ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 6, 2011

DOI: 10.1089/ars.2010.3771

FORUM REVIEW ARTICLE

Redox Control and Interplay Between p53 Isoforms: Roles in the Regulation of Basal p53 Levels, Cell Fate, and Senescence

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Abstract

The p53 tumor suppressor protein has achieved stardom in molecular oncology owing to frequent inactivation in a large range of cancers. Known as a factor activated by multiple forms of stress and causing a broad suppressive response to DNA damage, its regulation and functions in basal (non-stress) conditions has received relatively little attention. We summarize recent findings highlighting roles of p53 in physiological processes such as stem cell maintenance, development, aging and senescence, and regulation of basal oxidative cell metabolism. We suggest that these properties are regulated through two integrated biochemical systems: the redox-sensing capacity of the p53 protein (due to its structural features and its regulation by redox factors such as thioredoxin, metallothioneins, or the redox-repair enzyme APE1/ref-1), and the expression of p53 as multiple isoforms with antagonist effects. We propose that interactions between p53 and its isoforms Δ40p53 or Δ133p53 play critical roles in intracellular signaling by reactive oxygen species. We also discuss evidence that p53 controls energy production by repressing glycolysis and enhancing mitochondrial oxidative metabolism. Together, these mechanisms suggest that p53 acts not only as a "guardian of the genome" against DNA damage but also as a finely-tuned regulator of redox-dependent physiological processes. *Antioxid. Redox Signal.* 15, 1655–1667.

Introduction

THIRTY YEARS AFTER ITS DISCOVERY, the p53 protein has achieved stardom status among tumor suppressors (25). This protein owes its name to its apparent molecular weight of 53 kDa and was initially identified by two parallel approaches. First, p53 was shown to complex with the Large T antigen of the SV40 virus, thus representing critical cellular target for transformation induced by this virus. Second, it was found that the serum of mice (and later, patients) carrying tumors often contained antibodies reacting with the same 53 kDa cellular phosphoprotein. Ten years later, in 1989, the *TP53* gene was identified as the site of mutations or loss of alleles in almost every known type of human cancer. Today, alterations in this gene remain by far the most universal cancer-related genetic defect, with high mutation prevalence (over 50%) in common cancers such as lung, ovary, head and neck, or esophagus, intermediate prevalence in breast, bladder, pancreas, or prostate cancer, and relatively infrequent mutations in melanoma, testis, or cervical cancers (53) (IARC TP53 database). These somatic mutations show a remarkable diversity in their locations in the coding sequence and in their types. In several instances, it has been possible to demonstrate that mutations were the direct consequence of site-specific DNA damage by mutagens such as polycyclic aromatic hydrocarbons from tobacco smoke in lung cancers of smokers, or aflatoxin in liver cancer in regions where this mycotoxin is a common food contaminant. Furthermore, the *TP53* gene can also be mutated in the germline, causing a Mendelian syndrome of predisposition to multiple cancers called Li–Fraumeni syndrome. It is assumed that *TP53* mutations may occur at the rate of 1 in 2500 births, making it a common cause of familial predisposition to cancer (54).

The biological basis for frequent alteration of p53 in cancer is now relatively well understood. The p53 protein is a transcription factor with multiple roles in antiproliferative cell responses, inducing cell cycle arrest, apoptosis, senescence, DNA repair or differentiation, the type of response depending from both the initial stimulus and from the nature of the cell (Fig. 1). It has long been considered that p53 was a "latent" factor, that is, a factor virtually absent from normal cells and tissues. Indeed, although expressed ubiquitously, p53 is rapidly degraded by a complex, proteasome-dependent machinery. The main regulator of p53 stability is Hdm2 (the human homolog of the product of the Murine Double Minute 2 gene). Hdm2 operates as a p53 E3-ubiquitin ligase that

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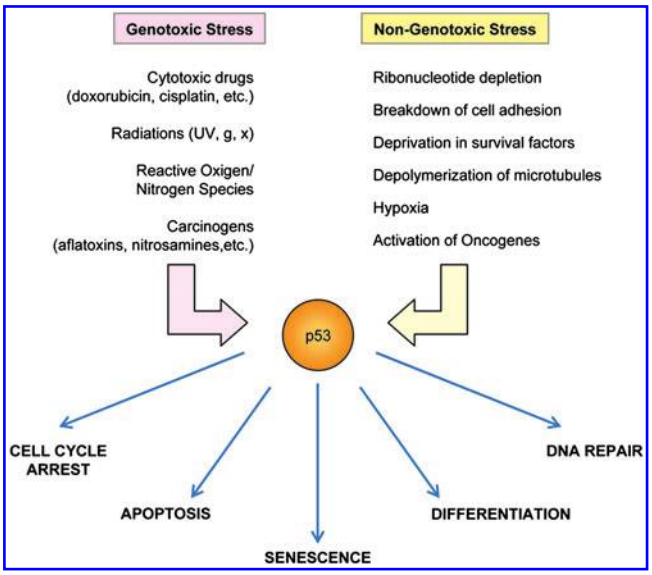

FIG. 1. p53, a sensor of multiple forms of stress. Signals that induce p53 activation are presented in two broad categories, genotoxic and nongenotoxic stress. After activation, p53 may induce a large range of suppressive responses, depending upon signal, cell type, and tissue context. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

targets p53 for rapid degradation by the proteasome, thus preventing its accumulation unless p53 is specifically induced by various activating signals. A number of other factors can also regulate p53 degradation, either by complementing or counteracting Hdm2 activity, or through distinct, Hdm2-independent, pathways (26, 45).

In response to a vast panel of genotoxic or nongenotoxic stress conditions, p53 becomes stabilized by multiple post-translational changes, escapes degradation, and accumulates in the nucleus, where it binds with high affinity to the regulatory regions of a large variety of target genes. Stresses that activate p53 include those that produce genotoxic damages (UV and γ-rays, chemicals, reactive oxygen species (ROS)), extreme physiological changes (hypoxia, depletion of microtubules or of ribonucleotides) as well as untimely or excessive growth signals (so-called "oncogenic stress", caused by constitutive activation of tyrosine kinase receptor or ras pathways) (Fig. 1). This large variety of inducing signals confers to p53 the properties of a multi-purpose, stress-induced suppressor. When activated in an acute way, p53 is an extremely potent factor that can lead cells to dramatic apoptosis in a matter of hours. However, when present at lower levels, p53 has a much more subtle range of effects that are now becoming to emerge.

This review focuses on understanding how p53 is regulated in this vast range of low-to-moderate stress conditions, and how cells may control p53 activity to avoid its untimely and counterproductive activation. These conditions are of particular importance because they represent changes that occur within the normal physiological range, as well as changes associated with normal developmental and aging processes. We develop the concept that the mechanisms responsible for the regulation of p53 in these conditions are at least partially distinct from those that drive rapid and acute p53 induction in response to high levels of DNA damage. In particular, we propose that, in such "low stress" conditions, p53 activity is tightly regulated by two complementary mechanisms, redox control of p53 protein activity and transcriptional control of p53 expression through the production of p53 isoforms. While activation of p53 by stress generally results in the accumulation of the major 53 kDa peptide which has given its name to the protein, the patterns of protein expression in "low-stress" conditions are complex, with a wide family of products with electrophoretic mobility ranging from 33 to 53 kDa. In addition, the p53 protein is extremely sensitive to oxidation/reduction, changing its tertiary and quaternary structure according to its capacity to maintain a number of critical cysteines in a reduced form. In turn, p53 regulates a complex network of cellular pathways in which ROS are involved as effectors or second messengers (8, 21, 38). In this review, we summarize current knowledge on these "subinduction" levels of p53 and their regulation. Furthermore, we discuss how these regulatory mechanisms may participate in critical functions of p53 in physiological processes such as the control of energy metabolism and of senescence, two mechanisms in which ROS signaling plays a key role, as well as in the maintenance of stem cells pools.

