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Structural Evolution and Dynamics of the p53 Proteins

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The family of the p53 tumor suppressive transcription factors includes p73 and p63 in addition to p53 itself. Given the high degree of amino-acid-sequence homology and structural organization shared by the p53 family members, they display some common features (i.e., induction of cell death, cell-cycle arrest, senescence, and metabolic regulation in response to cellular stress) as well as several distinct properties. Here, we describe the structural evolution of the family members with recent advances on the molecular dynamic studies of p53 itself. A crucial role of the carboxy-terminal domain in regulating the properties of the DNA-binding domain (DBD) supports an induced-fit mechanism, in which the binding of p53 on individual promoters is preferentially regulated by the K_{OFF} over K_{ON}.

STRUCTURE OF INDIVIDUAL p53 DOMAINS

The p53 tumor-suppressor gene encodes a DNA-sequence-dependent transcription factor that preserves genome integrity through the regulation of relevant cellular pathways, including cell cycle, apoptosis, and cellular senescence (Dötsch et al. 2010; Joerger and Fersht 2010). The *TP53* gene encodes nine different protein isoforms as a result of alternative splicing, alternative promoter usage, and alternative initiation sites of translation (Fig. 1A) (Bourdon et al. 2005; for more details, see Joruiz and Bourdon 2016). p53 is a nuclear phosphoprotein that is capable of either transactivation through the binding to specific DNA responsive elements (REs) (el-Deiry et al. 1992), or repressing transcription of promoters that do not contain binding sequences (see Fig. 1). Specificity, affinity, and cooperativity of p53 binding to DNA are all tightly related to its structural features.

In its active conformation, the p53 protein is a tetramer formed by four identical chains of 393 residues each (Joerger and Fersht 2008). The p53

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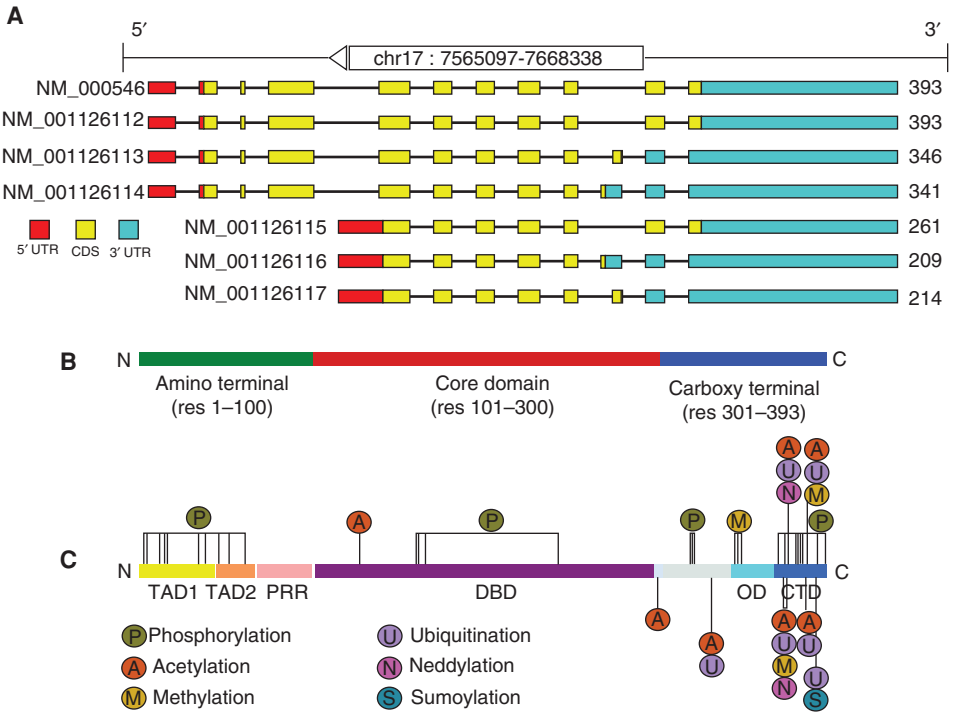

Figure 1. Structural organization of human p53. (*A*) Alternative splicing forms of the *Trp53* gene. (*B*) Organization of the 393 residues of p53 protein into three major regions. (*C*) The sites of the most frequent post-translational modifications are distributed all along the sequence, with the greatest concentrations at the transactivation domains (TADs), in the amino-terminal and carboxy-terminal domain (CTD) in the carboxy terminal. They include phosphorylation, acetylation, ubiquitination, methylation, neddylation, and sumoylation. PRR, Proline-rich region; DBD, DNA-binding domain; OD, oligomerization domain.

polypeptide displays a modular domain structure (Fig. 1B), comprising an amino-terminal transactivation domain (TAD, residues 1–61), a proline-rich region (PRR, residues 61–92), a central DBD (residues 94–292), a short tetramerization region (OD, residues 326–353), and a carboxy-terminal regulatory domain (CTD, residues 353–390) (Joerger and Fersht 2008).

The structure of the full-length p53 tetramer has been elucidated by a combination of experimental approaches, including NMR, electron microscopy, small-angle X-ray scattering, and FRET (Okorokov et al. 2006; Huang et al. 2009; Melero et al. 2011). In the absence of DNA, p53 forms an open cross-shaped structure with loosely coupled dimers interacting via the core domain. On DNA binding, the structure of p53 rigidifies, becoming more compacted. Because the high proportion of intrinsically disordered regions hampers crystallization, the majority of the structural studies have addressed only single domains or fractions of them.

