Decision Making by p53: Life versus Death

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Abstract

Cellular response to DNA damage is multifacted in nature and involves a complex signaling network in which p53 functions as a "molecular node" for converging signals. p53 has been implicated in a variety of cellular processes primarily functioning as a transcription factor and also in a transcription-independent manner. It is rapidly activated following DNA damage with phosphorylation as one of the initial signals. Cellular context as well as the type and severity of DNA damage determine p53 activation code, and its activities are regulated predominantly through protein degradation, post-translational modification and interactions with various cellular co-factors. These events are crucial in decision making by p53 as it has the ability to receive, assess and integrate different signals and route them accordingly to induce cell death or promote cell survival. In this decision making process, its transcriptional role to activate a specific subset of target genes linked to inducing cell cycle arrest or apoptosis is critical that is further fine-tuned by its transcription-independent function. This article reviews the current state of knowledge about the role of p53 in determining the fate of cells that have incurred DNA damage.

Keywords

Cell Cycle Arrest; DNA damage; Apoptosis; p53; Mdm2; Post-translational modifications

Introduction

DNA, the carrier of genetic inform ation, may be the most prized possession of a cell. However, DNA is vulnerable to damage caused by a variety of toxic insults including those mediated by intrinsic and extrinsic genotoxic agents. While unicellular organisms respond to this challenge by tolerating or repairing the DNA damage, multi-cellular eukaryotes have an additional choice of apoptosis, which facilitates the removal of severely damaged cells. Evolutionarily, this altruistic strategy makes sense because the severely damaged cells carrying DNA beyond repair pose great danger to the well-being of neighboring cells and the organism as a whole. It remains an important question as to how higher eukaryotes determine whether and when the damaged cell should commit suicide. While most of the key players involved in the DNA damage response are conserved in all eukaryotes, p53 and its associated regulatory factors appear to mostly exist in multi-cellular organisms. Several lines of evidence suggest that it is the insertion of a control module involving p53 that confers upon multi-cellular organisms the ability to exercise an additional option of apoptosis in response to severe DNA damage (1). This article will focus on the current state of knowledge about the role of p53 in deciding the fate of cells that harbor damaged DNA.

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p53: Structure, Function and Regulation

Structure and Function

The human p53 gene resides at chromosome 17p13.1, encoding a 393 amino acid protein of 53 kDa. p53 contains modular domains that highlight the structure of a typical transcription factor (Figure 1). The nuclear localization signal (NLS) at amino acid position 305–321 signals the transport of p53 into the nucleus where it exerts its transcriptional functions. The tetramerization domain involving residues 339–350 promotes p53 to form a homotetramer, which is the active form of p53. Once in the nucleus in its active form, the sequence specific DNA-binding domain, residing within residues 102-292, binds to DNA in a sequence specific manner (p53 responsive element) and then the transactivation domain present within amino acids 1-44 recruits the basal transcriptional machinery and co-activators or co-repressors to regulate the target gene expression (2,3). In addition, there is also a negative regulatory domain at the C-terminus residing at amino acid position 368-393, which represses p53 DNA binding. Two nuclear export signals (NES) at positions 11-27 and 339–350 reside at the N- and C-termini respectively (3).

p53 functions primarily as a transcription factor to activate or repress a large number of target genes, some of which are involved in the three major DNA damage responses including DNA damage repair, cell cycle arrest and apoptosis (4-8) (Figure 2). p53 function is also linked to other cellular processes such as senescence, metabolism and autophagy and these topics are beyond the scope of this review. In addition to its transcriptional-dependent functions, p53 also physically interacts with several important cellular proteins to mediate its effects in a transcription-independent manner (9,10). For example, in response to severe DNA damage, while the nuclear p53 promotes apoptosis by inducing transcription of pro-apoptotic proteins, such as PUMA, Bax, and several others, the cytoplasmic p53 also contributes by either directly activating Bid through inducing a conformational change in Bid or indirectly by sequestering Bcl-XL to release Bid from the inhibitory control of Bcl-XL (11).