Biological Significance of "Subinduction" Levels of p53

Early studies on mice with *TP53* alleles inactivated by homologous recombination (p53-null mice) led to the conclusion that p53 did not play any significant role in basal conditions in the absence of stress signals. These mice were found to develop normally and did not show any major physiological defect, although they were prone to the development of multiple early cancers (31, 40, 51). This view of p53 as "dispensable" for normal life has been challenged by a number of observations showing that p53 may exert important functions in other contexts than response to DNA damage (Fig. 2). First, it was found that inactivation of the p53 regulatory gene *MDM2* was embryonically lethal in mice. *MDM2*-null embryos were eliminated at an early preimplantation stage. This phenotype was however rescued in double knock-out mice lacking both *MDM2* and *TP53* alleles (33, 51). This observation suggests that p53 plays a critical, rate-limiting role during

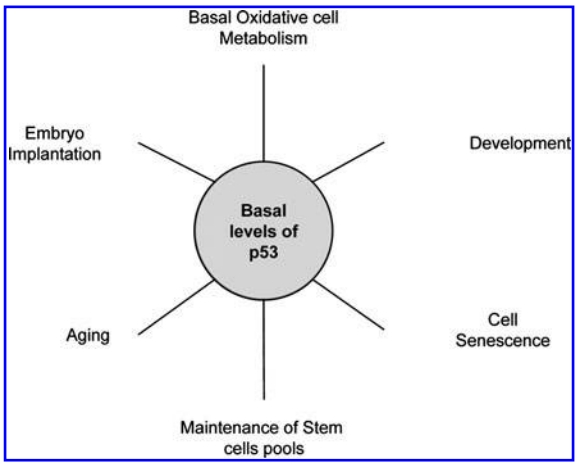

FIG. 2. Basal p53 activities. In addition to its role in growth suppression in response to acute stress signals, p53 also exerts pleiotropic effects when activated at low to moderate levels and thus play an important role in several physiological processes.

early development in the absence of typical DNA-damaging stress and that this role must be kept under control by Mdm2 in order for normal development to occur. Second, recent studies have uncovered novel roles of low levels of p53 in the regulation of basal oxidative cell metabolism. The p53 protein regulates the transcription of a number of genes involved in the control of energy metabolism (reviewed in (10)). In particular, it represses glycolytic enzymes, downregulates glucose transport, and participates in the maintenance of oxidative phosphorylation. These activities may contribute to oppose the metabolic shift (Warburg effect) that characterizes cancer cells. Third, p53 has also emerged as an important regulator of cellular and organismal senescence. Mice with ultra-short telomeres ($Terc^{-/-}$) show a prematurely aging phenotype that can be rescued by inactivation of p53 (16). Even more significant, p53 acts as a barrier to the reprogramming of differentiated cells into induced pluripotent stem cells (46). This role of p53 is related to its function in response to DNA damage, since telomere erosion represents a form of DNA damage that activates p53. Furthermore, telomere erosion is a hallmark of aging which is promoted by increased production of ROS. Nevertheless, this mechanism operates in conditions that preserve cell viability, that is, conditions in which the levels of p53 are not dramatically increased to high levels that would result into the induction of massive apoptosis. A fourth physiological mechanism in which subinduction levels of p53 may play a role is reproduction. The leukemia inhibitory factor LIF is a transcriptional target of p53, the production of which at sufficient levels is required for the implantation of blastocysts or early embryos in the uterus (70). Close examination of reproduction patterns in p53-null mice have shown that female embryos have reduced pregnancy rates and litter sizes due to embryo implantation failure (27). In humans, a common single nucleotide polymorphism at codon 72 (R or P) in *TP53* shows geographic variations in allele frequency in relation to temperature (68). The R72 allele is most common in regions with cold winter temperatures and there is evidence that p53 carrying R72 is more potent in activating LIF transcription. This polymorphism may therefore represent an adaptation facilitating reproduction in unfavorable climates. Thus, it is becoming clear that p53 has much broader functions than induction of apoptosis when accumulated to high levels in response to acute DNA damage. Indeed, these recent observations suggest that low-to-moderate levels of p53 are required for the maintenance of many vital functions, including in particular the maintenance of stem cells pools and of tissue regeneration capacities.

The nature of the signals that activate p53 to such low-to-moderate levels is not clearly identified. An interesting hypothesis is that low (physiological) levels of oxidative stress may play a signaling role. There is growing evidence that p53, itself a redox-sensitive protein, controls a complex network of redox dependent reactions involved in the maintenance of cell homeostasis, of cell metabolism, and of cell senescence and tissue aging. Recent studies on the role of p53 isoforms provide strong clues for an essential role of basal p53 activity in the regulation of senescence and aging.

The p53 Isoform Network: Transcriptional Control of p53 Expression and Activity

Until recently, p53 was considered to be expressed as a single protein isoform of apparent molecular weight of 53 kDa, with variants corresponding to distinct polymorphic and/or post-translationally modified forms. However, in recent years, it has emerged that *TP53* has a complex expression pattern, with alternatively spliced forms, alternative initiation codons as well as transcripts initiated from different promoters (4, 12). The consequence of these variable expression patterns is that p53 can be expressed as multiple protein isoforms that differ from the canonical p53 by their proximal and/or distal domains. The biological and pathological relevance of these isoforms remain a matter of debate. Based on their expected functional domains, they may exert profound regulatory effects on p53 functions.

The p53 protein is composed of three main structural domains (Fig. 3A) (see reviews in Refs. 12, 26, 43, 45). The proximal, acidic N-terminus (residues 1–95) contains the major transactivation (TA-I) domain (1–42) and a secondary, weaker transactivation domain (TA-II) located around residues 43 to 73, which operates essentially as a modulator of the specificity of transactivation by the major domain. The TA-I domain also contains the binding site for Hdm2 (residues 17–29), the main regulator of p53 degradation (3). Further to these two domains lies a proline-rich region structured as a –SH3 binding domain, which provides a rigid link between the N-terminus and the DNA-binding core domain (DBD). The latter (residues 96–292) is made up of two anti-parallel beta-sheets linked together by flexible loops and helixes, adopting the well-known "jelly roll" structure (11). The DNA-binding surface is made up of two parts, one defined by loops 2 and 3 that are bridged together by an atom of zinc and that bind to the minor groove of DNA, and the other made of a sheet-loop-helix motif that binds to the major groove of DNA (Fig. 3B). The distal part of the protein is made up of at least three subregions, the first forming a link that contains the main nuclear localization signal (residues 296–324), the second defining a complex oligomerization domain (OD; residues 325–365) and the third, in the C-terminus, representing a critical negative regulatory region. This extreme C-terminus is rich in basic residues. Both the acidic N-terminus and the basic C-terminus contain the majority of the post-translational regulatory sites (phosphorylation, acetylation, sumoylation, neddylation), whereas the central domain represents a more hydrophobic, tightly packed region which is relatively devoid of post-translational modifications.