The TAD, which is relevant for interaction with transcriptional coactivators and corepressors (Raj and Attardi 2016), is natively unfolded (Dawson et al. 2003; Chillemi et al. 2013). This region can be further divided into two subdomains: TAD1 (residues 1–40) and TAD2 (residues 41–60), which can independently activate transcription (Candau et al. 1997). The presence of intrinsically disordered segments in the TAD allows this domain to bind to a range of interacting proteins with high specificity. Binding partners of the p53 TAD include the transcription factors TFIID and TFIIHA, TATA box-binding, protein-associated factors (TAFs), and several protein modifiers, such as MDM2, CBP/p300, and PCAF (Chang et al. 1995;

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Di Lello et al. 2006; Ferreon et al. 2009). Residual secondary structure can be observed in regions containing functionally relevant hydrophobic residues. In response to DNA damage, the TAD can be phosphorylated at multiple sites by distinct kinases (Fig. 1C), which regulate p53 protein stability, subcellular localization, and function (Meek and Anderson 2009).

The PRR that links the TAD to the DBD in human p53 contains 12 proline residues, including four copies of the sequence PXXP (Walker and Levine 1996). These motifs create a binding site for Src-homology-3 (SH3) domains that mediates protein–protein interactions in signal transduction (Yu et al 1994). Unlike other domains of p53, the PRD is relatively unconserved. Functionally, it has been shown that the PRD is necessary for apoptosis and efficient growth suppression elicited by p53 (Venot et al. 1998).

p53 binds DNA as a homotetramer, whereas its oligomerization is mediated by the OD. The DBD and the OD domain are folded regions that are connected through a flexible linker (Fig. 2A,C). The DBD core domain adopts an immunoglobulin-like β sandwich architecture that provides a scaffold for a DNA-binding surface, consisting of a loop–sheet–helix motif and two loops stabilized by a zinc ion (Cho et al. 1994; Joerger and Fersht 2010). Several crystal structures of a p53 core tetramer bound to DNA have been resolved (Ho et al. 2006; Kitayner et al. 2006; Malecka et al. 2009). The p53 RE contains two decameric half-site palindromes of the general sequence 5′-RRRCWWGYYY-3′ (R = A, G; W = A, T; Y = C, T), separated by 0–13 base pairs (el-Deiry et al. 1992). Two core domains associate with a half-site DNA motif, forming a symmetrical dimer (Fig. 2A,B). The dimers assemble to form a tetramer, which is stabilized by protein–protein and base-stacking interactions. The four p53 DBDs bind to DNA in a highly cooperative manner (Nagaich et al. 1999). With this stoichiometry, the affinity of the tetramer for DNA is increased up to 100-fold as compared with the single monomer.

The most frequently mutated region of p53 in human cancers is the DBD, with ∼90% of the oncogenic mutations lying in this domain (see Hainaut and Pfeifer 2016). This observation highlights the importance of sequence-specific DNA binding by the DBD for p53 to display its tumor-suppressor activities.

The carboxy-terminal region of p53 crucially controls the structure and function of the entire molecule (Fig. 2A). Tetramerization

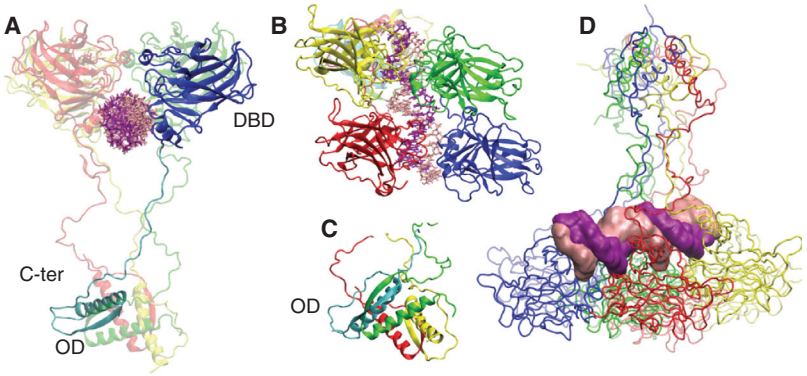

Figure 2. 3D structure of the p53–DNA complex in tetrameric form. (*A*) Monomer 1 is divided in the DNA-binding domain (DBD) (blue), in interaction with DNA, and carboxy terminal with the OD (oligomerization domain) (cyan). The other three monomers are depicted in transparent mode. (*B*) Immunoglobulin-like β sandwich architecture of DBD adopted by the four monomers. (*C*) Tetramerization helices in the carboxy terminal. (*D*) Projection of the tetramer MD trajectory along eigenvector 1. Specific conformations visited by the tetramer correlate with the DNA deformation measured by the roll and twist parameters (D'Abramo et al. 2015). Therefore, p53 is capable of creating moderate deformities of the DNA, even in the absence of additional molecular partners. C-ter, Carboxy terminal.

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is a function of the OD that can by itself form tetramers in solution (Jeffrey et al. 1995). The monomeric OD domain comprises a short β-strand (Glu326-Arg333), and an α-helix (Arg335-Gly356) linked by a tight turn (Gly-334) and two monomers form a dimer through antiparallel interaction of β-strands. Two dimers associate through their helices to form a tightly packed tetramer, which can bind efficiently to DNA (Jeffrey et al. 1995). The oligomeric structure of p53 is thought to position in close proximity of the DBD and the CTD and to promote DNA bending when the four core domains bind the four REs (Fig. 2C) (Chen et al. 2012; D'Abramo et al. 2015). In addition, the OD of p53 contains a nuclear export signal (NES) sequence (residues 340–351), which is masked on tetramer assembly (Stommel et al. 1999). Hence, p53 tetramerization and nuclear export are functionally connected (Fig. 2D).

