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REVIEW ARTICLE

Regulation of p53 - insights into a complex process

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Abstract

The p53 protein is one of the most important tumor suppressor proteins. Normally, the p53 protein is in a latent state. However, when its activity is required, e.g. upon DNA damage, nucleotide depletion or hypoxia, p53 becomes rapidly activated and initiates transcription of pro-apoptotic and cell cycle arrest-inducing target genes. The activity of p53 is regulated both by protein abundance and by post-translational modifications of pre-existing p53 molecules. In the 30 years of p53 research, a plethora of modifications and interaction partners that modulate p53's abundance and activity have been identified and new ones are continuously discovered. This review will summarize our current knowledge on the regulation of p53 abundance and activity.

Keywords: *p53; Mdm2; ionizing radiation; ultraviolet light; protein degradation; post-translational modifications*

Introduction

The p53 protein is one of the most important tumor suppressor proteins. Functionally, p53 is a transcription factor, particularly for genes that control progression through the cell cycle or that initiate apoptosis. Nevertheless, p53 can also function as a transcriptional repressor for some genes (Gomez-Lazaro *et al.*, 2004; Laptenko and Prives, 2006). In resting cells, p53 is rapidly degraded, which keeps its protein levels low, and the few remaining p53 molecules are kept transcriptionally inactive. Cellular stress such as DNA damage or hypoxia leads to rapid activation of the tumor suppressor protein. Under these conditions, the p53 protein is rescued from degradation, accumulates to high level and is post-translationally modified at several sites. These alterations enable p53 to initiate cell cycle arrest, apoptosis or senescence, which all prevent the propagation of DNA lesions to daughter cells. Which fate the cell finally encounters depends on the cell type, the incoming signal and the resulting post-translational modification of p53.

The importance of p53 function for tumor suppression is demonstrated by the high incidence and early onset of cancer in mice and men with a genetic deletion or germ line mutation of *p53* (Donehower *et al.*, 1992; Srivastava *et al.*, 1992). In addition, p53 is often mutated in many different types of cancer. About half of all human tumors possess alterations in the *p53* gene and tumors which retain wild-type p53 often show mutations in genes that are upstream or downstream of p53 in the DNA damage response (reviewed in: Vousden and Lu, 2002).

Consistent with its function as a transcription factor, the majority of p53 is localized in the nucleus. Part of the p53 protein is, though, also present in the cytoplasm where it promotes apoptosis via direct interactions with pro- and anti-apoptotic proteins, and where it inhibits autophagy, possibly by inhibiting AMP-dependent kinase and by activating mammalian target of rapamycin (mTOR; reviewed in Moll *et al.*, 2006; Green and Kroemer, 2009).

The p53 protein possesses several functional domains including an N-terminal transactivation domain, a proline-rich domain, a central DNA binding domain (DBD), a nuclear localization sequence (NLS), a tetramerization domain (TET), a nuclear export sequence (NES) and a C-terminal regulatory domain (REG; Figure 1). All these domains are subjected to extensive post-translational modifications, which ensures a rapid response to

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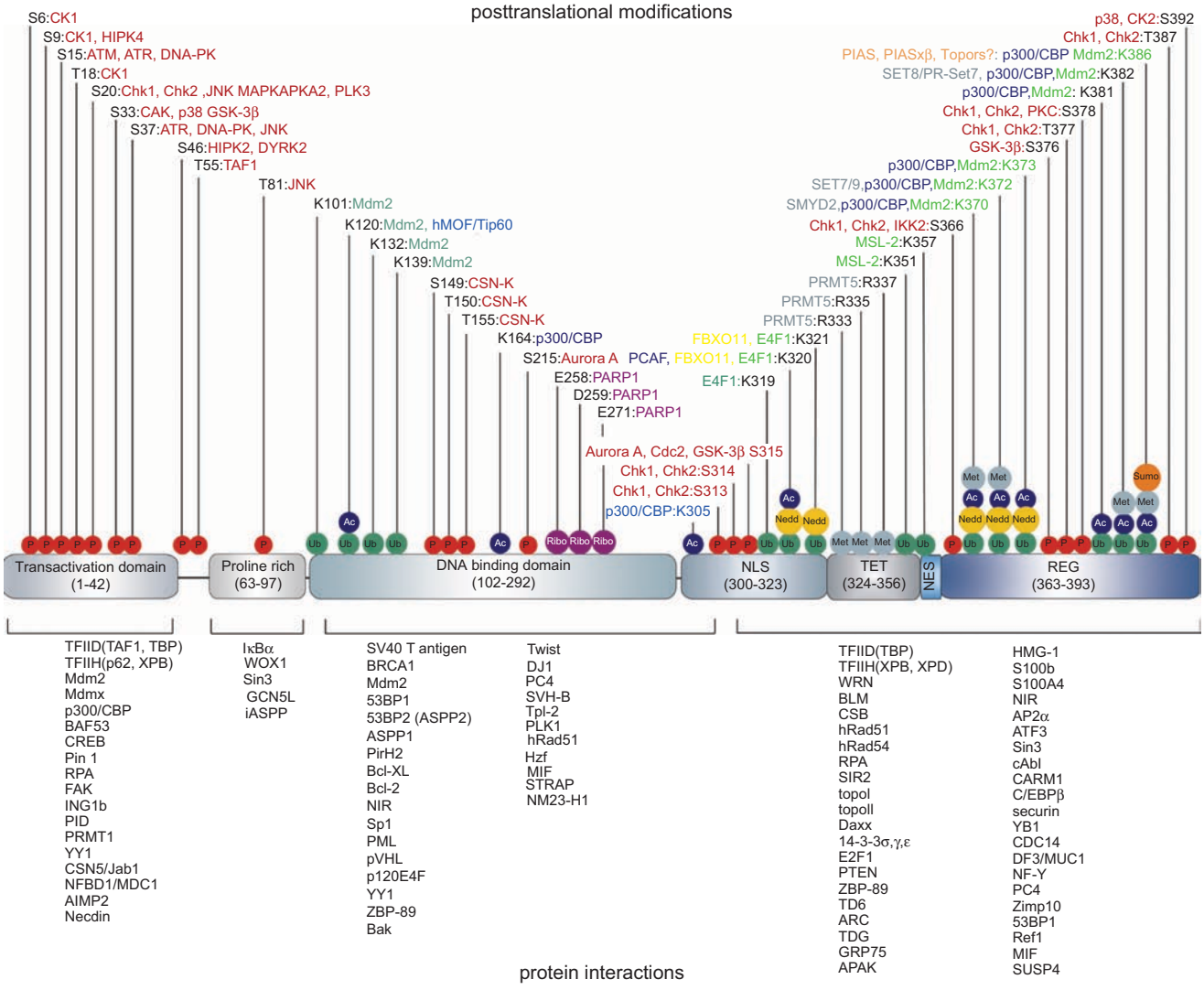

Figure 1. Schematic drawing of the p53 protein. The p53 protein consists of several functional domains, which are all intensively subjected to post-translational modifications. Moreover, an increasing number of proteins has been identified to interact with these individual domains of the tumor suppressor protein. NLS: nuclear localization signal; TET: tetramerization domain; NES: nuclear export signal; REG: regulatory domain.

changing conditions. In this review we will summarize current knowledge on how these modifications control p53 protein stability and activity.

Control of p53 abundance

The anti-proliferative activity of p53 necessitates a tight control. Part of this control is performed by maintaining p53 protein levels low. If p53 activity is dispensable, the protein is very efficiently targeted for degradation by cellular 26S proteasomes. In addition, translation of p53 RNA is kept at a low level due to binding of the ribosomal protein L26 to the 5′ untranslated region of the p53 RNA, which reduces translation efficiency (Takagi *et al.*, 2005). Cellular conditions that require p53 activity rescue the tumor suppressor from proteasomal degradation and attenuate the inhibitory effect of L26 (Maltzman and Czyzyk, 1984; Takagi *et al.*, 2005; Ofir-Rosenfeld *et al.*, 2008), resulting in a strong accumulation of the tumor suppressor protein.

Regulation of p53 stability by Mdm2

Degradation of p53 in cellular proteasomes requires prior polyubiquitination of the tumor suppressor protein, a reaction that is catalyzed by several ubiquitin ligases. Despite the identification of additional ubiquitin ligases in more recent years, the oncoprotein Mdm2 appears to be the most important regulator of p53. This has been demonstrated convincingly with mice where both *mdm2* alleles have been genetically deleted. These animals died

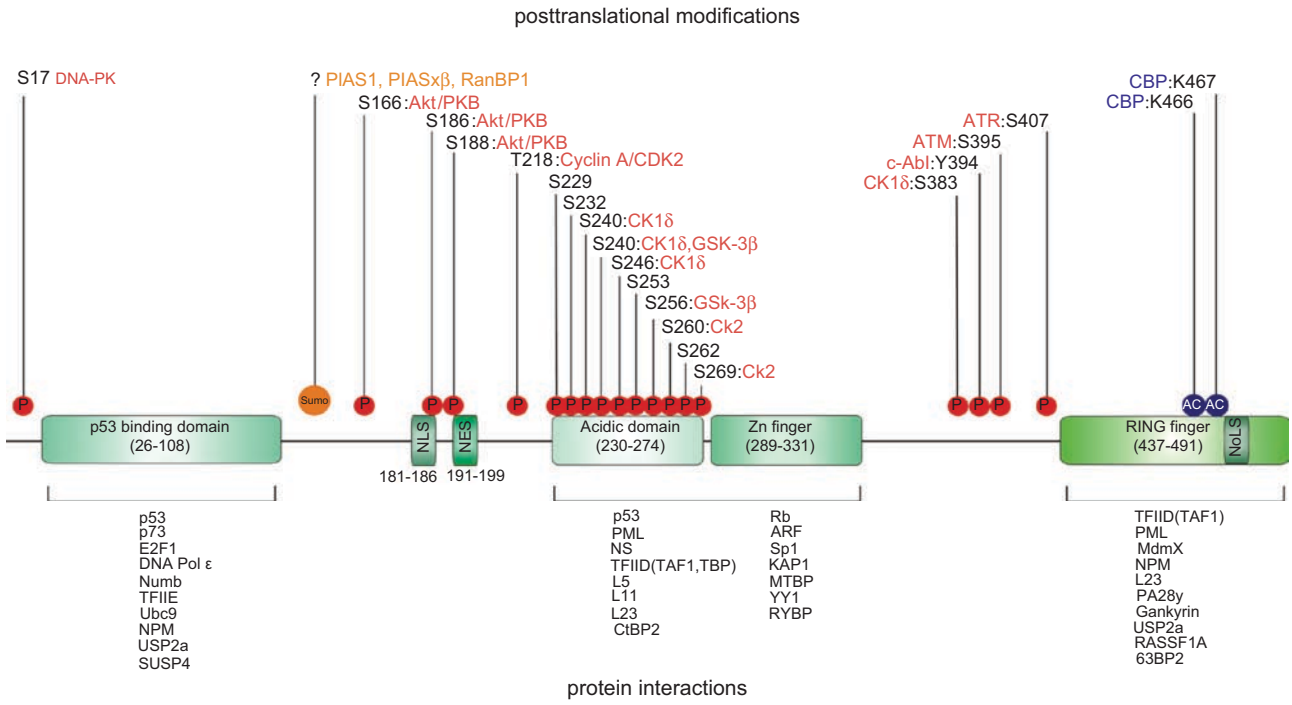

Figure 2. Schematic drawing of the Mdm2 protein. The graph shows the functional domains of the Mdm2 protein, its sites for post-translational modifications, modifying enzymes and interaction partner. NLS: nuclear localization signal; NES: nuclear export signal; Zn finger: zink finger; NoLS: nucleolar localization signal.