Regulation

In agreement with its diverse and important functions in cells, p53 is also regulated through multiple mechanisms. At the core of its regulation is the relative abundance of p53 in a given cell. In unstressed cells, p53 is a very short-lived protein with a quick turn over (half-life of only 20 minutes). Thus, while p53 is continuously synthesized at a high rate, it is also rapidly degraded at an equally high rate resulting in a low steady-state level of p53 under physiological conditions (12). It is therefore, not surprising that interruption of p53 degradation leads to its rapid accumulation. At the same time, p53 activity is also modulated by its post-translational modification and via its interactions with various cellular co-factors.

Degradation—p53 is degraded through the ubiquitin-proteasome pathway. To date at least nineteen E3 ubiquitin ligases for p53 have been reported (13), of which the murine double minute 2 (Hdm2 in human, hereafter referred to as Mdm2) is thought to be the major E3 ligase (14). Some of the other E3s ligases include Pirh2, COP1 (constitutively photomorphogenic 1), CHIP (chaperone associated ubiquitin ligase), topors (human topoisomerase I- and p53-binding protein) and ARF-binding protein (ARF-BP1) (2). Recent studies have shown that the ubiquitination reaction can also be reversed by Herpes virus-associated ubiquitin-specific protease (HAUSP), which deubiquitinates p53 both in vitro and in vivo (15,16).

While Mdm2 negatively regulates p53 protein level, p53 positively regulates the transcription of Mdm2. Thus, it forms a negative Mdm2-p53 feedback loop which functions

to maintain p53 at a low level in unstressed cells. This negative feedback loop is the core of the regulatory circuit of p53 stabilization. Disruption of p53-Mdm2 interaction, e.g., by competition or posttranslational modifications of p53 and/or Mdm2, leads to a rapid accumulation of p53 after stress. For example, upon oncogenic activation, the tumor suppressor ARF, the product of the alternative reading frame from *CDKN2A*, can bind to and sequester Mdm2 in the nucleoli and thereby prevent it from action (17). However, ARF is not essential for the p53 response following DNA damage, where post-translational modifications of p53 and/or Mdm2 play a major role (see below).

Post-translational modifications—p53 is subjected to a complex and diverse array of post-translational modifications, including ubiquitination, phosphorylation, acetylation, methylation, sumoylation and neddylation (18). At the N-terminus of p53 that contains the transactivation domain, a NES and the Mdm2 interaction region is intensively phosphorylated by diverse kinases (Figure 3). Phosphorylation in this region in most cases upregulates p53 activity through the following mechanisms: (i) it reduces Mdm2 interaction with p53, such as phosphorylation on Thr18 and Ser20 residues, thus leads to p53 accumulation (18,19); (ii) it blocks p53 export presumably by masking the NES in the N-terminus, for example, phosphorylation on Ser15, Thr18 and Ser20 residues (18,20); (iii) it recruits p300/CBP and other transcription co-activator, like Ser15, Thr18 and Ser20 phosphorylation (18,19,21). All of the above mentioned phosphorylation events are induced after DNA damage. There is an exception however, that involves Thr55 phosphorylation by TAF1 during G1 cell cycle progression, which negatively regulates p53 by promoting p53 ubiquitination and nuclear export (22,23). Interestingly, this phosphorylation event is reduced after DNA damage thus contributing to accumulation of p53 (22,23). In addition to regulation of p53 abundance, localization and activity, N-terminal phosphorylations also contributes to modulation of p53 transactivation specificity through promoter selection, for example, with Ser46 phosphorylation p53 appears to favor the transcription of pro-apoptotic genes (24).

While the N-terminus of p53 is primarily phosphorylated, the C-terminus undergoes multiple modifications (Figure 3). The p53 ubiquitination sites reside in this region and while ubiquitination usually leads to p53 degradation, acetylation at the C-termius stabilizes p53 presumably by masking the ubiquitination sites (18). C-terminal acetylations also increase p53 DNA binding activity possibly by acetylation-induced conformational change which relieves the inhibitory effect of the p53 C-terminal negative regulatory domain on its tetramerization (25-27). In addition to phosphorylation and acetylation, the C-terminus of p53 is also subjected to methylation, sumoylation and neddylation. C-terminal methylation stabilizes p53 (28) while C-terminal sumoylation enhances its transactivation (29,30), both possibly through conformational change which relieves the inhibitory effect of the p53 C-terminal negative regulatory domain on its tetramerization. Finally, p53 is also neddylated at the C-terminus by Mdm2, which, in contrast to other C-terminal modifications, inhibits p53 transcriptional activity, providing yet another mechanism by which Mdm2 negatively regulate p53 activity (31).

