# A closer view of an oncoprotein–tumor suppressor interaction

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**The cellular response to DNA damage is coordinated by the p53 protein. Mdm2, an oncoprotein, inhibits p53 and promotes p53 degradation. A recent high-resolution structure of the Mdm2–p53 complex may aid the design of small molecules to disrupt this interaction, for use in investigating the interaction further and for designing anticancer drugs.**

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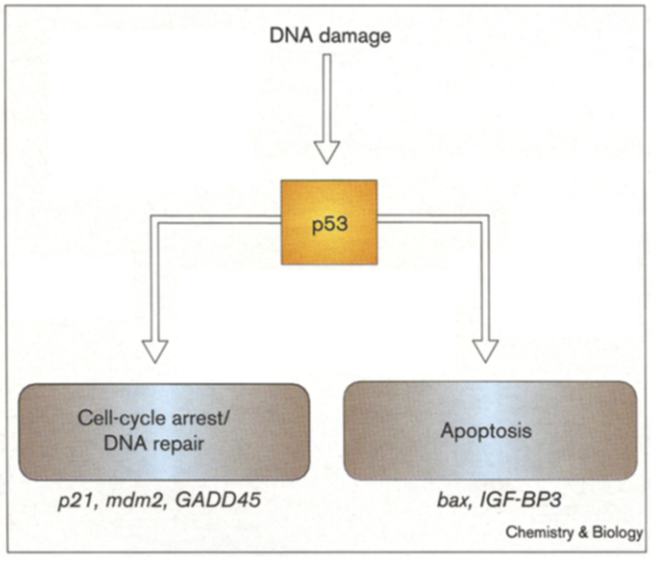
Mutations in the *p53* gene have been identified in virtually half of all reported cancer cases [1]. As a result, *p53* has been the subject of extraordinary levels of interest in the cancer-biology community. Research efforts have focused on p53 protein functions, including tumor suppression and transcriptional activation [2]. As various stresses, such as DNA damage, are imposed on a cell, p53 orchestrates a response that leads to either cell-cycle arrest and DNA repair, or programmed cell death (apoptosis; Figure 1). In normal cells, rapid degradation suppresses p53 levels. Recent reports by Haupt *et al.* [3] and Kubbutat *et al.* [4] have strongly implicated the proto-oncogene Mdm2 as being responsible for initiating the expedient proteolysis of p53, thereby heightening the importance of Mdm2 in p53 regulation (as discussed in more detail below). When DNA damage is sensed, degradation of p53 is inhibited and the cellular levels of p53 rapidly elevate. As a transcription factor, p53 is able to cause a halt in cell-cycle progression, primarily at the G1 stage, by upregulation of certain gene products including $p21^{CIP1/WAF1}$, which has been identified as a potent inhibitor of several cyclin–Cdk complexes [5]. The mechanism by which p53 is able to initiate apoptosis is less clear, however. The *bax* gene, which has been shown to be transcriptionally activated by p53, may play an important role in this response by antagonizing the effects of the anti-apoptotic gene *bcl2* [6]. This minireview will summarize recent developments in understanding the molecular basis of the regulatory control exerted upon the p53 tumor suppressor protein by Mdm2. In addition, the potential for disruption of the p53–Mdm2 interface by small molecules and the use of these molecules for studying and controlling p53 function is discussed.

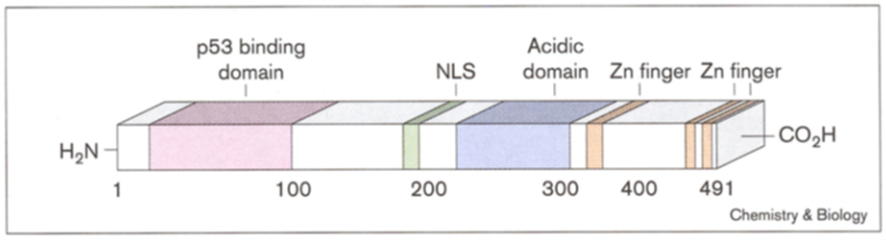
**Figure 1**




The role of p53 in cell-cycle arrest and apoptosis. Upon DNA damage, p53 is stabilized, resulting in either cell-cycle arrest and subsequent DNA repair, or apoptosis. Shown at the bottom of the figure are several of the genes expressed following p53 stabilization and transcriptional activation.

