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Minireview

Of flies and men; p53, a tumour suppressor

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Abstract The completion of the *Drosophila* genome sequencing project [Science 287 (2000) 2185] has reconfirmed the fruit fly as a model organism to study human disease. Comparison studies have shown that two thirds of genes implicated in human cancers have counterparts in the fly [Curr. Opin. Genet. Dev. 11 (2001) 274; J. Cell Biol. 150 (2000) F23], including the tumour suppressor, p53. The suitability of the fruit fly to study the function of the tumour suppressor p53 is further exemplified by the lack of p53 family members within the fly genome, i.e., no homologues to p63 and p73 have been identified. Hence, there is no redundancy between family members greatly facilitating the analysis of p53 function. In addition, studying p53 in *Drosophila* provides an opportunity to learn about the evolution of tumour suppressors. Here, we will discuss what is known about *Drosophila* p53 in relation to human p53.

Keywords: p53; Apoptosis; Cell cycle; DNA repair; *Drosophila melanogaster*

1. Human p53

The human p53 transcription factor is a 393 amino acid protein consisting of a N-terminal transactivation domain (amino acids 1–83), a central sequence-specific DNA binding domain (amino acids 102–292), and a C-terminal oligomerization domain (amino acids 324–355). Hundreds of studies are dedicated to elucidating the functions of p53. This is because although p53 is not essential for normal growth and development, it plays a pivotal role in tumour suppression. Indeed, loss of normal p53 function occurs in almost all human cancers [4].

At present there is agreement on a model describing the general mode of p53 function: p53 is inactive in normal cells. It is rapidly activated by various types of stress signals which cause oncogenic alterations, such as DNA damage, hypoxia or nucleotide depletion. p53 activation results in the execution of transcriptional programmes which either result in cell cycle arrest, allowing time for damage repair, or cause the offending cell to be destroyed by apoptosis. However, the molecular mechanisms underlying p53 activation, cell cycle arrest and apoptosis have not been fully elucidated.

1.1. Function of p53

As mentioned above, p53 functions as an inhibitor of cell growth, by inducing cell cycle arrest or apoptotic cell death [5]. Hence, the activity of p53 must be tightly regulated in order to allow both normal growth and tumour suppression, and the transition between these states. p53 is regulated at both the translational and transcriptional level [6]. However, there are three mechanisms, which shall be discussed below, which are the major determinants of p53 activation; controlling the protein levels of p53, its cellular localization, and lastly its ability to function as a sequence-specific transcription factor. All of which are regulated by posttranslational modifications.

1.2. p53 protein stability

In normal unstressed cells, p53 is a short-lived protein, which is maintained at low, often undetectable levels in the cell. Tight regulation of the abundance of p53 is required for normal growth and development. In the absence of stress-inducing signals, p53 is kept at low levels via its interaction with mouse double minute 2 (MDM2), an ubiquitin ligase. Upon binding to the N-terminus of p53, MDM2 transfers ubiquitin moieties to several sites within the C-terminus of p53. The ubiquitinated p53 is subsequently exported from the nucleus and degraded by the proteasome [7]. The expression of MDM2 is under the control of p53, hence, this negative feedback loop provides a means of keeping the level of p53 low in unstressed cells, and reducing the level of p53 in stressed cells, once the damage has been repaired [8,9].

It should be noted that proteasome-independent p53 degradation pathways do exist in human cells as well: for example, calpains, a family of ubiquitous cysteine proteases that is conserved between flies and man, degrade p53. Furthermore, calpain inhibitors can induce apoptosis under certain circumstances [10–12].

