1-1-2015

Cell regulation by phosphotyrosine-targeted ubiquitin ligases

Jonathan A. Cooper
Fred Hutchinson Cancer Research Center

Tomonori Kaneko
Western University

Shawn S.C. Li
Western University, sli@uwo.ca

MCB Journals.ASM.org

MINIREVIEW

Cell Regulation by Phosphotyrosine-Targeted Ubiquitin Ligases

Jonathan A. Cooper,[a] Tomonori Kaneko,[b] Shawn S. C. Li[b]

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA[a]; Department of Biochemistry, Western University, London, Ontario, Canada[b]

Three classes of E3 ubiquitin ligases, members of the Cbl, Hakai, and SOCS-Cul5-RING ligase families, stimulate the ubiquitination of phosphotyrosine-containing proteins, including receptor and nonreceptor tyrosine kinases and their phosphorylated substrates. Because ubiquitination frequently routes proteins for degradation by the lysosome or proteasome, these E3 ligases are able to potently inhibit tyrosine kinase signaling. Their loss or mutational inactivation can contribute to cancer, autoimmunity, or endocrine disorders, such as diabetes. However, these ligases also have biological functions that are independent of their ubiquitination activity. Here we review relevant literature and then focus on more-recent developments in understanding the structures, substrates, and pathways through which the phosphotyrosine-specific ubiquitin ligases regulate diverse aspects of cell biology.

Phosphorylation and ubiquitination are among the commonest and best-studied posttranslational modifications of proteins. A phosphate group or ubiquitin molecule can trigger or obstruct protein-protein interactions, alter subcellular localization, stabilize a particular protein conformation, or have myriad other effects. Phosphorylation is directly catalyzed by protein kinases, but ubiquitination is more complex, requiring sequential activity of E1, E2, and E3 ubiquitin-activating, -conjugating, and -ligating enzymes (1–5). E3 ubiquitin ligases fall into two major groups: HECT domain ligases receive ubiquitin from an E2 enzyme and transfer it to a bound substrate, while RING-type ligases position an E2-ubiquitin conjugate near a substrate protein to facilitate ubiquitin transfer. Both phosphorylation and ubiquitination are reversible; protein phosphorylation is reversed by protein phosphatases and ubiquitination by deubiquitinating enzymes (DUBs) (6–8). Therefore, both phosphorylation/dephosphorylation and ubiquitination/deubiquitination can allow repeated cycles of protein modification. Reversible ubiquitination is particularly important in DNA repair and NF-κB signaling. However, many ubiquitination events lead irreversibly to protein destruction, allowing regulation of protein turnover. For example, K48 polyubiquitin chains primarily route cytosolic proteins to the proteasome, while modification of many Lys residues with single ubiquitin molecules (multimonoubiquitination) has several functions, including routing membrane proteins for destruction in the lysosome (9–11). The irreversibility of proteolysis means that the ubiquitin-proteasome and ubiquitin-lysosome pathways directly control protein life spans.

Protein phosphorylation and ubiquitination cross talk at many levels (12). In this review, we focus on situations where phosphorylation of a substrate creates a binding site for an E3 ligase, rendering ubiquitination dependent on prior phosphorylation of that substrate (13). Such phosphorylation-dependent substrate selection has particular importance because it can layer negative feedback onto an otherwise reversible phosphorylation event (Fig. 1). In principle, increasing the kinase activity in a simple kinase/phosphatase cycle simply increases the steady-state level of the phosphorylated substrate (Fig. 1A and C), but adding phospho-specific ubiquitination and proteolysis reactions alters the kinetics, causing the level of phosphorylated substrate to decay back to baseline as the total substrate pool is depleted (Fig. 1B and C). This constitutes a negative-feedback loop that is "hardwired"; no other regulatory inputs are required. In principle, the E3 ligase limits the duration of signaling by a substrate and may introduce a refractory period during which signaling cannot recur. Perhaps for this reason, phosphorylation-dependent ubiquitin ligases are key controllers of different steps in the cell cycle and in DNA repair, signal transduction, and other fundamental cellular events (12).

This review focuses on the subset of phosphorylation-dependent ubiquitin E3 ligases that require phosphotyrosine (pY) in their substrates. These ligases fall into two well-studied groups, Cbl family proteins and cullin 5 (Cul5)-RING ligase complexes (CRL5s) bound to suppressor of cytokine signaling (SOCS) protein adaptors, as well as a less-studied ligase named Hakai. Here we introduce these ligases and discuss their strategies for binding pY, their substrates, and their biological functions. Because tyrosine protein kinases are master regulators of signal transduction cascades, pY-dependent ubiquitination might regulate cell growth, proliferation, motility, survival, and differentiation. Indeed, genetic evidence reviewed here shows the importance of Cbl and SOCS proteins in cancer, autoimmunity, and endocrine disorders, including diabetes. However, some Cbl and SOCS proteins have additional binding, scaffold, or adaptor functions independent of their ubiquitin ligase activity. This greatly complicates the identification of critical pY substrates and our understanding of how pY-dependent E3 ligases regulate cell biology.

A BRIEF HISTORY OF Cbl, Hakai, AND SOCS FAMILY PROTEINS

The c-Cbl gene was identified as the cellular homolog of the v-Cbl oncogene in Cas-Br-M mouse retrovirus (14). c-Cbl and its relatives Cbl-b and Cbl-3 contain an N-terminal pY-binding tyrosine kinase-binding (TKB) domain and a central zinc-binding C_3HC_4 RING motif (15–17). c-Cbl suppresses transformation in part by binding and downregulating receptor tyrosine kinases (RTKs),

Accepted manuscript posted online 16 March 2015

Citation Cooper JA, Kaneko T, Li SSC. 2015. Cell regulation by phosphotyrosine-targeted ubiquitin ligases. Mol Cell Biol 35:1886–1897. doi:10.1128/MCB.00098-15.

Address correspondence to Jonathan A. Cooper, jcooper@fhcrc.org.

doi:10.1128/MCB.00098-15

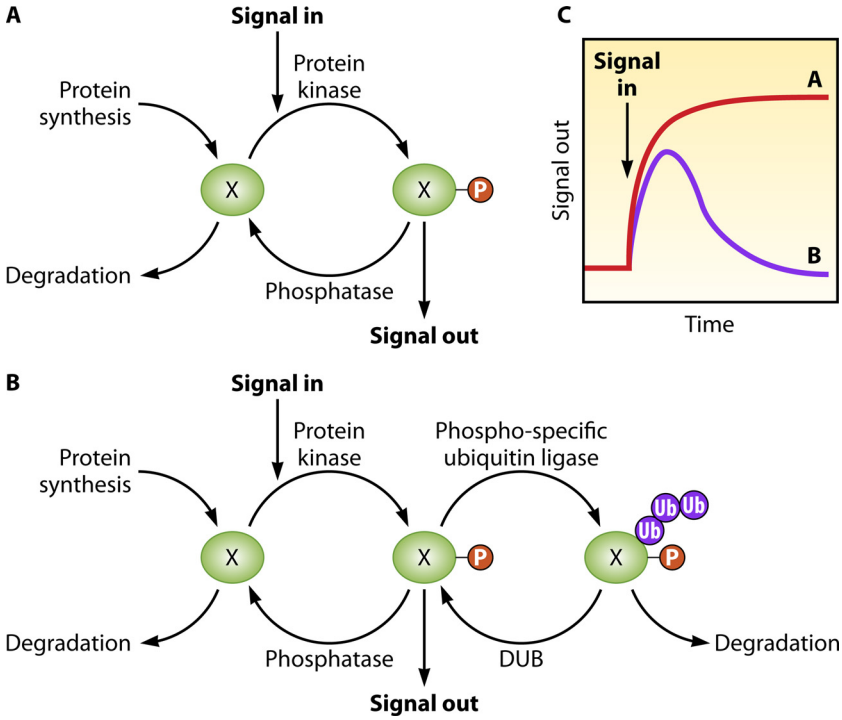

FIG 1 Regulation by phosphorylation-dependent ubiquitin ligases. Phosphorylation-dependent ubiquitination can provide negative feedback. (A) A simple system with substrate protein X undergoing constitutive slow synthesis and equal slow degradation is acted on by a regulated protein kinase and a constitutive phosphatase. (B) A phosphorylation-dependent ubiquitin ligase can promote the ubiquitination of the phosphosubstrate and target it for lysosomal or proteasomal degradation. Ubiquitination may be reversed by deubiquitinases (DUBs). (C) Signal outputs over time for schemes A and B. In the simple kinase/phosphatase system (curve A), kinase activation causes a sustained increase in the level of phosphosubstrate. With the addition of a ubiquitin ligase and DUBs (curve B), kinase activation leads to a transient increase in the level of phosphosubstrate. Phosphorylation/dephosphorylation is assumed to be faster than ubiquitination/deubiquitination. Note that the ubiquitinated substrate can also be dephosphorylated and rephosphorylated, but this does not affect the negative feedback.

but the mechanism was unclear until Cbl's RING domain was found to stimulate ubiquitin transfer from an E2-ubiquitin donor to nearby proteins (18). pY substrates for Cbl family members bind to the TKB domain and receive ubiquitin from an E2 bound to the RING domain. The C termini of c-Cbl and Cbl-b contain proline-rich regions and phosphorylation sites that allow them to also act as signaling scaffolds (17).

Hakai (also called Cbl-like-1 and Cbll1) was first identified as a RING-containing, E-cadherin-binding protein that resembled Cbl (19). However, we now know that Hakai and Cbl have different mechanisms of pY substrate binding. Hakai dimerization creates an unusual HYB domain that binds pY substrates (20). The RING domains are directly adjacent to the HYB domain, allowing positioning of E2 and ubiquitin close to the pY residues in the substrate.

The SOCS proteins SOCS1-7 and CIS (cytokine-inducible SH2 protein) were discovered as cytokine-inducible inhibitors of signaling, STAT-induced STAT inhibitors, and cytokine-inducible SH2 proteins (21–25). They were initially shown to compete with STATs for binding to pY sites on cytokine receptors and to bind to and inhibit cytokine-activated kinases called JAKs. However, CIS bound to erythropoietin receptors through a pY site that was dispensable for signaling, calling into question the simple competition model. Moreover, erythropoietin induced CIS ubiquitination and proteasome inhibitors prolonged erythropoietin signaling (26). Later, SOCS1 was found to be induced by RTKs and to inhibit signaling at the level of the RTK and intracellular signaling proteins, such as Vav1 (27). Subsequently, SOCS1 was found to stimulate ubiquitination and degradation of tyrosine-phosphorylated Vav1 (28). We now know that all eight SOCS proteins contain a consensus sequence (a SOCS-BC box) that mediates their association with a multisubunit CRL5 complex (29–31). The other subunits of a SOCS-CRL5 complex which comprises elongin B, elongin C, cullin 5, and Rbx2 (RNF7) are invariant (32). (Under some circumstances, cullin 5 can form a complex with Rbx1 [33–35], but Rbx2 is more common). Rbx2 contains a RING domain and binds E2-ubiquitin conjugates. SOCS-CRL5 thus combines a substrate recognition domain (SH2) and a ubiquitin transfer domain (RING) in different subunits of a multiprotein complex. The RING protein is quite distant from the SH2 domain, potentially facilitating polyubiquitination.

pY SUBSTRATE BINDING AND UBIQUITINATION BY Cbl PROTEINS

Cbl family proteins contain a tyrosine kinase-binding or TKB domain and a RING domain, linked by a well-conserved linker helix region (LHR). c-Cbl and Cbl-b also share a proline-rich region and a C-terminal ubiquitin-associated (UBA) domain (16) (Fig. 2A). The TKB domain harbors a 4-helix bundle (4H), a Ca-binding EF hand, and an atypical SH2 domain (15) (Fig. 2B). The sequence of the SH2 domain is highly divergent, yet it forms a classic SH2 fold and binds pY peptides in a conventional way (15).

