Protein Degradation

Vol. 4: The Ubiquitin-Proteasome System and Disease

Edited by
R. John Mayer, Aaron J. Ciechanover, and Martin Rechsteiner

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Preface

There is an incredible amount of current global research activity devoted to understanding the chemistry of life. The genomic revolution means that we now have the basic genetic information in order to understand in full the molecular basis of the life process. However, we are still in the early stages of trying to understand the specific mechanisms and pathways that regulate cellular activities. Occasionally discoveries are made that radically change the way in which we view cellular activities. One of the best examples would be the finding that reversible phosphorylation of proteins is a key regulatory mechanism with a plethora of downstream consequences. Now the seminal discovery of another post-translational modification, protein ubiquitylation, is leading to a radical revision of our understanding of cell physiology. It is becoming ever more clear that protein ubiquitylation is as important as protein phosphorylation in regulating cellular activities. One consequence of protein ubiquitylation is protein degradation by the 26S proteasome. However, we are just beginning to understand the full physiological consequences of covalent modification of proteins, not only by ubiquitin, but also by ubiquitin-related proteins.

Because the Ubiquitin Proteasome System (UPS) is a relatively young field of study, there is ample room to speculate on possible future developments. Today a handful of diseases, particularly neurodegenerative ones, are known to be caused by malfunction of the UPS. With perhaps as many as 1000 human genes encoding components of ubiquitin and ubiquitin-related modification pathways, it is almost certain that many more diseases will be found to arise from genetic errors in the UPS or by pathogen subversion of the system. This opens several avenues for the development of new therapies. Already the proteasome inhibitor Velcade is producing clinical success in the fight against multiple myeloma. Other therapies based on the inhibition or activation of specific ubiquitin ligases, the substrate recognition components of the UPS, are likely to be forthcoming. At the fundamental research level there are a number of possible discoveries especially given the surprising range of biochemical reactions involving ubiquitin and its cousins. Who would have guessed that the small highly conserved protein would be involved in endocytosis or that its relative Atg8 would form covalent bonds to a phospholipid during autophagy? We suspect that few students of ubiquitin will be surprised if it or a ubiquitin-like protein is one day found to be covalently attached to a nucleic acid for some biological purpose.

We are regularly informed by the ubiquitin community that the initiation of this series of books on the UPS is extremely timely. Even though the field is young, it has now reached the point at which the biomedical scientific community at large needs reference works in which contributing authors indicate the fundamental roles of the ubiquitin proteasome system in all cellular processes. We have attempted to draw together contributions from experts in the field to illustrate the comprehensive manner in which the ubiquitin proteasome system regulates cell physiology. There is no doubt then when the full implications of protein modification by ubiquitin and ubiquitin-like molecules are fully understood we will have gained fundamental new insights into the life process. We will also have come to understand those pathological processes resulting from UPS malfunction. The medical implications should have considerable impact on the pharmaceutical industry and should open new avenues for therapeutic intervention in human and animal diseases. The extensive physiological ramifications of the ubiquitin proteasome system warrant a series of books of which this is the forth one. The focus of this book is on the role of the UPS in disease.

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1
Ubiquitin Signaling and Cancer Pathogenesis

Kaisa Haglund and Ivan Dikic

1.1
Introduction

Post-translational modifications of proteins allow cells to respond dynamically to intra- and extracellular stimuli to control cellular processes [1]. A modification that has been given special attention among all possible modifications is protein ubiquitination, due to the frequency of its occurrence and the key role it plays in the inducible and reversible control of signaling pathways which regulate cellular homeostasis [2–4]. Tagging of proteins with ubiquitin occurs in a three-step process through the sequential action of the ubiquitin activating (E1), conjugating (E2) and ligase (E3) enzymes [5, 6]. Ubiquitination is a dynamic and reversible modification, and the rapid removal of ubiquitin from substrates and the processing of ubiquitin chains is catalyzed by de-ubiquitinating enzymes (DUBs) [7]. The regulation of DUBs is attracting increasing interest, since they serve to switch off the ubiquitin signal or to initiate a shift between different modifications of the same lysine residue. Moreover, there seems to be an interesting interplay between E3 ubiquitin ligases and DUBs. Interactions between E3s and DUBs have been shown to regulate the stability of E3s which undergo autoubiquitination. This type of interaction also leads the DUB to its substrate and regulates the target stability [7].

