# Dial 9-1-1 for p53: Mechanisms of p53 Activation by Cellular Stress

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**Abstract**

**The tumor suppressor protein, p53, is part of the cell's emergency team that is called upon following cellular insult. How do cells sense DNA damage and other cellular stresses and what signal transduction pathways are used to alert p53? How is the resulting nuclear accumulation of p53 accomplished and what determines the outcome of p53 induction? Many posttranslational modifications of p53, such as phosphorylation, dephosphorylation, acetylation and ribosylation, have been shown to occur following cellular stress. Some of these modifications may activate the p53 protein, interfere with MDM2 binding and/or dictate cellular localization of p53. This review will focus on recent findings about how the p53 response may be activated following cellular stress.** *Neoplasia* (2000) **2,** 208–225.

**Keywords:** phosphorylation, blocked RNA polymerase II, nucleocytoplasmic shuttling, MDM2, proteasome.

## Introduction

The p53 gene product plays an important role in tumor suppression. The evidence for this stems from the fact that the *p53* gene is the most frequently mutated gene found in human cancers [1]. Furthermore, individuals with the Li-Fraumeni syndrome, who have inherited a faulty allele of the *p53* gene, or mice strains in which the *p53* gene has been knocked out, are at much higher risk for contracting cancers than normal humans or normal mice. In some types of cancers, p53 inactivation occurs early in tumorigenesis [2] while in others, it is a late event [3]. Not only does functional inactivation of p53 predispose individuals to the induction of cancer, successful outcome of chemotherapy and radiotherapy has been suggested to depend on functional p53 [1,4,5]. Thus, elucidation of the function and regulation of p53 are of great importance for the understanding of the process of carcinogenesis, as well as for finding new avenues for therapeutic intervention.

## The p53 Response — A Two-Edged Sword

The mechanism of tumor suppression by p53 is thought to be related to its function as a transcription activator [6]. This is based on the fact that the great majority of p53 mutations observed in human tumors are found in the DNA-binding domain of the protein. This DNA-binding domain is required for its transactivation function [7]. However, transcription-independent functions appear to be important in tumor suppression as well [8,9]. The induction of p53 involves the stabilization of the protein itself [10,11], transformation of the protein from a latent to an active form [12,13] and localization of the protein to the nucleus [14].

Some 100 genes are thought to be transactivated by p53 [15–18], but the consequences of this gene activation are not fully understood. In addition to transactivation of target genes, p53 concurrently reduces transcription of other genes [19]. This can be accomplished either by an indirect mechanism involving sequestering of transcription factors [19–22] or through a direct mechanism involving inhibition of specific genes by histone deacetylation [23]. It is well-known that the $p21^{WAF1}$ gene product, which is induced by p53, is a potent inhibitor of $G_1$ cyclin-dependent kinases [24]. It has been suggested that it is through the $p21^{WAF1}$ protein that cells arrest at the $G_1$/S border of the cell cycle following exposure to ionizing radiation [25]. Activated p53 also plays an important role in other protective functions by stimulating nucleotide excision repair [26–31] and participating in the induction of senescence [32] (Figure 1). Recently, we have shown that wild-type p53 function is important for the efficient recovery of mRNA synthesis following ultraviolet (UV) irradiation [33].

In contrast to its protective functions, the p53 protein has also been implicated in the induction of apoptosis in certain cell types following DNA damage [8,11,34–36]. The role of p53 in apoptosis, which is thought to be mediated through caspase-9 and Apaf-1 [37], may involve induction of distinct target genes such as *bax* [38], *fas* [39] and *killer* [40]. However, the observation that transactivation-deficient p53 mutants also appear capable of inducing cells to undergo apoptosis suggests that p53 may trigger apoptosis through both transactivation-dependent and transactivation-independent mechanisms [41]. Transactivation-independent mechanisms may involve generation of reactive oxygen species [42–44] and abrogation of mitochondrial membrane potential [44]. The p53-mediated transport of Fas receptors from the Golgi complex to the cell surface is also thought to influence the induction of apoptosis [45]. Aggregation of these receptors following UV irradiation [46,47] triggers a

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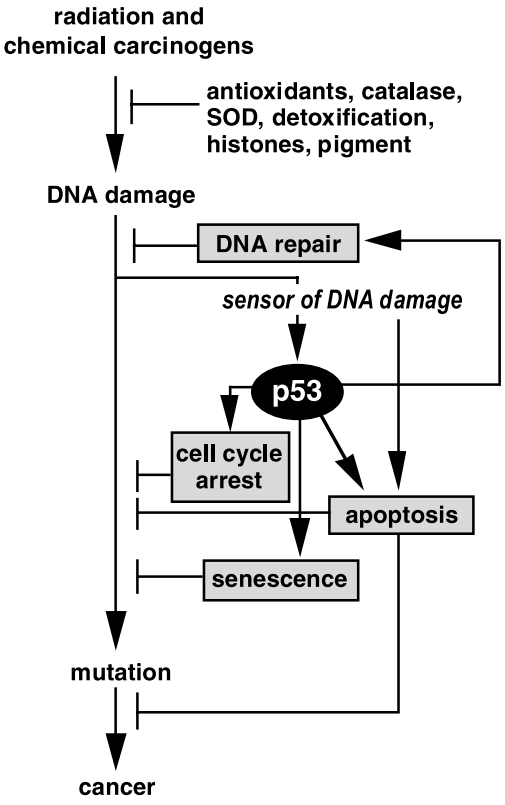


**Figure 1.** *Model describing some of the roles of p53 as a tumor suppressor. Cancer is a disease of multiple mutations most of which originate from unrepaired DNA damage induced by radiation or chemical carcinogens. Many defense mechanisms have evolved to safeguard the genetic material and to avoid the formation of mutations. In addition to antioxidants and DNA repair systems, mammalian cells can induce the p53 response to modify the behavior of a cell to avoid the formation of mutations. Following the induction of DNA damage, a "sensor" will trigger the induction of the p53 response that will manifest itself as cell cycle arrest, senescence, enhancement of nucleotide excision repair or apoptosis. All of these scenarios will reduce the probability of mutations arising in the exposed cells, and thus reduce the likelihood of the organism to develop cancer.*

cascade of events leading to the activation of caspase-8 with subsequent activation of a cascade of caspases leading to apoptosis [48,49].

As outlined above, p53 can play both protective and apoptosis-promoting roles following exposure to DNA-damaging agents. The decision whether to save or eliminate the cell depends on many factors such as cell type, severity of damage, and the oncogenic status of the cell [50]. It has recently been shown that wild-type p53 plays a protective role against the induction of apoptosis following moderate doses of UV light [33]. This protection by wild-type p53 expression in human fibroblasts correlated with a p53-dependent enhancement of the recovery of mRNA synthesis following UV irradiation. Furthermore, stimulation of recovery of mRNA synthesis and the protection against apoptosis by wild-type p53 appears to require p53-mediated transactivation *prior* to UV irradiation (McKay *et al.*, submitted). Thus, p53-mediated transactivation by basal levels of p53 prior to the insult confers protection, while induction of p53 following irradiation augments the UV-induced apoptotic process.

**Wasteful Waiting — Suppression of the p53 Response**

The p53 tumor suppressor protein negatively regulates cell growth through the induction of cell cycle arrest or apoptosis. Thus, in dividing tissue, there must be mechanisms put in place to downregulate the function of p53. It is now known that in proliferating cells, p53 is kept at a very low level by a mechanism involving proteasome-mediated degradation [51–55]. The proteasome-mediated protein degradation pathway plays an important role in the regulation of various cellular processes such as cell cycle progression, cell differentiation, signal transduction, stress responses and apoptosis [56–59]. The degradation of p53 involves a cascade of enzymatic reactions leading to the ubiquitylation and degradation of p53 by the 26*S* proteasome (Figure 2).

