STAT92E is a positive regulator of *Drosophila* inhibitor of apoptosis 1 (DIAP/1) and protects against radiation-induced apoptosis

Aurel Betz*, Hyung Don Ryoo†, Hermann Steller†, and James E. Darnell, Jr.*§

*Laboratory of Molecular Cell Biology and †Howard Hughes Medical Institute, Laboratory of Apoptosis and Cancer Biology, The Rockefeller University, 1230 York Ave, New York, NY 10065; and ‡Department of Cell Biology, New York University Medical Center, New York, NY 10016

Contributed by James E. Darnell, Jr., July 15, 2008 (sent for review April 1, 2008)

The proapoptotic factors Reaper, Hid, Grim, and Sickle regulate apoptosis in *Drosophila* by inhibiting the antiapoptotic factor DIAP1 (*Drosophila* inhibitor of apoptosis 1). Heat, UV light, x-rays, and developmental signals can all increase the proapoptotic factors, but the control of transcription of the *diap1* gene is unclear. We show that in imaginal discs the single *Drosophila* STAT protein (STAT92E) when activated can directly increase DIAP1 through binding to STAT DNA-binding sites in the *diap1* promoter. The STAT92E contribution to DIAP1 production is required for cell survival after x-irradiation but not under unstressed conditions. Because DIAP1 prevents apoptosis after a variety of stresses, STAT92E may have a role in regulating stress responses in general.

Jak STAT | stress | cancer | survival

The regulation of apoptosis is highly conserved in animals and is thought to limit the risk of genomic instability and cancer. Its function depends on the balanced levels of proapoptotic and antiapoptotic proteins (1).

The *Drosophila* proapoptotic factors Reaper, Hid, Grim, and Sickle [also called IAP-binding motif (IBM) proteins] are specifically increased by a variety of agents, both intrinsic developmental signals and extrinsic events (e.g., heat, UV, and ionizing radiation) (2). Inhibition by the IBM proteins of the antiapoptotic activity of DIAP1 (*Drosophila* inhibitor of apoptosis 1) (3, 4) releases Dronc (the *Drosophila* caspase 9 ortholog) and drice/dcp1 (caspase 3 ortholog), triggering apoptosis. Protein–protein interactions of DIAP1 with the IBM proteins and with caspases have received extensive examination (2, 5). However, to our knowledge, direct transcriptional regulation by specific transcription factor(s) of the widely expressed *diap1* gene has not been described.

Two of the seven human STAT family members are antiapoptotic and their overactivity is correlated with cancer, heightening the previously unrecognized antiapoptotic role of STAT92E. The simpler Jak-STAT pathway in *Drosophila* consists of the ligands unpaired 1, 2, and 3 and the receptor *domeless*, with which one Jak family kinase, *hopscotch*, is associated. Mutations in the pathway affect a wide variety of processes including a positive effect on the cell cycle (6–8).

We now report that the full-length tyrosine-phosphorylated STAT92E isoform participates in blocking apoptosis *in vivo* by acting through highly conserved STAT DNA-binding sites in the *diap1* promoter to maintain normal DIAP1 levels. In developing eye and wing discs, STAT92E, although not critical for maintaining basal levels of DIAP1 for survival under unstressed conditions, increases DIAP1 to levels required to combat stress-induced apoptosis.

Results

STAT92E Blocks X-Ray-Induced Apoptosis. To investigate any involvement of *stat92E* in apoptosis, we generated *stat92E* mutant cell clones through somatic recombination by using the

