Expression Profiling of a Hypercontraction-induced Myopathy in Drosophila Suggests a Compensatory Cytoskeletal Remodeling Response

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Mutations that alter muscle contraction lead to a large array of diseases, including muscular dystrophies and cardiomyopathies. Although the molecular lesions underlying many hereditary muscle diseases are known, the downstream pathways that contribute to disease pathogenesis and compensatory muscle remodeling are poorly defined. We have recently identified and characterized mutations in Myosin Heavy Chain (Mhc) that lead to hypercontraction and subsequent degeneration of flight muscles in Drosophila. To characterize the genomic response to hypercontraction-induced myopathy, we performed expression analysis using Affymetrix high density oligonucleotide microarrays in Drosophila Mhc hypercontraction alleles. The altered transcriptional profile of dystrophic Mhc muscles suggests an actin-dependent remodeling of the muscle cytoskeleton. Specifically, a subset of the highly up-regulated transcripts is involved in actin regulation and structural support for the contractile machinery. In addition, we identified previously uncharacterized proteins with putative actin-interaction domains that are up-regulated in Mhc mutants and differentially expressed in muscles. Several of the up-regulated proteins, including the dystrophin-related protein, MSP-300, and the homolog of the neuronal activity-regulated protein, ARC, localize to specific subcellular muscle structures that may provide key structural sites for cytoskeletal remodeling in dystrophic muscles. Defining the genome-wide transcriptional response to muscle hypercontraction in Drosophila has revealed candidate loci that may participate in the pathogenesis of muscular dystrophy and in compensatory muscle repair pathways through modulation of the actin cytoskeleton.

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** The online version of this article (available at http://www.jbc.org) contains supplemental Tables I and II.

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2 The abbreviations used are: TS, temperature-sensitive; ARC, activity-regulated cytoskeleton-associated protein; SAM, significance analysis of microarrays; GFP, green fluorescent protein; RT, reverse transcription; d, Drosophila.
response mechanisms to muscle dysfunction and could potentially reveal novel targets for therapeutic treatment of muscle diseases.

**EXPERIMENTAL PROCEDURES**

*Drosophila Genetics—*Drosophila* were maintained on standard culture media at room temperature. All crosses were performed at 25 °C.

*Tissue Processing for Microarrays—*Tissue was derived from 3- to 4-day-old adult male flies of the indicated genotype raised at 25 °C. Flies were frozen in liquid nitrogen and vortexed in order to obtain isolated heads and bodies (wings and legs were discarded). Circadian differences were minimized by processing tissue between 11 a.m. and 2 p.m. A total of eight independent microarrays were analyzed (four control, two *MhcS1*/+, and two *MhcS2*/+). Total RNA was isolated using TRIzol (Invitrogen). 7–12 flies were processed in 200 μl of TRIzol. Five batches (1 ml) of processed tissue were then used to isolate the total RNA. From the total RNA, mRNA was isolated using a Qiagen Oligotex mRNA extraction kit. cDNA was created with random hexamer and T7-poly(T) oligonucleotides using the cDNA kit from Invitrogen. cDNA was then purified by phenol/chloroform extraction and Phase Lock Gel extraction tubes (Eppendorf) as suggested by Affymetrix®. Biotin-labeled cRNA was made with the Enzo High Yield Bioarray kit using the supplied protocol with the modification of running half-reactions. Reactions were cleaned with the Qiagen RNeasy kit, precipitated overnight, and resuspended into 13 μl of diethyl pyrocarbonate-treated double distilled H2O. Fragmentation was done with fragmentation buffer (200 mM Tris acetate, pH 8.1, 500 mM KOAc, 150 mM NaCl, 10 μM EDTA) at 94 °C for 1 min and then brought up into 33 μl total volume. cRNA was sent to the Affymetrix® processing lab at Massachusetts Institute of Technology for hybridization and signal detection.

