

Cardiomyopathy Is Associated with Ribosomal Protein Gene Haplo-Insufficiency in *Drosophila melanogaster*

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ABSTRACT The Minute syndrome in *Drosophila melanogaster* is characterized by delayed development, poor fertility, and short slender bristles. Many Minute loci correspond to disruptions of genes for cytoplasmic ribosomal proteins, and therefore the phenotype has been attributed to alterations in translational processes. Although protein translation is crucial for all cells in an organism, it is unclear why Minute mutations cause effects in specific tissues. To determine whether the heart is sensitive to haplo-insufficiency of genes encoding ribosomal proteins, we measured heart function of Minute mutants using optical coherence tomography. We found that cardiomyopathy is associated with the Minute syndrome caused by haplo-insufficiency of genes encoding cytoplasmic ribosomal proteins. While mutations of genes encoding non-Minute cytoplasmic ribosomal proteins are homozygous lethal, heterozygous deficiencies spanning these non-Minute genes did not cause a change in cardiac function. Deficiencies of genes for non-Minute mitochondrial ribosomal proteins also did not show abnormal cardiac function, with the exception of a heterozygous disruption of *mRpS33*. We demonstrate that cardiomyopathy is a common trait of the Minute syndrome caused by haplo-insufficiency of genes encoding cytoplasmic ribosomal proteins. In contrast, most cases of heterozygous deficiencies of genes encoding non-Minute ribosomal proteins have normal heart function in adult *Drosophila*.

THE Minute phenotype in *Drosophila melanogaster* is characterized by delayed development, poor fertility, short slender bristles, and smaller body size (Lambertsson 1998; Marygold *et al.* 2007). The distribution of Minute loci throughout the genome and the uniformity of the phenotype have been of great interest to geneticists for nearly a century. Many Minute loci correspond to cytoplasmic ribosomal genes, suggesting that the basis of the Minute phenotypes involves disruption of ribosomal function (Hart *et al.* 1993; Andersson *et al.* 1994; Mckim *et al.* 1996; Saeboe-Larssen and Lambertsson 1996; Schmidt *et al.* 1996; Saeboe-Larssen

et al. 1997; Van Beest *et al.* 1998; Torok *et al.* 1999; Fauvarque *et al.* 2001; Marygold *et al.* 2005; Alexander *et al.* 2006; Tyler *et al.* 2007).

Eukaryotic cells contain cytoplasmic ribosomes and mitochondrial ribosomes, each containing many subunits. While all of these subunits are translated from nuclear genes, cytoplasmic ribosomes translate proteins in the cytosol and on the endoplasmic reticulum, whereas mitochondrial ribosomes synthesize proteins from mitochondrial genes in the mitochondrial matrix. Recent genetic and bioinformatic efforts have shown that *Drosophila melanogaster* have 88 genes encoding 79 different cytoplasmic ribosomal proteins (CRPs) and 75 nuclear-encoded mitochondrial ribosomal proteins (MRPs) (Marygold *et al.* 2007). While mutations in many CRP genes result in a Minute phenotype, mutations in MRP genes are not associated with the Minute syndrome (Marygold *et al.* 2007).

Drosophila CRPs correspond with all 79 human CRPs, while MRPs are more divergent between species (Marygold

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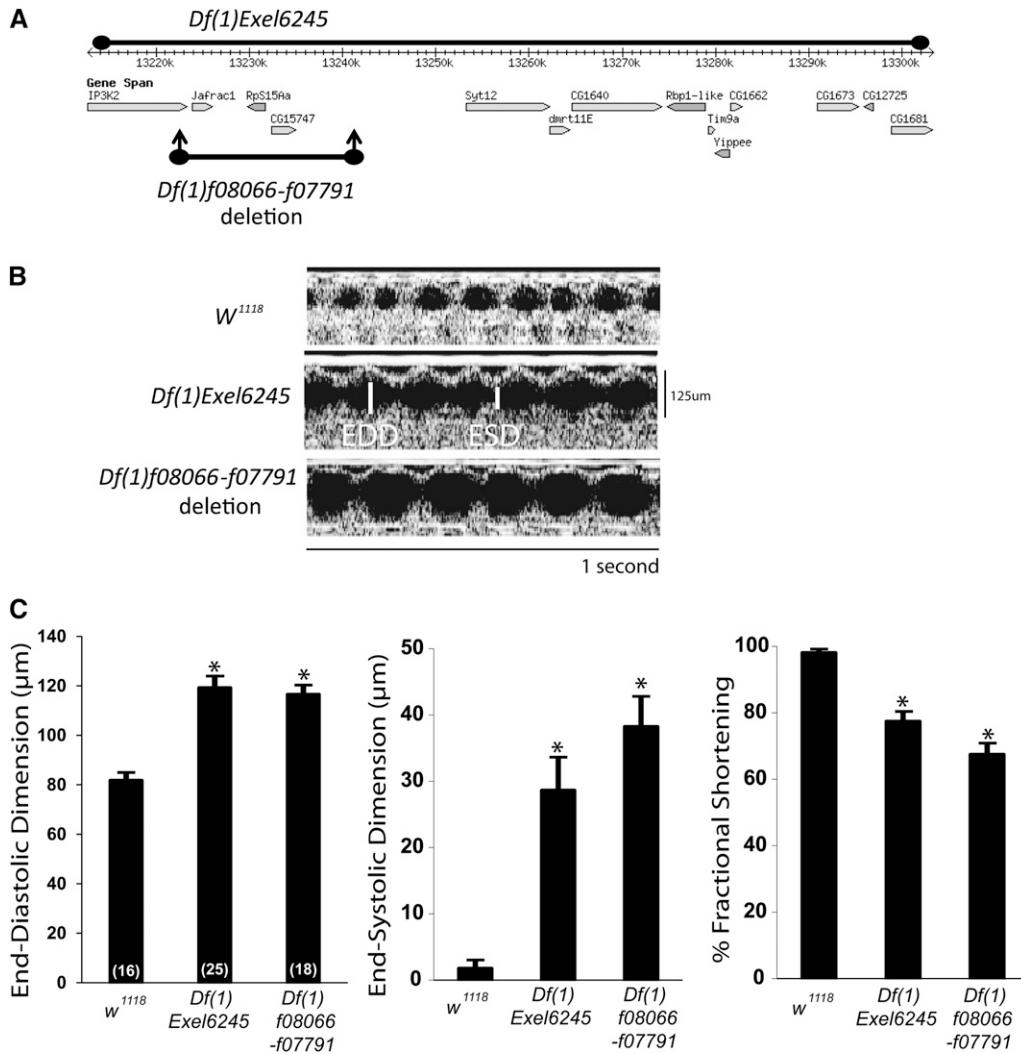


