Drosophila as a model for the identification of genes causing adult human heart disease

Matthew J. Wolf*, Hubert Amrein†, Joseph A. Izatt‡, Michael A. Choma‡, Mary C. Reedy§, and Howard A. Rockman*†§¶

Departments of *Medicine, §Cell Biology, †Molecular Genetics and Microbiology, and ‡Biomedical Engineering, Duke University, Durham, NC 27110

Edited by Oliver Smithies, University of North Carolina, Chapel Hill, NC, and approved December 14, 2005 (received for review August 26, 2005)

Drosophila melanogaster **genetics provides the advantage of molecularly defined P-element insertions and deletions that span the entire genome. Although *Drosophila* has been extensively used as a model system to study heart development, it has not been used to dissect the genetics of adult human heart disease because of an inability to phenotype the adult fly heart *in vivo*. Here we report the development of a strategy to measure cardiac function in awake adult *Drosophila* that opens the field of *Drosophila* genetics to the study of human dilated cardiomyopathies. Through the application of optical coherence tomography, we accurately distinguish between normal and abnormal cardiac function based on measurements of internal cardiac chamber dimensions *in vivo*. Normal *Drosophila* have a fractional shortening of 87 ± 4%, whereas cardiomyopathic flies that contain a mutation in troponin I or tropomyosin show severe impairment of systolic function. To determine whether the fly can be used as a model system to recapitulate human dilated cardiomyopathy, we generated transgenic *Drosophila* with inducible cardiac expression of a mutant of human δ-sarcoglycan (δsg^{S151A}), which has previously been associated with familial dilated cardiomyopathy. Compared to transgenic flies overexpressing wild-type δsg, or the standard laboratory strain *w*^{1118}, *Drosophila* expressing δsg^{S151A} developed marked impairment of systolic function and significantly enlarged cardiac chambers. These data illustrate the utility of *Drosophila* as a model system to study dilated cardiomyopathy and the applicability of the vast genetic resources available in *Drosophila* to systematically study the genetic mechanisms responsible for human cardiac disease.**

optical coherence tomography | cardiomyopathy

The recent generation of ≈19,000 high-density P-element and PiggyBac element insertions throughout the *Drosophila* genome provides the unique advantage to screen an entire genome and rapidly map candidate genes, features not yet applicable to any other species including the mouse genome (1, 2). *Drosophila* has emerged as a powerful model system for a number of human diseases including neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (3, 4), diabetes (5), and cancer (6). However, *Drosophila* has not been used to study cardiomyopathy in the postdevelopmental heart because of the inability to phenotype normal and abnormal cardiac function and structure in the adult fly. In the larvae, the dorsal vessel gives rise to the adult *Drosophila* mature heart (7). Although the fly has been used extensively to study heart development, previous studies of adult *Drosophila* cardiac structure were limited to low magnification (7) or focused on age-related changes (8, 9). Prior studies of adult *Drosophila* cardiac function have relied solely on measurements of heart rate parameters, often derived from edge-measurement microscopy or electrical pacing methods (10–13). These methods generate valuable information about heart rate and rhythm, but are based on measurements of the external surface of the heart and do not provide information about the internal cardiac chamber dimensions and cardiac function that would be required to identify cardiomyopathic phenotypes. The ability to accurately phenotype dilated cardiomyopathy in adult *Drosophila* would provide an avenue to the vast genetic resources available in *Drosophila* genetics, including the enormous collection of P-element insertions and genomic deletions (1, 2).

Optical coherence tomography (OCT) (14, 15) is a noninvasive, nondestructive imaging technique that can provide microscopic tomographic sectioning of biologic tissues. By measuring singly backscattered light as a function of depth, OCT provides subsurface imaging with high spatial resolution (≈10 μm) *in vivo* with no contact needed between the probe and the tissue (14, 15), obviating the need for anesthetizing agents that can seriously confound the interpretation of data because of anesthetic-induced arrhythmias and alterations in cardiac systolic and diastolic properties (16–19).

In this study, we applied OCT to noninvasively image the heart in adult awake *Drosophila* and demonstrate the acquisition of image information that is analogous to clinical parameters obtained by echocardiography in humans. We show that this strategy can readily distinguish normal cardiac function from cardiomyopathy in adult *Drosophila* that possess a point mutation in troponin I, lack an isoform of tropomyosin 2, or have a genomic deletion of dystrophin. Furthermore, we created *Drosophila* that carry a transgene encoding δsg^{S151A}, a mutant human δsg associated with human dilated cardiomyopathy (20), and demonstrate that *Drosophila* can develop inducible heart failure, thus recapitulating characteristics of human cardiomyopathy. These data illustrate that *Drosophila* can be used as a model system to explore the role of novel human mutations in the development of dilated cardiomyopathy. Therefore, we postulate that the application of OCT would provide the means to accurately phenotype cardiac function in adult awake *Drosophila*, thus opening the study of human cardiomyopathies to *Drosophila* genetics.

