

The contribution of the Trp/Met/Phe residues to physical interactions of p53 with cellular proteins

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Abstract

Dynamic molecular interaction networks underlie biological phenomena. Among the many genes which are involved, p53 plays a central role in networks controlling cellular life and death. It not only operates as a tumor suppressor, but also helps regulate hundreds of genes in response to various types of stress. To accomplish these functions as a guardian of the genome, p53 interacts extensively with both nucleic acids and proteins. This paper examines the physical interfaces of the p53 protein with cellular proteins. Previously, in the analysis of the structures of protein–protein complexes, we have observed that amino acids Trp, Met and Phe are important for protein–protein interactions in general. Here we show that these residues are critical for the many functions of p53. Several clusters of the Trp/Met/Phe residues are involved in the p53 protein–protein interactions. Phe19/Trp23 in the TA1 region extensively binds to the transcriptional factors and the MDM2 protein. Trp53/Phe54 in the TA2 region is crucial for transactivation and DNA replication. Met243 in the core domain interacts with 53BP1, 53BP2 and Rad 51 proteins. Met384/Phe385 in the C-terminal region interacts with the S100B protein and the Bromodomain of the CBP protein. Thus, these residues may assist in elucidating the p53 interactions when structural data are not available.

1. Introduction

Normal maintenance of cellular functions and division is controlled by a large number of genes and proteins. Molecular events accompanying cellular life are neither random nor static; dynamic molecular interaction networks underlie biological phenomena. Among the many genes which are involved in dynamic molecular interaction networks, p53 plays a central role controlling cellular life and death. p53 was discovered as a tumor suppressor protein [1, 2]. After 26 years of study, it is now clear that the p53 protein not only operates

as a tumor suppressor (50% of the cancers relate to p53 mutations), but also helps regulate hundreds of genes in response to various types of stress. p53 is one of the most connected hubs in the cells [3]. It transcriptionally regulates more than 160 genes [4] and is a guardian in maintaining genome stability [4]. p53 activation and deactivation are under fine control, showing oscillatory and non-oscillatory dynamic behavior and response to a variety of environmental stimuli [5].

p53 interactions with cellular proteins can be classified as post-translational chemical modification of p53, physical and functional interactions. Post-translational chemical

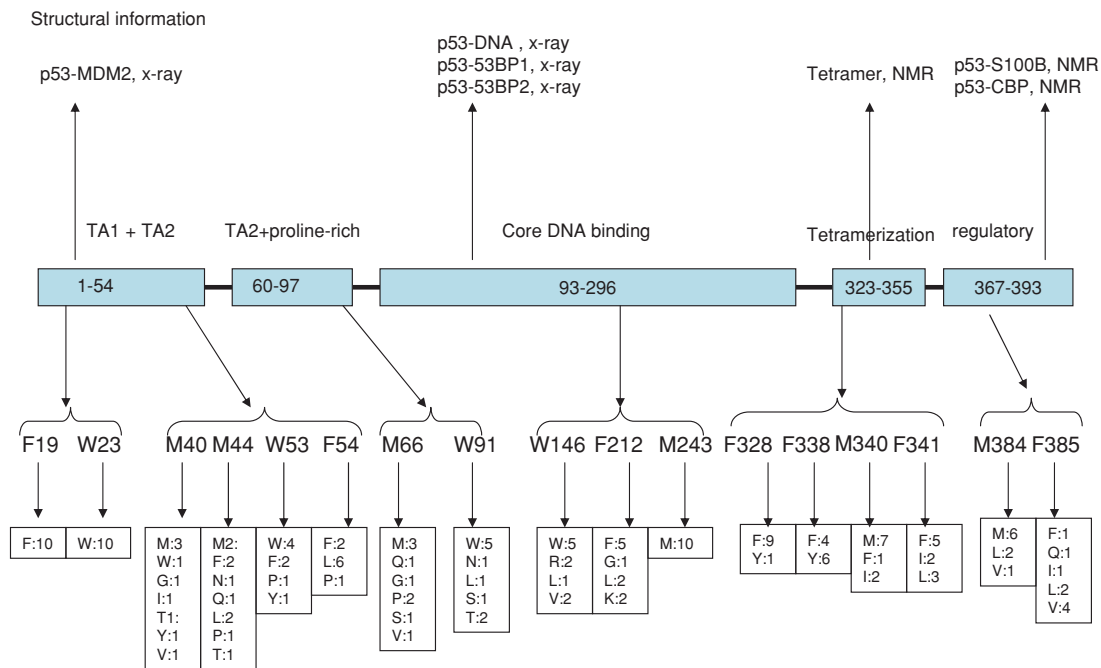


Figure 1. Sequence domains of the p53 protein and the conservation of exposed Trp-Met-Phe residues from 10 species (1. Homo sapiens (Human); 2. *Platichthys flesus* (European flounder); 3. *Oryzias latipes* (Medaka fish) (Japanese ricefish); 4. *Xenopus laevis* (African clawed frog); 5. *Oncorhynchus mykiss* (Rainbow trout) (*Salmo gairdneri*); 6. *Brachydanio rerio* (Zebrafish) (*Danio rerio*); 7. *Mus musculus* (Mouse); 8. *Cricetulus griseus* (Chinese hamster); 9. *Bos taurus* (Bovine); 10. *Felis silvestris catus* (Cat)).

modifications of p53 in cells have recently been reviewed by Bode and Dong [6]. In many cases, chemical modifications and physical interactions are coupled, impacting p53 transcriptional activation, as illustrated in the p53 phosphorylation by kinases and acetylation and deacetylation of Lys residues by CBP/p300-p53 interactions [7–10]. On the other hand, proteins physically interacting with p53 can also regulate p53 function without chemical modifications.