The CTD is highly basic, is intrinsically disordered (Bell et al. 2002; Chillemi et al. 2013), and provides a platform for the relevant post-translational modifications and protein–protein interactions, which regulate p53 activity. The CTD can undergo disorder-to-order transitions as a result of binding to protein interactors or nonspecific DNA (Rustandi et al. 2000; Weinberg et al. 2004). In vitro nonspecific binding of the p53 CTD to DNA relies on the low-affinity electrostatic interactions between several carboxy-terminal lysine residues and DNA. Interaction of the p53 CTD with no specific DNA sequences regulates binding of the core domain to specific recognition sequences in promoters (Weinberg et al. 2004). Evidence supportive for both negative and positive regulation of sequence-specific DNA binding by the CTD has been reported in the last few decades (Hupp et al. 1992; McKinney et al. 2004; Kitayner et al. 2006; Laptenko et al. 2015). According to the model proposed by Kitayner et al. (2006), the sequence-specific complex of p53 with DNA is stabilized by multiple nonspecific electrostatic interactions between the positively charged proximal CTD and the DNA backbone. A recent report by Laptenko et al. (2015) has shown that the unmodified CTD is required in vivo for the core domain of p53 to recognize the full repertoire of its REs, comprising those that significantly diverge from its consensus sequence. An additional mechanism by which the CTD stabilizes p53 association to its binding sites is the ability of the CTD to induce conformational changes within the DBD following association with DNA (Laptenko et al. 2015). These structural changes would stabilize cooperative contacts between single-core domains within the tetramer, which are required for the stability of the p53–DNA complexes. In line with these observations, molecular dynamic simulations have revealed that the CTD of p53 can create an induced-fit mechanism on the DBD (D'Abramo et al. 2015). Further structural details of the CTD and of the intramolecular interactions among individual domains of p53 will be discussed in the next sections.

EVOLUTION OF THE p53 PROTEIN FAMILY

Although greater emphasis has probably been placed on the structural and functional characteristics of p53, largely as a result of its relevance to human cancer, p63 and p73 are evolutionarily older homologs of p53. *TP63* and *TP73* genes are expressed as multiple protein isoforms generated by the presence of alternative promoters and splicing sites (Murray-Zmijewski et al. 2006). Both p63 and p73 proteins display domain structures similar to that of p53, and significant amino-acid sequence homology in the TAD, DBD, and OD domains, with the DBD showing the highest degree of conservation (Tomasini et al. 2008a; Dötsch et al. 2010; Levine et al. 2011; Melino 2011).

Knockout mouse studies of all three members of the p53 protein family have shown that p63, p73, and p53 serve different functions in mammals (Mills et al. 1999; Yang et al. 1999, 2000; Tomasini et al. 2008b; Wilhelm et al. 2010; Rufini et al. 2012). The bewildering complexity of these different functions in light of the very high-sequence identity of the DBD—leading to similar transcriptional targets—has sparked speculation about the evolutionary origin of this protein family. The discovery that p63 is not only expressed in the basal compartment of stratified epithelial tissues but also in

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oocytes (Suh et al. 2006) has suggested that the original ancestor of the mammalian family members was a quality-control factor of germ cells (Fig. 3). Indeed, p53-like proteins have been identified in short-lived animals that are not threatened by the development of cancer and therefore do not need a tumor suppressor (Brodsky et al. 2000; Ollmann et al. 2000; Derry et al. 2001; Nedelcu and Tan 2007; Joerger et al. 2014). In the nematode *Caenorhabditis elegans*, the p53-like protein Cep-1 is expressed in its germ cells in which it is required for DNA damage–induced apoptosis in the late-stage pachytene phase of meiotic cells (Greiss et al. 2008). Structural investigations of Cep-1 have revealed that it contains a SAM domain, similar to mammalian p63 and p73, suggesting that Cep-1 is more closely related to p63/p73 than to p53 (Ou et al. 2007). In agreement with the hypothesis that p63-like proteins developed first as quality-control factors of germ cells, the eggs of the tunicate species, *Ciona intestinalis*, express two different p53-like isoforms (Noda 2011; Heering et al. 2015). Because germ cells are transferred from generation to generation, they must be kept under very tight quality control to ensure the survival even of short-lived species.

