Recovery of Dominant, Autosomal Flightless Mutants of *Drosophila melanogaster* and Identification of a New Gene Required for Normal Muscle Structure and Function

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ABSTRACT

To identify further mutations affecting muscle function and development in *Drosophila melanogaster* we recovered 22 autosomal dominant flightless mutations. From these we have isolated eight viable and lethal alleles of the muscle myosin heavy chain gene, and seven viable alleles of the indirect flight muscle (IFM)-specific *Act88F* actin gene. The *Mhc* mutations display a variety of phenotypic effects, ranging from reductions in myosin heavy chain content in the indirect flight muscles only, to reductions in the levels of this protein in other muscles. The *Act88F* mutations range from those which produce no stable actin and have severely abnormal myofibrillar structure, to those which accumulate apparently normal levels of actin in the flight muscles but which still have abnormal myofibrils and fly very poorly. We also recovered two recessive flightless mutants on the third chromosome. The remaining five dominant flightless mutations are all lethal alleles of a gene named *lethal(3)Laker*. The *Laker* alleles have been characterized and the gene located in polytene bands 62A10,B1-62B2,4. *Laker* is a previously unidentified locus which is haplo-insufficient for flight. In addition, adult wild-type heterozygotes and the lethal larval *trans*-heterozygotes show abnormalities of muscle structure indicating that the *Laker* gene product is an important component of muscle.

THE indirect flight muscles (IFMs) of *Drosophila melanogaster* provide a unique model system with which to genetically dissect muscle structure and function (see SPARROW *et al.* 1991a). These muscles, which power flight, also share a number of structural and functional characteristics with vertebrate skeletal muscles. Mutations affecting *Drosophila* IFMs are easily detected by impairment of flight ability, and a large number of flightless mutations have already been identified (see BERNSTEIN *et al.* 1993). Many, but by no means all, are in myofibrillar protein genes.

MOGAMI and HOTTA (1981) isolated dominant autosomal flightless mutants following ethyl methanesulfonate (EMS) mutagenesis. They argued that wild-type heterozygotes for any mutation which prevents synthesis of a myofibrillar protein will accumulate only half as much of the protein as a wild-type fly. If as a consequence the flight muscles of mutant heterozygotes do not produce sufficient power for flight, the flies will exhibit a dominant flightless phenotype. The mutations they isolated were clustered in two regions of the genome. Subsequent analysis has shown the second chromosome mutations, with only one exception, are in the single muscle myosin heavy chain gene located at 36B (ROZEK and DAVIDSON 1983; BERNSTEIN *et al.* 1983; MOGAMI *et al.* 1986; CHUN and FALKENTHAL 1988; HOMYK and EMERSON 1988; O'DONNELL *et al.* 1989; KRONERT *et al.* 1991). The

mutations on the third chromosome are in two genes located in polytene chromosome region 88F: the IFM-specific actin gene, *Act88F* (FYRBERG *et al.* 1983; KARLIK *et al.* 1984b, 1987; SAKAI *et al.* 1991), and the *TmI* tropomyosin gene (BAUTCH *et al.* 1982; KARLIK and FYRBERG 1985). Note there is some inconsistency in the numbering of the *Tm* genes. We have used that of HANKE and STORTI (1988).

Selection for dominant autosomal flightless mutants is a good way to obtain muscle protein gene mutations in *Drosophila*, but a number of questions remain. First, the genes for the myosin light chains (FALKENTHAL *et al.* 1984; PARKER *et al.* 1985; TOFFENETTI *et al.* 1987), paramyosin (VINOS *et al.* 1991; BECKER *et al.* 1992) and a second tropomyosin gene, *TmII*, which encodes IFM-specific isoforms (KARLIK *et al.* 1984a; HANKE and STORTI 1988) have all been cloned and localized to the third chromosome, yet flightless mutations were not recovered in these genes by MOGAMI and HOTTA (1981). Since MOGAMI and HOTTA (1981) studied only mutations which altered the pattern of accumulation of thoracic proteins, one must ask whether all genes which can mutate to give autosomal dominant flightlessness have been detected in the *Drosophila* genome.

Second, the existing mutations show a variety of phenotypic effects. For instance, both lethal (MOGAMI *et al.* 1986) and homozygous viable (O'DONNELL *et al.* 1989; CHUN and FALKENTHAL 1988) mutations of the single myosin heavy chain gene have been recovered. *Act88F*

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TABLE 1
Chromosomal rearrangement stocks used in this study

Stock	Cytology	Reference ^a	Source
<i>Df(2L)H20/CyO</i>	Df(2L) 36A8-9;36E3-4	1	S. I. BERNSTEIN
<i>Df(3L)R^E/TM3</i>	Df(3L) 62B8-9;62B12-C1	2	T. J. SLITER
<i>Df(3L)R^{G2}/TM3</i>	Df(3L) 62B2-4;62B11-12	2	T. J. SLITER
<i>Df(3L)R^{G5}/TM3</i>	Df(3L) 62A10-B1;62C4-D1	2	T. J. SLITER
<i>Df(3L)R^{G7}/TM3</i>	Df(3L) 62B8-9;62F2-5	2	T. J. SLITER
<i>T(Y;3)D8</i>	T(Y;3) Y ^L ;62A	4	D. LINDSLEY
<i>T(Y;3)A158</i>	T(Y;3) Y ^L ;62F2-63A1	4, 5	D. LINDSLEY
<i>TM2, ry Ubx¹³⁰ e⁺ P[1ArB]C40.1S3/Df(3R)P13</i>		3	C. J. O'KANE

^a References: 1, SIMPSON (1983); 2, SLITER *et al.* (1989); 3, BELLEN *et al.* (1989); 4, LINDSLEY *et al.* (1972); 5, R. M. CRIPPS (unpublished results).

mutations are known which show an array of allele-specific phenotypic effects, particularly at the subcellular and molecular levels (KARLIK *et al.* 1984b; MAHAFFEY *et al.* 1985; HIROMI *et al.* 1986; OKAMOTO *et al.* 1986; DRUMMOND *et al.* 1991; SPARROW *et al.* 1992). Might new classes of mutant alleles be recovered in those genes in which mutations have already been found?

In this study we report on the selection and analysis of further EMS-induced autosomal dominant flightless mutants in an attempt to answer these questions. We show that, as reported by MOGAMI and HOTTA, mutations in *Mhc* and *Act88F* are easily recovered, showing a range of phenotypic effects. In addition, we report the isolation of mutations in a previously unidentified gene, *lethal(3)Laker*. This gene shows haplo-insufficiency for flight and also appears to encode a muscle protein.

MATERIALS AND METHODS

Flies: Flies were grown at 25° on either yeast-sucrose medium (CARPENTER 1950) in small vials, or on yeast-maizemeal-molasses medium in half-pint bottles. Gene and chromosome symbols used are as described in either LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1992), unless otherwise specified. *TE* denotes a transposon carrying *w⁺* and *rst⁺* (ISING and RAMEL 1976). Details of specific *TE* insertions, stocks of which were kindly provided by G. ISING, are taken from ISING and BLOCK (1981), except *TE159*, which is an insert at 62D7, mapping at 2.8 ± 0.5 map units from *ru* (G. ISING, personal communication). Details of rearrangement strains used in this study are described in Table 1.

