Cell Death and Differentiation (2006) 13, 885–889

www.nature.com/cdd

News and Commentary

The versatile interactions of p53 with DNA: when flexibility serves specificity

E Kim[1,2] and W Deppert*[,2]

[1] Neuro-Oncology Research Group, Department of Neurosurgery, University of Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, D-23538 Luebeck, Germany
[2] Heinrich-Pette-Institute, Martinistrasse 52, D-20251 Hamburg, Germany
* Corresponding author: W Deppert, Heinrich-Pette-Institute, Martinistrasse 52, D-20251 Hamburg, Germany. Tel: +49 40 48051261; Fax: +49 40 48051117; E-mail: wolfgang.deppert@hpi.uni-hamburg.de

Cell Death and Differentiation (2006) **13,** 885–889.
doi:10.1038/sj.cdd.4401909; published online 17 March 2006

The tumor suppressor p53 exerts versatile interactions with DNA. Of importance is the binding of wild-type p53 to p53-responsive elements in promoters of p53 target genes. This interaction is complex and determined by DNA sequence and structure, and involves the p53 core DNA binding and the C-terminal domain. In addition, wild-type p53 binds linear DNA with high affinity in a sequence-independent manner, and non-B DNA in a DNA strictly structure-selective mode that is not dependent on the presence of a p53-responsive element. Mutant p53 has lost sequence-specific DNA binding and high-affinity binding to linear DNA, but has retained the ability to interact with DNA in a structure-selective manner. We discuss the interactions of p53 with DNA, which are characterized by a high flexibility, both on the side of p53 and DNA, thereby providing p53 with the specificity required for its functions.

The major molecular property of p53 is that of a DNA binding protein. Consequently, its interaction with DNA is central to various p53-mediated activities. Best known and analyzed is the sequence-specific DNA binding of p53 (p53-SSDB), followed by transcriptional activation of genes involved in regulation of the cell cycle, DNA repair or apoptosis, upon activation of the p53 pathway by endogenous or exogenous stress factors.[1] Another p53 stress response is the binding of p53 to damaged sites in DNA, considered to serve as a platform for recruiting repair factors to the sites of the lesions (reviewed by Sengupta and Harris[2]). However, also in non-stressed cells, some p53 activities involved in the 'routine' maintenance of genomic integrity rely on p53 interactions with genomic DNA, particularly at sites of active metabolic processes that render DNA vulnerable or prone to potential structural re-arrangements. In this respect, it has been shown that p53 binds to recombination intermediates, Holliday junctions (reviewed by Sengupta and Harris[2]) or telomeric t-loops.[3] Reflecting the diversity of p53 functions that involve its interaction with DNA, p53 binds to DNA in very different modes that differ for the type of target DNA and in their modes of DNA recognition. Recognition and binding affinity of the various p53 DNA interactions are determined either by the presence of specific sequence motifs (sequence-specific DNA binding, p53-SSDB) or by specific structural determinants presented by DNA in non-B DNA conformations (DNA structure-selective binding, p53-DSSB). Regardless of whether the p53 target site is determined by the presence of a specific sequence motif or by the three-dimensional structure of the DNA, either mode of DNA recognition ensures the specificity of the interaction. Last but not least, in addition to the different modes of recognition that underlie the site-specific targeting of p53 to DNA, as in p53-SSDB or p53-DSSB, high-affinity binding of wild-type p53 to unspecific linear double-stranded DNA represents yet another mode of interaction that probably is important for targeting p53 to its specific response elements in the genome.[4,5]

