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Tickets for p53 journey among organelles

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1. ABSTRACT

A broad range of stressors – intrinsic and extrinsic to the cell – stabilize and activate p53, affecting it by a series of post-translational modifications such as phosphorylation, acetylation, ubiquitination, methylation and sumoylation. p53 is able to integrate each kind of post-translational modification and to adequately respond by inducing cell cycle arrest, senescence or apoptosis. p53 controls the cell fate at the level of different compartments, and its trafficking among organelles is modulated by different types of post-translational modifications. Thus, miss-location or sequestration of p53 within a compartment might obstruct its function as tumor suppressor leading to cell immortalization and tumorigenesis. The aim of this contribution is to give a unified overview of several reports in the literature, concerning the post-translational modifications endured by p53 which regulate its cellular trafficking and distribution at different organelles.

2. INTRODUCTION

The p53 protein, first described about 30 years ago, was characterized as tumor suppressor at the beginning of 1990 decade (1), and is nowadays subject of almost 47 000 articles[1]. The p53 tumor suppressor protein plays a central role in the regulation of apoptosis, cell cycle and senescence as a response to a broad range of stresses such as DNA damage, oncogene activation and hypoxia; *p53 gene* or its product was found to be inactivated in more than 50% of all human cancers. The crucial tumor-suppressor activity of p53 involves both transcription-dependent and -independent mechanisms (2). Thus, p53 activates the transcription of genes that encode apoptotic effectors, such as Puma, Noxa, Bax, Bid, p53AIP1 proteins (3, 4), and it represses the transcription of anti-apoptotic genes such as *bcl-2* and *survivin* (5, 6). Beside these well known activities, p53 has lately been described as regulating a wide spectrum of processes such as the

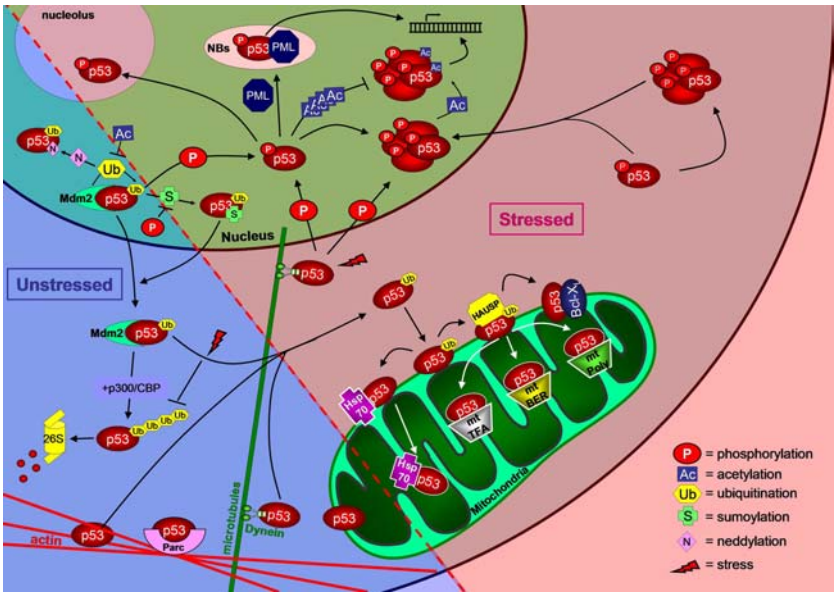

Figure 1. Modifications required for p53 trafficking among organelles. Several post-translational modifications which regulate p53 subcellular location are depicted here. For details see text.

metabolism (glycolysis, ROS damage), autophagy, cell invasion and motility, angiogenesis, bone remodeling, etc (7). For more than a decade now, many papers emerged describing the transcriptional-independent pro-apoptotic activities of p53, and its capacity to regulate the function of proteins involved in apoptosis commitment (8-10). For example, p53 modulate mitochondria outer-membrane permeabilization by inducing the Bax translocation at mitochondria (11). Moreover, recently numerous publications report that p53 itself relocates and induces apoptosis directly at mitochondria, *via* the interaction with members of Bcl2 family proteins (12, 13).

Overall, the regulation of p53 function within a cell involves different mechanisms, such as the control of stabilization and activity mainly by post-translational modifications; however, the sub-cellular localization is another way to regulate p53's activity. The aim of this contribution is to review the panoply of p53 locations within the cell, in both stress-induced and unstressed conditions, paralleled by the description of the pattern of post-translational changes that occurs in p53 and regulate its cellular distribution (Figure 1).

