Cell. Mol. Life Sci. 66 (2009) 391 – 406
1420-682X/09/030391-16
DOI 10.1007/s00018-008-8336-3

Cellular and Molecular Life Sciences

Review

p53 isoforms – A conspiracy to kidnap p53 tumor suppressor activity?

V. Marcel and P. Hainaut*

International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08 (France), Fax: + (33) 4 72 73 83 22, e-mail: hainaut@iarc.fr

Received 17 June 2008; received after revision 02 September 2008; accepted 08 September 2008
Online First 16 October 2008

Abstract. For 25 years, the p53 tumor suppressor protein was considered the only protein expressed by the *TP53* gene. However, in several studies the existence of p53 alternative transcripts in mouse and human cells has been documented, while their expression patterns and functions remained a mystery. Since 2002, several groups have identified and described the existence of up to 10 p53 isoforms and have demonstrated their roles in modulation of p53 suppressive activity. It is now clear that the patterns of p53 expression are much more complex than previously recognized and that these isoforms have the potential to act either synergistically or antagonistically, depending on their structure and mechanism of production. This review focuses on the different ways to produce p53 isoforms, on their specific properties, on their effect on p53 suppressive activity as well as on their implication in a new potential mechanism involved in p53 deregulation in cancer.

Keywords. p53, isoforms, cancer, mutations, p53 response.

Introduction

The p53 tumor suppressor protein is ubiquitously expressed in latent form in normal cells. In response to stresses, including environmental DNA-damage or endogenous metabolic stresses, p53 is subjected to rapid activation and nuclear accumulation, thus regulating the transcription of genes involved in cell cycle arrest, DNA repair and apoptosis [1]. These processes provide an anti-proliferative response preventing accumulation of genomic alterations due to cell replication in improper conditions. Furthermore, p53 growth suppression has a direct impact on cell differentiation and senescence. The balance between different p53-dependent responses varies according to cell type and differentiation status.

The *TP53* gene belongs to a family containing two other members, *TP63* and *TP73*, which play roles in development and differentiation. *TP53* appears to be the only family member specialized in stress responses [2]. Both *TP63* and *TP73* are expressed as multiple isoforms that share a common "p53-like" DNA-binding domain with a different N- or C-terminus, generated by multiple promoter usage and alternative splicing. This observation prompted a re-investigation of the expression status of *TP53.* In 2002, two studies identified a shorter form of the p53 protein, lacking the N-terminus [3, 4]. Since then, the systematic work of Bourdon and colleagues has shown that *TP53* expresses a complex pattern of isoforms, and data have started to accumulate on their potential functions [5]. Here, the current knowledge on the mecha-

* Corresponding author.

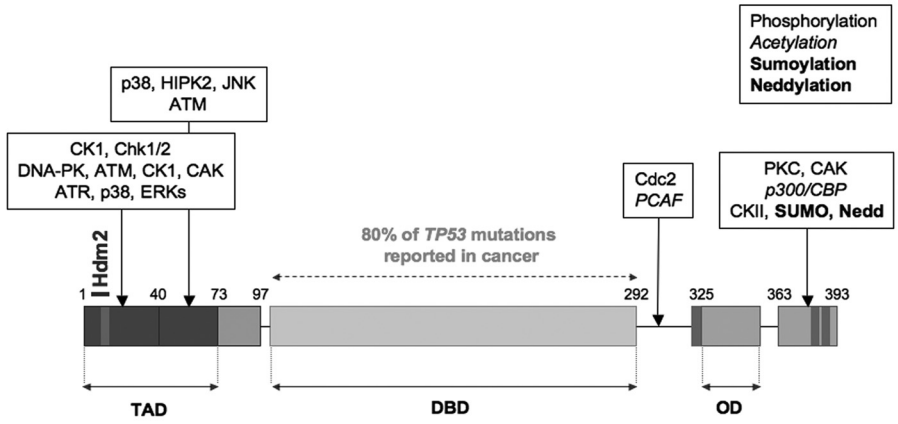


Figure 1. Organization of the p53 transcription factor. The transactivation domain (TAD) contains two sub-domains (TADI and TADII) following by a proline-rich region. The central domain is a DNA-binding domain (DBD). The C-terminus encompasses the oligomerisation domain (OD) and a basic region. One nuclear export signal (NES) is present in N-terminus, whereas three nuclear localization signals (NLS) are located in C-terminus (gray boxes). A single Hdm2-binding site is present in TAD. Several proteins are known to induce post-translational modifications on p53.

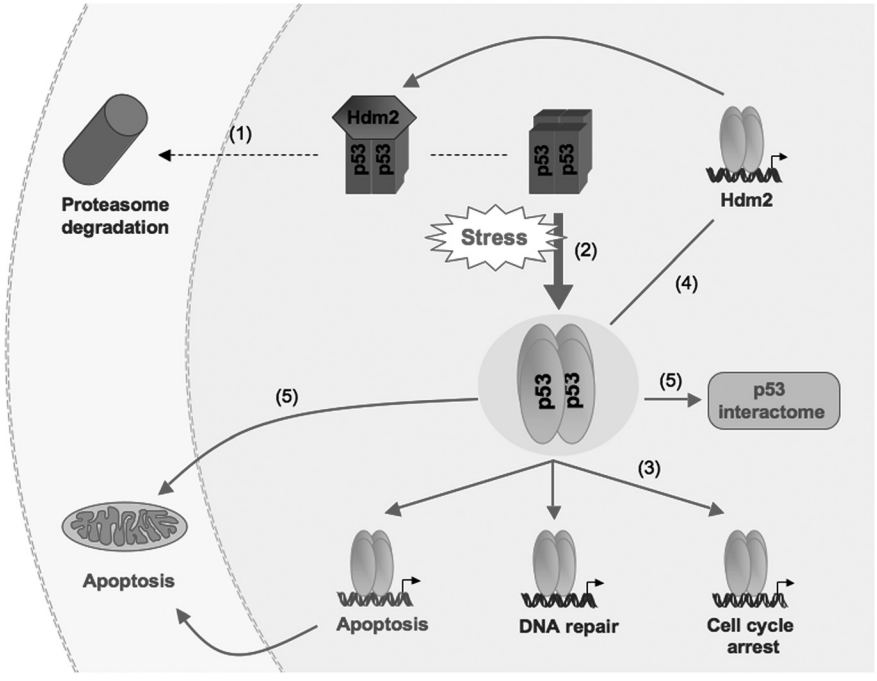


Figure 2. The classical p53 pathway. In normal cells, p53 is bound by Hdm2, which mediates its cytoplasmic re-localization and its proteasome-mediated degradation (1). After genotoxic or non-genotoxic stress, p53 escapes to Hdm2, leading to its stabilization (2). Thus, p53 regulates several targets genes involved in apoptosis, DNA repair and cell cycle arrest (3). p53 also induces expression of Hdm2, this auto-regulatory loop leading to the return to normal p53 expression level (4). The p53 tumor suppressor activity is also mediated by protein interactions, such as in mitochondria to induce apoptosis (5).

nisms regulating the expression of p53 isoforms is summarized and their impacts on p53 suppressive capacity as well as on cancer development are discussed.

p53 protein: domain organization and functions

The p53 transcription factor has a classical organization, including an N-terminal transactivation domain (a major sub-domain, TADI, residues 1–40; and a minor one, TADII, residues 43–73); a proline-rich domain (residues 65–97); a central DNA-binding domain (DBD, residues 102–292); a hinge region containing the main nuclear localization signal (NLS, residues 300–325); a C-terminal oligomerisation domain (OD, residues 325–356); and a basic region (residues 363–393) (Fig. 1) [6]. Under normal conditions, p53 is present at a low intracellular level due to its interaction with Hdm2 (Human double minute 2), which acts as a p53-specific E3-ubiquitin ligase [7, 8]. In the nucleus, Hdm2 binds residues 18–23 of the p53

TADI and induces p53 mono- and poly-ubiquitination, respectively related to cytoplasmic re-localization and proteasome-mediated degradation (Fig. 2 (1)) [9–12]. In response to stress, phosphorylations within the N-terminus, including Ser15 and Thr18 sites, prevent p53/Hdm2 interaction that results in both p53 stabilization and nuclear localization by altering the accessibility of the nuclear export signal (NES, residues 11–27) (Fig. 2 (2)) [13, 14]. This initiates a complex cascade of post-translational modifications, including phosphorylation, acetylation, sumoylation or neddylation (Fig. 1) [15]. These modifications stabilize p53 as a tetramer, with high affinity for specific DNA sequences and high capacity to attract transcriptional co-activators at the promoter of target genes [16, 17].

