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Review

Regulation and function of p53: A perspective from *Drosophila* studies

María Clara Ingaramo[a,1], Juan A. Sánchez[a,1], Andrés Dekanty[a,b,*]

[a] Instituto de Agrobiotecnología del Litoral, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional del Litoral (UNL), Santa Fe 3000, Argentina
[b] Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (UNL), Santa Fe 3000, Argentina

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ABSTRACT

Tp53 is a central regulator of cellular responses to stress and one of the most frequently mutated genes in human cancers. P53 is activated by a myriad of stress signals and drives specific cellular responses depending on stress nature, cell type and cellular context. Additionally to its classical functions in regulating cell cycle arrest, apoptosis and senescence, newly described non-canonical functions of p53 are increasingly coming under the spotlight as important functions not only for its role as a tumour suppressor but also for its non-cancer associated activities. *Drosophila melanogaster* is a valuable model to study multiple aspects of normal animal physiology, stress response and disease. In this review, we discuss the contribution of *Drosophila* studies to the current knowledge on p53 and highlight recent evidences pointing to p53 novel roles in promoting tissue homeostasis and metabolic adaptation.

1. Introduction

The p53 transcription factor is a central regulator of cellular responses to stress and a major tumour suppressor gene in humans with more than 50% of all human cancers showing alterations in p53 signalling. Consistent with its function as a tumour suppressor, germline mutation of Tp53 is associated with the hereditary Li-Fraumeni syndrome, characterized by early onset of tumourigenesis, and p53 knockout mice are highly prone to develop spontaneous and damage-induced tumours (Kastenhuber and Lowe, 2017). The p53 signalling pathway is activated in response to diverse stress signals ranging from DNA damage, hypoxia, oncogene activation and nutrient deprivation. Among p53 most studied functions are its capacity to delay the cell cycle, repair DNA lesions and induce apoptosis, all of these functions being largely mediated through direct transcriptional regulation of specific target genes (Kastenhuber and Lowe, 2017). Alongside with its role as the 'guardian of the genome', more recent work has brought new insights to p53 nexus with other cellular processes that might be important not only for its role as a tumour suppressor but also for non-cancer-associated functions of p53.

The single *Drosophila* orthologue of mammalian p53 (Dmp53) shares significant structural and functional features with human p53 (Fig. 1; Brodsky et al., 2000; Jin et al., 2000; Ollmann et al., 2000). Besides its conserved function in regulating apoptosis upon DNA damage, Dmp53 has been proved to be essential for tissue and metabolic homeostasis (Barrio et al., 2014; Mesquita et al., 2010a; Wells and Johnston, 2012). Upon tissue damage, Dmp53 regulates compensatory cell proliferation resulting in tissues and structures with normal size and pattern (Dichtel-Danjoy et al., 2013; Wells et al., 2006). In addition, Dmp53 has been involved in cell competition, a process by which cells with a growth disadvantage are eliminated from the tissue during development (De La Cova et al., 2014). Some of these novel functions of *Drosophila* p53 are conserved in vertebrates (Bondar and Medzhitov, 2010; Zhang et al., 2017). This review summarizes the contribution of *Drosophila* studies to the current knowledge on p53 and highlights differences and similarities in the way p53 is regulated between mammals and *Drosophila*. Moreover, we describe various functions of p53 that are important for tissue homeostasis and tumour suppression and discuss recent evidences for a role of p53 in enabling metabolic adaptation and organismal survival upon nutrient stress (Barrio et al., 2014).

2. Gene structure and function of p53 family

In mammals, *p53* belongs to a gene family comprising two other members, *p63* and *p73*, all showing a high degree of structural and functional similarity (Murray-Zmijewski et al., 2006). Although these last two members were more recently identified, a p63/p73-like gene is in fact considered to be the common ancestor of the p53-family (Murray-Zmijewski et al., 2006). Distinct phenotypes of knockout mice

* Corresponding author at: CCT-Santa Fe, Colectora Ruta Nº168 km 0, Santa Fe 3000, Argentina.
E-mail address: adekanty@santafe-conicet.gov.ar (A. Dekanty).
[1] These authors contributed equally.

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Fig. 1. Structure and regulation of human and *Drosophila* p53.
Multiple mRNA variants (A) and protein isoforms (B) are generated from *Homo sapiens* (Hs) and *Drosophila melanogaster* (Dm) p53 genes based on alternative promoters, splicing sites and translation initiation codons. (A) Hsp53 encodes for at least 9 protein isoforms. The full-length transcript encodes for p53 and Δ40p53, whereas Δ133p53 variants are generated from an internal promoter. Additionally, alternative splicing of exon 9 can produce isoforms α, β and γ. Dmp53 gene can potentially produce 3 protein isoforms. Full-length transcript encodes for Dmp53B and C, whereas Dmp53A is generated from an internal promoter. Exons are represented by boxes (non-coding exons in grey). (B) Scheme of p53 protein isoforms where amino acid positions defining p53 domains are indicated. Full-length p53 proteins includes: transactivation domain (TAD, in red or brown colours), DNA-binding domain (in green), nuclear localization signal (NLS, in orange), oligomerization domain (in light blue) and C-terminal regulatory domain (CTR, in dark blue). Dmp53B includes a full TAD domain equivalent to the human full-length p53 isoform (Hsp53α); Dmp53A (also known as DΔNp53) contains a truncated TAD domain thus resembling human Δ133p53 and Δ40p53 isoforms; Dmp53E (also known as Dmp53ΔC) encoded by a short transcript leading to a putative C-terminally truncated isoform bearing only the TAD. (C) Overview of post-translational modifications of Human and *Drosophila* p53. General scheme of p53 protein with the main functional domains indicated. Modification sites are plotted along the proteins and the enzymes responsible for each type of modification in each organism are detailed on the right and in the corresponding colour.

for each of these members indicate unique roles in development for p63 and p73 not shared by p53. Whereas $p53^{KO}$ mice are developmentally normal, $p63^{KO}$ or $p73^{KO}$ mice have defects in epithelial differentiation and neuronal development, respectively (Mills et al., 1999; Yang et al., 2000, 1999). The single *Drosophila* p53 gene encodes proteins homologous to p53, p63 and p73 (Lu et al., 2009). While *Dmp53* gene structurally and functionally resembles mammalian *p53* it has also been suggested to play a role in cell differentiation independently of its p53-like pro-apoptotic role.