3. STRESSORS WHICH ACTIVATE p53

The outstanding tumor suppressor activity of p53 is distinguished by its capacity to contribute to genome stability and repair, primarily at the level of G1/S and G2/M cell-division checkpoints (14). p53 is present in normal or unstressed cells at a low basal level having a short half-life, and its turnover is mediated by the interaction with Mdm2 protein (15-17). A variety of different stresses – intrinsic and extrinsic to the cell – stabilize and activate p53, affecting p53 at post-translational level, mainly to facilitate the dissociation of p53-Mdm2 complex, rather than transcriptional control (18). The most often described signals that activate p53 are DNA-damaging agents, such as ultraviolet light (19) or ionizing radiations which were among the first stresses described as increasers of p53 level (20). Base alkylation, DNA depurination, oxidative free radicals also alter DNA in different ways and activate p53. Another category of stresses which are non genotoxic are: hypoxia (such as heart and brain ischemias) (21), nutrient deprivation, heat- and cold-shock conditions which result in denatured proteins and RNA aggregation, nitric oxide, oncogenic stimuli such as c-myc or Ras, tumor suppressor gene inactivation including retinoblastoma (Rb), telomeric repeats, spindle damage, cytokine stimulation, cell to cell contact, metabolic drugs (22); all of them activate p53 leading to different cell fates. For all these damaging agents, the cell has developed different detection mechanisms and the p53 protein is able to integrate these signals and to give an adequate response: cell cycle arrest, senescence or apoptosis. This explains why the loss or mutation of p53 gene or its product is connected to a wide variety of cancers.

4. P53 LOCALIZATION IN STRESSED AND UNSTRESSED CELLS

4.1. Nuclear Localization

Since the main function of p53 is the regulation of transcription, its nuclear localization is crucial to determine p53's response. The first reported observations of p53 localization depended on the cell status; p53 displayed cytoplasmic localization in normal/untransformed cells, but was found to be nuclear in transformed or immortalized cells (23, 24). Later, the nucleo-cytoplasmic trafficking of p53 has been shown to be associated with cell progression through the division cycle, in which p53 presents a cytoplasmic accumulation in G1 phase, enters the nucleus during G1/S transition and goes

back to the cytoplasm afterwards (25, 26). p53 cellular trafficking is finely regulated; defects in p53 nuclear translocation or export would impair its biological function leading either to immortalization or to massive cell death (27). After stress induction, p53 is rapidly accumulated into the nucleus either by its importing or by its retention *via* the impediment of Mdm2-mediated cytosolic trafficking (Mdm2-mediated turnover). Thus, p53 activity may be regulated *via* nuclear import versus export signals. The C-terminal regulatory domain of p53 enclose both nuclear localization signal (NLS) and nuclear export signal (NES) sequences (28, 29). The nuclear localization signal of p53 human protein, which is a bipartite sequence composed mostly by clusters of basic amino acids, is initiated by the binding of specific complex of proteins, such as importin α/β (30, 31). The translocation of p53 into the nucleus is terminated by the dissociation of NLS/importin α/β complexe. The nuclear export signal of p53 is composed of a highly conserved leucine-rich sequence, and p53's export requires exportin 1 binding (or CRM1). The p53 human protein contains two such NES sequences, one in the C-terminal oligomerization domain (32), and the other one in N-terminal Mdm2-binding region (33). As one NES signal is situated in the oligomerization domain of p53, it was proposed that the tetramerization of p53 inhibits its nuclear export by masking the NES sequence and blocking the exportin 1 binding, but NES is functional when p53 is either a monomer or a dimer (34). Recently, it has been stated that ARC, an anti-apoptotic protein abundant in some breast cancers, is able to bind wild type p53 within the nuclei (35). ARC binding inhibits p53 tetramerization domain, disabling its function as a transcription factor, and exposes the p53's NES to CRM1 binding which triggers its nuclear export (35). However, the tetramerization domain of p53 is also necessary for its nuclear import regulation (29). Nevertheless, it is not clear if p53 is oligomerized before or after entering the nucleus, because it is very difficult to determine the oligomeric state in unstressed cells due to the low level of p53 (34).

Once entered the nucleus, p53 may reside in specific sub-nuclear structures, such as nucleoli (36) or else in nuclear bodies (NBs) (37, 38); both being important for its transcriptional activation property. This specific sub-nuclear distribution depends on the interaction of p53 with the PML protein which also enhances p53's transcriptional activity to induce apoptosis or senescence (39-43). Indeed, the PML bodies serve as "assembly platforms" for different proteins to favor protein-protein interaction, especially in stress conditions (44). In this way, the recruitment of p53 at NBs facilitates post-translational modification of p53 by the co-recruitment of kinases, acetyltransferases or other enzymes (40, 45, 46). The nucleolus also plays a role in the regulation of p53's activity. In stress conditions, p53 sequestration within the nucleolus seems to participate at its re-activation by preventing p53's ubiquitination and subsequent proteasomal degradation (47-49). Within nucleoli, p53 can interact and undergo complex formation with proteins such as nucleolin, at sites of RNA synthesis, were p53 can efficiently monitor the genome for DNA damage (36). Moreover, Daniely *et al.* suggest that following stress, p53/nucleolin complex formation participates in nucleolin translocation from the nucleolus to the nucleoplasm, to transiently inhibit DNA repair and replication (50). Under stress conditions, at nucleolus p53 can also form complexes by the interaction with nucleophosmin (51), a phosphoprotein which shuttles between the nucleus and the cytoplasm, with PARP-1 (52) and topoisomerase I (53); being important to increase the stability and transactivation property of p53.