Interaction of Wild-type p53 with Specific DNA

Activation of p53-SSDB is a hallmark of the p53 transcriptional response induced in cells experiencing acute stress that poses a threat to the structural integrity of genomic DNA. Until recently, p53-SSDB had been considered to be independent from all other modes of p53 DNA interactions. The strict partition between p53-SSDB and 'unspecific' DNA binding was compatible with the then established view according to which p53-SSDB was seen as being determined solely by the recognition of a specific sequence motif. The delineation of the p53 consensus (PuPuPu-C(A/T)(A/T)G-PyPyPy)[6] has been instrumental for understanding the parameters required for a given sequence to be recognized as a p53-specific binding site. The initial DNA binding studies had revealed that resemblance to the p53 consensus, and the presence of more than one cognate motif (also called half-sites) are obligatory parameters of p53-SSDB (reviewed by Kim and Deppert[7]). However, the explosive identification of 'natural' p53 response elements (PREs) surprisingly revealed that genomic p53 binding sites do rarely conform to the p53 consensus with the stringency that would be expected from an interaction based solely on the recognition of a specific sequence. In fact, a considerable divergence from the p53 sequence is common to most functional PREs as they typically contain only one half-site, if at all, that corresponds well to the consensus, whereas the other half-site(s) contains a varying number of non-complying bases (reviewed by Kim and Deppert[7]). The extreme sequence heterogeneity of PREs is puzzling and, intuitively, might be seen as being incompatible with the highly specific interaction such as p53-SSDB. However, the discrepancy may be only seeming, if one considers the possibility that the recognition of specific DNA by p53 is based not exclusively on the recognition of a specific sequence. In this respect, architectural features of the p53 promoter DNA have emerged as an important parameter that contributes to both the affinity and the specificity of p53-SSDB.

Initially, the long known ability of p53 to sense structurally distorted DNA was considered to underlie primarily the interaction of p53 with aberrant sites in damaged DNA that may occur anywhere in the genome and therefore cannot be sequence-dependent. The fact that *in vitro*, distinct domains of the p53 protein elicit different modes of DNA binding independently from each other seemed to further support the idea that the different modes of p53 DNA binding are functionally unrelated: whereas p53-SSDB is a major function of the p53 core DNA binding domain (p53-DBD),[8] p53 binding to aberrant DNA structures has been firmly associated with the p53 C-terminal domain (p53-CTD), which can bind efficiently different types of aberrant DNA sites in the absence of the p53-DBD.[9] Moreover, it has been postulated that the p53-CTD can exert inhibitory effects on p53-SSDB either by allosteric inhibition of the conformationally flexible p53-DBD[10] or by negative interference owing to the high-affinity binding of the p53-CTD to unspecific DNA.[11] Thus the relations between the DNA binding activities of the p53-CTD and the p53-DBD were generally seen as antagonistic.

The idea that the p53-CTD is inhibitory to p53-SSDB was originally derived from *in vitro* analyses of p53-SSDB, which appears to be cryptic unless the p53-CTD is removed or modified either covalently or non-covalently. Allosteric inhibition of the p53-DBD by the p53-CTD has been delineated in the 'p53 latency' concept as a mechanism that operates in cells to switch p53 from a DNA binding inactive form ('latent' p53) to the active form ('activated' p53).[10] However, the last few years have been marked by a dramatic change in the overall picture of the interactions of p53 with DNA, the quintessence being the realization that there is no 'latency' with respect to p53-SSDB (reviewed by Kim and Deppert[7]). The initial observations that shook the paradigm of 'p53 latency' were obtained from comparative analyses of p53-SSDB on different DNA templates (reviewed by Kim and Deppert[7]). It turned out that the manifestation of a 'latent' p53 phenotype is a particularity of p53-SSDB assays using short linear DNA targets that is not observed in the context of more complex templates such as supercoiled, nonlinear or chromatinized DNA. These findings indicated that the 'latent' p53 phenotype may be a phenomenon specific for the particular type of DNA template rather than a general mechanism regulating p53-SSDB. Indeed, p53 'latency' in p53-SSDB can be reversed by altering the structure of the target DNA,[12] indicating that DNA topology, and not a conformational switch of the p53 protein, is the major factor determining the interactions of p53 with specific DNA targets. Thus, p53 is not 'latent' for p53-SSDB *per se*, but it appears like being 'latent' with certain types of DNA templates. Further supporting the conclusion, comparative structural analyses revealed that the global conformation of the p53-DBD is largely uninfluenced by the p53-CTD,[13] a finding that undermined a stronghold of the 'p53 latency' concept postulating that p53-CTD inhibits p53-SSDB by conformationally inhibiting the p53-DBD. Finally, the decisive evidence eliciting the impact of the p53-CTD on p53-SSDB under physiological conditions was provided recently in a recent study by McKinney *et al*.[5] demonstrating that deletion of the p53-CTD impairs p53-SSDB and the potential of p53 to activate transcription *in vivo*. Altogether, these findings revealed that the p53-CTD is essential for p53-SSDB, strongly contrasting the previously established view of the p53-CTD as an inhibitory domain.

