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The dual role model for p53 in maintaining genomic integrity

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Abstract. The tumour suppressor p53 is a potent mediator of cellular responses against genotoxic insults. In this review we describe the multiple functions of p53 in response to DNA damage, with an emphasis on p53's role in DNA repair. We summarize data demonstrating that p53 actively participates in various processes of DNA repair and DNA recombination via its ability to interact with components of the repair and recombination machinery, and by its various biochemical activities. An important aspect in evaluating p53 functions is provided by the finding that the core domain of p53 harbours two mutually exclusive biochemical activities, sequence-specific DNA binding required for its transactivation function, and 3′–5′ exonuclease activity, possibly involved in aspects of DNA repair. Based on the finding that modifications of p53 which lead to activation of its sequence-specific DNA-binding activity result in inactivation of its 3′–5′ exonuclease activity, we propose that p53 exerts its functions as a 'guardian of the genome' at various levels: in its noninduced state, p53 should not be regarded as a 'dead' protein but, for example, via its exonuclease activity might be actively involved in prevention and repair of endogenous DNA damage. Upon induction through exogenous DNA damage, p53 will exert its well-documented functions as a superior response element in various types of cellular stress. This dual role model for p53 in maintaining genomic integrity significantly enhances p53's possibilities as a guardian of the genome.

Key words. p53; DNA repair; DNA damage; DNA recombination; DNA replication; sequence-specific DNA binding; 3′–5′ exonuclease.

Introduction

The tumour suppressor p53 has become one of the most famous molecules in the area of cancer research, best reflected and acknowledged by its nomination as 'molecule of the year' in 1993 by *Science* magazine [1]. The importance of p53 was deduced from the finding that about 50% of all human cancers contain mutations within the p53 gene, rendering it the most frequently mutated single gene in human cancer known so far [2, 3]. During the past 5 years the functional consequences of the loss of p53 emerged. In the many experiments leading from its initial classification as an oncogene to its reclassification as a tumour suppressor, it had been observed that overexpression of wild-type p53 leads to growth arrest or apoptosis (programmed cell death). The puzzle of p53 function then was ready to be solved, when an earlier observation was rediscovered, namely, that DNA damage will trigger the accumulation of p53 [4–6]. This hinted at the possibility that p53 might play an important role in cellular responses to DNA damage, and led to the now famous coining of p53 as the 'guardian of the genome' by David Lane [7].

Although the main features of p53's role in maintaining the integrity of the genome seem to be outlined, there is still a lot to be learned about exactly how p53 acts to prevent the accumulation of mutational events within a

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cell. Despite some uncertainties regarding the various mechanisms of its induction, elimination of damaged cells by p53-induced apoptosis is an easily understood mechanism for achieving this goal. Much less is known about the role of p53 in DNA repair. So far, the main emphasis has been given to pathways leading to the activation of p53 by signals emanating from damaged DNA, with p53 integrating these signals and triggering a cascade of responses leading to either growth arrest or apoptosis (fig. 1). These mechanisms have been summarized in detail in several recent reviews (e.g., see [8–11]); thus this topic will be addressed here only very briefly. The next steps in this cascade, namely, the activation of the repair pathways themselves and the role p53 plays in their activation are far less clear. Furthermore, it is still not known whether and how p53 directly participates in DNA repair processes, despite some evidence pointing to this possibility. Last but not least, a possible role of p53 in the control of genomic integrity in its noninduced state, that is, in the absence of DNA damage, has not yet been considered so far. In contrast, the general assumption seems to be that nonactivated p53 has no function [3, 12].

In this review we will focus on p53-induced pathways that lead to or are part of DNA repair processes, with an emphasis on the known biochemical activities of p53 which might play a direct role in repair processes. Based on recent evidence from our laboratories showing that at least one of these activities, the $3' \rightarrow 5'$ exonuclease activity, is regulated in a manner opposite to that of sequence-specific DNA binding, we propose a model according to which p53 exerts basic functions in maintaining genomic integrity also in its noninduced state, that is, in the absence of conditions which lead to its activation. Upon various cellular stress conditions, such as hypoxia, nutrient depletion or DNA damage, the well-known functions of p53 in integrating the respective signals become activated, triggering the p53 responses which lead to growth arrest and DNA repair, or apoptosis [13].

Activation of p53 upon DNA damage

Eukaryotic cells have developed a network of highly conserved surveillance mechanisms (checkpoints) which ensure that damaged chromosomes are repaired before they are replicated or segregated. These mechanisms are essential for maintaining genomic integrity and cell viability. The tumour suppressor p53 is one of the critical mediators of cellular responses to various types of genotoxic stress (reviewed, e.g., in ref. 13), acting at different levels of control during the cell cycle.

In response to DNA damage, such as ionizing irradiation (IR) [4], ultraviolet (UV) irradiation [6, 14], hypoxia [15] and ribonucleoside triphosphate depletion [16], an accumulation of the p53 protein is observed. An increase in p53 protein levels has also been demonstrated after introduction of DNA restriction enzyme or nuclease into the nuclei of cultured cells, and the process of DNA transfection itself can induce p53 accumu-

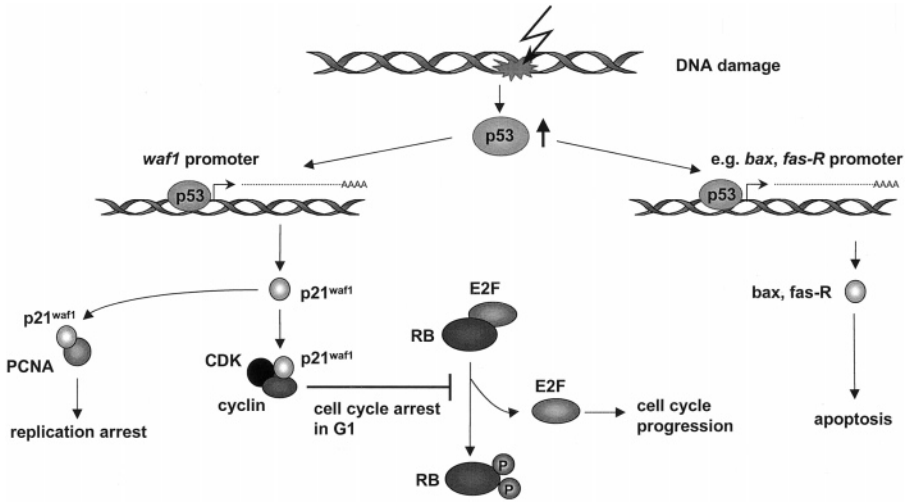

Figure 1. p53: guardian of the genome. According to the current model for p53 function, latent p53 becomes activated after DNA damage and, as its major function, transactivates genes involved in cell-cycle control and apoptosis. Transactivation of the *waf1* gene is thought to be central in mediating p53-induced growth arrest.

