STRUCTURE AND ASSEMBLY OF THE MYOFIBRIL

Organizers: Richard Lymn, Donald Fischman and Henry Epstein

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Structure and Assembly of the Myofibril

Principles of Self-Assembly

CA 001 What can we Learn from the Genetic Analysis of Protein Folding and Subunit Assembly in Prokaryotes? Jonathan King, Dept. of Biology, MIT, Cambridge, MA 02139.
For prokaryotic organelles such as bacterial viruses and flagella, all of the genes and proteins involved in assembly have been identified. This information combined with the discrete nature of the structures has permitted detailed dissection of their assembly pathways. In considering myofibril assembly the following results from prokaryotic assembly processes may be of interest: 1) Amino acid sequences controlling the folding of polypeptide chains are dispersed through the polypeptide chain; 2) In vivo folding and assembly intermediates have their own conformations, not just a subset of the native structure; 3) Subunit assembly processes often involve incompletely folded precursors, such that the mature structure is formed only after association; 4) Scaffolding proteins and chaperonins interacting with such precursors are common; 5) With polymeric structures getting started generally requires additional information compared to keeping going; 6) Control of polymerization involves a conformational switching of the subunits; 7) The conformational switching does not occur in solution but on the growing sites of the growing structure; 8) The precise lengths of polymeric phage tails are determined by extended protein molecules which are not extended prior to the polymerization process; 9) Enzymatic activity of components of large structures are often modulated by conformational transformations of the entire structure (hundreds of subunits); 10) Where function requires mobile subunit interfaces - moving parts - assembly jigs and templates are often required to insure correct assembly.

Assembly Properties of Contractile Proteins

CA 002 MATCHING MOLECULES IN MUSCLE ASSEMBLY, Carolyn Cohen, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254
Fibrous proteins are frequently characterized by periodic features which provide precise recognition sites for complementary arrays. The assembly and functioning of muscle exemplify this notion. Tropomyosin filaments wind in the grooves of the actin helix so that a recognition site on each half-turn of the tropomyosin coiled coil makes equivalent interactions with a complementary site on one actin monomer. Troponin is also precisely positioned on the thin filament, possibly through periodic ionic interactions of its elongated tail portion with tropomyosin. Switching from the "off" to the "on" state of contraction involves large-scale molecular motions of tropomyosin: both conserved and variable linkages are determined by the strength and number of these recognition sites. The assembly and functioning of myosin thick filaments involve similar principles. Common periodicities in the acidic and basic residues of myosin and paramyosin in invertebrates are identical so that both self-assembly and co-assembly appear to be largely specified by intermolecular ionic interactions. Similarly, both conserved and variable linkages are involved in changes in the myosin/paramyosin interactions in different functional states. Other proteins which assemble with the thick filament may be expected to show matching periodicities. The matching and mismatching between the periodicities of myosin and actin filaments also account for certain aspects of contractile activity.

CA 003 FOLDING AND ASSOCIATION OF TROPOMYOSIN PEPTIDES, Alfred Holtzer and Marilyn Emerson Holtzer, Department of Chemistry, Washington University, St. Louis, MO 63130.

Extant and new experiments on the thermal unfolding equilibria of coiled coils, particularly tropomyosin and isolated segments of it, are summarized. These data are examined according to two extant models of the equilibrium, i.e. the "all-or-none-segments" (AONS) model in which substantial segments are supposed to undergo a two-state unfolding and the "continuum-of-states" (COS) model in which all conformational states are supposed to be possible, subject to statistical weighting, and in which loop entropy is accounted for. It is shown that the AONS model is demonstrably in disagreement with some of the data while the COS model is not. Neither model is entirely satisfactory. Both agree that local segment stabilities depend not only upon local intrinsic properties, but also upon neighboring segments, possibly through their influence on the details of side-chain packing in the helix-helix interface. The profound implications this has for the development of a guiding physical picture of the thermodynamic stability of coiled coils are discussed. Helix-helix interactions can also be examined by study of hybridization (heterodimer formation) of homodimeric coiled coils. Extant and new evidence is summarized showing that a) native protein coiled coils are kinetically as well as thermodynamically stabilized at or below room temperature; b) heterodimeric coiled coils may be more or less stable than their homodimeric counterparts; c) just above room temperature, the mechanism of hybridization is by dissociation-reassociation; d) the rate constant for the dissociation of the tropomyosin coiled coil into chains at 36°C is ~ 8×10^{-5} s^{-1}. Some implications of these data for the mechanism of folding and unfolding the coiled coil are discussed.