Figure 1 Identification of a deletion on the X chromosome causing dilated cardiomyopathy. (A) Schematic of the *Df(1)Exel6245* deletion containing 14 genes on the X chromosome. *Df(1)f08066-f07791* represents the flp-mediated recombination deletion made with transposons *f08066* and *f07791*, covering 3 genes including *RpS15Aa*. (B) Representative M-mode recordings of *Drosophila* cardiac function reflecting the change in cardiac chamber dimension over time. Control *w*¹¹¹⁸ and representative dilated images of *Df(1)Exel6245/FM7c* and *Df(1)f08066-f07791/FM7c* are shown. End-diastolic and end-systolic positions are delineated with vertical, white lines. (C) Summary of cardiac function for *w*¹¹¹⁸, *Df(1)Exel6245/FM7c*, and *Df(1)f08066-f07791/FM7c*. Mean \pm SEM for each group, $n = 16-25$ per group shown in parentheses. * $P < 0.01$ compared to *w*¹¹¹⁸, one-way ANOVA.

et al. 2007). Mutations in human CRP genes as well as genes for other proteins involved in ribosomal biogenesis have been shown to cause a variety of syndromes (reviewed in Freed *et al.* 2010), most notably CRP gene mutations in Diamond Blackfan anemia, characterized by congenital anemia and a variety of developmental abnormalities. Several MRP genes also map to loci associated with human disorders as well (reviewed in O'Brien *et al.* 2005).

Since ribosomal proteins are important in every cell of an organism, we tested whether abnormal heart function is part of the Minute syndrome. We addressed this question using optical coherence tomography (OCT) (Wolf *et al.* 2006; Kim and Wolf 2009; Kim *et al.* 2010; Yu *et al.* 2010) to examine *in vivo* cardiac size and function in adult *Drosophila* with ribosomal deficiency. We show that haploinsufficiency of *RpS15Aa*, a predicted Minute gene, results in characteristic short, slender bristles as well as significant dilation and dysfunction of the *Drosophila* heart. By examining the heart phenotype of many other deficiencies across CRP and MRP genes we also show that cardiac abnormalities are common in Minute flies, indicating that the heart is

sensitive to deletion of a single copy of some ribosomal genes.

Materials and Methods

Drosophila stocks

All ribosomal deficiency stocks were obtained from the Bloomington Drosophila Stock Center. All UAS-RNAi stocks were obtained from the Vienna Drosophila RNAi Center (Dietzl *et al.* 2007): 19198 (*RpS15Aa*), 106321 (*RpS3*), 36060 (*RpS5*), 105182 (*mRpS33*).

*wy*¹; +/+; *p{tinC-Gal4}/p{tinC-Gal4}* stock was provided by Manfred Frasch (Yin and Frasch 1998). *wy*¹; *p{tubP-GAL80^{ts}}/p{tubP-GAL80^{ts}}; p{tinC-Gal4}/p{tinC-Gal4}* stock was created as described (Kim and Wolf 2009; Yu *et al.* 2010). All stocks were maintained on standard media at room temperature.

Custom genomic deficiencies

PBac{WH}f08066, *PBac{WH}f07791*, *PBac{RB}e00904*, and *P{XP}d02459* insertions stocks were obtained from the

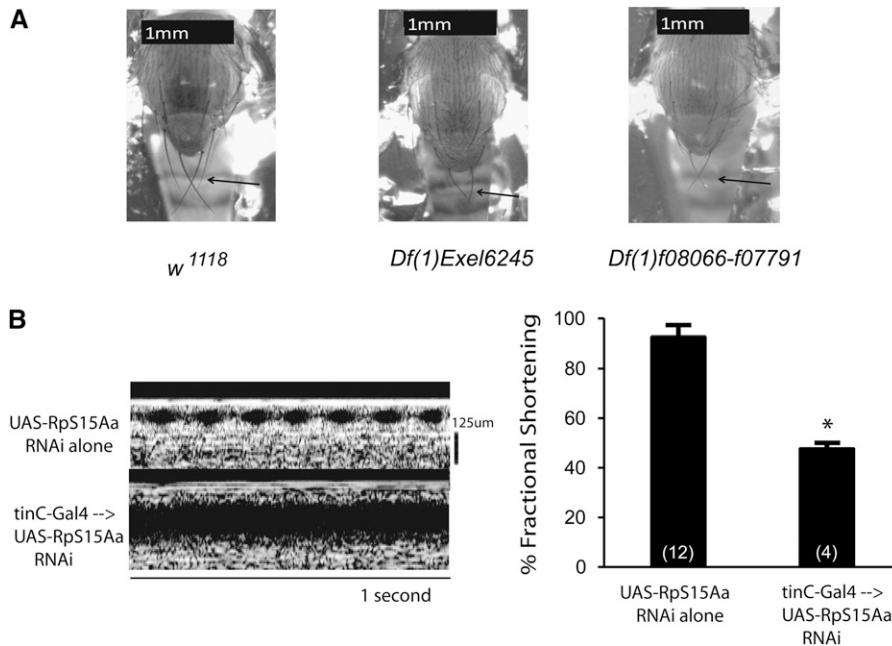


Figure 2 Heart-specific knockdown of *RpS15Aa* severely impairs cardiac function in adult *Drosophila*. (A) Representative images of bristles in *w¹¹¹⁸* control, *Df(1)Exel6245/FM7c*, and *Df(1)f08066-f07791/FM7c*. Both deletions have characteristically Minute bristles that are shorter and thinner than wild-type bristles. (B) Heart-specific tinC-Gal4 driving expression of RNAi against *RpS15Aa* causes a severe cardiac phenotype and severely reduced eclosion. Representative OCT M-mode images of the few eclosed adults ($n = 4$) showed severely impaired heart function compared to control of UAS-RNAi alone without driver ($n = 12$). * $P < 0.001$ vs. no driver control, unpaired t-test.