Cellular cofactors—While post-translational modifications are important to modulate p53 accumulation, they may not be sufficient to increase its activity as cellular cofactors are also believed to play a critical role. Several such cellular cofactors including transcriptional co-activators e.g., histone acetyltransferases and histone methyltransferases and co-repressors e.g., histone deacetylase and ubiquitin ligases for histones, modulate the transcriptional activity of p53. A second group of p53 cofactors provides p53 with promoter selectivity for a subset of its target genes. The members of apoptosis stimulating proteins of p53 (ASPP) family, consisting of ASPP1, ASPP2 and iASPP fall into this group. ASPP1 and ASPP2 are implicated in p53-dependent proapoptotic functions whereas the inhibitory member iASPP

is involved in anti-apoptotic activities. All ASPP family proteins share the same binding domain on p53. Upon binding, ASPP1 and ASPP2 enhance while iASPP inhibits the transactivation of pro-apoptotic genes by p53 (27-29). Finally, p53 transcription-independent functions are also modulated via its interactions with cellular cofactors. It has been reported that p53 can translocate to the mitochondria and directly interact with and sequester the anti-apoptotic proteins such as Bcl-2 and Bcl-XL and thereby free the pro-apoptotic proteins Bax and Bid to induce apoptosis (32).

Clearly, the above three events: protein degradation, post-translational modifications and binding to cellular co-factors, cooperatively regulate p53 in different modes including feedback loops and signaling cascades, resulting in a 'finely-tuned' p53 response to a specific stress affected also by cellular microenvironment.

DNA Damage Responses

The integrity of genomic DNA is always under threats from both inside and outside the cell, including but not limited to intrinsic replication error, endogenous metabolic and environmental agents such as, ionizing and ultraviolet radiations, and natural and synthetic chemicals (33). Different genotoxic agents lead to different types of DNA damage, including single-strand breaks, double strand breaks (DSBs) and DNA adducts. Although the severity of DNA damage could vary (34), the consequences of DNA damage, if left unrepaired, are diverse and often adverse (35).

To counteract the deleterious consequences of those genomic insults, multi-cellular organisms have developed mechanisms to repair or tolerate the damage, or eliminate the damaged cell. The multi-cellular organisms deploy three major pathways towards this end including (a) cell cycle arrest that allows time for repair before the damage is passed onto daughter cells (b) DNA damage repair through the removal of damaged DNA and restoration of the continuity of the DNA duplex and (c) apoptosis that helps eliminate cells with excessive DNA damage (Figure 2) (34). Additionally, under certain specific conditions, cells also employ DNA damage tolerance mechanism to allow replication bypass of damaged template during DNA replication, which operates at the expense of replication fidelity (36).

Like other signal transduction pathways, the conceptual framework of the DNA damage response pathways involves three components including sensors, transducers and effectors. The DNA damage signal is first sensed by sensor proteins, then mediated via transducers to numerous downstream effectors involved in specific pathways (37-39). In mammalian cells, Ataxia telangiectasia mutated (ATM) (38,40,41)and ataxia telangiectasia and Rad3-related (ATR) (41) kinases have been considered the primary activators of DNA damage response. ATM and ATR belong to PI3-kinase-related protein kinases (PIKKs) family (41). While ATM primarily responds to DSBs, ATR has a mild preference for UV-induced damage and stalled replication forks (41). They function downstream of sensors at or close to the sites of primary DNA damage, as primary transducers. When activated upon DNA damage, they selectively phosphorylate downstream substrates such as Chk1 and Chk2 to further convey the signal accordingly to the respective effectors in different DNA damage response pathways (41). Interestingly, p53 is activated and plays an essential role in all three major DNA damage response pathways (42). Thus p53 is proposed to be the "molecular node" of the DNA damage signaling network, responsible to converge signals and also decide which effector role to assume (42).