The emerging new role of the p53-CTD as an auxiliary domain that facilitates p53-SSDB led to a re-consideration of the impact of the DNA binding activities associated with the p53-CTD. The ability of the p53-CTD to bind with high affinity to structurally aberrant sites in DNA earlier had been exclusively associated with p53 functions in DNA repair and recombination, but it now appears that it is also utilized in p53-SSDB. While obscuring p53 binding to specific linear DNA,[4] the p53-CTD promotes binding of p53 to target sites present in a nonlinear DNA conformation.[12] Thus, the sequence-specific interactions of p53 with nonlinear DNA, in addition to the binding of the p53-DBD to the consensus sequence, might require the interaction of the p53-CTD with structural determinants on the DNA either within or in close vicinity to the p53 consensus element. In light of the findings that p53-SSDB is less efficient if the p53-CTD is missing,[5] it can be envisioned that 'sensing' the DNA topology by the p53-CTD might be as important for p53-SSDB as sequence-specific recognition mediated by the core domain. The formation of a stable p53 complex with specific DNA thus may be influenced by the initial interaction of the p53-CTD with nonlinear DNA. In such a scenario, structural distortions of the DNA duplex may dock p53 via its CTD and thereby promote binding of the p53-DBD to the nearby located canonic p53 binding site. Supporting the hypothesis, p53-SSDB can be strongly promoted by local distortions of the DNA duplex in close vicinity to the p53-specific binding site (unpublished data). Importantly, recognition of the distorted DNA is not sequence-specific and is mediated by the p53-CTD (unpublished data). The proper recognition of PREs, in addition, seems to require yet another DNA binding activity associated with the p53-CTD, its high-affinity binding to unspecific linear DNA. This type of p53 interaction does not discriminate between specific and unspecific DNA, thereby explaining the failure of wild-type p53 to specifically recognize specific sequences in linear DNA unless the DNA binding properties of the p53-CTD are inactivated. Unspecific high-affinity binding to linear DNA may allow 'sliding' of p53 along genomic DNA, and it has been proposed that such 'linear diffusion' may be an important step in p53-SSDB that allows searching for specific binding sites as the protein scrolls along unspecific DNA.[5] A premise for the 'linear diffusion' model is that p53 should be 'docked' at unspecific DNA strongly enough to keep the protein in close proximity to the duplex yet not too strongly to enable linear diffusion. From all known modes of p53 interaction with different types of DNA, unspecific binding to linear dsDNA would perfectly fit the requirements. Indeed, wild-type p53 binds to unspecific linear dsDNA with a relatively high affinity ($\sim 30.2 \pm 8.7$ nM), which, however, is lower than that of p53-SSDB (1.1 ± 0.2 nM).[4]

Interaction of Mutant p53 Proteins with DNA: Specificity Determined by DNA Structure