It is well-established that the MDM2 protein is involved in the degradation of p53 [60,61]. The MDM2 protein binds tightly to the N-terminus of p53 and this interaction leads to the ubiquitylation and subsequent proteasome-mediated degradation of the p53 protein. The MDM2 protein has been shown to have ubiquitin ligase activity and probably acts as the E3 ligase for p53 [62,63]. Results suggest that in addition to the N-terminal domain to which MDM2 binds, the C-terminal domain is important for MDM2-mediated degradation [64,65].

In addition to MDM2, the jun kinase (JNK) has been shown to target p53 for ubiquitin-mediated degradation in non-stressed cells [66,67]. This targeting is dependent on the binding of JNK to the amino acids 97–155 of p53 and occurs independently of MDM2. While MDM2 is found to complex with p53 specifically in the S and $G_2$/M phases of

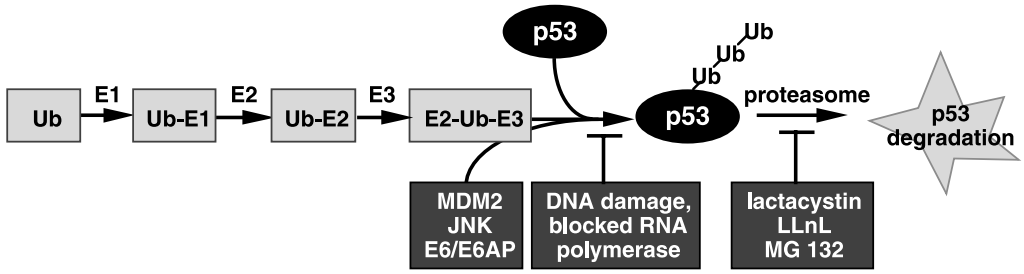


**Figure 2.** *Ubiquitin-dependent proteolysis of p53. In a sequential enzymatic reaction (E1–E3), ubiquitin (Ub) is activated and added onto the p53 protein. The ubiquitylated p53 protein is than targeted for proteolysis by the 26S proteasome. The proteins involved in directing ubiquitylation of p53 are the cellular proteins, MDM2 and JNK. The HPV 16 protein E6 can also target p53 for degradation by acting as a ubiquitin ligase. Following cellular stresses, proteasome-mediated degradation of p53 is stopped. The p53 protein can also accumulate by default following inhibition of proteasome activity with certain drugs.*

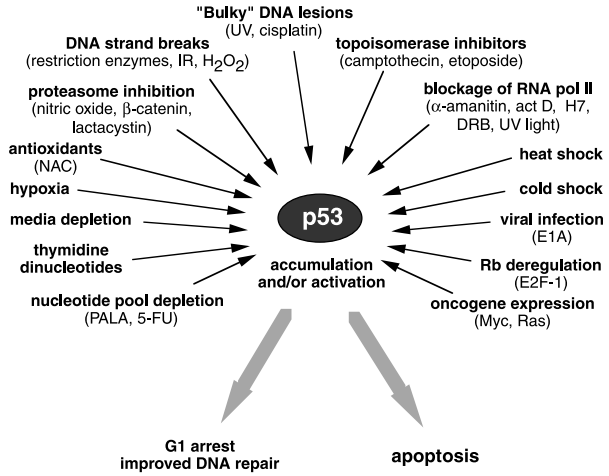


**Figure 3.** *The p53 response is triggered by many different stresses involving both DNA-damaging and non-DNA-damaging agents. These include ribonucleotide synthesis inhibitors resulting in perturbations of nucleotide pools [270], thymidine dinucleotides [271], media depletion [272], hypoxia [273], antioxidants [274,275], inhibition of ubiquitylation or the proteasome proteolysis pathway [53,107,108], DNA strand breaks [95], bulky DNA lesions [10,11], DNA topoisomerase inhibitors [11,276], blockage of RNA polymerase II [71–75], heat shock [273,277], cold shock [278], viral infection [217,279], pRb deregulation [216,280] and oncogene expression [214,215].*

the cell cycle, JNK–p53 complexes are preferentially found in the $G_0/G_1$ phases [66]. This suggests that degradation of p53 by MDM2 and JNK occurs at different stages of the cell cycle and that MDM2 and JNK act independently of each other. Interestingly, cell extracts depleted of both MDM2 and JNK can still support some degradation of p53, suggesting that there may be additional factors involved in targeting p53 for degradation [66]. Some studies suggest that in addition to proteasome-mediated degradation, the p53 protein can be targeted for proteolysis by calpain proteases [68–70].

## The Alarm Goes Off — Mobilization of the p53 Response

In order for p53 to accumulate in cells and to transactivate target genes, the degradation of p53 must be inhibited, the p53 protein must accumulate in the nucleus and the sequence-specific binding activity must be induced. Numerous agents have been found to cause the mobilization of p53 in cells (Figure 3). Some of these agents cause DNA damage and some do not. What sensor protein(s) does the cell use to learn about the inflicted damage or stress? How are these sensors signaling p53? Can the signals be fed through different signaling pathways depending on the type of insult inflicted and if so, does activation of different signaling pathways lead to distinct p53 modifications that tailor their function to best address the specific stress situation?

Much has been learned lately about the triggering mechanisms for the p53 response. The emerging picture is that multiple, distinct sensors and signaling pathways are triggered following exposure to different stresses [71] (Figure 4). The mechanism of p53 induction by two different DNA-damaging agents, ionizing radiation and UV light, will be contrasted below. Although these two physical agents both induce damage to DNA and trigger p53 accumulation, the types and amounts of DNA lesions induced are very different as are the cellular responses inducing the mobilization of the p53 response (Table 1). Also, certain agents appear to trigger p53 accumulation by directly interfering with the degradation pathway of p53. Finally, recent studies have implicated mismatch repair proteins in signaling p53 following induction of specific types of DNA damage.

## Stuck on the Tracks — Blockage of RNA Polymerase II by UV-Induced DNA Lesions Signals p53

It has been proposed that blockage of RNA polymerase may be the trigger for p53 accumulation following exposure to UV

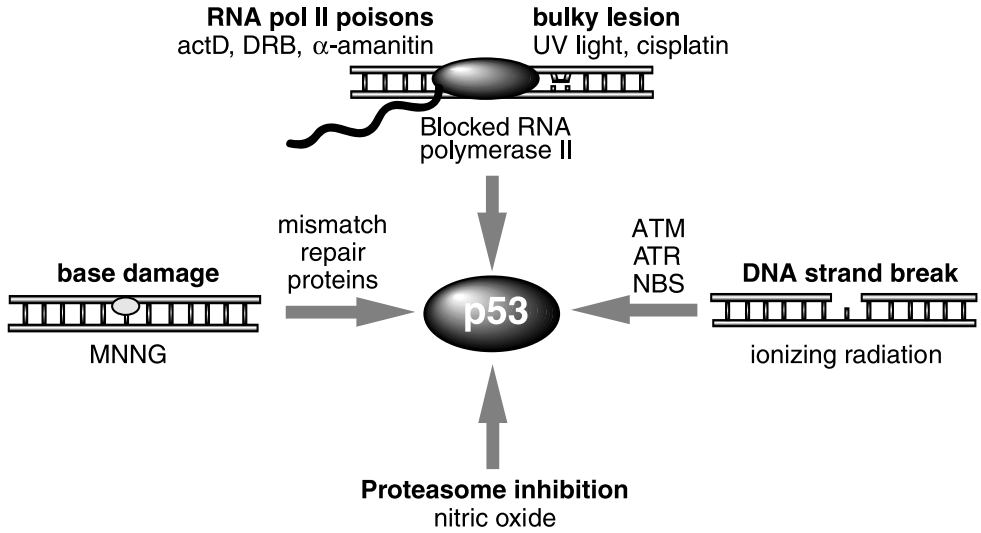


**Figure 4.** *The p53 response is activated through multiple pathways. Top: UV light and cisplatin induce bulky lesions in DNA that if formed in the transcribed strand of an active gene will impede the elongation of RNA polymerases. Also, certain drugs that poison the activity of RNA polymerase II, such as actinomycin D, DRB and α-amanitin, trigger the p53 response. Right: DNA strand breaks induced by ionizing radiation may be recognized by the proteins ATM, ATR and NBS which directly or indirectly initiate p53 induction. Bottom: Inhibition of the proteasome by specific drugs, nitric oxide or the overexpression of β-catenin leads to accumulation of p53. Left: $O^6$-methylguanine and cisplatin adducts are recognized by mismatch repair proteins resulting in the induction of p53 and p73 (see text for details).*