during early embryogenesis due to p53-mediated apoptosis. Only when *p53* was deleted as well did the animals develop normally (Jones *et al.*, 1995; Montes de Oca Luna *et al.*, 1995). Mdm2 induces ubiquitination, nuclear export and degradation of the p53 protein. In addition, Mdm2 binding results in a conformational change of p53 (Sasaki *et al.*, 2007) whose physiologic relevance is, though, as yet unclear. These activities require the N-terminus of Mdm2 with the major p53 binding site, the central acidic domain that contains a second p53 binding site and the C-terminal domain where the ubiquitin ligase activity is localized (Figure 2; Chen, J. *et al.*, 1993; Haupt *et al.*, 1997; Fang *et al.*, 2000; Kawai *et al.*, 2003; Meulmeester *et al.*, 2003; Kulikov *et al.*, 2006). As a RING-finger ubiquitin ligase, Mdm2 has no intrinsic enzymatic activity. For ubiquitination of p53, it cooperates with the ubiquitin conjugating enzymes (E2) UbcH5B and UbcH5C (Saville *et al.*, 2004). In the presence of Mdm2, these E2s transfer ubiquitin moieties directly onto p53. *In vitro*, Mdm2 mediates ubiquitination of the six most C-terminal lysines as well as lysine 101, lysine 120, lysine 132 and lysine139 (to avoid confusion, all numberings in this review relate to the human proteins, regardless of whether murine or human proteins have been investigated) in the DNA binding domain (DBD) of p53 (Figure 1; Table 1; Chan *et al.*, 2006). It is, however, unclear whether all these lysines are also ubiquitinated under physiologic conditions, particularly since mice in which all seven C-terminal lysines of p53 have been removed are phenotypically normal. Moreover, mutant p53 lacking the seven C-terminal lysines displays a normal rate of turnover (Feng *et al.*, 2005; Krummel *et al.*, 2005). The reason for this discrepancy is unclear. Eventually, there are redundant sites that can substitute for the most C-terminal lysines. However, such sites are not as yet identified. Alternatively, an ubiquitin-independent degradation process may play a more important role for p53 degradation than hitherto known. In this context, it is particularly interesting that p53 has also been shown to be degraded by a mechanism driven by the NAD(P)H quinone oxidoreductase 1 (Asher *et al.*, 2002).

Mdm2 can induce both monoubiquitination and polyubiquitination of p53. Which one of these activities is performed depends on the level of Mdm2 activity. Low levels of Mdm2 activity induce monoubiquitination and nuclear export of p53, which allows p53 degradation in cytoplasmic proteasomes, whereas high levels of Mdm2 promote p53 polyubiquitination and degradation in the nucleus (Li, M. *et al.*, 2003; Xirodimas *et al.*, 2001). Polyubiquitination of p53 further requires the association of Mdm2 with a chain assembly factor (E4), e.g. p300 (Grossman *et al.*, 2003). Mdm2 mutants that are defective for binding to p300 retain the ability to promote p53 monoubiquitination but fail to promote p53 polyubiquitination and are also unable to target p53 for degradation (Zhu *et al.*, 2001; Grossman *et al.*, 2003). Ubiquitination is not only required for the recognition of p53 as a target for the 26S proteasome,

Table 1. Sites of post-translational modifications of p53. The table includes sites of modification of human and murine p53, the modifying enzyme and the physiologic consequence.

Modification site human	Modification site mouse	Modifying enzyme	*Cellular function*
Phosphorylation			
—	S7	?	?
S6	S9	CK1	DNA damage induced; enhanced p300/CBP binding
S9	S12	CK1, HIPK4	DNA damage induced; enhanced p300/CBP binding; creation of a binding site for Smad2
S15	S18	ATM, ATR, DNA-PK	DNA damage induced; enhanced p300/CBP binding; regulation of abundance?
T18	T21	CK1	DNA damage induced; enhanced p300/CBP binding
S20	S23	Chk1, Chk2, JNK, MAPKAPK2, PLK3	DNA damage induced; enhanced p300/CBP binding; regulation of abundance?
S33	—	CAK, p38, GSK3β	DNA damage induced; enhanced p300/CBP binding; creation of a binding site for Pin1
S37	S37	ATR, DNA-PK, JNK1,2,3	DNA damage induced; enhanced p300/CBP binding
S46	S58	HIPK2, DYRK2	DNA damage induced; stimulation of CBP mediated acetylation at K382; upregulation of pro-apoptotic target genes; creation of a binding site for Pin1
T55	—	TAF1	Cytoplasmic localization; enhanced association with CRM1; DNA damage induced
—	T76	ERK1/2	?
T81	—	JNK	Creation of a binding site for Pin1
—	T86	ERK1/2	?
S149	S145	CSN-K	Induction of proteasomal degradation
T150	T147	CSN-K	Induction of proteasomal degradation
T155	S152	CSN-K	Induction of proteasomal degradation
S215	S212	Aurora A	Enhanced stability
S313	S310	Chk1, Chk2	?
S314	—	Chk1, Chk2	?
S315	S312	Aurora A, GSK3β, p60-cdc2, Cyclin B-cdc2	Phosphorylated in a cell cycle dependent manner; cytoplasmic localization; stimulation and alteration of DNA binding; creation of a binding site for Pin1
S366	S363	Chk1, Chk2, IKK2	Creation of a binding site for β-TrCP; proteasomal degradation
S376	S373	GSK3β	Phosphorylated in proliferating cells; cytoplasmic localization; dephosphorylation creates binding site for 14-3-3 proteins
T377	T374	Chk1, Chk2	?
S378	S375	PKC, Chk1, Chk2	Phosphorylated in proliferating cells
T387	—	Chk1, Chk2	?

Table 1. continued on next page.

Table 1. Continued.

Modification site human	Modification site mouse	Modifying enzyme	*Cellular function*
S392	S389	p38, CK2	Phosphorylated after UV radiation; transcriptional activation
Ribosylation			
E258	E255	PARP-1	Prevention of interaction with CRM1; nuclear localization
D259	D256	PARP-1	Prevention of interaction with CRM1; nuclear localization
E271	E268	PARP-1	Prevention of interaction with CRM1; nuclear localization
Ubiquitination			
K101	K98	Mdm2	Targeting for proteasomal degradation
K120	K117	Mdm2	Targeting for proteasomal degradation
K132	K129	Mdm2	Targeting for proteasomal degradation
K139	K136	Mdm2	Targeting for proteasomal degradation
K319	K316	E4F1	Expression of cell cycle arresting genes
K320	K317	E4F1	Expression of cell cycle arresting genes
K321	K318	E4F1	Expression of cell cycle arresting genes
K351	K348	MSL-2	Cytoplasmic localization
K357	—	MSL-2	Cytoplasmic localization
K370	K367	Mdm2	Targeting for proteasomal degradation
K372	K369	Mdm2	Targeting for proteasomal degradation
K373	K370	Mdm2	Targeting for proteasomal degradation
K381	K378	Mdm2	Targeting for proteasomal degradation
K382	K379	Mdm2	Targeting for proteasomal degradation
K386	K383	Mdm2	Targeting for proteasomal degradation
Acetylation			
K120	K117	hMOF/Tip60	Induction of apoptosis
K164	K161	p300/CBP	DNA damage induced; impaired interaction with Mdm2 or MdmX
K305	K302	p300/CBP	?
K320	K317	PCAF	DNA damage induced; inhibition of apoptosis
K370	K367	p300/CBP	?
K372	K369	p300/CBP	?
K373	K370	p300/CBP	Enhanced interaction with TAF1; induction of apoptosis
K381	K378	p300/CBP	?
K382	K379	p300/CBP	Enhanced interaction with TAF1; induction of apoptosis
K386	K383	p300/CBP	?

Table 1. continued on next page.

Table 1. Continued.

Modification site human	Modification site mouse	Modifying enzyme	*Cellular function*
Neddylation			
K320	K317	FBXO11	Increased stability; decreased transcriptional activity
K321	K318	FBXO11	Increased stability; decreased transcriptional activity
K370	K367	Mdm2	Increased stability; decreased transcriptional activity
K372	K369	Mdm2	Increased stability; decreased transcriptional activity
K373	K370	Mdm2	Increased stability; decreased transcriptional activity
Methylation			
R333	R330	PRMT5	Enhanced stability; cell cycle arrest?
R335	R332	PRMT5	Enhanced stability; cell cycle arrest?
R337	R334	PRMT5	Enhanced stability; cell cycle arrest?
K370	K367	SMYD2	Monomethylation: reduced stability; dimethylation: enhanced interaction with 53BP1
K372	K369	Set7/9	Enhanced stability and activity; nuclear localization; blocked K370 methylation
K382	K379	Set8/PR-Set7	Enhanced stability; reduced transcriptional activity; binding of 53BP1
K386	K383	?	?
Sumoylation			
K386	K383	PIAS1, PIASxβ, Topors	Increased stability; decreased transcriptional activity

it moreover allows the exposure of a carboxy-terminal nuclear export sequence (NES) and dissociation of p53 from Mdm2 (Carter *et al.*, 2007). For efficient ubiquitination of p53, both proteins need to associate via two independent domains on the p53 and Mdm2 proteins. The strongest association of p53 and Mdm2 occurs via the N-terminal p53 binding pocket on the Mdm2 protein and the N-terminal transactivation domain of p53 (Chen, J *et al.*, 1993). In addition to the association via these N-terminal domains, p53 and Mdm2 also interact via the central acidic domain of Mdm2 and the core domain of p53. Deletion of the central acidic domain of Mdm2 significantly reduces p53 ubiquitination and degradation (Kulikov *et al.*, 2006; Ma *et al.*, 2006; Wallace *et al.*, 2006; Yu *et al.*, 2006). Moreover, the N-terminal and the central interaction sites enhance the association of the two proteins synergistically (Kulikov *et al.*, 2006). A significant proportion of cellular p53 is organized in dimers and tetramers (Lomax *et al.*, 1998; Kulikov *et al.*, 2006). Since only p53 capable of forming tetramers shows a synergistic effect in binding to the N- and C-terminal domain of Mdm2, it is most likely that different molecules of a tetramer bind to the two binding sites (Kulikov *et al.*, 2006). Consistently, point mutations in the p53 oligomerization domain reduce the ability of p53 to bind Mdm2 (Kubbutat *et al.*, 1997; Lomax *et al.*, 1998).

