A Direct Screen Identifies New Flight Muscle Mutants on the Drosophila Second Chromosome

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ABSTRACT

An ethyl methanesulfonate mutagenesis of *Drosophila melanogaster* was undertaken, and >3000 mutagenized second chromosomes were generated. More than 800 homozygous viable lines were established, and adults were screened directly under polarized light for muscle defects. A total of 16 mutant strains in which the indirect flight muscles were reduced in volume or disorganized or were otherwise abnormal were identified. These fell into seven recessive and one semidominant complementation groups. Five of these eight complementation groups, including the semidominant mutation, have been mapped using chromosomal deficiencies and meiotic recombination. Two complementation groups mapped close to the *Myosin heavy chain* gene, but they are shown to be in different loci. Developmental analysis of three mutations showed that two of these are involved in the early stages of adult myogenesis while the other showed late defects. This is the first report of results from a systematic and direct screen for recessive flight muscle defects. This mutant screen identifies genes affecting the flight muscles, which are distinct from those identified when screening for flightlessness.

THE mechanisms underlying myogenesis have been investigated intensely in *Caenorhabditis elegans* (Brenner 1974), leech (Jellies and Kristan 1988), grasshopper (Ho *et al.* 1983), Drosophila (Bate 1993; Bate and Baylies 1996; Ruiz Gomez and Bate 1997; Baylies *et al.* 1998; Roy and VijayRaghavan 1998), and vertebrates (Buckingham 1992; Cossu *et al.* 1996; Firulli and Olson 1997). In the fruit fly *Drosophila melanogaster*, muscle development takes place twice—in the embryo during the formation of the muscles of the larva and during pupal development when adult muscles are made (Hooper 1986; Bate 1990; Fernandes *et al.* 1991; Reedy and Beall 1993). Muscle development in the embryo involves the specification of the mesoderm (Thisse *et al.* 1988; Azpiazu *et al.* 1996; Riechmann *et al.* 1997), the choice of a muscle founder cell (Bate 1990; Rushton *et al.* 1995), and the consequent fusion of myoblasts. This forms a fiber that attaches to specific sites on the epidermis and is correctly innervated (Bate 1990; Broadie and Bate 1993). During embryonic myogenesis, signals from the ectoderm and the mesoderm result in the selection and specification of a muscle progenitor cell (Ruiz Gomez and Bate 1997; Carmena *et al.* 1998). This divides asymmetrically to give rise to two daughter cells, one of which becomes an adult muscle progenitor (Ruiz Gomez and Bate 1997). In the thoracic and head segments, the adult progenitor myoblasts associate with imaginal disc cells and proliferate during larval life (Poodry 1980; Lawrence and Brower 1982; Fernandes *et al.* 1991). During pupal development, adult myoblasts move to specific sites of muscle formation and fuse to form the adult fibers (Bate *et al.* 1991; Currie and Bate 1991; Fernandes *et al.* 1991; Fernandes and VijayRaghavan 1993; Roy and VijayRaghavan 1997).

Several important features make adult flight muscle development interesting and novel. Unlike other segments, the development of muscles in the second thoracic segment (T2) occurs in the absence of any autonomous requirement for homeotic gene function (Roy *et al.* 1997). Thus, in T2 of the embryo and in the dorsal T2 muscles of the adult, not only is there no expression of genes of the Antennapedia and Bithorax complex, but ectopic expression aborts muscle development (Roy and VijayRaghavan 1997). Therefore, as in vertebrate somite development, inductive influences play important roles in muscle patterning (Lance-Jones 1988; Ordahl and Ledouarin 1992; Fernandes *et al.* 1994; VijayRaghavan *et al.* 1996; Roy *et al.* 1997; Roy and VijayRaghavan 1998). Another difference between flight muscle myogenesis and that of the embryonic muscles in Drosophila is that the adult motor neurons develop by modification of larval neurons. The physiological properties of the indirect flight muscle (IFM) innervation are very different from those of the larval motor neurons. The mechanisms that underlie this transformation of neuronal physiology remain largely

We dedicate this article to the founder of our Drosophila Stock Centre, Professor H. A. Ranganath, on the occasion of his 50th birthday.

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unknown. It has been suggested that the developing muscle fibers may play a role in this process (Fernandes *et al.* 1994; Roy and VijayRaghavan 1998). The development of a male-specific muscle in the adult abdomen is dependent on the sex of the motor neuron, which is the only dramatic example of neuronal influence on muscle patterning so far reported (Lawrence and Johnston 1986; Currie and Bate 1995). Such similar influences between neuron and muscles remain yet to be identified. The IFMs provide an accessible system in which inductive influences, such as epidermis-muscle and nerve-muscle interactions, as well as the properties intrinsic to the mesoderm that affect muscle pattern and differentiation, can be studied.

Screens for adult flight muscle mutants have been few and far between. Many X-linked flightless mutants were isolated during the screening for mutants that showed wing position abnormalities (Deak 1977; Homyk and Sheppard 1977; Koana and Hotta 1978; Deak *et al.* 1982). Many of these genes have been analyzed during development, and the molecular analysis of some of them has also been fruitful (Costello and Wyman 1986; Homyk and Emerson 1988; de la Pompa *et al.* 1989; Fleming *et al.* 1989; De Couet *et al.* 1995). The autosomal screens for muscle mutants have, for obvious reasons, concentrated on isolating and studying dominant flightless phenotypes. These studies have identified mutations in genes that encode muscle structural components. The ease with which dominant flightless mutants have been recovered suggests either that the dose requirements of these gene products are stringent or that relatively small perturbations in muscle structure can reduce flight ability (Mogami and Hotta 1981; Okamoto *et al.* 1986; Warmke *et al.* 1989; Cripps *et al.* 1994a).

In this article, we describe the results of a direct screen for second chromosomal recessive mutations that result in IFM abnormalities. We have identified eight new complementation groups that affect muscle development. Our results demonstrate the advantage of a screen that directly examines the muscle phenotype. We discuss our results in relation to the possible roles of the identified genes and prospects for further screens.

MATERIALS AND METHODS

Fly stocks: Canton-S was used as the wild-type strain. The balancer chromosomes used are described in Lindsley and Zimm (1992), as are the other markers used in the crosses outlined below, unless otherwise specified. A total of 84 deficiency- and duplication-bearing strains covering almost 80% of the second chromosome were obtained from the Drosophila Stock Center (Mysore and Indore, India) and the Bloomington Stock Center in the U.S. Details of the rearrangement strains where our mutants are mapped and their breakpoints are given in the results of the respective mutants. All the stocks were cultured on standard cream of wheat-agar medium at 24 ± 1° in half-pint bottles.

Mutagenesis and isolation of mutants: Ethyl methanesulphonate (EMS) at 25 mm was administered to 2-day-old Canton-S adult male flies following the procedure described in Grigliatti (1986). EMS-treated males were crossed to *Tft/CyO* virgin females, and the F_1 male offspring, which had either curly or tufted phenotypes, were crossed separately to *Tft/CyO* virgin females. Progeny from these individual crosses carrying the *CyO* balancer chromosome were sib-mated to obtain the mutagenized chromosome in homozygous condition. The IFMs of the viable homozygotes were screened for possible muscle defects using polarized light. The genetic scheme is presented in Figure 1. Control mutagenesis experiments were carried out using *X^X/Y* stocks following the procedure in Grigliatti (1986). Furthermore, alleles of *ifm(2)RU3*, *ifm(2)RU4*, and *ifm(2)RU5* (see results for nomenclature) were isolated by crossing flies carrying a mutagenized chromosome with those carrying an appropriate noncomplementing deficiency-bearing chromosome.

Muscle analysis: For analysis of the IFMs in the mutant adults, whole-thorax mounts were prepared following the procedure described by Fyrberg *et al.* (1994). Briefly, the head and abdomen of each fly were removed, and the thoraces were dehydrated in alcohol series, cleared in methyl salicylate, and observed in plane-polarized light using a Leica Wild M3Z zoom stereomicroscope with polarizing filter attached. For photography, hemithoraces were made by freezing in liquid nitrogen, bisecting them with a razor blade, and then processing as described above. The sections were observed and photographed using a Leitz Aristoplan microscope using polarized light optics.