4.2. Mitochondrial Localization

Recently, the non-transcriptional apoptotic functions of p53 gain an increasing interest (54). Moll and co-workers have reported that during p53-dependent apoptosis a low fraction of p53 proteins rapidly translocates to mitochondria, in synergy to the nuclear translocation (12, 55-57). p53's translocation to mitochondria is sufficient to induce Bax, Bak and VDAC oligomerization causing mitochondria outer-membrane permeabilization and cytochrome c release, providing evidence for a direct pathway from p53 to the activation of caspase cascade (58). Collaterally, three groups proposed that p53 has a direct apoptotic effect at mitochondria *via* a physical interaction with members of the Bcl-2 family proteins; however there are some controversies between authors concerning the proteins involved in the interaction and concerning p53's binding-region. On the one hand, anti-apoptotic Bcl-2 and $BclX_L$ proteins (55, 59) were shown to interact with the central core of the DNA-binding domain and/or the proline-rich domain of p53 and, on the other hand, the pro-apoptotic Bax, Bak and Bad proteins (60-62) were described to bind the region of DNA-binding domain of p53, a region close to the proline-rich domain (63). Moreover, p53 stress-induced localization at mitochondria may also disrupt the integrity of the inner-membrane by forming a complex with cyclophilin D (58), a component of PTP (permeability transition pore) complex normally located at this site. Indeed, involvement of p53 in mitochondrial PTP has recently been proposed, by using a PTP inhibitor cyclosporine A (64), but further evidence is needed in order to confirm this result. Furthermore, in various cell types, a mitochondrial localization of p53 has been detected not only in apoptotic conditions but also associated to a growth arrest response to drug treatment; however the connection between the two events is not yet well established (65, 66).

The precise sub-mitochondrial localization of p53 is still subject of debate, the majority of literature data suggest that p53 is located to the outer membrane where it interacts with proteins of the Bcl-2 family, although some publications suggest that a part of p53 is found within the mitochondria matrix, for instance by forming a complex with chaperon proteins like Hsp70 (12, 67). Localization of p53 within the mitochondria matrix has been described by various authors, in which case p53 seems to be involved in the transcription and the replication of mitochondrial DNA (mtDNA) (68). In this way, Huang *et al.* suggests a novel role of p53 in the mitochondrial genetic stability through its direct interaction with mtDNA polymerase γ and consequently enhances the DNA replication function of polymerase γ (69). Moreover, p53 could bind directly the mtDNA (70), or bind to the mitochondrial base excision

repair machinery (mtBER) (71) to remove damaged base and stimulate the correct nucleotide incorporation, otherwise p53 is able to bind the mitochondrial transcription factor A (mtTFA) in order to regulate the mtDNA's transcription (72).

Furthermore, p53 can also be located at mitochondria in absence of stress. Knudsen and the co-workers reported this observation by studying the mtDNA genomic response in the presence of a dominant-negative p53 fused to a mitochondrial import signal and they proposed a direct positive influence of the mitochondria targeted p53 on mitochondrial biogenesis and function (73). We also confirm that wild type p53 can be localized at mitochondria in absence of any kind of stress, when cells are proliferating; and this localization has been observed in many cell types issued from different species, regardless of their status (tumor, immortalized or primary cells), all cells harboring wild type p53 (Ferecatu I and Vayssiere J.L, submitted paper). Moreover, when we studied the sub-mitochondrial localization of p53 we found the greater part in membranes compartments.

4.3. Other Locations

Mitochondrial localization of p53 was usually coupled with stress conditions and there are few literature data concerning the localization of p53 in the absence of stress. In living cells, p53 is maintained at a basal protein level *via* Mdm2, and the coactivator p300/CREB, induced poly-ubiquitylation and degradation by the proteasome (74, 75). Some studies have shown that Mdm2-mediated mono-ubiquitination exports p53 from the nucleus into the cytoplasm (76, 77). Once in the cytoplasm the major part of p53 is degradated by the proteasome, however low levels of p53 are still present in normal tissues. In mild stress, p53 has a protector role as a "guardian of the genome". To assume this function p53 is necessary in the nucleus, at the onset of S phase, but is kept cytosolic in the rest of the time (25, 26). During neuronal differentiation p53 is translocated to the nucleus and is then relocated to the cytoplasm in differentiated cells (78). What about its localization in normal/living conditions? Although few, some data exist about the localization of p53 in such conditions, ranking from exclusively cytoplasmic to exclusively nuclear, and depending mostly of the cell status (normal or tumor/transformed cells). Few data concern a cytoskeleton associated location of wild type p53 either with the actine filaments (79), or with the microtubule network (80). The interaction with microtubules is accomplished by the link with a motor protein (the dynein), which in stress conditions participates to the transport of p53 toward the nucleus using the microtubule network as a "highway" (80, 81).

