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Degradation of Activated Protein Kinases by Ubiquitination

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Abstract

Protein kinases are important regulators of intracellular signal transduction pathways and play critical roles in diverse cellular functions. Once a protein kinase is activated, its activity is subsequently downregulated through a variety of mechanisms. Accumulating evidence indicates that the activation of protein kinases commonly initiates their downregulation via the ubiquitin/proteasome pathway. Failure to regulate protein kinase activity or expression levels can cause human diseases.

Keywords

activation; downregulation; phosphorylation; ubiquitin

INTRODUCTION

Protein kinases are important regulators of intracellular signal transduction pathways that mediate the development and regulation of both unicellular and multicellular organisms. They play critical roles in cell growth, division, differentiation, adhesion, motility, and death. More than 500 protein kinases have been identified in the human genome (1). Based on their catalytic specificity, they can be subdivided into tyrosine (Tyr, Y)- and serine (Ser, S)/threonine (Thr, T)-specific kinases. Their activity is normally tightly regulated to mediate their cellular function and physiological responses. Perturbation of protein kinases by mutations, altered expression, or dysregulation can cause many human diseases, including cancer and diabetes (2). Normal cellular protein kinases can be positively upregulated by phosphorylation, dephosphorylation, protein cleavage, translocation, ligand/second-messenger or ion binding, dimerization or oligomerization, activating subunit interaction, and protein-protein or protein-lipid interactions. The activity of protein kinases is also governed by negative feedback systems, which can attenuate or terminate kinase activity and thus their induced downstream signals. Several mechanisms serve to negatively regulate kinase activity, including, in the case of ligand-induced receptor tyrosine kinase signaling, antagonistic ligands, hetero-oligomerization with truncated receptors, phosphorylation, dephosphorylation, endocytosis, protein degradation, and reduction of receptor mRNA, all of which serve as means of downregulation (3).

DISCLOSURE STATEMENT

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Regulated proteolysis is a common mechanism for downregulating activated protein kinases. In most cases this involves activation-dependent protein degradation mediated through the ubiquitin (Ub)/proteasome or lysosome pathways, although a few kinases, such as Src, AKT, and FAK, can be downregulated by caspase- or calpain protease–mediated protein cleavage in the process of apoptosis (4–9). The ubiquitin-proteasome system (UPS) controls the levels of many proteins, including protein kinases and other intracellular regulatory proteins, and is essential in eukaryotes. Conjugation of Ub to substrate proteins requires three enzymes: the Ub-activating enzyme (E1), an Ub-conjugating enzyme (E2), and an Ub ligase (E3). E1 activates Ub through the formation of a thiol-ester bond between the C terminus of Ub and the active site cysteine (Cys). The activated Ub is then transesterified to a conserved Cys of an E2. The E3 ligase interacts with both E2 and the substrate and facilitates ubiquitination of the substrate. There are two distinct types of E3 ligases: the enzymatic HECT (homologous to E6-AP C terminus) domain E3s and the non-HECT domain E3s. HECT domain E3s form a thiolester with Ub, which can then be transferred directly to the substrate. Thenon-HECT domain E3s, containing a RING, variant RING, or a U box, do not form a thioester with Ub but function as adaptors to facilitate interaction between substrates and a charged E2 (10). E3s have a key role in defining the substrate specificity of ubiquitination. The polyubiquitin chain on substrates is formed through isopeptide linkages between the ε-amino group of a lysine (Lys, K) in one Ub and the C terminus of the next Ub. Seven lysines in Ub can be used for Ub-chain formation. Polyubiquitin chains linked through K48 and, in some cases, K11 are recognized by the proteasome as a signal for degradation (11–13). Polyubiquitin chains linked through other lysines serve other functions. For example, TRAF6, a RING finger E3 ligase, acting together with E2 Ubc13/Uev1A, induces K63 polyubiquitination. TRAF6 polyubiquitination does not result in degradation, but rather is recognized by K63 linkage-specific binding proteins leading to activation of the TAK1 kinase and its downstream targets MKK6 and IκB kinase (IKK) (14,15).

There are at least 22 known classes of Ub-binding domains (UBDs) [e.g., Ub-associated (UBA) domain) and Ub-interacting motif (UIM)] (16–18). Of 12 human AMP-activated protein kinase (AMPK)-related kinases, 9, including Par-1 paralogs/microtubule-affinityregulating kinases 1–4, possess a UBA domain, which binds intramolecularly to the K29/33 Ub chains formed on AMPK family members. This binding causes dissociation of the UBA domain from the catalytic domain, thus reducing kinase activity, which requires UBA/catalytic domain interaction (19).

Besides Ub, 13 other small Ub-like (UBL) proteins (e.g., SUMO1-3 and NEDD8/Rub1) are structurally similar to Ub, and are conjugated to substrate proteins or lipids via their C termini, thereby regulating different cellular responses (16). In addition, UBLs can be found as an integral UBL domain (ULD) within the larger context of a protein (20). IKKα, IKKβ, IKK*i*/IKKε, and IKK-related kinase TANK-binding kinase 1 (TBK1/NAK/T2K) contain ULDs. The ULD of TBK1, IKK*i*, or IKKε is required for kinase domain activation, interaction with kinase substrates, and subsequent degradation of substrates (21,22).

Many Ser/Thr- and Tyr-specific protein kinases can be degraded through the UPS, and in most cases initiation of the degradation process is triggered upon activation of the protein kinase. The downregulation of activated protein kinases through degradation, and, in some cases, through degradation of regulatory subunits provides negative-feedback regulation of kinase activity, which is essential for the precise control of cellular functions. Not surprisingly, the phosphorylation and dephosphorylation events that regulate protein kinase activity are also involved in their degradation. Activation of the kinase catalytic domain, which commonly occurs through phosphorylation of the activation loop, results in a conformational change that reorients the catalytic center and thereby allows phosphate transfer from ATP to substrate. The activated conformation of a protein kinase is a prerequisite for initiating degradation (23), but

its subcellular localization often determines how it is processed for degradation. For instance, in contrast to nonreceptor tyrosine kinases, which are downregulated by the classical UPS-mediated mechanisms, most activated receptor tyrosine kinases are ubiquitinated and directed to the lysosome for degradation. This review focuses on the activation-dependent and UPS-mediated degradation of protein kinases of both the nonreceptor (Table S1: Follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org) and receptor types (Table S2), including those mediated by protein kinase-specific chaperones.

NONRECEPTOR PROTEIN KINASES

Eukaryotic protein kinases can be divided into two main types: receptor protein kinases and nonreceptor protein kinases. Receptor protein kinases are type I transmembrane proteins with an N-terminal extracellular domain, which can in principle bind activating ligands, a single transmembrane domain, and a C-terminal cytoplasmic domain that includes the catalytic domain. Nonreceptor protein kinases lack a transmembrane domain; most are soluble intracellular proteins, but in some cases, they associate with membranes via a membrane-targeting posttranslational modification, such as an N-terminal myristoyl group, and can act as the catalytic subunit for receptors that lack their own catalytic domain. Eukaryotic protein kinases are specific for either serine/threonine or tyrosine residues in their target protein. Receptor and nonreceptor protein kinases of both amino acid specificities are known (2,3).

Nonreceptor Serine/Threonine Kinases

Nonreceptor serine/threonine kinases are the largest group of eukaryotic protein kinases comprising 376 out of the total of 518 protein kinases in humans (1). Examples are protein kinase C (PKC), the MAP kinases, and AKT.

Protein kinase C—PKC isoforms are Ser/Thr kinases involved in signal transduction pathways that govern a wide range of physiological processes, including differentiation, proliferation, gene expression, membrane transport, and organization of cytoskeletal and extracellular matrix proteins. Some PKC isoforms are targets of tumor-promoting phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA). Initially, phorbol ester treatment activates conventional (α, βI, βII, and γ) and novel PKC isoforms (δ, ε, η, and θ), but then, upon prolonged exposure, causes their depletion by proteolytic degradation. Downregulation of PKCδ is responsible for the tumor-promoting effect of phorbol esters (24). The degradation of PKC isoforms induced by diacylglycerol (DAG), a physiological activator of PKC, TPA, or bryostatin-1 is mediated through the UPS. That PKC ubiquitination and degradation is prevented by inhibition of PKC activation demonstrated for the first time that a Ser/Thr protein kinase exhibits activation-dependent protein degradation (25), which acts as a feedback regulatory mechanism. Ubiquitination of several PKC isoforms may be mediated by common E2s and E3s, which can differ according to the nature of the upstream signal. For instance, an active PKCβII mutant interacts with and is ubiquitinated in vitro by E3 complexes composed of RING finger protein HOIL-1L and HOIL-1L–interacting protein, and HOIL-1 deficiency largely inhibits TPA–induced PKCα degradation (26). In contrast, RING finger protein that interacts with C kinase (RINCK) interacts with the C1A domain of PKCβII and ubiquitinates PKCβII. RINCK depletion increases expression of PKCα, PKCδ, and PKCζ in a cell line-specific manner, but has no effect on TPA-mediated downregulation of PKC (27).

In contrast to conventional and novel PKCs, PKCζ, an atypical PKC (aPKC), which is not responsive to TPA or DAG, can be activated by caspase cleavage upon treatment with tumor necrosis factor (TNF)-α (28). Although the physiological significance of PKCζ downregulation is unclear, the released PKCζ kinase domain, which has significantly increased kinase activity, undergoes UPS-dependent degradation (28). The von Hippel-Lindau (VHL) protein regulates

ubiquitination of PKCζII, a rapidly and constitutively degraded variant of PKCζ. VHL, together with elongins B/C, Cul-2, and the RING finger-containing protein Rbx1, forms a complex (VCB-Cul2) that functions as an E3 Ub ligase in which VHL recognizes the N-terminal PB1 domain of PKCζII. VHL also regulates ubiquitination of serumactivated PKCλ/ι, another aPKC (29).

Exactly how PKC kinase activation leads to ubiquitination and degradation is not fully understood. In its inactive state, PKC is auto-inhibited as a result of the binding of an N-terminal pseudosubstrate site to the catalytic domain, which prevents the catalytic domain from binding and phosphorylating substrates. A constitutively active form of PKCη, in which mutation of its pseudosubstrate site releases intramolecular inhibition, is rapidly degraded. In contrast, a kinase-dead variant of the PKCη pseudosubstrate-site mutant, which should adopt an active conformation, is resistant to degradation. However, this mutant, but not wild-type (WT) PKCη, which has an inactive conformation, is degraded when co-overexpressed with a constitutively active PKCη mutant (23). These results suggest that the active conformation of PKCη is required but not sufficient for its degradation. Additional modifications, such as auto-/transphosphorylation and/or transphosphorylation of substrates, dephosphorylation, and correct subcellular localization may be required to bring PKC together with the ubiquitination machinery. The importance of phosphorylation is evidenced by the correlation between enhanced PKCδ degradation and PKCδ hyperphosphorylation at T505 in the activation loop and autophosphorylation at S643 induced by treatment with calyculin A, a protein phosphatase PP1/PP2A inhibitor, TPA, or serum. Degradation of the PKCδ T505A mutant is slower than that of its WT counterpart (30). Furthermore, autophosphorylation of PKC βII is required for its ubiquitination by the HOIL-1L/HOIL-1L–interacting protein complex (26). Transphosphorylation can also be involved in PKC ubiquitination. Expression of c-Src or v-Src results in phosphorylation of PKCδ at Y311 and degradation, and mutation of Y311 prevents Src-induced phosphorylation and degradation of PKCδ (31). These results imply that phosphorylated residues on PKCδ may in general provide a docking site for recruiting E3 ubiquitination machinery.

MAP kinase pathways—Mitogen-activated protein kinases (MAPKs) transduce signals from the cell surface to the nucleus. MAPK activity is regulated through three-tiered cascades composed of a MAPK, a MAP2K [also named MEK (MAPK/ERK kinase) and MKK (MAPK kinase)], and a MAP3K [also named MAPK kinase kinase or MEKK (MEK kinase)] (Figure 1). When triggered by a mitogenic stimulus, the extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) MAPKs are phosphorylated and activated by MEK1/2. In response to epidermal growth factor (EGF) or serum treatment, this activated state is only transient, because ERK1/2 can be dephosphorylated byMAPKphosphatases, whose expression is induced downstream of ERK activation. In contrast, a hyperosmotic stimulus results in sustained activation of ERK1/2, which is eventually downregulated by ERK1/2 protein degradation mediated by an upstream MAP3K, MEKK1, which in addition to its Ser/Thr kinase catalytic domain has a variant RING domain [this was initially classified as a plant homeodomain (PHD)], with Ub ligase activity, that promotes ubiquitination of ERK as well as c-Jun and Fra2 (32,33). Abrogation of MEKK1 and ERK1/2 interaction through mutation of the ERK1/2 docking site reduces sorbitol-induced ERK1/2 degradation and thereby osmotic stress-induced apoptosis. MEKK1 activity is also required for JNK1-dependent activation of the HECT domain E3 Itch and subsequent JunB and c-Jun degradation in T cells (33). MEKK1 autoubiquitinates, and this depends on its kinase activity and RING integrity (33). In addition to autoubiquitination, the C-terminal fragment of MEKK1 containing the kinase domain interacts with and is ubiquitinated by the RING finger protein Deltex, which functions as a suppressor of T-cell activation through selective degradation of MEKK1 (34).

MEKK2, another member of the MEKK MAP3K family, interacts with and is ubiquitinated and degraded by Smurf1, a C2-WW-HECT domain E3 ligase. Loss of Smurf1 leads to accumulation of phosphorylated MEKK2 and activation of the downstream JNK signaling cascade, which subsequently enhances osteoblast activity and increases bone mass (35). The c-Raf-1 MAP3K is degraded by the UPS when cell adhesion is disrupted, and the c-Mos MAP3K, which is a regulator of oocyte maturation, is primarily ubiquitinated at K34 and degraded soon after fertilization or activation of *Xenopus* eggs (36,37).

ERK7, despite being expressed at mRNA levels comparable to those of ERK2, is present at considerably lower levels in tissues and cultured cells than ERK2; in part this is because, ERK7 is constitutively active, and is rapidly degraded by a Skp1-Cullin-F box (SCF) complex (38). The 20 residues at the N terminus of the kinase domain are a primary determinant of ERK7 degradation (38). Like ERK7, ERK3 is also an unstable protein and is constitutively degraded by the UPS; this is independent of its kinase activity but dependent on two regions in the N-terminal lobe of its kinase domain. ERK3 accumulates during cell differentiation, and expression of stabilized forms of ERK3, in which part of its N-terminal lobe is replaced by the corresponding ERK1-derived sequences, causes G_1 arrest (33).

The prototype MAP3K, Ste11 in *Saccharomyces cerevisiae*, mediates mating and high-osmolarity glycerol and filamentous growth responses. Ste11 phosphorylates and activates Ste7 (MKK), which, in turn, phosphorylates and activates two MAPKs, Fus3 and Kss1. Ste11, Ste7, Fus3, and Kss1 form a complex with the scaffold protein Ste5 in the mating pathway (39). Pheromone stimulation results in UPS-dependent degradation of Ste11 and Ste7. This requires Doa4 (Ubp4p) and Ubp3, respectively (40,41), which are deubiquitinases (DUBs) that remove and recycle Ub from ubiquitinated proteins before degradation. Ste5, which is a scaffold that assembles the kinase components of this pathway, has a RING finger domain, and this might play a role in the ubiquitination of Ste11 or Ste7. In *Drosophila*, the expression of Wallenda, a MAP3K homologous to vertebrate DLK and LZK, is regulated by a RING finger protein, highwire (42).