CA 004 **ACTIN FILAMENT REGULATION OF MYOSIN ASSEMBLY: IMPLICATIONS FOR MYOFIBRIL FORMATION.** Joel D. Pardee, Rohit K. Mahajan, Kevin T. Vaughan, and Julie A. Johns, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021.

Pre-fusion myoblasts contain non-muscle isoforms of actin and myosin that drive cell locomotion and are present during myotube formation and myofibrillogenesis. Because myofibril assembly presumably must take place within complex arrays of cortical actin filaments, we have investigated how actin, myosin interactions might give rise to primitive myofibrils or stress fiber-like structures in non-muscle cells. Using cytoskeletal proteins isolated from Dictyostelium amoebae, a model cytogel system was reconstituted in vitro that allowed for kinetic regulation of myosin assembly into thick filaments, and formed primitive myofibrillar structures as a result of controlled myosin assembly. When non-muscle myosin from Dictyostelium is assembled in the presence of skeletal muscle or non-muscle actin filaments, a 5-fold acceleration of myosin assembly ensues. Fluorescence energy transfer (FET) assays indicate that accelerated assembly requires transient binding of myosin molecules to actin filaments before incorporation into thick filaments. Association of unassembled myosin to F-actin is shown to be a key regulatory rate-limiting step for thick filament formation in the presence of actin filaments. Assembly is therefore controlled by nucleotide-dependent actin, myosin interactions and/or phosphorylation of myosin. The architecture of the actin filament network is also implicated in regulation of thick filament formation. Fragmentation of F-actin networks by Dictyostelium severin, or bundling by a 34kD filipodial protein present in Dictyostelium and cultured fibroblasts abolishes accelerated assembly. Of keen interest for morphogenesis of myofibrils are the structures generated during actin-mediated myosin assembly. When assembly is allowed to take place in the absence of actomyosin contraction (in AMPPNP or with specifically phosphorylated myosin), thick filaments form along actin filaments, spontaneously transforming random F-actin networks into structures that resemble primitive myofibrils. Subsequent addition of ATP or myosin dephosphorylation results in contraction. Actin filament bundles created by alpha-actinin or 34kD protein can also be laterally aligned during myosin assembly, forming fibers containing myosin thick filaments in parallel with actin filaments. Taken together, these data suggest a potential model for an initial stage of myofibrillogenesis in which site-directed assembly of non-muscle myosin on cortical actin networks causes rearrangement of F-actin into contractile fibers.

Structure and Assembly of the Myofibril

Genetic Analysis of Myofibrillar Assembly

CA 005 FUNCTIONS OF THE MYOSIN ATP- AND ACTIN-BINDING SITES ARE REQUIRED FOR C. elegans THICK FILAMENT ASSEMBLY, Amy Bejsovec and Philip Anderson, Dept. of Genetics, Univ. of Wisconsin, Madison, WI, 53706.

We have investigated C. elegans mutants in which missense substitutions of a myosin heavy chain gene disrupt thick filament assembly in body-wall muscle cells. These mutants exhibit a dominant, muscle-defective phenotype. As heterozygotes, the animals are muscle-defective and paralyzed, because altered myosin heavy chain B (MHC B), the product of the unc-54 gene, disrupts assembly of wild-type MHC B. This contrasts with mutations that completely eliminate MHC B, which as heterozygotes are phenotypically wild-type and assemble large numbers of well-organized thick filaments. The mutant MHC B product of dominant unc-54 mutations is unstable in vivo. Strongly dominant alleles accumulate an amount of mutant MHC B that can be less than 2% of that found in wild-type animals.

