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The complex interactions of p53 with target DNA: we learn as we go

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Abstract: The most import biological function of the tumor suppressor p53 is that of a sequence-specific transactivator. In response to a variety of cellular stress stimuli, p53 induces the transcription of an ever-increasing number of target genes, leading to growth arrest and repair, or to apoptosis. Long considered as a "latent" DNA binder that requires prior activation by C-terminal modification, recent data provide strong evidence that the DNA binding activity of p53 is strongly dependent on structural features within the target DNA and is latent only if the target DNA lacks a certain structural signal code. In this review we discuss evidence for complex interactions of p53 with DNA, which are strongly dependent on the dynamics of DNA structure, especially in the context of chromatin. We provide a model of how this complexity may serve to achieve selectivity of target gene regulation by p53 and how DNA structure in the context of chromatin may serve to modulate p53 functions.

Key words: tumor suppressor p53, sequence-specific DNA binding, DNA conformation, chromatin, chromatin remodeling.

Résumé : Le rôle le plus important du suppresseur tumoral p53 est d'être un transactivateur spécifique d'une séquence. En réponse à divers stress cellulaires, p53 induit la transcription d'un nombre toujours croissant de gènes cibles, entraînant soit un arrêt de la croissance et la réparation des dommages, soit l'apoptose. Longtemps considérée comme une protéine « latente » ne se liant à l'ADN qu'à la suite de son activation par une modification C-terminale, de récents résultats apportent un puissant élément de preuve indiquant que la liaison de p53 à l'ADN dépend fortement d'éléments structuraux de l'ADN cible, et que p53 est une protéine latente seulement lorsque la structure de l'ADN cible ne comporte pas un code signal donné. Dans cette revue, nous discutons des éléments de preuve en faveur d'interactions complexes entre p53 et l'ADN, fortement dépendantes de la dynamique de la structure de l'ADN, spécialement dans la chromatine. Nous présentons un modèle montrant comment cette complexité servirait à rendre sélective la régulation des gènes cibles par p53 et comment la structure de l'ADN dans la chromatine servirait à moduler les fonctions de p53.

Mots clés : suppresseur tumoral p53, liaison à une séquence spécifique de l'ADN, conformation de l'ADN, chromatine, remodelage de la chromatine.

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Introduction

Since its discovery in the late 70s (Lane and Crawford 1979; Linzer and Levine 1979), p53 remains in the centre of unabated attention in the field of cancer research. The past decade has revealed an extreme functional diversity of p53, the key to its central role in restraining tumor formation and progression. Amongst the many activities of p53, its function as a sequence-specific transactivator is the best studied and probably the most important one. A prerequisite for the transcriptional activity of p53 is its ability to interact in a sequence-specific manner with p53-responsive elements in promoters of target genes, a hallmark of functionally active p53 (Levine 1997). The central role of this activity for the functions of p53 is underscored by the fact that loss of sequence-specific DNA binding (SSDB) impairs p53-dependent growth suppression and is the most characteristic feature of mutant p53 proteins (Cho et al. 1994; Vogelstein et al. 2000; Soussi and Beroud 2001; Bullock and Fersht 2001). The mode of SSDB seemed clear for nearly a decade until recently, when SSDB of p53 again became a highly debated issue. This review will focus on SSDB of p53. We will discuss SSDB in the light of recently discovered aspects that led to the realization that the interactions of p53 with DNA are more complex than antici-

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pated. Furthermore, understanding the DNA binding activity of p53 requires that we consider the dynamic nature of DNA, especially in the context of chromatin. In addition, the role of DNA as a modulator of p53 functions will be addressed.

