

Function of the p53 Gene: Choice between Life and Death

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Abstract—Gene p53 is a central component of a system that eliminates pathologically damaged cells from an organism. Multiple signal pathways monitor the state of a cell and when damage or a fault is found that could cause heritable changes, p53 protein is activated to either coordinate the repair process or induce cell suicide. Thus, the p53 gene acts as a supreme judge that decides the fate of cells and guarantees their social behavior. Loss of the p53 gene results in uncontrolled accumulation of genetic damage causing failure of control by the organism, malignant cell growth, and death of the organism.

Key words: tumor suppressor, oncogene, cell cycle, apoptosis, genetic stability, transcription, protein conformation

One of the most significant achievements in molecular biology in recent years is the elucidation of a system that rapidly destroys cells that are damaged and potentially dangerous for the organism. This system monitors various kinds of damage as well as stress states which produce or may produce genetic changes and influences the choice of “suppression measures” for the cells involved in these processes. Therefore, the cells are either temporarily stopped at cell cycle checkpoints (see review by B. P. Koprin in this issue) for repair or completely purged away from subsequent divisions by apoptotic cell death or by cell senescence and necrotic death. The central component of this system is the p53 protein. The p53 protein collects numerous signals of the emergence of irregular situations and its level quickly increases, predominantly by the stabilization of the protein molecule, and also by transformation of the protein to a functionally active state. Consequently, several intracellular mechanisms are initiated to cause the self-restriction or suicide of a damaged cell.

CONSEQUENCES OF p53 INACTIVATION

The function of p53 is not strictly needed for normal growth and functioning of an organism. Mice with the p53 gene completely deactivated by gene knockout produce rather normal offspring. However, these mice rarely live more than six months due to the fact that they are extremely sensitive to tumor progression [1, 2]. Cultivated p53^{-/-} mouse cells are of high genome insta-

bility, and they display severe variations in karyotype even in the first days of *in vitro* passaging [3].

Damage to the p53 gene also plays a major role in human carcinogenesis. Point mutations or deletions in the p53 gene are observed in approximately 50% of malignant diseases although the frequency of damage depends on the tumor type [4, 5]. In human and in experimental animal tumors there are numerous other ways in which the p53 gene can be inactivated with no changes in the structure of the gene. In these cases there is damage to genes involved in regulation of the p53 gene. Either the p53 gene cannot be activated in response to various stresses or, in contrast, its expression level is increased but subsequent characteristic intracellular mechanisms cannot be initiated. Therefore, the maintenance of genome stability is lost to some extent as in the case of direct damage to the p53 gene.

Thus, defects of p53-dependent processes are observed in most tumor diseases. Only after the p53-dependent self-restriction of damaged cells ceases, conditions for the rapid accumulation of genetic changes are established that lead to the loss of negative control of cell division. In fact, tumor cells become out of the control of the whole organism and acquire autonomy. From this point of view, the cancer cell cannot be considered as a part of an organism, because the cancer cell competes with the organism. So, the tumor behaves like a separate, parasitic organism that is involved in evolution according to Darwin's selection laws. Consecutive accumulation of mutations provides enhanced proliferation and invasive growth, which ends only upon death of the host organism.

THE BIOLOGICAL ROLE OF THE p53 GENE

The p53 gene has been figuratively called the "genome guard" [6]. Indeed, this gene coordinates all the essential processes of maintaining genome stability in a multicellular organism. In spite of the fact that a genome is extremely complex, all cells of an organism are genetically uniform. This fact results from not only the high fidelity of the DNA replication process and the effectiveness of repair systems, but also to a considerable degree this fact is the consequence of a continuous p53-dependent process of removal of cells that develop genetic defects or that are subjected to an unfavorable influence that threatens the cells with the development of genetic defects. The p53-dependent control system is exceptionally responsible. Numerous signals monitoring the state and environment of a cell come together at p53. This is achieved by various modifications of the p53 molecule such as phosphorylation, acetylation, and binding with molecules regulating the activity of p53. The responses of the p53-dependent mechanisms must be adequate to a situation. Many processes in a cell are tissue-specific. Thus, the requirements for p53-dependent control in different cells may vary. For example, the high proliferative activity of colon epithelium cells and organs of hemogenesis demand the p53 system to be tuned to a high degree of sensitivity. Even slight damage to DNA can cause p53-dependent apoptosis in such cells. In contrast, the role of p53 is less remarkable in infrequently dividing cells, e.g., in hepatocytes, and in a series of other physiological states. Also, a similar influence can be either physiologic or pathologic depending on the situation. For instance, the activation of MAP-kinase cascades in response to growth factors is physiological, while the same process initiated by a permanently activated oncogene is pathological. Therefore, physiological processes at certain times order the p53-dependent control to be ceased temporarily. If an influence exceeds some level permissible for the cell, the p53-dependent processes must be resumed.

