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Cellular and Organismal Ageing: Role of the p53 Tumor Suppressor Protein in the Induction of Transient and Terminal Senescence

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Abstract In recent years, an impact of the p53 tumor suppressor protein in the processes of cellular and organismal ageing became evident. First hints were found in model organisms like *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster* where a clear connection between ageing phenotypes and pathways that are regulated by p53, were found. Interestingly, pathways that are central to the ageing process are usually also involved in energy metabolism and are highly conserved throughout evolution. This also supports the long known empiric finding that caloric restriction has a positive impact on the life span of a wide variety of organisms. Within the last years, on the molecular level, an involvement of the insulin-like growth factor and of the histone deacetylase SRIT1 could be shown. Insight on the impact of p53 on ageing at the organismal level came from mice expressing aberrant forms of the p53 protein. Obviously, the balance of the full length p53 protein and of the shorter p44/ΔNp53 isomer bear a strong impact on ageing. The shorter isoform regulates full length p53 and in cases where there is too much of the longer isoform, this leads to elevated apoptosis resulting in decreased tumor incidence but also in premature ageing due to exhaustion of the renewal potential. Therefore, modulating the expression of the truncated p53 isoform accordingly, might lead to increased health-span and elevated life-span. J. Cell. Biochem. 101: 1355–1369, 2007. © 2007 Wiley-Liss, Inc.

Key words: p53 stability; HIPK2; PML; Mdm2; p53 isoforms; cell cycle; CDK inhibitors; cell-cycle arrest; p53 ubiquitylation.

BACKGROUND

Although the topics of ageing and longevity were already of exceptional interest to human society long before scientific methods were established, it was only recently that science came closer to answering some fundamental questions concerning these topics based on strictly scientific methods and data. Knowledge about the influence of nutrition and exercise as well as the abuse of certain substances on mean life expectancy goes back a long time. However, these findings were purely observational and often intermingled with superstition and religious belief and did not meet the fundamental criteria for a scientific approach.

A few years ago, some of the key players and main pathways influencing the process of ageing have started to be elucidated. At first, model organisms like *Caenorhabditis elegans*, *Drosophila melanogaster*, and even *Saccharomyces* were used to acquire basic information about the regulation of life span. Interestingly, some of the fundamental pathways have been conserved throughout evolution from yeast to human. In mammalian cells, senescence

Abbreviations used: ARF, alternatively reading frame; CDK, cyclin-dependent kinase; COP1, constitutive polymorphogenic1 protein; CR, caloric restriction; HIPK2, homeodomain protein kinase 2; IGF, insulin-like growth factor; Mdm2, mouse double minute-2; MEFs, mouse embryo fibroblasts; NB, nuclear bodies; PARP-1, poly(ADP-ribose) polymerase-1; PML, promyelocytic leukemia; RING, really interesting new gene.

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(terminal growth arrest) induced by the p53 protein can occur when telomeres are exhausted and, as a result, become uncapped (i.e., replicative senescence), or as a consequence of detrimental cellular damage (i.e., stress-induced cellular senescence) [Serrano et al., 1997]. Ageing can be described as the reduced capability of cells or tissues to properly renew themselves and respond to various kinds of stress, some manifestations of which are osteoporosis or a decreased capacity of wound healing, found in older mice as well as humans [Gerstein et al., 1993]. Longevity is the ability to delay the effects of ageing and to increase the life span beyond the limit that is typically found in a certain species. From an evolutionary point of view it is most important for an organism to live long enough to reproduce itself and facilitate a survival of the genes. In this context, a significantly longer survival of an individual after the reproductive phase is, on the level of the species, not a primary goal and therefore, there is no strong selective pressure for an excessive elongation of life span also in species with an already relatively long mean life expectancy.

In yeast, ageing is measured through the replicative life span (RLS) of mother cells indicating the number of mitotic cycles completed before entering a phase of terminal growth arrest [Mortimer and Johnston, 1959]. Interestingly, in yeast, senescence can be caused by the accumulation of extrachromosomal rDNA circles (ERCs) in the nucleus of mother cells [Sinclair and Guarente, 1997] and this process is suppressed by the Sir2 protein [Gottlieb and Esposito, 1989]. It follows that deletion of Sir2 leads to a shortening of life span by approximately 50% [Kaeberlein et al., 1999]. The formation of ERCs during ageing is one process where yeast cells clearly deviate from the mechanisms found in the ageing of mammalian cells.

An additional factor positively correlated with extension of life span in yeast is caloric restriction (CR) which can be achieved by growing yeast in a growth medium containing only 0.5–2% glucose instead of the commonly used 5% [Lin et al., 2000]. A correlation between CR and life span was also found in mammalian cells, including a variety of human cells, further indicating that some basic mechanisms of ageing were conserved throughout evolution. Importantly, in contrast to older literature, a recent article clearly shows that the Sir2 protein reduces the accumulation of rDNA circles and increases the mean life span in yeast independently of CR [Kaeberlein et al., 2004]. Since a homolog of the Sir2 protein is also present in mammalian cells (SIRT1 in human cells) and this protein influences processes involved in determining life span of these cells, the finding that CR and Sir2 work in parallel pathways in yeast is highly interesting. SIRT1 is a class III histone deacetylase, influencing life span in several organisms [Guarente, 2000; Guarente and Kenyon, 2000; Nemoto et al., 2004]. SIRT1 decreases p53 stability through deacetylation of the protein [Luo et al., 2001; Vaziri et al., 2001; Langley et al., 2002].

Involvement of p53 in the Ageing Process

p53, a protein found by chance nearly four decades ago [DeLeo et al., 1979; Lane and Crawford, 1979; Linzer and Levine, 1979], is supposedly the best-characterized tumor suppressor protein and constitutes one of the key players in cellular stress response, apoptosis, cell-cycle regulation, and senescence. p53 and the other members of the p53 family, p63 and p73, additionally play an important role in the development of multicellular organisms. Recently, a central function of p53 in organismal ageing was established [Donehower, 2002] (Fig. 1).