Sequence requirements for SSDB: when perfect is not good enough

The premises for p53 being a sequence-specific DNA-binding protein were laid by early observations demonstrating that p53 exhibits double-stranded DNA binding activity (Lane and Gannon 1983), and that such binding is reduced in mutant p53 proteins (Steinmeyer and Deppert 1988). The sequence requirements for DNA binding were determined in 1991 as the result of a systematic search for specific sequences in genomic DNA that were bound by wild type p53 in vitro (Kern et al. 1991). Soon afterwards a consensus for p53 cognate motifs (p53CON) was established, comprising the sequence 5′-PuPuPu-C(A/T)(A/T)G-PyPyPy-3′ (el-Deiry et al. 1992). The initial DNA binding studies revealed the importance of two parameters required by a given sequence to be recognized by p53 as a specific binding site: compliance to the p53CON sequence, and the presence of more than one cognate motif (also called half-sites) (Wang et al. 1995). The explosive discovery of p53 regulated genes during the past decade allowed the identification of a large number of "natural" p53 response elements (PREs), mediating transcriptional activation of p53 target genes. Functional PREs exhibit a sequence heterogeneity that is characterized by a significant degree of divergence from the p53CON sequence. Typically, in most known PREs (a comprehensive list of known PREs can be found in Qian et al. 2002) only one half-site, if at all, corresponds well to the consensus, whereas the other half-site(s) contains a varying number of non-complying bases. Interestingly, initial studies in which the influence of sequence variations on p53 DNA binding was examined revealed that some sequences perfectly matching the p53CON sequence were not bound by p53 (Halazonetis and Kandil 1993). These surprising observations suggested that compliance to the consensus sequence might not necessarily suffice for a given sequence to function as a p53 binding site. Such a view was supported by later studies in which the potential to drive p53-dependent transactivation, a function directly dependent on SSDB, was compared among individual PREs (Qian et al. 2002; Martinez et al. 1998; Thornborrow and Manfredi 1999; Zhang et al. 1994; Inga et al. 2002). From these studies it appears that different PREs determine the strength of promoter activation by p53, in addition to the impact of the specific promoter context and the action of auxiliary cofactors. Interestingly, however, the difference in the ability of PREs to mediate p53 transactivation does not correlate well with the degree of concordance with the p53CON sequence (Qian et al. 2002; Contente et al. 2002; Inga et al. 2002). Perhaps the most clear demonstration of such a "more-than-consensus" phenomenon comes from a recent study, in which p53 response elements mediating SSDB and induction of the pro-apoptotic *pig3* gene by p53 were identified in vivo and in vitro. It was found that a polymorphic microsatellite repeat $(TGYCC)_n$, located within the transcribed region of the *pig3* gene, mediates transactivation by p53, while the previously identified promoter PRE resembling the p53CON sequence (Polyak et al. 1997) is dispensable for transactivation (Contente et al. 2002). It should be noted that the search for functional PREs in many studies was based on the assumption that elements mediating transcriptional activation by p53 must comply with the consensus sequence. However, it seems that such a formal approach might in fact obscure the identification of some functional PREs, especially if the gene-in-question contains multiple regions that formally comply with the p53CON sequence, but which are unable to mediate transactivation by p53 in the context of chromatin. This would explain why some putative p53 specific promoter elements isolated from p53 regulated genes fail to support transactivation of the gene by p53, even though they contain sequences that conform to the p53CON sequence and are bound by p53 in vitro (Igata et al. 1999; Schmidt et al. 1999; Qian et al. 2002; Thornborrow and Manfredi 1999). Indeed, *pig3* is not the only p53-regulated gene for which a discrepancy between the putative and the true functional PRE has been revealed. It was reported recently that another pro-apoptotic target gene of p53, *bax1*, is regulated by an intronic PRE (Thornborrow et al. 2002) and not by the previously identified p53CON-like sequence in the promoter region (Miyashita and Reed 1995) that was thought to be a functional PRE.

p53's "latency": lessons from the past and concerns of the present

An important feature of p53 as a DNA-binding protein is what is known as "latency" of its DNA binding potential (Hupp et al. 1992; Hupp and Lane 1994*a*). Most impressively manifested in gel-shift experiments, latency of p53 appears as the lack of appreciable DNA binding if the C-terminus of p53 is physically intact or unmodified. The C-terminus is an important administrator of SSDB of p53, as it bears two functional domains that are crucial for the ability of p53 to bind DNA both specifically and non-specifically: the oligomerization domain (amino acid residues 320–356) and the C-terminal regulatory domain (CRD, amino acid residues 363–393). p53 binds DNA as tetramer, and the ability to form tetramers is crucial for the transcriptional activity of p53 (reviewed in Chene 2001). Already early DNA binding studies had revealed that some modifications, such as the removal of the last 30 C-terminal amino acid residues, binding of the monoclonal antibody PAb421 (specific for an epitope located in the p53 C-terminus), or phosphorylation of some C-terminal amino acids residues, strongly enhance SSDB in vitro (Hupp et al. 1992; Hupp and Lane 1994*a*, 1994*b*). These findings led to the proposal that SSDB of p53 is normally inhibited by the C-terminus, presumably by keeping the DNA binding domain of the protein in a "locked" conformational state inherent to native ("latent") p53 (Hupp et al. 1992). It has been postulated that activation of SSDB might involve a conformational switch that converts "latent" p53 into its DNA binding active counterpart, "activated" p53. The finding that some post-translational modifications within the CRD that are also found on transcriptionally active p53 in vivo (Hupp and Lane 1995; Wang and Prives 1995; Gu and Roeder 1997), enhance SSDB of cellular p53 in vitro supported the idea. Such a mode for SSDB of p53