As well as mutations in the *p53* gene that alter the function of the p53 protein directly, p53 can be altered by changes in Mdm2. The Mdm2 protein has been shown to bind to p53 and disrupt transcriptional activation by associating with the transactivation domain of p53 [7]. In fact, Mdm2 overexpression has been implicated in the etiology of one third of soft tissue sarcomas [8], presumably as a result of sequestration of wild-type p53 and inactivation of its tumor suppression activity. Interestingly, in these sarcomas, *mdm2* overexpression has become an alternative to *p53* mutation. Mdm2 is a 490 amino-acid protein that contains a highly conserved central acidic region, three putative zinc finger motifs and an amino-terminal 100 amino-acid region shown to be necessary for p53 binding (Figure 2). Mutational studies of p53 have shown that Mdm2 binds to and inactivates the transactivation domain of p53, which interacts with subunits of the general transcription factor TFIID (the TAF31–TAF80 complex; Figure 3) thereby interfering with the transcriptional activation machinery of p53 [9]. Mdm2 transcription itself is stimulated by p53, suggesting that there is a negative-feedback loop between p53 and Mdm2. The relevance of this molecular antagonism was substantiated by studies in which mice with a targeted *mdm2* deletion were found to die early during embryonic development. The *mdm2* 'knockout' mice could be rescued from embryonic lethality by the simultaneous deletion of

**Figure 2**




The layout of the Mdm2 oncoprotein. NLS, nuclear localization signal.

p53, however [10,11]. These experiments have implicated Mdm2 as a negative regulator of the growth-suppressing activity of p53 during early development.

### Mdm2 is involved in p53 regulation

As mentioned earlier, recent studies reported by Haupt *et*

strong correlation has been found between the levels of both Mdm2 and p53 proteins. Rapid loss of p53 coincides with maximal Mdm2 induction during recovery from DNA damage, serving to ensure an effective termination of the p53 signal. In fact, an effective time window for p53 activity has been defined as the interval between p53 activation and Mdm2 accumulation. Mdm2 appears to regulate p53 levels post-transcriptionally, as cells co-transfected with Mdm2 contained normal levels of p53 messenger RNA. Furthermore, Mdm2 transfection promoted rapid degradation of p53 in cells that express p53 from a constitutive promoter [3,4]. This indicates that Mdm2 overexpression induced the destabilization of p53. Interestingly, p53 levels are maintained if cells are treated with the known proteasome inhibitors lactacystin or MG132, suggesting that expression of Mdm2 promoted the rapid proteasome-dependent degradation of p53 (Figure 3).

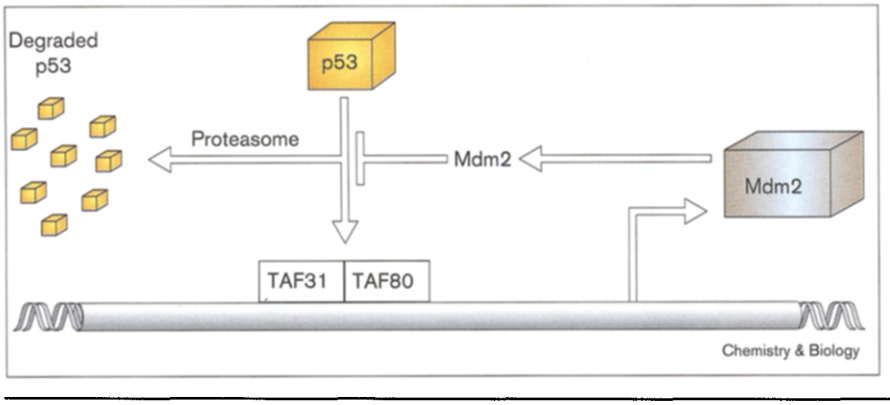
The molecular basis for Mdm2-dependent regulation of p53 has also been explored. Abolition of p53 binding to Mdm2 by amino-acid substitution on either protein results in p53 levels that are insensitive to Mdm2 overexpression. Binding of Mdm2 to p53 alone, however, is not sufficient for degradation of p53. Mdm2 mutants containing only the amino-terminal p53-binding region, as well as mutants lacking the amino-terminal domain, fail to cause degrada-

Mdm2 appear to be necessary for a proteasome-dependent response, their roles remain poorly defined and will certainly be the focus of future research [12].