Ubiquitin modification can occur in multiple ways, making it a very diverse modification with distinct cellular functions (Figure 1.1). In its simplest form, a single ubiquitin molecule is attached to a single lysine residue in a substrate, which is defined as monoubiquitination [8, 9]. Alternatively, several single ubiquitin molecules can be attached to several different lysines, which is referred to as multiple monoubiquitination or multiubiquitination [10, 11]. Moreover, ubiquitin contains seven lysines itself that can be used to form various types of ubiquitin chain in an iterative process known as polyubiquitination [5, 12]. Interestingly, all seven lysines (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63) have the potential to be used in chain formation, giving rise to chains with different linkages or branches [13].

Monoubiquitination is involved in endocytosis of plasma membrane proteins, the sorting of proteins into the multivesicular body (MVB), budding of

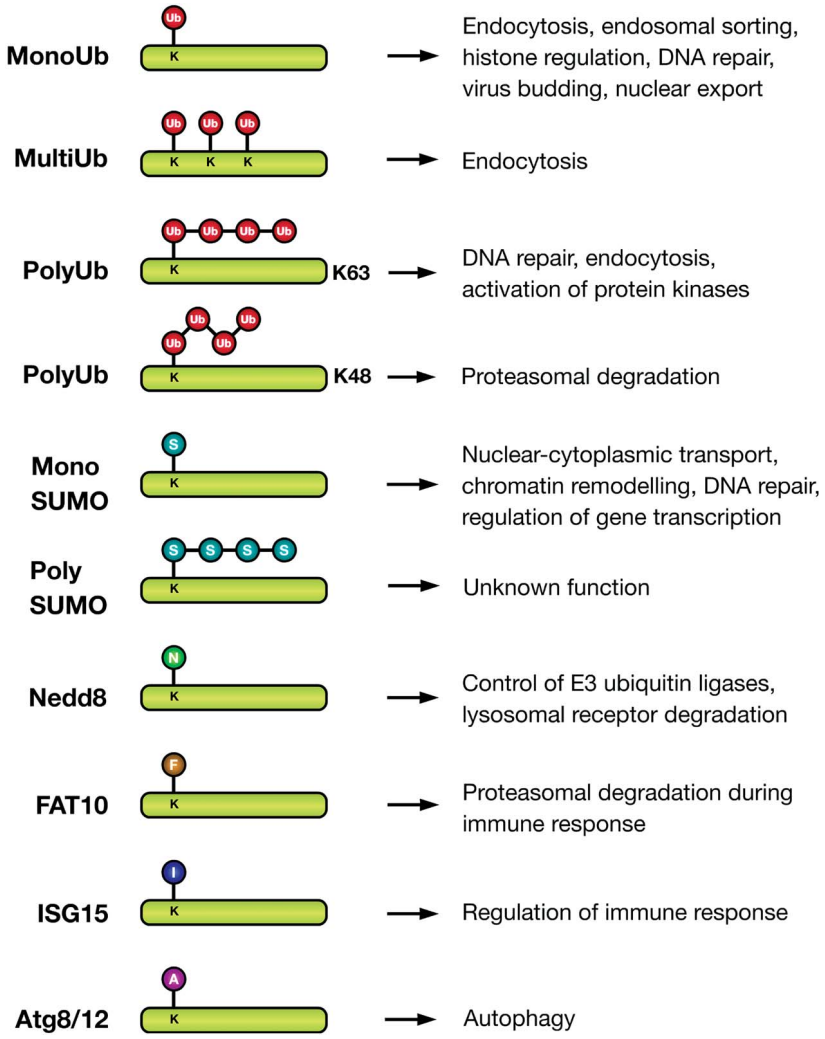

Fig. 1.1. Ubiquitin and ubiquitin-like protein (Ubl) modifications regulate a wide variety of cellular processes. Ubiquitin and Ubls share the same ubiquitin superfold and are collectively referred to as ubiquitons. All ubiquitons are attached via their C-terminal glycine residue to lysine residues in target proteins via a covalent isopeptide bond. Monoubiquitination (MonoUb) is essential for endocytosis and/or endosomal sorting of a variety of receptors, regulation of histones, DNA repair, virus budding and nuclear export. Tagging of several lysines with single ubiquitin molecules (MultiUb), is involved in endocytosis of certain RTKs and regulation of p53 localization. Polyubiquitination (PolyUb), the formation of ubiquitin chains via different lysines of ubiquitin, targets proteins for degradation in the 26S proteasome when linked via lysine 48, and has non-proteolytic functions, including control of DNA repair, endocytosis and activation of protein kinases when linked via lysine 63. Sumolyation controls several processes in the cell nucleus, including DNA repair, protein localization, chromatin remodeling and gene transcription. Neddylation regulates the activity of several E3 ubiquitin ligases, including Cbl, Mdm2 and cullins, and cooperates with ubiquitin to target EGFRs for lysosomal degradation [33, 34]. ISG15 and FAT10 are dimeric ubiquitons implicated in immune response [33, 34]. Atg8 and Atg12 play important roles in autophagy, the degradation of bulk cytoplasmic components, by contributing to the formation of autophagosomes during nutrient starvation of cells [33, 34]. Ub, ubiquitin; K, lysine; S, SUMO; N, Nedd8; F, FAT10; I, ISG15; A, Atg8/12.

