Molecular Genetics of *Drosophila* Alpha-Actinin: Mutant Alleles Disrupt Z Disc Integrity and Muscle Insertions

Eric Fyrberg,* Melissa Kelly,* Elizabeth Ball,* Christine Fyrberg,* and Mary C. Reedy‡

* Department of Biology, The Johns Hopkins University, Charles and 34th Streets, Baltimore, Maryland 21218; and ‡ Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

Abstract. We have isolated a *Drosophila melanogaster* alpha-actinin gene and partially characterized several mutant alleles. The *Drosophila* protein sequence is very similar (68% identity) to those of chicken alpha-actinin isoforms, but less closely related (30% identity) to *Dictyostelium* alpha-actinin. The gene is within subdivision 2C of the X chromosome, coincident with 15 *lethal (l)2Cb* mutations. At least four alleles, $l(1)2Cb^1$, $l(1)2Cb^2$, $l(1)2Cb^4$, and $l(1)2Cb^5$ are interrupted by rearrangement breakpoints and must be null. In all four cases, hemizygous mutants complete embryogenesis and do not die until the second day of larval growth, signifying that either the role of alpha-actinin in nonmuscle cells is redundant or that a distinct and only distantly related gene encodes the nonmuscle isoform. Allelic but less severely affected *fliA* mutants are apparently due to point mutations, and develop into adults having thoracic muscle abnormalities. EM of mutant muscles reveals that Z discs and myofibrillar attachments are disrupted, whereas epithelial "tendon" cells are less affected. We discuss these phenotypes in the light of presumed in vivo alpha-actinin functions.

To better understand muscle physiology and mechanical properties of the cytoskeleton, it is essential to learn in more detail how actin filaments are organized and anchored. The observation that distinct muscle types have rather differently arranged Z discs suggests that thin filament organization is a critical determinant of contractile properties (Auber and Couteaux, 1963; Goldstein et al., 1982; Yamaguchi et al., 1985). Likewise, considerable evidence suggests that proteins that anchor or cross-link actin filaments profoundly affect cytoskeletal functions (Smith, 1988).

Probably the best characterized actin filament cross-linking protein is alpha-actinin. The 100,000-mol wt polypeptide occurs as antiparallel homodimers, which approximate rods 7 nm wide by 48 nm long (Imamura et al., 1988). Both muscle and nonmuscle isoforms of alpha-actinin have been described (Endo and Masaki, 1982; Duhaiman and Bamburg, 1984). Skeletal muscle alpha-actinin is stably localized within Z discs (Masaki et al., 1967), whereas the distribution of nonmuscle isoforms is dynamic and complex, generally paralleling that of microfilaments. In avian fibroblasts, for example, alpha-actinin antibodies stain stress fibers in a periodic pattern and also decorate less orderly cortical actin filament arrays, especially in the vicinity of where cells are bound to the substrate (Lazarides and Burridge, 1975; Tokuyasu et al., 1981).

Recent sequence analyses have generated a hypothesis for alpha-actinin structure and function (refer to the review of Blanchard et al., 1989). Alpha-actinins include three distinct domains, an NH_2-terminal actin binding domain of $\sim$220 residues, four 122-amino acid central repeats, and a carboxy-terminal region containing two EF-hand-like sequences. The actin-binding regions, which interact most closely with residues 1–12 and 86–123 of actin, would be located at the ends of the rod-shaped antiparallel dimer (Mimura and Asano, 1987). The central repeats are thought to engender electrostatic interactions essential for formation of antiparallel dimers (Imamura et al., 1988). Finally, the EF handlike sequences probably determine whether actin binding of a particular isoform is calcium dependent. For some time it has been known that actin binding of cytoplasmic alpha-actinin isoforms is calcium sensitive, while that of muscle isoforms is not (Burridge and Feramisco, 1981). This functional distinction appears to be conferred by sequence differences in the EF hand domains (Arimura et al., 1988).

Immunolocalization studies and structural analyses have partially defined the in vivo roles of alpha-actinin. Within Z discs of skeletal muscle the protein anchors parallel and antiparallel thin filament arrays via several morphologically distinct types of cross-links recently visualized in image reconstructions of electron micrographs (Cheng and Deatherage, 1989; Deatherage et al., 1989). Sato et al. (1987) have suggested that alpha-actinin of nonmuscle cells may dynamically crosslink actin filament networks within the cortical cytoplasm and thus regulate their rates of deformation. However, there is as yet no evidence to suggest that alpha-actinin

© The Rockefeller University Press, 0021-9525/90/06/1999/13 $2.00

engenders comparable changes in microfilament networks in vivo. Immunolocalization experiments have demonstrated that alpha-actinin, vinculin, and talin are closely associated with microfilaments in the vicinity of focal adhesion plaques. This observation implies that alpha-actinin participates in linking microfilament networks to plasma membranes, although probably not directly (refer to Geiger et al., 1979; Chen and Singer, 1982).

The indirect flight muscles of *Drosophila* are an excellent system for examining functions of alpha-actinin in both muscle and nonmuscle cells. The versatility of *Drosophila* molecular genetic techniques allows one to perturb structures of contractile and cytoskeletal proteins within these fibers, then evaluate the functional consequences. Another attractive feature of flight muscles is the juxtaposition and well-defined relationship of muscle and nonmuscle cells. In contrast to the extracellular collagenous tendon used by vertebrates, in the *Drosophila* thorax specialized epithelial cells serve as "cellular tendons" connecting flight muscle fibers to cuticle (Auber, 1963). Within these specialized nonmuscle cells, microtubule bundles and cytoskeletal elements transmit force from muscle cell to cuticle. Ultrastructural examination of these cuticular insertions therefore allows both sarcomeric and cytoplasmic functions of alpha-actinins to be explored in a single system.

Here we describe isolation of a gene which encodes sarcomeric *Drosophila* alpha-actinin, document several mutant alleles, and illustrate some of the associated muscle abnormalities. Several apparent missense alleles perturb Z discs and severely disrupt myofibrillar insertions into muscle cell membranes. Surprisingly, null alleles do not affect embryonic development, suggesting that either cytoskeletal roles of alpha-actinin are largely redundant, or that cytoskeletal alpha-actinin of *Drosophila* is encoded by a different gene. We discuss these results in light of the proposed functions of alpha-actinin in muscle and nonmuscle cells.

Materials and Methods

Antibody Screening of Lambda gt11 Expression Libraries. A library of *Drosophila* head-specific cDNAs cloned in the lambda gt11 vector (Young and Davis, 1983) was obtained from Dr. Paul Salvaterra (City of Hope Medical Center, Duarte, CA). Phage were plated on *E. coli* strain Y1090 at a density of 50,000 plaques per 150-mm plate in soft agar on L-broth plates containing ampicillin. Plaques were allowed to form at 42°C for 3-4 h. Subsequently plates were removed to 37°C and fusion protein synthesis induced by covering with nitrocellulose filters that had been soaked for 1 h in 10 mM isopropyl-β-D-thio-galactopyranoside. Filters were removed after 3-4 h, washed with Tris-buffered saline containing 0.05% Tween 20, and incubated in hybridoma cell supernatant containing antibody MAC 274 1:200 dilution) for 3 h, then 2 h with peroxidase-conjugated rabbit anti-rat IgG (Cappel Laboratories, Malvern, PA). Second antibody binding was visualized by developing blots in a solution containing one part 3 mg/ml chloronapthol in methanol and five parts TBS (50 mM Tris, PH 7.5, 150 mM NaCl) plus 0.5% hydrogen peroxide.

Screening Lambda gt10 Drosophila cDNA Libraries. A cDNA library representing mid-pupal stage *Drosophila* mRNA (provided by Dr. Tom Kornberg, University of California at San Francisco) was screened with a partial cDNA isolated from a *Drosophila* cDNA expression library using the method of Benton and Davis (1977). From a screen of 1×10^5 recombinants we isolated four partial clones which collectively represented the entire *Drosophila* alpha-actinin cDNA sequence. All cDNAs were cloned in the pUC19 vector and sequenced using the dideoxy method of Sanger et al. (1977), or the chemical degradation method of Maxam and Gilbert (1980).

In Situ Hybridization to Polytene Chromosomes. Polytene chromosomes from larval salivary glands of wild-type (Canton-S strain) and *l(l)2Cb*^{2} heterozygotes were isolated and spread using the technique of Gall and Pardue (1971). Tritium-labeled cRNA was prepared from alpha-actinin gene containing recombinant lambda phage templates as described by Wensink et al. (1974). Denaturation and hybridization were as described by Karlik et al. (1984).

Hybridization to Southern-blotted DNA. Hybridizations were performed at 42°C in 50% formamide, 5× SSC, 5× Denhardt's solution, 50 mM sodium phosphate (pH 6.8) containing 40 μg/ml of denatured calf thymus DNA. Filters were washed two times at room temperature and three times at 68°C in 2× SSC, 0.1% sodium pyrophosphate, 0.1% SDS. Hybridizing fragments were visualized by exposure to x-ray film. In an attempt to detect additional alpha-actinin genes we performed some genomic blots at lower stringency. In these experiments formamide concentration was reduced to 30%, and blots were washed only at room temperature.

EM of Indirect Flight Muscles. Thoraces were surgically hemisected in the midsagittal plane so as to expose the dorsal longitudinal flight muscles without disturbing fibers or insertions. Hemisected thoraces were fixed in 3% glutaraldehyde, 0.2% tannic acid in buffered Ringer's solution (110 mM NaCl, 2 mM KCl, 3 mM $MgCl_2$, 20 mM K-MOPS, pH 6.8) at 4°C for 2 h, rinsed three times in buffered Ringer's solution, then twice in 0.2 M $NaPO_4$, pH 6.0. The hemithoraces were postfixed in 1% OsO_4, 0.2 M PO_4, 10 mM $MgCl_2$, pH 6.0 for 1 h on ice, rinsed in H_2O three times, block stained in 2% aqueous uranyl acetate for 1 h on ice, rinsed in H_2O, and dehydrated through an ethanol series. Hemithoraces were infiltrated with Araldite 506 mixture (Reedy, 1968; Reedy and Reedy, 1985) at which time further dissection into individual fibers or groups of fibers and their insertions was performed. Individual fibers or oriented fiber bundles with insertions were arranged on polyethylene sheets, and blocks were prepolymerized at 65°C. BEEM capsules with fresh resin were inverted over the oriented fibers and polymerized at 85°C for 48 h. 25-30-nm longitudinal and cross-sections were cut with a Diatome diamond knife on a Reichert OMU3 ultramicrotome and picked up on thin carbon films on 200-mesh copper grids. Sections were stained with potassium permanganate followed by Sato lead stain, according to Reedy and Reedy (1985) and photographed with a Siemens 101 electron microscope.

