Review

Guardian ancestry: fly p53 and damage-inducible apoptosis

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Abstract

The tumor suppressor, p53, is among the most commonly mutated genes in human cancers. Recent reports describe shared and divergent properties of a *Drosophila* p53 homolog Dmp53. Like its mammalian counterpart, Dmp53 also functions in damage-induced cell death. In this model system, the apoptosis activator reaper has emerged as an important target gene. Together with the wealth of genomic data available in *Drosophila*, continued studies on Dmp53 promise new insights into the regulation and function of this important gene family. *Cell Death and Differentiation* (2000) **7,** 1035–1038.

Introduction

The p53 tumor suppressor acts as a cellular guardian, responding to genotoxic stress by either arresting the cell cycle and promoting DNA repair or eliminating potentially damaged cells via apoptosis. Not surprisingly, disabling such a safeguard appears to be a common route toward oncogenic transformation as a predominance of human cancers are associated with mutations at the p53 locus. p53 functions as a transcription factor and several targets connected to cell cycle regulation, DNA repair and apoptosis have been characterized. The precise mechanisms that specify these distinct responses, however, remain largely ill defined.

The recent identification by Brodsky *et al.*, Ollman *et al.*, and Jin *et al.* of a *Drosophila* p53 homolog, Dmp53, provides a new and valuable avenue for dissecting p53's function in development and damage responses.[1–3] The 385 amino acid Dmp53 protein shares properties in four regions previously characterized for their discrete functions in mammalian p53. These domains divide p53 into a transcriptional activation domain at the N-terminus, a DNA binding domain, an oligomerization domain and basic domain at the C-terminus. Such domains were identified in Dmp53 with the DNA binding domain representing the strongest area of homology (43% similarity). More significantly, of the six sites most frequently found mutated in mammalian tumors, Dmp53 is identical in four and similar in the remaining two. Of these four identical residues, two have been shown to directly contact DNA from mammalian studies. Among six other residues involved in DNA binding, four are identical and one is similar in Dmp53. In addition, the four residues that contact zinc are also conserved.

Phylogeny

As the most evolutionarily remote representative of the p53 family, *Drosophila* p53 can provide insights into the gene family's ancestry. Prior to Dmp53's identification, squid was the most distantly related species for which a p53 homolog was isolated. However, squid p53 is more similar to the human p63/p73 subfamily of p53 because all contain a Sterile Alpha Motif (SAM) domain. This led to the proposal that extant p53 family members arose from a p73-like ancestor.[4] Dmp53 is equally distant from human p53, human p73, and squid p53, but lacks a SAM domain. With no p63/p73 homologs identified in the near complete *Drosophila* genome, Ollman *et al.* suggested that the p53 gene family originated from a p53-like ancestor and that the p63/p73 subfamily evolved after the divergence between vertebrate and invertebrate lineages. Current phylogenic relationships are at odds with this proposal, however, since the common ancestor of insects and molluscs is certainly more recent than the divergence between protostomes and deuterostomes. A more likely alternative is that the common ancestor of invertebrates and vertebrates contained both p53 and p73-like genes and that p73-like genes may have been selectively lost in the arthropod lineage. Moderate support for this scenario is suggested from lineage algorithms that use only the DNA binding domain of p53 family members. If only this domain is considered to be the evolutionary unit, Dmp53 shows a slightly closer resemblance to the members p63/p73 subfamily (see Table 1). This result might support the possibility of a p73-like ancestor and also implicates the existence of variants with and without the SAM domain in the common ancestor. An important missing piece of the puzzle, of course, is whether or not other invertebrate genomes contain both p73 and p53-like genes. In light of this, recent reports of p53 family members from the clam suggest that molluscs do indeed have p53 like genes with and without SAM domains.[5] Clearly, any definitive statements about p53's evolutionary origin requires more complete genomic information from a variety of species representing several metazoan radiations.

Although *Drosophila* p53 ranks as the most divergent member of the family yet described, numerous studies established that Dmp53 shares properties with its

Table 1 Sequence relationships between the DNA binding domains of p53 family genes

	Human p53	Human p73	Squid p53	Clam p53	Clam p73
Dmp53	25%	29%	29%	32%	32%

THe DNA binding domain of *Drosophila* p53 (Genbank Accession: AF224713) was compared against human p53 (NP 000537), human p63 (AF075433), human p73 (NM 005427), squid (U43595), *Xenopus* (X77546), zebrafish (U60804), clam p53 (AF253323), and clam p73 (AF253324) using Blast 2 (Basic Local Alignment Search Tool).[36] Identity scores are shown

mammalian counterparts. Both the full-length protein, as well as isolated domains predicted from protein alignment algorithms, were tested in numerous contexts. In two-hybrid experiments, the N-terminal region conferred transcriptional activation while the C-terminal region promoted oligomerization. Site specific DNA binding was demonstrated using mobility shift assays,[2,3] one-hybrid tests and cell culture transfections.[1] Together, these analyses established that Dmp53 can bind to human p53 binding sites.

