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Sliding into home: facilitated p53 search for targets by the basic DNA binding domain

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p53 is a unique DNA binding protein with two distinct DNA binding domains, the evolutionarily conserved central domain and the C-terminal basic DNA binding domain (BD domain). The presence of two separate DNA binding domains with distinct DNA binding properties distinguishes p53 from other DNA binding proteins. Transcription factors generally bind DNA with sequence specificity but not with DNA structure specificity. DNA repair proteins generally bind DNA with structure specificity without sequence specificity. Even in the p53 gene family, the BD domain is a unique feature of vertebrate p53 proteins that is absent in p63, p73 and p53 homologues in primitive species like squid, *Drosophila* and *Caenorhabdities. elegans*. A central question is why p53 requires two DNA binding domains for its role as a tumor suppressor.

Repression *Versus* Activation of p53 DNA Binding by the BD Domain

Whereas the function of the central domain has been well defined as the region that binds sequence-specific DNA (SS-DNA), the biological function of the BD domain has been a subject of debate since its identification as a separate DNA binding domain. [1] Initially, the BD domain of p53 was postulated as a conformational regulatory mechanism to repress the SS-DNA binding activity of the central domain, based on evidence that p53 binding to its consensus DNA is activated by multiple means: truncation of the entire basic domain in p53Δ30 (360–393); replacement of the basic domain by alternative splicing; phosphorylation at 376 and 378 by protein kinase C or cyclin-activated kinase; acetylation at 373 and 382 by p300/CBP; and association with other factors like c-Abl and PAb421 antibody, which recognizes an epitope between 370 and 378 of p53 protein. The conformation model is also favored in a more recent study with purified acetyl p53 showing that acetylation of p53 enhanced binding to SS-DNA detectable by chromatin immunoprecipitation (ChIP).[2] However, the conformation models have been challenged by nuclear magnetic resonance analysis arguing against conformational change-induced p53 binding to DNA. The apparent BD domain repression of central domain-mediated SS-DNA binding can be explained by interference with BD domain-mediated binding to non-sequence-specific DNA (NS-DNA). Evidence for this is the ability of BD-specific antibody PAb421 to block p53 binding to NS-DNA.[3,4] Furthermore, the BD domain has been demonstrated to have a favorable effect on p53 binding to SS-DNA in the context of long stretches of DNA and chromatin-assembled DNA,[5] nonlinear stem–loop DNA, and bent DNA.[6]

The BD Domain Favors p53 Binding to Genomic Targets

In an attempt to resolve apparent discrepancies between the BD domain as an enhancer or repressor of SS-DNA binding, Liu *et al*. [7] compared DNA binding activities of wild-type (wt) p53 and p53as, a physiological variant of p53 with altered DNA binding properties due to replacement of the BD domain by alternative splicing. Unlike p53as, p53 binding to SS-DNA is severely compromised by the presence of a subset of NS-DNA that is also altered in its structure-specific DNA as competitor, such as poly-dIdC or nonspecific plasmid DNA.[3,4] However, p53 binding to endogenous DNA targets is far more efficient than p53as, as measured by ChIP of the *CDKN1A* promoter encoding the $p21^{Waf1}$ cyclin-dependent kinase inhibitor.[7] Kinetic studies showed that p53 binds to the *CDKN1A* promoter earlier than p53as. However, both p53 and p53as proteins are able to achieve similar maximal binding at later time points. Cells containing the p53as that lacks the BD domain exhibited significantly delayed (at least 4 h) binding to the endogenous *CDKN1A* promoter compared to p53 with intact BD domain. At the same time as our study, McKinney *et al*.[8] and McKinney and Prives[9] demonstrated that the BD domain is required for efficient binding to endogenous p53 targets and transactivation. Previous studies to compare transcriptional activities between p53 and p53Δ30 have drawn different conclusions about the role of the BD domain in transactivation. A number of studies have shown that the last 30 amino acids are dispensable for p53-mediated transactivation.[10] Others favor the synergistic role of the BD domain in transactivation as demonstrated with the temperature-sensitive p53as mutant, *in vitro* transcription of *CDKN1A* chromatin, and the yeast reporter system.[11] Discrepancies could arise because of differences in the level of p53 protein as well as the time points of the analysis. As demonstrated in our dynamic studies, the favorable role of the BD domain in transactivation is seen early after protein induction and becomes negligible at later time points at which the p53 level is high. Indeed, the previous studies that showed no difference in transactivation activities between p53 and p53Δ30 analyzed time points between 48 and 72 h after transient transfection.[10]