Exelixis Collection at Harvard Medical School. The genomic deletion *Df(1)f08066-f07791* was generated using *PBac{WH}f08066* and *PBac{WH}f07791* stocks, and *Df(1)e00904-d02459* was generated using *PBac{RB}e00904* and *P{XP}d02459* stocks according to previously established protocols (Parks *et al.* 2004). The phenotype of these custom deletions are described in the *Results* for *Df(1)f08066-f07791* and shown in Table 1 for both *Df(1)f08066-f07791* and *Df(1)e00904-d02459*.

OCT measurement of adult cardiac function

In vivo adult *Drosophila* cardiac function was measured in 7-day-old awake female flies as described (Wolf *et al.* 2006; Kim and Wolf 2009; Kim *et al.* 2010; Yu *et al.* 2010), using an OCT microscopy system (Bioptigen, Inc., Durham, NC).

The cardiac chamber in the first abdominal segment was first visualized using two-dimensional B-mode OCT, then recorded as one-dimensional line scans (M-mode images) that represent systolic and diastolic changes in cardiac chamber size as a function of time. OCT M-modes were analyzed using ImageJ software and a 125- μ m standard. End-diastolic dimension (EDD) and end-systolic dimension (ESD) were calculated from three consecutive beats per M-mode trace. Fractional shortening (FS) was calculated as $(EDD - ESD)/EDD \times 100$.

Images of bristles

Bristles were directly visualized using a Leica M165FC stereomicroscope with a DFC310x camera.

Statistical analysis

Data are expressed as mean \pm SEM. Comparison of difference in heart dimensions was tested by either a student's *t*-test for two samples or an analysis of variances (ANOVA)

for multiple comparisons. GraphPad statistical software (GraphPad Software Inc.) was used for analyses. $P < 0.05$ was considered significant.

Results

Df(1)Exel6245 causes dilated cardiomyopathy

We used OCT to measure cardiac function in a screen of genomic deficiency mutants from the Exelixis and DrosDel collections (Parks *et al.* 2004; Thibault *et al.* 2004; Ryder *et al.* 2007). OCT allows assessment of *in vivo* heart function in awake adult *Drosophila* in a manner similar to that of echocardiography in mammals (Wolf *et al.* 2006; Kim and Wolf 2009; Kim *et al.* 2010; Yu *et al.* 2010). EDD measures the heart chamber in the fully relaxed state, and ESD measures the fully contracted state during each beat. Fractional shortening reflects the level of cardiac function of the heart and is calculated as the difference between EDD and ESD divided by EDD. When compared to *w¹¹¹⁸* controls, *Df(1)Exel6245/FM7c* adult females had increased EDD and ESD and a decrease in fractional shortening (Figure 1). These changes in cardiac parameters were consistent with an enlarged heart and reduced cardiac function.

The deficiency corresponding to *Df(1)Exel6245* spans 95 kb from cytology region 11E11 to 11F4 and encodes 14 genes on the X chromosome (Figure 1A). Due to homozygous lethality, the cardiac phenotype of *Df(1)Exel6245* was observed in heterozygous females maintained with an X chromosome balancer. The abnormal cardiac phenotype persisted even after removal of the balancer chromosome by crossing into a *w¹¹¹⁸* genetic background (data not shown). To narrow the candidate interval within *Df(1)Exel6245*, we engineered custom deletions using transposons containing FRT sites from the Exelixis collection (Parks *et al.* 2004).