heat-shock-inducible mosaic analysis with a repressible cell marker (MARCM) system (9) (see *Materials and Methods* and Fig. 1*A*), which allows expression of DIAP1 in clones in which STAT92E had been lost (called *stat92E*^{−/−}), thus testing the effect on cell survival. Areas in the wing disc pouch (Fig. 1*A*) that were *stat92E*^{−/−} or *stat92E*^{+/+} (twin spots) were measured in each individual disc, averaged, and compared to controls without x-ray treatment or without overexpressed DIAP1 (Fig. 1*B*). Without x-irradiation, *stat92E*^{+/+} areas averaged 28% of the pouch, whereas *stat92E*^{−/−} tissue occupied only 6.4% (Fig. 1*Ba*). No obvious difference in cell size was observed; the difference was presumably attributable to cell number and likely reflected the loss of positive activity of *stat92E* in the cell cycle (6–8). In contrast, when comparable larvae were irradiated (5,000 rad daily for 3 days), *stat92E*^{+/+} areas averaged 40% and *stat92E*^{−/−} areas only 1.1% of the total pouch area (Fig. 1*Bb*). When DIAP1 was overexpressed in *stat92E*^{−/−} clones in irradiated discs, the average area rose to 7.7% (Fig. 1*Bd*), equal to or slightly higher than the *stat92E*^{−/−} area (6.4%) without irradiation (Fig. 1*Ba*). Because DIAP1 is known not to affect the cell cycle (10) and had no significant effect on the unirradiated control (Fig. 1*Bc*), we conclude that the rescue of the size of the irradiated *stat92E*^{−/−} area by DIAP1 was a result of blocking x-ray-induced apoptosis. (Clones in hinge tissue were not scored, because they were too rare even without irradiation.) Furthermore, clones induced by the same method in eye discs behaved similarly, except their differences were even more pronounced (Fig. 1*C*).

To directly observe apoptosis, we used the same genetic system as described above, but cells were stained with an antibody against a cleavage product of caspase 3 as an apoptosis marker (11) (Fig. 2*A* and *B*) 5 h after x-irradiation (5,000 rad); the percentage of caspase-positive cells in wing disc pouch areas (*stat92E*^{+/+}, *stat92E*^{+/−}, and *stat92E*^{−/−}) was then recorded (Fig. 2*C*). Unirradiated controls only very rarely had caspase-positive cells in any area regardless of genotype (data not shown). However, *stat92E*^{−/−} tissue in discs from irradiated larvae (dark areas in Fig. 2*Aa'* and *a''*) showed the most cleaved caspase (44.3% of area), whereas *stat92E*^{+/−} tissue (gray regions in Fig. 2*Aa'* and *Aa''*) was intermediate at 20.3% and *stat92E*^{+/+} (bright-white regions in Fig. 2*Aa'* and *Aa''*) had the fewest positive cells (2.3%). The apoptotic nature of cleaved caspase-positive cells was also evident by their pyknotic morphology, because GFP-marked *stat92E*^{−/−} cells often appeared as small green spots (Fig. 2*Ab'* and *Ab''*),

Author contributions: A.B., H.D.R., H.S., and J.E.D. designed research; A.B. performed research; A.B. and J.E.D. analyzed data; and A.B. and J.E.D. wrote the paper.

The authors declare no conflict of interest.

§To whom correspondence should be addressed. E-mail: darnell@mail.rockefeller.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0806291105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA

Fig. 1. The difference of *stat92E*^{+/+} vs. *stat92E*^{-/-} areas is enhanced by x-irradiation and suppressed by DIAP1 overexpression. Statistically significant differences ($P < 0.001$) are marked with asterisks. (Aa) Developmental zones of the wing disc shown in Aa'–Aa'''. MARCM clones with the *stat92E*³⁹⁷ loss-of-function (LOF) mutant. Mutant (–/–), heterozygous (+/–), and twin-spot (+/+) tissues were marked with α -STAT92E antibody (Aa'), and –/– clones were marked with GFP (Aa''). DIAP1 was expressed exclusively inside *stat92E*^{-/-} clones (Aa''' and Aa'''). (B) Averaged clonal and twin-spot areas from 50 third-instar wing pouches as percentage occupancy of the pouch area, color-coded as in A; the twin-spot vs. clone ratio is shown in gray. (C) As described for B, except the eye discs were scored.

frequently colocalizing with the apoptosis marker. In contrast, in control discs in which DIAP1 was overexpressed in *stat92E*^{-/-} clones, apoptotic cells were very rare (0.7%) inside clones (compare Fig. 2Ba' and Ba'' with Fig. 2Ab' and Ab'' and graphed in Fig. 2Cf). In these discs, the majority of apoptotic cells occurred in *stat92E*^{+/+} regions, where no overexpression of DIAP1 occurred (22.1%; Fig. 2Ba'', red cells, and Ce), and again the presence of STAT92E protected against x-ray-induced apoptosis in twin spots (Fig. 2Cd). (Similar results were obtained with another independent mutant allele, *stat92E*^{85C9}, which is not shown.) These data suggest that after x-irradiation, but not under unstressed conditions, *stat92E* plays a quantitative role in cell survival.