Of course, the function of p53 has to be explained within the context of a very complex network of interacting proteins. The central role of p53 is further underscored by the huge number of up- and downstream interaction partners [Kohn, 1999; Haupt et al., 2003; Dartnell et al., 2005; Kohn and Pommier, 2005]. Also, various post-translational modifications of the p53 protein influencing its stability and activity [Wesierska-Gadek and Schmid, 2005; Wesierska-Gadek et al., 2005b] ensure that the functions of p53 are tightly regulated. One protein that can form complexes with p53 and also modifies it post-translationally is PARP-1, a molecule that is also involved in DNA-damage responses, apoptosis, cell-cycle regulation, and ageing [Burkle et al., 2004; Wesierska-Gadek et al., 2005b,c].

In vivo experiments conducted to elucidate the influence of p53 on cellular and organismal ageing that include the inactivation of the *p53* gene have been hampered by the fact that p53

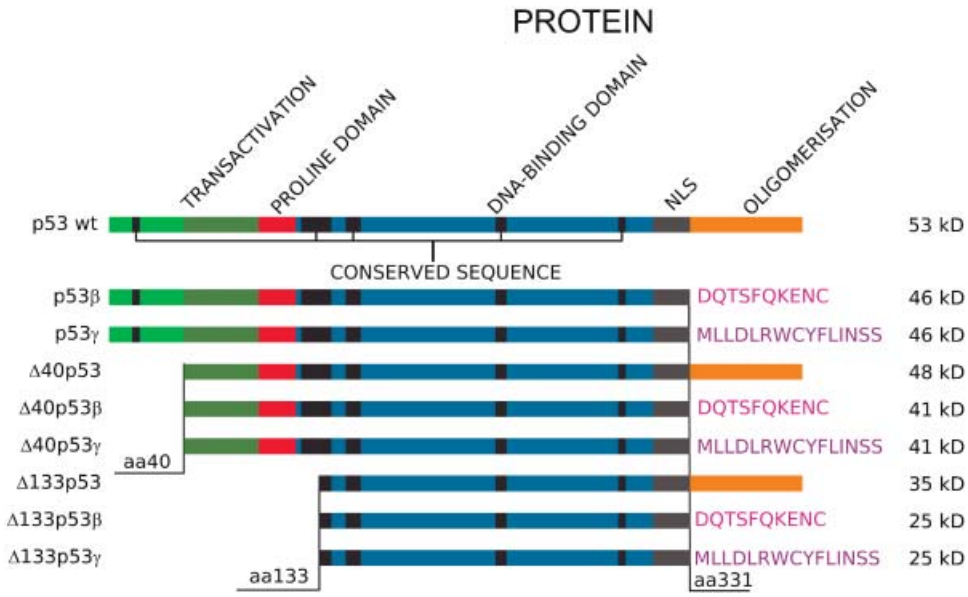

Fig. 1. Diagram depicting the organization and structure of known human p53 isoforms. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

knock-out mice are cancer-prone and exhibit pronounced genomic instability. Often, they succumb early to neoplastic lesions and are therefore not really preconditioned for ageing experiments [Clarke and Hollstein, 2003]. On the other hand, genetically modified mice overexpressing p53 protein die early in embryonic development because of severe defects caused by excessive apoptosis in certain tissues.

However, the establishment of several new strains of p53 mutant mice has shed light onto the complex question of the impact of the tumor suppressor protein on organismal ageing. One mutant mouse line expressed a temperature-sensitive (ts) p53 variant with an alanine to valine substitution at position 135 of the protein. This substitution enabled the protein to switch between wild-type (32°C) and mutant conformation (37°C), depending on the ambient temperature. As Tyner et al. [2002] showed in their recent study, the mice exhibited clear signs of premature ageing exclusively in tissues that were localized to regions of the animal exposed to a lower ambient temperature (e.g., skin). This unexpected effect can be explained by the specific properties of this p53 variant that is present in the non-functional mutant conformation in the core regions of the animal with elevated temperature, whereas it adopts wt conformation in the cooler parts located in close vicinity to the surface of the animal [Tyner et al., 2002].

Another p53 mutant mouse harbors a truncated *p53* gene, termed p53 m, solely expressing the five exons encoding the C-terminal part of the protein but lacking the remaining six exons at the 5′ end [Tyner et al., 2002; Maier et al., 2004] and some undefined sequences upstream of the coding region. The allele was the result of an aberrant gene-targeting event in embryonic stem cells [Tyner et al., 2002]. However, the resulting shortened tumor suppressor protein was significantly more stable than the full-length protein, apparently due to the fact that the N-terminal part of the protein that is usually modified with ubiquitin through the action of the mouse double minute-2 (Mdm2) protein, is missing. Therefore, the protein cannot be marked for degradation via the proteasome pathway. p53 m is capable of increasing the transcriptional activation and growth suppression activity of its wt counterpart. In these mice a strong decrease in neoplastic transformations was apparent. However, this beneficial effect was counteracted by an induction of accelerated ageing. The mutant mice suffered from early ageing-associated phenotypes and the life span was reduced to approximately 80% of their wt littermates. Interestingly, onset of premature ageing

became evident solely in animals older than 1 year.

Regulation of p53 Activity and Stability by Mdm2

Mdm2, in human tissues also termed Hdm2, is the central intra-cellular negative regulator of the p53 tumor suppressor protein (Fig. 2). It is a ubiquitous protein with an intrinsic ubiquitin E3 ligase activity. The full-length protein is 491aa long and has a predicted molecular weight of 55 kD [Wu et al., 2006]. *mdm2* is defined as a proto-oncogene: studies revealed that multiple copies in soft tissue sarcomas, osteosarcomas, and gliomas lead to enhanced degradation of p53, thereby abolishing p53-regulated growth control [Oliner et al., 1992]. Strikingly, the importance of Mdm2 for p53 regulation is underscored by the fact that Mdm2 deficiency is able to rescue the embryonic lethality of p53 knock-out mice [Jones et al., 1995; Montes de Oca Luna et al., 1995]. The transcription of the *mdm2* gene is simultaneously regulated with the transcription of *p53* and additionally by the Raf/MEK/MAP kinase pathway. It has been shown that upregulation of *mdm2* by the Raf/MEK/MAP kinase pathway abolished p53-mediated apoptosis after DNA damage [Ries et al., 2000]. To balance the levels of p53 and Mdm2, $p21^{Ras}$ induces the production of $p14^{ARF}$ which binds to Mdm2 and sequestrates it to the nucleolus, thereby stabilizing p53. Some other cellular (*c-myc*) and viral (*E1A*) oncogenes also induce $p14^{ARF}$ and sensitize cells to p53-mediated growth [Honda and Yasuda, 1999].