retroviruses, DNA repair, histone activity and transcriptional regulation [8, 9, 14–16]. Multiple monoubiquitination is also involved in endocytosis of receptor tyrosine kinases (RTKs) and in nuclear export of p53 [10, 11]. In the case of polyubiquitination, the functions of polyubiquitin chains linked via lysines 48 and 63 have been best characterized. Proteins that are polyubiquitinated with Lys48-linked chains are recognized by ubiquitin-binding subunits of the 26S proteasome and are targeted for proteasomal degradation [5, 17]. Chains linked via Lys63, on the other hand, are involved in regulating endocytosis, DNA repair and activation of NF-κB [2, 14, 18–20]. Thus, whereas Lys48-linked polybiquitination was the first proteolytic signal described, it is becoming clear that monoubiquitination and Lys63-linked polyubiquitination function in several non-proteolytic cellular processes to regulate signaling networks.

1.1.1 Ubiquitin Signaling Networks

Ubiquitination is similar to phosphorylation and functions as a signaling device in cellular signaling networks. First, ubiquitination is an inducible event, which can be triggered by signals such as extracellular stimuli, phosphorylation and DNA damage [2]. This is associated with the fact that E3 ubiquitin ligases are tightly regulated by signal-induced mechanisms, such as post-translational modifications, compartmentalization, degradation and oligomerization [21, 22]. A prominent example is the ubiquitin ligase Cbl, which is recruited to a particular phosphotyrosine residue in the epidermal growth factor receptor (EGFR) following its ligand-induced activation, and subsequently tyrosine phosphorylation of Cbl itself promotes its ubiquitin ligase activity and consequently ubiquitination of the EGFR [23–25].

Second, ubiquitination is a reversible signal that is modulated by the action of DUBs, which is critical for the dynamic regulation of ubiquitin networks in the cell. The regulation of DUB activity is only beginning to be understood, and structural data indicate that these enzymes are in an active conformation only when bound to ubiquitin. Some DUBs require formation of complexes with other proteins in order to become active, and it has been reported that some are inhibited by phosphorylation or degradation [7]. For example, CYLD, an important DUB in the NF-κB pathway, undergoes inhibitory phosphorylation after TNF-α stimulation, leading to the accumulation of one of its substrates, Lys63-ubiquitinated TRAF2 [26].