In mammals, the expression of the TAp63α isoform starts around day E18.5 and remains at a high level during the dictyate arrest stage (Suh et al. 2006). The expression of TAp63α is correlated with a dramatic increase in the sensitivity of the developing oocytes toward DNA double-strand breaks. Although prenatal oocytes tolerate hundreds of DNA double-strand breaks as part of the process of homologous recombination, postnatal oocytes expressing TAp63α are killed by fewer than 10 DNA lesions (Suh et al. 2006). The high expression level of p63 makes oocytes far more vulnerable than the surrounding follicular cells. Because oocytes are limited in number and are no longer generated after birth, several control mechanisms ensure that unintended cell death of oocytes is prevented. TAp63α is expressed in a closed and inhibited conformation in which the protein is transcriptionally inactive (Serber et al. 2002). Although all transcriptionally active forms of the mammalian p53 protein family are tetrameric (Luh et al. 2013), TAp63α in oocytes forms inactive dimers (Fig. 3B) (Deutsch et al. 2011). These dimers are stabilized by the interaction of the carboxy-terminal transactivation inhibitory (TI) domain and the amino-terminal TAD, which together block the tetramerization interface of the central tetramerization domain (Fig. 3C). This inhibition allows oocytes to survive for decades in humans despite the high concentration of the proapoptotic factor TAp63α. Detection of DNA double-strand breaks, however, activates p63 through phosphorylation (Fig. 3B,C). Several kinases, including ATM, c-Abl, and Chk2, have been reported to be involved in this process (Fig. 3C) (Suh et al. 2006; Gonfloni et al. 2009; Bolcun-Filas et al. 2014). Activation results in the formation of an open and tetrameric form of TAp63α with a 20-fold higher DNA-binding affinity that leads to the induction of apoptosis mediated by the two BH3-only proteins PUMA and NOXA, which are direct transcriptional targets of activated TAp63α (Fig. 3B,C) (Kerr et al. 2012). Presumably, the p63 quality-control system developed originally to ensure that oocytes, which still have DNA double-strand breaks after chromosomal repair following homologous recombination, undergo removal. The end-point of homologous recombination, however, overlaps with the beginning of the expression of TAp63α in oocytes. Phosphatases (PPases) ensure that p63 stays in the inhibited and closed conformation during that stage (Kim and Suh 2014) to prevent the premature induction of cell death in oocytes that are still actively repairing their chromosomes (Fig. 3C).

Following maturation into pre-antral follicles, TAp63α expression is lost, and instead p73 starts to play an important quality-control function. In vitro fertilization experiments with oocytes obtained from knockout mice lacking only the TA isoform of p73 have shown that the development of the blastocyst is severely impaired (Tomasini et al. 2008b, 2009), often resulting in the formation of multinucleated blastomeres (Fig. 3A). Detailed analysis suggested that this effect is because of problems in proper formation of the spindle assembly complex resulting in genomic instability associated with enhanced aneuploidy. The molecular

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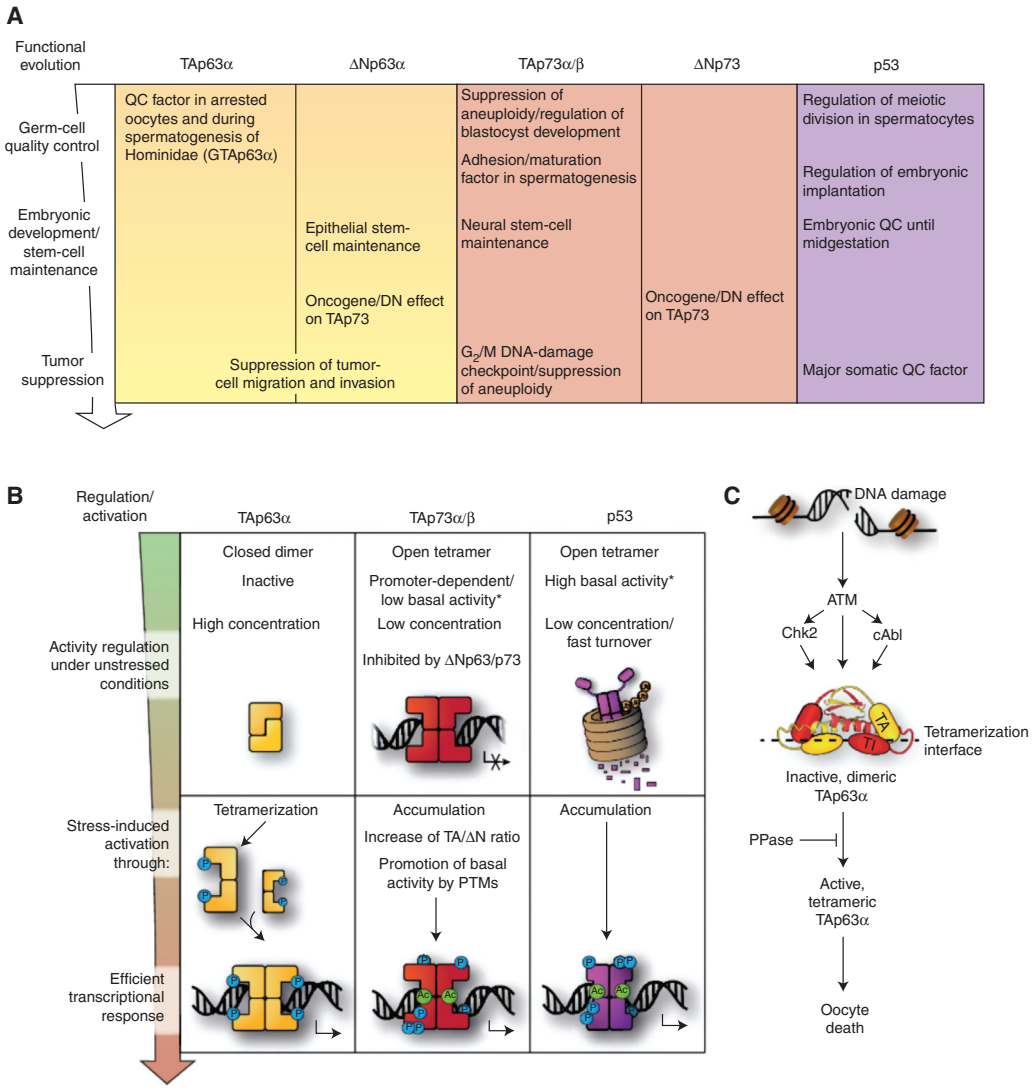