Despite the presence of some p53 and Mdm2 in the cytoplasm, ubiquitination of p53 by Mdm2 takes place in the nucleus (Yu *et al.*, 2000). Disruption of the nuclear localization signal (NLS) of p53 completely prevents it from entering the nucleus and also prevents Mdm2-dependent ubiquitination. After polyubiquitination, p53 can be destroyed both by nuclear and cytoplasmic proteasomes (Xirodimas *et al.*, 2001). For degradation in the cytoplasm, polyubiquitinated p53 must be transported into the cytoplasm, a function that has also been attributed to Mdm2 (Roth *et al.*, 1998) and that requires the C-terminal nuclear export sequence (NES) of p53 while the NES of Mdm2 is dispensable for this process (Boyd *et al.*, 2000; Geyer *et al.*, 2000). Disrupting the C-terminal NES of p53, in contrast, blocks p53 nuclear export and prevents it from Mdm2-mediated degradation in cytoplasmic proteasomes (O'Keefe *et al.*, 2003).

P53 and Mdm2 are connected in an autoregulatory feedback loop, where p53 induces the transcription of Mdm2 while Mdm2 promotes p53 degradation (Juven

Table 2. Sites of post-translational modifications of Mdm2. The table includes sites of modification of human and murine Mdm2, the modifying enzyme and the physiologic consequence.

Modification site human	Modification site mouse	*Modifying enzyme*	Function
Phosphorylation			
S17	S17	DNA-PK	Reduced binding to p53 *in vitro*
S166	S163	Akt/PKB	Reduced self-ubiquitination; translocation into the nucleus
S186	S183	Akt/PKB	Reduced self-ubiquitination
S188	S185	Akt/PKB	Reduced self-ubiquitination; translocation into the nucleus
T218	T216	CyclinA/Cdk2	Reduced binding to p53; increased binding to p14ARF (human)/ p19ARF (mouse)
S229	S227	?	?
S232	S230	?	?
S240	S238	CK1	Enhanced p53 degradation
S242	S240	CK1, GSK3β	Enhanced p53 degradation
S246	S244	CK1	Enhanced p53 degradation
S253	S251	?	Enhanced p53 degradation
S256	S254	GSK3β	Enhanced p53 degradation
S260	S258	CK2	?
S262	S260	?	?
S269	S267	CK2	?
S383	-	CK1	?
Y394	Y393	c-Abl	Phosphorylated after IR; impaired nuclear export and degradation of p53
S395	S394	ATM	Phosphorylated after IR; impaired nuclear export and degradation of p53
S407	S406	ATR	Phosphorylated upon inter-S-phase arrest; impaired nuclear export and degradation of p53
Acetylation			
K466	K464	CBP	Impaired ubiquitination of p53
K467	K465	CBP	Impaired ubiquitination of p53

et al., 1993; Haupt *et al.*, 1997; Kubbutat *et al.*, 1997). Nevertheless, the majority of *mdm2* mRNA (in mouse tissues 80–90%) is transcribed from a promoter upstream of the p53 binding site that is unresponsive to p53 (Jones *et al.*, 1996).

Alterations of Mdm2 abundance or activity have a major impact on p53 abundance and already a small decrease in Mdm2 activity leads to the stabilization of the tumor suppressor protein (Mendrysa *et al.*, 2003). Like p53, Mdm2 is a short-lived protein that is degraded by 26S proteasomes and regulation of Mdm2 abundance and activity occurs mainly at the post-transcriptional level. Nevertheless, a genome-wide screen identified more recently several genes (*HES1*, *HEY1*, *TFAP4*, *OSR1*) that repress *mdm2* gene transcription after overexpression (Huang *et al.*, 2004).

Degradation of Mdm2 is mainly controlled by ubiquitination. Like most ubiquitin ligases, Mdm2 also displays autoubiquitination activity. However, Mdm2 from genetically engineered mice, where a critical cysteine in the RING domain of Mdm2 has been mutated (C464A), was degraded as efficiently as wild-type Mdm2, suggesting that the autoubiquitination activity of Mdm2 plays a minor role *in vivo* (Itahana *et al.*, 2007). Another ubiquitin ligase for Mdm2 is the histone acetyltransferase PCAF (Linares *et al.*, 2007), while the deubiquitinating enzymes USP2a and HAUSP have been shown to remove ubiquitin moieties from Mdm2 (Li, M. *et al.*, 2004; Stevenson *et al.*, 2007). The activity of HAUSP towards Mdm2 can further be enhanced by the DAXX protein, which binds simultaneously to Mdm2 and HAUSP (Tang, J. *et al.*, 2006). Remarkably, after ultraviolet (UV) irradiation, which leads to strong destabilization of Mdm2 (Stommel and Wahl, 2004), DAXX dissociates from Mdm2, thus allowing efficient Mdm2 destruction (Tang, J., 2006). In addition, Mdm2 abundance can be regulated by the MEG3 noncoding RNA, which also reduces Mdm2 abundance and enhances p53 protein levels (Zhou, Y. *et al.*, 2007).

Like p53, Mdm2 is subjected to intensive posttranslational modifications including phosphorylation,

ubiquitination, acetylation and sumoylation (Figure 2, Table 2). Particularly important for p53 activity is a cluster of serine residues in the central domain of Mdm2. These sites are highly phosphorylated in unstressed proliferating cells (Blattner *et al.*, 2002). Kinases that phosphorylate this region include casein kinase 1 delta (CK1δ), which phosphorylates Mdm2 at serine 246 (Winter *et al.*, 2004), GSK-3, which phosphorylates Mdm2 at serine 242 and 256 (Kulikov *et al.*, 2005) and casein kinase 2 (CK2) which phosphorylates Mdm2 at serine S260 and S269 (Gotz *et al.*, 1999; Hjerrild *et al.*, 2001). While phosphorylation of serine 240, serine 242, serine 246, serine 253, serine 256 and serine 260 are important for p53 degradation (Blattner *et al.*, 2002), phosphorylation of Mdm2 at serine 269 has no influence on p53 stability (Gotz *et al.*, 1999; 2005). Phosphorylation of the central domain of Mdm2 regulates the association of the central domain of Mdm2 with the core domain of p53 and is required for p53 degradation (Blattner *et al.*, 2002; Kulikov *et al.*, 2006). The relevance of this phosphorylation becomes particularly clear in response to ionizing radiation. Ionizing radiation leads to a strong reduction in phosphorylation of the central domain of Mdm2, which is the major trigger for p53 stabilization in the presence of double stand breaks (Blattner *et al.*, 2002; Boehme *et al.*, 2008). Other sites of phosphorylation of Mdm2 are serine 17, serine 166, serine 186, serine 188 and threonine 218. Phosphorylation of serine 17 is catalyzed by DNA-PK and reduces the binding of Mdm2 to p53, at least *in vitro* (Mayo *et al.*, 1997). Whether this phosphorylation also occurs *in vivo* is still unclear. Serine166, serine186 and serine188 are all phosphorylated by AKT/PKB *in vitro* whereas serine 166 and serine 186 are the major Akt/PKB phosphorylation sites *in vivo*. Phosphorylation at these sites can, moreover, be enhanced after overexpression of 14-3-3ζ. Phosphorylation at serine 166 and serine 188 reduces Mdm2 self-ubiquitination and degradation and enables the translocation of Mdm2 from the cytoplasm into the nucleus. Due to the increase in Mdm2 abundance, p53 degradation is enhanced upon Akt/PKB activation (Mayo and Donner, 2001; Zhou *et al.*, 2001; Ashcroft *et al.*, 2002; Ogawara *et al.*, 2002; Feng *et al.*, 2004; Danes *et al.*, 2008). Threonine 218 is phosphorylated by cyclin A/CDK. This phosphorylation weakens the interaction of Mdm2 with p53 and augments the binding to p14ARF (Zhang and Prives, 2001). After ionizing radiation, Mdm2 is additionally phosphorylated by c-Abl at tyrosine 394 and by ataxia telangiectasia mutated (ATM) kinase at serine 395, (Khosravi *et al.*, 1999; Maya *et al.*, 2001; de Toledo *et al.*, 2000; Goldberg *et al.*, 2002). Moreover, upon inter-S phase arrest, Mdm2 also becomes phosphorylated at serine 407. This phosphorylation is performed by ATM- and Rad3-related (ATR) kinase and impairs nuclear export and p53 degradation (Shinozaki *et al.*, 2003).

Less is known about phosphatases that remove phosphate groups from Mdm2. The only phosphatases that are known for Mdm2 are PP2A, which reverts AKT/PKB-mediated phosphorylation of Mdm2, and Wip1, which removes phosphorylation of serine 395 and increases the half-life of the oncoprotein (Okamoto *et al.*, 2002; Lu *et al.*, 2007).

In addition to phosphorylation, Mdm2 activity can also be regulated by acetylation and sumoylation. The histone acetylase CBP, for instance, acetylates Mdm2 in the RING-finger domain, which reduces Mdm2-mediated p53 ubiquitination and degradation (Wang *et al.*, 2004). Sumoylation of Mdm2 can be performed by the SUMO-ligases PIAS and RanBP2. This reaction is stimulated by $p14^{ARF}$ and inhibited by the Mdm2 homolog MdmX (Mdm4) (Miyauchi *et al.*, 2002; Ghosh *et al.*, 2005).

The activity of Mdm2 towards p53 is furthermore regulated by proteins that bind to Mdm2 and either enhance or reduce the association of Mdm2 with p53 or its ubiquitination, or regulate its nucleo/cytoplasmic shuttling activity. In particular, nucleolar proteins e.g. $p14^{ARF}$ ($p19^{ARF}$ in mice), L5, L11, L23, S7, nucleophosmin (NPM) and nucleostemin (NS) reduce p53 ubiquitination and degradation by binding to the central region of Mdm2 in close proximity to the second p53 binding site (Bothner *et al.*, 2001; Bernardi *et al.*, 2004; Dai and Lu, 2004; Dai *et al.*, 2004; Jin, A. *et al.*, 2004; Kurki *et al.*, 2004; Chen *et al.*, 2007; Horn and Vousden, 2008). These proteins primarily sequester Mdm2 in the nucleolus which prevents its interaction with p53 (Kamijo *et al.*, 1998; Pomerantz *et al.*, 1998; Honda and Yasuda, 1999; Zhang and Xiong, 1999). However, due to fostered association of Mdm2 with L5 and L11, p53 levels are also elevated upon downregulation of NS (Dai *et al.*, 2008).

Individual nucleolar proteins show different additional activities. $P14^{ARF}$, for instance, impairs nucleo-cytoplasmic shuttling of Mdm2 (and thus of p53), possibly by preventing its trafficking through the nucleolus, which may be a prerequisite for nuclear export (Tao and Levine, 1999). Cyclin G1, another protein that associates with Mdm2, promotes this association of $p14^{ARF}$ with Mdm2 (Kimura and Nojima, 2002). NPM furthermore promotes p53 sumoylation which additionally hampers p53 degradation (Kurki *et al.*, 2004). Despite the fact that it is not a nucleolar protein, the promyelocytic leukemia tumor suppressor protein (PML) also sequesters Mdm2 in the nucleolus, but it requires the presence of the ribosomal protein L11 for this activity (Bernardi *et al.*, 2004). Upon nucleolar stress, which causes p53 accumulation, the interaction of Mdm2 with the ribosomal proteins L5 and L11 is enhanced and co-localization of Mdm2 with PML and L11 in the nucleolus is augmented, which reduces Mdm2 activity towards p53 (Bernardi *et al.*, 2004; Horn and Vousden, 2008; Sun *et al.*, 2008). In addition, NPM is distributed to the nucleoplasm where it binds to

Mdm2 and prevents the association of Mdm2 with p53. Other proteins that prevent p53 degradation may also associate with the central domain of Mdm2, but may not necessarily sequester Mdm2 in the nucleolus. The retinoblastoma protein (Rb), for example, stabilizes p53 by promoting the formation of a ternary complex with Mdm2 and p53 (Hsieh *et al.*, 1999). Conversely, the Ras-GTPase-activating protein-SH3-domain binding protein 2 (G3BP2) associates with the RING-finger of Mdm2 and reduces the ubiquitin ligase activity of Mdm2 towards p53 and itself. The association of G3BP2 with the ubiquitin-specific protease USP10 may further contribute to the stabilization of p53 (Kim *et al.*, 2007).