We have determined the positions and sequences of 31 such assembly-disruptive unc-54 mutations. All 31 alleles are missense substitutions affecting the globular head of myosin. The most strongly dominant alleles alter highly conserved residues of the myosin ATP binding site. Other alleles alter conserved residues throughout the myosin head, including residues in the site at which myosin binds actin. The positions and sequences of unc-54 dominant mutations indicate that functions of the ATP- and actin-binding sites are needed for C. elegans thick filament assembly.

CA 006 MOLECULAR GENETIC ANALYSIS OF MYOSIN HEAVY CHAIN ISOFORM FUNCTION IN *DROSOPHILA MELANOGASTER*. S.I. Bernstein, P.T. O'Donnell, W.A. Kronert, V.L. Collier, K.A. Edwards, K.D. Becker, N.K. Hess, W.N. Callen, E.S. Roche and L. Wells. Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, California 92182.

Drosophila melanogaster has a single muscle myosin heavy chain gene which produces multiple MHC isoforms via alternative RNA splicing. Sequence analysis of the entire gene and numerous cDNA clones revealed that most alternative exons encode regions of the globular head (these may differentially affect ATPase activity, force generation, actin or myosin light chain binding). In the rod coding region, a portion of the hinge is encoded by alternative exons as is the C-terminus of the protein. Isoforms that differ in these regions may have different assembly or force generation characteristics. Analysis of a series of flightless MHC mutants in conjunction with *in situ* tissue hybridization using alternative exon-specific probes has revealed remarkable complexity and muscle-specificity of MHC isoform accumulation. For instance, one of the two alternative hinge coding regions is only used in some adult muscles and is not used in larval muscles. A mutation in the splice junction preceding this alternative exon results in muscle-specific defects in thick filament assembly and muscle function. Mutations in an alternative exon encoding a highly conserved region near the actin binding sites of the globular head also affect MHC RNA or protein accumulation in a muscle-specific fashion. Interestingly a single amino acid substitution in this area that changes a uniformly conserved glutamic acid to a lysine results in MHC turnover, suggesting that this portion of the protein is important for thick filament assembly and/or stability. Using P element-mediated germline transformation with the entire MHC gene, we are attempting to rescue these mutant phenotypes. We have already defined a region at the 5' end of the gene that permits high level MHC gene transcription in all muscle types. Using this promoter in conjunction with portions of the MHC gene and various cDNAs, we are constructing MHC genes that are restricted in the alternative exons that they can use. Transformation of such a gene into an MHC mutant would produce the "incorrect" MHC isoform in a given muscle. Subsequent ultrastructural, biochemical and physiological measurements should elucidate the function of such isoform-specific protein domains.

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CA 007 DROSOPHILA MYOFIBRIL FORMATION: DEFECTS ENGENDERED BY MUTANT ALLELES OF CONTRACTILE PROTEIN GENES,
Clifford Beall, Mary C. Reedy*, and Eric Fyrberg, Department of Biology, The Johns Hopkins University, Charles and 34th Streets, Baltimore, MD 21218 and *Department of Cell Biology, Duke University Medical Center, Durham, NC 27710.
We are employing molecular genetic methods to investigate myofibril formation in flight muscles of Drosophila melanogaster. This approach allows us to perturb or eliminate particular contractile proteins and evaluate effects on myofibrillogenesis. We will describe defects associated with null alleles of genes encoding actin, myosin heavy chain, and alpha-actinin as well as those engendered by site directed mutation of actin.

CA 008 GENES IMPLICATED IN MUSCLE ASSEMBLY IN THE NEMATODE C. ELEGANS, R. Waterston, R. Barstead, J. Waddle and B. Williams. Department of Genetics, Washington University, St. Louis MO 63110

The nematode Caenorhabditis elegans allows the in vivo assessment of functions necessary for normal muscle assembly. The principal muscle of C. elegans is associated with the body wall and is necessary for movement and viability. This muscle can be readily observed in the whole animal with the light microscope, and a battery of specific monoclonal antibodies can be used to assay a variety of muscle components.