### Structural basis for the Mdm2–p53 interaction

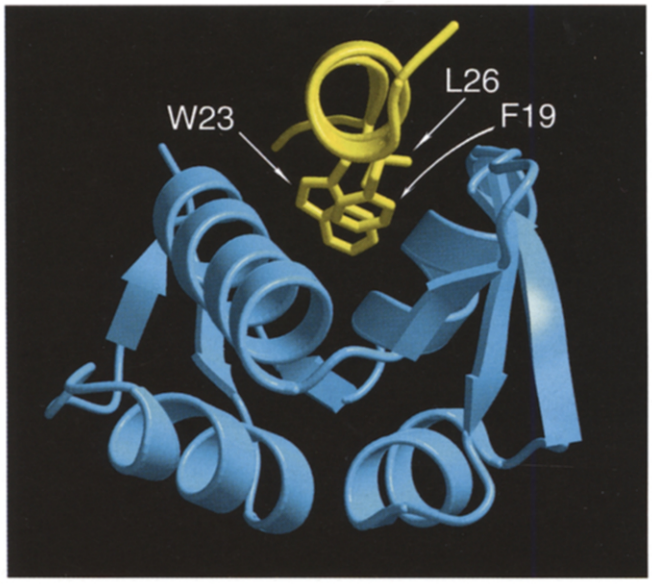
Recently, a molecular view of the Mdm2–p53 interaction was reported, at 2.3Å resolution, by Pavletich and co-workers [13]. The 12 kDa amino-terminal domain of Mdm2, represented by amino acids 17–125, co-crystallized with an 11 amino-acid peptide (residues 17–27) derived from p53 (Figure 4). The highly conserved p53 peptide had been shown, by mutational analysis, to be sufficient for Mdm2 binding with an apparent $K_d$ of 600nM, compared to a $K_d$ of 420nM for residues 1–57 of p53. Residues 17 to 27 had also been shown to be sufficient for the transactivation activity of p53 [14]. The amino-terminal domain of Mdm2 consists of two halves that show close structural similarity but little sequence homology to one another. A pseudosymmetric plane divides the two halves, forming a deep V-shaped cleft at an approximately 70° angle. The cleft is

**Figure 3**




The effect of Mdm2 on p53. Mdm2 can block the interaction of p53 with the TAF31–TAF80 complex, thereby inhibiting transcriptional activation. In addition, Mdm2 expression will cause degradation of p53 via a proteasome-dependent pathway.

**Figure 4**



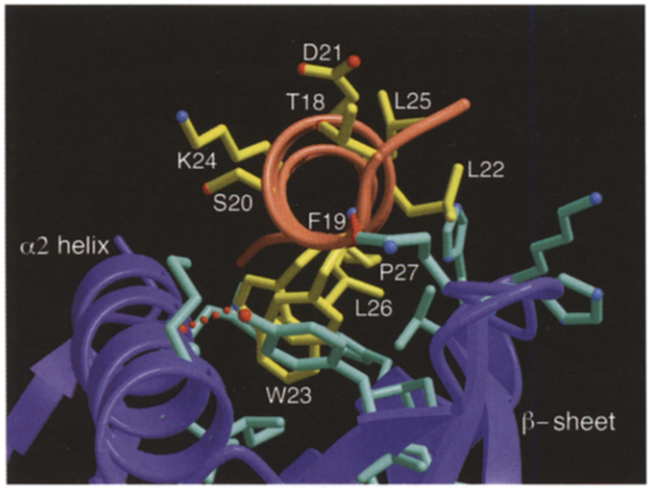

A side-on view of the Mdm2–p53 interaction. Mdm2 is blue and p53 is yellow; p53-derived residues critical to the complex are highlighted (Trp23, Leu26, and Phe19). Reprinted with permission from [13].

lined with several hydrophobic residues and measures 25 Å long, 10 Å wide (at the surface) and 10 Å deep. Short α helices form the floor of the cleft, whereas the sides are constructed of long α helices. An 11 amino-acid peptide derived from the transactivation domain of p53 forms an amphipathic α helix of 2.5 turns that inserts into the Mdm2 crevice (Figure 5). Phe19, Trp23 and Leu26 of p53 protrude into the Mdm2 cleft and Leu22 packs against the side wall of Mdm2 (Figure 6). As opposed to many of the other well-characterized protein–peptide interactions, including that of MHC class I [15], in which there is extensive hydrogen bonding between the protein and peptide, there are only two intermolecular hydrogen bonds between p53 and Mdm2. A large proportion of the interactions responsible for the tight association of p53 with Mdm2 are due to Van der Waals contacts which result in 1498 Å$^2$ of buried surface area. Mutational studies have shown that p53 residues Phe19, Trp23 and Leu26, when altered, are the most disruptive to the Mdm2–p53 interaction; these residues are also essential for transactivation by p53. The mutational studies, corroborated by the structural evidence provided by Pavletich and colleagues [13], indicate how Mdm2 is able to inhibit interaction of the p53 amphipathic helix with the TAF31–TAF80 complex, thereby quelling transcriptional activation by p53. It has been reported that the amino-terminal domain of Mdm2 also targets the E2F1–DP1 transcription-factor complex, and the interacting region of E2F1 contains a segment that exhibits high homology with the region of p53 contacting Mdm2 [16]. Unlike its effect on p53, however, Mdm2 stimulates the activation potential of the E2F1–DP1 complex. The retinoblastoma protein (Rb) has also been shown to interact physically and functionally with Mdm2, negatively regulating its activity [17]. It will be interesting to watch the emerging story of Mdm2 and its impact on growth regulation.