Complementation tests: Five-day-old males and virgin females from each of the newly isolated mutant lines were crossed reciprocally among each other, and the progeny were analyzed for IFM defects. All the newly isolated mutants were crossed reciprocally with four viable *Myosin heavy chain* (*Mhc*) alleles (Mhc^7, Mhc^{12}, Mhc^{13}, and Mhc^{19}), and the muscle phenotypes of the *trans*-heterozygotes (mutant/*Mhc*) were observed. The frequency of recombination between the *Mhc* locus and a specific mutation was estimated by scoring the number of recombinants with a wild-type IFM phenotype as seen using polarized light microscopy.

Flight test: This was done, with slight modifications, as described by Vigoreaux *et al.* (1993). Individual flies, 1–2 days old, were placed in an empty milk bottle and observed for wingbeat and flight. Flies were scored on a scale from 0 to 3 as flightless, weak fliers, moderate fliers, and normal fliers, respectively. Flies that never beat their wings and fell straight to the bottom when the bottle was tapped were termed flightless and assigned a score of 0. When the bottle was tapped, flies that landed on a wall or bottom with some wing vibration but that otherwise did not fly were assigned a score of 1 (weak fliers). Flies that vibrated their wing when the bottle was not tapped and flew sporadically were assigned a score of 2 (moderate fliers). Normal fliers with very active wingbeats were assigned a score of 3.

Fertility and viability: Five-day-old males and virgin females from each mutant line were crossed reciprocally to Canton-S males and virgin females. Twenty replicate crosses were made for each line. Each mating was observed for 3 hr. After 5 days, the parents were transferred to a fresh vial, then each vial was examined for viable larvae. Mutant males or females, which produced no viable larva, were termed sterile.

The viability of the newly isolated mutants was estimated by adopting the following method. Ten males and females aged at least for 5 days were separately placed in food vials for 2 days to mate. Serial transfers to a new set of fresh food vials were made every 2 days for a total of six transfers. The number of heterozygous and homozygous flies emerging in each of these vials was counted. Five such replicates were set up for

each mutant line, and the viability of the homozygous flies was calculated as a fraction of the total number of heterozygotes. Similar fertility and viability experiments of the mutants with the noncomplementing deficiency lines were also carried out.

Maceration of thorax: To observe myofibrils of IFM, the muscles were dissected from the thorax using fine forceps and needles in a drop of phosphate-buffered saline (PBS; relaxing) or 0.7% sodium chloride (contracting) solution. The muscle was then teased with fine needles to separate the myofibrils and then covered with a coverslip. The specimens were examined with a phase-contrast microscope (Leica) and photographed.

Rescue with *Mhc* transgene: *w; P[w$^+$Mhc$^+$]wm3* transgene flies (Cripps *et al.* 1994b) carry two extra copies of the wild-type *Mhc* gene inserted on chromosome 3. This strain was crossed to *w/w; Ifm(2)RUI/CyO; +/+* and *w/w; ifm(2)RU2^1/CyO; +/+* using standard genetic methods. The resulting noncurly F_1 progeny with orange, *i.e.*, *P[w$^+$]*, eyes of the genotypes *w; Ifm(2)RU1/+; P[w$^+$Mhc$^+$]wm3/+* and *w; ifm(2)RU2^1/+; P[w$^+$Mhc$^+$]wm3/+*, respectively, were tested for flight, and wing and muscle phenotypes. Virgin females and males of these were crossed to get an F_2 generation, and the resulting progeny were screened for flight, and wing and muscle phenotypes.

Developmental analysis: Appropriate crosses were made to create mutant flies carrying either the *MHC-lacZ* (Hess *et al.* 1989) or *Act88F-lacZ* (Hiromi *et al.* 1986) transgenic promoter-*lacZ* fusions. Developmental analysis of the IFMs of these flies was done by histological and histochemical methods following the protocols of Fernandes *et al.* (1991).

RESULTS

Mutagenesis and screening of the mutations: The mutagenesis scheme in Figure 1 was used to generate second-chromosome IFM mutants. Five independent mutagenesis experiments were conducted, from which a total of 3283 mutagenized chromosomes were generated. From these, 897 viable recessive lines were recovered in F_3. Flies homozygous for the mutagenized second chromosome from each line were analyzed for IFM defects, and 16 lines were identified in which the IFMs were reduced in volume or otherwise abnormal. Each mutant originated from a single male from an independent culture. One mutant was found to be semidominant, and the remaining 15 were completely recessive. Furthermore, 1600, 1280, and 900 mutagenized chromosomes were screened for new noncomplementing alleles of *ifm(2)RU3*, *ifm(3)RU4*, and *ifm(2)RU5*, respectively. This yielded six alleles for *ifm(2)RU3*, two alleles for *ifm(2)RU4*, and one allele for *ifm(2)RU5*.

Complementation analysis and nomenclature: Muscle phenotypes of progeny from the reciprocal crosses between all the lines revealed that the semidominant mutation showed a mutant phenotype *in trans* with all other mutations and *in trans* with wild-type flies. The semidominant mutation has been named *Indirect flight muscle (2) RU1* (*Ifm(2)RU1*). The other 15 recessive mutant lines fall into seven complementation groups and they have been designated as *ifm(2)RU2–8*. Nine alleles of *ifm(2) RU2* have been isolated and designated as *ifm(2)RU2$^{1–9}$*. The viable alleles for the *ifm(2)RU3* have been designated as *ifm(2)RU3$^{1–4}$* and *ifm(2)RU3l$^{5–7}$* for lethal alleles. Three alleles of *ifm(2)RU4* and two alleles of *ifm(2)RU5* were also recovered.

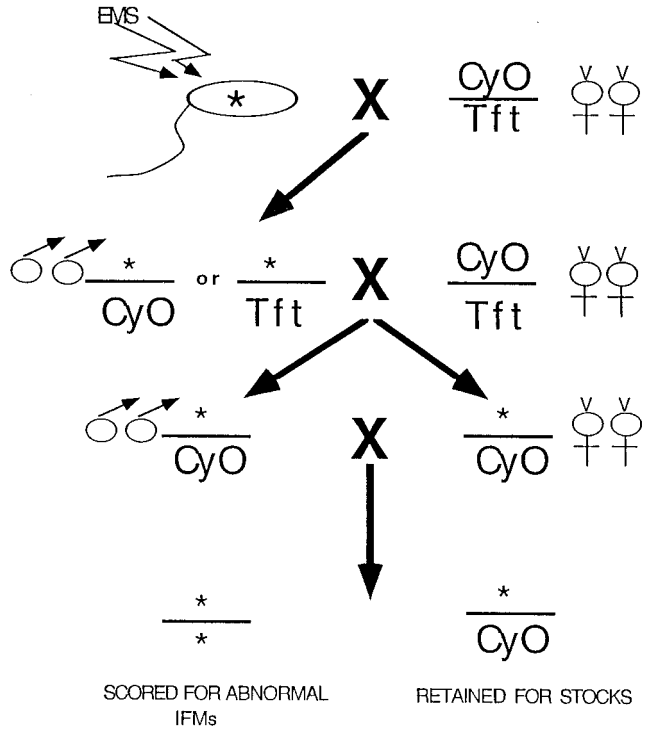

Figure 1.—Genetic scheme for screening indirect flight muscle mutations on the second chromosome. EMS-treated males were crossed to *CyO/Tft* virgin females. F_1 male offspring were aged for 2 days. Each of these males, which had either a *CyO* or *Tft* phenotype, were crossed separately to *CyO/Tft* virgin females. Male and virgin female progeny from the above cross carrying the *CyO* balancer chromosome were crossed to each other to bring the mutagenized chromosome in homozygous condition. IFMs of the viable homozygotes were screened for possible defects, and the heterozygotes were maintained for stocks.

