Variegated Expression of Hsp22 Transgenic Reporters Indicates Cell-specific Patterns of Aging in Drosophila Oenocytes

John Tower, Gary Landis, Rebecca Gao, Albert Luan, Jonathan Lee, and Yuanyue Sun

The cytoplasmic chaperone gene Hsp70 and the mitochondrial chaperone gene Hsp22 are upregulated during normal aging in Drosophila in tissue-general patterns. In addition, Hsp22 reporters are dramatically upregulated during aging in a subset of the oenocytes (liver-like cells). Hsp22 reporter expression varied dramatically between individual oenocytes and between groups of oenocytes located in adjacent body segments, and was negatively correlated with accumulation of age pigment, indicating cell-specific and cell-lineage-specific patterns of oenocyte aging. Conditional transgenic systems were used to express 88 transgenes to search for trans-regulators of the Hsp70 and Hsp22 reporters during aging. The wingless gene increased tissue-general upregulation of both Hsp70 and Hsp22 reporters. In contrast, the mitochondrial genes MnSOD and Hsp22 increased expression of Hsp22 reporters in the oenocytes and decreased accumulation of age pigment in these cells. The data suggest that cell-specific and cell lineage–specific patterns of mitochondrial malfunction contribute to oenocyte aging.

Key Words: Mitochondria—Age pigment—Mosaic—Cell lineage—Prepattern.

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IDENTIFYING biomarkers of aging that variegate between different individuals (1–5) and between different cells and tissues within an individual is of particular interest as it may help explain the large and apparently stochastic variation observed in life-span and aging phenotypes (6,7). The changes in gene expression that occur during normal aging in Drosophila are similar to an oxidative and proteotoxic stress response (1,8–11), including upregulated expression of the cytoplasmic chaperone Hsp70 (12) and the mitochondrial chaperone Hsp22 (13,14). Upregulation of Hsp70 and Hsp22 transgenes during aging requires functional heat shock factor (HSF)-binding sites (Heat Shock Elements, HSEs) in the gene promoters (13,15), consistent with activation of the unfolded protein response. The time course of Hsp gene upregulation during aging scales with life span as altered by temperature or oxidative stress (12). Moreover, the expression of Hsp70-GFP and Hsp22-GFP transgenic reporters in young individual flies is partially predictive of remaining life span (2), indicating that both these genes are biomarkers of Drosophila aging (16).

In addition to the common features of Hsp70 and Hsp22 expression, several results have suggested that Hsp22 may have an additional regulatory input during aging (13). For example, Hsp22 reporters exhibit a greater fold induction when the fly is challenged with oxidative stress (1). In addition, Hsp22 expression is increased during the first half of adult life in strains that have been genetically selected for increased life span (17). Finally, MnSOD overexpression can extend the life span of adult flies (18), and this is associated with increased expression of Hsp22, but not Hsp70 (19). These observations prompted additional analysis of the regulation of Hsp70 and Hsp22 expression during aging.

The oenocytes are large, postmitotic, and metabolically active cells that appear similar to hepatocytes because they carry out liver-like lipid processing reactions. These reactions include ketogenesis during the fasting response in larvae (20) and synthesis of cuticular hydrocarbons in adults, including ones that mark species and sexual identity (21,22) and that change in composition and function during aging (23–25). The oenocytes derive their name from the Greek word for wine (oenos) because these cells dramatically accumulate dark-colored age pigment as a byproduct of cellular metabolic activity. Pigment accumulation during aging is also observed in mammalian tissues including the liver (26–28). The oenocytes are located in the abdomen of the fly, immediately beneath the translucent cuticle, and are arranged in clusters along the ventral midline and in a row along the dorsal side of each abdominal segment. The large size, characteristic arrangement, and age pigment accumulation facilitates the visualization of these cells in live flies. In this study, we report that Drosophila oenocytes display variegated expression of the aging biomarkers Hsp22 and age pigment, indicating cell-specific and cell lineage–specific patterns of mitochondrial failure during aging.
METHODS

Drosophila Culture and Strains

The Hsp70-GFP, Hsp70-DsRED, Hsp22-GFP, and Hsp22-DsRED reporter strains are as previously described (2), and the Hsp22-LacZ reporter strain and in situ beta-galactosidase assay are as previously described (13). The Desat1-GAL4 driver lines were generously provided by J. Levine (21), and the UAS-DsRED[A] reporter line was obtained from Bloomington Drosophila Stock Center. The Tet-on system driver strain w[1118]; rtTA(3)[E2] and the Gene-Switch system driver strain w[1118]; Act-GS[255B] as well as conditions for use of the systems are as previously described (29). Strains containing multiple transgenes were generated by recombination and/or by appropriate crosses to double-balancer strains. Transgenic strains used in the genetic screen are listed in Supplementary Table S1.