Thus, activated MAPK signaling can be downregulated by UPS-mediated protein degradation at several levels.

Phosphatidylinositol 3-kinase, AKT, and mTOR—Phosphatidylinositol 3-kinase (PI3-K) is a lipid kinase consisting of an 85-kDa regulatory subunit bound to a 110-kDa catalytic subunit. PI3-K catalyzes the production of phosphatidylinositol-3,4,5-trisphosphate and thereby contributes to the activation of various signaling components involved in the regulation of gene expression and cell survival. Tyrosine-phosphorylated c-Cbl, whose identification as the first RING-type E3 ligase led to the idea that other RING finger-containing proteins may have the same function (43,44) (Figure 2), interacts with, ubiquitinates, and degrades the p85 subunit in multiple experimental systems (45,46). In addition, Cbl-b has been shown to downregulate bone formation through suppression of insulin-like growth factor (IGF)-1 signaling, including degradation of PI3-K and AKT in osteoblasts (47). p85 was also shown to be ubiquitinated by Cbl-b in a T-cell system, dependent on an interaction between a distal C-terminal proline-rich region in Cbl-b and the c-Src homology (SH)3 domain of p85. In this case, however, the ubiquitination of p85, instead of leading to p85 degradation, inhibits the recruitment of p85 to CD28 and to T-cell antigen receptor (TCR)ζ and CD28 (45).

AKT/protein kinase B is one of the major downstream targets of PI3-K. Platelet-derived growth factor (PDGF) and IGF-1 activateAKT in vascular smooth muscle cells accompanied by rapid degradation of AKT mediated by the UPS. Downregulation of AKT requires PI3-K activity but not intrinsic AKT activity (48). Deprivation of vascular endothelial growth factor (VEGF), inhibition of mTOR, or treatment with TNF-α results in both caspase-and proteasome-

dependent AKT degradation (49,50). Furthermore, AKT is preferentially degraded by the UPS in neuronal dendrites during the establishment of neuronal polarity, which is accelerated by inhibition of AKT activity (51). Cbl-b deficiency stabilizes the expression of AKT in osteoblasts during denervation, supporting a role for Cbl-b in AKT degradation. Undoubtedly, this important and conserved signaling pathway will prove to be regulated through ubiquitination at many levels, and a recent report indicates that mTOR, downstream of Akt, is degraded in a phosphorylation-dependent manner through SCF^{Fbw7}-mediated ubiquitination (52).

NIK—NF-κB-inducing kinase (NIK), a highly labile Ser/Thr kinase, is a critical regulator of the noncanonical NF-κB pathway. NIK phosphorylates and activates IKKα, which, in turn, phosphorylates the *NF-κB2* gene product p100. This subsequently results in processing of p100 to p52, an important functional member of NF-κB family. NIK is ubiquitinated and degraded by the RING finger E3 ligases, inhibitor of apoptosis (IAP) proteins c-IAP1 and c-IAP2, in a RING finger–dependent manner (53). c-IAP1 and c-IAP2 interact, via their baculovirus IAP repeat (BIR) domains, directly with TRAF2 and are recruited to TNF receptor 1- and 2-associated complexes, where they regulate receptor-mediated apoptosis (54). A c-IAP1 mutant that cannot associate with TRAF2 fails to affect NIK levels, suggesting that TRAF2 provides a critical scaffolding link between the E3 ligase c-IAP1 and its substrate NIK. Treatment with IAP antagonists or the TNF family cytokine TWEAK results in autoubiquitination and rapid proteasomal degradation of c-IAPs and leads to a remarkable increase in the levels of NIK, which initiates p100 processing to NF-κB2 (53).

In addition to regulating NIK stability co-ordinately by c-IAP and TRAF2, TRAF3 also interacts with NIK and targets proteasomal degradation of NIK, whereas noncanonical NF-κB stimuli induce degradation of TRAF3 and elevated NIK expression (55). TRAF3 deficiency results in NIK accumulation and constitutive noncanonical NF-κB activity. The rescue of early postnatal lethality of TRAF3 deficiency in mice by compound loss of the p100 gene further indicates that TRAF3 is a critical negative modulator of the noncanonical NF-κB pathway (56).

Serum- and glucocorticoid-induced protein kinases—Serum- and glucocorticoid-induced protein kinase (SGK, also referred to as SGK1) is a stress-induced Ser/Thr kinase that plays a critical role in insulin signaling, cation transport, and cell survival. SGK is ~50% homologous in the catalytic domain with AKT, cAMP-dependent protein kinase, and PKC, and can be phosphorylated and activated through a PI3-K-dependent signaling pathway. Steady-state SGK is rapidly degraded by the UPS. SGK degradation is independent of the catalytic activity and activation site phosphorylation; it requires a hydrophobic motif (GMVAIL; residues 19–24) and six lysines surrounding the GMVAIL motif. The hydrophobic motif is also necessary for SGK localized to the endoplasmic reticulum (ER), where SGK interacts with and is ubiquitinated by a cochaperone and Ub ligase, C terminus of Hsp70-interacting protein (CHIP) (57,58). The HECT domain Nedd4-2 E3 is another E3 that acts as a negative regulator of SGK. Moreover, SGK phosphorylation of Nedd4-2 potentiates Nedd4-2-mediated SGK ubiquitination and degradation (59).

Nonreceptor Tyrosine Kinases

Nonreceptor tyrosine kinases play a key role in transducing signals from surface receptors following ligand binding. Attenuation of receptor signaling is critical for precise control of cellular responses. This can be achieved by internalization and lysosomal degradation of the receptor proteins themselves, but there are also several mechanisms for downregulating nonreceptor tyrosine kinases following receptor-dependent activation, including

dephosphorylation and UPS-mediated degradation. The Cbl family of RING finger E3 ligases play a particularly important part in ubiquitination of activated nonreceptor tyrosine kinases.

Src—Src family tyrosine kinases (Src, Fyn, Yes, Lyn, Hck, Fgr, Lck, and Blk) are important signal transducers that modulate a wide variety of cellular functions downstream of surface receptors, and their constitutive activation leads to cellular transformation. c-Src is activated by the dephosphorylation of Y529 (Y527 in avian c-Src) at the C terminus and is inactivated through phosphorylation of this residue by the carboxyl-terminal Src kinase (Csk) and Csk-type protein tyrosine kinase. In *Csk*$^{-/-}$ cells, activated c-Src molecules are ubiquitinated and degraded. In addition, active c-Src Y527Phenylalanine (Phe, F) mutant, but not a kinase-dead c-Src mutant, is ubiquitinated and degraded (60,61). c-Cbl interacts with the Src SH3 domain through its proline-rich region, and phosphorylated Y419 (avian Y416) of c-Src through its TKB domain, mediating ubiquitination of active c-Src Y757F in vitro (62). Overexpression of c-Cbl or another family member, Cbl-3 induces the ubiquitination and downregulation of v-Src and suppresses v-Src-induced cell transformation (45).

Other Src family members are also regulated by Cbl. Src, Fyn, Yes, Lyn, and spleen tyrosine kinase (Syk) coimmunoprecipate with and phosphorylate c-Cbl, albeit at different levels (45, 63). c-Cbl interacts with the Lck SH3 domain, leading to Lck kinase activity–dependent ubiquitination of Lck. Consistently, c-Cbl–deficient T-cell lines have enhanced levels of active Lck, correlating with positive selection of $CD4^+$ thymocytes and thymocyte maturation, both of which are Lck-dependent events (63). Both active Fyn and Lyn are substrates of Cbls, and increased expression of Fyn and Lyn are detected in *c-Cbl*$^{-/-}$ cells (63,64). The expression levels of Lck and Lyn in thymocytes are elevated in mice with a loss-of-function mutation C379Alanine (Ala, A) in the c-Cbl RING finger domain, thus recapitulating the effects on Lck and Lyn in *c-Cbl* knockout mice (65). A membrane-anchored form of c-Cbl ubiquitinates and degrades Hck, reduces total cellular tyrosine phosphorylation levels, and inhibits Hck-induced cellular transformation (45).

c-Abl—c-Abl is another nonreceptor tyrosine kinase whose activity is tightly regulated. The N-terminal half of c-Abl is closely related in structure to Src family kinases. However, unlike Src, c-Abl is not regulated by an inhibitory phosphate that interacts with the SH2 domain, but rather a myristoyl switch involving the N-terminal myristoyl group that serves the same purpose. c-Abl is activated through autophosphorylation and transphosphorylation events. Active and Tyr-phosphorylated c-Abl is unstable and undergoes UPS-mediated degradation (66). c-Abl can be activated by several factors, including Src family kinases, integrin engagement, DNA damage, growth factors, and oxidative stress (67). The expression of v-Src, activated SrcY527F, or Fyn Y528F, which can phosphorylate and activate c-Abl, results in decreased c-Abl stability. Y245 and Y412 are the two major phosphorylated tyrosines on activated c-Abl, and phosphorylation of these sites significantly increases c-Abl activity in vitro (68). In the inactive Abl structure, the SH3-SH2 domains form a clamp in which the SH2 domain is docked onto the C-lobe of the kinase domain, and the SH3 domain binds to the PXXP motif of the SH2-kinase linker where the second Pro is replaced with Y245. Phosphorylation of Y245 will dislodge the SH3 domain and disrupt the assembled structure, and phosphorylation of Y412 in the activation loop will stabilize a conformation that is compatible with substrate binding and catalysis (69). Increased stability is observed in a double Y245/412F mutant of c-Abl but not in an activated mutant with open conformation in which the intramolecular interaction between the Pro-rich region and the SH3 domain is disrupted. This indicates that phosphorylation on Y245 and Y412 of c-Abl contributes to the conformational change and subsequent c-Abl degradation (66). Distinct from the way in which autophosphorylation regulates c-Abl protein stability, Y261 of Arg (the second member of the Abl family) can be autophosphorylated or transphosphorylated by c-Abl, and this phosphorylation both contributes to Arg kinase activity and blocks c- Arg degradation by the

UPS in response to oxidative stress (70). The Arg-binding protein 2 (ArgBP2) adaptor protein, which is a substrate for both c-Abl and Arg, links c-Abl to c-Cbl, facilitates phosphorylation of c-Cbl by c-Abl, and promotes Cbl-directed ubiquitination and degradation of c-Abl (45).

Syk and ZAP-70—Syk and zeta-chain–associated protein kinase 70 (ZAP-70) are twin SH2-domain cytoplasmic tyrosine kinases with a highly conserved structure that are essential elements in cascades coupling immune receptors to intracellular responses. Syk is present in all hematopoietic cells, whereas ZAP-70 expression is restricted to T lymphocytes and natural killer (NK) cells. NK cells have a multimeric receptor complex consisting of the ligand-binding α subunit (CD16), which associates noncovalently with homodimers or heterodimers of the TCR-ζ and -γ chains. CD16 engagement on human NK cells rapidly induces recruitment of both Syk and ZAP-70 to the receptor complex, leading to their phosphorylation and activation, ubiquitination, and degradation (45,63). Syk downregulation is also observed in human basophils in response to IgE stimulation (71).

Cbl has been implicated in the regulation of Syk and Lyn. The c-Cbl TKB domain binds to phosphorylated Y316 and Y323 of Syk and aspartate (D) 290 and Y292 of ZAP-70 (63). Nevertheless, enhanced phosphorylation and activation—but not protein levels—of ZAP-70 and increased Lck and ZAP-70 association are found upon crosslinking of CD3 and CD4 in thymocytes from *c-Cbl C379A* knockin mice (a loss-of-function mutation in RING finger) and *c-Cbl* knockout mice (65). These results suggest that Syk or ZAP-70 ubiquitination by c-Cbl/Cbl-b does not lead to their degradation and that c-Cbl or Cbl-b may function as a regulator of the interaction between Syk/ZAP-70 and a phosphatase or an upstream kinase (45,63). Besides Cbl, it has been reported that the HECT domain of Nedd4 E3 ligases is required for ubiquitination of Syk and Lyn in cells infected with the latent membrane protein 2A of Epstein-Barr virus (72).

FAK—FAK is a ubiquitously expressed tyrosine kinase that is instrumental in integrating signals from integrins, which recognize extracellular matrix proteins, and receptor tyrosine kinases in processes, such as cell adhesion, survival, proliferation, and motility. Coordinated activation of integrins and the PDGF receptor results in increased expression of the suppressors of cytokine signaling (SOCS)1 (also referred to as JAB and SSI-1) and SOCS3 and SOCS-FAK interaction, which depends on theSH2and kinase inhibitory region (KIR) domains of the SOCS protein and FAK Y397 phosphorylation. SOCS proteins interact with elongins B/C via the SOCS box, and thereby associate with cullin-5 and Rbx1, forming a complex with E3 ligase activity that recognizes target proteins through the SH2 domain of the SOCS subunit. Overexpression of SOCS1 and SOCS3 in fibroblasts inhibits kinase activity and tyrosine phosphorylation of FAK and promotes polyubiquitination and degradation of FAK in a SOCS box-dependent manner (73). Signal-transducing adaptor protein (STAP)2, acting together with c-Cbl, also negatively regulates FAKexpression in T cells. STAP2 associates with c-Cbl while concomitantly interacting with the FERM domain of activated FAK via an SH2-like domain in STAP2 and colocalizing with FAK at focal adhesions. STAP2 deficiency or reduced c-Cbl expression enhances FAK protein levels and cell adhesion, whereas STAP2 overexpression induces UPS-dependent FAK degradation and a decrease in integrin-mediated T-cell adhesion to fibronectin (74).

JAK—Many cytokines, hormones, and growth factors elicit their cellular functions through the Janus family of protein-Tyr kinase (Jak)-STAT pathways. Ligand-induced receptor dimerization results in auto- and transphosphorylation of JAK2 on its activation loop and stimulation of its catalytic activity. JAK2, in turn, phosphorylates and activates STAT family transcription factors (75). Activation of JAK2, either by cytokine stimulation or by oncogenic TEL-JAK2 fusion, induces polyubiquitination and degradation of Tyr-phosphorylated JAK2 (75). Phosphorylation of Y1007 in the JAK2 activation loop, which binds to the SH2 domain

and an additional N-terminal 12 amino acids of SOCS1, is required for proteasomal degradation of JAK2. This binding also directly inhibits JAK2 kinase activity, probably through preventing the JAK2 substrates or ATP access to the catalytic pocket (75). Conversely, dephosphorylation of Y1007 by the SH2 domain-containing protein-tyrosine phosphatase (SHP)-2 stabilizes JAK2 (76). The C-terminal SOCS box of SOCS1 interacts with elongins B/C and Cul-2, which function as a Ub ligase together with Rbx1 in an SCF-type E3 complex (75). The functional recruitment of the Cul-2 complex through the SOCS box accelerates UPS-dependent degradation of JAK2 (75).

Cyclin-dependent kinases and other cell-cycle-related protein kinases—In addition to direct downregulation of protein kinases by UPS degradation, activated protein kinases can in some cases be shut down by destruction of their activating regulators or upregulation of their inhibitors. A good example of this type of regulation is the cyclin-dependent kinases (CDKs), which govern cell cycle progression. CDKs are activated by cyclin binding and inhibited by CDK inhibitors (CKIs). Although CDKprotein levels do not change significantly, the dynamic activities of CDKs during the cell cycle are regulated through expression, ubiquitination, and degradation of cyclins and CKIs (which are not covered in this review), both temporally and spatially, in addition to phosphorylation and dephosphorylation. Two major types of E3 ligases are involved in the regulation of cell cycle progression: the SCF complexes, which mainly control the transition from G_1/S and G_2/M, and the anaphase-promoting complex or cyclosome (APC/C), primarily required for mitotic progression and exit as well as G_1/S progression.