Results

Characterization of Cardiac Function in Awake Adult *w*^{1118} *Drosophila* by OCT. Because the identification of mutations in *Drosophila* that cause dilated cardiomyopathy requires a rapid and precise method to measure cardiac function, we tested whether OCT could be applied to accurately phenotype cardiomyopathy in the awake adult fly. To determine normal adult *Drosophila* cardiac function, we examined males and females of a standard strain (*w*^{1118}) and wild-type *Oregon-R* wild-type strain at 7, 14, and 30 days of age. To avoid possible anesthetic-associated cardiac arrhythmias that can complicate the analysis of cardiac function (17, 19), we used awake *Drosophila* in all of our studies. Flies were briefly sedated by continuous CO_2 and gently placed on a soft gel support with the dorsal side facing the OCT source. Flies were then removed from CO_2 flow for at least 20 min and allowed to return to a fully awake state as ascertained by movement of the head, body, and extremities (Fig. 1*A*). OCT

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: OCT, optical coherence tomography; EDD, end-diastolic dimension; ESD, end-systolic dimension; FS, fractional shortening; HR, heart rate.

¶To whom correspondence should be addressed. E-mail: h.rockman@duke.edu.

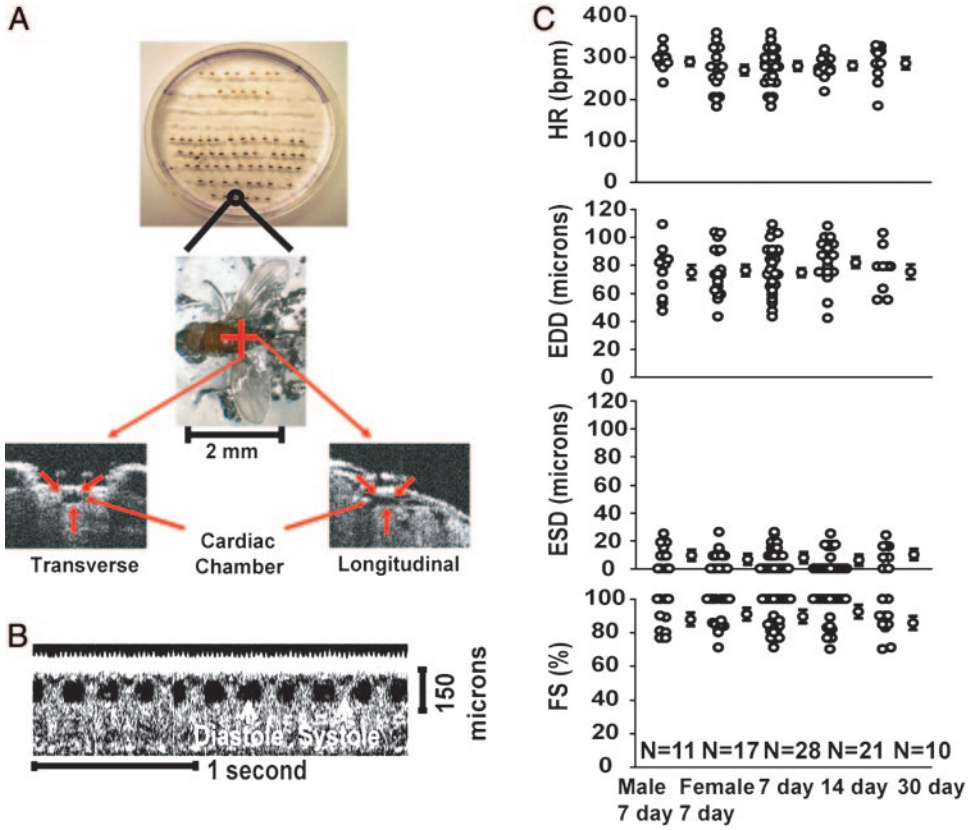

Fig. 1. Imaging of cardiac function in awake, adult *Drosophilia*. (*A*) Representative plate of immobilized *Drosophila* on transparent gel support and acquired B-mode images in longitudinal and transverse orientations. (*B*) Representative M-mode OCT image of a 7 day-old w^{1118}. Depth scale, 150 μm; time scale, 1 s. (*C*) Cardiac chamber parameters in w^{1118} at 7, 14, and 30 days of age at 22°C showing heart rate (HR) in beats per minute (bpm), end-diastolic dimension (EDD) in μm, end-systolic dimension (ESD) in μm, and fractional shortening (FS). All data are expressed as individual data points (left) and mean ± standard error (right); no statistically significant differences were found in HR, EDD, ESD, or FS by one-way ANOVA.

images were acquired with a sweep speed of 4,000 lines per second using 1,300-nm wavelength light directed either in the transverse or longitudinal orientation. Remarkably, OCT provided images that bore striking resemblance to the structures initially described in histological sketches of the *Drosophila* circulatory system by Miller in 1950 (21) and correlated with histological sections of intact flies (see Fig. 5, which is published as supporting information on the PNAS web site). The cardiac chamber was readily visualized along the midline at the A1–A3 abdominal segments underneath the dorsal cuticle surface. The cardiac chamber demonstrated regular rhythmic contractions with occasional episodes of irregular activity, consistent with prior studies of heart rhythm in the fly (11, 22) (see Movie 1, which is published as supporting information on the PNAS web site). M-mode OCT imaging provided detailed measurements of the internal cardiac chamber parameters with an end-diastolic dimension (EDD) of 76 ± 3 μm, an end systolic dimension (ESD) of 8 ± 2 μm, and a fractional shortening (FS) of 90 ± 2% (Fig. 1 *B* and *C* and Table 1, which is published as supporting information on the PNAS web site). All parameters were similar among flies of both sexes and different ages (7, 14, and 30 days). Furthermore, heart rate (HR), but not EDD, ESD, and FS, was temperature dependent (HR 271 ± 13 bpm at 22°C vs. 511 ± 26 bpm at 38°C; $P < 0.001$, see Table 1). Cardiac parameters of *Oregon-R* wild-type *Drosophila* were indistinguishable from those of w^{1118} (see Table 1, which is published as supporting information on the PNAS web site).