Some proteins stabilize p53 by competing with p53 for binding to Mdm2. SUSP4, a nuclear SUMO-specific protease, and RYBP are examples for this mode of action (Chen *et al.*, 2009; Lee *et al.*, 2006). SUSP4, furthermore, removes SUMO-1 from Mdm2 leading to Mdm2 self-ubiquitination and degradation. Interestingly, UV irradiation increases SUSP4 protein levels, which correlates with a decrease in Mdm2 protein levels and an increase in p53 abundance (Lee *et al.*, 2006). The van-Hippel-Lindau tumor suppressor protein (pVHL) also suppresses Mdm2 mediated ubiquitination and nuclear export of p53. In response to genotoxic stress, pVHL promotes the association of p53 with p300, which enhances p53 acetylation at lysine 373 and lysine 382 (Roe *et al.*, 2006).

While most proteins that interact with Mdm2 prevent p53 degradation by impinging on Mdm2 activity, the FK605-binding protein 25 (FKBP25) stabilizes p53 and activates transcription of p53 target genes by promoting Mdm2 ubiquitination and degradation. Although FKBP25 is primarily localized in the cytoplasm, a fraction of the protein is also present in the nucleus where it can bind, probably at several sites, to nuclear Mdm2. Interestingly, p53 suppresses expression of FKBP25 which connects these proteins in a negative feedback loop (Ochocka *et al.*, 2009).

Other proteins that interact with Mdm2 enhance p53 degradation. The Mdm2 homolog MdmX, for example, heterodimerizes with Mdm2 via their RING-domains and this hetero-RING complex has been shown to be a better ubiquitin ligase for p53 as Mdm2 homodimers (Kawai *et al.*, 2007). MTBP and TAF1, instead, bind to the central region of Mdm2 (Leveillard and Wasylyk, 1997; Allende-Vega *et al.*, 2005; Brady *et al.*, 2005). MTBP reduces Mdm2 autoubiquitination and degradation, which leads to Mdm2 accumulation and increased p53 degradation. Upon UV irradiation, MTBP turnover is increased which contributes to enhanced degradation of Mdm2 (Brady *et al.*, 2005). Gankyrin and PA28γ bind to Mdm2 in close proximity to the C-terminal RING-finger. They enhance the association of Mdm2 with p53 and facilitate p53 ubiquitination and degradation (Higashitsuji *et al.*, 2005; Zhang and Zhang, 2008). Another protein that enforces Mdm2-mediated ubiquitination and degradation of p53 is KAP1. Its mode of action is not entirely clear but it is speculated that it promotes Mdm2 function by competing with p14ARF (Wang *et al.*, 2005). Moreover, interplay between the hedgehog pathway and p53 has been discovered where constitutively active smoothened as well as the downstream factors Gli1 and Gli2 promoted p53 degradation. Although the mode of action of these factors is not entirely clear, it is most likely that they act on Mdm2 since hedgehog signaling induces activating phosphorylation of Mdm2 (Abe *et al.*, 2008).

To make the story even more complicated, proteins can regulate p53 stability by impinging on proteins that interact with Mdm2. One such example is Twist, a member of the family of basic helix-loop-helix transcription factors which enhances Mdm2 activity by reducing the expression of $p14^{ARF}$ (Maestro *et al.*, 1999). Also the tumor suppressor protein RASSF1A increases p53 levels indirectly. RASSF1A associates with Daxx and the C-terminal region of Mdm2 and interferes with the formation of a ternary complex between Mdm2, Daxx and the ubiquitin hydrolase HAUSP resulting in increased auto-ubiquitination and degradation of Mdm2. The association of Daxx and RASSF1A is enhanced in the presence of adriamycin, which may contribute to increased Mdm2 degradation in the presence of the drug (Song *et al.*, 2008).

Regulation of p53 stability by other ubiquitin ligases

Apart from Mdm2, p53 can be ubiquitinated by several other ubiquitin ligases (Figure 3). The RING-finger ubiquitin ligases COP1, PirH2 and synoviolin ubiquitinate p53 *in vitro* and *in vivo* and depletion of these E3 ligases stabilizes p53 and leads to G1 arrest or apoptosis (Leng *et al.*, 2003; Dornan *et al.*, 2004; Yamasaki *et al.*, 2007). Like Mdm2, COP1 is connected with p53 in a negative feedback loop. In contrast to Mdm2, COP1 or PirH2, synoviolin sequesters p53 in the cytoplasm, which certainly contributes to its negative effect on p53 function (Yamasaki *et al.*, 2007). The HECT-domain ubiquitin ligase ARF-BP1, a major component of ARF-containing nuclear complexes in human cells, CARP 1 and CARP 2 and Topors have also been reported to ubiquitinate p53 (Rajendra *et al.*, 2004; Chen *et al.*, 2005; Yang *et al.*, 2007). While COP1, CARP 1/2, PirH2 and ARF-BP1 have only been shown to link ubiquitin to p53, the ubiquitin ligase Topors adds both ubiquitin and SUMO residues to C-terminal lysines of p53 (Rajendra *et al.*, 2004; Weger *et al.*, 2005). In contrast to Mdm2, Cop1, PirH2, CARP1, CARP2 also ubiquitinate p53 and target it for degradation when it is phosphorylated at serine 20. The chaperone-associated ubiquitin ligase CHIP and the Skp1-Cullin-F-box complex-associated ubiquitin ligase β-TrCP have also been reported to ubiquitinate p53, at least *in vitro*, and enhance its degradation after over-

expression. However, β-TrCP only targets p53 for degradation when it is phosphorylated at serine 366 and eventually at serine 362 (Esser *et al.*, 2005; Xia *et al.*, 2009). While most ubiquitin ligases promote p53 degradation, ubiquitination of p53 by WWP1, a HECT-domain E3 ubiquitin ligase, forces cytoplasmic translocation of p53, increases its stability and prevents it from activating transcription of its target genes (Laine and Ronai, 2007). Another potential ubiquitin ligase for p53 is Cul4A. Cul4A associates with p53 and Mdm2 and depletion of Cul4A leads to the accumulation of p53 (Nag *et al.*, 2004). However, although Cul4A can ubiquitinate certain targets, it is more likely that it rather functions as an ubiquitin chain assembly factor (E4) for p53 than as an ubiquitin ligase (E3).

Ubiquitination of proteasomal substrates can be reverted by ubiquitin proteases. For p53 the only known ubiquitin hydrolase is HAUSP. Overexpression of HAUSP leads to the stabilization of p53, even in the presence of Mdm2 (Li *et al.*, 2002). Since HAUSP also deubiquitinates Mdm2, the stoichiometry of both proteins is important. A partial reduction in HAUSP levels destabilizes p53, while complete ablation of HAUSP stabilizes and activates the tumor suppressor protein (Li, M. *et al.*, 2004).

Regulation of p53 stability by other small ubiquitin-like proteins

In addition to ubiquitin, p53 can also be modified by SUMO (small ubiquitin-like modifier; Rodriguez *et al.*, 1999). The SUMO ligases PIAS1 and PIASxβ modify p53 at lysine 386 together with Ubc9 as conjugating enzyme (Schmidt and Muller, 2002). In addition, p53 can be sumoylated at lysine 386 by Topors (Weger *et al.*, 2005). Sumoylation is stimulated by nucleolar targeting of p53, e.g. by $p14^{ARF}$ (Chen and Chen 2003). Also the ubiquitin-like modifier Nedd8 can be attached to p53 (Xirodimas *et al.*, 2004). Lysine 370, lysine 372 and lysine 373 are neddylated by Mdm2, while FBXO11 neddylates lysine 320 and lysine 321 of p53 (Xirodimas *et al.*, 2004; Abida *et al.*, 2007). In resting cells, only a minority of p53 molecules are neddylated. However, in response to UV irradiation, neddylation of p53 is enhanced, at least at early time points after irradiation. At the same time ubiquitination

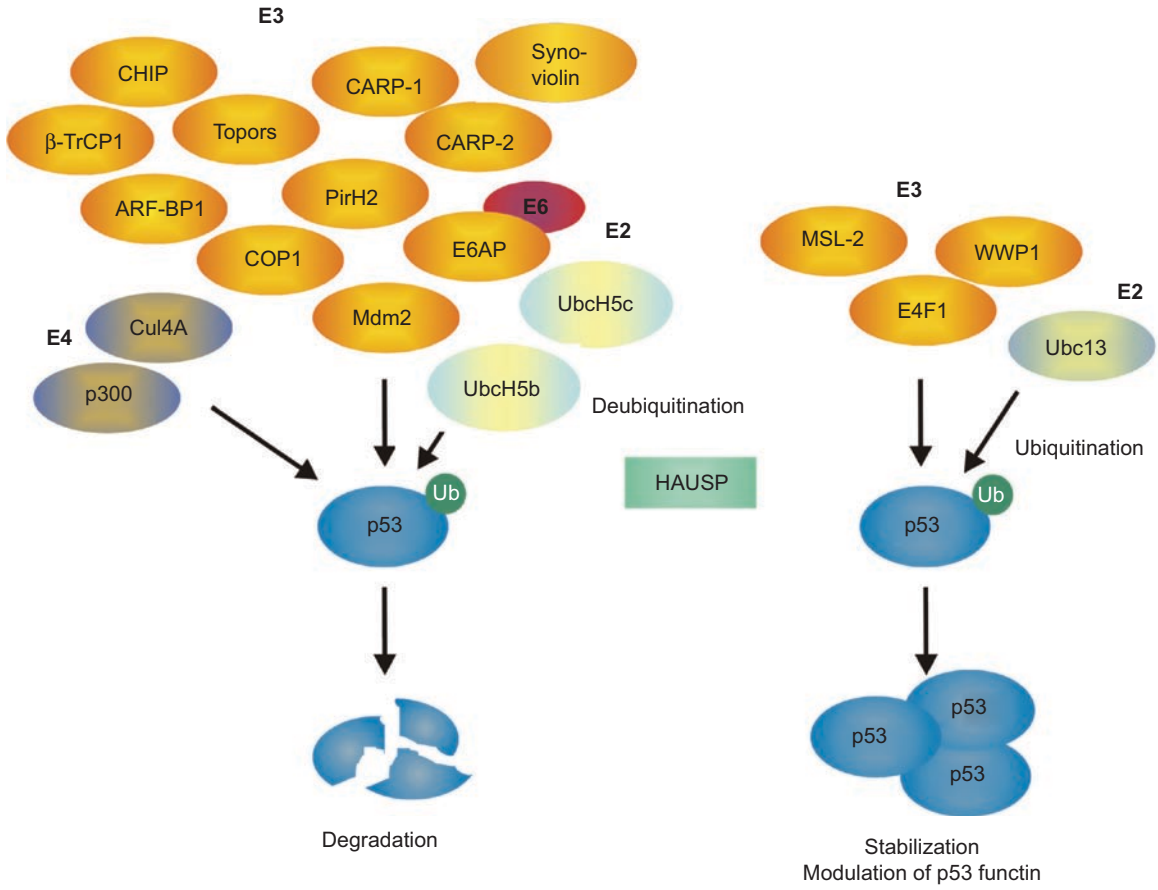

Figure 3. Ubiquitin-ligases that control p53 function and activity. The p53 protein is controlled by several ubiquitin ligases which can, depending on site and type of ubiquitination, target the tumor suppressor protein for rapid degradation by 26S proteasomes or even enhance its stability and modulate p53 function. The type of ubiquitin chains that are formed depends largely on the contribution of different E2s (ubiquitin conjugating enzymes) and E4s (chain assembly factors).

of the tumor suppressor protein is decreased, probably since ubiquitin and Nedd8 compete for the same lysines. At later time points after UV irradiation, neddylation is no longer detectable (Xirodimas *et al.*, 2004).