Genetic Screen for Trans-regulators of Hsp Reporter Expression During Aging

Each of the 88 transgenes screened was assayed for its effects on both Hsp22-GFP reporter expression and on Hsp70-GFP reporter expression, as follows. Each of 57 Tet-on system transgenic strains was crossed to each of two Tet-on system “tester” strains: w[1118]; Hsp22-GFP[1MI1], rtTA(3)[E2]/TM3 Sb and w[1118]; Hsp70-GFP[1MI2], rtTA(3)[E2]/TM3 Sb. Each of 31 Gene-Switch system transgenic strains was crossed to each of two Gene-Switch system “tester” strains: w[1118]; Act-GS[255B]; Hsp22-GFP[1MI1]/TM3 Sb and w[1118]; Act-GS[255B]; Hsp70-GFP[2MI4]/TM3 Sb. From each cross, 160 progeny were scored that contained the three desired transgenes (the driver, the Hsp reporter, and the target for overexpression). From these 160 flies, 40 males were maintained minus drug, 40 males plus drug, 40 females minus drug, and 40 females plus drug; at a density of approximately 20 flies per vial. Flies were passaged to fresh positive/negative drug media three times per week and were scored by fluorescent microscopy at ages 7, 30, and 60 days. Crosses of the “tester” strains to the w[1118] strain were used to generate control cohorts containing the driver transgene and Hsp reporter, but no overexpressed transgene, and these flies were used for comparison to experimental crosses and to control for any possible effects of drug treatment. All crosses were conducted with the “tester” strains as the female parent, except for crosses involving transgenes on the X chromosome. Strains scored as positive were confirmed in repeat experiments, and results were quantified as described below.

Fluorescent Imaging and Quantification

Fluorescent images and overlays were generated using live flies anesthetized with humidified CO2 gas, and the Leica MZFLIII fluorescence stereomicroscope and Spot imaging software (2). Quantification of fluorescence in flies was performed using more than or equal to six flies per sample. Flies were photographed from a ventral angle, and mean fluorescence intensity was determined using Image J software; the eye and any portions of the body occluded by the wing were excluded from analysis. The mean and standard deviation were determined for each sample, and experimental and control samples were compared using unpaired, two-sided t-tests. Additional quantification of fly and oenocyte fluorescence was conducted using Green Fluorescent Protein (GFP) video analysis and FluoroScore software (30), and by counting the number of Hsp22-GFP-positive oenocyte sectors in cohorts of flies. To estimate in vivo superoxide levels, transgenic flies containing the Hsp22-GFP[1MI1] reporter were dissected at 60 days of age according to the procedure of Krupp and Levine (31) to generate abdominal walls with attached oenocytes and fat-body tissue. The fat-body was partly dissected away to reveal regions of the oenocytes, and the tissues were incubated for 30 minutes at 25°C in media containing 5-μm MitoSOX-Red dye (Invitrogen Life Technologies).

RESULTS

Hsp Reporters are Upregulated During Aging in Tissue-General Patterns

The expression of the Hsp22-GFP and Hsp70-GFP reporters were examined in live, anesthetized flies at young age (6 day old) and old age (60 day old) (Figure 1A). Both the Hsp70-GFP reporter (Figure 1A) and the Hsp22-GFP reporter (Figure 1B) were upregulated in tissue-general patterns, particularly muscle and nervous tissue, as previously observed (2), and this expression pattern was slightly greater in males than in females (Supplementary Figure S1).