Such strict conditions for the selectivity of the action of p53 suggest that regulation mechanisms of its activity must exist. Indeed, a large number of facts concerning the complexity and refinement of the p53-dependent mechanisms and consolidating it into a system of many regulatory processes formerly deemed to be separate has accumulated to date. If the p53-dependent system of pathologic cell restriction is considered as a signal pathway, the processes recognizing damages, stresses, and faults occupy the upper levels. Then these influences must come together to p53 causing qualitative and quantitative changes in its expression and activity. The lower levels of this pathway are occupied by the effector genes that are influenced by the various states of p53. A cell is either stopped for a short time in a checkpoint or suffers apoptosis or cessation of division caus-

ing premature cell aging. The lower levels of the p53-dependent signal pathway have been studied most completely. These levels represent the mechanism of action of p53, which has been the subject of profound research for the last decade. Dozens of genes executing suppressor signals of activated p53 have been determined. This report is mainly devoted to the diversity of the mechanisms of activation of p53, i.e., the upper levels of the p53-dependent pathways. However, it is necessary to sum up briefly the essential data about p53 and its action mechanism before considering these questions. The readers can obtain more complete information in a series of recently published reviews [7-13].

FUNCTIONS OF THE p53 PROTEIN

p53 is a polyfunctional protein which functions in the nucleus. The p53 gene is continuously transcribed and translated, but the protein is rapidly subjected to ubiquitin-dependent degradation in proteosomes [14, 15]. Therefore, the concentrations of p53 in cells of most tissues are quite low and may be at the limit of detection. The activation of p53 as a response to various stresses and damages proceeds mainly post-translationally via a decrease in its degradation rate and a change of its conformation to generate increased functional activity. Several functions and activities are attributed to p53. The protein can act as a transcription repressor [16] and as an inhibition factor of the translation of some mRNAs [17]. There are reports indicating that p53 has 3'-5'-exonuclease activity [18], the capability to bind directly with single-stranded DNA [19, 20], unpaired bases [21], and single-stranded gaps [22]; this suggests that p53 participates in processes of recognition and immediate repair of damaged DNA [22]. Undoubtedly however, the main function of p53 is its action as a transcription factor [23].

The p53 molecule consists of 392 amino acids; it forms a tetrameric complex that recognizes a specific DNA sequence and stimulates transcription of several genes having an appropriate DNA element adjacent to the promoter. The DNA element with which p53 couples consists of two "semi-sites" of general structure PuPuPuC(A/T)(A/T)GPyPyPy positioned one after another at 0-13 nucleotides distance [24, 25]. A similar element has been found in many genes activated by p53. This element is present in the gene for the Mdm2 protein, which is a natural inhibitor of p53 [26]. Mdm2 blocks a transactivation domain of p53 [27] and at the same time aids in p53 export from the nucleus to the system of proteosomal degradation [28].

The p53 molecule contains several functionally significant domains that play important roles in regulation [29].

The N-terminal sequence (amino acids 1-50) contains a domain involved in the activation of transcrip-

tion of target genes. This locus is also responsible for the interaction with the protein inhibitor Mdm2 [30, 31]. The N-terminal domain plays a substantial role in the regulation of p53 activity due to several serines and threonines located there which are targets of regulatory kinases. Phosphorylation of the N-terminal amino acids changes the interaction p53 with Mdm2 and changes interactions with factors of the transcription apparatus. A second transcription domain is located between amino acids 43 and 73; its activity is essential for the activation of some p53 targets [32].

A flexible proline-rich domain is located between amino acids 63 and 97 in which the PxxP elements are found. This domain is needed for the full suppressor activity of p53 [33]. Although the mechanism of action of the proline-rich domain is not completely clear, it is supposed to take part in both transcription-dependent [34] and transcription-independent apoptosis [35].

The central domain between amino acids 100 and 300 participates directly in recognition and binding with specific DNA sequences [36]. A structure of this domain plays an exceptional role in the ability of p53 to display transcription factor activity. This domain is the site of most missense mutations of the p53 gene in various cases of human tumors. Here two characteristic peculiarities are observed. On one hand, almost every amino acid of this domain can be mutated with different frequencies in tumors giving proteins with defective activity. The fact that any change in the structure of this locus produces a protein of faulty function indicates the exceptional fragility of the p53 conformation determined by the central part of the molecule. On the other hand, there are hot mutation points in the central locus in which mutations occur at high frequency. The amino acids of these hot points were shown by X-ray analysis of DNA with the p53 DNA-binding domain to be involved in immediate contact with a specific DNA sequence [36]. The fragility of the p53 DNA binding domain results in changes in antigen epitopes. Many mutated proteins lose the epitope for monoclonal PAb1620 antibodies [37, 38] but at the same time acquire the epitope recognized by PAb240 antibodies [39]. This monoclonal antibody pair is an important diagnostic tool for detecting conformational changes of not only the mutated protein but also the intact p53 in various physiologic states [40].

A linker sequence between amino acids 305 and 323 is responsible for the nuclear localization of p53 [41, 42].