Phenotypes of the mutants: Table 1 summarizes the muscle abnormalities of the newly isolated mutants as moderate or severe. The main features of the mutants (in each section, the data of first allele have been described unless otherwise specified) are as follows:

All animals homozygous for the semidominant mutant *Ifm(2)RU1* show a drooping wing phenotype, in some cases so extreme that it interferes with walking. The IFMs in these flies are totally disorganized, with the muscle fibers appearing thin, disrupted, and constricted to a small region of the thorax (Figure 2B). Although the phenotype is completely penetrant, the expressivity varies. Thinning of the dorsal longitudinal muscles (DLMs) is seen toward either end of the thorax, whereas in some extreme cases, a whole DLM appears as thin strips. Phase-contrast micrographs show that myofibrils appear to be easily broken with no demarcation of the muscle bands (Figure 3C). *Ifm(2)RU1/+* heterozygous flies also show a slight drooping of the

TABLE 1

Wing and muscle abnormalities of the newly isolated IFM mutants

Mutant lines	Total no. of flies scored	Wing		Defects in IFMs[a]			
		Normal	Raised/drooping and extended	DLMs		DVMs	
				A	B	A	B
Ifm(2)RU1	118	Φ	118 (100)[b]	Φ	118	13	105
Ifm(2)RU1/+	315	110	205 (65)	180	135	216	99
ifm(2)RU2[1]	179	02	177 (98.8)	39	140	120	59
ifm(2)RU3[1]	124	105	19 (15.3)	10	51	16	10
ifm(2)RU4[1]	176	130	46 (26.1)	28	28	Φ	Φ
ifm(2)RU5[1]	145	100	45 (31)	10	30	02	01
ifm(2)RU6	124	114	10 (8)	55	05	28	04
ifm(2)RU7	144	115	29 (20.1)	05	37	Φ	Φ
ifm(2)RU8	206	206	Φ	28	20	Φ	Φ

A, moderate defect of the specific IFM; B, severe reduction of the specific IFM; Φ, normal wing muscles. The numbers in parentheses indicate the percentage of wing position abnormality.

[a] Number of flies showing reduction in the volume or variation in number or other abnormalities in the IFMs.

[b] Drooping wing phenotype only.

wings, and the DLMs are affected to varying extents. In some flies (180/315), one or two DLMs are disorganized, and in others (135/315), the posterior regions have degenerated (Figure 2C and Table 1). The dorsoventral muscles (DVMs) are also usually disorganized or degenerated (Figure 2B, Table 1). The myofibrils of the heterozygotes appear more or less like those of the homozygotes, except that the Z bands are preserved (Figure 3D). Homozygotes are clearly more severely affected than heterozygotes.

ifm(2)RU2 homozygotes (all alleles) have raised wings, but this phenotype is not completely penetrant (Table 1). The DLMs show thinning in the anterior parts to various degrees (Figure 2D). The DVMs are also disorganized and in extreme cases are totally degenerated (Figure 2D). The fibrils are split, spongy, and loosely packed (Figure 3E). *ifm(2)RU2*[1]/+ fibrils show normal banding patterns (Figure 3F), confirming this as a recessive allele.

The *ifm(2)RU3*[1] homozygotes show normal (105/124) to raised/held-out wing phenotypes (19/124). The number of DLM fibers varies, but usually (51/124) only three are present, the posterior ends of these fibers have degenerated, and the DVMs are disorganized (Figure 2E). The single myofibril in the phase-contrast micrograph (Figure 3G) appears to have a normal banding pattern, except for a few breaks and gaps. Subsequently isolated lethal alleles of *ifm(2)RU3*[1] show late pupal le-

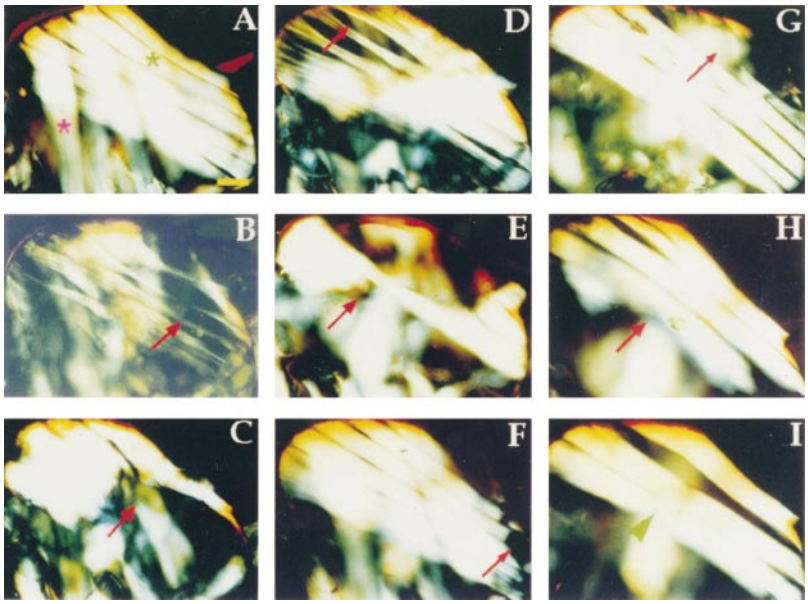

Figure 2.—Normal and mutant IFM morphology as seen under a polarized light microscope. (A) Normal IFMs. (B) *Ifm(2)RU1/Ifm(2)RU1*. (C) *Ifm(2)RU1/+*. (D) *ifm(2)RU2*[1]/*ifm(2)RU2*[1]. (E) *ifm(2)RU3*[1]/*ifm(2)RU3*[1]. (F) *ifm(2)RU4*[1]/*ifm(2)RU4*[1]. (G) *ifm(2)RU5*[1]/*ifm(2)RU5*[1]. (H) *ifm(2)RU6/ifm(2)RU6*. (I) *ifm(2)RU7/ifm(2)RU7* and *ifm(2)RU8/ifm(2)RU8* (both show a similar type of variation in the number of the DLMs). The green asterisk shows one of the six DLMs, and red represents the first set of the DVMs. The red arrows show the disorganized muscle fibers. The green arrowhead shows one of the DLMs of the mutants where only three DLMs are present. In all the figures anterior is toward the left-hand corner and dorsal is toward the top. Only one hemisegment is shown in all the figures. All figures are shown at the same magnification. Bar, 200 μm.

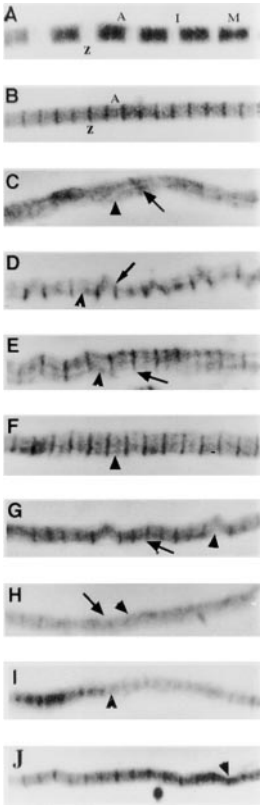

Figure 3.—Phase-contrast micrographs of normal (A and B) and mutant myofibrils (C–J). (A) Normal myofibril in the relaxed condition. (B) Highly contracted state of a normal myofibril. (C) *Ifm(2)RU1*/*Ifm(2)RU1*. (D) *Ifm(2)RU1*/+. (E) *ifm(2)RU2*^{1}/*ifm(2)RU2*^{1}. (F) *ifm(2)RU2*^{1}/+. (G) *ifm(2)RU3*^{1}/*ifm(2)RU3*^{1}. (H) *ifm(2)RU4*^{1}/*ifm(2)RU4*^{1}. (I) *ifm(2)RU5*^{1}/*ifm(2)RU5*^{1}. (J) *ifm(2)RU6*/*ifm(2)RU6*. The mutant myofibrils do not show band patterns as shown in A in the relaxing solution; they appear as homogenous rods. The arrows show the defective Z bands, and the arrowheads show the defective myofilaments. A, anisotropic band; I, isotropic band; Z, telophragma (fine transverse membrane attached to the sarcolemma); M, mesophragma (a delicate membrane traversing the median discs). All the figures are shown at the same magnification. Bar, 2.5 μm.