The discovery of p53 protein isoforms, both in vertebrates and *Drosophila*, revealed a major complexity of p53 regulation and function (Fig. 1; Dichtel-Danjoy et al., 2013; Olivares-Illana and Fåhraeus, 2010). Expression of each p53 isoform is differentially regulated and plays critical roles in controlling appropriate cellular responses. In this sense, abnormal isoform expression and abundance contribute to tumorigenesis and have profound effects on tumour response to therapy. Tp53 isoforms present in humans can be divided mainly into two groups: transactivating forms (p53) and N-terminally truncated isoforms (Δ40p53 and Δ133p53) lacking a complete TA domain (Fig. 1). These p53 isoforms can interact with each other in many ways and clarifying the interplay between them is crucial for cancer research. Dmp53 gene potentially encodes three protein isoforms (Fig. 1; Joruiz and Bourdon, 2016; Marcel et al., 2011). Most studies have focused on the amino-terminally truncated Dmp53A (also known as DΔNp53), the most abundant isoform and the primary mediator of apoptosis after ionizing radiation. However, Dmp53B has recently been shown to induce massive apoptosis when overexpressed and to mediate apoptosis-induced proliferation (see below). Given the resemblance of Dmp53 gene structure to its human counterpart, future studies in *Drosophila* may provide new insights on how the interaction between different p53 protein isoforms affect p53 transcriptional activity and participate in p53-mediated cellular responses.

3. Upstream signalling to P53

Multiple levels of regulation ensure that p53 is exclusively activated in response to stress. Together with transcriptional and post-transcriptional processes, several post-translational modifications including phosphorylation, ubiquitination, acetylation, sumoylation and glycosylation can regulate p53 protein stability, subcellular localization and transcriptional activity (Fig. 1; Dai and Gu, 2010). In unstressed cells, p53 is maintained at low physiological levels mainly by the action of the ubiquitin ligase MDM2, which promotes p53 nuclear export and degradation. In this manner, MDM2 inhibition by different effectors results in p53 stabilization. This is the case for the tumour suppressor ARF which responds to aberrant oncogene activation and directly inhibits MDM2. Stabilized and activated p53 is able to transcriptionally activate MDM2 establishing a negative feedback loop to limit p53 activity once stress is overcome. Many post-translational modifications other than ubiquitination and deubiquitination are also responsible for p53 stabilization. Following DNA damage, a series of protein kinases are able to phosphorylate and stabilize p53 including Ataxia telangiectasia mutated (ATM), Ataxia telangiectasia related (ATR), Checkpoint kinase 1 (Chk1) and Checkpoint kinase 2 (Chk2) (Dai and Gu, 2010). Likewise, upon glucose deprivation, AMP-activated protein kinase (AMPK)-dependent activation of p53 via serine-15 phosphorylation is required for p53 induction of a transient cell cycle arrest. Aside from phosphorylation, p53 has been shown to be regulated by acetylation and deacetylation. Acetylation can affect p53 protein stability, interaction with Mdm2, recruitment of transcriptional cofactors and promoter-specific activation of p53 targets (Dai and Gu, 2010). Deacetylases such as Sirtuins (Sirt) act as important negative regulators of p53-transcriptional activity. Finally, p53 signalling is also regulated by the micro-RNA (miRNA) machinery and many miRNAs have been described to repress either p53 or its regulators (Feng et al., 2011).

As its human counterpart, *Drosophila* p53 was shown to be regulated by ubiquitination, phosphorylation and sumoylation. Dmp53 activation following DNA damage relies on the activity of Lok, the *Drosophila* orthologue of mammalian Chk2. Lok/Chk2-dependent phosphorylation of Dmp53 on serine-4 activates a global transcriptional response to DNA damage that induces DNA repair as well as apoptotic pathways. For its part, Grapes, the *Drosophila* orthologue of mammalian Chk1, appears not to regulate Dmp53 (Brodsky et al., 2004; Peters et al., 2002). Although canonical ARF/MDM2 pathway is absent in non-vertebrate species, Dmp53 is activated by oncogene expression in germline tumour models, suggesting the existence of ancient pathways linking oncogene activation to p53 that may precede evolution of the ARF/MDM2 axis (Wylie et al., 2014). Despite evidence against existence of a MDM2 homologue, Synoviolin (dSyno), an E3 ubiquitin ligase implicated in endoplasmic reticulum-associated degradation (ERAD) was shown to ubiquitinate Dmp53 in vitro and reduce Dmp53 protein levels and Dmp53-dependent apoptosis when overexpressed (Yamasaki et al., 2007). In addition to dSyno, gene *companion of reaper* (*corp*) has also been identified as a negative regulator of Dmp53 protein levels (Chakraborty et al., 2015). Corp overexpression promotes cell survival after DNA damage in the soma but reduces survival in the germline, mimicking the effects observed in p53 mutants. Similarly to *Mdm2*, *corp* is transcriptionally regulated by Dmp53 suggesting a conserved feedback mechanism to limit the Dmp53 response (Akdemir et al., 2007; Brodsky et al., 2004). Indeed, Corp shares a protein motif with vertebrate MDM2 in a region that is essential for the interaction between MDM2 and p53 and, interestingly, this region of Corp mediates the interaction with Dmp53. Although it may be tempting to conclude that *corp* encodes for a functional analogue of vertebrate MDM2 in flies, the debate about the existence of a clear *Mdm2* homologue in *Drosophila* calls for further studies in order to positively demonstrate this hypothesis (Lane and Verma, 2012).