The primary target of Mdm2 is the tumor suppressor protein p53. Its modes of action include: inhibition of the transactivation activity of p53, translocation of p53 to the cytoplasm, and targeting of p53 for degradation [Meek and Knippschild, 2003]. Binding of the N-terminal domain of Mdm2 to the N-terminal domain of p53 leads to inhibition of the transactivation activity of p53. Recently it was shown that a second site of interaction is required for ubiquitylation of p53: the acidic and zinc-finger domain of Mdm2 interacting with p53 tetramers [Kulikov et al., 2006; Ma et al., 2006; Yu et al., 2006]. Consistent with these findings, deletion of the zinc-finger domain, or substitution of the amino acids placing the zinc atom leads to loss of the ubiquitylation activity on both p53 and Mdm2. Furthermore, binding of Mdm2 to p53 at this site is significantly enhanced by phosphorylation of the central domain of Mdm2. Another important Mdm2-mode of action is its self-ubiquitylation followed

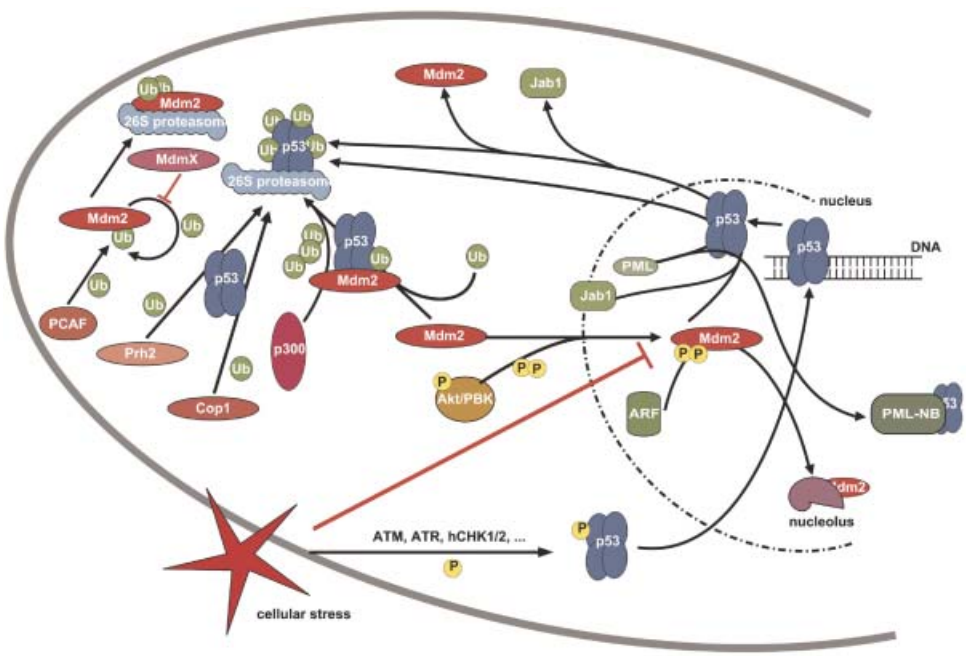

Fig. 2. Scheme illustrating distinct pathways involved in the stabilization of wt p53 protein in normal unstressed cells and in cells after stress stimuli. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

by degradation to maintain moderate levels of Mdm2 [Fang et al., 2000].

The localization of Mdm2 in the nucleus is dependent on the phosphatidylinositol 3-kinase (PI3K) pathway and its downstream serine-threonine kinase Akt/PKB. Activation of this pathway leads to the phosphorylation of Mdm2 at Ser166 and Ser186, two sites necessary for the translocation of Mdm2 from the cytoplasm to the nucleus. Dominant negative defects in the PI3K/AKT pathway, or mutation of the phosphorylation sites in Mdm2, ultimately increase p53 activity in the nucleus [Mayo and Donner, 2001]. Studies in knock-out-mice lacking Jab1/CSN5 (Jun activation domain-binding protein 1), led to the discovery of elevated p53 and $p27^{Kip1}$ levels [Tomoda et al., 2004]. This was further investigated and led to the findings that Jab1, as a nuclear/cytoplasmic shuttle protein, is also involved in translocating p53 to the cytoplasm. p53 is not translocated to the cytoplasm in Mdm2-deficient mouse embryo fibroblasts (MEFs) in the presence of Jab1, but concerted activity of Mdm2 and Jab1 greatly enhanced nuclear export and degradation as compared to a Jab1-deficient background [Oh et al., 2006].

Upon binding of Mdm2 to p53, which is normally localized to the nucleus, p53 is exported to the cytoplasm and subjected to proteasome-dependent degradation. Mdm2 mediates multiple mono-ubiquitylation reactions, through interaction with p300 (CREB-binding protein). p53 can also be polyubiquitylated [Grossman et al., 2003]. Phosphorylation of p53 at Ser15 after DNA damage is sufficient to reduce the binding ability of Mdm2 to p53 leading to stabilization of the tumor suppressor. Another mechanism important for p53 stabilization after oncogenic and oxidative stress is binding of Seladin-1 to the N-terminus of p53. As a result the ubiquitin E3 ligase Mdm2 is displaced from p53 and the protein is protected from degradation and able to respond to stress stimuli [Wu et al., 2004].

