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Article

The *archipelago* Tumor Suppressor Gene Limits Rb/E2F-Regulated Apoptosis in Developing *Drosophila* Tissues

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Summary

Background: The *Drosophila archipelago* gene (*ago*) encodes the specificity component of a ubiquitin ligase that targets the cyclin E and dMyc proteins for degradation. Its human ortholog, *Fbw7*, is commonly lost in cancers, suggesting that failure to degrade *ago*/*Fbw7* targets drives excess tissue growth.

Results: We find that *ago* loss induces hyperplasia of some organs but paradoxically reduces the size of the adult eye. This reflects a requirement for *ago* to restrict apoptotic activity of the *rbf1*/*de2f1* pathway adjacent to the eye-specific morphogenetic furrow (MF): *ago* mutant cells display elevated *de2f1* activity, express the prodeath dE2f1 targets *hid* and *rpr*, and undergo high rates of apoptosis. These phenotypes are dependent on *rbf1*, *de2f1*, *hid*, and the *rbf1*/*de2f1* regulators *cyclin E* and *dacapo* but are independent of *dp53*. A transactivation-deficient *de2f1* allele blocks MF-associated apoptosis of *ago* mutant cells but does not retard their clonal overgrowth, indicating that intact *de2f1* function is required for the death but not overproliferation of *ago* cells. *Epidermal growth factor receptor* (*EGFR*) and *wingless* (*wg*) alleles also modify the *ago* apoptotic phenotype, indicating that these pathways may modulate the underlying sensitivity of *ago* mutant cells to apoptotic signals.

Conclusions: These data show that *ago* loss requires a collaborating block in cell death to efficiently drive tissue overgrowth and that this conditional phenotype reflects a role for *ago* in restricting apoptotic output of the *rbf1*/*de2f1* pathway. Moreover, the susceptibility of *ago* mutant cells to succumb to this apoptotic program appears to depend on local variations in extracellular signaling that could thus determine tissue-specific fates of *ago* mutant cells.

Introduction

Genetic screens in the fruit fly *Drosophila melanogaster* have identified many genes that restrict growth of developing tissues (reviewed in [1, 2]). In some cases, orthologs of these *Drosophila* growth suppressor genes have subsequently been implicated as vertebrate tumor suppressors. One example is the *archipelago* (*ago*) gene, which was identified because *ago* mutations confer a growth advantage to imaginal disc cells [3]. *ago* encodes an F box/WD (tryptophan/aspartic acid) protein (Ago) that acts as the substrate adaptor for an Skp/Cullin/F box (SCF) E3 ubiquitin ligase. SCF-Ago targets the G1/S cell-cycle regulator cyclin E (CycE) and dMyc, the fly ortholog of the c-Myc proto-oncogene, for degradation in vivo [3, 4]. These proteins hyperaccumulate in *ago* mutant cells and drive balanced increases in rates of division and growth, producing enlarged clones composed of normally sized cells [4]. In addition to this mitotic role, *ago* also regulates hypoxia sensitivity and postmitotic morphogenesis of the tracheal system via degradation of the Trachealess transcription factor [5, 6].

Fbw7, the human *ago* ortholog, is frequently mutated in a wide array of human tumor types, including those of endometrial, colorectal, and hematopoietic origin (reviewed in [7]). Moreover, deletion of murine *Fbw7* increases cancer incidence and collaborates with *p53* mutations to promote epithelial carcinogenesis [8]. Growth suppression by *Fbw7* is linked to defective degradation of SCF substrates including CycE, c-Myc, the Notch intracellular domain, c-Jun, sterol regulatory element-binding protein (SREBP), and mTor kinase (reviewed in [7, 9]). Thus, *ago* and *Fbw7* both behave as antigrowth genes in vivo, and this property derives in part from their role in the timely destruction of common oncogenic substrates like CycE and Myc.

Many *Drosophila* mutations that accelerate the rate of cell proliferation also increase the size of the corresponding adult organ. Such mutations affect various regulatory networks, including the Tsc/Tor, IGF/PI3K, Sav/Wts/Hpo, ras/EGFR, and Notch pathways (reviewed in [1, 2]). Notably, ectopic expression of progrowth factors such as *dMyc* in larval discs also increases adult organ size [10]. It is therefore somewhat surprising that adult eyes composed mainly of *ago* mutant cells expressing very high levels of dMyc are not obviously enlarged [3, 4]. One explanation for this might be that excess dMyc causes *ago* cells to behave as "supercompetitors" that kill adjacent normal cells [11, 12], thus balancing the overall rate of growth of a mosaic organ. Alternately, an as yet unrecognized cell-autonomous mechanism might limit the ability of *ago* mutant cells to give rise to an enlarged organ.

