Post-translational modifications are used by cells to link additional information to proteins. Most modifications are subtle and concern small moieties such as a phosphate group or a lipid. In contrast, protein ubiquitylation entails the covalent attachment of a full-length protein such as ubiquitin. The protein ubiquitylation machinery is remarkably complex, comprising more than 15 Ubls (ubiquitin-like proteins) and several hundreds of ubiquitin-conjugating enzymes. Ubiquitin is best known for its role as a tag that induces protein destruction either by the proteasome or through targeting to lysosomes. However, addition of one or more Ubls also affects vesicular traffic, protein–protein interactions and signal transduction. It is by now well established that ubiquitylation is a component of most, if not all, cellular signalling pathways. Owing to its abundance in controlling cellular functions, ubiquitylation is also of key relevance to human pathologies, including cancer and inflammation. In the present review, we focus on its role in the control of cell adhesion, polarity and directional migration. It will become clear that protein modification by Ubls occurs at every level from the receptors at the plasma membrane down to cytoskeletal components such as actin, with differential consequences for the pathway’s final output. Since ubiquitylation is fast as well as reversible, it represents a bona fide signalling event, which is used to fine-tune a cell’s responses to receptor agonists.

Key words: adhesion, E3 ligase, integrin, migration, RhoGTPase, ubiquitin.
Figure 1  The multi-layered complexity of protein ubiquitylation

(A) Cells express limited numbers of E1 and E2 ubiquitin ligases, but approximately 600 E3 ligases have been identified, on the basis of sequence homology, most of which are of the RING-type. In addition, cells express a series of Ubls in addition to ubiquitin (U), such as SUMO (S) and Nedd8 (N). (B) Since ubiquitin monomers can be linked together, formation of chains with different lengths is a second means of introducing variation into the mode and extent of ubiquitylation. In addition, cells may become multi-ubiquitylated by the conjugation of ubiquitin (Ub) to different lysine residues. (C) Chain elongation can, in principle, occur on any of the seven lysine residues within ubiquitin of through the N-terminus, giving rise to linear or branched conformations. (D) Chain branching and mixing with other Ubls is another variant that increases the number of possible variations in protein ubiquitylation. (E) Finally, recognition by the different ubiquitin chains is mediated by UBDs, of which there are at least 20, occurring within a large number (>200) proteins. The UBDs display a certain level of specificity, imposed by structural constraints, and may have preference for linear over branched conformations.

UBIQUITYLATION-BASED PROTEIN MODIFICATION

The complexity of protein ubiquitylation and the machinery that regulates it is mind-boggling (Figure 1). Mammalian cells express two E1 ligases and approximately 30 E2 ligases in addition to over 600 potential E3 ubiquitin ligases [4]. Most E3 ligases are members of the RING family, whereas only a fraction (28) are of the HECT type [5,6]. So far, only a few of these (<50) have any experimental evidence to support their proposed ubiquitin-conjugating activity. The number of potential E3 ligases exceeds that of the superfamily of protein kinases. Similar to most protein kinases, ubiquitin ligases show variable specificity. The Cbl RING-type E3 ligase, for example, is remarkably promiscuous and targets not only receptor tyrosine kinases, but also integrins, Src-like kinases, FAK (focal adhesion kinase), adaptor proteins including WAVE (Wiskott–Aldrich syndrome protein) verprolin homologous) and small GTPases such as Rap1 (reviewed in [7]).

Ubiquitin is a member of a growing group of >15 Ubls (ubiquitin-like proteins) that can be covalently linked to target proteins, with variable substrate preferences [8,9]. Aside from ubiquitin, the best studied Ubl is SUMO (small ubiquitin-like modifier), which is also conjugated to lysine residues within substrates and of which four isoforms are expressed in vertebrates. SUMOylation occurs on various types of protein, including transcription factors as well as small GTPases [10,11] and induces different effects, such as regulation of nucleocytoplasmic shuttling or recruitment of ubiquitin ligases that harbour SIMs (SUMO-interacting motifs), resulting in the ubiquitylation of a SUMOylated protein. Nedd (neural-precursor-cell-expressed developmentally down-regulated) 8 is another Ubl, the best characterized substrates of which are the cullin proteins [12]. Cullins are adaptor proteins that associate with CRLs (cullin/RING/E3 ligases) and ‘Neddylation’ of cullins can increase the ubiquitin ligase activity of the CRLs. Neddylation plays an important role in cancer, as both tumour suppressors as well as oncogenes have been identified as Nedd8 substrates. Although there is limited information on direct effects of Nedd8 on cytoskeleton-regulating proteins, some of the cullins have been linked to the regulation of small GTPases and their activators (as discussed below).

In addition to the various types of Ubl, complexity results from differences in chain length and conformation (Figure 1). Subsequent polyubiquitylation causing lysosomal targeting and degradation. Alternatively, polyubiquitylation may result in recognition and degradation by the proteasome. However, the physiological relevance of protein ubiquitylation spans a much wider range, emerging as an essential aspect of cellular signalling pathways.