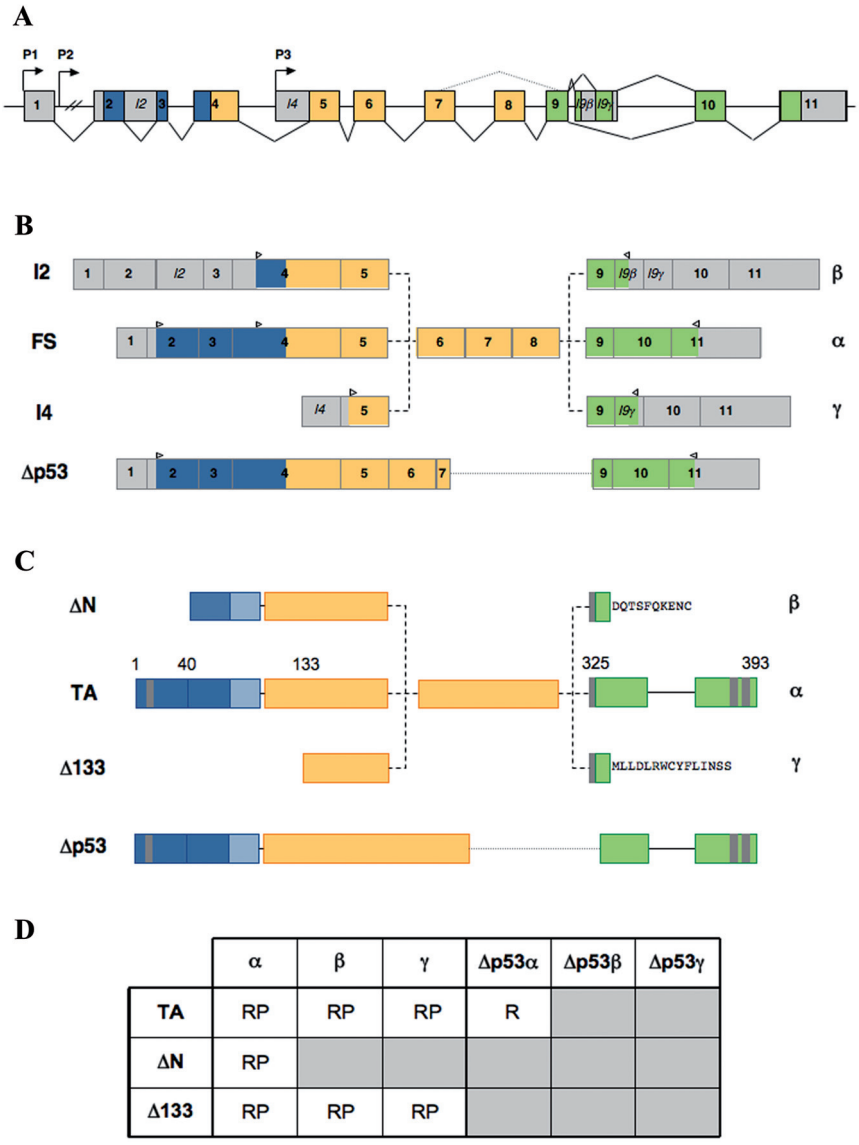
The p53 response-element (p53RE) corresponds to repeats of a palindromic sequence 5'-PuPuPuC(A/T)(T/A)GPyPyPy-3', present in over 300 copies in the genome [16, 18, 19]. Genes regulated through a p53RE include genes involved in cell cycle: *CDKN1A (p21(WAF1/CIP1))*, *GADD45*, *CCNG1 (Cyclin G)*, *CCNB1 (Cyclin B1)* and *14-3-3σ* ; apoptosis: *BAX*, *PIG3*, *IGFBP3*, *PUMA*, *NOXA*, *CD95(APO-1/FAS)*, *KILLER/DR5*, *CXCR4*; and DNA repair: O^6*MGMT*, *MSH2* (Fig. 2 (3)). *HDM2* is also a p53-target gene, leading to a regulatory loop that down-regulates p53 protein levels and suppressive activity to induce a progressive return to low basal level after stress (Fig. 2 (4)) [20]. Several other Hdm2-like proteins induce p53 proteasomal degradation, such as COP1, CHIP and Pirh2 [21–24]. In addition to gene regulation, p53 also acts through direct formation of protein complexes to induce mitochondrial changes leading to apoptosis, via Bak/p53 interactions, or by participating in machineries regulating transcription, replication or repair (Fig. 2 (5)) [25, 17, 26]. In cancer, p53 suppressive activity is inactivated by different molecular mechanisms. First, *TP53* is frequently mutated in cancer and 80% of these mutations occur in the DBD to inactivate DNA-binding capacity resulting in loss of p53 transactivation (IARC *TP53* database: www-p53.iarc.fr) [27]. Second, loss of wild-type *TP53* alleles is extremely common in cancer, sometimes in conjunction with mutation in the remaining allele [28]. Third, p53 functional inactivation can occur through alterations of the p53 pathway, such as over-expression of *HDM2* in sarcoma or retinoblastoma leading to constitutive inhibition of p53 suppressive activity, or through sequestration and degradation of p53 by viral proteins, such as the E6 protein produced by oncogenic forms of human papilloma viruses [29–31].

Regulation of p53 isoform expression

In human cells, recent studies described 10 different p53 isoforms. Most of them share a common DBD but differ in their N-terminus (TA, ΔN and Δ133 forms) and in their C-terminus (α, β and γ forms) (Fig. 3). The TA forms carry a complete TAD as compared to ΔN and Δ133 forms, lacking 39 or 132 residues, respectively [4, 5]. In C-terminus, the entire OD is conserved in α forms, while in β and γ forms, it is truncated and replaced by 10 or 15 additional residues [5]. The combination of the six different ends produces theoretically nine p53 isoforms: the canonical p53 protein (or TAp53α), p53β (TAp53β), p53γ (TAp53γ), ΔNp53 (ΔNp53α, also termed p44, p53/47 or Δ40p53), ΔNp53β, ΔNp53γ, Δ133p53 (Δ133p53α), Δ133p53β and Δ133p53γ. In addition, a tenth isoform, Δp53, has been described that lacks part of DBD and OD [32]. The production of these p53 isoforms involves regulatory mechanisms at the level of transcription, RNA processing and translation.

Transcriptional regulation of *TP53* gene. The proximal p53P1 promoter, located upstream of the non-coding exon 1, initiates the synthesis of TA and ΔN forms (Fig. 3A). p53P1 is a bi-partite promoter that lacks conventional TATA box [33, 34]. However, a 21 bp PE21 element (-79 to -60 from transcriptional initiation site) carries a bi-directional initiation activity toward a reporter gene and may be considered the transcriptional origin [35]. p53P1 promoter controls basal expression of *TP53* but its activity can be induced in response to stress, such as UV radiation or mitogen stimulation, whereas it is down-regulated by NF-kB and c-Jun [35–38]. These observations suggest that several stresses associated with environmental exposures, cell division or inflammatory responses may regulate p53P1 promoter activity and thus the expression of both TA and ΔN forms.

A second promoter, p53P2, has been described in intron 1, generating a 1,125 bp mRNA containing only sequences derived from intron 1 [39]. Although p53P2 activity was more important than p53P1 in reporter assays, the nature and function of the protein encoded by this sequence remains elusive [40]. These observations suggest that the *TP53* locus may contain more than one gene. In murine cell lines, a transcript of 1.3 kb encoding by the first intron was also described that accumulated in the nucleus during differentiation [41]. Recent *in silico* analysis supported the fact that *TP73* also contains a promoter located in intron 1, both in human and murine genes [42]. The presence of a promoter within the first intron of *TP53* is a conserved feature through evolution. It remains to



	α	β	γ	Δp53α	Δp53β	Δp53γ
TA	RP	RP	RP	R		
ΔN	RP					
Δ133	RP	RP	RP			

Figure 3. The human p53 isoforms. (*A*) Schematic representation of the *TP53* tumor suppressor gene. *TP53* contains 11 exons (boxes) and several introns (bold lines). Three promoters have been described (black arrows): p53P1 upstream exon 1 regulates transcription of FSp53 and p53I2 variants; p53P2 in the large intron 1 of 10 kb; p53P3 initiates transcription in intron 4 to produce p53I4 variant. Normal splice is shown by bold line under the gene's schema, while alternative splicing pattern is shown over the gene's schema. Grey boxes: non-coding sequence; blue boxes: sequence coding TAD; orange boxes: sequence coding DBD; green boxes: sequence coding OD; number 1 to 11: exons 1 to 11; *I2:* intron 2; *I4:* part of intron 4 retained in p53I4 mRNA; *I9β:* part of intron 9 encoding p53β; *I9γ:* part of intron 9 encoding p53γ. (*B*) Representation of p53 transcripts. p53P1 promoter generates two 5' regions: FSp53, which retains all exons correctly spliced, and p53I2, which retains the stop codon-rich intron 2. p53P3 promoter produces p53I4 mRNA containing 3' part of intron 4. In C-terminus, α forms retain all exons from 9 to 11, while p53β and p53γ retain *I9β* and/or *I9γ*. A deletion from 3' end of exon 7 to 5' end of exon 9 is present in Δp53 transcript. Grey boxes: non-coding sequence; open arrow in right direction: start site of translation; open arrow in left direction: stop site of translation. (*C*) Organization of p53 isoforms. TA forms contain an entire TAD, while ΔN forms lack TADI, due to initiation of translation at ATG 40 using both FSp53 and p53I2 transcripts, and Δ133 forms lack entire TAD and part of DBD because of usage of the alternative promoter p53P3. The α forms conserve an entire C-terminal domain, whereas β and γ forms lack several residues replaced by new amino acids. Δp53 isoform lacks a large part of the DBD and the C-terminal NES. Grey boxes: nuclear export or localization signal. (*D*) Pattern of p53 expression. The lines correspond to N-terminal forms, while columns correspond to C-terminal forms. R: RNA expression detected; P: protein expression detected.

be shown whether these other genes participate to the regulation of p53 functions.

Recently, reporter gene assays using as promoter a large fragment of *TP53* demonstrated the existence of a third internal promoter, p53P3 [5]. Primer extension indicated that the initiation site of transcription is located in the middle of intron 4, leading to the production of p53I4 mRNA (GenBank DQ186650). This transcript retains the 3' end of intron 4 followed by all exons correctly spliced. The use of AUG 133 in exon 5 generates the

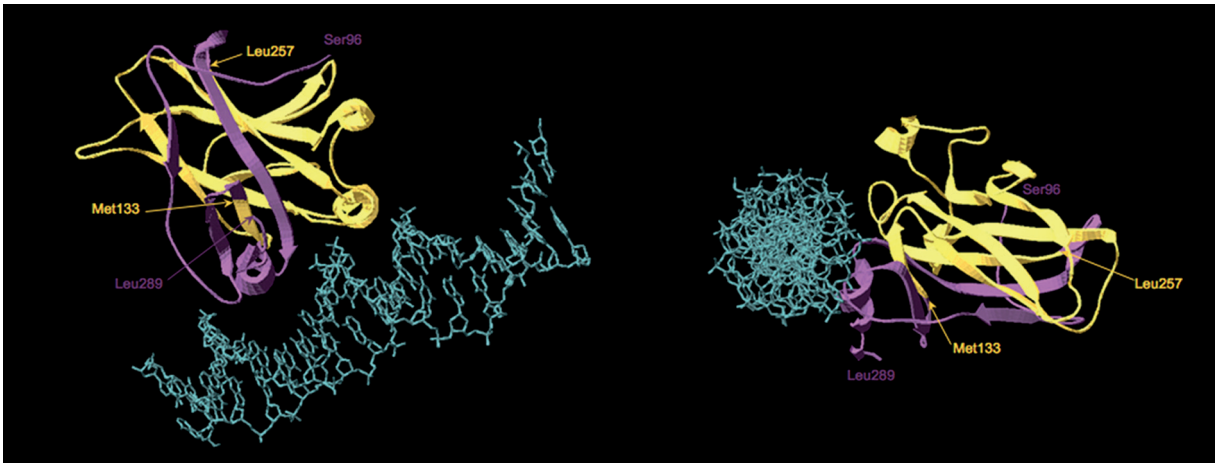


Figure 4. Structural representation of p53 core domain. This tri-dimensional model presents p53 monomer binding its consensus sequence (left panel: right view; right panel: back view). All p53 isoforms conserve a core domain containing residues Met133 to Leu257 (yellow) but could lack a large part of this domain in Δp53 isoform (violet). The core domain included in p53 isoforms contains the residues needed for Zinc atom binding and also the L2-L3 loops able to bind minor grove of DNA. This representation was realized using p53 sequence of Protein DataBase n°1TSR and Swiss PDB Viewer [69].