Activation of p53 in response to double strand breaks (DSBs)

Of the various stresses that induce p53, the mechanisms underlying p53 activation in response to DSBs are well studied. DSBs are generated by ionizing radiation, reactive oxygen species, radiomimetic chemicals and during replication as a consequence of replication fork stall or collapse. DSBs are also a normal result of meiotic recombination and the immunoglobin class switching processes (34,43). DSBs could activate cell-cycle checkpoints to allow time for damage assessment and repair by either homologous recombination (HR) or nonhomologous end-joining (NHEJ) mechanisms (44-46), while extensive DSBs would trigger apoptosis.

DSB responses also follow the "sensors—transducers—effectors" cascade mode. Upon double strand breaks, the sensor Mre11-Rad50-Nbs1 (MRN) complex senses the damage and binds to the double strand ends, which is required for the initial recruitment and activation of the primary transducer ATM, a PPIK family member. ATM then phosphorylates H2AX, which promotes the recruitment of MDC1 to phosphorylated-histones around the damage sites (37,41). In turn, MDC1 itself can use another domain to recruit more ATMs, thus forming a positive ATM-MDC1 feedback loop, driving the formation of growing foci at the damage site, to facilitate recruitment of additional damage-response proteins (37,41). While the above model proposed by Chen and colleagues (37) fits most of the reported results, contrasting findings are also reported, which show that ATM can become activated even without physical contact with the DSB ends (47). Although activation of ATM itself is undisputable, further studies are needed to clarify the exact mechanism of ATM activation. Activated ATM then transduces signals by phosphorylating downstream substrates, including p53 (41).

In addition to the primary transducer ATM, another PPIK, ATR is also activated in DSB response, albeit at a later stage and a slower rate (41,48). Although this induction of ATR is functionally redundant to ATM, in most cases, it acts independent of ATM in order to maintain the phosphorylation status of some of the ATM substrates (48). However, recently it was also shown that during the S and G2 phases of the cell cycle, the ATR response to DSBs (that are not following the replication fork stall) is downstream of ATM recruitment to the DNA ends (41,49-52). Nevertheless, upon DSBs insults, ATM and ATR are activated independently or cooperatively to induce p53 where ATM acts predominantly.

Phosphorylation of p53 serves to initiate its activation upon DSBs as p53 is rapidly phosophorylated at Ser15 by ATM, which is later maintained by the slowly activated ATR for several hours (Figure 4) (48). Ser15 phosphorylation in turn promotes Ser20 phosphorylation by Chk2, a protein kinase downstream of ATM. Both Ser15 and Ser20 phosphorylations are required for the subsequent Thr18 phosphorylation by CK1 (Figure 4) (18).

Phosphorylations of these three N-terminal sites are considered to be important in p53 activation as they lead to p53 accumulation in the nucleus both directly and indirectly by disrupting the p53-Mdm2 negative feedback loop. The Thr18 phosphorylation significantly reduces p53-Mdm2 interaction, leading to inhibition of p53 degradation (18,19). Ser15 and Ser20 phosphorylations block Mdm2-mediated p53 export possibly by masking the nuclear export signal around those two sites (18,20). Furthermore, Ser15, Ser20 and Thr18 phosphorylations also stimulate p53 interactions with its co-activators p300/CBP and PCAF (18,19,21), both of which are histone acetyltransferases. In addition to histones, they also acetylate multiple sites at the C-terminus of p53. As mentioned above, the C-terminal acetylation of p53 inhibits its ubiquitination by masking the C-terminal ubiquitination sites and thus leading to p53 accumulation (18).

In addition to p53, ATM and its downstream kinases also target Mdm2 and other p53 negative regulators to upregulate p53 protein level (53). For example, ATM phosphorylates Mdm2 at Ser395, which inhibits Mdm2-mediated p53 degradation (54,55). ATM also activates its downstream effector c-Abl protein kinase to phosphorylate Mdm2 at Tyr 394, which impedes Mdm2-mediated p53 export (56). Thus, by directly mediating or indirectly promoting post-translational modifications of both p53 and its negative regulator Mdm2, ATM disrupts the p53-Mdm2 negative loop through different mechanisms, resulting in a rapid accumulation of p53, especially in the nucleus. However, p53 accumulation itself does not account for the overall activation of the p53 functions. Because p53 usually exists in a latent form in unstressed cells, its activity also needs to be increased in order for it to cope with the challenge posed by double strand breaks.