lation [6, 17, 18]. The upregulation of the tumour suppressor gene product is mediated by posttranscriptional mechanisms and is accompanied by an increase in p53 transcriptional activity [4, 14, 19–21]. The mechanisms by which DNA strand breaks lead to upregulated p53 levels and to an enhancement of its transcriptional activity are not yet fully understood. The p53 protein is phosphorylated by a wide variety of protein kinases both at its transactivation domain, by casein kinase (ck) I, DNA-dependent protein kinase (DNA-PK), c-Jun N-terminal kinase (JNK) and mitogen-activated protein (MAP) kinases, and at its C-terminus, by cyclin-dependent kinases (Cdks), protein kinase C (PKC) and ck II (see refs 22, 23 and references therein). The different protein kinases can modify the sequence-specific DNA-binding activity of p53 and may therefore modulate the relative efficiency of activation of different p53 target genes [12, 24–27]. Recently, Kastan and co-workers [28], and Prives and co-workers [29] demonstrated that DNA damage leads to specific post-translational modifications of the p53 protein and to its activation as a transcription factor. Endogenous p53 becomes phosphorylated by DNA-PK de novo within the transactivation domain of the protein [29], abrogating its interaction with MDM2, a key player in negatively regulating p53 transcriptional activity and level (reviewed in ref. 30). The phosphorylation on serine-15 and possibly serine 37 within (human) p53 correlates with the enhanced transcription of downstream p53 target genes. Target genes which are transcriptionally activated include *waf1*, *mdm2* and *gadd45* [20, 31]. The ability of p53 to transactivate downstream target genes results in cell-cycle arrest at specific points in the cell cycle. As a consequence, the cell cycle stops either before DNA replication in G1, or before mitosis in G2, accompanied by a severe decrease in the amount of cells in S phase.

p53-dependent G1 arrest occurs mainly through transactivation of the *waf1* gene coding for the small kinase inhibitor $p21^{waf1}$. $p21^{waf1}$ interferes with cell-cycle progression and prevents S-phase entry by blocking the activity of cyclin-dependent kinases (Cdk) [20, 32–34]. Irradiated G1 phase cells accumulate high levels of Cdk2/cyclin E complexes, which are inactivated by the association with $p21^{waf1}$. Inhibition of G1 phase-specific kinase activity maintains a hypophosphorylated retino-blastoma susceptibility gene product pRb which blocks E2F-specific transcription of genes required for entry into S phase, thereby inhibiting cell-cycle progression. As a consequence, cells accumulate in late G1 (fig. 1).

In another checkpoint-response pathway which operates in S phase and slows down the rate of DNA replication, $p21^{waf1}$ binds to the proliferating-cell nuclear antigen (PCNA) and blocks its activity, interfering with cell-cycle progression by blocking the elongation step in DNA replication [35]. Stillman and co-workers suggested a dual role for PCNA in DNA replication and DNA repair which allows $p21^{waf1}$ to arrest DNA replication while permitting active DNA repair [36]. In DNA replication, PCNA together with replication factor C (RF-C) recognizes a primer-template junction and promotes loading of DNA polymerase δ. This trimeric protein complex also enhances the processivity of DNA polymerase δ during the elongation step of DNA replication [37]. The direct binding of $p21^{waf1}$ to PCNA causes a rapid dissociation of the polymerase δ-RF-C-PCNA complex from the replication fork, stalling replicative DNA synthesis. In repair DNA synthesis, PCNA helps the DNA polymerases δ or ε to localize the junction of incised DNA, and is also needed during DNA synthesis. Also discussed is participation of PCNA in DNA mismatch repair, since a two-hybrid screen has shown an interaction between PCNA and the mismatch repair proteins MLH1 and MSH2 [38]. However, this mismatch repair complex might rather be used in DNA replication to ensure fidelity of replication than in conventional repair mechanisms. In cells sustaining DNA damage, the interaction of PCNA with MLH1 and MSH2 is probably disrupted to allow $p21^{waf1}$ to complex with PCNA. This hypothesis is supported by the finding that a peptide comprising the PCNA-binding domain of $p21^{waf1}$ blocks DNA mismatch repair synthesis more efficiently than DNA replication, and that this inhibition can be overcome by an excess of free PCNA [38].

The trimeric replication protein A (RPA) is another cellular factor which binds to p53 and functions in DNA replication [39, 40], homologous recombination [41] and in nucleotide excision repair [42]. This multifunctional single-stranded DNA-binding protein complex, composed of 70-, 34- and 11-kDa subunits [39, 40, 43], is involved in DNA unwinding and DNA synthesis during the initiation and elongation stages of DNA replication [44]. RPA was also found complexed to two excision repair proteins, the xeroderma pigmentosum damage-recognition protein A (XPA) [45, 46] and the endonuclease XPG [47–49]. A complex of RPA and XPA binds to DNA lesions in a cooperative manner which indicates an early function of RPA in excision repair. RPA might also be involved in targeting the endonuclease to damaged DNA [50]. In addition, modification of RPA after DNA damage probably coordinates DNA repair with other events, such as inhibition of DNA replication and cell-cycle arrest. In many irradiated cells the p34 subunit of RPA is phosphorylated [51, 52], and RPA derived from UV-irradiated cells does not support simian virus 40 (SV40) DNA replication in vitro [53]. An interesting finding is the disruption of the RPA-p53 complex after UV radiation in vivo [54]. The authors

report that p53 induction after UV treatment correlates with the disappearance of RPA-p53 complexes, transducing the damage signal by activating the p53-dependent checkpoint control. At the same time the released RPA can participate in nucleotide excision repair (NER).