**Expression Analysis**—Statistical analysis was done with the Affymetrix® Microarray Suite software using the following normalization values: scaling target, 1500; alpha1, 0.04; alpha2, 0.06; tau, 0.015; gamma1L, 0.0025; gamma1H, 0.0025; gamma2L, 0.0030; gamma2H, 0.0030; and perturbation, 1.1. Gene changes were called based upon the stringency criteria that differential regulation must be reported by Affymetrix software for at least 75% of the pairwise comparisons. Additionally, an analysis utilizing SAM version120 was done in order to provide an independent analysis of the normalized data, giving higher stringency in differential regulation hits. Analysis with SAM was done as two-class, unpaired data sets, setting Δ to −25% false-positive rate.

**Semi-quantitative RT-PCR**—Semi-quantitative RT-PCR was utilized to verify select genes from the microarray analysis. PCR primers were designed to sequences used in the design of probe pairs by Affymetrix. Isolated cDNA was diluted into the equivalent amount isolated from 1 μg of total RNA per 1 μl. This solution was further diluted 1:100, and 0.1 to 1 μl was used in the reactions depending upon transcript abundance and primer efficiency. Reaction samples were taken from cycles 21 to 30 in order to determine linear regions of the reactions. If linear regions were overlapping, comparisons were made in those cycles. If linear regions were not overlapping, reactions were run until mutant reactions were within linear cycles.

**In Situ Analysis—*In situ* expression analysis was performed using standard procedures. Probes were designed to ~200-bp fragments, amplified by PCR using a 3’ primer that includes the T7 sequence. Sequences used for *in situ* hybridization coincide to sequences used by Affymetrix for probe pair design. In most cases, the same sequence used for semi-quantitative RT-PCR was used for *in situ* analysis.

**Immunostaining**—Immunostaining of third instar larval fillets was done as described previously (5). For detecting MSP-300, we utilized α-MSP-300 antiserum at 1:500 (17). Secondary goat α-rabbit conjugated to Cy2 was used at 1:500 (The Jackson Laboratories). F-actin was labeled with Texas Red-conjugated phalloidin at 1:500, incubated simultaneously with secondary antibody (Molecular Probes). Antiserum to dARCl was kindly provided by Leslie Griffith and used at 1:1000.

**Production of Transgenic UAS-dARCl and UAS-dARCl-GFP Lines**—The open reading frame of dARCl was PCR-amplified from cDNA libraries prepared for the microarray experiments and cloned into the pUAST vector. The dARCl-GFP fusion was created using standard PCR fusion techniques and cloned into the pUAST vector as well. The cloned constructs were sequence-verified at the Massachusetts Institute of Technology Biopolymers Facility (Cambridge, MA) and sent to Genetic Services, Inc. (Cambridge, MA) for production of transgenic animals.

**RESULTS**

**Expression Analysis of MhcS1 and MhcS2 Hypercontraction Mutants**—Both transcriptional and post-translational mechanisms are likely to be altered in dystrophic muscles, contributing to subsequent pathology. To identify transcriptional differences between hypercontracted and normal muscles in *Drosophila*, we performed expression profiling with Affymetrix high density oligonucleotide GeneChip arrays. The gene arrays contain sequences used for most of the 14,000 genes in *Drosophila*, allowing a survey of the majority of the genome for dystrophy-related alterations in gene expression. In our expression analysis, we utilized two independent alleles of *Mhc* (*MhcS1* and *MhcS2*) that cause flight muscle hypercontraction defects. These alleles are missense mutations (V235E and E187K) that localize near the ATP binding/hydrolysis domain of MHC (Fig. 1). The use of two independent alleles allowed us to look for conserved responses to MHC dysfunction by reducing background and allele-specific changes. In addition, these two mutations map to a region of the MHC protein in which at least nine different mutations have been identified that cause hypercontractile cardiomyopathy (18) (Fig. 1).