Whether Dmp53 is also regulated by acetylation has not been entirely elucidated. However, *Drosophila* deacetylase Sirt2 (dSir2) was shown to physically interact with Dmp53 and genetic evidence suggests that, as in mammals, Dmp53 is a downstream target of dSir2 (Bauer et al., 2009). Along with acetylation, Dmp53 can be efficiently sumoylated on two lysine residues (lysine 26 and lysine 302) and mutation of both sumoylation sites dramatically reduces the transcriptional activity of p53 and its ability to induce apoptosis when overexpressed (Mauri et al., 2008). Finally, *Drosophila* p53 has also been shown to be regulated by miRNAs in several tissues and interestingly, miRNA regulation of p53 in the *Drosophila* adipose tissue is modulated by nutrient availability and the TOR pathway (Barrio et al., 2014). Taken together all these results indicate that p53 is regulated similarly in *Drosophila* and mammals, which support the use of *Drosophila* as a valuable model to explore novel physiological function of p53.

4. Canonical functions of P53

The control of cell cycle progression, DNA repair and apoptosis are the most intensively studied functions of p53. In vertebrates, p53 has the ability to temporary block the cell cycle and promote DNA repair. Under certain circumstances, p53 is also able to induce senescence or promote apoptosis thus providing mechanisms against the accumulation of potentially malignant or defective cells. In this section we will focus on these canonical functions of p53 both in vertebrates and in *Drosophila*.

4.1. Apoptosis

In response to DNA damage, p53 induces apoptotic cell death mainly through transcriptional activation of classical pro-apoptotic genes, such as PUMA, BAX and NOXA along with other genes having pro-apoptotic functions such as death receptors (DRs) FAS, DR4 and DR5 (members of the TNF-receptor superfamily). Additionally, p53 can modulate apoptosis by its interaction with apoptotic modulators in the

cytoplasm or mitochondrial membrane (Berkers et al., 2013). In *Drosophila*, Dmp53 regulates DNA-damage induced apoptosis by transcriptionally activating classical pro-apoptotic genes such as *head involution defective* (*hid*), *reaper* (*rpr*), *grim* and *sickle* (Brodsky et al., 2000; Ollmann et al., 2000). Interaction of these pro-apoptotic proteins with *Drosophila* Inhibitor of Apoptosis Protein 1 (dIAP1) leads to activation of the initiator caspase Dronc (Caspase-2 and -9 homologue) and the effector caspases DrICE and Dcp-1 (Caspase-3 and -7 homologues, respectively) [reviewed in (Xu et al., 2009)]. Similar to mammalian SMAC/DIABLO and OMI/HTRA2, *Drosophila* pro-apoptotic proteins contain a short N-terminal motif, named IBM (IAP-Binding-Motif), necessary for IAP-binding and apoptosis induction (Shi, 2002). In addition, Dmp53 activates Jun-N-terminal kinase (JNK) signalling pathway in response to genotoxic stress which triggers a positive feedback loop implicating Dmp53, JNK and the pro-apoptotic genes *hid* and *rpr* (Shlevkov and Morata, 2012). Although the mechanism underlying p53-dependent JNK activation is not fully understood, experimental evidence suggests that both in humans and *Drosophila* direct interaction between p53 and phospho-JNK (Basket, in *Drosophila*) avoids the action of phosphatases leading to sustained JNK activity and apoptosis (Gowda et al., 2012). Another interesting crosstalk involves Dmp53 and the Hippo (Hpo) pathway. Phosphorylation and activation of Hpo following DNA damage depends on Dmp53 and depletion of the Hpo pathway significantly reduces the cell death response elicited by IR or Dmp53-overexpression (Colombani et al., 2006). Similarly, cytokinesis failure activates p53 via Hpo signalling and the PIDDosome multiprotein complex, thus preventing proliferation of tetraploid and potentially malignant cells (Fava et al., 2017; Ganem et al., 2014).

Apart from mediating DNA damage-induced apoptosis, genetic analyses suggest that Dmp53 contributes to eliminate a subset of primordial germ cells (PGC) and mitotic germ cells during early *Drosophila* embryogenesis and adult spermatogenesis, respectively (Napoletano et al., 2017; Yamada et al., 2008). Elimination of PGC occurs in many species and it is thought to be a mechanism for selecting the fittest or least damaged germ cells. *Cep-1*, the *C. elegans* homologue for *p53*, is required for proper chromosome segregation during meiosis and DNA-damage-induced PGC death (Baruah et al., 2014). In mice, p53 positively regulates PGC apoptosis and p53-deficient mice show reduced germ cell death and increased levels of abnormal sperm (Francis and Lo, 2006). Collectively, these results illustrate conserved mechanisms and function of p53 in mediating apoptotic cell death during both cellular stress and germ cell development.