Modulation of p53 by COP1

Constitutive polymorphogenic 1 protein (COP1) was recently identified as a novel and critical negative regulator of p53 [Dornan et al., 2004a]. COP1 is a RING (really interesting new gene) finger-containing protein that targets p53 for degradation by the proteasome and is involved in p53 turnover in normal and cancer cells [Dornan et al., 2004a]. COP1 was found to be overexpressed in breast and ovarian adenocarcinomas suggesting that it may promote tumorigenesis by inactivating the p53 tumor suppressor [Dornan et al., 2004a]. Besides the RING domain, COP1 possesses two additional major domains: a coiled-coil domain and a WD40 domain with varying numbers of repeats. The RING-finger domains can act as ubiquitin-protein E3 ligase to target proteins for degradation [Reyes, 2001]. COP1 uses its coiled-coil domain for self-dimerization in plants and mammals and represses c-Jun-mediated AP-1 transcription without affecting c-Jun protein levels [Yi and Deng, 2005]. COP1 interacts through its WD40 repeats with c-Jun and other proteins in mammalian cells [Yi and Deng, 2005]. COP1 shuttles between the nucleus and the cytoplasm and forms subnuclear speckles in both plants and mammals [Yi and Deng, 2005]. Two nuclear localization signals (NLSs) near the RING domain, and a nuclear export signal (NES) located within the coiled-coil domain, are crucial for COP1 shuttling between subcellular compartments. Moreover, an N-terminal extension of mammalian COP1 might be responsible for targeting COP1 to the nuclear envelope (NE).

p53 is rapidly turned over in unstressed cells by a proteasome-dependent pathway, and this is achieved by substrate recognition for the E3 ligase [Dornan et al., 2004a]. Protein ubiquitylation in general requires a specific E3 ubiquitin ligase, which can be a single protein or a multicomponent protein complex. An E3 ubiquitin ligase typically functions by recruiting an ubiquitin-conjugating enzyme (E2) through a RING-finger motif, and the substrate through another protein–protein interaction domain [Yi and Deng, 2005]. For COP1 the strongest evidence that its RING finger can act as an E3 ubiquitin ligase is the functional and genetic interaction with the signalosome, a sub-complex of the proteasome [Reyes, 2001]. The ability of COP1 to negatively regulate p53 by direct ubiquitylation, and c-Jun family members by transcriptional inhibition, places COP1 in the position of a master switch for the cellular regulation of growth arrest or apoptosis versus proliferation and growth [Dornan et al., 2004b].

The findings of Dornan et al. [2004b] illustrate that displacement of COP1, Pirh2, or Mdm2 results in an increase in p53 and $p21^{Waf1}$ steady state levels in normal BJ fibroblasts. This indicates that each ligase plays

a separate role in the negative regulation of p53 in normal cells [Dornan et al., 2004b]. So far, two COP1 ubiquitylation substrates have been identified in mammals: c-Jun and p53. COP1-mediated p53 degradation is an important regulatory mechanism for p53 function in the cell. Over-expression of COP1 inhibits p53-dependent apoptosis, whereas inactivation of COP1 by siRNA leads to p53 accumulation and cell-cycle arrest [Yi and Deng, 2005].

Mutual Regulation of p53 and Pirh2

The recently identified ubiquitin-protein ligase Pirh2 has also been reported to promote the accelerated degradation of the p53 protein [Leng et al., 2003]. The *pirh2* gene encoding a RING-H2 domain-containing protein is regulated by p53 [Leng et al., 2003]. Pirh2, possessing an intrinsic ubiquitin-protein ligase activity, interacts physically with p53 protein and promotes its ubiquitylation that is Mdm2-independent [Leng et al., 2003]. Thus, the expression of Pirh2 and wt p53 protein is inversely correlated [Leng et al., 2003]. An increased expression of Pirh2 decreases the level of p53. Interestingly, wt Pirh2 has a short half-life that can be extended by co-expression of TP60 (Tat-interactive protein of 60 kD) [Logan et al., 2004]. It has been found that Pirh2 is overexpressed in both human and mouse lung cancers [Duan et al., 2004, 2006], and it was detected primarily in the cytoplasm and plasma membrane of malignant cells [Duan et al., 2004]. Since Pirh2 has been reported to be trans-activated by p53, the mechanism by which a high level of Pirh2 expression is maintained in tumor cells despite a low level of wt p53 protein is still not clear [Duan et al., 2006]. Surprisingly, Pirh2 expression is not affected in cancer cells expressing wt p53 [Duan et al., 2006]. Pirh2 enhancement in human cells lacking p53 indicates the existence of additional molecular mechanisms for Pirh2 up-regulation and implicates that p53 is not the only target of Pirh2 ubiquitin ligase activity [Duan et al., 2006].

The Promyelocytic Leukemia (PML) Tumor Suppressor Protein and PML Bodies

Recently, a number of articles have provided evidence substantiating the relationship between wt p53 and the PML tumor suppressor protein. The *PML* gene is a tumor suppressor originally identified in acute promyelocytic leukemia (APL) patients with a reciprocal t(15;17) chromosomal translocation. In APL patients the *PML* gene was found to be fused to the *retinoic acid recepotor-α* (*RARα*) gene [Piazza et al., 2001]. PML is an essential constituent of discrete nuclear subcompartments termed PML nuclear bodies (PML-NBs). PML is either associated to NBs or unbound in the nucleoplasm. Under certain conditions, for example, virus infections, PML shuttles between the nucleus and the cytoplasm [Stuurman et al., 1997]. Although there are controversial studies about the function of NBs, they likely act as storage sites for regulatory proteins and organizing centers, where nuclear processes may be regulated [Borden, 2002]. In APL cells, where PML is fused to RARα, the NBs are disorganized which suggests that normal PML protein is indispensable for the organization and structural integrity of the PML-NBs [de The et al., 1991]. PML is composed of nine exons. Exons six to nine may be spliced alternatively, yielding at least seven isoforms, which differ in their COOH-domain. Exons one to three are common to all isoforms and harbor a tripartite motif called RBCC or TRIM (RING finger, B-box, coiled-coil) [Reymond et al., 2001]. All of the isoforms are able to recruit p53 into PML-NBs, but only one isoform, PML-IV, is able to regulate p53 activity [Fogal et al., 2000] and to induce premature senescence [Bischof et al., 2002] (Fig. 3).

