p53: A Glimpse at the Puppet Behind the Shadow Play

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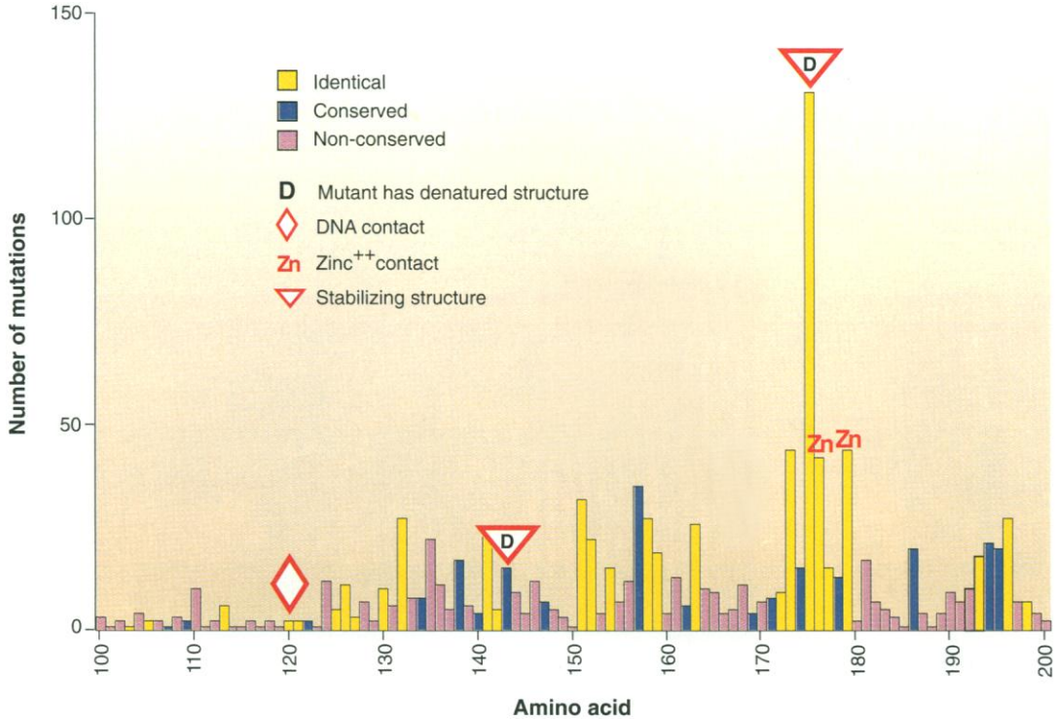
The parable of the cave in Plato's *Republic* describes prisoners who are quite satisfied that the shadows projected on the cave wall define reality—until their bonds are cut and they view the actual objects (*1*). A similar feeling of revelation comes from viewing the three-dimensional structure of the tumor suppressor protein p53, as presented in this issue of *Science* by the crystal structure of the p53 core domain (*2*) and the solution structure of its oligomerization domain (*3*).

Once p53 shed its cloak as a dominant oncogene in 1988, it quickly became evident that many human cancers were associated with changes in p53, most frequently missense mutations (*4*). These seemingly ubiquitous mutations suggested that the inactivation of p53 must be critical for the formation of many malignancies. The importance of p53 as a cancer susceptibility gene was soon afterward verified in Li-Fraumeni syndrome (*5*). As its biological importance in human cancer has been repeatedly substantiated, p53 has acquired many potential roles within the cell. In some circumstances it controls a G_1/S checkpoint in the cell cycle, and at other times it functions as an essential trigger of apoptosis (*6*–*8*). Equally important has been the recognition that p53 is part of a pathway responsible for DNA damage repair and, as such, helps to ensure genomic stability (*8*, *9*).

The p53 protein accomplishes these many tasks with a diverse assortment of functions. These include its ability to be a site-specific transactivator, as well as a repressor, of transcription (*10*, *11*). The p53 protein may also bind double-stranded RNA and act as a regulator by binding to the replication protein RPA (*12*). The crystal structure of p53 has therefore been anticipated with excitement—not only by structural biologists, but also by those interested in its myriad cellular functions and the vast clinical potential of restoring p53 function.