4.2. Cell cycle arrest

Although p53-dependent cell death prevents accumulation of damaged cells, p53 has also the ability to delay cell cycle progression and promote DNA repair. Cell cycle arrest is induced by p53 either by induction of cyclin-dependent kinase inhibitor p21 or repression of the phosphatase Cdc25c impairing cell cycle progression at the G1-S and G2-M transitions, respectively. Although *Drosophila* genome encodes for most known cell-cycle regulators including p21/p27 and Cdc25 homologues Dacapo and String (Ollmann et al., 2000), the role of Dmp53 in regulating the cell cycle is very much controversial. Cell cycle arrest and apoptosis responses to DNA damage are independently regulated in *Drosophila*. On one hand, Mei-41/ATR and its downstream target Grapes/Chk1 control cell cycle arrest upon DNA damage. On the other hand, Tefu/ATM and its downstream targets Mnk/Chk2 and Dmp53 regulate IR-induced apoptosis but are dispensable for IR-induced cell cycle arrest (Brodsky et al., 2000, 2004; Jaklevic and Su, 2004; Ollmann et al., 2000). However, Dmp53 does contribute to cell cycle arrest in other biological contexts. Disruption of the mitochondrial electron transport chain causes a decrease in ATP levels sensed by AMPK which, in turn, acts through Dmp53 to activate a G1-S checkpoint (Mandal et al., 2010). In this fashion, Dmp53 regulates the levels of Cyclin E (CycE) through its known transcriptional target Archipelago (Ago) which contributes to proteasomal degradation of CycE thereby arresting the cell cycle. Indeed, it is through Ago/CycE axis that Dmp53 regulates ectopic neural stem cell (NSC) formation caused by *numb* loss-of-function in the *Drosophila* brain (Ouyang et al., 2011). These results clearly indicate a major role of Dmp53 in promoting apoptosis in response to DNA damage and support a function of Dmp53 in controlling cell cycle progression in other biological contexts.

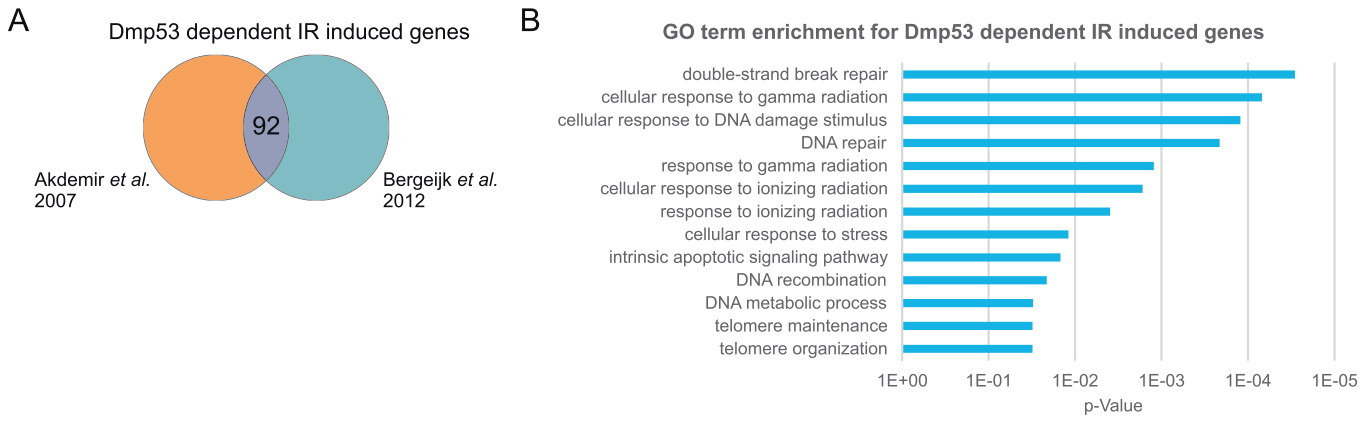
4.3. DNA repair and genomic stability

In response to DNA damage, p53 induces a reversible G1 phase checkpoint along with DNA repair genes allowing cells to correct DNA lesions previous to further cell division. Studies in developing *Drosophila* retina showed that Dmp53 protects cells from undergoing apoptosis in response to UV radiation most probably by enhancing nucleotide excision repair (Jassim et al., 2003). Dmp53 is also required to resolve IR-induced DNA damage as phosphorylated H2Av, a marker for DNA double-strand breaks, persists in Dmp53 mutant flies exposed to IR (Wells and Johnston, 2012). Additionally, Brodsky et al. showed Dmp53-dependent induction of genes involved in homologous recombination (HR) and non-homologous end joining (NHEJ) in IR treated embryos (Brodsky et al., 2004). More recent transcriptome analysis of *Drosophila* p53 regulatory network revealed a group of 92 genes that is consistently up-regulated upon IR in a p53-dependent manner (Fig. 2; Akdemir et al., 2007; van Bergeijk et al., 2012). Gene ontology analysis of these 92 genes showed enriched terms associated with DNA double strand-break repair, cellular responses to gamma radiation and apoptosis signalling pathways (Fig. 2). Despite only 8 of these genes have human orthologues known to be p53 target genes, the fact that most radiation-induced genes were clearly dependent on Dmp53 strongly suggest that Dmp53 orchestrate the acute response to DNA damage (Fig. 2; Fischer, 2017).

Unrepaired DNA damage can result in cells with an abnormal number of chromosomes or aneuploidy. In contrast to mammalian cells, Dmp53 is not required to eliminate aneuploid cells from *Drosophila* tissues (Mcnamee and Brodsky, 2009). In fact, apoptosis in response to chromosomal instability (CIN) generated by mutations in spindle assembly checkpoint genes, albeit relying on JNK signalling activation, is p53-independent (Dekanty et al., 2014, 2012). Therefore, p53 has a conserved function in supporting repair and survival of cells with moderate DNA damage while eliminating cells that have sustained and/or irreparable DNA damage. Curiously, however, CIN-induced apoptosis seems to be differentially regulated between flies and mammals.