A locus between amino acids 323 and 356 is responsible for tetramerization of p53 molecules. This locus has a clear α -helical structure [43]. Loss of the ability of p53 to oligomerize occurs if the α -helical domain is damaged, and this causes functional inactivation [43, 44]. p53 molecules with inactivating mutations in the DNA-binding domain are also able to form complexes by virtue of the α -helical domain. Hybrid complexes of wild

type and mutant proteins are inactive. This explains the transdominant (dominant-negative) character of missense mutations of the p53 gene [45].

An alkaline domain essential for the regulation of p53 activity is located immediately at the C-terminus of the molecule (amino acids 363-393). This domain is a target for a series of modifying enzymes. The unmodified alkaline domain prevents formation of the complex of DNA and the central DNA-binding domain of the p53. Removal of the alkaline domain stimulates the DNA-binding activity of p53 *in vitro* [46]. Treatment of p53 with PAb421 monoclonal antibodies recognizing a part of the C-terminal domain [47] and addition of synthetic peptides matching this p53 fragment [48, 49] produce the same effect. In the latter case, the activation may take place due to destruction by the peptide of a complex of the C-terminal and the central p53 domains. The C-terminal domain is essential for regulation of p53 activity *in vivo*. Modification of this element by kinases, acetylases, glycosylases, and binding with other proteins causes a delicate transformation of the DNA-binding and transactivation ability of p53. Also, the C-terminal p53 fragment is able to bind nonspecifically with single-stranded DNA elements, unpaired bases, and DNA ends [50] indicating its possible involvement in the process of recognition of damaged DNA.

The main consequences of the activation of the p53 gene are delay of progression through the cell cycle and apoptosis. However, the biological effects of p53 are not limited to these two processes; it is clear that p53 can play a role in DNA repair and in some substantial intracellular processes, for example, in the control of centrosome replication [51] and regulation of secretion of angiogenic and other peptide factors acting at the level of the organism [52-54]. The list of known p53 activities will probably grow with time.

p53 TRANSCRIPTION TARGETS

p53 can exert a complex influence on the transcription of genes, activating the expression of some and repressing others. Transcription repression is a function of the C-terminal fragment of the p53 molecule [55]; the repression is partially due to the capability of this protein to couple with a basal component of the transcription apparatus—the TBP factor (TATA-Box Binding Protein) [56]—and due to suppression of the activity of the TFIID and TFIID complex [57]. Hence, the activity of RNA-polymerase II and RNA-polymerase III is partially reduced. Also, p53 represses the activity of several transcription factors such as Spi1 [58], HIF-1 [59], a thyroid hormone receptor [60], estrogen receptor [61], and transcription factor STAT5 [62]. Therefore, p53 can repress transcription of many genes such as the *BCL2* [63] and *RELA* [64] genes playing a role in apoptosis,

MDR1 [65], *Hsp70* [66], and actin gene [67], a gene of the MAP4 protein bound with microtubules [68], and *p19-ARF* [69], a product of which prevents degradation of p53.

Most of the data concerning the mechanisms of p53-induced processes was obtained by identification of the effector genes whose expression increases under the influence of p53. Many p53-regulated genes have been identified in addition to the *MDM2* gene that negatively regulates p53. These can be conditionally separated into several groups.

Genes whose activation induces cell cycle arrest. A main effector of the p53 gene is the p21-WAF1 protein, a powerful inhibitor of cyclin-dependent kinases (cdk) [70]. p21 is responsible for the arrest at the G1 phase as a response to hyperexpression of p53 [71] and DNA damage [72, 73]. p21-WAF1 mainly couples with cyclin-cdk complexes of the G1 and S phases but poorly binds with the cyclin B-cdc2. Besides, p21 associates with a "proliferating cells nuclear antigen" (PCNA) and, therefore, can diminish the processivity of DNA-polymerase, possibly switching of replicative synthesis to the dominantly reparative synthesis [74].

Three p53-responsive genes are known to take part in the G2 phase arrest. The *GADD45* gene product as well as p21 can bind with the PCNA [75]. Also, this product associates with and inhibits a cyclin B-cdc2 complex causing arrest at G2 [76]. Another p53 responsive gene encodes 14-3-3- σ protein [77]. This protein catches a phosphorylated form of cdc25c to take it away from its cycle, preventing activation of the cyclin B-cdc2 complex. B99, a product of a p53-responsive gene, is localized predominantly on microtubules in the G2 phase [78] and its expression in p53-negative cells induces a halt at this phase. Thus, the influence of p53 on the G2 phase is mediated by the activities of several genes applying various mechanisms.

Genes participating in p53-dependent apoptosis. The number of potential mediators of the p53-dependent apoptosis is great and is increasing in contrast to the small number of p53-regulated genes involved in control of the cell cycle. This variety could be possibly explained by implementation of alternative programs of p53-dependent apoptosis in cells of different tissues and in cells under different physiological conditions.