SCF complexes: The SCF complexes consist of three invariant subunits—SKP1, CUL1 [cell division cycle (Cdc) 53p in yeast], and the RING finger protein Rbx1/Roc1 (Hrt1 in yeast)—as well as a variable component known as an F-box protein. F-box proteins, of which there are ~70 in humans (77), bind to SKP1 through their F box, and recognize substrates through other domains in the F-box protein (78). Three classes of F-box proteins are defined based on their substrate recognition motifs: FBL [Leucine (Leu)-rich repeat (LRR) domain] and FBW (WD40 repeats), which can recognize target degrons in a phosphorylation-dependent manner through their WD40 or LRR domains, and others, where the nature of the recognition domain and the degron that is recognized has only been identified in a few cases (79). SCF complexes, working in concert with the UBC3/Cdc34 E2 (which binds to Rbx1), control G_1-S progression and target G_1 cyclins (Cln1/2p in yeast and cyclins D and E in mammals) and CKIs (Sic1 and Far1 in yeast and p27 and p21 in mammals) for degradation (80,81).

Cyclin D: The activation of CDK4/6 by cyclin D1 binding in response to mitogenic signaling results in phosphorylation of Rb, thereby initiating E2F-mediated gene expression and sequestration of p27, which is required for progression into S phase. Whereas free and nonphosphorylated cyclin D1 can also be ubiquitinated, glycogen synthase kinase (GSK)-3β-mediated phosphorylation of T286 of nuclear cyclin D1 during S phase results in its translocation to the cytoplasm, where it is ubiquitinated and degraded by the proteasome. Binding of cyclin D1 to CDK4 enhances T286 phosphorylation by GSK-3β and ubiquitination of cyclin D1 (82). Pin1, a *cis*/*trans* peptidyl-prolyl isomerase that acts only on phosphorylated Ser/Thr-Pro bonds, directly binds to cyclin D1 phosphorylated at Thr286–Pro, increases nuclear cyclin D1 levels, and stabilizes cyclin D1. Cyclin D1 expression is reduced in many tissues in *Pin1*$^{-/-}$ mice (83). Although whether cyclin D1 is a direct substrate of SCF^{SKP2} is not yet clear, T286-phosphorylated cyclin D1 is recognized and ubiquitinated during S phase by $SCF^{Fbx4/\alpha B\text{-crystallin}}$, in which the heat shock protein αB-crystallin functions in concert with the Fbx4 F-box protein (84). Knockdown of either Fbx4 or αB-crystallin attenuates cyclin D1 ubiquitination, enhances cyclin D1 expression, and accelerates cell-cycle progression from G_1 to S phase. In addition, GSK3β phosphorylates Fbx4 at S12, which leads to Fbx4

dimerization and cyclin D1 ubiquitination. The detection of mutations in the N-terminal regulatory domain including the GSK3β phosphorylation site in Fbx4 or reduced expression of Fbx4 and αB-crystallin in a subset of primary human cancers that overexpress cyclin D1 (84,85) implies that impairment of $SCF^{Fbx4/\alpha B\text{-}crystallin}$ function is a potential mechanism underlying cyclin D1 overexpression in human cancer.

Cyclin E: Cyclin E accumulates at the G_1/S-phase boundary, where it is required for activation of CDK2 and initiation of DNA replication, and is degraded as cells progress through S phase (86,87). Both free and CDK2–complexed cyclin E are ubiquitinated and proteasomally degraded. Free cyclin E is ubiquitinated in a phosphorylation-independent manner by a CUL3-containing E3 complex (88). In contrast, cyclin E in association with CDK2 is ubiquitinated in a phosphorylation-dependent manner by a CUL1-containing SCF complex (86). The different tissue-expression patterns of upregulated cyclin E protein in *CUL1*$^{-/-}$ and *CUL3*$^{-/-}$ mouse embryos suggests that different E3s predominantly control the abundance of cyclin E in different tissues (88,89). Human cyclin E in complex with CDK2 is phosphorylated on at least four different sites: T62 (murine T74), S372, T380 (murine T393), and S384; T380 and S384 are primarily phosphorylated by GSK3 and CDK2, respectively (86). Phosphorylation of cyclin E on all these residues except S372 is important for its binding to the WD40 domains of the Fbw7 F-box protein and the turnover of cyclin E mediated by SCF^{Fbw7} (86). *Cyclin E T393A* or *T74/393A* knockin mice exhibited increased cyclin E stability and proliferative anomalies in hematopoietic and epithelial lineages, consistent with a failure to degrade cyclin E appropriately (86).

The three Fbw7 splice variants, α, β, and γ, form homodimers or heterodimers through the D domain, and Fbw7 dimerization enhances the turnover of a weakly associated cyclin E S384A mutant (86). Among the Fbw7 isoforms, $SCF^{Fbw7\alpha}$ serves as an essential cofactor for Pin1-dependent prolyl isomerization of cyclin E, which potentiates the ubiquitination of cyclin E by $SCF^{Fbw7\gamma}$. Knockout of *Fbw7*α or *Pin1* elevated cyclin E expression (86). Consistently, highly expressed cyclin E accompanied with Fbw7 mutations that result in a nonfunctional polypeptide or protein have been detected in several types of human cancers (86). These findings suggest that deregulation of CDK activity, resulting from defective ubiquitination and degradation of their positive regulators, may contribute to tumor development. Cyclin E is also reportedly regulated by other E3 ligases, including SCF^{SKP2} (87).

Checkpoint kinase 1: Checkpoint kinase 1 (Chk1) is a major effector of S-phase checkpoint signaling during the cellular response to genotoxic stress and is regulated by SCF complexes. Replicative stress induces polyubiquitination and degradation of Chk1, which is dependent on phosphorylation of Chk1 at S345 by ATR. Cul1 and Cul4A selectively associate with Chk1 following genotoxic stress, which induces S345 phosphorylation. Co-expression of both cullins caused an additive increase in Chk1 ubiquitination, and depletion of both proteins had an additive effect on stabilization of Chk1, suggesting that the activation-dependent ubiquitination of Chk1 is carried out by an SCF containing either Cul1 or Cul4A (90). A Cul1 complex containing the Fbx6 F-box protein may in part be responsible for degradation of activated Chk1.

APC/C: Sharing remarkable structural similarities with the SCF complex, the APC/C is a large multisubunit E3 ligase complex, consisting of catalytic core components [including APC11 (an Rbx1-related RING finger protein) and APC2 (a CUL1-related scaffold protein)] as well as a variable component known as an activator (77,91). Cdc20 and Cdh1 (also named HCT1), two known activators in mitotically cycling cells, and a meiosis-specific adaptor, Ama1 (in budding yeast), confer substrate specificity in the same way that F-box proteins do in the SCF complex, and recognize substrates containing D box, KEN box, A box, or O box motifs (92). In yeast two E2s act in sequence to promote the two steps in polyubiquitin chain assembly on targets of APC/C: Ubc4 (also known as UbcH5) monoubiquitinates APC/C substrates at

multiple lysines, whereas Ubc1 (in budding yeast) or UbcH1/E2-25K (a human homolog of Ubc1) catalyzes K48-linked polyubiquitin-chain assembly on preattached Ubs in a manner dependent on the UBA domain in a Ubc1 (93).

APC/C regulates mitosis and exit from mitosis by ubiquitination and degradation of D box–containing mitotic cyclins (cyclin A and B in mammals) and other mitotic proteins (Figure 3). APC^{Cdc20} is active from mid- to late mitosis, whereas APC^{Cdh1} is activated at late mitosis, remains active through G_1 phase, and is extinguished at the G_1–S boundary (77). In vertebrates, binding of cyclin A to Cdc2/CDK1 is required for entry into mitosis, and association of cyclin A with CDK2 is required for progression through S phase. The activation of APC^{Cdc20} at the onset of prometaphase occurs through CDK1-mediated APC/C phosphorylation and/or $SCF^{\beta\text{-}TrCP}$-mediated degradation of the APC/C inhibitor early mitotic inhibitor 1 (Emi1), following phosphorylation by polo-like kinase (Plk) 1 (94,95). Further enhancement of APC/C activation can be achieved by UPS degradation of the CDK1 inhibitor $p21^{CIP1}$ by APC/C^{Cdc20}, which forms a positive feedback for activation of CDK1 (96). The interaction of tumor suppressor Ras association domain family 1 (RASSF1A) with Cdc20 is another means of inhibiting APC/C, and the *RASSF1A* gene is commonly silenced in lung cancer and other sporadic tumors (97). Activation of APC/C^{Cdc20} initiates the degradation of cyclin A, thereby inhibiting CDK1. A nondegradable cyclin A mutant with an N-terminal domain deletion maintains hyperactivated CDK1 and arrests cell cycling at mitosis (82). In contrast to cyclin B1, the proteolysis of cyclin A is not delayed by the spindle assembly checkpoint (98). The mitotic checkpoint complex (MCC) composed of Cdc20, Mad2, Mad3 (BubR1 in metazoans), and Bub3 prevents Cdc20 from activating APC/C, thus blocking anaphase onset. Mad2 stimulates the association between Mad3 and Cdc20 (99). Dissociation of Mad2 and BubR1 from Cdc20 mediated by UbcH10 and $p31^{comet}$, a protein that binds Cdc20-bound Mad2, promotes Cdc20 to activate APC/C and exit from the mitotic checkpoint. This dissociation process involves Cdc20 multi-ubiquitination by APC/C and is counteracted by the deubiquitinating enzyme Ub-specific protease 44 (USP44) (100).

APC/C^{Cdc20} and APC/C^{Cdh1} mediate the degradation of cyclin B (Clb2 in yeast) (92,101). Cyclin B degradation begins in metaphase and inactivates its binding partner CDK1, which allows the removal of inhibitory phosphate residues from Cys protease separase by protein phosphatases. The dephosphorylation of separase together with the APC/C-dependent proteolysis of the separase-binding protein securin (Pds1 in budding yeast/Cut2 in fission yeast) results in activation of separase, which, in turn, cleaves the cohesin protein complex. Dissolving cohesion between sister chromatids allows their separation (77,92). Cyclin B binds to separase and inhibits its activity. Conversely, separase that is free of securin binds to cyclin B and inhibits CDK1 (102). Expression of a nondegradable cyclin B with a D-box mutation blocks CDK1 inactivation and exit from mitosis (103). The high levels of cyclin B and arrest in metaphase at the two-cell stage of embryos lacking Cdc20 function indicate an essential role of Cdc20 in degradation of cyclin B and in mitosis. Cdc20 and securin double-mutant embryos cannot maintain a metaphase arrest, suggesting that securin participates in preventing mitotic exit (104). Securin is ubiquitinated and regulated by both APC/C^{Cdc20} and APC/C^{Cdh1} (105).

The binding of dephosphorylated Cdh1 to the APC during mitotic exit mediates the destruction of Cdc20 (92). The dephosphorylation of Cdh1 can be a combinatorial consequence of inactivation of CDK1 by APC/C^{Cdc20}-mediated degradation of cyclin A and B and removal of inhibitory phosphorylation from Cdh1 by Cdc14 phosphatase (106). At the G_1-S transition, Cdh1 is rephosphorylated by CDKs and disassociates from APC, allowing reaccumulation of cyclins until APC/C binds Cdc20 in the subsequent mitosis (92,103) (Figure 3). Cdh1 can be phosphorylated at different sites in the same domain and inhibited by CDK1 and the CDK-related kinase Ime2. These two kinases overlap functionally as inhibitors of APC/C^{Cdh1} and license chromosome replication during meiosis (107). Aside from inhibition of Cdh1 by

rephosphorylation, Emi1 may also contribute to inhibition of APC/C^{Cdh1}. The expression of Emi1 is stimulated at the G_1-S transition by the E2F transcription factor and stabilized in S/G_2 phase by Evi5-mediated antagonism of $SCF^{\beta\text{-}TrCP}$-dependent Emi1 ubiquitination and destruction (100). In addition, inactivation of APC/C^{Cdh1} can also be facilitated by degradation of its E2 UbcH10 by APC/C^{Cdh1} (108), autoubiquitination of Cdh1 in G_1 and G_0 (109), and reduction of Cdh1 at S phase by SCF family-mediated ubiquitination (110).

Other APC/C-regulated protein kinases include Bub1, Aurora A/B, Nek2A, and Plk family members such as Plk1 (vertebrate), Polo kinase (*Drosophila*), Plx1 (*Xenopus*), and Cdc5 (budding yeast) (100,111). Plk1 regulates both the Wee1 kinase and the Cdc25C phosphatase, which, in turn, control CDK1 activity at the G2 to M transition. Plk1 and cyclin B/CDK1 phosphorylate and inhibit Wee1, which inactivates CDK1 by phosphorylation of T15 and T14 on CDK1. Plk1 and cyclin B/CDK1 can constitute another autoactivating feedback loop by phosphorylation and activation of Cdc25C, which dephosphorylates and activates CDK1 (112). Plk1 and Cdc5 accumulate during late G_2/M phase and their degradation is initiated at the start of anaphase, which is regulated by APC/C^{Cdh1}. Nondegradable Plk1 or Cdc5 causes a reduction in APC/C activity, a defect in the destruction of mitotic cyclins, and a delay in mitotic exit (113,114). Bub1, which inhibits APC/C^{Cdc20} activity by phosphorylating Cdc20, cooperates with Aurora kinase to promote binding of the mitotic checkpoint complex to the APC/C^{Cdc20} (115,116). The protein levels and activities of the Bub1 and Aurora kinases peak in mitosis and drop drastically in G_1 through the action of APC/C^{Cdh1} (117,118).

Interplay between SCF complexes and APC/C: Cdh1 is actively proteolyzed during S phase. Depletion of Cul1 or expression of a dominant-negative Cul1 results in Cdh1 accumulation, implicating the SCF family in the degradation of Cdh1 at S phase (110). Conversely, both the SKP2 F-box protein and its cofactor Cks1 are unstable in G_1 phase, ubiquitinated and rapidly degraded by APC/C^{Cdh1} upon activation of transforming growth factor (TGF)β/Smad signaling (119–121). In contrast, depletion of Cdh1 in G_1 cells stabilizes SKP2 and Cks1, with consequent degradation of $p21^{CIP1}$ and $p27^{Kip1}$ and an increased percentage of cells in S phase (119,120). Phosphorylation of SKP2 by CDK2 and CDK1 stabilizes SKP2 by interfering with its recognition and degradation by APC/C^{Cdh1}, which is reversed by dephosphorylation by the mitotic phosphatase Cdc14B during the M to G_1 transition (122). Expression of a stable SKP2 mutant that cannot bind APC/C^{Cdh1} induces premature entry into S phase. Thus, the induction of SKP2 and Cks1 degradation in G_1 represents a mechanism by which APC/C^{Cdh1} prevents the unscheduled degradation of $SCF^{SKP2\text{–}Cks1}$ substrates and maintains the G_1 state (119, 120).