4.4. Cellular senescence

Animal models of p53 restoration have demonstrated that in some cases tumour suppression depends on the ability of p53 to induce senescence, a permanent cell cycle arrest. P53-mediated senescence can be triggered by telomere loss, replicative stress or oncogenic signalling and relies on transcriptional activation of *p21*. Sustained p21 expression leads to up-regulation of $p16^{INK4A}$ and activation of the Retinoblastoma protein (pRb), which promotes a senescence program by which cells become unable to re-enter the cell-cycle regardless of the presence of proliferative signals, thus preventing unrestricted growth. Cells undergoing senescence produce a variety of secreted factors, collectively known as 'senescence-associated secretory phenotype' (SASP), which can promote clearance of tumour cells by the innate immune system but may also promote tumourigenesis by inducing growth and invasion (Krizhanovsky et al., 2008; Lujambio et al., 2013). Likewise, senescence induced in *Drosophila* epithelial cells by simultaneous activation of Ras oncogene (Ras^{V12}) and mitochondrial dysfunction is accompanied by Dmp53 activation, cell cycle arrest and secretion of proliferative molecules leading to overgrowth of neighbouring tissue (Nakamura et al., 2014; Ohsawa et al., 2012). 'Ras^{V12};$mito^{-/-}$'cells show increased Dacapo expression and decreased CycE levels. As discussed before, Dmp53



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Drosophila Gene	Mammal Orthologue	Conserved Function
act57B	ACTA2	Role in cell motility, structure and integrity
iscu	ISCU	Component of the iron-sulfur (Fe-S) cluster scaffold
loco	RGS16/RGS20	Regulator of G protein signaling family
mus205	REV3L	Catalytic subunit of DNA polymerase zeta
p53	TP53/TP63/TP73	p53 family of transcription factors involved in responds to diverse stresses
rcd2	VWCE	Von Willebrand Factor C And EGF Domains, may be a regulatory element in the beta-catenin signaling
spn47C	SERPINE1	Serine proteinase inhibitor superfamily
traf4	TRAF4	Links members of the tumor necrosis factor receptor (TNFR) family to different signaling pathways

Fig. 2. Analysis of Dmp53-dependent gene expression upon IR.
(A) Venn Diagram showing the total number of overlapped genes (92 genes) between two previously reported transcriptome analyses of p53-dependent IR-response (Akdemir et al., 2007; van Bergeijk et al., 2012) (B) Gene ontology analysis of the identified 92 coincident genes. (C) Venn Diagram showing overlap between Dmp53-dependent induced genes upon IR and a list of validated Hsp53 target genes as described by Fischer, 2017. (D) Detailed list and function of conserved p53-target genes obtained in (C).

activation does not regulate expression of p21/dacapo, but it does regulate CycE protein stability. Thus, Dmp53-dependent and -independent mechanisms cooperatively impair cell cycle progression during senescence in *Drosophila*. Together, these findings indicate that cellular senescence and SASP are evolutionarily conserved in insects and support a function of Dmp53 in controlling senescence. Future studies in *Drosophila* could provide novel mechanistic insights into these phenomena.

5. Non-canonical functions of P53

Although the fundamental role of p53 in tissue homeostasis has been largely appreciated from its canonical functions, recent observations have shown that p53 participates in many other physiological processes that are relevant to maintain tissue homeostasis and organismal survival. In this regard, *Drosophila* research has contributed significantly to identify novel and previously unappreciated functions of p53 including its role in tissue regeneration and apoptosis-induced proliferation, coordination of growth, cell competition and adaptive responses to nutrient stress at organismal level (Fig. 3).

5.1. Apoptosis-induced proliferation

Regeneration is a homeostatic process that enables the maintenance of tissues and organs. It involves a tight coordination of cell proliferation, senescence, apoptosis and differentiation, all cellular processes known to be regulated by p53. Indeed, variations in p53 activity have been observed during salamander limb regeneration, as well as during liver and nerve regeneration in mice (reviewed in Charni et al., 2017). Apoptosis-induced proliferation (AiP), an essential process for regeneration, was first described in *Drosophila* developing tissues and shown to be conserved in vertebrates afterwards (Jung et al., 2010; Li et al., 2010). Following *Drosophila* tissue damage, apoptotic cells secrete signalling molecules belonging to Wnt, TGF-b and Hedgehog families, which promote proliferation of surrounding non-apoptotic cells so that dying cells are replaced and tissue size recovered (Fig. 3B). In order to understand tissue responses to apoptotic stimuli, the so-called “undead cells” were originally used. Undead cells are generated by expression of pro-apoptotic proteins, such as Hid or Rpr, together with the baculovirus caspase inhibitor p35. As a consequence, undead cells show high levels of caspase activity without executing a real apoptotic program. Under this condition, these cells produce mitogenic molecules and promote cell proliferation, hyperplastic growth of the tissue and an invasive behaviour (Huh et al., 2004; Pérez-Garijo et al., 2004; Ryoo et al., 2004). Interestingly, Dmp53B acts downstream of the initiator caspase Dronc to induce ectopic expression of Wg and hyperplastic growth (Dichtel-Danjoy et al., 2013; Wells et al., 2006). Along with *Drosophila* studies, the role of caspases and p53 in regulating the expression of mitogenic molecules and promoting non-autonomous cell proliferation has been observed in other model organisms including freshwater Hydra, Xenopus tadpole, planarians as well as in different mouse tissues (Chera et al., 2009; Pearson and Alvarado, 2010; Zhao et al., 2006).

5.2. Coordination of tissue growth

Disrupting insulin/TOR signalling, ribosomal biogenesis or protein translation activates p53 in both mammalian cells and *Drosophila* tissues. Specifically in *Drosophila* developing tissues, adjacent cell populations grow in a coordinated manner buffering local variations in growth to maintain tissue homeostasis and produce well-proportioned organs (Dekanty and Milán, 2011). Mesquita et al. showed that