Facilitated Search of Genomic Targets by the BD Domain

The more rapid binding of p53 than p53as as to the endogenous *CDKN1A* promoter raises the question of why the BD domain that interferes with p53 binding to its consensus DNA in the presence of competitor NS-DNA *in vitro* is required for efficient binding to p53 target sites in the genome *in vivo*. As the p53 target sites in cells are embedded in 3 billion base pairs of genomic DNA, p53 binding to endogenous target DNA should be far weaker than that of p53as if NS-DNA binding interferes with p53's SS-DNA binding. Two models that could answer this question are a conformational model and a facilitated search model. The conformation model of Kim and Deppert[11,12] proposes that p53 binding to SS-DNA is activated by the C-terminal binding to altered DNA conformation at specific binding sites of the target genes. This model is based on the observation that p53 binding to SS-DNA can be enhanced by DNA structural changes in the SS-DNA, such as non-B DNA structure, stem–loop structure, and DNA bending. Given that the conformational changes can be induced by DNA damage, sensing altered DNA conformation in the SS-DNA could be a mechanism to activate p53 in response to DNA damage. However, a model of locally altered DNA structure does not explain how a finite number of key p53 SS-DNA sites undergoing conformational changes upon DNA damage are being recognized by p53 protein within the 3×10^9 bp genome. The model also does not reconcile the observation that both p53 and p53as are bound similarly to the endogenous *CDKN1A* promoter (detectable by ChIP) at later times,[7] implying comparable binding affinity to the *CDKN1A* promoter.

In the facilitated search model proposed by von Hippel and Berg,[13] DNA binding proteins (such as DNA repair proteins that sense DNA structure) do not bind to their specific target sites directly, but rather through two equilibria, first from their free state to binding randomly and non-sequence-specifically to genomic DNA (NS-DNA), followed by sliding or intersegment transfer on chromatin DNA to their target S-DNA binding sites (free state ⇆ NS-DNA bound → S-DNA bound). As calculated by different mathematic models, the specific binding rate facilitated by NS-DNA could be 10^6 or more faster than that by random search.[14] The NS-DNA binding facilitated model has been indirectly supported by the evidence from *in vitro* DNA binding kinetic studies with procaryotic DNA binding proteins such as restriction enzymes, lac repressor, restriction methylases, and T4 endonuclease as well as structural comparisons between NS-DNA-bound state *versus* S-DNA-bound state using restriction enzyme *Bam*HI and lac repressor.[14] By time-lapse atomic force microscopy, Jiao *et al.*[15] provided evidence to support p53 sliding on immobilized DNA strands. Presentations at the 12th International p53 Workshop addressed the issues of p53 search for SS-DNA and the requirement for the C-terminal BD domain. We showed that the p53 BD domain facilitates a more rapid search for SS-DNA than p53as molecules lacking the BD domain and that this occurs through NS-DNA binding.[7] McKinney *et al.*[8] and McKinney and Prives[9] showed that p53 is capable of sliding on the DNA strands as measured by competition assay with biotin-flanked DNA in the presence or absence of streptavidin. Furthermore, they demonstrated that the BD domain is required for efficient p53 linear sliding along DNA.