Isolation of flightless mutants: EMS mutagenesis of adult males was done according to the method of LEWIS and BACHER (1968). In mutant screen I (M-series mutations) the mutagenized males were homozygous for the *TE52* second chromosome; in screen II (N-series mutations) they were heterozygous for the *TE89* and *TE39* third chromosomes (Figure 1). In each case the progeny of mutagenized *TE* males crossed to *w* females were flight-tested (see below). Individual flightless males were mated to three *w* females and the progeny of each culture were flight tested. Flies from cultures containing a significant proportion of flightless progeny were crossed and recovered as second (*CyO*) or third (*TM3*) chromosome balanced strains. Note that in the second backcross to *w* females the presence of only red-eyed flightless flies or both red and white-eyed flightless flies served to immediately identify the autosome upon which each dominant flightless mutation was located.

Flight testing: Flightlessness was selected for using a simple

flight-testing column (GREEN *et al.* 1986). Flies were at least 24 hr old before testing, since newly emerged flies are poor fliers. Flightless flies were selected as those which fell to the bottom of the column. Flight ability was measured by marking the column into six zones, 1-6 (top to bottom). The number of flies sticking in each zone was scored, as was the number of flies collected at the bottom of the column (B). An alternative flight-testing method, which gives a better estimate of intermediate flight abilities was also used, in which flies were released into a Perspex box. The ability of each fly to fly either up (U), horizontal (H), down (D), or to drop without flying (N) was scored [see DRUMMOND *et al.* (1991) for details].

Electron microscopy: For indirect flight muscles, longitudinally bisected half thoraces were fixed in 3% glutaraldehyde in 0.2 M sodium phosphate buffer, pH 7.0, for 1 hr at room temperature. After washing in the buffer, they were post-fixed in 1% osmium tetroxide in 0.2 M sodium phosphate buffer, pH 7.0 for 1 hr on ice. After washing in buffer, samples were dehydrated with an alcohol series and embedded in Epon E218 araldite. Sections were stained with lead citrate and uranyl acetate and viewed in a JEOL 1200EX transmission electron microscope.

For embryonic body wall muscles, late stage embryos were removed from the chorion and vitelline membranes. They, and early first instar larvae, were then transferred to fixative (3% glutaraldehyde, 2% paraformaldehyde, 100 mM sucrose, 2 mM EGTA, in 100 mM sodium phosphate, pH 7.2). After 2 hr of fixation at room temperature, samples were washed in buffer and subsequently treated in the same manner as the thoraces.

Electrophoresis: Muscle samples were prepared in two different ways during the course of this work. In earlier experiments, longitudinally bisected half thoraces were placed in a glycerol-Triton "skinning" solution at -20°, a solution used in muscle physiology experiments to chemically demembranate the tissues (PECKHAM *et al.* 1990). After skinning, the muscles were dissected out and heated at 100° for 4-7 min in sample buffer (1% 2-mercaptoethanol, 1% sodium dodecyl sulfate (SDS), 20 mM Tris-Cl, pH 7.8, 17.5 mM sodium phosphate, 10% glycerol, 0.03 µg/ml bromphenol blue). In other experiments, fresh dorso-longitudinal muscles (DLMs) were dissected and chemically skinned by homogenizing and washing the myofibril pellet formed after centrifugation in an homogenization buffer containing 0.5% Triton X-100 [see CRIPPS and SPARROW (1992) for details].

One-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) was performed by a method derived from that of LAEMMLI (1970) using a Bio-Rad Mini-Protein II minigel apparatus. Sample equivalent to the muscles dissected from a single fly was applied to each lane. Gels were stained with Coomassie Brilliant Blue. For quantitative studies the destained gels were scanned using a Joyce-Loebl Chromoscan 3 with a 546-nm filter.

FIGURE 1.—Mutagenesis schemes used for recovery of dominant flightless mutations. * indicates mutagenized chromosomes; TE## refers to either TE39 or TE89.

Recovery of flightless mutants: A total of 40,700 F_1 progeny were screened (Figure 1) for dominant flightless mutants. Of these, 720 putative flightless F_1 males were recovered and mated with white-eyed females. Their progeny were flight-tested and a culture considered to be flightless if >20% of the flies tested fell through the column. Originally more than 40 lines still showed dominant flightlessness but in nearly half of these it was close to 20%. Subsequent analysis of these low level flight ability lines proved impossible. Finally, 22 lines with high levels of flightlessness were obtained. Each originated from a single male from different parental cultures. Segregation of the TE chromosomes and flightlessness showed that eight flightless mutations were on chromosome 2 and 12 were on chromosome 3. Homozygous lines were obtained whenever viable. Third chromosome mutants of the second (N-series) screen were unfortunately induced in a chromosome carrying a lethal mutation close to the 88F region. Only *N180* has been recovered as a homozygous line. Dominant flightless mutants were recovered at frequencies of 0.045% (7/15, 500) for screen 1 and 0.052% (13/25, 200) for screen 2.

Flight testing the mutants: The mutants were flight tested as wild-type heterozygotes with *Texas* using both the column and box methods (Table 2). *Texas/Canton-S* heterozygotes, wild-type heterozygotes of the amorphic *Mhc* allele *Mhc^l* (O'DONNELL and BERNSTEIN 1988) and the amorphic *Act88F* allele *Act88F^{KM88}* (HIROMI and HOTTA 1985), are included as controls. As expected from the method of mutant selection, many, if not most of the mutant heterozygotes drop through the column, although in many cases significant numbers stick high up on the column sides. This is probably due to flies tumbling or gliding after release into the column, since by the flight box method all are poor fliers (Table 2).

N6 and *N201*, initially recovered as dominant flightless mutants, are now flightless only as *TM3, Sb Ser* heterozygotes (Table 2). We have confirmed that they are third chromosome recessive mutants. The recessive flightless mutation is present only on one of our *TM3* chromosomes, since the mutations give a flighted phenotype when heterozygous with *TM3, Ser ry* (data not

shown). The gene(s) causing this behavior in the *N6* and *N201* strains has not been mapped. The *N6* and *N201* chromosomes are homozygous lethal as a result of TE-associated lethal mutations. We do not know if the mutations themselves are lethal. *N6/N201* flies are viable since the two mutations were induced in different TE chromosomes. These flies have moderate flight ability, but significant numbers are only able to fly down (Table 2) though not significantly more than *TM3/+*. We conclude that these two mutations are probably weak alleles of a recessive flightless mutation present on the *TM3, Sb Ser* chromosome.

Second chromosome dominant flightless mutants: Flightlessness of all the second chromosome mutants mapped to proximal 2L (see Table 3) with map positions consistent with locations in polytene chromosome region 36B, the region containing the single muscle myosin heavy chain gene, *Mhc* (BERNSTEIN *et al.* 1983; ROZEK and DAVIDSON 1983). Further analysis (see below) indicates that they are all *Mhc* alleles.

Of these mutations, *M125*, *Mhc¹⁷*, *Mhc¹⁸*, *Mhc²⁰* and *Mhc²¹* are lethal as heterozygotes with both *Df(2L)H20* (which deletes a number of genes including *Mhc*) and with *Mhc^l* (Table 3). In addition, each reduces the myosin heavy chain (MHC) content of both legs and thoracic muscles of adult wild-type heterozygotes (Table 3) to 60–80% of wild-type values. Similar MHC contents have been detected in wild-type heterozygotes of *Mhc* null mutations (MOGAMI *et al.* 1986). We therefore conclude that each of these mutants are alleles of *Mhc*. Note: *M125* has subsequently been lost and not ascribed a number as a member of the *Mhc* allelic series.