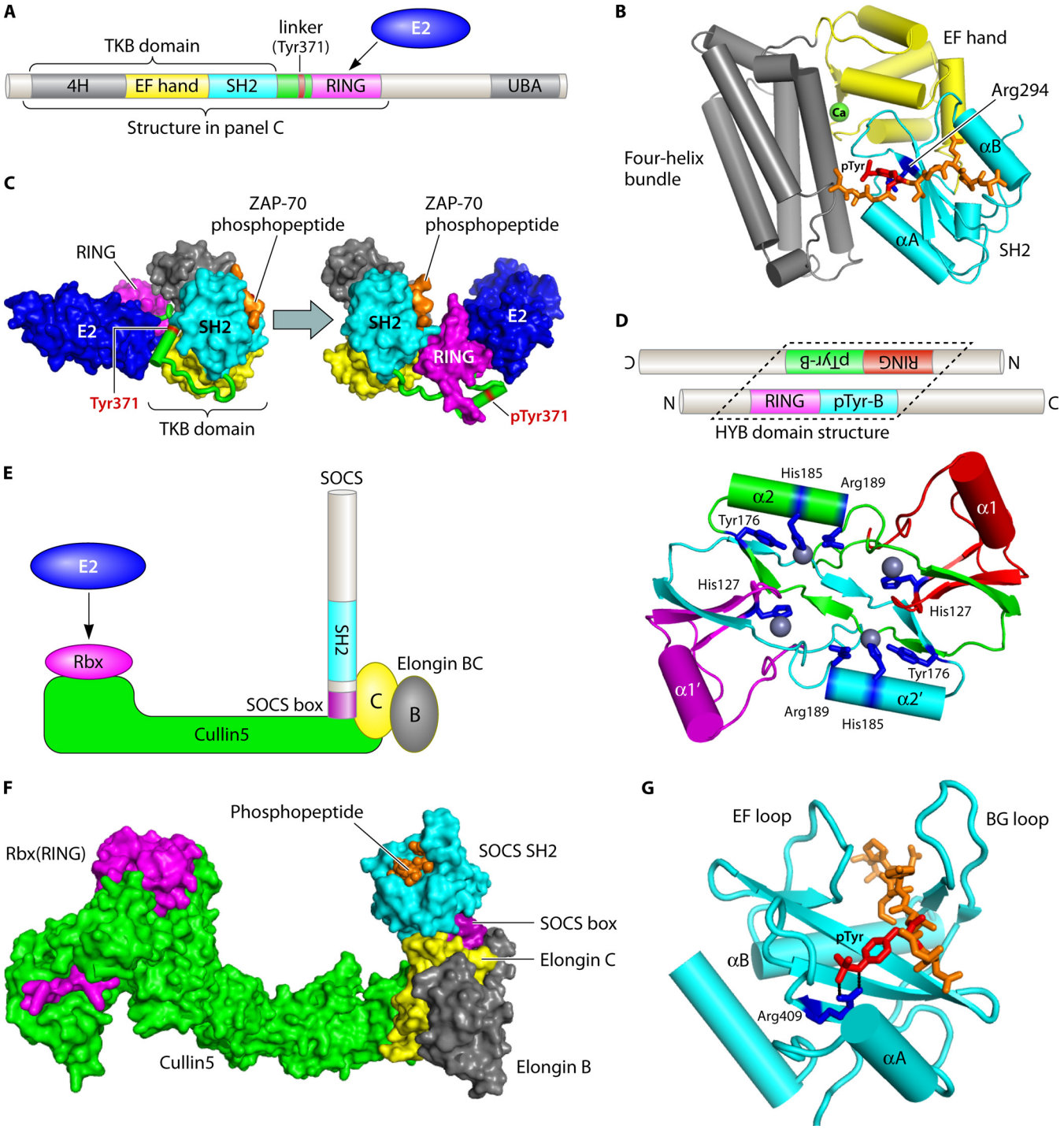

FIG 2 Structures of three families of E3 ligases that recognize tyrosine-phosphorylated ligands. (A) Domain structure of c-Cbl and Cbl-b. Cbl-c is similar but terminates after the RING domain. (B) Crystal structure of the c-Cbl TKB domain in complex with a tyrosine-phosphorylated peptide derived from ZAP-70 (PDB code 2CBL) (15). In this panel and in panels D and G, α helices and β strands are shown in columns and ribbons, respectively. The peptide is shown as orange sticks, except for the pTyr residue, which is red. The regions in the TKB domain are colored according to the colors in panel A. The arginine residue of the SH2 domain (Arg294), essential for binding to the phosphate group of pTyr, is blue. (C) Activation of Cbl via a conformational change triggered by tyrosine phosphorylation of the LHR linker region. Shown here is the structure, in surface representation, of a c-Cbl–E2–substrate peptide complex in an inactive conformation (left, PDB code 1FBV [36]) or when activated through phosphorylation of Tyr371 in the linker region (right, PDB code 4A4C [37]). The E2 and RING domains are blue and magenta, respectively. The SH2 domain is cyan. A ZAP-70-derived phosphopeptide is orange. The LHR linker between the SH2 and the RING domains is represented by a green noodle, with Tyr371 highlighted in red. (D) Homodimeric structure of the Hakai HYB domain (PDB code 3VK6 [20]). The dimer coordinates six zinc ions (gray balls), two of which are located behind α1 and α1′ helices and are thus invisible in this figure. Four residues (i.e., His127, Tyr176, His185, and Arg189) from each monomer that contribute to phosphopeptide recognition are shown as blue sticks. (E) Diagram of a SOCS-CRL5 complex. (F) Structural model of a SOCS-CRL5 complex. The model was constructed according to reference 149, based on the following crystal structures: the cullin 5 C-terminal domain in complex with Rbx1 (PDB code 3DPL [150]), the cullin 5 N-terminal domain in complex with elongin B, elongin C, and Socs2

The TKB domain allows Cbl to bind to pY proteins, such as active RTKs. Cbl may then become phosphorylated and bind other proteins, serving as an adaptor for protein complex assembly. In addition, pY proteins bound to the TKB domain can be ubiquitinated by an E2 enzyme bound to the RING domain. However, the structure of the TKB-LHR-RING region of c-Cbl revealed that an E2 enzyme bound to the RING domain would be unable to access the pY substrate by the TKB domain (36) (Fig. 2C, left). The mystery was solved when it was found that phosphorylation of Tyr371 in c-Cbl (Tyr363 in Cbl-b) within the LHR linker region induces a drastic conformational change that, in effect, reorients the substrate so that it is now close to the RING and E2 domains (37–40) (Fig. 2C, right). This phosphorylation switch may help explain how Cbl can alternatively promote signaling as an adaptor or inhibit signaling as an E3 ligase, as described below.

pY SUBSTRATE SELECTION BY Hakai

Hakai was discovered as a Cbl-like protein that binds to the membrane protein E-cadherin (19). Hakai targets a cytoplasmic NVYYY motif in E-cadherin when it is phosphorylated on the second tyrosine (19, 20). Interestingly, the same motif is also the binding site for the Numb PTB domain, although Numb binds when the motif is nonphosphorylated (41). A NVYpYY peptide binds to an unusual structure formed by Hakai dimerization. The Hakai sequence contains a classic C_3HC_4 RING followed by a zinc-binding C_2H_2 motif known as a pTyr-binding (pTyrB) domain. In the dimer, two pTyrB regions come together in an antiparallel arrangement, mediated by strand exchange and stabilized by Zn ions and the RING domains, to create a basic channel that binds pY (20) (Fig. 2D). This dimeric structure is known as an HYB (Hakai pY-binding) domain. The HYB domain recognizes, in addition to the NVYpYY target sequence, the flanking acidic residues. In the same vein, Hakai has been shown to bind other acidic pY-containing sequences found in Cortactin and Dok1, but the physiological significance of these interactions is not defined (20). The Hakai-related proteins ZNF645, LNX1, and LNX2 also have RING-pTyrB homology, but the functions and structures of these domains are unknown (20).

pY SUBSTRATE RECOGNITION BY SOCS PROTEIN ADAPTORS FOR CRL5

The C termini of all SOCS proteins contain an SH2 domain that binds pY, followed closely by a SOCS-BC box for binding to Cul5 and the elongin BC subunits of CRL5 (Fig. 2E). The SH2 domain positions a pY substrate on the same side of Cul5 as Rbx2, which contains a RING domain and positions an E2-ubiquitin conjugate for ligation (Fig. 2F). SOCS SH2 domains bind pY peptides through modified SH2-pY interactions, as shown for the SOCS6 SH2 domain in complex with a juxtamembrane phosphorylation site, Tyr568, in c-Kit (42) (Fig. 2G). Although the SOCS SH2 domain adopts a typical SH2 fold, it features an unusually long BG loop, and together with the EF loop, they form an elongated channel to allow interactions with residues between 3 and 6 residues C terminal to the pY in the c-Kit peptide. This unique binding mode dictates that only certain phosphopeptides (and the corresponding proteins) would be targeted by SOCS6.

SOCS proteins may also recruit non-pY substrates to CRL5. SOCS7 contains a proline-rich region that binds the SH3 domains of Nck, Grb2, PLCγ, and vinexin (43, 44). SOCS7 shuttles between the nucleus and cytoplasm and acts as a bridge between septins and Nck (45). However, there is no evidence that tyrosine phosphorylation or ubiquitination is involved. Tyrosine phosphorylation of SOCS3 creates a binding site for the SH2 domains of Nck and Crk/CrkL (46). It is again unclear if Nck or Crk/CrkL is a substrate for ubiquitination. Finally, using overexpressed proteins, the N-terminal region of SOCS6 was shown to bind to the SH2 kinase region of active (but not inactive) Lck and less well to other Src-related kinases (47).

SUBSTRATES AND FUNCTIONS OF Cbl

The first insights into Cbl function came from *Caenorhabditis elegans* genetics (48). Mutation of Cbl restored normal development to worms containing a weakly active mutant epidermal growth factor receptor (EGFR), implying that c-Cbl inhibits the EGFR. Subsequent animal cell studies showed that EGF stimulates the binding of c-Cbl to EGFRs and Cbl tyrosine phosphorylation (49). While EGF-activated EGFRs are targeted to the lysosome, an EGFR relative, ErbB4, which does not bind Cbl, simply recycles (50). This suggested a role for c-Cbl in trafficking between endosomes and lysosomes. Indeed, overexpression of c-Cbl stimulated EGF-dependent EGFR ubiquitination, internalization, and degradation, which was dependent on the Cbl RING domain (39, 51). Following the discovery that the c-Cbl RING domain has intrinsic E3 ligase activity (18), the results suggested that EGF stimulation induces c-Cbl binding and EGFR ubiquitination and that ubiquitination then signals lysosomal trafficking. Subsequent studies have filled in critical details about how c-Cbl binds and ubiquitinates the EGFR and about the importance of ubiquitination for lysosomal degradation (52).