Functional studies also validated an orthologous role in damage inducible apoptosis. Like its mammalian counterpart, overexpression of Dmp53 triggers ectopic apoptosis and variants that are disrupted for DNA binding activity exert dominant negative effects. In the developing wing disc, dominant-negative Dmp53 expression blocked radiation-induced apoptosis but had no apparent affect on normal development. Similar observations were documented in transfection experiments where mutant Dmp53 poisoned transactivation by the wild-type protein. A likely explanation for these observations is that the mutant Dmp53, like other members of this family, forms inactive tetrameric complexes with the wild-type protein.

New insights

The availability of a p53 homolog in the *Drosophila* model system holds the potential for rapid elucidation of novel pathways controlled by p53. Such promise has already been partially realized with the identification of *reaper* as a likely p53 transcriptional target by Brodsky and colleagues. *reaper* is a pro-apoptotic gene whose expression is governed by developmental signals and damaging stimuli.[6] Later studies showed that induction of *reaper* by ionizing radiation is under transcriptional control, implicating an upstream factor satisfying a role orthologous to p53 in mammals.[7] Further delineation of *reaper*'s regulatory region identified a minimal radiation responsive element that matched the consensus p53 responsive element. The consensus p53 binding element (p53 RE) consists of two repetitions of a 10 mer. While *in vitro* characterizations of the p53 RE suggest a tolerance for up to a 13 bp separation between the 10 mers, native *in vivo* targets such as those found upstream of the human target genes *mdm-2*[8] and *p21/WAF1*[9] typically contain adjacent sites. Like these mammalian targets, the radiation responsive element upstream of *reaper* also contains adjacent sites. In this case, two mismatches exist at the outermost nucleotides of the p53RE, thus preserving an invariant 18 base pair core.

The identification of Dmp53 as a potential regulator of *reaper* expression provides a satisfying link between genotoxic stress and apoptosis in *Drosophila*. In this model system, *reaper*, *hid*, and *grim* respond differentially to various apoptotic stimuli including ionizing radiation.[10] These promote caspase activation by acting positively through Dark, the Apaf-1/Ced-4 homolog,[11–13] and inhibiting anti-apoptotic functions such as the inhibitor-of-apoptosis proteins (IAPs).[14,15] Released from the inhibiting grip of IAPs, caspases are then presumably free to activate downstream caspases, committing the cell to an apoptotic fate (Figure 1).

The *reaper* protein, at only 65 amino-acids in length, shares no obvious sequence similarity with genes outside of other insects. The possible existence of a mammalian *reaper* homolog, however, is supported by the observations that Reaper can induce apoptosis in mammalian cell culture systems as well as bind mammalian IAPs.[16–19] *Reaper's* small size has strained the limits of algorithms used to identify prospective homologs in genomic databases. The connection between p53 and *reaper* in flies may assist computational searches for mammalian *reaper* homologs. Likewise, it will be interesting to see whether Smac/Diablo, a recently identified inhibitor of IAPs (with no obvious homology to *reaper*)[20,21] might be responsive to p53.

Missing players

As mentioned above, functional studies on DmP53 in the eye and wing discs established profound effects upon apoptosis but uncovered no apparent affect upon cell-cycle progression. While it is possible that both primordial wing and retinal tissues are atypically insensitive to p53 induced arrest, it is more likely that Dmp53 simply does not engage the cell cycle machinery in the same way that mammalian members of this family do. This is a surprising result given the extensive cell cycle arrest properties documented for mammalian p53 proteins (reviewed in[22]) and the conservation of orthologous cell cycle regulators in the fly. In mammals, transactivation of p21 by p53 defines a major pathway that inhibits the activity of cyclin-dependent kinases (CDKs) and prevents phosphorylation of the retinoblastoma (pRb) protein.[23] p21 can also trigger G1 arrest in *Drosophila*[24] but despite the preservation of this checkpoint pathway, existing evidence suggests that Dmp53 does not appear to use it (although transactivation of fly *p21* could possibly occur without triggering a checkpoint arrest).