Results

To isolate *Drosophila* alpha-actinin genes, we screened a cDNA expression library with an mAb that recognizes waterbug alpha-actinin. The *Drosophila* library (provided by Dr. Paul Salvaterra) was constructed by inserting cDNAs representing mRNA of adult heads into the lambda gt11 vector of Young and Davis (1983). Because large muscles and extensive neuronal arrays are found within *Drosophila* heads, we expected that most cDNA's encoding muscle or cytoskeletal proteins would be represented. We screened the library with antibody MAC 274 (provided by Drs. Belinda Bullard and Geoff Butcher) directed against alpha-actinin of *Lethocerus griseus* and *Lethocerus uhleri* flight muscles. In preliminary work the antibody had been shown to bind specifically to Z discs of both *Lethocerus* and *Drosophila* muscles and to recognize a 98-kD protein on immunoblots (Bullard, B., personal communication). The antibody recognizes a polypeptide of the same molecular weight in immunoblots of *Drosophila* thoracic proteins (data not shown). On the basis of the Z disc staining pattern and the molecular weights of the reactive proteins, we presumed that the antibody recognizes *Lethocerus* and *Drosophila* alpha-actinins. From a screen of $\sim 1 \times 10^5$ plaques we recovered one, MK384, in which the fusion protein reacted consistently with the antibody. The insert was subcloned into plasmid pUC19 and analyzed further.

We sequenced portions of cDNA MK384 and found that the encoded protein sequence was very similar to those of chicken alpha-actinin isoforms (Baron et al., 1987*a*,*b*; Arimura et al., 1988). To obtain a series of overlapping

```
Dros   1          mmMenglsmeygdgYMeqEEeWeRegLLDPAWEKQQkKTFTAWCNSHLRKAGTaIdNIEED
CSM    1            Mdhhydpqqtnd YMqpEEdWDRDLLLDPAWEKQQRKTFTAWCNSHLRKAGTQIENIEED
CSK    1 mnsmnqietnmqytynyeedeYMtqEEeWDRDLLLDPAWEKQQRKTFTAWCNSHLRKAGTQIENIEED
con      ---------m---------d-YM-qEEeWdRdlLLDPAWEKQQrKTFTAWCNSHLRKAGTqIeNIEED

Dros  62 FRnGLKLMLLLEVISGEtLpKPdRGKMRfHKIaNVNKALDFIASKGVhLVSIGAEEIVDGNlKMTLGM
CSM   60 FRdGLKLMLLLEVISGERLaKPeRGKMRvHKIeNVNKALDFIASKGVKLVSIGAEEIVDGNVKMTLGM
CSK   69 FRnGLKLMLLLEVISGERLpKPdRGKMRfHKIaNVNKALDyIASKGVKLVSIGAEEIVDGNVKMTLGM
con      FRnGLKLMLLLEVISGErLpKPdRGKMRfHKIaNVNKALDfIASKGVkLVSIGAEEIVDGNvKMTLGM

Dros 130 IWTIILRFAIQDISVEEmtAKEGLLLWcQRKTAPYKNVNvQNFHlSfKDGLaFCALIHRHRPdLIDYa
CSM  128 IWTIILRFAIQDISVEETSAKEGLLLWyQRKTAPYKNVNIQNFHiSWKDGLGFCALIHRHRPeLIDYg
CSK  137 IWTIILRFAIQDISVEETSAKEGLLLWcQRKTAPYrNVNIQNFHlSWKDGLGlCALIHRHRPdLIDYs
con      IWTIILRFAIQDISVEEtsAKEGLLLWcQRKTAPYkNVNiQNFHlSwKDGLgfCALIHRHRPdLIDY-

Dros 198 KLsKDnPLeNLNTAFDVAEKYLDIPrMLDpdDlinTpkPDErAIMTYVScyYHAFqGAQqAETAANRI
CSM  196 KLrKDDPLtNLNTAFDVAEKYLDIPKMLDAEDIVgTarPDEkAIMTYVSeFYHAFsGAQkAETAANRI
CSK  205 KLnKDDPigNiNlAmeiAEKhLDIPKMLDAEDIVnTpkPDErAIMTYVScFYHAFaGAeqAETAANRI
con      KL-KDdPl-NlNtAfdvAEKyLDIPkMLDaeDivnTpkPDErAIMTYVScfYHAF-GAqqAETAANRI

Dros 266 CKVLkVNQENErLMEeYErLASDLLEWIRRTmPWLnsRqadNslagvQkKLEeyRtYRRkHKPPrVeq
CSM  264 CKVLAVNQENEqLMEdYEkLASDLLEWIRRTIPWLENRaPENTMQAMQqKLEDFRDYRRlHKPPKVQE
CSK  273 CKVLAVNQENErLMEeYErLASeLLEWIRRTIPWLENRtPEkTMQAMQkKLEDFRDYRRkHKPPKVQE
con      CKVLaVNQENErLMEeYErLASdLLEWIRRTiPWLenR-pentmqamQkKLEdfRdYRRkHKPPkVqe

Dros 334 KakLEtNFNTLQTKLRLSNRPAylPtEGKtVSDIsNsWkGLElAEKafEEWLLaEtqRLERLeHLAqK
CSM  332 KCQLEINFNTLQTKLRLSNRPAFMPSEGKMVSDInNAWgGLEQAEKGYEEWLLNEIRRLERLdHLAEK
CSK  341 KCQLEINFNTLQTKLRiSNRPAFMPSEGKMVSDIagAWqrLEQAEKGYEEWLLNEIRRLERLeHLAEK
con      KcqLEiNFNTLQTKLRlSNRPAfmPsEGKmVSDI-naW-gLEqAEKgyEEWLLnEirRLERLeHLAeK

Dros 402 FkhKAdaHEdWTrGKEeMLQsqDfrqykLnElKALkKKHEAFESDLAAHQDRVEQIAAIAQELNtLeY
CSM  400 FRQKASiHEsWTdGKEaMLQQKDYEtAtLsEiKALLKKHEAFESDLAAHQDRVEQIAAIAQELNELDY
CSK  409 FRQKAStHEqWayGKEqiLlQKDYEsAsLtEvrAmLrKHEAFESDLAAHQDRVEQIAAIAQELNELDY
con      FrqKAs-HE-Wt-GKE-mLqqkDye-a-L-E-kAllkKHEAFESDLAAHQDRVEQIAAIAQELNeLdY

Dros 470 hDcvcVNARCQrICDQWDrLGALTQrRRtALdeaEriLEkIDiLhLEfAKRAAPFNNWldGtrEDLvD
CSM  468 yDspSVNARCQKICDQWDnLGALTQKRREALERTEKLLETIDQLyLEyAKRAAPFNNWMEGAMEDLQD
CSK  477 hDaaSVNdRCQKICDQWDsLGtLTQKRREALERTEKLLETIDQLhLEfAKRAAPFNNWMEGAMEDLQD
con      hD--sVNaRCQkICDQWD-LGaLTQkRReALertEklLEtIDqLhLEfAKRAAPFNNWmeGamEDLqD

Dros 538 mFIVHTmvEIQGLiqAHdQFKATLgeADKEfnlIvnlvrEVesIVkqhqipggleNPYTTlTandmtr
CSM  536 tFIVHTIEEIQGLttAHeQFKATLPdADKERQAILgIhNEVsKIVQtYhvnmagtNPYTTiTpqEIng
CSK  545 mFIVHsIEEIQsLisAHdQFKATLPeADgERQAILsIqNEVeKviQsYsmrisasNPYsTvTveEIrt
con      mFIVHtieEIQgLi-AHdQFKATLpeADkErqaIl-i-nEVekivq-y-------NPYtT-T--ei--

Dros 606 KWsdVRQLVPqRDQtLanErrkQQnNEmLRrQFaekANivGPWIerqMdvvtplvwdlqGsLEDQLhr
CSM  604 KWEhVRQLVPrRDQaLmEEhARQQqNERLRkQFgAQANVIGPWIQTKMEEIgRiSIEMhGtLEDQLMh
CSK  613 KWEkVkQLVPqRDQsLqEElARQhaNERLRrQFaAQANVIGPWIQTKMEEIaRsSIEMtGpLEDQmMq
con      KWe-VrQLVPqRDQ-L-eE-arQq-NErLRrQFaaqANviGPWIqtkMeei-r-siem-G-LEDQln-

Dros 674 LkeYEqavhaYKPnIeeLEkiHQavQEsmIFeNryTNYTMEtlRVGWEQLLTsInRnINEVENQILTR
CSM  672 LrQYEksIvNYKPkIDqLEGDHQqIQEALIFDNKHTNYTMEHIRVGWEQLLTTIARTINEVENQILTR
CSK  681 LkQYEqnIiNYKhnIDkLEGDHQlIQEALvFDNKHTNYTMEHIRVGWElLLTTIARTINEVEtQILTR
con      LkqYEq-i-nYKpnId-LEgdHQ-iQEaliFdNkhTNYTMEhiRVGWEqLLTtIaRtINEVEnQILTR

Dros 742 DaKGISQEQlNEFRsSFNHFDknrTGrlspEeFksCLvSlGYsigkerqGdldFqRIlavVDPNntGy
CSM  740 DAKGISQEQMNEFRASFNHFDRkKTGmMDcEDFRACLISMGY       nmGEAEFARIMSiVDPNrmGv
CSK  749 DAKGItQEQMMdFRASFNHFDRrKnGlMDhdDFRACLISMGY       dlGEAEFARIMSlVDPNgqGt
con      DaKGIsQEQmNeFRaSFNHFDr-ktG-md-edFraCLiSmGY-------GeaeFaRIms-VDPN--G-

Dros 810 VhFdAFlDFMtREstDTDTAeQVidSFrILAaDKpYIlpDELRRELPPDQAEYCIqRMpPYkGpngVP
CSM  803 VTFQAFIDFMsRETADTDTAdQVmASFkILAgDKnYItvDELRRELPPDQAEYCIaRMaPYnGrdaVP
CSK  812 VTFQsFIDFMtRETADTDTAeQViASFrILAsDKpYIlaDELRRELPPeQAnYCIkRMpqYtGpgsVP
con      VtFqaFiDFMtREtaDTDTAeQViaSFrILA-DKpYIl-DELRRELPPdQAeYCI-RMppY-Gp--VP

Dros 878 GALDYMSFSTALYGEtDL
CSM  871 GALDYMSFSTALYGESDL
CSK  880 GALDYtSFSsALYGESDL
con      GALDYmSFStALYGEsDL
```

