. (1999). *EMBO J.*, **18,** 2401–2410.
Ko HW, Jiang J and Edery I. (2002). *Nature*, **420,** 673–678.
Koepp DM, Schaefer LK, Ye X, Keyomarsi K, Chu C, Harper JW and Elledge SJ. (2001). *Science*, **294,** 173–177.
Koike J, Sagara N, Kirikoshi H, Takagi A, Miwa T, Hirai M and Katoh M. (2000). *Biochem. Biophys. Res. Commun.*, **269,** 103–109.
Kondo K, Klco J, Nakamura E, Lechpammer M and Kaelin Jr WG. (2002). *Cancer Cell*, **1,** 237–246.
Kroll M, Margottin F, Kohl A, Renard P, Durand H, Concordet JP, Bachelerie F, Arenzana-Seisdedos F and Benarous R. (1999). *J. Biol. Chem.*, **274,** 7941–7945.
Kudo Y, Kitajima S, Sato S, Miyauchi M, Ogawa I and Takata T. (2001). *Cancer Res.*, **61,** 7044–7047.
Kurz T, Pintard L, Willis JH, Hamill DR, Gonczy P, Peter M and Bowerman B. (2002). *Science*, **295,** 1294–1298.
Lassot I, Ségéral E, Berlioz-Torrent C, Durand H, Groussin L, Hai T, Benarous R and Margottin-Goguet F. (2001). *Mol. Cell. Biol.*, **21,** 2192–2202.
Latres E, Chiarle R, Schulman B, Pellicer A, Inghirami G and Pagano M. (2001). *Proc. Natl. Acad. Sci. USA*, **98,** 2515–2520.
Latres E, Chiaur DS and Pagano M. (1999). *Oncogene*, **18,** 849–855.
Li X, Zhao Q, Liao R, Sun P and Wu X. (2003). *J. Biol. Chem.*, **278,** 30854–30858.
Luque I and Gelinas C. (1997). *Semin. Cancer Biol.*, **8,** 103–111.
Maniatis T. (1999). *Genes Dev.*, **13,** 505–510.
Mantovani F and Banks LM. (2003). *J. Biol. Chem.*, **278,** 42477–42486.
Maranchie JK, Vasselli JR, Riss J, Bonifacino JS, Linehan WM and Klausner RD. (2002). *Cancer Cell*, **1,** 247–255.
Margottin-Goguet F, Hsu JY, Loktev A, Hsieh HM, Reimann JD and Jackson PK. (2003). *Dev. Cell*, **4,** 813–826.
Marti A, Wirbelauer C, Scheffner M and Krek W. (1999). *Nat. Cell Biol.*, **1,** 14–19.
Maruyama S, Hatakeyama S, Nakayama K, Ishida N and Kawakami K. (2001). *Genomics*, **78,** 214–222.
Masuda TA, Inoue H, Sonoda H, Mine S, Yoshikawa Y, Nakayama K and Mori M. (2002). *Cancer Res.*, **62,** 3819–3825.
Matise MP and Joyner AL. (1999). *Oncogene*, **18,** 7852–7859.
Mendez J, Zou-Yang XH, Kim SY, Hidaka M, Tansey WP and Stillman B. (2002). *Mol. Cell*, **9,** 481–491.
Moberg KH, Bell DW, Wahrer DC, Haber DA and Hariharan IK. (2001). *Nature*, **413,** 311–316.
Nag A, Bondar T, Shiv S and Raychaudhuri P. (2001). *Mol. Cell. Biol.*, **21,** 6738–6747.
Nakayama K, Nagahama H, Minamishima Y, Matsumoto M, Nakamichi I, Kitagawa K, Shirane M, Tsunematsu R, Tsukiyama T, Ishida N, Kitagawa M, Nakayama K and Hatakeyama S. (2000). *EMBO J.*, **19,** 2069–2081.
Nayak S, Santiago FE, Jin H, Lin D, Schedl T and Kipreos ET. (2002). *Curr. Biol.*, **12,** 277–287.
Nikolaev AY, Li M, Puskas N, Qin J and Gu W. (2003). *Cell*, **112,** 29–40.