Variations within p53 cellular distribution were also mentioned in tumor cells expressing wild-type p53. In this case, one efficient way to control the activity of transcription factors is their shuttling out of nucleus (82). Thus, in certain human cancers such as breast cancers, colon cancers and neuroblastoma, wild-type p53 is sequestrated in the cytoplasm (83-85). Cytoplasmic sequestration of p53 in these cancers has been associated with their poor response to chemotherapy and radiation therapy (84, 85). In this case, proteins such as Parc have been shown to be the cytoplasmic anchor for wild type p53, and preventing its transport in the nucleus (86, 87). This is the case for neuroblastoma cells which express high level of Parc protein (88), therefore cytoplasmic accumulation of p53 may occur because of Parc and Mdm2-mediated high nuclear export activity and cytoplasmic retention (89). Moreover, evidences suggest that Parc is able to bind p53 *via* the tetramerization domain (90). Nevertheless, since Parc displays ubiquitin ligase activity, ubiquitination could also partially explain the stabilization and sequestration of p53 (86). As well, cytoskeletal proteins such as vimentin (an intermediate filament protein) contribute to cytoplasmic sequestration of a temperature-sensitive mutant p53, and this sequestration requires intact intermediate filament protein scaffold (91). Nevertheless, cytoplasmic sequestration of p53 can also be associated to a physiological occurrence in some cell types such as mammary gland during lactation (83) and embryonic stem cells (92) to permit transient proliferation.

Nowadays, lysosomes destabilization is considered to participate in apoptosis induction by different stressors, such as p53 overexpression, staurosporin and oxidative stress, *via* a lysosome-mitochondria pathway (93-95). Interestingly, recent evidence suggests that in the presence of a phytoactive ingredient of marijuana, p53 is phosphorylated and relocates to lysosome of cultured rat cortical neurons, were it stimulates the cytosolic release of cathepsin-D enzyme, which subsequently causes mitochondrial outer-membrane permeabilization leading to apoptosis commitment (96). This paper represents the first evidence of such a localization of p53. As for the mechanism of p53 induced lysosomal membrane permeabilization, the authors speculate a Bax-induced permeabilization, such as for mitochondria, since Bax is equally relocated to lysosomal membrane following staurosporine-induced apoptosis. However, further investigation is required in other to evaluate the significance of p53 phosphorylation when located at lysosome, and to generalize this localization to other types of stress inducers.

5. POST-TRANSLATIONAL MODIFICATIONS OF P53

p53 stabilization and activation is achieved by a series of post-translational modifications that include phosphorylation, acetylation, methylation, ubiquitination, sumoylation neddylation, glycosylation and rybosylation. p53's post-translational modifications are mediated by kinases, histone acetyl-transferases, methylases, ubiquitin ligases and sumo ligases, which respond to different stress stimuli. Each type of p53 modification at distinct amino-acid residues, reflects a different type of stress and determines different p53 response as cell fate (18, 97). Until today, post-translational modifications of p53, discovered following DNA-damage, occur on at least 30 sites mostly positioned in N-terminal transactivator and prolin-rich domains, and in C-terminal regulatory domain, whereas less site modifications in the central DNA-binding