Hsp22 Reporters Are Upregulated During Aging in the Oenocytes

The Hsp22-GFP reporters were also found to be dramatically upregulated during aging in a subset of the oenocytes (Figure 1B; indicated with white arrow) and this pattern was not observed with multiple Hsp70 reporters. Induction of Hsp22 reporters in the oenocytes was slightly greater in females than in males (Supplementary Figure S1B; quantification in Supplementary Figure S5). Hsp22-GFP expression in oenocytes was absent in young flies (0–20 days) and first became apparent at approximately 20–30 days in a semirandom pattern involving individual oenocytes scattered throughout the abdomen (Figure 2B; Supplementary Figure S1B), as well as in groups of adjacent oenocytes; often all of the oenocytes in a row along one (or sometimes both) side of a (random) abdominal segment were positive (Figure 1B; see also Figure 2E and quantification in Supplementary Figure S5). The intensity of reporter expression continued to increase with age,
and additional positive cells sometimes became apparent at later ages. The number of positive oenocytes varied greatly from fly-to-fly, and flies were never observed with all the oenocytes marked. Which oenocytes and which abdominal segment(s) were affected appeared to be random in males, whereas in females there was a slight preference for oenocytes located near the ventral midline of the most ventral segments (Supplementary Figure S1B, indicated with asterisk). When multiple oenocytes in an abdominal segment were marked, the oenocytes most distal to the dorsal midline (ie, at the end of the row of oenocytes) tended to have the greatest levels of expression (see Figures 1B, 2E and 4A). A similar pattern of expression was observed with additional independent transgenic lines of the Hsp22-GFP reporter and with multiple independent transgenic lines of the Hsp22-DsRED reporter (data not shown), as well as with Hsp22-LacZ reporter strains that contain more extensive Hsp22 gene sequences (Supplementary Figure S2C), thereby demonstrating that expression of Hsp22 reporters in a subset of the oenocytes is not simply a consequence of a particular chromosomally insertion site, construct design, or genetic background. The dramatic upregulation of Hsp22 reporters in a subset of the oenocytes was unique to aging, as it was not observed upon acute induction of the Hsp22 reporters with heat stress ([2] and additional data not shown).

**Variegated Expression of Hsp22 Reporters by Cell and by Body Segment**

To confirm the identification of the Hsp22-GFP-positive cells as oenocytes, the oenocytes were specifically marked with Discosoma sp. red-fluorescent protein (DsRED) fluorescence by adding the oenocyte-specific driver construct Desat1-GAL4 [21] and a UAS-DsRED reporter construct (Figure 2A). A ventral view of the abdomen is presented to highlight the clusters of oenocytes arranged along the ventral midline (arrows). Dramatic induction of the Hsp22-GFP reporter was observed in a subset of the oenocytes at age 35 days (Figure 2B, indicated with asterisks). Overlay
of the DsRED and GFP images reveals Hsp22-GFP-positive oenocytes in yellow color adjacent to oenocytes with no expression of Hsp22-GFP (Figure 2C), thereby demonstrating cell-specific patterns of Hsp22 induction during aging in the oenocytes. Similarly, Hsp22-GFP induction often occurred in all of the oenocytes of one abdominal segment (Figure 2E, indicated with asterisk), whereas oenocytes in adjacent body segments remained largely or completely negative (arrows). Because the oenocytes in each abdominal segment represent a distinct developmental cell lineage (32–34), this pattern suggests cell lineage–specific induction of the Hsp22 reporter in the oenocytes during aging.

A Genetic Screen for Trans-regulators of Hsp Gene Expression During Aging

To further characterize the regulation of Hsp gene induction during aging, 88 transgenes (Supplementary Table S1) representing 57 genes were overexpressed specifically in adult flies in a tissue-general pattern. Overexpression was produced using the Tet-on and/or Gene-Switch conditional systems, in genetic backgrounds containing the Hsp22-GFP and Hsp70-GFP reporters, in cohorts of more than or equal to 160 flies for each transgene. Overexpression of wingless increased the tissue-general pattern of Hsp70-GFP and Hsp22-GFP reporter expression during aging (Figure 3A; Supplementary Figure S3), consistent with the ability of wingless overexpression in adult flies to dramatically decrease life span (35) and the implication of the wingless pathway in senescence pathways in other systems (36–38); although notably the oenocyte-specific induction of Hsp22-GFP during aging was not increased by wingless (Figure 3A and additional data not shown). None of the other transgenes tested had a detectable effect on the tissue-general expression pattern of the Hsp reporters, including several that dramatically decrease life span when overexpressed (eg, ras-activated-form, doublesex-F, fruitless-MA) (35,39) and several previously reported to have favorable effects on life span.