In the present review, we provide an overview of the regulation and consequences of ubiquitylation of proteins that control cytoskeletal dynamics, cell adhesion and cell migration. We briefly sketch the complexity that is a key feature of ubiquitin-based protein modifications. Next, we address ubiquitylation of cell-surface proteins and intracellular adaptor proteins linked to adhesion and migration, and focus on small GTPases, key regulators of the cytoskeleton. We also touch upon recent findings that link DUBs (deubiquitylating enzymes) to cytoskeletal dynamics and finally briefly discuss examples of specific disorders related to defects in cell adhesion or migration that are caused by mutations within the ubiquitylation machinery.
Ubiquitin contains seven lysine residues, each of which can be used for chain elongation, in addition to the N-terminal methionine residue. This can be homotypic, e.g. Lys48-based, leading to a polyubiquitylated protein that is recognized by the proteasome, or Lys63-based, which affects endocytosis and vesicular sorting of the substrate. In addition, chain elongation can be heterotypic, mixing the use of different lysine residues for elongation by ubiquitin monomers, giving rise to bifurcations or differences in ‘open’ and ‘closed’ conformations of the ubiquitin chain. Finally, these chains can become heterogeneous owing to incorporation of other Ubls such as SUMO [13].

Ubiquitylated proteins are recognized by proteins that encode UBDs (ubiquitin-binding domains). Approximately 200 intracellular proteins encode one or more of the 20 different ubiquitin-binding motifs identified so far [14,15]. UBDs can bind either mono-ubiquitin, or di- or poly-ubiquitin chains, and may have a preference for open or closed ubiquitin chains, depending on their structure. UBD-encoding proteins function in a range of cellular processes, including internalization and sorting of ubiquitylated proteins, DNA damage responses, NF-κB (nuclear factor κB) signalling and proteasomal degradation [14]. Of particular interest to the regulation of cell adhesion and migration is the finding that a subset of SH3 (Src homology 3) domains, such as in the adaptor protein CIN85 (Cbl-interacting protein of 85 kDa) and the BAR (Bin/amphiphysin/Rvs)-domain protein amphiphysin, bind mono-ubiquitin and compete with proline-rich regions in signalling proteins [16].

Ubiquitylation is reversible. There exists a family of proteins known as DUBs or USPs (ubiquitin-specific proteases) that remove ubiquitins from substrates (see below). Finally, the ubiquitylation machinery is tightly regulated as well, for example by ubiquitylation. This can occur in cis, as in auto-ubiquitylation by E3 ligases, or in trans, such as for the Cbl RING ligase, which is a target of the HECT E3 ligase Nedd4-1 [17,18]. This ubiquitylation can block or allow subsequent protein–protein interactions, but may also trigger proteasomal degradation [17,19,20]. Another example is the phosphorylation-directed SUMOylation of substrates carrying a PDSM (phosphorylation-dependent SUMOylation motif), as in transcription regulators such as heat-shock factors and GATA-1 [21]. Thus ubiquitylation is an abundant, reversible and tightly controlled event regulating many, if not all, signalling pathways, providing the cell with an almost infinite repertoire of tunable signals to respond to internal and external cues.

UBIQUITYLATION OF CELL-SURFACE PROTEINS

**Growth factor and chemokine receptors**

Cytoskeletal dynamics and cell migration are triggered by classical chemokines acting through G-protein-coupled receptors as well as by growth factors binding to receptor tyrosine kinases. It is well established that growth factor receptors become ubiquitylated after ligand binding. This topic was reviewed previously [22,23] and we will therefore not discuss it in the present review in much detail. Ubiquitylation of growth factor receptors correlates with, but may not be essential for, ligand-induced internalization of the receptor [24,25]. Following internalization, ubiquitylation can induce targeting of growth factor receptors to lysosomes for degradation. However, upon deubiquitylation, the receptor can be sorted to recycling endosomes for re-expression at the cell surface. Similarly, G-protein-coupled receptors are subject to regulation by ubiquitylation. The receptor for CXCL12 (CXC chemokine ligand 12), CXCR4 (CXC chemokine receptor 4), is ubiquitylated by the HECT E3 ligase AIP4 (atrophin-interacting protein 4) [26], which regulates its intracellular sorting and degradation. This is likely to control chemotaxis as well, although this aspect has not been studied in much detail yet.

**Integrins**

Integrins are heterodimeric glycoprotein receptors which comprise single-transmembrane α and β subunits [27]. Integrins connect extracellular matrix proteins (such as fibronectin) to the actin cytoskeleton and control cell adhesion and migration, as well as proliferation and differentiation. Therefore integrin function has to be strictly regulated. In addition to phosphorylation, it has recently become clear that ubiquitylation of the cytoplasmic domains also regulates integrin function [28].

**α-Subunits**

Ubiquitylation of integrins was first described for the α5 subunit of the fibronectin receptor α5β1 integrin [29]. In human calvarial osteoblasts, ligand binding and activation of FGFR2 (fibroblast growth factor receptor 2) induces recruitment of the RING E3 ligase Cbl to the leading edge of membrane ruffles, where Cbl ubiquitylates α5 integrin, but not αv and β1 integrins, targeting the protein for proteasomal degradation (Figure 2). Loss of α5 integrin leads to impaired osteoblast adhesion to fibronectin and, as a consequence, caspase-dependent cell apoptosis. Moreover, degradation of the activated FGFR2 is also driven by Cbl-mediated ubiquitylation, making Cbl a central regulator in a negative-feedback loop [30].

In human fibroblasts, fibronectin binding induces ubiquitylation of the α5 subunit, which mediates sorting of the internalized α5β1 integrin together with fibronectin into MVEs (multivesicular endosomes) before targeting the complex to lysosomes [31] (Figure 2). This pathway may regulate α5β1 integrin turnover to avoid recycling of ligand-bound integrin, which would lead to dysfunctional adhesion and impaired migration. On the other hand, α5β1 degradation may also allow limiting of integrin-mediated signalling towards cell migration [31,32]. The sorting process is dependent on the activity of another α5β1 integrin-binding partner, the ESCRT (endosomal sorting complex required for transport) machinery, that is also involved in endosomal sorting of other ubiquitylated membrane proteins [33]. Mutational studies demonstrate the requirement of the cytoplasmic lysine residues in the α5 subunit for ubiquitylation and, moreover, there are indications that α5 integrin may be multi- or poly-ubiquitylated [31]. However, the relevant E3 ubiquitin ligase remains to be identified.