The third line of defence against induced chromatid damage occurs in G2. A possible role for p53 in the G2/M checkpoint was suggested by Li et al. [55], who reported that $p21^{waf1}$ can associate with cyclin A and cyclin B complexes during the later phases of the cell cycle, suggesting a functional interaction with the respective associated kinases. Furthermore, a bimodal periodicity for *waf1* messenger RNA (mRNA) levels in human fibroblasts with peaks in G1 and G2/M was observed, indicating that $p21^{waf1}$ may play a role at the onset of mitosis. $p21^{waf1}$ is absent from the nucleus during S phase and transiently reenters the nucleus during late G2 phase. In late G2, half of the Cdk2/cyclin A is complexed with $p21^{waf1}$. $p21^{waf1}$ may thereby either directly inhibit the active kinase or prevent its activation by the Cdk-activating kinase (CAK) [56]. Another possibility is that complex formation with $p21^{waf1}$ may block interaction of substrates with Cdk2/cyclin A [57]. In *Xenopus* egg extracts Cdk2 serves as a positive regulator for the activation of Cdk1/cyclin B complexes and prevents entry into mitosis when complexed to $p21^{waf1}$ [58]. The biological significance of the nuclear accumulation of $p21^{waf1}$ and the resulting inactivation of Cdk-cyclin complexes at the onset of mitosis might be part of a $p21^{waf1}$-dependent mitotic attenuation mechanism [59]. However, p53 activity and the resulting $p21^{waf1}$ accumulation are required for DNA damage-induced G1 arrest, and $p21^{waf1}$ clearly is not essential for the immediate G2 checkpoint response [60–62]. Nevertheless, several studies have suggested that the G2/M arrest following DNA damage is also p53-dependent [63–67]. Recently, Vogelstein and co-workers used a wild-type p53-expressing human colorectal cancer cell line to analyse gene expression following γ-irradiation. These cells arrested mostly in G2, and the block was accompanied by changes in gene expression. Quantitative analysis of gene expression patterns revealed a strong induction of the 14.3.3σ gene. The induction of 14.3.3σ is mediated by a p53-responsive element [68]. The 14.3.3 family of proteins are found in a wide variety of mammalian tissues and in other eukaryotic organisms including plants and yeast [69], and diverse biochemical properties have been ascribed to them. Mammalian cells contain a minimum of seven 14.3.3 isoforms [70, 71]. In *Schizosaccharomyces pombe*, the two 14.3.3σ homologues Rad24 and Rad25 function at the radiation checkpoint and ensure that DNA damage is repaired before mitosis is attempted. In addition, *S. pombe rad24* null mutants or *rad25* null mutants enter mitosis prematurely, which indicates that 14.3.3 proteins have a role in determining the timing of mitosis [72]. Thus induction of 14.3.3σ by p53 might be part of p53's control of G2/M transition. This link between DNA damage control and cell-cycle checkpoint control at G2/M could be further substantiated by the following observations. In irradiated cells the protein kinase Chk1, which is required for this DNA damage checkpoint [73], undergoes a Rad3-dependent phosphorylation (Rad3 is a kinase related to the ataxia-telangiectasia-mutated (ATM) protein) [72]. The ultimate target of this checkpoint signal is thought to be Cdk1, the cyclin-dependent kinase that induces mitosis [74], as Chk1 directly phopshorylates Cdc25C, a regulator of Cdk1 phosphorylation [73, 75, 76]. This phosphorylation event promotes the binding of 14.3.3 to Cdc25C and thereby its sequestration. In this state Cdc25C cannot activate Cdk1. Thus independent p53-mediated effector pathways block cell-cycle progression after γ-irradiation (and possibly other DNA damage-inducing events). Entry into S phase is inhibited by $p21^{waf1}$, whereas 14.3.3σ prevents cells that have completed S phase from entering mitosis. Therefore, the p53-dependent induction of 14.3.3σ connects DNA damage with the Cdk1-driven G2/M progression in the same way that the p53-dependent $p21^{waf1}$ induction connects DNA damage with the cdks required for G1/S progression.

DNA repair after activation of p53 by DNA damage

Activation of p53 after DNA damage leads to p53-induced growth arrest or apoptosis [7]. Whereas induction of apoptosis bypasses any need to repair DNA damage, p53-induced transient growth arrest will fulfill its intention only if it achieves accurate repair of the damaged DNA. Sequence-specific transactivation of p53 target genes is regarded as the major function of p53 after its activation. Therefore, upregulation of genes involved in DNA repair might be envisioned as a first step in initiating repair of DNA damage. However, with the exception of the *gadd45* gene [31], which has been linked to DNA repair by circumstantial evidence only, no such direct effect of p53 is known. One thus has to conclude that DNA repair during p53-induced growth arrest occurs in a p53-independent manner or, alternatively, that p53 participates in DNA repair by other activities. Although there is definitely p53-independent DNA repair (as can be demonstrated in p53-deficient cells), there is also evidence for participation of p53 in repair processes, and for p53-dependent activation of DNA repair. The assumption of a direct involvement

of p53 in DNA repair leads to the prediction that a mutation in p53 or the lack of p53 should lead to a defect in DNA repair. If p53 actively participates in DNA repair, p53 should interact with proteins that are part of DNA repair pathways and/or possess biochemical activities by which it could be part of repair pathways.

To test for direct involvement of p53 in DNA repair, Ford and Hanawalt examined Li-Fraumeni fibroblasts which were homozygous for a mutated p53 and found that these cells were indeed deficient in the rate and extent of NER of genomic DNA [77, 78]. In contrast, they did not observe a defect in transcription-coupled NER. This point, however, remains somewhat controversial, since other investigators found a deficiency in transcription-coupled NER [79, 80]. Reduced NER was also observed when the normal p53 function was disrupted by targeting p53 for degradation by the human papillomavirus E6 oncogene, or by expression of a dominant-negative mutant p53 [81]. Furthermore, Li-Fraumeni syndrome cells with mutated p53 are impaired in their recovery for both RNA and DNA synthesis after UV treatment, an anomaly which they share with the DNA repair disorders xeroderma pigmentosum and Cockayne's syndrome [80]. So, in this respect p53 behaves like other proteins that are directly involved in DNA repair. But not only does a defect in p53 function result in defective DNA repair; the induction of p53 after treatment mimicking UV damage even leads to enhanced DNA repair [82].