The importance of interplay between SCF complexes and APC/C is also exemplified by the regulation of Wee1. Wee1 family protein kinases that inhibit CDK1 during the G_2 phase of the cell cycle are ubiquitinated and downregulated at the onset of mitosis (123). The F-box protein Tome-1 interacts with and ubiquitinates phosphorylated Wee1 in *Xenopus* egg extracts. Depletion of Tome-1 inhibits Wee1 degradation and mitotic entry; this mitotic entry defect can be rescued by removing Wee1, suggesting that Wee1 is a substrate of $SCF^{Tome\text{-}1}$. Tome-1, in turn, is a substrate of APC^{Cdh1} and is degraded during the G_1 phase, which allows Wee1 accumulation during interphase (124). In mammalian cells, $SCF^{\beta\text{-}TrCP}$, another SCF E3, appears to play a more critical role in Wee1 degradation and entry into mitosis than Tome-1; phosphorylation of S53, S121, and S123 of Wee1 A by Plk1, CK2, and CDK1, respectively, is required for direct binding to β-TrCP, ubiquitination, and degradation (125,126). Dynamic control of Wee1 and Tome-1 expression by SCF and APC/C complexes contributes to the precise regulation of cyclin B/CDK1 activity and thus mitotic entry and exit.

RECEPTOR PROTEIN KINASES

Ligands bind to and thereby activate receptor protein kinases by inducing dimerization and trans-autophosphorylation, resulting in the activation of a plethora of cellular processes. The receptors must then be inactivated so that the signaling can be switched off. Defects in this inactivation process can lead to cancer. Activation–dependent receptor internalization and ubiquitination-dependent degradation, integrated with feedback from serine/threonine phosphorylation and tyrosine dephosphorylation of receptors, play key roles in switching off ligand-induced activation of receptor protein kinases. Following ligand activation and autophosphorylation, the major mechanism of receptor kinase downregulation depends on receptor ubiquitination, which triggers internalization and trafficking of receptors to the lysosome for degradation rather than for UPS degradation (Table S2). In addition, K63-linked polyubiquitination of the receptors may be required for targeting to lysosomes, as has been reported for EGFR (127). Although the mechanisms for degrading activated nonreceptor and receptor tyrosine kinases are different, the Cbl family E3 ligases play an important role in both systems. Mutations that abrogate interaction with Cbl are a common type of oncogenic mutation in receptor tyrosine kinases. The Nedd4 and Itch family HECT domain E3 ligases, which can associate with membranes via their C2 domains, also regulate multiple receptor kinases.

Receptor Tyrosine Kinases

Receptor tyrosine kinases comprise over half of the 90 tyrosine kinases in the human genome (1). Most of them bind to specific protein ligands, such as growth factors and cytokines, via their extracellular domain, which results in activation of the cytoplasmic catalytic domain upon ligand-mediated oligomerization and phosphorylation of cytoplasmic proteins, thereby transducing extracellular signals across the plasma membrane (3).

EGFR family—The EGF receptor family of tyrosine kinases has four members—EGFR, ErbB2, ErbB3, and ErbB4. EGFR, ErbB3, and ErbB4 bind ligands in the EGF family. EGFR, ErbB2, and ErbB4 are active kinases, whereas ErbB3 lacks catalytic activity. Upon ligand binding, these receptors can heterodimerize, thus generating distinct intracellular signals depending on the combination. For instance, ligand-induced interaction of any of the three active receptors in combination with ErbB3 results in activation of the PI3-K pathway (2).

EGFR ubiquitination by c-Cbl: Ligand-induced downregulation of active EGFR has been extensively studied. EGF induces ubiquitination and downregulation of EGFR. The finding that kinase-dead EGFR is resistant to ubiquitination and is degraded more slowly illustrates the importance of EGFR activation for priming its own degradation (128). Sli-1, the Cbl ortholog in *Caenorhabditis elegans*, was first identified as a negative regulator of the Let-23 receptor, an EGFR homolog (129). This suggested that c-Cbl could negatively regulate EGFR function. The c-Cbl protein has an N-terminal tyrosine kinase–binding (TKB) domain, a linker region, a RING finger domain, a RING finger C-terminal flank or tail domain, extensive Pro-rich regions, poly-Pro-Arg (PXXXPR) motifs, and a UBA/LZ domain in its C-terminal half. The TKB domain consists of three interacting domains: a four-helix bundle (4H), a Ca^{2+}-binding EF hand, and a variant SH2 domain (130) (Figure 2). Mutation of the conserved Cys (RING) reduces c-Cbl–induced degradation and ubiquitination of EGFR (44), demonstrating an essential role of the c-Cbl RING finger in EGFR degradation. c-Cbl E3 activity is autoinhibited by the TKB domain and linker regions. c-Cbl phosphorylation at Y371 and Y368 may lead to a conformational change that relieves autoinhibition and activates c-Cbl (131). The c-Cbl UBA domain homo- or heterodimerizes with Cbl-b along a large hydrophobic surface formed by helices 2 and 3 and binds Ub at the first helix of the UBA. Cbl-b dimerization

is regulated by Ub binding and is required for tyrosine phosphorylation of Cbl-b and ubiquitination of Cbl-b substrates (132,133).

Upon EGF stimulation, phosphorylated c-Cbl is recruited to he cell surface before the EGFR is internalized (134–136). c-Cbl associates with the EGFR at the plasma membrane and colocalizes in late endosomes and multivesicular bodies (MVBs), suggesting that c-Cbl associates with EGFR throughout the endocytic route (137). The E2 ubiquitin-conjugating enzyme Ubc4/5 that cooperates with c-Cbl is relocated to the plasma membrane, and then to Hrs-positive endosomes, strongly suggesting that EGFR continues to be ubiquitinated after internalization (138). The kinase activity of EGFR and phosphorylation of EGFR at Y1045 that binds to the TKB domain of c-Cbl is necessary for ubiquitination of the EGFR (139, 140). In addition, EGF-induced Y1045 phosphorylation and ubiquitination of EGFR and c-Cbl phosphorylation at Tyr residues require p38 activity (141). The direct link of Y1045 to cancer was evidenced by identification of a truncated EGFR in human glioblastoma that had an intact kinase domain but was missing Y1045 and the internalization signals (142). Mutation of Y1045 in the constitutively active EGFRVIII, which lacks a large part of the extracellular domain and has been detected in various cancer types, inhibits its ubiquitination and downregulation by Cbl-b and enhances its ability to transform cells (143).

Mass spectrometry analysis has identified EGF-induced ubiquitination on six distinct lysines within the EGFR kinase domain, and mutation of Y1045 greatly reduced but did not abolish ubiquitination on these residues (127). The residual ubiquitination could be at-tributable to other E3 ligases or to c-Cbl recruited in a Y1045-independent manner. The latter possibility is supported by the observation that Grb2, which binds directly to phosphorylated Y1068 and Y1086 in activated EGFR, interacts with c-Cbl proline-rich domains via its SH3 domain and recruits Cbl to EGFR. In essence, Cbl may also be recruited to EGFR indirectly by Shc, which forms a complex with Grb2 and binds to phosphosphorylated Y1148 and Y1173 of EGFR (45,144). Mutation of the six Lys residues in the kinase domain largely prevented EGFR ubiquitination and degradation but not its Tyr phosphorylation and internalization (127). Consistently, *c-Cbl*$^{-/-}$ cells show a deficiency in EGFR ubiquitination and degradation, but not in its internalization into early endosomes (145). Moreover, a kinase-dead EGFR mutant was resistant to ubiquitination but retained the ability to internalize EGF and escape degradation (146). These results strongly suggest that receptor ubiquitination by Cbl is not required for initial internalization but is required for sorting the receptors at the early endosome and transit to the lysosome for degradation instead of recycling back to the cell membrane. In addition to using Ub to regulate the EGFR, c-Cbl also mediates EGFR modification with NEDD8. EGF stimulates EGFR neddylation, which requires the RING and TKB domains of Cbl, as well as Y1045 of EGFR. EGFR neddylation, in turn, enhances the subsequent ubiquitination and sorting of EGFR for degradation. Multiple Lys residues within the EGFR kinase domain serve as attachment sites both for NEDD8 and Ub (147). These results suggest that EGFR neddylation, in coordination with ubiquitination, accelerates sorting of EGFR for lysosomal degradation.

EGFR can be phosphorylated and inhibited by ligand-induced activation of PKC. PKC-mediated phosphorylation at T654 inhibits ligand-induced phosphorylation, ubiquitination, and degradation of EGFR and diverts internalized EGFR from degradation to the recycling pathway (148,149). Downregulation of EGFR can be inhibited by the binding of RALT to EGFR and promoted by the binding of the transmembrane protein LRIG1 to ERFR and c-Cbl, and the association between ACK1 and activated EGFR (45,150,151).

EGFR regulation by Cbl-b, Cbl-3, and oncogenic Cbls: Cbl-b and Cbl-3, the other two members of the Cbl family, also accelerate EGFR degradation (140). EGFR activation results in coordinated degradation of Cbl-b along with its substrate Shc and Grb2, which requires an

intact RING finger and the TKB domain of Cbl-b and binding of Cbl-b to the activated EGFR (152). Notably, activated EGFR interacts with Shc and Grb2 not only at the plasma membrane but also in the endosomal compartment, where EGF induces augmented recruitment of Shc and Grb2. This implies that the three proteins remain associated during the early phase of the endocytic process (153,154). Nedd4 and Itch, WW domain HECT E3s, also bind Cbl-b and target it for proteasomal degradation. Nedd4 reverses Cbl-b's effects on EGFR ubiquitination and downregulation of EGFR as well as active c-Src (155), thus providing an additional level of indirect regulation for Cbl substrates. c-Cbl and Cbl-b have important overlapping functions including in ubiquitination and degradation of activated EGFR, and c-Cbl- or Cbl-b-deficient mice are viable and fertile, but the loss of both is embryonically lethal (156,157).

v-Cbl, a truncated protein consisting of only the first 355 amino acids, and 70Z-Cbl, a deletion mutant lacking 17 internal amino acids that overlap the RING finger, are two RING finger–defective oncogenic forms of c-Cbl (44,139). However, RING finger mutations in c-Cbl that abolish E3 ligase activity are insufficient for cell transformation. In contrast, mutations within a highly conserved α-helical structure linking the SH2 and RING finger render Cbl proteins oncogenic. The linker helix mutations also cause a loss of ubiquitination and downregulation of EGFR, possibly through disruption of contacts with both the TKB domain of Cbl and the Ubc (158). Thus, in addition to its pivotal role in E3 function, the linker helix of c-Cbl is a critical determinant of Cbl's ability to transform cells possibly through precisely regulating the position and orientation of the SH2-bound substrate relative to the RING finger-bound E2 enzyme (159). The other possible explanation is that linker helix mutations allow the TKB domain of oncogenic Cbl to bind with higher affinity to targets, like EGFR, than the c-Cbl TKB domain does, and therefore compete more effectively for EGFR binding. As a result, the negative regulation of endogenous c-Cbl on receptor tyrosine kinases is much reduced.

EGFR in the early phases of endocytosis: Whereas the SH2 domain and RING finger are critical for ubiquitination of EGFR, the C terminus including the poly-Pro-Arg motif of c-Cbl functions separately from its Ub ligase activity, interacting with SH3 domains at the N terminus of CIN85 and recruiting the constitutively associated CIN85-endophilin complex to the activated EGFR. Endophilin, which binds to CIN85 via its SH3 domains, modulates plasma membrane invagination during the early steps of endocytosis (144). Sprouty2 forms a tertiary complex with CIN85 and c-Cbl, thereby preventing CIN85-mediated clustering of c-Cbl and blocking EGFR ubiquitination, whereas c-Cbl concurrently ubiquitinates Sprouty2 leading to degradation (144). The interplay between c-Cbl and Sprouty2 acts to fine-tune EGFR regulation. Likewise, the SH3 domain of β-Pix in complex with Cdc42 competes with CIN85 for binding to the poly-Pro-Arg motif of c-Cbl to inhibit c-Cbl-mediated EGFR downregulation. c-Cbl, in turn, promotes ubiquitination and subsequent degradation of β-Pix (45,160). In addition, ALG-2-interacting protein X (Alix), a CIN85-interacting protein, and the UBA-and SH3-containing protein T-cell Ub Ligand (TULA) also inhibit EGFR ubiquitination by binding to CIN85 or c-Cbl (161).

EGF induces c-Cbl/Cbl-b-mediated monoubiquitination of CIN85 and its homolog CMS (p130Cas ligand with multiple SH3 domains). Impaired monoubiquitination of CIN85 does not affect Cbl-directed EGFR ubiquitination, but blocks EGFR internalization and delays receptor degradation (144). Consistent with the role of monoubiquitination in endocytosis, the endocytic proteins, Eps15, Eps15R, epsins, spartin, HGF-regulated substrate (Hrs) [yeast ortholog vacuolar protein sorting (Vps) 27], Sts1, Sts2, and Ymer are mono- or multiubiquitinated in response to EGF. Eps15 and other two UIM-containing proteins, Eps15R and Epsin, are indispensable for EGFR internalization (162,163). The RING finger protein Parkin, whose mutation is responsible for a common familial form of Parkinson's disease, and Nedd4 are involved in the monoubiquitination of Eps15 and Hrs (164). The monoubiquitination of Eps15 depends on the binding of UIMs in Eps15 to the Parkin Ub-like domain. Knockout

of *Parkin* accelerates EGFR endocytosis and degradation, implying that Eps15 ubiquitination may interfere with the ability of the Eps15 UIMs to bind and control the downregulation of ubiquitinated EGFR (165,166). Monoubiquitination of UBD-containing proteins Eps15, Sts1, Sts2, and Hrs results in inhibitory intramolecular interactions between Ub and their UBD, thereby preventing them from binding in *trans* to ubiquitinated targets (167). Thus, monoubiquitination of UBD-containing proteins can terminate their interaction with the ubiquitinated receptor complex and allow other UBD-containing proteins to bind the ubiquitinated cargo as they move along the sorting pathway (168).

Among the regulators for EGFR endocytosis, Hrs and signal-transducing adaptor molecule (STAM), each containing a UIM domain as well as a VHS (Vps27, Hrs, and STAM) domain for STAM, associate with each other and localize on the cytoplasmic face of the early endosomal membrane. The Hrs-STAM complex is proposed to be the sorting receptor that recognizes the Ub moieties of activated receptor tyrosine kinases and introduces them into MVBs from early endosomes. Upon EGF stimulation, Hrs is phosphorylated on Y329 and Y334 in a Cbl E3 ligase–dependent manner, and phosphorylation of Hrs is required for the joint degradation of EGFR and Hrs. Depletion or deficiency of Hrs or STAM inhibits receptor tyrosine kinase downregulation in mammalian cells (144,161).

Both clathrin-dependent and lipid raft-dependent endocytic routes have been proposed for ubiquitinated EGFR molecules (144). EGFR internalized via clathrin-dependent endocytosis is not targeted for degradation, but instead is recycled to the cell surface. By contrast, lipid raft-dependent internalization preferentially commits the receptor to degradation (169).