A total of eight independent arrays were performed as follows: four CantonS, two *MhcS1*/+, and two *MhcS2*/+. For experiments, we used circadian-matched CantonS males of identical age and rearing conditions as controls. For each chip used in our analysis, we isolated RNA from the heads and bodies of 50 animals to reduce individual variability in gene expression. The data were processed using the Affymetrix sta-
FIGURE 2. Expression analysis of the \( \text{Mhc}^{11} \) and \( \text{Mhc}^{12} \) mutants using Affymetrix high density oligonucleotide GeneChip microarrays. A, schematic depicting the pairwise comparison matrix used to analyze the microarray data with the Affymetrix statistical algorithm. B, sample pairwise comparison between \( \text{CantonS} \) and \( \text{Mhc}^{11/+} \) expression analyses. Green lines indicate a 2-fold difference in expression. C, graphical representation of the output of SAM analysis version 1.20 using a two-class unpaired data set and \( \Delta \) value set to give \( \sim 25\% \) false detection rate. D, semi-quantitative RT-PCR shows similar vector changes identified by the microarrays. E–L, quantification of pixel intensity following normalization for background in separate RT-PCR experiments (black, blue, and green lines indicate separate experiments) for \( \text{CantonS} \) (CS) and Mhc hypercontraction mutants for the indicated genes.
stistical expression algorithm in a total of 16 pairwise comparisons between mutant and wild type arrays (Fig. 2, A and B). To analyze the mutant chips as a group against the controls, SAM version 1.20 analysis was performed using the two-class unpaired response type (Fig. 2C) (19). To increase the chances of making relevant gene change calls, we set stringent conditions to analyze the data. For a gene to be differentially regulated, it must have been labeled up- or down-regulated by the Affymetrix algorithm in 75% of the pairwise comparisons. To further rank the results, we subdivided the data into the following two categories: 1) differentially regulated in 75% of all pairwise comparisons, and 2) differentially regulated in only one of the two mutants. We then analyzed statistical overlap between genes identified by the Affymetrix statistical expression algorithm and the SAM analysis. Using these criteria, 228 genes were up-regulated and 118 were down-regulated in response to hypercontraction-induced myopathy (Table 1 and supplemental Tables S1 and S2).

To verify that vectorial changes in gene expression identified by microarrays were correct, a subset of the differentially regulated genes was tested using semi-quantitative RT-PCR. We quantified the results from three separate RT-PCRs for the genes encoded from the Act57B, GHO5741, MSP-300, CG12505 (dARC1), CG32030 (dFHOS), Mlp60A, and CG2330 (dNeurochondrin) loci. As a control, we compared expression to the GAPDH2 locus, whose RNA levels were unchanged in mutant animals. RT-PCR results from these genes revealed similar expression changes compared with the microarray analysis, corroborating expression levels reported by microarray analysis (Fig. 2, D–L).

To categorize genes that were transcriptionally altered in dystrophic muscles, we performed a BLAST analysis for each transcript to identify known homologs and previously characterized structural domains. The genes were then categorized according to known or putative functions based on current literature or sequence similarity (see Table 2 and sup-

### TABLE 1
Summary of differentially regulated genes from the expression analysis

| Up-regulated genes | Category | No. |
|--------------------|----------|-----|
| Muscle structure/function | 35 |
| Amino acid proteases | 24 |
| Novel | 54 |
| Chaperones | 8 |
| Nuclear biology | 13 |
| Metabolism and energy | 55 |
| Immune response | 15 |
| Signaling | 8 |
| Other | 16 |
| Total | 228 |

| Down-regulated genes | Category | No. |
|----------------------|----------|-----|
| Metabolism and energy | 77 |
| Muscle structure/function | 6 |
| Other | 22 |
| Novel | 13 |
| Total | 118 |