The nematode vinculin gene has been recovered through antibody screening of an expression library, and its position on the physcial map ascertained. Using this information, we have identified two mutations affecting vinculin, one resulting in the lack of the protein and the other apparently producing a truncated protein. The nature of the vinculin mutations and their effects on the assembly of other muscle components are currently being determined. We anticipate that the mutants will also be useful in identifying genes for other interacting proteins.

CapZ proteins bind the barbed end of actin filaments and have been postulated to be involved in nucleating or otherwise regulating the assembly of thin filaments in vertebrate striated muscle. Using the polymerase chain reaction we have recovered candidate genes for both CapZ subunits in the nematode. Localization of these genes on the physical map of C. elegans will permit a genetic dissection of their function in muscle assembly.

Although more than 40 muscle affecting genes have been identified in C. elegans, recent experiences with the myosin and vinculin genes shows that other genes remain to be identified. Mutants of the vinculin and myosin genes result in arrest at the same developmental stage, and cause an almost complete loss of the ability to move. We have begun looking for additional mutants which confer the same phenotype with the expectation of identifying other components essential for muscle development and assembly. Several of the mutants isolated are more severe alleles of previously known genes, and show that these genes are essential for muscle function. In addition, several new genes have been identified, and are candidates for genes playing critical roles in the assembly or function of striated muscle. The similarity in phenotype of these animals indicates that muscle function is required for the completion of normal embryogenesis.

Structural Analysis of Invertebrate Filaments and Fibrils

CA 009 THICK FILAMENTS OF CAENORHABDITIS ELEGANS: COMPLEX STRUCTURES ASSEMBLED VIA INTERMEDIATE ASSEMBLAGES, Henry F. Epstein, Philip R. Deitiker, Kenneth C. Lerner, Douglas L. Casey and Irving Ortiz, Departments of Neurology and Biochemistry, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

The thick filaments of 95 body-wall muscle cells in the nematode C. elegans contain two myosin isoforms, homodimeric for A (myo-3) and B (unc-54) heavy chains. Myosins A and B are assembled into central and polar zones, respectively. The differential assembly is dependent upon a third major protein, paramyosin (unc-15) which forms a substratum. Paramyosin, in turn, is organized about core structures which contain several additional proteins. Core proteins are being identified by specific monoclonal antibodies. The assembly of the core proteins, paramyosin and the two myosins requires an intermediate structure, the multifilament assemblage (MFA). MFAs contain at least two nascent thick filaments extending from at least one end of central paracrystalline paramyosin structure. Mutants in four thick filament-related genes accumulate MFAs of varying lengths and filament number. These genes are unc-15, unc-54 and myo-3, already described, and unc-82, a putative paramyosin kinase gene. Mutant MFAs (e73, unc-15) are adsorbed to glass and visualized by immunofluorescence microscopy following reaction with antimyosin and antiparamyosin antibodies. Incubation with homologous 25000 g supernatants containing mutant paramyosin, myosin-actin, 5 mM ATP and 5 mM $MyCl_2$ leads to a 1.7 fold increase in thick filaments per MFA over 120 min. In contrast to previous studies of myosin polymerization in vitro, no changes in physical-chemical conditions including pH, ionic strength or protein concentration were required. Thick filaments or soluble proteins alone do not show any increase in filament number under otherwise identical conditions. When e73 MFAs were incubated with similar fractions from wild-type in which paramyosin is native, a 3.8 fold increase in thick filaments per MFA is observed. This in vitro rescue of a mutant structure by wild-type protein suggests that the MFAs are likely assembly intermediates.

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CA 010 MYOFIBRIL ASSEMBLY IN DROSOPHILA FLIGHT MUSCLE: MUTANTS AND DEVELOPMENTAL MODELS. MARY C. REEDY*, CLIFF BEALL@ AND ERIC FYRBERG@ *Department of Cell Biology, Duke University Medical Center, Durham, N.C. 27710. @ Department of Biology, Johns Hopkins University, Baltimore, Md. 21218.