Δ133 forms, which lack the entire TAD and a part of the DBD (Fig. 3B, C). A computational analysis identified several response elements (REs) in intron 4, such as TFIID-RE, SP1-RE, AP1-RE and NFkB-RE [43]. Their presences suggest that p53I4 mRNA synthesis may be controlled by factors involved in the regulation of cell proliferation, survival and migration. However, the contribution of these factors to the expression of Δ133 forms remains to be investigated.

p53P1 and p53P3 promoters, regulating the production of TA and N-truncated forms, are conserved among distant species (zebrafish, drosophila and human) as well as in *TP63* and *TP73* [44, 5, 45, 46]. The high conservation of p53P3 internal promoter among the *TP53* family, if not in terms of promoter sequence, at least with respect to its position within the gene, suggests an essential role of N-truncated isoforms in the activity of *TP53* family members. In addition, it suggests that expression of TA and ΔN forms is tightly coordinated, since they are regulated by the same proximal promoter. Unfortunately, the focus on post-translational regulation as the main mechanism for induction of p53 in response to stress has eclipsed studies on transcriptional regulation of basal gene expression during the normal cell cycle, differentiation and senescence. The discovery of complex promoter activities that work either in alternation (e.g. p53P1 *vs.* p53P3) or in an integrated manner (e. g p53P1 in the control of both TA and ΔN forms) suggests that basal p53 levels may be under sophisticated transcriptional controls.

Alternative splicing of p53 pre-RNA. In the N-terminus, TA and ΔN forms are produced by an alternative splicing of intron 2 (Fig. 3B). The transcript initiated from p53P1 promoter shares two different 5' regions in human cells, the fully spliced p53 variant (FSp53, GenBank DQ286964) and the p53I2 variant [47]. FSp53 retains all 5' exons and use of AUG 1 results in synthesis of TA forms. The first evidence supporting the existence of p53I2 mRNA came from the screening of a p53 cDNA library of normal human foreskin fibroblasts [48]. This study detected a second p53 mRNA containing sequences derived from intron 2 but did not succeed in identifying its complete 5' end. Seventeen years later, additional investigations showed that p53I2 retained the entire intron 2 [47]. This additional exon introduces three stop codons in the reading frame of AUG 1, favoring the use of AUG 40 to generate ΔN forms. The p53I2 variant is less abundant than the FSp53 variant but its accumulation in polysomes is the same as FSp53, suggesting that the two transcripts are equally used for translation [47].

In the region encoding the DBD, a non-canonical alternative splicing has been described that produces a transcript lacking 198 bp, corresponding to a fragment from the 3' end of exon 7 to the 5' end of exon 9 [32]. These exons contain two identical cassettes of 7 bp that have been predicted to be ESE (Exon Splicing Enhancers), a sequence bound by the SF2/ASF splicing factor [49]. The reading frame initiated from ATG 1 is not altered, inducing a deletion of 66 amino acids that corresponds to a part of the DBD and the entire NLS (residues 257–322). The resulting protein, Δp53, is predicted to lack a major α-helix motif that binds p53RE in the minor groove, indicating a protein with functional properties that are very different from

those of p53 (Fig. 4) [49]. However, the existence of this isoform is controversial [50].

In the C-terminus, three alternatively spliced forms have been identified and termed α, β and γ. The α forms correspond to the canonical C-terminal domain encoding by exons 9 to 11, while alternative splicing in intron 9 generates the β and γ forms (Fig. 3). The p53 transcript encoding β forms was described for the first time in acute lymphoblastoid leukemia cells and later in lymphocytes of healthy blood donors using a functional assay in yeasts (FASAY) [51, 52]. This p53+I9 mRNA contains 133 additional bp from intron 9 and corresponds to the transcript of p53β isoform described more recently by Bourdon and collaborators (GenBank DQ186648) [5]. The retention of an additional exon 9β leads to the replacement of the entire OD by a new motif (DQTSFQKENC), which has been used for developing a specific antibody, KJC8. The level of p53β is decreased in the presence of wortmannin, used as an inhibitor of the Non-Mediated Decay pathway (NMD), which eliminates transcripts carrying a premature stop codon in the open reading frame [53]. However, wortmannin has other biochemical activities, such as inhibition of PI3-kinases, and the NMD-dependent regulation of p53β remains to be clarified. As β forms, the p53γ isoform lacks the OD, substituted by 15 new residues (MLLDLRWCYFLINSS), due to the retention by alternative splicing of 58 bp corresponding to the distal portion of the 133 bp additional segment of p53β mRNA (GenBank DQ186649) [5].

Alternative splicing is a well-conserved mechanism among *TP53* family members in the same species but not necessarily among *TP53* genes of different species. For example, the murine ASp53 transcript, which retains 96 bp of intron 10 at position +1091, has no equivalent in humans, since the additional 5' splice site used to produce ASp53 is conserved in humans but is non-functional [54–57]. In addition, the human splicing of intron 2 generating p53I2 mRNA has not yet been reported for other species. In chicken cell lines, at least 28 truncated mRNAs have been described, lacking sequences that encode parts of proline-rich domain and DBD [58]. In human cells, the EASI method (Enrichment of Alternatively Spliced Isoforms) has recently allowed the identification of up to 14 human p53 alternative transcripts in addition to those discussed here [59]. The main difficulty will be determining which of these mRNAs leads to the production of proteins with meaningful activities.

Translational regulation of p53 transcripts. The generation of ΔN forms also occurs through alternative initiation of translation at AUG 40 located in exon 4, using both FSp53 and p53I2 transcripts (Fig. 3A). The first clue supporting internal initiation was obtained in 1987, when a conserved Kozak sequence was described at codon 40 [60]. Recent transfection of a p53 cDNA resulted in the production of two distinct p53 proteins, a 53 kDa protein corresponding to the canonical p53 and a shorter form of 40 kDa, recognized by PAb1801 antibody (residues 46–55) but not by DO-7 (residues 19–26), that correlates with characteristics of the ΔNp53 isoform [4]. Mutation of ATG 40 in p53 cDNA abolished expression of ΔNp53 but not of p53, while mutation of ATG 1 eliminated expression of p53 but not of ΔNp53 [3, 4]. In contrast, mutation of another ATG located at position 44 had only minor effects, suggesting that this latter ATG has no significant role in translational initiation. These observations suggest the presence of two initiation sites of translation on FSp53 transcript, AUG 1 and AUG 40 that generate, respectively, TA and ΔN forms. On the other hand, AUG 40 also serves as the only initiation site on p53I2 transcript, which retains the stop codons-rich intron 2, thus preventing the synthesis of a protein using AUG 1 (Fig. 3). There is no strict Kozak consensus environment at AUG 133, suggesting that Δ133p53 cannot be produced by internal initiation of translation from FSp53 transcript (Kozak consensus: GCC GCC (A/G)CC ATG G) [61].

Recent studies demonstrated that the dual translational initiation using FSp53 mRNA is regulated by cap-dependent (e.g. recognition of 5' mRNA cap by 40S ribosomal subunit) and cap-independent mechanisms (e.g. using IRES signal, Internal Ribosome Entry Sites) [62, 63]. In particular, two IRES elements have been described. The first, (-1)5'UTR, is located in the 5' UTR just upstream of AUG 1 and enhances translational initiation of TA forms. The second, p53(+39), is located in the first coding 120 nucleotides upstream of AUG 40 generating ΔN forms [63]. Previous studies showed that 5' UTR sequence of FSp53 transcript has an impact on the translation of p53, initiated at AUG 1. The deletion of 132 bp-long 5' UTR resulted in an increase in p53 expression, suggesting that this region inhibits FSp53 translation [64]. This inhibition may result from the binding of regulatory proteins such as nucleolin or p53 itself, on the 5' UTR of FSp53 mRNA [65, 66]. It has also been shown that DNA-damaging stress increased binding of RPL26 ribosomal protein on 5' UTR of FSp53, leading to a higher p53 expression. In this context, the return to a low basal level of p53 protein appears to depend upon antagonistic effects between nucleolin and RPL26 ribosomal protein [65]. Recently, it was shown that hnRNPC1/C2 protein binds the 5' UTR of FSp53 mRNA, leading to an increase in p53 protein level in response to cisplatin treatment [67].

Table 1. Biochemical properties of p53 isoforms. The intra-cellular localization of p53 isoform and p53 in the presence of p53 isoform are shown. N: nucleus; C: cytoplasm; nd: not determined. The DNA-binding and oligomer formation capacities of p53 isoforms are presented.

p53 isoform	Localization		DNA-binding	Oligomerisation		Reference
	isoform	isoform and p53		isoform	isoform and p53	
p53β	N	nd	Yes	nd	Yes	[5]
ΔNp53	C	C	Yes	Yes	Yes	[4, 47]
Δ133p53	N	nd	nd	nd	nd	[5]
Δp53	C	N	Yes	Yes	No	[32, 71]

The Kozak sequence around AUG 40 is conserved in several species such as human, mouse, rat and chicken, but not in cow, xenopus, rhesus and pig, suggesting that alternative initiation of translation is not fully conserved through evolution [4]. Mice do not express a p53I2 transcript produced by alternative splicing, but may generate ΔN forms through internal initiation of translation. This mechanism is also conserved among *TP53* family members, as one IRES signal has been described in exon 2 of *TP73* that regulate alternative translational initiation to produce ΔNp73 [68]. The diversity of the mechanisms, which can be used to produce ΔN forms (alternative promoter, splicing or translational initiation), is a clue to the functional importance of these forms in cell physiology.

The three mechanisms described above result in the generation of three different N- and C-termini. In theory, the combinations of one N-terminal variant with another C-terminal variant, in addition to taking account of an entire core- or truncated-domain as described in Δp53, could result in the generation of 18 distinct p53 isoforms. However, only eight forms have been consistently observed at RNA and protein level (Fig. 3D). The lack of detection of some isoforms may be due to the poor performance of available antibodies that are currently not specifically dedicated to detecting them. For example, the p53β isoform has been undetectable until an antibody against its 10 C-terminal residues was raised, allowing its detection against a background of other isoforms [5].