The targeting of cellular proteins for proteasomal degradation is also exploited by certain viruses to facilitate the infection cycle within the host cell, affecting host cell motility. Particles of human Ad5 (adenovirus type 5) interact with cell-surface receptors, including α3β1 integrin, to be internalized into the host cell. In human lung carcinoma cells, the α3 subunit is, after viral entry, ubiquitylated through the viral Ad5–cullin-5-based E3 ligase complex which also has to contain viral proteins, such as the adaptor E4orf6 and the substrate-targeting protein E1B55K [34]. Virus-mediated targeting of α3 integrin for proteasomal degradation leads to an impaired adhesion of the host cell to the extracellular matrix and to apoptosis, similar to what was described for Cbl-mediated ubiquitylation of α5 integrin in osteoblasts [29]. The induction of apoptosis is probably used by adenoviruses to stimulate release of viral particles, thereby spreading the infection.
β-Subunits

In contrast with the α-subunits, much less is known about ubiquitylation of β integrins and how this regulates cell adhesion and migration. Most details are known regarding the Ang-2 (angiopoietin-2)/Tie-2-induced ubiquitylation of the αvβ3 integrin in human ECs (endothelial cells) [35] (Figure 2). Ang-2 is a ligand of the receptor tyrosine kinase Tie-2, which is important for angiogenesis and maintenance of blood vessels. Since Ang-2 acts as an antagonist, it reduces adhesion of ECs with consequences for cell migration and endothelial permeability during vascular remodelling. Thomas et al. [35] suggest that these observations are downstream effects of the Ang-2/Tie-2 pathway which triggers disassembly of FAs (focal adhesions) as well as internalization of the αvβ3 integrin receptor, followed by ubiquitylation and lysosomal degradation.

Intriguingly, recent findings suggest a link between integrins and the ERAD (endoplasmic-reticulum-associated degradation) pathway [36]. E3 ligases in the ERAD machinery act as part of a protein quality-control system through recognition of misfolded or unassembled proteins in the ER (endoplasmic reticulum). In Caenorhabditis elegans, unassembled β-integrin PAT-3 is ubiquitylated in the ER through the ER-associated RING E3 ligase RNF (RING finger protein) 121 and targeted for proteasomal degradation in the cytoplasm through the ERAD pathway (Figure 2). Cells are thus able to adapt to ER stress caused by, for instance, unassembled proteins. In human sarcoma cells, a similar link has been shown for the ER-associated RING E3 ligase gp78 [also known as AMFR (autocrine motility factor receptor) or RNF45] which ubiquitylates and targets the tetraspanin and metastasis suppressor KAI1/CD82 for proteasomal degradation through the ERAD pathway [37]. This way, gp78 promotes metastasis of tumour cells, which correlates with its negative regulation of E-cadherin expression [38]. KAI1/CD82 regulates β1 as well as β3 integrins and causes impaired migration due to increased integrin activation [39]. However, whether gp78 can also target KAI1/CD82-associated integrins remains to be established.

**Cadherins**

Cadherins control cell–cell contact and polarity, consequently affecting developmental processes including cell motility, differentiation and tissue morphogenesis [40]. The prototypical E-cadherin is a transmembrane glycoprotein that has five extracellular cadherin domains which mediate calcium-dependent homophilic cell–cell contacts. The cytoplasmic domain binds α- and β-catenins, that in turn connect the cadherin complex in a dynamic fashion to the actin cytoskeleton. As for other cell-surface proteins, ubiquitylation of E-cadherin is associated with its internalization and targeting for recycling or degradation. The best-characterized E-cadherin-regulating ubiquitin ligase is the RING ligase Hakai [41]. Hakai down-regulates E-cadherin in epithelial cells and in human colon carcinoma and breast cancer cell lines [41–45]. Down-regulation of E-cadherin leads to loss of cell–cell contact and consequent increased cell motility and is a hallmark of EMT (epithelial–mesenchymal transition). Hakai is structurally and functionally related to the RING E3 ligase c-Cbl, which ubiquitylates integrins, growth factor receptors and other proteins [41].

Binding of HGF (hepatocyte growth factor) to the c-Met receptor [44], as well as the calcium-dependent activation of Cdc42 (cell division cycle 42) and subsequent EGFR
(epidermal growth factor receptor) activation [43], stimulates Src in breast and colon cancer cells which in turn induces tyrosine phosphorylation of E-cadherin. This recruits Hakai, followed by Hakai-driven mono-ubiquitination, internalization and endosomal sorting of E-cadherin before its degradation in lysosomes [41,42]. Furthermore, activation of the Robo (Roundabout) receptor in colorectal carcinoma cells by its ligand, the chemorepellent Slit, also recruits Hakai to E-cadherin [45].

Components of the cadherin–catenin complex itself are also involved in the regulation of E-cadherin ubiquitylation. siRNA (small interfering RNA)-mediated depletion of p120-catenin increases E-cadherin turnover in human A431 cervical carcinoma cells, resulting in reduced cell–cell contact [46]. This is because the binding sites for p120-catenin and Hakai in the E-cadherin juxtamembrane region overlap, and loss of p120 will therefore recruit Hakai [47]. Another pathway that regulates E-cadherin involves the adaptor protein RACK1 (receptor for activated C-kinase 1), which functions in integrin-driven cell adhesion [44]. RACK1 suppresses tyrosine phosphorylation of the E-cadherin–catenin complex and the subsequent Hakai-driven ubiquitylation and internalization of E-cadherin, reducing colon cancer cell invasion.