The changing picture of p53-SSDB also put the issue of the DNA binding properties of mutant p53 proteins into a new

perspective. The search for common sequence denominators that could serve as specific response elements for mutant p53 proteins has been only moderately successful, and no uniform consensus sequence has been delineated so far (reviewed by Kim and Deppert[14]). However, examination of mutant p53 proteins for the potential to bind various DNA structures yielded interesting results. We have found that many mutant p53 proteins, including all 'hot spot' mutants, while having lost the ability to bind linear DNA, have retained the potential to bind selectively to nonlinear DNA in a DSSB mode, which we termed mutant p53-DSSB.[15] Interestingly, both the p53-CTD and the p53-DBD contribute to DNA binding of mutant p53 proteins. Furthermore, it appears that an 'open' conformation of the p53-DBD, as exhibited by the group of 'conformational' p53 mutants, favors binding to nonlinear DNA.[15] The biological implications of mutp53-DSSB have been discussed in detail in a recent review.[14]

A characteristic feature of mutant p53-DSSB is that it is sequence-independent as far as the presence of the p53 cognate motif is concerned. This implies that mutant p53 proteins should be able to bind canonic PREs when they are present in a suitable nonlinear DNA conformation. This indeed is the case.[15] However, analyses of mutant p53-DSSB by chromatin immunoprecipitation so far have failed to identify known PREs among genomic DNA sequences bound by mutant p53 proteins (unpublished data). Out of several possible explanations that could resolve this apparent inconsistency between *in vivo* and *in vitro* data, we will discuss two particularly intriguing ones. One is based on the finding that all p53 mutant proteins analyzed so far have selectively lost high-affinity binding to linear dsDNA.[15,4] The inability of mutant p53 proteins to bind linear dsDNA would preclude their linear diffusion and, consequently, diminish their chances of finding non-canonic DNA structures formed by p53-response elements (p53-RE). Alternatively, or in addition, the stability of secondary structures formed within PREs may be a limiting factor for their recognition by mutant p53 proteins. Considering that self-complementarity within the p53 cognate motifs is not continuous but, as a rule, is interrupted by individual non-matching bases, stem–loop structures formed by PREs are unlikely to be stable. Therefore, it is an intriguing possibility that formation of a preferred, nonlinear conformation within a given wild-type PRE requires an additional supportive activity, which is not provided in cells expressing mutant p53. In such a scenario, one would have to assume that an activity that promotes alterations in the local DNA topology within or nearby p53 binding sites should be present in cells with wild-type p53, but not in cells with mutant p53. In fact, such a hypothetic activity may be inherent to the wild-type p53 protein itself. In this regard, the finding that wild-type p53 induces global relaxation of chromatin[16] and can influence local chromatin structure by recruiting chromatin-modifying activities[17] provides important hints for future investigations. It will be interesting to find out whether there might be a connection between the ability of wild-type p53 for 'linear diffusion' and its potential to cause global chromatin relaxation, and whether mutant p53 proteins also have lost the potential to induce chromatin relaxation. Intuitively, 'mobile' wild-type p53 would be expected to be more effective in eliciting global effects on chromatin structure than a protein that cannot diffuse along genomic DNA, like mutant p53.

Specific Interaction of Wild-type and Mutant p53 Proteins with 'Unspecific' DNA: Commonality that can Make a Difference