Figure 3. Functional evolution and regulation within the p53 family highlighting the unique regulatory mechanism of the most ancient family member TAp63α. (*A*) All family members and isoforms have acquired distinct regulatory roles in the functional evolution from germ-line quality control via embryonic development and stem-cell maintenance to tumor suppression. (*B*) Although activation leads to a very similar transcriptional program inducing cell-cycle arrest and apoptosis, the transactivation (TA) isoforms display fundamental differences in the regulation of their transactivation potential. (*C*) In TAp63α, the TA and transactivation inhibitory (TI) domains of a dimer interact on top of the tetramerization interface establishing a closed, dimeric autoinhibitory conformation. TAp63α monomers are indicated in different colors for clarity. DNA-damage-induced phosphorylation disrupts the interdomain interaction network allowing formation of active tetrameric protein that subsequently induces oocyte death. Protein phosphatases (PPases) may revert activating phosphorylations, preventing the disruption of the autoinhibitory conformation. DN, Dominant negative; PTM, posttranslational modification.

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basis of this effect seems to be the direct interaction of TAp73α with several components of the spindle assembly checkpoint (SAC) complex (Tomasini et al. 2009). Interestingly, and consistent with the mouse studies, morpholino knockdown experiments with the two p53-like proteins from the tunicate *C. intestinalis* showed that both proteins are involved in the development of the blastopore by inhibiting gastrulation movement, important for closing of the blastopore (Noda 2011).

In mammals, at an even later phase, during the implantation stage of the embryo, p53 also gets involved by controlling the expression of leukemia inhibitory factor (LIF), a cytokine important for implantation (Fig. 3A). Indeed, in p53 null mice, the number of pregnancies and the litter size are reduced because of impaired implantation, demonstrating that all three family members are involved in controlling different stages of maternal reproduction (Hu et al. 2007). In addition, p73 is also involved in the process of spermatogenesis. Specific knockout of the TA isoforms result in male infertility because of severe impairment of spermatogenesis (Inoue et al. 2014). Detailed analysis has revealed that TAp73α functions as a critical factor for adhesion and maturation of the seminiferous epithelium (Holembowski et al. 2014). In Hominidae, a special isoform of p63 with an amino-terminal elongation is also involved in quality control of male germ cells (Mattia et al. 2007; Beyer et al. 2011). This special variant, GTAp63α, was created relatively recently during evolution by fusing the 5′ end of the p63 gene with the long terminal repeat (LTR) region of the human endogenous retrovirus 9 (ERV9). GTAp63α is strongly expressed in spermatogenic precursors but not in mature spermatozoa, indicating that this isoform serves a similar quality-control function as TAp63α in the female germ line.

Although germ-cell quality control is likely to be the original function of the p53 family, it is not the only developmental process in which it is involved. The p63 and p73 knockout mouse studies have revealed that both proteins are master regulators of the development of epithelial tissues and neuronal cells, respectively (Fig. 3A). As the average lifetime of organisms increased and started to exceed the average lifetime of individual cells, evolution developed renewable tissue, which required the establishment of stem cells. Because germ cells are the prototype of a stem cell and p63 (and p73) was already involved in the maintenance of the genetic quality of germ cells, it probably got reused for the new tasks of controlling the proliferative potential of stem cells. In the basal layer of mammalian epithelial tissue, the ΔNp63α isoform plays a central role by orchestrating a transcriptional program that is essential for the maintenance of stratified epithelial tissues (Mills et al. 1999; Yang et al. 1999). Likewise, p73 has developed into an essential factor for neuronal stem-cell maintenance (Fujitani et al. 2010). With the appearance of renewable tissue, however, tumorigenesis became an increasing problem with ∼80% of human cancers originating from epithelial tissues. In the most recent stage of the evolution of the p53 protein family, the tumor-suppressor function was added to its repertoire with p73 (Tomasini et al. 2008) and, in particular, p53 (Levine 1997; Vousden and Lane 2007) being assigned to the surveillance of the genetic and cellular quality. Although p63 seems to play a minor role as a tumor suppressor, the ΔNp63α isoform has been shown to suppress metastasis. In addition, recent studies have also assigned stem-cell maintenance functions to p53 (Cicalese et al. 2009; Levine et al. 2016), demonstrating that all three family members have similar functions in quality control in reproduction, stem-cell maintenance, and tumor suppression, albeit with different specializations acquired during evolution.

STRUCTURE AND MOLECULAR DYNAMICS OF p53

Molecular dynamics (MD) has been extensively used in p53 research, with the great majority of in silico studies dealing with the DNA-binding region (Lu et al. 2007; Madhumalar et al. 2008) and its cancer-related mutations (Rohani et al. 2015; Thayer and Quinn 2015).

The structural and dynamic characterizations of p53 mutants is a first step toward the rational drug design of anticancer molecules

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that stabilize the mutants, thus recovering wild-type activity (Boeckler et al. 2008; Basse et al. 2010), but MD also has the potential to find new drug targets, sampling transient protein states not detected by X-ray crystallography (Joerger et al. 2015).

Mutation R175H, located in the helix H1 in the DBD, is close to the Zn-binding residue H179. Therefore, we modeled two possible scenarios, the first in which the binding site for the ion is conserved (purple line in Fig. 4) and the other in which it is coordinated to H175 (orange line in Fig. 4). These two full-length models were compared with the native proteins in the monomeric state.