E-cadherin can also interact with the RING E3 ligase MDM2 (murine double minute 2) in human MDA-MB-453 breast cancer cells [48]. Functional studies in different epithelial and fibroblasts cells confirm that MDM2, like Hakai, ubiquitylates E-cadherin, resulting in reduced cell–cell adhesion, increased cell motility and invasion of tumour cells [48]. In contrast with the mono-ubiquitylation by Hakai, MDM2 mediates polyubiquitylation of E-cadherin at the plasma membrane [41,42,48]. Ubiquitylated E-cadherin is then internalized and sorted with MDM2 into early endosomes before lysosomal degradation [48]. Indirectly, MDM2 may also stabilize E-cadherin by ubiquitylation and degradation of the E-cadherin suppressor Slug in H1299 and MCF7 cancer cells [49]. Thus MDM2 has a dual regulatory role, as it can cause both the down- and up-regulation of E-cadherin protein expression. Ubiquitylation of E-cadherin is also described in the context of the ERAD pathway [50]. Owing to the mutations R749W and E757K, which are associated with tumour development in gastric cancer, E-cadherin is retained in the ER rather than localized at the plasma membrane. However, the ER-associated E3 ligase that targets these E-cadherin mutants for proteasomal degradation remains to be elucidated.

UBIQUITIN-MEDIATED REGULATION OF ADAPTOR PROTEINS AND KINASES

Downstream of cell-surface receptors and adhesion molecules, various adaptor proteins and protein kinases that function in pathways controlling cytoskeletal dynamics are regulated by ubiquitylation. A few examples are briefly discussed below.

Talin

Talin is an actin-binding adaptor protein that resides in FAs and binds and activates integrins through its N-terminal head domain [51,52]. Interestingly, the head domain, but not full-length talin, was found to be a substrate for Smurf (Smad ubiquitin regulatory factor) 1-mediated ubiquitylation. In line with this, a small fraction of ectopic Smurf1 localizes to a subset of FAs [53]. Phosphorylation of the talin head domain by Cdk5 (cyclin-dependent kinase 5) prevents binding to Smurf1 and protects talin from ubiquitylation and proteasomal degradation. Expression of a phosphorylation-deficient talin mutant increased FA disassembly and blocked cell migration of human neuroblastoma as well as CHO (Chinese-hamster ovary)-K1 cells, showing that Cdk5-mediated protection of the talin head from degradation is required for efficient cell motility.

Paxillin

The adaptor protein paxillin localizes primarily to FAs and is required for cell adhesion and migration [54]. The RING E3 ligase RNF5 ubiquitylates paxillin, probably through Lys63-linked conjugation, and does not affect paxillin degradation, but rather its localization [55]. RNF5-mediated ubiquitylation induces a loss of paxillin from FAs and a concomitant increase in cytosolic paxillin. As a consequence, expression of RNF5 in NIH 3T3 fibroblasts inhibits cell migration. In Xenopus, the RNF5 orthologue XRNFl85 associates with paxillin and, in these cells, does regulate its stability [56]. Paxillin is ubiquitylated in response to non-canonical Wnt signalling, and one of its components, Dishevelled, associates with XRNFl85 [56]. However, these authors suggested that XRNFl85 serves as a linker, recruiting the proteasome to paxillin in FAs, rather than acting as a paxillin ubiquitin ligase [56]. Down-regulation of XRNFl85 leads to impaired migration of mesoderm cells and reduced FA turnover, supporting the notion that proper regulation of the dynamics of paxillin within FAs, by it via degradation or mislocalization, is important for cell motility.

Filamin

Filamins are actin-cross-linking proteins which bind to a wide range of cell-surface receptors (including integrins) and signalling proteins, and control cell shape, adhesion and migration [57]. Three homologous isoforms, filamin A, B and C, are known in mammals. In myeloid leukaemia cells, the filamins are ubiquitylated and are targeted for proteasomal degradation by the haemopoietic-specific ASB2α [ankyrin repeat-containing protein with a SOCS (suppressor of cytokine signalling) box 2α] which is part of a CRL complex [58–61]. This complex also contains cullin-5, Rbx-2 (RING-containing protein box-2) and elongin BC. ASB2α-mediated polyubiquitylation takes place in the ABD (actin-binding domain) of the filamins [60]. Overexpression of ASB2α, like depletion of filamin, impairs fibronectin-dependent cell spreading and migration of NIH 3T3 fibroblasts, HT1080 human fibrosarcoma cells and Jurkat T-lymphoblasts [58,61] and increases fibronectin-dependent adhesion of myeloid leukaemia cells [59]. Moreover, ASB2α depletion in these cells delays filamin degradation and inhibits haemopoietic differentiation [58].