The combination of functional biological approaches and structural biological studies has provided much information about the structural-functional relationship of the p53 protein. Structures of several individual isolated domains have been solved. However, the complexity of p53 protein-protein interactions has made the elucidation of a detailed picture a very difficult task. Here, we attempt to take some steps in this direction through a close examination of available structures of p53 complexes and mapping of the interaction regions. In particular, we focus on the contributions of three amino acids (Trp, Met and Phe) to the physical interactions of p53 with cellular proteins.

Amino acids contribute differently to protein-protein interactions. Trp, Met and Phe have been identified as the most important residues involved in protein-protein interactions in general [11]. Trp/Met/Phe are conserved only at protein-protein interaction sites, not on the exposed protein surface. The conservation of the Trp/Met/Phe residues on the protein surface implies potential binding sites [11]. Our current examination of p53 interactions with cellular proteins confirms the important contributions of these three structural ‘hot spots’ in the p53 interaction network.

2. The structure and dynamics of full length p53

p53 contains several domains (figure 1). The domains have been classified as the transactivation domain 1 (TA1), transactivation domain 2 (TA2), core DNA-binding domain, tetramerization domain and the carboxyl terminal regulatory domain. DNA binding is critical for the biological functions of p53. Proper p53-DNA binding requires a well-folded core binding domain (CBD) and a p53 homotetramer. In more than 50% of all human cancers, there is a loss of p53 function by mutations that affect the DNA-binding motif, the CBD folding stability or the oligomerization state. The inter- and intra-domain interactions of p53 are critical for its association with other molecules [12]. The N-terminal domain, CBD and the carboxyl terminal regulatory domain interact with each other in the monomeric state. It can be demonstrated computationally that residues 369–382 form an energetically favorable complex with the (Ala-Pro)₃ repeat of residues 84–89 [13]. The C-terminal domain [14] and proline-rich domain [15] also interact with the CBD, possibly only cooperatively [16]. The tetramer domain (TD) provides a direct means for the p53 oligomerization by forming a dimer of dimers [17]. The TD is particularly important for the p53 binding of DNA loops [18, 19] or of supercoiled DNA [19]. Without the TD domain, the CBD binds DNA with a weaker affinity, although still in a cooperative manner [20].

p53’s integration of cell signals is accomplished by its multiple domains and oligomerization states. In the monomeric p53 only the DNA-binding domain is folded, while other regions are naturally disordered [21]. However, these

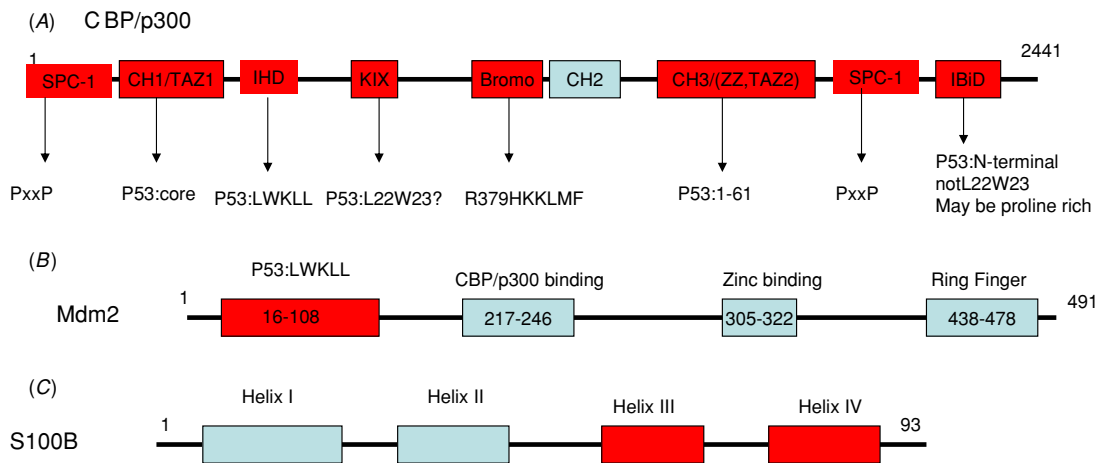


Figure 2. The sequence domains of CBP/p300 protein, MDM2 protein and S100B protein. The domains interacting with p53 are colored red.

naturally disordered regions can adopt well-folded structures upon oligomerization and interaction with other proteins.