The realization that wild-type p53 is a bona fide DNA structure-dependent protein strongly suggests that the pool of genomic sites targeted by p53 might be much larger than estimated from searches that were based solely on the analyses of sequences that match the p53 consensus. Furthermore, the finding that most mutant p53 proteins have retained the potential for high-affinity structure-selective DNA binding[15] points towards the possibility that some sites in genomic DNA might be common targets for wild-type and for mutant p53 proteins, contrasting the general view that target sites bound by wild-type p53 cannot be recognized by mutant p53 proteins. The latter indeed is true for the targeting of wild-type p53 to PREs for p53-SSDB, which requires the presence of a specific sequence as a mandatory parameter.[12] However, the postulate may not apply for those sites whose targeting by wild-type p53 is determined solely by the presence of a suitable DNA structure. Until recently, the existence of such sites remained hypothetical, as most of the conventional approaches utilized for identifying putative p53 binding sites had been based on analyses of sequence similarity with the p53 consensus. However, we recently found that simple trinucleotide (CTG:CAG)*n* repeats comprise a novel type of p53 target sites that are bound both by wild-type and some mutant p53 proteins in naked DNA as well as in the context of chromatin.[18] Intriguingly, the interaction of p53 with (CTG:CAG)*n* repeats is determined solely by the structure of the DNA and can occur by various modes depending on the conformation adopted by the (CTG:CAG)*n* DNA. In the canonical B-form, CTG:CAG repeat DNA is unspecifically bound by wild-type p53, but not by mutant p53 proteins. However, hairpin structures formed by CTG:CAG repeat DNA were bound with high affinity both by wild-type p53 and by at least some mutant p53 proteins. Interestingly, formation of a hairpin structure altered the mode of p53 binding, which then exhibited features of a specific interaction, as p53 proteins occupied a clearly defined site that is determined not by its specific sequence but by its location within the DNA structure.[18] Although the physiological relevance of the p53 interaction with (CTG:CAG)*n* tracts remains to be elucidated, the notorious instability and high recombinogenic potential of trinucleotide repeats (reviewed by Bacolla and Wells[19]) suggests the possibility that wild-type p53 may be involved in the regulation of (CTG:CAG)*n* tract stability. Supporting the notion, our results indicate that wild-type p53 proteins can resolve mismatched duplexes that contain multiple T:T or A:A mismatches by inducing local melting of homoduplexes formed by individual CTG or CAG strands.[18] Considering that there is a strong causative relationship between the formation of hairpin structures by CTG:CAG tracts and the occurrence of DNA breakpoints (reviewed by Bacolla and Wells[19]) one possibility to be considered is that wild-type p53 by binding to

nonlinear structures formed by CTG:CAG tracts can prevent re-arrangements that may occur during DNA replication and transcription.

The existence of sites in genomic DNA that can be targeted both by wild-type p53 and by mutant p53 proteins at first glance suggests the possibility that the DNA structure-selective interaction of p53 with genomic DNA may reflect biological activities conserved in wild-type and mutant p53 proteins. This seems plausible considering that p53-DSSB involves the p53-CTD, which is physically intact in most mutant p53 proteins, therefore suggesting that many of the interactions occurring at the p53-CTD are preserved in mutant p53 proteins. In this regard, the interaction with and activation of the catalytic activity of topoisomerase 1[20] can be considered as an example of an activity that is common to wild-type and mutant p53 proteins. The apparent mechanistic commonality between wild-type and mutant p53 proteins such that they both interact with damaged/aberrant DNA *in vitro*[20] is in striking contrast to their distinctly different impacts on genomic integrity *in vivo*, with wild-type p53 being the 'guardian of the genome' and mutant p53 promoting genomic instability. However, this apparent discrepancy might be resolved if we consider the *dynamics* of the interaction of wild-type p53 and mutant p53 proteins with non-canonic DNA structures. We postulate that both wild-type p53 and mutant p53 proteins are capable of binding to aberrant or potentially dangerous secondary DNA structures (i.e., those formed by CTG:CAG tracts) with comparable efficacy. However, the functionality of the complexes formed by wild-type or by mutant p53 proteins might differ owing to the different composition of the multiprotein complexes assembled at sites of unusual/aberrant DNA structure. Complexes formed by wild-type p53 will recruit DNA repair factors including those which themselves are targets of wild-type p53 p53-SSDB, such as GADD45. Once the potentially dangerous structure has been resolved with the aid of DNA repair factors, wild-type p53 will move on owing to its ability to slide along DNA.[5] Alternatively, or in addition, wild-type p53 can be displaced from DNA by repair factors targeted to unusual DNA structures independently from p53. Indeed, there seems to