Like ASB2α, the muscle-specific β-isofrom ASB2β also forms an E3 ligase complex with cullin-5, Rbx-2 and elongin BC [62]. In contrast, ASB2β targets only filamin B, and not filamin A or C, for polyubiquitylation and degradation during differentiation of C2C12 mouse myoblast cells. Depletion of ASB2β impairs filamin B down-regulation and myogenic differentiation, which can be rescued by additional reduction of filamin B levels in these cells. Ubiquitylation of certain filamin isoforms is also mediated by the RING E3 ligase MuRF3 (muscle-specific RING finger protein 3), which is expressed in cardiac and skeletal muscle [63]. In C2C12 myoblast cells, MuRF3 interacts with filamin C, but not filamin A. Filamin C levels are significantly increased in hearts of mice lacking MuRF3, whereas filamin C expression is reduced in C2C12 cells overexpressing MuRF3. This suggests that MuRF3-mediated degradation of filamin C is part of its regulation of cardiac integrity and function following myocardial infarction.
**Focal adhesion kinase**

FAK is a central regulator of cell adhesion, motility and proliferation. FAK binding to integrins results in its activation and (auto)phosphorylation. Sekine et al. [64] showed that the adaptor protein STAP2 (signal-transducing adaptor protein-2) recruits activated FAK and the E3 ligase Cbl in human T-cells. Cbl polyubiquitylates FAK and targets FAK for proteasomal degradation, which leads to disassembly of FAs and impaired T-cell adhesion on fibronectin. Interestingly, Cbl also controls the STAP2 protein levels through proteasomal degradation, suggestive of Cbl-mediated negative feedback [65]. FAK activation can also be induced by CXCL12, a ligand of CXCR4. SDF-1 (stromal-cell-derived factor 1)-activated FAK is targeted by SOCS3, which is part of a cullin-5-based E3 ligase complex that ensures subsequent polyubiquitylation and proteasomal degradation of FAK [66]. SOCS3-mediated attenuation of the CXCR4/FAK pathway induces impaired adhesion and migration of B-cell precursors in the bone marrow. Finally, FAK has been reported to be SUMOylated on Lys152 by the E3 ligase PIAS [protein inhibitor of activated STAT (signal transducer and activator of transcription)] 1, in an activity-independent fashion [67]. This promoted its autophosphorylation, suggesting that SUMOylation may enhance FAK-induced signalling.

**Src**

The non-receptor tyrosine kinase Src regulates cell adhesion and migration in several ways, including its phosphorylation and regulation of FAK [68]. Activity-dependent ubiquitylation of Src was first reported in 1999 [69]. In this study, inactivation of Src following co-expression of Csk (c-Src kinase) increased protein levels of Src and Fyn, whereas expression of constitutively active SrcY527F induced its prominent ubiquitylation [69]. Activated Src can be ubiquitylated by RING-type E3 ligases, including c-Cbl [70] and a SCF (Skp1/cullin/F-box protein) complex comprising cullin-5. Depleting cullin-5 by siRNA in fibroblasts expressing activated Src co-operated with the consequently increased Src levels by inducing morphological transformation, reducing cell spreading and, upon injection in mice, increasing tumour incidence [71]. Most recently, the endosome-associated U-box E3 ligase CHIP [C-terminal of constitutive HSC70 (heat-shock cognate 70)-interacting protein] also known as Stub1) was shown to ubiquitylate Src, primarily through Lys63-linked conjugation in the context of TLR (Toll-like receptor) 4/9 signalling in T-cells [72]. CHIP-mediated modification of Src was required for TLR signalling, suggesting that, in this system, Lys63-linked polyubiquitylation activates Src. Intriguingly, CHIP associates with HSP (heat-shock protein) 70 and HSP90, and is considered, similar to gp78 (see above), to be part of a cellular quality-control system [73].

Finally, Src activity itself appears to regulate the expression level of the actin-severing protein cofilin, a key regulator of directional cell motility [74]. Activated v-Src phosphorylates cofilin on Tyr3, and this modification is required to induce polyubiquitylation and degradation of cofilin. This effect mediates v-Src-induced inhibition of cell spreading [75].

**c-Raf**

The serine/threonine kinase c-Raf is activated by the small GTPase Ras and is an upstream activator of the ERK (extracellular-signal-regulated kinase) 1/2 pathway [76]. In addition to regulating cell proliferation, Raf also promotes cell migration. Silencing expression of the ubiquitin E3 ligase XIAP (X-linked inhibitor of apoptosis protein) or cIAP (cellular inhibitor of apoptosis protein) increases protein levels of c-Raf in various cell lines and promoted Raf/ERK-dependent cell migration [77]. This work showed further that it is not XIAP itself that acts as a Raf E3 ligase, but that XIAP recruits the CHIP ubiquitin ligase, leading to Raf ubiquitylation. In line with this, silencing CHIP in HeLa cells promoted cell migration in a Raf-dependent manner [77].

**REGULATION OF SMALL GTPASES BY UBIQUITYLYATION**

The superfamily of small GTPases controls cell adhesion and migration, differentiation and cell division. Under resting conditions, RhoGTPases are present in their inactive GDP-bound form and able to bind RhoGDIs (guanine-nucleotide-dissociation inhibitors) which function as cytosolic chaperones. GEFs (guanine-nucleotide-exchange factors) facilitate the exchange of GDP to GTP and are likely to be relevant for GDI release. Thus GEFs promote binding of GTP and are required for the activation of RhoGTPases. This step is accompanied by membrane translocation, as GDI dissociation exposes the C-terminal lipid anchor, and by conformational changes in the switch regions of the GTPase, which allows binding to downstream effector proteins. It is generally accepted that the main pathway to terminate downstream GTPase signalling requires GTP hydrolysis, stimulated by GAPs (GTPase-activating proteins). However, mounting evidence suggests an additional mode of inactivation that involves ubiquitylation and proteasomal degradation [78–81] (see below).