Computational studies have also been performed on the p53 amino-terminal (Mavinahalli et al. 2010) and carboxy-terminal fragments (Gordo et al. 2008; Allen et al. 2010). The complexity of posttranslational regulations targeting p53 (Fig. 1C) makes it the ideal system to be studied by MD, especially when structural information is not obtainable by any experimental technique. Comparison of the full-length p53 in monomeric form with the tetramer–DNA complex shows, for example, that the OD regions in the carboxy-terminal domain are fully structured only in the tetrameric form, whereas the DBD secondary structure is already formed in the isolated monomeric form (OD and DBD in Fig. 5; compare panels A and B).

LONG-RANGE COMMUNICATION BETWEEN DIFFERENT DOMAINS: OD AND DBD

We have recently performed an MD simulation of the p53 protein as a tetramer bound to DNA, in which we highlighted the existence of long-range interactions between different domains of p53 (Fig. 5). In particular, the p53 carboxyl terminus, including the OD, shows clear intramonomer-3 (M3) interactions with the DBD as well as intermonomer level interactions between M2-OD and M3-DBD and also between M3-OD and M4-DBD (Fig. 5C). As previously

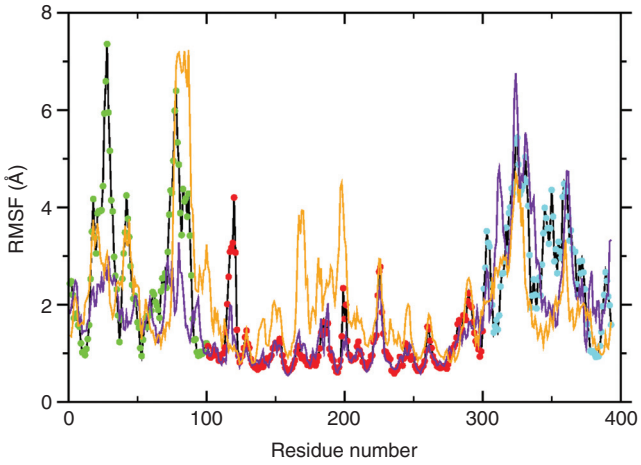

Figure 4. Per-residue root-mean-square fluctuation (RMSF) for wild-type p53 and the R175H mutant. The average fluctuation observed by molecular dynamics (MD) (200 nsec) shows different degrees of movement depending on the distinct region of p53 in the wild-type form (black line); point color code as in Fig. 1 (amino terminal, green; DBD [DNA-binding domain], red; carboxyl terminal, blue). The two TADs (transactivation domains), PRR (proline-rich domain), OD (oligomerization domain), and CTD (carboxy-terminal domain) are clearly distinguishable. The DBD is highly stable. Mutant p53 isoforms shows a drastic change in flexibility. The p53 R175H mutant that maintains the native Zn binding (yellow line) has increased the flexibility of DBD, indicating a potential abnormal binding to the selected promoter. The p53 R175H mutant that has an alternative Zn-binding site (purple line) has reduced flexibility of the carboxyl terminus, suggesting abnormal transactivation properties. (From D'Abramo et al. 2015; modified, with permission.)

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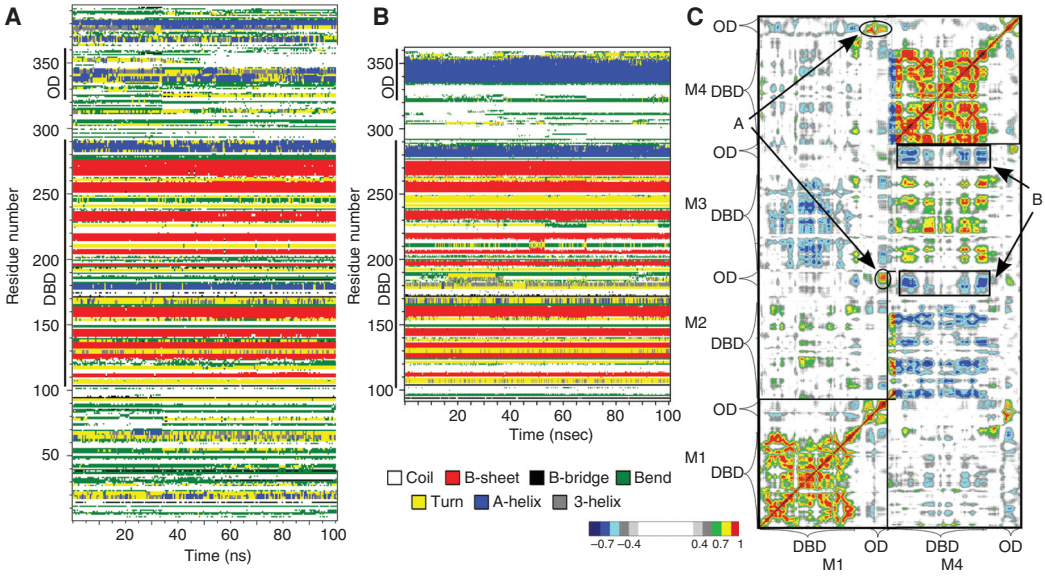