Regulation of p53 stability by other post-translational modifications

In addition to ubiquitination, sumoylation and neddylation, the p53 protein is extensively modified by phosphorylation, acetylation and methylation. All these modifications are very important for p53 activity, but less critical for p53 stability. Interestingly, while these modifications clearly regulate p53 stability under experimental conditions, several of them play a minor role under physiologic conditions. This is exemplified by phosphorylation of p53 at serine 15 or serine 20. Under experimental conditions, these phosphorylations interfered with the binding of p53 to Mdm2 (Chehab *et al.*, 2000; Sakaguchi *et al.*, 2000; Shieh *et al.*, 2000; Schon *et al.*, 2002). However, p53 also accumulates in cells where these phosphorylation sites have been mutated or in cells that are deficient in kinases that phosphorylate these sites (Hengstermann *et al.*, 1998; Ashcroft *et al.*, 1999; Blattner *et al.*, 1999; Thompson *et al.*, 2004; Boehme *et al.*, 2008). Likewise, mice in which serine 15 or serine 20 has been mutated show no measurable defect in p53 accumulation after DNA damage (Wu *et al.*, 2002; Sluss *et al.*, 2004). A possible explanation for this discrepancy is provided by the fact that p53 is only functional as a tetramer. Under experimental conditions all p53 molecules are modified while under physiologic conditions, even after irradiation, not all p53 molecules are phosphorylated (C. Blattner, unpublished observations). This lower level of phosphorylation might be insufficient for blocking the association of all four p53 molecules of a tetramer with Mdm2. However, the association of only one or two p53 molecules per tetramer with Mdm2 is sufficient for p53 ubiquitination and degradation (Inoue *et al.*, 2001).

To date 23 phosphorylation sites have been reported for human p53 (Figure 1, Table 1). While p53 is extensively phosphoryated on serines and threonines, tyrosine phosphorylation of p53 has not been described as yet. Most of the phosphorylation sites are conserved between mice and men. There are, however, a few exceptions. In mice phosphorylation sites corresponding to serine 33, threonine 55, threonine 81, serine 314 and threonine 387 are missing, while serine 7, threonine 76 and threonine 86 of murine p53 lack equivalent phosphorylation sites in human p53 (reviewed in Xu, 2003). Consistently, ERK1/2 phosphorylates murine p53 at threonine 76 and threonine 86 while no ERK1/2 phosphorylation has been reported for human p53 (Jardine *et al.*, 1999).

Phosphorylation sites that have been reported to regulate p53 stability apart from serine 15 and serine 20 are serine 149, threonine 150, threonine 155, serine 215, serine 315 and serine 366. Serine 215 and serine 315 are phosphorylated by Aurora A and enhance p53 stability while the COP9 signalosome kinase (CSN-K) can phosphorylate serine 149, threonine 150 and threonine 155 and promote p53 degradation (Bech-Otschir *et al.*, 2001; Katayama *et al.*, 2004; Liu *et al.*, 2004). Serine 366 (and eventually also serine 362) can be phosphorylated by the IκB kinase 2 (IKK2), a kinase that is constitutively active in pathogenic states and cancer cells. Phosphorylation of p53 by IKK2 creates a binding site for β-TrCP, an E3 ligase that subsequently targets p53 for degradation (Xia *et al.*, 2009).

p53 stability can further be regulated by methylation. Lysine 370, lysine 372, lysine 382, lysine 386, arginine 333, arginine 335 and arginine 337 are targets for this modification. Lysine 370 can be methylated by SMYD2, which reduces the stability of p53 (Huang *et al.*, 2006). This site can, in addition, become dimethylated. However, the enzyme that dimethylates this site has not been identified as yet. Lysine 372 is methylated by Set7/9, a methyl transferase also known to methylate histone H3. Like lysine 370, this site can also be dimethylated. Methylation of lysine 372 restricts p53 to the nucleus and increases its stability and activity (Chuikov *et al.*, 2004). P53 stability is also increased when p53 is dimethylated at lysine 382. This dimethylation creates a binding site for 53BP1 and enhances p53 stability in a 53BP1-dependent manner (Adams and Carpenter, 2006). Dimethylation of p53 at this site is especially increased in response to neocarzinostatin, a drug that mimics double strand breaks (Kachirskaia *et al.*, 2008). Lysine 382 can also be monomethylated, a modification that is implemented by Set8. This modification, though, does not affect p53 abundance (Shi *et al.*, 2007). In addition, p53 can be dimethylated at lysine 386, but dimethylation at this site is also less important for p53 stability (Kachirskaia *et al.*, 2008). Arginine methylation of p53 appears to be exclusively performed by PRMT5. Since overexpression of PRMT5 increases p53 levels, it is most likely that methylation of at least one of these sites can also regulate p53 stability (Jansson *et al.*, 2008).

Regulation of p53 stability by protein–protein interactions

P53 stability is furthermore controlled by proteins that associate with the tumor suppressor protein without having enzymatic activity (Figure 1). Although these proteins cannot modify p53 themselves, they can impinge on p53 modifications by enhancing or reducing the association of p53 with modifying enzymes. P53 interaction partners that reduce Mdm2-mediated degradation are, for example, the human homolog of yeast alteration/deficiency in activation 3 (hAda3), AIMP2, macrophage migration inhibitory factor (MIF), Ras-GTPase activating

protein-SH3-domain-binding proteins 1 and 2 (G3BP1 and G3BP2), Sin3, ING1b, activating transcription factor 3 (ATF3), S100b, NM23-H1, serine-threonine kinase receptor-associated protein (STRAP), c-Abl and WOX1 (Baudier *et al.*, 1992; Scotto *et al.*, 1998; Sionov *et al.*, 1999; 2001; Zilfou *et al.*, 2001; Chang *et al.*, 2005; Kim *et al.*, 2007; Nag *et al.*, 2007; Han *et al.*, 2008; Jung *et al.*, 2008). WOX1, however, prevents p53 degradation only upon prior phosphorylation of p53 at serine 46 (Chang *et al.*, 2005). Although all these proteins prevent p53 degradation, they may do so in different ways. MIF1, G3BP1 and G3BP2, for instance, promote cytoplasmic retention of p53 which reduces its degradation, but also causes transcriptional inactivation of p53 (Kim *et al.*, 2007; Jung *et al.*, 2008). This cytoplasmic sequestration does, though, not exclude ubiquitination of p53. In fact, MIF1 even enhances p53 ubiquitination, probably by stabilizing the interaction of p53 and Mdm2 (Jung *et al.*, 2008). Other p53-interaction partners such as ING1b, NM23-H1, STRAP, S100b and ATF3 disrupt the interaction between Mdm2 and p53 and thus reduce p53 ubiquitination (Leung *et al.*, 2002; Fernandez-Fernandez *et al.*, 2005; Yan *et al.*, 2005; Jung *et al.*, 2007). Some of these p53 interaction partners have additional activities, which may or may not contribute to their stabilizing activity. HAda3, for instance, also enhances p53 acetylation due to associated histone acetyl transferases. Other protein–protein interactions only occur in response to DNA damage. The association of p53 with hAda3, for example, is particularly enhanced after treatment of cells with adriamycin, which may contribute to p53 activation under these conditions (Nag *et al.*, 2007). The association of p53 with AIMP2, which usually forms higher order complexes with aminoacyl-tRNA synthetases in the cytoplasm, or with ATF3, only becomes possible after DNA damage, e.g. when AIMP2 is released from cytoplasmic complexes, which allows its translocation into the nucleus and association with nuclear p53 (Yan *et al.*, 2005; Han *et al.*, 2008). Likewise, the association of p53 with NMH23-H1 and STRAP is increased upon treatment of cells with 5FU (Jung *et al.*, 2007). It should also be noted that although c-Abl is a kinase that becomes activated in response to certain types of cellular stress, its p53-stabilizing activity is independent of its kinase activity (Sionov *et al.*, 1999; 2001).

Other proteins, such as Yin Yang1 (YY1) or the focal adhesion kinase (FAK), increase p53 ubiquitination and degradation, mostly by stabilizing the interaction between p53 and Mdm2, an activity that can be counteracted by $p14^{ARF}$, at least in the case of YY1 (Gronroos *et al.*, 2004; Sui *et al.*, 2004; Lim *et al.*, 2008).

Another protein that associates with p53 and impinges on its stability is MdmX. Depending on the experimental conditions, MdmX can both increase and decrease p53 stability. The stabilizing activity is due to its shared binding site with Mdm2. Like Mdm2, MdmX associates with the N-terminus of p53, but has no intrinsic ubiquitin ligase activity. It therefore competes with Mdm2 for binding to p53 and inhibits nuclear export and degradation of the tumor suppressor protein (Barboza *et al.*, 2008; Jackson and Berberich, 2000). However, due to the formation of heterodimers between Mdm2 and MdmX, p53 has also been found included in ternary complexes with Mdm2 and MdmX which enhances its ubiquitination, at least after overexpression (Tanimura *et al.*, 1999).

Regulation of p53 activity

While other activities have also been reported for p53 (reviewed in Mihara *et al.*, 2003; Sengupta and Harris, 2005; Moll *et al.*, 2006), the p53 protein is primarily known as a sequence-specific transcription factor. Upon activation, p53 is recruited to promoters of its target genes. However, for some target genes, it has been reported that p53 occupies the promoter even in the absence of an activating stimulus (Espinosa and Emerson, 2001; Kaeser and Iggo, 2002). In this case, the control of DNA binding will be insufficient and additional principles must apply to switch p53 on.