Analysis of *TP53* gene expression identified up to ten isoforms affecting both N- and C-terminal domains of the p53 protein (Fig. 4) (35). Three C-terminus isoforms have been described (4). p53α corresponds to the classical full-length p53, which contains the entire OD domains (4, 12). p53β and p53γ isoforms are produced by alternative splicing in intron 9, leading to truncated p53 proteins ending with distinct tails of respectively 10 and 15 new residues (Fig. 4C) (15). These three C-terminal isoforms could in theory be combined with three configurations of the N-terminal domain: TAp53, Δ40p53, and Δ133p53. TAp53 has the longest N-terminus, containing the entire sequence of the main TA domain, whereas Δ40p53 and Δ133p53 are shorter forms missing either the first 39 or 132 residues, respectively. In theory, these N- and C-terminal variants may combine with each other to generate up to 9 protein isoforms. A tenth isoform, Δp53, has been described as the product of noncanonical alternative splicing between the exons 7 and 9 (63). So far, it is still not clear whether all of these isoforms are expressed in all tissues. It is most likely that their

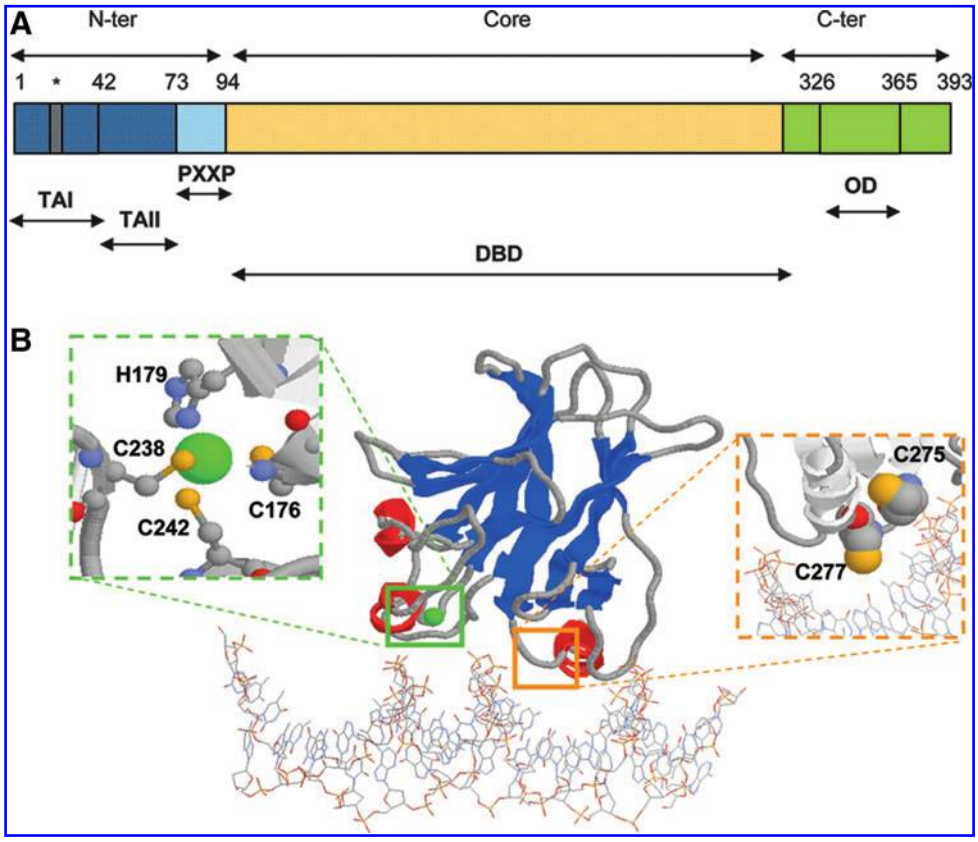

FIG. 3. Structure and redox-sensitive sites of human p53 protein. (A) Schematic view of the domain structure of human p53. The protein contains an N-terminal transactivation domain subdivided in a major subdomain (TAI) and a minor one (TAII), a proline-rich domain (PXXP), a central DNA-binding domain (DBD), a C-terminal oligomerization domain, and a basic autoregulatory region. *Gray bar* marked with an *asterisk*: Hdm2 binding site. **(B)** Structure of the DBD in complex with consensus DNA. A cartoon representation of the DNA-binding domain shows the helix (*red*)-loop (*gray*) structures binding in both major and minor groove of DNA. These loops are supported by a scaffold of beta-sheets (*blue*). The location of important redox-sensitive sites is shown, including the zinc (*green*)-binding cluster (Cys 176, 238, 242, and His 179) and two cysteines (Cys 275 and 277), the latter binding to DNA in the major groove. Models were generated using RASMOL software based on data from Cho et al., 1994 (11). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

expression is restricted to specific phases of cell proliferation, differentiation, senescence, or response to stress.

While the role of C-terminal isoforms is still far from being elucidated, recent results have provided insights on the role of N-terminal isoforms. It should be noted that these N-terminal isoforms present structural and functional similarities with the so-called ΔN isoforms of p63 and p73, the products of two *TP53* gene homologues that are involved in the differentiation of normal epithelial tissues, as well as in specific aspects of the regulation of neural and haematopoietic cells (12).

The Δ133p53 isoform

In contrast to the "canonical" form of p53, which is transcribed from a promoter P1 devoid of typical TATA-box and located upstream of the noncoding exon 1, the Δ133p53 isoform is expressed by internal initiation of transcription at an intragenic promoter P3, located in intron 4 (a P2 promoter, located in intron 1, has been shown to regulate an open-reading frame of unknown significance) (4, 44). Initiation at P3 produces a specific mRNA, p53I4. In this mRNA, the first AUG that can be used for initiation of translation corresponds to codon 133 of full-length p53. Thus, the corresponding protein isoform lacks the first 132 residues and is deleted from the entire TA domain, as well as from the first 30 residues of the DBD. Consistent with these structural features, it lacks DNA-binding capacity towards p53 response elements *in vitro* and counteracts the capacity of p53 to suppress cell growth. We have recently shown that human p53 can transactivate the internal P3 promoter, thus regulating the expression of its Δ133p53 isoform (4,44). In turn, Δ133p53 can neutralize the suppressive activity of p53, suggesting a regulatory feedback mechanism by which p53 can modulate its own biological effects. This modulation may consist of selective interference with the capacity of p53 to transactivate target genes, thus contributing to shape the repertoire of p53-dependent responses in a manner that depends upon cell type and/or biological context. Similar conclusions have been drawn from studies in Zebrafish. The Zebrafish homolog of Δ133p53 is directly transactivated by full-length p53 in response to developmental as well as to DNA-damaging signals, resulting in enhanced expression of the isoform (9). This expression, in turn, could antagonize p53-induced apoptosis via activating bcl2L (closest to human Bcl-x(L)), a factor preventing mitochondrial permeabilization and release of ROS in the cytosol) (9).

Although the exact role of Δ133p53 remains to be elucidated, a recent study has provided a clue for its involvement in the regulation of replicative senescence in normal fibroblasts (18). This study observed that replicative senescence of normal fibroblasts was accompanied by diminished expression of Δ133p53. Knocking-down Δ133p53 induced accelerated senescence, perhaps due to the fact that loss of Δ133p53 results in an untimely increase in basal p53 activity. In this study, rather than direct interaction and competition with p53, Δ133p53 appeared to repress miR34a, a microRNA transactivated by p53 and which in turn downregulates many genes required for cell proliferation and survival (Fig. 5). Since