Binding to Genomic DNA as Intermediate Step in the Search for Specific Targets

In the facilitated search model applied to p53, there are two equilibria for p53 binding to its SS-DNA (free p53 ⇆ NS-DNA bound → SS-DNA bound), one from free state to NS-DNA-bound state and the other from NS-DNA-bound state to SS-DNA-bound state by means of sliding on the DNA track (Figure 1). As sliding on a DNA track could be highly efficient,[16] initial random binding to genomic NS-DNA could be a rate-limiting step for searching. Using an unbiased global ChIP assay, we demonstrated that the BD domain facilitates p53 binding to genomic DNA from its free state.[7] Sequence analysis of p53-bound genomic DNA segments isolated by the ChIP revealed that only 5% of p53-bound genomic DNA fragments contained p53 consensus sites. The majority of p53-bound genomic DNA segments were randomly distributed NS-DNA. This was similar to the results of a recent microarray analysis of p53 binding to genomic DNA of chromosomes 21 and 22 in which only 2% of p53 binding events were associated with p53 consensus sites.[17] A plausible explanation for the presence of the abundant

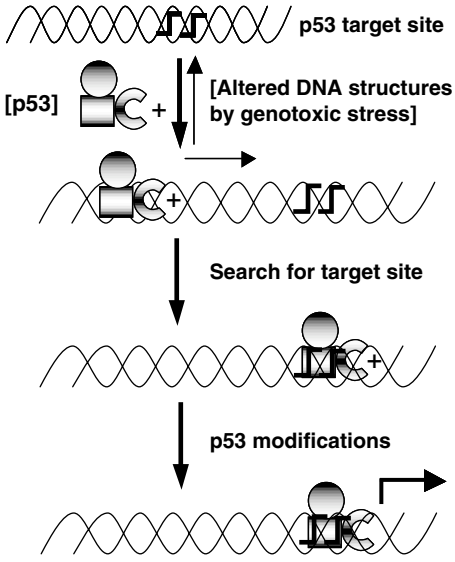

Figure 1 A model of p53 searching for specific target sites facilitated by the BD domain. The equilibrium of p53 from free state to genomic DNA-bound state is driven by increasing concentration of p53 with intact BD domain (denoted by + symbol) and increasing DNA structural alterations following cellular stress (denoted by expanded DNA). The p53 with BD domain senses global changes in DNA and rapidly binds DNA non-sequence-specifically. Once bound to the genomic DNA, p53 slides along the DNA track or transfers between strands to find its specific targets in the genome. p53 isoforms and p53 family members that lack the BD domain bind DNA without structural preference and take longer to find and transcriptionally activate the p53 target genes. Optimum p53 response invokes a dynamic model in which the positively charged BD domain facilitates rapid binding of p53 to altered DNA and search for specific target sites in cells under stress, then undergoes modifications (denoted by the absence of + symbol) at the target promoter site by p300 or other factors for transcriptional activation

NS-DNA-bound by p53 or p53as is that it represents a 'snapshot' of their sliding and/or intersegment transfer within the genomic DNA. Although it remains to be confirmed with high-throughput global ChIP sequence analysis of DNA-bound by not only p53 but also other specific DNA binding proteins, the data provided represent the first *in vivo* evidence to support NS-DNA binding as an intermediate state in searching for specific sites in the eucaryotic system.

So far there is no experimental evidence to resolve how the BD domain facilitates p53 binding to genomic DNA. However, a unique feature of p53 as a DNA binding protein is that p53 binds to a variety of DNA structures mediated through the BD domain.[11] In general, weak NS-DNA binding is an intrinsic feature associated with SS-DNA binding proteins. However, it is not common for an SS-DNA binding protein to bind NS-DNA with structure specificity. Based on the property of the p53 BD domain in binding to a variety of DNA structures, we speculate that the mechanism by which the BD domain enhances p53 binding to genomic DNA is through its binding to altered DNA structures in the genome under genotoxic stress. It has been demonstrated that only a few DNA strand breaks in a cell are sufficient to activate the majority of ATM proteins within minutes.[18] This suggests that local DNA damage may generate changes that can be sensed globally in chromatin. The BD domain, by sensing these global changes in chromatin structure, favors p53 transition from free state to genomic DNA-bound state. Therefore, the BD domain binding to altered chromatin DNA structure is a mechanism to synergize with p53 protein induction to mediate rapid response to DNA damage.