thality (data not shown). The viable alleles show similar types of muscle defects, but those of *ifm(2)RU3*^{1} are especially variable in expressivity and penetrance (28–35%).

ifm(2)RU4^1 homozygotes show normal (130/176) to slightly extended wings (46/176). The DLMs have degenerated fibers that are split in the posterior end (Figure 2F). The DVMs appear normal. Where degeneration occurs, no banding pattern of the myofibrils is seen (Figure 3H). Two viable alleles, one showing a more severe phenotype and the other very similar phenotypically to *ifm(2)RU4*^{1}, were recovered during the allele screening.

ifm(2)RU5^1 homozygotes have a weak drooping or held-out wing phenotype, and their DLMs show many defects, including improper splitting, variation in fiber number, and degeneration. The DVMs are usually normal, but degeneration was seen in a few homozygotes (Figure 2G). An aberrant splitting occurs along the length of the myofibrils (Figure 3I), a phenotype also seen in another allele, *ifm(2)RU5*^{2}.

ifm(2)RU6 homozygotes have a slight drooping of the wings and IFMs degenerate to various degrees (Figure 2H, Table 1). A splitting and degeneration of myofibrils occurs in some regions, but in others, the myofibrils appear completely normal (Figure 3J).

ifm(2)RU7 and *ifm(2)RU8* homozygotes exhibit a held-out wing phenotype, and the DLMs range in number from three to six (Figure 2I), but the myofibrils appear normal.

Flight ability: Table 2 summarizes the flight ability of the mutants. Apart from *Ifm(2)RU1* and *ifm(2)RU2*^{1} homozygotes, which are completely flightless, all other mutants show some degree of flight ability—from *ifm(2)RU3*^{1} and *ifm(2)RU6*, which are either flightless or weak fliers, to *ifm(2)RU7* and *ifm(2)RU8*, where most homozygotes are fliers, though a few show moderate flight. Except for *Ifm(2)RU1* heterozygotes, which are flightless, heterozygotes of all the other mutants show normal flight.

Mapping of the mutants: The semidominant *Ifm(2)RU1* mutant mapped between *black* (48.5) and *cinnabar* (56.5) at 52.4, close to the *Mhc* gene (Table 3). This was confirmed using chromosomal deficiencies. *Ifm(2)RU1* was uncovered by the deficiency *Df(2L)H20* (36A8-9; 36E3-4) and showed wing and muscle phenotypes more severe than those seen in animals homozygous for this mutation (Figure 4, A and B). *Ifm(2)RU1 in trans* with all other 83 deficiencies, including *Df(2L)H68* (36B-C1; 37A1-B1), showed a phenotype similar to that of *Ifm(2)RU1*/+ animals. *Df(2L)H20*/+ flies, though haploinsufficient for flight, show normal muscle patterns, except for a reduced birefringence of the IFMs compared to those of wild type. These crosses confirm the semidominant nature of this mutation and map to the 36A8-9; 36B-C1 region.

The recessive mutations could not be readily assigned a map position by meiotic recombination because of their incomplete penetrance and expressivity. Therefore, mapping these mutations by using chromosomal deficiencies was attempted. Four of the seven recessive complementation groups were mapped using 84 deficiencies and duplication strains covering 80% of the second chromosome. The remaining three could not be mapped with these strains.

ifm(2)RU2/*Df(2L)H20* animals, independently of the *ifm(2)RU2* allele used, show wing and muscle phenotypes similar to *ifm(2)RU2*^{1} homozygotes (Figure 4, C and D). *Df(2L)H68*, however, complements the mutant phenotype. This suggests that this mutation is also located in the same region as *Ifm(2)RU1*; *i.e.*, 36A8-9; 36B-C1.

ifm(2)RU3^1 mutant phenotypes are uncovered by the deficiency *Df(2R)Px* (60B8-10; 60D1-2), but they are cov-

TABLE 2

Flight ability of the newly isolated mutants

Genotype	Flightless	Weak flight	Moderate flight	Normal flight
+/+ (Canton-S)	—	—	2	48
Ifm(2)RU1/Ifm(2)RU1	50	—	—	—
Ifm(2)RU1/+	45	5	—	—
ifm(2)RU2[1]/ifm(2)RU2[1]				
Raised wing	50	—	—	—
Normal wing	36	14	—	—
ifm(2)RU2[1]/+	—	—	10	40
ifm(2)RU3[1]/ifm(2)RU3[1]				
Expanded/raised wing	44	6	—	—
Normal wing	12	38	—	—
ifm(2)RU3[1]/+	—	—	4	46
ifm(2)RU4[1]/ifm(2)RU4[1]				
Expanded wing	—	26	24	—
Normal wing	—	—	6	44
ifm(2)RU4[1]/+	—	—	2	48
ifm(2)RU5[1]/ifm(2)RU5[1]				
Expanded wing	—	14	28	8
Normal wing	—	2	46	2
ifm(2)RU5[1]/+	—	—	4	46
ifm(2)RU6/ifm(2)RU6				
Expanded	1	40	4	5
Normal	—	14	28	8
ifm(2)RU6/+	—	5	17	28
ifm(2)RU7/ifm(2)RU7	—	—	12	38
ifm(2)RU8/ifm(2)RU8	—	—	16	34

The number of flies tested for each genotype was 50.

ered by the deficiencies *Df(2R)or-BR6* (59D5-10; 60B3-8) and *Df(2R)Px2* (60C5-6; 60D9-10). The anterior ends of the DLM are completely lost in *ifm(2)RU3[1]/Df(2R)Px* (Figure 4E), a phenotype that is not seen in *Df(2R)Px/+* flies. This indicates that *ifm(2)RU3[1]* maps to the 60B8-10; 60C5-6 region.

The recessive phenotype of *ifm(2)RU4[1]* is covered by the deficiency *Df(2R)bw[5]* (59D10-E1; 59E4-F1) and uncovered by *Df(2R)bw-S46* (59D8-11; 60A7), which maps *ifm(2)RU4* to the region 59E4-F1; 60A7. The muscle defects of the *ifm(2)RU4[1]/Df(2R)bw-S46* heterozygotes are less penetrant than mutant homozygotes (Figure 4F). Neither deficiencies show muscle defects in wild-type heterozygous conditions.

The recessive phenotype of *ifm(2)RU5[1]* is complemented by *Df(2L)al* (21B8-C1; 21C8-D1) and *Df(2L)net K1* (21A1; 21B4-B5), but *Df(2L)PMF* (21A1; 21B7-8) fails to complement. These deficiencies do not show muscle defects in heterozygous conditions. These results show that *ifm(2)RU5* is located in the 21B4-5; 21B7-8 region.

Fertility: Animals homozygous for *ifm(2)RU2[1]*, *ifm(2)RU5[1]* and *ifm(2)RU7* are sterile. While *Ifm(2)RU1* homo-

TABLE 3

Muscle defects of the newly isolated IFM mutants over noncomplementing deficiencies

Mutant lines	Deficiencies	No. of flies scored[a]	No. of flies with normal muscle[b]	Defect in IFMs[c]
Ifm(2)RU1	*Df(2L)H20*	77	—	77 (100)
ifm(2)RU2[1]	*Df(2L)H20*	46	4	42 (91)
ifm(2)RU3[1]	*Df(2R)Px*	250	201	49 (20)
ifm(2)RU4[1]	*Df(2R)bw-S46*	110	84	26 (24)
ifm(2)RU5[1]	*Df(2L)PMF*	375	351	24 (6)

[a] The number of heteroallelic survivors/total progeny obtained by crossing balancer heterozygotes (mutant/*CyO*) with the noncomplementing deficiencies.
[b] No visible muscle defect observed under polarized light.
[c] The values in parentheses are percentages.