### TABLE 2
Differentially regulated genes in Mhc mutants with known or predicted roles in muscle structure/function

| Gene | Description | CS Signal Average | CS SD | Mutant Signal Average | Mutant SD | Signal FC | Algorithm |
|------|-------------|------------------|------|----------------------|-----------|-----------|-----------|
| CG12505 | dARC1 | 578.33 | 473.21 | 3453.4 | 959.88 | 5.79 | ABCD |
| CG5797 | aPHOS | 3186.75 | 1207.21 | 22637.27 | 3663.28 | 7.15 | ABCD |
| Act57B | Actin | 10463.42 | 5668.01 | 40679.65 | 9187.02 | 4.45 | ABCD |
| CG32030 | dFHOS | 11098.03 | 2269.31 | 45266.9 | 4213.05 | 4.08 | ABCD |
| Mlp60A | Muscle LIM protein at 60A | 1224.45 | 225.45 | 5634.03 | 1359.43 | 3.48 | ABCD |
| Msp-300 | Nauploin/Msp-300 | 51.25 | 0.60 | 216.33 | 98.8 | 4.22 | ABCD |
| CG9025 | dTEMA | 681.72 | 163.00 | 1535 | 890.8 | 1.39 | ABCD |
| CG2471 | dCLP | 1747.93 | 110.18 | 28017.7 | 2258.4 | 1.06 | ABCD |
| Act57B | Actin | 36068.76 | 36799.79 | 63935.4 | 46245.0 | 0.82 | ABCD |

| Gene | Description | CS Signal Average | CS SD | Mutant Signal Average | Mutant SD | Signal FC | Algorithm |
|------|-------------|------------------|------|----------------------|-----------|-----------|-----------|
| CG12505 | dARC1 | 1542.88 | 346.60 | 2914.55 | 739.42 | 1.86 | ABCD |
| CG19242 | Ki67 | 25.17 | 19.90 | 50.45 | 50.53 | 0.20 | ABCD |
| CG11941 | dARC1 | 63.37 | 10.11 | 634.64 | 167.74 | 10.01 | ACD |
| KEEP | dATG | 5915.5 | 300.65 | 8158.55 | 1250.23 | 1.37 | ABC |
| ExDNA12243 | capulet | 2848.8 | 149.90 | 3278.85 | 373.77 | 0.95 | BD |
| BG-DS071980.2 | wnt-binding protein | 3368.77 | 437.94 | 5163.53 | 694.75 | 1.53 | BD |
| CG2330 | dneurochondrin | 1732.22 | 290.35 | 4316.98 | 1700.51 | 2.48 | B |
| CG3671 | ARM-repeat containing protein | 1003.53 | 305.05 | 2027.1 | 569.75 | 1.02 | B |
| CG3397 | K1 channel–subunit | 1164.35 | 262.79 | 1357.82 | 412.87 | 1.6 | B |
| Mlp60B | Muscle LIM protein at 84B | 10213.85 | 2715.28 | 13503.95 | 2411.89 | 1.32 | B |
| CG5090 | myosin-like | 3773.05 | 434.98 | 5810.4 | 2137.96 | 1.54 | B |
| CG13311 | peroxisome matrix structural protein | 1023.7 | 294.95 | 1722.98 | 444.21 | 1.68 | B |
| CG1678 | proline-rich protein | 2784.35 | 516.68 | 3091.07 | 829.48 | 0.82 | B |
| CG4020 | ARM repeat, ER chaperone | 381.52 | 65.20 | 587.03 | 124.5 | 1.34 | B |
| CG19772 | ARM-repeat, AIPS-like | 582.25 | 152.19 | 1352.2 | 478.28 | 1.6 | B |
| CG5600 | Myosin binding subunit | 921.72 | 322.79 | 1436.57 | 271.83 | 1.56 | B |
| CG8631 | Taks | 1285.95 | 255.85 | 1864.32 | 532.21 | 1.45 | B |
| CG5010 | proline-rich protein | 4339.55 | 1400.98 | 6745.4 | 2017.47 | 1.55 | B |
| CG18051 | Paxillin | 2506.05 | 413.85 | 3550.1 | 385.37 | 0.92 | B |
| CG13124 | ARM repeat, middle domain of dIF4G | 5646.87 | 1270.3 | 7262.42 | 1363.31 | 1.26 | B |
| CG0679 | LEM domain, antithrombin repeat | 138.7 | 17.30 | 209.45 | 19.82 | 1.02 | B |
| CG6208 | anti-sense | 2586.08 | 308.03 | 3621.89 | 277.15 | 1.42 | B |
| CG6416 | PDZ domain, zasp domain (e-active binding) | 2086.07 | 68.51 | 24118.45 | 1498.87 | 1.14 | B |
| CG6718 | Abl kinase repeats, calcium independent phosphatase A2 | 170.57 | 46.14 | 163.5 | 58.36 | 1.06 | B |
| CG5652 | YIP1-like | 2323.8 | 159.48 | 2720.08 | 71.7 | 1.16 | D |

a SD indicates standard deviation.

b Average signal FC indicates CS signal average/mutant signal average. See supplemental Table for comprehensive list.

c A indicates identified in 75% of total pairwise comparisons. B indicates identified in 75% of MhcS1 pairwise comparisons. C indicates identified in 75% of MhcS1 pairwise comparisons. D indicates identified by SAM analysis.