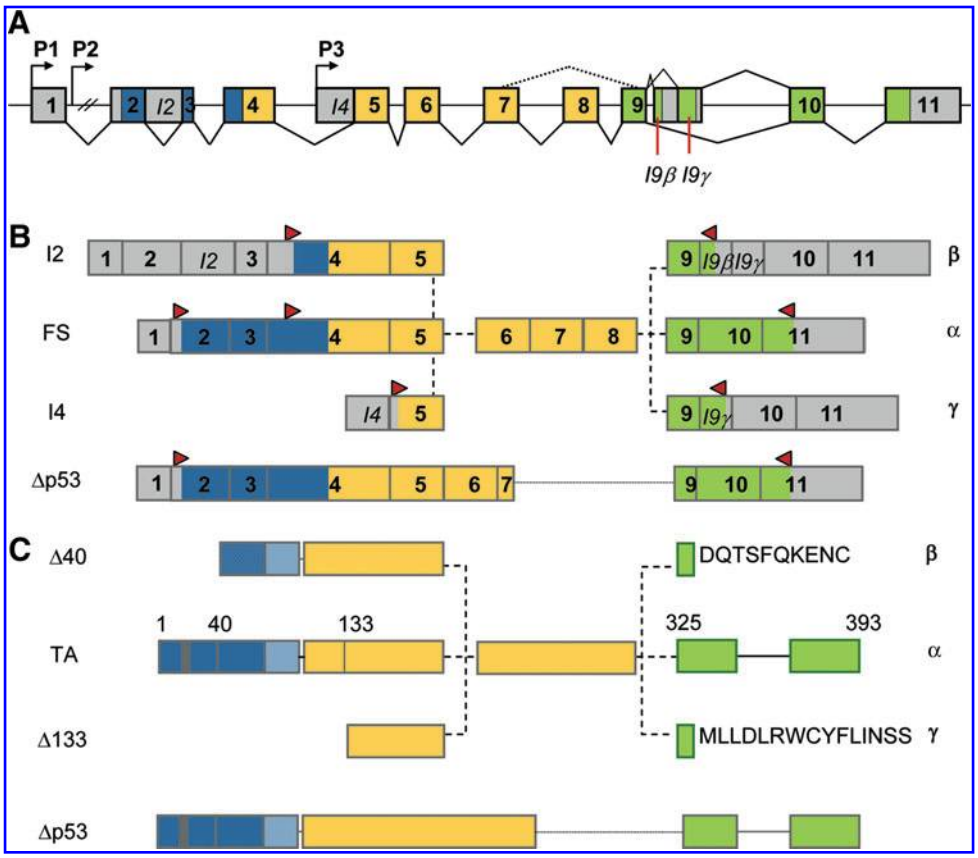

FIG. 4. Human p53 isoforms. (A) Intron/exon structure of the *TP53* gene showing the positions of P1, P2, and P3 promoters. p53P1 regulates the transcription of FSp53 (Full-Spliced) and p53I2 mRNA variants; p53P2, located in intron 1, controls the expression of an ORF of unknown function; p53P3 initiates the transcription in intron 4 to produce the p53I4 mRNA variant. Intron sizes are not to scale. *Gray boxes*: noncoding regions; *blue boxes*: regions encoding the N-terminal transactivation domain; *orange boxes*: regions encoding the central DBD; *green boxes*: regions encoding the C-terminal oligomerization domain. I2: intron 2 retained in p53I2 mRNA; I4: part of intron 4 retained in p53I4 mRNA; I9β: part of intron 9 encoding p53β; I9γ: part of intron 9 encoding p53γ. **(B)** Structure of p53 mRNA splicing variants. All variants have in common a part of the region encoding the DBD and differ from their size and sequence of 5′ and 3′ ends. Domains are color-coded as in **(A).** *Open arrow in right direction*: start site of translation; *open arrow in left direction*: stop site of translation. **(C)** Structure of p53 protein isoforms. The canonical p53 (TAp53) combines TA N-terminus and α C-terminus. Δ40p53 possesses a shorter N-terminus, lacking 39 amino acids and Δ133p53 lacks the whole N-terminus plus part of the DBD. The α form conserves an entire C-terminal domain, whereas β and γ forms are missing several residues replaced by new amino acids. Δp53 isoform lacks a large part of the DBD. Domains are color-coded as in **(A).** *Dark gray bar:* Hdm2 binding site. Based on Marcel and Hainaut, 2009, with modifications (43). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

ROS production plays critical role in the regulation of cell cycle and cell fate (7), it will be of particular importance to determine whether the Δ133p53 isoform may modulate the redox networks controlled by p53.

The Δ40p53 isoform

The Δ40p53 isoform is produced by two different mechanisms using as template the "canonical" p53 mRNA synthesized from the P1 promoter. First, Δ40p53 can be produced by internal initiation of translation at codon 40 using a completely spliced p53 mRNA (FSp53; fully spliced p53 mRNA) (13). FSp53 contains two internal ribosome entry sites (IRES, positions −1 and +39), used to initiate the translation of TAp53 or ΔΔ40p53 isoforms, respectively (62) (Figs. 4B and 4C). Second, Δ40p53 may also be produced from an alternatively spliced mRNA that retains intron 2, p53I2. The retention of this short intron introduces several nonsense codons downstream of AUG 1, precluding the assembly of a full-length p53. However, initiation of translation at codon 40 (located in exon 4) allows for the assembly of a protein that lacks the first 39 residues and thus, the main TA domain (19) (Figd. 4B and 4C). Δ40p53 appears to operate as a regulator of p53 activity in conditions when it is not activated in response to genotoxic damage. Contrary to TAp53, Δ40p53 does not accumulate after genotoxic stress. Due to the absence of the Hdm2-binding domain, Δ40p53 does not interact with Hdm2 and therefore escapes the rapid degradation mechanism that regulates TAp53 level (13). Similar to TAp53, Δ40p53 retains the capacity to bind DNA and may conserve a low but selective transactivation activity, as observed by luciferase assay on $p21^{WAF1}$ promoter, a TAp53 target gene. However, due to the lack of the first 40 residues containing the major transactivation domain (TAD), the transcriptional activity of Δ40p53 towards most p53 promoters is extremely weak (13). As shown by co-immunoprecipitation, Δ40p53 interacts with TAp53 through their conserved OD domain. This interaction leads to the formation of mixed tetramers, the transactivation capacity of which depends upon their specific composition (Hafsi and Hainaut, unpublished data).

There is strong evidence for a biological role of Δ40p53 in organismal aging and senescence. Studies by Maier and colleagues have investigated the effects of an N-terminally truncated p53 protein corresponding to Δ40p53, expressed as a transgene in mice (42). Mice expressing the transgene had a profoundly altered growth rate, with a short size, a reduction in size and weight of many organs, and a premature aging phenotype with typical lordokyphosis and accelerated