EGFR in the late phases of endocytosis: Precisely how ubiquitination contributes to receptors being targeted for lysosomal degradation, instead of being recycled back to plasma membrane, is still a puzzle. Recent studies of protein sorting in MVBs in yeast and mammals may provide a link between ubiquitinated protein complexes and their final destination, the lysosome. MVBs are a subset of late endosomes; their typically multivesicular appearance results from the invagination and budding of the limiting (outer) membrane with associated cargo proteins into the compartmental lumen. Fusion of the limiting membrane of the MVB with the lysosomal membrane leads to the lumenal vesicles and their contents being degraded in the lysosome (vacuole in yeast). Membrane proteins, including nonubiquitinated proteins that are excluded from these inner lumenal vesicles and remain in the limiting MVB membrane, can ultimately be transferred to the limiting membrane of the lysosome, recycled back to the plasma membrane, or transported to other sites in the cell. Attachment of a single Ub to non-MVB cargo proteins is sufficient to drive these proteins into the MVB pathway (170). Consistently, ubiquitinated EGFR is sorted with EGF into MVB lumenal vesicles, which are degraded in the lysosome following fusion of the limiting membrane of the MVB with the lysosomal membrane (171). Kinase-dead EGFR mutants, which lack c-Cbl–mediated ubiquitination and degradation, are still able to internalize and reach the limiting MVB membrane, but are excluded from lumenal MVB vesicles and predominantly recycled back to the plasma membrane (139,140,146). This suggests that kinase activity and c-Cbl-dependent ubiquitination of EGFR and/or EGFR-associated monoubiquitinated endocytic proteins play a key role for EGFR being delivered to the lumenal MVB vesicles for subsequent degradation.

Active EGFR is dephosphorylated and loses its activity before localizing to late endosomes/lysosomes (172,173). Inhibition of proteasome activity or prelysosomal/lysosomal proteolysis reduces EGFR degradation, indicating the involvement of both the proteasome and lysosomes (140). Furthermore, proteasomal inhibitors inhibit both translocation of activated EGFR from the outer limiting membrane to inner membranes of MVBs and the decline in the level of ubiquitinated EGFR, implying that proteasomal activity is required for lysosomal sorting of EGFR and deubiquitination of the EGFR before its lysosomal degradation (134,174).

Efficient transport via endosomes relies on endosomal sorting complex required for transport (ESCRT) complexes. In yeast, ESCRT-I, which is recruited transiently to the endosomal membrane from the cytoplasm, is a conserved 350-kDa protein complex composed of Vps23 (mammalian ortholog tumor susceptibility gene Tsg101), Vps28, and Vps37, which are members of the 17 known class E Vps gene products (170). Hrs, in addition to its function in early endosome sorting, binds to Tsg101 via a PSAP motif and mediates the initial recruitment of ESCRT-I to endosomes, thereby indirectly regulating MVB formation. The Ub-conjugating-like domain of Tsg101/Vps23 is required for ESCRT-I to bind Ub in vitro, implying a direct role for Tsg101/ESCRT-1 in the recognition and sorting of ubiquitinated MVB cargo proteins (175). The Golgi-localized, γ-ear-containing, Arfbinding (GGA) protein binds Tsg101 as well as Ub via its VHS and GAT (GGA and TOM) domains and may coordinate with Tsg101 in sorting EGFR. In addition, ubiquitination and degradation of Tsg101 by two E3 ligases, Mahogunin and Tsg101-associated E3 ligase (Tal), contribute to regulation of EGFR degradation (175,176).

Other ESCRT complexes, such as ESCRT-II (a 155-kDa complex composed of Vps22, Vps25, and Vps36) and ESCRT-III whose endosomal recruitment depends on ESCRT-II, act further downstream to regulate protein sorting in the MVB pathway. ESCRT-III is composed of two functional subcomplexes: a membrane-proximal subcomplex that consists of Snf7/CHMP4a and Vps20/CHMP6, and a peripheral associated subcomplex that consists of Vps2/CHMP2a and Vps24//CHMP3 (177). The Snf7 and Vps20 subcomplex-dependent association of Vps2 and Vps24 with the endosomal membrane results in recruitment of Ub isopeptidases or DUBs as well as Vps4 (in yeast) or SKD1 (in mammals), an AAA-type ATPase that has a role in catalyzing the dissociation of all three ESCRT complexes from endosomes (144,170,175). The interaction of CHMP3 with associated molecule with SH3 domain of STAM (AMSH) and recruitment of AMSH to the MVB is necessary for deubiquitination and degradation of EGFR. Besides, AMSH, other DUBs, such as the Ub-specific protease Y (UBPY) [also designated as Ub-specific protease 8 (USP8), an ortholog of the yeast Doa4, and AMSH-like protein (AMSH-LP)] can also remove Ub residues from target proteins before they enter the MVB and thereby recycle Ub to the cytoplasm (161,175). How these DUBs coordinately deubiquitinate EGFR at the different stage of endocytosis is unclear.

Although further work is needed to define the precise molecular mechanisms of EGFR internalization and degradation, the accumulated evidence suggests the following model of EGF-induced EGFR endocytosis: EGFR that is autophosphorylated at Y1045 within its C-terminal domain recruits and interacts with the TKB domain of c-Cbl. c-Cbl associates with and mediates ubiquitination of the EGFR at several Lys and monoubiquitination of endophilin-bound CIN85 and ubiquitinated EGFR/c-Cbl/CIN85 forms a complex with the UIM-containing endocytotic proteins Eps15, Eps15R, epsins, and Hrs, which are monoubiquitinated by Nedd4 and Parkin, an event that may regulate the assembly/activity of various components of the endocytotic and sorting machinery. EGFR/c-Cbl complexes bound to endocytic proteins travel from early endosomes to late endosomes/MVBs, where Vps23/ESCRT-I recognizes the ubiquitinated EGFR and/or monoubiquitinated CIN85 and/or endocytic proteins. ESCRT-III-recruited DUBs remove the Ub from cargo proteins, thereby resulting in invagination of cargo protein into the lumenal vesicles of MVB. The translocation process of activated EGFR from the outer limiting membrane to the inner membranes of MVBs and deubiquitination of EGFR involves proteasomal activity that targets lumenal vesicles of MVB for fusion with lysosome for final degradation of EGFR (Figure 4 and Figure 5).

Ligand-independent degradation of EGFR: SOCS36E is implicated as a negative regulator of the *Drosophila* ortholog of EGFR. SOCS5, the mammalian ortholog of SOCS36E, associates with EGFR and promotes EGFR degradation in a ligand- and c-Cbl-independent manner. EGFR degradation requires the SOCS box of SOCS5, which mediates the binding of

the elongin B/C complex to recruit an E3 Ub ligase (178). Ligand-independent and constitutive endocytosis of the receptor involves Hrs. Hrs overexpression enhances the ubiquitination of EGFR and leads to translocation of EGFR to Hrs-containing endosomes, both of which depend on the integrity of the lipid-binding motif of Hrs. Hrs itself is polyubiquitinated, which depends on its UIM that is endowed two functions: binding ubiquitinated proteins and recruiting Nedd4 Ub ligase for self-ubiquitination. The polyubiquitination or depletion of Hrs negatively modulates the endocytosis and degradation of EGFR (179).

EGF versus TGFα: Different EGF family ligands do not elicit EGFR downregulation in an identical fashion. EGF induces more sustained phosphorylation and ubiquitination of EGFR and subsequently more efficient degradation than does TGFα, though both ligands recruit c-Cbl to the plasma membrane and cause the same extent of initial ubiquitination and endocytosis of the EGFR. EGFR and c-Cbl colocalize in endosomes after EGF but not TGFα treatment. Moreover, EGF, which is more acidic than TGFα, remains complexed to the EGFR within MVBs, sustaining EGFR kinase activity and triggering lysosomal sorting. In contrast, most TGFα is dissociated in MVBs, allowing the EGFR to recycle to the plasma membrane (134).

ErbB2, ErbB3, and ErbB4: ErbB2 is associated with poor prognosis when overexpressed in cancers. ErbB2 can be activated by EGF through the formation of heterodimers with EGFR or by neuregulin through heterodimerization with ErbB3/ErbB4. ErbB2 ubiquitination and degradation can be induced by EGF, neuregulin 1β (a neuregulin-1 isoform), and tumor-inhibitory ErbB2-specific antibodies (e.g., trastuzumab). The binding of such antibodies to ErbB2 recruits c-Cbl to Y1112 of ErbB2, and a Y1112F mutation retards antibody-induced ErbB2 degradation (144,161,180). The involvement of c-Cbl in ligand-induced ErbB family protein degradation requires kinase activity and tyrosine phosphorylation in the C-terminal tails of ErbB proteins, and only the membrane fraction of receptors is targeted by the ligand-dependent mechanism (161). In contrast, the class of ErbB2 tyrosine kinase inhibitors that alkylate a prominent Cys residue uniquely positioned in the nucleotide-binding pocket of ErbB receptors and inhibit kinase activity, and an Hsp90 inhibitor, the ansamycin antibiotic geldanamycin (GA), both induce UPS-mediated degradation of both mature and nascent ErbB2. Moreover, this process appears to be mediated by an E3 ligase, CHIP, in complex with protein chaperones (181).

Nrdp1 (mouse FLRF) possesses an atypical RING finger domain and associates with and stimulates ErbB3 ubiquitination and degradation (161). In the presence of the ErbB3 ligand neuregulin-1, AKT phosphorylates the USP8 DUB at T907 and contributes to USP8 stability, which, in turn, suppresses the ubiquitination and degradation of Nrdp1. The accumulated Nrdp1 promotes ErbB3 ubiquitination (161). Overexpression of Itch does not alter levels of unstimulated EGFR, ErbB-2, or ErbB-3, but it interacts with ErbB4 receptors via its WW domains and promotes its polyubiquitination and degradation (161).

PDGF receptor family—The PDGF family consists of four protein chains that form five biologically active dimers (PDGF-AA, -AB, -BB, -CC, and -DD). Two types of receptors for PDGF, α (which binds PDGFA, -B, and -C chains) and β (which binds PDGF-B and -D chains), have been identified. The PDGFR can be downregulated through reduced mRNA expression, a process regulated by the proto-oncoprotein c-Myc (182) and by the immediate internalization and ubiquitination of the receptor. PDGFRβ was the first receptor tyrosine kinase found to be polyubiquitinated and degraded (183). A kinase-deficient or a C-terminal autophosphorylation site (Y1009/1021F) PDGFRβ mutant exhibits reduced ligand-induced ubiquitination and degradation and amplified mitogenic activity (184). In response to ligand stimulation, the c-Cbl TKB domain interacts with PDGFRα as well as phosphorylated Y1021 of PDGFRβ, a known phospholipase C (PLC)γ1 SH2 domain binding site. The TKB domain as well as an intact RING finger are required for c-Cbl to polyubiquitinate the PDGFR (185). PDGFRα

expression is elevated in the mammary glands of *c-Cbl*$^{-/-}$ mice and c-Cbl deficiency or ablation of the c-Cbl binding site on PDGFRβ enhances PDGF-induced PLCγ1 association with PDGFRβ and impedes receptor sorting to the lysosome (185,186).

PDGFR, like EGFR, is regulated by proteins associated with receptors and c-Cbl. CMS interacts with both c-Cbl and the small GTPase Rab4 and is involved in the regulation of early endosome morphology. Expression of a truncated form of CMS that retains the ability to interact with Rab4 but not with c-Cbl inhibits ligand-induced PDGFRβ degradation (187). In addition, ligand-induced ubiquitination and degradation of PDGFRβ is inhibited by the interaction of c-Cbl with Alix or the low-density lipoprotein receptor-related protein 1 (LRP1) (45,188).

Other members of the PDGFR family, the stem cell factor receptor c-Kit, and the CSF-1 receptor CSF-1R/c-Fms behave similarly in response to ligand stimulation. The ubiquitination and degradation of c-Kit is ligand-dependent and requires its intrinsic kinase activity and an involvement of c-Cbl (189,190). c-Fms in c-Cbl–deficient macrophages, which have higher proliferation rates, is ubiquitinated to a lesser extent, internalized at a slower rate, and is degraded by the lysosomal but not a proteasome-dependent mechanism (191). Direct binding of the c-Cbl TKB domain to phosphorylated Y568 and Y936 of c-Kit or Y973 (mouse; equivalent to Y969 in the human homolog) of c-Fms is required for ligand-induced ubiquitination, internalization, and degradation of c-Kit or c-Fms (189,192). Notably, Y568 and Y936 in c-Kit and Y973 in c-Fms are absent from oncogenic v-Kit and v-Fms, and deletion of c-Cbl binding sites from c-Kit greatly enhances their transforming ability (192,193). Consistent with these observations, mutations of the Cbl binding site are frequently observed in c-Fms in human myelodysplasia and acute myeloblastic leukemia, further implicating loss of c-Cbl binding in oncogenic deregulation of c-Fms in human cancer (194,195).

FGF receptors—In vertebrates, the 24 members of the FGF family have a high affinity for heparan sulfate proteoglycans and require heparan sulfate to activate one of four cell-surface FGFR tyrosine kinases. FGFs have diverse roles in embryonic development, bone morphogenesis, and tissue repair; are important for neuronal signal transduction in the central and peripheral nervous systems; and contribute to the pathogenesis of cancer when inappropriately expressed (196). Mutation at an autophosphorylation site (Y766) of FGFR1, which is essential for interaction with phospholipase C, results in decreased FGFR1 internalization and reduced ligand-induced FGFR1 degradation (197). Activated FGFR phosphorylates FRS2α, a docking protein containing a myristoyl anchor and a phosphotyrosine-binding (PTB) domain at the N terminus that associates with FGFR1 at the juxtamembrane region (amino acids 419–430), whereas the multiple tyrosine phosphorylation sites in the C-terminal tail of FRS2α serve as binding sites for adaptor proteins such as Grb2 and Shp2 (198–200). Grb2 constitutively binds to c-Cbl through both SH3 domains. This complex is recruited to the membrane and directly binds to the phosphorylated FRS2α through the SH2 domain of Grb2. This binding results in ubiquitination of FGFR and FRS2α itself in response to FGF stimulation. Thus, unlike EGFR and PDGFR, which form a direct complex with c-Cbl by way of its TKB domain, FRS2α functions as a link between FGFR and Grb2/c-Cbl complex. This contributes to the dual role of FRS2α for the recruitment of at least two multiprotein complexes, Grb2/c-Cbl and Grb2/SOS, which are responsible for both the activation and attenuation of signals. FGF-induced FGFR downregulation is only partially blocked in *FRS2α*$^{-/-}$ cells, indicating that attenuation of signaling by FGFR is regulated by redundant or multiple mechanisms (201). Several activating FGFR3 mutations, including G380R in the transmembrane domain as well as K650E and K650M associated with achondroplasia and thanatophoric dysplasia (TD) types II, may disrupt c-Cbl-mediated ubiquitination and degradation by altered conformation of receptor and allow the receptors to escape into a recycling pathway (202).