Identification of Dilated Cardiomyopathy in *Drosophila* That Have a Mutation in Troponin I. Next, we sought to identify abnormal cardiac function consistent with a phenotype of dilated cardiomyopathy by examining flies with mutations in sarcomeric contractile proteins. *held-up2* (*hdp2*) were initially characterized from a mutagenesis screen designed to produce abnormalities in flight. The troponin I in *hdp2* contains a single amino acid substitution that replaces alanine at position 55 with valine (23). *hdp2* flies have abnormal indirect flight musculature, with degradation of sarcomeric integrity within 48 h after emergence of the adult from the pupal case and, as a result, are flightless and hold their wings upright (23). No prior cardiac abnormality has been identified in *hdp2*. Through OCT imaging, we found that homozygous TnI mutants have a significantly enlarged diastolic chamber dimension with markedly impaired systolic function as shown in the B-mode images (Fig. 2*A* and Movie 1) and the M-mode analyses (Fig. 2*B*). Because the TnI flightless phenotype has been reported to be a recessive trait with respect to the indirect flight musculature, we generated heterozygote TnI flies to examine whether a single mutant allele would result in an abnormal cardiac phenotype. Surprisingly, we found a partially dominant effect because the heterozygote TnI demonstrated a normal EDD but a markedly enlarged ESD and a severe impairment in FS (Fig. 2*B*). Thus, TnI mutant heterozygotes demonstrate impaired systolic function with preserved diastolic dimensions, whereas the TnI mutant homozygotes have both impaired systolic function and cardiac enlargement recapitulating the clinical phenotype of human dilated cardiomyopathy; namely systolic dysfunction with or without cardiac chamber enlargement (24).

The Ultrastructural Changes in *hdp2* Hearts Are Distinct from the Changes Seen in the Indirect Flight Musculature. Because the TnI point mutation in *hdp2* flies has been reported to severely disrupt

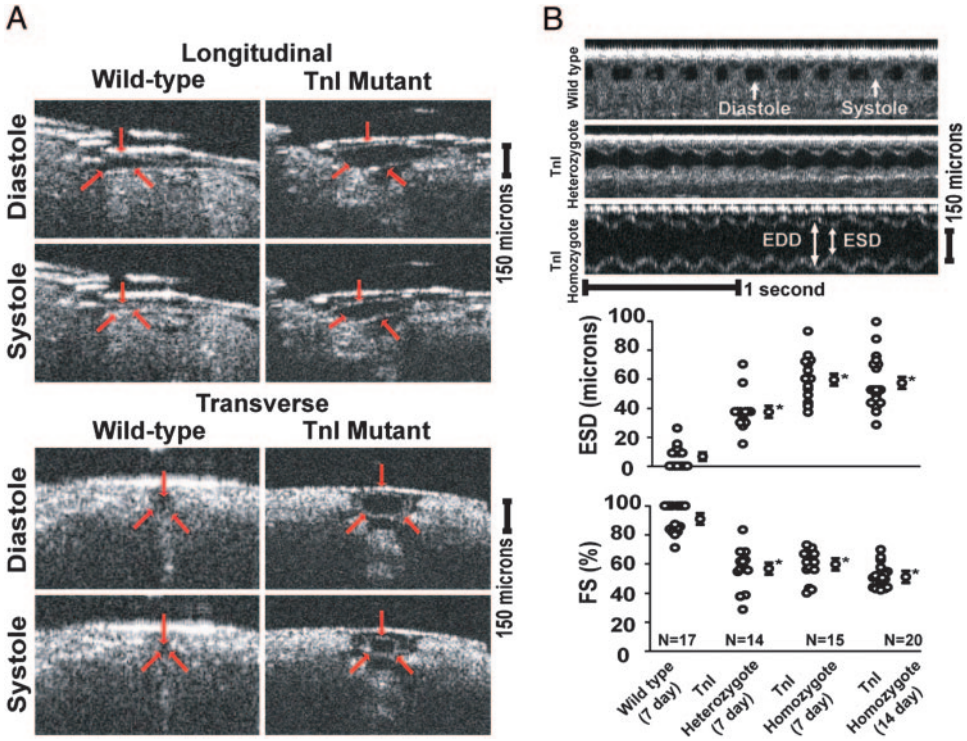

Fig. 2. A point mutation in Troponin I causes dilated cardiomyopathy in adult *Drosophilia*. (*A*) Representative longitudinal and transverse two-dimensional B-mode OCT images of the cardiac chamber during diastole and systole in wild-type w^{1118} and homozygous TnI mutant *Drosophila* at 7 days of age. Arrows outline the edges of the cardiac chamber in both phases of the cardiac cycle. (Scale, 150 μm.) (*B*) Representative M-mode OCT images and summary data for ESD and FS from 7-day-old w^{1118}, heterozygote, and homozygote TnI mutant *Drosophila*. Time scale, 1 s; dimension scale, 150 μm. *, $P < 0.005$ by ANOVA with Bonferroni correction for multiple comparisons.