The distribution of the three binding hot spots (Trp, Met and Phe) is coincident with the functional domains of p53 (figure 1). The TA1 region (also known as the mdm2-binding domain) has highly conserved Phe19 and Trp23. The TA2 and proline-rich domains have three methionine residues (Met40, Met44 and Met66), Trp53 and Phe54, which are more or less conserved. Trp91 is located at the end of the proline-rich domain. In the core DNA-binding domain, there are eleven Trp/Met/Phe residues. Among those, three are exposed surface residues (Phe146, F212 and Met243). The tetramerization domain has three phenylalanines (Phe328, Phe338 and Phe341) and Met340. The Met384 and Phe385 are located in the C-terminal regulatory domains. Most of these residues contribute to the p53 protein–protein interactions. In the following sections, we examine their roles in p53 interactions with other proteins.

3. Phe19 and Trp23: transactivation, CBP/p300 and MDM2 interactions

The primary role of the TA1 domain is to recruit the transcriptional machinery. The interacting proteins include the TATA box binding protein and the associated factor TFIID [22]. Even though there is no structural information about the contribution of Phe19 and Trp23, their involvement can be inferred from their critical roles in p53 transactivation [23]. Mutational studies of Leu22Glu/Trp23Ser indicated the inhibition of p53 transcriptional activity [24].

p53's interactions with CBP/p300 proteins are among the most important interactions in p53 activated transcriptions. The CBP/p300 proteins are transcriptional coactivators that have acetyltransferase activity and serve as scaffolds for the transcriptional machinery. The CBP/p300 interacts with p53 by as many as eight domains located throughout a more than two thousand residue sequence (figure 2, CBP has 2442 residues and p300 has 2414 residues).

It appears that CBP/p300 regulates p53 signaling physically and biochemically [25], as a critical part of the DNA damage response [26]. The physical binding of p53 TA1 domain to CBP C-terminus promotes acetylation of the p53 C-terminal lysine residues by CBP/p300. The acetylations of the lysine residues contribute to the p53 stabilization by disabling the lysines as the ubiquitylation targets. The acetylation of p53 also stimulates its sequence specific DNA-binding activity [27]. The phosphorylation of Ser15 of p53 is a critical step for the activation of transcription by CBP and p53 [28]. Phosphorylation of p53 Ser15 increases interaction with CBP [29], possibly by the synergistic hydrophobic and electrostatic interactions. The CH3 (TAZ2) domain of CBP has been identified as one of the sites for the p53 N-terminus interaction. A peptide consisting of residues 14–28 of the p53 protein has been shown to weakly bind with the TAZ2 domain (with dissociation constant K_d of 300 μ M) [30]. The TAZ2 domain is highly positively charged. NMR structure of the TAZ2 domain reveals that the p53 binding area on the TAZ2 surface has a hydrophobic patch and a lysine residue [30]. It is conceivable that hydrophobic residues like Phe19/Trp23 can interact with the hydrophobic patch of TAZ2 while the phosphorylation of the Ser15 further enhances the electrostatic interaction of p53 with the positively charged TAZ2 domain of CBP.

Trp23 is located within the LxxLL motif (L22WKLL26). The LxxLL motif is known for its role in many protein–protein interactions related to transcriptional regulation [31]. Two nearby regions (the IHD and KIX domains) in the CBP protein may interact with the LxxLL motif of p53 protein. Finlan and Hupp found that the IHD domain contacts the LxxLL motif directly, and the phosphorylation of Ser20 enhances the IHD-domain interaction with the p53 tetramer [32]. A Phage-peptide display study identified a consensus IHD binding sequence which has significant homology to the LxxLL motif of p53 [32]. The possible interaction of the LxxLL motif with the KIX domain of CBP was inferred by the double point mutation (L22Q/W23S) that completely abolished the p53–KIX interaction [33]. However, a single LxxLL motif cannot

bind to the IHD and KIX domains simultaneously. Therefore, it seems that either the L22Q/W23S mutant function does not imply local interaction of the L22W23 of p53 with the KIX domain, or the LxxLL motif interacts with the IHD domain of p300 and the KIX domain of CBP selectively. The latter case would imply that p300 and CBP have different p53 interaction patterns, even though they have similar sequences and functions in p53 regulation. The third possibility is that the IHD/KIX domains simultaneously interact with the two LxxLL motifs from the p53 tetramer.

The direct contribution of the Phe19/Trp23 to p53 protein–protein interactions is manifested in the crystal structure of the p53–MDM2 complex. The TA1 domain of p53 is also known for its role in binding the MDM2 protein to inactivate transcription [34], by blocking its ability to transactivate (i.e. bind to transcription activation factors) [35].

In 1992, it was found that MDM2 forms a tight complex with the p53 protein, and the *mdm-2* oncogene can inhibit p53-mediated transactivation [36]. MDM2 is a multifunctional protein with p53-independent activities as well [37]. The full length MDM2 has 491 amino acids with several domains (figure 2). The p53 interacting part is localized at the N-terminal region (16–108). The central part has two important regions, with residues 217–246 binding to p300 and residues 305–322 being a Zn-finger. The C-terminal of the MDM2 has the ring-finger domain.