The prevalent mode of activating pre-existing p53 molecules, both with regard to the targeting reaction as well as for allowing its activity at promoters, is certainly the addition of post-translational modifications. In particular phosphorylation, acetylation and methylation are widely used. Some of these modifications can be introduced into monomeric p53 while others require tetrameric p53 (Warnock *et al.*, 2008). Addition of small molecules such as SUMO or Nedd8 plays, though, a minor role for the activation of pre-existing p53 molecules. In addition, p53 activity can be regulated by protein–protein interactions. Particularly proteins that bind to the DNA-binding domain or to the transactivation domain of p53 can have a strong impact on p53 activity. Proteins that bind to the C-terminus of p53 often impinge on the oligomerization status of p53 which is also relevant for p53's function.

Regulation of p53 activity by phosphorylation

P53 is known as a phosphoprotein for a long time and phosphorylation is certainly the most investigated post-translational modification of p53. A few sites of p53 are phosphorylated in normal cells but most phosphorylation events occur in response to cellular stress (Figure 1, Table 1). One of the sites of p53 that is constitutively phosphorylated is serine 33. This site can be phosphorylated by GSK-3β and by the CAK-complex (Cdk7/cyclinH/Mat1; Ko *et al.*, 1997; Turenne and Price, 2001), both are active in unstressed cells. In resting cells, human p53 is also phosphorylated at threonine 55, a phosphorylation site that is absent in the murine homolog. TAF1 phosphorylates this

residue and enhances the association of p53 with CRM1, a protein that promotes cytoplasmic translocation of p53 (Gatti *et al.*, 2000; Li, H.H. *et al.*, 2004; Cai and Liu, 2008). Upon DNA damage, threonine 55 is dephosphorylated by the phosphatase B56γ-PP2A which mediates nuclear retention and gene transcription. This dephosphorylation depends strictly on phosphorylation of serine 15 (Li, H.H. *et al.*, 2007; Shouse *et al.*, 2008). Other sites that are phosphorylated in proliferating cells are serine 376 and serine 378 (Waterman *et al.*, 1998). Eventually, these sites can be phosphorylated by CAK since CAK can phosphorylate residues in the C-terminus of p53 which have, however, not been characterized in detail (Lu *et al.*, 1997). Serine 376 can furthermore be phosphorylated by GSK-3β (Qu *et al.*, 2004). Like threonine 55, serine 376 becomes dephosphorylated after cellular stress which, together with phosphorylation of serine 378, creates a binding site for 14-3-3 proteins and increases sequence-specific DNA binding. The mechanism of dephosphorylation is still unclear but ATM appears to contribute to this process (Waterman *et al.*, 1998). In addition, serine 315 is phosphorylated in unstressed cells. Phosphorylation at this site can stimulate and alter p53 DNA binding. P60-cdc2, cyclin B-cdc2, Aurora A and GSK-3β phosphorylate this residue while the phosphatase Cdc14 has been shown to dephosphorylate this site, at least *in vitro* (Wang, Y. and Prives, 1995; Bischoff *et al.*, 1998; Li *et al.*, 2000; Katayama *et al.*, 2004; Qu *et al.*, 2004).

Most of the phosphorylation events, though, occur in response to cellular stress where both the pattern and the kinetics of the modification reflect the inducing signal. Serine 392, for instance, is specifically phosphorylated after UV irradiation, but not after ionizing radiation (IR) radiation. Consistently, cells from mice with a homozygous knock-in of an alanine at this site show reduced transcriptional activation of p53 target genes and apoptosis after UV irradiation while IR-induced responses are not affected (Bruins *et al.*, 2004; Lu *et al.*, 1998). Similarly, threonine 18 is more efficiently phosphorylated after irradiation with IR than with UV, while phosphorylation of serine 9, serine 33 and serine 37 is stronger after UV irradiation than after IR (Saito *et al.*, 2003). Moreover, after IR, phosphorylation of p53 reaches a maximum at two to four hours, while after UV irradiation, p53 phosphorylation is highest between eight and 24 hours and at some sites, phosphorylations can even persist for several days.

Several kinases have been identified that phosphorylate p53 in response to UV irradiation or IR. Examples are casein kinase 1δ (CK1δ), ATM, ATR, checkpoint kinase 1 (Chk1), checkpoint kinase 2 (Chk2) and MAP-kinases. CK1δ phosphorylates serine 6, serine 9 and threonine 18 in the N-terminus of p53. However, serine 9 and threonine 18 require prior phosphorylation of serine 6 and serine 15 (Dumaz *et al.*, 1999; Higashimoto *et al.*, 2000). Moreover, serine 9 can be phosphorylated by HIPK4, a serine/threonine kinase that is expressed in lung and white adipose tissue (Arai *et al.*, 2007). Phosphorylation of serine 9 is required for the association of p53 with Smad2, which is required for a TGF-β-induced cytostatic program (Cordenonsi *et al.*, 2007). Phosphorylation of threonine 18 can be significantly enhanced by the presence of PML, which forms a ternary complex with p53 and CK1δ after DNA damage (Alsheich-Bartok *et al.*, 2008). ATM and ATR phosphorylate p53 at serine 15, and Chk1 and Chk2, which become activated by ATR and ATM, respectively, phosphorylate p53 at serine 20 (Tibbetts *et al.*, 1999; Hirao *et al.*, 2000; Shieh *et al.*, 2000; Hammond *et al.*, 2002; Saito *et al.*, 2002). Which of these kinases, ATM and Chk2 or ATR and Chk1, phosphorylate p53 depends on the activating stimulus. ATM and Chk2 phosphorylate p53 after IR while ATR and Chk1 come into play in response to other stimuli such as UV irradiation or hypoxia. Furthermore, serine 20 can be phosphorylated by polo-like kinase 3 (PLK3), the Jun-N-terminal kinase (JNK) and MAPKAP kinase 2, at least *in vitro*. The association of PLK3 with p53 is enhanced in response to DNA damage, while transcriptional activation of p53 is reduced in UV-irradiated cells from JNK1–/– and JNK2–/– mice, supporting the physiologic relevance (Xie *et al.*, 2001; She *et al.*, 2002). Like phosphorylation of threonine 18, phosphorylation of serine 20 is enhanced in the presence of PML (Louria-Hayon *et al.*, 2003). These phosphorylations enhance the association of p53 with CBP/p300, which leads to increased acetylation of the C-terminus of p53 (Lambert *et al.*, 1998; Dornan and Hupp, 2001). Phosphorylation of serine 15 and serine 20 has been shown to be necessary for the induction of p53-mediated apoptosis (Unger *et al.*, 1999; Chao *et al.*, 2003; Sluss *et al.*, 2004), which requires p53 acetylation by p300. These phosphorylations are, however, dispensable for transcriptional activation of *mdm2* or *p21* (Jackson *et al.*, 2004). Consistently, cells from knock-in mice showed reduced activation of many p53 target genes and restricted apoptosis following DNA damage (Wu *et al.*, 2002; Sluss *et al.*, 2004). Furthermore, ATR phosphorylates p53 at serine 37, a site that can also be phosphorylated by DNA-PK after IR and by JNK after UV irradiation (Milne *et al.*, 1995; Hu *et al.*, 1997; Tibbetts *et al.*, 1999). Chk1 and Chk2 also phosphorylate serine 313, serine 314, serine 366, serine 377, serine 378 and threonine 387 (Hirao *et al.*, 2000; Ou *et al.*, 2005).

After UV irradiation, p53 becomes phosphorylated at serine 33, serine 46 and serine 392. These modifications are introduced by CK2 and by p38, which translocates into the nucleus after irradiation and specifically enhances transcription of pro-apoptotic genes (Bulavin *et al.*, 1999; Keller *et al.*, 2001). Other reports have, however, failed to reproduce the ability of p38 to phosphorylate serine 46. Nevertheless, serine 46 can be phosphorylated by HIPK2 and by DYRK2 upon DNA damage (D'Orazi *et al.*, 2002;

Cecchinelli *et al.*, 2006; Dauth *et al.*, 2007; Taira *et al.*, 2007). HIPK2 co-localizes with p53 in PML nuclear bodies and phosphorylates the tumor suppressor protein after UV irradiation with PML acting as a cofactor (Moller *et al.*, 2003). DYRK2, in contrast, phosphorylates serine 46 in response to treatment of cells with adriamycin (Taira *et al.*, 2007). Phosphorylation of p53 at serine 46 stimulates CBP-mediated acetylation of p53 at lysine 382 and is associated with lethal DNA damage and upregulation of pro-apoptotic target genes of p53 (D'Orazi *et al.*, 2002; Hofmann *et al.*, 2002). Phosphorylation of serine 392, which can be introduced by CK2 or p38, enhances the formation of p53 tetramers and stimulates the sequence-specific DNA binding function of p53 (Hupp *et al.*, 1995; Huang *et al.*, 1999; Keller *et al.*, 2001; Bruins *et al.*, 2004).

Regulation of p53 activity by acetylation

The last decade showed that of all the numerous modifications that are attached to p53, acetylation has the highest impact on p53 activity. The implementation of acetyl modifications is directly linked to increased sequence-specific DNA binding and most acetylations are required for transcriptional activation of pro-apoptotic target genes. Complete loss of acetylation abolishes p53-dependent growth arrest and apoptosis. Interestingly, the p53–Mdm2 feedback loop appears to be independent of this modification (Luo *et al.*, 2004; Sykes *et al.*, 2006; Tang, Y. *et al.*, 2006; 2008). In proliferating cells, acetylation levels are usually very low, but they are strongly increased in response to cellular stress which correlates with the activation of p53 target genes. Acetylation and stability of p53 are tightly connected, particularly since eight of the 10 lysines of p53 that can be acetylated are also targets for ubiquitination, at least *in vitro*. In consequence, acetylation of the C-terminus of p53 interferes with its ubiquitination and rescues it from degradation and vice versa. In addition, Mdm2 regulates the abundance of PCAF by mediating ubiquitination and degradation of the acetylase and it attracts and presents the HDAC1 deacetylase to p53 (Kobet *et al.*, 2000; Ito *et al.*, 2001; 2002; Jin, Y. *et al.*, 2004).

Site-specific acetylation of p53 is also a powerful means to direct selective reactions in response to different incoming stimuli (Knights *et al.*, 2006; Roy and Tenniswood, 2007). Activation of the *bax* promoter, for example, requires acetylation of p53 at both, lysine 320 and lysine 373, whereas acetylation at one of these sites is sufficient for the induction of p21 (Roy *et al.*, 2005). These different outcomes may result from acetylation-induced conformational changes that alter the accessibility of phosphorylation sites, interacting proteins and promoter sequences (Knights *et al.*, 2006). In particular acetylation of lysine 320 and lysine 373 appears to function as a switch to direct the consequences of p53 acetylation either to cell cycle arrest or apoptosis. Most interestingly, acetylation also appears to function as a "sensor" for type and extent of DNA damage. Treatment of cells with the multifunctional alkylating agent bezelesin or with the radiomimetic drug etoposide results in a robust and extended acetylation of lysine 373, while treatment with the monofunctional acetylating compound adozelesin caused transient acetylation of lysine 373, but prolonged acetylation of lysine 320. Likewise, exposure of human cells to UV light results in a more distinct and prolonged acetylation of p53 in comparison with IR (Saito *et al.*, 2003; Knights *et al.*, 2006).