Structure-function comparisons for the central regions of p53. Amino acids 100 to 300 contain the site-specific DNA binding core domain. Shown are the sites frequently mutated in human tumors (*4*), and the residues that when mutated inactivate p53 function while either retaining overall structural stability (N) or disrupting global stability (D).

Oblivious to this anticipation, the p53 protein has been quite resistant to crystallization. The method taken by Pavletich and co-workers, which was appropriately sensitive to the unstructured floppy amino terminus, has been to crystallize a p53 fragment that extends from amino acid residue 94 to 312 and contains the critical site-specific DNA binding "core domain" (residues 102 to 292), the proteolysis-resistant core of p53 (*2*). However, this core domain does not contain the amino-terminal transactivating domain, with the binding site for the transcription factor mdm2, nor the carboxyl-terminal 101 amino acids, with both the oligomerization binding domain and an inhibitory domain. Despite these missing regions, the crystal structure of the p53 core domain is immensely informative: The p53 protein accomplishes its binding to DNA on its own terms, without the specific motifs that are found in other DNA binding proteins. For example, helix-turn-helix or helix-loop-helix motifs were not observed. The p53 structure also does not contain any TFIIA zinc fingers. Nevertheless, the structure follows many of the rules previously established for DNA binding proteins. Contacts with the major DNA groove are made primarily by side chains of an a helix extending from residues 278 to 286, and the minor groove is contacted by the arginine at position 248.

Exciting to p53 aficionados is how the crystal structure consolidates the role of p53 as a site-specific DNA binding protein. The six most commonly mutated sites in p53 all lie close to the DNA binding site, two actually contacting DNA, and each stabilizes this interaction. In this respect, it is quite satisfying that one of the most frequently altered sites in p53, Arg^{248}, performs the critical job of anchoring p53 to the DNA minor groove with four hydrogen bonds. In viewing the direct correlation between highly conserved amino acids, which are frequently altered in human tumors, and their key functions in this DNA binding core domain, one is immediately struck by the critical importance that DNA binding must have in the ability of p53 to act as a tumor suppressor.

The crystal structure of the core domain

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also suggests why p53 exhibits such a wide spectrum of missense mutations. Virtually all of the amino acids within the 190–amino acid DNA binding domain have been found to be mutated in one human cancer or another (see figure) (*4*). Therefore, far from having a unique Achilles heel, p53 is poised for inactivation by mutation at any point in this core domain. The crystal structure reveals that at the DNA protein binding interface there is little extended secondary structure. Instead, packing of the side chains in this region provides the necessary stability. Thus, missense mutations at any point, which would easily disrupt this packing, can alter overall p53 stability and, presumably, function.