### Synthetic chemistry may play a role in eventually modulating p53 regulation via Mdm2

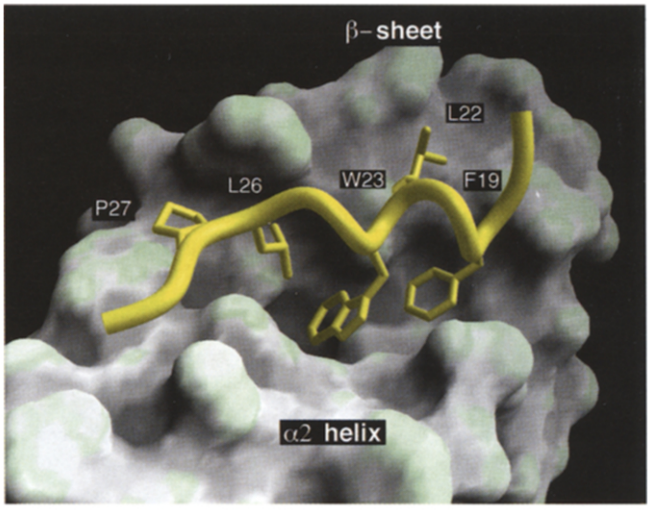
The Mdm2–p53 interaction represents an excellent opportunity for the use of synthetic chemistry to elucidate

**Figure 5**




A side-on detailed view of the Mdm2–p53 interface. Mdm2 is shown in blue and the residues that form contacts with the p53 peptide are light blue. The amphipathic p53 α helix (red) and its sidechains (yellow) are also shown. Reprinted with permission from [13].

**Figure 6**




The deep hydrophobic cleft of Mdm2 forms an interface with several hydrophobic sidechain residues of p53. Reprinted with permission from [13].

further the role Mdm2 plays in p53 regulation. In addition, it may facilitate the development of novel small-molecule therapies for cancer. Using structural information to design combinatorial libraries of small molecules, and high-throughput yeast-based screening techniques, it may be possible to develop small molecules that abrogate the Mdm2–p53 association. These small molecules would be expected to stabilize p53 and they may be useful in cases where Mdm2 overexpression is the primary means of p53 inactivation. In fact, success has already been registered by Lane and coworkers [18] who have identified novel Mdm2-binding peptides by 'phage display, and demonstrated their activity *in vivo* [19]. Clearly, the challenge now is to identify small molecules that have equally high affinities for Mdm2 but that are also cell-permeable. Such molecules may be extremely useful as inhibitors of Mdm2 which could be administered with temporal control to probe the role Mdm2 plays in non-transformed cells. Interesting questions concerning Mdm2 remain to be answered; for example, what functions do the domains other than the amino-terminal domain of Mdm2 perform, and how do they control the proteasome-dependent processing and regulation of p53? A small molecule that disrupted the Mdm2–p53 interaction might be quite useful in studies to answer these questions. As mentioned earlier in this review, many soft-tissue sarcomas overexpress Mdm2 in the presence of wild-type p53. These cancers may be held in check with small molecules that could intercept Mdm2, thereby preventing suppression of p53. Additionally, small-molecule disrupters of Mdm2–p53 interactions could be used as adjuvant therapy to help control and modulate the extent of the p53-dependent apoptosis response in conventional chemotherapy.

Regulation of p53 by Mdm2 continues to attract research efforts at several levels. Each discovery seems to provide an equal number of intriguing questions and answers. The recent disclosure by two independent laboratories [3,4] that an interaction between Mdm2 and p53 is responsible for regulating levels of p53 in a proteasome-dependent manner has enhanced our understanding of p53 regulation. High-resolution crystallographic analysis of the interaction has provided a glimpse at the molecular association between an oncogene and a tumor suppressor. This snapshot may reveal guiding principles for the development of small molecules that would be of value both in the laboratory and clinic.

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