sarcomeric structure in the indirect flight muscles (23), we used electron microscopy to compare the ultrastructure of w^{1118} and *hdp2* myocardium and indirect flight muscle. The myofibrils of the circular wall of the cardiac chamber in w^{1118} were circumferentially oriented in a shallow spiral, whereas the myofibrils of the ventral outer muscular layer were longitudinally oriented (Fig. 3*A*). In contrast to w^{1118}, the outer and inner muscle layers of the cardiac chamber of homozygote TnI were markedly abnormal (Fig. 3*B*). Examination of the myocardial ultrastructure in homozygote TnI flies showed a considerably thinner outer muscle layer and redundant basal lamina in the space between the outer and inner walls (Fig. 3 *A* and *B*). Moreover, the mutant myocardium showed considerable disorientation of myofibrils throughout the inner layer (Fig. 3*B*) and displayed swollen sarcoplasmic reticulum when compared to w^{1118}; however, the T-tubule dyads appeared unaffected in the mutant (Fig. 3 *C* and *D*), and the ratio of thin and thick filaments in the myocardium of homozygote TnI was similar to that in w^{1118} (Fig. 3 *E* and *F*), strikingly different from the marked deterioration of the myofibrils of the indirect flight muscle (Fig. 3 *G* and *H*) (23).

Adult *Drosophila* with a Mutation in Tropomyosin 2 Have Dilated Cardiomyopathy. We next determined whether a mutation in a gene encoding another flight muscle protein, tropomyosin 2 (TM2), present in $TM2^3$ flies, which creates a flightless phenotype due the absence of 1.7 and 1.9 kb TM2 mRNAs, also produces a cardiomyopathic phenotype (25). Homozygous and heterozygous $TM2^3$ mutant flies had heart rates that were similar to wild-type flies but possessed enlarged cardiac chambers and significant impairment in systolic function and FS compared to w^{1118} (Table 2, which is published as supporting information on the PNAS web site). Taken together, these data show that flight muscle proteins of *Drosophila* also may be essential in the physical integrity and performance of the heart.

Transgenic *Drosophila* That Carry a Gene Mutation in Human δ-Sarcoglycan (δsg) Recapitulate Human Familial Dilated Cardiomyopathy. Because *Drosophila* has used to explore several human diseases (6, 26), we next examined the effects of a mutation in human δsg that has been associated with, but not proven to directly cause, dilated cardiomyopathy (20). We generated transgenic *Drosophila* carrying either human wild-type δsg or δsg^{S151}, (UAS-δsg^{wt} or UAS-δsg^{S151A}) a mutation associated with familial dilated cardiomyopathy (20). Importantly, the serine at amino acid 151 is conserved between humans and *Drosophila*. Transgenic *Drosophila* that expressed δsg^{S151A} under control of cardiac tissue specific p[*tin-C*]-GAL4 demonstrated impaired systolic function with enlarged diastolic parameters as compared to either UAS-δsg^{wt}; p[*tinC*]-GAL4or w^{1118} flies (Fig. 4), indicating that δsg^{S151A} has a dominant-negative effect on cardiac dysfunction.

Because transgenes that are under the control of the *tin-C* promoter are expressed from early development through adulthood (27–29), we next evaluated an inducible transgenic model. p[*hsp70*]-GAL4; δsg^{wt} or p[*hsp70*]-GAL4; δsg^{S151A} flies had similar cardiac parameters before heat shock induction, although p[*hsp70*]-GAL4; δsg^{S151A} flies had a slightly larger ESD (Fig. 4). This finding is consistent with the known leakiness of the *hsp70* promoter (30). However, heat shock-induced transgene expression resulted in impaired systolic function and enlarged diastolic dimensions in δsg^{S151A}, but not in wt-δsg (Fig. 4).

Discussion

Although *Drosophila* has been used to study cardiac development (28, 31), the ability to investigate human dilated cardiomyopathies in the adult has been limited. A major problem has

