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The Tetramer of p53 in the Absence of DNA Forms a Relaxed Quaternary State

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Supporting Information

ABSTRACT: p53 is a tetrameric multidomain protein that triggers the anticancer cellular response to stress. We have calculated a three-dimensional reconstruction of full-length human p53 in the absence of DNA using single-particle electron microscopy. The reconstruction of DNA-free full-length p53 shows a square-shaped structure with four distinct domains and a hollow center. In comparison with the known compacted DNA-bound full-length p53 structures, the DNA-free p53 tetramer adopts a relaxed conformation with separated monomers and oligomerization interfaces different from those of the DNA-bound conformation.

p53 is a multidomain protein with anticancer activity found at the center of a complex network of signaling pathways that organize the cellular response to stress.[1,2] In the nucleus of normal cells, p53 binds DNA at response element (RE) sequences and acts as a transcription factor, activating more than 100 genes in the DNA repair, cell arrest, senescence, and apoptotic pathways.[1−4] Because of its central role in the cellular response to stress, p53 is the protein most commonly found mutated in cancerous cells with ∼50% of human tumors carrying a mutation in the p53 gene.[1]

Human p53 is a 393-amino acid tetrameric protein with each monomer organized in three regions (Figure 1A). The N-terminus (amino acids 1−92) is an inherently unfolded region that recruits transcriptional activators to the promoter site and acquires an α-helical structure in complex with their binding partners.[5−8] The largest structured region (amino acids 93−293) is the central DNA-binding domain (DBD) that has an immunoglobulin fold where loops from the β-sandwich and an α-helix at the end of the domain recognize the DNA.[9] As a dimer of dimers, p53 binds to 20 bp REs in a head−tail−head−tail manner to four quarter-sites with the consensus Pur-Pur-Pur-Cyt-Ade/Thy sequence.[10−14] Finally, the C-terminus also forms a dimer of dimers with a β-strand−turn−α-helix oligomerization domain (amino acids 325−360),[15] and an unstructured regulatory domain (amino acids 361−393) that binds DNA nonspecifically.[16,17] The inherent structural flexibility of the N- and C-termini allows p53 to interact with dozens of proteins and participate in a complex network of regulated cellular functions.[16,18] Such structural flexibility has limited the determination of the structure of the full-length structure of p53.

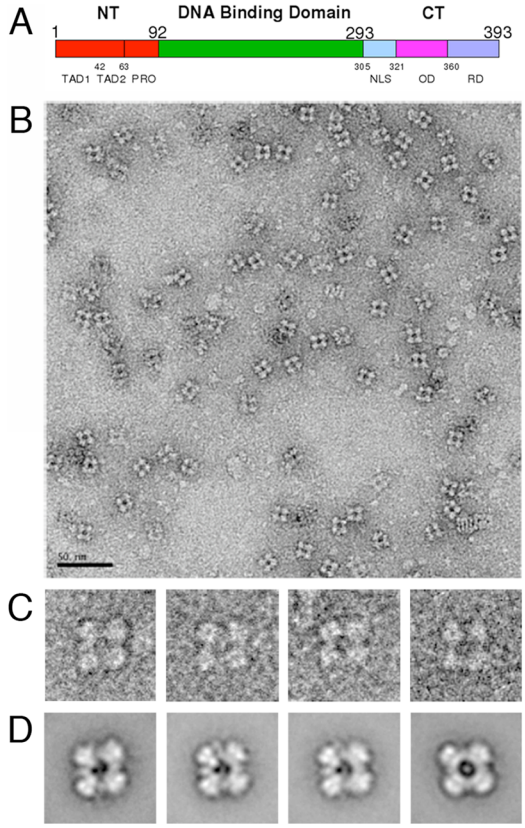

Figure 1. Gene structure and single-particle analysis of the DNA-free p53 tetramer. (A) Schematic human p53 gene structure. (B) Electron micrograph of negatively stained human full-length p53 at 50000× magnification. The scale bar is 50 nm. (C) Raw images of full-length p53. (D) Class averages of p53 tetramer particles. The last average results from the misalignment of particles that are rotated 90°. The window size is 304.8 Å per side.

A gap exists in our knowledge of p53 structure because we do not understand how its domains interact with each other to explain how its diverse functions are regulated. In this work, we present the three-dimensional reconstruction of the DNA-free p53 tetramer. By comparing the DNA-free p53 reconstruction with previously determined structures with DNA, we show that, in the absence of DNA, the quaternary structure of human full-

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length p53 is a relaxed tetramer that becomes compact when it binds DNA.