The *BAX* gene contains a p53-responsive element, and it can be activated by p53 [79]. The *BAX* gene encodes a proapoptotic protein of the Bcl2 group [80] that promotes the release of cytochrome *c* from mitochondria [81]. The cytochrome *c* in cooperation with APAF1 activates caspase 9, initiating the apoptotic cascade [82]. The expression of Bax protein has been found necessary at least in fibroblasts for apoptosis caused by DNA damage [83].

A Fas-receptor gene (*APO1*) is also activated by p53 [84]. This makes cells sensitive to the Fas-ligand

causing trimerization of Fas/APO1, after which its cytoplasmic "death domain" associates with a FADD adapter molecule. Then activated procaspase 8 is involved into the complex and induces the apoptotic cascade. Interestingly, p53 can initiate apoptosis with participation of the Fas/APO1-receptor by way of a transcription-independent mechanism making a rapid translocation of the APO1 from the Golgi apparatus to the cell surface [85].

p53 also activates the gene of KILLER/DR5 protein from another group of receptor proteins carrying a "death domain" [86]. KILLER/DR5 is one of the receptors activated by a TRAIL-ligand [87]. The KILLER/DR5 initiates a caspase cascade as well as other receptor inducers of apoptosis.

A protein inhibitor of the insulin-like growth factor IGF-BP3 is also encoded by a p53-dependent gene. The IGF cytokine is a strong anti-apoptosis factor [88]. IGF induces Mdm2, thus decreasing p53 activity [89]. IGF-BP3 eliminates the effects of this cytokine and, therefore, causes apoptosis. Interestingly, p53 simultaneously suppresses the expression of IGF [90], thus amplifying the proapoptotic effect. Thus, these four proteins operate in a complex regulatory loop.

PAG608 protein is related to a group of proteins containing a zinc-finger. This protein is mainly localized in nucleoli, and it produces morphologic changes characteristic of apoptosis when it is hyperexpressed. This protein is induced by a p53-dependent mechanism [91] and probably it is a carrier of p53-proapoptotic activity.

A rather large group of p53-induced genes was found by a broad screening of genes that are differentially expressed upon p53 activation. Genes of the *PIG* [92] group initiate the formation of oxygen radicals in a cell to damage mitochondria membranes and to start apoptosis. A p53-responsive element was found in one of these genes (*PIG3*); this element requires for its activation the intact proline-rich p53 domain, in contrast to the most of known p53-elements [34].

A protein p85 encoded by a p53-regulated gene is a regulatory subunit of the PI3 kinase [93]. The p85 is required for the p53-dependent apoptosis induced by hydrogen peroxide, though details of the participation of the protein remain unclear.

The G-cyclin gene, whose function is still unclear, carries a p53-responsive element. Hyperexpression of this gene suppresses proliferation [94] and increases apoptosis induced by various other influences [95].

Genes encoding angiogenesis inhibitors and other secreted factors. Cells containing activated p53 secrete protein factors that repress angiogenesis. Conversely, this secretion ceases upon the loss of the p53 gene [53]. p53 activates the expression of the thrombospondin 1 (Tsp1), an anti-angiogenic factor [53], along with other angiogenesis inhibitors—BAI1 [96] and GD-AiF [97]. Furthermore, other secreted growth inhibitors— β -

inhibin, TGF- β 2, inhibitors of serine proteases, etc.—were found among the genes activated by p53. A consequence of the action of these factors appears to be the repression of the proliferation of neighboring cells [54]. Thus, the activity of p53 as a tumor suppressor may extend to surrounding cells.

Genes whose functions remain unknown. This group includes a constantly increasing number of the genes known to be induced by p53 expression. These are generally insufficiently studied previously unknown genes found by screening of differentially expressed genes.

The diversity of the enumerated p53 gene targets illustrates well the strategy of this gene—redundancy in the ways it influences a cell. Several restriction mechanisms are activated simultaneously when danger arises to guarantee the elimination the consequences of the fault. Furthermore, the redundancy of the mechanisms is explained by the variety of the cells of different types: some mechanism acts on one type of cells but does not do so on others. The blockage of some paths and promotion of others enable different types of cells to adequately react to changing conditions. One fact remains an enigma: why with the great diversity in the ways in which the p53 gene is expressed is there no other gene with similar function? Indeed, a single mutation in the p53-coding region can cause complete deactivation of the finely regulated system, and the cell loses control of the intactness of its genome. As with everything in nature, this fact can hardly be considered an accident. The vulnerability of the most important regulator of life and death being just p53 is possibly of wider biological meaning. For example, a certain frequency in the death of some individuals before they achieve infertile age may be maintained in this way. However, other hypotheses can be suggested, but their discussion is beyond the scope of this report.