DIAP1 Expression Depends on Phosphorylated STAT92E. Because STAT92E, an activator of transcription, protects against x-ray-induced apoptosis, we investigated whether expression of DIAP1 was perhaps regulated by STAT92E. As described before, we compared wing disc areas of *STAT92E*^{+/+}, *STAT92E*^{+/–}, or *STAT92E*^{-/-} genotype with a DIAP1 antibody strain. There was more DIAP1 (Fig. 3A) in *stat92E*^{+/+} areas compared to adjacent *stat92E*^{+/–} tissue (compare twin spot marked +/+ to corresponding adjacent +/- tissue in Fig. 3Aa and Aa'). There was a possible further but not complete reduction of DIAP1 in the *stat92E*^{-/-} clones. (Compare *stat92E*^{-/-} regions, black in Fig. 3Aa and green in Fig. 3Aa'', in the intensity of red stain in Fig. 3Aa.) This remaining expression of DIAP1 in *stat92E*^{-/-} clones is consistent with the likelihood that a small amount of DIAP1 is controlled by some other transcription factor. This small amount of DIAP1 might still allow the survival of *stat92E*^{-/-} clones under unstressed conditions; *stat92E*^{-/-} clones are known to survive to adulthood and contribute to WT structures (7). In contrast, *diap1*-null clones are quickly eliminated through apoptosis (12, 13). Therefore, DIAP1 production depends to a significant degree, but probably not exclusively, on STAT92E.

Was there a direct role of STAT92E in DIAP1 expression? The most prominent STAT function in mammalian cells depends on tyrosine phosphorylation, dimerization, and DNA binding (supporting information (SI) Fig. S1), although some STAT transcriptional function as unphosphorylated molecules also occurs (14). To determine whether *diap1* gene activation depended on STAT92E phosphorylation, we expressed STAT92E or a *STAT92E*[Y→F] mutant specifically in the posterior wing disc compartment (see *Materials and Methods*). Overexpression of STAT92E resulted in increased DIAP1 levels in the posterior compartment (Fig. 3Ba and Bb), whereas overexpression of *STAT92E*[Y→F] did not (Fig. 3Ca and Cb). These results suggest that classic Jak-STAT signaling participates in transcription of the *DIAP1* gene.

STAT DNA-Binding Sites in the *diap1* Locus Are Required for STAT-Dependent DIAP1 Production. Using the Vista Genome Browser (15), we searched for evolutionarily conserved STAT DNA-binding sites in the *diap1* locus. Two canonical STAT-binding sites (TTCCNNGAA) (16) are present within a 1.2-kb segment 3.3 kb upstream from the most proximal putative *diap1* RNA start site. These are perfectly conserved within the Drosophilidae (Fig. 4A and B). These potential STAT DNA-binding sites were functionally tested by preparing transgenic flies bearing LacZ reporter constructs using the 1.2-kb segment with either WT STAT (WTSTDpZ) or mutant STAT (MTSTDpZ) sites (Fig. 4C).

Expression patterns of these reporter constructs in third-instar wing discs were compared (Fig. 5A). The WTSTDpZ expression in the wing disc hinge, pouch, and margin closely resembled (8 of 8 lines) the previously reported pattern of *diap1-lacZ* enhancer traps (17). In the MTSTDpZ lines, however, LacZ reporter expression (8 of 8 lines) was reduced greatly in the hinge region but not in the wing margin that traverses the pouch (Fig. 5Aa and Ab, gray arrow) with also some reduction in the main portion of the pouch. A pattern similar to that of STAT92E dependence was found when we