HGF receptor—Hepatocyte growth factor (HGF) [also known as scatter factor (SF)] binds and activates the widely expressed HGF receptor (HGFR)/Met receptor tyrosine kinase, mediating pleiotropic cellular responses. The human Met family has two members: Met (c-Sea in chickens) and RON (*recepteur d'origine nantais*) (203). Met is a cell surface–associated 190-kDa disulfide-linked heterodimer consisting of a 50-kDa α chain and a 140-kDa β chain. The α chain is entirely extracellular, and the β chain spans the membrane and possesses intrinsic, ligand-activated tyrosine kinase activity in its intracellular domain. In response to ligand binding, Met undergoes both multiple monoand polyubiquitination and degradation by proteasome- and lysosome-dependent mechanisms, which requires intact tyrosine kinase activity (203). Inhibition of proteasome activity does not affect initial HGF-dependent Met internalization. Instead, it promotes recycling from early endosomes at the expense of sorting to late endosomes, thereby ensuring the rapid return of internalized Met to the cell surface (204). Distinct from these reports, Met has also been shown to be downregulated in a proteasome-independent manner, which requires the formation of K48-linked polyubiquitin chains (205).

c-Cbl/Cbl-b can promote ubiquitination of the Met receptor, and this requires interaction of the c-Cbl/Cbl-b TKB domain with the phosphorylated juxtamembrane Y1003 residue in Met. Met juxtamembrane mutations that lead to decreased Cbl binding and prolonged Met protein stability and signaling have been identified in human lung cancer (206). An oncogenic form of Met, Tpr-Met, generated through chromosomal translocation of *Met*, and the oncogenic v-Sea receptor, derived from the chicken ortholog of Met, both lack the binding sites for c-Cbl and are not ubiquitinated (203). Although the Met Y1003F mutant is resistant to ubiquitination and degradation and is oncogenically active, it is internalized and undergoes endosomal trafficking with kinetics similar to those of WT Met (203,207), implying that phosphorylation at Y1003 is critical to targeting Met to components of the lysosomal sorting machinery, whereas phosphorylation of other Tyr residues may be important for the early stages of Met endocytosis. In agreement with this, phosphorylation of Y1349 and Y1356 of Met is essential for ligand-induced Met internalization. Phosphorylated Y1356 is a site for indirect c-Cbl binding via Grb2. Depletion of Grb2 or abrogation of c-Cbl E3 activity blocks Met internalization, which can be rescued by expression of a chimeric protein in which the SH2 domain of Grb2 is tagged at the C terminus c-Cbl. These findings indicate that Cbl E3 ligase activity is critical for Met internalization and suggest a role for Grb2 as an intermediary link between Cbl and this process (203). Met internalization is mediated by clathrin-dependent endocytosis, and expression of a dominant-negative GTPase mutant of dynamin, which inhibits pinching off of invaginating coated pits, reduces the rate of ligand-induced Met receptor downregulation. In addition, as is the case for EGFR, a complex of Met receptor, endophilins, CIN85, Hrs, and Cbl controls the endocytosis of Met (203).

The steady-state level of Met can be regulated by the transmembrane protein LRIG1. Met interacts with the LRIG1 independent of HGF stimulation, and LRIG1 destabilizes Met in a c-Cbl- and proteasome-independent, but lysosome-dependent manner (208). In addition to the regulation of Met by ubiquitination, Met activity is negatively regulated at the transcription level by the RING finger protein Skeletrophin, whose direct relevant substrate is as yet unidentified (209).

Ron is a receptor for macrophage stimulating protein (MSP, also known as HGF-like protein or SF2) that belongs to the plasminogen-prothrombin gene family, which includes plasminogen and HGF among others (203). c-Cbl interacts with Ron at Y1017 (Met Y1003), Y1353 (the multifunctional docking site), and Y1360 (Grb2 binding site) and promotes receptor ubiquitination and internalization in a ligand- and Ron kinase activity-dependent manner. Phosphorylation at Y1017 and Y1353 is required for c-Cbl ubiquitination of Ron, whereas phosphorylation at Y1360 is more important for c-Cbl binding to Ron. In addition, CHIP can

act as an E3 ligase for Ron ubiquitination and degradation in response to Hsp90 inhibition by GA (210,211).

IGF-I receptor—IGFs, which include IGF-I and IGF-II, and their tyrosine kinase receptors (IGF-IR and IGF-IIR) exert potent mitogenic and differentiating effects on most cell types. IGF-IR is a heterotetrameric protein (α2β2) consisting of two identical α extracellular subunits containing a cysteine-rich IGF-binding site and two β transmembrane subunits bearing intrinsic tyrosine kinase activity. IGF-IR activation depends on an ATP-binding site (K1003) and a cluster of three Tyr residues at positions 1131, 1135, and 1136, which are autophosphorylated upon ligand binding (212). Exposure to IGF-I leads to phosphorylation of the IGF-IR β chain, which is subsequently ubiquitinated, internalized, and degraded, in a process mediated by Grb10 that connects IGF-IR to Nedd4; this requires both proteasome and lysosome activity in some cell lines but is dependent only on UPS in other cells (164). The RING finger protein Mdm2, an E3 ligase for p53, associates with and ubiquitinates ligand-unstimulated IGF-IR with involvement of β-arrestin1, the adaptor molecule to bridge Mdm2 and IGF-1R (213, 214). UV radiation-upregulated expression of p53 indirectly induces the expression of IGF-IR by sequestering Mdm2 in the nucleus, thereby reducing Mdm2-mediated IGF-IR degradation. Conversely, inhibition of p53 expression results in Mdm2-dependent ubiquitination and degradation of IGF-IR (213,214). Also, the heat shock proteins Hsp10 and Hsp60 are involved in regulation of IGF-IR by inhibition of Ub conjugation on IGF-IR. Overexpression of Hsp10 and Hsp60 increases the abundance of IGF-IR by suppression of receptor polyubiquitination, IGF-I-stimulated receptor autophosphorylation, and activation of downstream signaling. Insulin induces expression of Hsp60, and diabetic myocardium is associated with decreased abundance of Hsp60, increased ubiquitination of IGF-1R, and reduced levels of IGF-1R protein (215,216).

Receptor Serine/Threonine Kinases

In contrast to the large number of receptor tyrosine kinases, there are only 12 receptor serine/threonine kinases (1). They are all members of a single extended family that binds ligands in the TGFβ family of cytokines and transduces signals that promote growth arrest and differentiation. Signaling by this type of receptor kinase is accomplished through the binding of aTGFβ family member, which induces the formation of a heterodimer containing a type ITGFβ receptor and a type II TGFβ receptor. Within this dimer, the constitutively active type II receptor phosphorylates the inactive type I receptor in the complex, and thereby activates it to phosphorylate downstream cytoplasmic target proteins (3).

TGFβ receptors—Heteromeric complexes of the TGFβ receptor type I, encoded by seven mammalian genes, and theTGFβ receptor type II, encoded by five mammalian genes, are transmembrane Ser/Thr kinase receptors. Twenty-eight members of theTGFβ superfamily, which includes TGFβ isoforms, activins, and bone morphogenetic proteins (BMPs), bind to heteromeric complexes of the receptors, which initiate signals controlling growth, differentiation, and apoptosis of cells and have important functions during embryonic development. Type I receptors, which are transphosphorylated and activated by type II receptors upon ligand binding, mediate specific intracellular signals. Type I receptors phosphorylate receptor-activated Smads (R-Smads including mammalian Smad1, -2, -3, -5, and -8) and initiate Smad signaling facilitated by the Smad anchor protein called Smad anchor for receptor activation (SARA) (217,218). The inhibitory Smads (I-Smads) Smad6 and Smad7 can negatively regulate TGFβ signaling through association with activated type I receptors, thereby preventing phosphorylation of R-Smads (217,218). Phosphorylated R-Smads form heteromeric complexes with common-partner Smads (Co-Smads) Smad4 and then translocate into the nucleus, where they modulate transcription either alone or, more commonly, by interacting with DNA-binding partners. Active R-Smads are continuously dephosphorylated

in the nucleus, resulting in their dissociation from Smad4. Smad2/3 and Smad4 recycle back to the cytoplasm via a nuclear export receptor, using CRM1-independent and -dependent mechanisms, respectively. The activity of TGFβ receptors is dynamically controlled after ligand stimulation, and if the receptors are still active at the plasma membrane, the R-Smads are rephosphorylated, form complexes with Smad4, and return to the nucleus. If the receptors are no longer active, the R-Smads are retained in the cytoplasm (219,220). These findings indicate that the Smads are constantly shuttling between the nucleus and the cytoplasm for the duration of active signaling and that the levels of receptors, which are regulated by ubiquitination, may directly determine how long a pool of activated Smads remains in the nucleus, which, in turn, may influence the biological response to TGFβ.

Type I receptor is sumoylated in response to TGFβ and its sumoylation requires the kinase activities of both type I and II receptors (221). Ligand-dependent endocytosis and downregulation of TGFβ receptor complex requires the kinase activity of type II but not type I receptors, even though the kinase activity of both receptors is required for TGFβ-induced signaling (222). This suggests that phosphorylation of the type I receptors or other substrates by type II receptors is necessary for efficient receptor trafficking and downregulation. Upon ligand stimulation, TGFβ receptors are internalized through both clathrin- and caveolae (lipid raft)-dependent mechanisms. Clathrin-dependent endocytosis internalizes receptors into early endosomes, where the Smad2 anchor SARA is enriched. Inhibition of this endocytosis blocks TGFβ-dependent signaling without affecting the half-life of the receptors. In contrast, the lipid raft domain mediates the internalization of Smad7-Smurf2 bound receptor, and interference with lipid raft function stabilizes the receptors and slightly enhances signaling and Smad2 activation. These results support the importance of clathrin-dependent endocytosis for TGFβ signaling through the Smad-dependent pathway, whereas lipid rafts are required for rapid degradation of TGFβ receptors (217).

TGFβ stimulates the expression of Smurf2, a C2-WW-HECT domain Ub ligase, via a PI3-K/AKT-dependent pathway (223). Nuclearly localized Smurfs bind to Smad7 and induce the nuclear export of Smad7, which depends on the nuclear export signal in a C-terminal region of Smurfs and CRM1 (224). The N-terminal conserved 2 (C2) domain of Smurfs associates constitutively with Smad7 primarily through an interaction with a PPXY sequence (PY motif) of Smad7. The recruitment of the complex, in which Smad7 functions as an adaptor, to the activated TGFβ receptor causes degradation of receptors and Smad7 through the catalytic activity of the Smurf2 HECT domain via proteasomal and lysosomal pathways (217,218). Loss of Smurf1 does not affect Smad-mediated intracellular TGFβ or BMP signaling (35), implying a functional redundancy between endogenous Smurf1 and Smurf2. Acting similarly to Smurfs, the other two C2-WW-HECT E3 ligases, TGIF-interacting Ub ligase 1 (Tiul1)/WWP1 and Nedd4-2, also constitutively associate with Smad7 and induce degradation of the activated type I receptor as well as Smad2, subsequently inhibiting TGFβ and BMP signaling (217,218).

In contrast to its role in complexing with an E3 ligase for degradation of TGFβ receptor, Smad7 can also act as an adaptor to recruit Ub C-terminal hydrolase 37 (UCH37), a DUB, to the type I TGFβ receptor for deubiquitinating and stabilizing the receptor (217). This finding suggests that competing effects of E3s and DUBs in complex with Smad7 can serve to fine-tune responses to TGFβ under various physiological and pathological conditions. The stability of Smad7 is also regulated by histone acetyltransferase p300-dependent acetylation. Acetylation of Smad7 at K64 and K70, which are also likely ubiquitination sites, or mutation of these Lys residues prevents ubiquitination and degradation of Smad7 mediated by Smurf1. Activation of TGFβ signaling decreases the acetylation of Smad7 and promotes Smad7 degradation (217), implying that acetylation and ubiquitination may compete at the same Lys residues for regulation of smad7.

The TGFβ signaling pathway is regulated at many other levels by ubiquitination, and this provides a good example of the complex manner in which signaling pathways are regulated by ubiquitination. For instance, in addition to degradation of TGFβ receptors and Smad7, Smurfs also exhibit E3 ligase activity toward other Smad proteins. When overexpressed, Smurf2 interacts with and ubiquitinates Smad1 and Smad2, leading to inhibition of their transcriptional activity. Similarly, overexpressed Smurf1 interacts with Smad1 and Smad5 in vitro, mediates their degradation in mammalian cells, and inhibits BMP signaling, thereby affecting embryonic pattern formation in *Xenopus* and BMP-induced osteogenic conversion (217,218). Distinct from regulation of Smad2 in response to TGFβ signaling by UPS, Itch promotes Smad2 ubiquitination, facilitates complex formation between TGFβ receptor and Smad2, augments Smad2 phosphorylation, and enhances TGFβ-induced transcription without altering protein levels of Smad2, Smad4, and Smad7. Knockout of *Itch* results in reduced susceptibility to TGFβ-induced cell growth arrest and decreased phosphorylation of Smad2 (217,218).

Aside from its role in desensitization of TGF signaling, Smurf2 also positively regulates TGF signaling by its involvement in downregulation of SnoN. SnoN and c-Ski, nuclear corepressor proteins of the Ski family regulating TGFβ-responsive genes, are rapidly ubiquitinated and degraded by Smurf2 in response to TGFβ signaling, which is mediated by Smad2-dependent interaction between Smurf2 and SnoN (217,218). TGF stimulation also induces the assembly of the complex consisting of Smad2/3, SnoN, and APC^{Cdh1}, in which Smad3, and to a lesser extent Smad2, interact with both the APC^{Cdh1} and SnoN. The APC^{Cdh1} regulates ubiquitination and degradation of SnoN with the possible involvement of the E2 UbcH5 (218). Arkadia, a RING finger E3 ligase, is another E3 interacting with SnoN and c-Ski in their free state as well as in the forms bound to Smad proteins that downregulated the levels of SnoN and c-Ski proteins. This regulation requires formation of a complex between Arkadia and phosphorylated Smad2 or Smad3 (225,226). Arkadia, cooperating with the scaffold protein Axin, is also involved in polyubiquitination and degradation of Smad7 (218). In addition to targeting three major negative regulators of TGFβ signaling—Smad7, SnoN, and c-Ski—Arkadia terminates TGFβ signaling by ubiquitinating and degrading phosphorylated and activated Smad2/3, which may promote a rapid resetting of TGFβ signaling after its activation. *Arkadia*$^{-/-}$ embryos have a similar phenotype to *Smad2*$^{-/-}$ embryos and cannot form foregut and prechordal plates (227). Thus, Arkadia fine-tunes TGFβ signaling by controlling both negative and positive regulators of this pathway.

Mass spectrometry analysis has shown Smad4 to be mono- or oligo-ubiquitinated at K507 of the MH2 domain. Mono- or oligoubiquitinated Smad4 has enhanced ability to oligomerize with R-Smads, whereas K507 mutation results in inefficient Smad4/R-Smad hetero-oligomerization and defective transcriptional activity. Thus, oligo-ubiquitination positively regulates Smad4 function (228). The importance of precise Smad4 regulation has been demonstrated in studies of early vertebrate development and tumor formation. TGFβ signals are involved in the formation of mesoderm and endoderm, whereas ectoderm specification requires attenuation of the TGFβ response. This is achieved by Ectodermin (Ecto), a RING finger E3 ligase, which binds and ubiquitinates Smad4, thus restricting the mesoderminducing activity of TGFβ/BMP signals to the mesoderm. Depletion of Ecto in human cells enforces TGFβ-induced cytostasis; overexpression of Ecto or missense mutations in the MH1 domain of Smad4, which leads to the proteasomal degradation of Smad4, has been found in colon cancer (217,218). Misregulation of Smad4 is also exemplified in other types of tumors. Smad4 interacts with the F-box proteins β-TrCP1 and SKP2 present in the $SCF^{β\text{-}TrCP1}$ and SCF^{SKP2} E3 ligases, leading to ubiquitination and degradation. Smad4 mutants derived from pancreatic tumors or acute myelogenous leukemia exhibit increased interaction with β-TrCP1 or SKP2 and significantly elevated UPS-dependent degradation, suggesting that downregulation of Smad4 by mutationinitiated ubiquitination participates in tumor development (217,218).