The basic steps in EGFR downregulation by c-Cbl are as follows (52). EGF binding activates the EGFR kinase domain and leads to autophosphorylation on Tyr1068 and Tyr1086. These sites then bind an adaptor protein, Grb2, which binds in turn to a proline-rich region in c-Cbl. The complex is further stabilized by a second, direct interaction of the c-Cbl TKB domain with the EGFR pY1045 site. c-Cbl then transfers ubiquitin onto multiple lysine residues within the EGFR (53). These lysines are mono-, not poly-, ubiquitinated (54, 55). While multimonoubiquitination can trigger receptor endocytosis, it is not required (52, 55). Rather, multimonoubiquitination stimulates sorting to multivesicular bodies and degradation in the lysosome. Sorting is presumably mediated by ubiquitin-binding domains contained in Hrs and other components of the ESCRT0 complex (52). Thus, a mutant EGFR lacking all ubiquitination sites is internalized but not degraded (53), and wild-type EGFRs are internalized but not degraded in c-Cbl knockout fibroblasts (56).

(PDB code 4JGH [151]), the structure of cullin 1, used here as a template for joining the N-terminal and C-terminal fragments of cullin 5 structures (PDB code 1LDJ [148]), and the Socs6 SH2 domain bound to a tyrosine-phosphorylated peptide (PDB code 2VIF [42]). The magenta molecule is Rbx1 (instead of Rbx2), since there are no available Rbx2-cullin complex structures. (G) SOCS6 SH2 domain in complex with a phosphorylated peptide derived from the juxtamembrane region of the receptor tyrosine kinase c-KIT (PDB code 2VIF [42]). The bound peptide is shown in orange sticks except for the pTyr residue, which is highlighted in red. The Arg409 residue of the SH2 domain, essential for pTyr recognition, is shown as blue sticks. The peptide is bound between the BG and EF loops.

c-Cbl-dependent multimonoubiquitination also downregulates other RTKs and the gp130 cytokine receptor, thereby inhibiting responses to a range of growth factors (57, 58). Removal of Cbl might thus be expected to increase mitogenic signaling. Indeed, c-Cbl is frequently mutated in lung cancer (59). EGFR is also frequently mutated in lung cancer, and some mutations in EGFR may decrease its downregulation by c-Cbl, leading to increased EGFR activity (60). EGFR ubiquitination is also implicated in breast cancer; an EGFR-specific DUB named Cezanne-1 is upregulated in many cases of breast cancer (61). c-Cbl and Cbl-b are also required for routing activated, tyrosine-phosphorylated T cell receptors to the lysosome for degradation, thus downregulating the immunological response and suppressing autoimmunity (62).

Despite the importance of c-Cbl in receptor trafficking, cancer, and immunity, mutant mice completely lacking c-Cbl are viable and fertile (63). The mild phenotype may be due to redundancy with Cbl-b or Cbl-c. Indeed, close examination revealed alterations in hematopoiesis, T cell response, mammary epithelial growth, and fat and energy metabolism, consistent with c-Cbl stimulating the downregulation of various receptors (63, 64). Curiously, point mutation of the c-Cbl RING domain caused a much stronger phenotype—death *in utero*—suggesting that c-Cbl has significant developmental roles (65). The c-Cbl RING mutant may compete with Cbl-b and Cbl-c, allowing the true scope of c-Cbl functions to be exposed. However, the interpretation is challenging because evidence suggests that the c-Cbl RING mutant has gain-of-function effects. First, mice expressing half the normal amount of the RING mutant were viable and showed the same fat and energy defects as the knockout mice, indicating that these phenotypes likely require ubiquitin transfer (65). Second, the RING mutant mice suffer complete thymic ablation due to hyperactivation of phosphatidylinositol 3′ (PI3′) kinase, not seen in the knockout mice (66). The results suggest that both signal inhibition by c-Cbl's RING and signal activation by c-Cbl's adaptor function occur *in vivo*.

Human cancer mutations also indicate that c-Cbl has both positive and negative effects *in vivo*. Many c-Cbl mutations found in myeloid neoplasms are heterozygous missense and frameshift mutations (67, 68). These mutations generally leave the TKB domain and LHR intact, so the mutant proteins may act as dominant-interfering alleles, competing with endogenous Cbl proteins for binding sites on the receptor. However, they may have alternatively gained mitogenic activity. Tyr371 substitutions, frequently found in spontaneous neoplasms and in a cancer predisposition syndrome called Noonan syndrome, present a curious case (67, 69). Since phosphorylation of Tyr371 stimulates ubiquitination of bound pY substrates (Fig. 2C), Tyr371 mutation is likely to inhibit ubiquitination. However, these alleles transform cells only when endogenous c-Cbl is absent (68, 69). This is difficult to explain as a dominant interfering effect and suggests that these c-Cbl mutants may have gained oncogenic activity. c-Cbl mutants that have decreased ubiquitination activity may be more stable, and increased c-Cbl protein levels may stimulate signaling through an adaptor function.

Some c-Cbl functions may be due to ubiquitination of cytoplasmic tyrosine kinases and pY proteins, but ubiquitination-independent mechanisms may also play a role. ZAP70 is a case in point. A major phenotype of c-Cbl knockout mice is lymphoid hyperplasia and increased T cell signaling via the T cell receptor-associated kinase ZAP70 (63, 70, 71). However, ZAP70 degradation is not altered. Similarly, when Cbl-b is overexpressed in T cells, the p85 subunit of PI3′ kinase is ubiquitinated, but its steady-state abundance and half-life are not affected (72). Nevertheless, ubiquitination inhibits the recruitment of p85 to the coreceptor CD28. Decreased p85 ubiquitination and increased PI3′ kinase activity may explain the hyper-activation of Cbl-b knockout T cells and consequent autoimmunity, but p85 degradation may not be involved (73, 74). Cbl-b also regulates the phosphatase PTEN, independently of its ubiquitin ligase activity (75). These results suggest ubiquitin-independent and degradation-independent effects of both c-Cbl and Cbl-b.

c-Cbl has also been implicated in the degradation of the Src kinase. Activation induces Src downregulation by ubiquitination and proteasomal degradation (76, 77). Overexpressed Cbl was found to stimulate ubiquitination of Src and Fyn (a Src-related kinase), implicating endogenous Cbl in normal cells (78, 79). Indeed, Fyn protein levels are increased in c-Cbl knockout cells (79), and overexpressed truncated Cbl TKB inhibits Src turnover, as expected if the TKB has a dominant negative effect (80). However, overexpressed Cbl TKB may inhibit the binding of other Src-specific E3 ligases. Indeed, other evidence, described below, suggests that SOCS-CRL5 complexes may be responsible for downregulating endogenous Src. Moreover, genetic experiments in osteoclasts are not consistent with Cbl inhibiting Src but are consistent with Src requiring Cbl for signaling (81–83). Src and other tyrosine kinases may phosphorylate Cbl at sites that bind PI3′ kinase and Crk, thus stimulating signaling (55). Phosphorylation of Cbl by Src also stimulates Cbl ubiquitination and degradation. Decreased Cbl means that there is less downregulation of RTKs and more RTK activity (78, 84). This may contribute to the synergy between Src and RTKs for transformation (85).

The original concept that Cbl family proteins target cytoplasmic proteins like p85, ZAP70, and Src for degradation is difficult to reconcile with the findings that c-Cbl catalyzes monoubiquitination of EGFR and gp130 (54, 55, 58). Monoubiquitination triggers lysosomal but not proteasomal degradation, and cytoplasmic proteins are not normally degraded by the lysosome. However, monoubiquitination can affect the binding or enzymatic properties of substrates independently of degradation. Indeed, monoubiquitination of CARMA1 by Cbl-b inhibits binding of CARMA1 to Bcl10 without inducing degradation, thereby inducing NKT cell anergy (86). In general, Cbl family proteins may stimulate degradation of transmembrane proteins by the lysosome but have other effects, not related to degradation, on cytoplasmic proteins.

CADHERIN REGULATION BY Hakai

E-cadherin is the best documented substrate for Hakai (19). Hakai overexpression increases E-cadherin ubiquitination, endocytosis, and lysosomal degradation, dependent on tyrosine phosphorylation and the NVYYY sequence. This sequence is part of a longer motif that binds p120Ctn, a protein that stabilizes E-cadherin on the cell surface (87). p120 binds only the nonphosphorylated sequence; phosphorylation thus switches E-cadherin from binding to p120 to binding to Hakai (19, 88–90). Competition between Hakai, p120Ctn, and Src for the same site makes it difficult to interpret the effects of E-cadherin mutations and knockdown or overexpression of Hakai or p120Ctn on E-cadherin stability. Nevertheless, there is good evidence that Hakai promotes E-cadherin downregulation from adherens junctions and induces the epithelial-mesenchymal transition (EMT) (91). Thus, removing calcium

induces downregulation of E-cadherin by a series of steps, including activation of Cdc42, EGF receptor, and Src; phosphorylation of E-cadherin; Hakai-dependent ubiquitination; and lysosomal degradation (92). Overexpressing Hakai, or stimulating E-cadherin phosphorylation by activating Src or RTKs, also drives E-cadherin downregulation and junction disassembly (19, 93). Transforming growth factor β (TGFβ), in combination with Ras pathway activation, stimulates Hakai gene expression and induces E-cadherin downregulation and EMT (94). Despite this evidence, it remains unclear whether endogenous Hakai regulates EMT *in vivo*. A Hakai knockout mouse has not been described. Knockdown of Hakai in cancer cell cultures does not restore cell-cell junctions but does inhibit cell proliferation, independently of E-cadherin (95). Hakai expression inversely correlates with E-cadherin in some cancers but not others (96). It will be important to determine whether Hakai is required for EMT during normal embryonic development and cancer progression.

CELL REGULATION BY SOCS-CRL5 COMPLEXES AND BY SOCS PROTEINS ACTING ALONE

SOCS proteins are best understood as inhibitors of cytokine receptors and RTKs. Cytokines induce the transcription of SOCS1, -2, and -3 and CISH genes by a cascade of cytokine receptor phosphorylation, JAK kinase binding and activation, and phosphorylation of STAT transcription factors (97–99). The phosphorylated STATs then move to the nucleus and activate expression of many genes, including SOCS genes. SOCS proteins then provide feedback inhibition by binding to cytokine receptors and JAK kinases. Accordingly, mutations of SOCS1 (100–102) and SOCS3 (103–105) have major effects on erythropoiesis, the immune system, and the placenta, while CISH deletion also affects immunity but in a more subtle way (106).

While SOCS proteins may inhibit signaling by CRL5-dependent downregulation of tyrosine-phosphorylated cytokine receptors or JAKs, several SOCS proteins have major effects that are independent of CRL5. Specifically, SOCS1 and -3 bind poorly to the remainder of the CRL5 complex (107) and are able to inhibit signaling through two ubiquitin-independent mechanisms. On the one hand, they bind through their SH2 domains to phosphorylated cytokine receptors, thereby competing for STAT binding, and on the other, they bind to and inhibit JAKs via the "kinase inhibitor regions" (KIRs) immediately N terminal to their SH2 domains (98). Indeed, germ line mutation of the SOCS1 BC-SOCS box has a much reduced effect on gamma interferon signaling *in vivo* than a complete SOCS1 gene ablation, suggesting that CRL5 binding is largely dispensable for *in vivo* function (108). Even so, under some conditions, SOCS1 and -3 can stimulate ubiquitination; SOCS1 stimulates polyubiquitination and proteasomal degradation of pY-JAK2 when overexpressed (109), and SOCS3 can stimulate ubiquitination of JAK2 and gp130 *in vitro* (110).