was compatible with the conformational flexibility of the p53 protein, revealed by early studies using conformation-dependent antibodies (Milner 1984; Milner 1995). Furthermore, structural and biochemical studies had revealed that the oligomerization domain of p53 exhibits a dihedral symmetry and therefore may be instrumental for conformational switches of the protein (Jeffrey et al. 1995; Waterman et al. 1995; McCoy et al. 1997). Further supporting the view of a "latent" p53, it was found that the inhibitory effects of the C-terminus on SSDB could be relieved by deletion of an N-terminal region encompassing the proline-rich domain (Muller-Tiemann et al. 1998). These findings suggested that inter-domain interactions between the CRD and the N-terminal region spanning residues 80–93 might be the structural basis for stabilizing native p53 in the latent conformation incompatible with SSDB. Thus a wealth of biochemical data supported by structural analysis left little doubt that the activation of "latent" p53 is the crucial step in initiating SSDB of p53, and thereby its transcriptional response. It was not until very recently that the concept of p53 latency and activation of SSDB as a mode for initiating a p53 transcriptional response was questioned after nearly a decade of dominance in the field.

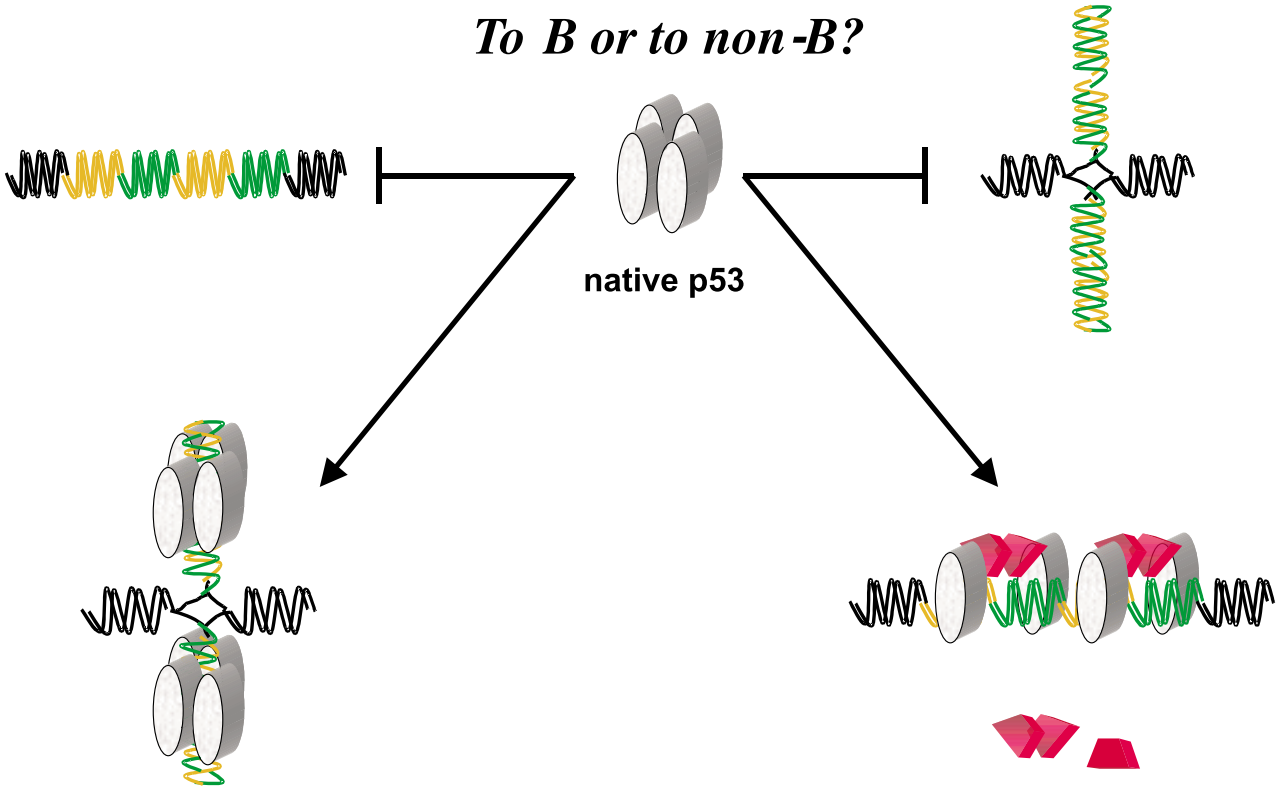
The initial hints disturbing the view of a latent p53 came from studies in which SSDB of p53 was analyzed applying alternative methods, such as DNase protection assay. It was found that the same p53 preparations that did not bind DNA without activation in a band shift assay showed strong binding to the same binding motif in larger DNA molecules when SSDB was analyzed by DNase protection assays (Kim et al. 1999; Cain et al. 2000). Thus activation of p53 for SSDB was not required for its interaction with more complex DNA molecules, such as large DNA fragments or plasmid DNA. The finding strongly indicated that p53 is an active, and not a latent, DNA-binding protein. This view was further supported by a recent study in which SSDB of p53 was examined with in vitro assembled chromatin containing the p53-responsive promoter of the *p21/waf1* gene (Espinosa and Emerson 2001). It was shown that p53 response elements embedded in a chromatin context were bound by p53 in the absence of additional activation. Importantly, binding by a non-activated p53 induced transcription of the *p21* promoter (Espinosa and Emerson 2001). Thus, after being long seen as a DNA binding inactive protein, latent p53 suddenly appeared as an excellent DNA binder that needs no activation. In vivo studies correlating SSDB of p53 with its transcriptional activity using chromatin immunprecipitation (ChIP) provided further evidence that is incompatible with the view of a "latent" p53. It was found that cellular, non-activated p53 binds to p53-regulated promoters with kinetics corresponding to an active, and not a latent, DNA-binding protein (Szak et al. 2001; Kaeser and Iggo 2002). Even more surprising was the finding that the C-terminus was required for the efficient binding of p53 to both naked DNA and to in vitro assembled chromatin (Espinosa and Emerson 2001), which is in contrast to the inhibitory role of the C-terminus postulated by the "activation" model. Underscoring the physiological significance of these findings, deletion of the CRD had deleterious effects on the p53 transactivation potential, as the coactivator p300 strongly augmented transactivation of wild type p53, but not of a deletion mutant lacking the CRD (Espinosa and Emerson 2001). Similarly, deletion of the CRD weakened the transactivation potential of p53 in a yeast system (Inga et al. 2002), further supporting the view that the C-terminus may positively regulate p53-dependent SSDB and transactivation. Thus, the requirement of the C-terminus for the interaction of p53 with its cognate sites either in naked DNA or in chromatin-assembled DNA argues against the previously proposed inhibitory role of the C-terminus. The findings raised the concern that the whole concept of latency of p53 in SSDB and transactivation needs to be reconsidered (Ahn and Prives 2001). However, before abandoning the concept of p53 "latency" as an experimental artifact, one has to consider that it still might reflect some important aspect of the interaction of p53 with DNA that becomes manifested only under specific experimental conditions.