Oberg C, Li J, Pauley A, Wolf E, Gurney M and Lendahl U. (2001). *J. Biol. Chem.*, **276,** 35847–35853.
Ohta T, Michel JJ, Schottelius AJ and Xiong Y. (1999). *Mol. Cell*, **3,** 535–541.
Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y and Nakamura Y. (2001). *Cancer Res.*, **61,** 2129–2137.
Orian A, Gonen H, Bercovich B, Fajerman I, Eytan E, Israel A, Mercurio F, Iwai K, Schwartz AL and Ciechanover A. (2000). *EMBO J.*, **19,** 2580–2591.
Ou CY, Lin YF, Chen YJ and Chien CT. (2002). *Genes Dev.*, **16,** 2403–2414.
Pagano M and Benmaamar R. (2003). *Cancer Cell*, **4,** 251–256.
Peters JM. (2002). *Mol. Cell*, **9,** 931–943.
Peters JM. (2003). *Mol. Cell*, **11,** 1420–1421.
Pintard L, Willis JH, Willems A, Johnson JL, Srayko M, Kurz T, Glaser S, Mains PE, Tyers M, Bowerman B and Peter M. (2003). *Nature*, **425,** 311–316.
Piva R, Liu J, Chiarle R, Podda A, Pagano M and Inghirami G. (2002). *Mol. Cell. Biol.*, **22,** 8375–8387.
Polakis P. (1999). *Curr. Opin. Genet. Dev.*, **9,** 15–21.
Polakis P. (2000). *Genes Dev.*, **14,** 1837–1851.
Pugh CW and Ratcliffe PJ. (2003). *Semin. Cancer Biol.*, **13,** 83–89.
Querido E, Blanchette P, Yan Q, Kamura T, Morrison M, Boivin D, Kaelin WG, Conaway RC, Conaway JW and Branton PE. (2001a). *Genes Dev.*, **15,** 3104–3117.
Querido E, Morrison MR, Chu-Pham-Dang H, Thirlwell SW, Boivin D, Branton PE and Morisson MR. (2001b). *J. Virol.*, **75,** 699–709.
Rayet B and Gelinas C. (1999). *Oncogene*, **18,** 6938–6947.
Reimann JD, Freed E, Hsu JY, Kramer ER, Peters JM and Jackson PK. (2001). *Cell*, **105,** 645–655.
Rui L, Yuan M, Frantz D, Shoelson S and White MF. (2002). *J. Biol. Chem.*, **277,** 42394–42398.
Saitoh T and Katoh M. (2001). *Int. J. Oncol.*, **18,** 959–964.
Schoenfeld AR, Davidowitz EJ and Burk RD. (2000). *Proc. Natl. Acad. Sci. USA*, **97,** 8507–8512.
Schwechheimer C and Deng XW. (2001). *Trends Cell Biol.*, **11,** 420–426.
Shigemasa K, Gu L, O'Brien TJ and Ohama K. (2003). *Clin. Cancer Res.*, **9,** 1756–1763.
Shim EH, Johnson L, Noh HL, Kim YJ, Sun H, Zeiss C and Zhang H. (2003). *Cancer Res.*, **63,** 1583–1588.
Shirane M, Hatakeyama S, Hattori K and Nakayama K. (1999). *J. Biol. Chem.*, **274,** 28169–28174.
Signoretti S, Di Marcotullio L, Richardson A, Ramaswamy S, Isaac B, Rue M, Monti F, Loda M and Pagano M. (2002). *J. Clin. Invest.*, **110,** 633–641.
Singer JD, Gurian WM, Clurman B and Roberts JM. (1999). *Genes Dev.*, **13,** 2375–2387.
Skowyra D, Craig KL, Tyers M, Elledge SJ and Harper JW. (1997). *Cell*, **91,** 209–219.

Soldatenkov VA, Dritschilo A, Ronai Z and Fuchs SY. (1999). *Cancer Res.*, **59,** 5085–5088.
Spencer E, Jiang J and Chen ZJ. (1999). *Genes Dev.*, **13,** 284–294.