The ubiquitination activities of SOCS1 and -3 may be more important in suppressing signaling through adhesion receptors and RTKs (111, 112). Overexpressed SOCS3 binds focal adhesion kinase (FAK) through a pY in the activation loop and stimulates FAK turnover (111). This may be important in the adhesive response of B lymphocytes to the chemokine CXCL12 (113). As B cells mature, SOCS3 expression increases and CXCL12-induced FAK activation and adhesion decrease. SOCS2 has a similar function in regulating the FAK-like kinase Pyk2 in NK cells (114). SOCS2 expression is induced in NK cells by interleukin 15 (IL-15) and binds Pyk2 through the activation loop pY. SOCS2 stimulates Pyk2 ubiquitination and degradation. Accordingly, the knockdown of SOCS2 caused the accumulation of pY-Pyk2 and blocked NK cell effector functions.

Perhaps the best-understood target for SOCS2 is the growth hormone (GH) receptor, which signals through a classical JAK-STAT mechanism. SOCS2 knockout causes mice to grow excessively, to 40% larger than their littermates, with symptoms of increased GH and insulin-like growth factor 1 (IGF1) signaling (115). Mutant cells have increased responses to GH but not IGF1, which is likely activated indirectly (116). Genetic experiments revealed that the SOCS2 phenotype requires increased STAT5b signaling from pY595 of the GHR (116). SOCS2 inhibition of GH signaling requires the SOCS box and SH2 domain, pointing to a role for CRL5 and suggesting that SOCS2 brings CRL5 to pY-GHR and stimulates ubiquitination and degradation (117). Curiously, overexpression of SOCS2 in a transgenic mouse also stimulated growth (118), suggesting that SOCS2 both stimulates and inhibits GH responses. One potential explanation is that overexpressed SOCS2 (and perhaps SOCS6 and SOCS7) negatively regulates other SOCS proteins (119). However, it is not clear whether this mechanism explains the increased growth of SOCS2 transgenic mice.

SOCS4, -5, -6, and -7 are more widely expressed than SOCS1, -2, and -3 and CISH (112). They have functions beyond cytokine signaling. For example, both SOCS4 and -5 are induced by EGF (120). Overexpressed SOCS5 inhibits EGFR signaling through its SOCS box and SH2 domain (120, 121). EGFR degradation is increased, but curiously, both inactive and active receptors are degraded (120). This suggests that SOCS5 might downregulate the EGFR independently of pY, but the recognition mechanism and biological significance are unknown. SOCS5 knockout mice have no apparent phenotype (122), and the SOCS4 mutant phenotype has not been reported. SOCS4 and -5 are closely related and potentially overlap in function, so double mutation may be necessary to detect phenotypes.

SOCS6 and -7 may also have overlapping functions. They are closely related in their SH2 domains, although they diverge considerably in their N-terminal regions. Both SOCS6 and SOCS7 bind to pYVϕϕ sequences, where ϕ is a hydrophobic residue (123). Both can bind to insulin receptor substrates 2 and 4 (IRS2 and IRS4, respectively) and to the p85 subunit of PI3′ kinase. However, deletion of SOCS6 has little effect on insulin sensitivity or other reported phenotypes, although the mice were a little smaller than controls (123). Potential overlapping functions with SOCS7 may conceal SOCS6 mutant phenotypes. SOCS6 also binds the juxtamembrane region of the RTK c-Kit, and overexpressed SOCS6 inhibits c-Kit activation and shortens its half-life (42, 124). However, it is unclear whether SOCS6 triggers c-Kit lysosomal or proteasomal degradation or whether endogenous SOCS6 regulates c-Kit.

Unlike with SOCS6, deletion of SOCS7 causes many phenotypes, depending on the particular allele and the genetic background. Systemic SOCS7 deletion was reported to hyper-activate mast cells, with increased proinflammatory cytokine production and severe skin disease (125). SOCS7 deletion caused hydrocephalus in other mouse strains (126). Systemic SOCS7 deletion can also increase insulin sensitivity by stabilizing IRS proteins, which mediate insulin signaling, in pancreatic β cells (127). Conditional

knockout of SOCS7 in neural progenitors during brain development altered the migration of specific classes of neurons, consistent with hyper-responsiveness to reelin, a migration-stimulatory factor (128). SOCS7 binds to Dab1, a protein that is phosphorylated at tyrosine in reelin-stimulated neurons, and stimulates ubiquitination of pY-Dab1 *in vitro*. Dab1 protein accumulates in misplaced neurons in SOCS7 and Rbx2 mutant brains, consistent with a requirement for SOCS7-CRL5-dependent turnover of pY-Dab1 for normal neuron migration.

CELL REGULATION BY CRL5: STUDIES WITH Cul5 AND Rbx2

Removal of one of the CRL5 invariant subunits, Cul5 or Rbx2, might be expected to inhibit all SOCS gene functions, unveiling phenotypes that might be concealed by redundancy when a single SOCS gene is deleted. However, SOCS proteins are not the only substrate adaptors for CRL5: approximately 30 other proteins that lack SH2 domains may also recruit non-pY substrates to CRL5 (129). Removing Cul5 or Rbx2 may affect these nonphosphorylated substrates as well as SOCS protein ligands. One way to determine which Cul5 or Rbx2 phenotypes might involve pY-dependent ubiquitination is to focus on phenotypes and substrates that also require tyrosine kinases.

Along these lines, the removal of Cul5 and/or Rbx2 from fibroblasts, epithelial cells, and developing brain increases the level of active but not inactive Src and Fyn proteins but not mRNA (128, 130–133). This suggests that CRL5 complexes regulate Src/Fyn stability, but changes in half-life have not been reported. Knockdown or knockout of Cul5 or Rbx2 also increases fibroblast transformation and epithelial cell proliferation and migration, depending on Src, suggesting that the pY substrates are involved (130–133). The specific pY substrates suppressed by Cul5 in fibroblasts are not known, but the Src substrate p130Cas is required for the increased proliferation and migration of Cul5-deficient epithelial cells (130). p130Cas turnover requires Src, Cul5, and SOCS6. Phosphorylated p130Cas binds SOCS6. This suggests that SOCS6-CRL5 inhibits proliferation and migration by targeting p130Cas for ubiquitination and degradation after it has been phosphorylated by Src. The full picture is more complicated, however. Combined knockdown of several SOCS proteins is required to stimulate cell migration to the same extent as Cul5 (130). SOCS7 knockdown stimulates migration of some cancer cell lines (134). In addition, SOCS3 overproduction inhibits keratinocyte proliferation and migration and wound healing *in vivo* (135). Therefore, several SOCS proteins probably target multiple pY proteins for ubiquitination, and together they inhibit cell proliferation and migration.

Such inhibitory effects suggest that SOCS-CRL5s may function as tumor suppressors. Indeed, SOCS6 is strongly downregulated in the most-severe cases of prostate cancer (136), hepatocellular carcinoma (137), lung cancer (138), and gastric cancer (139). SOCS3 is downregulated in head and neck cancer (140) and Barrett's adenocarcinoma (141). High expression of SOCS4 and -7 is associated with improved clinical outcome in breast cancer (142), loss of SOCS5 correlates with poor outcome in liver cancer (143), and decreased SOCS2 expression correlates with shorter recurrence-free survival in prostate cancer (136). In addition, SOCS1 and -2 are hypermethylated and underexpressed in more-severe cases of ovarian and breast cancer (144, 145). These findings are consistent with tumor suppression by SOCS proteins.

However, it should be noted that some evidence suggests that the Rbx2 gene may also function as an oncogene in certain contexts. Rbx2 levels increase in some tumors (35). Tan et al. reported that Rbx2 is required for an Rbx2-Cul1-Fbxw7 complex to stimulate ubiquitination and degradation of the tumor suppressor NF1, consistent with the Rbx2 gene being an oncogene (146). On the other hand, other research indicates that NF1 is degraded by a Cul3 mechanism (147) and that Fbxw7 normally binds to Cul1-Rbx1 via Skp1 as opposed to elongin BC (148). It is an open question whether Rbx2 may function separately from Cul5.

SUMMARY AND FUTURE PROSPECTS

Despite having different mechanisms for binding to their substrates and associating with E2-ubiquitin conjugates, Cbl, Hakai, and SOCS proteins all have the potential to promote pY-dependent ubiquitination and provide negative regulation of tyrosine kinase signaling pathways. This potential has been brought out by tissue culture and overexpression experiments. However, Cbl and SOCS proteins, and quite probably Hakai, also have ubiquitin-independent functions, making it difficult to tease out the biological importance of pY-dependent ubiquitination *in vivo*. Indeed, for Cbl and SOCS proteins, where knockout data are available, some of the phenotypes are likely due to loss of ubiquitin-independent functions. A continuing challenge for the future is the identification of bona fide *in vivo* pY proteins whose ubiquitination and degradation by pY-dependent ubiquitin ligases is critical for normal development or homeostasis. Given the low stoichiometry of many tyrosine phosphorylation events, pY-dependent turnover may not detectably change the bulk abundance or half-life of a substrate. New approaches for substrate identification will be key.

ACKNOWLEDGMENTS

J.A.C. is supported by grants NS080194 and GM109463 from the U.S. Public Health Service. S.S.C.L is the Canada Research Chair in Functional Genomics and Cellular Proteomics.

J.A.C. thanks lab members for inspiration.

REFERENCES

1. **Cohen P.** 2000. The regulation of protein function by multisite phosphorylation—a 25 year update. Trends Biochem Sci **25:**596–601. http://dx.doi.org/10.1016/S0968-0004(00)01712-6.
2. **Ubersax JA, Ferrell JE, Jr.** 2007. Mechanisms of specificity in protein phosphorylation. Nat Rev Mol Cell Biol **8:**530–541. http://dx.doi.org/10.1038/nrm2203.
3. **Pickart CM.** 2004. Back to the future with ubiquitin. Cell **116:**181–190. http://dx.doi.org/10.1016/S0092-8674(03)01074-2.
4. **Deshaies RJ, Joazeiro CA.** 2009. RING domain E3 ubiquitin ligases. Annu Rev Biochem **78:**399–434. http://dx.doi.org/10.1146/annurev.biochem.78.101807.093809.
5. **Rotin D, Kumar S.** 2009. Physiological functions of the HECT family of ubiquitin ligases. Nat Rev Mol Cell Biol **10:**398–409. http://dx.doi.org/10.1038/nrm2690.
6. **Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T.** 2004. Protein tyrosine phosphatases in the human genome. Cell **117:**699–711. http://dx.doi.org/10.1016/j.cell.2004.05.018.
7. **Shi Y.** 2009. Serine/threonine phosphatases: mechanism through structure. Cell **139:**468–484. http://dx.doi.org/10.1016/j.cell.2009.10.006.
8. **Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, Bernards R.** 2005. A genomic and functional inventory of deubiquitinating enzymes. Cell **123:**773–786. http://dx.doi.org/10.1016/j.cell.2005.11.007.
9. **Hicke L, Dunn R.** 2003. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu Rev Cell Dev Biol **19:**141–172. http://dx.doi.org/10.1146/annurev.cellbio.19.110701.154617.