Biochemical and biological functions of p53 isoforms

p53 isoforms differ by the absence of structural and functional domains that may alter biochemical properties known to be essential for p53 suppressive functions. Only one motif is common to all isoforms, the portion of the DNA-binding domain (residues 133–257), which is stabilized by a Zinc atom and provides the structural motif that binds in the minor groove of DNA (Fig. 4) [69]. In theory, p53 isoforms can exert their effects by two mechanisms: autonomous functional properties, different from those of p53, and/or modulation of p53 activity.

Intrinsic properties of p53 isoforms. Most studies on the biochemical properties of p53 isoforms have been performed by transfection of constructs encoding isoforms in p53-null cells, revealing that p53 isoforms have distinct biochemical properties (Table 1). However, these experimental conditions have serious limitation as compared to p53 activation by physiological or toxic stimuli. In normal cells, p53 presents a rapid turnover estimated at about 5 to 20 minutes because of its Hdm2-dependent cytoplasmic degradation (Fig. 2) [70]. In transfected cells, p53 expressed in excess escapes Hdm2-induced degradation leading to p53-dependent activation of reporter systems, even in the absence of additional induction by stress.

The controversial Δp53 isoform was described as a protein detected exclusively in the cytoplasm, perhaps due to absence of the main C-terminal NLS signal (residues 300–325) [71]. Indeed, addition of NLS sequences downstream of Δp53 coding sequences redirected it into the nucleus. Alternatively, Hdm2 may be involved in Δp53 cytoplasmic location, as Δp53 has been shown to have a higher affinity for Hdm2, a higher sensitivity to Hdm2-dependent ubiquitination and a higher proteasome-dependent degradation than p53 [71]. This intracellular location is consistent with the incapacity of Δp53 to transactivate p53 target genes, such as *P21(WAF1/CIP1)*, *HDM2* or *Cyclin B1* in non-stressed cells (Table 2) [71]. It has also been proposed that the lack of a major α-helix part of the DNA-binding domain may explain this deficiency in transactivation capacity [49]. However, under DNA damaging stress, transfected Δp53 appears to recover some DNA-binding and transactivation capacity, as it physically binds *P21(WAF1/CIP1)* and *14-3-3σ* promoters, and the products of these two genes are up-regulated [32]. No increase was observed on Hdm2, Bax or Pig3 protein expression or on the binding of Δp53 to promoters of these genes.

It has been reported that transfection of ΔNp53 resulted in its cytoplasmic location, due to its mono-

Table 2. Transactivation activity of p53 isoforms. The p53 transactivation activity is defined as 100 % (++). The intrinsic transactivation activity of p53 isoforms are based on p53 activity: 0: 0 to 30 % of p53 transcriptional activity; +: 30 to 60 %; ++: 60 to 100 %; nd: not determined. The residual transactivation activity in the presence of both p53 and p53 isoform are compared to the intrinsic activity of p53 alone: --: 0 to 50 % of p53 transcriptional activity; -: 50 to 100 %; 0: 100 %; +: 100 to 150 %; ++: higher than 150 %; nd: not determined.

p53 isoform	Target gene	p53	Isoform	Isoform and p53	Reference
p53β	P21 (WAF1/CIP1)	++	0	nd	[73]
				0	[5]
	BAX	++	0	nd	[73]
				++	[5]
	IGF-BP3	0	0	nd	[91]
ΔNp53	p53-RE	++	0	–	[4]
	P21 (WAF1/CIP1)	++	+	nd	[4]
		++	0	--	[47]
		nd	nd	++	[76]
	IGF-BP3	0	0	nd	[91]
	HDM2	++	0	nd	[4]
		nd	nd	++	[76]
	GADD45	++	0	nd	[4]
	Cyclin G	++	0	nd	[4]
	PIG3	++	0	nd	[4]
Δ133p53	CXCR4	–	0	nd	[92]
Δp53	P21 (WAF1/CIP1)	++	0	+	[71]
	HDM2	++	0	0	[71]
	Cyclin B1	–	0	0	[71]

ubiquitination status, while others and we observed a predominantly nuclear location [47]. The differences in cell culture and stress conditions may explain the contradictory observations. Indeed, one week after stable transfection, ΔNp53 was found to localize exclusively in the nucleus, but could be sent back to the cytoplasm by stress treatment using doxorubicin or second transfection of an empty vector, which is supposed to induce a form of DNA-dependent stress [47]. These observations suggest that, in normal cells, ΔNp53 is located in the nucleus but re-localizes in the cytoplasm in response to stress. This behavior seems opposite to that of p53, which is known to accumulate in the nucleus in response to stress. Another spectacular difference between p53 and ΔNp53 is its extended stability, with a half-life greater than 9.5 hours [60]. This stability is explained by the fact that ΔNp53 lacks Hdm2-binding site and does not form detectable complexes with Hdm2 in co-immunoprecipitation experiments [4]. The expression level of endogenous ΔNp53 is not affected by DNA-damage induced by hydrogen peroxide, UV-B, MMS or cisplatin, but is increased in some cell types after serum starvation or endoplasmic reticulum stress [4, 62]. Although ΔNp53 can form stable and specific complexes with p53 consensus DNA sequence, reporter assays showed that ΔNp53 does not carry an autonomous transcriptional capacity, perhaps due to the lack of the first transactivation domain (Table 2) [4, 47]. Surprisingly, a recent analysis of the transcriptome of Saos-2 cells expressing ΔNp53 indicated that this isoform could regulate 30 % of the p53 target genes, including *BAX* [72]. This observation is in agreement with observations showing that Bax protein levels increased after over-expression of ΔNp53 [3]. Moreover, the panel of genes regulated by ΔNp53 appeared to include genes non-regulated by p53, with a preferential activation of several pro-apoptotic genes [72]. Although binding tightly to the p53 consensus DNA sequence, ΔNp53 does not appear to promote transcription initiation alone and may require recruitment of co-factors on some promoters, containing p53REs or not.

The transfection of p53β or Δ133p53 results in their nuclear localization. This suggests that the C-terminal NLS signal carried by the p53β isoform is sufficient for nuclear location [5]. The expression levels of these isoforms are not affected by six hours of actinomycin D treatment at pro-apoptotic concentrations in breast and osteosarcoma cell lines, suggesting that accumulation of p53β and of Δ133p53 is not necessary for their biological activities. The p53β isoform binds *BAX* and *P21(WAF1/CIP1)* promoters with a six-fold higher affinity than the *HDM2* promoter but failed to transactivate either *BAX* or *P21(WAF1/CIP1)* promoters in a reporter system [5]. In addition, no induction of p21 expression was observed after cisplatin treatment in the neuroblastoma SK-N-AS cells, which express only the p53β isoform [73]. These observations suggest that p53β has no intrinsic transcriptional capacity towards promoter genes containing a p53RE, despite retaining the DNA-binding and the N-terminal transactivation domains of p53. Little information is available about the biochemical properties of the Δ133p53 isoform (Table 1). The

only evidence available so far shows that Δ133p53 had no effect on pG13-Luc reporter, which contains 13 p53RE upstream of a minimal promoter and luciferase gene [74].

All p53 isoforms discussed above appear to conserve some DNA-binding activity. The lack of transcriptional activity is likely due to their poor capacity to recruit components of the transcriptional machinery onto promoters, either because of the lack of a suitable protein interaction domain (e.g. ΔNp53 and Δ133p53) or to the lack of domains essential for high DNA-binding affinity (e.g. Δp53: core domain; p53β: oligomerisation and/or regulation domains). However, it cannot be ruled out that they may exert effects towards genes different from the known p53 target genes.

Modulation of the classical p53 transcriptional activity by p53 isoforms. Analysis of *HDM2* and *Cyclin B1* luciferase reporters in the presence of Δp53 showed that their activities were identical in cells transfected with p53 alone or with both Δp53 and p53 [71]. This observation suggests that Δp53 has no detectable impact on p53 transcriptional activity, in agreement with the results showing that the lack of the main NLS in Δp53 led to cytoplasmic location and rapid degradation by Hdm2. The addition of three C-terminal NLS to Δp53 forced Δp53-NLS to enter into the nucleus and co-expression of p53 and Δp53-NLS was associated with a dose-dependent decrease of luciferase activity of *HDM2* reporter system as compared to p53 transfected cells [71]. Δp53 is thus able to act as an inhibitor of p53, but only if Δp53 is forced to re-localize into the nucleus. Δp53 is not able to form oligomers with p53 but retains DNA-binding capacity, suggesting that the inhibitory effect results from competition between p53 and Δp53 for promoter binding [71, 32].

With Δ133p53, reporter assays using pG13-Luc have shown a dose-dependent inhibition of p53 transcriptional activity [74]. This is compatible with the lack of the N-terminal transactivation domain. However, no further studies have been reported on Δ133p53 biochemical properties.

ΔNp53 isoform can also inhibit p53 transcriptional activity. The p53-RE reporter system containing two p53RE showed that a ten-fold excess of ΔNp53 inhibited transactivation of p53 [4]. The same result was obtained on *P21(WAF1/CIP1)* reporter system, leading to a complete inhibition of p53-dependent transactivation [47]. Using co-immunoprecipitation, it has been shown that ΔNp53 binds p53 to form hetero-dimers and -tetramers [47, 4]. As ΔNp53 lacks the first transactivation domain, hetero-oligomers ΔNp53/p53 may be deficient for transactivation. In addition to this inhibitory effect, transfected ΔNp53 may also affect p53 activity by altering its cellular localization, as exogenous ΔNp53 accumulated in the cytoplasm and induced the cytoplasmic re-localization of p53, thereby decreasing its transcriptional activity [47]. Stable co-transfection of ΔNp53 and p53 led to accumulation of the two proteins in the nucleus. However, the two proteins migrated to the cytoplasm after doxorubicin treatment or transfection of an empty vector, treatment that is supposed to induce a form of DNA damage [47]. ΔNp53 may work as an inhibitor of p53 suppression by two complementary mechanisms, depending upon stress context. In non-stressed cells, ΔNp53 may work in the nucleus as a competitive inhibitor of p53 to prevent transactivation. In stressed cells, accumulation of p53 may favor formation of hetero-oligomers with ΔNp53 that are exported from nucleus into cytoplasm, preventing p53 from regulating gene expression.

In contrast with the above isoforms, p53β presents the capacity to increase p53 transcriptional capacity, as shown using a pG13-Luc reporter system [74]. This effect appears to be promoter-dependent, as these positive effects have been observed only with *BAX* but not with *P21(WAF1/CIP1)* reporter genes [5]. p53β may thus modulate p53 transcriptional activity to favor the transactivation of apoptotic target genes, such as *BAX*. While p53β lacks a large part of the oligomerisation domain, this isoform interacts with p53 as shown by co-immunoprecipitation of endogenous p53 and p53β in MCF7 cells [5]. This observation suggests that p53β may modulate p53 transactivation by hetero-oligomers formation, whereas their structure remains to be determined. There is some evidence that a p53 protein lacking the OD may nevertheless form unstable complexes that retain DNA-binding capacities [75]. It is not known whether this mechanism accounts for the biological effects of p53β on transactivation by p53 [5].