Spruck C, Strohmaier H, Watson M, Smith A, Ryan A, Krek W and Reed S. (2001). *Mol. Cell*, **7,** 639–650.
Spruck CH, Strohmaier H, Sangfelt O, Muller HM, Hubalek M, Muller-Holzner E, Marth C, Widschwendter M and Reed SI. (2002). *Cancer Res.*, **62,** 4535–4539.
Strohmaier H, Spruck CH, Kaiser P, Won KA, Sangfelt O and Reed SI. (2001). *Nature*, **413,** 316–322.
Tedesco D, Lukas J and Reed SI. (2002). *Genes Dev.*, **16,** 2946–2957.
Thayer SP, Pasca di Magliano M, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi PY, Gysin S, Fernández-del Castillo C, Yajnik V, Antoniu B, Mcmahon M, Warshaw AL and Hebrok M. (2003). *Nature*, **425,** 851–856.
Tsvetkov LM, Yeh KH, Lee S, Sun H and Zhang H. (1999). *Curr. Biol.*, **9,** 661–664.
Unoki M and Nakamura Y. (2001). *Oncogene*, **20,** 4457–4465.
van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R and Friend SH. (2002). *Nature*, **415,** 530–536.
von der Lehr N, Johansson S, Wu S, Bahram F, Castell A, Cetinkaya C, Hydbring P, Weidung I, Nakayama K, Nakayama KI, Soderberg O, Kerppola TK and Larsson LG. (2003). *Mol. Cell*, **11,** 1189–1200.
Wang Y, Penfold S, Tang X, Hattori N, Riley P, Harper JW, Cross JC and Tyers M. (1999). *Curr. Biol.*, **9,** 1191–1194.
Welsh JB, Zarrinkar PP, Sapinoso LM, Kern SG, Behling CA, Monk BJ, Lockhart DJ, Burger RA and Hampton GM. (2001). *Proc. Natl. Acad. Sci. USA*, **98,** 1176–1181.
Winston JT, Koepp DM, Zhu C, Elledge SJ and Harper JW. (1999a). *Curr. Biol.*, **9,** 1180–1182.
Winston JT, Strack P, Beer-Romero P, Chu CY, Elledge SJ and Harper JW. (1999b). *Genes Dev.*, **13,** 270–283.
Wu C and Ghosh S. (1999). *J. Biol. Chem.*, **274,** 29591–29594.
Wu G, Lyapina S, Das I, Li J, Gurney M, Pauley A, Chui I, Deshaies RJ and Kitajewski J. (2001). *Mol. Cell. Biol.*, **21,** 7403–7415.
Xu L, Wei Y, Reboul J, Vaglio P, Shin TH, Vidal M, Elledge SJ and Harper JW. (2003). *Nature*, **425,** 316–321.
Yaron A, Hatzubai A, Davis M, Lavon I, Amit S, Manning AM, Andersen JS, Mann M, Mercurio F and Ben-Neriah Y. (1998). *Nature*, **396,** 590–594.
Yasui K, Arii S, Zhao C, Imoto I, Ueda M, Nagai H, Emi M and Inazawa J. (2002). *Hepatology*, **35,** 1476–1484.
Yokoi S, Yasui K, Saito-Ohara F, Koshikawa K, Iizasa T, Fujisawa T, Terasaki T, Horii A, Takahashi T, Hirohashi S and Inazawa J. (2002). *Am. J. Pathol.*, **161,** 207–216.
Yu H, Peters JM, King RW, Page AM, Hieter P and Kirschner MW. (1998a). *Science*, **279,** 1219–1222.
Yu ZK, Gervais J and Zhang H. (1998b). *Proc. Natl. Acad. Sci. USA*, **95,** 11324–11329.
Zhang H, Kobayashi R, Galaktionov K and Beach D. (1995). *Cell*, **82,** 915–925.
Zhong W, Feng H, Santiago FE and Kipreos ET. (2003). *Nature*, **423,** 885–889.
Zollman S, Godt D, Prive GG, Couderc JL and Laski FA. (1994). *Proc. Natl. Acad. Sci. USA*, **91,** 10717–10721.