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# *Ubiquitination of Myc: Proteasomal Degradation and Beyond*

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**Abstract.** The level of Myc proteins is a critical determinant of cellular proliferation and apoptosis. Ubiquitination of Myc plays a key role in controlling protein levels by stimulating proteasomal degradation of the protein. Some experiments suggest that ubiquitination may also regulate Myc function in addition to turnover. This review attempts to summarize current knowledge about this field.

## 1 Introduction

Mammalian *MYC* genes comprise a small gene family that has five members, three of which have been implicated in the genesis of multiple human tumors; these are called *c-MYC*, *MYCN*, and *MYCL* and are thought to differ mainly in their expression pattern, although some functional differences have been reported. All three genes encode phospho-

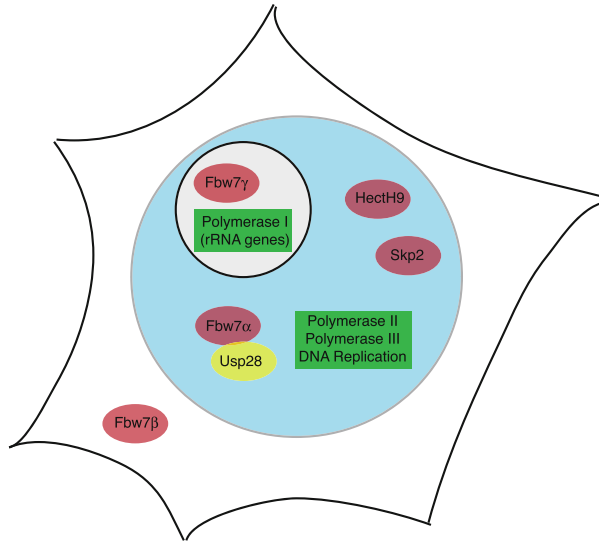


**Fig. 1.** Intracellular localization of Myc function and turnover. The diagram shows the intracellular localization of ubiquitin ligases and deubiquitinating enzymes that have been implicated in the turnover of Myc proteins and the functions of Myc proteins in each compartment

proteins that are predominantly localized in the nucleus (Adhikary and Eilers 2005; Oster et al. 2002). FRAP experiments show, however, that c-Myc, the most intensely studied of the Myc proteins, rapidly shuttles between nucleolus, nucleus, and the cytosol, arguing that the steady-state distribution of Myc proteins is the result of a highly dynamic and probably also a highly regulated process (Arabi et al. 2003) (see Fig. 1).

Myc proteins are transcription factors that both activate and repress distinct sets of target genes. In addition, they appear to have direct functions in controlling DNA replication (Dominguez-Sola et al. 2007). As transcription factors, Myc proteins appear to be unusual in two respects: first, they occupy a staggeringly large number of binding sites, recently estimated to be well over 10,000 sites per haploid genome (Chen et al. 2008; Kim et al. 2008). This generates a somewhat paradox situation in the sense that the number of Myc molecules in nontransformed cells

is probably significantly lower than the number of binding sites in the genome; if so, each binding site is likely to be occupied only transiently. Second, Myc proteins can activate transcription mediated by all three eukaryotic RNA polymerases, including RNA polymerase I, which transcribes genes encoding the ribosomal RNA precursor in the nucleolus (Arabi et al. 2005; Grandori et al. 2005). Consistently, Myc proteins accumulate in the nucleolus, most notably in cells treated with inhibitors of the proteasome (Arabi et al. 2003). The mechanisms, by which Myc affects the expression of its target genes, and the identity and relevance of target genes have been reviewed recently and will not be discussed here.

