Four p(53)s in a pod

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The tumor suppressor p53 protects the cell from cellular stress, and in so doing it decides between cell-cycle arrest and cell death. The high-resolution structure of four DNA binding domains of p53 in complex with DNA shows how the structural collaboration between protein and DNA may influence the biological outcomes of the tumor suppressor.

It is widely accepted that mutations within the core DNA binding domain of the p53 tumor suppressor protein (p53DBD) account for approximately 50% of all cancers. Therefore, given the importance of p53 in cancer biology, it is fitting that molecular and structural biologists have strived for many years to understand how the p53DBD recognizes and binds to the hundreds of p53 response elements in the genome to regulate the transcription of genes that put the brakes on cellular proliferation. On page 423, the study by Kitayner *et al.*[1] rationalizes the differential affinity of p53DBD for two classes of promoter sequences: those that are more frequently associated with cell cycle–arrest genes, and those that tend to regulate pro-apoptotic genes. The question of how the p53 stress response directs the cellular choice between temporary cell-cycle arrest or cellular suicide has been a long-standing one in the p53 field. The work by Kitayner *et al.*[1] suggests that part of this choice may result from a synergistic collaboration between the four p53DBDs within this tetrameric transcription factor and the spacing and physical properties of the high-affinity promoter sequences.

Since p53's discovery over 30 years ago, considerable effort has focused on the structure-function relationships of the p53DBD to understand how the full-length tetrameric protein might assemble on the DNA. El-Deiry *et al.*[2] first reported a p53 consensus-binding DNA sequence comprising two pairs of inverted repeats (5′-PuPuPuC(A/T)•(A/T)GPyPyPy-3′) that can be separated by 0–13 base pairs (bp)[2]. These variations in consensus-site architectures, referred to in Kitayner *et al.*[1] as type I (>0-bp insertions) and type II (no insertions; **Fig. 1**), were originally thought to have little impact on the biological activity of p53; however, more recent characterization of these genetic elements suggests otherwise. Kitayner *et al.*[1] compare a new crystal structure of four p53DBDs bound to a response element with two contiguous half-sites (essentially a class II site) with previous structures of the p53DBD bound to class I elements[3,4] and a covalently cross-linked p53DBD–DNA complex[5]. The new class II complex structure and that of a similar complex reported by Chen *et al.*[6] reveal two key features that were not seen or fully appreciated in the previous structures.

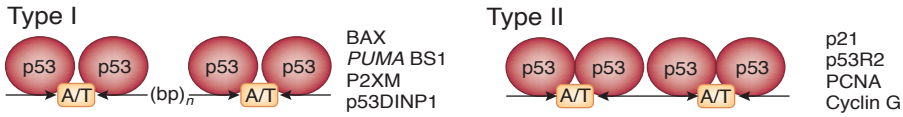

Figure 1 Schematic representation of type I and type II architectures. The differential affinities displayed by p53DBD for type I and type II response elements are proposed to be directly linked to differences in the quaternary configuration of the tetramer on the DNA. The lack of base pair insertions in the type II complex favors additional protein-protein interactions that stabilize the complex, and thus contributes to a tighter affinity between the two molecules.

First, whereas the current and previous structures all contained four p53DBDs arranged as pairs of dimers centered on each 10-mer half-site, the new, noncovalently linked class II structures of Kitayner *et al.*[1] and Chen *et al.*[6] have a much tighter association between p53DBDs at the dimer-dimer interface. Compared to type I complexes, these assemblies result in an increased buried surface area within and between p53DBD dimers. Kitayner *et al.*[1] posit that this may provide a structural explanation for why sequences that conform most closely to the type II format (for example, those with no insertions or that have fewer than two mismatches with respect to the consensus sequence) bind more tightly than those of the type I character[7,8]. Furthermore, the fact that lower-affinity, class II sequences are more frequently found in promoters of pro-apoptotic genes than those of cell cycle–arrest genes hints at the structural origins of the differential activity of p53 for these two types of promoters. The extensive protein-protein interactions among and between dimers are also consistent with the well-documented binding cooperativity of p53 subunits upon interaction with DNA.

Second, the structure of Kitayner *et al.*,[1] which is the highest-resolution structure yet for a p53–DNA complex, reveals the formation of Hoogsteen base pairs at the center of each half-site—the only significant departure from otherwise regular B-form DNA. This chemistry rationalizes the conservation and disposition of the A-T junction between inverted 5-mer repeats. A narrower minor groove at the A-T junction at the center of the p53 half-site, originally noted by Cho *et al.*, is involved in mediating tight interactions with key arginine residues in p53DBD[9]. Interestingly, in comparison to that of Watson-Crick B-DNA, the Hoogsteen base-pairing geometry accounts for an even narrower groove and a more pronounced acidic surface, which is highly complementary to the size and shape of the side chain of Arg248, a

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residue whose mutation accounts for roughly 10% of all p53-associated cancers. This residue has a fixed position in the complex structure and does not show multiple conformations as observed in type I complexes. Interestingly, Hoogsteen base pairs were not reported for the class II complex structure reported by Chen *et al.*[6] Instead, this structure, which contains a different consensus sequence, has an overall straight, B-form DNA except for a slight deformation at the A-T junction between half-sites and not between inverted repeats.