Downregulation of Smad4 by Smurf1/2 and by Tiul1/WWP1 and Nedd4–2, which requires complex formation with Smad2 or Smad6/7 that act as adaptor proteins, is also observed (217,218).

Aside from regulation by ubiquitination, endogenous Smad4 is sumoylated at K113 and K159 in the MH1 domain by the conjugating enzyme Ubc9 and by PIAS1 and PIASxβ, members of the PIAS family of SUMO ligases. Five PIAS members have been identified in mammals, specifically PIAS1, -3, -xα, -xβ, and -y. Sumoylation of Smad4 may serve to protect Smad4 from Ub-dependent degradation (229,230).

In contrast to Smad4, Smad3 as well as Smad7 associate with PIASy, stimulating sumoylation of Smad3 and suppressing TGFβ-mediated Smad3 activation (231). In addition to sumoylation, Smad3 is ubiquitinated by the $SCF^{\beta\text{-}TrCP1}$ E3 complex and PRAJA, a RING-H2 protein, in response to TGFβ signaling (217,218). Basal-expressed Smad3 as well as Smad1 and Smad4 can be directly ubiquitinated and degraded by CHIP independent of TGFβ signaling, whereas Axin facilitates GSK3β-mediated phosphorylation of Smad3, triggering Smad3 ubiquitination and degradation (217,218). In addition to regulation by the UPS and sumoylation, Smad3 is transcriptionally repressed upon TGFβ1 activation, which may represent another feedback loop controlling TGFβ signaling (232).

In summary, TGFβ signaling is regulated through the UPS pathway by several E3 ligases at multiple levels, initiated from TGFβ receptors, downstream Smads including R-, Co-, and I-Smad, and the Ski nuclear corepressors SnoN and c-Ski. The temporal and spatial control of this signaling has a pivotal role in many cellular functions and in embryonic development. Dysregulation or mutation of TGFβ signaling components such as Smad4 can contribute to cancer formation.

G protein–coupled receptor signaling and GRK2—The GRK2 Ser/Thr kinase is a central regulator of G protein-coupled receptor (GPCR) signaling and a critical regulator of a wide variety of biological processes. GRK2 is a short-lived protein and is degraded by the UPS; this requires GRK2 activity and can be enhanced upon agonist stimulation of GPCRs such as β_2-adrenergic receptor (AR), βAR, and CXCR. Interfering with GRK2 degradation potentiates GPCR desensitization. After agonist stimulation, the adaptor molecule β-arrestin binds to GPCRs and mediates GRK2 phosphorylation at Y13/86/92 by recruited c-Src, which facilitates phosphorylation of GRK2 S670 by ERK MAPK. Mutation of these phosphorylation sites retards GRK2 degradation (233,234). β_2AR activation promotes the binding of β-arrestin–mediated interaction between GRK2 and Mdm2. Mdm2-mediated ubiquitination of GRK2 at K19, K21, K30, and K31 is critical for GRK2 degradation. Moreover, IGF-IR activation enhances GRK2 stability by a mechanism involving the PI3-K/AKT pathway and the Mdm2 E3 ligase (235).

PROTEIN KINASE DEGRADATION MEDIATED BY PROTEIN KINASE–SPECIFIC CHAPERONES

Many protein kinases are associated with molecular chaperones, and the levels and activities of these kinases can be downregulated by the regulation of protein kinase/chaperone complexes. Among the many molecular chaperones, Hsp90 and Cdc37 are often observed in complex with signal-regulated protein kinases, and Cdc37 stabilizes client proteins following their interaction with Hsp90 and regulates protein kinase activity (236). Hsp90 associates with at least 105 protein kinases (237). Hsp90 recognizes a common surface in the N-terminal lobe within the kinase domain, and surface features, rather than a linear sequence motif, define the capacity of the Hsp90 chaperone machine to recognize client protein kinases (237,238).

Heterocomplexes of protein kinases with Hsp90/Cdc37 are assembled by a multiprotein chaperone system comprising Hsp90, Cdc37, Hsp70, Hop, Hsp40, and p23 (239). The Hsp90 family members contain an ATP-binding pocket, and the binding and hydrolysis of ATP are required for the refolding and release of the native protein from the chaperone complex (239). Hsp90 inhibitors, including radicicol, herbimycin-A, GA, and its derivative 17-AAG, bind tightly to the Hsp90 ATP/ADP pocket, prevent ATP binding and the completion of substrate protein refolding, and lead to disruption of protein kinase/chaperones complex. As a result, treatment with these inhibitors leads to UPS-dependent degradation of Hsp90-Cdc37 client proteins, including EGFR, ErbB2, PDGFR, IGF-I R, PI3-K, AKT, Src, Lck, Lyn, Raf1, Bcr-Abl, and CDK4/6 (240).

The expression of the functionally defective mutant Cdc37-1 in *S. cerevisiae*, the fission yeast *Schizosaccharomyces pombe*, and *Drosophila* decreases the levels of the cyclin-dependent protein kinase Cdc28, Spc1 stress-activated protein kinase, and Aurora B, respectively (241). That a transcription factor CCAAT/enhancer binding protein-α (C/EBPα) disrupts a chaperone CDK4/Hsp90-Cdc37 complex via direct interaction with CDK4 and induces UPS-mediated CDK4 degradation (242) further supports the notion that a functional Hsp90-Cdc37 complex is required for the stability and function of target protein kinases.

In addition to the complex formed between protein kinases and Hsp90/Cdc37, the cochaperone CHIP interacts with Hsp70 and Hsp90 through an N-terminal TPR domain and also displays E3 ligase activity mediated by its a C-terminal U box domain (239). Cochaperone Bag family members (BAG1-6 in humans) have a C-terminal BAG domain that associates with the ATPase domain of Hsp70, stimulating ADP-ATP exchange and client protein release (239). Bag1 associates with the 26S proteasome through an N-terminal UBL domain as well as through specific ubiquitin moieties appended to Bag1 by CHIP, in which the individual ubiquitins are linked through K11. The noncanonical polyubiquitin chain does not induce the degradation of Bag1; instead, it stimulates UBL domain-dependent association of Bag1 with the proteasome (243). Bag1 accepts substrates that can be activated by Bag1, such as Raf-1, from Hsp70, and presents Raf-1 to CHIP for ubiquitination (244–246). Thus, Bag1 acts as a physical link between the Hsp70 chaperone system and the proteasome and recruits Hsp70 to the proteasome, resulting in trafficking of ubiquitinated client proteins to proteasomal degradation (239,244). Similarly, Hsp70- and CHIP-mediated degradation of ErbB2 (181), apoptosis signal-regulating kinase 1 (ASK1) (247), SGK1 (57), and activated dual leucine zipper-bearing kinase (DLK) (248) has been observed.

The ability of Hsp90 inhibitors to downregulate cellular protein kinases has led to their being tested as potential antitumor agents (240). Ron is ubiquitinated in response to GA or 17-AAG by CHIP, which is recruited via Hsp90 and Hsp70. The constitutively active and oncogenic Ron M1254T mutant that escapes c-Cblnegative regulation upon MSP stimulation is recruited into the CHIP-chaperone complex even more efficiently than WT Ron is. Ron M1254T displays increased sensitivity to GA, enhanced interaction with Hsp90, and an increased degradation rate. The transforming potential evoked by Ron M1254T is abrogated upon Hsp90 inhibition (211). These findings imply that Hsp90 antagonists are suitable pharmacological agents for therapy for cancers in which altered Ron signaling is involved.

CONCLUDING REMARKS

The protein kinases encoded by the human genome regulate and maintain physiological responses and normal cellular functions, including cell growth, differentiation, adhesion, motility, and death. Protein kinase activity is regulated positively or negatively by phosphorylation/dephosphorylation, oligomerization, the binding of second messengers, activating subunits and inhibitor proteins, protein cleavage, and intracellular translocation, as

well as by expression level. In the majority of cases, protein kinase activity is reversibly modulated through either phosphorylation/dephosphorylation or the binding of small molecule or protein regulators. However, it is increasingly apparent that activation of protein kinases, particularly if it is sustained, can initiate irreversible downregulation by UPS-mediated protein degradation, and that this can be an important mechanism of signal termination.

Several general principles apply to the recognition of activated protein kinases or their regulators for ubiquitination and degradation (249). First, autophosphorylation of a protein kinase can create binding sites for E3 ligases. In the case of receptor tyrosine kinases, ligand engagement results in phosphorylation of receptor tyrosines leading to recruitment of Cbl family E3 ligases either directly or indirectly via Grb2. Second, transphosphorylation of a protein kinase can create a phosphodegron that is recognized by a phospho-dependent SCF ligase (e.g.,Wee1 by Plk1 and CDK1, Chk1 by ATR, and cyclin E by GSK3 and CDK). Third, activation of a protein kinase results in an altered, active conformation that can be recognized by an E3 ligase. For instance, a common surface on the N-terminal lobe of the activated catalytic domain promotes CHIP-mediated ubiquitination (237,238). In addition, activation may expose a degron outside the catalytic domain in the protein that is otherwise masked in the inactive state (e.g., Chk1). Finally, most protein kinases are targeted by more than one E3 ligase for ubiquitination, with one or more E3 ligases acting to provide constitutive turnover, and other E3 ligases recognizing the activated protein kinase, meaning that multiple E3 ligases often collaborate to terminate kinase action.

Disruption of ubiquitination that regulates protein kinase directly or indirectly and constitutive activation of the protein kinase inevitably lead to human diseases, including cancer. Mutations of protein kinases themselves, such as oncogenic mutants of EGFR and c-Fms, v-Fms, v-Kit, and Tpr–Met, which lack the autophosphorylation site that recruits c-Cbl (195), or the regulatory components of ubiquitination machinery such as oncogenic Cbl, in which a mutation abolishes the E3 Ub ligase activity, result in escape from the surveillance of this Ub-dependent downregulation. Constitutively active kinases can also elicit oncogenic effects through UPS-mediated downregulation of growth-inhibitory proteins and tumor suppressors. For example, BCR-ABL and v-Src tyrosine kinases trigger UPS-dependent destruction of the Abi proteins, a family of Abl-interacting proteins that bind to and antagonize the oncogenic potential of Abl; expression of Abi is lost in cell lines and bone marrow cells from patients with aggressive BCR-ABL-positive leukemias (250).

We can foresee that the accumulating knowledge about the mechanisms of ubiquitination and degradation that regulate protein kinases will permit development of potent and pharmacological modulators of these processes, which may afford a new approach for treating human diseases.

SUMMARY POINTS

1. The activation of protein kinases commonly initiates their downregulation via the ubiquitin/proteasome pathway.
2. Autophosphorylation of a protein kinase can create a binding site for an E3 ligase.
3. Transphosphorylation of a protein kinase can create a phosphodegron that is recognized by a phospho-dependent SCF ligase.
4. Activation of a protein kinase can result in an altered, active conformation that can be recognized by an E3 ligase.
5. Protein kinase activation can expose a degron outside the catalytic domain in the protein that is otherwise masked in the inactive state.

6. Multiple E3 ligases often collaborate to terminate protein kinase action.
7. Disruption of ubiquitination and degradation of protein kinases leads to human diseases, including cancer.

Supplementary Material

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Glossary

Protein kinases, enzymes that modify other proteins by chemically adding phosphate groups to them
Tyr, Y, tyrosine
Ser, S, serine
Thr, T, threonine
phosphorylation, the addition of a phosphate (PO_4) group to a protein or other organic molecule
Ub, ubiquitin
UPS, ubiquitin-proteasome system
E3, Ub ligase
Ubiquitination, the posttranslational modification of a protein by the covalent attachment of one or more ubiquitin monomers
UBDs, Ub-binding domains
UBA, Ub-associated domain
UIM, Ub-interacting motif
PHD, plant homeodomain

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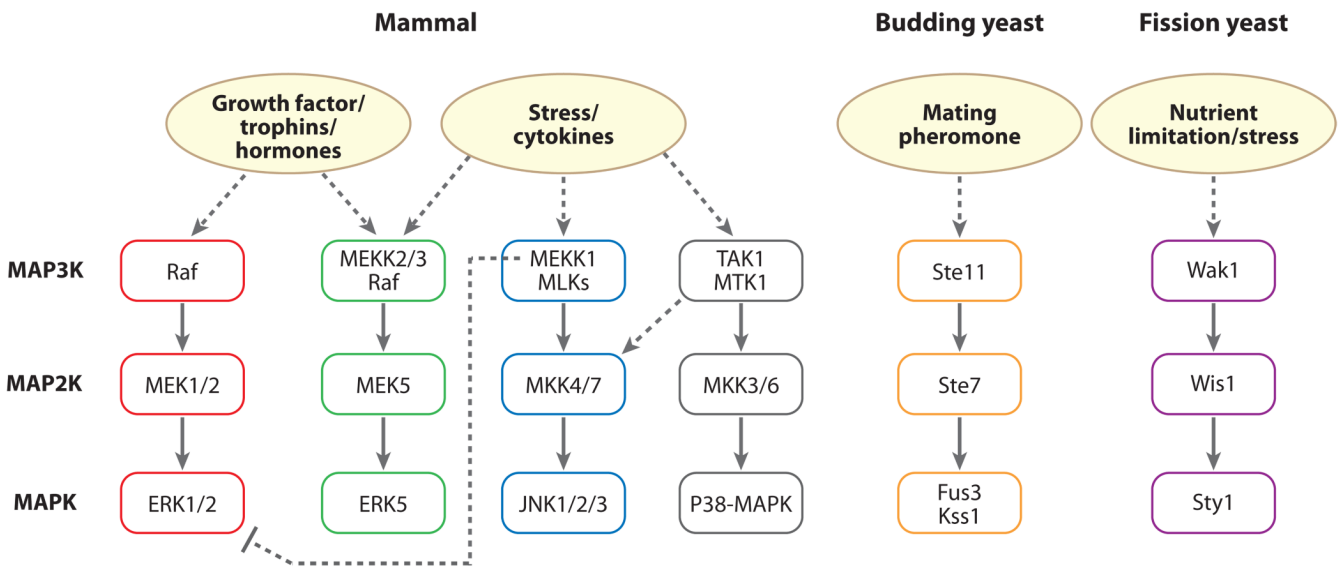

Figure 1.

Conventional (ERK1/2, JNK, and p38 MAP kinase) and ERK5 MAP kinase signaling pathways in mammalian cells and in yeast. Only representative signaling molecules and one of the multiple MAPK pathways for budding or fission yeast are shown. Solid arrows: main pathways; dashed arrows: branched pathways and indirect activation.