Phase contrast light microscopy of the IFM myofibrils of mutant heterozygotes for each *Mhc* mutation showed striated myofibrils which were much shorter and with less regular striations than wild type (data not shown). The mutant myofibrils frequently have "frayed" ends and splits within the myofibrils, whereas wild-type myofibrils have clean breaks. All of these features were previously described by MOGAMI and co-workers (MOGAMI and HOTTA 1981; MOGAMI *et al.* 1986) who argued they probably reflect structural weaknesses in the mutant myofibrils. Most of the mutant heterozygotes showed a "raised wing" phenotype, although none show the

TABLE 2

Flight abilities of the dominant flightless mutants isolated in the screens, measured both by the column and the box methods

Genotype	Column ^a								Box ^b				
	1	2	3	4	5	6	B	n	U	H	D	N	n
+/+	84	16	0	0	0	0	0	80	82	12	6	0	34
<i>Mhc</i>													
<i>Mhc</i> ¹ /+	10	19	19	10	6	10	26	48	0	0	7	93	46
<i>Mhc</i> ¹² /+	12	16	10	12	5	4	43	129	0	0	1	99	74
<i>Mhc</i> ¹³ /+	9	19	13	5	4	5	45	150	0	0	7	93	107
<i>Mhc</i> ¹⁷ /+	9	8	8	5	5	5	60	63	0	0	0	100	40
<i>Mhc</i> ¹⁸ /+	5	11	14	13	14	5	38	85	0	0	2	98	52
<i>Mhc</i> ¹⁹ /+	10	24	9	3	5	5	44	89	0	0	6	94	35
<i>Mhc</i> ²⁰ /+	13	18	10	6	2	6	45	84	0	0	2	98	87
<i>Mhc</i> ²¹ /+	8	10	7	4	7	6	58	75	0	0	0	100	72
<i>Act88F</i>													
<i>KM88</i> /+	10	15	13	9	5	2	46	99	0	1	33	66	80
<i>A138V</i> /+	8	15	13	6	5	2	50	188	0	0	9	91	115
<i>E93K</i> /+	8	14	8	8	4	8	50	178	0	0	5	116	121
<i>N119</i> /+	8	9	7	4	1	0	71	129	0	0	6	94	34
<i>N142</i> /+	8	13	10	6	4	9	50	92	0	0	3	97	96
<i>N155</i> /+	8	9	13	4	1	9	56	78	0	0	0	100	42
<i>N180</i> /+	8	8	6	4	5	1	68	104	0	0	0	100	20
<i>N306</i> /+	16	10	10	5	6	5	48	74	0	0	5	95	39
<i>l(3)Lkr</i>													
<i>Lkr</i> ¹ /+	12	19	11	6	6	3	43	119	1	2	59	38	107
<i>Lkr</i> ² /+	7	23	6	7	4	5	48	187	14	4	39	43	98
<i>Lkr</i> ³ /+	3	7	7	4	1	2	76	168	2	2	46	50	96
<i>Lkr</i> ⁴ /+	7	5	4	4	3	2	75	139	0	0	32	68	111
<i>Lkr</i> ⁵ /+	7	5	10	4	4	4	66	133	0	0	4	96	111
Others													
<i>N6</i> /+	78	15	5	1	0	0	0	88	94	6	0	0	36
<i>N6/TM3</i>	7	30	13	9	10	5	26	175	0	4	29	67	24
<i>N201</i> /+	85	11	4	0	0	0	0	71	94	3	0	3	67
<i>N201/TM3</i>	25	43	8	5	2	3	14	63	17	27	47	9	109
<i>TM3</i> /+	82	17	1	0	0	0	0	65	78	10	12	0	51
<i>N6/N201</i>	NT								67	13	16	4	63

Numbers are percentages of flies sticking in each column zone or displaying particular flight patterns in the box. *n*, number of flies tested. *Mhc*¹/+ and *Act88F*^{KM88}/+ are included as controls. In all cases "+" denotes a *Texas* wild-chromosome except for +/+ which is a hybrid between the *Canton-S* and *Texas* wild-type strains.

^a 1–6 correspond in descending order to column zones in which flies stick; B is the bottom of the column.

^b U, H, D, and N correspond to specific flight behaviors in the box; U, upward flight; H, horizontal flight; D, downward flight; N, no flight. NT, not tested.

"bashed thorax" phenotype of some other *Mhc* mutants (HOMYK and EMERSON 1988). By following the survival of fertilized eggs from a cross of *Mhc*¹/+ females to *SM5*/mutant males it was determined that for each mutant 22–27% of fertilized eggs ceased development during the embryonic period; for wild-type this was less than 1%. In each case this was the only significant mortality; between 70 and 78% of the fertilized eggs survived to adulthood. A large number of the unhatched embryos from each mutant cross had completed development, having made a segmented cuticle with denticle belts; pigmented mouthparts were present. However, these embryos never showed any movements of the type that are usual in embryos shortly before hatching.

The mutations *Mhc*¹², *Mhc*¹³ and *Mhc*¹⁹ are not lethal alleles of *Mhc* although all show significantly reduced viabilities over both *Df(2L)H20* and *Mhc*¹ (Table 3). The flightlessness of each mutation maps to positions indistinguishable from those of the lethal *Mhc* alleles.

Homozygous stocks of these viable alleles have been obtained. *Mhc*¹³ and *Mhc*¹⁹ do not detectably affect the MHC content of leg or thoracic muscle of mutant homozygotes (Table 3). However, both are *Mhc* gene mutations. Dissection of homozygous *Mhc*¹³ thoraces reveals a distinctive alteration in the shape of the DLMs in which the greater part of the muscle mass has "contracted" toward the anterior thoracic attachment site (Figure 2). Similar morphological changes occur in *Mhc*¹⁹ homozygotes. The phenotype is not seen in wild-type heterozygotes for either allele, nor in *Mhc*¹/+ heterozygotes, but is seen in *Mhc*¹/*Mhc*¹³ and *Mhc*¹/*Mhc*¹⁹ heterozygotes. This indicates that *Mhc*¹³ and *Mhc*¹⁹ are *Mhc* alleles with a novel recessive phenotype. *Mhc*¹³ was previously described as *N178* (FIECK *et al.* 1988). The severity of this recessive phenotype is lower in *Mhc*¹⁹/*Mhc*¹ than in *Mhc*¹³/*Mhc*¹ flies.

The reduced viability of *Mhc*¹³/*Mhc*¹ and *Mhc*¹⁹/*Mhc*¹ (Table 3) may indicate that the defective myosin heavy

TABLE 3
Summary of genetical and biochemical analyses of the second chromosome flightless mutants

Allele	Map position Chr. 2	Viability ^a		Myosin content ^b			
				Heterozygotes		Homozygotes	
		/Df(2L)H20	/Mhc ¹	Thorax	Legs	Thorax	Legs
<i>Mhc</i> ¹				69	77		
<i>M125</i> ^c		0/423	0/102	81	77		
<i>Mhc</i> ¹²	52.9	136/403	41/100	69	96	<20	112
<i>Mhc</i> ¹³	51.8	12/158	22/204	92	96	104	111
<i>Mhc</i> ¹⁷	54.8	0/119	0/196	68	78		
<i>Mhc</i> ¹⁸	53.5	0/125	0/124	64	63		
<i>Mhc</i> ¹⁹	51.9	8/99	28/138	92	107	104	105
<i>Mhc</i> ²⁰	52.1	0/159	0/125	71	67		
<i>Mhc</i> ²¹	53.8	4/327	0/177	74	80		

^a These figures are the number of heteroallelic survivors/total progeny obtained by crossing balancer (*SM5* or *CyO*) heterozygotes.