In response to cellular stress signals, p53 is activated, and this increase in activity is accompanied by an increase in the stability of the protein. As the p53/MDM2 interaction is important for maintaining a low concentration of p53, it is also critical for increasing the levels of p53. Hence, several pathways which activate p53 impinge upon the p53/MDM2 interaction. Upon cellular stress, kinases belonging to the phosphatidylinositol-3-kinase family are activated. For example, in response to ionizing radiation (IR), ataxia-telangiectasia-mutated (ATM) is activated and phosphorylates various proteins [13]. Ataxia-telangiectasia-and-Rad3-related (ATR) is upregulated in response to incomplete DNA replication [14]. How the activity status of these kinases is altered in response

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to stress signals is not fully understood. Both direct mechanisms, wherein ATM and ATR interact with damaged DNA, and indirect mechanisms, whereby they interact with DNA repair or maintenance proteins have been proposed [15]. These kinases can activate p53 by both direct and indirect methods: ATM/ATR can phosphorylate p53 at Serine 15, which results in an increase in p53 stability. Alternatively, they initiate a phosphorylation cascade; ATM and ATR target the effector kinases checkpoint-homologues Chk2 and Chk1, respectively, which function during checkpoint control during the cell cycle (for a recent review of Chk1 and Chk2 see [16]). Chk1 and Chk2 subsequently phosphorylate p53, resulting in the release of MDM2 from p53. Hence, the interaction between MDM2 and p53 is disrupted, and MDM2 can no longer target p53 for ubiquitination.

In addition to targeting p53 for phosphorylation, the ATM kinase can also modify MDM2. Although this phosphorylation occurs outside of the p53 binding region, this modification abrogates its interaction with p53 [17]. Indeed other kinases, such as DNA-PK, also phosphorylate MDM2 and abolish its ability to interact with p53 [18]. In addition to phosphorylation there are other means which result in p53 stabilization. For example, in response to DNA damage c-Abl can bind to and stabilize p53. This association does not prevent MDM2 from binding p53, but prevents p53 from being ubiquitinated by MDM2 [19].

1.3. Localization of p53

In addition to stability, p53 is regulated by where it is in the cell. As p53 functions as a transcription factor, localization of p53 to the nucleus plays a key role in regulating its activity. There are various mechanisms which can import p53 into, or export it out of the nucleus. The means for transporting p53 into the nucleus are two fold; firstly, p53 can be transported into the nucleus by dynein and the microtubule network, which requires the N-terminus of p53 [20]. Secondly, within the C-terminus of p53 reside various nuclear localization signals which are recognized by nuclear import factors [6]. Upon entering the nucleus there are mechanisms to export p53 back into the cytoplasm. These take the form of two export sequences: one within the C-terminal oligomerization domain [21] and another in the N-terminal MDM2 binding region [22]. Whilst the export of p53 is not MDM2-dependent, MDM2 does contribute to this effect, via its ubiquitin ligase activity. In the tetramerized form the C-terminal nuclear export signal is not accessible to the export pathway, hence, it could be speculated that, upon ubiquitination, the C-terminal export signal is exposed, and p53 can move to the cytoplasm [23].

The N-terminal nuclear export signal is regulated by phosphorylation, insomuch as phosphorylation inhibits the nuclear export sequence. Hence, the effects of N-terminal p53 phosphorylation to retain p53 in the nucleus are two fold: firstly by inhibiting the N-terminal export signal, and secondly by inhibiting binding by MDM2 and subsequently reducing ubiquitination and activation of the C-terminal export signal (Fig. 1).

1.4. Regulation of p53 activity

p53 exerts its effects by both transcriptional and non-transcriptional mechanisms [6]. Best understood is p53's role as a classical transcription factor, upregulating the expression of genes which function to arrest the cell cycle, for example the