The p53–MDM2 interaction not only blocks the first transactivation domain of p53, but also leads to subsequent degradation of the p53 protein. The concentration of p53 in the cell is maintained through the involvement of several factors. In normal cells, p53 is maintained at a low level by its high rate of proteolytic turnover. Cellular stress increases p53 expression by increasing the half life of the protein. p53 is negatively regulated by MDM2 [38]. However, MDM2 itself is also regulated by p53 forming an autoregulating feedback loop. p53 upregulates and activates MDM2 at the transcriptional level.

Phosphorylations of Ser15, Thr18 and Ser20 of p53 protein have been shown to strongly disrupt p53–MDM2 binding and trigger various biological consequences (reviewed in [6]). The Ser20 has critical role in the negative regulation of p53 by the MDM2. Phosphorylation of Ser20 mediates stabilization of p53 in response to the DNA damage [39]. Phosphorylation of Thr18, which requires a prior phosphorylation of Ser15, also disrupts the p53–MDM2 interaction; however, the phosphorylation of Ser15 alone does not prevent p53–MDM2 binding [40]. What are the structural mechanisms of the phosphorylations of Thr18 and Ser20 of p53 protein on its MDM2 binding?

The N-terminal part (residues 17–27) of p53 forms an α -helix which binds the cleft formed by two α -helices of MDM2 (figure 3) [34]. The interaction is mostly hydrophobic, with three p53 amino acids—Phe19, Trp23 and Leu26—contributing the most to the contact. The absolute conservation of Phe19 and Trp23 emphasizes the importance of ensuring both the strength and the binding mode. While phosphorylations of Thr18 and Ser20 of p53 will strongly disturb Phe19, the possible influences were not reflected

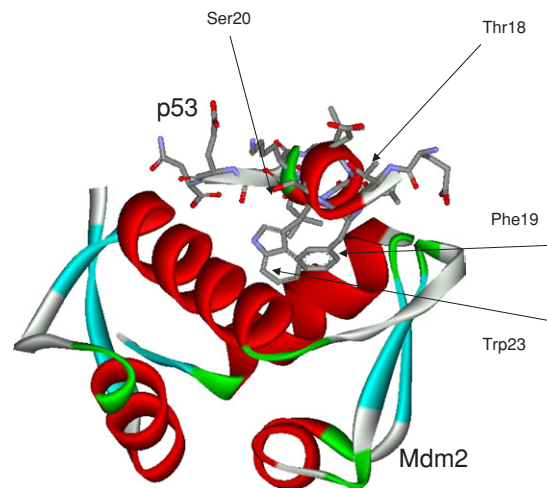


Figure 3. The contribution of Phe19/Trp23 to the p53–MDM2 interactions.

in the p53–MDM2 crystal structure, because both Thr18 and Ser20 do not contact with MDM2 directly. Peptides mimicking the p53 N-terminus show confusing results on their MDM2 binding. Unger *et al* have shown that the peptide (Ac-L14SQETF(S)DLWKLLPEN29-NH₂) bearing phosphorylated Ser20 does not compete with p53 on its MDM2 binding, consistent with the effects on the p53. In another study, using the peptide of S15QETFSDLWKLLPEN29, Schon *et al* found that phosphorylation of Ser15 and S20 did not affect MDM2 binding, but Thr18 phosphorylation weakened binding tenfold [41]. The different observations could be the results of the slight difference of peptide sequences used. The conformational preference of the N-terminus of p53 is extremely sensitive, due to the requirement to bind various proteins. Indeed, Schon *et al* also found that the shortened peptide (E17SDLWKLL26) bound MDM2 ten times more tightly than did the p53(15–29). In our study (Zanuy, Ma and Nussinov, unpublished results), we found that the helical preference of the N-terminus of p53 is sensitive to the phosphorylations of Ser15 and Thr18. The phosphorylation decreases the helical propensity of the N-terminus of p53, thus making the p53–MDM2 binding less favorable.

4. Second transactivation domain and proline-rich domain

Unlike the highly conserved TA1 region, the second transactivation domain has extensive sequence variability. In the region between residues 30–60, we observe Met40, Met44, Trp53 and Phe54. These residues are within two turns of the mostly unstructured region [42]. Turn 1 contains the hydrophobic surface of Met40, Leu43 and Met44; turn 2 has the hydrophobic surface from Ile50, Trp 53 and Phe54. The hydrophobic patches may imply roles in protein–protein interactions; however, knowledge of the contributions of these residues to the p53 protein interaction is limited. Met40 and

Met44 are identified as the alternative initiation sites [43] without any indication of the involvement of p53 protein–protein interaction.

Like the Phe19/Trp23's role in the function of the first transactivation domain, Trp53 and Phe54 are known for their critical roles in the second transactivation domain (TA2) [44]. W53Q/F53S mutations inactivate TA2 [24, 44]. Residues Trp53/Phe54 are also involved in the p53's interaction with the replication protein A (RPA) [45–47]. The p53–RPA interactions mediate suppression of homologous recombination [45] and WRN helicase activity [48].