Association of p53 with components of repair pathways

Clues as to how p53 is involved in DNA repair emerge from data which show a specific interaction of p53 with components of repair pathways. Most of these proteins are members of the transcription factor IIH (TFIIH) multiprotein complex, which initiates basal transcription of RNA polymerase II and couples transcription with NER [79, 83, 84]. p53 interacts with three components (XPB/ERCC3, XPD/ERCC2 and p62) of the TFIIH protein complex. p53 inhibits the helicase activity of XPB and XPD, probably through its strand-reannealing properties [79, 83]. The effect of TFIIH on p53 function is not yet known. It is possible that the interaction of TFIIH components with p53 serves to colocalize p53 at sites of DNA repair or transcriptional initiation. Recently another p53 binding factor of the TFIIH complex was identified as the $p36^{MAT1}$ subunit of the trimeric Cdk7/cyclin H complex [85], which is a CAK. CAK was originally identified as a cellular kinase required for the activation of a Cdk-cyclin complex [86–88], and it is also a component of the TFIIH multiprotein complex [89–92]. Lu and co-workers found that Cdk7/cyclin H phosphorylates p53 in a $p36^{MAT1}$-dependent manner and concluded that CAK phosphorylation of p53 may provide a mechanism by which the functions of p53 could be regulated by the basal transcriptional and/or DNA repair machinery.

p53 also binds to Cockayne syndrome B protein (CSB), which was identified as a repair protein by phenotypic complementation of an excision repair-deficient rodent cell line [93]. The encoded protein is functionally defect in human Cockayne syndrome type B, the most common form of this disease. Afflicted individuals are mentally retarded and sensitive to sunlight. Wang and co-workers were able to show that in vitro translated CSB also binds specifically to GST–wild-type p53 fusion protein [79]. CSB, like ERCC2 and ERCC3, also possesses an unwinding activity. Therefore, binding of p53 to helicases involved in NER (and possibly their inhibition) seems to be a common feature of p53, although no mechanistic explanation has been provided so far.

The already mentioned modulation of the interaction of p53 with RPA after UV damage provides another hint for direct involvement of p53 in NER. UV irradiation disrupts the RPA complex, correlating with an activation of p53, but only in cells which are capable of carrying out global nucleotide excision repair [54]. Since UV irradiation is a major signal for the activation and induction of p53, it seems likely that binding of p53 to RPA is involved in upstream regulation of p53-dependent damage response. Combined with the fact that a defect in p53 function leads to a defect in global NER, it may be more than coincidence that UV irradiation only leads to disruption of the binding of RPA to p53 in cells capable of global NER. A plausible model is that RPA in nonirradiated cells sequesters p53, but releases it after DNA damage. The released p53 could then act as a transcriptional activator, or could directly participate in global NER, for example, by interacting with TFIIH.

Biochemical activities of p53 related to DNA repair

An important feature of p53 in considering its direct involvement in DNA repair is its ability to interact with DNA in many different ways: p53 binds to double-stranded and single-stranded DNA in a non-sequence-specific manner [94], to ends of double-strand breaks [95], to Holliday junctions [96] and to DNA mismatches leading to DNA 'bulges' [97]. These activities are important for p53's ability to bind to damaged DNA. p53 thus can 'sense' and bind strongly to DNA damaged by ionizing radiation [98] and form highly stable complexes with insertion/deletion mismatches [97]. p53 in this respect shows parallels to the DNA repair factor MSH2. Binding to damaged DNA is mediated by the C-terminal domain of p53, which has been shown to be important for the regulation of p53 function. This 'sensing'

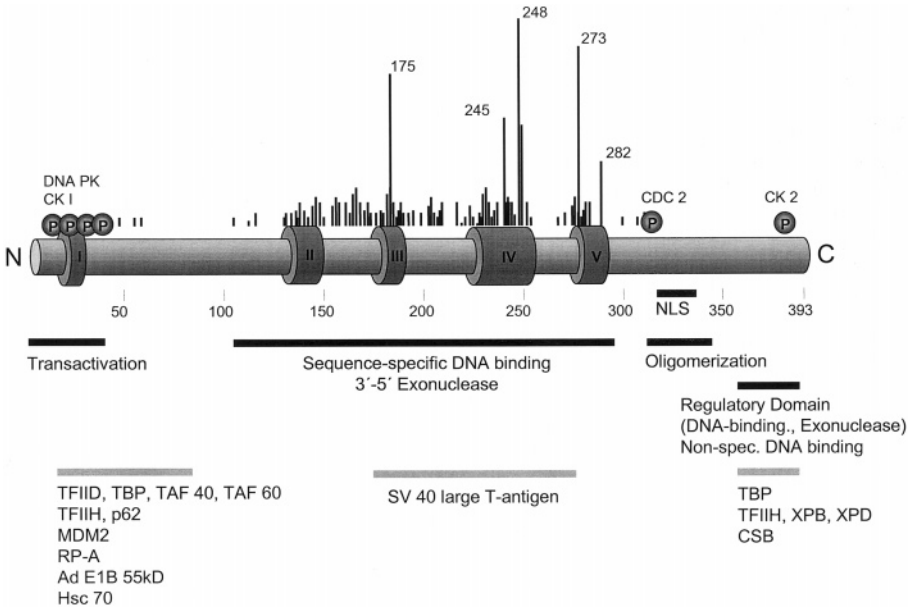

Figure 2. p53 landmarks. Roman numerals represent the five regions of p53 that are conserved within p53 from all vertebrates. Known phosphorylation sites are indicated (P). The vertical bars, clustered in the centre of the p53 molecule, indicate amino acid residues mutated in human tumours (hot spots are identified by amino acid number). Shown below and indicated by horizontal bars is the current information concerning various domains of p53 for biological activities, p53 DNA interactions and p53-protein complex formation. Abbreviations: CK 2, casein kinase 2; CSB, Cockayne's syndrome B protein; DNA PK, DNA-dependent protein kinase; NLS, main nuclear localization signal; RP-A, replication protein A; SV 40, simian virus 40; TAF, transcription activating factor; TBP, TATA-Box binding protein; TF, transcription factor; XPB, xeroderma pigmentosum B protein; XPD, xeroderma pigmentosum D protein.