**Table 1.** The DNA-Damaging Agents Ionizing Radiation and UV Light Induce Different Types of DNA Lesions Leading to Contrasting Cellular Responses.

| Agent | Lesions/cell to kill 63% of cells[a] | Bulky DNA lesions | DNA strand breaks | DNA repair | Inhibits transcription[b] | Induces p53 | Induces apoptosis[c] |
|---|---|---|---|---|---|---|---|
| IR | 1,000 | no | yes | fast | no | yes | no |
| UV | 400,000 | yes | no | slow | yes | yes | yes |

[a]Ref. [91].
[b]Ref. [71].
[c]In human fibroblasts [71].

light [71–75]. This has been based on the findings that cells defective in the removal of UV-induced DNA lesions from the transcribed strand of active genes induce p53 at significantly lower doses than cells with proficient repair. These results suggest that persistent UV-induced lesions in the transcribed strand of active genes, but not lesions elsewhere in the genome, appear to be the triggering signal. Since UV-induced DNA lesions in the transcribed strand block elongation of RNA polymerase II [76–78], these results suggest that blockage of RNA polymerase II elongation may trigger the activation of the p53 pathway following exposure to UV light.

Recent studies have suggested that inhibition of RNA polymerase II may be sufficient for the induction of p53 [71]. The RNA synthesis inhibitors, actinomycin D, DRB, H7 and $\alpha$-amanitin, were found to all induce the accumulation of p53 in the same dose range as was shown to cause inhibition of mRNA synthesis. Furthermore, the induction of p53 by these agents did not correlate to the induction of DNA strand breaks. Although these studies suggest that inhibition of mRNA synthesis is sufficient to trigger p53 accumulation, certain agents such as ionizing radiation or proteasome inhibitors were found to trigger p53 without affecting mRNA synthesis. Thus, inhibition of RNA polymerase II may be a common mechanism by which many, but not all, p53-inducing agents trigger the accumulation of p53 (Figure 4).

What proteins are involved in sensing that RNA polymerase II elongation is blocked and how is the signal transmitted to p53? There are a number of proteins that are part of the RNA polymerase II holoenzyme that could directly or indirectly modify p53 (see Tables 2–4). The Cdk-activating kinase (CAK) plays an important role in the cell cycle by regulating the activity of Cdks. As part of the transcription factor TFIIH, CAK regulates the phosphorylation of the carboxyl-terminal domain (CTD) of RNA polymerase II [79]. Recent studies have shown that CAK can directly phosphorylate p53 *in vitro* [80,81]. Thus, CAK could potentially be a very powerful sensor of transcription elongation activating both p53 and cell cycle arrest if transcription is inhibited. Another interesting component of the RNA polymerase II holoenzyme that potentially could regulate p53 is the $TAF_{II}250$, which possesses both acetyl transferase and kinase activity [82,83]. $TAF_{II}250$ may indirectly regulate p53 stability through its role in the expression of the *MDM2* gene [84]. Finally, the transcription activators p300/CBP and PCAF are also proteins with the capacity to modify p53 by acetylation [85,86]. Taken together, there are many proteins associated with the transcription machinery that potentially could act as sensors of blocked RNA polymerases and could transmit (or stop transmitting) a signal resulting in p53 modifications/accumulation (Figure 5).

Inhibition of RNA polymerase II results in dramatic alterations in nuclear architecture [87,88]. The nucleus increases in size, the chromatin aggregates at the nuclear periphery and the nucleoli disintegrate. It is therefore possible that the induction of p53 following inhibition of transcription could be triggered by nuclear architectural alterations rather than direct signaling from the blocked RNA polymerase complex. Alternatively, since nuclear export is thought to be critical for the normal turnover of p53, the nuclear dismay caused by inhibition of transcription could interfere with the nuclear export machinery leading to the entrapment of p53 in the nucleus. The inhibition of transcription may also indirectly interfere with the p53 ubiquitylation process [54], or by a decrease in the cellular level of MDM2 which in turn would result in p53 accumulation [89] (see below). Further studies are needed to decipher the mechanism(s) of how inhibition of transcription leads to p53 accumulation.

## Breaks in the Helix — Ionizing Radiation Induces a Rapid p53 Response

Ionizing radiation induces a number of different types of DNA lesions [90]. Among these are base damage, single- and double-strand breaks and DNA–protein cross-links. Compared to equitoxic doses of UV light, ionizing radiation induces relatively few lesions in DNA [91] (Table 1). Furthermore, the repair of radiation-induced DNA strand breaks is significantly more rapid than the removal of bulky UV-induced lesions [92,93]. It is well-established that ionizing radiation induces a rapid induction of p53 in mammalian cells [36,94] most likely triggered by radiation-induced DNA strand breaks [95]. Is it the strand break itself that is recognized by the cell or is it its effect on chromatin structure and/or function that sets off the alarm?

It has been shown that the DNA in mammalian cells contains unconstrained torsional tension [96]. Studies of the *DHFR* gene domain have revealed that this unconstrained negative superhelicity is localized to the promoter region of the gene when it is poised for transcription [97]. Interestingly, this localized tension can be abolished when DNA breaks are introduced, on the average, 30 kb away from the area of tension. It is tempting to speculate that the topological integrity of chromatin loops may be monitored by sensor molecules located like spiders in their webs. When tension is lost following introduction of a strand break in a domain,