**RhoGDI**

Several studies indicate that RhoGDI acts as a guardian, protecting RhoGTPases from ubiquitylation and degradation. Phosphorylation of RhoA at Ser188 in its hypervariable C-terminus by PKG (protein kinase G) or PKC (protein kinase C) promotes cytosolic localization and binding to RhoGDI in smooth muscle cells. This was accompanied by reduced susceptibility for ubiquitylation and proteasomal degradation [82]. More recently, Boulter et al. [83] showed that depletion of RhoGDI1 by siRNA in HeLa cells, fibroblasts and ECs results in a proteasome-dependent loss of RhoA, Rac1 and Cdc42, but not RhoB, which does not bind to RhoGDI. Although GTPase ubiquitylation was not analysed, these findings suggest that association with RhoGDI prevents RhoGTPase degradation. Degradation of RhoA that followed GDI depletion could not be rescued by expression of p190RhoGAP, indicating that, under these conditions, RhoA becomes degraded, irrespective of its state of activation.

**RhoGTPases**

The concept of activity-driven ubiquitylation of RhoGTPases emerged from the discovery and analysis of bacterial toxins that modulate host cell signalling. The CNF1 (cytotoxic necrotizing factor 1) toxin of *Escherichia coli* renders RhoGTPases constitutively active by deamidating Gln61 of Rac1 and Cdc42 and Gln63 of RhoA [84,85]. This constitutive activity makes the GTPases susceptible to ubiquitylation and proteasomal degradation, as confirmed in epithelial cells, ECs, primary fibroblasts and macrophages [86–88].

The HECT ligase Smurf1 was the first E3 ligase shown to target CNF1-activated RhoA for ubiquitylation and proteasomal degradation [6,79,87]. Smurf1 targets RhoA, but not Rac1 or Cdc42 [79], and ubiquitylates Lys6 and Lys7 in the RhoA N-terminus [89]. In line with this, overexpression of Smurf1 causes a loss of actin stress fibres and reduced cell motility in various epithelial cells, indicative of inhibition of RhoA [79,90,91].
Smurf1-mediated degradation of active RhoA has been proposed to occur at defined subcellular localizations, such as membrane protrusions and tight junctions [79,90,92]. Recruitment of Smurf1 by the atypical PKCζ targets Smurf1 to cellular protrusions, resulting in local degradation of RhoA and promoting cell polarity [79]. TGFβ (transforming growth factor β) signalling controls this pathway by inducing phosphorylation of the polarity protein Par6, which then associates with Smurf1. This leads to local degradation of RhoA and dissolution of tight junctions, which is required for EMT [89]. A recent study using Neuro2a cells showed that phosphorylation of Smurf1 at Thr306 by PKA (protein kinase A) also induced local degradation of RhoA, which is required for axon development [89,93]. Unphosphorylated Smurf1 mediates polyubiquitination and degradation of Par6 [93]. Thus the increased levels of pThr306 Smurf1 in axons suggests that, at these locations, Par6 is protected from Smurf1, whereas RhoA is degraded.

Smurfl2, which shares 83% sequence similarity with Smurf1 [94], also promotes neuronal polarity. However, unlike Smurf1, Smurfl2 targets GDP-bound Rap1B, but not RhoA, for proteasomal degradation in hippocampal neurons [95]. Rap1B shares 95% sequence homology with Rap1A and both encode a lysine residue at position 5, which, in Rap1B, is targeted by Smurfl2 [95]. However, whether Smurfl2 shows specificity towards either of the two Rap isoforms in the control of cell migration remains to be determined.

In contrast with the HECT ligases, the CRLs are complexes containing multiple adaptor proteins that serve to control E3 ligase activity [96]. Cullin-3 binds to adaptor proteins with a BTB (broad complex/tramtrack/bric-a-brac) domain which controls cullin-3 substrate specificity [97]. This links cullin-3 to the class of atypical RhoBTT GTPasses [98]. RhoBTT homologues bind cullin-3, which results in the ubiquitination and proteasomal degradation of RhoA, which is required for axon development [89,93]. Unphosphorylated Smurf1 mediates polyubiquitination and degradation of Par6 [93]. Thus the increased levels of pThr306 Smurf1 in axons suggests that, at these locations, Par6 is protected from Smurf1, whereas RhoA is degraded.

In contrast with the HECT ligases, the CRLs are complexes containing multiple adaptor proteins that serve to control E3 ligase activity [96]. Cullin-3 binds to adaptor proteins with a BTB (broad complex/tramtrack/bric-a-brac) domain which controls cullin-3 substrate specificity [97]. This links cullin-3 to the class of atypical RhoBTT GTPasses [98]. RhoBTT homologues bind cullin-3, which results in the ubiquitination and proteasomal degradation of RhoA, which is required for axon development [89,93]. Unphosphorylated Smurf1 mediates polyubiquitination and degradation of Par6 [93]. Thus the increased levels of pThr306 Smurf1 in axons suggests that, at these locations, Par6 is protected from Smurf1, whereas RhoA is degraded.

Cullin-3, with the BTB protein KLHL20, mediates ubiquitination of a GEF for RhoA, PDZ-RhoGEF [100] (Figure 3). Whereas depletion of KLHL20 in hippocampal neurons increases PDZ-RhoGEF expression and RhoA activity, overexpression of KLHL20 promotes PDZ-RhoGEF ubiquitination which stimulates, like Smurf1, neurite outgrowth [100]. KLHL20 contains a Kelch repeat domain that mediates binding and cross-linking of F-actin (filamentous actin) as shown for the KEL protein in Drosophila (dKEL) [101]. In Drosophila, binding of cullin-3 triggers dKEL degradation [102], suggesting that, in mammalian cells, cullin-3 uses the Kelch repeat domains of KLHL20 to control F-actin dynamics and cell migration.