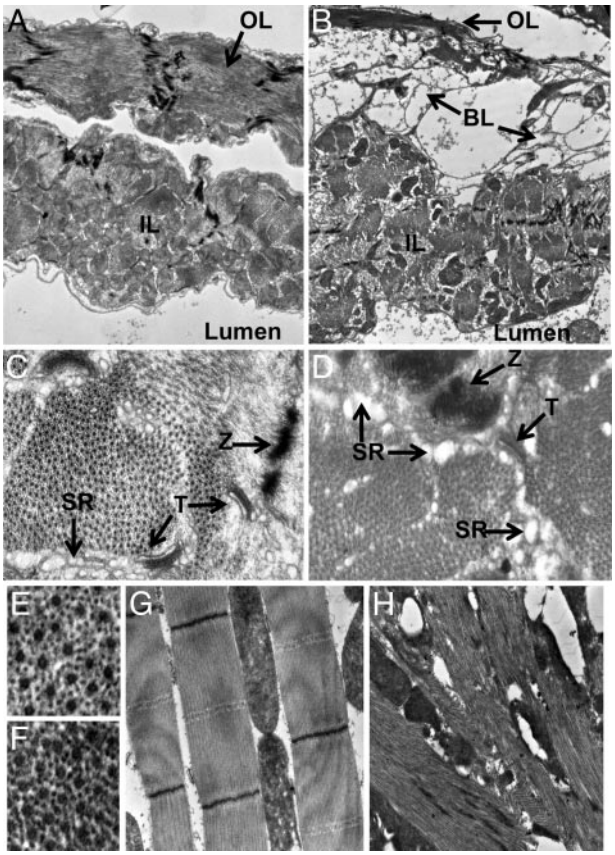

Fig. 3. Electron microscopy of the outer and inner layers of the cardiac chamber from w^{1118} (*A*) and homozygous TnI mutant *Drosophila* (*B*) at ×4,900 magnification; myocardial T tubule and sarcoplasmic reticulum in w^{1118} (*C*) and homozygous TnI mutant (*D*) at ×30,000 magnification; myocardial thin and thick filaments in w^{1118} (*E*) and homozygous TnI mutant (*F*); and indirect flight muscle from w^{1118} (*G*) and homozygous TnI mutant (*H*) at ×10,500 magnification. OL, outer layer; IL, inner layer; BL, basal lamina; SR, sarcoplasmic reticulum; T, T tubule dyad; Z, Z disk.

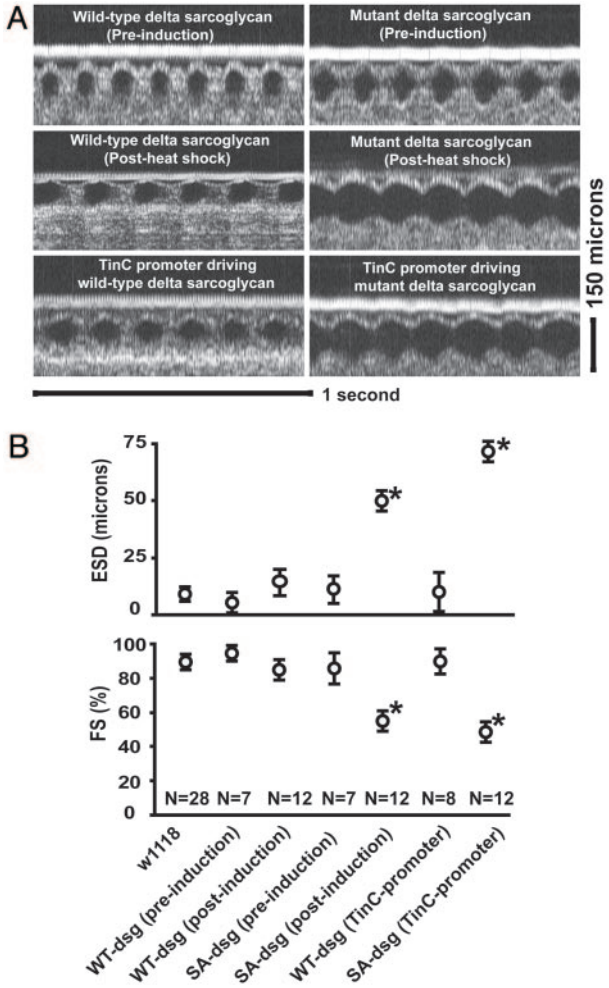

Fig. 4. Representative M-mode OCT images (*A*) and summary data (*B*) for FS and ESD from *Drosophila* harboring δsg^{wt} and δsg^{S151A} transgenes. δsg^{wt} or δsg^{S151A} was driven by either *tinC* promoter-GAL4 for constitutive expression or *hsp70* promoter-GAL4 for inducible expression and cardiac function was evaluated at 7 days of age. Time scale, 1 s; dimension scale, 150 μm. *, $P < 0.005$ by ANOVA with Bonferroni correction for multiple comparisons. dsg, δ-sarcoglycan.

been the lack of a method to accurately and rapidly identify abnormalities in cardiac function that correlate with clinical parameters used to characterize human cardiomyopathies. Prior studies have used partially dissected cardiac preparations, assumed internal chamber dimensions without direct measurement, or relied on artificial external electrical pacing methods under anesthesia (10, 11, 13, 32). Unfortunately, these methods do not directly measure the internal cardiac chamber of the adult fly and are not readily applicable as phenotying strategies in large genetic screens.

Our results are consistent with previous observations that adult *Drosophila* do not display an age-related alteration in EDD, ESD, and FS. Previous studies have found an age-related decline in HR (11, 13); however, we did not observe this change. Interestingly, the previous studies used 59-day-old flies and volatile amine anesthetics that may have partially contributed to the age-related decline HR. It is also possible that the awake flies in our study may have experienced increased stress and therefore did not manifest a decrease in HR at 21 days of age.

In our study, we applied OCT to adult awake *Drosophila* and accurately determined cardiac function based on cardiac cycle-dependent changes in internal chamber dimensions, analogous to echocardiography in humans. To validate our phenotyping method, we examined the cardiac function in *Drosophila* with sarcomeric protein mutations. The effects of the A55V mutation in troponin I have been described in the context of indirect flight muscle structure and function (23); however, the influence of this mutation on cardiac structure and function has not been previously examined. The troponin I mutation is recessive with respect to the flight phenotype; however, the cardiac phenotype is more complex. We demonstrate an allelic dose dependence because homozygote troponin I mutants show both increased diastolic dimensions and impaired systolic function, whereas heterozygotes have preserved diastolic dimensions and impaired systolic function. In addition, the homozygous troponin I mutant shows marked ultrastructural abnormalities.