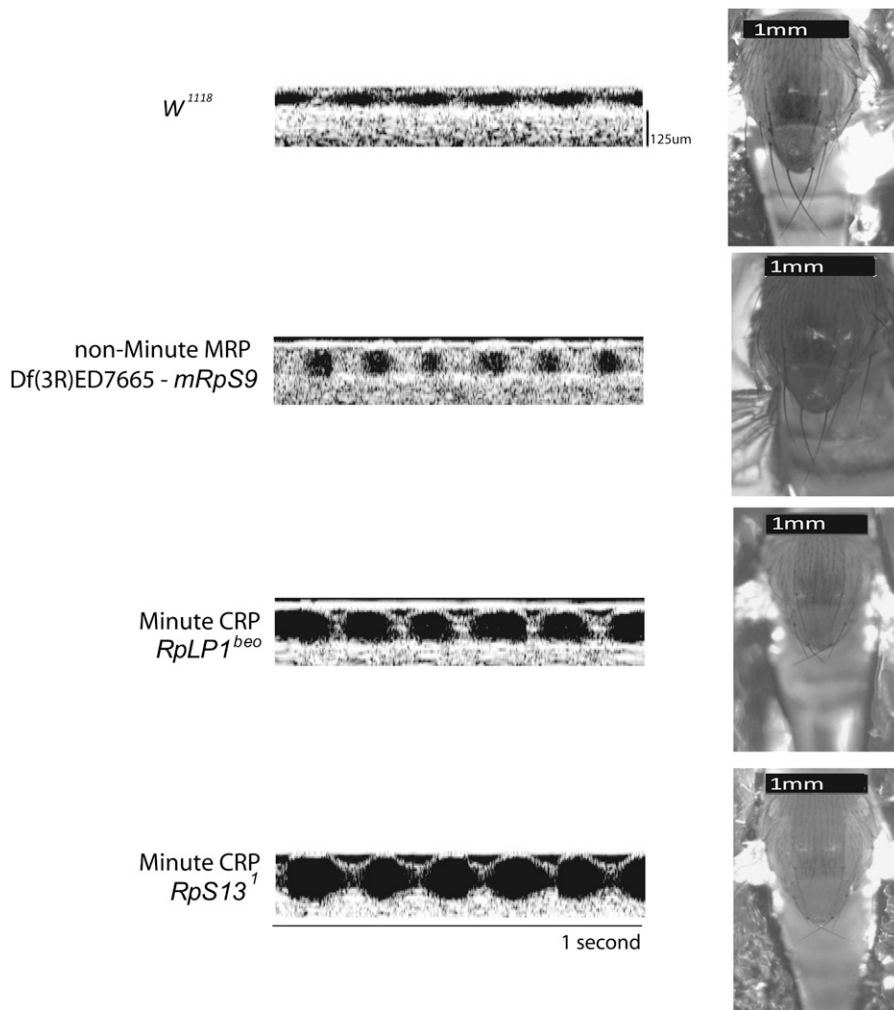


Figure 3 Minute deficiencies display cardiomyopathy while non-Minute mutants do not. Representative OCT images and the corresponding bristle images of *Drosophila* with mutations or deficiencies in genes for ribosomal proteins. Representative heterozygous *Minute* mutants display small thin bristles as well as significantly enlarged heart phenotype. Heterozygous deletion of a non-Minute MRP has normal bristle and heart phenotypes. Summary data are shown in Table 1.

One heterozygous 19-kb deletion, denoted as *Df(1)f08066-f07791/FM7c*, corresponded to a genomic region that encoded three genes (Figure 1A). Cardiac function in *Df(1)f08066-f07791/FM7c Drosophila* was abnormal and phenocopied the cardiac dysfunction seen in *Df(1)Exel6245*, indicating cardiomyopathy (Figure 1, B and C).

One of the genes within the narrowed region of *Df(1)f08066-f07791*, designated *RpS15Aa*, encoded a ribosomal protein subunit. Since *RpS15Aa* is predicted to be a causative gene for a *Minute* locus mapped to this region (Marygold *et al.* 2007), we examined these stocks for other *Minute* phenotypes. Both *Df(1)Exel6245/FM7c* and *Df(1)f08066-f07791/FM7c* had short, thin bristles compared to *w¹¹¹⁸* control, consistent with the expected phenotype of a haplo-insufficiency for this gene encoding a CRP (Figure 2A).

***RpS15Aa* disruption causes severe cardiomyopathy**

Next we examined the effects of ubiquitous and cardiac-specific knockdown of *RpS15Aa*. Cardiac expression of UAS-*RpS15Aa* RNAi using a heart-specific driver tinC-Gal4 (Yin and Frasch 1998; Qian and Bodmer 2009; Yu *et al.* 2010) resulted in very few progeny; however, flies that escaped

lethality had severely impaired heart function (Figure 2B). Ubiquitous expression of UAS-*RpS15Aa* RNAi by using an actin5C-Gal4 driver resulted in lethality during development. Flies with UAS-*RpS15Aa* RNAi in the absence of any Gal4 driver had normal cardiac function (Figure 2B). These data suggest that *RpS15Aa* expression in the heart is important for both heart function and viability of the fly.

Abnormal cardiac function is a common trait in *Minute* mutants

Since the *Minute* phenotypes are highly consistent across the numerous loci in the *Drosophila* genome (Lambertsson 1998), we examined other *Minute* mutants to determine whether cardiomyopathy is also associated with these stocks. We obtained publicly available *Drosophila* stocks that had genetic deficiencies spanning ribosomal protein genes and measured cardiac chamber size and function. All of the *Minute* mutants screened demonstrated severely impaired heart function with significantly decreased fractional shortening as compared to *w¹¹¹⁸* controls (Figure 3 and Table 1). While most *Minutes* correspond to mutations in CRP genes, a single *Minute* locus is caused by mutation of *eIF-2 α* , a translation initiation factor gene (Marygold *et al.* 2007). Cardiac

function of a mutant deficient for *eIF-2 α* (*Df(1)ED7364/FM7h*) showed enlarged EDD and ESD with reduced fractional shortening (Table 1). Although *eIF-2 α* is not a CRP gene, disruption of *eIF-2 α* likely leads to impaired protein translation resulting in a Minute phenotype and cardiomyopathy.

Deficiencies of non-Minute ribosomal genes are not generally associated with cardiomyopathy

Approximately 25% of CRP genes are not associated with a Minute phenotype (Marygold *et al.* 2007). Additionally, heterozygous deletions of MRP genes have not been associated with Minute phenotypes (Marygold *et al.* 2007). To test whether non-Minute CRP or MRP genes are associated with cardiomyopathy, we measured heart function in heterozygous deletions that span genes encoding MRPs and non-Minute CRPs. Of the 29 non-Minute deficiencies or insertions screened, 28 had normal heart function (Figure 3 and Table 1), while only *mRpS33*^{f01766} showed an abnormal heart phenotype (Figure 4). These findings suggest that, similar to the Minute syndrome, not all deficiencies of CRP genes cause a cardiac phenotype. Likewise, heterozygous deletions of MRP genes do not generally cause an abnormal heart phenotype. These data support our hypothesis that deficiencies that cause a Minute phenotype also cause cardiomyopathy, whereas deficiencies of non-Minute ribosomal protein genes do not generally have an altered heart phenotype.

Interestingly, a stock heterozygous for *PBac{WH}mRpS33*^{f01766}, an insertion in *mRpS33*, has significantly decreased heart function (Figure 4). Knockdown of *mRpS33* in the heart with RNAi driven by tinC-Gal4 also results in severely abnormal heart function without causing significant lethality (Figure 4). To date this is the only MRP gene we have found to cause a heart phenotype as a heterozygous mutant, and knockdown in the heart confirms that disruption of *mRpS33* causes severely impaired heart function.