10. **Hershko A, Ciechanover A.** 1998. The ubiquitin system. Annu Rev Biochem **67:**425–479. http://dx.doi.org/10.1146/annurev.biochem.67.1.425.
11. **Komander D, Rape M.** 2012. The ubiquitin code. Annu Rev Biochem **81:**203–229. http://dx.doi.org/10.1146/annurev-biochem-060310-170328.
12. **Hunter T.** 2007. The age of crosstalk: phosphorylation, ubiquitination, and beyond. Mol Cell **28:**730–738. http://dx.doi.org/10.1016/j.molcel.2007.11.019.
13. **Gao M, Karin M.** 2005. Regulating the regulators: control of protein ubiquitination and ubiquitin-like modifications by extracellular stimuli. Mol Cell **19:**581–593. http://dx.doi.org/10.1016/j.molcel.2005.08.017.
14. **Langdon WY, Hartley JW, Klinken SP, Ruscetti SK, Morse HC, III.** 1989. v-cbl, an oncogene from a dual-recombinant murine retrovirus that induces early B-lineage lymphomas. Proc Natl Acad Sci U S A **86:**1168–1172. http://dx.doi.org/10.1073/pnas.86.4.1168.
15. **Meng W, Sawasdikosol S, Burakoff SJ, Eck MJ.** 1999. Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase. Nature **398:**84–90. http://dx.doi.org/10.1038/18050.
16. **Thien CB, Langdon WY.** 2001. Cbl: many adaptations to regulate protein tyrosine kinases. Nat Rev Mol Cell Biol **2:**294–307. http://dx.doi.org/10.1038/35067100.
17. **Thien CB, Langdon WY.** 2005. c-Cbl and Cbl-b ubiquitin ligases: substrate diversity and the negative regulation of signalling responses. Biochem J **391:**153–166. http://dx.doi.org/10.1042/BJ20050892.
18. **Joazeiro CA, Wing SS, Huang H, Leverson JD, Hunter T, Liu YC.** 1999. The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. Science **286:**309–312. http://dx.doi.org/10.1126/science.286.5438.309.
19. **Fujita Y, Krause G, Scheffner M, Zechner D, Leddy HE, Behrens J, Sommer T, Birchmeier W.** 2002. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. Nat Cell Biol **4:**222–231. http://dx.doi.org/10.1038/ncb758.
20. **Mukherjee M, Chow SY, Yusoff P, Seetharaman J, Ng C, Sinniah S, Koh XW, Asgar NF, Li D, Yim D, Jackson RA, Yew J, Qian J, Iyu A, Lim YP, Zhou X, Sze SK, Guy GR, Sivaraman J.** 2012. Structure of a novel phosphotyrosine-binding domain in Hakai that targets E-cadherin. EMBO J **31:**1308–1319. http://dx.doi.org/10.1038/emboj.2011.496.
21. **Yoshimura A, Ohkubo T, Kiguchi T, Jenkins NA, Gilbert DJ, Copeland NG, Hara T, Miyajima A.** 1995. A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. EMBO J **14:**2816–2826.
22. **Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S, Kishimoto T.** 1997. Structure and function of a new STAT-induced STAT inhibitor. Nature **387:**924–929. http://dx.doi.org/10.1038/43219.
23. **Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyazaki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S, Yoshimura A.** 1997. A new protein containing an SH2 domain that inhibits JAK kinases. Nature **387:**921–924. http://dx.doi.org/10.1038/43213.
24. **Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA, Hilton DJ.** 1997. A family of cytokine-inducible inhibitors of signalling. Nature **387:**917–921. http://dx.doi.org/10.1038/43206.
25. **Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcalf D, Nicola NA.** 1998. Twenty proteins containing a C-terminal SOCS box form five structural classes. Proc Natl Acad Sci U S A **95:**114–119. http://dx.doi.org/10.1073/pnas.95.1.114.
26. **Verdier F, Chretien S, Muller O, Varlet P, Yoshimura A, Gisselbrecht S, Lacombe C, Mayeux P.** 1998. Proteasomes regulate erythropoietin receptor and signal transducer and activator of transcription 5 (STAT5) activation. Possible involvement of the ubiquitinated Cis protein. J Biol Chem **273:**28185–28190.
27. **De Sepulveda P, Okkenhaug K, Rose JL, Hawley RG, Dubreuil P, Rottapel R.** 1999. Socs1 binds to multiple signalling proteins and suppresses steel factor-dependent proliferation. EMBO J **18:**904–915. http://dx.doi.org/10.1093/emboj/18.4.904.
28. **De Sepulveda P, Ilangumaran S, Rottapel R.** 2000. Suppressor of cytokine signaling-1 inhibits VAV function through protein degradation. J Biol Chem **275:**14005–14008. http://dx.doi.org/10.1074/jbc.C000106200.
29. **Kamura T, Maenaka K, Kotoshiba S, Matsumoto M, Kohda D, Conaway RC, Conaway JW, Nakayama KI.** 2004. VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. Genes Dev **18:**3055–3065. http://dx.doi.org/10.1101/gad.1252404.
30. **Mahrour N, Redwine WB, Florens L, Swanson SK, Martin-Brown S, Bradford WD, Staehling-Hampton K, Washburn MP, Conaway RC, Conaway JW.** 2008. Characterization of Cullin-box sequences that direct recruitment of Cul2-Rbx1 and Cul5-Rbx2 modules to Elongin BC-based ubiquitin ligases. J Biol Chem **283:**8005–8013. http://dx.doi.org/10.1074/jbc.M706987200.
31. **Okumura F, Matsuzaki M, Nakatsukasa K, Kamura T.** 2012. The role of Elongin BC-containing ubiquitin ligases. Front Oncol **2:**10. http://dx.doi.org/10.3389/fonc.2012.00010.
32. **Petroski MD, Deshaies RJ.** 2005. Function and regulation of cullin-RING ubiquitin ligases. Nat Rev Mol Cell Biol **6:**9–20. http://dx.doi.org/10.1038/nrm1547.
33. **Heuze ML, Guibal FC, Banks CA, Conaway JW, Conaway RC, Cayre YE, Benecke A, Lutz PG.** 2005. ASB2 is an Elongin BC-interacting protein that can assemble with Cullin 5 and Rbx1 to reconstitute an E3 ubiquitin ligase complex. J Biol Chem **280:**5468–5474. http://dx.doi.org/10.1074/jbc.M413040200.
34. **Kamura T, Burian D, Yan Q, Schmidt SL, Lane WS, Querido E, Branton PE, Shilatifard A, Conaway RC, Conaway JW.** 2001. Muf1, a novel Elongin BC-interacting leucine-rich repeat protein that can assemble with Cul5 and Rbx1 to reconstitute a ubiquitin ligase. J Biol Chem **276:**29748–29753. http://dx.doi.org/10.1074/jbc.M103093200.
35. **Wei D, Sun Y.** 2010. Small RING finger proteins RBX1 and RBX2 of SCF E3 ubiquitin ligases: the role in cancer and as cancer targets. Genes Cancer **1:**700–707. http://dx.doi.org/10.1177/1947601910382776.
36. **Zheng N, Wang P, Jeffrey PD, Pavletich NP.** 2000. Structure of a c-Cbl-UbcH7 complex: RING domain function in ubiquitin-protein ligases. Cell **102:**533–539. http://dx.doi.org/10.1016/S0092-8674(00)00057-X.
37. **Dou H, Buetow L, Hock A, Sibbet GJ, Vousden KH, Huang DT.** 2012. Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. Nat Struct Mol Biol **19:**184–192. http://dx.doi.org/10.1038/nsmb.2231.
38. **Kobashigawa Y, Tomitaka A, Kumeta H, Noda NN, Yamaguchi M, Inagaki F.** 2011. Autoinhibition and phosphorylation-induced activation mechanisms of human cancer and autoimmune disease-related E3 protein Cbl-b. Proc Natl Acad Sci U S A **108:**20579–20584. http://dx.doi.org/10.1073/pnas.1110712108.
39. **Levkowitz G, Waterman H, Ettenberg SA, Katz M, Tsygankov AY, Alroy I, Lavi S, Iwai K, Reiss Y, Ciechanover A, Lipkowitz S, Yarden Y.** 1999. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. Mol Cell **4:**1029–1040. http://dx.doi.org/10.1016/S1097-2765(00)80231-2.
40. **Kassenbrock CK, Anderson SM.** 2004. Regulation of ubiquitin protein ligase activity in c-Cbl by phosphorylation-induced conformational change and constitutive activation by tyrosine to glutamate point mutations. J Biol Chem **279:**28017–28027. http://dx.doi.org/10.1074/jbc.M404114200.
41. **Wang Z, Sandiford S, Wu C, Li SS.** 2009. Numb regulates cell-cell adhesion and polarity in response to tyrosine kinase signalling. EMBO J **28:**2360–2373. http://dx.doi.org/10.1038/emboj.2009.190.
42. **Zadjali F, Pike AC, Vesterlund M, Sun J, Wu C, Li SS, Ronnstrand L, Knapp S, Bullock AN, Flores-Morales A.** 2011. Structural basis for c-KIT inhibition by the suppressor of cytokine signaling 6 (SOCS6) ubiquitin ligase. J Biol Chem **286:**480–490. http://dx.doi.org/10.1074/jbc.M110.173526.
43. **Matuoka K, Miki H, Takahashi K, Takenawa T.** 1997. A novel ligand for an SH3 domain of the adaptor protein Nck bears an SH2 domain and nuclear signaling motifs. Biochem Biophys Res Commun **239:**488–492. http://dx.doi.org/10.1006/bbrc.1997.7492.
44. **Martens N, Wery M, Wang P, Braet F, Gertler A, Hooghe R, Vandenhaute J, Hooghe-Peters EL.** 2004. The suppressor of cytokine signaling (SOCS)-7 interacts with the actin cytoskeleton through vinexin. Exp Cell Res **298:**239–248. http://dx.doi.org/10.1016/j.yexcr.2004.04.002.
45. **Kremer BE, Adang LA, Macara IG.** 2007. Septins regulate actin organization and cell-cycle arrest through nuclear accumulation of NCK me-