**Table 2.** Phosphorylation of p53 by Various Kinases.

| Kinases | General | Inducer | p53 modifications | Effect on p53 | References |
|---|---|---|---|---|---|
| ATM | binds double-stranded DNA preferentially at DNA ends in Ku-independent manner; no affinity for UV-irradiated DNA; phosphorylates BRCA1 following IR but not UV | IR, not UV; inhibited by caffeine and wortmannin | ser15, ser37 | blocks MDM2 binding; stimulates acetylation of C-terminus; plays a role in p53-mediated apoptosis | [36,86, 112–126] |
| ATR | protective function against IR and UV; involved in cell cycle checkpoint control | IR and UV; inhibited by caffeine but not very sensitive to wortmannin | ser15, ser37 | blocks MDM2 binding; stimulates acetylation of C-terminus; plays a role in p53-mediated apoptosis | [86,121–130] |
| DNA-PK | binds to and is activated by DNA ends; involved in double-strand break repair | IR, inhibited by wortmannin | ser15, ser37 *in vitro* only | not involved in p53 modification *in vivo*; required for efficient transactivation by p53 | [126,131–137] |
| JNK | associates with p53; direct ubiquitylation when not activated as a kinase | UV; ATM-dependent activation following IR | murine ser34, possibly ser37 on human p53 | blocks MDM2 binding | [66,104,131, 138–141] |
| c-Abl | interacts with p53 and DNA-PK after IR; phosphorylates p73 but not p53; neutralizes inhibition of p53 by MDM2; phosphorylates CTD of RNA pol II | IR (ATM-dependent activation), cisplatin | tyr99 of p73 after IR | stimulates p73-mediated transactivation and apoptosis | [100–103, 142–144] |
| CK I | expression may be induced by p53 | etoposide | ser4, ser6, ser9 | ? | [145] |
| CK II | strong affinity for binding to p53 | UV; constitutively phosphorylated? | ser392 | increases tetramerization and sequence-specific DNA binding; regulates p53-dependent transcriptional repression; inhibits p53-mediated renaturation of DNA strands; nuclear localization? | [13,146–151] |
| CAK | Cdk-activating kinase; phosphorylates CTD of RNA pol II | UV light downregulates CAK activity | ser33, C-terminus (ser371, 376, 378 and 392 potential targets) | enhances sequence-specific DNA binding *in vitro* | [80,81,131, 152] |
| PK-C | stimulates apoptosis | UVA, but not UVB or UVC | ser371, ser376, ser378 *in vitro* only | enhances sequence-specific DNA binding *in vitro* | [131,153–157] |
| Cdc2/cycl in B<br>Cdk2/cycl in A | regulates cell cycle progression; activated by CAK | UV light downregulates Cdc2 and Cdk2 | ser315 | increases sequence-specific DNA binding in vitro;targets p53 for degradation; attenuates tetramerization; target p53 for cytoplasmic localization? | [13,131,151, 158–166] |
| p38 | Interact with p53 | UV, not IR | ser33, ser46, ser392 | ser33 and ser46 phosphorylation stimulate phosphorylation of ser15 and ser37 and is important for UV-induced apoptosis; for effect of ser392 phosphorylation see above for CKII | [167,281] |
| PKR | ser/thr kinase modulating protein synthesis; requires double-stranded RNA for its activity; can bind p53 | interferon-inducible; UV?, IR? | ser392 | see above for p38 | [168,169] |
| CHK1 | may be an ATR-induced kinase | UV, IR | ser20 | blocks MDM2 interaction; plays a role in p53-mediated apoptosis | [124,170, 171, 282–286] |
| CHK2 | ATM-induced kinase; may be induced by ATR following UV light exposure, phosphorylates BRCA1 (ser988) following IR | UV, IR | ser20 | blocks MDM2 interaction; plays a role in p53-mediated apoptosis | [124,170, 171,282, 286–290] |
| Raf-1 | participates in the Ras signaling pathway initiated from the membrane | UV, IR | ser27 of mouse p53 | increases transactivation ability of p53 | [172–175] |

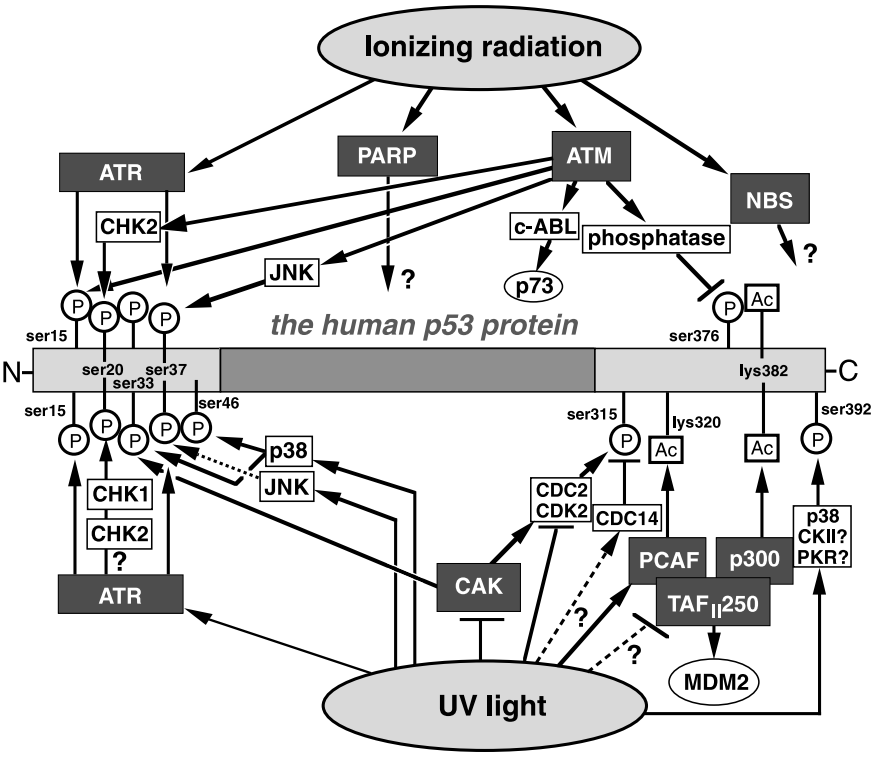


**Figure 5.** *Map of some of the modifications known to occur following ionizing radiation (top) and UV light (bottom). The p53 protein is divided in its three functional domains. The N-terminus contains the transactivation domain and binding sites for MDM2. The central core contains the sequence-specific binding domain. This is where most mutations are found in human tumors. The C-terminus harbors the NLS, NES and tetramerization domains. The p53-activating signal induced by IR is thought to be triggered by DNA strand breaks while UV light will induce p53 through a mechanism involving blocked RNA polymerase II at sites of DNA damage as well as from signals originating from the cell membrane (i.e., p38). The potential sensors are boxed in and mediators/effectors are listed. The dashed lines represents hypothetical pathways. Circled P represents phosphorylated residue while boxed Ac represents acetylated residue.*

these sensor molecules may signal p53. One such protein may be the ATM protein, which has been shown to be chromatin-associated and able to modify interactions between DNA and the nuclear matrix [98]. Although the "spider-in-the-web" hypothesis is an attractive model for how DNA strand breaks may rapidly trigger p53 mobilization, it has been shown that microinjection of exogenous DNA with free ends into mammalian cells appears to be sufficient to activate p53 [99]. This would argue against a mechanism involving the monitoring of DNA topology for p53 activation, although such a mechanism of p53 activation following ionizing radiation cannot be ruled out.

**Table 3.** Dephosphorylation of p53 by Various Phosphatases.

| Phosphatases | General | Inducers | p53 modification | Effect on p53 | References |
|---|---|---|---|---|---|
| PP2A | major kinase phosphatase in eukaryotic cells | ? | ser378 | reduces DNA sequence-specific binding of p53; reduces apoptosis | [153,176,177] |
| PP5 | participates in cell cycle checkpoint control | UV light reduces PP5 mRNA | ? | reduces p53's transactivation activity | [178] |
| hCDC14A&B | birds to C-terminal domain of p53; dephosphorylate Cdc2 and Cdk2 substrates; involved in exit of mitosis in yeast | ? | ser315 | expected to favor tetramerization by removing inhibitory ser315 phosphorylation; increased stability? nuclear localization? | [151,179,283] |
| ? | ATM-dependent activation of unknown phosphatase | IR | ser376 | creating a binding site for 14-3-3 protein resulting in increased sequence-specific DNA binding | [105] |