Knockdown of Minute-causing genes postdevelopmentally in the heart results in cardiomyopathy

We next tested the effect of postdevelopmental knockdown of *RpS15Aa* and two other known Minute genes, *RpS5a* and *RpS3*, specifically in the adult fly heart. We used the tubulin-Gal80^{ts}; tinC-Gal4 driver system to knock down these genes in a temporally and spatially restricted manner (Kim and Wolf 2009; Kim *et al.* 2010; Yu *et al.* 2010). *Drosophila* with tub-Gal80^{ts}, tinC-Gal4, and UAS-RNAi against *RpS15Aa*, *RpS5a*, or *RpS3* were kept at 18° throughout development, keeping knockdown off, and then either maintained at 18° or moved to 27° after eclosion to allow RNAi expression in the adult heart. The postdevelopmental expression of RNAi directed against *RpS15Aa*, *RpS5a*, and *RpS3* all resulted in abnormal heart size and reduced cardiac function compared to control flies maintained at 18° (Figure 5). These data show that CRP function remains important for adult cardiac function. Additionally, adult cardiac knockdown of the MRP gene *mRpS33* resulted in impaired heart function in adult

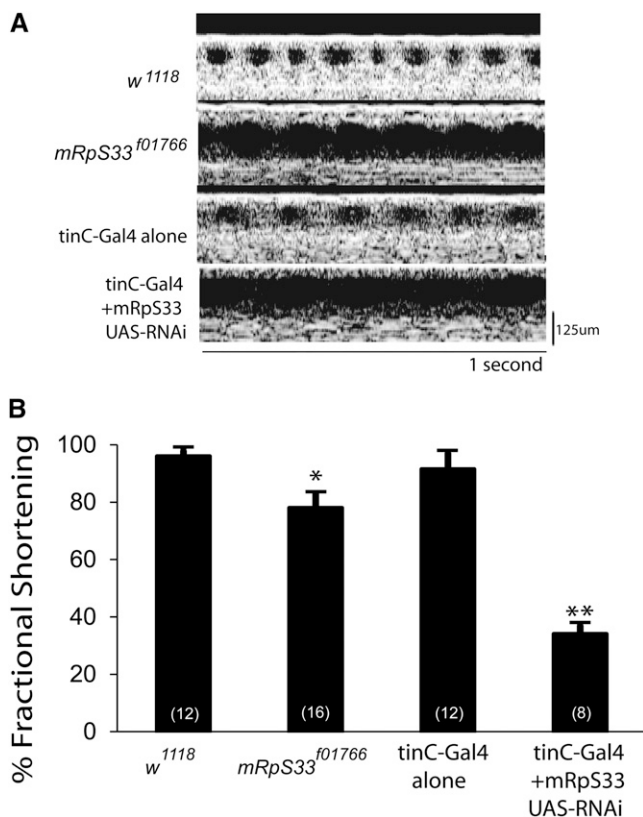


Figure 4 Disruption of *mRpS33* causes cardiomyopathy. (A) Representative OCT images of control *w*¹¹¹⁸, transposon insertion *PBac{WH}mRpS33*^{f01766}, tinC-Gal4 control, and UAS-RNAi against *mRpS33* driven by tinC-Gal4. (B) *PBac{WH}mRpS33*^{f01766}, and heart-specific *mRpS33* knockdown have significant decrease in heart function compared to controls. *n* = 8–16 per group shown in parentheses. **P* < 0.05, ***P* < 0.01 vs. *w*¹¹¹⁸, one-way Anova with Dunnett Multiple Comparisons test.

Drosophila indicating its importance for maintaining normal adult heart function (Figure 5).

Discussion

In this study we demonstrate that *RpS15Aa* is a candidate gene for cardiomyopathy and that cardiomyopathy is associated with the Minute phenotype observed in many CRP gene deficiencies. *Drosophila* heterozygous for CRP and MRP genes that did not cause a Minute phenotype in general did not demonstrate abnormal cardiac function. However, deficiency of *mRpS33* appears to be an exception since *mRpS33*^{f01766} had abnormal heart function. We also demonstrate that postdevelopmental, cardiac-specific knockdown of ribosomal protein genes that are associated with the Minute phenotype results in an impairment of *Drosophila* heart function.

We narrowed the candidate region of a Minute locus on the X chromosome to three coding regions using the custom deletion *Df(1)f08066-f07791* and showed that heart-specific RNAi knockdown of *RpS15Aa*, a gene within the deletion, caused severe cardiac dysfunction in adult *Drosophila*. On

Table 1 Cardiomyopathy correlates with the Minute phenotype in *Drosophila melanogaster*