diated by SOCS7. Cell **130:**837–850. http://dx.doi.org/10.1016/j.cell.2007.06.053.
46. **Sitko JC, Guevara CI, Cacalano NA.** 2004. Tyrosine-phosphorylated SOCS3 interacts with the Nck and Crk-L adapter proteins and regulates Nck activation. J Biol Chem **279:**37662–37669. http://dx.doi.org/10.1074/jbc.M404007200.
47. **Choi YB, Son M, Park M, Shin J, Yun Y.** 2010. SOCS-6 negatively regulates T cell activation through targeting p56lck to proteasomal degradation. J Biol Chem **285:**7271–7280. http://dx.doi.org/10.1074/jbc.M109.073726.
48. **Yoon CH, Lee J, Jongeward GD, Sternberg PW.** 1995. Similarity of sli-1, a regulator of vulval development in C. elegans, to the mammalian proto-oncogene c-cbl. Science **269:**1102–1105. http://dx.doi.org/10.1126/science.7652556.
49. **Galisteo ML, Dikic I, Batzer AG, Langdon WY, Schlessinger J.** 1995. Tyrosine phosphorylation of the c-cbl proto-oncogene protein product and association with epidermal growth factor (EGF) receptor upon EGF stimulation. J Biol Chem **270:**20242–20245. http://dx.doi.org/10.1074/jbc.270.35.20242.
50. **Waterman H, Sabanai I, Geiger B, Yarden Y.** 1998. Alternative intracellular routing of ErbB receptors may determine signaling potency. J Biol Chem **273:**13819–13827. http://dx.doi.org/10.1074/jbc.273.22.13819.
51. **Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, Yarden Y.** 1998. c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. Genes Dev **12:**3663–3674. http://dx.doi.org/10.1101/gad.12.23.3663.
52. **Sorkin A, Goh LK.** 2009. Endocytosis and intracellular trafficking of ErbBs. Exp Cell Res **315:**683–696. http://dx.doi.org/10.1016/j.yexcr.2008.07.029.
53. **Huang F, Kirkpatrick D, Jiang X, Gygi S, Sorkin A.** 2006. Differential regulation of EGF receptor internalization and degradation by multiubiquitination within the kinase domain. Mol Cell **21:**737–748. http://dx.doi.org/10.1016/j.molcel.2006.02.018.
54. **Mosesson Y, Shtiegman K, Katz M, Zwang Y, Vereb G, Szollosi J, Yarden Y.** 2003. Endocytosis of receptor tyrosine kinases is driven by monoubiquitylation, not polyubiquitylation. J Biol Chem **278:**21323–21326. http://dx.doi.org/10.1074/jbc.C300096200.
55. **Haglund K, Sigismund S, Polo S, Szymkiewicz I, Di Fiore PP, Dikic I.** 2003. Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. Nat Cell Biol **5:**461–466. http://dx.doi.org/10.1038/ncb983.
56. **Duan L, Miura Y, Dimri M, Majumder B, Dodge IL, Reddi AL, Ghosh A, Fernandes N, Zhou P, Mullane-Robinson K, Rao N, Donoghue S, Rogers RA, Bowtell D, Naramura M, Gu H, Band V, Band H.** 2003. Cbl-mediated ubiquitinylation is required for lysosomal sorting of epidermal growth factor receptor but is dispensable for endocytosis. J Biol Chem **278:**28950–28960. http://dx.doi.org/10.1074/jbc.M304474200.
57. **Peschard P, Park M.** 2003. Escape from Cbl-mediated downregulation: a recurrent theme for oncogenic deregulation of receptor tyrosine kinases. Cancer Cell **3:**519–523. http://dx.doi.org/10.1016/S1535-6108(03)00136-3.
58. **Tanaka Y, Tanaka N, Saeki Y, Tanaka K, Murakami M, Hirano T, Ishii N, Sugamura K.** 2008. c-Cbl-dependent monoubiquitination and lysosomal degradation of gp130. Mol Cell Biol **28:**4805–4818. http://dx.doi.org/10.1128/MCB.01784-07.
59. **Tan YH, Krishnaswamy S, Nandi S, Kanteti R, Vora S, Onel K, Hasina R, Lo FY, El-Hashani E, Cervantes G, Robinson M, Hsu HS, Kales SC, Lipkowitz S, Karrison T, Sattler M, Vokes EE, Wang YC, Salgia R.** 2010. CBL is frequently altered in lung cancers: its relationship to mutations in MET and EGFR tyrosine kinases. PLoS One **5:**e8972. http://dx.doi.org/10.1371/journal.pone.0008972.
60. **Shtiegman K, Kochupurakkal BS, Zwang Y, Pines G, Starr A, Vexler A, Citri A, Katz M, Lavi S, Ben-Basat Y, Benjamin S, Corso S, Gan J, Yosef RB, Giordano S, Yarden Y.** 2007. Defective ubiquitinylation of EGFR mutants of lung cancer confers prolonged signaling. Oncogene **26:**6968–6978. http://dx.doi.org/10.1038/sj.onc.1210503.
61. **Pareja F, Ferraro DA, Rubin C, Cohen-Dvashi H, Zhang F, Aulmann S, Ben-Chetrit N, Pines G, Navon R, Crosetto N, Kostler W, Carvalho S, Lavi S, Schmitt F, Dikic I, Yakhini Z, Sinn P, Mills GB, Yarden Y.** 2012. Deubiquitination of EGFR by Cezanne-1 contributes to cancer progression. Oncogene **31:**4599–4608. http://dx.doi.org/10.1038/onc.2011.587.
62. **Naramura M, Jang IK, Kole H, Huang F, Haines D, Gu H.** 2002. c-Cbl and Cbl-b regulate T cell responsiveness by promoting ligand-induced TCR down-modulation. Nat Immunol **3:**1192–1199. http://dx.doi.org/10.1038/ni855.
63. **Murphy MA, Schnall RG, Venter DJ, Barnett L, Bertoncello I, Thien CB, Langdon WY, Bowtell DD.** 1998. Tissue hyperplasia and enhanced T-cell signalling via ZAP-70 in c-Cbl-deficient mice. Mol Cell Biol **18:**4872–4882.
64. **Molero JC, Jensen TE, Withers PC, Couzens M, Herzog H, Thien CB, Langdon WY, Walder K, Murphy MA, Bowtell DD, James DE, Cooney GJ.** 2004. c-Cbl-deficient mice have reduced adiposity, higher energy expenditure, and improved peripheral insulin action. J Clin Invest **114:**1326–1333. http://dx.doi.org/10.1172/JCI21480.
65. **Molero JC, Turner N, Thien CB, Langdon WY, James DE, Cooney GJ.** 2006. Genetic ablation of the c-Cbl ubiquitin ligase domain results in increased energy expenditure and improved insulin action. Diabetes **55:**3411–3417. http://dx.doi.org/10.2337/db06-0955.
66. **Thien CB, Blystad FD, Zhan Y, Lew AM, Voigt V, Andoniou CE, Langdon WY.** 2005. Loss of c-Cbl RING finger function results in high-intensity TCR signaling and thymic deletion. EMBO J **24:**3807–3819. http://dx.doi.org/10.1038/sj.emboj.7600841.
67. **Kales SC, Ryan PE, Nau MM, Lipkowitz S.** 2010. Cbl and human myeloid neoplasms: the Cbl oncogene comes of age. Cancer Res **70:**4789–4794. http://dx.doi.org/10.1158/0008-5472.CAN-10-0610.
68. **Sanada M, Suzuki T, Shih LY, Otsu M, Kato M, Yamazaki S, Tamura A, Honda H, Sakata-Yanagimoto M, Kumano K, Oda H, Yamagata T, Takita J, Gotoh N, Nakazaki K, Kawamata N, Onodera M, Nobuyoshi M, Hayashi Y, Harada H, Kurokawa M, Chiba S, Mori H, Ozawa K, Omine M, Hirai H, Nakauchi H, Koeffler HP, Ogawa S.** 2009. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. Nature **460:**904–908. http://dx.doi.org/10.1038/nature08240.
69. **Niemeyer CM, Kang MW, Shin DH, Furlan I, Erlacher M, Bunin NJ, Bunda S, Finklestein JZ, Sakamoto KM, Gorr TA, Mehta P, Schmid I, Kropshofer G, Corbacioglu S, Lang PJ, Klein C, Schlegel PG, Heinzmann A, Schneider M, Stary J, van den Heuvel-Eibrink MM, Hasle H, Locatelli F, Sakai D, Archambeault S, Chen L, Russell RC, Sybingco SS, Ohh M, Braun BS, Flotho C, Loh ML.** 2010. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet **42:**794–800. http://dx.doi.org/10.1038/ng.641.
70. **Thien CB, Bowtell DD, Langdon WY.** 1999. Perturbed regulation of ZAP-70 and sustained tyrosine phosphorylation of LAT and SLP-76 in c-Cbl-deficient thymocytes. J Immunol **162:**7133–7139.
71. **Naramura M, Kole HK, Hu RJ, Gu H.** 1998. Altered thymic positive selection and intracellular signals in Cbl-deficient mice. Proc Natl Acad Sci U S A **95:**15547–15552. http://dx.doi.org/10.1073/pnas.95.26.15547.
72. **Fang D, Liu YC.** 2001. Proteolysis-independent regulation of PI3K by Cbl-b-mediated ubiquitination in T cells. Nat Immunol **2:**870–875. http://dx.doi.org/10.1038/ni0901-870.
73. **Chiang YJ, Kole HK, Brown K, Naramura M, Fukuhara S, Hu RJ, Jang IK, Gutkind JS, Shevach E, Gu H.** 2000. Cbl-b regulates the CD28 dependence of T-cell activation. Nature **403:**216–220. http://dx.doi.org/10.1038/35003235.
74. **Bachmaier K, Krawczyk C, Kozieradzki I, Kong YY, Sasaki T, Oliveira-dos Santos A, Mariathasan S, Bouchard D, Wakeham A, Itie A, Le J, Ohashi PS, Sarosi I, Nishina H, Lipkowitz S, Penninger JM.** 2000. Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b. Nature **403:**211–216. http://dx.doi.org/10.1038/35003228.
75. **Guo H, Qiao G, Ying H, Li Z, Zhao Y, Liang Y, Yang L, Lipkowitz S, Penninger JM, Langdon WY, Zhang J.** 2012. E3 ubiquitin ligase Cbl-b regulates Pten via Nedd4 in T cells independently of its ubiquitin ligase activity. Cell Rep **1:**472–482. http://dx.doi.org/10.1016/j.celrep.2012.04.008.
76. **Harris KF, Shoji I, Cooper EM, Kumar S, Oda H, Howley PM.** 1999. Ubiquitin-mediated degradation of active Src tyrosine kinase. Proc Natl Acad Sci U S A **96:**13738–13743. http://dx.doi.org/10.1073/pnas.96.24.13738.
77. **Hakak Y, Martin GS.** 1999. Ubiquitin-dependent degradation of active Src. Curr Biol **9:**1039–1042. http://dx.doi.org/10.1016/S0960-9822(99)80453-9.
78. **Yokouchi M, Kondo T, Sanjay A, Houghton A, Yoshimura A, Komiya S, Zhang H, Baron R.** 2001. Src-catalyzed phosphorylation of c-Cbl