LATENT AND ACTIVATED STATES OF p53

As stated above, the content and activity of p53 is low in a cell under normal conditions. Although p53 can be present in significant amounts in some cell types, the protein is in a latent inactive form. The latent p53 form is unable to activate transcription of the p53-responsive genes. This does not mean that the latent p53 is devoid of any activity. The latent form of the protein may play some role, for example, in DNA repair processes. In particular, the latent p53 form unable to associate with DNA was found to be able to recognize single-stranded DNA elements, unpaired bases, and to exhibit increased 3'-5'-exonuclease activity. In contrast, the activated form of p53 lacks exonuclease activity but acquires the ability to associate with DNA [98, 99]. The presence in some cell types of certain p53 levels in the absence of stress may indicate either that p53 performs an “every

day” function in such a system or that the latent form is accumulated in anticipation of sudden need to be rapidly transformed into an active form to restrict a damaged cell rapidly.

Gene p53 is activated under stressful conditions. Nevertheless, changes in p53 transcription occur rarely. However, the regulatory region of the p53 gene promoter carries elements binding some transcription factors that can induce transcription changes [100]. Nevertheless, these changes probably are not of urgent nature but proceed in the order of total tuning of the predisposition of the cell for rapid activation of p53 at a post-translation level. The hyperexpression of c-myc [101] and NF- κ B [102] produces a certain increase in p53 gene transcription. Substantial activation of the p53 gene at a transcription level was found only for premature embryos [103] and undifferentiated embryonic teratocarcinomas, where the p53 mRNA level has risen by an order of magnitude in comparison with that of the cells of an adult organism [104].

The fact that p53 accumulates in response to stresses even in the presence of RNA and protein synthesis inhibitors indicates its post-translational character [104, 105]. Thus, significant stabilization of p53 must occur [106]. However, changes at other levels may contribute to the entire effect of p53 induction, for example due to the intensification of mRNA translation [107].

Major qualitative changes of the protein globule occur during the transition from the latent to the functionally activated state in addition to quantitative accumulation of p53. These changes proceed by virtue of phosphorylation, dephosphorylation, acetylation, and glycosylation of various regions of the protein molecule together with the formation of covalent and noncovalent complexes with other proteins. The diversity of the modification sites and factors interacting with p53 provides fine regulation of its activity. The activation of p53 is reversible because mechanisms of reverse transformation return the p53 to the less active or latent state after the defects and faults have been eliminated.

Interaction of p53 with a specific DNA element is required for p53 to function as a transcription factor. A modification of the C-terminal part of the molecule initiated by various stress mechanisms is followed by a conformational rearrangement and acquisition of the ability of the protein to associate with DNA. A complex interaction of the p53 N-terminal transcription-activator region with components of the transcription apparatus such as proteins of the TFIID complex—TBP (TATA-Box Binding Protein) and TBP-associated factors TAFII—is essential [56, 108-111]. A small hydrophobic region of p53 around Leu-22 and Trp-23 is responsible for the simultaneous interaction with several factors of the transcription apparatus [30]. In addition, transcription coactivators, CREB-binding protein (CBP) and its closely related p300 protein, have to be

present in a complex with TFIID for activation of the specific promoters. p300/CBP displays histone-acetyltransferase activity. The acetylation of histones plays a significant role in chromatin structure modulation upon activation of transcription [112-114], facilitating accessibility of the chromatin to the transcription apparatus. p300/CBP is able to directly interact with the RNA-polymerase II complex conducting initiation of transcription in response to association with a transcription factor. The N-terminal transactivation domain of the p53 also interacts with the p300/CBP [115] simultaneously with its N- and C-terminal regions [116]. The p300/CBP can acetylate some transcription factors and increase their activity [117]. This complex acetylates Lys-382 of the C-terminal regulatory domain of p53 [118, 119]. The other transcription coactivator, PCAF, in a complex with p300/CBP, acetylates Lys-320 of the p53 region of a nuclear localization signal [119, 120]. The acetylation of each of these two sites generates a significant increase in the transactivation function of p53 [119]. The initiation of transcription from the p53-responsive promoter occurs.

The low level of p53 activity under non-stress conditions is maintained by a system of active ubiquitin-dependent degradation. The Mdm2 protein plays an essential role in this process. p53 activates a promoter of the *MDM2* gene, the product of which suppresses p53 activity [26, 27]. Mdm2 associates with the N-terminal region of p53 near the transcription activation domain. This association produces repression of transcription activity of p53 [121] and at the same time stimulation of proteosomal degradation. Activation and accumulation of p53 occur upon degradation of the Mdm2-p53 interaction introduced by mutations [122, 123]. The destruction of the Mdm2-p53 complex by monoclonal antibodies [124] or a competitor peptide [125] gives rise to activation of p53-dependent transcription and the resulting arrest of cell division; this fact shows the indispensability of constant repression of p53 for continuance of somatic cell proliferation.