Gene (synonym)	Bristle phenotype ^a	Heart phenotype	Allele	Genes affected	EDD ^b (μm)	ESD ^b (μm)	FS ^b (%)	n =
Control	Normal	Normal	<i>w¹¹¹⁸</i>		81 ± 4	2 ± 1	98 ± 1	16
Chromosome X								
<i>RpL36</i>	Minute	Abnormal	<i>Df(1)y74k24.1</i>	>30	123 ± 6	29 ± 9	78 ± 6	8
<i>RpL7A</i>	Minute	Abnormal	<i>Df(1)dx81</i>	>100	96 ± 5	23 ± 5	78 ± 5	14
<i>RpS15Aa</i>	Minute	Abnormal	<i>Df(1)f08066-f07791</i>	3	131 ± 12	32 ± 10	78 ± 5	9
<i>eIF-2α</i>	Minute	Abnormal	<i>Df(1)ED7364</i>	45	107 ± 4	22 ± 4	79 ± 4	4
<i>RpS5a</i>	Minute	Abnormal	<i>RpS5a¹</i>	1	104 ± 6	24 ± 5	78 ± 4	14
<i>mRpS14</i>	Normal	Normal	<i>Df(1)e00904-d02459</i>	9	70 ± 3	5 ± 1	93 ± 4	22
Chromosome 2								
<i>RpLP1</i>	Minute	Abnormal	<i>RpLP1^{beo}</i>	1	84 ± 4	17 ± 4	81 ± 4	16
<i>RpS21 (oho23B)</i>	Minute	Abnormal	<i>Df(2L)JS31</i>	~70	91 ± 10	29 ± 10	71 ± 11	4
<i>RpL40</i>	Minute	Abnormal	<i>Df(2L)BSC217</i>	20	82 ± 5	28 ± 4	67 ± 4	11
<i>RpS13</i>	Minute	Abnormal	<i>RpS13¹</i>	1	109 ± 5	35 ± 6	69 ± 5	12
<i>RpL27A</i>	Minute	Abnormal	<i>RpL27A¹</i>	1	90 ± 5	28 ± 5	69 ± 4	11
<i>RpS26</i>	Minute	Abnormal	<i>Df(2L)Exel8038</i>	26	89 ± 4	22 ± 6	75 ± 7	7
<i>RpL5</i>	Minute	Abnormal	<i>RpL5^{2d2}</i>	1	98 ± 7	22 ± 5	80 ± 4	7
<i>mRpL10</i>	Normal	Normal	<i>Df(2L)Exel7002</i>	18	82 ± 7	7 ± 3	93 ± 3	14
<i>mRpL48</i>	Normal	Normal	<i>Df(2L)Exel6005</i>	16	76 ± 6	5 ± 2	95 ± 2	16
<i>mRpS2</i>	Normal	Normal	<i>mRpS2^{EY10086}</i>	1	63 ± 3	1 ± 1	99 ± 1	14
<i>mRpL24</i>	Normal	Normal	<i>Df(2L)Exel7021</i>	22	92 ± 7	12 ± 5	87 ± 5	10
<i>mRpL28</i>	Normal	Normal	<i>Df(2L)Exel7021</i>	22	92 ± 7	12 ± 5	87 ± 5	10
<i>RpS28-like</i>	Normal	Normal	<i>Df(2L)ED678</i>	65	94 ± 3	1 ± 1	99 ± 0	8
<i>RpS2 (sop)</i>	Normal	Normal	<i>Df(2L)ED695</i>	40	76 ± 4	1 ± 0	99 ± 0	11
<i>mRpL52</i>	Normal	Normal	<i>Df(2R)Exel7092</i>	20	54 ± 5	11 ± 5	85 ± 6	12
<i>RpS15Ab</i>	Normal	Normal	<i>Df(2R)ED2098</i>	56	85 ± 4	4 ± 1	96 ± 2	12
Chromosome 3								
<i>RpS17</i>	Minute	Abnormal	<i>RpS17⁶</i>	1	92 ± 6	15 ± 4	83 ± 4	11
<i>RpS29</i>	Minute	Abnormal	<i>Df(3R)ED5454</i>	68	97 ± 2	19 ± 5	81 ± 5	9
<i>RpS25</i>	Minute	Abnormal	<i>Df(3R)ED5518</i>	80	86 ± 4	23 ± 5	73 ± 6	9
<i>RpS3</i>	Minute	Abnormal	<i>RpS3^{Plac92}</i>	1	84 ± 3	28 ± 4	64 ± 6	7
<i>mRpL17</i>	Normal	Normal	<i>Df(3L)Exel6084</i>	43	66 ± 6	18 ± 6	86 ± 7	7
<i>mRpL23</i>	Normal	Normal	<i>Df(3L)ED4287</i>	85	76 ± 9	9 ± 3	86 ± 5	7
<i>mRpS35</i>	Normal	Normal	<i>Df(3L)Exel6091</i>	13	69 ± 6	7 ± 2	93 ± 3	12
<i>mRpS6</i>	Normal	Normal	<i>Df(3L)ED4342</i>	48	83 ± 5	5 ± 3	94 ± 3	14
<i>mRpL12</i>	Normal	Normal	<i>mRpL12¹⁰⁵³⁴</i>	1	81 ± 3	6 ± 3	94 ± 3	11
<i>mRpL2</i>	Normal	Normal	<i>Df(3L)ED4470</i>	121	85 ± 5	7 ± 3	93 ± 3	11
<i>RpL10Ab</i>	Normal	Normal	<i>Df(3L)ED4470</i>	121	85 ± 5	7 ± 3	93 ± 3	11
<i>mRpL20</i>	Normal	Normal	<i>Df(3L)ED4486</i>	64	78 ± 6	3 ± 3	97 ± 3	15
<i>RpS12</i>	Normal	Normal	<i>Df(3L)ED4486</i>	64	78 ± 6	3 ± 3	97 ± 3	15
<i>mRpL39</i>	Normal	Normal	<i>Df(3L)ED217</i>	98	84 ± 4	4 ± 2	96 ± 3	16
<i>mRpS34</i>	Normal	Normal	<i>Df(3L)ED4606</i>	148	113 ± 5	1 ± 1	99 ± 1	14
<i>mRpS26</i>	Normal	Normal	<i>Df(3L)ED4710</i>	81	79 ± 4	2 ± 2	96 ± 3	14
<i>mRpS9</i>	Normal	Normal	<i>Df(3R)ED7665</i>	131	75 ± 5	7 ± 3	91 ± 3	12
<i>mRpL1</i>	Normal	Normal	<i>Df(3R)ED5230</i>	103	73 ± 3	4 ± 2	96 ± 3	21
<i>mRpL19</i>	Normal	Normal	<i>Df(3R)ED5330</i>	71	75 ± 6	3 ± 3	96 ± 4	5
<i>mRpS21</i>	Normal	Normal	<i>Df(3R)ED5612</i>	104	71 ± 5	1 ± 1	90 ± 1	14
<i>mRpS33</i>	Normal	Abnormal	<i>mRpS33¹⁰¹⁷⁶⁶</i>	1	97 ± 6	24 ± 6	78 ± 5	16
<i>mRpS11</i>	Normal	Normal	<i>Df(3R)ED5780</i>	100	87 ± 3	4 ± 2	96 ± 2	13
<i>mRpL55</i>	Normal	Normal	<i>Df(3R)ED5938</i>	73	69 ± 5	7 ± 3	91 ± 4	12
Chromosome 4								
<i>RpS3A</i>	Minute	Abnormal	<i>RpS3A^{57g}</i>	1	117 ± 5	42 ± 7	65 ± 5	10

^a Bristle phenotypes were confirmed as published (Duffy *et al.* 1996; Marygold *et al.* 2007) or visually confirmed in the case of custom deletions or obtained *P*-element stocks.