Table 1. Modifications of p53 at specific residues

Domains of human p53	Residue	Modification	Role	Enzymes
TAD and PxxP domains	Ser 6	P	Act	CK1
	Ser 9	P	Act	CK1, DNA-PK
	Ser 15	P	Act	AMPK, ATM, ATR, Cdk9, DNA-PK, ERK, mTOR, p38, RSK2
	Ser 18	P	Act	CK2, Chk2, DNA-PK, VRK1
	Ser 20	P	Act	Chk2, JNK, MAPKAPK2, PLK3
	Ser 33	P	Act	CAK, Cdk5, Cdk9, GSK3β, p38K
	Ser 37	P	Act	DNA-PK, ATR
	Ser 46	P	Act	Cdk5, HIPK2, p38K, PKCδ
	Thr p55	deP	Act	TAF1, ERK2
	Thr 81	P	Act	JNK
DBD domain	Ser 149	P / G	Act / Act	CSN-K /
	Ser 150	P	Act	CSN-K
	Ser 155	P	Act	CSN-K
	Ser 215	P	Act	AurK, STK15
CTR domain	Lys 305	Ac	Idc	p300CBP
	Ser 313	P	Act	Chk1, Chk2
	Ser 314	P	Act	Chk1, Chk2
	Ser 315	P	Act	AurK, Cdk2, Cdk9, GSK3β, STK15
	Lys 320	Ac / Ub / N	Idc / Inh / Inh	PCAF / E4F1 / N8(FBXO11)
	Lys 321	N	Inh	N8(FBXO11)
	Ser 366	P	Act	Chk2
	Lys 370	Ub / N / M	Inh / Inh / Inh	Mdm2 / N8(FBXO11) / Smyd2
	Lys 372	Ac / Ub / N / M	Idc / Inh / Inh / Act	p300CBP / Mdm2 / N8(FBOX11) / Set7, Set9
	Lys 373	Ac / Ub / N	Idc / Inh / Inh	p300CBP / Mdm2 /N8(FBXO11)
	Ser p376	deP	Act	PKC
	Ser 377	P	Act	Chk1, Chk2
	Ser p378	deP	Act	Chk1, Chk2, PKC
	Lys 381	Ac / Ub	Idc / Inh	p300CBP / Mdm2
	Lys 382	Ac / Ub	Idc / Inh	p300CBP / Mdm2
	Lys 386	Ub / S	Inh / Act	Mdm2 / SUMO-1
	Thr 387	P	Act	Chk1
	Ser 392	P	Act	Cdk9, CK2, FACT, p38K, PKR

Abbreviations: Act: activation; Inh: inhibition; Idc: indecisive; Ser: serine residue; Lys: lysine residue; Thr: threonine residue; P: phosphorylation; deP: de-phosphorylation; Ac: acetylation; Ub: ubiquitination; N: neddylation; S: sumoylation; M: methylation; G: O-glycolsylation; TAD: transactivation domain; PxxP: proline-rich domain; DBD: DNA-binding domain; CTR: C-terminal regulatory domain. A code of colours is used to distinguish each modification, which is also conserved in the Figure 1. Modified after (20, 22, 144, 145).

domain have been reported (98). While p53 phosphorylation occurs at serine and threonine residues in both N- and C-terminal of the molecule, other modifications such as acetylation, methylation, ubiquitination or sumoylation take place on lysines mostly at C-terminal regulatory domain of p53. For a complete list of human p53 amino acids residues subjected to modification see the website of Carl Anderson[2].

5.1. Phosphorylation

Phosphorylation of p53 has been the most commonly reported protein modification induced by DNA-damage. p53's amino-acid sites subjects for phosphorylation are described in Table 1 (99, 100). Subsequent studies revealed that post-translational modifications at Ser15, Ser18, Ser20 and Ser37 which are located in the Mdm2-binding site, prevent p53 from being targeted for degradation by dissociating the negative regulator Mdm2. However, some other sites, such as Thr p55, Ser p376, Ser p378 (pXX – meaning phosphorylated) are constitutively phosphorylated in absence of stress and undergo dephosphorylation after DNA-damage in cells exposed to ionizing radiation; thus dephosphorylating p53 may also contribute to its activation as stress response (101).

ATM plays a key role in phosphorylating both p53 (on Ser15, Ser20) and Mdm2 (on Ser395) in response to DNA double-stand breaks. Phosphorylated Mdm2 also destabilizes its interaction with p53, and thus appears to lose the capacity to nucleo-cytoplasmic shuttle p53 (102). There is less literature data related to the consequences of p53 dephosphorylation. Some groups argue that Ser p376 dephosphorylation increases the sequence specific DNA-binding activity of p53 (103) and other studies revealed an increased expression of $p21^{Waf/Cip1}$ and caspase-3 correlated with the induction of apoptosis (104).

5.2. Acetylation

Acetylation is also an important modification in p53 stabilization. Several lysines can be acetylated in C-terminal regulatory domain, by two different histone acetyltransferases, the coactivators p300/CBP (HAT) and PCAF (Table 1). Regarding the significance of p53 C-terminal acetylation, literature data are partially contradictory. Acetylation has mostly been described to enhance sequence-specific DNA-binding activity of p53

(105). By the use of EMSA assay, it has been shown that acetylation of p53 at C-terminal domain increased its ability to bind to short oligonucleotides *in vitro* (106, 107). But these data were recently refuted by the use of several more physiological models (105, 108, 109). In such studies, Barlev *et al.* showed that both wild-type or lysines mutant p53 (acetylation defective) are able to bind the endogenous p21 promoter (110). They also suggest that acetylation subsequently promotes the recruitment of coactivators to the promoters of p53 responsive genes. Thus, p53's acetylation do not seems to play a direct role in the transactivation function, but indirectly by facilitating the recruitment of coactivators p300/CBP and PCAF (105, 110). Thus, nowadays it is not clear whether p53 acetylation is required for its DNA-binding activity or whether this modification is necessary for its transcriptional activation function. Otherwise, a commonly accepted fact is that lysine acetylation protects p53 from proteasomal degradation since the same lysine residues may compete with Mdm2 promoted ubiquitination (111).