Electron microscopic examination of indirect flight muscle from the homozygous troponin I mutant demonstrated dramatically altered sarcomeric ultrastructure, consistent with prior studies (23, 33). In contrast to the indirect flight muscle, the cardiac muscle of the homozygous troponin I mutant had more subtle effects on the thin and thick filaments. The heart of the homozygous troponin I mutant had a thinner outer muscular layer and the appearance of a redundant basal lamina between the inner and outer muscular layers suggestive of repetitive injury and repair possibly in response to dilated cardiomyopathy. Interestingly, *Drosophila* heart muscle has perforated Z discs that allow for tremendous variation in sarcomere length (34) and

most likely contribute to the degree of dilation seen in the TnI mutants. The exact ultrastructural changes that are responsible for dilated cardiomyopathy will require further study.

Because the A55V troponin I mutation was present throughout *Drosophila* cardiac development, we explored the possibility of inducing dilated cardiomyopathy in adult *Drosophila* in the postdevelopmental period by examining the effects of a mutant human δsg. Mutations in sarcoglycans are associated with severe cardiomyopathy and skeletal dystrophies and mouse models that lack δsg manifest cardiac and skeletal muscle phenotypes that resemble human limb girdle muscular dystrophy (35, 36). Interestingly, δsg knockout mice have vascular smooth muscle irregularities and arterial vascular constrictions that result in focal myocardial damage and leads to global cardiomyopathy (37–39). Our findings that the expression of human δsg^{S151A}, either under the control of *tinC*-Gal4 or *hsp70*-Gal4, results in dilated cardiomyopathy have two important implications. First, the δsg^{S151A} mutation acts as a dominant-negative mutation because dilated cardiomyopathy is present when δsg^{S151A} is expressed in the context of endogenous *Drosophila* δsg. Second, the δsg^{S151A} mutation has a direct effect on myocyte function because the *Drosophila* myocardium lacks a coronary vascular system and therefore is not subject to the effect of vasospasm as described in the δsg knockout mouse (31–33).

The troponin I and δsg^{S151A} mutations provide an avenue to identify potential genetic polymorphisms that influence the severity of dilated cardiomyopathy by serving as genetic sensitizers in potential modifier screens (40). For example, flies with troponin I or δsg^{S151A} mutation can be used as a cardiomyopathic sensitizer gene and backcrossed into either *Canton-S* or *Oregon-R* strains to derive recombinant inbred strains to examine the impact of genetic strain differences on dilated cardiomyopathy. Then, single-nucleotide polymorphism mapping strategies can be conducted to identify quantitative trait loci corresponding to genomic regions that contain protective or detrimental genetic modifiers of dilated cardiomyopathy (41, 42). Identification of candidate genes from these modifier screens can be tested in populations of individuals with heart failure and thereby provide an unbiased avenue using *Drosophila* to find potential markers that correspond to human disease progression.

Drosophila genetic screens have been successfully used to address fundamentally important questions concerning embryonic development and cellular signaling mechanisms (40, 43). Large-scale *Drosophila* screens to identify genetic components that are responsible for adult cardiomyopathies have not been conducted because of the inability to rapidly and accurately phenotype normal and abnormal cardiac function in adult *Drosophila*. Our study opens the investigation of human cardiomyopathies to *Drosophila* genetics and illustrates that the fly could be used to identify genes involved in human dilated cardiomyopathy. Recent efforts to enhance the applicability of *Drosophila* as a genetic screening tool led to the generation of 19,000 P-element and PiggyBac element insertion strains that can be used to produce molecularly defined deletions throughout the fly genome (1, 2). To examine our phenotyping strategy, we initiated an initial screen of Exelixis stocks covering ≈200 genes and identified dilated cardiomyopathy in a stock that was haploinsufficient for dystrophin (see Fig. 6, which is published as supporting information on the PNAS web site). Because OCT data acquisition takes ≈1 min per fly, we can analyze two to three mutant stocks per day. We anticipate that the near complete evaluation of the *Drosophila* genome can be accomplished in ≈1 year, and predict that a high-throughput phenotype-driven screen for dilated cardiomyopathy will identify genes responsible for this prevalent human disease.

Materials and Methods

***Drosophila* Stocks and Breeding.** w^{1118}, *Oregon-R*, $TM2^3$, and Exelixis stocks were obtained from the *Drosophila* Stock Center (Bloomington, IN; http://flystocks.bio.indiana.edu). *hdp2 Drosophila* was a generous gift from Jim Vigoreaux (University of Vermont, Burlington). The *tinC*-GAL4 transgenic *Drosophila* (29) was generously provided by Manfred Frasch (Mount Sinai School of Medicine, New York). For the screen of Exelixis stocks, the hemizygote progeny from crosses of the Exelixis stocks with w^{1118} were evaluated to remove potential effects of the balancer chromosomes.