Fig. 2. STAT92E dosage in wing discs determines the degree of x-ray-induced apoptosis. α -Caspase 3 antibody (red), an antibody to STAT92E (white), distinguished *stat92E*^{-/-} clones (dark), twin spots (*stat92E*^{+/+}) (white), and (*stat92E*^{+/-}) tissue (gray). GFP marked the *stat92E*^{-/-} mutant clones. Overview (Aa) and enlarged frames from Aa (Aa' and Aa''): *stat92E* LOF clones (dark areas, one with pink arrow) harbor many apoptotic cells (see Aa''), whereas apoptotic cells appear only in intermediate numbers in the heterozygous cells and very rarely in the WT twin spots (one with turquoise arrow). (Ab) Overview of identical disc as in Aa, with frame enlarged in Ab' and Ab''. GFP-marked, clonal apoptotic cells can be seen as small dots (Ab') and associated with the activated caspase marker (Ab'') (red), some double-labeled in yellow. (Ba) Control overexpressing DIAP1 in *stat92E*^{-/-} clonal cells (enlarged frames in Ba' and Ba''). The clonal cells, GFP-marked in Ba', rarely stained with activated caspase antibody (red in Ba'') because they were protected from apoptosis by high concentrations of DIAP1. (Ba'') Apoptotic cells, in red, were mostly in *stat92E*^{+/-} areas (in the white channel, not shown). (C) Quantification of activated caspase-positive areas within areas of *stat92E*^{-/-} (Cc and Cf), heterozygous (Cb and Ce), or *stat92E*^{+/+} tissue (Ca and Cd) of the type shown in A and B (*n* > 20 wing disc pouches of each genotype). (Ca–Cc) Genotype as in A; (Cd–Cf) genotype as in B. *stat92E*^{-/-} clones overexpressing DIAP1 in f suppress apoptosis. The asterisks mark significant differences of *P* < 0.001.

generated *stat92E* mutant clones (genetically as before) in the WTSTDpZ background to test whether STAT92E was responsible for the observed difference in reporter expression (Fig. 5B).

In the hinge (left box of Fig. 5 *Ba*, *Ba'*, and *Ba''* and enlarged in Fig. 5 *Bb* and *Bb'*), WTSTDpZ reporter expression was moderately reduced in *stat92E*^{+/-} regions and strongly reduced

Fig. 3. STAT92E contributes to DIAP1 protein expression by a tyrosine 704-dependent mechanism. DAPI is in gray; *stat92E*^{-/-} MARCM clones are shown. (A) Partial dependence of DIAP1 expression levels on STAT92E dosage. (Aa and Aa') A reduction of DIAP1 can be seen in the pouch and the hinge in +/- regions compared with WT levels in twin spots (marked +/+) and a further, more moderate reduction of DIAP1 in -/- clones (one marked -/-). (B) en-Gal4 drives expression of *uas-stat92E* in the posterior engrailed domain (Ba') resulting in increased DIAP1 levels only in the posterior domain (Ba). (C) In contrast, en-Gal4-driven *uas-stat92E*[Y-F] overexpression in the posterior domain (Ca') does not increase DIAP1 (Ca).

Fig. 4. The *diap1* locus has two conserved STAT DNA-binding sites. (A) DNA alignment of seven *Drosophila* species with *Drosophila melanogaster*. The transcriptional direction is right to left, shown on the black ORF line (28). Neighboring genes are only partially shown. The STAT sites (green), located at -3.3 and -4.3 kb upstream from the most proximal conceptual transcription start site, are contained within a highly conserved cluster of ≈1.2 kb, delineated by the box. (B) DNA sequence alignment surrounding the two STAT-binding sites of the *Drosophila* species shown in A. (C) Transgenic *diap1*(1.2 kb)*lacZ* reporter constructs. The vector contains a basal hs promoter (arrow) and insulator elements to prevent position effects.

in mutant *stat92E*^{-/-} clones. In the pouch in Fig. 5 Bc and Bc', the reporter expression was moderately reduced in *stat92E*^{-/-} clones and in the *stat92E*^{+/-} section but was strong in the cells of the *stat92E*^{+/+} genotype.