In nontransformed cells, Myc proteins are thought to translate growth factor-dependent signals into changes in gene expression, thereby altering cell behavior (e.g., by stimulating cell proliferation and cell growth). This notion is supported by several key findings: first, expression of the different *MYC* mRNAs is tightly regulated by external growth factors in nontransformed cells and this control is frequently disrupted by oncogenic mutations during transformation (He et al. 1998). Second, the activation of conditional alleles of Myc in tissue culture and in vivo is often sufficient to induce strong changes in cell proliferation, cell growth, and also in apoptosis (Pelengaris et al. 2002). Third, Myc proteins are very short-lived in vivo and growth factor-dependent and cell cycle-dependent pathways regulate their turnover (Lüscher and Eisenman 1988). This latter aspect is the central topic of this review.

## 2 Turnover of Myc

In nontransformed cells, (c-)Myc rapidly turns over with a half-life of approximately 20–30 min; similar values have been reported for N-Myc. Degradation of Myc proteins is mediated by the proteasome, as documented by numerous observations showing that inhibition of the proteasome greatly extends the half-life of Myc (Bonvini et al. 1998; Gross-Mesilaty et al. 1998). The proteasome usually recognizes ubiquitinated proteins; consistent with this observation, Myc proteins are extensively ubiquitinated in vivo. The lysine residues of Myc that are ubiquitinylated have not been determined in vivo, although it has been

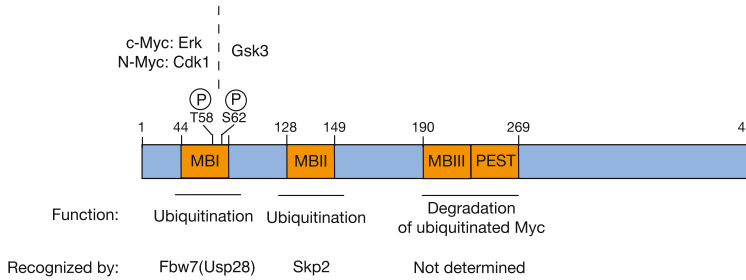


**Fig. 2.** MycBoxes and their function in Myc turnover. Localization of four sequence elements in Myc proteins that have been implicated in Myc ubiquitination and turnover. The primary sequence of PEST element defined in c-Myc is evolutionarily poorly conserved and therefore not considered a MycBox

described that a mutant allele of Myc lacking six lysine residues is less ubiquitinylated by one of the ubiquitin ligases of Myc, HectH9 (Adhikary et al. 2005). The half-life of Myc proteins can be extended in tumor cells, arguing that degradation of Myc is a target for oncogenic mutations (Malempati et al. 2006). Indeed, by now different mutations have been found that impinge on Myc turnover; several of these will be discussed below.

Three domains of the Myc protein are necessary to mediate its turnover: since they were initially discovered as short, evolutionarily highly conserved stretches of amino acids in an otherwise less conserved family of proteins, they were termed MycBoxes (see Fig. 2).

The best understood domain in terms of degradation is MycBoxI, located close to the amino-terminus of Myc: this is the binding site for one of the ubiquitin ligases that mediate Myc turnover, Fbw7 (Welcker et al. 2004b; Yada et al. 2004). Point mutations within MycBoxI therefore stabilize the Myc protein and reduce its ubiquitination (Bahram et al. 2000). Such point mutations in MycBoxI are frequently found in lymphomas; in contrast, no point mutations of Myc have been found in solid tumors.

In addition, both MycBoxII and MycBoxIII affect Myc turnover. Deletion of MycBoxIII stabilizes Myc and leads to the accumulation of ubiquitinated Myc in tissue culture experiments (Herbst et al. 2004,

2005). This would argue that MycBoxIII, in contrast to MycBoxI, promotes turnover at a postubiquitination step; similar observations have also been made for the deletion of a PEST sequence that is adjacent to MycBoxIII (Gregory and Hann 2000). The mechanism(s) that prevents degradation of ubiquitinated Myc in both deletion mutants have not been resolved. Finally, MycBoxII serves as a binding site for Skp2, an F-box ubiquitin ligase that has been implicated in Myc turnover (Kim et al. 2003; von der Lehr et al. 2003).