Figure 5. Secondary structure content as a function of simulation time. (*A*) Molecular dynamics (MDs) of the full-length p53 in isolated monomeric form (Chillemi et al. 2013). (*B*) MD of monomer 1 in the p53–DNA tetrameric complex, taken as representative of the secondary structure in the tetramer (D'Abramo et al. 2015). Comparison of the two systems shows that the oligomerization domain (OD) region is fully structured only in the tetrameric form, whereas nascent helix structure is observed in the monomeric form both in transactivation domains (TADs) and OD. (*C*) Dynamic cross correlation map of monomers 1 and 4 showing the intermonomeric motions M4-M1, M2-M1 (*A*), and the anticorrelated motions of the TET-DBD between M3-M4 and M2-M4 (*B*) (see D'Abramo et al. 2015).

described (D'Abramo et al. 2015), the protein shows a double interaction with DNA. First, distinct interaction is formed by nonspecific noncovalent interaction with DNA; this seems to occur via the β strands S9 in the β sandwich and the carboxyl terminus of the β strand S10 in the loop–sheet–helix motif (Wassman et al. 2013). Second, specific sequence recognition occurs at the level of the helix H2 and loop L1, which are within the loop–sheet–helix motif. When we analyzed the molecular dynamics of this interaction, the loop L1 indicates nonsymmetrical dynamics in the four p53 monomers; this is shown by the essential dynamics of the concatenated DBD trajectory along the second eigenvector. MD indicates a switch from a conformation where L1 tucks into the major groove with an outward projection away from the major groove, whereas the other monomers move in the opposite direction. These data provides a molecular mechanism for the regulation of p53 functions through posttranslational modifications of the carboxyl terminus over the DBD.

As suggested above by the MD analysis (D'Abramo et al. 2015) and by very recent evidence (Hamard et al. 2013; Laptenko et al. 2015), p53 carboxyl terminus has a significant effect on the transcriptional activity of p53 by regulating the function of the DBD, p53 stability and p53 subcellular localization, as well as on the recruitment of additional co-factors. The investigators attribute this effect to changes in the affinity of electrostatic interactions between the lysines at the carboxyl terminus during specific binding with other p53 regions (Laptenko et al. 2015). In agreement, the MD changes indicate the biological value of the lysine interaction on the stability of the p53–DNA complex through the facilitation of cooperative contacts with the DBD (D'Abramo et al. 2015). Both papers are in line with the hypothesis of the "induced-fit mechanism." Indeed, Petty et al.

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(2011) showed a conformational change within the DBD involving the L1 loop, which adopted either an extended or a recessive conformation. The dynamic flexible movement of the four monomers shows how the carboxyl terminus can modulate the electrostatic surface regardless of the presence of the lysines (Lys370, 372, 373, 381, 382, and 386 are absent in the MD model) according to the two different conformations at the extreme of the first essential eigenvector. This is the biophysical rationale for the understanding of the carboxyl terminus interaction with the DBD. The in vivo role for the carboxyl terminus of p53 is finally highlighted by the recent knockout mouse lacking the carboxyl terminal 24 amino acids (Hamard et al. 2013). These mice show premature death at 2 weeks with hematopoietic failure and impaired cerebellar development related to enhanced expression of the proapoptotic proteins Puma and Noxa in the bone marrow, while in the liver and in the spleen the phenotype is related to alternative gene expression mechanisms (Hamard et al. 2013).

The implications of the above-mentioned results are evident on the induced-fit theory (see Koshland 1958; Johnson 2008). The existence of an induced-fit conformational transition alters the kinetic equilibrium from Equation 1 to Equation 2:

$$\mathrm{p53} + \mathrm{DNA} \rightleftarrows \mathrm{p53/DNA}, \quad (1)$$

$$\mathrm{p53} + \mathrm{DNA} \rightleftarrows \mathrm{p53/DNA} \rightleftarrows \mathrm{p53m/DNA}. \quad (2)$$

Multiple structural switches ($\mathrm{p53_m}$) induce a better conformational fit of p53 to DNA, moving from Equation 1 to Equation 2 in which $K_2 > K_1$ and resulting in the stabilization of the bound conformation. Therefore, the selectivity of the promoter bound is more dependent on the K_{OFF}, rather than on the K_{ON}. Originally, Halazonetis identified a role for L1 in this mechanism (Emamzadah et al. 2014); however, we cannot disregard that the conformationally induced fit is caused by additional partners that are included in the transcriptional complex. Nonetheless, MD shows that the carboxyl terminus can per se induce a conformational change on the DBD, even in the absence of other partners.

MD OF p53 WITH OTHER INTERACTORS

Being able to accurately model the protein interactions of p53 with its molecular partners would greatly enhance our understanding of the biological processes involved. In which direction are the structural and MD research moving? Clearly, a full structural analysis by Cryo-EM would provide a more solid base to elucidate the entire p53 complex with the DNA. This would allow a more realistic MD analysis, in which both the DNA (different promoter sequences) and p53 (phosphorylation, ubiquitylation, and other posttranslational modifications) could be studied. Already now, the interaction of p53 with other interactors is under investigation.

Binding of the HMGB1 A box with the TAD2 region (Fig. 6A) plays a role in DNA binding (Rowell et al. 2012). In agreement, MD results of the full-length form show an anticorrelation movement between the TADs and DBD in the helix region around Lys120 (Fig. 6B).

The structural mechanism that allows the alternative methylation of Lys372 by Set9 (Chuikov et al. 2004) or Lys370 by SMYD2 (Fig. 5C) (Wang et al. 2011) is still not fully unraveled. A synergistic role for the carboxy-terminal lysine residues and Lys120 in the DBD has been highlighted by the MD simulation of the full-length monomeric form, in which conformations in the most visited essential subspace present a unique positive charged surface formed mainly by the lysines (Fig. 6D).