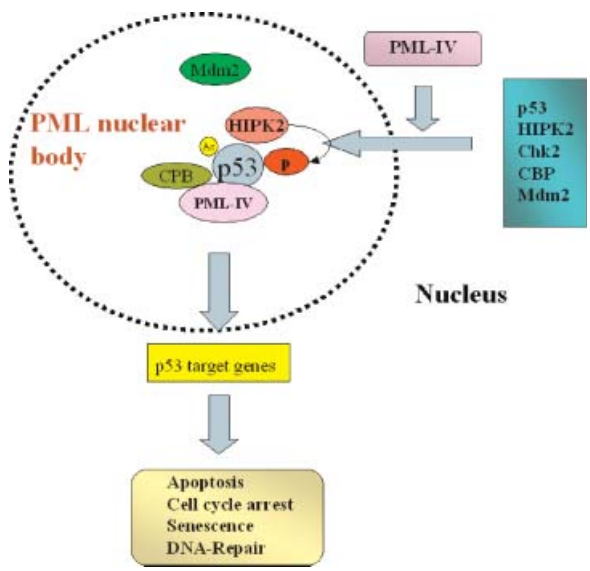

Fig. 3. Role of PML nuclear bodies in the regulation of p53 activity and function. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PML, An Upstream Regulator of p53, Plays a Key Role in Control of p53 Functions and Modulation of Cellular Senescence

Several studies indicate that PML acts as an upstream regulator of p53 under normal and stress conditions. In unstressed cells, p53 protein forms complexes with PML at basal levels. Upon stress stimuli concentrations of p53 protein increase, and as a consequence, proportionally increase the level of p53-PML complexes. p53 interacts with the COOH-terminal region of PML through its central core domain [Gu and Roeder, 1997; Fogal et al., 2000] (Fig. 3).

What is the consequence of the association of p53 with the PML protein? PML recruits p53 proteins to PML bodies. Overexpression of PML, γ irradiation of cells, or oncogenic signals such as Ras-overexpression can recruit p53 to PML-NBs and enhance its transcriptional activity [Ferbeyre et al., 2000; Fogal et al., 2000; Guo et al., 2000; Pearson et al., 2000; Bischof et al., 2002]. PML stimulates the phosphorylation of p53 at Ser20 [Louria-Hayon et al., 2003] which interferes with the Mdm2-p53 interaction after DNA damage [Unger et al., 1999]. Moreover, PML regulates p53 acetylation at Lys382 upon Ras expression. Ras induces re-localization of p53 protein and the CBP acetyltransferase and their recruitment to PML bodies, resulting in the formation of a trimeric p53-PML-CBP complex [Pearson et al., 2000]. PML-mediated recruitment of both proteins promotes p53 acetylation [Guo et al., 2000; Pearson et al., 2000].

Interestingly, PML can bind to p53 and its negative regulator Mdm2 and form trimeric complexes, thereby protecting p53 from Mdm2-mediated ubiquitylation and subsequent degradation [Louria-Hayon et al., 2003; Wei et al., 2003]. PML increases the accumulation of p53 protein and its targets such as $p21^{waf1}$ by antagonizing Mdm2 functions, which leads to an increase in the half-life of p53. All isoforms of PML have a binding site for Mdm2 around the coiled-coil motif and directly interact with Mdm2 [Bernardi et al., 2004]. Moreover, PML is also able to stabilize p53 by recruiting Mdm2, a negative regulator of p53, into the NBs, thereby preventing p53 from Mdm2-mediated degradation [Kurki et al., 2003; Louria-Hayon et al., 2003; Wei et al., 2003].

PML as a Direct Target of 53

p53 is able to induce PML as well as PML mRNA, and to increase the number and size of PML-NBs. It has been shown that PML contains three putative p53 responsive elements (REs) in its promoter region. One RE lies within intron one, being mainly responsible for p53 regulation of PML. These findings suggest that PML expression is directly regulated by p53 [de Stanchina et al., 2004]. Due to this fact it must be noted that PML also contributes to cellular p53-dependent processes like senescence, cell-cycle arrest, and p53-mediated apoptosis, and additionally PML arranges p53 tumor suppressor functions [de Stanchina et al., 2004]. Cells lacking PML are nevertheless able to induce p53 and p53 target genes, clearly illustrating a lower ability to induce the mentioned p53-dependent processes.

PML and Homeodomain-Interacting Protein Kinase 2 (HIPK2)

It is well established that PML-NBs recruit a wide variety of proteins that are associated with multiple functions, including transcription, DNA repair, protein modifications, and degradation. Homeodomain-interacting protein kinases are also components of PML-NBs. One major function of the HIPK2 is to phosphorylate p53 at the NH_2-terminal Ser46, which leads to p53-dependent gene expression, and ultimately to cell-cycle arrest and apoptosis [D'Orazi et al., 2002; Hofmann et al., 2002]. Interestingly, HIPK2 can be activated by inhibition of cyclin-dependent kinase-2 (CDK2) [Wesierska-Gadek et al., 2007]. Exposure of human breast cancer MCF-7 cells to roscovitine, a selective CDK inhibitor, resulted in HIPK2-mediated phosphorylation of p53 protein at Ser46 [Wesierska-Gadek et al., 2007]. This phosphorylated p53 isoform transcriptionally activated the mitochondrial p53 apoptosis inducing protein 1 (p53AIP1) [Oda et al., 2000] thereby facilitating the elimination of cancer cells by induction of apoptosis [Wojciechowski et al., 2003; Wesierska-Gadek et al., 2005a] (Fig. 3).

HIPK2 and its homologous kinase HIPK3 are found in so-called subnuclear HIPK domains. The formation of these domains does not depend on PML [Moller et al., 2003]. PML-IV, which is one isoform of PML involved in the regulation of the activity of p53 [Fogal et al., 2000] is also able to recruit endogenous HIPK2 into the

PML-NBs. The COOH-terminal portion of the kinase is crucial for this recruitment [Moller et al., 2003].