Ubiquitin mediates many of its functions by interacting with highly specialized ubiquitin-binding domains (UBDs) in downstream effector proteins. More than 15 UBDs (UBA, UIM, IUIM, UEV, GAT, CUE, PAZ, NZF, GLUE, UBM, UBZ, VHS etc.) have been discovered so far [13, 27–31]. The structures of most of these domains have been elucidated when they are complexed with ubiquitin and it appears that they have many different tertiary structures and bind ubiquitin with relatively low affinity (50–100 μM) [13, 30]. The low affinity of UBD–ubiquitin interactions allows rapid assembly and disassembly of interaction networks, which

facilitates dynamic biochemical processes [9, 13]. Moreover, it is thought that a local increase in the concentration of UBD-containing proteins and UBDs, for example by the formation of multimeric complexes or the presence of several UBDs within the same protein, might increase the rate at which UBD–ubiquitin interactions occur [9, 13, 30]. Furthermore, some UBDs can bind several ubiquitin molecules simultaneously, as has been reported for the UIM of the endocytic sorting protein Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) [32]. Due to its versatility, the numerous substrates that can be tagged with ubiquitin and the various proteins containing UBDs, ubiquitination is thus involved in complex networks of interactions in time and space that regulate key cellular functions, such as signaling, endocytosis, cell cycle and DNA repair.

1.1.2 Ubiquitin-like Proteins

The complexity of cellular signaling networks is further increased by modifications with ubiquitin-like (Ubl) proteins, including the small ubiquitin-related modifier (SUMO), Neural precursor cell-expressed developmentally downregulated 8 (Nedd8), interferon-stimulated gene 15 (ISG15), FAT10, Atg8 and Atg12 [33, 34], all of which regulate a variety of physiological processes (Figure 1.1). All Ubls share a similar three-dimensional structure, the ubiquitin superfold which is a β-grasp fold. Despite the varying degrees of sequence similarity, all proteins containing this fold are collectively known as ubiquitons [34].

In a manner similar to that involved in the tagging of proteins with ubiquitin, Ubls are covalently attached to their target proteins via a cascade of three enzymes (E1, E2, E3) which are partially specific for each of the Ubls [33]. As with ubiquitin, Ubls most frequently attach to lysines, although the free N-terminus can be an attachment site for both for ubiquitin and Ubls. In contrast to the ubiquitin system, Ubls generally form mono-conjugates with the substrates and not polymeric chains (Figure 1.1). SUMO conjugates have been observed, however, but their function is not yet known [35]. It is very likely that there are specialized interaction domains for all the Ubls, although they have only been described for a subset. SUMO-interacting motifs (SIMs) have been assigned [36–39], and some known UBDs interact not only with ubiquitin, but also with Nedd8 [40]. Moreover, it is interesting to note that UBDs and SIMs bind at distinct surface locations on ubiquitin and SUMO, respectively, resulting in highly specific interactions which provide some insights into the different cellular functions of these two proteins [1].

In many cases, there is an active interplay between ubiquitin and Ubls in the regulation of individual proteins and/or cellular pathways. For example, the same lysine residue can be modified with either ubiquitin or SUMO, leading to the activation of completely different downstream pathways. The modification of PCNA (proliferating cell nuclear antigen), that forms a clamp that recruits DNA polymerases to the replication fork, with either ubiquitin or SUMO induces error-prone DNA repair or DNA synthesis, respectively [14]. Moreover, there is apparent cooperation between ubiquitin and Nedd8 during downregulation of the epider-

mal growth factor receptor (EGFR). EGF stimulation triggers Cbl-mediated neddylation of the EGFR, which in turn promotes the subsequent Cbl-mediated ubiquitination of the receptor and its degradation [40].