Figure 1. Derived protein sequence of *Drosophila* alpha-actinin (*Dros*), aligned with the chicken smooth muscle (*CSM*) and chicken skeletal muscle (*CSK*) sequences. At the bottom of each line is the consensus (*con*) sequence. The *Drosophila* sequence is very similar to both chicken sequences, with 608 (skeletal) or 609 (smooth) of 896 residues identical. These sequence data are available from EMBL/Genbank/DDBJ under accession number X51753.

cDNAs we used the original gt11 isolate to screen a library of midpupal stage *Drosophila* cDNAs cloned in the lambda phage vector gt10. One clone that extended to the initiation codon, MKQ4, and three that included 3′ ends of the cDNA were thus isolated, and we determined their complete sequences. The encoded protein sequence, as well as those of chicken skeletal and smooth muscle alpha-actinins, are displayed in Fig. 1. Alignment of these sequences reveals extensive similarity. *Drosophila* and chicken isoforms, including a chicken fibroblast isoform not shown, have an average 68% amino acid identity. Independent comparisons of the *Drosophila* sequence with that encoded by the single *Dictyostelium* alpha-actinin gene revealed considerably more divergence, with only 266 identical residues (Noegel et al., 1986, 1987).

Comparisons of the *Drosophila* alpha-actinin sequence to those of chicken and *Dictyostelium* generally support prior proposals for alpha-actinin structure–function relationships (refer to our introduction and the review by Blanchard et al., 1989). The presumed actin binding region of the chicken smooth muscle sequence, including residues 25–244, is 86% identical to that of *Drosophila*. This high degree of sequence conservation is expected in light of the very slow evolution of actin. Note, however that there is only poor conservation between the NH_2 termini of chicken and *Drosophila* isoforms, suggesting that this region is not directly involved in actin binding. Central repeats are also highly conserved. Repeats 1, 2, 3, and 4 of the *Drosophila* sequence (residues 248–366, 367–481, 482–602, and 603–715, respectively) have 70, 65, 59, and 46% amino acid identity with their counterparts in the chicken smooth muscle isoform. One remaining highly conserved sequence at the COOH terminus, including residues 851–862, has an unknown function.

In light of the import of establishing whether actin binding by particular alpha-actinin isoforms is calcium sensitive, we have compared sequences of both EF-hand-like domains (residues 752–821 of the encoded *Drosophila* alpha-actinin) with those present within other alpha-actinin isoforms. EF handlike sequences of the five isoforms so far characterized are displayed in Fig. 2. In the NH_2-terminal *Drosophila* EF hand 13 of 16 residues are in accord with rules for calcium binding loops outlined by Kretsinger (1980), whereas the COOH-terminal sequences has 12 of 16 residues that conform to the consensus sequence. Since only 12 of 16 suitable residues are typically required for calcium binding, we predict that both EF-hand-like domains of *Drosophila* alpha-actinin would be functional. Furthermore, *Drosophila* and *Dictyostelium* isoforms have either aspartic acid or asparagine residues, both of which are able to coordinate calcium binding, at the liganding Y-position of the first EF hand. This may be especially significant because Blanchard et al. (1989)

```
                EL  LL  LD D DG ID  EL  LL  L           EL  LL  LD D DG ID  EL  LL  L
                **   *  ** * *   *  **   *  *            *  **  ** * ** **  **  **
                        X  Y Z-Y-X -Z                           X  Y Z-Y-X -Z
Dictyostelium.  EFKACFSHFDKDNDNKLNRLEFSSCLKSIGDEL-----TEEQLNQVISKIDTDGNGTISFEEFIDYMVSS
Drosophila.     EFRSSFNHFDKNRTGRLSPEEFKSCLVSLGYSIGKERQGDLDFQRILAVVDPNNTGYVHFDAFLDFMTRE
Chicken NM.     EFRASFNHFDRDHSGTLGPEEFKACLISLGYDIGNDAQGEAEFARIMSIVDPNRMGVVTFQAFIDFMSRE
Chicken SM.     EFRASFNHFDRKKTGMMDCEDFRACLISMGYNM-----GEAEFARIMSIVDPNRMGVVTFQAFIDFMSRE
Chicken SkM.    DFRASFNHFDRRKNGLMDHDDFRACLISMGYDL-----GEAEFARIMSLVDPNGQGTVTFQSFIDFMTRE
```

Figure 2. Comparisons of EF-hand-like sequences of *Dictyostelium*, *Drosophila*, and chicken nonmuscle, smooth muscle, and skeletal muscle isoforms. The figure compares sequences of the EF hand-like regions for five alpha-actinin isoforms. *Drosophila* and chicken nonmuscle sequences have five extra residues between the two EF handlike sequences, which necessitates adding five pad characters (−) to the remaining three sequences. There are 16 positions where amino acid identity is requisite for an EF hand structure. Positions denoted by "*E*" are typically glutamic acid, "*L*" can be phenylalanine, leucine, isoleucine, valine, methionine, alanine, or tyrosine, "*D*" can be aspartic acid, asparagine, glutamic acid, glutamine, serine, or threonine, "*G*" is typically gly, and "*I*" is isoleucine or valine. Functional EF hands have correct residues at twelve or more of these 16 positions. Asterisks denote the alignment of liganding oxygens corresponding to the octahedral vertices indicated by *X, Y, Z, −Y, −X, −Z*.

have suggested that the critical determinant of calcium sensitivity is whether the residue at the Y position of the first EF hand is able to coordinate calcium binding. Finally, note that the *Drosophila* sequence has five extra residues between EF hands, and in this respect more resembles the chicken nonmuscle isoform than either of the two chicken muscle isoforms. Collectively, these observations demonstrate that both EF handlike sequences of *Drosophila* alpha-actinin are theoretically capable of binding calcium, and that this portion of the *Drosophila* protein is more similar to nonmuscle isoforms of chicken and alpha-actinin of *Dictyostelium* than to vertebrate sarcomeric and smooth muscle isoforms.

To delineate the chromosomal arrangement of the *Drosophila* alpha-actinin gene we isolated an overlapping series of phages containing the entire gene by screening the *Drosophila* library of Maniatis et al. (1979) with our pool of cDNA clones and sequenced all hybridizing fragments. The protein coding region defined as such is organized as seven exons, none of which obviously corresponds to any of the hypothetical protein domains (refer to Table I). The only irregularity of the sequence arrangement is that the last 36 codons of exon six (which specify residues 370–405) are duplicated within the intronic sequence just preceding exon seven (data not shown). This duplication is very unusual in that all 108 nucleotides are identical. The intronic duplicate must not be alternatively utilized, however, as it is not preceded by an acceptable splice acceptor sequence. Finally, note that there must be at least one additional exon encoding remaining 5′ untranslated regions, as the sequence immediately upstream from the translational start is not colinear with the most distal sequences contained in our cDNAs (data not shown).

We ascertained the chromosomal location of the alpha-actinin gene in order to correlate it with map positions of extant mutant alleles. cRNA representing genomic DNA fragments spanning the entire structural gene was synthesized using *E. coli* RNA polymerase and ^{3}H-labeled ribonucleotide triphosphates. This probe was hybridized to denatured polytene chromosomes prepared from wild-type third instar

Table I. Codons Contained within Drosophila Alpha-Actinin Gene Exons

Exon No.	Codons
1	1–38
2	39–177
3	178–228
4	229–257
5	258–288
6	289–405
7	406–Term

Codons included within each exon are listed. In general, locations of intron/exon junctions do not correspond to borders of proposed alpha-actinin protein domains.

larvae, and slides were subsequently coated with nuclear track emulsion. After developing the emulsion it was clear that only one chromosomal locus was consistently labeled, the distal portion of polytene subdivision 2C (refer to Fig. 3 *A*). This particular chromosome region has been well characterized genetically, as Perrimon et al. (1985) previously determined the number and order of all complementation groups within subdivisions 2C and 2D. One of the loci thus defined, *l(1)EA82*, recently renamed *l(1)2Cb*, (refer to Lindsley and Zimm, 1986), has fifteen recessive lethal alleles and an additional four, termed *fliA* (refer to Homyk and Emerson, 1988), which render homozygotes and hemizygotes flightless due to flight muscle abnormalities.

To definitively establish whether mutations of the *l(1)2Cb* locus disrupt the alpha-actinin gene we analyzed chromosomal DNA of mutant heterozygotes using either in situ hybridization or Southern blots. One mutation judged likely to be due to genomic rearrangement was *l(1)2Cb*^{4}, which is associated with an X chromosome inversion encompassing bands 2C3 to 7B1. In an initial experiment we hybridized DNA which included the entire alpha-actinin gene to polytene chromosomes of *l(1)2Cb*^{4} heterozygotes. In these heterozygotes the inverted and normal X chromosomes are synapsed (held together by sequence-specific lateral associations), which forces the inverted segment to form a loop. We observed that grains were localized in two foci at the base of the loop (refer to Fig. 3 *B*), the expected result if portions of the alpha-actinin gene were located at opposite ends of the inverted segment. In preparations wherein the inverted and uninverted X chromosomes were separated during their preparation, we again saw two foci of hybridization on the inverted chromosome (refer to Fig. 3 *C*), and in this case they were clearly at opposite ends of the inverted region. These observations confirmed the chromosomal location of the cloned sequences and established that one of the inversion breakpoints divides the sarcomeric alpha-actinin gene into two portions.

