Suppression of Muscle Hypercontraction by Mutations in the Myosin Heavy Chain Gene of *Drosophila melanogaster*

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ABSTRACT

The indirect flight muscles (IFM) of *Drosophila melanogaster* provide a good genetic system with which to investigate muscle function. Flight muscle contraction is regulated by both stretch and Ca^{2+}-induced thin filament (actin + tropomyosin + troponin complex) activation. Some mutants in troponin-I (*TnI*) and troponin-T (*TnT*) genes cause a "hypercontraction" muscle phenotype, suggesting that this condition arises from defects in Ca^{2+} regulation and actomyosin-generated tension. We have tested the hypothesis that missense mutations of the myosin heavy chain gene, *Mhc*, which suppress the hypercontraction of the *TnI* mutant *held-up*^{2} (*hdp*^{2}), do so by reducing actomyosin force production. Here we show that a "headless" *Mhc* transgenic fly construct that reduces the myosin head concentration in the muscle thick filaments acts as a dose-dependent suppressor of hypercontracting alleles of *TnI*, *TnT*, *Mhc*, and *flightin* genes. The data suggest that most, if not all, mutants causing hypercontraction require actomyosin-produced forces to do so. Whether all *Mhc* suppressors act simply by reducing the force production of the thick filament is discussed with respect to current models of myosin function and thin filament activation by the binding of calcium to the troponin complex.

THE indirect flight muscles (IFM) of *Drosophila melanogaster* provide a powerful genetic system with which to understand muscle function, structure, and development. As flight is not required for survival under laboratory conditions, many mutants have been obtained in the genes for the major sarcomeric proteins by selection for flightlessness or for a "wings-up" phenotype (see Bernstein *et al.* 1993; Vigoreaux 2001). A number of Drosophila muscle protein gene mutations that give a dominant or recessive flightless phenotype produce an additional recessive phenotype in which IFM fibers undergo an auto-destructive contraction after the muscles have developed normally (Kronert *et al.* 1995). This leads to separation and accumulation of fiber material to one or both attachment sites or to fiber bunching with detachment from both ends. Intracellularly, the phenotype is very variable but is characterized by disruption of the myofibrillar lattice as well as by bulging and shortening of individual sarcomeres. In some mutants (*e.g.*, *Mhc*^{13} and *fln*^{0}) the phenotype includes muscle protein proteolysis (Kronert *et al.* 1995; Reedy *et al.* 2000).

As the term "hypercontraction" has been used in muscle pathology to describe similar types of muscle damage arising from excessive contraction in mutant *Caenorhabditis elegans* (Korswagen *et al.* 1997; Garcia-Anoveros *et al.* 1998), in reperfused rat hearts (Duncan 1987; Bhatti *et al.* 1989; Monticello *et al.* 1996), in Duchenne muscular dystrophy (Valentine *et al.* 1989; Tay *et al.* 1992; Cozzi *et al.* 2001), and in human muscle injuries (Roth *et al.* 2000; Finol *et al.* 2001), we have defined this Drosophila phenotype as "hypercontraction." We make the important distinction, implicit in the above, that the term is used only where the muscles develop normally, or nearly so, before the muscle damage occurs.

Extant mutants exhibiting the phenotype include *held-up*^{2} [*hdp*^{2}; an allele of the *wings-upA* gene, which encodes Troponin-I (TnI)], *up*^{101} [an allele of the *upheld* gene, which encodes Troponin-T (TnT)], some alleles of the IFM-specific actin gene, *Act88F* (An and Mogami 1996), three alleles of the myosin heavy chain gene, *Mhc*^{6}, *Mhc*^{13}, and *Mhc*^{19} (Kronert *et al.* 1995), and *fln*^{0}, an allele of the flightin gene, *fln* (Reedy *et al.* 2000). The fact that mutant alleles of the TnI and TnT genes produce hypercontraction suggests that defects in Ca^{2+} regulation can produce this phenotype. The recovery of three missense mutants of the *Mhc* gene (Kronert *et al.* 1995) and, more recently, of an allele of *flightin* (Reedy *et al.* 2000), all of which cause hypercontraction, suggests that a single explanation for hypercontraction is unlikely. The extant hypercontracting *Mhc* alleles are restricted to a small, five-amino-acid region of the light meromyosin domain. It is through this domain that

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TABLE 1

Muscle fiber phenotypes caused by thin and thick filament hypercontracting mutations

Genotype (protein)	Before 78 hr APF	After 78 hr APF	At eclosion	>2 days
hdp^2 (TnI)	Normal	HC	HC	HC
up^{101} (TnT)	Normal	HC	HC	HC
$Act88F^{R28C}$ (Actin)	Detached posterior fiber ends	HC initiated from posterior ends	PHC (one end detached)	HC
$Act88F^{E334Q}$ (Actin)	Loosely attached	PHC detached ends	PHC (bunched)	HC
$Act88F^{G268D}$ (Actin)	Wiggly fibers	Wiggly and detached	PHC (bunched)	HC
$Act88F^{R95C}$ (Actin)	Normal	Normal	Normal	PHC (pulled at posterior end)
Mhc^{13} (Myosin)	Normal	Normal	Normal	HC
fln^0 (Fln)	Normal	Normal	Normal	HC

HC, hypercontracted fibers; PHC, partially hypercontracted fibers. All the genotypes scored were homo- or hemizyogous (X-linked).

myosin dimerizes by the assembly of α-helical coiled-coil rods, which subsequently polymerize to form thick filaments. Flightin is likely a thick filament protein associated with the myosin rod domain (Vigoreaux *et al.* 1993; Reedy *et al.* 2000). These latter mutants suggest that structural defects within the sarcomere can also lead to hypercontraction. Since actin is the major component of the thin filament, the *Act88F* alleles that cause hypercontraction could produce either defects in the troponin-tropomyosin (Tn-Tm) complex regulation or, like the two thick filament proteins, structural defects of the sarcomere.

hdp^2 is a point mutation (A116V) within exon 5 of the *wupA* gene (Beall and Fyrberg 1991) and likely affects Ca^{2+} regulation. It corresponds to a highly conserved residue, alanine 25, of vertebrate skeletal muscle TnI, which is part of the N-terminal α-helix that interacts with TnC (Vassylyev *et al.* 1998). To investigate the protein interactions occurring within the Tn-Tm complex during Ca^{2+} regulation, Prado *et al.* (1995) recovered six suppressors of hdp^2 by their suppression of the wings-up phenotype. One suppressor is an intragenic mutation within the *wupA* gene (Prado *et al.* 1995), another is a missense mutation within the *Tm2* gene (Naimi *et al.* 2001), and the remaining four are *Mhc* gene mutations within the myosin head (Kronert *et al.* 1999).

The recovery of *Mhc* alleles as suppressors of a mutation affecting thin filament regulation might seem surprising, unless the forces produced by the interaction of myosin with actin are normally required to produce the hypercontraction phenotype. Support for this comes from experiments of Beall and Fyrberg (1991) that removed all the IFM myosin from hdp^2 flies using an IFM-specific *Mhc* null mutation, *Ifm(2)2* (now known as Mhc^7). The $hdp^2/Y; Mhc^7$ muscles did not hypercontract but also myofibrils did not develop. The investigators proposed that actomyosin interactions exacerbate the structural or functional defect resulting from the troponin-I mutation. We further propose that it is the actomyosin force generation that is largely responsible for the hypercontraction either in response to aberrant regulation of contraction or when sarcomeric structure is compromised. This proposal further predicts that *Mhc* suppressors will be neither allele nor gene specific in their interactions with hypercontracting alleles in other genes. We have examined this proposal using genetic approaches to reduce the concentration of myosin heads in the sarcomere and by characterizing new *Mhc* missense suppressors of hdp^2.

MATERIALS AND METHODS

Fly strains: All chromosome and gene symbols unless specifically mentioned are as described in FlyBase (http://flybase.bio.indiana.edu/). Canton-S was used as the control in all the experiments unless specified. The *Y57* and *Y97* transgenic lines express a myosin heavy chain polypeptide lacking the head (or motor) domain and were gifts from S. I. Bernstein and R. M. Cripps (Cripps *et al.* 1999). The *Mhc* suppressors of hdp^2, Mhc^{D1}, Mhc^{D41}, Mhc^{D45}, and Mhc^{D62} were obtained from A. Ferrus. fln^0 is described in Reedy *et al.* (2000). All flies used for experiments are 2–4 days old unless otherwise indicated. All stocks and crosses were maintained at 25° on a yeast-sugar-agar medium.