Generation of Transgenic *Drosophila* Harboring δsg^{wt} and δsg^{S151A}. The cDNA corresponding to human wild-type δsg (δsg^{wt}) (GenBank accession no. NM_000337) was obtained by RT-PCR from human myocardium, subcloned in to pCR-II vector (Invitrogen), and the DNA was sequenced. Next, oligonucleotide primers were designed to introduce a new BstBI restriction enzyme site and the corresponding S151A mutation. The δsg^{wt} and δsg^{S151A} constructs were subcloned into pUAST, sequenced, and injected into *Drosophila* embryos according to established methods to generate transgenic *Drosophila* (44). Eight and 10 independent lines with UAS-δsg^{wt} and UAS-δsg^{S151A} transgenes, respectively, were obtained and propagated.

For constitutive transgene expression, the UAS-δsg^{wt} or UAS-δsg^{S151A} was crossed with the tinC-GAL4 transgenic *Drosophila* (p[*tinC*]-Gal4), and the F_1 progeny were studied at 7 days of age. For inducible transgene expression, the UAS-δsg^{wt} or UAS-δsg^{S151A} was crossed with *hsp70*-GAL4 transgenic *Drosophila* (p[*hsp70*]-GAL4). The F_1 progeny were maintained at 25°C and exposed to a heat shock at 37°C for 1 h every 24 h over a 3-day period. Uninduced transgenic flies were maintained at 25°C and did not receive heat shock stimulus. All flies were analyzed at 7 days of age.

OCT Measurements and Analysis of Cardiac Function. Flies of similar body length (≈3 mm) and weight (22–25 mg per 20 flies) were preselected from each group under a calibrated microscope. Flies were subjected to continuous CO_2 for 3–5 min and then, under microscopic view, gently placed on a plate of GelWax (Yaley Enterprises, Redding, CA) for immobilization with the dorsal aspect facing the OCT source and allowed to fully awaken as ascertained by movement of the head, body, and extremities. Images were obtained at 22°C unless otherwise specified. The OCT system was built by Michael Choma and Joseph Izatt (Biophotonics Laboratory, Duke University) as described (15). Two-dimensional OCT imaging was composed of one-dimensional line scans acquired as the probe moved laterally across the specimen with a sweep speed of 4,000 lines per second by using a 1,300-nm light source (15). Two-dimensional B-mode images were obtained in the longitudinal direction to identify the cardiac chamber in the A1-3 abdominal segments and then the OCT image orientation was turned 90° to obtain transverse images. M-mode OCT images were obtained in the transverse plane by stopping the scanning mirror in the midline and collecting continuous image data. After obtaining M-mode images, repeat B-mode transverse images were obtained to ensure proper anatomic localization throughout image acquisition. All images were calibrated with a 150-μm standard.

M-mode images were used to determine cardiac parameters and derived by averaging a minimum of three cardiac cycles during normal regular rhythm. Cardiac chamber dimensions were measured from the edge of the superior and inferior walls during mid-diastole for EDD and mid-systole for ESD. Fractional shortening (FS) was calculated as $[(EDD - ESD)/EDD] \times 100$ and represents the extent of cardiac chamber area change

during systole. Heart rate was calculated from 2.5-s M-mode tracings during normal regular rhythm.

Histology and Electron Microscopy of *Drosophila* Myocardium. Seven-day-old female w^{1118} and TnI homozygote *Drosophila* were processed for electron microscopy as described, except all studies used Karnovsky fixative (3% formaldehyde/3% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.35) (Tousimis, Rockville, MD). Semithin araldite sections were stained 1% Toluidine Blue for electron microscopy (45).

Statistics Analysis. Data are shown as mean ± SEM. Comparisons of chamber dimensions were determined by analyses of variances with Bonferroni corrections for multiple corrections when necessary using SAS statistical software (SAS Institute, Cary, NC).

We thank Drs. Manfred Frasch and James Vigoreaux for generously providing the *tinC*-Gal4 and *hdp*^{2} *Drosophila*, respectively. This work was supported by National Institutes of Health Grants HL68963 (to H.A.R.), R037-AR14317 (to M.C.R.), and BES-0216403 (to J.A.I.).