**Table 4.** Acetylation and Ribosylation of p53.

| Modifier | General | Inducer | p53 modification | Effect on p53 | References |
|---|---|---|---|---|---|
| P300 | acetyl transferase; acetylation of p53 favored if N-terminus of p53 is phosphorylated; functions in the apoptotic response to IR | UV, IR | acetylation of lys382 both *in vitro/in vivo* | increases sequence-specific binding of p53; increases stability of p53 | [85,86,180–182] |
| PCAF | acetyl transferase; acetylation of p53 favored if N-terminus of p53 is phosphorylated | UV, IR | acetylation of lys320 both *in vitro/in vivo* | increases sequence-specific binding of p53 | [86,181] |
| PARP | abundant chromatin-bound enzyme activated by DNA strand breaks; ribosylates itself, histones and other proteins; plays role in strand break and base excision repair; interacts with p53 | IR, not UV | ribosylates sites within central core and C-terminal domain | increases p53 stability; increases transactivation of target genes | [183–194] |

Proteins thought to be the sensors of radiation-induced DNA strand breaks are the Ataxia Telangiectasia protein (ATM), the ATM-related protein (ATR), the Nejmeegen Breakage Syndrome (NBS) protein and poly(ADP-ribose)polymerase (PARP) (see Tables 2 and 4 and Figure 5). The ATM protein can directly (ser15) [119,120] or indirectly via CHK2 (ser20) [286–289], JNK (ser37) [104] or c-Abl [100–103] phosphorylate p53 following exposure to ionizing radiation. Furthermore, ATM activates a phosphatase that specifically dephosphorylates the ser376, which activates the sequence-specific binding of p53 by allowing for the binding of the 14-3-3 protein [105]. The p53 modifications known to be induced following ionizing radiation are summarized in Figure 5.

## Staying Around — Inhibition of Proteasome-Mediated Degradation Extends p53's Half-life

As described above, the p53 tumor suppressor protein is under normal conditions rapidly degraded by the 26*S* proteosome [52,53,55] in a process dependent on ubiquitylation mediated by MDM2 [60–62] and/or JNK [66]. A number of peptide aldehydes that inhibit the 26*S* proteasome, such as lactacystin, LLnL and MG132, have been developed [106]. When treating cell cultures with these compounds, the level of the p53 protein rapidly increases [52,53]. Certain cell types have also been shown to undergo apoptosis following treatment with proteosome inhibitors [284,285].

Nitric oxide is a signal mediator that has been implicated in the induction of p53 and apoptosis in macrophages [107]. Recently, it was suggested that the mechanism by which nitric oxide induces the accumulation of p53 is linked to its inhibitory effect on the 26*S* proteasome [107]. Similarly, it has been suggested that overexpression of the multifunctional protein $\beta$-catenin induces p53 accumulation through inhibition of the proteasome degradation pathway [108]. Taken together, there may be a group of compounds or gene products that induce p53 accumulation through the interference of the activity of the 26*S* proteasome.

## Tagging the Damage — Mismatch Repair Proteins Alert p53

Cisplatin adducts in DNA are efficient blocks for transcription both *in vitro* [109] and *in vivo* [110]. As discussed above, blockage of transcription is sufficient to induce p53 and apoptosis. Thus, this may be one mechanism of how cisplatin induces p53 and apoptosis in exposed cells. However, recent studies suggest that mismatch repair proteins may also be involved in mediating a signal leading to the induction of apoptosis [100]. This signal transduction pathway, which is initiated by a MLH1-dependent event, involves activation of c-Abl resulting in the phosphorylation and accumulation of the p53 family member p73.

Exposure of cells to the alkylating agent, MNNG, results in many types of DNA lesions including $O^6$-methylguanine. This lesion has been shown to induce p53 and apoptosis in normal cells but not in cells lacking the mismatch repair protein complex MutS$\alpha$ [111]. Thus, it appears that the mismatch repair system recognizes this lesion and induces a signal leading to p53 activation. The mechanism for p53 induction is unknown, but it is possible that the $O^6$-methylguanine lesion complexed with mismatch repair proteins causes an impediment for the elongating RNA polymerase II. Thus, by converting a DNA lesion from a form that most likely is bypassed by RNA polymerase II to a form that blocks elongation, the MutS$\alpha$ protein complex may alert the cells of the damage by using the transcription machinery to signal p53.

## Regulation of p53 at the Level of Transcription and Translation

The transcription of the p53 gene is regulated by both transcriptional activators and repressors. A synergistic

activation of transcription from the p53 promoter can be accomplished by the transcription factors AP-1, NF-κB and Myc/Max [195]. In addition, YY1 and NF1 [196] can bind to and stimulate *p53* promoter activity. In contrast, a number of members of the Pax transcription factor family, which is implicated in the control of mammalian development, have been shown to inhibit transcription of the *p53* gene [197]. In addition, the virally encoded Tax protein [198], and overexpression of c-Jun [199] leads to the repression of p53 transcription. Interestingly, the p53 protein itself has been suggested to negatively regulate its own transcriptional expression through an indirect mechanism [200] (Figure 6).

Since the stress-inducible transcription factors AP-1 and NF-κB stimulate transcription from the p53 promoter, it is possible that this increased transcription from the *p53* gene contributes to the accumulation of p53 following cellular stresses. In fact, studies using a CAT reporter plasmid assay found that a number of different genotoxic agents could stimulate expression from the human p53 promoter [201]. This effect was attributed to a novel genotoxic-responsive p53 promoter element. However, increased levels of endogenous p53 mRNA were not observed in cells exposed to ionizing radiation, UV light, cisplatin or etoposide [11,94]. Since blockage of transcription leads to p53 accumulation [71], regulation of p53 transcription is unlikely to be a universal mechanism by which p53 accumulates following cellular stresses. Thus, increased transcription of the *p53* gene may play some role in the accumulation of p53 under certain circumstances but clearly is not a major mechanism by which the p53 response is launched following cellular stress.

The 3′ untranslated region (UTR) of human p53 mRNA [202] and the 5′ UTR of murine p53 mRNA [203] contain sequences that potentially could form stable secondary structures. The human 3′ UTR sequence has the ability to repress translation presumably through RNA-binding factors acting at the 3′ UTR [204]. Interestingly, the p53 protein itself has been found to bind tightly to the 5′ UTR of murine p53 mRNA resulting in the inhibition of its own translation [203]. In addition, the thymidylate synthase protein has been shown to bind to and inhibit translation from the p53 mRNA [205]. It has been hypothesized that following DNA damage-induced stress, the suppression of p53 mRNA translation is reversed. In fact, ionizing radiation, but not UV light, has been shown to partially overcome the repression exerted by the human 3′ UTR of p53 [202]. Taken together, regulation of p53 transcription and translation may contribute to p53 accumulation following certain types of stresses. Moreover, the suppressive role of p53 on its own transcription and translation sets up an interesting negative feedback loop where accumulation of p53 following cellular stress would lead to the shutdown of both its own transcription and translation (Figure 6). This would ensure that the p53 response is turned off shortly after the damage responsible for triggering the response has been repaired.

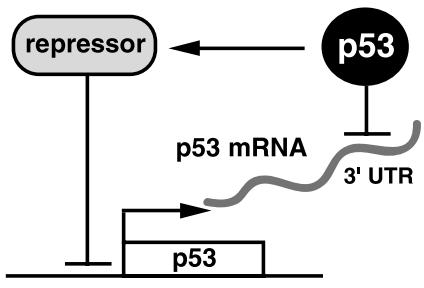


**Figure 6.** *A p53 autoregulatory feedback loop. Following accumulation, p53 can direct the inhibition of its own transcription by an indirect mechanism and inhibition of its own translation by binding to the 3′ UTR of the p53 mRNA.*

**Breaking the Loop — Inhibiting MDM2- and JNK-Mediated Degradation**

It has been proposed that MDM2 suppresses p53 in nonstressed cells in at least three ways [206]. First, MDM2 binds to the same region of p53 as do components of the transcription factor TFIID and thus, the ability of p53 to transactivate target genes is diminished. Second, the MDM2 protein may direct nuclear export of the p53/MDM2 complex. Third, MDM2 can act as a ubiquitin ligase targeting p53 for proteasome-mediated degradation [62]. Mutation of the nuclear export sequence (NES) of MDM2 or inhibition of the export machinery by the drug leptomycin B inhibits the nucleocytoplasmic shuttling of MDM2, resulting in nuclear accumulation of p53 [207].