mechanism forms the basis for a model in which p53 via its C-terminus senses DNA damage, leading to activation of sequence-specific DNA binding of p53 and in turn to transactivation of target genes which then participate in and enhance DNA repair [99]. Although intriguing at first glance, this model presents a major problem: it is not easy to envision how p53 will bind tightly to damaged DNA with a half-life of more than 2 h [97], and at the same time transactivate genes which in all likelihood are located in a distant part of the genome, that is, megabases away from the DNA damage locus. In addition, as already indicated above, so far no p53 target genes have been identified, which could account for the global repair defect when p53 is mutated. As an alternative model we therefore suggest that p53 could be directly involved in DNA repair processes by several biochemical activities in addition to damage recognition, namely, by its non-sequence-specific DNA-binding activity, its DNA-reannealing activity, its ability to promote DNA strand transfer, and its $3'-5'$ exonuclease activity. Especially the p53 intrinsic $3'-5'$ exonuclease activity, localized in the core domain [100] (F. Janus, N. Albrechtsen, F. Grosse and W. Deppert, unpublished data), could be an important player in repair activities of p53. Exonucleases are required for DNA replication, DNA repair and recombination and often enhance the fidelity of these processes. As mutant p53 is exonuclease-deficient, and cells expressing a mutant p53 are defective in global NER, this correlation might point to a possible role of the p53 exonuclease activity in DNA repair. The various biochemical activities of p53 and its interactions with viral and cellular proteins are summarized and related to p53 structure in figure 2.

Involvement of p53 in control of homologous recombination

Recombination processes are subjected to complex surveillance mechanisms ensuring high fidelity of DNA repair and of genetic transmission during meiosis [102, 103]. Regulatory circuits must also exist in order to establish differences in DNA exchange frequencies between distinct genomic loci during meiosis, and in mitotically growing cells, where homologous recombination must be suppressed by a factor of 1000. p53 accumulates and becomes functionally activated [18, 104]

upon the introduction of double-strand breaks (DSB) into DNA [6]. DSB arise spontaneously due to errors in replication, recombination or mitosis and can be induced experimentally by ionizing radiation, radiomimetic drugs, or by the introduction of restriction endonucleases. DSB trigger both repair-associated and targeted recombination processes, a connection which in turn render p53 a likely candidate for a regulatory factor in DNA-exchange processes. During recent years we [105] and others [106–108] demonstrated that p53 suppresses spontaneous inter- and intrachromosomal homologous recombination events at least by one to two orders of magnitude.

Inhibition of p53 recombination control by SV40

In many laboratories SV40 was chosen as a model system for probing recombination, taking advantage of the small chromatin-packaged viral genome, which is amplified episomally, and which promotes high-frequency exchange rates for easy detection of recombination events [105, 109–111]. The SV40 tumour antigen (T-Ag) was found to be the causative agent for the elevated recombination frequencies of cellular and viral DNAs as well as for the stimulation of the closely related gene amplification events [105, 112–114]. SV40 T-Ag targets p53 in SV40 infection and transformation by forming a tight complex with p53. A possible role for p53-T-Ag complex formation in eliminating a function of p53 in the control of recombination was found by comparing the recombination rates between SV40 genomes expressing a wild-type T-Ag or a T-Ag containing a point mutation which specifically blocks T-Ag–p53 interactions [105]. As recombination frequencies in cells expressing a T-Ag unable to bind p53 were reduced at least by one order of magnitude, we conclude that suppression of homologous recombination events by wild-type p53 can be alleviated by complex formation with SV40 T-Ag [105].

Elimination of p53-mediated suppression of recombination by T-Ag might also explain the finding that immortalization of human fibroblasts after stable transformation with T-Ag increases chromosomal recombination in a Rad51-dependent manner [115]. From this, one might assume that, in the absence of T-Ag, p53 binds Rad51, but the association of T-Ag with p53 in these cells protects Rad51 from complex formation, thereby supporting unrestrained strand exchange by Rad51. A correlation between p53 neutralization by complex formation and elevated recombination rates can also be drawn from data concerning other viral binding partners, such as HPV16 E6 [106, 116], and possibly from the genetically destabilizing hepatitis B virus (HBV) X antigen [117].

Evidence for checkpoint functions of p53 in the control of recombination

However, the picture of p53 as a general inhibitor of DNA-exchange processes seems to be oversimplified. Data by Yang and co-workers [118] showed a p53-dependent stimulation of DNA end-joining activities in thyroid cells. Furthermore, Gersten and Kemp did not observe elevated rates for the targeted types of DNA exchange during meiosis and during antigen-receptor rearrangements in p53 knockout mice [119]. p53 mRNA is prominently expressed in zygotene to pachytene spermatocytes [120, 121], and it will be a future challenge to understand whether p53 serves to eliminate defective meiotic spermatocytes or contributes to some as yet unidentified surveillance mechanism during meiosis. Evidence for an indirect involvement of p53 in V(D)J recombination came from studies with mice suffering from severe combined immunodeficiency (scid), whose failure to join DNA ends during T-cell receptor (TCR) rearrangement can be partially rescued either by a p53-dependent bypass mechanism upon γ-irradiation [64, 122] or by knocking out p53 in scid mice [123, 124]. Deficiency of both DNA-PK and p53 activities in these mice potentiates lymphoma formation beyond the susceptibility of p53 single knockouts. These observations support the notion that p53 exerts a checkpoint function most importantly in situations of abnormal DNA structures and damage in order to permit controlled DNA repair activities.

Accumulating biochemical and genetic data indicates a close functional relationship between p53 and the human RecA counterpart HsRad51 [125–127]. p53 and HsRad51 protein interact physically [128] and probably share the same degradation control by association with the UBE2I ubiquitin-conjugating enzyme [129]. Furthermore, wild-type p53 is needed for the inducible accumulation of a newly described mammalian RecA homologue, Kin17, after treatment with ionizing radiation [130]. With respect to the p53 expression pattern during chiasmata formation in meiosis, it is interesting to note that Rad51 from vertebrates is present in early recombination nodules, which in yeast appear coincident with the appearance of DSBs [131–133]. Mice carrying a mutation in Rad51 display an early embryonic lethal phenotype, which can be alleviated by a mutation in p53 [134, 135].

Tight coupling between p53 and Rad51 even extends to Rad51 complex partners such as the products of the genes *Brca1* and *Brca2* [136], which are frequently mutated in breast and ovarian cancers, and which represent putative tumour suppressors. Early embryonic lethality in *Brca1* or *Brca2* mutant mice can be postponed by a *p53* null mutation [137, 138]. Other mitotic checkpoint factors, Atm, the product of the gene mutated in patients with ataxia telangiectasia [139], and its relative Atr, belong to the family of phosphatidylinosi-

tol 3-kinase (PIK)-like protein kinases which are good candidates for signal-amplifying molecules after sensing DNA aberrations [140] and for upstream functions within the signalling response towards p53 [17, 29, 31]. Compared with *Atm*-deficient mice, *Atm* and *p53* double knockout mutants are blocked later during meiotic prophase at the pachytene stage. Therefore, the block executed by p53 can be at least partially attributed to its growth arrest-signalling functions after sensing nonrepaired DNA lesions [137, 141].