We analyzed chromosomal DNA of several *l(1)2Cb* heterozygote strains by hybridization to cloned portions of the *Drosophila* alpha-actinin gene. Results of experiments are summarized in Fig. 4. The figure illustrates the organization of the protein-encoding exons, locates restriction sites within chromosomal DNA, and delineates the approximate locations of chromosome rearrangements associated with particular mutants. These breakpoint locations were deduced by a series of Southern blots, in which DNA of mutant heterozygotes was probed with various portions of the alpha-

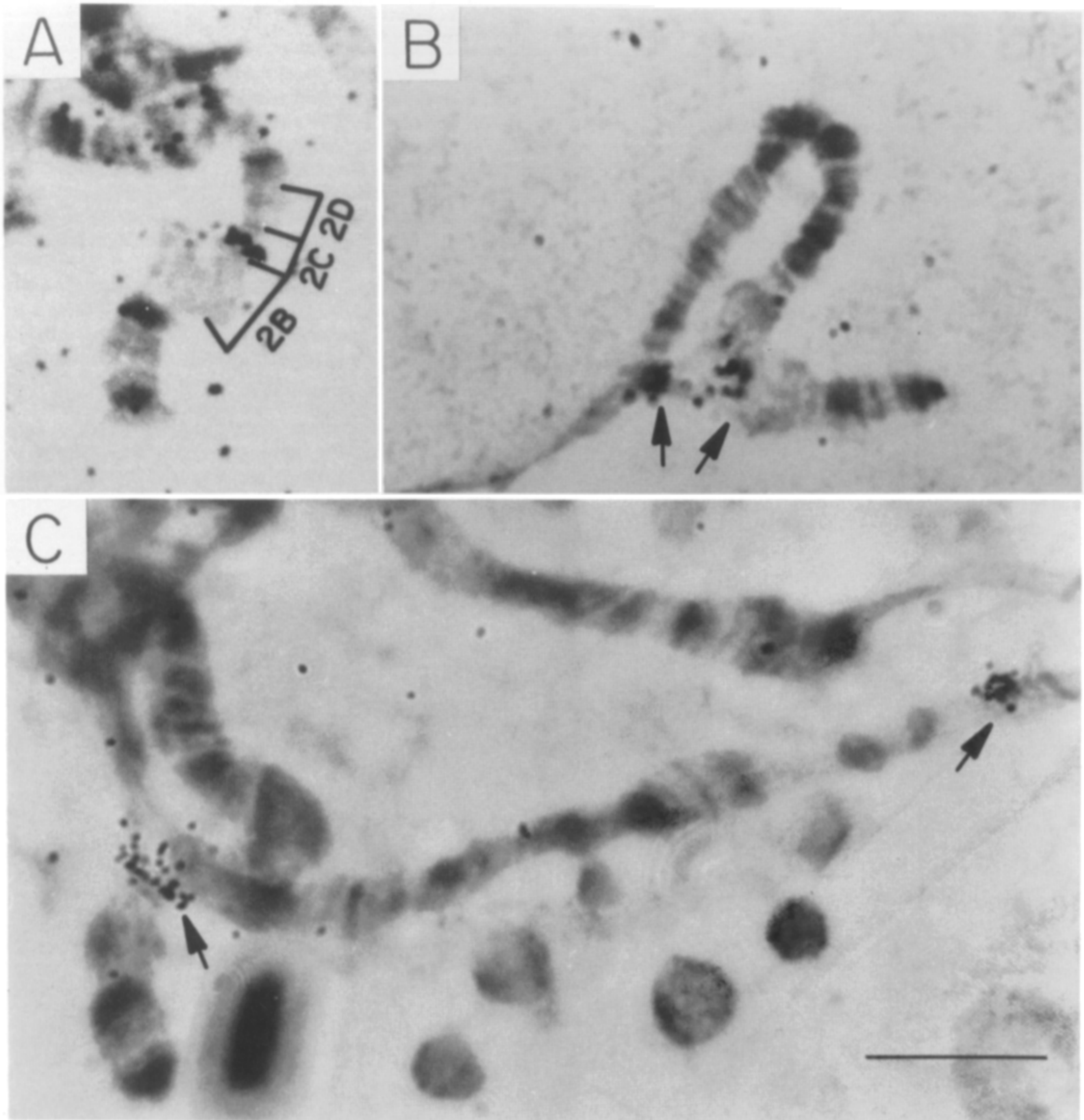

Figure 3. In situ localization of the *Drosophila* alpha-actinin gene. *A* illustrates hybridization of cRNA representing the alpha-actinin gene to an overstretched X chromosome. Brackets denote the locations of the 2B, 2C, and 2D subdivisions. A cluster of grains is seen within the distal portion of subdivision 2C. *B* illustrates hybridization to the synapsed X chromosomes of a *l(1)2Cb*^{2}/+ heterozygote. This mutant allele is associated with an inversion of the 2C3-7B portion of the X chromosome, which forms a loop when paired with an uninverted chromosome. When the same probe is hybridized, we observe grains at the base of the loop, where the opposite ends of the inversion are juxtaposed. *C* illustrates a heterozygote mutant chromosome wherein the inverted and uninverted (not visible in photo) X chromosomes have been separated during preparation. The probe hybridizes to both 2C and 7B chromosome regions, demonstrating that the inversion is within the alpha-actinin gene sequences used as probes. Bar, 20 μm.

actinin gene (data not shown). We found that at least four alleles, *l(1)2Cb*^{1,2,4} and ^{5} have breakpoints within the 5′ half of the alpha-actinin gene. These rearranged alleles may be capable of synthesizing portions of the alpha-actinin amino terminus, but such truncated peptides are seldom stable, and all four are almost certainly null. Substantiating this hypothesis is the fact that hemizygotes for all four mutants are lethal, and all die within precisely the same period of *Drosophila* larval development, the expected consequence if the four mutations eliminate, rather than alter, the encoded protein.

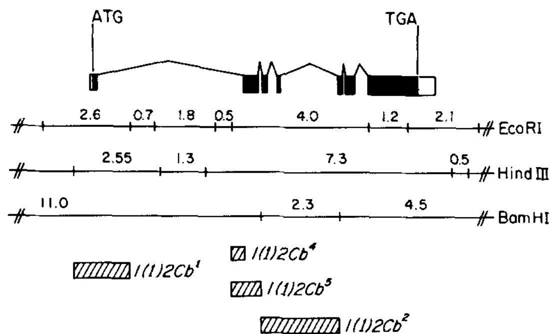

Figure 4. Localization of chromosomal breakpoints associated with particular *l(1)2Cb* alleles. The top portion of the diagram depicts the chromosomal arrangement of the *Drosophila* alpha-actinin gene. Lines immediately below illustrate the relative positions of Eco RI, Hind III, and Bam HI restriction sites. Sizes of restriction fragments, in kilobase pairs, are indicated by numbers. Hatched areas delimit the chromosome regions within which we have localized rearrangement breakpoints associated with particular *l(1)2Cb* alleles.

Because *fliA* mutants display less severe phenotypic consequences than do the allelic *l(1)2Cb* mutants, it seemed logical that they would have milder lesions at the DNA level. When we compared patterns of hybridizing bands in Bam HI-digested DNA derived from four homozygous *fliA* mutants to that of Canton-S, no differences in band sizes or intensities were observed (data not shown), suggesting that in all four instances muscle abnormalities are due to point mutations which perturb alpha-actinin structure and function, rather than to mutations which abolish protein accumulation as do *l(1)2Cb* alleles. Preliminary immunoblotting experiments further substantiate this notion. We find levels of alpha-actinin within thoraces comparable to those seen in wild type in *fliA*^{1,2}, and ^{3}, while *fliA*^{4} has an $\sim$50% reduction of steady-state alpha-actinin level (data not shown). Thus, all four *fliA* mutant homozygotes accumulate high levels of alpha-actinin.

We examined normal and mutant flight muscles using EM to visualize effects of mutant alpha-actinins on muscle structure and function. The most conveniently analyzed mutant