To unfold the p53 response following cellular stress, the inhibitory activities of the MDM2 protein must be overcome. This could be accomplished by modifications of the p53 protein itself so that MDM2 can no longer bind to it. Alternatively, the MDM2 protein, or other proteins involved in the ubiquitylation and degradation of p53, may be modified so that they no longer can interact with p53. There is evidence suggesting that both of these scenarios may play roles in the accumulation of p53 in stressed cells (Figure 7).

Mutated p53 proteins found in tumors are often much more stable than wild-type p53. This has been suggested to be caused by the inability of the mutant p53 to transactivate the *MDM2* gene [65]. Inhibition of MDM2 expression is also accomplished by blockage of RNA polymerase II elongation. It has been shown that following UV irradiation or incubation in the presence of the RNA polymerase II inhibitors DRB and H7, the level of MDM2 protein rapidly decreases [14,89]. Thus, this represents an indirect mechanism by which p53 may be stabilized in cells by the loss of expression of its ubiquitin ligase MDM2. However, induction of p53 can occur at very low doses of UV light in DNA repair-deficient xeroderma pigmentosum cells (XP-A) without concurrent decrease of MDM2 protein levels [73]. Although the synthesis of total mRNA was reduced by about 50% following an exposure of XP-A cells to 4 J/m$^2$ of UV light, the protein level of MDM2 was found to be significantly increased. This would argue that induction of p53 following UV irradiation is more complex than simply resulting from the inhibition of MDM2 expression.

In addition to transcriptional regulation of MDM2, the activity of MDM2 can be regulated through posttranslational modifications. The DNA-dependent protein kinase (DNA-

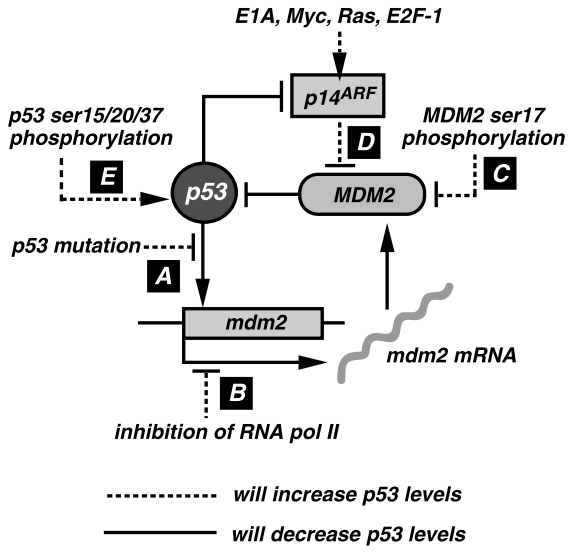


**Figure 7.** *The p53–MDM2–ARF regulatory feedback loops. Under non-stressed conditions, the MDM2 and p53 proteins balance each other and are found in low amounts. In order for p53 to accumulate in cells, it has to escape the inhibitory action of MDM2. This can be accomplished by (A) mutations in the p53 gene resulting in a transactivation-deficient p53 protein; (B) inhibition of general transcription by, e.g., UV light; (C) modifications of the MDM2 protein so that it binds p53 less efficiently; (D) induction of the p14ARF protein which reverses MDM2's inhibitory action on p53 or (E) modifications of the N-terminus of p53 leading to reduced binding to MDM2. DNA-damaging agents may cause p53 accumulation by stimulating the processes denoted with dashed lines, while solid lines denote processes repressing p53 accumulation. Following p53 accumulation, transactivation of the MDM2 gene ensures that the p53 response can be turned off soon after the signal that triggered p53 is removed.*

PK), which is activated by DNA strand breaks, can directly phosphorylate MDM2 at ser17 leading to the attenuation of the ability of MDM2 to interact with p53 [208]. Another damage-inducible protein that negatively regulates MDM2-mediated degradation of p53 is the protein tyrosine kinase, c-Abl [103]. The mechanism for how c-Abl neutralizes the inhibitory effect of MDM2 on p53 is not known but could involve blockage of MDM2-mediated nuclear export of p53 or direct interference of the p53 degradation process [103].

The ability of MDM2 to bind to and to ubiquitylate p53 can also be abrogated by expression of the tumor suppressor $p14^{ARF}$ [209–211]. The $p14^{ARF}$ protein has been shown to physically interact with MDM2 and this interaction interferes with the ability of MDM2 to act as a p53 ubiquitin ligase [212]. Expression of the $p14^{ARF}$ is not thought to be induced by DNA-damaging agents [213] but it is induced by oncogenes such as Myc [214], Ras [215] and E2F-1 [216], as well as the viral protein E1A [217]. However, a recent study shows that ARF $^{-}/_{-}$ mouse lymphoma cells have an attenuated induction of p53 and $p21^{WAF1}$ following treatment with the alkylating agent, cyclophosphamide [218]. In addition, these cells were more chemoresistant compared to corresponding wild-type ARF-expressing cells. Thus, DNA-damage signaling to p53 may in part be mediated by the $p14^{ARF}$ protein. Interestingly, wild-type p53 expression leads to the inhibition of $p14^{ARF}$ transcription, setting up a feedback loop between p53 and $p14^{ARF}$ [219] (Figure 7).

An important mechanism by which DNA-damaging agents may interrupt MDM2 binding to p53 is by modifications of the MDM2-binding domain of p53. Both ionizing radiation and UV light have been shown to induce phosphorylations of serines 15, -33 and -37 of p53 [170,171,220,221] (Figure 5). Ionizing radiation and UV light also induce phosphorylation of ser20, [170,282]. These modifications have been suggested to result in the abrogation of p53–MDM2 interactions leading to increased stability and activity of the p53 protein [220,221,282] (Figure 7). Phosphorylation of only serine 15 will cause inhibition of p53 transactivation activity by abolishing the interaction between p53 and the TATA-binding protein TBP [125], but phosphorylation of both serines 15 and 37 restores transactivation but abrogates the interaction between MDM2 and p53 [125].

JNK has also been shown to mediate ubiquitylation and degradation of p53 in nonstressed cells [66]. JNK can directly interact with p53 within a region spanning amino acids 97–155 of p53. JNK may be an adapter molecule that links p53 to an ubiquitin–ligase complex [66]. Complexes of JNK–p53 are preferentially observed in the $G_0/G_1$ phases of the cell cycle, while MDM2–p53 complexes are found preferentially in $S/G_2/M$. Following cellular stresses that induce the activation of JNK, the JNK-mediated ubiquitylation and degradation of p53 are abolished [140]. Activated JNK actually phosphorylates p53 leading to the attenuation of the interaction between p53 and MDM2. Thus, kinases upstream of JNK may be critical regulators of p53 stability by controlling both JNK- and MDM2-mediated ubiquitylation of p53 (Figure 8).