Mismatch recognition: a specific role for p53 in recombination control?

We recently discovered that p53 inhibits DNA exchange most dramatically upon recognition of certain mismatches within nascent recombination intermediates [142]. Parallels to the functions of classical mismatch repair factors in recombination immediately arise, as the mammalian MutS counterpart MSH2, like p53, binds to Holliday junctions [143] and abolishes DNA exchange between divergent sequences [144]. Therefore, it is tempting to speculate that p53 monitors the fidelity of recombination in concert with the mismatch repair system. The mammalian MutL homologues PMS2 and hMLH1 are essential for stabilizing chiasmatas during meiosis, as can be deduced from abnormal chromosome pairing or chiasmata resolution in spermatocytes of the respective nullizygous mice [145–147]. MSH2 and p53 do not seem to perform structural but rather regulatory functions during DNA-exchange processes. Consistent with the hypothesis of two surveillance pathways acting in parallel, the inactivation of both pathways causes a synergism in tumourigenesis, evidence for which was recently given by the analysis of *Msh2* and *p53* double knockout mice [148]. Therefore, MSH2 and p53 must be considered to be members of different genetic epistasis groups dedicated to the regulation of recombination processes. This view is consistent with our biochemical data suggesting complementary fidelity control due to an opposing mismatch specificity for MSH2-GT-binding protein complexes, which most efficiently recognize G-T single-base mispairings [149], and for p53 tetramers, which display highest affinities for A-G mismatches [142]. It remains to be established whether p53 upon encountering mismatches in heteroduplices transmits signals to downstream molecules, such as $p21^{waf1}$, or blocks the progress of DNA exchange, possibly even by participating in error removal. Interestingly, purified multiprotein complexes performing homologous recombination in vitro contain DNA polymerase ε, DNA ligase and low levels of $5' \rightarrow 3'$ and $3' \rightarrow 5'$ exonuclease activities [150].

Is there a role for p53 in DNA repair in its noninduced state?

The active participation of p53 in DNA repair processes might not be restricted to situations activating a p53 response, like exogenously inflicted DNA damage. On the contrary, expanding p53's role as 'guardian of the genome' by endowing it with a basic function in DNA repair processes in its noninduced state instead of regarding it as just a 'dead' protein should greatly enhance its possibilities for preserving the integrity of the genome. Actually, some inconsistencies regarding the role of the *waf1* gene as a major target in the p53 response to DNA damage already point to such a basic function. If indeed p53-mediated induction of the *waf1* gene, and the ensuing growth arrest and halt of PCNA-dependent DNA replication, play the major role in p53's activities to ensure repair of DNA lesions, one would have to postulate that *waf1* nullizygote mice should display a similar phenotype regarding tumour predisposition as p53 null mice. This, however, is not the case, despite the finding that cells from *waf1* nullizygote mice have largely lost the ability to arrest in G1 after DNA damage [151]. One thus has to assume that some other function of p53 is preventing the accumulation of mutations in these mice. According to our model, this function could be the 'basic function' of p53, preventing the accumulation of mutations by its involvement in the repair of endogenous DNA damage.

With the exception of sequence-specific transactivation of p53 target genes, which seems to be dependent on posttranslational modification of p53 after DNA damage, all of its other activities outlined above could, in principle, be exerted also by a noninduced p53. Possible limitations would only arise from the low amounts of p53 usually present in normal cells. However, even this limitation is questionable, since it has been demonstrated that the transactivation function of p53 can be induced without a detectable rise in p53 levels [152]. Although there is no in vivo evidence for such a basic function of p53, comparative analysis of the major biochemical activities exerted by the p53 core domain, sequence-specific DNA binding and $3'–5'$ exonuclease activity (fig. 2), prompts us to suggest a direct participation of p53 in the repair of endogenous DNA damage, in preventing faulty DNA repair and possibly also in DNA replication.

The localization of the $3' \rightarrow 5'$ exonuclease activity of p53 to the same domain as the sequence-specific DNA-binding activity poses the problem of how these activities are regulated, as it seems rather unlikely that p53 will act as an exonuclease and simultaneously bind to DNA in a sequence-specific manner. During deletion mapping analyses we found that C-terminal truncation of the p53 molecule activated its exonuclease activity by

about a factor of 10. Deletion of the C-terminal 30 amino acids was sufficient for this activation, indicating that the C-terminal regulatory domain of p53 negatively influences the p53 exonuclease activity (fig. 3). The same, however, has been shown for the sequence-specific DNA-binding activity of p53, underscoring the importance of the p53 C-terminus for the regulation of p53 functions. Yet, whereas both activities were negatively regulated by the p53 C-terminus, treatments which altered the regulation of p53 activities by the p53 C-terminus had opposing effects on sequence-specific DNA binding and the exonuclease activity of p53: treatments which activated sequence-specific DNA binding of p53, such as addition of the monoclonal antibody PAb421, which binds to an epitope within the C-terminal regulatory domain, or enhanced the phosphorylation status of p53, strongly inhibited the p53 exonuclease activity (F. Janus, N. Albrechtsen, F. Grosse and W. Deppert, unpublished data). The intriguing point of these experiments is that – with the exception of C-terminal truncation – so far all treatments which activated sequence-specific DNA binding led to inactivation of p53 exonuclease activity. This points to the exciting possibility that p53 can exist in at least two different functionally active states. Since activation of sequence-specific DNA binding is considered to be a hallmark of p53 activation after DNA damage, we conclude that p53 loses its exonuclease activity when the sequence-specific DNA binding of p53 becomes activated after DNA damage. Conversely, however, one can also conclude that nonactivated p53 exerts exonuclease activity, implying that nonactivated p53 is not equal to nonfunctional p53.