Recent EM observations of myofibrillar defects associated with mutations of flight-muscle-specific actin or sarcomeric α-actinin genes reveal varying degrees of disruption of sarcomere assembly, especially affecting Z-lines. In one site-directed actin mutant, $Act88F^{G6AA7T}$, the conversion of amino acids 6 and 7, Gly-Ala to Ala-Thr, results in multilevel Z-lines which are bypassed at the fibril periphery by several untethered thick and thin filaments. In the actin mutant $Act88F^{I76Val}$, peripheries of many Z-lines extend into stress-fiber-like appendages which trail alongside the myofibrils. In several α-actinin mutants, the terminal sarcomeres and myofibril insertions are more disrupted than the rest of the sarcomeres. Each of these assembly defects fits best with a different contending developmental model, i.e., that new flight muscle sarcomeres are formed by transverse splitting of existing Z-bands (1), that myofibrils assemble on stress-fiber templates (2), or that new sarcomeres are added at the ends of the muscle (3). It is not known whether any of these models describe sarcomere assembly in flight muscle(IFM) of Drosophila, since no modern ultrastructural studies of developing Drosophila IFM in vivo have been reported. We have therefore conducted an EM study of the development of the IFM of normal Drosophila, in order to establish a foundation which will allow evaluation of effects single-site mutations on sarcomere assembly and function.

(1) Auber, J. 1969. J.Microsc.8: 197-232. (2)Dlugosz.A., Antin,P.,Nachmias, V. and Holtzer,H. 1984 J.Cell.Biol. 99:2268-2278. (3)Peristanis,G. and Gregory,D. 1971. J. Insect Physiol. 17: 1005-1022.

CA 011 STRUCTURE AND INTERACTIONS OF INVERTEBRATE THICK FILAMENTS.

Peter Vibert and Loriana Castellani, Rosenstiel Center, Brandeis University, Waltham, MA 02254.

The thick filaments in scallop striated muscles, which are about 2 μ long and 350 Å in diameter, exemplify one class of invertebrate filaments that contain less than 10% paramyosin. Their structure differs both from the tubular form of insect and crustacean filaments (also containing little paramyosin) and from the much thicker filaments of smooth and obliquely striated muscles, which contain large amounts of paramyosin and in some cases other core proteins. Native scallop filaments and synthetic filaments assembled from purified scallop myosin contain subfilaments of about 100 Å diameter. These appear to be equal in number to the rotational symmetry of the filament (eg. 7-fold for native filaments from the scallop *Placopecten magellanicus*), and to coil around each other. The location of paramyosin is not yet apparent. Native scallop filaments terminate in protrusions similar to the "end-filaments" associated with vertebrate striated muscle thick filaments, possibly reflecting the presence of the invertebrate form of titin. In the relaxed state, the myosin heads on the filament surface are arranged in a stable helical array. When native filaments are activated by Ca^{2+}, the myosin heads leave their helical positions in a reversible, cooperative transition. Synthetic filaments show similar behavior. This disordering occurs also when ATP is removed, when regulatory light chains are dissociated from the myosin heads, or when a myosin head sulfhydryl is modified. The order-disorder transition may thus be significant for light chain-dependent regulation, with head-head and/or head-rod interactions restricting the ability of myosin heads to interact with actin in the relaxed state.

Vertebrate Thin Filaments

CA 012 TROPOMYOSIN AND TROPONIN T ISOFORMS IN DEVELOPING THIN FILAMENTS, Jim J.-C. Lin, Jian-Ping Jin, Sue-Mei Wang*, and Jenny L.-C. Lin, Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242. *Dept. of Anatomy, National Taiwan Univ., Taipei, Taiwan, ROC.