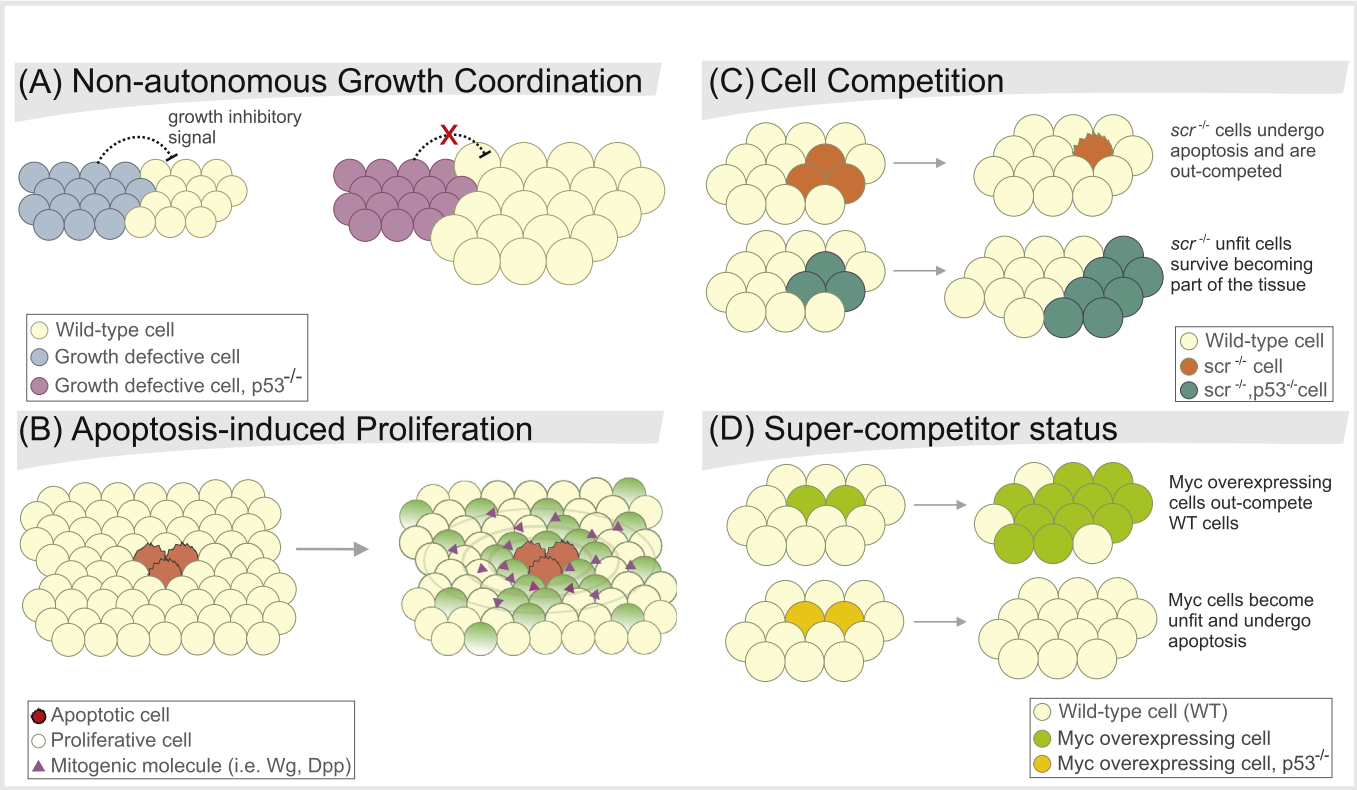

Fig. 3. p53 non-canonical functions.
(A) Tissue growth and proliferation rates are regulated non-cell-autonomously by Dmp53 activity. Impairing growth in defined territories of the developing wing primordium activates Dmp53, which in turn decreases growth and proliferation rates among adjacent wild-type (WT) cells. (B) Following tissue damage, apoptotic cells secrete signalling molecules that promote proliferation of surrounding non-apoptotic cells (AiP); Dmp53 acts downstream of Dronc to induce ectopic expression of Wg and cell proliferation. (C) Mammalian epithelial cells silenced for the polarity gene *scribble* are hypersensitive to cellular crowding and are eliminated in a p53-dependent manner when interacting with WT cells. (D) Aberrant Myc expression induces a super competitor state able to eliminate surrounding WT cells; in the absence of Dmp53 dMyc overexpressing cells are not able to out-compete WT cells, have increased genomic instability and undergo apoptosis.

depletion of growth promoting genes or disruption of the protein biosynthetic machinery in defined territories of the developing wing primordium induces a decrease in growth and proliferation rates among adjacent wild type cells (Mesquita et al., 2010b). Activation of Dmp53 upon growth impairment is required for the non-autonomous response of neighbouring cells as the absence of Dmp53 uncouples growth of adjacent cell populations and ultimately gives rise to asymmetric wings. Hence, tissue growth and proliferation rates are regulated non-cell-autonomously by the activity of Dmp53 (Fig. 3A).

Non-autonomous mechanisms of p53 have also been documented in vertebrates, mainly in the framework of tumour suppression. In stromal fibroblast, p53 modulates the production of secreted proteins that affect survival and spreading of adjacent tumour cells. In presence of chronic liver damage, ablation of p53 in hepatic stellate cells increases liver fibrosis and enhances the transformation of adjacent epithelial cells (Lujambio et al., 2013). As described before, activation of p53 induces a senescence program (SASP) characterized by the secretion of growth factors and inflammatory cytokines, that limits the extent of tissue damage and facilitates wound resolution (Krizhanovsky et al., 2008). These results imply that p53 activation can restrict cancer through non-cell-autonomous mechanisms and underscore the interplay between p53 and tissue microenvironment. In light of the results obtained in *Drosophila*, these evidences raise the possibility that p53 might control tissue growth, at least in part, by regulating the expression of signalling molecules that eventually act non-cell-autonomously to suppress proliferation and induce apoptosis. Future experiments in *Drosophila* developing tissues could shed light on the molecular mechanisms downstream of p53 involved in these processes.