In contrast, in the wing margin (the bright-red vertical stripe marked by the gray arrow in Fig. 5 Ba, Bc, and Bc'), several cells still expressed the reporter with little if any reduction (hence their yellow color). Thus, the WTSTDpZ expression partially depended on STAT92E, and this tissue-specific dose-response behavior to variations in STAT92E concentration was similar to that of the DIAP1 protein (Fig. 3A). In younger wing discs (second to third instar), WTSTDpZ expression in the pouch appeared somewhat more dependent on STAT92E than in the third-instar tissue (Fig. S2), possibly reflecting the underlying shift in the STAT92E activation pattern based on the gradually shifting distribution of the ligand *upd* during development (7, 18). A control in which *stat92E*^{-/-} clones were induced in the MTSTDpZ background had no effect on reporter expression (data not shown).

To further test the specificity of the STAT DNA-binding sites, we compared the response of WTSTDpZ and MTSTDpZ to STAT92E expression in the posterior wing disc domain (Fig. 5C). WTSTDpZ expression, but not MTSTDpZ expression, was increased in response to *en>STAT92E* (compare red α -LacZ in Fig. 5 Ca and Cb). Together these data indicate that signals from the LacZ reporter partially depended on STAT92E in a domain-specific manner and that the conserved STAT DNA-binding sites in the *diap1* promoter are required for full *diap1* transcriptional activity *in vivo*.

Overexpressed STAT92E Is Antiapoptotic in Eye Discs. After establishing that STAT92E-dependent DIAP1 production occurred in several other tissues including the eye disc (Fig. S3), we tested whether overexpressed STAT92E was able to block

apoptosis in the fast dividing cells of the first mitotic wave in the eye disc. These cells normally undergo rapid apoptosis when irradiated with x-rays (19, 20) (Fig. 6Ab). When STAT92E[Y→F] was overexpressed in clones (*SI Text*) crossing this line of cells, no statistically significant effect on apoptosis frequency was seen (Fig. 6Ab'-Ab''') and scored in Fig. 6 Bc and Bd). In contrast, when WT STAT92E was expressed in the same manner, apoptosis was significantly suppressed in the cells located in the mitotic wave (Fig. 6 Aa-Aa''', scored in Fig. 6 Ba and Bb). A control experiment in which cell cycle activity was scored with a phospho-histone H3 antibody showed that the reduction of apoptosis was not perhaps caused by an indirect effect of a decreased cell cycle (Fig. S4a scored in Fig. S4b). An analogous irradiation experiment in wing discs where *en>stat92E* or *en>stat92E*[Y→F] was overexpressed yielded a similar result (data not shown). We conclude that in fast dividing cells after x-irradiation, overexpressed STAT92E can provide a significant increase in apoptotic resistance to levels exceeding those of WT through the direct up-regulation of *diap1* transcription.

Discussion

This study reveals a role for STAT92E in protecting imaginal disc cells against x-ray-induced apoptosis. Earlier studies showed that x-rays stimulate the p53 response, leading to direct transcriptional increase of Reaper. Reaper then causes DIAP1 degradation, a process that activates caspases, leading to apoptosis (refs. 21 and 22 and Fig. S5). Our experiments show that tyrosine-phosphorylated STAT92E directly regulates DIAP1 levels in several tissues through conserved STAT DNA-binding sites in the *diap1* promoter, significantly contributing to total WT DIAP1 production (Fig. S6). Thus,

Fig. 5. STAT92E directly activates the *diap1* promoter via two conserved STAT DNA-binding sites. (A) Comparison of expression patterns of the transgenic *diap1*(1.2 kb)*lacZ* reporter constructs. (Aa) Expression pattern from WTSTDpZ. (Ab) LacZ expression from MTSTDpZ is almost completely lost in the hinge region (compare white arrow in Aa to star in Aa) and is partially reduced in the pouch but is unchanged in the wing margin (horizontal stripe marked by the gray arrow). DAPI is in gray. (B) MARCM *stat92E* clones in the background of the WTSTDpZ reporter. (Ba–Ba'') Lower power of which enlargements of white frames are shown in Bb, Bb', Bc, and Bc'; OF, out of focus area. (Bb and Bb') In the hinge, reporter expression levels strongly depend on STAT92E. (Bc and Bc') In the pouch, expression was moderately reduced from WT *stat92E*^{+/+} to *stat92E*^{+/-} tissue along with a further subtle reduction in *-/-* clones. The gray arrow marks the wing margin. (C) WTSTDpZ activity requires the WT STAT DNA-binding sites. Overexpressed STAT92E in the posterior wing disc activates WTSTDpZ (Ca and Ca') but not the MTSTDpZ reporter (Cb and Cb').