The repression of the transcription function of p53 by Mdm2 proceeds via a complex interaction with components of the transcription apparatus. Mdm2 simultaneously binds with the N-terminal region of p53, TBP, TAFII250 [126, 127], and p300/CBP [116]. The N- and C-terminal regions of p300/CBP together interact with the p53 transcription-activator domain. Mdm2 couples with the N-terminal regions of p300/CBP and p53 and hinders formation of the complex of these proteins causing repression of p53-dependent transcription [116]. Interestingly, a similar mechanism of p53-dependent transcription repression is used by viral proteins that suppress the function of p53. The product of the adenoviral oncogene *E1A* can associate with p300/CBP, displace it from the complex with PCAF, and repress the ability of both proteins to acetylate p53 [128]. The prod-

uct of oncogene *E6* of a human papilloma virus represses p53-dependent transcription activation by virtue of the interaction with p300/CBP together with its ability to promote degradation of p53 [129].

The promotion of p53 degradation upon binding with Mdm2 proceeds via two mechanisms. Both p53 [130] and Mdm2 carry regions responsible for export from the nucleus [131, 132]. Due to these two signals, Mdm2 exports p53 from the nucleus [133, 134] and directs it into proteasomes, where both proteins are subjected to ubiquitin-dependent degradation [135]. Moreover, Mdm2 itself displays ubiquitin ligase activity and participates in the ubiquitinylation of p53 [136]. The Mdm2-p300/CBP complex plays some role in the degradation of p53 [137].

An alternative system utilizing non-activated JNK (Jun N-terminal kinase) operates in cells not subjected to stresses in addition to the Mdm2-dependent degradation. JNK binds with p53 in the region of amino acids 97-117 and facilitates ubiquitinylation and destabilization of p53 by an unknown mechanism [138, 139].

p53 ACTIVATION AS A RESPONSE TO STRESSES

Faults of the described above mechanisms of the p53 repression occur as a response to numerous stress situations. p53 stabilization is achieved by a covalent modification of the N-terminal region of p53 or by the association of p53 and Mdm2 with interaction regulators [11]. The Mdm2 binding site is phosphorylated as a response to DNA damage. Another mechanism involves binding of p53 and Mdm2 with the ARF protein (mouse p19-ARF and human p14-ARF), a product of an alternative frame of the *p16-INK4a* locus. Although these two mechanisms are carried out through different signal pathways and activated by different stimuli, they give the same result. Stress influences induce modifications of the C-terminal region of the p53 molecule followed by general amplification of functional activity.

The N-terminal domain of p53 contains seven potential phosphorylation sites, some of them being actually subjected to phosphorylation *in vivo*. Several kinases were found to be able to modify these sites *in vitro*. However, only indirect data are available for some of these sites indicating their possible participation. DNA damage is followed by rapid phosphorylation of Ser-15 [140, 141], which is a phosphorylation substrate of the "DNA activated protein kinase" DNA-PK [142]. DNA-PK binds with DNA gap regions and its kinase activity increases [143, 144]. Replacement of the Ser-15 by Ala produces a loss of suppressor properties of p53 [145], which indicates possible activator function of phosphorylation of this amino acid. p53 is unable to bind with DNA and to be activated after the influence of ionization radiation in mouse cells totally deficient in

DNA-PK [146, 147]. Thus, DNA-PK is necessary for p53 activation after the occurrence of DNA damage. The DNA-PK isolated from non-irradiated cells does not activate p53 *in vitro*. The presence of an additional factor in the irradiated cell extracts is required for the activation to take place [146]. Thus, DNA-PK only is insufficient for the p53 activation in irradiated cells. DNA-PK phosphorylates a p53-binding site of Mdm2 together with phosphorylation of Ser-15 of p53 and, therefore, the produced Mdm2 as a response to the higher p53 level is unable to suppress the p53 activity. A non-phosphorylated form of Mdm2 is able to inactivate p53 forms only after reparation and decrease of the DNA-PK level [148].

Ser-15 can be phosphorylated by another kinase—the ATM protein [149, 150]. ATM belongs to the same protein class as that of DNA-PK. The *ATM* gene is damaged in ataxia-telangiectasia (AT), a rare autosomal recessive disease characterized by a hypersensitivity to ionizing radiation, immune deficiency, elevated risk of tumor diseases, cerebellar ataxia caused by neuronal degradation, etc. [151]. Cultivated cells of AT patients display p53 activation defects in response to the influence of ionizing radiation [152-154] and faults of the cell cycle checkpoints [155-157]. The phosphorylation rate of the p53 Ser-15 in AT cells is significantly decreased [140]. Introduction of recombinant ATM into AT cells recovers the ability of these cells to phosphorylate Ser-15 upon ionizing radiation exposition [158]. Thus, ATM also conducts a signal from DNA damage to p53 induction.

Another protein of the wide *ATM*-like group of genes, ATR/FRP-1, participates in the cell cycle checkpoints as well as ATM [159, 160]; this protein is also able to phosphorylate the Ser-15 of p53 [161], but its activity is an order of magnitude less than that of ATM [150].