In APL cells, where PML is fused to RARα, the HIPK2/p53 mediated anti-proliferative and pro-apoptotic activity is negatively affected [Moller et al., 2003]. These data reveal that prior to anti-cancer therapy that affects the p53 pathway, the PML-NBs in APL cells have to be restored by supplementation with retinoic acid.

It is well documented that oncogenic *ras* by itself is not able to transform primary human or rodent cells. *Ras* expression results in a permanent G_1 arrest resembling cellular senescence [Serrano et al., 1997]. However, contribution of the tumor suppressors p53 and Rb is necessary for premature senescence induced by oncogenic *ras* [Serrano et al., 1997; Lin et al., 1998; Zhu et al., 1998; Ferbeyre et al., 2000].

As a response to oncogenic signals, the number and size of NBs increase. This coincides with an elevation of PML concentration, re-localization of p53, CBP, and Rb, and their recruitment to NBs. Not only p53, but also the CBP acetyltransferase, is re-localized to the PML-NBs. Furthermore, PML, p53, and CBP form a ternary complex which leads to acetylation of p53 at Lys382. This specific $p21^{Ras}$-induced post-translational modification enhances the activity of p53 and leads to premature senescence [Ferbeyre et al., 2000; Fogal et al., 2000; Pearson et al., 2000].

Impact of p53 and p16 on Senescence

It is well established that p53 is responsible for induction and maintenance of the senescent phenotype [Papazoglu and Mills, 2007], but in case of p53 loss, only a high level of the CDK inhibitor $p16^{Ink4a}$ is capable of making this process irreversible. If however, the $p16^{Ink4a}$ level is not sufficiently high, a reversal of the senescent phenotype is possible [Beausejour et al., 2003]. Additionally, increased expression of $p16^{Ink4a}$ was described as a marker for ageing in certain murine cell types [Beausejour and Campisi, 2006]. In a series of recent studies, the impact of $p16^{Ink4a}$ on the ageing process in hematopoietic stem cells [Janzen et al., 2006], pancreatic cells [Krishnamurthy et al., 2006], and brain cells [Molofsky et al., 2006] was shown. It is clear that the tumor-suppressive functions of p53 are partly executed through the induction of senescence [Campisi, 2001; Campisi, 2005], and that senescence also contributes to the process of ageing [Campisi, 2005; Papazoglu and Mills, 2007]. However, the exact mechanism by which p53 and the other members of the p53 family influence ageing and longevity, is still under vivid discussion [Keyes et al., 2005; Wesierska-Gadek and Schmid, 2005; Wesierska-Gadek et al., 2005c; Keyes and Mills, 2006; Papazoglu and Mills, 2007] (Fig. 4).

Short Isoforms of p53—the Key for Understanding Its Influence on Ageing?

Recently, a short p53 variant was independently described by two groups [Courtois et al., 2002; Yin et al., 2002], and each group speculated on the function of this variant. The protein had actually been found earlier in Balb/c mice [Lavigueur et al., 1989] but did not receive much attention, although the authors found a connection between the short protein and an increased tumor incidence in the mice. The truncated variant, termed p44 in mice and ΔNp53/DeltaNp53 in humans, is expressed in various cells and tissues and shows a strong similarity with p53 m and also leads to an elevated p53 stability and activity [Courtois et al., 2002]. The truncated p53 is expressed via initiation of translation at an internal AUG start codon at amino acid position 40 (human) or 41 (mouse) relative to the full-length protein [Courtois et al., 2002; Scrable et al., 2005]. Additionally, it was evidenced by Yin et al. [2002], that transcription of the *p53* gene is regulated by Mdm2 through usage of alternative initiation sites. p53 and the truncated p44, lacking the Mdm2-binding site and N-terminal transactivation domain, are the products. Mdm2 binds to the nascent p53 polypeptide followed by degradation of full-length p53, thereby shifting the ratio toward p44 [Yin et al., 2002].

Interestingly, the short isoform of p53 more closely resembles the primitive p53 isoforms found in lower organisms, suggesting that features of p53 accomplished by the N-terminal portion were acquired later in evolution. Most importantly, it should also be mentioned, that the two other members of the p53 family, namely, p63 and p73 are expressed as several isoforms, also bearing or lacking the N-terminal portion. In most cases, the longer isoforms have functions similar to full-length p53, and the ΔNp63 and ΔNp73 isoforms counteract these activities. This is another analogy to p53

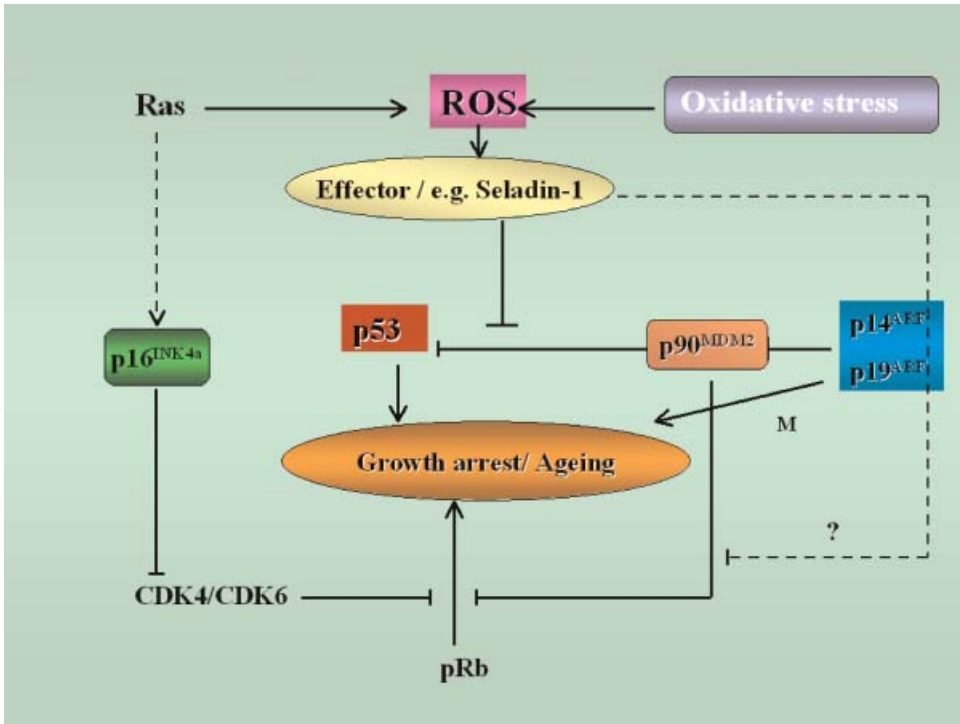

Fig. 4. Link between oxidative stress, growth arrest, and the ageing processes (adapted from Wu et al., 2004). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

where the short isoform is thought to counteract or fine-tune the action of the full-length protein, depending on the context [Maier et al., 2004].