Even if some intrinsic biochemical capacities have been attributed to p53 isoforms, most of the studies report their biochemical role towards p53 transcriptional activity either as inhibitory regulators (ΔNp53, Δ133p53 and Δp53) or as enhancer (p53β). In both instances, the biochemical mechanisms include DNA-binding modulation, hetero-oligomers formation and/or p53 sequestration in the cytoplasm. These three mechanisms may be dependent on the isoform, on the cellular context and also on the target gene considered, leading to a wide diversity of regulatory options, either positive or negative, by p53 isoforms. In this way, one of the most important factors is the relative expression level of the isoforms as compared to p53 itself, which may vary from one tissue or cell type to another [5]. These observations based on *in vitro* data

suggest that modulation of p53 transcriptional activity by its isoforms may elicit a complex panel of biological responses that have a profound impact on the consequences of p53 activation.

Biological properties of p53 isoforms. *ΔNp53: suppressor or enhancer of p53 functions?* Clonogenic assays in cells co-transfected with ΔNp53 and p53 revealed that ΔNp53 could partially alleviate growth suppressive activity of p53 [4, 47]. This inhibitory activity may contribute to transiently suppress p53 activity during normal cell-cycle progression. In the human fibroblast cell line WI38, endogenous levels of ΔNp53 increased at the G1/S transition while p53 expression level decreased, resulting in ratios that are in favor of ΔNp53 [4]. In parallel, levels of p21 were reduced throughout S phase, while normal p53 and p21 expression patterns were restored in cells progressing into G2/M. These experiments suggest that up-regulation of ΔNp53 may be part of a mechanism by which normal cells can switch off p53 activity during cell cycle progression. It was demonstrated that variations of p53 and ΔNp53 expression levels were dependent upon two IRES motifs that share different and independent capacities [63]. The (-1)5'UTR IRES motif regulates p53 expression and is the most active during G2/M transition, whereas the p53(+39) IRES motif, in charge of ΔNp53 expression, is more active during G1/S transition [63]. In addition, coordinated changes in the relative levels of p53 and ΔNp53 may be brought about by changes in p53 mRNA splicing patterns but remain to be investigated.

Based on the above biochemical and biological evidence, it would be expected that excess levels of ΔNp53 *in vivo* down-regulates p53 suppressive activity during development, stress responses and tumorigenesis. Surprisingly, this is not the case. In a mouse model, over-expression of an ΔNp53 transgene appeared to have a biological effect only in the presence of functional *TP53* allele [76]. The transgenic mice presented a decrease of both lifespan and body size, not due to a reduction of cell size or increased apoptosis, but due to a slowdown in cell proliferation rate associated with an increase in senescence. Maier and collaborators speculated that over-expression of ΔNp53 may interrupt an IGF-1 dependent proliferative signaling system, at the origin of the reduced lifespan and accelerated senescence [76]. However, it should be noted that transgenic mice expressed a higher p53 level than the non-transgenic mice, suggesting that ΔNp53 may contribute to stabilize p53 *in vivo* by helping p53 to escape Hdm2-dependent degradation and that, in such circumstances, ΔNp53 may enhance the basal activity of p53. This hypothesis is compatible with the observed biological effects in mice (i.e. proliferation slowdown, cellular senescence and premature ageing) and with reporter assay in normal MEF cells, where transfection of ΔNp53 in variable amounts resulted in opposite effects on p53 transcriptional capacity [76]. Low levels of ΔNp53 had inhibitory effects on p53 transactivation but, at high levels, ΔNp53 exerted enhancing effects on p53. These opposite results may be explained by the impaired Hdm2-dependent degradation of p53 in cells co-transfected with both p53 and ΔNp53 as compared to p53 alone [3]. The positive effect of ΔNp53 on p53 may be due to ΔNp53-dependent escape of p53 from Hdm2 degradation [4, 47].

There are only few clues on physiological and/or pathological conditions in which ΔNp53 may enhance p53 activity. Recent studies have shown that treatment of cancer cell lines with low doses of the novel platinum(IV) complex LA-12, which restores sensitivity to cisplatin-resistant cells, induced a p53-dependent inhibition of cell proliferation together with increased levels of p53 and ΔNp53 [77]. This observation is compatible with other recent results showing that ΔNp53 may modulate the folding, oligomerisation and post-translational regulation of p53 to induce different p53 properties depending upon cellular stress [78]. Thus, the role of post-translational modification status of p53, ΔNp53, or both, may have a major influence in either enhancing or counteracting p53 function by ΔNp53.

Aside from p53-dependent functions, it has been proposed that ΔNp53 may exert some autonomous activities. When expressed alone in clonogenic assays using p53-null cells, ΔNp53 induced an increase in cell proliferation rate as compared to empty vector, suggesting that ΔNp53 may have an intrinsic p53-independent proliferative activity [4, 47]. The mechanism behind this activity is however, not understood. It could result from interference between ΔNp53 and products of *TP63* or *TP73*, which use similar response elements to regulate a range of genes partially overlapping with p53 target genes [45, 46]. In contrast, when transiently transfected in a p53-null cell background, ΔNp53 over-expression was able to induce apoptosis, suggesting that this isoform retains an intrinsic capacity to transactivate pro-apoptotic gene [3]. This hypothesis was supported by the fact that at least some of the pro-apoptotic p53 target genes were found to be over-expressed in a micro-array based, transcriptome profiling of cells expressing ΔNp53 [72]. In addition, tunicamycin treatment inducing endoplasmic reticulum stress was shown to increase ΔNp53 expression in a cap-independent manner (i.e. use of IRES motif for translational initiation), leading to an ΔNp53-dependent apoptosis [62].

Other p53 isoforms. In p53-null transfected cells exposed to UV, Δp53 was observed to bind the *P21(WAF1/CIP1)* promoter and to increase its expression, leading to a decrease of cyclin A-Cdk2 activity and of progression into S phase [32]. This has been interpreted as a mechanism that may allow for better coordination of DNA repair before replication. In the same cell system, expression of p53 was responsible for inhibition of cyclin A-Cdk1 activity, resulting in cell cycle arrest at S/G2 transition.

Regarding Δ133p53, its co-expression with p53 was associated with a complete inhibition of p53-induced apoptosis that may be explained by inhibition of p53 transcriptional activity by Δ133p53 [5, 74]. In earlier studies, p53 cDNA construct retaining the entire intron 4 actually behaved as a better oncogene than p53 cDNA natural construct in *RAS*-mediated transformation of primary fibroblasts [79, 80]. Such a cDNA would be predicted to retain the capacity to express not only ΔNp53 but also Δ133p53. However, it has been shown that ΔNp53 expressed alone in a *RAS*-transformed assay has no effect on cell transformation, suggesting that combined effect of N-truncated p53 isoforms may have pro-oncogenic properties, such as ΔNp73 [81, 82]. In addition, induction of Δ133p53 expression in *def* -/- zebrafish embryos is concomitant to *P21(WAF1/CIP1)* gene activation, leading to cell cycle arrest [44].

Over-expression of p53β in the presence of p53 induced an increase by 20 % of apoptosis as compared to p53 alone, an effect that correlates with a quantitative increase in transactivation of the *BAX* promoter [5].

Although limited, current observations suggest some biological functions for p53 isoforms. Both N-truncated isoforms, ΔNp53 and Δ133p53, appear to be strong suppressors of p53. However, ΔNp53 seems to have more subtle effects since it enhances p53 activity in non-stressed conditions, whereas its effects range from suppression to enhancement of p53 activity in stressed cells. In addition, ΔNp53 may exert p53-independent function, being involved in cell cycle progression. The p53β isoform, which differs from p53 only by its C-terminus, may either cooperate with p53 or take over some specialized p53 suppressor functions, depending upon stress and cell type contexts.

p53 isoforms and clinical considerations

TP53 is frequently inactivated by gene deletion or mutation. In many cancers retaining a wild-type *TP53*, others types of alterations in the p53 pathway have been described resulting in the down-regulation of p53 function [27]. N-truncated p63 and p73 isoforms have been proposed to act as dominant-negative inhibitors of p53. In particular, ΔNp73 can operate as an oncogene through neutralization of p53 suppressive activity [46]. p53 isoforms modulate p53 activity, suggesting that their deregulation and/or differential expression may contribute to inhibit wild-type p53 suppressive activity. In this way, attenuation of p53 response may result in a higher sensitivity of cells to genetic damage, facilitating transformation and/or progression of tumorigenesis.

Deregulation of p53 isoforms expression in human cancers. Clinical studies have focused on the differential expression of p53 isoforms, in particular p53, Δ133p53 and their C-terminal variants. In eight normal breast tissues, p53 (β and γ) mRNA, but not Δ133p53 (α, β and γ), were detected, while in 30 human breast tumors only p53β (33 %) and Δ133p53α (80 %) were expressed [5]. This suggests an association between breast cancer and deregulation of p53 isoform expression, namely over-expression of Δ133p53α and reduction of p53β. Whether these changes occur preferentially in cancers that retain wild-type allele remains to be assessed. Two other studies were based on analysis of p53 isoforms expression by using nested RT-PCR, which amplified the entire p53 mRNA. The first analyzed biopsies of 21 Squamous Cell Carcinoma of the Head and Neck (SCCHN) and 16 adjacent tissues. The expression of p53β is observed in 93 % of all samples, including cancer and non-cancer, whereas p53γ and Δ133p53 (α, β and γ) were detected in only some samples and at low levels [83]. This study thus concluded that p53β is the most expressed, both in normal tissue and in SCCHN. However, numbers of cases were two small to draw a conclusion about a possible significant difference in expression. The second study analyzed the expression of p53 (α, β and γ) and Δ133p53 (α, β and γ) in 20 biopsies from patients with Oral Lichen Planus (OLP), a chronic inflammatory disease of skin and mucosa that is considered a pre-malignant condition [84]. This study found that p53β and Δ133p53α were the most expressed isoforms in OLP. Finally, one study analyzed the expression of isoforms in melanoma, a type of cancer where *TP53* mutation is infrequent. In nine primary cultures of melanoma cells, p53β mRNA was expressed in 55 % of samples, whereas p53I2 mRNA, encoding ΔNp53, was expressed in all samples [85]. In addition, in a panel of 16 melanoma cell lines, p53β and ΔNp53 isoforms presented an unconventional intracellular distribution. p53β was accumulated in both the nucleus and cytoplasm, while ΔNp53 was accumulated in the cytoplasm, instead of being restricted to the nucleus as observed in non-melanoma cell lines [85]. These

data suggest that p53 isoforms may exhibit differential pattern of expression in tumors. However, data available are too limited to draw definitive conclusions, due to lack of statistical power. In addition, no systematically analyses of all known p53 isoforms at both mRNA and protein levels were done because of the lack of suitable antibodies, in particular for N-terminally truncated isoforms.