activated STAT92E presumably participates in controlling the general sensitivity of cells to proapoptotic stimuli.

In *stat92E*^{-/-} cells, the DIAP1 protein, a *diap1-lacZ* enhancer trap (17), and the WTSTDpZ reporter are all expressed at much lower but still detectable levels, and these cells are viable. It is known that a low constitutive level of proapoptotic activity is generated by the mitochondrial pathway and Dronc even under unstressed conditions (2). Therefore, it is reasonable to assume that in unstressed cells, low levels of DIAP1 are still sufficient to prevent caspase activation and that under these conditions the Jak-STAT pathway is not implicated in preventing apoptosis. This is consistent with our finding that unlike *diap1*-null clones, which are known to undergo apoptosis even under unstressed conditions (4), STAT92E-deficient clones (even though reduced in size presumably by a slowed cell cycle) survive normally and without increased apoptosis (Fig. S6). In contrast, under stress conditions, which lead to up-regulation of proapoptotic IBM proteins, the contribution of the Jak-STAT pathway to the full WT complement of DIAP1 becomes crucial for survival (Fig. S7).

diap1 activation by STAT92E was clearly tissue- or cell-specific, possibly reflecting the differential activation state of the Jak-STAT pathway. In younger wing discs, WTSTDpZ reporter expression seemed to depend more on STAT92E than in the late third-instar wing discs; in the hinge region, expression depended on STAT92E throughout development, yet there was little or no dependency on STAT92E in the developing wing margin. Because both *diap1* enhancer traps and the MTSTDpZ reporter pattern indicated strong STAT92E-independent expression in the wing margin, other transcription factors probably

contribute to *diap1* transcription, even from the 1.2-kb *diap1* enhancer sequence (and likely through enhancers outside this region).

In fact, one other pathway that affects *diap1* transcription in third-instar wing discs has been implicated indirectly. The serine kinase Hippo negatively regulates the output of *diap1-lacZ* enhancer traps, likely through inhibiting the transcriptional coactivator Yorkie. However, the positive-acting DNA-binding transcription factor that would work in conjunction with Yorkie has not been identified (23). Because the Hippo pathway and, as we show here, the Jak-STAT pathway regulate both the cell cycle and apoptosis, it is possible that STAT92E and Yorkie act combinatorially to regulate common transcriptional targets or, alternatively, that STAT92E is a DNA-binding partner of Yorkie.

The previous model of apoptosis in *Drosophila* holds that regulation of apoptosis is achieved mostly through the magnitude of proapoptotic stimuli signaling via the IBM proteins, which result in DIAP1 degradation followed by release of caspase inhibition (2). However, our results call attention to the importance of regulating DIAP1 concentrations, thus determining the sensitivity to the proapoptotic stimuli. DIAP1 is well known for its ability to block apoptosis induced by a wide variety of proapoptotic stimuli (in addition to x-rays used by us for experimental simplicity) transmitted by all major proapoptotic pathways (e.g., the death receptor, the mitochondrial, and the IBM pathway) and in most cell types (2, 5). Therefore, it is highly likely that as long as STAT92E makes a critical contribution to the total DIAP1 level in a tissue, protection against apoptosis from these intrinsic and extrinsic stimuli also requires the STAT92E-dependent stimulation of *diap1* transcription. There-