One of the most interesting and also puzzling aspects of the Myc biology is the tight link between Myc turnover and its biological functions. One example for this is provided by the mutations in MycBoxI, which are found in lymphomas (Hemann et al. 2005). Such mutations not only enhance Myc stability as described above, but also strongly decrease the ability of Myc to induce apoptosis. As a result, lymphomas that are generated using these mutant alleles of Myc lack mutations in either the p53 gene or its upstream regulators that are invariably found in lymphomas arising with wild-type Myc. Two explanations can account for these observations: first, it is possible that the phosphodegron in MycBoxI is also recognized by other proteins that mediate effector functions of Myc. Consistent with this suggestion, phosphorylation at S62 not only regulates Myc stability, but also controls the spectrum of target genes that are regulated by Myc: for example, upregulation of gamma-glutamyl-cysteine synthetase expression by Myc requires Erk-dependent phosphorylation of S62 (Benassi et al. 2006). Alternatively, ubiquitinated Myc may itself have effector functions that differ from nonubiquitinated Myc.

## 3 Ubiquitin Ligases and Their Regulation

There are three ubiquitin ligases for which there is evidence that they can ubiquitinate Myc in vivo.

Best understood is the $SCF^{Fbw7}$ complex; this complex binds to the phosphodegron that is contained in MycBoxI and, upon binding, targets Myc for degradation. Several pieces of evidence document a role for the Fbw7 subunit of the $SCF^{Fbw7}$ complex in Myc turnover: for example, cells deficient for Fbw7 show elevated steady state levels of Myc

and an increased half-life of the protein (Onoyama et al. 2007; Yada et al. 2004). Consistent with this role, human *FBW7* has properties of a tumor suppressor gene; point mutations in the substrate recognition domain and missense mutations are found in colon carcinoma and other tumor entities (Mao et al. 2004). These mutations often affect only one of the two *FBW7* alleles, suggesting that *FBW7* may be a haploinsufficient tumor suppressor gene. Since Fbw7 proteins form homodimers, an alternative model proposes that the protein encoded by the mutated allele might have dominant-negative properties since it sequesters the wild type protein onto nonfunctional heterodimers (Welcker and Clurman 2008). A third possibility is to suggest that some degree of ubiquitination by Fbw7 has a positive function in Myc biology.

Notably, there are three isoforms of Fbw7, which arise by alternative splicing of the same primary RNA transcript (Grim et al. 2008; Welcker et al. 2004a). All three isoforms share a common carboxyl-terminus, which contains the substrate-recognition domain and therefore are identical in their ability to bind phosphorylated substrates. They differ, however, in their amino-terminus and subcellular localization: Fbw7α is localized in the nucleus, Fbw7β localizes to the cytosol and Fbw7γ is found in the nucleolus; furthermore, the isoforms differ in their ability to interact with the ubiquitin-specific protease, Usp28 (see the next section). The specific localization of Fbw7 isoforms may also be responsible for the observation that Myc proteins accumulate to very high levels in the nucleolus after inhibition of the proteasome. This has been taken as evidence that the rate of turnover of Myc in the nucleolus is very high.

To initiate degradation by Fbw7, a threonine residue (T58) in Myc-BoxI needs to be phosphorylated. In both c-Myc and N-Myc, glycogen synthase kinase 3 (Gsk3) is the kinase responsible for the phosphorylation of this residue (Welcker et al. 2004b; Yada et al. 2004). Since Gsk3 in turn is phosphorylated and inhibited by Akt, the stability of Myc proteins depends on an active PI3kinase/Akt pathway (Sears et al. 1999). Conversely, there is evidence that maintaining Myc stability is a key function of this pathway during oncogenic transformation (Yeh et al. 2004). Gsk3 needs a priming phosphorylation and this is provided by phosphorylation of serine 62. The mitotic cyclin B/Cdk1 complex phosphorylates S62 in N-Myc, and as a result degradation of N-Myc is

initiated during mitosis (Sjostrom et al. 2005). This mitotic degradation of N-Myc is important in terminating the proliferation of neuroblasts during the development of the central and most likely also the peripheral neural system (Otto et al., unpublished observations). In contrast, Map-kinases phosphorylate S62 in c-Myc (Benassi et al. 2006).