leads to the interdependent ubiquitination of both proteins. J Biol Chem **276:**35185–35193. http://dx.doi.org/10.1074/jbc.M102219200.
79. **Andoniou CE, Lill NL, Thien CB, Lupher ML, Jr, Ota S, Bowtell DD, Scaife RM, Langdon WY, Band H.** 2000. The Cbl proto-oncogene product negatively regulates the Src-family tyrosine kinase Fyn by enhancing its degradation. Mol Cell Biol **20:**851–867. http://dx.doi.org/10.1128/MCB.20.3.851-867.2000.
80. **Kaabeche K, Lemonnier J, Le Mee S, Caverzasio J, Marie PJ.** 2004. Cbl-mediated degradation of Lyn and Fyn induced by constitutive fibroblast growth factor receptor-2 activation supports osteoblast differentiation. J Biol Chem **279:**36259–36267. http://dx.doi.org/10.1074/jbc.M402469200.
81. **Sanjay A, Houghton A, Neff L, DiDomenico E, Bardelay C, Antoine E, Levy J, Gailit J, Bowtell D, Horne WC, Baron R.** 2001. Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha(v)beta(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. J Cell Biol **152:**181–195. http://dx.doi.org/10.1083/jcb.152.1.181.
82. **Tanaka S, Amling M, Neff L, Peyman A, Uhlmann E, Levy JB, Baron R.** 1996. c-Cbl is downstream of c-Src in a signalling pathway necessary for bone resorption. Nature **383:**528–531. http://dx.doi.org/10.1038/383528a0.
83. **Meng F, Lowell CA.** 1998. A beta 1 integrin signaling pathway involving Src-family kinases, Cbl and PI-3 kinase is required for macrophage spreading and migration. EMBO J **17:**4391–4403. http://dx.doi.org/10.1093/emboj/17.15.4391.
84. **Bao J, Gur G, Yarden Y.** 2003. Src promotes destruction of c-Cbl: implications for oncogenic synergy between Src and growth factor receptors. Proc Natl Acad Sci U S A **100:**2438–2443. http://dx.doi.org/10.1073/pnas.0437945100.
85. **Ishizawar R, Parsons SJ.** 2004. c-Src and cooperating partners in human cancer. Cancer Cell **6:**209–214. http://dx.doi.org/10.1016/j.ccr.2004.09.001.
86. **Kojo S, Elly C, Harada Y, Langdon WY, Kronenberg M, Liu YC.** 2009. Mechanisms of NKT cell anergy induction involve Cbl-b-promoted monoubiquitination of CARMA1. Proc Natl Acad Sci U S A **106:**17847–17851. http://dx.doi.org/10.1073/pnas.0904078106.
87. **Anastasiadis PZ, Reynolds AB.** 2000. The p120 catenin family: complex roles in adhesion, signaling and cancer. J Cell Sci **113:**1319–1334.
88. **Hartsock A, Nelson WJ.** 2012. Competitive regulation of E-cadherin juxtamembrane domain degradation by p120-catenin binding and Hakai-mediated ubiquitination. PLoS One **7:**e37476. http://dx.doi.org/10.1371/journal.pone.0037476.
89. **Ishiyama N, Lee SH, Liu S, Li GY, Smith MJ, Reichardt LF, Ikura M.** 2010. Dynamic and static interactions between p120 catenin and E-cadherin regulate the stability of cell-cell adhesion. Cell **141:**117–128. http://dx.doi.org/10.1016/j.cell.2010.01.017.
90. **Potter MD, Barbero S, Cheresh DA.** 2005. Tyrosine phosphorylation of VE-cadherin prevents binding of p120- and beta-catenin and maintains the cellular mesenchymal state. J Biol Chem **280:**31906–31912. http://dx.doi.org/10.1074/jbc.M505568200.
91. **Bonazzi M, Veiga E, Pizarro-Cerda J, Cossart P.** 2008. Successive post-translational modifications of E-cadherin are required for InlA-mediated internalization of Listeria monocytogenes. Cell Microbiol **10:**2208–2222. http://dx.doi.org/10.1111/j.1462-5822.2008.01200.x.
92. **Shen Y, Hirsch DS, Sasiela CA, Wu WJ.** 2008. Cdc42 regulates E-cadherin ubiquitination and degradation through an epidermal growth factor receptor to Src-mediated pathway. J Biol Chem **283:**5127–5137. http://dx.doi.org/10.1074/jbc.M703300200.
93. **Palacios F, Tushir JS, Fujita Y, D'Souza-Schorey C.** 2005. Lysosomal targeting of E-cadherin: a unique mechanism for the down-regulation of cell-cell adhesion during epithelial to mesenchymal transitions. Mol Cell Biol **25:**389–402. http://dx.doi.org/10.1128/MCB.25.1.389-402.2005.
94. **Janda E, Nevolo M, Lehmann K, Downward J, Beug H, Grieco M.** 2006. Raf plus TGFbeta-dependent EMT is initiated by endocytosis and lysosomal degradation of E-cadherin. Oncogene **25:**7117–7130. http://dx.doi.org/10.1038/sj.onc.1209701.
95. **Figueroa A, Kotani H, Toda Y, Mazan-Mamczarz K, Mueller EC, Otto A, Disch L, Norman M, Ramdasi RM, Keshtgar M, Gorospe M, Fujita Y.** 2009. Novel roles of hakai in cell proliferation and oncogenesis. Mol Biol Cell **20:**3533–3542. http://dx.doi.org/10.1091/mbc.E08-08-0845.
96. **Aparicio LA, Valladares M, Blanco M, Alonso G, Figueroa A.** 2012. Biological influence of Hakai in cancer: a 10-year review. Cancer Metastasis Rev **31:**375–386. http://dx.doi.org/10.1007/s10555-012-9348-x.
97. **Palmer DC, Restifo NP.** 2009. Suppressors of cytokine signaling (SOCS) in T cell differentiation, maturation, and function. Trends Immunol **30:**592–602. http://dx.doi.org/10.1016/j.it.2009.09.009.
98. **Ilangumaran S, Ramanathan S, Rottapel R.** 2004. Regulation of the immune system by SOCS family adaptor proteins. Semin Immunol **16:**351–365. http://dx.doi.org/10.1016/j.smim.2004.08.015.
99. **Krebs DL, Hilton DJ.** 2000. SOCS: physiological suppressors of cytokine signaling. J Cell Sci **113:**2813–2819.
100. **Marine JC, Topham DJ, McKay C, Wang D, Parganas E, Stravopodis D, Yoshimura A, Ihle JN.** 1999. SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. Cell **98:**609–616. http://dx.doi.org/10.1016/S0092-8674(00)80048-3.
101. **Naka T, Matsumoto T, Narazaki M, Fujimoto M, Morita Y, Ohsawa Y, Saito H, Nagasawa T, Uchiyama Y, Kishimoto T.** 1998. Accelerated apoptosis of lymphocytes by augmented induction of Bax in SSI-1 (STAT-induced STAT inhibitor-1) deficient mice. Proc Natl Acad Sci U S A **95:**15577–15582. http://dx.doi.org/10.1073/pnas.95.26.15577.
102. **Starr R, Metcalf D, Elefanty AG, Brysha M, Willson TA, Nicola NA, Hilton DJ, Alexander WS.** 1998. Liver degeneration and lymphoid deficiencies in mice lacking suppressor of cytokine signaling-1. Proc Natl Acad Sci U S A **95:**14395–14399. http://dx.doi.org/10.1073/pnas.95.24.14395.
103. **Marine JC, McKay C, Wang D, Topham DJ, Parganas E, Nakajima H, Pendeville H, Yasukawa H, Sasaki A, Yoshimura A, Ihle JN.** 1999. SOCS3 is essential in the regulation of fetal liver erythropoiesis. Cell **98:**617–627. http://dx.doi.org/10.1016/S0092-8674(00)80049-5.
104. **Roberts AW, Robb L, Rakar S, Hartley L, Cluse L, Nicola NA, Metcalf D, Hilton DJ, Alexander WS.** 2001. Placental defects and embryonic lethality in mice lacking suppressor of cytokine signaling 3. Proc Natl Acad Sci U S A **98:**9324–9329. http://dx.doi.org/10.1073/pnas.161271798.
105. **Croker BA, Krebs DL, Zhang JG, Wormald S, Willson TA, Stanley EG, Robb L, Greenhalgh CJ, Forster I, Clausen BE, Nicola NA, Metcalf D, Hilton DJ, Roberts AW, Alexander WS.** 2003. SOCS3 negatively regulates IL-6 signaling in vivo. Nat Immunol **4:**540–545. http://dx.doi.org/10.1038/ni931.
106. **Yang XO, Zhang H, Kim BS, Niu X, Peng J, Chen Y, Kerketta R, Lee YH, Chang SH, Corry DB, Wang D, Watowich SS, Dong C.** 2013. The signaling suppressor CIS controls proallergic T cell development and allergic airway inflammation. Nat Immunol **14:**732–740. http://dx.doi.org/10.1038/ni.2633.
107. **Babon JJ, Sabo JK, Zhang JG, Nicola NA, Norton RS.** 2009. The SOCS box encodes a hierarchy of affinities for Cullin5: implications for ubiquitin ligase formation and cytokine signalling suppression. J Mol Biol **387:**162–174. http://dx.doi.org/10.1016/j.jmb.2009.01.024.
108. **Zhang JG, Metcalf D, Rakar S, Asimakis M, Greenhalgh CJ, Willson TA, Starr R, Nicholson SE, Carter W, Alexander WS, Hilton DJ, Nicola NA.** 2001. The SOCS box of suppressor of cytokine signaling-1 is important for inhibition of cytokine action in vivo. Proc Natl Acad Sci U S A **98:**13261–13265. http://dx.doi.org/10.1073/pnas.231486498.
109. **Ungureanu D, Saharinen P, Junttila I, Hilton DJ, Silvennoinen O.** 2002. Regulation of Jak2 through the ubiquitin-proteasome pathway involves phosphorylation of Jak2 on Y1007 and interaction with SOCS-1. Mol Cell Biol **22:**3316–3326. http://dx.doi.org/10.1128/MCB.22.10.3316-3326.2002.
110. **Babon JJ, Laktyushin A, Kershaw NJ.** 2013. In vitro ubiquitination of cytokine signaling components. Methods Mol Biol **967:**261–271. http://dx.doi.org/10.1007/978-1-62703-242-1_19.
111. **Liu E, Cote JF, Vuori K.** 2003. Negative regulation of FAK signaling by SOCS proteins. EMBO J **22:**5036–5046. http://dx.doi.org/10.1093/emboj/cdg503.
112. **Kazi JU, Kabir NN, Flores-Morales A, Ronnstrand L.** 2014. SOCS proteins in regulation of receptor tyrosine kinase signaling. Cell Mol Life Sci **71:**3297–3310. http://dx.doi.org/10.1007/s00018-014-1619-y.
113. **Le Y, Zhu BM, Harley B, Park SY, Kobayashi T, Manis JP, Luo HR, Yoshimura A, Hennighausen L, Silberstein LE.** 2007. SOCS3 protein developmentally regulates the chemokine receptor CXCR4-FAK signaling pathway during B lymphopoiesis. Immunity **27:**811–823. http://dx.doi.org/10.1016/j.immuni.2007.09.011.
114. **Lee SH, Yun S, Piao ZH, Jeong M, Kim DO, Jung H, Lee J, Kim MJ, Kim MS, Chung JW, Kim TD, Yoon SR, Greenberg PD, Choi I.** 2010. Suppressor of cytokine signaling 2 regulates IL-15-primed human NK cell function via control of phosphorylated Pyk2. J Immunol **185:**917–928. http://dx.doi.org/10.4049/jimmunol.1000784.