In addition to p53 abundance, phosphorylation of p53 in response to DSB also plays an indirect role in upregulation of its transactivation. As mentioned previously, cellular cofactors bind to p53 to either regulate or mediate its activity. In most cases, phosphorylation and/or other modifications on p53 modulate its interaction with other proteins. For example, the interaction between p53 and its co-activators p300/CBP and PCAF is stimulated by Ser15, Ser20 and Thr18 phosphorylations on p53 (18,19,21). Those two histone acetyltransferases acetylate histones around the promoter region of p53 response genes thus enhancing their transcription (25,27). In the meantime, C-terminal acetylation of p53 by these two histone acetyltransferases also stimulates its transcriptional functions by promoting conformation changes that relieve the inhibitory effects of the C-terminal negative regulatory domain of p53 (25-27).

It is clear; p53 is regulated by an extensive and complicated network that involves a growing list of proteins. Why did the cell evolve to incorporate so many pathways to regulate just one single protein? One possibility is that these pathways are redundant and serve as a failsafe mechanism to ensure that a backup is always available in case one pathway fails to function properly. It is also possible that these pathways offer various alternatives for cells to prepare and respond to varying conditions. It is now becoming obvious that while some of these pathways are considered dispensable for p53 activation, p53 is also activated differentially even if exposed to the same genotoxic agent to varying extent. An example is the Ser46 phosphorylation on p53, which is believed to occur only in response to severe DNA damage and induces transactivation of proapoptotic gene targets (57).

In summary, DSBs insults activate ATM, which acts cooperatively with its other downstream kinases to phosphorylate both p53 and its regulators in order to (i) ensure p53 accumulation in the nucleus mainly by disrupting p53-Mdm2 negative feedback loop, (ii) activate p53 by influencing its co-factor binding directly or indirectly, and (iii) also confer p53 promoter selectivity under certain conditions. Thus, p53 is activated either to arrest cell cycle and assist DNA repair or to induce apoptosis under severe DNA damage. But how does p53 decide to induce cell cycle arrest associated with DNA repair versus apoptosis is a key question that remains to be fully elucidated.

Decision Making by p53: Life versus Death

p53 is regulated via various mechanisms that affect its relative abundance, post-translational modifications and ability to interact with co-factors. Therefore, it seems that these events are crucial in decision making by p53 to transactivate a specific subset of target genes to either arrest cell cycle and assist DNA repair or to induce apoptosis under severe DNA damage (Figure 5) (58).

Contribution of p53 abundance

Although all p53 target genes contain p53 binding element, p53 binds to them with variable affinity. The p53-binding element constitutes a bipartite RRRCWWGYYY (R = A,G; W = A,T; Y = C,T) sequence with a spacer in between. A recently resolved p53-DNA complex crystal structure related spacer length to p53-DNA binding affinity (59). In general, cell cycle arrest related genes with shorter spacers bind to p53 with high affinity. Some of apoptosis-related gene targets such as *IGF-BP3* (Box A), *p53DINP1, P2XM*, and *PUMA* (site 1) also bind p53 tightly while *Noxa, p53AIP1, PIDD*, and *PUMA* (site 2) with longer spacers bind p53 with very low affinity (60-62). As a result, these apoptosis related targets are expected to be fully activated when p53 abundance reaches a threshold. Thus, under conditions of severe DNA damage, p53 accumulates at higher levels and that enables it to overcome the low affinity binding within the promoter regions of certain apoptotic genes.

Post-translational modifications

In response to DNA damage, p53 undergoes heavy post-translational modifications. Combinatorial effects of varying modifications at different sites could serve as a specific code for the type and severity of DNA damage, which could in turn confer upon p53 the ability to act specifically as is the case for Ser46 phosphorylation (24). Phosphorylation on this site is believed to specifically activate the transcription of apoptosis-related genes and mutation of this site has been found to reduce p53-induced apoptosis but not cell cycle arrest after DNA damage (24,63).