^b The figures for myosin heavy chain content are obtained by scanning one-dimensional SDS-PAGE gels to quantify the myosin heavy chain and actin bands. Each figure is the myosin/actin ratio, normalized to wild type = 100. Each is the mean of a minimum of three separate determinations.

^c The *M125* mutant line was lost before flight analysis (Table 2) was complete.

chain polypeptides are expressed in non-flight muscles during development, even though the most dramatic effects are seen in the IFMs. Although the similar phenotypes of these two mutants suggest that they may be the same mutations, they are independent alleles since they were isolated from separate parental crosses.

*Mhc*¹² reduces the myosin heavy chain content of thoracic muscle samples from both heterozygotes and homozygotes (Table 3) but does not affect the MHC content of the leg muscles. The low levels of thoracic MHC detected in homozygotes are undoubtedly due to direct flight muscles and some leg muscles in these preparations. IFMs dissected from *Mhc*¹² homozygotes contain no MHC (Figure 3, panel A, lane 2). Since the *Mhc* gene exhibits alternate transcript splicing (BERNSTEIN *et al.* 1986; ROZEK and DAVIDSON 1986; WASSENBERG *et al.* 1987; GEORGE *et al.* 1989; COLLIER *et al.* 1990; KRONERT *et al.* 1991), the viability of *Mhc*¹² and the presence of myosin in other muscle types are explicable if *Mhc*¹² is a mutation which affects the expression of IFM-specific protein. This has been confirmed by showing that the *Mhc* transcripts are absent from the IFMs of *Mhc*¹² pupae but present in other muscle types (W. N. CALLEN and S. I. BERNSTEIN, personal communication).

Third chromosome dominant flightless mutants: Dominant flightlessness of these mutants mapped to two different regions; one group to chromosome arm 3R and the other group to the tip of 3L.

3R region mutations: Seven of the third chromosome dominant flightless mutants, *M320* (renamed *A138V*; SPARROW *et al.* 1992), *M342* (renamed *E93K*; SPARROW *et al.* 1991b) *N119*, *N142*, *N155*, *N180* and *N306*, were mapped between *p*^h at 3-48.0 and *ss* at 3-58.5. MOGAMI and HOTTA (1981) found a number of such mutants in this region which are now known to be *Act88F* gene mutations. To determine whether these mutations affect the accumulation of myofibrillar actin, heterozygotes

were made with the *Act88F* amorphic allele *KM88*. The myofibrillar proteins from the demembrated IFMs of these flies were separated by one-dimensional SDS-PAGE (Figure 3, panel C). The differences between *KM88/+* (lane 1) and *KM88/KM88* (lane 2) are due to the absence in the latter genotype of a single functional *Act88F* gene. Not only is there no actin, but arthrin, a conjugate of actin and ubiquitin (BALL *et al.* 1987), troponin-H, troponin-I, troponin-T and tropomyosin are also missing. Troponin-I, troponin-T and tropomyosin have been shown to be thin filament proteins in many muscle types, as has troponin-H in the indirect flight muscles of the water bug, *Lethocerus* (NEWMAN *et al.* 1992). Arthrin is also probably associated with the thin filaments (BULLARD *et al.* 1985). These pleiotropic effects of *KM88* and other *Act88F* mutations upon the accumulation of actin-associated myofibrillar proteins have been described previously (HIROMI and HOTTA 1985).

Mutants *N119*, *N142* and *N155* (Figure 3, panel C, lanes 3-5) clearly reduce the amount of actin accumulated compared to *KM88/+* (lane 1). *A138V* shows very little actin (panel B, lane 2) but a faint protein band is detectable at the actin position. Other thin filament proteins are either reduced or absent in all these mutants. *N180/KM88* (Figure 3, panel C, lane 6) muscles contain more actin and there are detectable protein bands coincident with those identified as TnH, TnT, TnI and tropomyosin. Note that arthrin, which normally appears as a band with similar levels of staining to TnT, is not apparent in this lane, suggesting that although some stable actin is formed in these muscles, it is not readily ubiquitinated. *In vivo* labeling experiments with wild-type flies have shown that actin must be assembled into myofibrils before ubiquitination occurs (R. GILLIVER, D. R. DRUMMOND and J. C. SPARROW, unpublished data). The effects of the *N180* mutation on actin accumulation may therefore affect its ubiquitination. This allele is the

FIGURE 2.—Scanning electron micrographs of bisected thoraces from **a**, wild-type flies, **b**, *Mhc¹³* homozygotes. Anterior is to the right, dorsal to top.

FIGURE 3.—One-dimensional SDS-PAGE of the indirect flight muscle myofibrillar proteins of dominant flightless mutants. (Panel A) lane 1, wild-type; lane 2, *Mhc¹²* homozygotes. (Panel B) lane 1, wild-type; lane 2, *A138V* homozygotes. (Panel C) lane 1, +/*KM88*; lane 2, *KM88/KM88*; lane 3, *N119/KM88*; lane 4, *N142/KM88*; lane 5, *N155/KM88*; lane 6, *N180/KM88*; lane 7, *N306/KM88*. ; H, high molecular mass proteins standards—200, 116, 93, 66 and 45 kD; L, low molecular mass standards—76, 66, 45, 30 and 14 kD. M, myosin heavy chain; P, paramyosin; Aa, α-actinin; TnH, troponin-H; Ar, arthrin; TnT, troponin-T; A, actin; TnI, troponin-I; Tm, tropomyosin; MLC-2, myosin light chain-2.

first one which has been shown to accumulate Actin88F protein but which is not ubiquitinated. *N306/KM88* muscles (Figure 3, panel C, lane 7) contain actin, arthrin and other thin filament proteins in quantities and positions indistinguishable from wild-type muscles on one-dimensional gels. Two-dimensional gels show that *N306* produces a charge change in both the actin and arthrin (data not shown). Certain *Act88F* alleles induce heat-shock protein synthesis (OKAMOTO *et al.* 1986; KARLIK *et al.* 1984; DRUMMOND *et al.* 1991; HIROMI and HOTTA 1985). Heat-shock protein accumulation is not apparent from the one-dimensional gels (Figure 3). However, the heat-shock proteins are most readily detected by autoradiography following injection of radiolabeled amino acids into flies. We have only performed such experiments on *E93K* and *A138V*. Neither induce the heat-shock response.