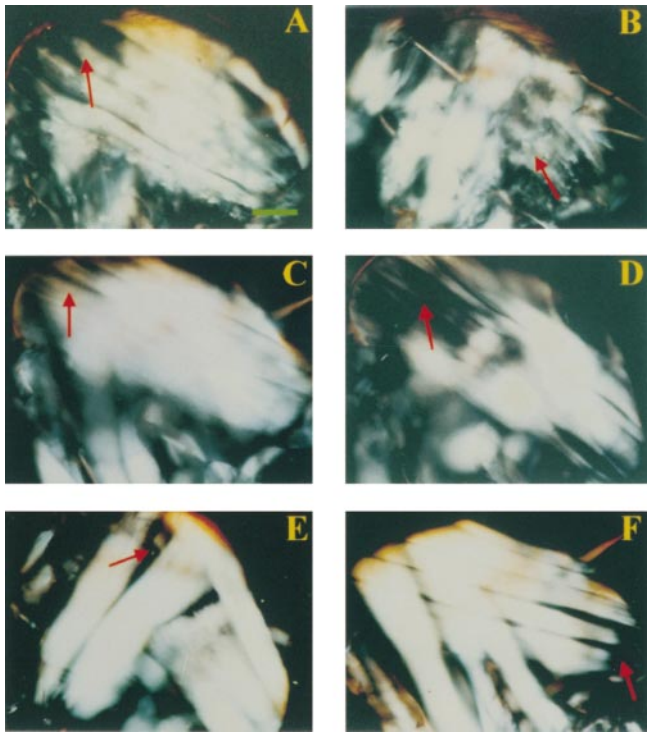

Figure 4.—Muscle phenotypes of the mutations *in trans* with the noncomplementing deficiency and duplication strains. (A and B) *Ifm(2)RU1*/*Df(2L)H20* showing moderate to severe expression of muscle defects, respectively. (C and D) *ifm(2)RU2*^{1}/*Df(2L)H20* showing moderate and severe muscle defects, respectively. (E) *ifm(2)RU3*^{1}/*Df(2R)Px* showing the degenerative muscle phenotype. (F) *ifm(2)RU4*^{1}/*Df(2R)bw-S46* showing muscle defect at the posterior ends of the DLM fibers. Red arrows show the muscle defects. Bar, 200 μm.

zygous females are fertile, homozygous males are sterile, as shown by their inability to mate with Canton-S females. The sterility could result from defects in courtship or other events preceding copulation. All the infertile lines are maintained as balanced stocks. The other original lines, *ifm(2)RU3*^{1}, *ifm(2)RU4*^{1}, and *ifm(2)RU6*, are fertile.

Viability: Viability data of the mutants are presented in Table 4. The viabilities of *ifm(2)RU4*^{1}, *ifm(2)RU5*^{1}, *ifm(2)RU7*, and *ifm(2)RU8* homozygotes are not affected significantly. The *ifm(2)RU2*^{1} gene alleles show allele-specific effects on viability. Alleles *ifm(2)RU2*$^{1-3}$ and *ifm(2)RU2*^{8} are fully viable, and the other five alleles show significant reductions in homozygous viability. The original lines *Ifm(2)RU1* and *ifm(2)RU6*, as well as all viable alleles of *ifm(2)RU3*, show significant reductions in viability. Except for *ifm(2)RU4*^{1} and *ifm(2)RU5*^{1}, all mutants show very low frequency of viability with the noncomplementing deficiencies. This indicates that most of the mutations *in trans*-combination with a deficiency for the region show stronger adult muscle and lethal phenotypes than homozygotes, except for *ifm(2)RU4*^{1} (see mapping section above).

Interactions with Myosin heavy chain alleles: *Ifm(2)RU1* and *ifm(2)RU2*^{1} interact with viable *Mhc* alleles (amorphs *Mhc*^{1} and *Mhc*^{7} and a hypomorph *Mhc*^{13}) *in trans* to give muscle phenotypes that are intermediate between the two or show severe IFM degeneration (Figure 5, B–I). However, wild-type recombinants were recovered with such a frequency between *Mhc* and the *Ifm(2)RU1* or *ifm(2)RU2* alleles in the F_2 generation (Table 5) that we do not consider *Ifm(2)RU1* and *ifm(2)RU2* alleles of *Mhc* (see discussion). All other mutations complemented the *Mhc* alleles.

Rescue with *Mhc* transgene: Flies carrying two copies of the *w; P[w*$^{+}$*Mhc*$^{+}$*]wm3* transgene rescue flight defects and muscle phenotypes of amorphic *Mhc* alleles (Cripps *et al.* 1994b). To ascertain whether the two mutations *Ifm(2)RU1* and *ifm(2)RU2*, which map to the *Mhc* region, are allelic to *Mhc* or not, animals of the genotype *w; Ifm(2)RU1*/+; *P[w*$^{+}$*Mhc*$^{+}$*]wm3*/+ were generated. Such animals are flightless, and their wing and muscle phenotypes are similar to those of *Ifm(2)RU1*/+. This shows that the presence of the one extra copy of the *Mhc*$^{+}$ does not rescue the semidominant mutant phenotype. Similarly, F_2 progeny of the appropriate genotype, *i.e.*, heterozygous or homozygous *Ifm(2)RU1* mutant animals, each carrying either one or two copies of the transgene, were examined. The transgene in one or two copies failed to rescue the homozygous wing and muscle phenotypes of mutants in the F_2 progeny. Animals carrying the *Mhc*$^{+}$ transgene and one copy of *ifm(2)RU2*^{1} show normal flight, and wing and muscle phenotypes, an expected result since it is completely recessive. However, all the F_2 homozygous *ifm(2)RU2*^{1} progeny with one or two copies of the transgene are flightless and show mutant wing and muscle phenotypes.

Developmental analysis: Table 6 summarizes the developmental analysis of three mutant lines, namely *Ifm(2)RU1*, *ifm(2)RU2*^{1} and *ifm(2)RU3*^{1}. *Ifm(2)RU1* homozygotes show uneven expression of the *Act88F-lacZ* and *MHC-lacZ* transgenes in the early developing IFMs; the fibers appear spongy with aberrant structures (Figure 6B). At ~30–32 hr after puparium formation (APF), the defect is very prominent in the margins of the muscle fibers, where attachment to the epidermis takes place (Figure 6D). In the later stages, the fibers pull apart to give the adult thinning muscle phenotype (see Figure 2B).

The early stages of IFM development in the *ifm(2)RU2*^{1} take place normally; defects are first seen in the form of uneven expression of *MHC-lacZ* and *Act88-lacZ* around 28 hr APF at the regions that lead to defective thinning of myofibers in the adults (Table 6, Figure 2D). Defects in the splitting of the DLMs lead to the development of a variable number of DLMs in *ifm(2)RU3*^{1} homozygotes (Table 6), while the degeneration of the muscles (Figure 2E) takes place a few hours before the eclosion. The same degeneration process occurs at a similar time in *ifm(2)RU4*^{1} and *ifm(2)RU5*^{1} homozygotes. Detailed developmental studies of the mutant alleles of

TABLE 4

Viability of the new IFM mutants

Mutant lines	Homozygotes			Upon mapped deficiency		
	No. of flies[a]	%	χ^2	No. of flies[b]	%	χ^2
Ifm(2)RU1	24/511	4.7	23.7*	77/564	13.7	10.9*
ifm(2)RU2^1	226/880	25.7	1.51	46/429	10.7	14.7*
ifm(2)RU2^2	197/635	31	0.09	61/534	11.4	13.8*
ifm(2)RU2^3	137/506	27	1.01	42/290	14.5	10.1*
ifm(2)RU2^4	128/568	22.5	13.8*	20/375	5.3	22.7*
ifm(2)RU2^5	118/570	20.1	4.39*	27/317	8.5	17.7*
ifm(2)RU2^6	116/792	20.9	4.25*	28/337	8.3	18.0*
ifm(2)RU2^7	130/772	16.8	7.69*	22/335	6.6	20.6*
ifm(2)RU2^8	176/726	24.2	2.22	42/517	8.1	18.3*
ifm(2)RU2^9	158/842	18.7	5.97*	09/281	3.2	26.3*
ifm(2)RU3^1	48/456	10.5	14.93*	250/425	58.8	18.7*
ifm(2)RU4^1	180/510	35.3	0.07	110/254	43.3	2.7
ifm(2)RU5^1	168/528	31.8	0.03	375/863	43.5	2.8
ifm(2)RU6	66/405	16.3	8.17*	—	—	—
ifm(2)RU7	44/216	20.37	4.64*	—	—	—
ifm(2)RU8	145/312	46.47	4.82*	—	—	

*$P < 0.05$ indicates a significant difference from the expected ratio and all others are insignificant at the 5% level.