The BD Domain for Rapid Response to Halt Cell Cycle

As a guardian of genome, p53 needs to mediate a cell cycle 'emergency brake' in order to prevent DNA-damaged cells from entering S phase where potential genetic alterations could be generated and propagated to subsequent generations. Sensing altered DNA structure through its basic domain could be a mechanism unique to p53 to facilitate rapid transactivation of its downstream genes in response to genotoxic stress. Although the BD domain contributes only a few hours advantage for p53 to mediate transactivation, those few hours may be critical for cell cycle arrest before entry into S phase, particularly for cells already past the restriction point. As rapid growth arrest in response to DNA damage is critical to prevent the development of oncogenic mutations, the loss of p53 binding to NS-DNA could be an oncogenic event to render genomic instability in tumor cells. Indeed, there is a naturally occurring p53 mutant (360-del) with a deletion of the entire BD domain identified from a nonpolyposis colorectal cancer.[19] This mutant has a 1-bp deletion at codon 360 that creates a stop codon resulting in the substitution of the last 33 amino acids with 7 different amino acids. This mutant is virtually identical to Δ30, an artificial p53 mutant widely used to study the function of the p53 C-terminal domain. Transformation activity of p53 without the BD domain has been evaluated *in vitro.*[10] Although Δ30 showed no difference from wt p53 in inhibiting the colony-forming efficiency of SAOS-2 cells, its ability to suppress transformation of rat embryo fibroblasts by activated H-Ras and HPV16 E7 genes is significantly compromised.[10] This indicates that the BD domain plays a critical role in the emergency cell cycle checkpoint to prevent malignant transformation. The generation of mice with targeted deletion or alternative splicing of the last 30 amino acids will be essential to evaluate the role of the BD domain in the cell cycle checkpoint and tumor suppression.

Summary and Future Challenges

At the 12th p53 Workshop, evidence was presented that rapid search and binding of p53 target promoters is facilitated by the p53 BD domain[7] and that p53 can slide along DNA strands, mediated by the BD domain. [8] Other evidence presented at the meeting, summarized by Braithwaite *et al.*,[20] indicated that certain isoforms of p53 lacking the BD domain (and the N-terminal acidic domain) had dramatic differences in promoter sequence specificity and that p53 proteins can already be present on endogenous promoters before cellular stress, as presented by Emerson and Espinosa.[21] These and other published findings expose unanswered questions. Does the BD domain facilitated search seen for the *CDKN1A* promoter extend to apoptotic and other promoters or is it limited to essential early cell cycle arrest genes? Is the p53 that activates transcription in response to stress a newly activated subset of p53 protein that binds to DNA and replaces resident p53 protein? The p53 BD domain can be extensively modified by acetylation, phosphorylation, ubiquitination, neddylation, sumolation, and methylation. It is largely unknown whether p53 searching for target genes is regulated by these post-translational modifications. We have evidence to show that p53 binding to the *CDKN1A* promoter is delayed by p53 with acetyl-mimic mutations (unpublished data). This is contrary to the notion that p53 acetylation enhances its transactivation activity.[5] The evidence from Dornan *et al.*[22] indicates that p53 binding to specific DNA occurs before its acetylation, as p53 acetylation is enhanced by its binding to specific DNA. Thus, we speculate that p53-mediated transactivation is a dynamic process with sequential-specific modifications to regulate p53 activities. Modifications at the C-terminus are excluded in the initial targeted searching, which requires the positively charged BD domain. Once bound to the promoter of its target genes, the positive charge in the p53 C-terminus is neutralized by acetylation to activate transcription. Additional modifications may occur to specify interactions with other proteins, to modulate transcription, and to regulate p53 turnover. Refinements to the model will require coordinated studies of *in vitro* and in-cell DNA binding of endogenous promoter sites by different p53 isoforms and of p53 BD domain post-translational modification states and p53 family members. They will require early time courses, including target promoters of growth arrest and apoptosis-associated p53 downstream genes and concurrent assessment of DNA-bound p53 forms and active transcription from each promoter site. Definitive discrimination between 'sliding into home base' and interstrand transfer or 'jumping' from one 'base' (position on DNA) to another as the mechanism of facilitated search by DNA binding proteins for their specific targets will be even more challenging. However, the p53 protein, with its two

separate DNA binding domains that specialize in sensing DNA structure *versus* DNA sequence, provides a unique molecule to test the more global strategies for how DNA binding proteins find their targets.

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