Phase contrast light microscopy has been used to study the effects of these actin mutants. Electron mi-

crographs of some of the mutants will be found elsewhere [BALL (1987); *E93K*, SPARROW *et al.* (1991b); and *A138V*, SPARROW *et al.* (1992)]. The light microscopy shows that the mutants can be grouped into at least three phenotypic groups. (1) Myofibrils are absent in *N119/KM88* and *N155/KM88* heterozygotes as well as *A138V* homozygotes; the myofibrils of *N119/+*, *N155/+* and *A138V/+* heterozygotes are indistinguishable from those of *KM88/+* flies showing striated fibers which have a tendency to fray. Electron micrographs of *KM88/+* myofibrils are described in BEALL *et al.* (1989). The myofibrillar structure and reduced IFM actin content lead to the conclusion that *A138V*, *N155* and *N119* are hypomorphic or possibly antimorphic alleles of *Act88F*. (2) *N142/KM88* and *N180/KM88* myofibrils show an absence of myofibrillar material. The same phenotype is also seen in *N142/+* heterozygotes but *N180/+* heterozygotes show the presence of large masses of fibrillar-like material which is not organized into discrete myofibrils. Both of these mutants are clearly antimorphic although the ways in which this is manifested at the structural level are clearly different. (3) The *N306* and *E93K* mutations are very similar. The myofibrils of *N306/+* and *E93K/+* are indistinguishable from *KM88/+*. Those of *N306/KM88* and *E93K* homozygotes show the presence of myofibrils which have a great tendency to fray and completely lack Z-discs. This and other aspects of the *E93K* mutation have been described in detail (SPARROW *et al.* 1991b); *E93K*, like *N306*, produces a similar charge change in the actin on two-dimensional gels. These mutations produce normal levels of actin. As heterozygotes they are not antimorphic for structure but *E93K/+* and *E93K/+* are flightless (D. R. DRUMMOND, and J. C. SPARROW, unpublished) demonstrating a functional antimorphicity.

From these analyses of all the new dominant flightless mutants mapping to chromosome arm 3R we conclude they are all mutations of the *Act88F* gene since *N306* and *E93K* specifically affect the charge of the Actin88F protein and the remaining alleles have reductions in the

FIGURE 4.—Polytene chromosome region 62A to 63A [redrawn from LINDSLEY and GRELL (1968)] showing the breakpoints of deficiencies and translocations used in this study. Open boxes indicate uncertainty of breakpoint localization. Filled boxes indicate localization of DNA probes. Details of chromosome breaks are given in Table 1. α Spec, α -spectrin; DM203, a muscle specific cDNA probe (Dm203; STORTI and SZWAST 1982). E, G2, G5 and G7 are chromosomal deletions as described in Table 1.

levels of Actin88F protein visible in the absence of another functional *Act88F* allele. It is unlikely that they are mutations in a closely linked locus since in that case *mutant/KM88* heterozygotes would be expected to accumulate the same amount of actin as *KM88/+* heterozygotes. In all cases *mutant/KM88* flies accumulate less actin. Furthermore *KM88* also uncovers the recessive myofibrillar defects present in the alleles. This has been supported by sequencing two of the alleles, *A138V* and *E93K*. *A138V* produces a single amino acid change of alanine to valine in codon 138 (SPARROW *et al.* 1992); *E93K* results in a change of glutamic acid to lysine in codon 93 (SPARROW *et al.* 1991b).

3L region mutations: The flightlessness of five of the third chromosome dominant flightless mutants mapped to the tip of 3L. Using the markers *ru* (3-0.0) and *h* (3-26.5) the flightlessness of all five mutants mapped 2-4 map units centromere proximal to *ru*. Flightless recombinants were recovered which contained only the region of the original chromosomes distal to *h*. In all cases, these recombinants were homozygous lethal. Complementation tests between these recombinants showed that each mutant chromosome carried an allelic lethal mutation. This cannot be due to a common mutation in the chromosome before mutagenesis since the alleles were isolated from both screen I and screen II. Further analysis (see below) shows that these five mutations are dominant flightless, recessive lethal alleles of a gene we have called *lethal(3)Laker*, *l(3)Lkr*. For brevity the alleles will be referred to as *Lkr^x* in the remainder of this report.

A precise map position of one of the alleles, *Lkr³*, was obtained by crossing *w/w; ru Lkr³ +/+ + TE46* to *w/Y; ru h st p^b ss e^s* males and testing each *ru-TE* recombinant (either *ru Lkr³ TE46/ru h st p^b ss e^s* or *+ Lkr³ +/ru h st p^b ss e^s*) in an *F₂* cross both for recessive lethality against a *Lkr⁵ e* chromosome, and for dominant flightlessness in *TM3/recombinant* flies. *TE46* maps at 3-10.4

± 0.5 (ISING and BLOCK 1981). The recessive lethality mapped to $3-2.65 \pm 0.45$. In all of the 103 recombinants between *ru* and *TE46*, the dominant flightless and recessive lethal phenotypes remained linked, indicating that if they arise from separate lesions, they are very tightly linked. Since both phenotypes occur in all five mutants, it is much more likely that they arise from mutations in a single gene. The map position places *l(3)Lkr* approximately in polytene chromosome region 62. The lethality of the *Lkr³* allele over *Lkr⁵* was shown to be 0.91 map units from *TE159* (at 62D7). By crossing *w; ru Lkr³ + TE46/+ + TE159* females to *w; + Lkr⁴ +/+ + TM3* males, we showed that *Lkr* lies centromere-distal to *TE159*, since all of the 3,139 non-TM3 progeny were red-eyed.

A cytological position (Figure 4) was obtained by complementation tests for the lethal phenotype of *Lkr²* relative to the four deficiencies, *Df(3L)R^E*, *Df(3L)R^{G2}*, *Df(3L)R^{G5}* and *Df(3L)R^{G7}*, recovered by SLITER *et al.* (1989). *Lkr²* was lethal in combination with *Df(3L)R^{G5}* but with none of the others, indicating that *Lkr* lies in the region 62A10-B1;62B2,4. The other alleles are also lethal with this deficiency. None of the complementation groups identified by SLITER *et al.* (1989) are in this region.

A genomic clone, named Dm203, containing a putative muscle protein gene has been localized to 62CD (STORTI and SZWAST 1982). This could possibly encode the Laker protein. An *EcoRI* restriction fragment length polymorphism (RFLP) for this clone in Southern blots of DNA from *Oregon* and *TE52* strains was discovered. This polymorphism is uncovered by both *Df(3L)R^{G5}* and *Df(3L)R^{G7}* (CRIPPS 1990), and since *Lkr* is included only in the former deficiency, λ Dm203 is not a clone of *Lkr*. Further, this localizes the Dm203 clone to 62B12,C1-C4,D1.

To determine whether *Lkr* shows haplo-insufficiency for flight, wild-type heterozygotes for each of the deficiencies were flight-tested (Table 4).

TABLE 4

Flight abilities of flies heterozygous for 3L deficiencies and Texas wild-type chromosomes

Genotype	U	H	D	N	n
<i>Df(3L)R^E/+</i>	97	1	1	1	148
<i>Df(3L)R^{G5}/+</i>	4	17	75	5	108
<i>Df(3L)R^{G7}/+</i>	16	35	29	20	116

See legend to Table 1 for genetic details and Table 2 for symbol explanation.

Df(3L)R^{G5}/+ flies exhibit dominant flightlessness which can be explained as a haplo-insufficiency for the *l(3)Lkr* locus; *Df(3L)R^{G7}/+* flies are also flightless although this deficiency does not delete the *Lkr* locus. This paradox may be explained by either of two observations. First, *Df(3L)R^{G7}* is *Minute*, a phenotype produced by mutations at many genomic locations which may be caused by reductions in protein synthesis (SINCLAIR *et al.* 1981). This hemizyosity for *M(3)LS2* may reduce muscle protein accumulation in the fly, and consequently reduce muscle power output. Second, *Df(3L)R^{G7}* deletes the 62CD region, which contains the Dm203 putative muscle protein gene, which may also be haplo-insufficient for flight. Since the muscle phenotypes of these two deficiencies are dissimilar (see below), we conclude that the flightless phenotypes are due to effects on different genes. *Df(3L)R^E/+* flies, in which none of these three loci are deleted, fly normally.