The impact of DNA conformation on SSDB: to B or to non-B?

Considering the obvious discrepancies and apparent contradictions, it seems that some critical parameters required for efficient SSDB by p53 in the various assay so far had not been understood and hence were not considered. An emerging view is that such parameters may be contained within the target DNA itself, which appears as a modulator of SSDB and not just a landing platform for the p53 protein. Indeed, alternate patterns of SSDB become apparent in experiments that use different types of DNA templates. SSDB needs activation with small DNA molecules such as short double-stranded oligonucleotides, commonly used in band shift assays. In contrast, activation of SSDB is no longer relevant with more complex DNA substrates such as large naked DNA or chromatin.

Is it only the size of DNA that matters? Recent studies provided the answer to this question and revealed that the phenomenon is more complex, but also much more interesting than that. As early 1997, Kim et al. (1997) demonstrated that the DNA binding activity inherent to native (unmodified) p53 is masked, if the PREs are presented in a linear (B-) DNA conformation. However, as many PREs display an internal symmetry, they also are capable of assuming a non-B DNA conformation, e.g., under conditions of superhelical stress. It appears that the presentation of PREs in a non-linear (stem-loop or bent DNA) conformation endows p53 with the capability to strongly and specifically bind such target DNA without the need to activate the protein (Kim et al. 1997; Gohler et al. 2002; McKinney and Prives 2002). Even short oligonucleotide DNA, when artificially stabilized in a non-linear DNA conformation, allows strong SSDB by p53 that is detectable by a variety of assays, such as band shift, DNase protection or electron microscopy (Kim et al. 1997; Gohler et al. 2002). Importantly, binding to non-linear PREs remains sequence-dependent, indicating that the presence of a cognate motif is the major determinant of specificity, whereas structural features within PREs modulate the affinity of binding (Gohler et al. 2002; McKinney and Prives 2002). These findings explain the observation of discrepant patterns of SSDB when either short (linear) oligonucleotide DNA or structurally more complex DNA is used in various assays. It rather is the level of structural complexity rising

Fig. 1. Differential modes of p53 interaction with linear or non-linear DNA. Native p53 is a DNA-binding active protein that binds with high affinity to its cognate sites when they are present in a non-linear DNA conformation. Yellow and green regions correspond to individual half-sites comprising full PREs. Covalent or non-covalent modifications at the C-terminus (depicted as red blocks) render p53 capable of binding to linear DNA.