5.3. Ubiquitination

Other types of modifications occur in unstressed cells and are essential for p53 physiologic regulation by promoting its proteasomal degradation *via* the polyubiquitin pathway. In this way, p53 is maintained at a low basal level via the interaction with Mdm2, a RING domain ubiquitin E3 ligase, and the coactivator p300/CBP which catalyze the addition of polyubiquitin chains (112). Apart from the N-terminal region of p53, which requires interaction with Mdm2, the C-terminal domain holds a cluster of lysines, likely to be affected by Mdm2-induced ubiquitination. The generation of mutant p53 molecules containing single and multiple lysine-to-arginine substitutions, which are resistant to Mdm2-mediated degradation, suggests that p53 C-terminal lysine residues are the main sites for ubiquitin ligation (Table 1) (113). Nevertheless, after genotoxic insult, the proteolytical degradation is inhibited and p53 level rises dramatically.

Interestingly, all these modifications follow a specific order, termed "the phosphorylation-acetylation cascade" (106). For example, in response to ionizing radiation, ATM induces a rapid phosphorylation of p53 at Ser15 residue, which in turn acts as a precursor for further N-terminal serine phosphorylation (Ser 18, Ser20, etc.) Then, N-terminal phosphorylation enhance the recruitment of other factors including p300/CBP or PCAF acetyltrasferases that promote the acetylation of C-terminal Lys382 or Lys320, which in turn prevent ubiquitination and degradation of p53.

5.4. Other modifications

Sometimes, the same lysines targeted for ubiquitination, such as Lys386, can be modified by conjugation with SUMO-1 (114), a small ubiquitin-related protein which was found to covalently bind various proteins in a process called protein "sumoylation", a process which increases p53 stability and transcriptional activity (115-117). In unstressed conditions the sumoylation of p53 can be abrogated by phosphorylation (118). In response to DNA-damage, p53 is also neddylated (Table 1) – by the ubiquitin-like protein NEDD8 – but there are few evidence concerning the implication of neddylation in regulation of p53 function, or if this process occurs in normal conditions (119, 120). The neddylation is mediated by the ubiquitin ligase Mdm2, which is also neddylated in the process. Recently, Abida *et al.* show that p53 can be also neddylated by FBXO11 (a member of F-box protein family) on both Lys320 and Lys321, this modification inhibiting its transcriptional activity (121).

Methylation of p53 occurs within the nucleus and is mediated either by histone methyltransferase Set7/9, for methylation at Lys372 residue, or by methyltransferase Smyd2, at Lys370. p53's methylation at Lys372 increases p53 stability and targets gene activation, and is required for acetyltransferase binding to promote further acetylation of p53 at multiple sites (122, 123). In contrast, p53's methylation at Lys370 represses its transcriptional activity (124). Thus, lysine methylation is responsible for both activating and repressing p53. Moreover, a few papers suggest that p53 may equally be a target for glycosylation or ribosylation, affecting its stability and function. In these papers, the glycosylation seems to activate DNA-binding property of p53 (125) and p53's rybosylation may be involved in the inhibition of topoisomerase I activities (126, 127).

6. REGULATION OF P53 LOCATION BY POST-TRANSLATIONAL MODIFICATIONS

One of the key signals involved in cellular trafficking of a protein between organelles are post-translational modifications (Figure 1), although other proteins may also be involved in this process.

6.1. Post-Translational Modifications In Nuclear Import-Export Regulation

Phosphorylation events are the principal modification of p53, which permit the regulation of cellular localization and nuclear accumulation. In response to UV irradiation, the main sites of p53 which by phosphorylation determines p53's retention into the nucleus are at Ser15 and Ser20 (33). Evidence support that the phosphorylated p53 is unable to leave the nucleus, and thus may be retained in this compartment either because it inhibits the N-terminal NES sequence or because it inhibits Mdm2-binding (33). At C-terminus domain, it has been shown that phosphorylation of Ser392 enhances tetramer formation (128, 129), but phosphorylation of Ser315 has the opposite effect (130, 131), the two of them regulating p53 cellular location. Although it is evident that p53's phosphorylation retains p53 into the nucleus, nowadays is unclear if p53 is phosphorylated before or after entering the nucleus. Together with p53 phosphorylation, some proteins might be involved in p53 nuclear retention. Indeed, phosphorylated p53 (Ser392) undergoes complex

formation with PARP-1, an enzyme involved in DNA-repair and maintenance of genomic integrity, resulting in masking of the NES signal, thereby preventing its nuclear export (52, 132).