All of the *l(3)Lkr* mutations have abnormal IFM myofibrils when heterozygous with Texas wild type. Longitudinal section electron micrographs of *Lkr⁴/+* and *Lkr⁵/+* (Figure 5, c and e) show the myofibrillar aberrations which are present in the IFMs of all the *Lkr* mutants. Obvious effects are the frequent aberrations in Z-disc shape and Z-discs which do not span the myofibril. "Anastomoses" of neighboring myofibrils are also seen (Figure 5e). Transverse sections of *Lkr⁴/+* and *Lkr⁵/+* muscles (Figure 5, d and f) show that the cores of the myofibrils have almost normal hexagonal packing arrangements of thick and thin filaments, but that the periphery of the lattice is always more disorganized. These are common features of mutant heterozygotes for a number of major myofibrillar protein genes (KARLIK and FYRBERG 1985; WARMKE *et al.* 1988; O'DONNELL and BERNSTEIN 1988; BEALL *et al.* 1989) and on this basis the *l(3)Lkr* gene is a muscle protein gene.

Electron micrographs of IFMs from *TM3/Df(3L)R^{G5}* flies show structural aberrations of the myofibrils indistinguishable from the *Lkr/+* heterozygotes which are consistent with this deficiency behaving as an amorphic *Lkr* allele (Figure 5g). Myofibrils from the IFMs of *TM3/Df(3L)R^{G7}* flies have a wild-type appearance (Figure 5h). The implication of this is that one copy of either or both of *M(3)LS2* and the putative muscle protein gene identified by lambda Dm203 (STORTI and SZWAST 1982), does not cause haplo-insufficiency for myofibrillar structure

and by this criterion accounts neither for the dominant flightlessness of this deficiency nor for the abnormal myofibrillar phenotype associated with *Df(3L)R^{G5}/+* flies. Note that the myofibrils of *Df(3L)R^{G5}/+* appear slightly larger than those of *Df(3L)R^{G7}/+*. The most likely explanations of this are either differences in growth conditions of the flies used for electron microscopy or that haplo-insufficiency for *M(3)LS2* affects myofibril size in *Df(3L)R^{G7}/+* adults.

Analysis of the IFM myofibrillar proteins of all *Lkr/+* genotypes by one- and two-dimensional SDS-PAGE did not reveal differences in either pattern or intensity between mutant and wild-type proteins (data not shown).

Wild-type terminal duplications of the 3L region were generated to study the effects upon dominant flightlessness of the *Lkr* alleles. *T(Y;3)A158* and *T(Y;3)D8* strains were used to generate duplications for 61A1-62F2,63A1 and 61A1-62A, respectively. The final crosses produced *C(1)M3, y² bb/Y; lkr^x/+* females and *+/T(Y;3) A158 Y^p; 3L^p, y⁺; lkr^x/+* duplicated males. For each duplication, the effects of additional dosages upon wild-type (denoted *+/+* and *+/+/+*) were also tested. All flies were flight tested using the flight box, and the data are summarized in Table 5. Both terminal duplications had no effect on flight in the control genotypes *+/+* (females) and *+/+/+* (males) despite the sex difference. The 61A1-62F2,63A1 duplication fully rescues the dominant flightlessness of *Lkr²*, *Lkr⁴* and *Lkr⁵*; it reduces, but does not fully rescue, the dominant flightlessness of *Lkr¹* and *Lkr³*. In contrast the 61A1-62A duplication does not rescue or reduce the dominant flightlessness of *Lkr¹* and *Lkr⁴*; the other three alleles have not been tested. The results indicate that duplication of the *Lkr* region, 62A-62F2,63A1 can rescue the dominant flightless phenotype of *Lkr²*, *Lkr⁴* and *Lkr⁵* suggesting they are hypomorphic or amorphic alleles. The partial rescue of *Lkr¹* and *Lkr³* dominant flightlessness may indicate that these alleles are weakly antimorphic.

The α -spectrin gene is located in chromosome region 62B1-7 (BYERS *et al.* 1987), and so may be synonymous with the *Lkr* gene (Figure 4). Southern blots of *TM3* heterozygotes of the *l(3)Lkr* alleles, probed with an α -spectrin probe failed to reveal any differences in restriction fragment lengths as compared to *w; TE52*. This may not be surprising since these mutations were induced by EMS, and although EMS-induced rearrangements of *Mhc* have been reported (MOGAMI *et al.* 1986), the myosin heavy chain mutants described for the first time here show no restriction map changes (S. I. BERNSTEIN, personal communication) and those actin alleles we have sequenced are point mutations. We have not ruled out the possibility that the *l(3)Lkr* gene is synonymous with the α -spectrin gene. However, α -spectrin is distributed throughout the embryo from a very early stage (PESACRETA *et al.* 1989), so specific effects of mutations of this gene upon muscle might be surprising, though they

FIGURE 5.—Electron micrographs of dorso-longitudinal indirect flight muscles. **a**, wild-type longitudinal section (LS); **b**, wild-type transverse section (TS); **c**, *Lkr*⁴/+ LS; **d**, *Lkr*⁴/+ TS; **e**, *Lkr*⁵/+ LS; **f**, *Lkr*⁵/+ TS; **g**, *Df(3L)R*^{G5}/+ TS; **h**, *Df(3L)R*^{G7}/+ TS. All micrographs are at same magnification. Bar line = 1 μ m.

might be explained by alternative splicing of transcripts.

The lethal phase of the *Lkr* alleles was obtained by making reciprocal crosses of *TM3/Lkr*^x with *+ /Lkr*^y and collecting eggs. The fate of the zygotes was followed by scoring for unhatched eggs, pupal cases, unhatched pupae and eclosed adults. In each case there was significant embryonic lethality (less than 25% of fertilized eggs). Examination at 48 hr after egg lay (AEL) showed that most of the unhatched eggs contained fully formed, phenotypically normal larvae except that although capable of movement, they moved slowly compared to 22-hr wild-type embryos. Several newly hatched larvae were found to have died with a "collapsed" appearance. When the unhatched larvae were gently removed from the vitelline membrane they moved slowly on the medium but within a few hours had died with the collapsed

phenotype. Live, fully formed but unhatched embryos were found up to 90 hr AEL, although by this time most had died. This phenotype is consistent with muscle dysfunction in the *Lkr* heteroallelic embryos, and correlates with similar phenotypes observed for mutations in *Mhc* (MOGAMI *et al.* 1986; this study). Confirmation that these are the *Lkr*^x/*Lkr*^y embryos was obtained by crossing *TM2, P[lArB] C40.1S3/Lkr*¹ with *TM2, P[lArB] C40.1S3/Lkr*⁵. Only 3 of 104 unhatched embryos still alive at 48 hr stained for β -galactosidase activity due to the presence of *P[lArB]* indicating that the lethal, slow moving phenotype is a recessive *Lkr* phenotype. The lethality probably arises from muscle dysfunction which either prevents hatching or, if hatching is achieved, from an inability to feed. The heteroallelic lethality of *Lkr* alleles can be prevented by a single wild-type copy

TABLE 5

Comparison of the flight abilities of *Lkr*/+ heterozygote genotypes with and without wild-type terminal duplications of 3L

Genotype	Duplication 61A1 to 62F2,63A1 [<i>T(Y;3)A158</i>] ^a					Duplication 61A1 to 62A [<i>T(Y;3)D8</i>] ^b				
	U	H	D	N	n	U	H	D	N	n
+/+	97	1	2	0	141	100	0	0	0	37
+/+/+	94	2	2	2	82	96	0	2	2	58
<i>Lkr</i> ¹ /+	4	10	46	40	77	0	0	26	74	58
<i>Lkr</i> ¹ /+/+	62	14	20	4	50**	0	0	14	86	44
<i>Lkr</i> ² /+	0	21	54	25	85					
<i>Lkr</i> ² /+/+	90	4	2	4	50					
<i>Lkr</i> ³ /+	1	11	60	28	80					
<i>Lkr</i> ³ /+/+	76	8	12	4	73**					
<i>Lkr</i> ⁴ /+	0	5	45	50	100	0	0	17	83	53
<i>Lkr</i> ⁴ /+/+	86	3	10	1	63	0	0	18	82	45
<i>Lkr</i> ⁵ /+	0	0	13	87	106					
<i>Lkr</i> ⁵ /+/+	86	9	5	0	58					

Numbers are percentages of flies in each class; n, number of flies tested.