with increasing DNA size that is responsible for the different results observed. Short double-stranded DNA molecules are rigid and conformationally more uniform than large DNA molecules. As the size of DNA increases, so does its plasticity and the potential to engage into intra- and intermolecular DNA interactions. Thermodynamically less favorable compared with Watson–Crick base pairing in the canonical DNA duplex, such interactions are transient and may be influenced by multiple factors such as DNA size, sequence context, or electrochemical properties of the environment. In more complex DNA, such as supercoiled naked DNA or chromatin DNA, specific structural profiles may be further influenced either by supercoiling or by chromatin remodeling activities maintaining the structural fluidity of the chromatin (reviewed in Kingston and Narlikar 1999). Local perturbations in the DNA duplex would endow DNA with specific structural "landmarks" that may facilitate or inhibit p53 binding. Indeed, it was reported that p53 preferentially binds to supercoiled DNA (Palecek et al. 1997; Palecek et al. 2001; Brazdova et al. 2002), and that the affinity of p53 for PREs directly correlates with the degree of the bending angle or sequence-dependent kinks in specific binding sites (Nagaich et al. 1997; Balagurumoorthy et al. 2002). Thus, it appears that p53 behaves as a "latent" protein only when structural "signature" elements are missing in the target DNA. If however the requirement for the presence of structural cognate elements is fulfilled, SSDB occurs efficiently in the absence of additional activation. Strikingly different modes of p53 binding to specific DNA in linear or non-linear form of DNA are obtained when the C-terminus of p53 is either deleted or modified non-covalently, e.g., by the addition of the antibody PAb421. While strongly enhancing p53 binding to linear DNA templates, such modifications abolish non-linear DNA binding, indicating that the p53 C-terminus regulates the interaction with structurally distinct forms of target sites (Gohler et al. 2002; McKinney and Prives 2002). Which mode of DNA binding is exerted by transcriptionally active p53 in vivo is unknown. However, the striking similarity between SSDB patterns manifested with non-linear DNA in vitro and those found in vivo or with transcriptionally active chromatin templates strongly suggests that transactivation may be mediated by p53 binding to PREs present a non-linear DNA form.

An important implication of the finding that p53 exhibits various modes of binding to structurally distinct DNA forms is the possibility that at a given time and physiological state the cellular p53 will not bind to all PREs simultaneously. Instead, only those PREs that exhibit a structural architecture that is compatible with SSDB of p53 in a certain conformational state will be preferentially bound. Post-translational modifications of the p53 protein would coordinate such a DNA conformation-dependent recognition of PREs by modulating the equilibrium among different sub-populations in the pool of cellular p53 that either have high affinity to linear DNA (C-terminally modified p53) or preferentially bind to non-linear DNA (C-terminally unmodified p53) (Fig. 1).

Although the physiological significance of the dependence of SSDB on DNA conformation still has to be elucidated,

the exciting possibility exists that p53 binding to different PREs might be regulated by their presentation in alternate conformational states. This would endow transcriptional control by p53 with high plasticity and provide means for the selective activation of different promoters by p53, depending on the conformational status of PREs. The large number of functionally distinct genes that can be induced by p53 via SSDB can be roughly divided in two classes: cell cycle regulators and apoptosis-associated genes (Zhao et al. 2000). Mechanisms ensuring a selective induction of p53-regulated genes must exist to avoid conflicts among incompatible signaling pathways, caused by an ill-timed induction of genes with opposing functions. How selective activation of target genes is achieved is not yet understood, but it most likely involves versatile mechanisms that all converge on the interaction of p53 with PREs. DNA structure-dependent SSDB thus may be one plausible mechanism by which the responsiveness of PREs to p53 may be regulated temporally and corresponding to the needs of the cell. In support of this hypothesis, preliminary evidence indicates that different DNA structures may exert different effects on SSDB. For example, we observed that an asymmetric secondary structure was formed in the *mdm2* promoter in supercoiled DNA that inhibited SSDB of p53 in vitro (Kim et al. 1999). In contrast, DNA supercoiling enhanced SSDB to the *p21* promoter (E. Palecek, personal communication) suggesting that a favorable structure may be formed by DNA supercoiling in the *p21* promoter.