5. Versatile core domain: Trp146, Phe212 and Met243

Currently, there is no indication for the contribution of Trp146 for the p53 protein–protein interaction. Phe212 contributes to the p53–p53 core domain dimeric interactions observed in the p53–DNA complex [49]. It is important for the dynamic stability of the p53–p53 core domain dimer [50]. There are no data showing the direct involvement of the phe212 in other p53 interactions. Met243 is highly conserved [50] in p53 and has been observed or implicated in several p53 interactions with other proteins.

Met243 is implicated in the p53–BclXL interactions. One of the recently focused issues is p53's apoptogenic role in the mitochondria [51]. The BclXL protein belongs to the Bcl-2 family and is an anti-apoptotic protein. The BclXL/Bcl2 proteins block the release of cytochrome c from mitochondria. By forming a complex with the BclXL/Bcl2 proteins, p53 induces the permeabilization of the mitochondrial membrane and release of cytochrome c. Biological evidence has shown that the BclXL interacts with the core domain of p53 protein. However, there are no structural data for the complex. Molecular modeling is used to deduce the atomic details of the p53–BclXL interactions. Starting from the separated crystallographic structures of the BclXL and p53 proteins, molecular docking attempts to find the best interacting sites between the two proteins. The BclXL protein has four sequence-conserved domains, BH1, BH2, BH3 and BH4. The N- and the C-termini of the BclXL are unstructured and the core part is an α -helical bundle. The cleft formed by BH4 and BH3 has been shown to bind to the p53-core domain DNA-binding motif. The major contact of p53 and the BclXL protein extends over residues 239–248. The molecular docking of the p53-core domain and the BclXL structure confirms the possibility of the biological mapping of the interaction [51]. However, there is no direct evidence of the contact of Met243 with the BclXL proteins.

Met243 may also contribute to the p53–Rad51 interactions. Rad51 proteins (for eukaryotes; RecA for bacteria) are needed in the homologous recombination (HR) of DNA. Homologous recombination can lead to gene conversion. Aberrant HR can cause genome instability. The p53 protein controls the HR process through its interactions with Rad51 [52–55]. Human Rad51 has 339 residues and three domains: N-terminal, core p53 binding and a C-terminal domain. The Rad51–p53 interactions were deduced from

biological experiments. For p53, the interaction sites are around residues 94–160 and 264–315, which are on the DNA-binding surface [56]. Recently, an NMR study confirmed the binding region and found additional interactions at N239, M243 and G244 [57]. The binding sites on the RAD51 protein have been localized to residues 179–190. The region overlaps the Rad51 oligomerization site. Thus, it seems that p53 binding should prevent the RAD51 polymerization.

More direct evidence for the importance of Met243 in p53 protein–protein interactions is obtained from crystal structures of two p53 binding proteins, as detailed in the following subsections.

5.1. p53 binding protein 1: (53BP1) and breast cancer tumor suppressor protein (BRCA1)

The factor 53BP1 was originally identified during a yeast two-hybrid screen for p53-interacting proteins and was subsequently characterized as an activator of p53-dependent gene transcription [58, 59]. The protein has a total of 1972 residues [60]. Currently, most of the identified functional regions are in the C-terminal portion (figure 4). The p53 binding region BRCT domains span from 1722 to 1972. 53BP1 binds the tumor suppressor protein p53 and appears to have a role in DNA damage response. 53BP1 enhances the p53-mediated transcription activation, indicating a more dynamic interaction between these proteins in the cell [60]. 53BP1 acts to recognize DNA double strand break events [61, 62], thus facilitating subsequent p53 binding to this lesion. The recruitment of the 53BP1 to nuclear loci is necessary for the phosphorylation of p53 [63–66] at that site. Although the biological contributions of the p53–53BP1 interactions remain to be studied further, a strong interaction has been shown in their crystal complex.

The crystal structures of the BRCT domains bound to p53 have been obtained [67, 68] (figure 4). The BRCT region has two repeats. The first BRCT repeat binds to the L3 loop and the helix 1 region. In the p53–DNA complex, the L3 loop binds DNA, and the helix 1 region has been identified as a region important for p53 tetramerization [50]. The interactions between p53 and the BRCT repeat have both hydrogen bonds and hydrophobic interactions contributing to the binding energies. In the H1 region, the p53–R181 forms a salt bridge with 53BP1–D1833. Figure 4(B) shows the p53 L3 region binding with 53BP1. R248 forms a hydrogen bond network with D1861 and L1847. R249 also forms a salt bridge with D1845. A notable feature in the highlighted region is the hydrophobic cluster by M243 of p53 and V1829 and Y1846 of 53BP1.

5.2. p53 binding protein 2: (53BP2) and ASPP family

Factor 53BP2 was originally identified in a yeast two-hybrid screen together with the 53BP1 [58, 59], and was found to have a similar function with 53BP1 [60]. In 1996, a longer sequence of the 53BP2 was identified and named Bbp for Bcl2-binding protein [69]. Now it is known that 53BP2 and longer sequences of Bbp are known to be truncations of the ASPP2 protein, the second protein of the ASPP family (figure 5) [70].