One important and still unresolved issue is the exact phosphorylation status of Myc proteins that are degraded by Fbw7; if differentially phosphorylated forms of Myc regulate different groups of target genes, the selective degradation of distinct phosphorylated Myc-species by Fbw7 may be an important tool to regulate Myc function in vivo. For c-Myc, several pieces of evidence suggest that isomerization of the Proline 61-peptide bond by the prolyl-isomerase Pin1 and subsequent dephosphorylation of S62 by protein phosphatase 2A (PP2A) is required for degradation by Fbw7, arguing that the substrate for Myc turnover is exclusively phosphorylated at T58 (Arnold and Sears 2006; Yeh et al. 2004). However, Fbw7 also recognizes a peptide corresponding to the doubly phosphorylated site in vitro (Yada et al. 2004*)*. In vivo, Fbw7 recognizes both the mono- and the biphosphorylated phosphodegron of cyclin E, another target of the ligase, yet the mode of recognition is different (Welcker and Clurman 2007). The biphosphorylated degron has a higher affinity for Fbw7 and can be degraded by monomeric Fbw7, whereas the monophosphorylated degron binds Fbw7 with lower affinity and needs Fbw7 dimers to be degraded; this would argue that dephosphorylation at S62 reduces the affinity of Fbw7 for Myc. Furthermore, Fbw7-dependent degradation of N-Myc does not require Pin1, although the sequence of the phosphodegron is identical (Sjostrom et al. 2005). Clearly, more work appears to be necessary to resolve this issue.

The second ubiquitin ligase that can both ubiquitinate and induce proteasomal degradation of Myc is Skp2. Binding of Skp2 to Myc does not require MycBoxI; instead, the integrity of MycBoxII is required for this interaction, although the binding may be indirect (Kim et al. 2003; von der Lehr et al. 2003). Consistent with the idea that Skp2 and Fbw7 recognize Myc via different domains, both proteins regulate Myc turnover independently and additively, as witnessed by the analysis of cells that are either singly or doubly depleted for Fbw7 and Skp2 (Yada et al. 2004). The analysis of the interaction of Skp2 with Myc provides a second clear example for the close link between ubiquitination and

Myc function: on one hand, Skp2 has clearly been implicated in Myc degradation; on the other hand, Skp2 itself has oncogenic properties. For example, deregulated expression of Skp2 is sufficient to transform primary cells in conjunction with oncogenic alleles of Ras (Gstaiger et al. 2001). A possible explanation for this paradox could be that Skp2 is also essential for both transcriptional activation and repression by Myc, suggesting that ubiquitinated Myc has a specific role in both processes (Kim et al. 2003; von der Lehr et al. 2003).

The third ubiquitin ligase implicated in Myc ubiquitination is the Hect-domain protein HectH9/Huwe1/Arf-Bp1/Mule (Adhikary et al. 2005). HectH9 binds both endogenous c- and N-Myc proteins at an yet unidentified binding site.

HectH9 polyubiquitinates c-Myc via lysine 63 (K63) linkage and appears not to be involved in turnover. Instead, ubiquitination of c-Myc is required to enhance transcriptional activation of several Myc target genes and to recruit the p300 co-activator protein to target promoters (Adhikary et al. 2005). Consistent with these observations, expression of HectH9 is strongly upregulated in multiple human tumors and depletion of HectH9 leads to inhibition of tumor cell proliferation in all phases of the cell cycle (Adhikary et al. 2005). Surprisingly, this situation is different in primary cells that depend on N-Myc for proliferation, such as ES cells. In contrast to its interaction with c-Myc, HectH9 assembles a predominantly K48-linked chain on N-Myc and has a role in catalyzing N-Myc turnover; as a result, inhibition of HectH9 enhances proliferation of these cells (Zhao et al. 2008).