Isolation, mapping, and sequencing of dominant *Mhc* suppressor mutations: Ethyl methanesulfonate (EMS) mutagenesis and isolation of dominant suppressors of the hdp^2 wings-up phenotype were as described in Prado *et al.* (1995). Mutants mapping to the second chromosome were assigned as *Mhc* suppressors by noncomplementation with lethal *Mhc* alleles, including *Df(2L)H20* (except $Mhc^{Su(2)D}$), and designated as $Mhc^{Su(2)X}$, where *X* is an allele identifier. They are referred to as *Su(2)X* mutations in the text. Oligonucleotide primers used

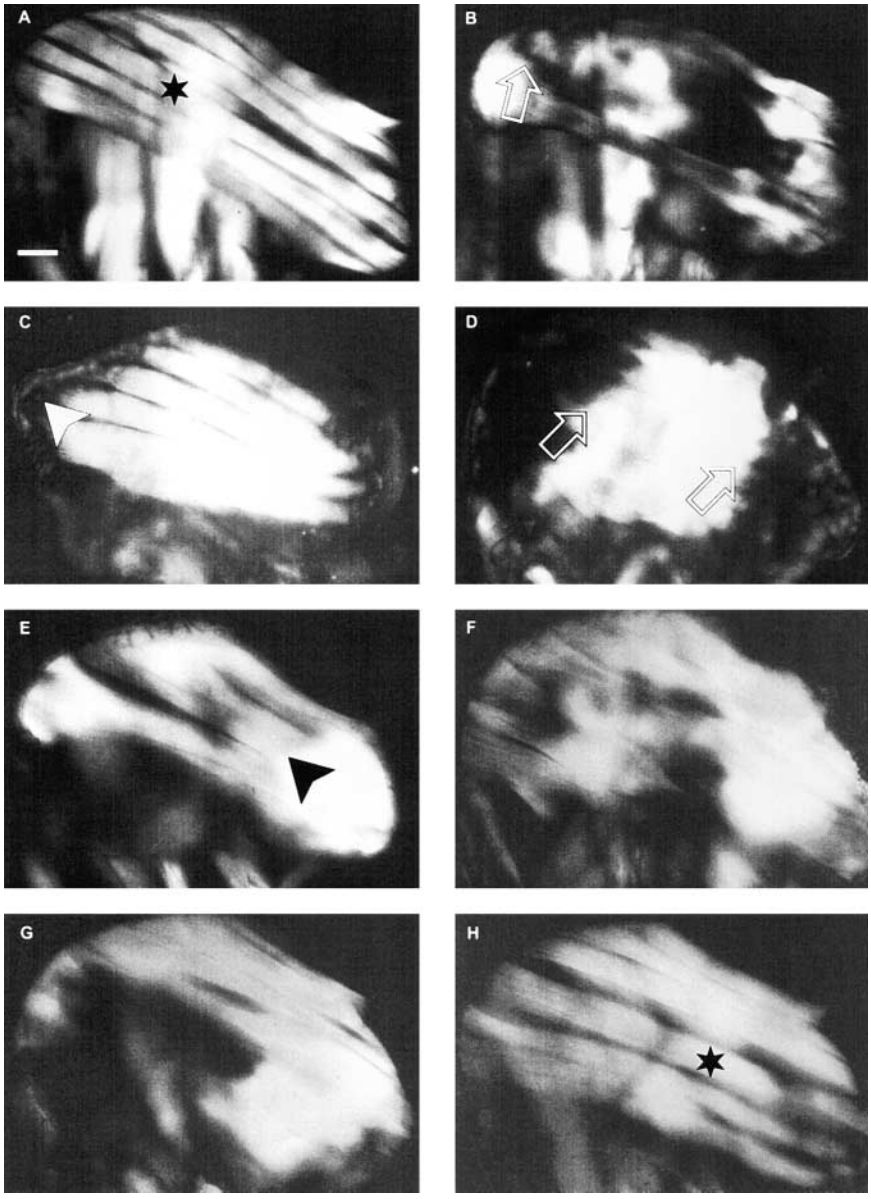

FIGURE 1.—Polarized light micrographs of hypercontracting IFM. (A) IFM of wild-type fly. A star indicates one of the DLMs. (B) hdp^2 showing IFM hypercontraction; fibers are bunched to the cuticle. Arrow indicates the bunched fibers at one end of the thorax. (C) DLMs of the $Act88F^{E334Q}$ fly just before the eclosion showing partially hypercontracted phenotype. Note the initiation of hypercontraction at the attachment site of the fibers (arrowhead). (D) Late $Act88F^{E334Q}$ showing the DLMs pulled to the center (arrows). (E) up^{101} showing the partially pulled DLMs (arrowhead). (F) hdp^2/Y; *Y97* partial suppression of the hdp^2 phenotype with a copy of the myosin headless construct. (G) hdp^2/Y; $Mhc^{10}/+$ showing that a reduction in MHC partially suppresses the hdp^2 phenotype. (H) hdp^2/Y; $Mhc^{10}/+$, *Y97*, a copy of Mhc^{10}, and the headless myosin construct completely suppress the hdp^2 hypercontraction (star). In all frames the anterior fly thorax is at the left corner, and the dorsal side is toward the top. All flies are 2–4 days old unless otherwise indicated and at the same magnification. Bar, 0.125 mm.

for PCR and sequencing were as described in KRONERT *et al.* (1999).

Hemithorax mounts for polarized light microscopy: IFMs were prepared for polarized light microscopy as described by NONGTHOMBA and RAMACHANDRA (1999). Briefly, fly thoraces were frozen in liquid nitrogen, longitudinally bisected with a razor blade, dehydrated through an alcohol series and then cleared in methyl salicylate and mounted in Canada balsam/DPX. Photographs were taken with a Leica microscope using polarized light optics.

Transmission electron microscopy: Fly half thoraces were prepared following the protocol of KRONERT *et al.* (1995) and embedded in Epon E218. Sections stained with lead citrate and counterstained with uranyl acetate were examined using a JEOL 1200 EX transmission electron microscope.

Behavioral studies: Measurements of adult flight, walking, larval crawling, and feeding behaviors were conducted as described in NAIMI *et al.* (2001). The flight index is calculated as the percentage of flies that flew up or horizontally, rather than down or not at all.

RESULTS

The hypercontracted IFM phenotype: Hypercontraction can take a number of forms. Table 1 summarizes the thick and thin filament protein mutants showing hypercontraction and the developmental stage at which this phenotype occurs. In many mutants hypercontraction ends with the muscles parted, or seemingly so, in the middle with the bulk of the fiber bunched at one or both attachment sites (Figure 1B). In other mutants the fibers separate from the attachment sites and bunch in the middle of the fiber (Figure 1, C and D). On the basis of these characteristics, only a handful of IFM mutants are classified as hypercontracted. In some mutants, $Act88F^{R28C}$ and $Act88F^{E334Q}$ (Figure 1D), the phenotype is less extreme in terms of either the degree of muscle shortening or the number of fibers showing the

TABLE 2

Wing and muscle phenotypes of hypercontracting thin and thick filament mutants and their suppression with headless myosin constructs

		Wing phenotypes (%)			Muscle phenotypes (%)		
Genotype	*n*	Upheld	Down	Normal	HC	PHC	N
hdp^2/Y	50	100	0	0	100	0	0
up^{101}/Y	50	86	6	8	88	12	0
Mhc^{13}	50	56	40	4	84	16	0
fln^0	50	16	24	60	76	24	0
Mhc^{10}/Mhc^{10}	50	52	42	6	0	0	100
$Mhc^{10}/+$	50	4	0	96	0	0	100
Y97/Y97	50	56	30	14	0	0	100
Y97/+	45	0	18	82	0	0	100
$Mhc^{10}/+$; *Y97/+*	32	0	44	56	0	0	100
hdp^2/Y; $Mhc^{10}/+$	51	49	51	0	29	71	0
hdp^2/Y; *Y97/+*	31	51	39	10	61	39	0
hdp^2/Y; $Mhc^{10}/+$; *Y97/+*	55	13	0	87	0	13	87
up^{101}/Y; $Mhc^{10}/+$	45	100	0	0	80	20	0
up^{101}/Y; *Y97/+*	58	100	0	0	50	50	0
up^{101}/Y; $Mhc^{10}/+$; *Y97/+*	45	22	18	60	0	0	100
Mhc^{13}/Mhc^{10}	72	76	24	0	39	61	0
Mhc^{13}/Mhc^{13}; *Y97*	82	51	49	0	13	87	0
Mhc^{13}/Mhc^{10}; *Y97*	58	12	26	62	0	21	79
Y57; Mhc^{10}/Mhc^{10}; fln^0/fln^0	50	20	24	56	0	8	92

All the genotypes are flightless. HC, hypercontracted muscle phenotype; PHC, partially hypercontracted; N, normal/suppressed muscle phenotype; *n*, number of flies scored.

phenotype (Figure 1E), and we refer to this as partial hypercontraction (Naimi *et al.* 2001).