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Overall, the most striking gene set identified in the microarray analysis was the up-regulation of genes known or predicted to be involved in muscle structure and function at the level of the actin cytoskeleton, suggesting a remodeling of the cytoskeleton or its structural support in response to hypercontraction (Fig. 3; Table 2). Of the 10 most up-regulated genes across all statistical analyses, 6 fall within this category. Differentially regulated transcripts included in this category are Mlp60A, Mlp84B, Kettin, MSP-300, flw, sqh, capulet, sling-shot, act88F, act57B, Mbs, and talin. Mlp60A, Mlp84B, Kettin, MSP-300, flw, capulet, and talin have all been shown to provide or modulate structural supports of the muscle cytoskeleton (20–25), suggesting that hypercontraction and subsequent degeneration trigger a transcriptional response that may remodel the muscle cytoskeleton. Such a response may represent a functionally beneficial compensation mecha-
nism by which the muscle responds to increased forces induced by hypercontraction.

The differential regulation in the Mhc^51 and Mhc^52 mutants also includes an up-regulation of developmentally controlled genes that are required for earlier stages of muscle development, similar to transcriptional responses observed in cardiomyopathies (7, 26). Mlp60A and Mlp84B, which normally show high expression during the initial setup of embryonic and adult musculature, are up-regulated in Mhc mutants (Fig. 3A) (27). Likewise, Act57B, the predominant actin of the larval body wall musculature, also shows up-regulation (Fig. 3A) (28). A second predominant class of up-regulated genes includes chaperones and serine proteases, further supporting a remodeling of the muscle architecture (Table S1).

In addition to the potential muscle remodeling response, we observed an up-regulation of immune response genes as has been observed in mammalian muscular dystrophy models (26). The largest class of down-regulated transcripts in dystrophic muscles consists of nuclear encoded mitochondrial genes and genes involved in energy and metabolism, suggesting metabolic dysfunction in damaged muscles. An additional down-regulated transcript of interest encodes the calcium pump responsible for transporting calcium back into the sarcoplasmic reticulum, Ca-P60A (11). This down-regulation may be responsible for potential Ca^{2+} homeostasis defects in Mhc flight muscles. Indeed, our previous analysis suggested that intracellular Ca^{2+} is dysregulated in Mhc mutant fibers, as larval body wall muscles exhibit spontaneous contraction cycles even in the absence of external Ca^{2+} (5).

A subset of the genes that were identified as differentially regulated in our hypercontraction-induced myopathy model have been implicated previously in activity-dependent synaptic plasticity in mammals. These include the Drosophila homologs of the mammalian proteins ARC (CG12505 and CG13941), neurochondrin/norbin (CG2330), and CPG2 (Msp-300) (29–32). These activity-regulated genes are thought to modulate actin dynamics to strengthen activated synapses during plasticity (29, 31), suggesting similar actin-dependent cytoskeletal rearrangements may occur during muscle remodeling.

A Subset of Novel Differentially Regulated Genes Is Expressed in Somatic Musculature—Most of the genes identified in our analysis as differentially regulated in dystrophic muscles are largely uncharacterized. To determine whether these genes may be important for muscle function, we analyzed the expression patterns of several novel genes encoding proteins with domain structures suggestive of a role in actin biology. These included CG32030 (CG5797 and CG5775) (dFHOS), CG12505 (dARC1), CG2330 (dNeurochondrin), CG2471 (dSCLP), CG6972 and CG9025 (dFEM-1). As performing in situ hybridization to adult tissues is technically difficult, we chose to examine the expression pattern of the transcripts in whole mount embryos where we could follow gene expression during myogenesis, as well as in differentiated embryonic muscles. Although we expect these patterns of expression to reflect the adult musculature, further analysis will be required to confirm tissue-specific expression of the transcripts in adults.