Electron Microscopy of the DNA-Free p53 Tetramer. We purified human full-length p53 to homogeneity. Electron micrographs of particles negatively stained with uranyl formate were taken in a 200 kV transmission electron microscope. The particles were homogeneous with clear contrast and a 13 nm diameter (Figure 1B). The p53 tetramers resemble a four-leaf clover with a hollow center, and they were deposited in the carbon-coated grid with a preferred orientation. The p53 tetramer particles were manually picked from the electron micrographs and extracted as square windows, and the 8468 particles were classified into 20 groups (see the Supporting Information). Image classification of 0° images confirmed the homogeneity, preferred orientation, and overall square-shaped p53 particles observed in the raw electron micrographs. Raw images and class averages show the same characteristic four-leaf clover shape with a separate density for each p53 monomer and a hollow center. The tetramer is arranged as a dimer of dimers; the density connecting two monomers to form a dimer is stronger than the density between the dimers in the tetramer (Figure 1C,D).

DNA-Free p53 Tetramer Three-Dimensional Reconstruction. We collected images at 0° and 60° of full-length p53 single particles and calculated the three-dimensional reconstruction using the random conical tilt approach. Particle projections used for the reconstruction showed the expected Euler angle distribution of 60° images, and the final map has a resolution of ~28 Å (see the Supporting Information). The crystal structures of the p53 DBD and oligomerization domain have different molecular symmetry, *C*2 and *D*2, respectively.[10−15] Thus, it is possible that the p53 tetramer is an oligomer with mixed domain symmetries. To avoid any biased assumption in the final reconstruction, no symmetry constraints were imposed at any time during the structure calculations.

The final p53 tetramer reconstruction shows an overall square shape with equal sides of 133 Å and a 46 Å diameter hole in the center (Figure 2). The distance between the centers of two adjacent monomers is 84 Å, and each monomer is connected to the two adjacent monomers by two protruding densities oriented at a 90° angle from each other. Monomers across the tetramer appear not to be in contact. Each monomer has a height of 66 Å, which is likely to be underestimated because of some likely flattening of the sample during staining. Because the height of the four main volumes is higher than that of the connecting density, two perpendicular grooves between monomers are formed at the bottom of the reconstruction. The similarity between the raw transmission electron micrographs, the class averages at 0° and 60°, the three-dimensional reconstruction, and the calculated projections from the reconstruction volume at 0° and 60° support the correctness of the final map (Figures 1 and 2 and the Supporting Information). The class averages and the symmetry-unrestrained reconstruction have *C*2 features that are consistent with a single 180° symmetry rotation axis. As a control, we calculated reconstructions with *C*2, *C*4, and *D*2 symmetries (see the Supporting Information). We discarded the possibility of assigning a *D*2 symmetry for the entire p53 tetramer,[19] as seen in the oligomerization domain crystal structure,[15] because such reconstruction has two-layer features that were not observed in the class averages of tilted particles or in the symmetry-unrestrained reconstruction. Although at a ~28 Å resolution the *C*2 and *C*4 reconstructions are very similar, we consider that the DNA-free p53 tetramer is likely a *C*2 oligomer as DNA-binding proteins that recognize inverse repeats are, i.e., restriction endonucleases.[20]

The DNA-Free p53 Tetramer Has a Relaxed Quaternary Structure Distinct from the DNA-Bound Structure. The comparison between the reconstruction of the DNA-free full-length p53 tetramer and the known structures of DNA-bound p53 shows that p53 can adopt two distinct quaternary structures. In the absence of DNA, the p53 tetramer has an overall volume with dimensions of 133 Å × 133 Å × 66 Å (Figure 3A). NMR and SAXS solution studies of full-length p53 also describe the DNA-free p53 structure as a loosely tethered tetramer susceptible to conformational changes;[21] such a

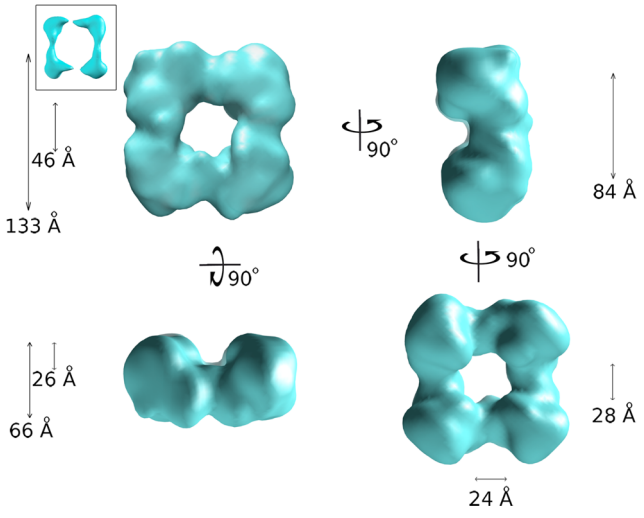

Figure 2. Three-dimensional reconstruction of the human full-length p53 tetramer. No symmetry averaging was applied to the reconstruction. Rendered at a high threshold, the top left inset shows the *C*2 symmetry reconstruction features.