Important for p53 acetylation are the three acetylases p300/CBP, PCAF and hMOF/TIP60. p300/CBP, p53 and PML form a ternary complex in nuclear bodies which is required for p53 acetylation by p300/CBP (Pearson *et al.*, 2000). Acetylation of p53 by PCAF and hMOF/TIP60, in contrast, appears to be independent of a specific environment. Acetylation of p300 target sites can be further enhanced by the presence of PTEN which associates with p300 and stimulates its activity. The formation of this complex is significantly increased in response to DNA damage (Li *et al.*, 2006).

Most acetylations are performed by p300/CBP, including acetylation of lysine 164, lysine 305, lysine 370 lysine 372, lysine 373, lysine 381, lysine 382 and lysine 386. Acetylation of lysine 370, lysine 372 and lysine 373 enhances the interaction of p53 with promoters possessing imperfect p53-binding sites which are particularly found in promoters of pro-apoptotic genes. Acetylation of p53 by p300 therefore principally stimulates its pro-apoptotic activity (Knights *et al.*, 2006). Acetylation by p300/CBP requires the association of p300/CBP with the N-terminus of p53 which is significantly enhanced by N-terminal phosphorylations (Polley *et al.*, 2008; Jenkins *et al.*, 2009). Correspondingly, in Chk1 and Chk2 knockout cells, acetylation of p53 is severely diminished (Ou *et al.*, 2005). Most interestingly, acetylation at these sites also appears to stimulate N-terminal phosphorylation. An acetyl-lysine mimicking mutant of p53 was constitutively hyperphosphorylated at serine 15 in comparison to the wild-type counterpart, suggesting a functional link between C-terminal acetylation and N-terminal phosphorylation of p53 (Knights *et al.*, 2006). The activity of p300 can be further stimulated by p300-specific co-factors. In response to DNA damage, p300 associates with JMY, which augments p53-dependent apoptosis. This association of p300 and JMY is additionally enhanced by STRAP, which binds to p300 and JMY particularly during the DNA damage response and enforces acetylation of p53 at lysine 382 (Demonacos *et al.*, 2001). Acetylation of p53 at lysine 382 is further increased in the presence of HIPK2 which stimulates p53-dependent transcription of pro-apoptotic target genes (Hofmann *et al.*, 2002). Also,

the arginine methyl transferases PRMT1 and CARM1 as well as the ankyrin repeat domain 11 (ANKRD11) protein cooperate with p300 to enhance p53 activity (An *et al.*, 2004; Neilsen et al, 2008). Acetylation of lysine 164 specifically blocks the interaction of p53 with its repressors Mdm2 and MdmX (Luo *et al.*, 2004; Tang *et al.*, 2008), while acetylation of lysine 373 and lysine 382 enhances the association of p53 with TAF1, a component of the TFIIH complex, and its recruitment to promoters of p53 target genes where the TFIIH holo-complex is formed (Li, A.G. *et al.*, 2007).

Lysine 320, which lies within the nuclear localization signal, is acetylated by PCAF (Sakaguchi *et al.*, 1998) and this modification enhances cytoplasmic localization of p53 by increasing its nuclear export. It furthermore reduces phosphorylation of N-terminal residues and stimulates in particular the association of p53 with promoters containing high-affinity binding sites such as the p21 promoter. In addition, it protects from cell death by interfering with apoptotic activities of p53 after DNA damage (Liu *et al.*, 1999; Di Stefano *et al.*, 2005; Chao *et al.*, 2006; Knights *et al.*, 2006). The kinase HIPK2 contributes to this activity by directing PCAF to p53 (Di Stefano *et al.*, 2005). While acetylation of p53 at lysine 373 and lysine 382, occurs particularly after severe and irreparable DNA damage, milder stress conditions stimulate acetylation of lysine 320 by PCAF primarily resulting in the expression of p21 and induction of cell cycle arrest.

hMOF/Tip60 acetylates lysine 120, a modification that appears to be specifically required for p53-mediated apoptosis, although requirement for acetylation of lysine 120 has also been reported for p53-mediated induction of growth arrest. Arginine substitution of lysine 120 or depletion of hMOF/Tip60 from cells inhibited the ability of p53 to activate transcription of *bax* and *puma* and diminished p53 dependent apoptosis. Moreover, arginine mutations of this site have been found in human cancers, supporting a physiologic role of this modification (Sykes *et al.*, 2006; Tang, Y. *et al.*, 2006).

Acetyl groups can be removed from p53 by two deacetylase complexes. hSIR2/SIRT1 deacetylates p53 in PML nuclear bodies particularly at lysine 382 which decreases p53 transcriptional activity and represses p53-dependent senescence and apoptosis after IR (Vaziri *et al.*, 2001). SIRT1 activity can be enhanced by Necdin, the product of a maternally imprinted gene that facilitates the interaction of p53 with SIRT1 and potentiates p53 deacetylation (Hasegawa and Yoshikawa, 2008). A second deacetylase that associates with and deacetylates p53 is the histone deacetylase complex 1 (HDAC1) (Luo *et al.*, 2000). HDAC1 deacetylates primarily lysine 373 and lysine 382. Its activity can be stimulated by Mdm2 and KAP1, which recruit the HDAC1 complex to p53, and by HSCO, a co-activator of HDAC1 (Juan *et al.*, 2000; Luo *et al.*, 2000; Ito *et al.*, 2002; Wang, C. *et al.*, 2005; Higashitsuji *et al.*, 2007). Eventually, the transcriptional co-repressor CtBP2, which binds to Mdm2 and which recruits histone deacetylases including HDAC1, contributes to this activity. The presence of CtBP2 reduces p53-dependent transcription of the *bax* promoter, but not of the *p21* promoter upon overexpression (Mirnezami *et al.*, 2003; Subramanian and Chinnadurai, 2003).

Regulation of p53 activity by methylation and ribosylation

Methylation does not only affect p53 stability, it also regulates p53 activity. Part of this activity may be due to competition for the same lysines of methylation and acetylation. Methylation at lysine 382, for example, reduces p53 occupancy at *p21* and *puma* promoters and induction of p53-target. Upon DNA damage, Set8, which methylates this site, is downregulated, thus allowing acetylation of lysine 382 and subsequent p53-dependent gene transcription (Shi *et al.*, 2007). Some of the lysines of p53 can be monomethylated as well as dimethylated. These two modifications influence p53 activity differently. Monomethylation at lysine 370, for instance, represses p53 function while dimethylation at this site fosters the association of p53 with its co-activator 53BP1 and enhances its activity. Methylation of lysine 370 can be removed by LSD1, which associates with p53 and removes *in vitro* mono- and dimethylation. *In vivo*, LSD1 has a stronger preference for dimethylated lysine 370. LSD1 activity reduces the association of p53 with 53BP1 and the presence of p53 at the *p21* promoter (Huang *et al.*, 2007). Another residue that can be methylated is lysine 372; this modification, though, does not alter p53 activity itself. It rather appears to create a binding site for additional modifying enzymes since cells lacking methylation of lysine 372 are also deficient in acetylation of lysine 320, lysine 373, and lysine 382 as well as methylation of lysine 370 of p53. In the absence of methylated lysine 372, cells are unable to induce p53 target genes upon DNA damage and mice lacking Set7/9, which methylates this site, are predisposed to oncogenic transformation (Huang *et al.*, 2006; Kurash *et al.*, 2008).

Methylation of arginines appears to have a rather modulatory role. Depending on the level of PRMT5, methylation of p53 can induce cell cycle arrest or apoptosis. Overexpression of PRMT5 enhances cell-cycle arrest while reduced levels of the enzyme promote apoptosis (Jansson *et al.*, 2008).

Another modification that affects p53 function is poly-(ADP-ribosyl)ation. Glutamic acid 258, aspartic acid 259 and glutamic acid 271 are major sites for this reaction. Only PARP-1 is able to poly-(ADP-ribosyl)ate p53 which prevents its interaction with CRM1 and retains p53 in the nucleus where it activates gene transcription (Kanai *et al.*, 2007).

Regulation of p53 by ubiquitination, sumoylation and neddylation

In addition to targeting the tumor suppressor protein for degradation, ubiquitination can also modulate p53 function. Ubiquitination can even rescue the tumor suppressor protein from proteasomal degradation, especially when ubiquitin chains are linked via residues other than lysine 48 of ubiquitin or when ubiquitin chains are attached to internal lysines of p53. E4F1, for instance, mediates oligo-ubiquitination in the hinge region of p53 which recruits p53 to chromatin and enhances specifically its cell cycle arrest inducing activity (Le Cam *et al.*, 2006). Also ubiquitination of p53 by the male-specific lethal-2 (MSL-2) protein, which ubiquitinates lysine 351 and lysine 357, or the presence of the ubiquitin conjugating enzyme Ubc13, which forces the formation of K63-linked ubiquitin chains, do not promote p53 degradation. Instead they enhance cytoplasmic localization of p53, which sequesters the tumor suppressor protein from its target genes, and reduces its degradation (Laine *et al.*, 2006; Kruse and Gu, 2009). Ubiquitination can also trigger apoptosis. In particular the mono-ubiquitinated form of p53 translocates to mitochondria where it associates with Bak and Bcl-XL to promote permeabilization of mitochondrial membranes and cell death (Marchenko *et al.*, 2007).

The attachment of Nedd8 or SUMO also affects p53 activity. However, while neddylation clearly decreases p53 activity, sumoylation can, dependent on the context, both increase and decrease p53 activity (Gostissa *et al.*, 1999; Xirodimas *et al.*, 2004; Abida *et al.*, 2007).

Regulation of p53 activity by protein-protein interactions

A great number of proteins that bind to p53 modulate p53's transcriptional activity. Most of these proteins interfere with p53 function by associating with the N-terminal transactivation domain or the DNA binding domain. Others regulate p53 activity by attracting or repelling co-activators or modifying proteins while a third group of interaction partners impinge on p53 tetramerization (Figure 1).

Among the proteins that interact with p53, Mdm2 and MdmX are best known. Both Mdm2 and MdmX associate with p53 via the N-terminal transactivation domain. However, despite the conservation of basic features between the interaction of Mdm2 and MdmX with p53, the hydrophobic clefts of Mdm2 and MdmX, to which p53 binds, are not identical, which results in measurable differences in binding to p53 (Popowicz *et al.*, 2007). Therefore, prevention of gene transcription simply caused by binding of Mdm2 to the transactivation domain of p53 appears to be incomplete. This became particularly clear after the analysis of mice where cysteine 464 in the RING finger has been replaced with an alanine. Although mutant Mdm2 was completely competent to bind p53, it was unable to suppress p53 activity to a degree that allowed embryonic development (Itahana *et al.*, 2007). Accordingly, although genetic deletion of both Mdm2 and MdmX results in embryonic lethality which can be rescued by deletion of p53, the timing of embryonic cell death differs upon genetic deletion of either protein (Barboza *et al.*, 2008; Parant *et al.*, 2001).

Other proteins that bind to the N-terminal domain and reduce p53 transcriptional activity are BAF53 and nuclear factor with BRCT domain1/mediator of DNA damage checkpoint protein1 (NFBD1/MDC1) (Nakanishi *et al.*, 2007; Wang, M. *et al.*, 2007). BAF53 decreases p53 activity by reducing acetylation of p53 at lysine 382 and by diminishing binding of the tumor suppressor protein to promoters of target genes. The precise mechanism for inhibition of p53 activity by NFBD1/MDC1 is, though, as yet unclear (Wang *et al.*, 2007). Upon DNA damage NFBD1/MDC1 protein levels are reduced which releases p53 from this control (Nakanishi *et al.*, 2007).