Possible functions of p53 exonuclease activity

Under the premises that activation for sequence-specific DNA binding and p53 exonuclease activity are mutually exclusive, and that noninduced DNA-binding-negative p53 exerts exonuclease activity, what could be the role of this activity in DNA repair, and are there other activities which could be performed by such p53 molecules? Exonucleases are required for nearly all processes of DNA metabolism, such as DNA replication, long-patch DNA repair, postreplicative mismatch repair and DNA recombination. An important type of error avoidance mechanism requiring exonuclease activities is the mismatch repair pathway. In bacteria this system invokes the coordinated action of the MutSLH damage recognition/endonuclease complex along with the UvrD helicase, DNA polymerase III, DNA ligase,

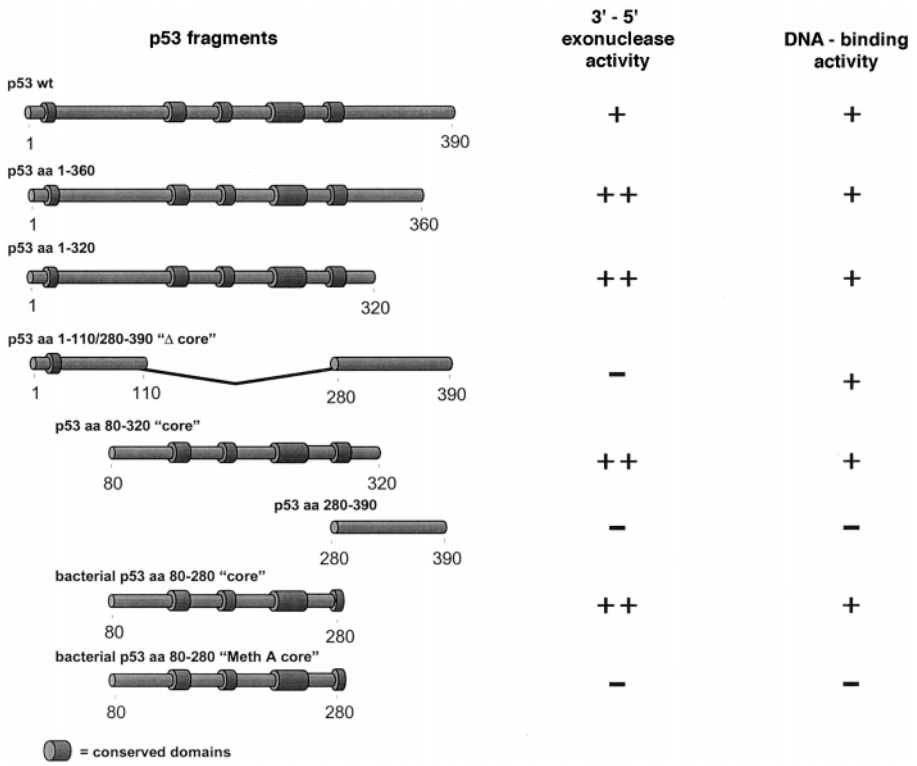

Figure 3. Sequence-specific DNA binding and 3′–5′ exonuclease activity are mediated by the p53 core domain and negatively regulated by the p53 C-terminus. Deletion mapping of sequence-specific DNA binding and 3′–5′ exonuclease activity. C-terminal truncation leads to p53 constitutively active for DNA binding and enhances 3′–5′ exonuclease activity by a factor of 10 (indicated by ++).

single-strand DNA-binding protein, and any one of the exonucleases Exo I ($3' \rightarrow 5'$ exo), Exo VII ($3' \rightarrow 5'$ and $5' \rightarrow 3'$ exo) or RecJ ($5' \rightarrow 3'$ exo) [153, 154]. An involvement of p53's exonuclease in the postreplicative mismatch repair pathway would be of particular interest, because this would link one of the enzymatic functions of p53, that is, its exonuclease activity, to the tumour suppressor function of the mammalian MutSLH homologues hMSH2, hMLH1, hPMS1 and hPMS2. A role complementary to these mismatch recognition proteins has already been proposed for p53 in mismatch recognition during recombination events (see above). Functional loss of either of these proteins leads to an increased incidence of colon carcinomas (for reviews, see refs 155–157). Furthermore, mutations in any of these genes display a generalized increase in spontaneous mutation rates, a replication error-positive (RER +) phenotype and resistance to alkylating agents [158–160]. In this respect, it might be more than coincidence that p53-deficient animals show a relative resistance to alkylating agents [161]. Moreover, there is an inverse correlation between RER + status and p53 mutation in colorectal cancer cell lines [162], pointing to a synergistic action of gene products involved in mismatch repair with p53. This synergism is further corroborated by the finding that male mice nullizygous for both *Msh2* and *p53* develop tumours significantly earlier than either of the single mutants [148]. Furthermore, there is some evidence that, like the p53 protein itself (see above), an intact mammalian MutSHL system displays an 'antirecombinogenic' effect [163]. In line with the possibility of a direct involvement of p53 in mismatch repair is the recent demonstration that wt p53 can 'sense' DNA mismatch lesions by tightly binding to DNA containing insertion/deletion mismatches [97].

Another possibility is p53's involvement in error avoidance by contributing a certain type of 'proofreading' activity. There are at least six different cellular DNA polymerases, designated as DNA polymerases α, β, γ, δ, ε, and ζ, that are involved in different aspects of DNA synthesis. Two of these, namely, DNA polymerases α and β, are devoid of a $3' \rightarrow 5'$ exonuclease activity that excises mismatched nucleotides immediately after the incorporation step. Therefore, DNA polymerases α and β are particularly error prone, and it is still not known how mismatched nucleotides incorporated by these two polymerases are removed [164]. Moreover, both polymerases have a strong tendency to introduce frame-shift mutations by misinsertion or deletion of nucleotides [165]. Since p53 recognizes DNA bulges caused by insertion/deletion mismatches [97], it might be particularly suited to excise them via its $3'-5'$ exonuclease. Last but not least, p53 might act as a direct proofreader for the proofreader-deficient DNA polymerases α and β [165a] (C. Melle, H.-P. Nasheuer and F. Grosse, manuscript in preparation).