^a Null hypothesis: the flight ability of *Lkr*/+/+ flies with the *T(Y;3)A158* duplication does not differ significantly from the wild-type duplicated flies. The H, D and N classes were aggregated. *Lkr*¹ $\chi^2_{[1]} = 21.25$, $P < 0.01$; *Lkr*² $\chi^2_{[1]} = 0.68$, $P > 0.05$; *Lkr*³ $\chi^2_{[1]} = 10.53$, $P < 0.01$; *Lkr*⁴ $\chi^2_{[1]} = 2.74$, $P > 0.05$; *Lkr*⁵ $\chi^2_{[1]} = 2.39$, $P > 0.05$.

^b Null hypothesis: the flight ability of *Lkr*/+/+ flies with the *T(Y;3)D8* duplication does not differ significantly from that of the *Lkr*/+ flies. The U, H and D classes were aggregated. *Lkr*¹ $\chi^2 = 2.29$ $P > 0.05$; *Lkr*⁴ $\chi^2_{[1]} = 0.01$, $P > 0.05$.

** Chi-square significant; $P < 0.01$.

of the 61A1-62F2,63A1 region introduced using the *T(Y;3)A158* strain. A duplication of 61A1-62A, using *T(Y;3)D8*, does not have this effect.

Comparison of electron micrographs of larval body wall muscle from wild-type and heteroallelic combinations of *l(3)Lkr* mutations show distinct differences in the appearance of the mutant muscle (Figure 6). The striated nature of the larval muscles is not well developed at this stage but in wild-type the thick and thin filaments have a regular, parallel, interdigitated appearance. In the mutant muscles (Figure 6, c and d) there are areas of normal packing of these filaments visible in both transverse and longitudinal sections. The filament orientations are often severely disorganized and both transverse and longitudinal views of filaments are seen in the same section of the same muscle (Figure 6c); even in more normal areas (Figure 6d) distinctive "chevron" patterns of thick filaments are often seen, which have not been observed in wild-type muscles. The electron dense Z-discs or Z-bodies are fairly constant in size and appearance in wild-type muscles (Figure 6b); in the mutant muscles they are irregular in appearance (Figure 6, c and d). The presence of areas of thick and thin filament interdigitation in the mutant muscle presumably accounts for the slow, weak movements seen in those mutant larvae that manage to hatch.

DISCUSSION

A strict stoichiometry of myofibrillar proteins is implicit in the very regular structure of the IFMs. The haplo-insufficiency for flight of a number of IFM protein genes can be explained both by decreases in the number of functional crossbridges and by myofibrillar aberrations (BEALL *et al.* 1989). In addition, since muscle is a

macromolecular complex consisting of a large number of different proteins, many of which interact functionally, there is considerable scope for mutant proteins to have antimorphic effects upon myofibril assembly and function.

We have recovered dominant flightless mutants in the two autosomal genomic regions identified as hotspots for such mutations by MOGAMI and HOTTA (1981). Genetical evidence suggests that the second chromosome mutations are in the muscle myosin heavy chain gene, *Mhc*. Recessive lethal *Mhc* mutations are the largest class of *Mhc* mutations we isolated. Homozygous viable alleles with specific effects upon myosin accumulation in the IFMs have been described previously (CHUN and FALKENTHAL 1988; O'DONNELL *et al.* 1989; COLLIER *et al.* 1990; KRONERT *et al.* 1991), and our allele *Mhc*¹², in which no MHC accumulates in the IFMs, clearly falls into this class.

Among all homozygous viable *Mhc* mutant alleles, *Mhc*¹³ and *Mhc*¹⁹ are novel in showing a phenotype in which the normally isometric dorso-longitudinal and dorso-ventral fibers appear to have "super-contracted" early in adult life. This causes most of the muscle mass to be at one of the attachment sites of the muscles on the cuticle. It is intriguing that mutations within the *Mhc* gene can cause such a change in muscle behavior; a further analysis of these mutations may identify specific regions of the MHC protein which are important for this phenotype.

The *Act88F* mutant alleles described here have a range of phenotypic effects. In addition to dominant flightlessness there are changes in actin accumulation in the IFMs, from *A138V* which appears to be a null mutation, to *N180* with a reduction in IFM actin content,

FIGURE 6.—Electron micrographs of embryonic body wall muscles. **a**, Wild-type transverse section (TS); **b**, wild-type longitudinal section (LS); **c**, *Lkr*²/*Lkr*⁵ T/LS. **d**, *Lkr*²/*Lkr*⁵ LS. Bar lines = 0.25 μ m.

to *E93K* and *N306* which accumulate normal levels of actin but have charge changes in the protein (SPARROW *et al.* 1991b; this study). Without exception these mutations have dominant effects on muscle structure (BALL 1987), a feature found to date with all *Act88F* mutations recovered by selecting for dominant flightlessness. In addition, those actin mutants we have obtained as homozygotes invariably produce severe aberrations of myofibrillar structure. *E93K* and *N306* are novel mutations which produce myofibrils which have lost Z-discs and sarcomeric structure (SPARROW *et al.* 1991b; this study).

The recovery of dominant flightless *Act88F* mutants which invariably have such severe effects suggests either that these are the commonest mutations that occur in the gene or that the selection process enriches for extreme mutations. Heterozygotes for the functionally "null" *Act88F* allele *KM88* show some defects of myofibril organization and dominant flightlessness (BEALL *et al.* 1989). Antimorphic effects are also a common feature of *Act88F* mutations (HIROMI *et al.* 1986; KARLIK *et al.* 1984b, 1987; DRUMMOND *et al.* 1991) which is normally explained in terms of the mutant actin monomers interfering with the assembly of wild-type protein into functional thin filaments. Actin is one of the most highly conserved proteins known, so most mutations might be expected to have severe phenotypic effects. However, there is still a disparity between the observation that all *Act88F* mutations recovered by dominant flightlessness have severe effects on myofibrillar structure, while in a

study where random mutations generated *in vitro* were introduced into the cloned gene and transformed into flies, two of the seven mutations studied had almost normal myofibrillar structure. One of these flew only moderately, the other not at all (DRUMMOND *et al.* 1990, 1991).