For Mhc^6, Mhc^{13}, Mhc^{19}, and fln^0, the hypercontraction phenotype develops progressively during the first 24 hr after adult eclosion (Kronert *et al.* 1995; Reedy *et al.* 2000); for hdp^2, we have shown (Naimi *et al.* 2001) that the IFM form normally up to 78 hr after puparium formation (APF), when twitching of the pupal legs is first observed and the IFM begin to shorten and break, a process that is complete at, or just before, eclosion.

Why is this hypercontraction phenotype produced at different developmental stages? Although the development and maturation of myofibrils continue for a few hours after eclosion, it is likely that functional myofibrils are formed by 75 hr APF (Reedy and Beall 1993; Reedy *et al.* 2000). Thus the TnI mutation, hdp^2, causes a pupal development of the phenotype while the four mutants of two thick filament proteins, MHC and FLN, lead to the progressive adult phenotype. We have investigated whether this correlation extends to other hypercontracting muscle protein mutations. The up^{101} mutation shows normal development until 78 hr APF when, like hdp^2 (Table 1), the IFM begin to hypercontract, a process that is complete by eclosion with the exception of a few flies that still remain partially hypercontracted (Figure 1E; Table 2). Actin $Act88F^{R28C}$ and $Act88F^{E334Q}$ mutants show almost normal myofibril development until 78 hr APF with the initiation of fiber detachment from one end (Figure 1C), followed by fibers bunching to one end of the thorax (not shown) or in the middle (Figure 1D). $Act88F^{G268D}$ develops "wavy" fibers but partially hypercontracts only after 78 hr APF, while in $Act88F^{R95C}$ partial hypercontraction develops only after eclosion (Table 1).

Hypercontraction is suppressed by reducing the amount of functional myosin heads: The appearance of hypercontraction in muscles that have developed normally suggests *a priori* that forces developed by actomyosin crossbridges cause the damage. We began by extending the experiment of Beall and Fyrberg (1991) on the hypercontraction of hdp^2, but rather than removing all the myosin (which also removes the thick filament and myofibrillar lattice), we used two "headless" *Mhc*-expressing transgenic lines, *Y57* (on the first chromosome) and *Y97* (on the third chromosome; Cripps *et al.* 1999) to reduce the myosin head concentration in the thick filaments. The headless myosin molecules retain the regulatory light chain binding site and all domains C-terminal of this. They coassemble with endogenous full-length myosin in the thick filaments of IFM (Cripps *et al.* 1999).

In combination with the IFM-specific Mhc^{10} null allele, the *Y97* construct acts as a dose-dependent suppressor of hdp^2 (Figure 1, F–H; Figure 2, E–J). In hdp^2 flies, although the muscle and myofibrils develop normally before 78 hr APF, by eclosion the fibers are completely pulled apart (Figure 1B). Hypercontraction completely disrupts the myofibrillar lattice, leaving fields of disor-

dered thick and thin filaments (Figure 2D) in which misaligned Z-bands and M-lines are seen. Few sarcomeres are seen and these are short (see Table 3) and often have a bulging appearance (Figure 2C). The presence of one copy of *Y97* partially suppresses the *hdp*^{2} phenotype at gross fiber (Figure 1F) and sarcomeric (Figure 2, E and F) levels. A slight suppression of the hypercontraction phenotype of *hdp*^{2} occurs due to *hdp*^{2}*; Mhc*^{10}/+ (Figure 1G and Figure 2, G and H). In *Mhc*^{10}/+ flies the removal of one functional *Mhc* gene copy will lead to ~60% of wild-type myosin accumulation (O'Donnell and Bernstein 1988; Cripps *et al.* 1994). Suppression of hypercontraction increases as the gene dose of *Y97* increases and that of *Mhc*$^+$ decreases until in *hdp*^{2}*/Y; Mhc*^{10}*/+; Y97* flies suppression is almost complete (Table 2; Figure 1H). Myofibrillar organization is partially restored with sarcomere lengths approaching those of wild type (Figure 2I). A remaining difference is the increased gap between neighboring myofibrils (Figure 2J) compared to wild-type controls (Figure 2B). Apart from the wild-type controls (Canton-S), all of the genotypes are flightless. This is hardly surprising as both *hdp*^{2} and a reduction in *Mhc* gene dosage cause flightlessness. The headless myosin cannot replace the power generation of wild-type MHC.

Overall genotypes that suppress the wings-up phenotype also suppress IFM hypercontraction, but in individual flies that is not invariably the case. Flies with *hdp*^{2}*/Y; Mhc*^{10}/+ or *hdp*^{2}*/Y; +/+; Y97* genotypes with partially suppressed hypercontraction show the full range of wing position from "wings up" to "wings held beside the abdomen" to "normal wing position" (Table 2), as do *Mhc*^{10}/*Mhc*^{10} homozygotes, which never show hypercontraction. In general we find in these and other studies that the correlation between the wings-up and IFM hypercontraction phenotypes is poor.

***Mhc* suppressor mutations of *hdp*^{2} hypercontraction:** Six dominant EMS-induced suppressor mutations of the *hdp*^{2} wings-up phenotype were recovered on the second chromosome from a screen of 25,000 progeny. Five mapped to the region between *black* (48.5) and *cinnabar* (57.5), which includes the *Mhc* gene. Four suppressors, *Su(2)A*, *Su(2)B*, *Su(2)C*, and *Su(2)F*, are *Mhc* alleles by their failure to complement the recessive lethality of *Mhc*^{l} and *Df(2)H20*. *Mhc*^{l} is a null allele due to a 1-kb internal deletion (O'Donnell and Bernstein 1988); *Df(2)H20* spans from 36A8-9 to 36F1 (Steward and Nusslein-Volhard 1986), which includes the *Mhc* gene. Lethality of these four *Mhc* suppressors as homozygotes or in heterozygous combination with either *Mhc*^{l}