^b Cardiac chamber measurements: end-diastolic dimension (EDD), end-systolic dimension (ESD), and fractional shortening (FS: (EDD – ESD)/EDD × 100). Values are expressed as the mean ± SEM. Abnormal heart function is noted by a significant increase in ESD and a significant decrease in fractional shortening compared to *w¹¹¹⁸* control. One-way Anova with Dunnett Multiple Comparisons test.

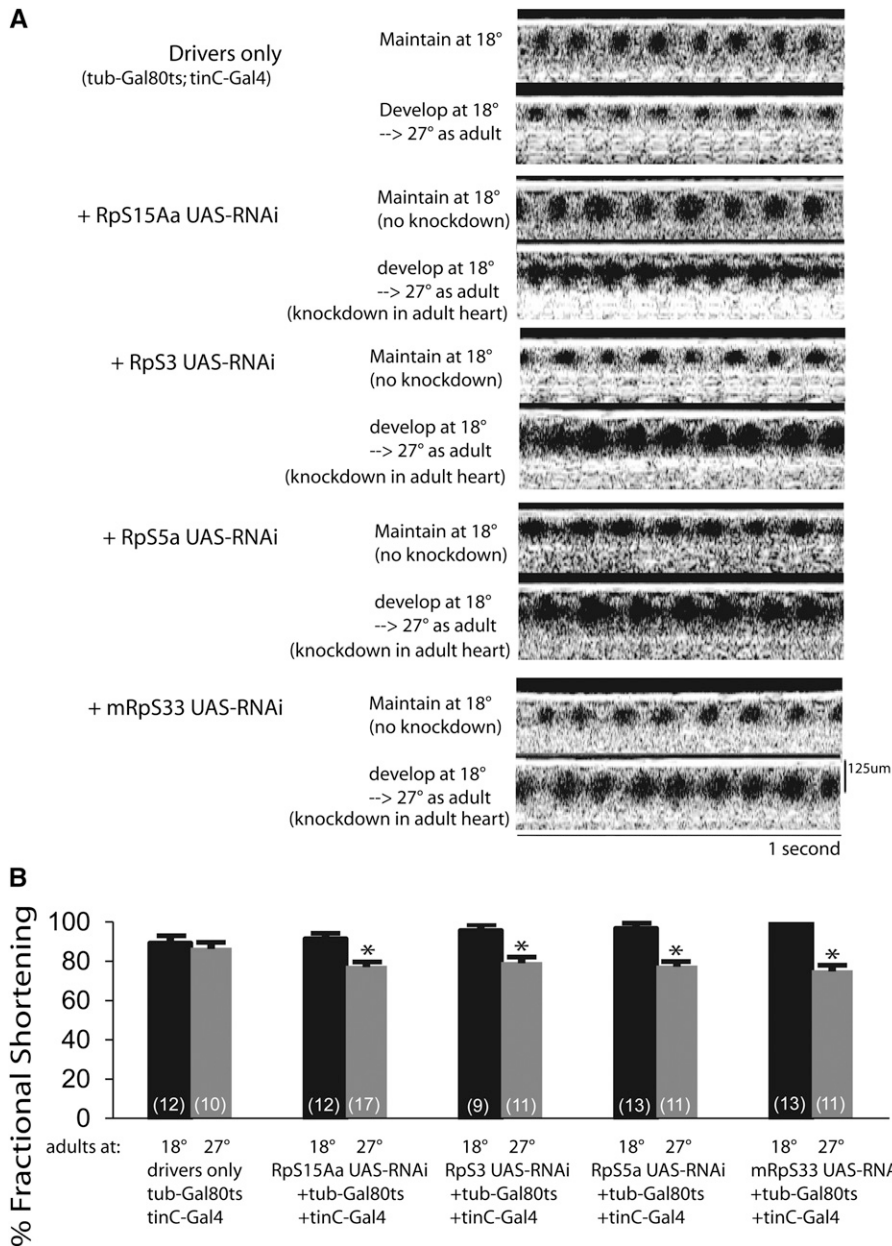


Figure 5 Cardiac-specific knockdown of ribosomal proteins in the adult results in postdevelopmental dilated cardiac phenotype. (A) Representative OCT images of *Drosophila* containing the following transgenes: tub-Gal80^{ts}, tinC-Gal4, and UAS-RNAi for *RpS15Aa*; tinC-Gal4 and UAS-RNAi for *RpS3*; tub-Gal80^{ts}, tinC-Gal4, and UAS-RNAi for *RpS5a*; tub-Gal80^{ts}, tinC-Gal4 and UAS-RNAi for *mRpS33*. Flies developed at 18° keeping the RNAi transgene from being expressed. Adult flies were either kept at 18° (no knockdown) or moved to 27° to turn on heart-specific knockdown in the adult. (B) Summary data showing that with drivers only, heart function remains normal when adults are moved to 27°; however, heart function is significantly impaired when RNAi expression is induced in the adult heart. $n = 9-17$ flies per group shown in parentheses. * $P < 0.05$ vs. 18° control, unpaired t -test. No error bars are shown on mRpS33 at 18° since all measurements of FS were 100%.

the basis of these observations, we show that many *Minute* stocks exhibit cardiomyopathy in addition to the previously described characteristics that define the *Minute* phenotype. The *Minute* stocks screened included deletions of CRP genes as well as the one non-CRP gene *eIF-2 α* (Marygold *et al.* 2007). Many *Minute* stocks have poor viability, and deletions across these *Minute* loci are often not available in many deletion collections. Our results suggest that cardiac abnormalities may contribute to the poor viability of *Minute* flies. While we have not screened all the *Minute* loci (17 of 61 loci were screened), we postulate that cardiomyopathy is likely a common phenotype of the *Minute* syndrome.