Fig. 6. STAT92E in the eye disc suppresses x-ray-induced apoptosis. (Aa) After x-irradiation, the activated caspase antibody (red) stains apoptotic eye disc cells in the first mitotic wave. (Aa') A clone overexpressing STAT92E[Y→F] (green in white box) crossing the line of the first mitotic wave does not inhibit apoptosis. However, when WT STAT92E is overexpressed in the same manner, apoptosis is suppressed (compare arrowhead in Aa and location of STAT92E gain-of-function (GOF) clone in Aa') (green in white box). (B) Quantification of A and B; gray columns were set at 100%. Significant suppression in the number of cleaved caspase-positive cells can be seen inside STAT92E GOF clones (column b) compared with equivalent outside WT areas (column a) ($P < 0.001$; $n = 50$). No significant difference was seen between inside STAT92E[Y→F] GOF clones (column d) and outside areas (column c) ($n = 30$).

fore, at least in flies, we propose a general *in vivo* role for the Jak-STAT pathway in protecting against stress-induced apoptosis (Figs. 1 and S7).

Our major conclusions about the role of the *Drosophila* STAT leading to resistance to x-ray-induced apoptosis suggests such a possibility in mammalian cells. Abnormally high levels of IAPs and persistently activated STAT3 and STAT5 are known to be oncogenic in human cancers, and such cells display enhanced resistance to stress-induced apoptosis (e.g., by chemotherapeutic agents) (24, 25). Because conserved STAT DNA-binding sites can be found in some of the mammalian IAP promoters, it will be worthwhile to investigate the possibility of an evolutionarily conserved STAT-IAP connection.

Materials and Methods

Fly Strains and Crosses. To induce MARCM, clones *FRT82B*, *stat92E*³⁹⁷ or *FRT82B*, *stat92E*^{89C5} (26) with or without *uas-diap1* on the second chromosome were crossed to *uas-GFP*, *hs-Flp*; *tub-Gal4*, *FRT82B*, *tub-Gal80/TM6B* flies, and F₁ first-instar larvae were heat-shocked at 37°C for 2 h. Twenty-four hours later we gave the first of three (wing discs) or four (eye discs) daily pulses of x-rays (5,000 rad). At the third-instar stage, wing imaginal discs were assayed.

diap1 was subcloned into pUAST to generate *uas-diap1*. The *uas-stat92E*[Y704→F] mutant was cloned and injected by standard methods or by BestGene. *uas-stat92E* was provided by Douglas A. Harrison (University of Kentucky, Lexington, KY). Flip-out clones generating *uas* transgenic lines (15) were crossed to a *yw*, *hs-flp122*; *tubulin*α > *y*+, *GFP*>*Gal4* line (Bloomington Stock Center), heat-shocked for 30 min at 37°C. The pPelican vector for reporter strains was from the Posakony Laboratory at UCSD, San Diego, CA.

Immunohistochemistry. The following antibodies were used: a guinea pig α-coiled-coil Stat92E antibody (1:30), a rabbit α-DIAP1 antibody (1:1,000) (17), and a rat α-NT-Stat92E antibody directed against the N terminus (27). Commercially available antibodies were the polyclonal mouse anti-β-galactosidase (Sigma) (1:2,000), the rabbit α-human cleaved caspase 3 antibody (1:50) (Cell Signaling Technology), and secondary fluorescent antibodies (Jackson ImmunoResearch).

ACKNOWLEDGMENTS. We thank Denise Montell for the *stat92E*³⁹⁷ and 85C9, Melissa Henriksen for the *uas-stat92E*[Y→F] construct, and Doug Harrison for the *uas-stat92E* stock. This work was supported by the Howard Hughes Medical Institute (H.S.) and by National Institutes of Health Grants AI34420 and AI32489 (to J.E.D.).