At present, there is no evidence available as to the conformation of PREs bound by p53 in vivo and it may be difficult, if not impossible, to directly assess the impact of structural alterations in DNA on the interaction of p53 with PREs in the context of a dynamically changing chromatin. However, several considerations indicate that DNA conformation-dependent binding may indeed be one of the mechanisms governing the transcriptional activity of p53. (*i*) SSDB observed with non-linear PREs in vitro shows striking similarities to the binding pattern of p53 observed either in vivo or with in vitro assembled chromatin, in so far as deletion of the C-terminus or addition of the antibody PAb421 strongly inhibits binding of p53 (Kim et al. 1997; Gohler et al. 2002; McKinney and Prives 2002; Espinosa and Emerson 2001). (*ii*) Structural transitions in chromatin occur concomitantly with DNA replication or transcription, processes that involve a local separation of DNA strands and may thereby facilitate formation of secondary structures in DNA (van Holde and Zlatanova 1994; Ward et al. 1990; Pearson et al. 1996). It was found that blocking DNA replication reduced transactivation of some but not all target genes by p53, even though the transcriptional activity of the p53 protein remained unaffected (Gottifredi et al. 2001). One plausible explanation for such a selective inhibition may be that structural transitions in DNA are blocked when replication forks are stalled, thereby preventing efficient binding of p53 because of the inability of some PREs to adopt a favorable conformation. (*iii*) p53 interacts with several DNA topology-dependent proteins such as the cruciform binding protein HMG1, topoisomerases, and various proteins of chromatin remodeling complexes (Jayaraman et al. 1998; Gobert et al. 1999; Cowell et al. 2000; Wang et al. 2001; Murphy et al. 1999; Lee et al. 2002). Although the physiological significance of such interactions in vivo awaits elucidation, it was shown that HMG1 can strongly enhance SSDB of p53 in vitro by bending its target DNA (McKinney and Prives 2002). These findings suggest that cellular DNA topology-modulating proteins may play an instrumental role in SSDB of p53 by modulating DNA structure. In line with such an idea is the finding that p53 induces a transient, subnuclear redistribution of topoisomerase I, which coincides with the transcriptional response mediated by p53 (Mao et al. 2002). Normally, topoisomerase I is predominantly localized in the nucleoli, where it maintains high rates of rRNA synthesis. Mao et al. (2002) demonstrated that wild type p53 induces the release of topoisomerase I from nucleoli into the nucleoplasm. The finding that transcriptionally inactive mutant p53 failed to cause such a subnuclear redistribution of topoisomerase I suggests that topoisomerase I may be involved in the regulation of the transcriptional response mediated by p53. (*iv*) Chromatin remodeling and chromatin modifying factors modulate the transcriptional response mediated by p53, and one of the mechanisms appears to involve local changes of DNA topology near functional PREs (discussed in more details in the next section).

DNA binding of p53 and chromatin modifying activities

Cells are equipped with a specialized machinery of chromatin-modifying complexes that maintain the fluidity of chromatin structure and function coordinately with transcription (reviewed in Kingston and Narlikar 1999 and Horn and Peterson 2002). The strong impact of DNA structure on p53 SSDB in vitro predicts that the ability of p53 to bind its target sites will be strongly influenced by factors capable of modifying chromatin structure in a living cell. Indeed, transcriptional activation by p53 is strongly enhanced by the chromatin-remodeling complex SWI/SNF (Lee et al. 2002), histone modifying acetylases (HATs) and deacetylases (HDACs) (Gu et al. 1997; Scolnick et al. 1997; Avantaggiati et al. 1997; Lill et al. 1997; Liu et al. 1999; Murphy et al. 1999; Koumenis et al. 2001), or by adaptor proteins such as hADA3 (Wang et al. 2001) that may mediate the interaction between p53 and HATs. The mechanisms by which these interactions modulate transactivation by p53 await elucidation, but they seem to involve the ability of all these factors to modulate chromatin structure. Perhaps the clearest evidence demonstrating the importance of a chromatin-modifying activity for p53-dependent transcription was derived from the analysis of the effects of the transcriptional coactivator p300 on SSDB and transactivation of the *p21* promoter by p53 in in vitro assembled chromatin. p300/CPB and PCAF interact with the N-terminal transactivation domain of p53 and acetylate p53 at the C-terminus (Scolnick et al. 1997, Gu and Roeder 1997). Acetylation of the C-terminus of p53 by p300 can enhance SSDB of p53 in vitro (Gu and Roeder 1997) in a similar fashion as activation of SSDB by other covalent C-terminal modifications. Consistent with then prevailing idea that DNA binding of p53 needs activation, the authors interpreted their findings as to indicate that p300 facilitates p53 transactivation by p53 acetylation. Meanwhile, however, several studies reported that mutating the acetylation sites in the C-terminus of p53 affects neither SSDB nor the transactivation

potential of p53 (Nakamura et al. 2000; Barlev et al. 2001). While the impact of p53 acetylation by p300 on SSDB of p53 thus seems questionable, Dornan and co-authors (Dornan et al. 2002) showed that acetylation of p53 by p300 was enhanced upon specific DNA binding of p53 in vitro, indicating that p53 may need first to bind to DNA before being acetylated by p300. The physiological significance of p53 acetylation upon DNA binding is unclear. However, considering that acetylation-deficient p53 mutants are transcriptionally competent (Nakamura et al. 2000; Barlev et al. 2001), it is unlikely to be involved in the enhancement of SSDB and the transcriptional potential of p53. The clue to a possible mechanism by which p300 may influence p53 transcription came from a study in which SSDB and transactivation of the *p21* promoter by p53 was examined in in vitro assembled chromatin (Espinosa and Emerson 2001). The study revealed that p300 acetylates nucleosomal histones in the vicinity of PREs in a p53-dependent manner, supporting the idea that acetylation of histones by transcriptional coactivators recruited to the promoter site by p53 may be crucial for p53 transactivation (Barlev et al. 2001). Interestingly, p300-dependent nucleosomal acetylation occurred unevenly throughout the *p21* promoter and strongly prevailed at one of two p53-binding sites to which p53 binds with a low affinity in vitro (Resnick-Silverman et al. 1998; Espinosa and Emerson 2001). The finding can be interpreted as to indicate that chromatin modifications near some PREs may be important for the formation of DNA structures favorable for p53 binding.