Similarly, the *ATM/Chk1* pathway is conserved in *Drosophila*[25] and is required for G2/M arrest in irradiated wing discs.[26] In mammals, ATM phosphorylates p53 on serines located in serine-glutamate pairs at positions 15 and 37.[27–29] Dmp53 contains a serine 4-glutamate 5 site which could serve as a phosphorylation target for Mei-41, the *Drosophila* ATM homolog. One model for ATM function suggests that phosphorylation of p53 by this kinase impairs binding and disposal by Mdm2.[30] Relating this scenario to Dmp53 is problematic, however, considering that orthologs of Mdm2 and its upstream regulator p19ARF are absent from the fly genome.[26] Perhaps equally important is the fact that key residues essential for binding to Mdm2 by p53 are absent in Dmp53. Thus, it is likely that alternate
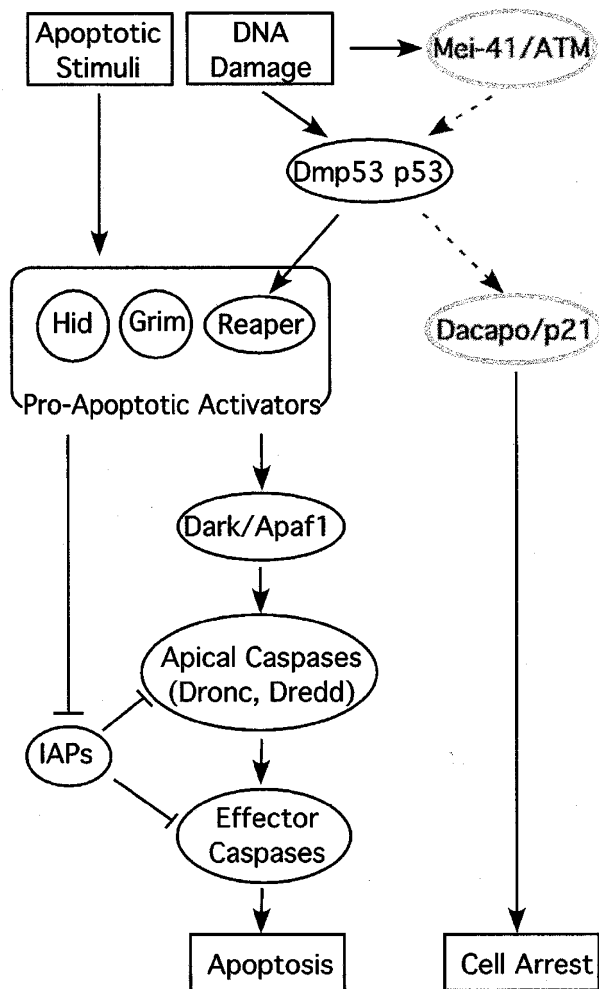
Figure 1 Regulation of apoptosis by Dmp53. In response to DNA damage, both mammalian p53 and its *Drosophila* ortholog (Dmp53) can respond by initiating apoptosis. In *Drosophila*, Dmp53 transactivates expression of *reaper*, one of three closely linked pro-apoptotic genes that respond to various apoptotic stimuli. Reaper leads to the activation of apical caspases possibly through the inhibition of IAPs (inhibitor of apoptosis proteins) and/or activation of the Apaf-1 homolog, Dark.[11] The apoptotic program continues with the subsequent activation of downstream effector caspases. As indicated in gray, Mei-41 and Dacapo, the fly homologs of ATM and p21, respectively, have demonstrated involvement in cell cycle arrest. Unlike mammalian p53, however, no role for Dmp53 in cell cycle arrest has been detected by either Brodsky *et al.*, Ollman *et al.*, or Jin *et al.*

mechanisms connecting genotoxic stress signals to Dmp53 action probably exist.

Future studies will continue to elucidate the extent to which the insect p53 pathways have diverged from vertebrates. It remains to be seen, for instance, whether Dmp53 has been completely divested of a role in cell cycle arrest. The limited results available thus far do not rule out the possible necessity of cooperative factors or 'co-signals' in promoting cell cycle arrest. Likewise, these interpretations rely upon dominant negative proteins and a tenuous presumption that Dmp53's homotetramerization is a conserved requirement for its cell cycle arrest function. The production of single gene mutations in Dmp53 should

help address these and other important issues. Given ~550 million years of evolutionary distance separating the two lineages, it is quite likely that significant upstream and downstream differences will emerge. In this regard it will be interesting to see if homologs for mammalian p53 targets such as *p21*[31] and *bax*[32] are targets for Dmp53 as well.

Future directions

Studies of fly counterparts to known participants in the mammalian p53 response pathways promise to reveal core elements in relevant damage responses. At the same time, points of divergence, such as the absence of Mdm-2, should highlight which features of p53 regulation are distinct in mammals. Beyond investigating known *Drosophila* homologs, the nearly complete genome sequence for both organisms provides exciting new possibilities for elaborating as yet undiscovered pathways. Already, high throughput analysis concentrating on mammalian genes regulated by p53 has produced a wealth of data by which to compare and contrast such efforts in *Drosophila*.[33–35] Toward this end the p53 responsive element identified in the *reaper* gene may serve as a valuable tool for identifying genes involved in the damage response.

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