Cofactor interactions

Finally, certain cofactors bound to p53 also affect p53's ability to selectively regulate certain promoters. For example, members of ASPP family including ASPP1 and ASPP2 enhance while iASPP inhibits the transactivation of pro-apoptotic genes by p53 (64-66). There are also co-factors such as YB1 or MUC1 that negatively regulate transactivation of pro-apoptosis gene by p53 (67,68).

It is plausible that the aforementioned three mechanisms work cooperatively to regulate p53 affinities for the promoters of a certain subset of genes. When persisted severe damage leads to high level of p53 above certain threshold, it may also deactivate/activate some p53 modifying enzymes to achieve loss of pro-survival and gain of pro-apoptotic modifications, which would in turn promote binding of pro-apoptotic cofactors and dissociation of pro-survival cofactors (Figure 5) (58). Decoding of the complex p53 post-translational modification code and the understanding of how these modifications regulate specific co-factors binding, although still remain challenging, are subjects of future investigations. Adding to the complexity is that in addition to its primary function as a transcription factor, p53 also mediates transcription-independent functions in the cytoplasm. How nuclear and cytoplasmic p53 are synchronized to function in the same direction in determining cell fate following DNA damage also remains a key unanswered question. Overall, in cellular context many factors influence the decision making process including (i) the type, time and extent of the DNA damage, (ii) cell type, (iii) extracellular stimuli, (iv) cellular proliferative potential and (v) efficiency of DNA repair (58). The ratio of pro-apoptotic to anti-apoptotic factors in the cell also sets a death threshold for p53.

Conclusion

Cellular response to DNA damage is multifaceted in nature and involves a complex signaling network in which p53 functions as a "molecular node" for many of the converging signals. Our current understanding of this field covers only part of this complex signaling network as many missing pieces of the puzzle still remain to be identified. It is clear however, that p53

has the ability to receive, assess and integrate different signals and route them accordingly to induce cell death or promote cell survival. The exact mechanism by which p53 achieves this is not clear and remains to be fully explored. Similarly, the mechanisms by which the function of this master modulator is controlled under different conditions also remain to be fully elucidated. Such studies are expected to provide valuable insights into the role of p53 at the key transition point that determines whether a cell with damaged genetic material should live or die.

Acknowledgments

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Figure 1. Functional domains of the p53 protein
Amino acid numbers covering each domain are indicated above or below each corresponding domain. Abbreviations: NES, nuclear export signal; NLS, nuclear localization signal. Drawn based on information from (3).
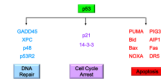
Figure 2. Transcription-dependent functions of p53 in response to DNA damage
Representative targets of p53 in the three major pathways are indicated in matching colors.
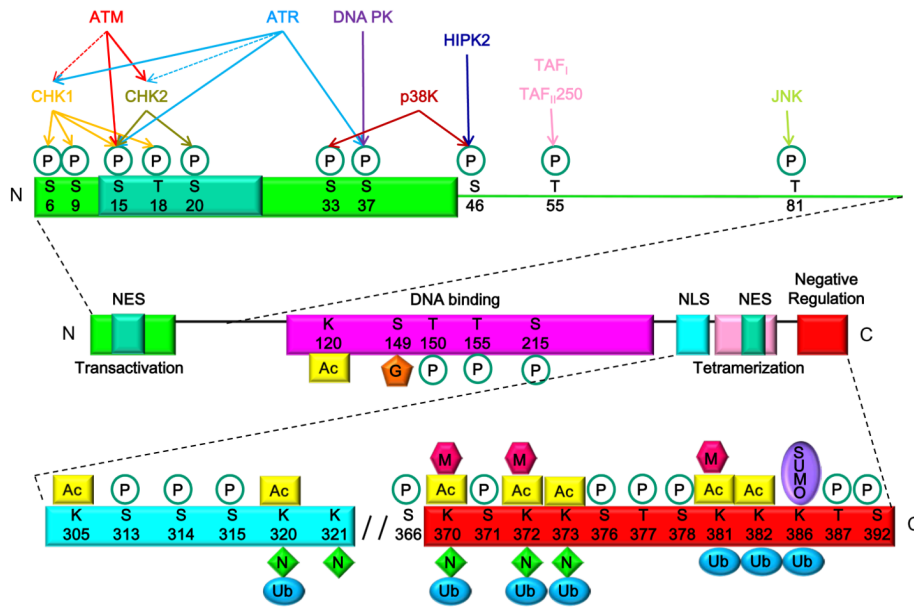Drawn based on information from (69).