Obviously, the ATM protein is not the only kinase phosphorylating the Ser-15 as a response to DNA defects. The type of kinase phosphorylating this site may depend on the nature of the DNA-damaging influence. For example, the normal activation subsequent to ultraviolet radiation is retained in the ATM gene deficient cells upon the failure of p53 activation as a response to ionizing radiation [154]; this normal activation is followed by phosphorylation of Ser-15 of p53 [140]. In this case, Ser-15 is probably phosphorylated by DNA-PK or another species from the PI3K-like proteins group (ATR, etc.).

Since Ser-15 is a target of several kinases on DNA damages, the phosphorylation of this amino acid is probably essential for p53 activity to be increased in response to DNA damage. Indeed, such modification significantly diminishes the ability of Mdm2 to form a complex with p53 and, therefore, to suppress the p53-induced transcription activation of the responsive genes [141]. The other phosphorylation sites in the N-terminal region of

p53 may also participate in the regulation of the Mdm2 association process as a response to the DNA damage [119, 162], but their role remains to be determined.

DNA-damaging factors also induce modifications at other sites in the p53 molecule. Ser-389 of mouse p53 (corresponding to the Ser-392 of the human p53) is phosphorylated in response to ultraviolet irradiation [163, 164]. This process is apparently carried out by casein-kinase II (CKII) phosphorylating this region *in vitro* [165]. Other DNA-damaging influences do not produce phosphorylation of this site, so the actual regulation mechanisms of CKII remain unclear. The C-terminal region of p53 carries several additional kinase sites whose phosphorylation activates the DNA-binding function of p53, but the stress responsive role of these sites remains uncertain. For instance, phosphorylation of Ser-315 by cyclin-dependent kinases may produce not only the activation of p53 but also alteration of the interaction specificity with various subtypes of p53-binding sequences. This route may implement the modulation of p53-dependent effects at different stages in the cell cycle [166].

Interestingly, the removal of all potential phosphorylation sites does not cause a loss of ability of p53 to be stabilized and activated in response to DNA damage [167, 168]. Hence, phosphorylation is not the only mechanism of p53 activation. Indeed, by means of ATM activation, Ser-376 is dephosphorylated and its region is covalently bound with a protein of the 14-3-3 group to substantially amplify the transcription activity of the p53 subsequent to ionizing radiation [169]. Protein kinase C phosphorylates Ser-376. It was demonstrated earlier that the phosphorylation of this site gives the latent p53 form the ability to bind with DNA on one hand and to repress the transcription activity on the other hand [170-172]. Therefore, PKC perhaps acts on p53 for temporary termination of its activity. ATM-induced dephosphorylation and association with the 14-3-3 protein recovers the activity of p53.

Acetylation of the p53 C-terminal domain plays an important role in the activation of this protein [120]. Phosphorylation of Ser-15 and its induced Mdm2 dissociation causes significant activation of Lys-382 acetylation carried out by p300/CBP [173]. The Lys-320 acetylation by PCAF is also increased [120]. One of the PCAF associated proteins, PAF400 (400 kD, PCAF-associated factor), is considerably homologous to the ATM-like protein superfamily (FRAP, ATM, ATR, and DNA-PK catalytic subunit). Although PAF400 does not exhibit kinase activity in contrast to the mentioned proteins, it may as a response to DNA defects participate to a certain extent in the stimulation of acetylation of p53 [174]. Thus, the mechanism of DNA-damage-induced transcription activation of p53 and of genes regulated by it can be postulated; this activation involves a signal pathway connecting the activities of the N-terminal kinases

(DNA-PK, ATM) and acetylases performing a modification of the transcription apparatus.

Numerous points remain indistinct in the mechanism of p53 activation in response to DNA damage. In particular, the participation of poly(ADP-ribose)-polymerase (PARP) in the recognition of DNA damage and repair is still unclear. Loss of PARP is followed by repression of p53 induction in some cell models [175-177], while in other models this effect is less distinct. Normal function activation of p53 was observed in PARP^{-/-} fibroblasts with the absence of notable protein stabilization [178].

Another mechanism of ATM- and p53-dependent restriction of proliferation in response to DNA damage was discovered recently; this mechanism involves a product of the *c-Abl* oncogene. Induction of *c-Abl* tyrosine-kinase activity in irradiated cells was found to require *ATM* gene function [179] to immediately phosphorylate the *c-abl* protein [180]. The activated *c-Abl* associates with p53, amplifies its transcription activity [181], and neutralizes Mdm2 inhibition [182]. The association of *c-Abl* and p53 causes significant *cdk2* synthesis repression, which is enough to stop the division of p21^{-/-} mouse fibroblasts [183]. The mechanism of this repression of *cdk2* remains uncertain.