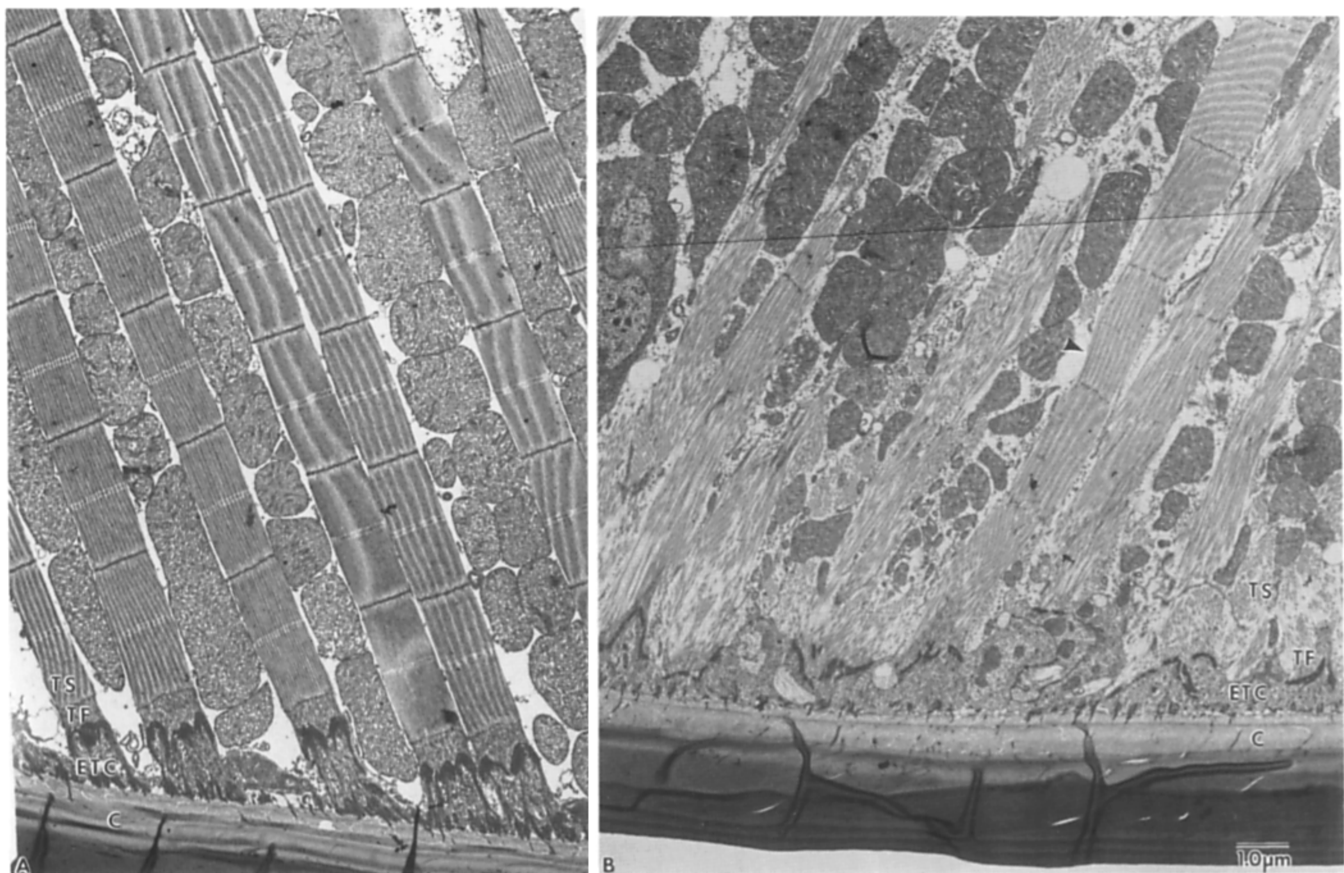

Figure 5. Longitudinal sections of wild-type and *fliA*^{3} flight muscles. *A* shows a low magnification view of a longitudinal section of wild type *Drosophila* flight muscle, including its cuticular insertion. The layered cuticle (*C*) and apposed epithelial tendon cells (*ETC*) can be seen at the bottom of the micrograph. The ETC is flattened against the cuticle except where it forms junctions with the muscle cell. Within the junction electron-dense faces of muscle and tendon cell membranes interdigitate to form a zigzag pattern. On the muscle side of the junction a modified Z disc forms a terminal feltwork (*TF*) at the end of the terminal sarcomere (*TS*). The light and dark variably spaced stripes (moiré) marking the myofibrils in these and following micrographs result when the 25-nm section alternately samples regions containing thick filaments and those that contain only thin filaments. *B* shows a comparable section of one mutant *fliA*^{3}, illustrating the disruption of both Z discs and muscle insertions. Note that disruption of the filament lattice can involve only the terminal sarcomere, as in the myofibril marked by the arrowhead, or can involve the terminal several sarcomeres, as in other myofibrils. Bar, 1.0 μm.

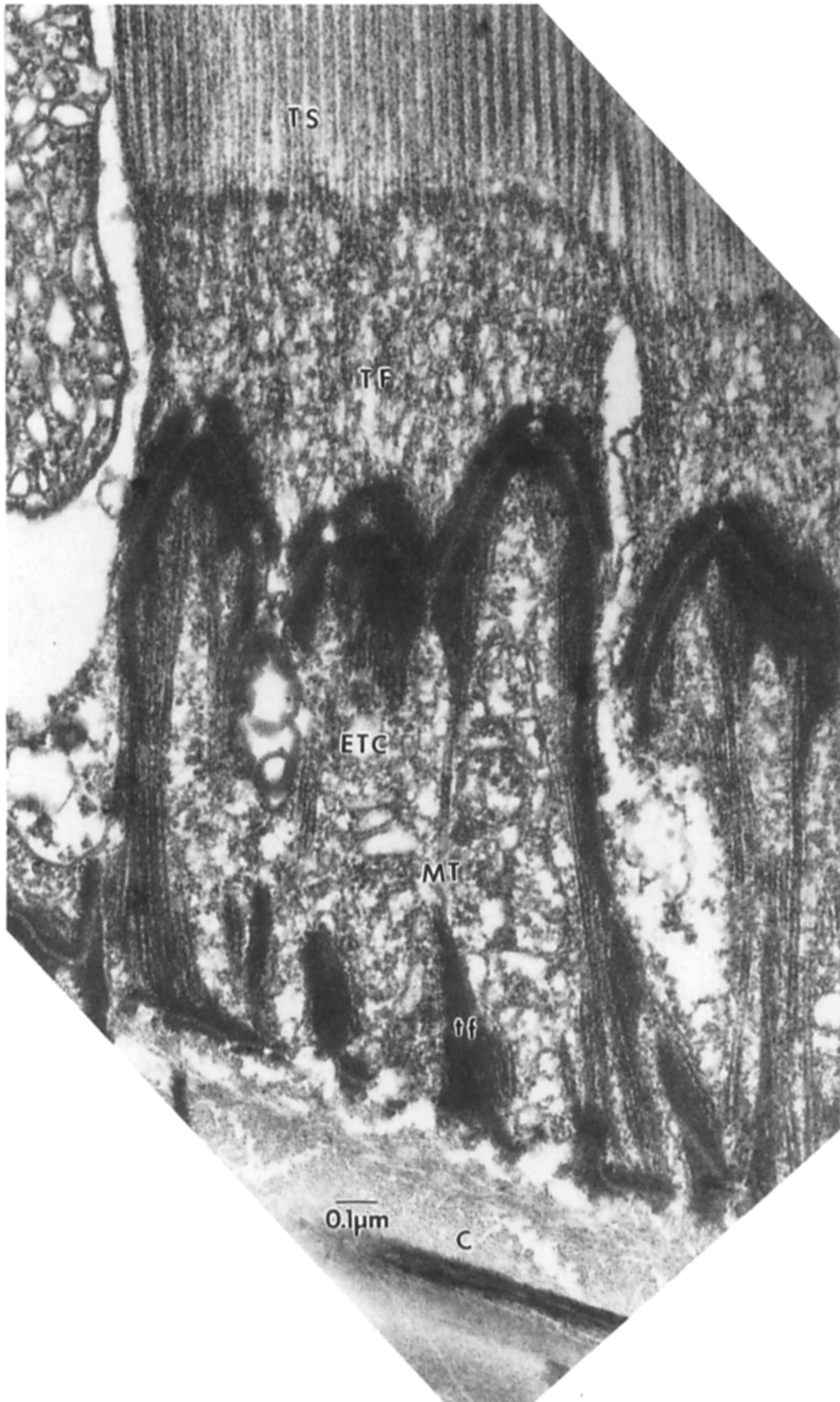

Figure 6. Higher magnification view of a junctional area of a wild-type myofibril illustrating the fascia adherens. Note the modified terminal Z disc (terminal feltwork, *TF*) of the terminal sarcomere (*TS*) and the epithelial tendon cell (*ETC*). Also note microtubule bundles (*MT*) crossing the tendon cell, and the dense tonofibrillae (*tf*) extending into the cuticle. Bar, 0.1 μm.

phenotypes are those engendered by the *fliA* alleles, in part because *fliA* homozygotes survive to adulthood, and in part because these mutations are known to affect indirect flight muscles of the thorax (Homyk and Emerson, 1988), which have been previously examined in detail using EM (Beall et al., 1989; Reedy et al., 1989). Fig. 5 *A* displays a longitudinal section of a *Drosophila* flight muscle and its cuticular insertion, whereas Fig. 6 illustrates the insertion of one myofibril at higher magnification. At low magnification, the most striking feature of wild type muscles is the regularity of both Z disc structure and myofibrillar insertions. Cuticular attachments of *Drosophila* flight muscles appear similar to those described for *Calliphora* (Auber, 1963; for descriptions of similar muscle attachments see Lai-Fook, 1967;

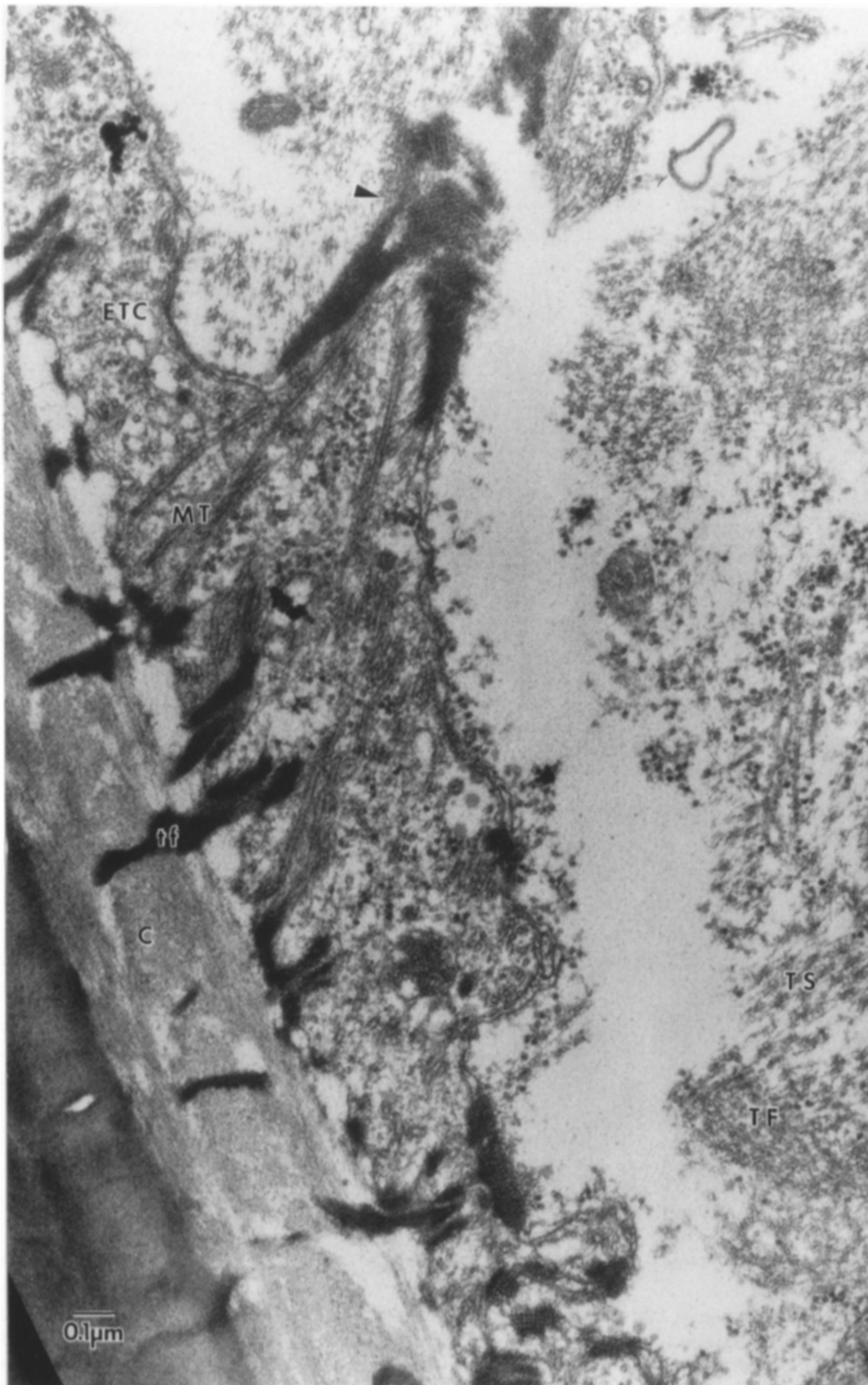

Figure 7. Higher magnification view of the junctional area of *fliA*[2] homozygotes. The section should be compared to the wild-type insertion illustrated in Fig. 6. Note that only vestiges of terminal feltwork (*TF*) are apparent and that thick and thin filaments in the terminal sarcomere (*TS*) are misoriented and disrupted. This general appearance is typical of many *fliA*[2] insertions, and is not due to any fixation artifact. Despite these muscle cell abnormalities microtubules (*MT*) nevertheless extend from the junctional area to the cuticular surface and tonofilaments penetrate the cuticle. The overall morphology of the tendon cell appears almost normal. Bar, 1.0 μm.