Many studies have characterized p53DBD-DNA interactions before. For instance, Cho *et al.*, who provided us with a 'quarter view' of the p53DBD–DNA complex, and a series of others, including previous work by Kitayner *et al.* and Tidow *et al.*, have refined and built upon this initial model[4,9,10]. However, the two most recent complex structures are an important advance in the field because they address a fundamental gap in knowledge concerning the assembly of the p53DBD tetramer on DNA and how this mode of binding correlates to the cooperative and differential binding affinity of p53DBD for its response elements.

Nevertheless, there are still many other questions that surface when one considers the mechanisms and implications of both type I and type II p53DBD interactions. First, it is well known that monomeric p53 has much lower affinity than tetrameric p53 for DNA and is inactive *in vivo*[11]. What role does the tetramerization domain have in promoting binding of the tetramer to class I and class II sites? Does it have a structural role, or is it simply a tether that facilitates the cooperative assembly of p53DBDs? The long linker between the DBD and tetramerization domains presumably limit the number of base pairs accommodated by type I DNA sites, but does the linker region contribute to class II complexes? Second, what effects do post-translational modifications have in the assembly of p53 subunits? For example, Lys120, a residue whose acetylation by hMOF and Tip60 leads to apoptosis[12,13], interacts with DNA in both the Chen *et al.*[6] and Kitayner *et al.*[1] type II complexes in a manner that appears to be incompatible with acetylation. Might acetylation of Lys120 therefore influence binding to different p53 response elements? Finally, does the differential exposed protein surface of a type II versus a type I complex lead to the recruitment of different binding partners at the p53 response elements? For example, it is thought that p53-induced apoptosis requires the recruitment of additional coactivators such as apoptosis-stimulating protein of p53 1 (ASPP1) and ASPP2 to the p53DBD[14]. Do ASPP proteins stabilize binding of p53 to type I sequences, perhaps overcoming the intrinsic lower affinity of p53 for these sites via the binding energy from protein-protein interactions?

Clearly, the p53 field has grown by leaps and bounds since Cho *et al.* determined the first crystal structure of a p53DBD in complex with DNA[9]. Future work should extend the dissect-and-build approach to include the p53 oligomerization domain, interacting proteins and post-translational modifications and/or should consider the role of histones and chromatin on DNA structure and p53 interactions[15]. Because many of the mutations responsible for p53-induced cancers localize to the p53DBD core, such analyses could help identify therapeutic strategies yet to be recognized.

COMPETING FINANCIAL INTERESTS
The author declare no competing financial interests

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Coil-in-to snRNP assembly and Cajal bodies

Je-Hyun Yoon & Roy Parker

Eukaryotic cells have numerous non–membrane bound bodies whose functions are often unclear. On page 403 of this issue, Strzelecka and colleagues provide evidence that the ability to form Cajal bodies increases the rate of small nuclear ribonucleoprotein (snRNP) biogenesis and/or function. This supports the hypothesis that some cellular bodies form to increase the rates of assembly of multicomponent cellular machines.

Eukaryotic cells have a plethora of subcellular bodies in the cytoplasm and nucleus, some of which are involved in RNA biology. These non–membrane bound structures include P bodies and stress granules in the cytoplasm as well as Cajal bodies, promyelocytic leukemia bodies and nucleoli in the nucleus[1,2].

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An unresolved issue for the study of many of these RNA-protein bodies is the function of the higher-order assembly of these bodies, as mutations that limit or affect the assembly of some of these bodies often do not yield strong phenotypes. For example, *Arabidopsis thaliana* or *Drosophila melanogaster* individuals lacking the coilin protein, a conserved component of Cajal bodies, are largely defective in Cajal body formation, but they develop normally[3,4]. Coilin appears to be more important in mice: coilin-knockout mouse lines show semilethality, with ~50% dying in gestation and the viable offspring showing fertility defects[5,6], although the biochemical basis for these phenotypes has not been detailed. Thus, an important goal is to develop easily manipulated experimental systems where defects in RNA-protein body assembly give strong phenotypes so that the role of assembly can be determined.

Cajal bodies are a good experimental system for studying the function of RNP-body assembly because their assembly is largely, but not entirely, dependent on the conserved coilin protein[2]. Cajal bodies are proposed to be involved in the biogenesis of RNA-processing complexes including snRNPs, and are highly enriched in snRNPs, in small Cajal body–specific RNAs