[a] The figures are the number of homozygotes obtained from the cross between balancer heterozygotes (*/*CyO*).

[b] These figures represent the number of heteroallelic survivors/total progeny obtained by crossing balancer heterozygotes (mutant/*CyO*) with the noncomplementing deficiencies as in Table 3.

the other three new genes have not yet been conducted because of their low penetrance and expressivity.

DISCUSSION

Extant adult Drosophila muscle mutants have been identified using wing position, thoracic morphology, or flight ability as selective phenotypes (Deak 1977; Homyk and Sheppard 1977; Koana and Hotta 1978; Fyrberg *et al.* 1980; Mogami and Hotta 1981; Deak *et al.* 1982; Warmke *et al.* 1989; Cripps *et al.* 1994a,b; De Couet *et al.* 1995; An and Mogami 1996). Autosomal screens for dominant mutations led to the identification of genes encoding structural components of muscles (Fyrberg *et al.* 1980; Mogami and Hotta 1981; Okamoto *et al.* 1986; Cripps *et al.* 1994a). Very few genes have been identified that affect the early events in adult flight muscle development. Mutations of the *erect-wing (ewg)*

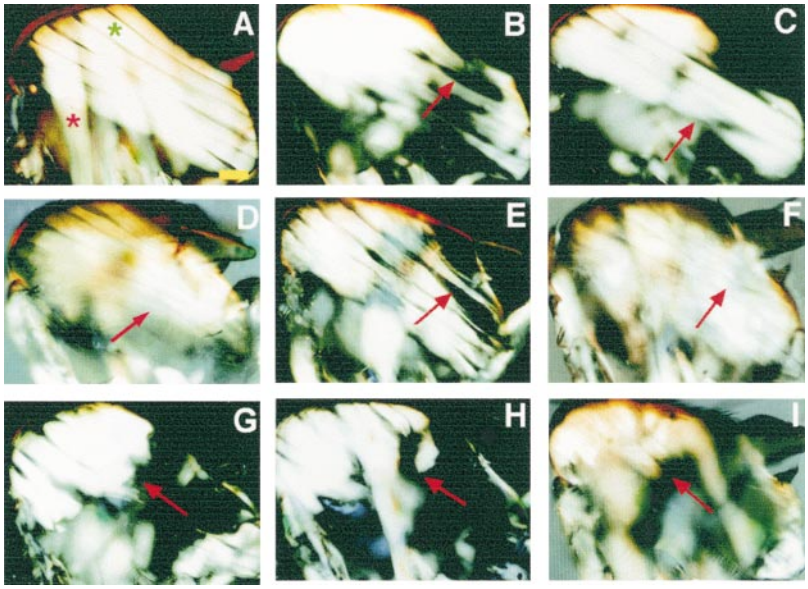

Figure 5.—Interaction muscle morphology of *Ifm(2)RU1* and *ifm(2)RU2* with the *Mhc* alleles. (A) Normal IFMs. (B) *Ifm(2)RU1*/*Mhc*^{1}. (C) *ifm(2)RU2*^{1}/*Mhc*^{1}. (D) *Mhc*^{7} homozygote. (E) *Ifm(2)RU1*/*Mhc*^{7}. (F) *ifm(2)RU2*^{1}/*Mhc*^{7}. (G) *Mhc*^{13} homozygote. (H) *Ifm(2)RU1*/*Mhc*^{13}. (I) *ifm(2)RU2*^{1}/*Mhc*^{13}. Red arrows show the disorganized IFMs. Red asterisk indicates one set of DVMs, while the green asterisk shows one of the DLMs. In A–I, anterior is to the left and dorsal is to the top. Only one hemisegment is shown. Bar, 200 μm.

TABLE 5

Frequencies of wild-type recombinants recovered from mutant *Ifm(2)RU1* and *ifm(2)RU2* alleles crossed to four different viable *Mhc* alleles

Mutant lines	*Ifm(2)RU1*	Mhc^{1}	Mhc^{7}	Mhc^{12}	Mhc^{13}	Mhc^{19}
Ifm(2)RU1		16/679	1/633	1/648	2/655	1/671
$ifm(2)RU2^{1}$	0/306	—	1/328	0/560	2/359	0/383
$ifm(2)RU2^{2}$	0/396	—	0/330	0/680	0/326	0/264
$ifm(2)RU2^{3}$	0/441	—	1/281	0/357	1/516	0/339
$ifm(2)RU2^{4}$	0/502	—	0/310	0/680	0/328	0/264
$ifm(2)RU2^{5}$	0/611	—	2/278	0/388	0/336	0/364
$ifm(2)RU2^{6}$	0/607	—	0/263	0/325	0/316	2/317
$ifm(2)RU2^{7}$	0/248	—	1/297	1/503	0/293	0/341
$ifm(2)RU2^{8}$	0/610	—	0/311	0/435	0/277	0/308
$ifm(2)RU2^{9}$	0/496	—	2/303	0/484	0/282	0/382

Mhc^{1}/*ifm(2)RU2* alleles cause sterility.

gene affect early events of differentiation (Deak *et al.* 1982; De Simone *et al.* 1996; Roy and VijayRaghavan 1998), whereas those in the *stripe* (*sr*) gene affect the attachment of developing muscles to the thoracic epidermis (Costello and Wyman 1986; de la Pompa *et al.* 1989; Lee *et al.* 1995; Fernandes *et al.* 1996).

Studies of the events and mechanisms in adult muscle development (Crossley 1978; Fernandes *et al.* 1991, 1994; Fernandes and VijayRaghavan 1993; Roy and VijayRaghavan 1997, 1998; Roy *et al.* 1997) indicate a role for many regulatory events (Baylies *et al.* 1998; Cripps *et al.* 1998). It has been possible recently to elucidate a regulatory cascade of gene interactions that operate during flight muscle formation. *Twist* and *D-mef2*, two genes that encode transcription factors, play critical roles in IFM formation. *Twist* is a direct regulator of *D-mef2* expression in myoblasts that will give rise to IFMs. In turn, *D-mef2* is involved in the execution of the proper differentiation program of these muscles (Cripps *et al.* 1998; Taylor 1998). Expression patterns of several other transcription factors and regulatory molecules have been documented in the IFM myoblasts and during muscle development itself (Ruiz Gomez and Bate 1997; Anant *et al.* 1998). It is clear that we need to decipher the roles of these and other molecules that orchestrate the process of myogenesis. In close association with muscle development, the related events of muscle attachment and innervation take place. For the IFMs, these processes have been described in terms of the expression patterns of a variety of genes (Fernandes and VijayRaghavan 1993; Fernandes *et al.* 1996; Roy and VijayRaghavan 1998). The *sr* gene affects muscle attachment and is expressed in the epidermis, but we know little about the mechanisms by which it regulates this process. The molecular and cellular bases for the nerve-muscle interactions that are thought to occur early in IFM development (Fernandes and Keshishian 1998; Roy and VijayRaghavan 1998) also remain elusive.

There are several reasons for the paucity of adult flight muscle mutants that affect IFM development. The first and most straightforward explanation is that there have been no systematic screens for recessive adult muscle mutants to date, either by using flightlessness as a

TABLE 6

Summary of developmental studies of the newly isolated mutants

	Ifm(2)RU1		*ifm(2)RU2*[1]		*ifm(2)RU3*[1]		
Developmental stages (hours APF)	No. of individuals screened[a]	No. of individuals with muscle defects (%)	No. of individuals screened[a]	No. of individuals with muscle defects (%)	No. of individuals screened[a]	No. of individuals with muscle defects (%) 3F	4F
8–12	42	71	36	—	48	—	—
13–20	42	90	48	—	45	11	9
21–28	51	92	50	—	60	13	7
29–72	54	96	50	64	45	18	11
Adult	45	100	40	100	45	19	11

Dashes indicate muscle defects. F, muscle fibers.