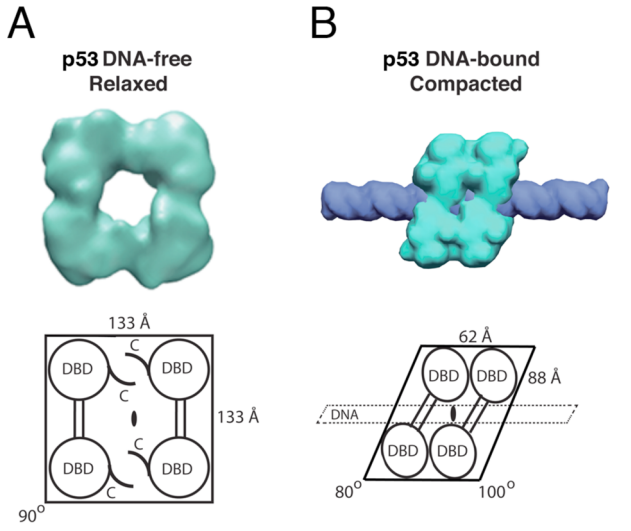

Figure 3. DNA-free and DNA-bound p53 quaternary structures. (A) In the absence of DNA, the p53 tetramer adopts a relaxed quaternary structure. Monomers arrange as a dimer of dimers. We suggest that the C-termini might stabilize the tetramer. (B) The low-pass-filtered crystal structure of p53 DBD bound to DNA (Protein Data Bank entry 3kz8) shows the tetramer in a compacted quaternary structure.[13]

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description agrees with the relaxed form of the p53 tetramer described in this paper. In comparison, the electron microscopy and crystal structures of the p53 tetramer bound to DNA show a compact tetramer.[10−14,21−23] For example, the low-pass-filtered crystal structure of the p53 DBD tetramer bound to DNA is denser than the structure without DNA (Figure 3B).

The p53 tetramer reconstruction has four clearly defined volumes and four bridging densities that link the bulkier volumes with each other. Most likely, each of the four large volumes of the reconstruction corresponds to the large DBDs. None of the determined structures in complex with DNA can explain the structure of the DNA-free tetramer. To explain the large described differences between the DNA-free and DNA-bound forms, any model of the DNA-free p53 tetramer requires the separation of the DBDs by at least 20 Å from the positions observed in the DNA−protein crystal structures (see the Supporting Information).

Some overall conclusions can be reached via comparison of the dramatic conformational change in the p53 tetramer upon binding of DNA and the fitting into the EM map of the two domains whose crystal structures have been determined. First, the DBD in complex with DNA has multiple dimerization and tetramerization contacts that maintain a tight DNA−protein complex (Figure 3B). Instead, in the absence of DNA, the contacts between the individual DBDs cannot form because distances between monomers increase between 45 and 71 Å (Figure 3A). Second, in the presence of DNA, the oligomerization domain seen in the crystal has been fitted as a tetramer into the EM maps.[21−23] Instead, in the absence of DNA, not all the monomers appear to be in contact with each other and the oligomerization domain was fit as two separate dimers (see the Supporting Information). Third, mutations that block DNA binding and often occur in cancer are unlikely to disturb the formation of the relaxed DNA-unbound quaternary structure. We postulate that the relaxed tetramer described here might represent the gain-of-function p53 mutant that accumulates in the nucleus of cancerous cells.[24] In conclusion, we propose that the p53 tetramer requires a large conformational change to bring the four DNA-binding and oligomerization domains in close contact to form the active p53 conformation that promotes transactivation.

Structural Data. The DNA-free p53 tetramer EM map has been deposited in the EMDB as entry EMB-5342.

■ ASSOCIATED CONTENT

Supporting Information

Details of protein expression and purification, EM data collection, reconstruction calculation, symmetry of the p53 tetramer, and the map interpretation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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