One puzzling aspect of the biology of this ubiquitin ligase is that it has been isolated in a diverse number of biological contexts: initially described as a protein that degrades histones during spermatogenesis (Liu et al. 2005), HectH9 has been identified as (a) a protein that interacts with the Arf tumor suppressor protein and degrades p53 (Chen et al. 2005) (b) as a ubiquitin ligase that degrades the anti-apoptotic Mcl1 protein during DNA damage-induced apoptosis (Zhong et al. 2005), (c) as a substrate for the Atm protein kinase (Mu et al. 2007), and (d) as a ubiquitin ligase that degrades Cdc6 after DNA damage (Hall et al. 2007). Whether these diverse functional descriptions, which also imply that HectH9 can be active in different subcellular compartments, reflect a common biochemical pathway or whether they indicate that the same

protein carries out largely unrelated biological functions is currently an open question.

## 4 Reversibility of Ubiquitination

Ubiquitination of Myc by Fbw7 can be reverted by the ubiquitin-specific protease, Usp28, which was identified in a siRNA-screen searching for proteins that are required for Myc function (Popov et al. 2007b). Depletion of Usp28 destabilizes Myc by facilitating turnover by Fbw7. Usp28 is localized in the nucleus, but not in the nucleolus, and can form a ternary complex with Myc and Fbw7α, but not withFbw7γ, providing a potential explanation why Myc is highly unstable in the nucleolus. The findings suggest that in proliferating cells nuclear Myc may undergo one or more futile cycles of ubiquitination and deubiquitination, before it is degraded by the proteasome. This model is similar to what has been proposed for the regulation of p53 by Mdm2 ubiquitin ligase and the Hausp deubiquitinating enzyme (Li et al. 2004). In both cases, the suggestion would be that this ensures a rapid regulation of protein levels in response to environmental changes. In support of this notion, regulation of Myc protein levels in response to DNA damage involves Usp28 (Popov et al. 2007a). Notably, expression of Usp28 itself is regulated in a proliferation-dependent manner in normal colon and strongly upregulated during carcinogenesis; tissue culture experiments suggest that *USP28* may be a transcriptional target of the Wnt pathway (Popov et al. 2007b). Upregulation of Usp28 may therefore be one mechanism explaining how Myc stability is extended during tumor development.

A second ubiquitin-specific protease that has been linked to Myc function is Usp22. Usp22 is a transcriptional co-activator of Myc, which reverses the monoubiquitination of histone H2B at Myc target genes (Zhang et al. 2008).

## 5 Ubiquitination of *Myc*: Beyond Turnover?

As indicated throughout this review, a number of observations demonstrate that ubiquitination of Myc is tightly coupled to the regulation of

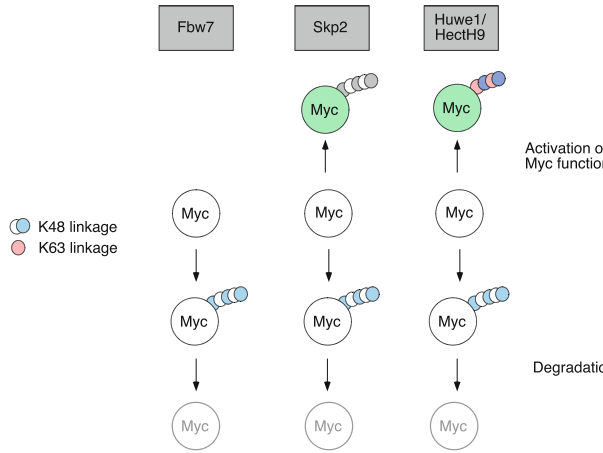


**Fig. 3.** Evidence for functional roles of ubiquitinated Myc. The diagram shows the three main ubiquitin ligases that have been implicated in turnover of Myc proteins together with a summary of what is known about nonproteasomal roles of Myc

its function. There appear to be two major issues that remain to be resolved (see Fig. 3).