We have identified a new gene, *l(3)Lkr*, by its dominant flightless mutations which are also recessive lethal. It is located in polytene bands 62A10,B1-62B2,4. Although we have not identified the product of this gene, it is undoubtedly a muscle protein. The gene and its mutations share many properties with all major myofibrillar proteins genes so far identified which are expressed in the IFMs. These include dominant flightlessness, dominant effects on myofibrillar structure and haplo-insufficiency for flight. In many of the muscle protein genes, this dominance can be explained by either haplo-insufficiency or antimorphic effects. Two of the *Lkr* alleles are antimorphic for flightlessness, being only partly rescued by the addition of two wild-type copies; the other three alleles appear to be either hypomorphs or amorphs. Heterozygotes for both a deficiency of *Lkr*⁺ and hypomorphic alleles of *Lkr* are flightless, demonstrating haplo-insufficiency. In addition, all the *Lkr* alleles, including its deficiency, have dominant effects on IFM myofibrillar structure and the lethal phase of heteroallelic combinations at the end of embryogenesis correlates to a dysfunction and structural aberrations of the larval body wall muscles.

By these criteria, *Lkr* must encode a muscle protein, although whether this needs to be a structural protein of the myofibrils or a protein involved in myofibrillogenesis is not clear. α -Spectrin is located in the same chromosome region as *Lkr*, but it is not yet known whether these two genes are coincident. Spectrin is involved in the attachment of myofibrils to the plasma membrane. Localization of the protein during *Drosophila* embryogenesis suggests that this might not be the case for α -spectrin (PESACRETA *et al.* 1989). Most of the genes encoding major muscle components in *Drosophila* have already been identified; *Lkr* might encode one of the very large Z-disc proteins found in *Drosophila*, such as Z(210) or Z(400–600), for which the genes have not yet been identified (SAIDE *et al.* 1989; VIGOREAUX *et al.* 1991).

The frequency of dominant flightless mutants recovered was 0.045% for screen I and 0.052% for screen II. These cannot be compared to those of MOGAMI and HOTTA (1981), since they give neither the total number of flies screened, nor the number of flightless males collected. One interesting observation concerns the different types of *Mhc* mutations identified in this study and by MOGAMI and co-workers (MOGAMI and HOTTA 1981; MOGAMI *et al.* 1986). All of our *Mhc* alleles have normal restriction maps (S.I. BERNSTEIN, personal communication), whereas four of the seven EMS-induced *Mhc* alleles isolated by MOGAMI and co-workers contain DNA rearrangements. The most likely cause of this is the differences in parental strains used. In the case of the *Act88F* mutations which have been sequenced from both studies, all are point mutations (KARLIK *et al.* 1984b, 1987; SAKAI *et al.* 1991; SPARROW *et al.* 1991b, 1992).

All the dominant flightless mutations we have recovered are alleles (≥ 5 per gene) of one of three haplo-insufficient genes. On this basis we could claim to have saturated the autosomes for genes which can mutate to dominant flightlessness. However, this is not the case as we discuss below.

Our approach differs from that of MOGAMI and HOTTA (1981) in two major ways. We used a different method for selecting flightless flies and have analyzed all the flightless mutants recovered rather than only those with changes on two-dimensional SDS-PAGE of myofibrillar proteins. Despite this, we have recovered mutations in some of the same genes and with similar properties as those described by MOGAMI and HOTTA (1981). The two exceptions are our recovery of the *Lkr* alleles and the absence of any tropomyosin mutations. Since there are no obvious effects of *Lkr* upon the accumulation of myofibrillar proteins as visualized by SDS-PAGE, this might explain our recovery of these novel mutations.

MOGAMI and HOTTA (1981) recovered a single mutation, *Ifm(3)3*, which results from the insertion of a *springer* transposable element. This affects the splicing pattern of the *TmI* gene such that synthesis of the IFM-specific product of this gene is reduced (KARLIK and FYR-

BERG 1985). This type of mutation may be rare, since they recovered one allele and we recovered none. It is not clear why we did not recover any dominant flightless mutations in this gene. A possibility is the difference in stringency of the selection procedures we have used as compared to that used by MOGAMI and HOTTA (1981).

A large number of myofibrillar protein genes have now been located on the autosomes. Apart from *Mhc*, *Act88F* and *TmI*, these include a second tropomyosin gene, *TmII*, known to be expressed in the IFMs (KARLIK *et al.* 1984a; HANKE and STORTI 1988), the myosin light chain-2 gene, *mlc-2* (PARKER *et al.* 1985), the alkali myosin light chain gene, *mlc-alk* (FALKENTHAL *et al.* 1984), the projectin gene (AYME-SOUTHGATE *et al.* 1991), also called *l(4)102CDa* (FYRBERG *et al.* 1992), and the paramyosin gene (VINOS *et al.* 1991; BECKER *et al.* 1992). While the *mlc-2* gene shows haplo-insufficiency for flight (WARMKE *et al.* 1988) it is not known if the *mlc-alk*, paramyosin or projectin genes are haplo-insufficient for flight. The *l(4)CDa* mutant and its allele *bt^p* show dominant flight reduction and effects on IFM myofibrillar structure (FYRBERG *et al.* 1992) although it is not known if in either case these effects are due to haplo-insufficiency.

One reason why we may not have recovered mutations in other myofibrillar protein genes may be the severity of our selection procedure. In fact recent flight-testing studies on flies heterozygous for a null allele of *mlc-2* using a replica of our flight-box (WARMKE *et al.* 1992) showed significantly reduced flight but the flies would have been unlikely to be recovered at the bottom of our flight-testing column.

The two IFM-specific tropomyosin-like products of the *TmII* gene (named troponin-H, or TnH) differ only in the alternative exons which encode the C-terminal halves of the molecules. Any redundancy might prevent the recovery of EMS-induced dominant flightless mutations affecting only one TnH isoform. The C-terminal exons show large sequence differences between the two isoforms, TnH-33 and TnH-34, despite their remarkable overall similarity in amino acid composition, consisting almost entirely of glutamic acid, glycine, alanine and proline (KARLIK *et al.* 1984a; HANKE and STORTI 1988). We have found frequent size and charge polymorphism in laboratory strains in TnH-34 (CRIPPS and SPARROW 1992) and more recently in TnH-33 (A. HARRISON and J. C. SPARROW, unpublished observations), but these polymorphisms do not affect flight ability in the laboratory. These natural mutations may indicate that substantial changes can occur without phenotypic effects and explain our inability to recover dominant flightless mutations by EMS mutagenesis.

Autosomal dominant flightless mutations which do not affect the myofibrils of the IFMs are known, and include many well known mutations which affect wing shape and wing posture. Two flies with curled wings were

recovered in this screen (BALL 1987). Other autosomal dominant flightless mutations such as *Df(2R)vg^D* (CRIPPS and SPARROW 1989) probably affect the nervous system, in this case probably by overexpression of the *Su(z)2* gene (BRUNK *et al.* 1991). Such mutations are likely to be rare.

It is clear that the great majority of dominant flightless mutations recovered occur in genes which encode major structural proteins of the IFM myofibrils and as such it may be worthwhile screening existing deletions for loci which are haplo-insufficient for flight or myofibrillar structure. This correlation is also true for the X chromosome flightless mutations, which affect the genes encoding α -actinin (FYRBERG *et al.* 1990b; ROULIER *et al.* 1992), troponin-T (FYRBERG *et al.* 1990a) and troponin-I (BARBAS *et al.* 1991; BEALL and FYRBERG 1991), although not all of the alleles of these genes confer dominant flightlessness (HOMYK and EMERSON 1988). We conclude that selection for dominant flightlessness is an efficient method for identifying the genes encoding major muscle proteins and for obtaining new alleles of these genes. However, not all major muscle protein genes appear to mutate readily to give dominant flightlessness and other techniques must be applied in order to identify additional genes and mutations within them.

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