115. **Metcalf D, Greenhalgh CJ, Viney E, Willson TA, Starr R, Nicola NA, Hilton DJ, Alexander WS.** 2000. Gigantism in mice lacking suppressor of cytokine signalling-2. Nature **405:**1069–1073. http://dx.doi.org/10.1038/35016611.
116. **Greenhalgh CJ, Bertolino P, Asa SL, Metcalf D, Corbin JE, Adams TE, Davey HW, Nicola NA, Hilton DJ, Alexander WS.** 2002. Growth enhancement in suppressor of cytokine signaling 2 (SOCS-2)-deficient mice is dependent on signal transducer and activator of transcription 5b (STAT5b). Mol Endocrinol **16:**1394–1406. http://dx.doi.org/10.1210/mend.16.6.0845.
117. **Greenhalgh CJ, Rico-Bautista E, Lorentzon M, Thaus AL, Morgan PO, Willson TA, Zervoudakis P, Metcalf D, Street I, Nicola NA, Nash AD, Fabri LJ, Norstedt G, Ohlsson C, Flores-Morales A, Alexander WS, Hilton DJ.** 2005. SOCS2 negatively regulates growth hormone action in vitro and in vivo. J Clin Invest **115:**397–406. http://dx.doi.org/10.1172/JCI2271.
118. **Greenhalgh CJ, Metcalf D, Thaus AL, Corbin JE, Uren R, Morgan PO, Fabri LJ, Zhang JG, Martin HM, Willson TA, Billestrup N, Nicola NA, Baca M, Alexander WS, Hilton DJ.** 2002. Biological evidence that SOCS-2 can act either as an enhancer or suppressor of growth hormone signaling. J Biol Chem **277:**40181–40184. http://dx.doi.org/10.1074/jbc.C200450200.
119. **Piessevaux J, Lavens D, Montoye T, Wauman J, Catteeuw D, Vandekerckhove J, Belsham D, Peelman F, Tavernier J.** 2006. Functional cross-modulation between SOCS proteins can stimulate cytokine signaling. J Biol Chem **281:**32953–32966. http://dx.doi.org/10.1074/jbc.M600776200.
120. **Kario E, Marmor MD, Adamsky K, Citri A, Amit I, Amariglio N, Rechavi G, Yarden Y.** 2005. Suppressors of cytokine signaling 4 and 5 regulate epidermal growth factor receptor signaling. J Biol Chem **280:**7038–7048. http://dx.doi.org/10.1074/jbc.M408575200.
121. **Nicholson SE, Metcalf D, Sprigg NS, Columbus R, Walker F, Silva A, Cary D, Willson TA, Zhang JG, Hilton DJ, Alexander WS, Nicola NA.** 2005. Suppressor of cytokine signaling (SOCS)-5 is a potential negative regulator of epidermal growth factor signaling. Proc Natl Acad Sci U S A **102:**2328–2333. http://dx.doi.org/10.1073/pnas.0409675102.
122. **Brender C, Columbus R, Metcalf D, Handman E, Starr R, Huntington N, Tarlinton D, Odum N, Nicholson SE, Nicola NA, Hilton DJ, Alexander WS.** 2004. SOCS5 is expressed in primary B and T lymphoid cells but is dispensable for lymphocyte production and function. Mol Cell Biol **24:**6094–6103. http://dx.doi.org/10.1128/MCB.24.13.6094-6103.2004.
123. **Krebs DL, Uren RT, Metcalf D, Rakar S, Zhang JG, Starr R, De Souza DP, Hanzinikolas K, Eyles J, Connolly LM, Simpson RJ, Nicola NA, Nicholson SE, Baca M, Hilton DJ, Alexander WS.** 2002. SOCS-6 binds to insulin receptor substrate 4, and mice lacking the SOCS-6 gene exhibit mild growth retardation. Mol Cell Biol **22:**4567–4578. http://dx.doi.org/10.1128/MCB.22.13.4567-4578.2002.
124. **Bayle J, Letard S, Frank R, Dubreuil P, De Sepulveda P.** 2004. Suppressor of cytokine signaling 6 associates with KIT and regulates KIT receptor signaling. J Biol Chem **279:**12249–12259. http://dx.doi.org/10.1074/jbc.M313381200.
125. **Knisz J, Banks A, McKeag L, Metcalfe DD, Rothman PB, Brown JM.** 2009. Loss of SOCS7 in mice results in severe cutaneous disease and increased mast cell activation. Clin Immunol **132:**277–284. http://dx.doi.org/10.1016/j.clim.2009.04.003.
126. **Krebs DL, Metcalf D, Merson TD, Voss AK, Thomas T, Zhang JG, Rakar S, O'Bryan MK, Willson TA, Viney EM, Mielke LA, Nicola NA, Hilton DJ, Alexander WS.** 2004. Development of hydrocephalus in mice lacking SOCS7. Proc Natl Acad Sci U S A **101:**15446–15451. http://dx.doi.org/10.1073/pnas.0406870101.
127. **Banks AS, Li J, McKeag L, Hribal ML, Kashiwada M, Accili D, Rothman PB.** 2005. Deletion of SOCS7 leads to enhanced insulin action and enlarged islets of Langerhans. J Clin Invest **115:**2462–2471. http://dx.doi.org/10.1172/JCI23853.
128. **Simo S, Cooper JA.** 2013. Rbx2 regulates neuronal migration through different Cullin5-RING ligase adaptors. Dev Cell **27:**399–411. http://dx.doi.org/10.1016/j.devcel.2013.09.022.
129. **Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM, Hilton DJ.** 2002. The SOCS box: a tale of destruction and degradation. Trends Biochem Sci **27:**235–241. http://dx.doi.org/10.1016/S0968-0004(02)02085-6.
130. **Teckchandani A, Laszlo GS, Simo S, Shah K, Pilling C, Strait AA, Cooper JA.** 2014. Cullin 5 destabilizes Cas to inhibit Src-dependent cell transformation. J Cell Sci **127:**509–520. http://dx.doi.org/10.1242/jcs.127829.
131. **Simo S, Jossin Y, Cooper JA.** 2010. Cullin 5 regulates cortical layering by modulating the speed and duration of Dab1-dependent neuronal migration. J Neurosci **30:**5668–5676. http://dx.doi.org/10.1523/JNEUROSCI.0035-10.2010.
132. **Laszlo GS, Cooper JA.** 2009. Restriction of Src activity by Cullin-5. Curr Biol **19:**157–162. http://dx.doi.org/10.1016/j.cub.2008.12.007.
133. **Feng L, Allen NS, Simo S, Cooper JA.** 2007. Cullin 5 regulates Dab1 protein levels and neuron positioning during cortical development. Genes Dev **21:**2717–2730. http://dx.doi.org/10.1101/gad.1604207.
134. **Sasi W, Ye L, Jiang WG, Sharma AK, Mokbel K.** 2014. In vitro and in vivo effects of suppressor of cytokine signalling 7 knockdown in breast cancer: the influence on cellular response to hepatocyte growth factor. Biomed Res Int **2014:**648040. http://dx.doi.org/10.1155/2014/648040.
135. **Linke A, Goren I, Bosl MR, Pfeilschifter J, Frank S.** 2010. The suppressor of cytokine signaling (SOCS)-3 determines keratinocyte proliferative and migratory potential during skin repair. J Investig Dermatol **130:**876–885. http://dx.doi.org/10.1038/jid.2009.344.
136. **Zhu JG, Dai QS, Han ZD, He HC, Mo RJ, Chen G, Chen YF, Wu YD, Yang SB, Jiang FN, Chen WH, Sun ZL, Zhong WD.** 2013. Expression of SOCSs in human prostate cancer and their association in prognosis. Mol Cell Biochem **381:**51–59. http://dx.doi.org/10.1007/s11010-013-1687-6.
137. **Qiu X, Zheng J, Guo X, Gao X, Liu H, Tu Y, Zhang Y.** 2013. Reduced expression of SOCS2 and SOCS6 in hepatocellular carcinoma correlates with aggressive tumor progression and poor prognosis. Mol Cell Biochem **378:**99–106. http://dx.doi.org/10.1007/s11010-013-1599-5.
138. **Sriram KB, Larsen JE, Savarimuthu Francis SM, Wright CM, Clarke BE, Duhig EE, Brown KM, Hayward NK, Yang IA, Bowman RV, Fong KM.** 2012. Array-comparative genomic hybridization reveals loss of SOCS6 is associated with poor prognosis in primary lung squamous cell carcinoma. PLoS One **7:**e30398. http://dx.doi.org/10.1371/journal.pone.0030398.
139. **Lai RH, Hsiao YW, Wang MJ, Lin HY, Wu CW, Chi CW, Li AF, Jou YS, Chen JY.** 2010. SOCS6, down-regulated in gastric cancer, inhibits cell proliferation and colony formation. Cancer Lett **288:**75–85. http://dx.doi.org/10.1016/j.canlet.2009.06.025.
140. **Weber A, Hengge UR, Bardenheuer W, Tischoff I, Sommerer F, Markwarth A, Dietz A, Wittekind C, Tannapfel A.** 2005. SOCS-3 is frequently methylated in head and neck squamous cell carcinoma and its precursor lesions and causes growth inhibition. Oncogene **24:**6699–6708. http://dx.doi.org/10.1038/sj.onc.1208818.
141. **Tischoff I, Hengge UR, Vieth M, Ell C, Stolte M, Weber A, Schmidt WE, Tannapfel A.** 2007. Methylation of SOCS-3 and SOCS-1 in the carcinogenesis of Barrett's adenocarcinoma. Gut **56:**1047–1053. http://dx.doi.org/10.1136/gut.2006.111633.
142. **Sasi W, Jiang WG, Sharma A, Mokbel K.** 2010. Higher expression levels of SOCS 1,3,4,7 are associated with earlier tumour stage and better clinical outcome in human breast cancer. BMC Cancer **10:**178. http://dx.doi.org/10.1186/1471-2407-10-178.
143. **Yoon S, Yi YS, Kim SS, Kim JH, Park WS, Nam SW.** 2012. SOCS5 and SOCS6 have similar expression patterns in normal and cancer tissues. Tumour Biol **33:**215–221. http://dx.doi.org/10.1007/s13277-011-0264-4.
144. **Farabegoli F, Ceccarelli C, Santini D, Taffurelli M.** 2005. Suppressor of cytokine signalling 2 (SOCS-2) expression in breast carcinoma. J Clin Pathol **58:**1046–1050. http://dx.doi.org/10.1136/jcp.2004.024919.
145. **Sutherland KD, Lindeman GJ, Choong DY, Wittlin S, Brentzell L, Phillips W, Campbell IG, Visvader JE.** 2004. Differential hypermethylation of SOCS genes in ovarian and breast carcinomas. Oncogene **23:**7726–7733. http://dx.doi.org/10.1038/sj.onc.1207787.
146. **Tan M, Zhao Y, Kim SJ, Liu M, Jia L, Saunders TL, Zhu Y, Sun Y.** 2011. SAG/RBX2/ROC2 E3 ubiquitin ligase is essential for vascular and neural development by targeting NF1 for degradation. Dev Cell **21:**1062–1076. http://dx.doi.org/10.1016/j.devcel.2011.09.014.
147. **Hollstein PE, Cichowski K.** 2013. Identifying the ubiquitin ligase complex that regulates the NF1 tumor suppressor and Ras. Cancer Discov **3:**880–893. http://dx.doi.org/10.1158/2159-8290.CD-13-0146.
148. **Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, Chu C, Koepp DM, Elledge SJ, Pagano M, Conaway RC, Conaway JW, Harper JW, Pavletich NP.** 2002. Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature **416:**703–709. http://dx.doi.org/10.1038/416703a.

149. **Bulatov E, Martin EM, Chatterjee S, Knebel A, Shimamura S, Konijnenberg A, Johnson C, Zinn N, Grandi P, Sobott F, Ciulli A.** 2015. Biophysical studies on interactions and assembly of full-size E3 ubiquitin ligase: suppressor of cytokine signaling 2 (SOCS2)-Elongin BC-cullin 5-ring box protein 2 (RBX2). J Biol Chem **290:**4178–4191. http://dx.doi.org/10.1074/jbc.M114.616664.
150. **Duda DM, Borg LA, Scott DC, Hunt HW, Hammel M, Schulman BA.** 2008. Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. Cell **134:**995–1006. http://dx.doi.org/10.1016/j.cell.2008.07.022.
151. **Kim YK, Kwak MJ, Ku B, Suh HY, Joo K, Lee J, Jung JU, Oh BH.** 2013. Structural basis of intersubunit recognition in elongin BC-cullin 5-SOCS box ubiquitin-protein ligase complexes. Acta Crystallogr D Biol Crystallogr **69:**1587–1597. http://dx.doi.org/10.1107/S0907444913011220.