The impact of p53 isoforms expression was also examined for therapeutic considerations. The expression patterns of cells isolated from peripheral venous blood of five patients diagnosed with Acute Myeloid Leukemia (AML) were analyzed by RT-PCR and two-dimensional protein electrophoresis before and 18 h after chemotherapy with idarubicine and cytaraline [86]. A group of p53 isoforms shorter than p53 was detected, perhaps corresponding to p53β, p53γ or Δp53. In cells before treatment, these shorter isoforms were expressed at levels higher than p53, while after treatment, the ratio p53/shorter isoforms increased, the shorter isoforms being expressed less after than before treatment [86]. This shift in p53 isoform patterns suggests that p53 response to chemotherapy requires that p53 accumulates at levels higher than the ones of short isoforms. This interpretation is consistent with the hypothesis of a negative regulatory role of p53 isoforms, which may have an impact on p53-responses to chemotherapy.

Δp53: a modulator of clinical outcome in breast cancer with *TP53* mutations? In a case-only series of 88 breast carcinomas, it was shown that Δp53 exhibited an expression pattern similar to p53 in relation to *TP53* mutational status [49]. In wild-type *TP53* tumors, p53 mRNA expression was higher than in tumors with missense or in frame mutations, but lower than in those with nonsense, frameshift and splicing mutations. Since some tumors harbored mutations located in region from exon 7 to 9, lacking in Δp53, this study investigated whether tumors with "double mutants" (e.g. mutation affecting both p53 and Δp53) had a different clinical outcome than those with "single mutants" (e.g. mutation affecting only p53). No difference in survival rate, clinical or biological parameters were observed. This observation suggests that Δp53 does not compensate for the functional impact of mutant *TP53*. Analysis of survival curves showed a trend for a worse prognosis of cancers with mutΔp53, and Δp53 mutational status showed a tendency to differ among breast cancer subtypes as classified according to gene expression patterns [49]. Wild-type Δp53 expression tended to be more common in Luminal A subtype, whereas mutΔp53 expression was more common in Basal and ERBB2 subtypes. These results suggest that mutΔp53 may be associated with a worse prognosis of cancer. However, with these data being the first available to implicate Δp53 in breast cancer, this type of analysis remains to be confirmed by a more important study.

Mutations affecting p53 splicing patterns. Several studies reported that intronic or exonic mutations gave rise to particular truncated p53 proteins with strong similitude to p53 isoforms. Molt-4 T-lymphoblastic leukemia cell lines express an unexpected p53 transcript of higher molecular weight than the usual p53 one [51]. A deletion of 8 bp was identified in intron 9 leading to an insertion of 133 additional nucleotides derived from intron 9. The authors suggested that this deletion induced formation of an alternative 5' acceptor splice site in intron 9. However, this transcript corresponds to p53β mRNA and we can speculate that the 8 bp deletion may favor the use of the alternative splice site producing p53β. In one case of colorectal cancer, a substitution of A to G in the 3' part of intron 9 led to the expression of a p53 protein, which conserves residues 1 to 332 but presents new residues from 332 to 358 [87]. This truncated protein presents some similitude with p53β but has a longer C-terminal domain.

On the other hand, mutations sometimes occur at splice junctions, leading to the production of aberrant p53 truncated protein. In one study of chronic lymphocytic leukemia, 85 % of patients expressed a Δex6 p53 transcript, which lacks the first 113 nucleotides of exon 6 leading to a premature stop codon at residue 189 that results in a C-truncated p53 product [88]. This transcript was not found in healthy patients and no mutation on *TP53* was detected, suggesting a deregulation of splicing giving rise to the production of a new p53 isoform. In Li-Fraumeni syndrome, defined as family carrying germline *TP53* mutations, three atypical mutations have been described, all affecting acceptor or donor splicing sites [89]. For example, mutation of 5' acceptor splice site in intron 3 resulted in the skipping of exon 4, suggesting that these cells were unable to produce p53 and ΔNp53. Another study presented similar results and compiled data of other studies to demonstrate the importance of mutations occurring in splice sites of *TP53* [90]. In 108 samples issued from patients with lung cancer, they described two aberrant transcripts produced by mutations occurring in splicing sites not detected by using their classical primers. This study suggested that mutations occurring in splice sites of *TP53* may occur at a higher frequency than currently estimated, raising the hypothesis that truncated p53 protein may have an impact on p53 function.

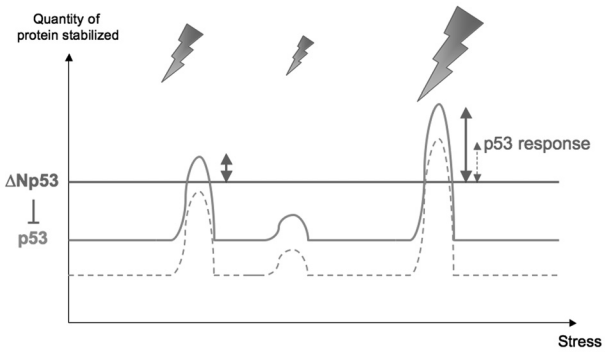


Figure 5. ΔNp53, a "buffer" for regulating p53 response. ΔNp53 is more stable than p53 as it escapes to Hdm2-degradation and is not activated by a stress as compared to p53. As ΔNp53 inhibits p53 transcriptional activity, a p53 response would be observed only when the quantity of stabilized p53 is higher than the quantity of ΔNp53. A change in p53 ratio would induce a decrease of p53 response (dotted line).

Conclusion: Biological integration of the p53 isoforms network

Several lessons can be extracted from current data on p53 isoforms. First, the diversity of processes leading to their production is a clue to the functional significance of p53 isoforms, as cells have adopted number of strategies to produce them in different regulatory contexts. Second, most p53 isoforms are deficient for p53-like suppressive function but appear to modulate p53 activity [4, 74, 71]. Third, N-truncated p53 isoforms escape Hdm2-mediated degradation and may provide a regulatory system for controlling p53 activity independently of Hdm2 [4, 11]. Fourth, patterns of p53 isoform appear to be deregulated in different cancer types and may provide an alternative mechanism to inactivate p53 suppressive function in wild-type *TP53* tumors or to modulate mutation penetrance and phenotype in mutant *TP53* tumors [5, 49, 84, 83].

A model illustrating the possible biological significance of p53 isoforms can be drawn (Fig. 5). Cells are permanently submitted to transient environmental (i.e. radiation, hypoxia) or endogenous (i.e. metabolic) stresses that induce p53-dependent cell death, an undesirable response in many physiological contexts. The model suggests that, to avoid small but potentially damaging variations in p53 activation, cells have developed sensors based on two main components. First, the p53-Hdm2 loop maintains p53 at a low level in normal conditions but is transiently disrupted in response to stress to allow rapid accumulation of p53 [11, 14]. Second, N-truncated isoforms, such as ΔNp53, act as repressors of p53 transcriptional response, working as a "buffer" against untimely p53 activation. Indeed, ΔNp53 is expressed at a higher level than p53 in non-stressed cells since ΔNp53, being deficient for Hdm2 binding, escapes Hdm2-induced degradation [4, 47]. Upon DNA-damage, ΔNp53 is not activated in contrast to p53, which then becomes more abundant than ΔNp53 [4]. This transcriptionally inactive protein may occupy p53RE in promoters of p53 target genes, so that a certain threshold of p53 accumulation must be reached to out-compete them and allow the binding of the transcriptionally active form (Fig. 5).

This hypothesis has several consequences: (1) the critical factor in determining p53 suppressive response will be the ΔNp53/p53 ratio, suggesting that only quite drastic stress would induce a p53 response (Fig. 5); (2) the balance between ΔNp53 and p53 may provide a subtle mechanism for selective induction of p53 target genes and thus for inducing the appropriate response, since p53RE may have different binding affinity for p53 and ΔNp53; (3) alteration of the ΔNp53/p53 ratio may deregulate the sensor system that integrates stress signals involved in the regulation of cell proliferation, DNA repair and cell survival, explaining the phenotype of premature aging observed in mice over-expressing ΔNp53 in a p53 background [76]; (4) different individuals may have different intrinsic levels of ΔNp53 expression, thus providing an additional mechanism to account for individual differences in susceptibility to stress, mutagenesis and carcinogenesis.

In this model, Δ133p53 isoform may have similar p53RE-blocking properties to ΔNp53. However, Δ133p53 is expressed from an independent promoter and in specific tissues, suggesting that its blocking properties may be specifically expressed in a cell type and time restricted manner, such as during development or differentiation [5]. Δp53 appears to be present in the cytoplasm of normal cells, suggesting a function independent of p53RE binding. Interestingly, Δp53 retains the portion of the DNA-binding domain that binds the p53-BP2 protein. Thus, this non-canonical isoform could be specialized in protein interactions. Finally, p53β may represent a truly suppressive form of p53, which may be particularly tuned to induce apoptotic responses.

The complex effects of p53 isoforms on p53 response may have significant consequences for cancer development. Aside from a possible involvement in individual susceptibility, isoform expression may contribute to p53 inactivation in cells retaining wild-type *TP53* alleles. However, based on the hypotheses detailed above, the biggest impact of isoforms may be on p53-dependent responses to cytotoxic therapies, as the capacity of cancer cells to undergo drug-induced apoptosis may be largely dependent upon p53 isoform expression patterns. For example, cancer cells ex-

pressing high levels of ΔNp53 or Δ133p53 may have poor apoptotic responses, whereas cells expressing high levels of p53β may be very good responders to treatment. Thus, studies on p53 isoforms will provide a rich field of new concepts and paradigms in the regulation of p53 activities.

Acknowledgments. We thank Magali Olivier for advice and for providing helpful comments on the manuscript. V. M. was supported by a fellowship of La Ligue Nationale Contre le Cancer (France).