CG5797 and CG5775, encoding the 5' and 3' regions of the CG32030 locus (hereafter referred to as dFHOS), represented the third highest up-regulated transcript (7-fold) identified in the microarray screen. The locus encodes an FH1- and FH2-domain containing protein with armadillo repeats in the N terminus. This gene is the Drosophila homolog of FHOS, a human “formin-homology containing protein overexpressed in the spleen” (33). Recent evidence has shown that formin-containing proteins regulate polymerization of nonbranched actin filaments (34, 35), suggesting that FHOS may function in assembly of the muscle sarcomere. In situ analysis with dFHOS probes reveals strong somatic muscle expression and staining in the putative midline mesodermal cells (Fig. 4B).

dARC1 is one of three Drosophila homologs (CG12505, CG13941, and CG10102) of the mammalian activity-regulated cytoskeleton-associated ARC gene and the most highly up-regulated transcript identified in our analysis. In situ hybridization with dARC1 probes reveal a broad staining pattern, with differential expression in both somatic and visceral musculature (Fig. 4C). Additionally, higher expression is observed in the central nervous system. Most interestingly, the central nervous system expression pattern is localized to synaptic regions of the ventral ganglion. In the mammalian central nervous system, ARC mRNA is trafficked to activated synapses and locally translated in the dendrites (36). The localization of dARC1 mRNA to synaptic regions, together with our previous identification of dARC1 as a seizure-induced gene in Drosophila (37), suggests ARC function may be conserved in synaptic plasticity. In addition, the identification of dARC1 expression in somatic muscle, together with its dramatic up-regulation in Mhc mutants, suggests dARC1 may also play a previously unrecognized role in muscular dystrophy.

Mammalian neurochondrin was isolated as an up-regulated transcript in neurons that have undergone tetraethylammonium-induced long term potentiation. Subsequent studies have revealed that neurochondrin over-expression in Neuro2a cells induces neurite outgrowth (31). The Drosophila homolog, dNeurochondrin (CG2330), is strongly up-regulated in Mhc mutants and shows expression in both somatic and visceral musculature (Fig. 4D), suggesting it functions in muscles in Drosophila.

dSCLP, a leucine-rich repeat-containing protein, is a homolog of SCLP, a gene originally identified in Manduca as an up-regulated transcript in skeletal muscle cells undergoing programmed cell death (38). dSCLP is up-regulated in Mhc mutants, and in situ probes show strong expression in the mesoderm (Fig. 4E), suggesting its primary function resides in muscles. CG6972 is an armadillo repeat protein also up-regulated in Mhc mutants. Expression analysis reveals RNA localization only in somatic musculature (Fig. 4F), again suggesting a function in muscle. FEM-1 was identified as an ankyrin repeat protein important in sex determination via programmed cell death in Caenorhabditis elegans (39). dFEM-1 RNA localization was primarily detected in the central nervous system (Fig. 4G), suggesting it may represent a class of transcripts that are up-regulated in non-muscle cells in response to muscle degeneration. With the exception of dFEM-1, the somatic muscle expression of these genes occurs during late mesodermal differentiation, turning on around stage 13 of embryogenesis, when the body wall myoblasts begin to fuse and form the body wall musculature, suggesting these genes are more likely to be involved with the structure or function of muscle cells, rather than the specification and determination of mesodermal derivatives (Fig. 4). In summary, five of six novel genes we selected for in situ analysis were expressed in muscles, suggesting many up-regulated transcripts in Mhc hypercontraction mutants likely represent transcriptional recording in damaged muscles.