Two genes were found to dramatically increase the preferential expression of Hsp22-GFP reporters in the aging oenocytes: the mitochondrial gene MnSOD (also called SOD2; Figure 3B) and Hsp22 itself (Supplementary Figure S4; quantification in Supplementary Figure S5). Both MnSOD overexpression and Hsp22 overexpression increased the number of Hsp22-GFP-positive oenocytes, and appeared to increase the amount of Hsp22-GFP expression in each oenocyte, with relatively greater effects observed for MnSOD.

Hsp22 Reporter Expression Is Negatively Correlated With Accumulation of Age Pigment

The accumulation of brown age pigment in the oenocytes is readily apparent by age 30 days (Supplementary Figure S2A) and continues to accumulate with advancing age (Figure 4A). The age pigment in the oenocytes did not fluoresce upon stimulation with 365 nm light, suggesting that it is chemically distinct from the fluorescent advanced-glycination end-products that accumulate with age in other tissues and are readily detected by fluorescence (Supplementary Figure S2B) (26,40). Strikingly, in every case, Hsp22-GFP-positive oenocytes had reduced accumulation of age pigment relative to Hsp22-GFP-negative oenocytes (Figure 4A). Oenocytes with reduced age pigment were also observed in old flies from additional laboratory and wild-type strains (data not shown), indicating that this variability in age pigment is not unique to a particular genetic background. In addition, both MnSOD and Hsp22 overexpression dramatically decreased age pigment accumulation in the oenocytes (Figure 3B; Supplementary Figure S4). No other transgenes, including Cu/ZnSOD, were found to affect age pigment accumulation in the oenocytes, either positively or negatively. To rule
out any possible effects of fluorescent protein expression on age pigment accumulation, both the Tet-on and GAL4/UAS systems were used to drive high-level expression of GFP and DsRED reporters in the oenocytes, and this had no detectable effect on age pigment; moreover, MnSOD overexpression decreased oenocyte age pigment in the absence of GFP expression (data not shown).

To investigate cellular levels of oxidative stress, abdominal wall tissues from 60-day-old flies containing the Hsp22-GFP reporter were stained with the superoxide indicator MitoSOX-Red dye (Supplementary Figure S2D–F). Hsp22-GFP-positive oenocytes had no detectable enrichment for MitoSOX-Red staining (Supplementary Figure S2G–I), and often appeared to have reduced signal relative to adjacent Hsp22-GFP-negative oenocytes (Supplementary Figure S2J–L).

**DISCUSSION**

Both Hsp70 and Hsp22 transgenic reporters are upregulated during aging in tissue-general patterns and this pattern requires functional HSEs in the gene’s promoters, consistent with activation through HSF and the cytoplasmic unfolded protein response (16). In addition, the Hsp22 reporters were also dramatically upregulated during aging in a subset of the oenocytes in a cell-specific and cell lineage–specific pattern. This pattern suggests that the affected oenocytes are aging more quickly and/or differently than their neighbors. The number of positive oenocytes varied dramatically from fly-to-fly, and it will be of interest in the future to determine if this variation might correlate with individual animal life span. Because Hsp22 encodes a mitochondrial chaperone (14) that is robustly induced in response to disrupted mitochondrial protein synthesis (41), and Hsp22 reporter expression in the oenocytes was negatively correlated with the metabolic markers age pigment and superoxide, the data indicate cell-specific patterns of mitochondrial malfunction in the oenocytes during aging.

The results of the genetic screen further support the link between Hsp22 expression, age pigment accumulation, and mitochondrial function during oenocyte aging. The wingless gene increased the tissue-general expression of the Hsp70 and Hsp22 reporters during aging, but did not increase the preferential expression of Hsp22 reporters in the oenocytes. Similarly, overexpression of dHSF increases the tissue-general expression of Hsp70 and Hsp22, but not the oenocyte expression pattern of Hsp22 (unpublished observations). The oenocyte pattern of Hsp22 expression was increased only by two mitochondrial genes, MnSOD and Hsp22 itself, thereby supporting the link between Hsp22 expression and mitochondrial function; moreover, both MnSOD and Hsp22 overexpression correspondingly reduced oenocyte age pigment accumulation. Hsp22 localizes to the mitochondrial matrix (14); however, its specific targets in that compartment remain unknown. The negative correlation between Hsp22-GFP expression and markers of metabolic activity does not inform on cause and effect; however, one possibility is that Hsp22 and other components of the mitochondrial unfolded protein response act to repress mitochondrial metabolic activity and therefore reduce production of age pigment and superoxide (Figure 4B). Such a model is consistent with the observation that MnSOD overexpression induces Hsp22 expression preferentially in the oenocytes (Figure 3B) and reduces adult fly metabolic activity (19).