Figure 3. Post-translational modifications of p53
Abbreviations: P, Phosphorylation; Ac, Acetylation; G, O linked N-acetylglusamine; M, Methylation; SUMO, Sumoylation; N, Neddylation; Ub, Ubiquitination. Modified from (69).
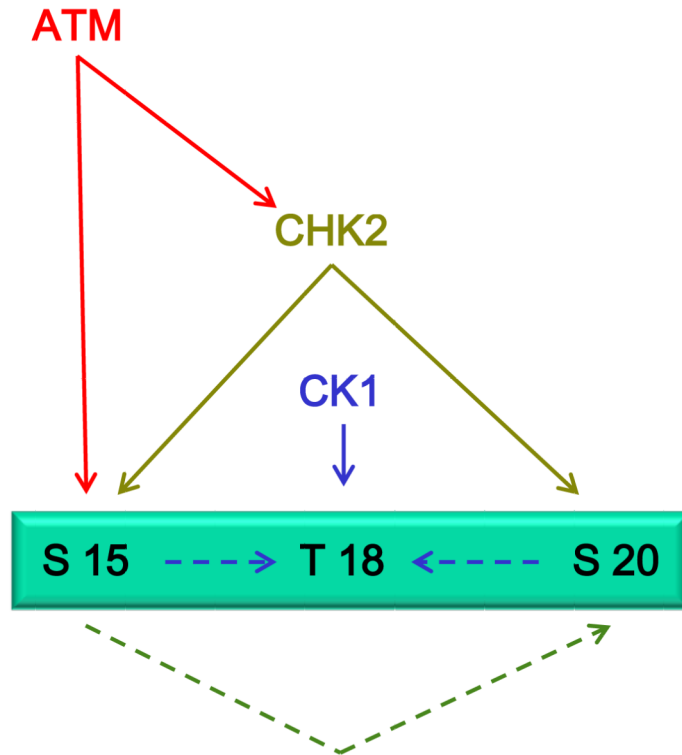
Figure 4.
Sequential phosphorylation cascade in p53 activation in response to DNA double strand breaks.
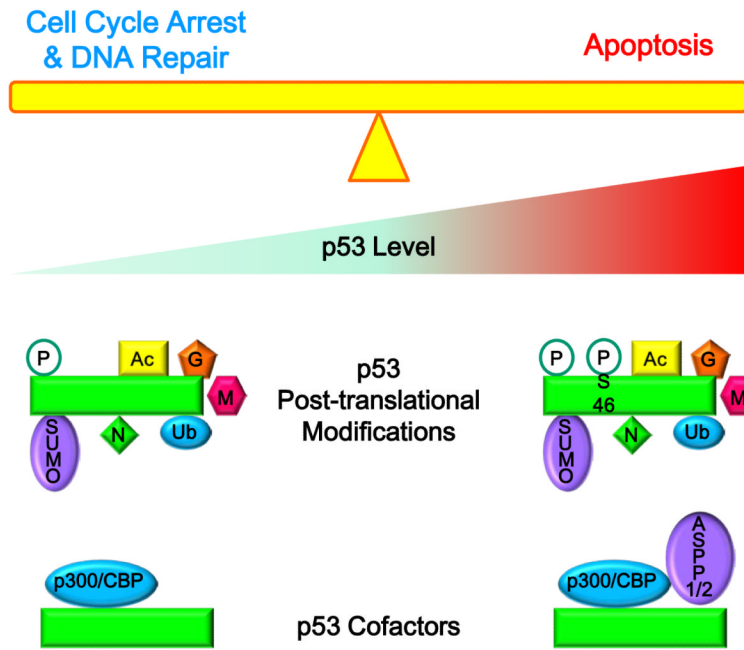
Figure 5. Determinants in p53 decision making
Abbreviations: P, Phosphorylation; Ac, Acetylation; G, O linked N-acetylglusamine; M, Methylation; SUMO, Sumoylation; N, Neddylation; Ub, Ubiquitination. Adopted from (58).