The mechanism for decreased cardiac function in *Minute* stocks is not known. Previous work has suggested that a specific balance of all subunits is needed in the ribosome

such that ribosome assembly and translation capacity is very sensitive to the least abundant component available (Marygold *et al.* 2007). Recent work indicates that mutations in genes that specifically affect ribosomal assembly result in marked decrease of functional ribosomes (Freed *et al.* 2010). Prior work has also investigated possible extra-ribosomal functions of some ribosomal proteins (Warner and McIntosh 2009), as well as tissue specific ribosomal activity (Kondrashov *et al.* 2011; Noben-Trauth and Latoche 2011). Alternatively, it has been proposed that differences in basal expression of various ribosomal protein genes could correspond with the differential sensitivity of the genes to haplo-insufficiency (Marygold *et al.* 2007). Therefore, heart size and function may be sensitive to decreases in CRP-mediated translation, or subsets of CRPs could have

heart-specific function as well. It is interesting that knock-down of CRPs in the adult fly can cause a postdevelopmental phenotype, indicating that decreasing ribosomal subunit expression in the adult fly can adversely affect heart function. The mechanism for the heart phenotype may be due to a decrease in the translational capacity of cardiac cells, or as previously mentioned, CRPs may have extra-ribosomal functions important for the integrity of the heart.

MRPs are essential for cell growth and proliferation, and mutant alleles of several MRP genes have been characterized in the fly, with defects in cell growth and development (Frei *et al.* 2005; Tselykh *et al.* 2005; Zhan *et al.* 2010). In our screen, we observed that 28 of 29 disruptions of non-Minute CRP or MRP genes retained a normal adult cardiac function, representing 5 of 22 non-Minute CRPs and 24 of 75 MRPs. While all of these genes are required to produce proteins for proper ribosome formation and protein translation, only a subset of ribosomal genes cause a Minute phenotype, including cardiomyopathy. *mRpS33* is the only exception we identified in which a heterozygous deficiency of a MRP gene causes significantly dilated cardiomyopathy.

Human CRP and MRP genes have been established as candidates for causing human syndromes and diseases (Draptchinskaia *et al.* 1999; Ruggero and Pandolfi 2003; O'Brien *et al.* 2005; Da Costa *et al.* 2010; Freed *et al.* 2010; Ito *et al.* 2010; Narla and Ebert 2010). Diamond Blackfan anemia is associated with mutations in several haplo-insufficient CRP genes (Draptchinskaia *et al.* 1999; Da Costa *et al.* 2010; Ito *et al.* 2010) and is characterized by congenital defects, including cardiac abnormalities (Ito *et al.* 2010; Doherty *et al.* 2010). Mutations in the gene encoding ribosomal protein RPS19 have been identified in approximately 25% of Diamond Blackfan anemia families and haplo-insufficiency of several other CRP genes have subsequently been found in affected patients (Da Costa *et al.* 2010). Studies in transgenic mice expressing a mutated RPS19 gene suggest that one mechanism by which mutations in RPS19 can cause Diamond Blackfan anemia is by its effect as a dominant negative protein (Devlin *et al.* 2010).

Other diseases that have decreased ribosomal biogenesis and function are commonly associated with increased susceptibilities to cancer (Ruggero and Pandolfi 2003; Bilanges and Stokoe 2007; Montanaro *et al.* 2008; Narla *et al.* 2011). From human studies, disease-causing mutations often appear to disrupt the biogenesis of the ribosome, and thus mutation in one gene is able to greatly influence the translational capacity of the cell. In humans, hematopoietic tissues seem to be especially vulnerable to these ribosomopathies, which could be explained by the high proliferation rate in these cells; however, the tissue specificity of these diseases of ubiquitous proteins is intriguing (Freed *et al.* 2010). In addition, several human MRP genes map to loci associated with disorders consistent with impaired oxidative phosphorylation (O'Brien *et al.* 2005). Mutations of human MRPS22 and MRP16 have been shown to cause severe disease in the homozygous state (Miller *et al.* 2004; Saada *et al.* 2007; Smits

et al. 2010), showing that complete lack of a MRP is extremely detrimental. Future investigation of human ribosomal protein gene mutations, both in the heterozygous and homozygous states, may reveal insights into ribosomal biology and cardiovascular disease.

Drosophila is a valuable model system for investigation into human disease, including cardiomyopathy. Previous work has found that alterations in structural and contractile proteins can alter contractility in the *Drosophila* heart (Wolf *et al.* 2006; Allikian *et al.* 2007; Taghli-Lamalle *et al.* 2008). Additionally, signaling in such pathways as Notch, Rhomboid 3, SMAD, and insulin can change heart function in flies (Wessells *et al.* 2004; Kim *et al.* 2010; Yu *et al.* 2010; Goldstein *et al.* 2011). In addition to the CRP genes that we identify in this study, large-scale RNAi screening has identified protein complexes important for *Drosophila* heart function (Neely *et al.* 2010).

Our data support the concept that alterations in ribosomal function can cause cardiac dysfunction as shown by the marked cardiomyopathy in *Drosophila* with a Minute phenotype. Additional investigation is needed to address the mechanism underlying the sensitivity of cardiac tissues in *Drosophila* to mutation in haplo-insufficient ribosomal protein genes.

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