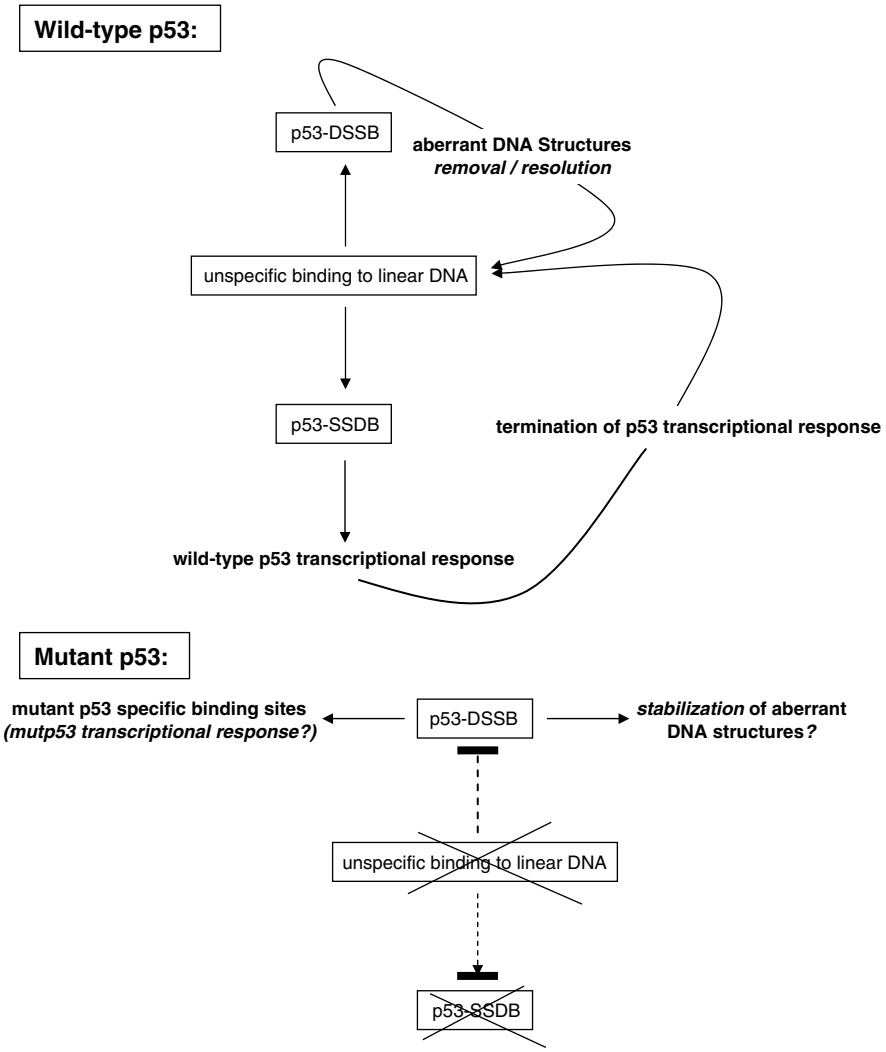

Figure 1 The cartoon depicts the different interactions of wild-type and mutant p53 with DNA, emphasizing the dynamics especially of the interaction of wild-type p53 with DNA. For details see text

be a dynamic interplay between wild-type p53 and other cellular factors that bind unusual DNA structures and can either stimulate or inhibit wild-type p53 binding to some types of secondary DNA structures. In contrast, mutant p53 proteins that are deficient for linear DNA binding are 'immobilized' at the sites of aberrant/unusual DNA structure. Such a constitutive DNA-bound state can be additionally favored by their nuclear abundance and the increased stability of mutant p53 proteins. The persistent occupancy of a structurally flexible DNA site by mutant p53 may stabilize unusual DNA structures and create loci of high recombinogenic activity, which can be further promoted by recombinogenic factors such as topoisomerase I and II that associate with mutant p53 proteins. The (dis)balance between DNA repair and DNA recombinogenic activities elicited by mutant p53 proteins interacting with unstable DNA regions will be further shifted towards the latter owing to the impaired p53-SSDB of mutant p53 proteins and their ensuing inability to activate transcription of DNA repair factors such as GADD45. Thus, the mechanistically common feature of p53-DSSB shared by wild-type p53 and mutant p53 proteins may elicit entirely distinct effects on biological end points, with genomic (in)stability being probably only one of the aspects that is influenced.