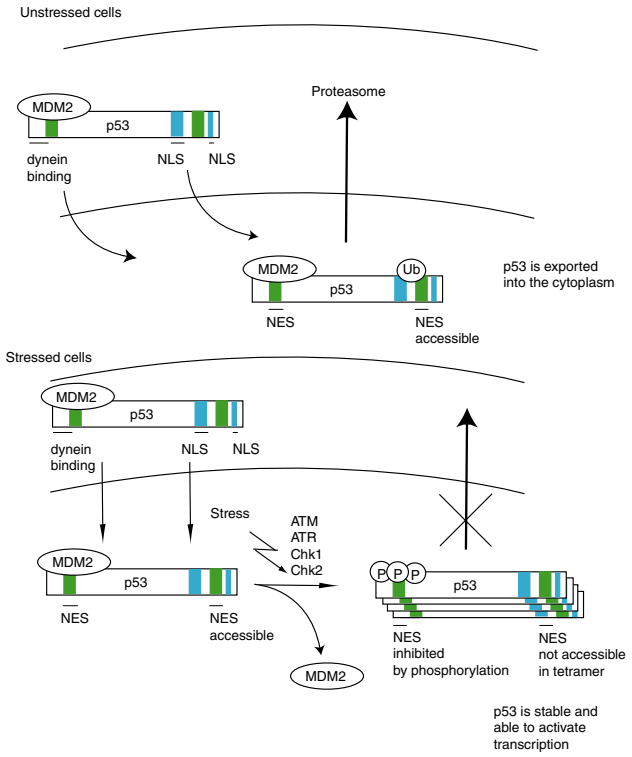

Fig. 1. Regulation of p53 stability and subcellular localization upon response to stress signals, p53 is phosphorylated, causing the release of MDM2, and inhibition of nuclear export signals resulting in stabilization and activation of p53.

cyclin-dependent kinase inhibitor p21 (CIP/KIP), or induce apoptosis, such as Bax1. How p53 distinguishes between invoking cell cycle arrest or apoptosis is at present ill-understood. However, it has been suggested that cofactors which associate with p53 help make this decision, such as JMY (junction-mediated and regulatory protein [24]), and apoptosis stimulating protein p53-1 (ASPP1) and ASPP2 [25]. Expression of ASPP2 stimulates recruitment of p53 to the pro-apoptotic Bax gene following UV treatment. By contrast, p53 binding to the cell cycle arrest gene p21 is not affected under these conditions.

The stimulation of p53's transcription factor function; binding DNA and interacting with the transcriptional machinery, is mediated, in part by posttranslational modifications (for a comprehensive review see [26]).

Within p53 there are two specific regions which are targeted for posttranslational modifications, one in the N-terminus which can undergo phosphorylation, and one in the C-terminus which can be acetylated, phosphorylated, glycosylated and sumoylated, each modification affects p53 activity [6].

As mentioned above, phosphorylation within the N-terminus of p53 not only stabilizes p53 by abolishing its interaction with MDM2, and regulates cellular localization by inhibiting nuclear export, it can also stimulate the transactivation activity of p53. Moreover, phosphorylation of p53, within its N-terminus, enhances the binding between p53 and histone acetyltransferases (HATs) such as CREB binding protein (CBP)/p300 and p300/CBP associated factor (P/CAF).

Whereas the N-terminus has only been shown to be phosphorylated, the C-terminus is targeted for a variety of posttranslational modifications. For example, a DNA

damage-induced complex containing casein kinase 2 and the chromatin transcriptional elongation factor is responsible for UV-induced phosphorylation of serine 392 in the C-terminus of p53 [27], leading to activation of DNA binding [28]. In addition, SUMO modifications of p53 at lysine 386 also activate p53 [29–31]. As mentioned above, stress-induced acetylation of p53 is undertaken by HATs [32–34]. These HATs acetylate distinct sites within the C-terminus: p300/CBP acetylates lysines 373 and 382, and to a lesser extent lysines 372 and 381, whilst P/CAF recognizes lysine 320 [35]. Acetylation of p53 increases the transcriptional activation of p53 by accentuating its sequence-specific DNA-binding activity [36–39].

Recently, new evidence has come to light which challenges this model. Several observations suggest that acetylation of p53 promotes coactivator recruitment, rather than DNA binding, leading to histone acetylation and transcriptional activation of target genes. Barlev and coworkers mutated the acetylation sites within p53, and performed chromatin immunoprecipitations to analyse the status of the p21 promoter in cells. They observed that mutated p53 was defective for transcriptional activation and G1 arrest, however, its DNA binding capacity was unaffected. Further analysis revealed that the level of histone acetylation in the presence of acetylation-site defective p53 was dramatically reduced compared to the level observed in the presence of wild-type p53. Hence, they concluded that p53 acetylation is required for coactivator/HAT recruitment [40].