1. Parks, A. L., Cook, K. R., Belvin, M., Dompe, N. A., Fawcett, R., Huppert, K., Tan, L. R., Winter, C. G., Bogart, K. P., Deal, J. E., *et al.* (2004) *Nat. Genet.* **36,** 288–292.
2. Thibault, S. T., Singer, M. A., Miyazaki, W. Y., Milash, B., Dompe, N. A., Singh, C. M., Buchholz, R., Demsky, M., Fawcett, R., Francis-Lang, H. L., *et al.* (2004) *Nat. Genet.* **36,** 283–287.
3. Feany, M. B. & Bender, W. W. (2000) *Nature* **404,** 394–398.
4. Muqit, M. M. & Feany, M. B. (2002) *Nat. Rev. Neurosci.* **3,** 237–243.
5. Rulifson, E. J., Kim, S. K. & Nusse, R. (2002) *Science* **296,** 1118–1120.
6. Bier, E. (2005) *Nat. Rev. Genet.* **6,** 9–23.
7. Molina, M. R. & Cripps, R. M. (2001) *Mech. Dev.* **109,** 51–59.
8. Burch, G. E., Sohal, R. & Fairbanks, L. D. (1970) *Arch. Pathol.* **89,** 128–136.
9. Burch, G. E., Sohal, R. S. & Fairbanks, L. D. (1970) *Nature* **225,** 286–288.
10. Dulcis, D. & Levine, R. B. (2005) *J. Neurosci.* **25,** 271–280.
11. Paternostro, G., Vignola, C., Bartsch, D. U., Omens, J. H., McCulloch, A. D. & Reed, J. C. (2001) *Circ. Res.* **88,** 1053–1058.
12. Wessells, R. J. & Bodmer, R. (2004) *BioTechniques* **37,** 58–60, 62, 64.
13. Wessells, R. J., Fitzgerald, E., Cypser, J. R., Tatar, M. & Bodmer, R. (2004) *Nat. Genet.* **36,** 1275–1281.
14. Huang, D., Swanson, E. A., Lin, C. P., Schuman, J. S., Stinson, W. G., Chang, W., Hee, M. R., Flotte, T., Gregory, K., Puliafito, C. A., *et al.* (1991) *Science* **254,** 1178–1181.
15. Yelbuz, T. M., Choma, M. A., Thrane, L., Kirby, M. L. & Izatt, J. A. (2002) *Circulation* **106,** 2771–2774.
16. Rockman, H. A., Choi, D. J., Rahman, N. U., Akhter, S. A., Lefkowitz, R. J. & Koch, W. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 9954–9959.
17. Roth, D. M., Swaney, J. S., Dalton, N. D., Gilpin, E. A. & Ross, J., Jr. (2002) *Am. J. Physiol.* **282,** H2134–H2140.
18. Vatner, S. F. & Braunwald, E. (1975) *N. Engl. J. Med.* **293,** 970–976.
19. Yang, X. P., Liu, Y. H., Rhaleb, N. E., Kurihara, N., Kim, H. E. & Carretero, O. A. (1999) *Am. J. Physiol.* **277,** H1967–H1974.
20. Tsubata, S., Bowles, K. R., Vatta, M., Zintz, C., Titus, J., Muhonen, L., Bowles, N. E. & Towbin, J. A. (2000) *J. Clin. Invest.* **106,** 655–662.
21. Demerec, M. (1994) *The Biology of Drosophila* (Cold Spring Harbor Lab. Press, Plainview, NY).
22. Burch, G. E. (1971) *Am. Heart J.* **82,** 574–575.
23. Beall, C. J. & Fyrberg, E. (1991) *J. Cell Biol.* **114,** 941–951.
24. Zipes, D. P. & Braunwald, E. (2005) *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine* (Saunders, Philadelphia).
25. Karlik, C. C. & Fyrberg, E. A. (1985) *Cell* **41,** 57–66.
26. Reiter, L. T., Potocki, L., Chien, S., Gribskov, M. & Bier, E. (2001) *Genome Res.* **11,** 1114–1125.
27. Bodmer, R. (1993) *Development (Cambridge, U.K.)* **118,** 719–729.
28. Frasch, M. (1999) *Semin. Cell Dev. Biol.* **10,** 61–71.
29. Lo, P. C. & Frasch, M. (2001) *Mech. Dev.* **104,** 49–60.
30. Steller, H. & Pirrotta, V. (1984) *EMBO J.* **3,** 165–173.
31. Bodmer, R. & Venkatesh, T. V. (1998) *Dev. Genet.* **22,** 181–186.
32. Curtis, N. J., Ringo, J. M. & Dowse, H. B. (1999) *J. Morphol.* **240,** 225–235.
33. Bullard, B., Leonard, K., Larkins, A., Butcher, G., Karlik, C. & Fyrberg, E. (1988) *J. Mol. Biol.* **204,** 621–637.
34. Bernstein, S. I., O'Donnell, P. T. & Cripps, R. M. (1993) *Int. Rev. Cytol.* **143,** 63–152.
35. Cox, G. F. & Kunkel, L. M. (1997) *Curr. Opin. Cardiol.* **12,** 329–343.
36. Finsterer, J. & Stollberger, C. (2003) *Cardiology* **99,** 1–19.
37. Coral-Vazquez, R., Cohn, R. D., Moore, S. A., Hill, J. A., Weiss, R. M., Davisson, R. L., Straub, V., Barresi, R., Bansal, D., Hrstka, R. F., *et al.* (1999) *Cell* **98,** 465–474.
38. Wheeler, M. T., Allikian, M. J., Heydemann, A., Hadhazy, M., Zarnegar, S. & McNally, E. M. (2004) *J. Clin. Invest.* **113,** 668–675.
39. Wheeler, M. T., Korcarz, C. E., Collins, K. A., Lapidos, K. A., Hack, A. A., Lyons, M. R., Zarnegar, S., Earley, J. U., Lang, R. M. & McNally, E. M. (2004) *Am. J. Pathol.* **164,** 1063–1071.
40. St. Johnston, D. (2002) *Nat. Rev. Genet.* **3,** 176–188.
41. Berger, J., Suzuki, T., Senti, K. A., Stubbs, J., Schaffner, G. & Dickson, B. J. (2001) *Nat. Genet.* **29,** 475–481.
42. Teeter, K., Naeemuddin, M., Gasperini, R., Zimmerman, E., White, K. P., Hoskins, R. & Gibson, G. (2000) *J. Exp. Zool* **288,** 63–75.
43. Nusslein-Volhard, C. & Wieschaus, E. (1980) *Nature* **287,** 795–801.
44. Brand, A. H. & Perrimon, N. (1993) *Development (Cambridge, U.K.)* **118,** 401–415.
45. Reedy, M. C. & Beall, C. (1993) *Dev. Biol.* **160,** 443–465.