The expression of the short p53 isoform seems to be regulated at the transcriptional level as well as at the translational level [Scrable et al., 2005]. An alternative p53 transcript (p53EII) was found in human foreskin fibroblasts [Matlashewski et al., 1987] and the ΔNp53 protein can be translated from this truncated transcript [Ghosh et al., 2004]. However, strong evidence indicates that the expression level of ΔNp53 is predominantly regulated at the post-transcriptional level. Transfection of p53-deficient mouse cell lines leads to the expression of the truncated, and the full-length isoforms [Gannon and Lane, 1991; Courtois et al., 2002] and in-frame disruption of human p53 cDNA at the alternative start codon AUG at position 40, completely impedes the expression of ΔNp53 in these cells [Courtois et al., 2002].

It is also suggested, that the p53 protein is capable of regulating its own translation by binding to the 5' UTR of its mRNA [Mosner et al., 1995]. However, only full-length p53 can perform this mRNA-binding because the N-terminal part of the protein, that is missing in the ΔN isoform, is obligatory for this kind of interaction [Mosner et al., 1995]. A large excess of the truncated p53 isoform seems to decrease the ability of the full-length isoform to transactivate most of its target genes [Courtois et al., 2002; Yin et al., 2002], whereas a modest level of the short protein enhances the trans-activational potential of the longer isoform [Maier et al., 2004]. It was also reported [Ghosh et al., 2004], that an elevated ΔNp53 level causes a predominantly cytoplasmic localization of full-length p53, thereby also impeding its transactivational properties and other nuclear functions of the protein. Finally, the short isoform could influence the cytoplasmic activities of p53 (e.g., cytochrome *c* release), thereby impeding its pro-apoptotic properties [Mihara et al., 2003; Scrable et al., 2005].

Recently, new data were published illustrating that excess levels of wild-type p53 protein can, under specific circumstances, protect mice against cancer and ageing. This line of evidence gives rise to a model where increased levels of p53 may protect organisms against neoplastic transformations without the unwanted collateral effects of premature ageing [Garcia-Cao

et al., 2002]. A Spanish group recently generated novel transgenic mice, called "super p53" [Garcia-Cao et al., 2002]. The super p53 mice produced, like normal mice, wt p53 from their two naturally occurring alleles, and additionally possessed one or two copies of a wt *p53* gene, inserted as a transgene in the form of a large genomic fragment. Importantly, the additional p53 gene copies were expressed from their natural promoter. This guarantees that the transgenetically expressed p53 was regulated in the same pattern as the endogenous p53 gene. The super p53 mice were more sensitive to DNA damage because higher levels of p53 led to an enhanced rate of apoptosis, and they were also more efficiently protected from chemically induced cancers.

Most importantly, in clear contradiction to the findings acquired from experiments employing the mutant p53 m animals, these "super" p53 mice did not display any signs of accelerated ageing. One reason for the lack of detrimental side effects described as premature ageing in "super" p53 mice may be that the transgenetically expressed p53 was regulated from its natural promoter and therefore the tumor suppressor protein was expressed exactly as required in the physiological and cellular context. These data may be a first glimpse of the idea that protection of cells against neoplastic lesions might be achieved by introducing the correctly expressed tumor suppressor gene into progenitor or stem cells.

Why does increased p53 activity lead to premature ageing and a reduced life span of mammalian organisms? The data from the literature clearly imply that the enhanced p53 activity leads to an elevated induction of apoptosis and cell-cycle arrest. This in turn seems to exhaust the organisms capability to renew certain tissues, most of all organs and tissues with a high turn-over rate that depend on the capability of stem cells to replace lost cells and tissues. It appears that in organisms in which this cellular homeostasis is disturbed, the negative effects become manifest in the form of changes commonly summarized under the term ageing.

The short isoform of p53 seems to have a strong influence on ageing and on the size of the mammalian organism [Maier et al., 2004]. It does this by changing the ability of the full-length protein to function as a transcription factor. This also alters insulin-like growth factor (IGF) signaling and causes a premature ageing phenotype in mice [Maier et al., 2004]. An involvement of the insulin/IGF1 pathway in the regulation of ageing has been described in many studies [Gems and Partridge, 2001; Richardson et al., 2004] and it is clear that the effects of CR are also, at least partly, mediated via this pathway. Additionally, it was shown that mice with a reduced expression of the insulin receptor or IGF1 receptor, as well as the growth hormone-deficient Ames and Snell mice, possess a significantly elevated life span [Gems and Partridge, 2001].

What is the connection between p53, p44/ΔNp53, insulin/IGF1, and ageing? A recent study [Maier et al., 2004] shed more light onto this scientific field. Two decades ago, the short p53 isoform was shown to be growth suppressive in the presence of the full-length isoform [Rovinski and Benchimol, 1988; Lavigueur et al., 1989], but tumorigenic in the absence of its longer counterpart [Mowat et al., 1985]. When the balance of the two p53 isoforms is disturbed by the over-expression of p44/ΔNp53, the growth suppression mediated by the truncated protein leads to reduced proliferation, small body size, cellular senescence, aberrant IGF signaling and consequently, to premature organismal ageing [Maier et al., 2004].