Both MnSOD and Hsp22 have either positive or negative effects on fly life span depending on the level, life cycle stage, and tissue-specificity of overexpression (16,29,42), and it is possible that the life span increases may involve hormetic effects of these genes on mitochondrial function. Consistent with this idea, when life span is extended by either MnSOD overexpression (19) or Hsp22 overexpression (42), it is associated with increased expression of...
mitochondrial genes that are normally downregulated during aging (1,8). Similarly, in Caenorhabditis elegans, tissue-general overexpression of the mitochondrial MnSOD gene sod-2 increases life span through a mechanism dependent on the stress–response transcription factor DAF-16, which is a positive regulator of Hsp gene expression (43). Interestingly, manipulations that increase mammalian life span are often associated with changes in liver mitochondrial gene expression and morphology (44,45) supporting the importance of this tissue for aging phenotypes.

Mitochondrial malfunction is implicated in aging in several Drosophila tissues, including gut (46), muscle, and nervous tissue (47,48). One appealing model is that the consequences of mitochondrial malfunction are partly tissue specific, for example, a mitochondrial unfolded protein response and reduced metabolic activity in a subset of the oenocytes versus increased oxidative stress and a cytoplasmic unfolded protein response in muscle tissue (12,16,47,49,50). The tissue-general expression of Hsp70-GFP and Hsp22-GFP reporters was relatively greater in males (Supplementary Figure S1), whereas the oenocyte pattern for Hsp22-GFP was relatively greater in females, in two different control genetic backgrounds (Supplementary Figure S5). Sex-specific metabolism is common across species and may be particularly costly for aging (44,48,51–55), and therefore the fact that the oenocytes are especially sex dimorphic in their metabolism (21–25) may contribute to the observed mitochondrial failure. Investigating possible tissue-specific responses to mitochondrial malfunction will be an interesting area for further research.

The cell lineage patterns of Hsp22 and age pigment observed in aging oenocytes suggest that mitochondrial failure is due, at least in part, to a change that is heritable at the cellular level and that occurs during the development of these postmitotic cells (Figure 4C). The alternative, and equally intriguing, explanation for such patterns is a non–cell autonomous mechanism wherein mitochondrial failure in one cell favors mitochondrial failure in adjacent cells (56,57). Although non–cell autonomy cannot be definitively ruled out at this time, the frequent observation of intensely positive cells in direct contact with completely negative cells tends to argue against such a model. The nature of a heritable change might be epigenetic, a nuclear mutation, or a mitochondrial mutation. We favor the idea of mitochondrial mutation for several reasons, including the high mutation rate of Drosophila mitochondrial genomes (58), and the increasing evidence from mammals for a developmentally produced load of mitochondrial mutations (59) that is subject to developmental bottlenecks (60) and that can expand during aging (61–63). Evidence for a change in mitochondrial DNA deletion frequency during Drosophila development and aging is mixed (64–66); however, those results may have been influenced by the use of mixed tissues, and point mutations have not yet been assayed. The Drosophila oenocytes should provide a tractable model system in which to further investigate mechanisms for cell-specific patterns of mitochondrial failure and aging.

**Supplementary Material**

Supplementary material can be found at: [http://biomedgerontology.oxfordjournals.org/](http://biomedgerontology.oxfordjournals.org/)

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**References**

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL METHODS

Tet-on system transgenic strains are as described in (1–8). Gene-Switch system transgenic strains were obtained from Bloomington Drosophila stock center, Harvard Drosophila stock center and VDRC as indicated in Supplemental Table S1, EcR strains are as described in (9), fru and dsx lines were provided by Michelle Arbeitman (10), l(2)efl line by H. Jasper (11).

Supplemental Table S1. List of all transgenic lines screened.

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Geneswitch
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Bl=Bloomington, d=Harvard, VDRC=Vienna, OE = over-expression, DN = dominant negative, AF= active form, <-+>> = higher activity, <-+ > = lower activity
SUPPLEMENTAL REFERENCES

2. Landis GN, Bhole D, Tower J. A search for doxycycline-dependent mutations that increase *Drosophila melanogaster* life span identifies the *VhaSFD, Sugar baby, filamin, fwd* and *Cctl* genes. Genome Biol. 2003;4:R8.

SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure S1.** Quantification of *Hsp70* and *Hsp22* reporters in males and females. The *Hsp70-GFP* reporter (A), and the *Hsp22-GFP* reporter (B), were analyzed in male (M) and female (F) flies, as indicated, at age 60 days. GFP fluorescence is shown
in upper panels, and visible light/GFP overlay in the lower panels; exposure times were limited to highlight differences between male and female. (A) Genotype yw;Hsp70-GFP[2MI4]/+. (B) Genotype yw; Hsp22-GFP[1MI1]/+. Expression of the Hsp22-GFP reporter in oenocytes was generally more extensive in females than in males, and is indicated with white arrows (quantification presented in Supplemental Figure S5). (C) GFP was quantified using Image J software in groups of 6 flies for each sample, as indicated. Mean and standard deviation are plotted, and the data for females was compared to males using unpaired, two-sided t-tests, and statistically significant differences (p < 0.05) are indicated with asterisk.

Supplemental Figure S2. Oenocyte marker characterization. (A) Male flies were photographed using visible light at ages 6 days and 30 days, as indicated. Arrow indicates age pigment accumulation in oenocytes. Genotype wild-type (Oregon R strain). (B) Male flies were photographed at ages 6 days and 60 days, as indicated, using visible (white) light illumination and using 365nm light illumination, and the visible light/fluorescence image overlay is presented. Increased bluish-lavender fluorescence in the old fly head, thorax and abdomen is indicated with white arrows; no fluorescence was detected from oenocytes. Genotype wild-type (Oregon R strain). (C) Expression of Hsp22-LacZ reporter in oenocytes. Dissected abdomens from 6 day old and 30 day old flies were stained for beta-galactosidase activity. Arrow indicates reporter expression in oenocytes in one abdominal segment. Genotype w[1118]; Hsp22-LacZ[3A]. (D-L) Tissue staining with MitoSOX-red dye. Flies at 60 days of age containing the Hsp22-GFP reporter were dissected and the tissues stained with MitoSOX-Red dye to indicate superoxide levels. (D-F) Dorsal view of dissected abdominal wall, with oenocytes attached (arrows) and partial fat-body tissue attached (asterisk). The posterior of the abdomen is to the left and anterior is to the right. (G-L) Individual abdominal segments were dissected with oenocytes attached (arrows) and partial fat-body tissue attached (asterisks). (D, G, J) GFP fluorescence imaging of oenocytes with high-level expression of Hsp22-GFP reporter (arrows). (E, H, K) Flourescence imaging of MitoSOX-Red staining. Fat-body tissue had relatively higher-level signal and is indicated with asterisks.
Oenocytes had relatively lower-level signal and are indicated with arrows. (F, I, L)
Overlay of Hsp22-GFP and MitoSOX-red images.

**Supplemental Figure S3.** Quantification of Hsp22-GFP reporter induction in oenocytes with over-expression of MnSOD and Hsp22. (A) Sector numbering. Groups (“Sectors”) of oenocytes in each segment and along the midline were numbered as indicated. A sector containing one or more intensely positive cells was counted as positive. (B) MnSOD over-expression. Male and female flies containing the Hsp22-GFP reporter, and either no target transgene (Control) or the tetO-MnSOD[12] transgene or the tetO-MnSOD[22] transgene, were cultured in the absence (-) and presence (+) of drug to age 60 days, as indicated. (C) Hsp22 over-expression. Male and female flies containing the Hsp22-GFP reporter, and either no target transgene (Control) or the tetO-Hsp22[26] transgene, the tetO-Hsp22[22A] transgene, or the tetO-Hsp22[23] transgene were cultured in the absence (-) and presence (+) of drug to age 60 days, as indicated. The number of GFP-positive groups of oenocytes was counted in cohorts of 35 flies for each sample and plotted. The mean value is indicated with a red line, and (+) was compared to (-) for each genotype using Mann-Whitney test, and statistically significant differences are indicated with an asterisk. Note that the greater degree of trans-activation apparent in males relative to females is believed to be due in part to the fact that the rtTA(3)E2 driver has ~50% greater activity in males than in females (data not shown).
A  Hsp70-GFP
M  F

B  Hsp22-GFP
M  F

C

Tower_Fig S1

Mean GFP

Hsp70 M  Hsp70 F  Hsp22 M  Hsp22 F
Tower_Fig S2

A 6 days 30 days

B 6 days 60 days

C

D

E

F

G

H

I

J

K

L