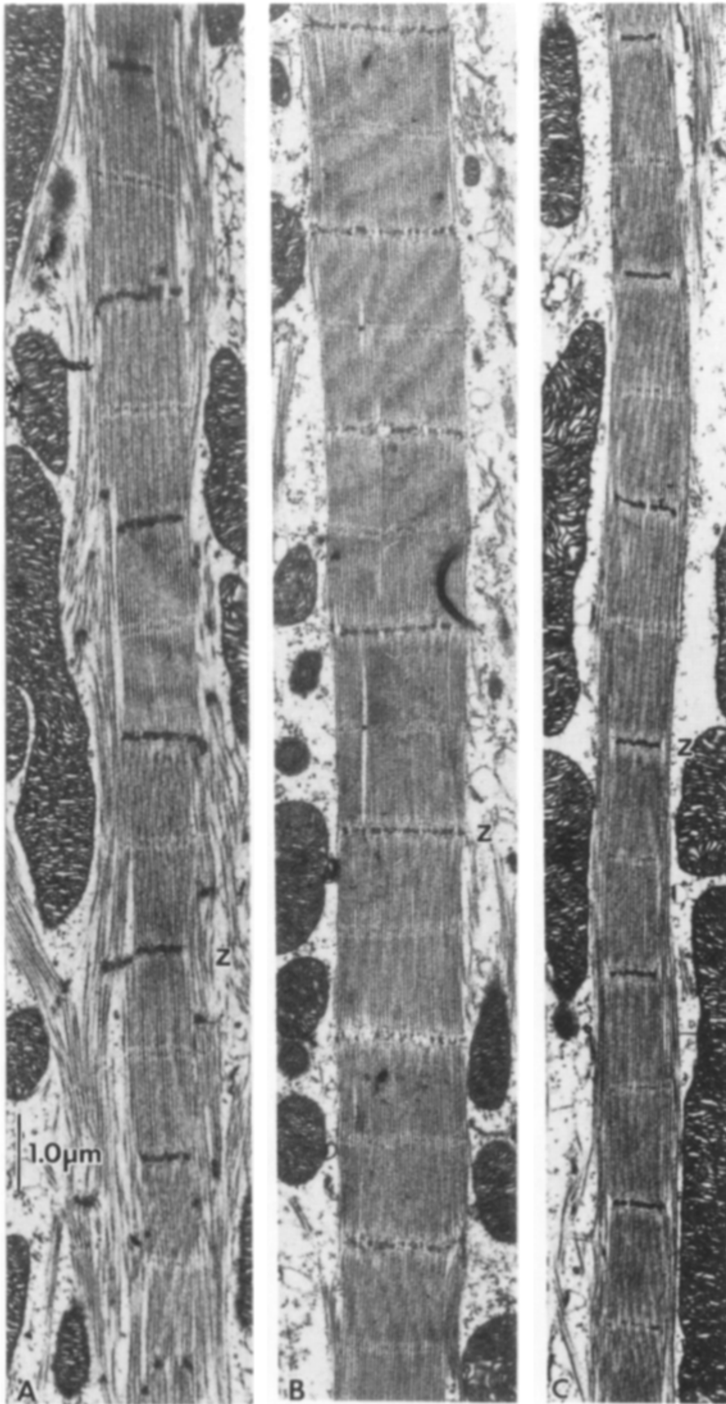

Figure 8. Low magnification views of 25 nm longitudinal sections of *fliA*^{2}, *fliA*^{3}, and *fliA*^{4} homozygotes. Note that each mutant has a distinct pattern of Z disc and myofibrillar lattice disruptions. *A* shows *fliA*^{2}, which has the most disrupted muscles. Z discs are nonplanar, and consequently the myofibrillar lattice is profoundly disrupted. *B* illustrates a *fliA*^{3} myofibril. The myofibrillar lattice is only marginally disrupted despite the pronounced punctate appearance of Z discs. *C* illustrates a *fliA*^{4} myofibril. Misregistered thick and thin filaments can be seen in peripheral regions of myofibrils. Bar, 1.0 μm.

Caveny, 1969; Smith et al., 1969). Each myofibril is anchored to the plasma membrane of the muscle cell by a modified terminal Z disc, which extends into a feltwork of filaments and dense material. The interdigitated "fascia adherens" junction formed between the membranes of the muscle and epithelial "tendon" cells forms a dense zigzag pattern. Bundles of microtubules extend from the dense cytoplasmic face of the "tendon" cell membrane opposite each terminal Z disc to the dense cytoplasmic surface of the "tendon" cell membrane apposed to the cuticle. Shafts of very dense material (tonofibrillae) anchor the folded membrane of the "tendon" cell to the cuticle.

The flight muscles of *fliA* mutants appeared unusual even in the course of preparing them for microscopy. We noted that muscles of all four *fliA* strains were easily detached from cuticle and readily dissociated into single fibers. This contrasts to wild-type flight muscles which are fused together and difficult to dislodge from the cuticle. Consistent with the observed mechanical weakness, EM revealed that flight muscle insertions of all *fliA* mutants are disrupted (refer to Figs. 5 *B* and 7), and that myofibrils, especially those closest to the sarcolemma, have structural defects.

Fig. 8 illustrates the myofibrillar abnormalities in more detail. *fliA*^{2} homozygotes appear most disrupted (Fig. 8 *A*).

Z-discs are discontinuous and nonplanar, and sarcomeres are irregular. The muscle side of myofibrillar insertions are completely disrupted, as can be appreciated by inspection of Fig. 7. In the terminal sarcomeres, thick and thin filaments are not held in a lattice and only a small remnant of the terminal feltwork is apparent. *fliA³* myofibrils (Fig. 8 *B*) have a rather different phenotype. Z discs appear to be planar arrays of punctate densities, and the myofibrillar lattice exhibits few discontinuities. However, the terminal sarcomeres are very disrupted (Fig. 5 *B*); the thick and thin filaments lose the hexagonal packing characterizing the rest of the fibril and the terminal feltwork is abbreviated and misshapen. *fliA⁴* flight muscles are the least disrupted of the homozygote mutants examined (Fig. 8 *C*). The principal defect is that a few peripheral filaments bypass the Z disc in most myofibrils. Again, despite these comparatively minor defects, insertions are disrupted; the terminal feltwork is irregularly shaped, and thick and thin filaments are misoriented (not illustrated).

We also examined flight muscles of heterozygotes (null/+) of the lethal *l(l)2Cb²* and *l(l)2Cb⁴* alleles. Heterozygotes are capable of flight, but we wished to directly ascertain whether flight muscles have completely wild-type structure. We found that in both mutants, the distinction between internal sarcomeres and those near insertions was striking. Sarcomeres throughout the myofibril appear normal, while the terminal sarcomeres are variably disordered. In *l(l)2Cb⁴* heterozygotes, illustrated in Fig. 9, the terminal four or five sarcomeres weave in and out of the section plane and myofilaments in the ultimate and penultimate sarcomeres spread out as if released from constraints of a Z band. The terminal feltwork varies in size and shape in both mutants. However, this perturbation of the insertions in heterozygotes is not sufficient to prevent flight.

In general, epithelial tendon cell morphology does not appear to be as disturbed as muscle structure in any of the mutants (Figs. 5 *B*, 7, and 9). One unusual feature observed in mutants was the abundance of fine, short filaments along the membrane and microtubules, which frequently give a "feathered" appearance to the microtubules, particularly in *fliA* mutants. However, the dense junctional membranes of the "tendon" and muscle cells remain folded into a zigzag pattern, even in the absence of an ordered terminal sarcomere or a terminal feltwork. Microtubules cross the "tendon" cell opposite a myofibril insertion even when the terminal sarcomere is disrupted. The density of the junctional membrane areas does not appear reduced in any of the mutants, despite the disruption or reduced density of Z discs and the apparently weakened connection of the myofibrils to the sarcolemma. Apparently the epithelial tendon cell can develop almost normally even when insertions of myofibrils into the sarcolemma are very abnormal.

Discussion

Alpha-actinin is an important component of both myofibrils and cytoskeleton. In both contexts, its role is probably to cross-link actin filaments. However, the in vivo consequences of these cross-links or the means by which they are quantitatively and qualitatively regulated are not well understood. A more comprehensive view of cytomechanics therefore necessitates a more detailed knowledge of the structure and function of alpha-actinin and related microfilament cross-linking proteins.

Molecular genetic techniques used in conjunction with ultrastructural methods offer a means by which to perturb and assess in vivo function of alpha-actinin and other contractile and cytoskeletal proteins. The combined approach offers sufficient resolution to refine our understanding of the cellular roles of the various proteins, and in addition may help to delineate functions of particular polypeptide domains. As a first step toward developing such an approach we have reported here the isolation of a gene which encodes alpha-actinin of *Drosophila*, and partial characterization of several mutant alleles.

The sequence encoded by the *Drosophila* alpha-actinin gene is very similar to chicken smooth muscle, skeletal muscle, and fibroblast isoforms, having roughly 68% residue identity with each. The proposed actin binding sequence, spanning residues 25–244 of the chicken smooth muscle sequence, is very highly conserved (∼86% residue identity) between *Drosophila* and the chicken isoforms, and highly conserved (60% residue identity) even when compared with the primitive eukaryote, *Dictyostelium*. This degree of sequence similarity is expected in light of the slow evolution of actin. Significant sequence similarities are also seen in central repeats and EF handlike sequences. In the case of central repeats the observed levels of sequence similarity (repeats 1, 2, 3, and 4 are 70, 65, 59, and 46% conserved, respectively, between *Drosophila* and the chicken smooth muscle isoform), as well as the observation that alpha-actinin central repeats are related to those of spectrin and dystrophin (see Blanchard et al., 1989) may imply that these repeats interact with cellular macromolecules that, like actin, are slowly evolving. It is also clear from the collective data that individual repeats, and in particular repeat 4, are under significantly different functional constraints, as they are evolving at different rates.