[a] Total number of individuals analyzed using both *Act88F-lacZ* and *MHC-lacZ* transformants.

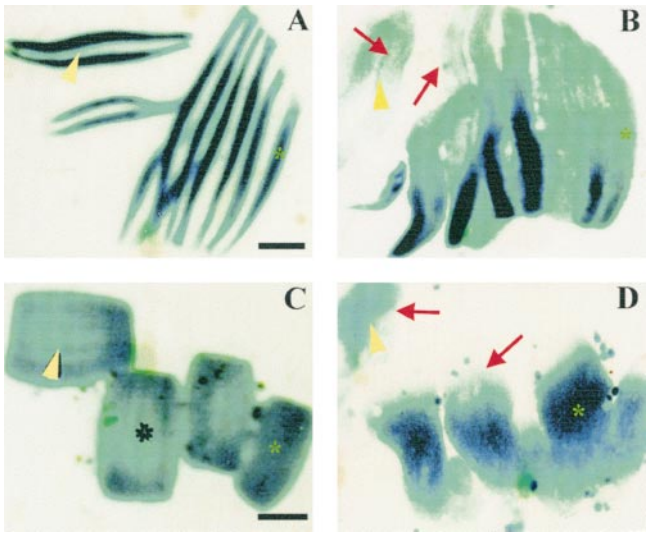

Figure 6.—Developmental stages of *Ifm(2)RU1* showing muscle abnormalities. Pupae of the desired ages carrying the mutant chromosome with the *Act-lacZ* transformant were dissected and stained with X-gal to study the developmental defects. (A) Normal IFMs at 22 hr APF. (B) *Ifm(2)RU1* at 22 hr APF. (C) Normal IFMs at 32 hr APF. (D) *Ifm(2)RU1* at 32 hr APF. Red arrows show the defective DLMs and DVMs. Yellow arrowhead indicates DVM-1. Green asterisks mark the dorsalmost DLM in the entire panel. Black asterisk indicates the ventralmost DLM set. Anterior is the top, and dorsal midline is toward the left. Bar, 10 μm.

screen or by direct examination of muscle. Second, many of the genes that have roles in adult flight muscle development may have earlier essential functions and, thus, loss-of-function mutations may lead to recessive lethality. Hypomorphic alleles at such loci, for example, as with *ewg* (Costello and Wyman 1986; Fleming *et al.* 1989) or *sr* (de la Pompa *et al.* 1989; Lee *et al.* 1995), could be isolated but would require systematic screens. Interaction screens, *i.e.*, dominant screens for suppressor or enhancer phenotypes *in trans*-combination, could also identify genes that have roles in flight muscle development in addition to other functions with extant flight muscle mutants as has been attempted with *ewg* (M. S. Sunanda, S. Sane and K. VijayRaghavan, personal communication).

Why have the screens for dominant flightless mutants identified genes that encode structural components? The screens that were undertaken demanded a stringent flight response. Flies with only slightly impaired flight ability would not have been identified. Given that muscle structural components are required in correct stoichiometry for complete flight ability (Cripps *et al.* 1994a; An and Mogami 1996), it appears reasonable in hindsight that most structural genes were likely to be identified. However, such a screen did not recover flightless mutations in the genes for *myosin light chain*, *paramyosin*, and a second *tropomyosin gene* 1 (Tm1, previously *Tm*II), which have already been cloned, although in the latter two cases it is known that these genes are not haploinsufficient for flight (Cripps *et al.* 1994a).

We have reported on the first direct visual screen for recessive mutations on the autosomes that cause muscle defects. The flight testing of the new mutant lines revealed that flies with slightly extended wing position and muscle defects [*ifm(2)RU6*, *ifm(2)RU7*, and *ifm(2)RU8*] could retain flight, while mutant flies with normal wing position can show flightlessness, as in the case of *ifm(2)RU3*[1]. This indicates that flightlessness and wing position defects are two often independent phenotypes. Although our direct screen is labor intensive, it has resulted in the identification of several new genes that affect flight muscle development. Given the increasing density of the Drosophila physical map, it should be possible to more precisely map the genes we have identified and characterize them at the molecular level. Another "direct screen," but for embryonic muscle defects, has also successfully used polarized light to screen for X-chromosome mutants (Drysdale *et al.* 1993).

Our screen identified 16 mutant strains that fell into 7 recessive complementation groups together with one semidominant allele. The frequency of occurrence of the viable recessive muscle mutations is 0.5% (7/3283). Five of the 8 complementation groups, including the semidominant mutation, have been mapped using meiotic recombination and/or chromosomal deficiencies.

It is very interesting to note that the muscle phenotype of *Ifm(2)RU1/Df* is more extreme than those seen in *Ifm(2)RU1* homozygotes. One of the possibilities is that this deficiency covers many genes involved in the muscle formation, namely the *Mhc* region, together with our *Ifm(2)RU1* and *ifm(2)RU2*[1] mutations. Therefore, these mutant flies produce more severe muscle phenotypes. It is also possible that *Ifm(2)RU1* produces some functional gene product (preliminary observation) that is involved in the formation of the *Ifm(2)RU1* muscle phenotype. This gene product is haploinsufficient in *Ifm(2)RU1/+* flies, therefore showing a less defective muscle phenotype than the homozygotes. The characterization of this gene product, however, is in progress.

Though *Ifm(2)RU1* and *ifm(2)RU2*[1] of the new gene map closely to the *Mhc* region, they appear to be distinct from the *Mhc* gene. The Drosophila MHC protein is encoded by a single gene that has the potential to generate 480 isoforms through alternative processing of its primary messenger RNA (George *et al.* 1989). Both lethal and viable alleles have been isolated (Mogami and Hotta 1981; Bernstein *et al.* 1983; O'Donnell *et al.* 1989; Cripps *et al.* 1994a). Many of the viable alleles show a recessive structural muscle disorganization phenotype, but all the alleles are haploinsufficient for flight (O'Donnell *et al.* 1989; Cripps *et al.* 1994a). Haploinsufficiency results from a reduction in the amount of MHC protein that is sufficient to affect muscle structure and power output for flight (O'Donnell *et al.* 1989; Cripps *et al.* 1994a). This is applicable for most of the

genes that code for the structural proteins of muscle, such as myosin light chain-2 (Warmke *et al.* 1989), Act88F actin (Hiromi *et al.* 1986), and tropomyosin-2 (Karlik and Fyrberg 1985). All *Mhc* mutant heterozygotes show some disorganization of muscle fibers, a phenotype that may be enhanced when *in trans*-combination with mutations in other structural genes (Homyk and Emerson 1988). The Mhc^{7} and Mhc^{12} alleles produce no MHC in the IFMs (Fyrberg and Beall 1990). Our observations that the IFMs of Mhc^{7} and Mhc^{12} alleles are nonbirefringent correlate with the absence of thick filaments in these muscles (Fyrberg and Beall 1990). Birefringence in polarized light depends on the presence of repeated protein structures in a tissue. In the absence of MHC thick filaments and sarcomeres, birefringence does not form.

Ifm(2)RU1 shows a muscle "thinning" phenotype that is not seen in any of the *Mhc* alleles as well as a reduction in the birefringence of the muscle. The *trans*-heterozygous muscle phenotypes of *Ifm(2)RU1* with Mhc^{7} or Mhc^{12} show birefringence of the fibers. This is only possible if the *Ifm(2)RU1* chromosome contains an *Mhc* allele, which expresses an MHC, which can assemble into thick filaments and produce normal or near-normal sarcomeres and myofibrils. An electron microscopy study of muscle fibers showed the presence of thick (myosin-containing) and thin (actin-containing) filaments in *Ifm(2)RU1*/*Ifm(2)RU1* animals (U. Nongthomba and N. B. Ramachandra, unpublished data). *Ifm(2)RU1* animals show a very high frequency of recombination with the *Mhc* alleles (Table 5). Assuming that 1% of recombination is equal to ~275 kb of DNA (Homyk and Emerson 1988) and that the *Mhc* gene is 22 kb long (Bernstein *et al.* 1983), the recombination frequency between *Ifm(2)RU1* with *Mhc*, which ranges from 0.15 to 2.4%, adequately separates this mutant from the *Mhc* locus. Homyk and Emerson (1988) found much lower recombination frequencies when performing their intragenic mapping of *Mhc* alleles.