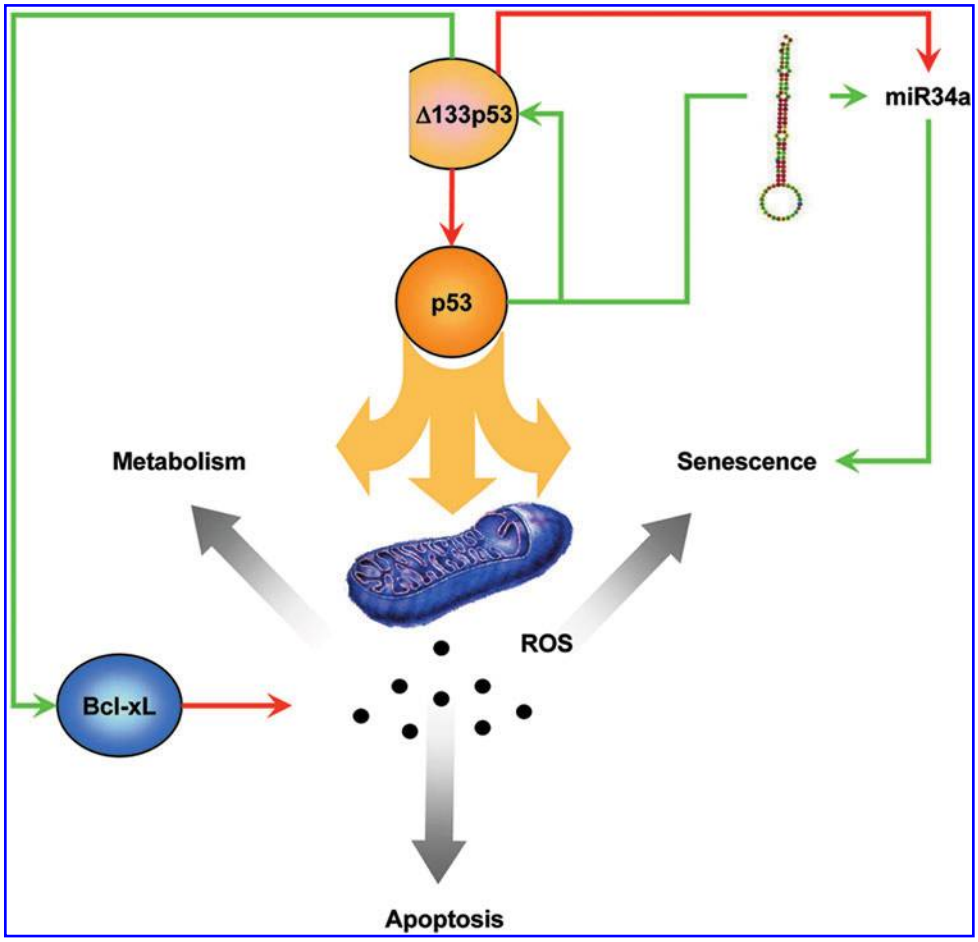

FIG. 5. Role of Δ133p53 in regulating senescence, apoptosis, and metabolism. A model integrating recent data on the role of Δ133p53 is proposed. Δ133p53 may act either directly by down-regulating mir34a or by inducing Bcl-xL. It may also act indirectly by binding to and inhibiting TAp53, thus perturbing its function as growth suppressor. We suggest that the effects of Δ133p53 converge to modulate the release of reactive oxygen species (ROS) by the mitochondria, with consequences on senescence, apoptosis, and metabolism. *Green lines*: positive effects (increased expression and/or activity); *Red lines*: negative effects (decreased expression and/or activity). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

decrease in bone density. Interestingly, this phenotype was not observed when the transgene was expressed in a p53-null mouse background (42). Mice lacking p53 are prone to multiple early cancers and expression of Δ40p53 did not rescue that tumor-prone phenotype. Thus, the striking growth retardation and accelerated aging induced by Δ40p53 was strictly dependent upon the presence of a normal p53 gene. These observations are consistent with the notion that Δ40p53 is an important regulator of the suppressive and senescence-inducing functions of p53. The mechanisms by which Δ40p53 induces these effects is not completely understood (Fig. 6). Maier and colleagues have described that Δ40p53 transgenic mice have an altered, hyperactive IGF-I signaling pathway (42). Recently, the role of this pathway has been further documented by the demonstration that mice overexpressing the Δ40p53 isoform ($p44^{+/+}$ mice) display cognitive decline and synaptic impairment early in life (57).

At the molecular level, the first and most significant effect of overexpression of Δ40p53 may be to affect the stability of p53 and to modulate its basal level in many cells and tissues, resulting in altered control of basal p53 activity. This increased basal activity may lead to enhanced aging as a consequence of impairment of cell renewal and activation of cell death pathways. Accelerated aging has also been reported in mice co-expressing wild-type p53 and a deletion mutant lacking sequences encoded by the first six exons (73). As increased production of ROS is one of the hallmarks of the aging process, such aging phenotypes may be due to defects in the capacity of p53 to control ROS production, or to be enhanced by suppressor activity in response to overproduction of ROS. Indeed, p53 deficiency has been shown to rescue several accelerated aging phenotypes in mouse models engineered with deletions of Terc or the metalloproteinase Zmpste24 (16, 76).

The p53 Redox Network: Sensing Redox Changes and Controlling Cell Metabolism

p53 as a redox- and metal-dependent transcription factor

The remarkable redox-sensitivity of the p53 protein first emerged as a very inconvenient trait for those researchers who were trying to produce and purify synthetic p53. The protein has a frustrating propensity to aggregate as soon as it is isolated from a healthy cellular environment. It even becomes rapidly oxidized in solution in contact with air, explaining the rapid disappearance of p53 activity from solutions kept for a few hours in a 4°C fridge.

p53 was initially identified as a redox-active transcription factor when it appeared that high affinity binding to consensus oligonucleotides *in vitro* required a strong reducing environment, equivalent to 10 m*M* dithiothreitol (DTT) (21, 22, 28). In contrast, treatment of p53 with oxidizing agents such as diamide completely prevented binding of p53 to specific DNA (23). These observations anticipated those deduced from the crystal structure of the DNA binding core domain of p53, which was described by Pavletitch and colleagues in 1993 in the form of a complex with DNA (11).

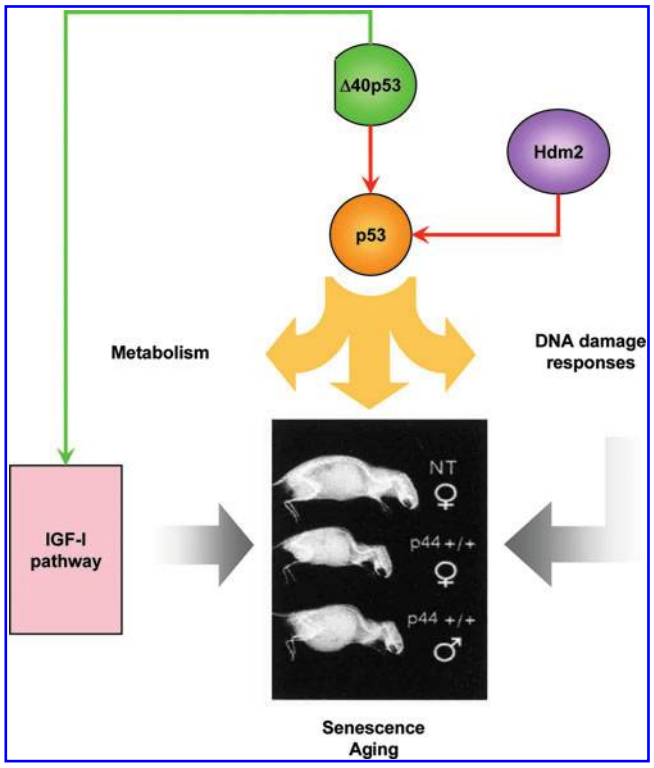

FIG. 6. Role of Δ40p53 in regulating aging and metabolism. A model integrating recent data on the role of Δ40p53 is proposed. This model proposes that Δ40p53 may have a direct effect on the IGF-I signaling pathway, thus modulating important growth and survival factors in many tissues. Alternatively, Δ40p53 may act as a regulator of p53 function by binding to the protein and affecting two of its properties, (1) its capacity to transactivate target genes, and (2) its capacity to bind Hdm2 and to undergo proteasome-mediated degradation. The *lower panel*, showing radiological analysis of senescent mice, is taken from Maier et al., 2004 (42). *Arrows* are color-coded as in Figure 5. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

The DBD of p53 contains 10 cysteines that cluster within the surface of the protein that makes direct contact with DNA (Fig. 3). Three of these cysteines, C176, C238, and C242, together with histidine H179, are involved in the binding of a divalent zinc ion (61). This zinc atom plays a structural role in bridging together loops L2 and L3 of the protein and in forming a stable structure interacting with the minor groove of DNA. Removal of zinc, or its replacement by other cations with high affinity for thiols such as cadmium (Cd^{2+}) or copper (Cu^{2+}), destabilizes p53 protein structure and disrupts DNA binding (49). Several nonzinc-binding cysteines may also be targets for redox regulation (61). C277 provides a hydrogen bond to DNA within the major groove and its oxidation prevents DNA binding. Three other cysteines are located within short distance of each other in that portion of the protein. Although their spatial arrangement does not appear to fit a tetrahedral model, it has been proposed that these residues may also be involved in binding metals (24, 79).