Regarding the EF handlike sequences, one difference between *Drosophila* and chicken muscle specific isoforms is that both EF handlike sequences of *Drosophila* could theoretically be functional. Inspection reveals that the amino-terminal *Drosophila* EF hand, unlike its chicken smooth and skeletal muscle counterparts, could coordinate calcium binding at the calcium-liganding Y position. Both *Drosophila* sequences also meet the requirements for functional EF hands outlined by Kretsinger (1980), whereas in both chicken muscle isoforms one of the EF-hand-like pairs fails this same test. This result suggests that alpha-actinin encoded by this *Drosophila* gene could function in nonmuscle cells, where actin binding is typically calcium sensitive. Conversely, it is not clear why calcium sensitive actin binding would be tolerated in a sarcomeric context, where alpha-actinin is thought to stably cross-link actin filament arrays. Direct testing of the ability of the *Drosophila* sequences to bind calcium will be required to substantiate whether or not the *Drosophila* EF handlike sequences are indeed functional.

That the *l(l)2Cb* mutants are due to chromosome rearrangements within the sarcomeric *Drosophila* alpha-actinin gene is clear from our described Southern blotting experiments. We conclude that *l(l)2Cb* hemizygotes produce no alpha-actinin, as rearrangement breakpoints associated with

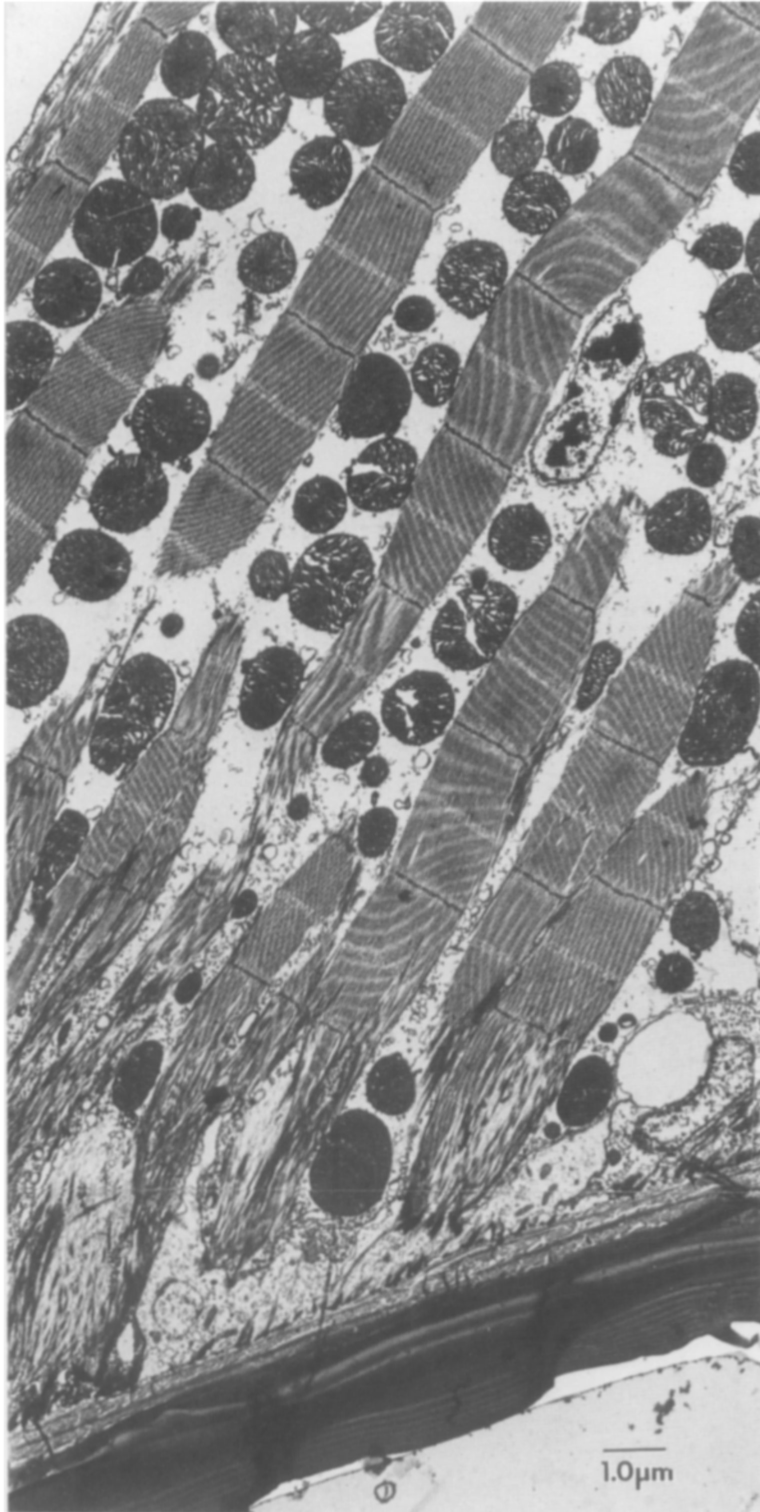

Figure 9. Disruption of myofibrillar termini in *l(1)2Cb*[4] heterozygotes. Note that the terminal four or five myofibrils weave in and out of the plane of section, and that the terminal feltwork is reduced. Despite these abnormalities, the muscles can apparently generate enough tension to permit some flight. Bar, 1.0 μm.

all four mutants analyzed are located within 5′ portions of the genes, and all *l(1)2Cb* mutants have identical lethal phases. One unexpected finding which will require further investigation concerns the effects of these null alleles on development. Perrimon et al. (1985) observed that *l(1)2Cb* hemizygotes complete embryogenesis and die as second instar larvae even when maternal RNA transcripts of the gene are eliminated from oocytes. That embryonic development is unaffected demonstrates that alpha-actinin encoded by the herein described gene is not required for the differentiation of larval tissues, including muscles, or for initial larval movements requisite for hatching from the embryonic eggshell. We have noted, however, that these larvae become progressively paralyzed within 24 h and die soon thereafter.

Why do these null mutations not impede embryogenesis or initial muscle function? There are two obvious explanations, and choosing between them will necessitate further studies of the accumulation and utilization of *Drosophila* alpha-actinin and related proteins. On the one hand, it is possible that nonmuscle alpha-actinin is encoded by a distinct gene. If this is the case, the encoded alpha-actinin must have a rather different sequence than that described here, because Southern blots performed at low stringency have not revealed evidence for any additional closely related genes. On the other hand, it is possible that demands for alpha-actinin function during embryogenesis can be met by other proteins having similar roles. Only after embryogenesis would a sarcomeric form of alpha-actinin become absolutely essential, perhaps because the amounts or specific functions of alpha-actinin required for skeletal muscle assembly could not be met by other functionally overlapping or redundant proteins. With respect to this hypothesis it is interesting to note that comparisons to all available polypeptide sequences revealed that alpha-actinin is related to spectrin and dystrophin (Byers et al., 1989). All three proteins may cross-link microfilaments in submembranous meshworks, opening the possibility that they may be able to functionally complement one another. Indeed, prior work has suggested that *Dictyostelium* mutants lacking alpha-actinin are not functionally impaired (Walraff et al., 1986; Schleicher et al., 1988; Noegel and Witke, 1988), and certain muscular dystrophy patients lacking most of dystrophin sequence manifest a relatively mild Becker syndrome (Koenig et al., 1988). A straightforward explanation for both observations is functional complementation of one member of the protein superfamily by another.

Electron micrographs of flight muscles in *fliA* hemizygotes and *l(1)2Cb* heterozygotes complement one another in highlighting the differential effects of the mutations on central versus terminal Z discs. Each hemizygous *fliA* mutant is associated with both disrupted myofibrillar attachments and distinctive Z disc defects. The distinctive phenotypes conferred by particular *fliA* alleles are consistent with our tentative conclusion that they are point mutations within the structural gene encoding sarcomeric alpha-actinin. It will be of interest to further investigate these phenotypes and correlate them with the amino acid replacements conferred by each, especially if the different mutants are located within distinct alpha-actinin domains.

EM of the *l(1)2Cb* null allele heterozygotes reveals that only the muscle insertions are affected. This may be due to lack of proper (stoichiometric) levels of alpha-actinin in heterozygotes, similar to effects observed in actin and myosin heavy chain heterozygotes (Beall and Fyrberg, 1989). However, in light of models for myofibril formation in which new sarcomeres are assembled at the ends of the muscle fiber (Goldspink, 1972; Peristanis and Gregory, 1971), the preferential disruption of terminal sarcomeres might reflect a more dynamic and biochemically distinct situation, perhaps constant incorporation of new alpha-actinin subunits, or requirements for interactions of proteins not present elsewhere in the myofibril.

Despite the described defects in flight muscle fibers, the epithelial tendon cell appears much less affected in the alpha-actinin mutants (Figs. 5, 7, and 9). The persistence of both dense junctional membrane folds and oriented microtubules, even in the absence of the modified terminal Z disc, suggest that the effects on the tendon cell are secondary to the disruption of muscle insertions by mutations within the described alpha-actinin gene.

The cloned *Drosophila* alpha-actinin gene and its mutant alleles represent an excellent model system for future studies of alpha-actinin in both muscle and nonmuscle cells. The muscle phenotypes associated with extant alleles can be evaluated and correlated with particular amino acid replacements determined by sequencing mutant alleles. In addition, it is now possible to manufacture mutant alleles using site-directed mutagenesis. These can be introduced into the null alpha-actinin background using P element-mediated transformation. Effects on assembly and function of site-directed mutations within the actin-binding, spectrin repeat, and EF-hand-like domains can be systematically evaluated in both muscle and nonmuscle cells.