Interestingly independent studies, undertaken by Espinosa and Emerson, show that p53 is an active DNA binding protein, which does not require C-terminal modifications in order to interact with DNA. They find that the C-terminal region does not exert an inhibitory effect, but rather is required for the binding of p53 to certain promoters. In addition, they show that p53 and p300 act synergistically to activate transcription, as p300 is recruited by chromatin-bound p53, resulting in localized nucleosomal acetylation with regional spreading. Hence, p300 mediates expression by p53-targeted nucleosomal acetylation rather than through p53 acetylation [41]. Further studies are required to ascertain which of these models is correct.

Although the exact effect acetylation has on the transactivation activity of p53 is still under debate, the effect of acetylation of particular lysine residues upon p53 coactivator recruitment has been investigated. It was demonstrated that the coactivator CBP binds specifically to acetylated lysine 382. This interaction is responsible for p53 recruitment of CBP in vivo upon DNA damage. This interaction appears to be crucial for p53-induced transcriptional activation of p21 in cell cycle arrest [42].

The importance of acetylation of p53 with regard to its function was further demonstrated by reciprocal experiments, investigating the role of histone deacetylases (HDACs) in p53 regulation. Such findings show that acetylated p53 interacts with the nucleosome-remodelling and deacetylation complex via its metastasis-associated-factor 2 [43]. Indeed, overexpression of MTA2 dampens both p53's cell cycle arrest and apoptotic activities, presumably as a result of p53 deacetylation. Moreover, MDM2 mediates deacetylation of p53, by recruiting a complex containing HDAC1 [44]. Furthermore, overexpression of SIRT1, a human homologue of silent information regulator (Sir2), deacetylates p53 and reduces p53-mediated transactivation [45].

2. Drosophila p53

2.1. Identification of Drosophila p53

The identification of *Drosophila* p53 (dmp53) was reported by three groups [46–48]. Of the three defined domains within p53, the N- and C-termini show little conservation, the greatest homology between p53 and dmp53 resides in the central DNA binding domain. Indeed, dmp53 can specifically bind to a DNA probe containing binding sites for human p53. Moreover, in transient transfection assays dmp53 can transactivate a reporter containing human p53 binding sites. This suggests that like human p53, dmp53 can function as a DNA binding transcriptional activator. Further studies utilizing yeast one- and two-hybrid assays, uncovered a transactivation domain in the N-terminal region of dmp53, and oligomerization activities in the C-terminal domain. Thus, human p53 and fly p53 share similar domains: an N-terminal transactivation domain, a central DNA binding domain and a C-terminal oligomerization domain.

2.2. In vivo function of dmp53: G1 cell cycle arrest

Within the DNA binding domain of human p53 there are mutational hotspots, which upon being mutated result in a dominant negative p53 protein. Introducing corresponding mutations into dmp53 creates a dominant negative protein that prevents wild type dmp53 from binding to DNA in vitro. In vivo analysis to elucidate the function of dmp53 was undertaken, wherein both the wild type and the dominant form of dmp53 were overexpressed [46–48]. Overexpression of wild type p53 does not induce G1 arrest. It fails to activate *dacapo*, the *Drosophila* homologue of the CIP/KIP-type inhibitors responsible for p53-mediated G1 cell cycle arrest in human cells. Moreover, expression of dominant negative dmp53 does not interfere with X-ray irradiation induced cell cycle arrest in third instar wing discs, suggesting that this block is dmp53-independent. So far, there is no evidence that dmp53 shares the ability of human p53 to induce a G1 cell cycle arrest in stressed cells.