Here, we uncover a requirement for *ago* to maintain eye size that reflects a cell-autonomous role for *ago* upstream of the *rbf1*/*de2f1* pathway in cells just anterior to the eye-specific morphogenetic furrow (MF). *ago* mutant cells fail to downregulate *de2f1*-dependent transcription, express elevated levels of prodeath dE2f1 target genes, and undergo high rates of apoptosis. Blocking this death causes *ago* mutant discs to grow into enlarged adult eyes, indicating that *ago* loss requires a collaborating antiapoptotic event to drive eye hypertrophy. *ago* apoptotic phenotypes are also sensitive to *EGFR* and *wingless* signaling, indicating that extracellular pathways can alter the threshold for *e2f1*-driven death of *ago* mutant cells. These data identify *ago* as a required upstream regulator of the *rbf1*/*de2f1* pathway in eye disc cells and show that apoptosis mediated by this pathway can act as a significant brake on the growth of developing tissues lacking the *ago* tumor suppressor.

Results

Loss of *ago* Has Different Effects on the Size of Tissues in the Adult Head

Drosophila with eyes and heads composed almost entirely of *ago* mutant tissue were generated with the ago^{1} allele and

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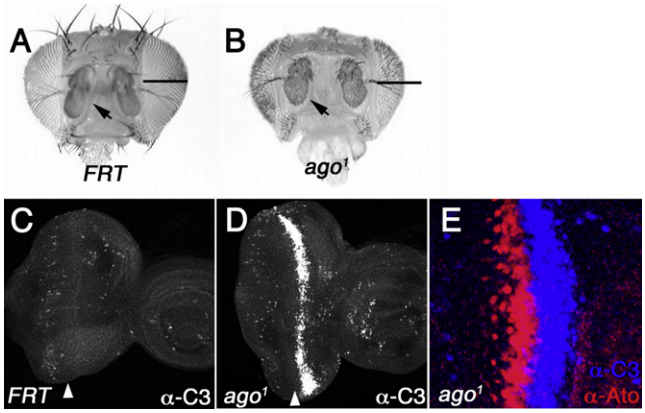

Figure 1. *ago* Loss Reduces Eye Size and Elevates Apoptosis

(A and B) Images of *FRT80B/M(3)* (A) and *ago*^{1}*/M(3)* (B) adult female heads. Black bar denotes depth of *FRT80B/M(3)* eyes. Arrows indicate increased antenna size in *ago*^{1}*/M(3)* heads.

(C–E) Merged confocal sections of *FRT80B/M(3)* (C) and *ago*^{1}*/M(3)* (D and E) larval eye-antennal discs stained for C3 or Atonal as indicated. Arrowheads indicate position of the morphogenetic furrow (MF). In these and all following images, posterior is to the left.

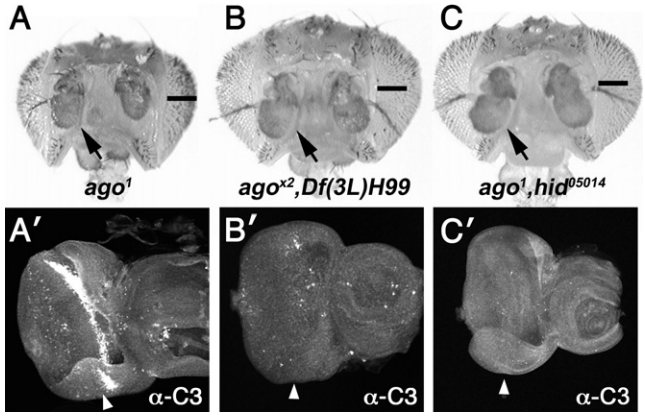

Figure 2. Blocking Death of *ago* Cells Produces Enlarged Organs

ago^{1}*/M(3)* (A), *ago*^{x2}*,Df(3L)H99/M(3)* (B), and *ago*^{1}*,hid*^{05014}*/M(3)* (C) adult female heads and merged confocal sections of α-C3 staining in corresponding larval eye discs (A′–C′). Black bar is standardized to the depth of *ago*^{1}*/M(3)* eyes; arrows indicate increased antennal size. Arrowheads indicate position of the MF.

the 3L *Minute* (*M*) allele *RpL14*^{1} [13] as a recessive cell-lethal mutation to kill *M/M* cells, allowing *ago*^{1}*/ago*^{1} cells to populate the disc and adult structures derived from it. *ago*^{1} encodes a prematurely truncated protein that cannot bind CycE and dMyc and increases levels of these proteins in vivo [3, 4]. Thus, the *ago*^{1}*/M(3)* genotype provides a model of organ development in the absence of normal *ago* activity. Adult *ago*^{1}*/M(3)* eyes were smaller than control eyes (Figures 1A and 1B). This phenotype was specific to the eye: other organs in *ago*^{1}*/M(3)* heads, such as the antennae (arrows, Figures 1A and 1B) and interocular cuticle, grew larger. A similar effect was observed with other *ago* alleles (data not shown). Thus, although *ago* behaves as a growth suppressor in some tissues, it is required for the developing eye to reach its normal size.