We thank Dr. Belinda Bullard, Geoff Butcher, and the AFRC Monoclonal Antibody Centre, Cambridge, for providing mAb MAC 274, Drs. Norbert Perrimon and Ted Homyk for providing *l(1)2Cb* and *fliA* stocks, Drs. Paul Salvaterra and Tom Kornberg for making available *Drosophila* cDNA libraries, and Lang Walker for photographic assistance. Some of the work reported here was carried out at the ICRF Developmental Biology Unit, Oxford University, and we thank Dr. David Ish-Horowicz for kindly providing laboratory space and supplies.

This work was supported by grants from the National Institutes of Health and Muscular Dystrophy Association of America to E. A. Fyrberg, and by grants from the same agencies to Mary C. Reedy and Michael K. Reedy.

Received for publication 5 December 1989 and in revised form 9 February 1990.

Note Added in Proof. We have recently discovered that an intron of 75 nucleotides resides between codons 843 and 844 of the *Drosophila* alpha-actinin gene.

References

Arimura, C., T. Suzuki, M. Yanagisawa, M. Imamura, Y. Hamada, and T. Masaki. 1988. Primary structure of chicken skeletal muscle and fibroblast alpha-actinin deduced from cDNA sequences. *Eur. J. Biochem.* 177:649–655.

Auber, J. 1963. Jonction myo-epidermique des Dipteres. *J. Microscopie (Paris).* 2:325–336.

Auber, J., and R. Couteaux. (1963). Ultrastructure de la strie dans des muscles de dipteres. *J. Microscopie (Paris).* 2:309–324.

Baron, M. D., M. D. Davison, P. Jones, B. Patel, and D. Critchley. 1987*a*. Isolation and characterization of a cDNA encoding chick alpha-actinin. *J. Biol. Chem.* 262:2558–2561.

Baron, M. D., M. D. Davison, P. Jones, and D. R. Critchley. 1987*b*. The sequence of chick alpha-actinin reveals homologies to spectrin and calmodulin. *J. Biol. Chem.* 262:17623–17629.

Beall, C. J., M. A. Sepanski, and E. A. Fyrberg. 1989. Genetic dissection of *Drosophila* myofibril formation: effects of actin and myosin heavy chain null alleles. *Genes & Dev.* 3:131–140.

Benton, W. D., and R. W. Davis. 1977. Screening lambda gt recombinant clones by hybridization to single plaques in situ. *Science (Wash. DC).* 196:180–182.

Blanchard, A., V. Ohanian, and D. Critchley. 1989. The structure and function of alpha-actinin. *J. Muscle Res. Cell Motil.* 10:280–289.

Burridge, K., and J. R. Feramisco. 1981. Non-muscle alpha-actinins are calcium-sensitive actin-binding proteins. *Nature (Lond.).* 294:565–567.

Byers, T. J., A. Husain-Chisti, R. R. Dubreuil, D. Branton, and L. S. B. Goldstein. 1989. Sequence similarity of the amino-terminal domain of *Drosophila* beta spectrin to alpha-actinin and dystrophin. *J. Cell Biol.* 109:1633–1642.

Caveny, S. 1969. Muscle attachment related to cuticle architecture in Apterygota. *J. Cell Sci.* 4:541–559.

Chen, W. L., and S. J. Singer. 1982. Immunoelectron microscopic studies of the sites of substratum and cell-cell contacts in cultured fibroblasts. *J. Cell Biol.* 95:205–222.

Cheng, N., and J. F. Deatherage. 1989. Three dimensional reconstruction of the Z disk of sectioned bee flight muscle. *J. Cell Biol.* 108:1761–1774.

Deatherage, J. F., N. Cheng, and B. Bullard. 1989. Arrangement of filaments and cross-links in the bee flight muscle Z disk by image analysis of oblique

sections. *J. Cell Biol.* 108:1175–1782.

Duhaiman, A. S., and J. R. Bamburg. 1984. Isolation of brain alpha-actinin. Its characterization and comparison of its properties with those of muscle alpha-actinins. *Biochemistry.* 23:1600–1608.

Endo, T., and T. Masaki. 1982. Molecular properties and functions in vitro of chicken smooth muscle alpha-actinin in comparison with those of striated muscle alpha-actinins. *J. Biochem. (Tokyo).* 92:1457–1468.

Gall, J. G., and M.-L. Pardue. 1971. Nucleic acid hybridization in cytological preparations. *Methods Enzymol.* 21:470–480.

Geiger, B., K. T. Tokuyasu, and S. J. Singer. 1979. Immunocytochemical localization of alpha-actinin in intestinal epithelial cells. *Proc. Natl. Acad. Sci. USA.* 76:2833–2837.

Goldspink, G. 1968. Sarcomere length during post-natal growth of mammalian muscle fibres. *J. Cell Sci.* 3:539–548.

Goldstein, M., J. Schroeter, and R. Sass. 1982. The Z-band lattice in a slow skeletal muscle. *J. Muscle Res. Cell Motil.* 3:333–348.

Homyk, T., and C. P. Emerson, Jr. 1988. Functional interactions between unlinked muscle genes within haploinsufficient regions of the *Drosophila* genome. *Genetics.* 119:105–121.

Imamura, M., T. Endo, M. Kuroda, T. Tanaka, and T. Masaki. 1988. Substructure and higher structure of chicken smooth muscle alpha-actinin molecule. *J. Biol. Chem.* 263:7800–7805.

Karlik, C. C., J. W. Mahaffey, M. D. Coutu, and E. A. Fyrberg. 1984. Organization of contractile protein genes within the 88F subdivision of the *D. melanogaster* third chromosome. *Cell.* 37:469–481.

Koenig, M., A. P. Monaco, and L. M. Kunkel. 1988. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell.* 53:219–228.

Kretsinger, R. H. 1980. Crystallographic studies of calmodulin and homologs. *Ann. NY Acad. Sci.* 356:14–19.

Lai-Fook, J. 1967. The structure of developing muscle insertions in insects. *J. Morphol.* 123:503–528.

Lazarides, E., and K. Burridge. 1975. Alpha-actinin: immunofluorescent localization of a muscle structural protein in non-muscle cells. *Cell.* 6:289–298.

Lindsley, D., and Zimm, G. 1986. The genome of *Drosophila melanogaster*, Part 2: lethals; maps. *Dros. Inf. Serv.* 64:8–9.

Maniatis, T., R. D. Hardison, E. Lacy, J. Lauer, C. O'Connell, D. Quon, G. K. Sim, and A. Efsradiatis. 1978. The isolation of structural genes from libraries of eucaryotic DNA. *Cell.* 15:687–701.

Masaki, T., M. Endo, and S. Ebashi. 1967. Localization of the 65 component of alpha-actinin at Z-band. *J. Biochem. (Tokyo).* 62:630–632.

Maxam, A., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* 65:499–560.

Mimura, N., and A. Asano. 1987. Isolation and characterization of a conserved actin binding 27 kd fragment of actinogelin and alpha-actinins and mapping of their binding sites on the alpha-actinin molecule by chemical cross-linking. *J. Biol. Chem.* 262:4717–4723.

Noegel, A. A., and W. Witke. 1988. Inactivation of the alpha-actinin gene in *Dictyostelium. Dev. Genet.* 9:531–538.

Noegel, A., W. Witke, and M. Schleicher. 1986. cDNA clones coding for alpha-actinin of *Dictyostelium discoideum. FEBS (Fed. Eur. Biochem. Soc.) Lett.* 204:107–109.

Noegel, A., W. Witke, and M. Schleicher. 1987. Calcium-sensitive non-muscle alpha-actinin contains EF-hand structures and highly conserved regions. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 221:391–396.

Peristanis, G., and D. Gregory. 1971. Early stages of flight muscle development in the blowfly *Lucilia cuprina*: a light and electron microscope study. *J. Insect. Physiol.* 17:1005–1022.

Perrimon, N., L. Engstrom, and A. P. Mahowald. 1985. Developmental genetics of the 2C-D region of the *Drosophila* X-chromosome. *Genetics.* 111:23–41.

Reedy, M. K. 1968. Ultrastructure of insect flight muscle. Screw sense and structural grouping in the rigor cross-bridge lattice. *J. Mol. Biol.* 31:155–176.

Reedy, M. K., and M. C. Reedy. 1985. Rigor crossbridge structure in tilted single filament layers and flared-X formations from insect flight muscle. *J. Mol. Biol.* 185:145–176.

Reedy, M. C., C. Beall, and E. Fyrberg. 1989. Formation of reverse rigor chevrons by myosin heads. *Nature (Lond.).* 339:481–483.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

Sato, M., W. H. Schwartz, and T. D. Pollard. 1987. Dependence of the mechanical properties of actin/alpha-actinin gels on deformation rate. *Nature (Lond.).* 325:828–830.

Schleicher, M., A. Noegel, T. Schwartz, E. Wallraff, M. Brink, J. Faix, G. Gerish, and G. Isenberg. 1988. A *Dictyostelium* mutant with severe defects in alpha-actinin: its characterization using cDNA probes and monoclonal antibodies. *J. Cell Sci.* 90:59–71.

Smith, D. S., U. Jarlfors, and F. E. Russell. 1969. The fine structure of muscle attachments in a spider (*Latrodectus mactans Fabr.*) *Tissue & Cell.* 1:673–687.

Smith, S. J. 1988. Neuronal cytomechanics: the actin-based motility of growth cones. *Science (Wash. DC).* 242:708–714.

Tokuyasu, K. T., A. H. Dutton, B. Geiger, and S. J. Singer. 1981. Ultrastructure of chicken cardiac muscle as studied by double immunolabeling in electron microscopy. *Proc. Natl. Acad. Sci. USA.* 78:7619–7623.

Walraff, E., M. Schleicher, M. Modersitski, D. Regier, G. Isenberg, and G. Gerisch. 1986. Selection of *Dictyostelium* mutants defective in cytoskeletal proteins: use of an antibody that binds to the ends of alpha-actinin rods. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:61–67.

Wensink, P. C., D. G. Finnegan, J. E. Donelson, and D. S. Hogness. 1974. A system for mapping sequences in the chromosomes of *Drosophila melanogaster. Cell.* 3:315–325.

Yamaguchi, M., M. Izumimoto, R. Robson, and M. Stromer. 1985. Fine structure of wide and narrow vertebrate muscle Z-lines. A proposed model and computer simulation of Z-line architecture. *J. Mol. Biol.* 184:621–644.

Young, R. A., and R. W. Davis. 1983. Yeast RNA polymerase II genes: isolation with antibody probes. *Science (Wash. DC).* 222:778–782.