A recent study, in which the impact of p53 on the transcription of the α-fetoprotein promoter was analyzed during chromatin assembly in vitro (Ogden et al. 2001), provided hints for another intriguing role of structural chromatin rearrangements in p53-regulated transcription. Ogden and co-authors observed that p53 was able to establish a closed chromatin structure leading to transcriptional repression of the α-fetoprotein promoter. Importantly, the structural alterations mediated by p53 required its binding to the specific p53 cognate motif in the α-fetoprotein promoter, distal from the core promoter and did not involve modifications of nucleosomal histones (Ogden et al. 2001). Thus it appears that by binding to DNA, p53 itself is able to alter chromatin structure.

To date, the plethora of data on p53 SSDB and transcription in the context of chromatin, while revealing the complexity of these processes raise more questions than answers, as they provide contradictory evidence arguing against one or the other scenario. In fact, the finding that some p53-regulated promoters exhibit constitutive hypersensitivity to DNase I in vivo corresponding to an "opened" conformation of the chromatin (Graunke et al. 1999; Xiao et al. 1998; Braastad et al. 2002) has led to the conclusion that chromatin alterations may not at all be required for SSDB and transactivation by p53 (Braastad et al. 2002). However, an important concern regarding these studies is that DNase I hypersensitivity of the chromatin was examined in isolated nuclei and not in intact cells. It thus is highly questionable whether chromatin structure could be conserved during preparation of the nuclei (Braastad et al. 2002). Despite the apparent controversies and the lack of a general agreement on the mutual impact of chromatin structural rearrangements and DNA binding of p53, it seems to be a valid conclusion that SSDB can not be understood, if considered independent from the structural features of DNA in the context of chromatin.

Structural transitions in DNA as a signaling mechanism for p53 activation

The inherent ability of p53 to bind certain types of DNA structures in the absence of cognate motifs is well known from studies in which the roles of p53 in DNA repair and recombination have been examined (reviewed in Khanna and Jackson 2001). It was proposed that direct binding to structurally aberrant sites in DNA may be a mechanism underlying the functions of p53 in DNA recombination and repair (Lee et al. 1995, 1997; Degtyareva et al. 2001; Yang et al. 2002), functions that are independent from the transcriptional activity of p53 (Saintigny et al. 1999; Khanna and Jackson 2001). Therefore, DNA structure-dependent DNA binding and sequence-dependent DNA binding traditionally have been considered independently from each other as functionally unrelated activities. A family of specific structural elements recognized by p53 includes mismatched bases (Lee et al. 1995; Degtyareva et al. 2001), Holliday junctions and cruciform bases (Lee et al. 1997; Jett et al. 2000), DNA loops formed by tetrameric repeats (Stansel et al. 2002), bent DNA (McKinney and Prives 2002; Nagaich et al. 1997), and structurally flexible MAR/SAR DNA (Muller et al. 1996; Deppert 1996; Will et al. 1998). In this light, the recent finding that DNA structure also has a strong impact on SSDB of p53 is not too surprising. The C-terminal domain of p53 appears to mediate the recognition of structural elements in DNA in both SSDB and sequence-unspecific binding to non-linear DNA (Gohler et al. 2002; McKinney and Prives 2002; Lee et al. 1995). The finding that modifications at the C-terminus are inhibitory to non-linear DNA binding by p53 in vitro raises the question as to how such (covalent and non-covalent) modifications may influence p53 functions that rely on its DNA binding activity. An important point in considering this question is that several hundred putative p53 binding sites with homology to the p53CON sequence have been predicted to exist in the genome (Tokino et al. 1994; Bourdon et al. 1997; Hoh et al. 2002). Probably only a minor portion of these sites corresponds to functional PREs in a sense that they can mediate p53-dependent transcription of target genes. However, such sites, spread throughout the chromatin, would have the potential to bind p53 under conditions facilitating p53 binding to such non-PRE binding sites. Considering the possibility that many of such sites will be in B-DNA conformation (i.e., "linear"), any enhancement of p53 binding to such p53CON-like sequences, e.g., by post-translational modifications or drugs, will have consequences for the structure and function of the chromatin surrounding such binding sites, and might even induce long range chromatin remodeling. For instance, one could envision that an increased p53 binding to regions of relaxed DNA in the chromatin may stabilize such regions in an "open chromatin"-like conformation, which may be important during initial stages of apoptosis. Chromatin undergoes structural changes in the course of apoptosis, and DNA cleavage of more accessible open regions in the

chromatin by apoptosis-associated endonucleases takes place at the initial stages of apoptosis, followed by histone deacetylation and chromatin condensation at later stages. Although purely speculative for the moment, such binding could contribute to the so far completely unknown transcription-independent functions of p53 in apoptosis.