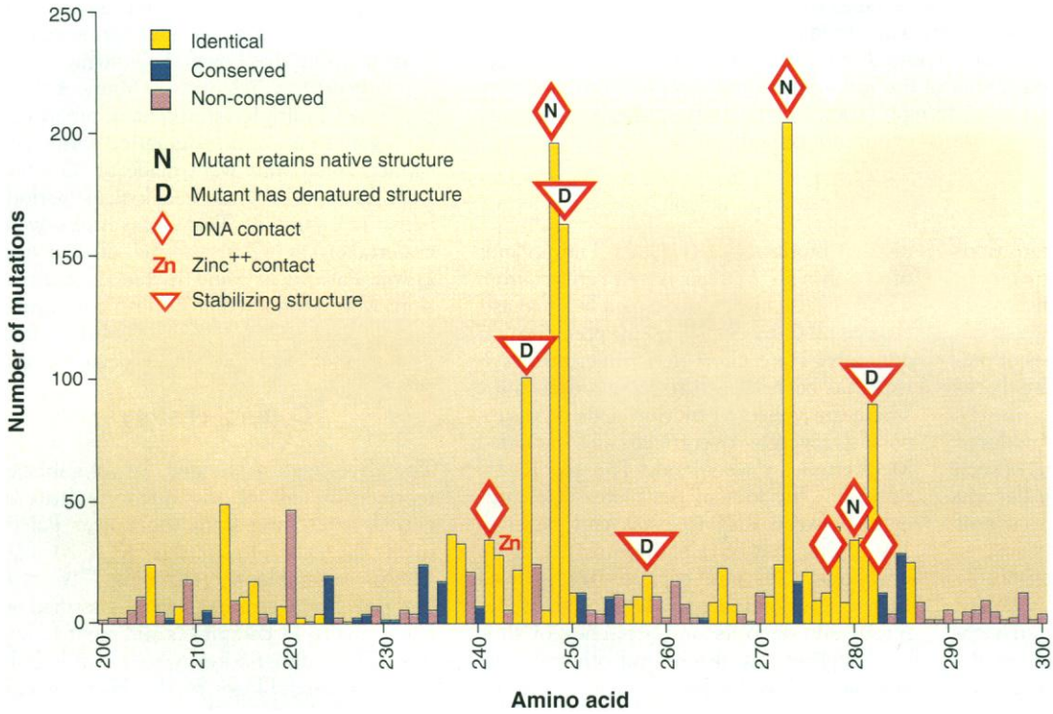
A few mutations of p53 completely disrupt the native conformation of the structure, whereas others do not alter overall structure at all (see residues labeled N and D in the figure) (*13*, *14*). The primary structure does little to explain why this is the case. The crystal structure is much more revealing (*2*). The inactivating mutations that allow p53 to retain its native structure appear to represent a class of mutations so essential to the DNA binding function of p53 (in three cases they actually contact the DNA) that they disrupt p53 function by interfering only with DNA binding and, unlike most other p53 mutations, do not alter the conformation of the protein.

Complementing the crystal structure of the p53 DNA binding domain (*2*) is the work by Clore and co-workers in this issue, who have used multidimensional nuclear magnetic resonance to solve the solution structure of the p53 oligomerization domain that exists between residues 319 and 360 (*3*). Definitive data is presented for a novel tetramer topology consisting of a dimer of dimers, each comprised of two antiparallel helices linked by an antiparallel β sheet. This structural data suggests that the carboxyl-terminal basic tail of p53 may be brought in close proximity to the DNA binding core of p53.

Numerous academic and industrial laboratories have been pursuing therapeutic strategies to cancer treatment based on the goal of restoring p53 wild-type activity with small molecules. This notion is based primarily on two concepts. The first is that some mutant p53 structures are alternate conformations that could perhaps be made less favorable if there were ways to stabilize the wild-type conformation (*15*). This approach is supported by the fact that the DNA binding activity of many mutant p53 molecules can be restored at 25°C (*16*). A second approach is based on the idea that p53 has inhibitory regulatory domains, which if disrupted in vitro can restore wild-type activity (*17*).

The three-dimensional structure of p53 allows a more sophisticated evaluation of these therapeutic approaches than has been possible. The crystal structure shows that the core DNA binding region or business end of the molecule forms a coherent structure that excludes the regulatory portions of the molecule. In order to restore DNA binding, modifications by small molecules in the regulatory domains would therefore have to be transmitted back to the central core domain. This is more difficult than if the regulatory domains had been found to be part of the DNA-protein interface. Additional three-dimensional data regarding proximity of the basic carboxyl-terminal region and the DNA binding domain will be needed to assess the feasibility of squelching this inhibitory basic domain. Second, many mutations cause a global loss of stability (such as arginine to histidine at position 175), not just an altered conformation. Therefore, restoration of the native conformation from a virtually denatured p53 molecule will be a Herculean task for any small molecule. Although such pessimism does not preclude the success of small molecule–based approaches to p53-directed therapy, the crystal structure has set a higher hurdle for the designers of cancer therapeutics.

Now that we have glimpsed the actual three-dimensional structure of the core domain of p53 and taken a look at the oligomerization domain, it will be hard to think of p53 as a linear arrangement of functional domains—and hard to wait for additional three-dimensional details.



Also shown are the structural roles of several key residues involved in DNA binding, zinc atom contacts, or structural stabilization, along with color codes denoting residues identical, conserved, or nonconserved among p53 proteins from diverse species.

References

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