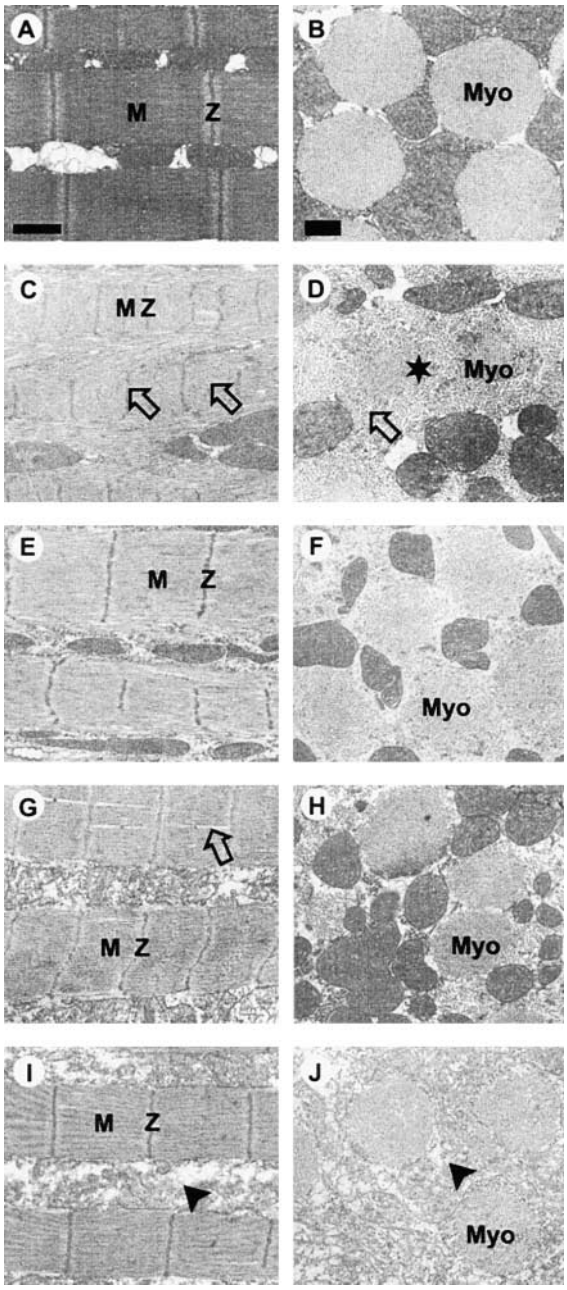

Figure 2.—Electron micrographs of *hdp*^{2} myofibrils suppressed by combinations of *Mhc*^{10} and the Y97 headless myosin transgenic insert. (A) Wild-type, longitudinal section (LS). (B) Wild-type, transverse section (TS). Myofibrils showing highly ordered myofibrillar lattices and borders. (C) *hdp*^{2} LS from newly eclosed flies. Sarcomere length is greatly reduced with streaming of Z- and M-bands (arrows). (D) *hdp*^{2} TS disruption of the myofibrillar borders and lattices (arrow); star indicates the region where thick and thin filament integrity is still preserved. (E and F) *hdp*^{2}*/Y; Y97* partial suppression of the *hdp*^{2} phenotype with a copy of the *Y97* headless construct. Sarcomere structure is slightly improved. (G and H) *hdp*^{2}*/Y; Mhc*^{10}/+. Partial suppression of the *hdp*^{2} phenotype by reduction of MHC. Breaks within the sarcomeric lattice are evident (arrow) and sarcomere length is shorter than normal. (I and J) *hdp*^{2}*/Y; Mhc*^{10}/+, *Y97*. One copy of *Mhc*^{10} and one copy of the *Y97* headless myosin construct completely suppress the *hdp*^{2} hypercontraction. Sarcomere length is nearly normal and there are minimal breaks and disruptions of the myofibrils; however, increased gaps filled with sarcoplasmic material separate the myofibrils (arrowheads). M, M-band. Z, Z-Band. Myo, myofibril. Bar, 1 μm for all the LS (all are of same magnification) and 0.5 μm for all the TS (all are at same magnification).

TABLE 3

Sarcomere lengths (in micrometers) in suppressed muscle fibers

Canton-S	hdp^2	2B/+	hdp^2; 2B/+	2F/+	hdp^2; 2F/+	2D/2D	hdp^2; 2D/+	hdp^2; 2D/2D
2.9 ± 0.3	1.0 ± 0.2	1.8 ± 0.2	2.4 ± 0.1	3.2 ± 0.2[a]	2.4 ± 0.1	2.6 ± 0.1	1.8 ± 0.1	2.6 ± 0.2

Lengths of the sarcomere were made by measuring the distance between neighboring Z-discs from electron micrographs. Mean lengths are calculated from measurements of 30 sarcomeres from three different samples.

[a] Indicates nonsignificant difference (Student's *t*-test) from the wild type. All other genotypes show significant differences in the length of the sarcomeres (at $P < 1\%$) with respect to Canton-S and hdp^2.

or *Df(2)H20* occurs early in development. Some die shortly after egg hatch after some crawling or in late first/early second instar at the first larval molt (double sets of mouthparts are seen in most larvae so molting is incomplete). All four mutants are dominant suppressors, which fully suppress hdp^2 hypercontraction (Table 4) but not the flightless phenotype. Each is dominant flightless in the absence of the hdp^2 mutation. This suggests that each *Mhc* suppressor mutation severely affects myosin function. Since they affect embryonic muscles and effect suppression of IFM hypercontraction, they are likely in constitutive exons of the *Mhc* gene since embryonic and IFM cDNAs differ in all alternative exons with the exception of exon 3, which is used in some embryonic muscles (George *et al.* 1989; Bernstein and Milligan 1997; Kronert *et al.* 1999; Zhang and Bernstein 2001). Genomic DNA from *Mhc* homozygous larvae was obtained by selecting *yellow* embryos/larvae from sib-mated *y/y* or *y/Y; CyO,* y^+/Mhc^x flies (see Kronert *et al.* 1999) and the constitutive and IFM-specific exons were sequenced following PCR. The *Mhc*-coding region of each mutant strain contained a single amino acid change encoded within a constitutive exon. The changes are given with the Drosophila codon numbers and the chicken skeletal muscle MHC homolog (in parentheses) to allow direct comparisons with *Mhc* mutants in other species.

Suppressors *Su(2)A*, *Su(2)B*, and *Su(2)C* were found to have the same point mutation (C → T) causing amino acid 401 (404 in chicken skeletal muscle myosin) to change from proline to serine in constitutive exon 8. This encodes part of the actin-binding region of the myosin head (Figure 3). These mutants could have arisen from a single mutational event and we have assumed that they did so. Suppressor *Su(2)F* is also a missense mutation (G → A) changing alanine at 462 (465) to threonine, a position close to the ATP-binding site (Figure 3).

Su(2)D is homozygous viable in combination with hdp^2 and on its own. *Su(2)D* heterozygotes without hdp^2 can fly as well as wild type [flight index of 79.6 ± 2.8 (SD), $n = 54$; in comparison to wild type, 78 ± 6.7, $n = 51$]. Homozygous *Su(2)D* flies, though, fly (flight index 63.9 ± 3.2) significantly less well (at 5% level) than wild type. *Su(2)D* heterozygotes partially suppress the hdp^2 wing position and muscle phenotypes but do so com-

TABLE 4

Suppression of hypercontraction mutant phenotypes with newly isolated missense mutations in myosin head

		Wing phenotypes (%)			Muscle phenotypes (%)		
Genotype	*n*	Upheld	Down	Normal	HC	PHC	N
Mhc^{2B}/+	41	0	0	100	0	0	100
Mhc^{2D}/+[a]	56	0	0	100	0	0	100
Mhc^{2D}/Mhc^{2D}[a]	85	0	0	100	0	0	100
Mhc^{2F}/+	64	8	0	92	0	0	100
hdp^2/Y; Mhc^{2B}/+	92	0	0	100	0	0	100
hdp^2/Y; Mhc^{2D}/+	148	49	0	51	10	85	5
hdp^2/Y; Mhc^{2D}/Mhc^{2D}	68	0	0	100	0	0	100
hdp^2/Y; Mhc^{2F}/+	53	28	0	72	0	11	89
up^{101}/Y; Mhc^{2B}/+	68	0	0	100	0	0	100
up^{101}/Y; Mhc^{2D}/+	29	52	34	14	0	100	0
up^{101}/Y; Mhc^{2D}/Mhc^{2D}[a]	30	0	0	100	0	0	100
up^{101}/Y; Mhc^{2F}/+	56	7	0	93	0	0	100
Mhc^{13}/Mhc^{2B}	55	4	38	58	0	35	65
Mhc^{2B}/+; fln^0	50	22	0	78	0	0	100

HC, hypercontracted; PHC, partially hypercontracted; N, normal/suppressed; *n*, number of flies scored.

[a] Flighted genotypes; all other genotypes are flightless.

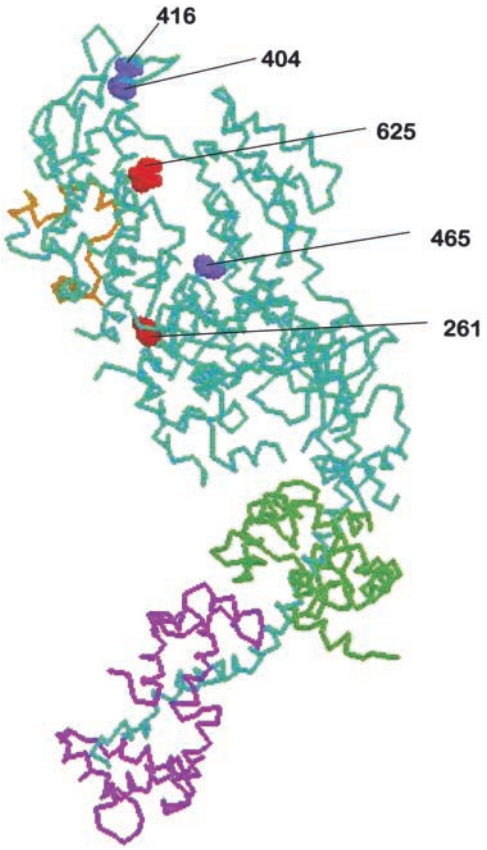

FIGURE 3.—The locations of the new suppressor mutations (blue) and those previously reported (red) within the atomic structure (2MYS.PDB) of chicken myosin S1 (RAYMENT *et al.* 1993b) depicted using Protein Explorer (http://www.umass.edu/microbio/chime/explorer). Suppressors *Su(2)A*, *Su(2)B*, and *Su(2)C* show the same amino acid change P401S (404 in chicken skeletal muscle myosin). Suppressor *Su(2)F* is the mutation A462T (465) in the same actin-binding loop. *Su(2)D* leads G413S (416). Mutations *D1* (D625G) and *D45* (A261T) are as described in KRONERT *et al.* (1999) and occur in the actin-binding loop and near the ATP-binding pocket, respectively. Exon 7 (orange) is alternatively spliced in *D41* as a result of a 2-bp insertion. *D62* causes an eight-amino-acid deletion near the actin-binding loop and is not shown, as the loop is not seen in this atomic structure (see KRONERT *et al.* 1999 for more details).