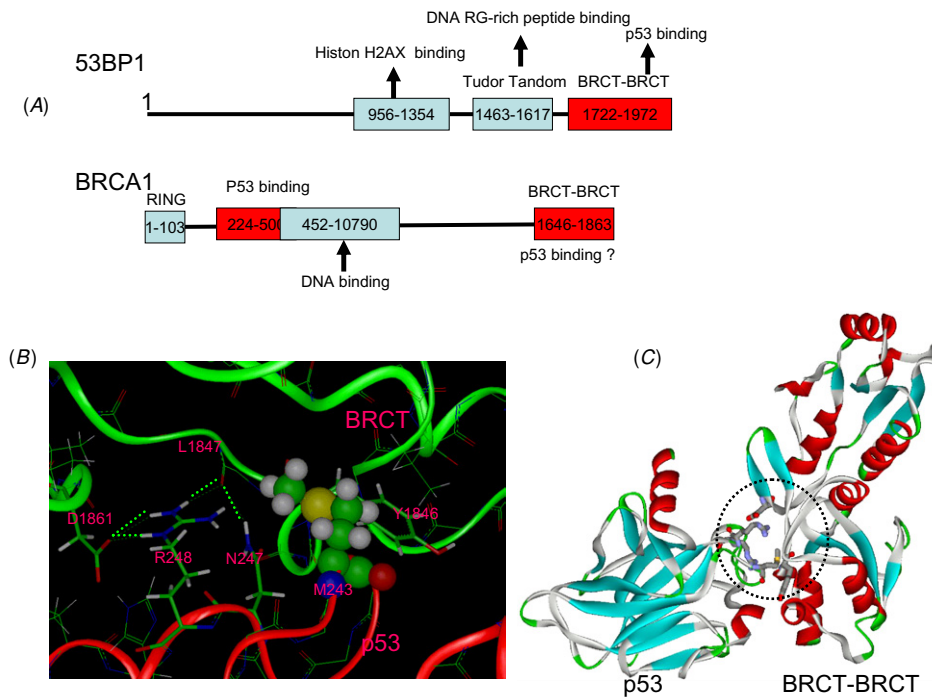


Figure 4. (A) The sequence domains of 53BP1 and BRCA1. (B) The interface of the p53–BRCT interaction. (C) The overall structure of the p53–BRCT complex. The circled region is highlighted in figure 3(B).

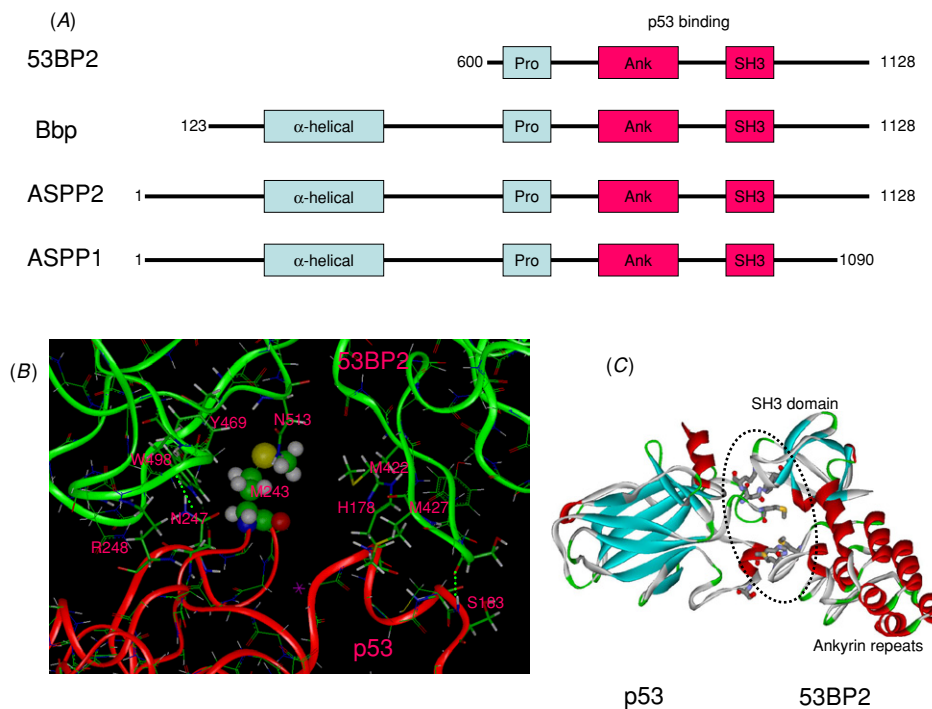


Figure 5. (A) The sequence domains of the ASPP family. (B) The interface of the p53–53BP2 interaction. (C) The overall structure of the p53–53BP2 complex. The circled region is shown in figure 4(B).

The name ASPP refers to proteins that have ankyrin repeats, SH3 and proline-rich domains, and they further function as the apoptosis stimulating protein of p53 [70]. ASPP1 has 1090 amino acids and the total length of ASPP2 is 1128. The p53

binding part of the ankyrin repeats and the SH3 domain are in the C-terminal part.