An exciting possibility, how DNA structure can modulate p53 function, can be deduced from the recent observation that global relaxation of DNA can trigger p53-activating pathways even in the absence of physical damage in DNA. Bakkenist and Kastan (2003) showed that DNA relaxation by the DNA intercalating drug chloroquine or by hypotonic conditions activated the ATM kinase, one of the major upstream regulators of p53, as efficiently as double-stranded DNA breaks (DSBs) induced by ionizing irradiation. The DNA damage dependent ATM–p53 pathway thus can be activated without physical damage in DNA by conditions that influence the topology of DNA, and thereby chromatin structure. Previously it had been proposed that p53 can sense and bind directly to individual sites of DNA damage, and that such an initial anchoring of p53 to DNA may be the underlying mechanism for the activation of p53 by ATM upon DNA damage. Unexpectedly, however, ATM activated by DSBs did not co-localize with sites of damaged DNA, but was distributed throughout the nucleus. The finding strongly indicates that the response to DNA damage is global and does not depend on binding of ATM to damaged sites in DNA (Bakkenist and Kastan 2003). The findings of Bakkenist and Kastan thus provide novel insight into how DNA damage can trigger the ATM–p53 pathway; it appears that topological changes in DNA caused by physical damage may be the activating signal sensed by ATM.

In a normal cell, structural changes in the chromatin must be strictly coordinated as it transverses through the cell cycle. This implies that the topological patterns of DNA change coordinately with the structural alterations in the chromatin. Such changes may comprise a specific signature code characteristic for certain stages of the cell cycle. Any unprogrammed disturbance (temporal or spatial) in conformational DNA profiles therefore may be recognized as a signal that structural changes in the chromatin are no longer under control. This would activate sensory pathways such as the ATM pathway, which appears to be an extraordinarily sensitive and rapid sensor of uncontrolled topological alterations in DNA (Bakkenist and Kastan 2003). Thus "the guardian of the genome p53" (Lane 1992) has a "watch dog", the ATM kinase that can sense topological changes in the chromatin caused by as few as 4–18 DSBs in the three billion base pairs comprising the human genome (Bakkenist and Kastan 2003). If uncontrolled structural transitions in the DNA of chromatin may be recognized as "alarm" signal, one might expect that the presence of unusual structures formed by naked non-chromatin DNA may also activate the cellular defense systems. Consistent with such a view, there is some evidence that sequences highly prone to adopt a non-linear DNA conformation may induce p53. It was shown that telomeric repeats, known to form unusual DNA structures, caused the stabilization and transcriptional activation of cellular p53, when delivered to cells ectopically (Milyavsky et al. 2001). Although it is unknown whether the activation of p53 involved a direct binding to telomeric repeats, this is a likely scenario considering that p53 is able to bind t-loops formed by telomeric DNA in vitro (Stansel et al. 2002). Raj and co-authors reported the intriguing observation that p53 activity could be induced in cells by hairpins formed by adenoviral DNA (Raj et al. 2001). It was observed that "empty" adenoviral DNA, i.e., DNA not coding for any recombinant protein caused activation of p53 and G2 arrest in infected cells. Intriguingly, G2 arrest caused by the virus was strictly dependent on the presence of p53 and did not occur in cells lacking p53 (Raj et al. 2001). Although the mechanisms by which viral hairpin triggered a p53 dependent response is unknown, it was proposed that such DNA could be recognized as damaged DNA and thereby activate a p53 response. Such a possibility cannot be excluded considering the inherent affinity of p53 to secondary DNA structures.

The last two years have been marked by the discovery that DNA topology has an immense impact on the interaction of p53 with DNA. In contrast to the previously established view that SSDB (to B-DNA) and recognition of structural features in DNA represent two distinct modes of p53 DNA binding, it by now has become clear that sequence-specific binding of p53 is also greatly influenced by DNA conformation. The findings indicate that p53 is not only a sequence-specific DNA binding transcription factor, but also is a DNA structure-dependent binding protein. The different modes of DNA binding used by p53 might explain how p53 can exert the variety of functions that are vital for cells, but only if they are strictly coordinated according to the cell's needs. The picture emerges that the different modes of p53 DNA binding could provide specificity to the recognition of PREs in promoters of target genes, thereby allowing the selective activation of the p53 target genes required in a given physiological situation. Non-sequence-specific recognition of certain DNA structures will allow p53 to participate in various repair processes, and global or even local changes in DNA and (or) chromatin structure might be powerful signals coordinating the various p53 activities not only in the presence but also in the absence of DNA damage. Despite the already very complex picture of p53 DNA interactions, we clearly have only scratched the surface. But, we will learn more as we go.

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