pletely in homozygotes (Table 4). Results from complementation analysis of *Su(2)D* and Mhc^1 are ambiguous because Mhc^1/+ flies are viable and show partial IFM hypercontraction (NONGTHOMBA and RAMACHANDRA 1999). *Su(2)D* maps to position 54.2 on chromosome 2, *i.e.*, within the *Mhc* region. Sequencing of the constitutive and IFM-specific *Mhc* exons of homozygous flies following PCR showed a single base pair change (G → A) leading to an amino acid change from glycine to serine in codon 413 (416), close to the *Su(2)B* suppressor mutant (Figure 3). *Su(2)E* is a very weak suppressor for both wing and muscle phenotypes of hdp^2, making it difficult to map. We have not included more detailed data on this mutation.

Abnormal myofibrillar structure in hdp^2 IFM suppressed by myosin suppressor mutations: *Su(2)B* completely suppresses fiber hypercontraction as seen in polarized light, except for some thinning of the dorsal-longitudinal muscle (DLM) fibers (Figure 4A) in a few flies. Electron micrographs of hdp^2/Y; *Su(2)B*/+ flies (Figure 4, B and C) show a complete recovery of wild-type myofibrillar structure, although the sarcomere length remains slightly, but significantly, shorter than that of wild type (Table 3). hdp^2 hypercontraction is completely suppressed by *Su(2)F*; sarcomere structure is comparable to wild type (Figure 4E) except that mean sarcomere length remains significantly shorter than that of wild type (Table 3) and a few days after eclosion muscle fibers become thin in many areas and contract (Figure 4D). In cross sections the periphery of the myofibrils show loosely packed thick and thin filaments (Figure 4F), quite similar to hdp^2 myofibrils when they start to hypercontract (Figure 2D).

Su(2)D heterozygotes partially suppress hdp^2. Half of the flies still show a wings-up phenotype and >80% of them have a partially suppressed muscle phenotype (Table 4). The fibers are thin and hypercontract from the posterior ends of the thorax (Figure 5A). Sarcomere structure is improved compared to hdp^2 (Figure 5B), but sarcomere length is barely half that of wild type (Table 3) and at the periphery the myofibrillar lattice is perturbed, suggesting that hdp^2 suppression is only partial at this level (Figure 5C). Homozygous *Su(2)D* completely suppresses hdp^2 hypercontraction (Figure 5D; Table 4) but still fails to restore wild-type sarcomere length (Table 3); homozygous *Su(2)D* sarcomeres are significantly shorter than those of wild type. For 6–7 days after eclosion the muscle structure remains completely normal, but after this the central myofibrillar lattice becomes disordered (Figure 5, E and F), with characteristic Z-band streaming and gaps in the lattice. We have previously reported on a similar age-related progressive myopathy with hdp^2 and the *Tm2* suppressor mutant, *D53* (NAIMI *et al.* 2001). Electron micrographs of myofibrils from the *Mhc* suppressors as hetero- or homozygotes (in the case of *2D*) without hdp^2 have a completely wild-type appearance (data not shown).

Suppression by the myosin suppressors of other behaviors affected by hdp^2: The hdp^2 mutation is in the constitutively expressed exon 5 of the TnI gene. Its effects on other muscle groups have been observed as changes in behavior (adult jumping and walking and larval crawling and feeding), including an age-dependent myopathy of the legs, associated with ultrastructural defects (NAIMI *et al.* 2001). All the new myosin suppressors fully suppress the effects of hdp^2 on walking (Figure 6) and all other behaviors (data not shown), consistent with their position within constitutive *Mhc* gene exons. In *Su(2)D* heterozygotes the hdp^2 walking behavior is partially suppressed while in homozygotes the suppression is complete. Suppressor *Su(2)B* itself

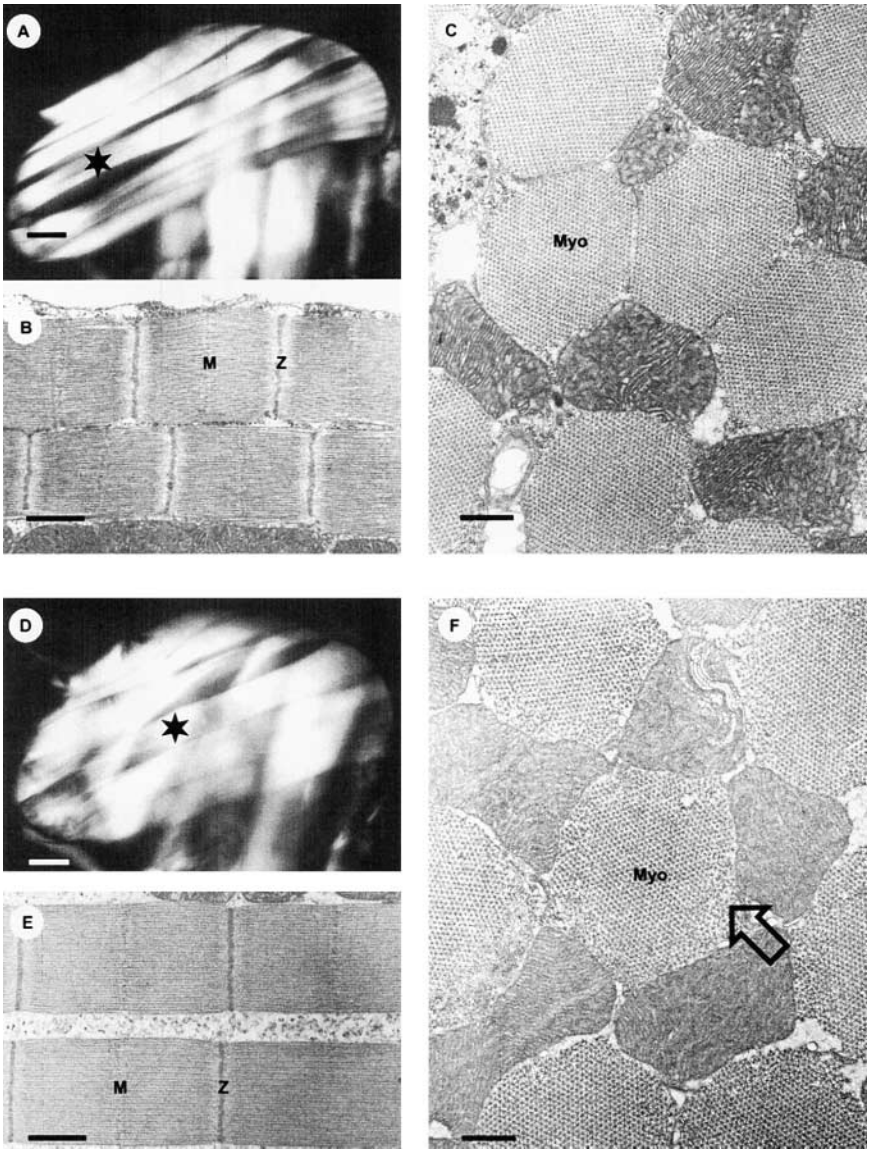

FIGURE 4.—Suppression of hdp^2 hypercontraction by new *Mhc* mutations. (A) Polarized light micrograph of 2-day-old hdp^2/Y; $Mhc^{2B}/+$. Fibers appear completely normal except for some damage (star) in one or two fibers of some of the flies. (B and C) Electron micrographs of hdp^2/Y; $Mhc^{2B}/+$ myofibrils. Sarcomere length appears slightly shorter than that of wild type and myofibrils (Myo) are packed very close to each other. (D) Polarized light micrograph of 10-day-old hdp^2/Y; $Mhc^{2F}/+$. Fibers (star) show breaks; 2- to 3-day-old flies show completely normal fibers (not shown). (E) LS of 2-day-old hdp^2/Y; $Mhc^{2F}/+$ showing completely normal myofibrils. Sarcomere lengths are quite normal; compare with Figure 2A. (F) TS of 10-day-old hdp^2/Y; $Mhc^{2F}/+$ showing the aged-related disruption of the myofibrils (Myo). Anterior-posterior axis of the thoraces runs from right to left. Bar, 0.125 mm for A and D; 1 μm for B and E; and 0.5 μm for C and F.

shows a progressive myopathy of the leg muscles, but hdp^2/Y; *Su(2)B*/+ walks as well as wild type (Figure 6).