First, two ubiquitin ligases, Skp2 and HectH9, are capable of both activating Myc's transcriptional functions and promoting Myc turnover (Adhikary et al. 2005; Kim et al. 2003; von der Lehr et al. 2003). In the case of HectH9, the assembly of K63-linked polyubiquitin chain on Myc does not target Myc to the proteasome; instead, it leads to enhanced activation. In contrast, HectH9 mediates degradation of N-Myc via the assembly of a K48-linked chain (Zhao et al. 2008); the topology of the chain that is assembled by Skp2 has not been determined. Two alternate models can explain these observations. first, assembly of a nondegradable polyubiquitin chain may prevent turnover and thereby the removal of chromatin-bound Myc from specific promoters. Consequently, this may enhance the time-of-residence of Myc at a given site on DNA. Under conditions where the total number of Myc molecules is smaller than the total number of binding sites (and therefore these sites would not be immediately refilled once Myc is removed), alterations in

residence time may have a major impact on gene regulation. Alternatively, ubiquitinated Myc may have direct effector functions that differ from nonubiquitinated, such as recruitment of the p300 histone acetyl transferase, which binds to polyubiquitin chains (Grossman et al. 2003).

Second, mutations in MycBoxI affect Myc function in a manner that does not simply reflect their effect on Myc protein levels (Hemann et al. 2005). One possible explanation would be that proteins other than Fbw7 recognize MycBoxI in a phosphorylation-dependent manner and that these proteins mediate transcriptional effector functions of Myc. This view is supported by two observations: first, the residues in Myc that are mutated in lymphomas include the phosphodegron residues that are recognized by Fbw7. However, other residues that are not thought to be involved in Fbw7 binding are also mutated, albeit at lower frequencies (Bahram et al. 2000). This would argue that the critical interaction that is disrupted by the mutations may not be the Myc/Fbw7 interaction. Second, phosphorylation at S62 in response to oxidative stress has widespread effects on the spectrum of Myc-target genes (Benassi et al. 2006). Although this observation does not rule out a model in which altered turnover has differential effects on Myc target genes, both observations together suggest that critical effector proteins bind to MycBoxI in a phosphorylation-dependent manner. If so, the specific removal of Myc species that are phosphorylated at T58 by Fbw7 might generate different functional states of Myc in vivo, a model first proposed by Amati and colleagues (Amati 2004).

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## References

Adhikary S, Eilers M (2005) Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 6:635–645

Adhikary S, Marinoni F, Hock A, Hulleman E, Popov N, Beier R, Bernard S, Quarto M, Capra M, Goettig S et al (2005) The ubiquitin ligase HectH9 regulates transcriptional activation by Myc and is essential for tumor cell proliferation. Cell 123:409–421

Amati B (2004) Myc degradation: Dancing with ubiquitin ligases. Proc Natl Acad Sci U S A 101:8843–8844

Arabi A, Rustum C, Hallberg E, Wright AP (2003) Accumulation of c-Myc and proteasomes at the nucleoli of cells containing elevated c-Myc protein levels. J Cell Sci 116:1707–1717

Arabi A, Wu S, Ridderstrale K, Bierhoff H, Shiue C, Fatyol K, Fahlen S, Hydbring P, Soderberg O, Grummt I et al (2005) c-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. Nat Cell Biol 7:303–310

Arnold HK, Sears RC (2006) Protein phosphatase 2A regulatory subunit B56alpha associates with c-myc and negatively regulates c-myc accumulation. Mol Cell Biol 26:2832–2844

Bahram F, von der Lehr N, Cetinkaya C, Larsson LG (2000) c-Myc hot spot mutations in lymphomas result in inefficient ubiquitination and decreased proteasome-mediated turnover. Blood 95:2104–2110

Benassi B, Fanciulli M, Fiorentino F, Porrello A, Chiorino G, Loda M, Zupi G, Biroccio A (2006) c-Myc phosphorylation is required for cellular response to oxidative stress. Mol Cell 21:509–519

Bonvini P, Nguyen P, Trepel J, Neckers LM (1998) In vivo degradation of N-myc in neuroblastoma cells is mediated by the 26S proteasome. Oncogene 16:1131–1139