Mice in which transgenic p44 was stably expressed showed a shortened mean life expectancy, early infertility (especially in male mice), and signs of premature ageing. It should also be mentioned that there was some sexual dimorphism, and that the infertility and some signs of ageing were more pronounced in male mice [Maier et al., 2004]. The early ageing phenotype was also manifested in lordokyphosis (hunchback) starting at 5 months of age, in significantly reduced bone mineral density, a lower trabecular bone volume, and an overall low bone turnover process in p44 mice [Maier et al., 2004]. On the protein level it was shown by Western blotting, that the expression level of p44 in the transgenic mice was approximately twice as high as in the wild-type mice. The growth-suppressive properties of elevated p44 levels resulted in transgenic mice that were about half the size of their wild-type littermates. In the absence of p53, p44 had no effect on the size of the mice, indicating that the full-length protein is needed for this growth-suppressive effect on the cellular and organismal level [Maier et al., 2004]. Interestingly, the growth

of the mice was already slowed in early embryogenesis and continued post-natally.

Most importantly, the insulin/IGF1 pathway was influenced by an increase in p44 protein. Cells from p44 transgenic mice showed several-fold higher levels of IGF1 receptor and of activated Akt, a down-stream target of IGF1 [Maier et al., 2004]. Additionally, p53 phosphorylation at Ser15 was elevated in p44 mice, and the expression of two major p53 transcriptional targets, namely $p21^{waf1}$ and Mdm2, was also increased [Maier et al., 2004]. For all these effects the presence of the full-length p53 protein was necessary, so that p44 could achieve the described changes.

It is known that IGF signaling can affect cell size and cell number in lower organisms, therefore, it was important to show which effect accounts for the smaller size of p44 mice. MEFs isolated from wild-type mice showed a proliferative burst within the first passages in cell culture, whereas cells from p44 mice did not show this effect [Maier et al., 2004]. The MAP kinase pathway is able to over-ride sustained proliferative signaling mediated by Akt in growth factor-stimulated cells [Roovers and Assoian, 2000]. Under conditions of permanent activation, ERK, the target of the MAP kinase pathway, is able to induce $p21^{Ras}$, which in turn leads to a G_1 phase cell-cycle arrest. This is a security mechanism similar to the induction of apoptosis when *Ras* is permanently activated. The most important finding from these experiments is that in MEFs isolated from p44 mice, the ongoing activation of IGF1 receptor leads to sustained ERK activation, $p21^{waf1}$ induction and consequently to cell-cycle arrest [Maier et al., 2004]. In this model, the sustained activation of the IGF1 pathway leads to induction of $p21^{waf1}$ and cell-cycle arrest, which in turn impedes proliferation of the cells. The small size of the animals is therefore caused by a decreased cell number and not by a smaller cell size [Maier et al., 2004]. The premature ageing phenotype is therefore caused by the inability of certain cells and tissues to renew themselves and to replace lost cells.

In conclusion, these findings clearly demonstrate that the p53 protein plays a central role in organismal ageing in addition to its importance in DNA damage response, cell-cycle regulation, and senescence. Interestingly, the ratio of full-length p53 to the short isoform p44/ΔNp53 appears to have a strong influence on the regulation of ageing. Therefore, an approach to modulate the level of the truncated p53 isoform as a tool to increase mammalian health-span [Scrable et al., 2005] and life span might be a reasonable as well as desirable goal to pursue. Nevertheless, additional investigations will be required to unravel the extremely complex signaling network involved in the various processes and manifestations of ageing and longevity.

A recent study [Baur et al., 2006] further underscores the impact of Sir2 on organismal ageing, and also points out the connection to obesity. Obese mice have a significant decrease in mean life span and display signs of premature ageing. Most importantly, when the diet of these obese mice was supplemented with resveratrol, a compound also found in relatively high concentrations in red wine, nearly all of the signs of premature ageing, including liver damage, disappeared, and the mice reached their species-specific life span [Baur et al., 2006; Kaeberlein and Rabinovitch, 2006]. However, resveratrol has no positive impact on slim mice, indicating that it is only able to prevent the adverse influence of obesity, but not to further improve the health and life span of normal-weight rodents. Interestingly, resveratrol is capable of activating the Sir2 protein in yeast [Howitz et al., 2003], supporting the notion that the sirtuins play a central role in the regulation of both life span and ageing-related illnesses.

Interestingly, resveratrol seems to exert an effect on the cellular levels of wt p53 protein and $p21^{waf1}$, its downstream target [Kim et al., 2004]. Further investigations revealed that resveratrol induces the expression of p53 in a concentration-dependent manner [Kim et al., 2004]. Narayanan et al. [2003] observed that resveratrol leads to the expression of a whole set of p53 target genes (e.g., $p21^{waf1}$, p300/CBP, Apaf1, and BAK), which are strongly involved in the induction of cell-cycle arrest and apoptosis. Thus, it seems that resveratrol affects the stability of cellular p53 through the modulation of its post-translational modifications [Zhang et al., 2004]. Interestingly, in vascular endothelial cells, resveratrol activated p53 by phosphorylation at Ser15. However, the site-specific phosphorylation of p53 was neither accompanied by an increase of its total concentration nor by up-regulation of its target $p21^{waf1}$ [Haider et al., 2003]. Thus, resveratrol induces a block in DNA synthesis that is not followed by apoptosis [Haider et al., 2003]. Regulation of p53 by resveratrol has been also proposed to occur via

MAPK activation (especially ERKs and p38) [She et al., 2001]. Additionally, resveratrol regulates p53 activity by modulation of p300 expression [Narayanan et al., 2002]. Possibly, p300 and p53 are part of an apoptotic loop of gene regulation that mediates resveratrol-induced apoptosis.

CONCLUSIONS

Our present article gives an overview about the central involvement of p53 on cellular and organismal ageing. Molecular studies in lower organisms, biochemical experiments, and findings from various p53 mutant mice clearly show the influence of p53 on ageing and longevity, and the connection to pathways involved in CR. Most importantly, an increased life span, and protection from cancers no longer appear to be opposing goals. Obviously, the interaction of a truncated and a full-length p53 variant is the key to increase mammalian health-span and life span concomitantly. However, more detailed studies about the mechanistic details and the development of proper tools to regulate the key factors will still be needed before this goal can be reached and patients can benefit from the scientific achievements.

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