In contrast with RhoA, an E3 ubiquitin ligase for Rac1 has yet to be described. Active, but not inactive, Rac1 is ubiquitylated at Lys147 [103] and this requires interaction with Rac1 effector proteins [104,105]. This is followed by polyubiquitination and proteasomal degradation, a process that requires the Rac1 hypervariable C-terminus [104]. Rac1 degradation may occur in the nucleus [106], in line with the recent finding that Rac1, through its C-terminus, binds to the nuclear transporter karyopherin-α [107]. Our group showed recently that Rac1 polyubiquitination at Lys147 is integrin-dependent and requires caveolin 1 [81], which may link Rac1 ubiquitination to caveolin-1-dependent internalization of Rac1 [108]. Activated Rac1 and caveolin 1 co-localize at FAs, but whether Rac1 is locally degraded in polarized cells, as RhoA is, is unknown. Rac1 ubiquitination is induced by HGF treatment of MDCKII (Madin–Darby canine kidney II) cells, preceding epithelial cell scattering [105]. Scattering could be prevented not only by proteasome inhibitors, but also by expression of Rac2, which is not degraded upon activation by CNF1 [104], suggesting that the selective degradation of Rac1 is instrumental in EMT. A recent study showed that, in response to HGF, Rac1 can also be SUMOylated in its C-terminus by the Rac1-interacting E3 SUMO ligase PIAS [11]. Using rescue experiments in Rac1-deficient fibroblasts, it was shown that Rac1 SUMOylation by PIAS is required for efficient cell migration and invasion.

Downstream events

Downstream events are only limited information on ubiquitin modification of proteins regulating cell adhesion and migration downstream of the small GTPases. The Rac1/Cdc42 effector PI3K (p21-activated kinase), a serine/threonine kinase that regulates cell adhesion and motility [109] subject to ubiquitination and degradation following its activation by the GTPase Chp, an inhibitor of T-cell chemotaxis. Since proteasome inhibitors inhibited T-cell chemotaxis across small pore-size filters, PK degradation appears to be required for efficient motility [110].

A few studies have linked ubiquitylation with the regulation of contractility and migration. PDGF (platelet-derived growth factor)-induced ubiquitylation and proteasomal degradation of MRLC (myosin regulatory light chain) through the E3 ligase MIR (MRLC-interacting protein) is required for migration of NIH 3T3 cells [111]. Another E3 ligase for myosin light chain is MuRF1, which also ubiquitylates actin and is up-regulated during muscle atrophy [112,113]. In addition, a regulatory MLCP (myosin light chain phosphatase) is controlled by ubiquitylation by the E3 ligase SIAH (seven in absentia homologue) 2 [114]. SIAH1 also associates with Par3, part of the Par3–Par6–PKCζ polarity complex. SIAH induces Par3 ubiquitylation and degradation in HEK (human embryonic kidney)-293 cells. In cerebellar granule neurons, SIAH expression reduces Par3 expression as well as direction. This determines which path cerebellar neurons will follow during migration in the developing cortex [115].

Intermediary filament proteins such as keratins and vimentin are SUMOylated, a modification which is increased during apoptosis and oxidative stress [116]. HyperSUMOylation reduces keratin solubility, which has consequences for their function in cellular stress responses. Tubulin is subject to multi-ubiquitylation and is a target of the E3 ligase parkin [117,118]. Tubulin, as well as actin, ubiquitylation was shown recently to correlate with loss of their expression during reticulocyte maturation [119]. Tubulin ubiquitylation impairs its polymerization [120], but whether this mechanism is also involved in the control of motility is unknown.

DEUBIQUITYLATED ENZYMES

In humans, ~80 DUBs have been identified which, based on the sequence of the catalytic domain, can be divided into five families, including the UCH (ubiquitin C-terminal hydrolase) and
UBIQUITIN AND DISEASE

Because ubiquitin-based regulation of cell signalling is so common, it may not be surprising that there have been an increasing number of proteins discovered that are part of the ubiquitin–proteasome system which play an important role in human disorders. A comprehensive discussion is beyond the scope of the present review, so we will mention just a few examples.

An intensely studied protein is parkin, a RING-type E3 ubiquitin ligase that regulates mitochondrial turnover, collaborates with HDAC6 in protein quality control [135] and plays a role in autosomal recessive juvenile parkinsonism. Parkin also ubiquitylates Eps15 in an EGF-dependent fashion, interfering with the Eps15–EGFR interaction and EGFR endocytosis, resulting in prolonged EGF-induced signalling [136]. The parkin–Eps15 interaction was shown to depend on the Eps15 UIM (ubiquitin-interacting motif) binding to the Ubl domain of parkin. This same domain was later shown to associate with a series of SH3 domains from endocytic BAR domains, including endophilin and amphiphris, linking parkin to the regulation of vesicular trafficking [137].