A model for p53 function in the maintenance of genetic integrity by DNA repair

Different subclasses of *p53* may perform different functions within the same cell

An important aspect for evaluating the activities of p53 in its induced and noninduced state is that not necessarily all p53 molecules need to be in the same functionally active state, that is, they need not all to exert the same function. Functional heterogeneity of multifunctional regulatory proteins is well known. Probably one of the best-documented examples is the SV40 T-Ag, the major regulatory protein of SV40. Analyses of its subcellular localization and of its biochemical activities in SV40 lytically infected cells clearly demonstrated the coexistence of several functionally different subclasses of T-Ag molecules, involved in various aspects of viral replication (reviewed in ref. 166). Assuming a similar situation for the multifunctional p53 molecule, one could envision that even after DNA damage only a certain fraction of the p53 molecules becomes activated for sequence-specific transcription, whereas other molecules remain in their noninduced state. Specificity for selective activation (or nonactivation) of p53 subclasses can be provided by subcellular compartmentalization of the p53 protein or by its association with different cellular proteins. The existence of functionally different subclasses of p53 within the same cell so far has not been proven. However, previous experiments from this laboratory at least demonstrated the presence of p53 in different nuclear compartments [167]. Therefore, one could envision that the p53 population not engaged in transcriptional regulation could exert functions other than induction of growth arrest or apoptosis, and directly participate in processes of DNA repair via its various biochemical activities described above. The exonuclease activity, for example, could be involved in repair processes such as DSB repair, which is thought to require DNA helicases, like the Ku autoantigen [168, 169], and exonucleases. Furthermore, p53 might act as an external proofreader for errors produced by cellular DNA polymerases involved in DNA replication and DNA repair also under DNA damage conditions. DNA polymerase α is certainly involved in nuclear DNA replication [170] but also has some function in DNA repair [171, 172], whereas DNA polymerase β has been solely assigned to DNA repair processes [173]. When DNA damage has occurred, there is an apparent shut-off of PCNA-dependent DNA replication [35, 174]. Although PCNA-dependent DNA repair synthesis still might continue under conditions of $p21^{waf1}$ induction [36], DNA repair synthesis could also be performed by the PCNA-independent and proofreader-free DNA polymerases α and/or β, for which p53 could provide the proofreader function. If one

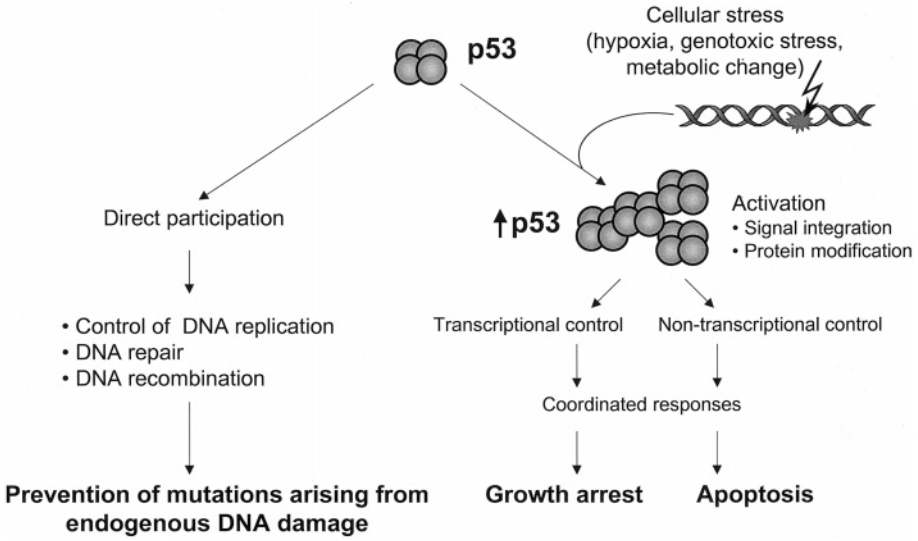

Figure 4. Dual role of p53 as guardian of the genome. The current view of p53 as a superior control element in the responses to various types of cellular stress is depicted in the right half of the figure (adapted from [13]). The left half provides an extension of this model, according to which a non-induced p53 actively participates in the prevention of mutations arising from endogenous DNA damage. Note that functions of a non-induced p53 can also operate under conditions of cellular stress, if one assumes that after cellular stress different functional subclasses can exist within the same cell (for details see text).

assumes that after damage induction different functional subclasses of p53 will exist within the same cell, then the ensuing increase of p53 protein levels not only will activate the potential of p53 to transcribe p53 target genes, leading to growth arrest and to shut-off of PCNA-dependent DNA replication, but will also increase the amount of p53 with a $3' \rightarrow 5'$ proofreading exonuclease activity. Such p53 then could enhance the accuracy of DNA repair synthesis performed by the more error-prone DNA polymerases α and β.

The 'dual role' model for *p53*

The major conclusion which can be drawn from the above considerations is that the current picture of p53 as a guardian of the genome solely activated by cellular stress or DNA damage might be an oversimplification. We assume that a non-induced p53 is not a dead protein. p53 in its noninduced state at least performs exonuclease activity, which could be involved in a variety of possible functions of p53 in DNA repair which all contribute to avoid mutations in the genome, as outlined above. We consider that the basic function of p53 is the repair of endogenous DNA damage, and the prevention of mutational events resulting from such damage. Superimposed are the up-to-now better characterized functions of p53 as a superior control element in integrating cellular stress signals, followed by the induction of either growth arrest or apoptosis. This model of a dual role of p53 as a guardian of the genome is outlined in figure 4. Clearly, the basic function of p53 to directly engage in repair processes must not be restricted to its noninduced state, but might also be exerted by a subclass of p53 after induction, if sequence-specific transcription was activated by a fraction of p53 only. Thereby p53 after DNA damage would then be able to exert its full range of possible biochemical activities.

Clearly, this model is highly speculative, but it is testable. For example, the postulated proofreader activity of p53 predicts a functional association of an exonuclease-active p53 with the proofreader-deficient polymerases α and β. Preliminary data from biosensor studies suggest that p53 binds to DNA polymerase α with considerable affinity [174a], and a specific complex between p53 and polymerase α could be detected in vivo (I. D., unpublished observation). Further experimentation is necessary to confirm these initial findings. Important clues regarding the validity of the dual role model for p53 might be obtained from the analysis of the recently discovered p53 homologues p73 [175] and KET [176]. The p73 core domain shares about 63% homology with p53, but with regard to its structure is more closely related to the p53

homologue previously identified in squid than to vertebrate p53 [176]. These homologues thus might be considered as ancestral p53 molecules, which possibly do not yet exhibit the bewildering multifunctionality of p53. Analysis of their function(s) should hint as to the primordial function of p53.

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