Headless transgenic construct and newly isolated myosin suppressors suppress other hypercontracting alleles: If the IFM hypercontraction phenotype requires actomyosin force production, then *a priori* all myosin suppressor mutations, including the transgenic headless myosin constructs, should suppress all those mutations that can generate the phenotype. We have tested this by making genotypes containing hypercontracting alleles and the *Mhc* suppressors and/or the headless myosin constructs. Both headless myosin constructs (*Y97* and *Y57*) suppress the up^{101}, Mhc^{13}, and fln^0 genes in a dose-dependent manner (Table 2). A single copy of *Y57* is enough to suppress fln^0 hypercontraction in the absence of any full-length endogenous MHC. Fiber morphology looks normal although with less birefringence, possibly a result of the highly disrupted myofibrillar organization at eclosion (data not shown). Such myofibrillar disruption is usually seen a few days after eclosion in fln^0 mutants and then only after hypercontraction has occurred (REEDY *et al.* 2000). The fln^0 fiber phenotype is also suppressed by a copy of the *Mhc* mutation *Su(2)B* (Table 4), indicating that force is required for fln^0 hypercontraction.

Other myosin suppressors of hdp^2 also suppress up^{101}: KRONERT *et al.* (1999) described four *Mhc* suppressors of hdp^2 in which the mutants localized within the myosin head domain. These mutations were dominant flightless in combination with hdp^2 (PRADO *et al.* 1995) but fully or partially flighted in heterozygous condition without hdp^2, indicating that the mutant myosins can assemble into normal myofibrils. We find that these mutations completely suppress the hypercontraction phenotype in flies up^{101} and up^{101},+/+, hdp^2 (Table 5). The degree of suppression appears to relate to how severely a mutation affects the molecule; the intragenic deletion mutations (*D41*, *D62*) suppress more strongly than either point mutation (*D1* or *D45*). Similar results were obtained with fln^0. All four myosin alleles suppress hypercontrac-

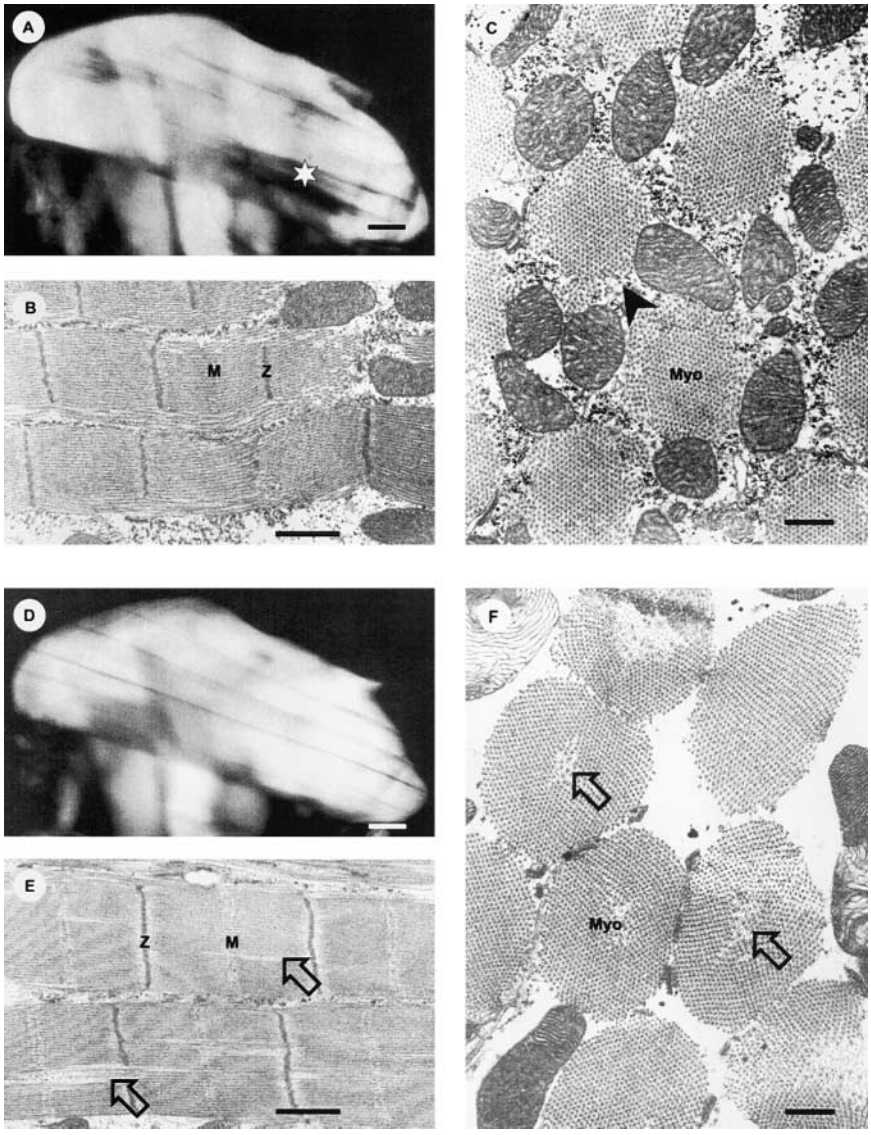

Figure 5.—Suppression of *hdp*^{2} hypercontraction by mutations in *Mhc* S1. (A) Polarized light micrograph of 2-day-old *hdp*^{2}*/Y; Mhc*^{2D}/+ flight muscles. Fibers are broken at many places, particularly in E and F of DLM fibers (star). (B and C) Electron micrographs of *hdp*^{2}*/Y; Mhc*^{2D}/+ myofibrils. Sarcomeres appear disrupted and shorter; the periphery of the myofibrils (Myo) is loosely packed with disrupted thick and thin filament lattices (arrowhead). (D) Polarized light micrograph of 10-day-old *hdp*^{2}*/Y; Mhc*^{2D}*/Mhc*^{2D} showing completely normal fibers. (E and F) LS and TS of 10-day-old *hdp*^{2}*/Y; Mhc*^{2D}*/Mhc*^{2D} showing age effects [2- to 3-day-old flies show completely normal myofibrils (not shown)]. Normal length sarcomeres show disruptions and gaps (arrows) that are clearly visible in the centers (arrows) of myofibrils (Myo), which is different from the *hdp*^{2}*/Y; Mhc*^{2F}/+ suppressor. Anterior-posterior axis running from left to right for the thoraces. Bar, 0.134 mm for A and D; 1 μm for B and E; and 0.5 μm for C and F.

tion of the flightin mutant but *D62* showed the strongest effect (data not shown).

Mhc suppressors cause hypercontraction in the absence of a functional troponin complex: The *wupA* allele *hdp*^{3} causes a missplicing of the IFM-specific transcript and no functional TnI is produced (Barbas *et al.* 1993). The result is that the IFM fail to form and only small muscle remnants are seen in the thoraces of later pupal and adult stages (Figure 7A; Beall and Fyrberg 1991; Barthmaier and Fyrberg 1995). *A priori* an absence of TnI is expected to prevent thin filament inhibition of muscle contraction. Unregulated contraction during myogenesis will lead to the observed phenotype. We have used this circumstance to explore whether the MHC produced in myosin suppressor homozygotes produces sufficient force *in vivo* to cause the muscle destruction previously seen in *hdp*^{3} flies. These experiments were performed to resolve the issue (see below) as to whether the *Mhc* suppressor mutations affect only force production or the role of the myosin in the muscle activation processes itself, since some current models (reviewed in Gordon *et al.* 2000) suggest that binding of the myosin head to the actin of the thin filament plays a role in muscle activation. The results (Table 6; Figure 7) show that in *hdp*^{3} flies lacking myosin, *hdp*^{3}*/Y; Mhc*^{12}*/Mhc*^{12}, the muscles do not hypercontract, nor do they do so in flies heterozygous, *Mhc*^{X}*/Mhc*^{12}, for any of the suppressors (*Mhc*^{X}) and the *Mhc*^{12} null mutant. However, in *hdp*^{3} genotypes containing *Mhc* suppressors heterozygous with *Mhc*^{12}, *i.e.*, *hdp*^{3}*/Y; Mhc*^{X}*/Mhc*^{12}, hypercontraction occurs (Figure 7C), although in some cases it is only partial (alleles *Su(2)B*, *Su(2)F*, and *D62*; see Figure 7B). These latter results suggest that these alleles show the largest reduction in force production so that, even in the absence of normal muscle regulation, in a single gene dose they can barely produce sufficient force to cause hypercontraction.