Chen D, Kon N, Li M, Zhang W, Qin J, Gu W (2005) ARF-BP1/Mule is a critical mediator of the ARF tumor suppressor. Cell 121:1071–1083

Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J et al (2008) Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 133: 1106–1117

Dominguez-Sola D, Ying CY, Grandori C, Ruggiero L, Chen B, Li M, Galloway DA, Gu W, Gautier J, Dalla-Favera R (2007) Non-transcriptional control of DNA replication by c-Myc. Nature 448:445–451

Grandori C, Gomez-Roman N, Felton-Edkins ZA, Ngouenet C, Galloway DA, Eisenman RN, White RJ (2005) c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. Nat Cell Biol 7:311–318

Gregory MA, Hann SR (2000) c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. Mol Cell Biol 20:2423–2435

Grim JE, Gustafson MP, Hirata RK, Hagar AC, Swanger J, Welcker M, Hwang HC, Ericsson J, Russell DW, Clurman BE (2008) Isoform- and cell cycle-dependent substrate degradation by the Fbw7 ubiquitin ligase. J Cell Biol 181:913–920

Gross-Mesilaty S, Reinstein E, Bercovich B, Tobias KE, Schwartz AL, Kahana C, Ciechanover A (1998) Basal and human papillomavirus E6 oncoprotein-induced degradation of Myc proteins by the ubiquitin pathway. Proc Natl Acad Sci U S A 95:8058–8063

Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y, Livingston DM (2003) Polyubiquitination of p53 by a ubiquitin ligase activity of p300. Science 300:342–344

Gstaiger M, Jordan R, Lim M, Catzavelos C, Mestan J, Slingerland J, Krek W (2001) Skp2 is oncogenic and overexpressed in human cancers. Proc Natl Acad Sci U S A 98:5043–5048

Hall JR, Kow E, Nevis KR, Lu CK, Luce KS, Zhong Q, Cook JG (2007) Cdc6 stability is regulated by the Huwe1 ubiquitin ligase after DNA damage. Mol Biol Cell 18:3340–3350

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW (1998) Identification of c-MYC as a target of the APC pathway. Science 281:1509–1512

Hemann MT, Bric A, Teruya-Feldstein J, Herbst A, Nilsson JA, Cordon-Cardo C, Cleveland JL, Tansey WP, Lowe SW (2005) Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. Nature 436:807–811

Herbst A, Salghetti SE, Kim SY, Tansey WP (2004) Multiple cell-type-specific elements regulate Myc protein stability. Oncogene 23:3863–3871

Herbst A, Hemann MT, Tworkowski KA, Salghetti SE, Lowe SW, Tansey WP (2005) A conserved element in Myc that negatively regulates its proapoptotic activity. EMBO Rep 6:177–183

Kim J, Chu J, Shen X, Wang J, Orkin SH (2008) An extended transcriptional network for pluripotency of embryonic stem cells. Cell 132:1049–1061

Kim SY, Herbst A, Tworkowski KA, Salghetti SE, Tansey WP (2003) Skp2 regulates myc protein stability and activity. Molecular cell 11:1177–1188

Li M, Brooks CL, Kon N, Gu W (2004) A dynamic role of HAUSP in the p53-Mdm2 pathway. Mol Cell 13:879–886

Liu Z, Oughtred R, Wing SS (2005) Characterization of E3Histone, a novel testis ubiquitin protein ligase which ubiquitinates histones. Mol Cell Biol 25:2819–2831

Lüscher B, Eisenman RN (1988) c-myc and c-myb protein degradation: effect of metabolic inhibitors and heat shock. Mol Cell Biol 8:2504–2512

Malempati S, Tibbitts D, Cunningham M, Akkari Y, Olson S, Fan G, Sears RC (2006) Aberrant stabilization of c-Myc protein in some lymphoblastic leukemias. Leukemia 20:1572–1581