Further complexity in Ubl signaling networks results from the fact that Ubl domains can be found within the genetically-encoded sequence of proteins. Many proteins containing Ubl domains interact with the proteasome, but there are also several examples in which the ubiquitin fold is involved in mediating protein–protein interactions in signal transduction cascades, consistent with the important role of ubiquitin and Ubls in both degradation and signaling pathways [34].

1.2 Ubiquitin in Cancer Pathogenesis

The development of cancer is a multi-step process which results from mutations in the cellular pathways that control signaling, endocytosis, cell-cycle and cell-death and interactions between the tumor and its surrounding tissue [41]. Deregulation of components of the ubiquitination machinery appears to be a common theme in the development of cancers [4, 42–44]. Mutations or overexpression of numerous E3 ubiquitin ligases can convert them to potent oncogenes and some E3s and DUBs act as tumor suppressors (Table 1.1). Several substrates that are affected by alterations in E3 and DUB activity play key roles in the cell cycle, DNA repair, NF-κB signaling, RTK signaling and angiogenesis and their levels or activity are precisely regulated by ubiquitination (Table 1.1; Figure 1.2). In the following sections we will highlight the nature of role that the ubiquitin system plays in maintaining the homeostatic balance of these processes and why its deregulation promotes the development of different types of tumors.

1.2.1 Ubiquitin in Cell Cycle Control

Deregulation of cell-cycle control is a fundamental characteristic of cancer. Uncontrolled proliferation of cancer cells occurs because the precise regulation of the cell cycle has been disrupted [41]. Progression through the cell cycle is mediated by cyclin-dependent kinases (CDKs) whose activity is regulated by cyclins and CDK inhibitors (CDKIs) [43]. These undergo ubiquitin-mediated proteolysis which results in their periodic expression, ensuring that the cell cycle proceeds at normal speed. Cyclins act as accelerators of the cell cycle, whereas CDKIs function as brakes. Therefore, cyclins (D1 and E) are frequently overexpressed in human cancers and the CDKI p27 is a prominent tumor suppressor [43, 45, 46].

Three structurally-related cullin-dependent E3 ubiquitin ligases, SKP1-CUL1-F-box-protein (SCF)/Skp2, SCF/Fbw1 and anaphase-promoting complex/cyclosome (APC/C), are involved in regulating the levels of cyclins and CDK inhibitors by promoting their polyubiquitination and degradation in the proteasome [43].

Table 1.1. Summary of pathways and proteins regulated by ubiquitination and whose deregulation leads to the development of cancer.

Pathway	Deregulated protein	Function	Type of deregulation	Substrate	Ubiquitin modification	Cancer type	References
Cell cycle	SCF/Skp2	E3 ubiquitin ligase subunit, oncogene	Overexpression	p27, cyclin E	Lys48-linked polyubiquitination	Lung cancer, malignant melanoma, lymphoma	43
	SCF/Fbw7	E3 ubiquitin ligase subunit, tumor suppressor	Mutation	Cyclin E	Lys48-linked polyubiquitination	Ovarian cancer, breast cancer, endometrial cancer	43
	APC/C	E3 ubiquitin ligase, tumor suppressor	Mutation	Cyclin B, securin	Lys48-linked polyubiquitination	Colorectal cancer	46
DNA repair	Mdm2	E3 ubiquitin ligase, oncogene	Overexpression	p53	Lys48-linked polyubiquitination	Non-small cell lung cancer, soft-tissue carcinoma, colorectal cancer	59
	HAUSP	DUB, tumor suppressor	Mutation	p53, Mdm2	Deubiquitination	Non-small-cell lung cancer	67
	BRCA1	E3 ubiquitin ligase, tumor suppressor	Germline mutation	γ-tubulin	Polyubiquitination	Breast cancer, ovarian cancer	68, 69
	FANCL	E3 ubiquitin ligase, tumor suppressor	Mutation	FANCD2	Monoubiquitination	Fanconi anemia-related cancers	68, 74
NF-κB signaling	CYLD	DUB, tumor suppressor	Mutation	NEMO, TRAF2, TRAF6, Bcl-3	Deubiquitination of Lys63-linked ubiquitin chains	Cylindromatosis	53
RTK signaling	Cbl	E3 ubiquitin ligase, proto-oncogene	Mutation	RTKs	Multiple monoubiquitination	Breast cancer, glioblastoma, head and neck cancer, lymphoma	81–84, 86, 87
Angiogenesis	SCF/VHL	E3 ubiquitin ligase subunit, tumor suppressor	Mutation	HIF1α	Lys48-linked polyubiquitination	Kidney cancer, blood vessel tumors in the CNS	78