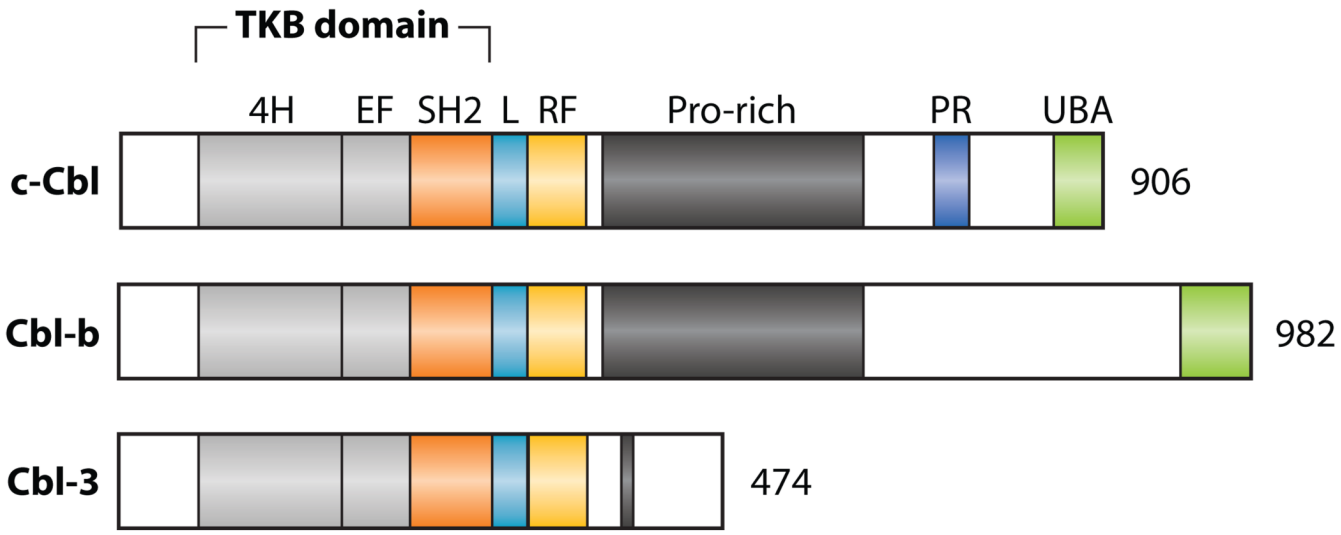

Figure 2.
Cbl protein family in mammals [c-Cbl, Cbl-b, and Cbl-3 (also known as Cbl-c)]. The Cbl proteins contain, from N to C terminus, a TKB domain, a linker region (L), RING finger domain (RF), Pro-rich regions, poly-Pro-Arg motif (PR), and UBA domain. The TKB domain consists of a four-helix bundle (4H), EF hand (EF), and a variant Src homology region 2 (SH2) domain. Cbl-3 lacks the PR motif and UBA domain.

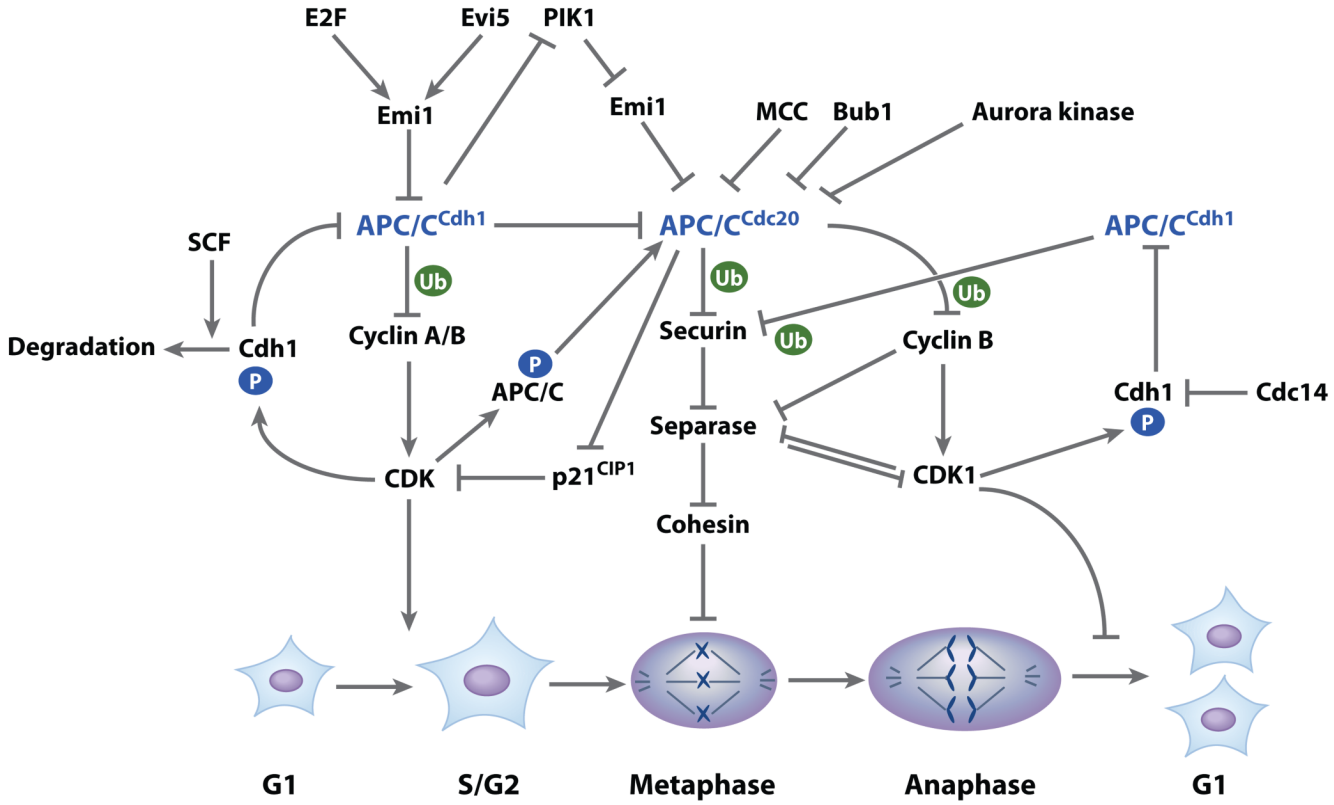

Figure 3.

Function and regulation of the APC/C E3 ligase during somatic cell cycle. At the G1-S transition, accumulated cyclin A and B dependent on the inhibition of APC/C^{Cdc20} by APC/C^{Cdh1} will activate CDKs and promote entry into S phase. In coordination with increased expression of Emi1 regulated by E2F and Evi5, activated CDKs phosphorylate Cdh1, inhibit APC/C^{Cdh1}, and cause subsequent accumulation of Plk1. During prometaphase and metaphase, APC/C^{Cdc20} is activated by combinatorial factors including downregulation of APC/C^{Cdh1}, CDK1-dependent APC/C^{Cdc20} phosphorylation, dissociation of MCC from APC/C^{Cdc20}, degradation of Bub1 and Aurora kinases by APC/C^{Cdh1} in G1 phase, and proteolysis of Emil by Plk1-dependent phosphorylation. Further enhanced activation of APC/C^{Cdc20} can be achieved by degradation of CDK1 inhibitor $p21^{CIP1}$ by APC/C^{Cdc20}. Activated APC/C^{Cdc20} initiates the degradation of cyclin A in prometaphase and cyclin B in metaphase and is involved in destruction of securin, which, in turn, reduces CDK1 activity and results in the dephosphorylation of Cdh1, in conjunction with Cdc14 phosphatase dephosphorylation, and activation of APC/C^{Cdh1}. Dephosphorylation of separase together with the APC/C^{Cdh1}-dependent proteolysis of securin (Pds1/Cut2) results in the activation of separase, cleavage of cohesin, and anaphase onset. Activated APC/C^{Cdh1} orchestrates the degradation of a wide spectrum of substrates, facilitating mitotic exit and cytokinesis.

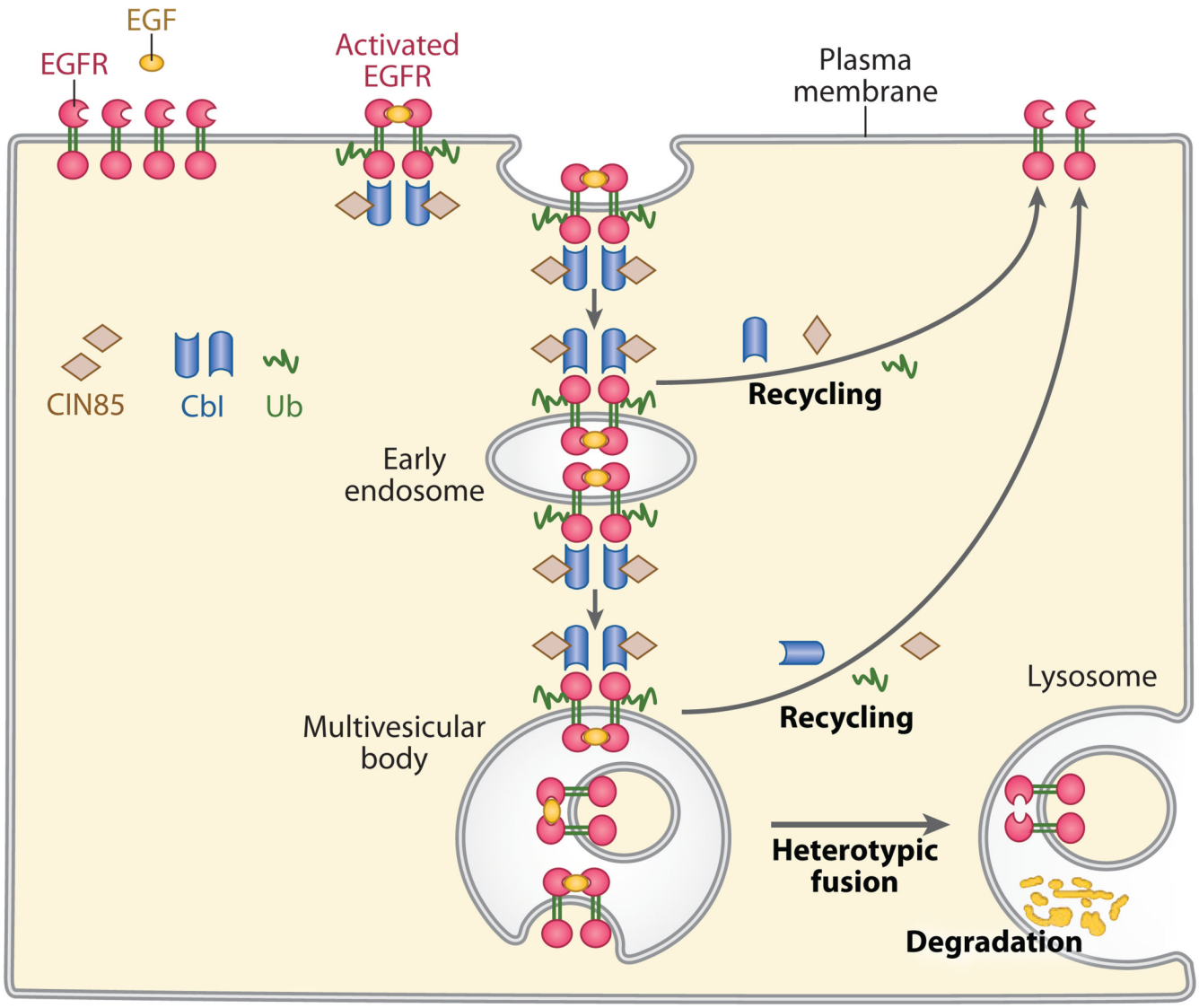

Figure 4.

An EGFR internalization model. Homodimerization and autophosphorylation of EGFR induced by EGF binding recruits and interacts with Cbl/CIN85 complex through the variant SH2 domain of Cbls. Cbls mediate the ubiquitination of EGFR and monoubiquitination of CIN85. EGFR/Cbl/CIN85 with other monoubiquitinated endocytic proteins such as Eps15, Eps15R, epsins and Hrs travel from early endosomes to late endosomes and MVBs. Subsequent invagination of cargo proteins into the lumenal vesicles of MVBs targets MVBs for fusion with lysosomes and then final degradation of EGFR. A certain proportion of EGFR will be deubiquitinated by DUBs and recycled back to the cell membrane.

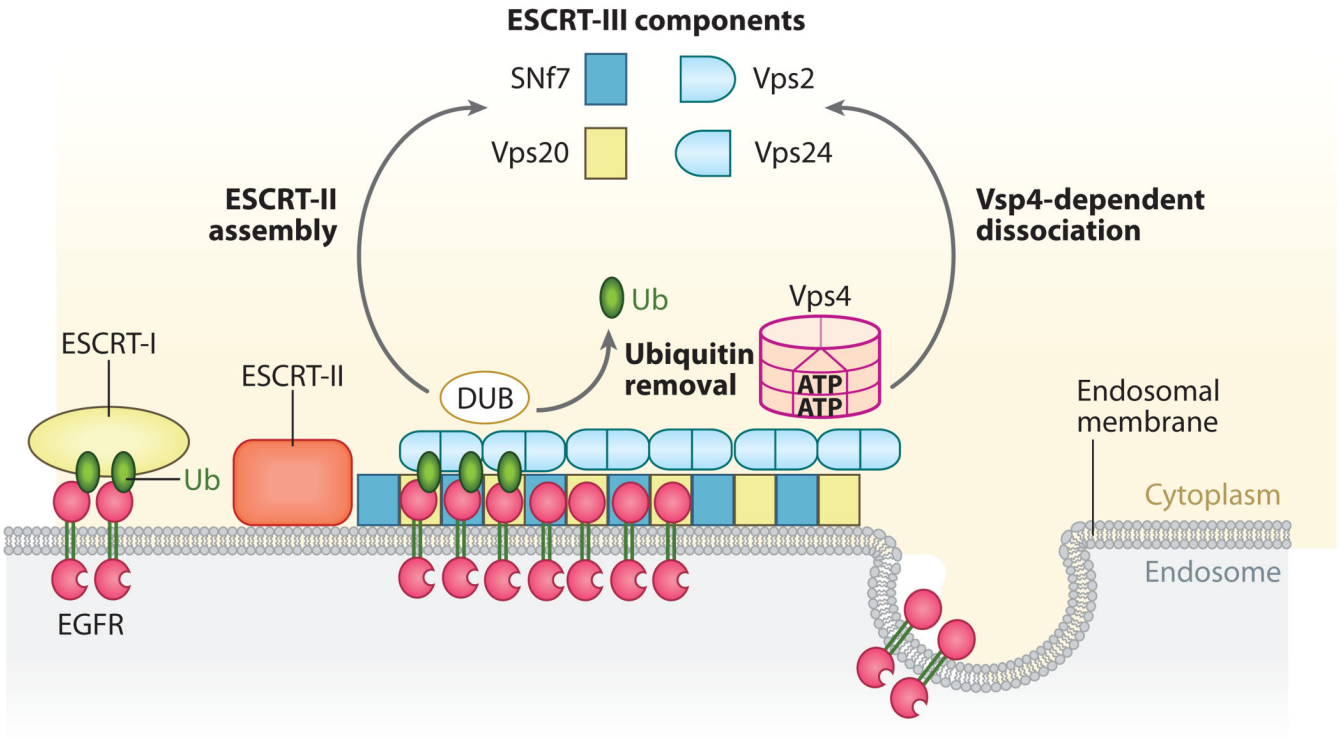

Figure 5.

A model of ESCRT functioning in EGFR sorting. The UBC-like domain of Vps23, which is a component of ESCRT-I, likely binds to multiubiquitinated EGFR at the membrane of MVBs. ESCRT-II, acting downstream of ESCRT-I, is recruited, and then putatively directs the ESCRT-III complex (which is composed of Vps20, Snf7, Vps2, and Vps24) to the appropriate MVB membrane. ESCRT-III-recruited DUBs remove Ub from cargo proteins, resulting in the invagination of cargo proteins into MVB lumenal vesicles. Vps4 is required for the disassembly and release of the entire MVB sorting machinery, which allows the ESCRT machinery to recycle back into the cytoplasm for further rounds of MVB sorting (170).