2.3. In vivo function of dmp53: induction of apoptosis

Overexpression of wild type dmp53 in the *Drosophila* eye stimulates apoptosis. Moreover, expression of the dominant negative dmp53 suppresses X-ray induced apoptosis in the wing disc. The involvement of dmp53 in radiation induced apoptosis was further confirmed by analysing proapoptotic genes, such as the reaper gene. This gene has a radiation inducible control element containing a putative dmp53 response element [46]. Yeast-one hybrid assay shows that dmp53 can mediate transactivation from this response element, moreover, this response element confers radiation-responsive transcriptional activation upon a reporter gene in vivo. These studies gave rise to the following model with regard to irradiation-induced dmp53 activation: irradiation results in dmp53 activation, which can then bind and activate proapoptotic genes such as *reaper*, but not cell cycle arrest genes such as *dacapo*. Indeed, this model is confirmed by the observation that mutating dmp53 abolishes irradiation-induced apoptosis and *reaper* induction [49,50].

Jassim and coworkers examined UV-mediated cell death in the *Drosophila* retina. *Reaper* was not found to have a role during the retina's response to UV. Instead another proapoptotic gene, *head involution defective*, was activated. Moreover,

p53 was shown to protect cells from UV-induced apoptosis. This was ascribed to its ability to direct DNA damage repair. Indeed, *dmp53* mutants are more sensitive to UV radiation [51].

2.4. How is dmp53 activity regulated?

As mentioned above, one of the major determinants of p53 activity in human cells is its regulated degradation, which is initiated by MDM2 polyubiquitination. The sequencing of the *Drosophila* genome has not identified an obvious MDM2 homologue [1–3], moreover, the MDM2 binding site is not conserved in dmp53. Curiously, however, ectopic expression of human MDM2 does lead to apoptosis in the fly but it is not clear what the targets of MDM2 in this context are [52]. How then is dmp53 activated following cellular insults? Are post-translational modifications such as those which regulate p53 in human cells involved?

The *Drosophila* homologue of Chk2 is required for DNA-damage induced cell cycle arrest and apoptosis [53]. Recent studies show that, not only does the overexpression of dmp53 in the eye result in apoptosis, but that this effect is enhanced by the coexpression of *Drosophila* Chk2, but not a kinase-dead form of *Drosophila* Chk2 [54]. This effect is specific, as the overexpression of wild type and mutant Chk1 did not affect dmp53-induced phenotypes. No changes in dmp53 steady state levels were detected in these experiments, suggesting that Chk2-dependent phosphorylation of p53 might influence properties such as DNA binding activity or interaction with cofactors.

Recently, Brodsky and coworkers demonstrated that upon cellular stress, dmp53 displays a phosphatase-sensitive change in gel mobility. This was attributed to phosphorylation of p53 by the *Drosophila* Chk2 homologue MNK. Furthermore, no alteration in p53 protein levels was observed, suggesting that p53 activity can be regulated without a MDM2-like activity [55]. A genome-wide analysis demonstrated that all IR-induced increase in transcript levels requires both dmp53 and MNK/Chk2. As befitting the previous observations that upon stress dmp53 does not induce cell cycle arrest, *dacapo* is repressed following IR treatment. Interestingly, DNA repair genes were also induced, such as *Ku70* and *Ku80* which have defined functions in DNA break repair and participate in the non-homologous end-joining pathway [55]. These findings corroborate earlier observations by Jassim and colleagues, who hypothesized that upon UV treatment, dmp53 enhances DNA excision repair [51].

3. Conclusion

At a first glance it may appear that human and fly p53 do not share many structural or functional similarities, however, close inspection reveals parallels between the two. An initial comparison between the sequences of human and fly p53 demonstrated that the greatest homology is located within the central DNA binding domain (25% identity, 43% similarity). In addition, this DNA binding domain is able to mimic its human counterpart and bind human p53 response elements. The other two functional domains, namely the N-terminal transactivation domain and the C-terminal oligomerization domain, do not show so much sequence homology, they do, however, perform similar activities, namely, transactivation and oligomerization, respectively.