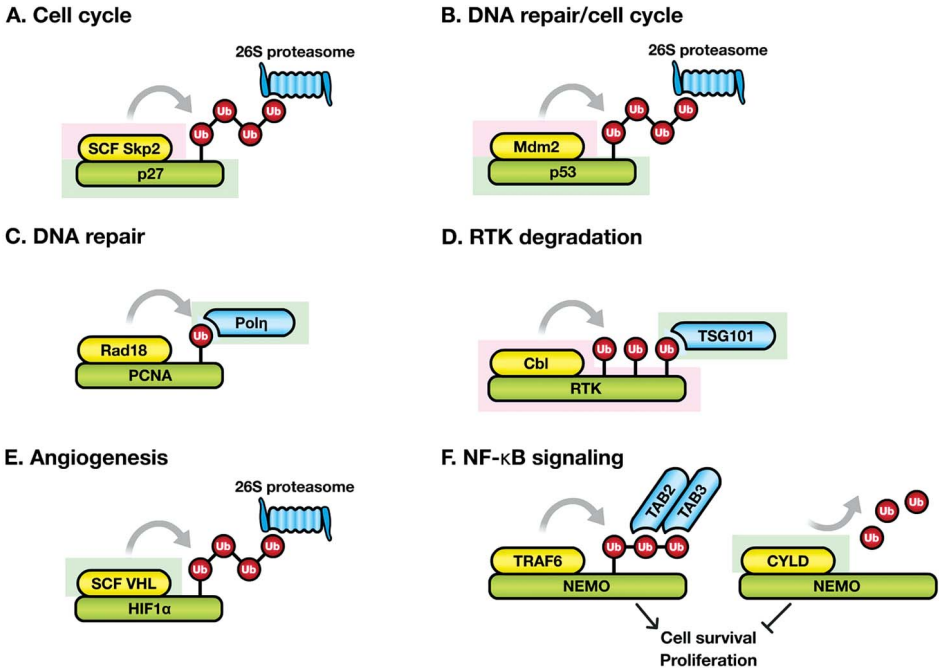

Fig. 1.2. Overview of cancer-relevant ubiquitin-dependent pathways. SCF/Skp2, Mdm2, Rad18, Cbl, SCF/VHL and TRAF6 all are E3 ubiquitin ligases (yellow) that mediate specific types of ubiquitination of their respective substrates which are indicated in the figure (p27, p53, PCNA, RTKs, HIF1α and NEMO) (green). The proteasome, which has UBD-containing subunits, and UBD-containing proteins (Polη, TSG101, TAB2/3) are shown in blue. (A, B, E) Lys48-linked polyubiquitination of p27, p53 and HIF1α leads to their proteasomal degradation, promoting cell cycle progression (p27, p53) or block of production of pro-angiogenic factors (HIF1α). SCF/Skp2 and Mdm2 act as oncogenes, because their overexpression leads to increased proliferation and the development of cancer. SCF/VHL, on the other hand, acts as a tumor suppressor, since its mutation leads to the accumulation of HIF1α, aberrant angiogenesis and tumorigenesis. (C) Rad18 mediates monoubiquitination of PCNA, a modification responsible for recruiting ubiquitin binding domain (UBD)-containing TLS polymerases to the site of DNA damage. Mutation of TLS polymerase Polη leads to a variant of a skin tumor syndrome called Xeroderma pigmentosum. (D) Cbl mediates multiple monoubiquitination of RTKs, which is recognized by ubiquitin-binding domains in proteins of the endocytic sorting machinery, including TSG101. Mutation of the Cbl binding site in RTKs, mutations of Cbl that abolish its ubiquitin ligase activity, or mutation in TSG101 all lead to defective receptor sorting and degradation, causing constitutive signaling and tumorigenesis. (F) TRAF6 mediates Lys63-linked polyubiquitination of NEMO, which recruits the UBD-containing proteins TAB2/3, leading to activation of the protein kinase TAK1 that is required for NF-κB activation. CYLD, the DUB that removes Lys63-linked chains from NEMO, is mutated in a cancer syndrome called cylindromatosis. Tumor suppressors are indicated in turquoise and oncogenes or proto-oncogenes in pink.