There is a complex interplay between zinc binding and redox regulation of p53. Zinc not only stabilizes the tertiary folding of p53 but also protects thiols from becoming oxidized and forming disulfides that cross-link p53 either with itself or with other redox-active proteins. In the absence of zinc and of free thiols, p53 becomes oxidized into disulfide-linked aggregates. This intrinsic redox and metal sensitivity may provide a biochemical mechanism by which p53 DNA binding activity undergoes subtle variation in relation to changes of intracellular redox conditions (78, 79). Thus, p53 may behave as a "sensor" of redox changes within the cell. Such biochemical properties are not unique to p53. Two other transcription factors with a wide spectrum of target genes, AP1 and NF-κB, share similar redox-dependent regulation (20, 80). Similarly to p53, NF-κ and AP1 are directly involved in controlling transcriptional programs in cells exposed to oxidizing agents.

In vivo *sensitivity of p53 to oxidative stress*

The biochemical properties summarized above suggest that p53 activity *in vivo* may be controlled by intracellular redox status and zinc bioavailability. Since oxidation of thiols can inactivate p53 by disrupting its wild-type conformation *in vitro*, it has been suggested that exposure to strong thiol oxidants *in vivo* may inactivate p53 function. In fact, this prediction has proven difficult to demonstrate since oxidative stress also induces DNA damage, which stabilizes and activates the p53 protein. This activation occurs at much lower levels of stress than those required to inactivate the p53 protein through direct oxidation. Thus, very high doses of H_2O_2 (1 m*M*) may lead to the inactivation of p53-dependent transcription, but such doses are cytotoxic and the physiological significance of this mechanism is not clear (55). Of greater physiological relevance are experiments using agents that induce depletion of glutathione (GSH), a critical factor in regulating cellular redox homeostasis, both as a direct ROS scavenger and as substrate for glutathione peroxidase (GPX), which contributes to remove H_2O_2. Reduced (GSH) and oxidized (GSSG) glutathione have been shown to bind to p53 in cultured tumor cells (77). In basal conditions, the protein was only marginally glutathionylated but oxidant and DNA-damaging treatments greatly enhanced GSH modification. Mass spectrometry of GSH-modified p53 protein identified cysteines 124, 141, and 182, which are all located in the DNA-binding domain, as the main sites of glutathionylation, with C141 appearing to be the most reactive site (77). This residue is not involved in making direct contacts with target DNA but lies at the interface between p53 dimers, suggesting that its glutathionylation may decrease p53 activity by interfering with oligomerization.

Control of p53 activity by metal- and redox-dependent mechanisms

Inside cells, zinc availability and fluxes of ROS are tightly controlled by overlapping sets of effectors. ROS fluxes are regulated by electron transfer reactions between donor and acceptor molecules defining redox cycles and redox-signaling pathways. The main mechanism that controls the reduced status of thiol-containing proteins is the thioredoxin (TRX)-thioredoxin reductase (TRR) system (24). Zinc availability is controlled through metal transfer reactions mediated by metallothioneins (MT), a class of small cysteine-rich proteins that operate as intracellular buffers for Zn^{2+} and several other divalent metal ions (Cu^{2+}, Cd^{2+}). There is evidence that both

systems, TRX–TRR and MT, can regulate p53 redox status and biological activity *in vivo* (Fig. 7).

Trx is a protein of low molecular weight (12 kDa) present at high levels in the cytoplasm, nucleus, membrane, and mitochondria. Trx has two basic functions: it operates as hydrogen donor for ribonucleotide reductase (RNR) providing deoxyribonucleotides for DNA replication and as protein disulfide reductase for many oxidized proteins. Trx is itself reduced by TRR, using NADPH as source of reducing equivalents (52). Studies in yeasts and in human cells have shown that inhibition of TRR results in the accumulation of oxidized Trx, which in turn induces the formation of disulfides in p53 and inactivates DNA binding (56). However, in mammalian cells, there is scant evidence that Trx directly interacts with p53 and it is likely that its effect may be indirect (66, 74). Inhibition of TRR1 with siRNA resulted in accumulation and at least partial activation of p53, despite accumulation of oxidized Trx (66). Recent evidence suggests that suppression of TRX-1, the major isoform of Trx, induced premature senescence in normal human fibroblasts through a pathway that involved upregulation of both p53 and p16/ink4a pathways (82).

The main candidate as "intermediate" between Trx and p53 is APE1/Ref1, a bifunctional enzyme with two separate cat-

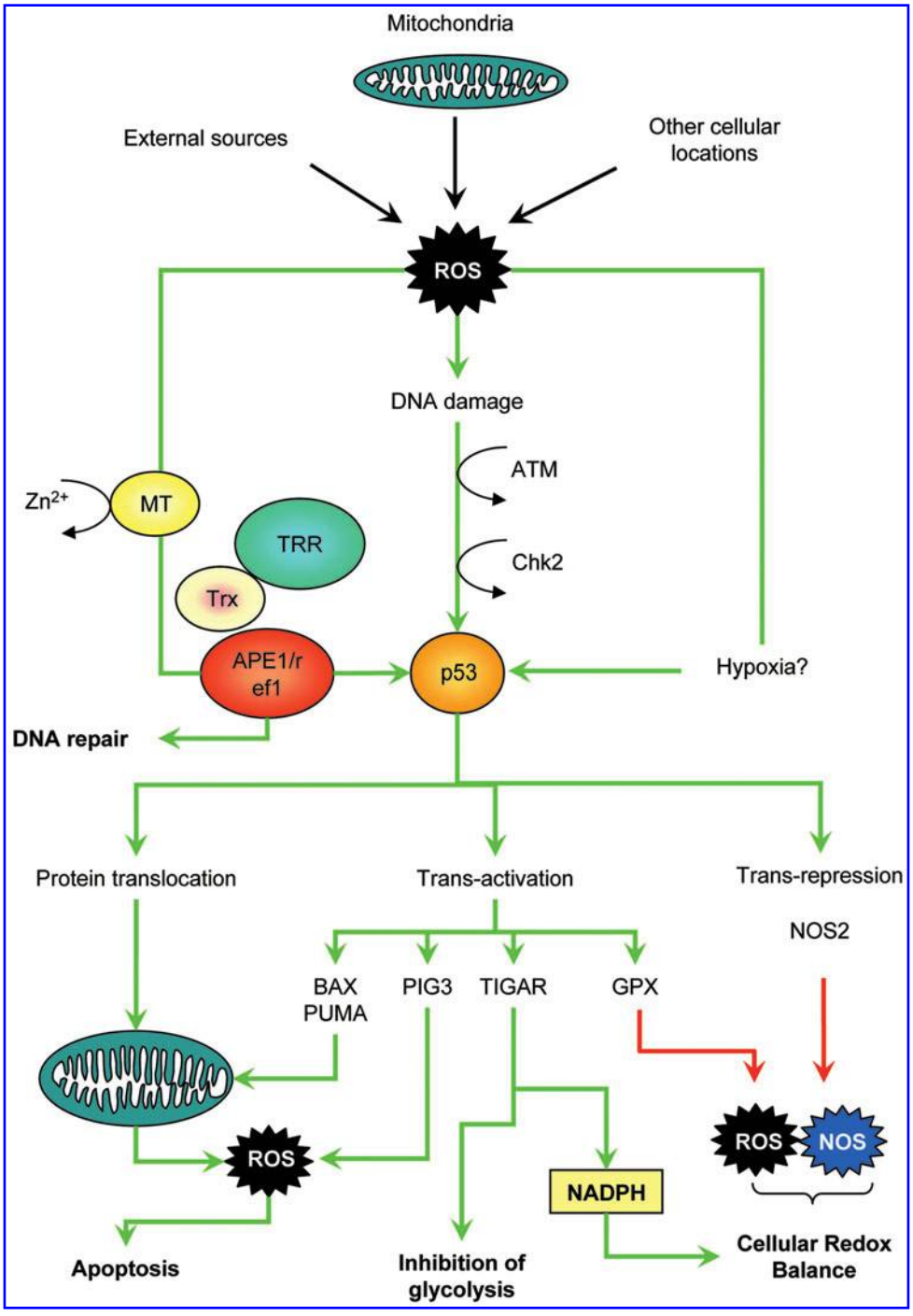

FIG. 7. An integrated model of the roles of p53 in ROS regulation and in the control of ROS-mediated processes. The *upper part* of the figure shows how ROS produced by several processes may contribute to activate p53. Three main pathways are proposed: (1) the DNA-damage pathway, in which ATM and Chk2, among other factors, signal to p53 the presence of ROS-induced lesions into DNA; (2) the redox-regulator pathway, in which changes in zinc binding and/or in thiol reduction may modulate the conformation and activity of p53 (MT, metallothioneins; TRR, thioredoxin reductase; Trx, thioredoxin). The *bottom part* shows the diversity of redox-dependent pathways controlled by p53, including (1) effects on the mitochondria; (2) effects on oxidative metabolism; (3) effects on intracellular redox balance. *Arrows* are color-coded as in Figures 5 and 6. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