Nuclear export of p53 is essential for forward p53 degradation. p53's ubiquitination is crucial in its nucleo-cytoplasmic shuttling, and is mediated by the Mdm2 protein. Some reports proposed that p53 is ubiquitinated by Mdm2 within the nucleus, and then p53's NES becomes unmasked permitting p53 to exit through the nuclear pores into the cytoplasm where it mediates its degradation by the proteasome (34, 133-135). However, Mdm2 alone catalyzes only the addition of single ubiquitin moieties (monoubiquitination of p53 at one or multiple sites) and consequently p53 poly-ubiquitination requires the presence of a cytosolic cofactor, p300, which mediates the formation of a complex containing both p53 and the proteasome (75, 112). Moreover, Li *et al.* reported that p53's fate may depend on Mdm2 levels: low levels of Mdm2 induce monoubiquitination and nuclear export of p53 whereas high levels induce polyubiquitination and degradation by nuclear proteasomes (76). In this study Li *et al.* produced p53-Ub fused proteins that mimics monoubiquitinated p53 and observed that these mutants accumulated into the cytoplasm, indicating that monoubiquitination may be a possible trafficking signal. Recently, another research group suggested that initial ubiquitination can promote further ubiquitination, sumoylation and neddylation of p53 (136). In the same study, in order to see the consequences on the p53 localization, they constructed and used p53-SUMO fusioned proteins to reveal that sumoylation participate in p53 nuclear export by regulating the strength of the p53–MDM2 interaction (136). However, p53–NEDD8 fusion protein, as well as the wild-type unfused protein, was located in the nucleus suggesting that this process is involved neither to p53 import nor to its export.

Acetylation also affects p53's sub-cellular localization, although the existing data are puzzling. Acetylation may regulate the stability of p53 by inhibiting Mdm2-induced ubiquitination as they occur at the same sites (111). By using p53 proteins carrying point mutations at putative acetylation sites, which also impair ubiquitination and render p53 resistant to MDM2-mediated degradation, Namakura *et al.* show that lysine mutants are localized in both nucleus and the cytoplasm as compared to nuclear location of wild type p53. The cytoplasmic localization of p53 lysine mutants was due to insufficient ubiquitination but not to inefficient transport toward the cytoplasm (137). In contrast, one year later, Barlev *et al* using the same lysine mutants did not detect any cytoplasmic localization in their study (110). Recently, Kawaguchi *et al.* highlighted the role of lysine acetylation in controlling p53 oligomerization and subcellular trafficking. They showed that p53 hyperacetylation or the overexpression of p300/CBP acetyltransferase, prevents p53 oligomerization (by lysine "charge patch" neutralization) and determine the cytoplasmic accumulation of p53 by exposing the NES (138). The contradiction between the involvement of acetylation in p53 activation but also in its cytoplasmic shuttling may be explained by the number of lysine involved in these processes. Kawaguchi *et al* support the idea that when fewer than three lysines residues are acetylated p53 would be stabilized in the nucleus. In order to export p53 to the cytoplasm, p53 has to be acetylated on more than four lysines (138). However, no functional importance has yet been associated to cytoplasmic p53 in such hyper-acetylated status. In this context the natural question that arises is if acetylation or other kind of modification are required for the novel role of p53 at mitochondria, or if these modifications determine its mitochondrial targeting?

6.2. Post-Translational Modifications In Mitochondrial Targeting

Not long ago, the idea that p53 is exported to cytoplasm because it requires its degradation has been questioned. Some authors suggested that p53 export may represent a safety measure to ensure the normal cell functioning, because the continuous presence of p53 in the nucleus may represent an obstacle for natural cell cycle progression (139). Interestingly, a secondary role of p53 within the cytoplasm has been suggested recently, in which p53 may have a direct role at mitochondria.

Mitochondria or endoplasmic reticulum shuttling usually requires specific targeting sequences that are removed during the import. Subsequently, since the structure of p53 does not harbor a typical mitochondrial leader sequence, the mechanisms of p53 mitochondrial translocation still remain unclear. Due to their implication in p53 accumulation and activation, the main investigations have been turned toward phosphorylation and acetylation modifications of p53 as putative mitochondria translocation signals. Moll *et al.* investigated such modification patterns at both nuclear and mitochondria after genotoxic stress. Even though p53 translocates at mitochondria after stress, the nuclear and mitochondrial p53 proteins were similarly charged, and there was no difference between the phosphoserine patterns, and only a small difference in the acetylation pattern, indicating that neither the acetylation nor the phosphorylation seems to be involved in p53 targeting to mitochondria (140). The same year, data from another group were contradictory. Yoo *et al.* sustained that Ser15 phosphorylation is responsible for mitochondria targeting and contributes to interaction with Bcl2 and Bcl-xL (141).