Despite the totally different folds of the 53BP1 and 53BP2, p53 binds to both proteins with very similar modes and binding

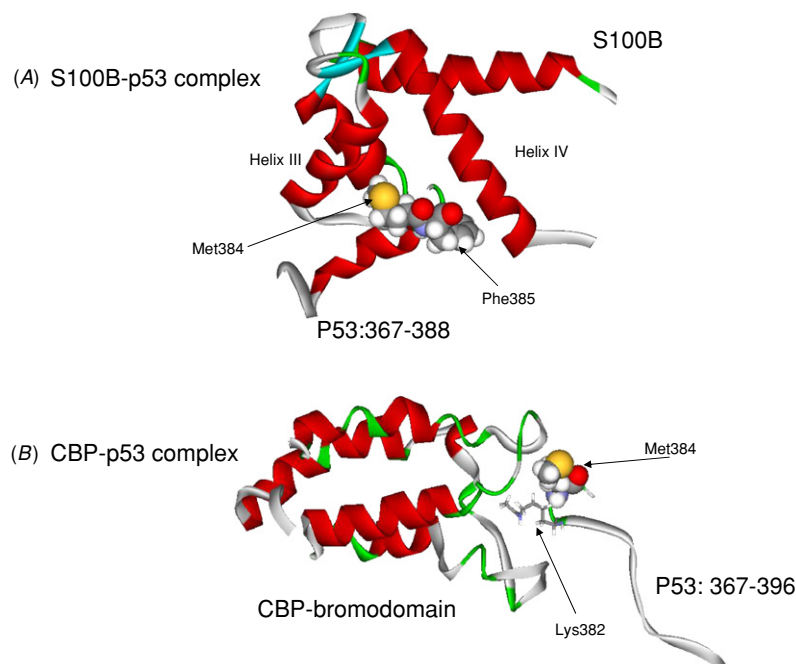


Figure 6. The contributions of Met384/Phe385 to (A) p53–S100B interactions and (B) p53–CBP interactions.

sites. The crystal structure of the p53 binding with the ankyrin repeats and the SH3 domain were solved in 1996 [71]. p53 binds the SH3 domain with the L3 loop and the ankyrin repeats with the H1 helix region. In the L3 loop, residue N247 again contributes to the hydrogen bonds with the SH3 domain. W498 forms a hydrogen bonding with the backbone oxygen of S241. M243 from p53 interacts with N513 of 53BP2. Met residues from the ankyrin repeats contribute significantly to the H1 helix interaction. H178 forms a second binding cluster with M422, Y424 and M427. In this region, there is also a backbone–backbone hydrogen bond between S183 and S425.

6. C-terminal region: Met384 and Phe385

The Trp–Met–Phe residues in the tetramerization domain interact extensively in the dimerization and tetramerization of p53. There are indications that the tetramerization domains also interact with the S100B protein. However, there is no information relating to the specific involvement of the Trp/Met/Phe in the tetramerization domain.

Many cellular proteins are known to interact with p53 via binding the extreme C-terminal region. Breast cancer tumor suppressor protein (BRCA1, figure 4) binds to the C-terminal domain of p53 [72, 73]. The p53–WRN protein (a helicase implicated in the Werner syndrome) interaction involves the carboxyl-terminal part of WRN and the regulatory carboxyl terminus of p53 [74]. The p53 C-terminus also binds BLM, a helicase impaired in the Bloom syndrome [75, 76].

The basic region can also regulate transcription via binding the TATA binding protein (TBA) [77] and the transcriptional factor IIH (TFIIH) [78]. The C-terminal region interacts with Hepatitis B virus X [79] and the Hepatitis C

virus core protein [80]. The interactions of p53 with the 14-3-3 protein are located around Ser376 and Ser378, a little away from the Met384 and Phe385 [81]. The mapping of the p53 interactions with Cyclin A leads to the motif of K381KLMF [82]. The derived peptide containing the KKLMF motif inhibits p53 phosphorylation by Cyclin A–cdk2; however, the mutant peptide with KKLMA failed to block the interaction [83], indicating the importance of the Phe385.

The basic region containing the Met384 and Phe385 can adopt multiple conformations when it binds with different proteins. This has been shown in two NMR structures with the p53 C-terminal peptide complexed with SB100 and the Bromodomain of CBP (figure 6). The biological functions of the CBP and S100B are opposite to each other. As discussed earlier, CBP is a transactivation coactivator; however, the SB100–p53 interactions inhibit C-terminal phosphorylation and prevent the p53 transactivation. In solution, the peptide derived from the region of p53 residues 367–388 has no regular structure [84]. The region becomes helical upon binding to S100B($\beta\beta$) [84]; however, it assumes a long loop and β turn structure upon binding to the Bromodomain of the CBP [85]. In the SB100B–p53 complex, Met384 binds helix III and Phe385 binds helix IV of SB100B. In the CBP/Bromodomain–p53 complex, Met384 is in the turn position and binds CBP directly, while Phe385 points away from CBP. In the CBP/Bromodomain–p53 complex, the acetylated Lys382 plays an important role in the CBP recognition. The CBP/Bromodomain–p53 interaction is a canonical interaction directed at the acetylated lysine.