An alternative mechanism of stabilization and activation of p53 functions is carried out by replacement of p53 from its complex with Mdm2 by means of the ARF protein. The locus *INK4a-ARF* is known to encode two alternative transcripts determining the synthesis of the inhibitor of the cyclin D-CDK complex (p16-INK4a) and that of the protein also displaying antiproliferative activity, p19-ARF (p14-ARF in human cells) [184]. ARF being overexpressed is able to increase the level of p53 and to induce p53-characteristic effects [185]. Mice in which the *ARF* gene is selectively inactivated are characterized by increased sensitivity to tumor diseases [186]; embryonic fibroblasts taken from such mice appear to be immortal. However, the ability of ARF^{-/-} cells to stop their cell cycle in response to DNA damage remains intact. The DNA damage induced p53 activation of target genes also remains intact [184, 187]. Thus, ARF acts above p53 but exploiting a separate signal pathway. This protein can associate *in vivo* with Mdm2 and form a ternary complex comprising ARF, Mdm2, and p53 [69, 185, 188, 189]. The formation of such complexes is possible because the Mdm2 C-terminal segment binds with the ARF N-terminus [185, 188] and the Mdm2 N-terminal segment forms a complex with p53 [126]. Therefore, ARF prevents export of the Mdm2-p53 complex from the nucleus to the cytoplasm [190, 191] and suppresses the Mdm2 ubiquitin-ligase activity in relation to p53 [189, 192]. The issue of possible enhancement of the ARF-induced Mdm2 degradation remains doubtful [13, 188] and contradicts observations indicating the amplification of p53 level following ARF-induced increase in

p53 transcription activity [185, 193, 194]. ARF can bind with p53 without Mdm2 [185]; this fact indicates the ability of this protein to separate the p53-Mdm2 complex by having an effect on both components. Thus, the ability of ARF to increase the p53 level is based on the mechanistic replacement of Mdm2 and elimination of obstacles to p53 function caused by this protein. p53 in turn suppresses expression of ARF [69, 185], thus forming another regulatory loop.

ARF participates in the functional activation of p53 in response to viral and activated cellular oncogenes. Hyperproliferative signals cause ARF induction followed by p53-dependent cell division arrest or induction of p53-dependent apoptosis. For instance, p53 activation and cell cycle arrest are observed on the occurrence of *RAS* oncogene expression in primary fibroblasts [195]. The introduction of *RAS* transforms *ARF* gene deficient cells, which grow indefinitely [184], the accumulation of p53 being not observed [196]. Introduction of the exogenous *ARF* gene stops the proliferation [197].

ARF deficient cells are immortal [184]. The absence of ARF in this system is some kind of imitation of the "immortalizing oncogenes" *myc* and *E1A*. However, it was unknown for a long time why these immortalizing oncogenes appeared to be powerful apoptosis inducers upon their introduction into cells. With the discovery of ARF it became clear that both oncogene *E1A* [193] and oncogene *myc* [194] induce ARF expression and result in p53 activation and strengthening of apoptosis. The cells lost either p53 or ARF function rapidly to survive under a selection pressure producing immortality.

Some details of the signal pathway from the *myc* oncogene to ARF remain unclear [194]. *RAS* may activate ARF via the pathway: Ras-Raf-MAPK-E2F [196]. The signal pathway leading to ARF activation induced by *E1A* is depicted as follows. The *ARF* gene promoter is activated by E2F transcription factor [198]. E2F in turn is controlled by the Rb protein. Hence, *E1A*, as well as other oncogenes inactivating Rb, increases the E2F level and ARF induction.

ARF-dependent mechanisms apparently play a significant role in the restriction of cells with defects of expression of proliferation regulating genes. *ARF* may act as tumor gene suppressor. Many types of human tumors are known to have point mutations and deletions in the *INK4a-ARF* locus [199]. However, this locus encodes at least two different genes; it is necessary to determine which observed mutations relate to the ARF gene. Nevertheless, a new oncogene has already been discovered in the ARF-p53 signal pathway, and its expression may be related to human carcinogenesis. This is the *TWIST* gene, whose product represses the *ARF* gene promoter and suppresses p53-dependent effects [200]. Expression of the *TWIST* gene is amplified in half of rhabdomyosarcoma cases, indicating its possible participation in this pathology.

In spite of substantial progress in the comprehension of mechanisms of p53 induction generated by DNA damage, very little is known about the pathways of p53 activation in response to other stresses. p53 induction may be produced by the following influences: hypoxia [201, 202], hyperthermia [203], defects of tubulin [204] and actin cytoskeleton, faulty chromosome segregation creating micronuclei [205], detachment of cells from a substrate [206], cessation of DNA and RNA synthesis, nucleotide exhaustion [10], and other causes. It is now clear that some of these influences may induce p53 by substantially different mechanisms. For instance, p53 activation and stabilization in hypoxia may be carried out by means of physical binding of p53 with an α -subunit of the HIF-1, the transcription factor inducing a series of adaptive genes specific to the hypoxic state [207].

Further understanding of signal pathways leading to p53 activation is the subject of intensive study. The results of these studies will certainly lead to the discovery of new and not less interesting mechanisms providing cells of an organism with a balance between life and death.

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