alytic domains, a thiol reductase which is recycled by Trx/TRR and an apurinic/apyriminidic endonuclease (2, 72) (Fig. 7). APE1/Ref1 provides the second major enzymatic step in base-excision repair (BER), the first one being the elimination of damaged bases by DNA glycosylases. The thiol reductase activity of APE1/Ref1 has been shown to control DNA binding by NF-κB and AP1. APE1/Ref1 interacts with p53 and stimulates DNA binding *in vitro* (32). In mammalian cells, silencing of APE1/Ref1 accelerates the turnover of p53 and strongly decreases basal p53 levels (66). However, silencing of APE1/Ref1 has relatively little impact on p53 activation in response to DNA damage, leading to the hypothesis that this mechanism may essentially operate to control p53 protein activity in basal conditions. Through its bifunctional properties, APE1/Ref1 may provide a mechanism that keeps p53 function in check during DNA base-excision repair, preventing excessive activation of p53 that may lead to apoptosis in response to levels of DNA damage that can be corrected by the basal DNA repair machinery.

Metallothioneins (MT) are small proteins (7 kDa) containing about 30% of cysteines organized in two structural clusters capable of binding seven Zn^{2+} equivalents per MT molecules. Transfection of MT has been shown to regulate the Zn^{2+}-dependent folding of p53 in cultured cells (49). Recent studies on the regulation of p53 by the Homeodomain-interacting protein kinase-2 (HIPK2) have shed light on the physiological significance of this regulation (59, 60). HIPK2 phosphorylates p53 at Ser46, a necessary step to induce p53-dependent apoptosis in response to severe DNA damage. Knockdown of *HIPK2* inhibited Ser46 phosphorylation as well as acetylation of p53 on Lys382. In fact, *HIPK2* appears to be required for the recruitment of p53 and coactivators of transcription onto promoters of pro-apoptotic genes. In addition to this inhibitory effect on p53 posttranslational modifications, knockdown of *HIPK2* also leads to p53 misfolding. This effect was due to enhanced expression of MT2a in response to *HIPK2* knockdown and could be rescued either by supplementation with zinc or by silencing MT2a. These observations demonstrate that control of p53 folding by MT and zinc is tightly integrated with posttranslational modification of p53 and in the regulation of the capacity of p53 to selectively activate different categories of promoters, such as, for example, the promoters of pro-apoptotic genes (Fig. 7). In this system, overexpression of MT2a appears to operate as a mechanism that prevents excessive p53 activation and thus protects cells against apoptotic responses (59).

Control of oxidative metabolism by p53

The p53 protein lies at the center of a vast network of pathways that use ROS and nitrogen oxygen species (NOS) as important signaling molecules (21, 38). In this network, p53 plays a dual role (Fig. 7), either as inducer of genes involved in protection against damage by ROS, or as regulator of genes whose products potentially generate ROS and whose action presumably contributes to p53-mediated cell death. Among genes that may contribute to the detoxification and protection against ROS, p53 has been shown to regulate glutathione peroxidase 1 (GPX1) (71), mitochondrial superoxide dismutase 2 (SOD2) (29), and aldehyde dehydrogenase 4 A1 (ALDH4A1) (81), which all encode products presumably acting as antioxidants. In addition, p53 also represses the expression of inducible nitric oxide synthase 2 (*NOS2*), thus protecting cells against excess damage by NO and derivatives (17). Other potential antioxidant products include two p53-regulated sestrins, PA26 and Hi95 (encoded by SESN1 and SESN2, respectively), which are essential for regeneration of overoxidized peroxiredoxins (5, 6), the enzymes involved in the decomposition of hydrogen peroxide3. Studies by Chumakov and colleagues have provided evidence that these potential antioxidant target genes are activated at low, quasi-basal levels of p53 and that loss of p53 function may sensitize cells to the damaging effects of low levels of ROS (64).

Conversely, p53 can operate as a trigger for the production of ROS and for ROS-dependent signaling. Candidate p53-target genes for such a role include *PIG3* (encoding a NADPH quinone oxidoreductase homolog) (58) and *FDXR*, encoding ferredoxin reductase, which transfers electrons from NADPH to cytochrome P450 via ferredoxin in mitochondria (30, 39). This increase in ROS production may participate in the initiation of apoptosis. Another ROS-dependent pro-apoptotic effect of p53 is the transcriptional activation of *BAX1* (50, 67) and *PUMA* (14), two factors that control mitochondrial permeability and facilitate the leakage of ROS from the mitochondria into the cytoplasm.

These various and apparently contradictory roles of p53 may all contribute to subtle effects on the regulation of energy metabolism, through which p53 may favor oxidative metabolism against glycolysis (reviewed in Ref. 10)). Figure 8 summarizes a number of recent observations on the role of p53 in energy metabolism. The role of p53 in downregulating glycolysis involves first the downregulation of glucose transporters at the plasma membrane through direct inhibition of *GLUT-1* and *GLUT-4* (65), and through indirect inhibition of *GLUT-3* by a mechanism involving NF-κB (34). Next, p53 regulates the synthesis of two enzymes acting as rate-limiting factors in the glycolytic pathway. It induces *TIGAR* (*TP53*-induced glycolysis and apoptosis regulator), an enzyme with fructose bi-phosphatase activities that counteracts the activity of the glycolytic enzyme 6-phosphofructo-1 kinase (1, 37). In contrast, it downregulates phosphoglycerate mutase (*PGM*), an enzyme involved in the conversion of 3-phosphoglycerate into 2-phosphoglycerate during the late ATP-generating steps of glycolysis (36). This antiglycolytic activity is accompanied by the upregulation of enzymes that divert the metabolism of glucose to the pentose phosphate pathway (PPP), in particular glucose-6-phosphate dehydrogenase (41). In turn, hexokinase 2 (*HK2*), the rate-limiting enzyme generating glucose-6-phosphate, contains p53-response elements in its promoter and is another potential target for upregulation by p53 (47). The PPP is an important contributor of reducing equivalents in the form of NADPH, as well as an essential step for the biosynthesis of ribose and subsequent synthesis of nucleic acids.