Muscle Remodeling May Occur on the Actin Cytoskeleton or at Key Structural Sites—To characterize the role of differential gene regulation in muscle dysfunction, it is important to identify the possible sites of action where the proteins may act within the muscle. To begin this analysis, we characterized the immunolocalization of MSP-300, the Drosophila nesprrin (20, 32). As reported previously, localization of MSP-300 was found in four distinct subcellular compartments in control larval body wall muscles, including the Z-line, the muscle attach-
ment sites, the nucleus, and at putative attachment sites between muscles 6 and 7 (Fig. 5, A–D). The localization of MSP-300 was not altered in the Mhc mutant background (Fig. 5, E–G), indicating up-regulation of MSP-30 targets the protein to its normal subcellular compartments, as opposed to novel sites. We next assayed whether the product of the most up-regulated gene, dARC1, also localizes to the muscle cytoskeleton. To examine dARC1 targeting, we generated transgenic flies expressing dARC1 fused to the N terminus of GFP, or nontagged dARC1, under the UAS/GAL4 expression system. Antisera generated to dARC1 demonstrated that dARC1 associated with larval body wall sarcomeres when overexpressed in muscle, co-localizing with the myosin-rich thick filament (Fig. 6, A–C). The GFP-tagged dARC1 protein did not fold correctly when overexpressed alone, but when co-expressed with untagged dARC1, dARC1-GFP localized to similar structures in muscle (Fig. 6D), confirming the antisera localization. Co-expression of the proteins in the central nervous system revealed GFP fluorescence at synapses as well (Fig. 6, E–H), consistent with localization studies in mammals (29, 40). dARC1 localization suggests the protein likely functions in synapses and muscle sarcomeres, where it may modulate MHC function or localization at thick filaments.

**DISCUSSION**

*Drosophila* hypercontraction mutants display abnormal muscle function that is reflected in TS behavioral paralysis and myogenic seizures in adults and Ca\(^{2+}\)-independent contraction cycles in larval body wall muscles. Using genome-wide microarray analyses, we have begun to
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define this altered state and identify differences that may contribute to compensation responses to altered contractile properties and pathology of hypercontracted muscles. The overall microarray profile in Mhc hypercontraction mutants suggests a transcriptionally regulated remodeling of the cytoskeletal architecture. This remodeling is largely directed at the level of the actin cytoskeleton and at key subcellular sites of muscle-induced stress, including muscle attachment sites, the nuclear membrane, and at the Z-line structure of the sarcomere.

A remodeling response in Mhc mutants is suggested by the up-regulation of genes that act at the level of the actin cytoskeleton. Specifically, Mlp60A, Mlp84B, Kettin, MSP-300, flw, sqh, capulet, slingshot, act88F, act57B, Mbs, and talin have been documented previously to function in muscle cytoskeleton assembly or stabilization (20–25). In addition to genetic evidence, localization studies support a localized remodeling response. The muscle LIM protein Mlp84B accumulates at muscle attachment sites and Z-lines (27). The protein encoded by *flp wing* is important for the integrity of muscle attachment sites and the organization of the Z-lines in the indirect flight muscles (22). *Drosophila Kettin* (titin) is required for both myoblast fusion and the structural integrity of the sarcomere, with immunolocalization to Z-lines (23, 41, 42).

Likewise, we find that the protein products of MSP-300 and dARC1, two of the most up-regulated genes identified in our screen, also localize to specific sites in the sarcomere. MSP-300 is enriched in the actin-rich thin filament, whereas dARC1 localizes to the myosin-rich thick filament. Cytoskeletal remodeling may represent a functionally beneficial compensation mechanism. The up-regulation of *flp* supports a compensatory effect. In wild type animals, *sqh* normally acts as a regulatory light chain of the non-muscle type II myosin, *zipper* (43). *sqh* up-regulation may alter the overall activity and force output of the muscle by changing the properties of the contractile machinery. In contrast, the down-regulation of *fln* may reflect a deleterious alteration in transcription. Loss-of-function mutations in *fln* lead to sarcomeric instability and defects in thick filament assembly (25). Besides the increased forces produced by mutant MHC, decreasing *fln* transcription may lead to further destabilization of the thick filament structure.