DISCUSSION

Our aim was to test the following proposals: that actomyosin force generation is required to produce the hy-

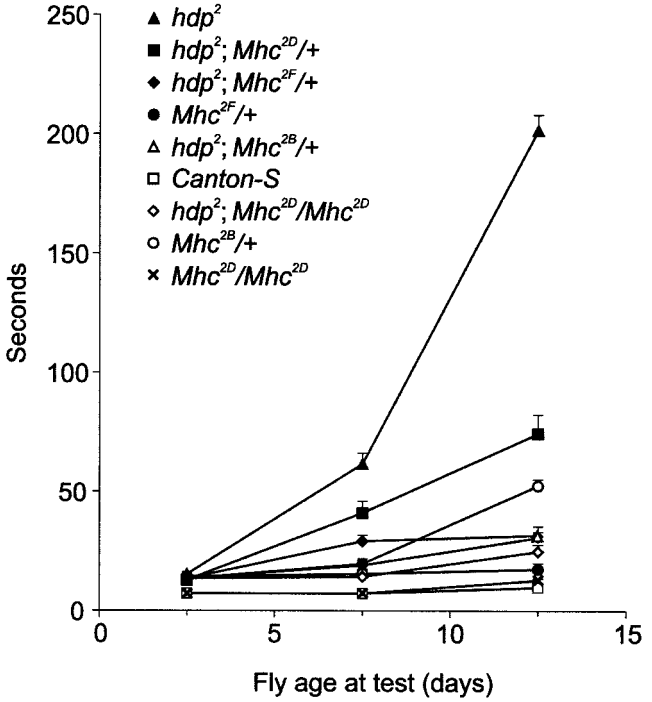

FIGURE 6.—Suppression of the hdp^2 walking behavior. hdp^2 flies show age-related myopathy in their walking behavior, which is partially or completely suppressed by all the suppressors. As in the fiber morphology, $Mhc^{2D}/+$ partially suppress the hdp^2 myopathy. All the suppressor genotypes show normal walking behavior that is not significantly different from wild type except $Mhc^{2B}/+$, which shows significant (5%) levels of age-related myopathy. "Seconds" is the mean time (10 tests/sample) taken for 50% of the flies to walk upward >80 mm.

percontraction phenotype and that force reduction explains the action of *Mhc* suppressors. Additionally, we intended to distinguish between these proposals, also made by KRONERT *et al.* (1999), and their alternative explanation that *Mhc* suppressors may indicate direct interactions between the myosin head and the TnI component of the troponin complex.

If the role of the myosin head in hypercontraction is to produce the forces that destroy the fibers in response to aberrant regulation of contraction or when the sarcomeric structure is compromised, then all hypercontracting mutants should be suppressed by reductions in myosin head concentration. In addition, suppression by *Mhc* alleles should be neither allele nor gene specific. The headless *Mhc* gene construct suppresses the hypercontraction muscle phenotypes of the hdp^2, up^{101}, Mhc^{13}, and fln^0 mutants in an *Mhc* gene dose-dependent manner consistent with this expectation. In addition, all of the *Mhc* suppressors described previously (KRONERT *et al.* 1999) and newly described here suppress the hypercontraction of all these hypercontracting mutants. There is no evidence for gene or allele specificity in the known *Mhc* suppressors. Although the fiber morphology shows complete suppression by the headless myosin constructs, normal sarcomeric structure is not restored. This is not surprising since the headless myosin does not produce wild-type myofibrils when expressed alone or in combination with wild-type myosin (CRIPPS *et al.* 1999). Incomplete suppression of structural aspects suggests that reduced force production is not sufficient to allow normal myofibrillogenesis or to prevent microdamage within the sarcomeres.

If suppression of hypercontraction by *Mhc* alleles is by reduced force production, then the stronger suppressors should be those *Mhc* alleles with more extreme phenotypes. Suppression by the new *Mhc* alleles is complete by the criterion of fiber structure in each case and most are stronger suppressors than the earlier ones (KRONERT *et al.* 1999) where suppression was incomplete. Some of these earlier suppressors now fly and homozygotes survive, a feature originally true only for *D1* (KRONERT *et al.* 1999). Stronger hdp^2 suppressors have a more extreme phenotype with respect to myosin function. So *Su(2)B* and *Su(2)F* have embryonic lethal and dominant flightless phenotypes, whereas *Su(2)D* is viable and flighted on its own, but only partially suppresses hdp^2. The *D1* suppressor is at odds with this relationship between suppression and myosin dysfunction; it is homozygous viable and flighted, even allowing the flight of some hdp^2/Y; *D1*/+ flies (Table 5).

The *Mhc* suppressor mutations all occur in the head domain (Figure 3). *D1* (D625G, chicken myosin S1 numbering system) and D62 (a 24-bp in-frame deletion) are in the actin-binding loop (KRONERT *et al.* 1999); *Su(2)B* (P404S) and *Su(2)D* (G416S) are located fairly close together in a region known to be involved in actin binding (RAYMENT *et al.* 1993a; UYEDA *et al.* 1994; ROVNER *et al.* 1995). *D45* (A261T) and *D41* (a 2-bp insertion that affects splicing) cause changes near the ATP-entry and the ATP-binding sites (KRONERT *et al.* 1999), while *Su(2)F* (A465T) is also close to the ATP-binding site. The mutant residues do not form the single cluster expected if they affect a specific binding of the myosin head to a component of the Tm-Tn complex, perhaps TnI, as suggested by KRONERT *et al.* (1999), nor has any such interaction been detected in the large volume of research on thin filament regulation (see review by GORDON *et al.* 2000). However, all the mutant residues, including the new *Mhc* suppressors, are in head regions important for actin binding and nucleotide exchange/hydrolysis, supporting the argument that all the mutants affect the crossbridge cycle and force production, changes sufficient to explain suppression.

Su(2)B (P404S) is next to residue R405, a hot spot for mutations causing human hypertrophic cardiomyopathy (HCM). Arginine 405 is part of a myosin loop that could directly interact with actin (RAYMENT *et al.* 1995). Myosins from myopathy patients move actin filaments with decreased velocity in an *in vitro* motility assay (CUDA *et al.* 1993) and muscle fibers display diminished power output (LANKFORD *et al.* 1995), although more recent *in vitro* studies of the same HCM myosins

TABLE 5

Suppression phenotypes of *hdp²* and *up¹⁰¹* with *D* series mutant suppressors of KRONERT *et al.* (1999)

Genotype	*n*	Wing phenotypes (%)			Flight (%)			
		Normal	Upheld	Down	U	H	D	N
hdp^2; Mhc^{D1}/+	94	93	7	0	0	17	56	27
hdp^2; Mhc^{D41}/+	82	94	2	4	0	0	17	83
hdp^2; Mhc^{D45}/+	41	93	7	0	15	7	34	44
hdp^2; Mhc^{D62}/+	64	84	16	0	0	0	3	97
up^{101}; Mhc^{D1}/+	45	78	18	4	0	0	18	82
up^{101}; Mhc^{D41}/+	40	80	15	5	0	0	10	90
up^{101}; Mhc^{D45}/+	47	96	4	0	9	57	17	17
up^{101}; Mhc^{D62}/+	31	100	0	0	0	0	10	90
up^{101},+/+, hdp^{2a}	46	0	96	4	0	0	8	92
up^{101},+/+, hdp^2; Mhc^{D1}/+	41	100	0	0	29	32	17	22
up^{101},+/+, hdp^2; Mhc^{D41}/+	36	89	11	0	0	0	6	94
up^{101},+/+, hdp^2; Mhc^{D45}/+	52	100	0	0	23	52	25	0
up^{101},+/+, hdp^2; Mhc^{D62}/+	43	100	0	0	0	0	26	74

U, flies flying up toward a light source; H, horizontal; D, down; N, flightless. *n*, number of flies tested. Also see KRONERT *et al.* (1999) for details on these mutations.