1 Vousden, K. H. and Lane, D. P. (2007) p53 in health and disease. Nat. Rev. Mol. Cell. Biol. 8, 275–283.
2 Courtois, S., de Fromentel, C. C. and Hainaut, P. (2004) p53 protein variants: structural and functional similarities with p63 and p73 isoforms. Oncogene 23, 631–638.
3 Yin, Y., Stephen, C. W., Luciani, M. G. and Fåhraeus, R. (2002) p53 stability and activity is regulated by Mdm2-mediated induction of alternative p53 translation products. Nat. Cell. Biol. 4, 462–467.
4 Courtois, S., Verhaegh, G., North, S., Luciani, M. G., Lassus, P., Hibner, U., Oren, M. and Hainaut, P. (2002) DeltaN-p53, a natural isoform of p53 lacking the first transactivation domain, counteracts growth suppression by wild-type p53. Oncogene 21, 6722–6728.
5 Bourdon, J. C., Fernandes, K., Murray-Zmijewski, F., Liu, G., Diot, A., Xirodimas, D. P., Saville, M. K. and Lane, D. P. (2005) p53 isoforms can regulate p53 transcriptional activity. Genes Dev. 19, 2122–2137.
6 May, P. and May, E. (1999) Twenty years of p53 research: structural and functional aspects of the p53 protein. Oncogene 18, 7621–7636.
7 Honda, R., Tanaka, H. and Yasuda, H. (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett. 420, 25–27.
8 Haupt, Y., Maya, R., Kazaz, A. and Oren, M. (1997) Mdm2 promotes the rapid degradation of p53. Nature 387, 296–299.
9 Picksley, S. M., Vojtesek, B., Sparks A. and Lane, D. P. (1994) Immunochemical analysis of the interaction of p53 with MDM2;–fine mapping of the MDM2 binding site on p53 using synthetic peptides. Oncogene 9, 2523–2529.
10 Li, M., Brooks, C. L., Wu-Baer, F., Chen, D., Baer, R. and Gu, W. (2003) Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. Science 302, 1972–1975.
11 Kubbutat, M. H., Jones, S. N. and Vousden, K. H. (1997) Regulation of p53 stability by Mdm2. Nature 387, 299–303.
12 Geyer, R. K., Yu, Z. K. and Maki, C. G. (2000) The MDM2 RING-finger domain is required to promote p53 nuclear export. Nat. Cell. Biol. 2, 569–573.
13 Craig, A. L., Burch, L., Vojtesek, B., Mikutowska, J., Thompson, A. and Hupp, T. R. (1999) Novel phosphorylation sites of human tumor suppressor protein p53 at Ser20 and Thr18 that disrupt the binding of mdm2 (mouse double minute 2) protein are modified in human cancers. Biochem. J. 342, 133–141.
14 Zhang, Y. and Xiong, Y. A. (2001) p53 amino-terminal Nuclear Export Signal inhibited by DNA damage-induced phosphorylation. Science 292, 1910–1915.
15 Pluquet, O. and Hainaut, P. (2001) Genotoxic and non-genotoxic pathways of p53 induction. Cancer Lett. 174, 1–15.
16 Halazonetis, T. D. and Kandil, A. N. (1993) Conformational shifts propagate from the oligomerization domain of p53 to its tetrameric DNA binding domain and restore DNA binding to select p53 mutants. EMBO J. 12, 5057–5064.
17 Thut, C. J., Chen, J. L., Klemm, R. and Tjian, R. (1995) p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60. Science 267, 100–104.
18 el-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W. and Vogelstein, B. (1992) Definition of a consensus binding site for p53. Nat. Genet. 1, 45–49.
19 Hoh, J., Jin, S., Parrado, T., Edington, J., Levine, A. J. and Ott, J. (2002) The p53MH algorithm and its application in detecting p53-responsive genes. Proc. Natl. Acad. Sci. USA 99, 8467–8472.
20 Wu, X., Bayle, J. H., Olson, D. and Levine, A. J. (1993) The p53-mdm-2 autoregulatory feedback loop. Genes Dev. 7, 1126–1132.
21 Dornan, D., Wertz, I., Shimizu, H., Arnott, D., Frantz, G. D., Dowd, P., O'Rourke, K., Koeppen, H. and Dixit, V. M. (2004) The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature 429, 86–92.
22 Esser, C., Scheffner, M. and Höhfeld, J. (2005) The chaperone-associated ubiquitin ligase CHIP is able to target p53 for proteasomal degradation. J. Biol. Chem. 280, 27443–27448.
23 Leng, R. P., Lin, Y., Ma, W., Wu, H., Lemmers, B., Chung, S., Parant, J. M., Lozano, G., Hakem, R. and Benchimol, S. (2003) Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. Cell 112, 779–791.
24 Brooks, C. L. and Gu, W. (2006) p53 ubiquitination: Mdm2 and beyond. Mol. Cell. 21, 307–315.
25 Leu, J. I., Dumont, P., Hafey, M., Murphy, M. E. and George, D. L. (2004) Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. Nat. Cell. Biol. 6, 443–450.
26 Abramova, N. A., Russell, J., Botchan, M. and Li, R. (1997) Interaction between replication protein A and p53 is disrupted after UV damage in a DNA repair-dependent manner. Proc. Natl. Acad. Sci. USA 94, 7186–7191.
27 Petitjean, A., Mathe, E., Kato, S., Ishioka, C., Tavtigian, S. V., Hainaut, P. and Olivier, M. (2007) Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Human mutation 28, 622–629.
28 Petitjean, A., Achatz, M. I., Borresen-Dale, A. L., Hainaut, P. and Olivier, M. (2007) TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene 26, 2157–2165.
29 Leach, F. S., Tokino, T., Meltzer, P., Burrell, M., Oliner, J. D., Smith, S., Hill, D. E., Sidransky, D., Kinzler, K. W. and Vogelstein, B. (1993) p53 Mutation and MDM2 amplification in human soft tissue sarcomas. Cancer Res. 53, 2231–2234.
30 Laurie, N. A., Donovan, S. L., Shih, C. S., Zhang, J., Mills, N., Fuller, C., Teunisse, A., Lam, S., Ramos, Y., Mohan, A., Johnson, D., Wilson, M., Rodriguez-Galindo, C., Quarto, M., Francoz, S., Mendrysa, S. M., Guy, R. K., Marine, J. C., Jochemsen, A. G. and Dyer, M. A. (2006) Inactivation of the p53 pathway in retinoblastoma. Nature 444, 61–66.
31 Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J. and Howley, P. M. (1990) The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 63, 1129–1136.
32 Rohaly, G., Chemnitz, J., Dehde, S., Nunez, A. M., Heukeshoven, J., Deppert, W. and Dornreiter, I. (2005) A novel human p53 isoform is an essential element of the ATR-intra-S phase checkpoint. Cell 122, 21–32.
33 Lamb, P. and Crawford, L. (1986) Characterization of the human p53 gene. Mol. Cell. Biol. 6, 1379–1385.
34 Tuck, S. P. and Crawford, L. (1989) Characterization of the human p53 gene promoter. Mol. Cell Biol. 9, 2163–2172.
35 Noda, A., Toma-Aiba, Y. and Fujiwara, Y. (2000) A unique, short sequence determines p53 gene basal and UV-inducible expression in normal human cells. Oncogene 19, 21–31.
36 Boggs, K. and Reisman, D. (2006) Increased p53 transcription prior to DNA synthesis is regulated through a novel regulatory element within the p53 promoter. Oncogene 25, 555–565.
37 de Moraes, E., Dar, N. A., de Moura Gallo, C. V. and Hainaut, P. (2007) Cross-talks between cyclooxygenase-2 and tumor suppressor protein p53: Balancing life and death during inflammatory stress and carcinogenesis. Int. J. Cancer 121, 929–937.

38 Schreiber, M., Kolbus, A., Piu, F., Szabowski, A., Möhle-Steinlein, U., Tian, J., Karin, M., Angel, P. and Wagner, E. F. (1999) Control of cell cycle progression by c-Jun is p53 dependent. Genes Dev. 13, 607–619.

39 Reisman, D., Greenberg, M. and Rotter, V. (1988) Human p53 oncogene contains one promoter upstream of exon 1 and a second, stronger promoter within intron 1. Proc. Natl. Acad. Sci. USA. 85, 5146–5150.

40 Reisman, D., Bálint, E., Loging, W. T., Rotter, V. and Almon, E. (1996) A novel transcript encoded within the 10-kb first intron of the human p53 tumor suppressor gene (D17S2179E) is induced during differentiation of myeloid leukemia cells. Genomics 38, 364–370.

41 Khochbin, S., Brocard, M. P., Grunwald, D. and Lawrence, J. J. (1992) Antisense RNA and p53 regulation in induced murine cell differentiation. Ann. N. Y. Acad. Sci. 660, 77–87.

42 Sayan, A. E., Rossi, M., Melino, G. and Knight, R. A. (2004) p73: in silico evidence for a putative third promoter region. Biochem. Biophys. Res. Commun 313, 765–770.

43 Shamsher, M. and Montano, X. (1996) Analysis of intron 4 of the p53 gene in human cutaneous melanoma. Gene 176, 259–262.

44 Chen, J., Ruan, H., Ng, S. M., Gao, C., Soo, H. M., Wu, W., Zhang, Z., Wen, Z., Lane, D. P. and Peng, J. (2005) Loss of function of def selectively up-regulates Delta113p53 expression to arrest expansion growth of digestive organs in zebrafish. Genes Dev. 19, 2900–2911.

45 Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M. D., Dötsch, V., Andrews, N. C., Caput, D. and McKeon, F. (1998) p63, a p53 homolog at 3q27–29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol. Cell 2, 305–316.

46 Ishimoto, O., Kawahara, C., Enjo, K., Obinata, M., Nukiwa, T. and Ikawa, S. (2002) Possible oncogenic potential of DeltaNp73: a newly identified isoform of human p73. Cancer Res. 62, 636–641.

47 Ghosh, A., Stewart, D. and Matlashewski, G. (2004) Regulation of human p53 activity and cell localization by alternative splicing. Mol. Cell. Biol. 24, 7987–7997.

48 Matlashewski, G., Pim, D., Banks, L. and Crawford, L. (1987) Alternative splicing of human p53 transcripts. Oncogene Res. 1, 77–85.

49 Baumbusch, L. O., Myhre, S., Langerød, A., Bergamaschi, A., Geisler, S. B., Lønning, P. E., Deppert, W., Dornreiter, I. and Børresen-Dale, A. L. (2006) Expression of full-length p53 and its isoform Deltap53 in breast carcinomas in relation to mutation status and clinical parameters. Molecular cancer 5, 47–67.