In addition to the up-regulation of cytoskeleton-associated proteins, other cellular processes accompanying muscle hypercontraction are suggested by the transcriptional response. The up-regulation of immune response genes indicates an infiltration of immune cells involved in degradation of degenerating muscles. The down-regulation of nuclear encoded mitochondrial genes and genes involved in energy and metabolism suggests a general metabolic crisis in muscles. Whether this response accompanies fiber degeneration or is causative to myopathy is unknown. The down-regulation of mitochondrial genes may indicate Ca\(^{2+}\)-overloading of mitochondria and subsequent Ca\(^{2+}\)-activated cell death. Mitochondrial staining in Mhc mutant larval body wall muscles appears normal (data not shown), indicating the down-regulation does not correspond to decreased mitochondrial number, but suggesting a loss of metabolic function and energy depletion in muscles.

An additional component of the expression profile is the up-regulation of a subset of genes that have been isolated in mammalian systems.
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as activity-regulated transcripts in the nervous system, including dARCl, dNeurochordrin, and MSP-300. In addition to conservation of protein sequence homology across evolution, transcriptional modulation has also been conserved for these genes, indicating this regulation is likely critical for their function. The mammalian homologs (ARC, neurochordrin/norbin, and CPG2) are up-regulated in response to synaptic activity and are thought to modulate morphological properties of mammalian synapses (29–31, 36, 44–47). Activity-dependent synaptic modification is dependent upon Ca2+ signals through N-methyl-D-aspartate receptors at synapses (48). Potentially, Ca2+-dependent signaling in muscular dystrophies and cardiomyopathies may impinge upon an endogenous activity-dependent transcriptional response used to remodel actin-dependent synaptic structures during neuronal plasticity. It will be of interest to determine whether additional uncharacterized transcripts encoding cytoskeletal regulators identified in our screen are also activity-regulated in neurons.

The transcriptional response to hypercontraction-induced myopathy in Drosophila suggests a conserved cellular response to muscle dysfunction from flies to humans. For both skeletal and cardiac dysfunction, dysregulation of intracellular Ca2+ homeostasis has been suggested to be important for pathogenesis and altered transcriptional regulation (15, 49–52). In mammalian cardiomyocytes, differential gene regulation has focused on the activation of fetal protein isoforms involved in cardiac function (14, 53). Similar responses were observed in Drosophila hypercontraction mutants. The up-regulated muscle LIM proteins, for example, have peak expressions at the terminal stages of muscle development in the embryo and pupa (21). In addition, the strongly up-regulated Act57B is the predominant actin for larval somatic musculature, not adult muscles (54). Expression analysis of muscular dystrophies has also revealed a down-regulation of nuclear-encoded mitochondrial genes (26, 55–57), similar to our observations in Drosophila. As is the case in vertebrate muscular dystrophies, Drosophila hypercontraction mutants exhibit an up-regulation of immune response genes. In Duchenne muscular dystrophy, this response is thought to reflect infiltration of degrading muscle tissue by activated dendritic cells, as immunohistochemical analysis of muscle biopsies show increases in dendritic cell infiltration into the muscle (26). Known and putative metabolic genes form a foundation for the transcriptional response. For example, genes such as the novel genes have sequences that indicate a role in cytoskeletal remodeling and show similar regulatory patterns in response to myopathy, but they also are preferentially expressed in muscle. One of these genes, dFHOS, is a formin domain containing protein. Recent data have shown that formin domains form unbranched actin polymers in an ARP2/3-independent manner (34, 35), suggesting that dFHOS may function to polymerize unbranched actin filaments in the sarcomere. In addition to gene expression differences in dystrophic muscles, it is likely that the expression profiling will reveal compensation responses occurring in other tissues, including neurons. By using molecular genetic tools available in Drosophila, we can begin to understand the function consequences of altered transcription in the context of muscle disease.

In summary, the expression profiling data indicate that hypercontraction in Myhc mutants leads to a specific transcriptional response that is similar to that of human muscular dystrophies and hypertrophic cardiomyopathies. This response does not represent a reactivation of the entire developmental program of the mesoderm, as early mesodermal determinants are not up-regulated. Rather, the transcriptional response activates a program of genes that are required for the structure or function of muscle cells. Future studies will determine which differentially regulated genes contribute to dysfunction and which are involved in remodeling of damaged muscle tissue.

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