- Wahl GM, Carr AM (2001) The evolution of diverse biological responses to DNA damage: Insights from yeast and p53. *Nat Cell Biol* 3:E277–E286.
- Kornbluth S, White K (2005) Apoptosis in *Drosophila*: Neither fish nor fowl (nor man, nor worm). *J Cell Sci* 118:1779–1787.
- Goyal L, McCall K, Agapite J, Hartwig E, Steller H (2000) Induction of apoptosis by *Drosophila* reaper, hid and grim through inhibition of IAP function. *EMBO J* 19:589–597.
- Wang SL, Hawkins CJ, Yoo SJ, Müller HA, Hay BA (1999) The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* 98:453–463.
- Tittel JN, Steller H (2000) A comparison of programmed cell death between species. *Genome Biol* 1:REVIEWS0003.
- Bach EA, Vincent S, Zeidler MP, Perrimon N (2003) A sensitized genetic screen to identify novel regulators and components of the *Drosophila* janus kinase/signal transducer and activator of transcription pathway. *Genetics* 165:1149–1166.
- Mukherjee T, Hombria JC, Zeidler MP (2005) Opposing roles for *Drosophila* JAK/STAT signalling during cellular proliferation. *Oncogene* 24:2503–2511.
- Tsai YC, Sun YH (2004) Long-range effect of upd, a ligand for Jak/STAT pathway, on cell cycle in *Drosophila* eye development. *Genesis* 39:141–153.
- Wu JS, Luo L (2006) A protocol for mosaic analysis with a repressible cell marker (MARCM) in *Drosophila*. *Nat Protoc* 1:2583–2589.
- Hay BA, Huh JR, Guo M (2004) The genetics of cell death: Approaches, insights and opportunities in *Drosophila*. *Nat Rev Genet* 5:911–922.
- Srinivasan A, et al. (1998) *In situ* immunodetection of activated caspase-3 in apoptotic neurons in the developing nervous system. *Cell Death Differ* 5:1004–1016.
- Hay BA, Wassarman DA, Rubin GM (1995) *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. *Cell* 83:1253–1262.
- Ryoo HD, Gorenc T, Steller H (2004) Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. *Dev Cell* 7:491–501.
- Yang J, et al. (2005) Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation. *Cancer Res* 65:939–947.

- Loots GG, Ovcharenko I, Pachter L, Dubchak I, Rubin EM (2002) rVista for comparative sequence-based discovery of functional transcription factor binding sites. *Genome Res* 12:832–839.
- Yan R, Small S, Desplan C, Dearolf CR, Darnell JE, Jr (1996) Identification of a Stat gene that functions in *Drosophila* development. *Cell* 84:421–430.
- Ryoo HD, Bergmann A, Gonen H, Ciechanover A, Steller H (2002) Regulation of *Drosophila* IAP1 degradation and apoptosis by reaper and ubcd1. *Nat Cell Biol* 4:432–438, and erratum (2002) 4:546.
- Bach EA, et al. (2007) GFP reporters detect the activation of the *Drosophila* JAK/STAT pathway *in vivo*. *Gene Expr Patterns* 7:323–331.
- Jassim OW, Fink JL, Cagan RL (2003) Dmp53 protects the *Drosophila* retina during a developmentally regulated DNA damage response. *EMBO J* 22:5622–5632.
- Ahmad K, Golic KG (1999) Telomere loss in somatic cells of *Drosophila* causes cell cycle arrest and apoptosis. *Genetics* 151:1041–1051.
- Brodsky MH, et al. (2000) *Drosophila* p53 binds a damage response element at the reaper locus. *Cell* 101:103–113.
- Steller H (2000) *Drosophila* p53: Meeting the grim reaper. *Nat Cell Biol* 2:E100–E102.
- Dong J, et al. (2007) Elucidation of a universal size-control mechanism in *Drosophila* and mammals. *Cell* 130:1120–1133.
- LaCasse EC, Baird S, Korneluk RG, MacKenzie AE (1998) The inhibitors of apoptosis (IAPs) and their emerging role in cancer. *Oncogene* 17:3247–3259.
- Bowman T, Garcia R, Turkson J, Jove R (2000) STATs in oncogenesis. *Oncogene* 19:2474–2488.
- Silver DL, Montell DJ (2001) Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in *Drosophila*. *Cell* 107:831–841.
- Henriksen MA, Betz A, Fucillo MV, Darnell JE, Jr (2002) Negative regulation of STAT92E by an N-terminally truncated STAT protein derived from an alternative promoter site. *Genes Dev* 16:2379–2389, and erratum (2002) 16:2729.