Mao JH, Perez-Losada J, Wu D, Delrosario R, Tsunematsu R, Nakayama KI, Brown K, Bryson S, Balmain A (2004) Fbxw7/Cdc4 is a p53-dependent, haploinsufficient tumour suppressor gene. Nature 432:775–779

Mu JJ, Wang Y, Luo H, Leng M, Zhang J, Yang T, Besusso D, Jung SY, Qin J (2007) A proteomic analysis of ataxia telangiectasia-mutated (ATM)/ATM-Rad3-related (ATR) Substrates identifies the ubiquitin-proteasome system as a regulator for DNA damage checkpoints. J Biol Chem 282:17330–17334

Onoyama I, Tsunematsu R, Matsumoto A, Kimura T, de Alboran IM, Nakayama K, Nakayama KI (2007) Conditional inactivation of Fbxw7 impairs cell-cycle exit during T cell differentiation and results in lymphomatogenesis. J Exp Med 204:2875–2888

Oster SK, Ho CS, Soucie EL, Penn LZ (2002) The myc oncogene: marvelous lY Complex. Adv Cancer Res 84:81–154

Pelengaris S, Khan M, Evan GI (2002) Suppression of myc-induced apoptosis in beta cells exposes multiple oncogenic properties of myc and triggers carcinogenic progression. Cell 109:321–334

Popov N, Herold S, Llamazares M, Schulein C, Eilers M (2007a) Fbw7 and Usp28 regulate myc protein stability in response to DNA damage. Cell cycle 6:2327–2331

Popov N, Wanzel M, Madiredjo M, Zhang D, Beijersbergen R, Bernards R, Moll R, Elledge SJ, Eilers M (2007b) The ubiquitin-specific protease USP28 is required for MYC stability. Nat Cell Biol 9:765–774

Sears R, Leone G, DeGregori J, Nevins JR (1999) Ras enhances Myc protein stability. Mol Cell 3:169–179

Sjostrom SK, Finn G, Hahn WC, Rowitch DH, Kenney AM (2005) The Cdk1 complex plays a prime role in regulating N-myc phosphorylation and turnover in neural precursors. Dev Cell 9:327–338

von der Lehr N, Johansson S, Wu S, Bahram F, Castell A, Cetinkaya C, Hydbring P, Weidung I, Nakayama K, Nakayama KI et al (2003) The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. Mol Cell 11:1189–1200

Welcker M, Clurman BE (2007) Fbw7/hCDC4 dimerization regulates its substrate interactions. Cell Div 2:7

Welcker M, Clurman BE (2008) FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. Nat Rev 8:83–93

Welcker M, Orian A, Grim JA, Eisenman RN, Clurman BE (2004a) A nucleolar isoform of the Fbw7 ubiquitin ligase regulates c-Myc and cell size. Curr Biol 14:1852–1857

Welcker M, Orian A, Jin J, Grim JA, Harper JW, Eisenman RN, Clurman BE (2004b) The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. Proc Natl Acad Sci U S A 101:9085–9090

Yada M, Hatakeyama S, Kamura T, Nishiyama M, Tsunematsu R, Imaki H, Ishida N, Okumura F, Nakayama K, Nakayama KI (2004) Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. EMBO J 23:2116–2125

Yeh E, Cunningham M, Arnold H, Chasse D, Monteith T, Ivaldi G, Hahn WC, Stukenberg PT, Shenolikar S, Uchida T et al (2004) A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. Nat Cell Biol 6:308–318

Zhang XY, Varthi M, Sykes SM, Phillips C, Warzecha C, Zhu W, Wyce A, Thorne AW, Berger SL, McMahon SB (2008) The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. Mol Cell 29:102–111

Zhao X, Heng JI, Guardavaccaro D, Jiang R, Pagano M, Guillemot F, Iavarone A, Lasorella A (2008) The HECT-domain ubiquitin ligase Huwe1 controls neural differentiation and proliferation by destabilizing the N-Myc oncoprotein. Nat Cell Biol 10:643–653

Zhong Q, Gao W, Du F, Wang X (2005) Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. Cell 121:1085–1095