Ubiquitylation controls cell polarity and E3 ligases are consequently important for the development of cancer and metastasis, through the regulation of, for example, EMT and tumour cell migration. A recent study showed that the tumour suppressor and E3 ligase BRCA1 (breast cancer early-onset 1) negatively regulates cell spreading and motility [138]. A BRCA1 mutant that lacked ubiquitin ligase activity promoted cell migration, as did expression of a C-terminal fragment, which dislocates the endogenous BRCA1 from the plasma membrane by competing for binding to ERM proteins [138]. Despite the large number of studies on BRCA1, the target proteins that are relevant for its control of cell migration remain to be identified. See [139] for a recent expert review on the role of RING E3 ligases in cancer. Another intriguing example linking ubiquitylation to cell migration in disease is the regulation of the RhoA ligase Smurf1 in a group of familial diseases called CCMs (cerebral cavernous malformations). CCMs are associated with defects in the cerebral vasculature, characterized by increased permeability and vessel dilation. These defects are prone to causing seizures and haemorrhagic stroke. Mutations causing CCMs have been found in several proteins, namely CCM1 (or KRIT1), CCM2 (malcavernin or OSM [osmosensing scaffold for MEKK3 (MAPK/ERK kinase kinase 3)]) and CCM3 (or PDCD10 [programmed cell death 10]). Deletion of CCM2 in mice is lethal, owing to severely impaired angiogenesis [140], indicative of a function of CCM2 in ECs. Depletion of CCM2 increased actin stress fibres and endothelial permeability in human microvascular ECs. The latter effect was accompanied by, and in part also dependent on, increased activity of RhoA, identifying CCM2 as a negative regulator of RhoA signalling. Crose et al. [141] revealed a part of the underlying mechanism by showing that the HECT domain of Smurf1 associates with a PTB domain in CCM2 and that this association mediates the targeting of Smurf1 to plasma membrane-localized CCM2. In line with the data by Whitehead et al. [140], Crose et al. [141] showed that reducing CCM2 expression up-regulated RhoA and that co-expression of CCM2 with wild-type, but not catalytically inactive, Smurf1 results in selective degradation of RhoA. Since loss of CCM2 increased endothelial permeability and reduced migration of brain microvascular ECs, these findings do support the notion that the CCM2–Smurf1 complex regulates the actin cytoskeleton through the localized degradation of RhoA (Figure 3).

On the basis of earlier work that CCM1/KRIT1 promotes endothelial barrier function downstream of a cAMP/Epac (exchange protein directly activated by cAMP)/Rap1 pathway [142], Stockton et al. [143] showed recently that CCM1/KRIT1 acts in conjunction with CCM2 in promoting endothelial integrity. Down-regulating CCM1/KRIT1 expression in HUVECs (human umbilical vein endothelial cells) increased RhoA activity >-3-fold and stimulated ROCK (Rho-associated kinase)-dependent MRLC

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Ubiquitin links to cytoskeletal dynamics, cell adhesion and migration

There are several connections between the protein ubiquitylation machinery and RhoA signalling in cells. The cullin-3 ubiquitin ligase, possibly in complex with the adaptor protein BACURD, can mediate ubiquitylation and degradation of GDP-bound RhoA (A). The cullin-3 complex can also target actin-regulating Kelch proteins (B). Complexed with KLHL20, cullin-3 can also ubiquitylate POZ-RhoGEF, thereby reducing RhoA activation (C). The RING ligase Smurf1 associates with the polarity complex via Par6 and can locally ubiquitylate GTP-bound RhoA, promoting cell polarity (D). Smurf1 can also induce degradation of the talin head domain (TH), enhancing turnover of FAs (E). U, ubiquitin.

CONCLUDING REMARKS

A recent proteomic analysis estimated that the human ubiquitinome comprises \(~19,000\) sites in approximately \(5000\) proteins [144]. Such an abundant protein modification is likely to affect most, if not all, cellular processes. As the present review has attempted to convey, regulation of cell adhesion and migration is no exception. Yet this field is murky, to say the least, and most of what has been reviewed represents rather scattered observations, with a few common themes emerging.

First, conjugation with ubiquitin or Ubls regulates receptors and signalling proteins at all levels in pathways that control cytoskeletal dynamics and cell migration. Functional consequences include internalization, modulation of signalling activity, protein–protein interactions and controlled degradation of activated proteins. The finding that some SH3 domains can bind ubiquitin (i.e. to ubiquitylated substrates) predicts unexpected potential for additional protein interactions in cell signalling. Also, ubiquitylation of Ras on Lys147 was suggested recently to stimulate GTPase activity [145]. In contrast, Nedd4-1-mediated ubiquitylation of Rap2A inhibited its interaction with effector proteins [146]. Thus cells use ubiquitylation to ‘tune’ their responses towards external cues.

Secondly, there are still many ‘orphan’ E3 ubiquitin ligases, forcing investigators in this area to keep an open mind as to which ligase could be most relevant for their favourite protein. Admittedly, this issue is complicated by ligase promiscuity.
Finally, localized ubiquitylation and consequent degradation has emerged as a relevant mechanism that operates in parallel to more established ways of terminating protein activity. This is true for some kinases, usually regulated by (de-)phosphorylation, but also for small GTPases that are in most schemes depicted as eternal cyclists that can be reactivated following GAP-stimulated GTP hydrolysis.

In several of the studies discussed above, control of signalling by ubiquitylation is, directly or indirectly, linked to the cellular systems of quality control, such as ERAD. Although very speculative, this could indicate that cells use the same machinery that deals with removal of newly synthesized, but misfolded, proteins to also modify signal transduction proteins. Since ubiquitylation occurs frequently on activated proteins (e.g. EGFR or Rac1), these may well be, as a result of an activation-associated conformational change, considered ‘misfolded’ and recognized by the ubiquitylation machinery. In this context, observations on so-called plasma membrane–ER contact sites [147,148] are particularly intriguing.

Ubiquitylation is fast, localized and reversible. Its dynamics require a timescale of milliseconds [149], which easily matches most cellular responses to extracellular stimuli. The number of these ubiquitin ligases are HACE1 (HECT domain and ankyrin repeat-containing E3 ubiquitin–protein ligase 1) [150], and both XIAP and cIAP1 [151].

Note added in proof (received 12 December 2011)

While the present review was being prepared for publication, two studies were published identifying the first E3 ligases for Rac1. These ubiquitin ligases are HACE1 (HECT domain and ankyrin repeat-containing E3 ubiquitin–protein ligase 1) [150], and both XIAP and cIAP1 [151].

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REFERENCES


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