Eye development is uniquely dependent on the MF, a moving compartment boundary that sweeps posterior to anterior across the larval disc and separates it into areas: asynchronously dividing cells anterior to the MF, G1 phase-arrested cells within the MF, and largely postmitotic cells posterior to the MF [14]. *ago* loss did not substantially alter the pattern of S phase entry in the vicinity of the MF (see Figure S1 available online), but it did produce an intense "stripe" of apoptosis, as detected with an antibody to the cleaved form of caspase-3 (C3), that extended laterally across the entire third-instar eye disc (Figures 1C and 1D). A second *ago* allele, *ago*^{3} [3], produced a similar pattern of apoptosis (Figure S2). Costaining for C3 and Atonal protein, which marks cells within the MF [15–17], showed that this death extended ~5–10 cell diameters from the anterior edge of the MF into the anterior portion of the disc (Figure 1E). This pattern was strongest at the lateral margins of *ago*^{1}*/M(3)* eye discs but was still penetrant enough to appear as a contiguous stripe in medial areas. Notably, C3 was also detected in *ago*^{1} clones that crossed the MF (see below), indicating that this apoptosis is autonomous to *ago* cells and not a product of the cell-lethal technique used to generate *ago*^{1}*/M(3)* discs. Thus, in addition to its growth suppressor function, *ago* is also required to protect cells from death in a region just anterior to the MF.

Blocking Death Increases *ago* Mutant Eye Size

The C3 data suggest that the MF is preceded by an intense wave of cell death that culls many cells from developing *ago*^{1}*/M(3)* eyes. To test whether blocking this could reverse the *ago* small-eye phenotype, the *ago*^{1} allele was combined with the *Df(3)H99* genomic deletion, which removes the proapoptotic genes *rpr*, *grim*, and *hid* [18]. This completely blocked the C3 stripe (compare Figures 2A′ and 2B′) and produced adult heads that were much larger than *ago*^{1}*/M(3)* or *H99/M(3)* heads (compare Figures 2A and 2B). *H99/M(3)* heads were similar in size to control *FRT80B/M(3)* heads (see below and data not shown). Thus, the *ago*^{1} and *H99* mutations cooperatively increase organ size. This effect was apparent in other head organs as well (e.g., antennae in Figure 2 and Figure S3). Combining *ago*^{1} and the *hid*^{05014} allele [19] was also sufficient to block C3 accumulation and increase organ size (Figures 2C and 2C′; Figure S3). Together, these data indicate that *hid*- and *H99*-dependent apoptosis of *ago*^{1} mutant cells restrains the oncogenic effect of *ago* loss in multiple tissues and that in the larval eye, this death is concentrated in an area just anterior to the MF.

ago Mutations Elevate dE2f Activity in the Furrow

ago MF-associated apoptosis resembles the pattern of death among cells lacking the *Drosophila* retinoblastoma (Rb) gene homolog *rbf1* [20], suggesting that these genes function within a common antiapoptotic pathway in cells just anterior to the MF. Like mammalian Rb, Rbf1 binds to the dE2f1 transcription factor and inhibits the expression of dE2f1 target genes (reviewed in [21]). This inhibition is reversed by G1 cyclin-dependent kinases that phosphorylate Rbf1 and dissociate it from dE2f1, allowing dE2f1 to transactivate target gene promoters. *rbf1* mutations thus upregulate expression of dE2f1 targets, including *PCNA* [22] and the proapoptotic genes *hid* [23] and *rpr* [24], which leads to MF-associated death of *rbf1* mutant cells [20].

To test whether *ago* controls *de2f* activity in the eye, we placed the dE2f reporter transgene *PCNA-GFP* [25] into the background of *ago*^{1} mosaic eye discs (Figure 3C). dE2f1 protein levels normally increased in cells just anterior to the MF (Figures 3A and 3A′), presumably as a result of a requirement for dE2f1 in Rbf1-mediated transcriptional repression.