In parallel with its capacity to limit glycolysis, p53 maintains and promotes oxidative phosphorylation through at least two mechanisms. First, it activates AIF (apoptosis inducing factor), a bifunctional protein with oxidoreductase function contributing to the assembly and function of Complex I of the respiratory chain (69, 75). The other function of AIF is extramitochondrial: under different apoptosis-inducing conditions, AIF translocates through the outer mitochondrial membrane to the cytosol and to the nucleus,

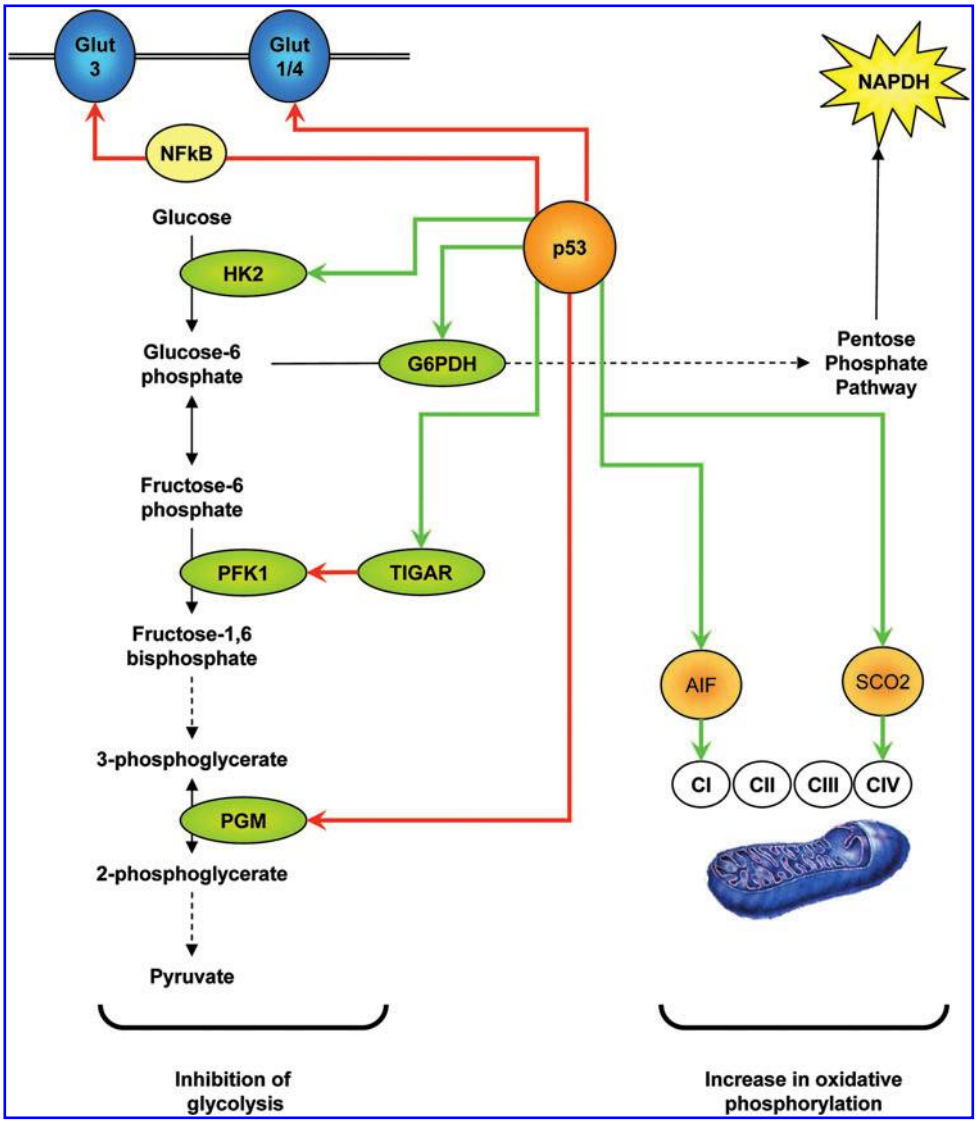

FIG. 8. p53 and regulation of metabolism. This figure summarizes recent data on the role of p53 as a transcriptional regulator of genes involved in the control of energy metabolism. *Left:* components of glucose transport (*blue*) and metabolism (*green*) regulated by p53. *Right*: components of mitochondrial oxidative phosphorylation chain regulated by p53 (*orange*). *Arrows* and *lines* are color coded as in Figures 5–7. Based on Cheung and Vousden, 2010, with modifications (10). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

where it induces chromatin condensation as well as DNA fragmentation. Second, p53 transactivates *SCO2* (synthesis of cytochrome c oxidase 2), a copper-dependent chaperone protein that is required for the assembly of cytochrome c oxidase (complex IV of the respiratory chain) (48).

Overall, these metabolic effects of p53 antagonize the Warburg effect that characterizes the metabolism of most cancer cells. The latter cells tend to become over-dependent upon glycolysis and to reduce their oxidative phosphorylation, oxygen-dependent metabolism. This effect is one of the biochemical adaptive mechanisms by which cancer cells survive in hypoxic and nutrient-deprived conditions. It is therefore possible that mutation of *TP53* and loss of p53 function contributes to the Warburg effect.

Conclusions: p53 as a Regulator of ROS in Stem Cell Maintenance

Until recently, studies on p53 have been mainly concerned by its role in the response to acute genotoxic stress induced by xenobiotics, radiations, or other exogenous DNA-damaging substances. Under such exposures, most cells undergo rapid nuclear accumulation of p53, followed by a range of growth suppressive responses aimed at preventing the accumulation of genetic damage. There is now growing evidence that p53 activity is also involved in the response to levels of stress signals generated during normal physiological processes. The main biological evidence for such an effect comes from studies in mouse models showing that p53 deficiency rescues many engineered phenotypes of premature aging. This suggests that organismal aging, which is largely mediated by overproduction of reactive oxygen species, is at least in part mediated through p53 activation and inhibition of cell growth or renewal.

These observations shed a new light on p53 regulatory mechanisms that have been so far less well studied than those involved in the pathways of DNA damage signaling. Of particular relevance in this context is the central role of p53 at the core of a network of redox signaling mechanisms. Indeed, p53 is both a sensor of DNA damage induced by ROS, a direct sensor of ROS homeostasis through its intrinsic redox dependence, and a regulator of multiple ROS controlling

mechanisms, either part of cellular antioxidant defense systems, or part of metabolic pathways involved in energy production. The model emerging from these observations is that small amounts of ROS generated during physiological processes may turn on p53 activity and trigger autoregulatory feedback mechanisms with either antioxidant response or, conversely, an escalating cycle of redox dysfunction leading to metabolic switch, mitochondrial release of ROS, genotoxic damage, and further activation of p53.

How cells handle the decision between antioxidant response and escalating cycles of redox dysfunction is a major focus for future research. One key mechanism in this respect may be the production of p53 isoform lacking the N-terminus. This hypothesis is essentially based on the observation that mice overexpressing the Δ40p53 isoform have an accelerated aging phenotype similar to those induced by excess oxidative stress. This observation places N-terminally truncated forms of p53 in the position of operating as controllers of levels of p53 activation in response to endogenous oxidative stress, thus linking redox regulation, isoforms, and organismal senescence. Redox regulation plays essential role in the maintenance and renewal of normal stem cells. It is therefore possible that deletion of p53 function by mutation may result in perturbations of p53-dependent redox control, thus facilitating the formation and expansion of cancer stem cells.

Acknowledgments

Research on p53 isoforms at IARC is supported by la Ligue Nationale Contre le Cancer and by the French National Cancer Institute (INCa).

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Date of first submission to ARS Central, November 9, 2010; date of final revised submission, December 7, 2010; date of acceptance, January 1, 2011.

Abbreviations Used

AIF = apoptosis inducing factor
BER = base-excision repair
DBD = DNA-binding domain
DTT = dithiothreitol
GPX = glutathione peroxidase
Hdm2 = human double minute-2
HIPK2 = homeodomain-interacting protein kinase-2
LIF = leukemia Inhibitory Factor
Mdm2 = murine double minute-2
MT = metallothioneins
NOS = nitrogen oxygen species
NOS2 = nitric oxide synthase 2
OD = oligomerization domain
PGM = phosphoglycerate mutase
PPP = pentose phosphate pathway
RNR = ribonucleotide reductase
ROS = reactive oxygen species
SCO2 = synthesis of cytochrome c oxidase 2
TA = transactivation
TIGAR = TP53-induced glycolysis and apoptosis regulator
TRR = thioredoxin-reductase
TRX = thioredoxin

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