50 Bourdon, J. C. (2007) p53 and its isoforms in cancer. Br. J. Cancer 97, 277–282.

51 Chow, V. T., Quek, H. H. and Tock, E. P. (1993) Alternative splicing of the p53 tumor suppressor gene in the Molt-4 T-lymphoblastic leukemia cell line. Cancer Lett. 73, 141–148.

52 Flaman, J. M., Waridel, F., Estreicher, A., Vannier, A., Limacher, J. M., Gilbert, D., Iggo, R. and Frebourg, T. (1996) The human tumor suppressor gene p53 is alternatively spliced in normal cells. Oncogene 12, 813–818.

53 Anczuków, O., Ware, M. D., Buisson, M., Zetoune, A. B., Stoppa-Lyonnet, D., Sinilnikova, O. M. and Mazoyer, S. (2008) Does the nonsense-mediated mRNA decay mechanism prevent the synthesis of truncated BRCA1, CHK2, and p53 proteins? Hum. Mutat. 29, 65 -73.

54 Arai, N., Nomura, D., Yokota, K., Wolf, D., Brill, E., Shohat, O. and Rotter, V. (1986) Immunologically distinct p53 molecules generated by alternative splicing. Mol. Cell. Biol. 6, 3232–3239.

55 Han, K. A. and Kulesz-Martin, M. F. (1992) Alternatively spliced p53 RNA in transformed and normal cells of different tissue types. Nucleic Acids Res. 20, 1979–1981.

56 Will, K., Warnecke, G., Bergmann, S. and Deppert, W. (1995) Species- and tissue-specific expression of the C-terminal alternatively spliced form of the tumor suppressor p53. Nucleic Acids Res. 23, 4023–4028.

57 Laverdière, M., Beaudoin, J. and Lavigueur, A. (2000) Species-specific regulation of alternative splicing in the C-terminal region of the p53 tumor suppressor gene. Nucleic Acids Res. 28,1489–1497.

58 Takagi, M., Ohashi, K., Morimura, T., Sugimoto, C. and Onuma, M. (2006) The presence of the p53 transcripts with truncated open reading frames in Marek's disease tumor-derived cell lines. Leuk. Res. 30, 987–992.

59 Venables, J. P. and Burn, J. (2006) EASI–enrichment of alternatively spliced isoforms. Nucleic Acids Res. 34, e103.

60 Rovinski, B., Munroe, D., Peacock, J., Mowat, M., Bernstein, A. and Benchimol, S. (1987) Deletion of 5'-coding sequences of the cellular p53 gene in mouse erythroleukemia: a novel mechanism of oncogene regulation. Mol. Cell. Biol. 7, 847–853.

61 Kozak, M. (1987) At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. J. Mol. Biol. 196, 947–950.

62 Candeias, M. M., Powell, D. J., Roubalova, E., Apcher, S., Bourougaa, K., Vojtesek, B., Bruzzoni-Giovanelli, H. and Fåhraeus, R. (2006) Expression of p53 and p53/47 are controlled by alternative mechanisms of messenger RNA translation initiation. Oncogene 25, 6936–6947.

63 Ray, P. S., Grover, R. and Das, S. (2006) Two internal ribosome entry sites mediate the translation of p53 isoforms. EMBO Rep. 7, 404–410.

64 Mokdad-Gargouri, R., Belhadj, K. and Gargouri, A. (2001) Translational control of human p53 expression in yeast mediated by 5'-UTR-ORF structural interaction. Nucleic Acids Res. 29, 1222–1227.

65 Takagi, M., Absalon, M. J., McLure, K. G. and Kastan, M. B. (2005) Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. Cell 123, 49–63.

66 Mosner, J., Mummenbrauer, T., Bauer, C., Sczakiel, G., Grosse, F. and Deppert, W. (1995) Negative feedback regulation of wild-type p53 biosynthesis. EMBO J. 14, 4442–4449.

67 Christian, K. J., Lang, M. A. and Raffali-Mathieu, F. (2008) Interaction of hnRNPC1/C2 with a novel cis-regulatory element within p53 mRNA as a response to cytostatic drug treatment. Mol. Pharmacol. 73, 1558–1567.

68 Sayan, A. E., Roperch, J. P., Sayan, B. S., Rossi, M., Pinkoski, M. J., Knight, R. A., Willis, A. E. and Melino, G. (2007) Generation of DeltaTAp73 proteins by translation from a putative internal ribosome entry site. Ann. N. Y. Acad. Sci. 1095, 315–324.

69 Cho, Y., Gorina, S., Jeffrey, P. D. and Pavletich, N. P. (1994) Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science 265, 346–355.

70 Hinds, P. W., Finlay, C. A., Quartin, R. S., Baker, S. J., Fearon, E. R., Vogelstein, B. and Levine, A. J. (1990) Mutant p53 DNA clones from human colon carcinomas cooperate with ras in transforming primary rat cells: a comparison of the "hot spot" mutant phenotypes. Cell Growth. Differ. 1, 571–580.

71 Chan, W. M. and Poon, R. Y. (2007) The p53 Isoform Deltap53 lacks intrinsic transcriptional activity and reveals the critical role of nuclear import in dominant-negative activity. Cancer Res. 67, 1959–1969.

72 Ohki, R., Kawase, T., Ohta, T., Ichikawa, H., and Taya, Y. (2007) Dissecting functional roles of p53 N-terminal transactivation domains by microarray expression analysis. Cancer Sci. 98, 189–200.

73 Goldschneider, D., Horvilleur, E., Plassa, L. F., Guillaud-Bataille, M., Million, K., Wittmer-Dupret, E., Danglot, G., de Thé, H., Bénard, J., May, E. and Douc-Rasy, S. (2006) Expression of C-terminal deleted p53 isoforms in neuroblastoma. Nucleic Acids Res. 34, 5603–5612.

74 Murray-Zmijewski, F., Lane, D. P. and Bourdon, J. C. (2006) p53/p63/p73 isoforms: an orchestra of isoforms to harmonise

cell differentiation and response to stress. Cell Death Differ. 13, 962–972.
75 Wang, Y., Schwedes, J. F., Parks, D., Mann, K. and Tegtmeyer, P. (1995) Interaction of p53 with its consensus DNA-binding site. Mol. Cell Biol. 15, 2157–2165.
76 Maier, B., Gluba, W., Bernier, B., Turner, T., Mohammad, K., Guise, T., Sutherland, A., Thorner, M. and Scrable, H. (2004) Modulation of mammalian life span by the short isoform of p53. Genes Dev. 18, 306–319.
77 Hrstka, R., Powell, D. J., Kvardova, V., Roubalova, E., Bourougaa, K., Candeias, M. M., Sova, P., Zak, F., Fåhraeus, R. and Vojtěsek, B. (2008) The novel platinum(IV) complex LA-12 induces p53 and p53/47 responses that differ from the related drug, cisplatin. Anticancer Drugs 19, 369–379.
78 Powell, D. J., Hrstka, R., Candeias, M., Bourougaa, K., Vojtesek, B. and Fåhraeus, R. (2008) Stress-dependent changes in the properties of p53 complexes by the alternative translation product p53/47. Cell Cycle 7, 950–959.
79 Hinds, P., Finlay, C. and Levine, A. J. (1989) Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation. J. Virol. 63, 739–746.
80 Lozano, G. and Levine, A. J. (1991) Tissue-specific expression of p53 in transgenic mice is regulated by intron sequences. Mol. Carcinog. 4, 3–9.
81 Unger, T., Mietz, J. A., Scheffner, M., Yee, C. L. and Howley, P.M. (1993) Functional domains of wild-type and mutant p53 proteins involved in transcriptional regulation, transdominant inhibition, and transformation suppression. Mol. Cell Biol. 13, 5186–5194.
82 Stiewe, T., Zimmermann, S., Frilling, A., Esche, H. and Pützer, B. M. (2002) Transactivation-deficient DeltaTA-p73 acts as an oncogene. Cancer Res. 62, 3598–3602.
83 Boldrup, L., Bourdon, J. C., Coates, P. J., Sjöström, B. and Nylander, K. (2007) Expression of p53 isoforms in squamous cell carcinoma of the head and neck. European journal of cancer 43, 617–623.
84 Ebrahimi, M., Boldrup, L., Coates, P. J., Wahlin, Y. B., Bourdon, J. C. and Nylander, K. (2008) Expression of novel p53 isoforms in oral lichen planus. Oral Oncol. 44, 156–161.
85 Avery-Kiejda, K. A., Zhang, X. D., Adams, L. J., Scott, R. J., Vojtesek, B., Lane, D. P. and Hersey, P. (2008) Small Molecular Weight Variants of p53 Are Expressed in Human Melanoma Cells and Are Induced by the DNA-Damaging Agent Cisplatin. Clin. Cancer Res. 14, 1659–1668.
86 Anensen, N., Oyan, A. M., Bourdon, J. C., Kalland, K. H., Bruserud, O. and Gjertsen, B.T. (2006) A distinct p53 protein isoform signature reflects the onset of induction chemotherapy for acute myeloid leukemia. Clinical cancer research 12, 3985–3992.
87 Kashkin, K. N., Khlgatian, S. V., Gurova, O. V., Kuprash, D. V. and Nedospasov, S. A. (2007) New mutations in the human p53 gene–a regulator of the cell cycle and carcinogenesis. Biochemistry Mosc. 72, 282–292.
88 Pekova, S., Cmejla, R., Smolej, L., Kozak, T., Spacek, M. and Prucha. M. (2008) Identification of a novel, transactivation-defective splicing variant of p53 gene in patients with chronic lymphocytic leukemia. Leuk. Res. 32, 395–400.
89 Varley, J. M., Attwooll, C., White, G., McGown, G., Thorncroft, M., Kelsey, A. M., Greaves, M., Boyle, J. and Birch, J. M. (2001) Characterization of germline TP53 splicing mutations and their genetic and functional analysis. Oncogene 20, 2647–2654.
90 Holmila, R., Fouquet, C., Cadranel, J., Zalcman, G. and Soussi, T. (2003) Splice mutations in the p53 gene: case report and review of the literature. Hum. Mutat. 21, 101–102.
91 Harms, K. L. and Chen, X. (2005) The C terminus of p53 family proteins is a cell fate determinant. Mol. Cell Biol. 25, 2014–2030.
92 Mehta, S. A., Christopherson, K. W., Bhat-Nakshatri, P., Goulet, R. J., Broxmeyer, H. E., Kopelovich, L. and Nakshatri, H. (2007) Negative regulation of chemokine receptor CXCR4 by tumor suppressor p53 in breast cancer cells: implications of p53 mutation or isoform expression on breast cancer cell invasion. Oncogene 26, 3329–3337.