Moreover, like human p53, dmp53 can induce apoptosis upon overexpression and in response to cellular insults. However, unlike human p53, it is unable to invoke cell cycle arrest. Perhaps cell cycle arrest has not been observed because it is cell type specific and not all tissue types have been analysed. Despite the apparent differences in cell cycle regulation, both human and fly invoke DNA repair. As maintaining the integrity of the genome is more important for a long living organism, human cells are perhaps given more time to repair DNA damage. p53's cell cycle arrest function might have evolved more recently (Fig. 2).

After the isolation of a p53 homologue in *Drosophila*, comparison studies have been undertaken in other invertebrates. *Caenorhabditis elegans* p53 (Cep53) is a 429 amino acid protein, which displays similarity and conservation with the N-terminal transactivation and central DNA binding domain of human p53, respectively. Cep53 functions both during normal development, to ensure proper meiotic chromosome segregation, and in response to cellular stress, such as DNA damage, starvation or hypoxia. Cep53 is ubiquitously expressed in embryos, however, its abundance must be tightly regulated as elevated amounts are lethal [56]. As such Cep53 is functionally more similar to dmp53 than to human p53. Moreover, other p53 family members have not been identified in the genomes of either of these invertebrates, suggesting that the p63/p73 subfamily evolved after the separation of the arthropod and vertebrate lineages [48].

There is a plethora of information concerning the post-translational modifications which target human p53, how these are induced, and their subsequent effects. In contrast, very little is known about what modifications target dmp53 and how they regulate dmp53 function. Recently, it was shown that dmp53 is phosphorylated upon stress, and that modification is required for subsequent transactivation of genes involved in apoptosis and DNA repair [55]. The sites within dmp53, which are phosphorylated, are yet to be identified. Furthermore, the molecular consequences of phosphorylation are yet to be determined. However, it is not known if dmp53 is acetylated or sumoylated. Presently, studies are being undertaken in order to elucidate the means by which p53 decides what set of genes to

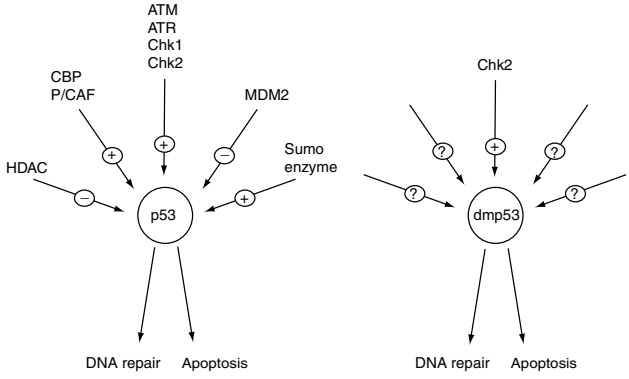

Fig. 2. Comparison of the posttranslational modifications regulating human and fly p53. There are numerous mechanisms which contribute to the regulation of human p53 activity. In comparison dmp53 is so far only known to be regulated by Chk2.

transactivate. These determinants have taken the form of co-factors which interact with p53 and target its transactivation potential to particular genes. It is possible that such cofactors exist in the *Drosophila* system. Finally, the regulation of the cellular localization of dmp53 is yet to be elucidated.

At present therapeutic attempts to kill cancer cells use radiation and chemotherapeutic agents which induce DNA damage. This damage is recognized within the cell and the apoptotic pathway is initiated, resulting in the offending cell being removed. However, in the majority of human cancers, a vital component of this pathway, namely p53, is mutated. In this form it is unable to act as a transcriptional activator and is rendered unable to induce apoptosis. If dmp53, as the initial observations suggest, indeed turns out to be a stable protein that can transactivate human p53 responsive genes, there may be therapeutic advantages to introducing dmp53 into human cells which have lost p53 function. Dmp53 not only provides an opportunity to extend out knowledge about the evolution of tumour suppressors, but may aid the quest for therapeutic alternatives.

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