The $ifm(2)RU2^{1}$ complementation group also maps closely to *Mhc* and shows defects in both DLMs and DVMs. This mutant *in trans* with *Mhc* alleles also shows more severely disorganized muscles than *Mhc* heterozygotes. Because (1) the $ifm(2)RU2^{1}$ mutant also produces recombinants with *Mhc* alleles (Table 5), (2) $ifm(2)RU2^{1}/+$ animals have normal flight, (3) appear to have normal fibers under phase-contrast optics (Figure 3, E–F), and (4) show normal thin-thick filaments as seen through electron microscopy (U. Nongthomba and N. B. Ramachandra, unpublished data), it seems likely that $ifm(2)RU2^{1}$ is a mutant in a separate locus from *Mhc*.

Although *Ifm(2)RU1* and $ifm(2)RU2^{1}$ differ phenotypically from each other, they map to the same chromosome region and could be alleles. Support for this comes from the observations that they fail to complement each other and no wild-type recombinants were recovered from >6000 progeny. The absence of complementation is complicated, however, as *Ifm(2)RU1* is a semidominant allele, and $Ifm(2)RU1/ifm(2)RU2^{1}$ *trans*-heterozygotes show the same phenotype as *Ifm(2)RU1*/+. In *Ifm(2)RU1* and $ifm(2)RU2^{1}$ homozygotes, muscle birefringence is concentrated at particular areas. Among all the new mutant genes identified in this study, this is a distinct muscle phenotype that is found only in these two genes. Further alleles and analysis are required to determine whether *Ifm(2)RU1* and $ifm(2)RU2^{1}$ represent two genes or alleles of a single muscle gene.

Independent and strong confirmation that *Ifm(2)RU1* and $ifm(2)RU2^{1}$ are not mutations in the *Mhc* locus comes from the complementation studies with the Mhc^{+} transgene. The *Mhc* transgene rescue experiments prove that the two mutations near the *Mhc* locus are not amorphic or hypomorphic alleles of *Mhc*. However, *Ifm(2)RU1* could show a neomorphic phenotype in the presence of the wild-type copy of *Mhc* because it is a semidominant mutation. The presence of one or two copies of the wild-type Mhc^{+} could not rescue the mutant phenotypes of either *Ifm(2)RU1* or $ifm(2)RU2^{1}$. Therefore, in the absence of further information, we conclude that the *Ifm(2)RU1* and $ifm(2)RU2^{1}$ genes (or gene) map closely to, but are distinct from, *Mhc*. Homyk and Emerson (1988) suggest that some structural muscle protein genes can interact with each other to enhance muscle disorganization compared to their own heterozygous phenotype (mutant/+). This appears also, but not necessarily, to be the case between *Mhc* and the *Ifm(2)RU1* and $ifm(2)RU2^{1}$ mutants.

To date, no mutations that affect IFM development other than those in muscle structural genes have been reported in the deficiencies covering $ifm(2)RU3^{1}$, $ifm(2)RU4^{1}$, and $ifm(2)RU5^{1}$. The $ifm(2)RU3^{1}$ mutation mapped to the 60B8-10; 60C5-6 region. Prout *et al.* (1997) have reported that the *piopio (pio)* gene was mapped to 60C6-60D11, which partially overlaps the deficiency region where $ifm(2)RU3^{1}$ mapped. Mutations of *pio* affect myo-epidermal junctions or muscle function in embryos, produce wing blisters in adults, and were proposed to be involved in integrin-dependent cell-to-cell adhesion (Prout *et al.* 1997). The two mutants $ifm(2)RU3^{1}$ and *pio* are unlikely to be alleles because their muscle pattern and developmental effects are very different. They do, however, represent a new region on the second chromosome that affects the IFMs. In addition to this, $Ifm(2)RU3^{1}/Df$ flies show more severe phenotypes than those of $ifm(2)RU3^{1}$ homozygotes, indicating that $ifm(2)RU3^{1}$ is able to produce some amount of functional gene product and is a hypomorph. The biochemical analysis of this mutation is in progress.

A large number of the new mutants are sterile. Outcrossing and backcrossing suggest that this is a property of the muscle mutants themselves. This is surprising, because most of the existing mutants that affect the structural components or development of the IFMs are

fertile. We conclude that many of these newly isolated mutants have pleiotropic effects on fertility. Thus, these mutants may directly or indirectly affect muscles involved in the production and/or release of gametes, but could also cause indirect effects on courtship and mating. All but *ifm(2)RU4* and *ifm(2)RU5* of the new genes contain lethal alleles or partially viable alleles. This is not a new phenomenon in the sense that muscles are an integral part in the life cycle of the fly. The IFMs are not required for viability, however, suggesting that these new genes have effects on the development of other muscles.

What are the developmental functions of the genes identified by our screen? The semidominant mutant *Ifm(2)RU1* represents a gene that is required for early IFM development and encodes, or regulates the expression of, a structural component of flight muscle. The reasons for these conclusions are that the early developing IFMs show an uneven pattern of expression of the *Act88F-lacZ* and *MHC-lacZ* transgenes and show fuzzy, spongy aberrant muscle structures at ~30–32 hr APF, the stage when differentiation of myofibers begins (Fernandes *et al.* 1991). The phenotype of the *Ifm(2)RU1* is strikingly similar to *heldup*^{3}, an X-chromosome mutant wherein specific isoforms of troponin-I, a protein that regulates acto-myosin interactions, are not synthesized (Fyrberg *et al.* 1990; Beall and Fyrberg 1991; Barbas *et al.* 1993). *heldup*^{3} animals showed abnormalities just after myoblast fusion and are later seen as small muscle remnants (Barthmaier and Fyrberg 1995). Although *ifm(2)RU2*^{1} homozygote IFMs also show uneven expression of *Act88F-lacZ* and *MHC-lacZ* transgenes at ~30–32 hr APF, degeneration of the fibers takes place a few hours before the eclosion.

In the case of one complementation group, *ifm (2)RU3*, we have identified both viable and lethal alleles, thereby allowing a study of the role of the gene at earlier and later developmental stages. Because the independently isolated lethal alleles fail to complement each other for recessive lethality in addition to failing to complement the adult phenotype of viable alleles, it can be concluded that they represent genuine lethal mutations at the *ifm(2)RU3* locus. *ifm(2)RU3* is also likely to be involved in early events of IFM development, as *ifm(2)RU3*^{1} homozygotes show defects in the process of splitting of the larval templates used for DLM development. Three DLM fibers result from the absence of template splitting, while four or five fibers result from the splitting of one or two template fibers. Just before the eclosion, degeneration of DLMs and DVMs was seen, which also suggests a possible role for *ifm(2)RU3*^{1} in the maintenance of IFM integrity.

Animals homozygous for mutations in the other genes we have identified [*ifm(2)RU4–8*] show phenotypes that indicate a late requirement during flight muscle development, though it must be kept in mind that it is difficult to predict the function of the gene product in the absence of multiple alleles. However, there are many important regulatory events in the maturation and maintenance of muscle about which we know little, and these newly identified genes may allow an analysis of such important and late regulatory events. We isolated several viable alleles in two complementation groups, *ifm(2)RU4* and *ifm(2)RU5*, which will be useful in determining the roles of these genes during the development of muscles.

This report and continuing analysis of the genes identified in our study with an ongoing extensive mutagenesis will yield valuable resources for the study of various aspects of adult muscle development in Drosophila.

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