## The Cellular Postal Office — Regulating Nucleocytoplasmic Shuttling

The functions of many cell cycle regulators and transcription factors are regulated by nucleocytoplasmic shuttling. To be allowed access into the nucleus, a protein needs to carry a specific zip code, or nuclear localization signal (NLS). Similarly, proteins to be

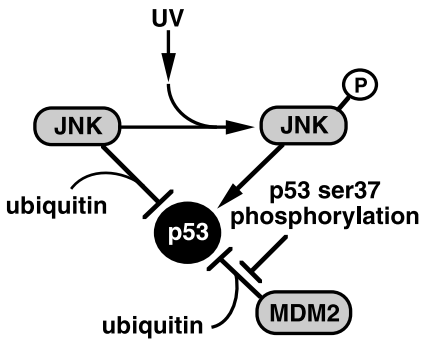


**Figure 8.** *The regulation of p53 by JNK. In addition to MDM2, JNK can direct ubiquitylation of p53. Following exposure to agents that induce JNK kinase activity, the JNK no longer can direct degradation of p53 but rather, phosphorylates p53 at the N-terminus. This phosphorylation leads to inhibition of MDM2 binding to p53. Thus, by activating the JNK, UV light knocks out two systems that normally direct degradation of p53.*

exported need to display a nuclear export signal (NES) or piggyback onto other proteins containing NES. The p53 protein has been shown to shuttle between the cytoplasmic and nuclear compartments in a cell-cycle-dependent fashion [222–224].

The nuclear import of p53 is an active process involving the association of the importin complex with the NLS of p53. In addition to a functional NLS, additional sites on p53 contribute to nuclear import. Mutation of a single lysine or arginine residue (lys305 or Arg306), or changing the positioning of these two amino acids relative to the NLS, has been shown to result in cytoplasmic localization of the p53 protein [225–227]. The importin complex brings proteins to be imported in contact with the nuclear envelope, and translocation through the nuclear pores is facilitated by the small and abundant GTPase protein, Ran [228–230]. In the cytoplasm, Ran is bound to GDP, but following completion of nuclear import of a protein, the GDP is exchanged for GTP. For nuclear export, Ran–GTP will complex with the exportins and their cargo proteins to facilitate nuclear export. Nuclear export of p53 is thought to be carried out by the export receptor, CRM1 [231]. The export of p53 can be blocked by the drug leptomycin B, resulting in the accumulation of p53 in the nucleus [207,231].

In certain tumors, such as breast cancer and neuroblastoma, p53 is commonly found localized to the cytoplasm [232,233]. This is not due to mutations in the *p53* gene. In fact, mutant p53 is most frequently found in the nucleus of cancer cells. The nuclear exclusion of p53 in these cancer cells was initially interpreted as being the result of an inability of p53 to translocate to the nucleus [233]. However, it was recently shown that the cytoplasmic "sequestering" of p53 could be reversed following inhibition of the nuclear export machinery with leptomycin B [231]. Thus, the cytoplasmic localization of p53 in these cells is not due to the inability of p53 to enter the nucleus, but rather is the result of an hyperactive nuclear export of p53.

How is nucleocytoplasmic shuttling of p53 regulated? First, p53 may be anchored in the cytoplasm by specific cytoplasmic proteins or structures. In order for p53 to be imported to the nucleus, the interaction with anchoring proteins must be overcome [227]. Second, the accessibility of the NLS or NES of the p53 protein may be regulated by proteins that bind to this region and block the interaction with the importin complex. It has been shown that the apoptosis antagonist, Bcl-2, in combination with c-Myc, appears to interfere with the nuclear import of p53 although the mechanism for this is unclear [234,235]. Third, the NLS, NES or adjacent sequences of p53 may be modified by phosphorylation, acetylation, ribosylation or other modifications that either stimulate or inhibit nuclear import [236].

Many proteins that shuttle between the cytoplasm and nucleus are regulated by phosphorylation mediated by CK II or Cdc2/cyclin B at sites near their NLS and NES domains [236]. While phosphorylation of these proteins by CKII is associated with nuclear location, phosphorylation by Cdc2/cyclin B is associated with cytoplasmic localization (see Table 2). The C-terminal domain of p53 has both Cdc2/cyclin B and CK II phosphorylation sites. Phosphorylation of ser392 by CK II has been shown to stimulate tetramerization of p53 [151]. Since tetramerization of p53 has been suggested to hide the NES of p53 and thus block nuclear export [231], phosphorylation of p53 by CK II may stimulate nuclear accumulation of p53 by blocking p53's export. In contrast, phosphorylation induced by Cdc2/cyclin B is associated with cytoplasmic localization of many different types of proteins. It has been shown that the tetramerization-stimulating effect of ser392 phosphorylation by CK II can be blocked by phosphorylation of ser315 by Cdc2/cyclin B [151]. Thus, it is possible that the nucleocytoplasmic shuttling of p53 could be regulated, in part, by these two kinases where CK II may stimulate nuclear import and Cdc2/cyclin B may stimulate nuclear export (Figure 9).

In addition to its potential role in cytoplasmic localization of p53, phosphorylation of ser315 may stimulate degradation of the p53 protein [163]. Whether this increased degradation is due to the phosphorylation itself or its stimulation of nuclear export is not clear. Unexpectedly, studies in which single or combinational mutations have been engineered in the p53 gene to eliminate phosphorylation sites showed no evidence for a role of any of the phosphorylation sites in regulating the stability of p53 [237–239]. Interestingly, deletion of the tetramerization domain of p53 was found to result in a p53 form that was not further stabilized by cellular stresses such as UV light. This suggests that the ability to form tetramers may be important for the stabilization of p53 following cellular stress [237–239]. However, these tetramerization-defective p53 mutants were expressed to higher levels than wild-type p53 in transient transfection assays, even in unirradiated cells. The high level of protein expression of these mutants may be due to diminished MDM2 binding since it has been suggested that an intact tetramerization domain is essential for the binding of MDM2 to the N-terminus of p53 [13]. Thus, the results from these studies regarding the role of the tetramerization domain in stabilizing p53 following stress are difficult to interpret.

The recently identified human protein phosphatases Cdc14A and Cdc14B, which were cloned by homology to the yeast Cdc14 [179], have been found to dephosphorylate p53 [283]. These phosphatases bind to the C-terminus of p53 and specifically dephosphorylate the ser315 site of p53. In yeast, Cdc14 specifically dephosphorylates proteins that are substrates for the yeast Cdc2 homologue, Cdc28/clb. One example of a protein that is subject to phosphorylation by Cdc28/clb and dephosphorylation by Cdc14 is the transcription factor, Swi5. Phosphorylation of Swi5 by Cdc28/clb stimulates its nuclear export, while Cdc14-mediated dephosphorylation of the same site leads to its nuclear localization [240]. By analogy to the yeast Cdc28/clb and Cdc14, the human Cdc2/cyclin B and Cdc14 may dictate the cellular localization of p53 by regulating the phosphorylation status of the ser315 site of p53 (Figure 9). Whether the activity or cellular localization of the human