In keeping, the analysis of mutant isoforms of p53 (see Fig. 4) may provide additional structural information on the defective function of p53 itself in cancer.

CONCLUDING REMARKS

Although the role of the p53 core domain in DNA binding has been extensively established, the contribution of its carboxy-terminal domain remained elusive until recently. Latest data on the structural properties of p53 have shed light on the regulatory mechanisms exerted by the

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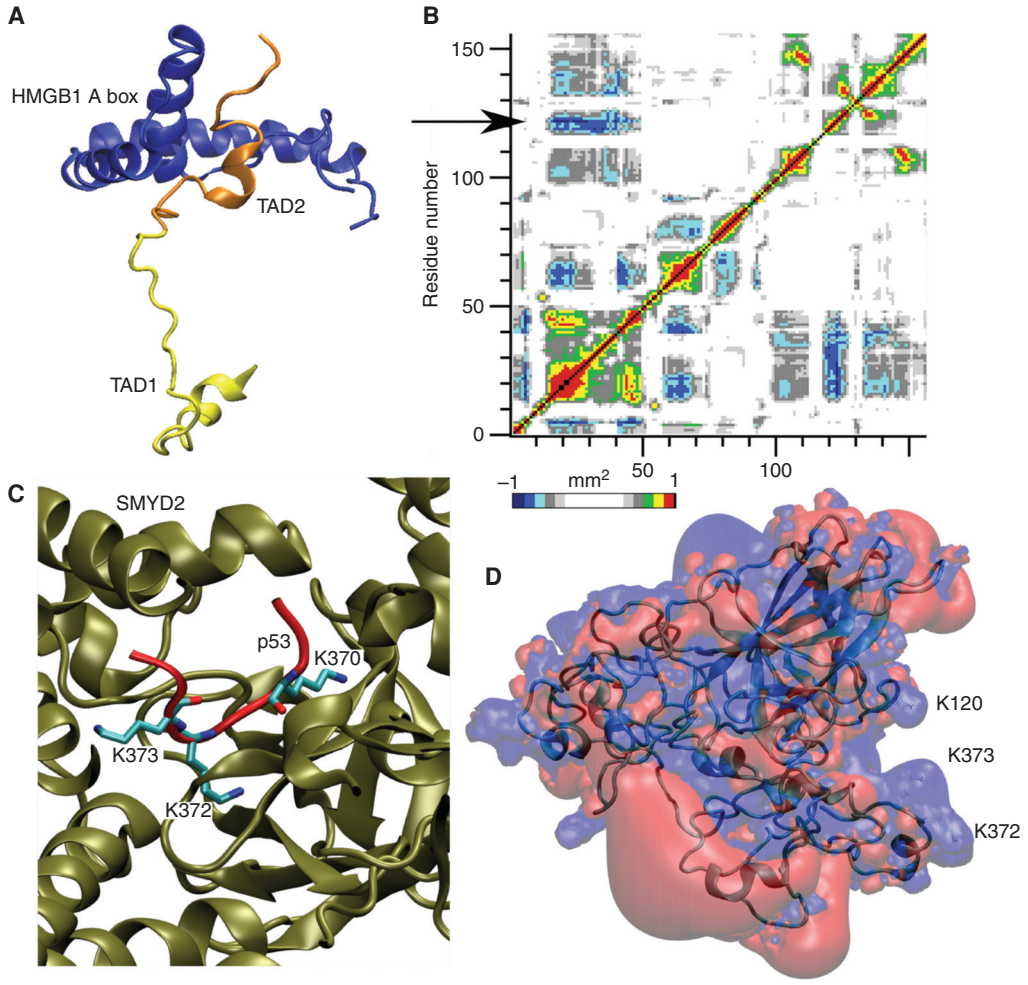

Figure 6. Examples of molecular partners of p53 with different biological roles. (*A*) Binding of the HMGB1 A box (blue) with TAD2 influences the p53 (orange) binding with DNA (not shown). (*B*) Long-range communications between p53 transactivation domains (TADs) and DNA-binding domains (DBDs) have been observed in the full-length molecular dynamic (MD) simulation with an anticorrelation motion (highlighted by the arrow) that structurally connect TADs with the Lys120 region in DBD. Red, correlation motion; blue, anticorrelation motion (see color legend). (*C*) Among the posttranslational modifications, methylation of lysine residues in CTD (carboxy-terminal domain) plays an important role. Lys370 is specifically methylated by SMYD2. (*D*) Lysine residues are important also for DNA binding. The most stable conformations in the eigenvector 1–2 essential subspace present a positive charged iso-surface participated by Lys120, besides the CTD lysines.

carboxy-terminal domain of p53 on DNA binding by the DBD. The MD simulation of the p53 tetramer in complex with DNA highlighted the presence of long-range communication between different domains. These novel data showed a dynamic flexible movement of the four monomers and showed that the carboxyl terminus can modulate the electrostatic surface between DNA and the transcription factor. The unstructured carboxy-terminal domain of p53 is indeed able to regulate the stability of site-specific DNA binding and facilitate contacts between the core DBDs of the tetramer. As a result, the carboxyl terminus of p53 has a strong

biological effect on its transcriptional function by controlling the DNA-binding activity, the stability, and the subcellular localization as well as the recruitment of transcriptional cofactors.

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