In addition to tissue local responses, growth perturbation or imaginal disc damage delays metamorphosis in order to ensure growth is systemically coordinated among tissues and all organs attain proper final size. Production of dILP8, a *Drosophila* insulin like-peptide, by damaged tissue activates $Lgr3^{+}$ neurons in the central brain to control synthesis of the moulting hormone ecdysone, thereby coordinating growth with developmental timing (Garelli et al., 2015). dILP8 expression is directly regulated by transcriptional co-activator of Hpo pathway, Yorkie (Yki; YAP in mammals), and its DNA-binding partner Scalloped (Boone et al., 2016). The fact that Hpo pathway is activated downstream of Dmp53 in response to IR and that Yki physically interacts with Dmp53 raises the possibility of a potential role for p53 in dILP8 regulation (Colombani et al., 2006). Indeed, it is known that a developmental delay is induced by Dmp53 following massive DNA damage (Wells and Johnston, 2012). Taken together, these evidences suggest that a crosstalk between Dmp53 and Yorkie along with a possible Dmp53 role in regulation of dILP8 production might be important for cellular responses upon stress and tissue homeostasis.

5.3. Cell competition

Cell competition is a homeostatic mechanism by which a tissue can eliminate genetically different or suboptimal cells to promote its proper function and therefore organismal health (Morata and Ripoll, 1975). Although initially described in several *Drosophila* tissues, this phenomenon has been observed in mammalian cell culture, liver and hematopoietic cells, in different stem cell compartments as well as during mouse embryogenesis (Di Gregorio et al., 2016). Cell competition occurs when two cell populations with different metabolic or growth properties confront each other resulting in growth and expansion of the

stronger cell population ("winner" cells) at the expense of weaker cells ("loser" cells). Many genetic conditions have been shown to induce cell competition, including heterozygous mutations in ribosomal genes (collectively known as *Minutes* mutations), mutations in apicobasal polarity genes (such as *scribble*, *lgl* and *disc large*) and different doses of the proto-oncogene *myc* (Di Gregorio et al., 2016). Although several molecules and pathways have been implicated in remotion of loser cells, the very first signal that define loser state and drives apoptosis is still unknown. In a recent report, Kucinski et al. identified several genes and pathways that are differentially active in loser cells carrying functionally unrelated loser mutations (in *Minute* and *mahjong*, a component of the polarity-associated/Cul4-DDB1 complex). In this way, activation of p53, Toll, JNK and JAK/STAT pathways along with genes involved in the oxidative stress response were identified as the main components of loser state (Kucinski et al., 2017). However, unlike JNK, Dmp53 has been shown not to be required for the elimination of *Minute* cells during cell competition (Kale et al., 2015).

Mammalian epithelial cells silenced for the polarity gene *scribble* ($scrib^{KO}$) are hypersensitive to cellular crowding and are eliminated when interacting with wild-type (WT) cells (Wagstaff et al., 2016). As opposed to *Drosophila* studies, p53 activation is necessary and sufficient to eliminate $scrib^{KO}$ cells (Fig. 3C). Likewise, *Mdm2* and *Mdm4* double-heterozygous cells present a growth disadvantage and are out-competed during mice embryogenesis by wild-type cells in genetically mosaic embryos (Zhang et al., 2017). Similarly to $scrib^{KO}$ cells, double-heterozygous cells exhibit mild p53 activation and elimination of *Mdm2+/− Mdm4+/−* cells was abrogated in p53 heterozygous mice. In other report, Bondar and Medzhitov characterized a new form of cell competition induced by stress and mediated by p53 (Bondar and Medzhitov, 2010). By using genetic mosaic mouse models and bone marrow chimeras, they showed that cells with higher levels of p53 activity are outcompeted in a repopulation assay. In this case, competition appears to be mediated by a non-cell-autonomous induction of growth arrest and expression of senescence-related genes in loser cells (Bondar and Medzhitov, 2010). While these studies positively demonstrate a role for p53 activity in cell competition both in vitro and in vivo in mammalian models, accomplishing a complete understanding of a potential Dmp53 function in this process will clearly require further work.

Despite constituting an important physiological and homeostatic mechanism, under certain conditions, cell competition may become pathogenic and contribute to cancer initiation. Oncogene activation, such as aberrant Myc expression, can derive in a super competitor state able to eliminate surrounding WT cells and ultimately colonize the tissue (Fig. 3D). De la Cova et al. showed that increased *Drosophila* Myc (dMyc) expression and thus, super competitor status, involves a Dmp53-dependent metabolic reprogramming in *Drosophila* developing tissues where Dmp53 is activated in dMyc overexpressing cells to regulate mitochondrial respiration. Furthermore, in the absence of Dmp53, dMyc overexpressing cells are not able to out-compete wild type cells, have increased genomic instability and undergo apoptosis (De La Cova et al., 2014). Altogether, these results demonstrate that p53 plays a protective role in Myc overexpressing cells and suggest that emerging cancer cells might use adaptive metabolic functions of p53 to compete with WT neighbouring cells. Whether p53 could play similar roles in mammalian cells in order to promote tumour formation is uncertain.