SCF/Skp2 targets among others p27 and cyclin E, and SCF/Fbw1 targets cyclin E for polyubiquitination and proteasomal degradation, events that regulate the G1–S transition (Figure 1.2) [47]. APC/C, on the other hand, promotes polyubiquitination and degradation of mitotic cyclins and securin, which are required for termination of the mitotic cycle and separation of the sister chromatids, respectively [46]. In this way APC/C maintains the normal chromosome number, alterations of which are a prevalent form of genetic instability in human cancers. These E3 ubiquitin ligases thus act at different time points during the cell cycle and importantly they appear to interplay in a regulatory loop [43].

Due to their central function in cell cycle progression, aberrant expression or mutations of SCF/Skp2, Fbw1 or APC/C have been found in several human cancers (Table 1) [43, 45, 46]. Skp2 has oncogenic properties in transgenic mouse models, is frequently overexpressed in lung cancers and its overexpression is correlated with poor prognosis in a wide range of cancer types [43]. Fbw1, on the other hand, acts as a tumor suppressor. Mutations in the *FBW1* gene have been reported in ovarian, breast and endometrial cancer, often correlated with increased cyclin E levels [43]. APC/C also functions as a tumor suppressor and is mutated in more than 70% of colorectal carcinomas [46]. Thus, cumulative evidence indicates that deregulation of the ubiquitin system in cell-cycle control is closely linked to the development of cancer.

1.2.2 Ubiquitin in the NF-κB Pathway

The NF-κB family of transcription factors triggers the expression of genes that are central mediators of cell survival, proliferation, and innate and adaptive immune responses. The role of NF-κB in cancer is connected to its constitutive activation of anti-apoptotic signals in both pre-neoplastic and malignant cells, and its emerging role in regulating tumor angiogenesis and invasion [48]. NF-κB activation is controlled by ubiquitination of several of the components of the NF-κB pathway [2, 18, 49]. A key step in the activation of NF-κB is its release from the inhibitor IκB and its subsequent translocation from the cytoplasm to the nucleus where it triggers the expression of its target genes. A central regulator of this process is the IκB kinase (IKK) complex, which consists of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ/NEMO). IKK promotes IκB phosphorylation which recruits the E3 ubiquitin ligase SCF-βTRCP to IκB which in turn promotes Lys48-linked ubiquitination and proteasomal degradation, thereby releasing NF-κB [18, 49].

Another type of ubiquitin modification is exemplified by Lys63-linked polyubiquitination which also plays a central role in NF-κB activation by activating protein kinases. Both IKK and the kinase that activates IKK, TGFβ-activated kinase (TAK1), require Lys63-linked chains synthesized by the E3 ubiquitin ligase TNF receptor associated factor 6 (TRAF6) for their activation [18]. IKK activation requires the modification of the regulatory subunit NEMO with Lys63-linked chains [50]. TAK1 activation depends on the interaction between the UBDs of the TAK1-binding