7. Conclusion and outlook

The p53 protein extensively interacts with other cellular proteins. Modular proteins with multiple binding sites are

common in proteins involving DNA processing [86]. This also holds for the p53 protein. The physical interactions of p53 with other proteins involve all sequence regions of p53, including the N-terminus, core domain and C-terminus. The protein–protein interactions of p53 must have evolved for its biological functions, while still adhering to the general principles governing this class of interactions. Previously, a general database analysis showed that the Trp/Met/Phe residues are important for protein–protein interactions. In this work, we have shown that these residues are critical for p53 functions. Among the Trp/Met/Phe residues highlighted in figure 1, the following residues are particularly involved in many of the p53 protein–protein interactions: Phe19/Trp23 in the TA1 region, Trp53/Phe54 in the TA2 region, M243 in the core domain and Met384/Phe385 in the C-terminal region. These results suggest that in the absence of structural data, when one is elucidating p53 interactions, these residues deserve special attention. At the same time, to date, the contributions of some Trp/Met/Phe residues, specifically Met66/Trp91 in the proline-rich region and Trp146/Phe212 in the core domain, have not been substantiated experimentally.

Interestingly, analysis of the contributions of the Trp/Met/Phe residues to the p53 protein–protein interactions has shown that these residues always interact with multiple binding partners. This trend may be the outcome of evolutionary pressure. The p53 protein is a hub in the cell interaction network. Thus, the ‘best’ residues to perform this multi-binding partner task would be those which have features suitable for general protein–protein interactions. In this sense, it is not surprising that Trp/Met/Phe residues are important for p53.

The biological consequences of the Trp/Met/Phe residue mutations depend on their location. The mutational effects come from the balance of overall protein interaction network and transactivation network. The trickiest part is the Phe19/Trp23 in the TA1 region. To compete for the same Phe19/Trp23 motif, p53–MDM2 binding will very likely block p53’s interaction with the C-terminus of CBP/p300 protein. Only a few tumor site mutations have been observed in the N-terminal and none of them is Phe19 or Trp23 [87]. Mutation of the Phe19/Trp23 may disrupt the transactivation, as indicated in the p53’s interactions with CBP/p300 and other transactivation factors. On the other hand, the mutation of the Phe19/Trp23 will stop p53–MDM2 binding, which in turn increase p53 concentration. However, the different binding mechanisms of p53 to CBP/p300 and to MDM2 may indicate different mutational effects. Indeed, it was just reported that p53QS(Leu22Glu/Trp23Ser) shows stress-specific apoptotic activity, with the p53QS unable to respond to DNA damage while active to hypoxic signal due to deficiency of mdm2 interaction [88]. Inhibiting p53–MDM2 interaction is an important target for cancer therapy (reviewed in [89]). Both peptidic and small molecules may be used as drugs to block the p53–MDM2 interaction. Flexible peptides similar to the p53 N-terminus may interfere not only with p53’s interaction with MDM2, but also with other proteins. Restricting the peptides to the MDM2-binding conformation may increase both MDM2-binding affinity and specificity.

Three core domain residues Trp146/Phe212/Met243 may not affect the folding stability of the core domain; however, the mutations of these sites still can affect p53’s interactions with other proteins. The frequency of the tumorigenic mutations of the Trp146/Phe212/Met243 is far less than the hot spot mutation residues. Besides mutation of Trp146 to the stop codon and deletion of Phe212, there are 12 Trp146 mutations, 13 Phe212 mutations and 45 Met243 mutations reported in the IARC TP53 database [87]. The 45 Met243 mutants cover possible amino acids from single missense mutation, including Ile, Leu, Thr, Lys and Arg. If we compare the p53’s interaction interfaces with 53BP1 and 53BP2 proteins, we see that Met243 of p53 binds 53BP1 in the hydrophobic region while it binds 53BP2 in the polar region. Therefore, it is possible that the Met243Ile and Met243Leu might perturb the p53–53BP1 binding less than the p53–53BP2 binding, while polar mutants of Met243 have opposite effects.

The complexity of the p53 interactions has led to difficulty to fully elucidate its biological effects. In the examples discussed in this work, some have well-proved biological significance, like p53’s interaction with MDM2, CBP/P300. As for other interactions with less biological information (such as 53BP1, 53BP3 and S100), the available structural information of p53’s interactions can provide guidance for us to understand the atomic mechanisms responsible for the interactions. We hope to combine the available structural and biological information to fully untangle p53 interaction network in future.

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Glossary

Gene regulation. The control of gene expression is the core of biological process. Gene expression leads to the formation of RNA (transcription) and protein (translation) molecules. The structure and function of a cell are controlled by the proteins expressed in the cell. Thus the gene regulation controls the cell’s fate via regulating the RNA polymerase binding to DNA. The regulation could activate or repress transcription of gene encoding particular sets of protein. p53 protein regulates gene expression by its transactivation in response of the cell’s stimulus.

Protein binding site. A protein functions usually through its interactions with other molecules. The interactions are

specific for its biological function. The molecular interaction leads to the binding of the protein with other molecule, at the specific area on the protein surface. The protein binding site decides the molecular recognition of the protein with the other molecule.

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