5.4. Metabolic homeostasis

Over the last decade, p53 has emerged as a key regulator of metabolic homeostasis and p53 activation has been regularly observed in response to different metabolic stimuli such as changes in oxygen tension, redox state or nutrient availability (Berkers et al., 2013). Upon nutrient deprivation, p53 has been shown to promote cell survival by interacting with nutrient-sensing pathways and also by promoting efficient nutrient utilization through modulation of multiple metabolic pathways and autophagy (Berkers et al., 2013; Maddocks et al., 2013). Inherent to its tumour suppressor role, it is well recognized that p53 regulates energy metabolism by inhibiting glycolysis and promoting mitochondrial respiration (Liang et al., 2013). On one side, p53 reduces expression of glucose transporters (GLUT1/3/4) and the glycolytic enzyme phosphoglycerate mutase (PGM) at the same time upregulating TIGAR, which indirectly regulates the rate-limiting enzyme in glycolysis phosphofructokinase 1 (PFK1). On the other side, p53 maintains mitochondrial integrity and promotes oxidative phosphorylation by regulating expression of synthesis of cytochrome *c* oxidase 2 (SCO2, a key regulator of the complex IV assembly), mitochondrial glutaminase 2 (GLS2) and apoptosis-inducing factor (AIF) (Berkers et al., 2013). Hence, p53 regulates glucose metabolism to favour energy production through oxidative phosphorylation. Conversely, p53 deficient cells show higher rates of glycolysis and reduced mitochondrial respiration, a distinctive metabolic profile seen in cancer cells and highly proliferating normal cells known as the Warburg effect (Liang et al., 2013). Interestingly, recent studies in mice indicate that p53-regulation of metabolic homeostasis under normal physiological conditions might be relevant for tumour suppression (Kastenhuber and Lowe, 2017). *Drosophila* p53 has also been associated with several metabolic pathways. As mentioned in the previous section, Dmp53 helps to preserve energy by inducing cell cycle arrest and inhibiting cell growth in response to low ATP levels generated by mitochondrial dysfunction (Mandal et al., 2010). In dMyc overexpressing cells, Dmp53 activation regulates glycolysis and OXPHOS as part of a homeostatic mechanism that promotes cell fitness (De La Cova et al., 2014). Dmp53 can also regulate glycolysis under nutrient deprivation by repressing the expression of two glycolytic enzymes, PGM and Hex-C (Barrio et al., 2014). The fact that PGM is also negatively regulated by p53 in cultured mammalian cells (Berkers et al., 2013) may support a conserved role of p53 in regulating glycolysis in vertebrate and invertebrate tissues.

Alongside with its capacity of controlling metabolism at a cellular level, Dmp53 has recently been shown to participate in organismal metabolic adaptation. Depletion of Dmp53 activity specifically in the *Drosophila* fat body, a functional analogue of vertebrate liver and adipose tissue, accelerates the consumption of the main energy stores, reduces sugar levels and compromises organismal survival upon starvation (Barrio et al., 2014). As mentioned before, Dmp53 is regulated in the fat body by miR-305 in a nutrition-dependent manner. Whereas in well fed animals TOR signalling contributes to miR-305 mediated inhibition of Dmp53, nutrient deprivation reduces the levels of miRNA machinery components leading to lower levels of miR-305 and subsequent de-repression of Dmp53 (Barrio et al., 2014). The mechanism by which Dmp53 regulates metabolic homeostasis and organismal survival under nutrient stress is not entirely understood but might involve regulation of specific metabolic pathways. In this regard, Dmp53-mediated downregulation of glycolysis in fat body cells may enhance metabolic adaptation by efficiently managing energy stores under nutrient restricted conditions. Regarding regulation of metabolism at a systemic level, Dmp53 has been also associated with regulation of Insulin/insulin-like growth factor signalling (IIS). IIS is a conserved nutrient-sensing system that systemically regulates metabolism and growth according to nutrient availability. Dmp53 has been proposed to modulate IIS through the regulation of at least one of the eight *Drosophila* insulin-like peptides (dILPs). Altering Dmp53 function in the insulin-producing cells (IPCs) has been shown to extend the life span of *Drosophila* adult flies (Bauer et al., 2007). This phenomenon is associated with a reduction in the levels of dILP2 and a decrease in IIS in the fat body (Bauer et al., 2007). The ability of Dmp53 to regulate IIS has been also observed upon starvation or ribosomal stress where Dmp53 activation within the IPCs contributes to regulate dILP2 secretion (Hasygar and Hietakangas, 2014).

Together these results indicate that p53 plays tissue specific roles in *Drosophila* integrating nutrient status with metabolic and physiological responses at an organismal level. It will be interesting to see whether future studies show similar roles of p53 in regulating physiological and

metabolic responses in mammals and if these systemic roles of p53 may have any implications in metabolic diseases.

6. Concluding remarks

Since its discovery, the transcription factor p53 has been intensively studied being recognized as the most popular gene in the human genome (Dolgin, 2017). After thorough study, p53 showed to contribute to tumour suppression mainly by inducing canonical cellular responses such as cell cycle arrest, DNA repair and apoptosis. In the last few years, however, significant interest has been raised in understanding non-canonical functions of p53 that might have potential roles in tumour suppression, including regulation of metabolism, autophagy and stem cell biology. In this review, we have discussed how the use of *Drosophila melanogaster* has contributed to the study of p53 regulation and function in normal animal physiology and upon various types of stress. Despite the debate on the existence of an Mdm2 homologue in *Drosophila*, evidences indicate that p53 is similarly regulated in *Drosophila* and mammals. In response to DNA damage, Dmp53 play a main role in promoting apoptosis and DNA repair but is dispensable for cell cycle arrest. However, Dmp53 is able to regulate cell cycle progression in other biological contexts, such as mitochondrial dysfunction and metabolic stress. Interestingly, *Drosophila* studies have contributed to identification of novel and previously unappreciated functions of p53 in regulating apoptosis-induced proliferation, cell competition and coordination of growth. In sharp contrast to its cell-autonomous canonical functions, p53 plays non-cell-autonomous roles in both *Drosophila* and mammals. Hopefully, future studies will provide novel insights into the molecular mechanisms that mediate Dmp53 non-autonomous responses in coordinating AiP and growth control. Finally, studies in *Drosophila* have identified tissue specific roles of Dmp53 in regulating insulin signalling and adaptive metabolic responses impacting on animal aging and stress survival. The role of p53 in metabolism as known in higher organisms, such as humans, has a greater complexity than what is currently found in *Drosophila*. Connections between important p53 upstream and downstream metabolic regulators such as AMPK, GSK3, HIF1alpha and/or autophagy related genes have not yet been demonstrated in *Drosophila*. Interestingly, however, many high-throughput genome-wide studies have suggested physical or genetic interactions of Dmp53 with genes involved in autophagy, OXPHOS and lipid and glucose metabolism. Future and more comprehensive studies on the relationship between Dmp53 and each of these metabolic pathways and genes will allow us to better understand the role of Dmp53 in regulating metabolism and, eventually, elucidate its potential role in metabolic diseases.

Competing interests

The authors have declared that no competing interests exist.

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