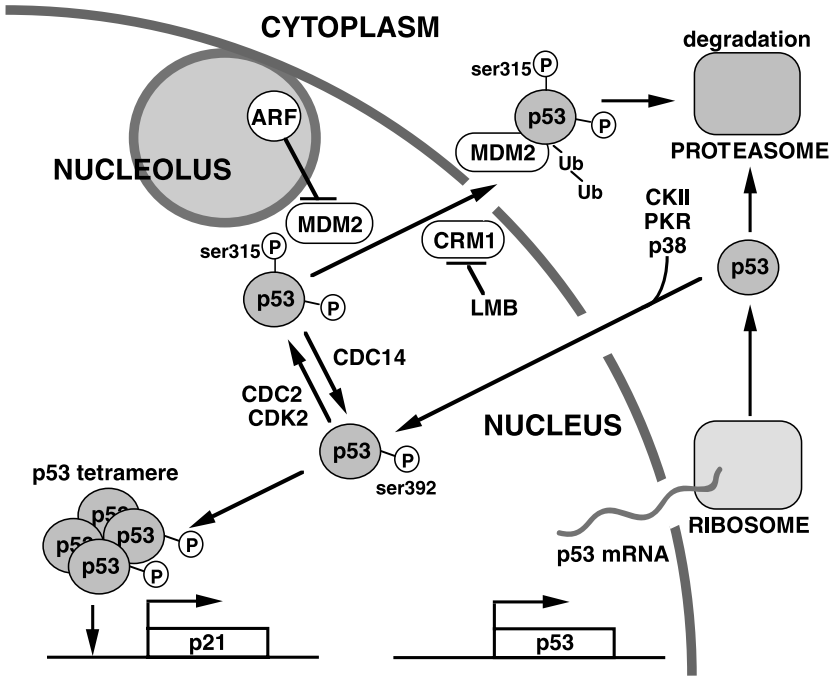


**Figure 9.** *Nucleocytoplasmic shuttling of p53. Following its synthesis, the cytoplasmic p53 protein may be directly degraded by the proteasome or imported into the nucleus. Phosphorylation of ser392 may stimulate nuclear localization by favoring tetramerization. p53 is also subject to nuclear export via the CRM1 nuclear export protein complex, a process that can be inhibited by the drug, leptomycin B (LMB). The nuclear export process may be stimulated by CDK-mediated phosphorylation of ser315 and by the MDM2 protein. The phosphorylation of ser315, which can be reversed by the Cdc14 phosphatases, inhibits p53 tetramerization. The p14$^{ARF}$ protein may interfere with the shuttling of both MDM2 and p53 by sequestering MDM2. Following export, the p53 protein is directed to the 26S proteasome by MDM2. Whether some of the exported p53 are allowed to re-enter the nucleus is not clear.*

Cdc14 phosphatases is altered following certain cellular stresses is currently being investigated.

### Activation of DNA-Binding Activity and the Battle for Cofactors

As discussed above, stabilization of the p53 protein and nuclear localization are important mechanisms required for the activation of the p53 response. However, to achieve efficient transactivation of target genes, two more events need to take place. First, the sequence-specific DNA-binding domain needs to be activated, and second, the p53 protein needs to interact with cofactors to stimulate transcription of the target genes.

The p53 protein is thought to contain a cryptic central DNA-binding domain negatively regulated by the C-terminal domain [12]. It has been shown that antibodies or small peptides directed against the C-terminal domain, inhibitors of molecular chaperones, as well as phosphorylation or acetylation of the C-terminal domain, result in the activation of the sequence-specific activity of the DNA-binding domain [13,241]. However, recent findings show that nuclear accumulation of p53 *per se* may be sufficient for transactivation of target genes [207,242]. In these studies, both p21$^{WAF1}$ and MDM2 proteins accumulated in the nucleus of cells treated with the nuclear export inhibitor, leptomycin B. Furthermore, the accumulated p53 proteins isolated from cells incubated with proteasome inhibitors were fully capable of binding DNA in a sequence-dependent manner [242]. It is possible that the "latent" form of p53 has some residual sequence-specific DNA-binding activity, that if present in large enough amounts in the nucleus will result in the activation of target genes. Alternatively, accumulation of p53 in the nucleus favors the p53 tetramerization which is thought to lead to activation of the DNA-binding domain by induced conformational alterations [13]. It is possible that some of the "activating" modifications of the C-terminus, such as phosphorylation of ser392, are related to stimulation of tetramerization which in turn activates the DNA-binding domain [148]. Association of p53 with noncovalent binding activators such as REF-1 and HMG-1, which are abundant nuclear proteins, may lead to the activation of the DNA-binding properties of p53 without the need for covalent modifications of its C-terminus [131,243,244]. Thus, induction of p53-mediated transactivation may be achieved either by specific modifications of the C-terminus leading to the induction of the sequence-specific DNA-binding activity, or in a less sophisticated manner, by brute force accumulation of p53 proteins in the nucleus.

To stimulate transcription of its target genes, p53 needs to interact with various transcription cofactors in order to stimulate transcription. It has been shown that the acetyl transferase CBP, which stimulates transcription by acetylating histones, plays an important role in p53-mediated transactivation [245–248]. Other proteins that p53 interacts with that could stimulate transcription are transcription factors TBP [20,249,250], TFIIH [251,252], TAFs [253], Sp1 [254], the p300 cofactor JMY [255] and DNA

topoisomerase I [256]. Since these enzymes and factors must be shared between many different genes, activated p53 and p53-inducible genes must compete for these factors with other transcription activators and genes. As a result of p53 accumulation following cellular stress, transcription factors will be sequestered by p53 leading to the activation of genes containing p53-binding sequences, while transcription from genes lacking p53-binding sequences will be attenuated [19–22]. In addition to the nonspecific inhibition of many genes by the sequestering transcription factors, p53 has recently been shown to specifically repress certain genes by bringing a histone deacetylase complex to these genes [23]. The deacetylase activity will reverse histone acetylation resulting in the compaction of the chromatin and repression of transcription.

Various stresses that induce accumulation of p53, such as UV light, concurrently induce the transcription factors NF-$\kappa B$ and AP-1 [257]. NF-$\kappa B$ binds, like p53, to the histone acetyl transferase CBP. It has been suggested that the battle between p53 and NF-$\kappa B$ for CBP sets up a transcriptional cross-talk between these two stress pathways [258,259]. The survival-promoting functions of NF-$\kappa B$ could perhaps be due, in part, to the inhibition of the apoptosis-promoting function of p53 by the sequestering of CBP [258,259]. Viral proteins, such as the adenovirus E1A protein, can bind to and suppress the activity of p300/CBP and PCAF, thereby interfering with p53 function [260,261]. To favor interactions between p53 and CBP following stress, stress-induced modifications of the p53 protein may increase the binding affinity of CBP for p53. In fact, phosphorylation of ser15 of p53 has been found to stimulate CBP-binding to p53 [123]. Binding of CBP to the N-terminus will also be favored by phosphorylations that exclude MDM2 binding [220,245]. Taken together, viral proteins and parallel stress signaling pathways may limit the transactivation activity of p53 by inactivating or competing for transcriptional cofactors while stress-induced modifications of the p53 protein may stack the cards in favor of p53.

## Conclusions and Future Directions

It is not difficult to appreciate that a protein that has been bestowed so much power over the fate of a cell must adhere to intricate and rigid regulation. Its role in transactivation is regulated by protein accumulation, nucleocytoplasmic shuttling, induction of its sequence-specific DNA-binding activity and through the competition for transcription cofactors. Relying predominantly on inhibition of its own degradation for induction, p53-mediated apoptosis can be achieved even after insults that severely limit the ability of the cell to perform transcription. If a damaged cell survives long enough to fully repair itself, multiple feedback systems are in place to eliminate p53 so that the repaired cell can re-enter the cell cycle. Following UV irradiation, e.g., the cell has to completely remove all UV-induced transcription blocks in the MDM2 gene before the p53 response can be turned off. Thus, the monitoring of the recovery of MDM2 mRNA synthesis is a way for the cell to assess the severity of the insult and determine whether apoptosis would be an appropriate solution.

It would be of great interest to better understand the alternative mechanisms used by tumor cells to inactivate their p53 function in addition to mutations in the p53 gene. What regulates the hyperactive nuclear export and cytoplasmic localization of "wild-type" p53 in cells of many tumor types [231]? What is different about the regulation of p53 in epithelial cells compared to fibroblasts [262–264]? How important are the inhibitory effects of p53 function by environmental pollutants such as arsenic [265] and cadmium [266] for human health?

More than 15,000 papers featuring p53 have been published since its discovery in 1979 [267–269]. About 10 papers are currently published daily. With this productivity in both basic and clinical research, there are good prospects that our further understanding of the regulation and function of p53 soon will lead to fruitful new efforts in both the prevention and treatment of cancer.

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