Proteins that bind to the proline-rich region of p53 can also interfere with its activity. IASPP, one of the evolutionary most conserved inhibitors of p53 binds to this domain and inactivates preferentially p53 with a proline at position 72, a site with a frequent polymorphism. Downregulation of iASPP specifically enhances binding of p53 to the *bax* promoter, but not to the *p21* promoter (Bergamaschi *et al.*, 2006).

Proteins that bind to the DBD of p53 can either promote or reduce p53 activity. Repression of p53-mediated transcription often occurs due to competition of interaction partners with promoters of target genes. Proteins that reduce p53 activity by binding to the DBD include the splicing variant involved in hepatocarcinogenesis B (SVH-B), the tumor progression locus-2 (Tpl-2) kinase, the macrophage migration inhibitory factor (MIF), Twist, polo-like kinase 1 (PLK1) and the oncogene DJ-1 (Ando *et al.*, 2004; Zhou, X. *et al.*, 2007; Fan *et al.*, 2008; Jung *et al.*, 2008; Shiota *et al.*, 2008; Khanal *et al.*, 2009). Tpl-2 reduces p53 activity by recruiting PP2A phosphatase which removes the phosphorylation at serine 15 while binding of MIF diminishes p53-dependent gene transcription directly through binding to the DBD (Jung *et al.*, 2008; Khanal *et al.*, 2009). In both cases, the contribution of alternative mechanisms is not entirely excluded. Twist suppresses binding of p53 to target gene promoters and reduces p300-mediated acetylation (Hamamori *et al.*, 1999; Stasinopoulos *et al.*, 2005; Shiota *et al.*, 2008). Other interaction partners of p53 that bind to the DBD enhance p53's transcriptional activity. Some of them specifically promote transcription of pro-apoptotic genes while others support the induction of growth arrest. In particular ASPP1 enhances the binding of p53 to pro-apoptotic

promoters, while the activity of ASPP2 is not entirely clear. At higher concentrations, ASPP2 can even displace p53 from target gene promoters (Samuels-Lev *et al.*, 2001; Patel *et al.*, 2008). Hzf and Muc1, in contrast, promote transcription of cell cycle-arrest inducing target genes (Das *et al.*, 2007; Wei, X. *et al.*, 2005). Interestingly, prolonged activation of p53 leads to Hzf degradation which supports the transcription of pro-apoptotic genes after severe DNA damage (Das *et al.*, 2007).

In addition, proteins that associate with the C-terminus of p53 can regulate its activity differentially. The ATM and p53-associated KZNF protein (APAK) specifically downregulates pro-apoptotic genes and suppresses p53-mediated apoptosis, probably by recruiting KAP1/HDAC1 complexes to p53, which reduce p53 acetylation (Tian *et al.*, 2009). Interestingly, this restrain is released upon DNA damage through ATM-mediated phosphorylation of APAK, which reduces its affinity for p53 (Tian *et al.*, 2009). The Bloom (BLM) and Werner (WRN) helicases specifically promote p53-mediated apoptosis (Spillare *et al.*, 1999; Garkavtsev *et al.*, 2001), while the interaction of p53 with Zimp10, a PIAS-like protein, enhances *mdm2* and *p21* transcription (Lee *et al.*, 2007). Likewise, c-Abl only enhances p53-dependent transcription from promoters with perfect p53 binding sequences such as the *p21* or *gadd45* promoters, while it fails to stabilize p53/DNA complexes when the promoters do not comply perfectly with the consensus sequence (Nie *et al.*, 2000; Wei, G. *et al.*, 2005). Other proteins that bind to the C-terminus of p53 enhance or repress transcription of all p53 target genes. The DNA repair protein thymine DNA glycosylase (TDG), for instance, enhances p53 abundance at all p53-dependent promoters (Kim and Um, 2008), while the Hsp70 family member GRP75 reduces p53 abundance at all target genes, probably by retaining p53 in the cytoplasm (Wadhwa *et al.*, 2002). Another protein that associates with p53 is Daxx. This association is rather weak, but it can be enhanced by proteins such as Mdm2 or Axin. Nevertheless, as both activation and repression of p53 activity has been reported after overexpression of Daxx, its function remains unclear (Zhao *et al.*, 2004; Li, Q. *et al.*, 2007).

Many of the proteins that associate with the C-terminus of p53 impinge on p53 tetramerization. Tetramerization is required for full activity of p53. However, the tetrameric form of p53 is less efficient in entering the nucleus than monomeric p53, but once p53 is present in the nucleus as a tetramer, its NES is occluded and the protein is retained in the nucleus (Stommel *et al.*, 1999; Liang and Clarke, 2001). Proteins that facilitate tetramerization of p53 include 14-3-3 proteins and the Redox factor 1 (Ref1), while S100 proteins and the apoptosis repressor with caspase recruitment domain (ARC) reduce p53 oligomerization (Jayaraman *et al.*, 1997; Yang *et al.*, 2003; Fernandez-Fernandez *et al.*, 2005; Foo *et al.*, 2007; Rajagopalan *et al.*, 2008). However, 14-3-3 proteins such as 14-3-3γ or 14-3-3ε only promote tetramerization when p53 is phosphorylated at its C-terminus and the association with 14-3-3σ only occurs upon dephosphorylation of serine 376 (Rajagopalan *et al.*, 2008; Stavridi *et al.*, 2001; Waterman *et al.*, 1998; Yang *et al.*, 2003). Although the interaction of Ref1 with p53 is rather weak, it stimulates the assembly of p53 dimers in tetramers as well as the de-stacking of p53 multimers. In addition, it reduces disulfide bonds in the DNA binding domain of p53, which further enhances p53 activity (Jayaraman *et al.*, 1997; Hanson *et al.*, 2005). ARC, in contrast, stimulates the export of p53 into the cytoplasm (Foo *et al.*, 2007).

Some of the proteins that associate with p53 have chromatin-modifying and remodeling activities. The BRG1 and hSNF5 proteins directly bind to p53 and recruit the SWI/SNF complex to p53 and to p53-regulated promoters thus enhancing p53-dependent gene transcription (Lee *et al.*, 2002; Xu *et al.*, 2007). The STAGA complex also associates with p53 via several components including the acetyltransferase GCN5L, ADA2b and TRRAP. This complex is recruited to p53-regulated promoters especially upon stress (Ard *et al.*, 2002; Gamper and Roeder, 2008). HCAS/CSE1L is found together with p53 at promoters of selected p53 targets where it cooperates with p53 to form active chromatin. This ass ociation is specifically connected to cell death (Tanaka *et al.*, 2007). The presence of chromatin-associated protein HMG-1, PC4 and PTEN causes a significant bend in the DNA helix which is required for sequence-specific binding of p53 to its target sites (Jayaraman *et al.*, 1998; McKinney and Prives, 2002; Freeman *et al.*, 2003; Batta and Kundu, 2007). In addition, p53 can associate with the novel INHAT repressor (NIR), which reduces p53-dependent transcription under normal growth conditions and in response to cellular stress. This activity is most likely due to a reduction of histone H3 and H4 acetylation (Hublitz *et al.*, 2005).

Other proteins that interact with p53 are E2F1, DP1, C/EBPβ, AP2α, Wilms Tumor 1 (WT1), NF-Y, PML and Pin1 (Maheswaran *et al.*, 1993; O'Connor *et al.*, 1995; Imbriano *et al.*, 2005; Schneider-Merck *et al.*, 2006; Stabach *et al.*, 2006). While the interaction of p53 with WT1 and AP2α enhances the transcriptional activity of p53, its association with C/EBPβ, E2F1 DP1, NF-Y, YB1, securin or the focal adhesion kinase (FAK) reduces its activity. The nuclear proteins YB1, securin and the non-receptor kinase FAK bind to p53 and inhibit p53-dependent cell death while YB1 does not interfere with the induction of *p21* or *Mdm2* (Bernal *et al.*, 2002; Golubovskaya *et al.*, 2005; Homer *et al.*, 2005). PML associates with Mdm2 and p53 and co-localizes with both proteins in nuclear bodies. Especially after UV irradiation, PML potentiates p53 function by enhancing

Chk2-dependent phosphorylation and CBP-dependent acetylation of p53 (Kurki *et al.*, 2003). The peptidyl-prolyl-isomerase Pin1 associates with p53 only upon prior phosphorylation at serine 33, serine 46, threonine 81 or serine 315. Binding of Pin1 leads to cis-trans-isomerization of the tumor suppressor protein. Particularly important is the binding of Pin1 to phosphorylated serine 46 and threonine 81. Isomerization at proline 81 is required for phosphorylation of p53 by Chk2 and p53 acetylation, while isomerization of serine 46 triggers the dissociation of p53 from iASPP, an inhibitor of apoptosis. Accordingly, p53 activity is compromised in cells that lack Pin1 function (Wulf *et al.*, 2002; Zacchi *et al.*, 2002; Mantovani *et al.*, 2007).

As a transcription factor, p53 also associates with proteins of the basal transcription machinery. Interactions with the TFIID subunit TBP, the TFIIH subunits XPD, XPB and p62, and with several TAFs have been described (Wang, X.W. *et al.*, 1995; Farmer *et al.*, 1996; Leveillard *et al.*, 1996).

Concluding remarks

This review provides an overview of the vast signal transduction and protein–protein interactions network that controls p53 abundance and activity. In this review, we have mostly concentrated on post-translational modifications and protein–protein interaction that involve the tumor suppressor protein itself (1st class p53-regulators). Nevertheless, one should bear in mind that a plethora of further proteins also impinge on p53 activity by modifying and regulating these 1st class p53-regulators. Inclusion of all these 2nd class p53 regulators would go beyond the scope of this review. However, to give a flavor of the multitude and variety of these additional regulators we also listed those proteins that affect p53 abundance and activity by regulating the ubiquitin ligase Mdm2.

In addition to cellular proteins, p53 activity is also regulated by a number of viral factors. These factors also have been completely omitted from this review.

This high number of factors that influence p53 activity, however, also leaves us with the critical question of whether these post-translational modifications and interaction partners are indeed all required for the control of p53 in a single cell. Certainly, most of them are. However, some modifications or interactions may be restricted to certain cell types. The interaction of p53 with GRP75, for instance, has so far only been observed in cell lines, but not in primary fibroblasts. Likewise, HIPK4, a serine/threonine kinase that phosphorylates p53 at serine 9, is predominantly expressed in lung and white adipose tissue. Moreover, some post-translational modifications and protein–protein interactions may be used to fine-tune the response, e.g. to increase or decrease the activity of p53 according to the strength of the inducing stimuli. Other modifications and protein–protein interactions may be used to direct the cell fate after p53 activation. Implementation of cell cycle arrest, for example, requires a different set of post-translational modifications of the p53 protein than induction of apoptosis. Last but not least, several post-translational modifications and protein–protein interactions may serve as safeguard mechanisms to ensure the function of this important tumor suppressor protein in all situations.

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