[a] Shows hypercontraction muscle phenotype; all other genotypes show normal muscle morphology under polarized light.

showed enhanced myosin activity (PALMITER *et al.* 2000; YAMASHITA *et al.* 2000). The partial suppression of hdp^3 hypercontraction by *Su(2)B* suggests that mutations in this region of the myosin molecule can reduce force *in vivo*, but do not directly address changed functions in the HCM mutations.

An absence of clustering of suppressor mutations and the fact that their effects can be explained by effects on myosin ATP hydrolysis and actin interactions reducing force production is not consistent with the proposed direct interaction between myosin and TnI (KRONERT *et al.* 1999). However, current models (reviewed in GORDON *et al.* 2000) suggest that myosin is involved in both force production and thin filament regulation. GEEVES and LEHRER (1998) have developed a model of thin filament regulation based on the kinetic studies of MCKILLOP and GEEVES (1993) in which the Tm-Tn complex can exist in three states on the F-actin thin filament core. In the absence of calcium, TnI binding to actin holds the Tm-Tn complex in the "closed" state in which the myosin-binding site is occluded, preventing myosin from binding actin. Calcium released into the muscles following neural stimulation binds to TnC, which undergoes a conformational change that alters its relationship with TnI, resulting in release of TnI binding from actin. This represents the "blocked" state, but in this state small movements of the Tm/Tn across the F-actin surface allow small numbers of myosin heads to bind F-actin, leading to the displacement of this complex to the "open" state. In this state myosin heads can bind to any available actin "target" site and muscle activation is achieved. In this model, therefore, a small fraction of myosin heads play an important role in the blocked to open transition. In doing so they bind to F-actin. There is no reason to assume that this binding is in any way different from the binding of a myosin head that also produces force. It is thus formally very difficult to determine whether the *Mhc* mutations that suppress hdp^2 do so by affecting regulation rather than force production. The reduced ability of the myosin suppressor mutants to hypercontract the IFM in the absence of a functional regulatory system (hdp^3) and any wild-type myosin certainly suggests that the suppressor mutations produce less force, but does not allow us to deduce that this is the primary effect of these mutants when acting as suppressors. At present we cannot perform with Drosophila proteins the type of sophisticated biochemical and biophysical experiments needed to resolve this issue. However, considerable progress has been made recently with the purification and assay of Drosophila IFM actin (RAZZAQ *et al.* 1999; SCHMITZ *et al.* 2000) and myosin (SWANK *et al.* 2001, 2002), including ATPase assays, *in vitro* motility, single molecule studies, and rapid kinetics.

IFM hypercontraction appears to develop from either misregulation of muscle contraction (hdp^2, up^{101}) or structural defects arising from reduced sarcomeric integrity (Mhc^6, Mhc^{13}, fln^0). It may be significant that in the former mutant group hypercontraction occurs during late pupal stages and is complete shortly after eclosion (NAIMI *et al.* 2001), while in the latter the phenotype arises during the first day or so of adult life (KRONERT *et al.* 1995). On this basis different hypercontracting *Act88F* alleles may affect regulation or sarcomeric integrity. Despite the different etiology the same *Mhc* suppressors suppress both groups. This does not

FIGURE 7.—*Mhc* suppressors cause hypercontraction in the absence of a functional troponin complex. (A) Polarized light micrograph of 2-day-old hdp^3/Y; thorax appears completely empty without IFMs and tergal depressor of trochanter (star). Occasionally skeins of fibers are seen (arrow). (B) hdp^3/Y; Mhc^{2B}/Mhc^{12}. The bulk of IFM fibers develop (squares) but show partially pulled fibers from attachment sites (arrowheads), indicating partial contact between thick and thin filaments with reduced force. (C) hdp^3/Y; Mhc^{2D}/Mhc^{12}. Only a few bits of fiber remain, indicating hypercontraction caused by the actomyosin force. hdp^3 requires very little functional myosin to cause hypercontraction (U. NONGTHOMBA and J. C. SPARROW, unpublished results). Anterior-posterior axis running from left to right for the thoraces. Bar, 0.125 mm.

allow us to distinguish between force production and regulation of muscle contraction as the primary effect of the mutant myosins. With few exceptions (see NAIMI *et al.* 2001) hypercontraction phenotypes are restricted to the IFM. It is intriguing that this phenotype, which presumably arises from excessive shortening, shows up in the IFM, muscles where contraction is typically isometric.

Not all *Mhc* alleles that cause myosin dysfunction are suppressors. Two exceptions are Mhc^6 and Mhc^{13}, which have hypercontraction phenotypes themselves. In both, the mutant amino acids are within the myosin α-helical coiled-coil domain that associates to form the thick filaments. Mhc^5 acts as an enhancer of the hdp^2 phenotype as hdp^2/Y; $Mhc^5/+$ males are lethal as young larvae. However, this allele on its own produces only a hypercontraction phenotype in adults and the synthetic lethality is almost certainly due to epistatic interactions. The Mhc^5 mutation substitutes G200 with aspartate (G200D); residue G200 is at the beginning of a helix that interacts with bound nucleotide (KRONERT *et al.* 1999). Mhc^5 myosin must be able to produce sufficient force for hypercontractive destruction of the muscle, but also affect regulation so that the phenotype occurs during late pupal/early adult life. The occurrence of hypercontraction at this time is consistent with an effect on regulation. How can Mhc^5 affect regulation? One possibility is that it does so by altering myosin kinetics so that a fraction of heads remains bound, keeping the thin filament in the "open" state in the absence of calcium. The Mhc^8 mutation (Y832H, a mutation in the myosin lever arm) is recessive lethal and, like Mhc^5, its survival as a wild-type heterozygote is severely reduced in combination with hdp^2 (KRONERT *et al.* 1999), so it is an enhancer of hdp^2. How a lever arm mutation achieves this is not clear. The lever arm binds the essential and regulatory light chains, so Mhc^8 may affect regulation rather than compromising its force-producing capacity.

Since most *Mhc* missense mutants that reduce the efficacy of myosin function suppress hypercontraction, they will not be very informative about troponin/tropomyosin complex function. However, the small number of mutations with unexpected interactions with troponin mutations (*e.g.*, Mhc^5 and Mhc^8) are likely to be informative about troponin/tropomyosin complex function. Clearly, selection for hypercontraction suppressors effi-

TABLE 6

Suppression phenotypes of hdp^3 with *Mhc* mutant suppressors

		Wing phenotypes (%)			Muscle phenotypes (%)		
Genotype	*n*	Upheld	Down	Normal	HC	PHC	N
hdp^3/Y	50	100	0	0	100[a]	0	0
Mhc^X/Mhc^{12}	50	0	0	100	0	0	100
hdp^3/Y; Mhc^{12}/Mhc^{12}	44	41	0	59	0	0	100
hdp^3/Y; Mhc^{2B}/Mhc^{12}	48	100	0	0	0	100	0
hdp^3/Y; Mhc^{2D}/Mhc^{12}	61	41	0	59	100	0	0
hdp^3/Y; Mhc^{2F}/Mhc^{12}	44	100	0	0	0	100	0
hdp^3/Y; Mhc^{D1}/Mhc^{12}	52	100	0	0	100	0	0
hdp^3/Y; Mhc^{D41}/Mhc^{12}	35	100	0	0	100	0	0
hdp^3/Y; Mhc^{D45}/Mhc^{12}	39	61	0	39	100	0	0
hdp^3/Y; Mhc^{D62}/Mhc^{12}	33	100	0	0	94	6	0

All the genotypes are flightless. HC, hypercontracted; PHC, partially hypercontracted; N, normal/suppressed.
^{X}, representing any suppressor (all produce the same data).
[a] No muscle fibers are visible; see text for details.

ciently isolates Drosophila *Mhc* mutations with important effects on myosin function.

Many human familial myofibrillar myopathies of skeletal and cardiac muscle have been linked with sarcomeric proteins (reviewed in Coonar and McKenna 1997; Redwood *et al.* 1999; Seidman and Seidman 2001) including myosin, actin, tropomyosin, and the troponins. The hypertrophic cardiomyopathies show very variable penetrance and expressivity, which are likely to have a genetic component (Coonar and McKenna 1997). The study of interacting mutations in Drosophila, where muscle structure and sarcomeric proteins are very similar to their vertebrate counterparts, should shed light on the direct effects of muscle dysfunction and on the genetic interactions that are important in the occurrence of human muscle disease.

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