Protein ubiquitination can occur in both nucleus and cytoplasm compartments, and may also be a protein trafficking signal. Although polyubiquitinated proteins are directly targeted to proteasome and degradated, protein multi-

monoubiquitination (the addition of single ubiquitin moieties at multiple sites) is a signal for intracellular trafficking among organelles. Protein recognition and degradation by the proteasome requires a minimum of four ubiquitin subunit within a chain per lysine (142), and thus multi-monoubiquitanated proteins are more stable. Recently, Moll and co-workers pointed up the monoubiquitylation of p53 as a possible mechanism for mitochondrial translocation (143). In this model, monoubiquitination switches the fate of p53 from degradation to mitochondria translocation and activation as stress response. Upon arrival at mitochondria, p53 undergoes deubiquitination by HAUSP, a process indispensable for complex formation with Bcl2 family members or with mtHsp70, because only non-ubiquitinated p53 forms such complexes. Moreover, they found that p53 protein which relocates at mitochondria do not arrive from nucleus but from a cytoplasmic pool. Last, they support the idea of two independent pools of p53 in unstressed cells, cytoplasmic and nuclear, which simultaneously respond to stressed conditions to induce apoptosis either at nuclear or at mitochondrial level.

7. CONCLUDING REMARKS

Altogether, p53 may be located at different cellular compartments (nucleus, mitochondria or cytoskeleton association), its localization depends on cell status (normal, immortalized or tumoral) and on cell condition (proliferation or stress). In stress conditions, nuclear localization of p53 is essential for the transcriptional regulation of its target genes, however recent investigations indicate that p53 can also induce apoptosis in the absence of its transcriptional activities by mitochondria relocation and direct interaction with members of Bcl2 family protein. Thus, p53 acts simultaneously at nuclei and mitochondria level to induce apoptosis as stress response. In absence of stress, there is a basal pool of p53 either associated to cytoskeleton proteins, which also serve as a railway for p53 migration toward nucleus, or at mitochondria having an active role in its biogenesis and function.

To display a specific location, p53 is shuttle among organelles and its traffiking can be modulated by different types of post-translational modifications. To summarize, in general phosphorylations are involved in nuclear import-export processes, acetylations and ubiquitinations in nuclear exports, and ubiquitinations in mitochondria shuttling. All these data indicate that the sub-cellular localization of p53 is subject to distinct regulatory mechanisms within the cell.

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Footnotes: [1] A search in PubMed database, with "p53" as query target, returns more than 46,000 results as of June 2008, [2] http://www.biology.bnl.gov/cellbio/human_p53.html

Abreviations: AMPK: adenosine monophosphate-activated protein kinase; ARC: apoptosis repressor with caspase recruitment; ATM: ataxia telangectasia mutated kinase; ATR: ataxia telangectasia and Rad3-related protein; AurK: Aurora kinase A; Bad: Bcl2 Antagonist of Cell Death; Bax: Bcl2-Associated X Protein; Bcl2: B-Cell CLL/Lymphoma 2; BER: base excision repair; CAK: CDK-activating kinase; Cdk2: Cdk5 and Cdk9, cyclin-dependent kinase 2, 5 and 9; Chk1 and Chk2: checkpoint kinase 1 and 2; CK1 and CK2: casein kinase 1 and 2; CREB: cAMP response element binding protein; CRM1: Chromosome Region Maintenance 1; CSN-K: cop-9 signalosome associated kinase complex; DNA-PK: dsDNA-activated protein kinase; EMSA: electrophoretic mobility shift assay; ERK: extracellular signal-regulated kinase; GSK3β: glycogen synthase kinase 3β; HAT: histone acetyl-trasfersase; HAUSP: herpes virus-associated ubiquitin-specific protease; HIPK2: homeodomain-interacting protein kinase 2; Hsp70: Heat-Shock Protein 70; JNK: c-JUN-NH2-terminal kinase; Lys: lysine residue; MAPKAPK2: mitogen-activated protein kinase-activated protein kinase 2; mtDNA: mitochondrial DNA; mtHSP: mitochondrial heat shock protein; mtTFA: mitochondrial transcription factor A; NBs: nuclear bodies; NES: nuclear export signal; NLS: nuclear localization signal; p38K: p38 MAP kinase; p300CBP: p300 CREB binding protein; PARP-1: poly(ADP-ribose) polymerase-1; PCAF: p300/CBP associated factor; PKC: protein kinase C; PKR: dsRNA-activated kinase; PLK3: pol-like kinase 3; PML: promyelocytic leukaemia protein; PTP: permeability transition pore complex; ROS: reactive oxygene species; RSK2: ribosomal S6 kinase 2; Ser: serine residue; Set7/9: Set7/9 methyltransferase; Smyd2: Set/Mynd domain-containing methyltransferase 2; SUMO-1: small ubiquitin-related modifier; TAF1: TATA-binding protein-associated factor 1; Thr: threonine residue; UV: ultraviolet light; VDAC: voltage-dependent anion channel; VRK1: vaccinia-related kinase 1

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