Concluding Remarks

Exciting new developments have significantly broadened our understanding of the complex and versatile interaction of p53 with DNA. If one would like to depict the most remarkable feature of the interactions of p53 with DNA in a single word, 'flexibility' would be the appropriate one. Indeed, when put together, the recently discovered new aspects of the various modes of p53 interaction with DNA reveal a very complex and highly dynamic picture as these modes can switch from one to the other (unspecific DNA binding (sliding) $\ggg$ SSDB; DSSB $\ggg$ unspecific linear DNA binding (sliding), or complement each other (DNA structure-dependent p53-SSDB), as depicted in Figure 1. A crucial step forward was the realization that in the center of such a remarkably dynamic relationship is the flexibility of the DNA itself, which by exhibiting specific structural features can influence the mode of p53 binding, and thereby determine which activity of p53 will be elicited. Although it took a long time before the impact of DNA topology on p53 DNA binding was realized, taking it into account has been rewarding and has brought us much closer to an understanding of p53 activities in the framework of chromatin. To achieve this ultimate goal, there is still a lot of work ahead and many open questions, but we will learn more as we will go on.

Acknowledgements

Research in the laboratory of WD was supported by the Deutsche Forschungsgemeinschaft, the Deutsche Krebshilfe (Dr. Mildred Scheel Stiftung), the Fonds der Chemischen Industrie and the EU (FP5 and FP6). We thank Mrs. Martina Hintz-Malchow for technical assistance during preparation of the manuscript. We apologize that owing to format limitations not all important studies that provided insight into the complex interactions of p53 with DNA could be cited individually. To compensate, we refer to recently published reviews that contain a more detailed compilation of studies addressing specific aspects of p53 DNA binding.

1. Vogelstein B *et al.* (2000) Nature 408: 307–310.
2. Sengupta S and Harris CC (2005) Nat. Rev. Mol. Cell Biol. 6: 44–55.
3. Stansel RM *et al.* (2002) J. Biol. Chem. 277: 11625–11628.
4. Wölcke J *et al.* (2003) J. Biol. Chem. 278: 32587–32595.
5. McKinney K *et al.* (2004) Mol. Cell 16: 413–424.
6. El-Deiry WS *et al.* (1992) Nat Gen. 1: 45–49.
7. Kim E and Deppert W (2003) Biochem. Cell Biol. 81: 141–150.
8. Cho Y *et al.* (1994) Science 265: 346–355.
9. Reed M *et al.* (1995) Proc. Natl. Acad. Sci. USA 92: 9455–9459.
10. Hupp TR *et al.* (1992) Cell 71: 875–886.
11. Anderson ME *et al.* (1997) Mol. Cell. Biol. 17: 6255–6264.
12. Göhler T *et al.* (2002) J. Biol. Chem. 277: 41192–41203.
13. Ayed A *et al.* (2001) Nat. Struct. Biol. 8: 756–760.
14. Kim E and Deppert W (2004) J. Cell. Biochem. 93: 878–886.
15. Göhler T *et al.* (2005) Nucleic Acids Res. 33: 1087–1100.
16. Rubbi CP and Milner J (2003) EMBO J. 22: 975–986.
17. Espinosa J and Emerson B (2001) Mol. Cell 8: 57–69.
18. Walter K *et al.* (2005) J. Biol. Chem. 280: 42497–42507.
19. Bacolla A and Wells RD (2004) J. Biol. Chem. 279: 47411–47414.
20. Albor A *et al.* (1998) Cancer Res. 58: 2091–2094.