Muscle development proceeds by a sequential and/or overlapping expression of many myofibrillar protein isoforms. The regulation of isoform switching, as well as the assembly and function of different isoforms are not completely understood. Using a monoclonal antibody against striated tropomyosin isoforms, we are able to selectively isolate a class of skeletal tropomyosin-enriched microfilaments from cultured muscle cells.[1] Biochemical characterization of this microfilaments isolated from myoblasts and myotubes revealed that various isoforms of actin and tropomyosin can assemble into the same set of microfilaments, presumably pre-existing microfilaments, to form the skeletal tropomyosin-enriched microfilaments, which will eventually become the thin filaments of myofibrils. By using the same approach, we have now been able to isolate the striated tropomyosin-enriched microfilaments from skeletal and cardiac muscles of chicken embryos at various stages of development. Biochemical characterization of these isolated microfilaments also revealed a coexistence of nonmuscle and muscle isoforms of actin and tropomyosin in the earlier developmental samples. The colocalization of different isoforms of tropomyosin in the same cells was further confirmed by double-label immunofluorescence with isoform-specific monoclonal antibodies. Furthermore, chicken cardiac troponin T found in the isolated microfilaments appeared to exist in embryonic and adult isoforms. The isoform switching in chicken hearts starts as early as in 5-d-old embryo and completes switching posthatch. Using DNA cloning, Cooper and Ordahl[2] have previously shown that two isoforms of cardiac troponin T are derived from a single gene via developmentally regulated alternative splicing. Recently, we have also found two isoforms of troponin T in developing rat hearts, although isoform switching did not occur until postpartum and the switching completes within 2 weeks after birth.[3] To study the regulation of troponin T isoform switching in rat hearts, cDNA and genomic cloning have been carried out. Through the nucleotide sequencing of cDNA clones and the S1 nuclease analysis of mRNA, we have concluded that two isoforms of rat cardiac troponin T are also generated from the same gene by differential RNA splicing.[4]

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2. Cooper, T. and C. Ordahl. (1985) J. Biol. Chem. 260:11140-11148.
3. Jin, J.P. and J.J.-C. Lin. (1988) J. Biol. Chem. 263:7309-7315.
4. Jin, J.-P. and J.J.-C. Lin. (1989) J. Biol. Chem. 264:14471-14477.

CA 013 REGULATION OF THIN FILAMENT ASSEMBLY IN EMBRYONIC MUSCLE. TAKASHI OBINATA, HIROSHI ABE and SUMIKO OHSHIMA, Department of Biology, Faculty of Science, Chiba University, Yayoi-cho, Chiba 260, Japan.
There is a large pool of non-polymerizable actin in embryonic skeletal muscle which contains α-, β- and γ- actins, while in adult muscle, only α-actin is present and the majority of this protein is assembled in thin filaments of myofibrils. Since purified embryonic muscle actin is polymerizable to the same degree as adult muscle actin, it was postulated that certain factors inhibit actin polymerization in embryonic muscle. We purified and characterized three actin binding proteins, the M_r of which are 16 kDa, 19 kDa and 20 kDa, respectively, from chicken embryonic skeletal muscle. As judged by their size and functional properties, we concluded that they are the same or akin to actin binding proteins of non-muscle tissues, that is profilin (16 kDa), ADF (an actin-depolymerizing factor)(19 kDa), and cofilin (20 kDa), respectively. The sequences of the ADF- and cofilin-like proteins exhibited extraordinarily high homology in spite of their different properties. Profilin and ADF-like proteins are detectable only at embryonic stages in skeletal muscle as complexes with G-actin. In contrast, the cofilin-like protein exists through developmental stages. This protein also formed a complex with G-actin in embryonic muscle but in addition, it was localized in actin filament bundles (or rods) in cultured muscle cells by immunocytochemistry, while in adult, it was free from actin molecules. Profilin exhibited a much higher affinity for β- and γ-actins than for α-actin in the embryonic muscle and inhibited the polymerization of β- and γ-actins more effectively in vitro, suggesting that the assembly of three actin isoforms is regulated differently in the developing muscle. Cofilin- and ADF-like proteins inhibited polymerization of -actin strongly, but the inhibitory action was removed by myosin. We conclude that actin polymerization in muscle cells is suppressed by the inhibitory proteins at embryonic stages, but is accelerated during development, because actin isoform changes from β- and γ- to α-type, the inhibitory proteins decreases in amount and the myosin amount increases with age. Myosin-dependent actin assembly may be important for ordered assembly of actin atthe early phase of myofibrillogenesis.

CA 014 STUDIES ON THE STRUCTURE AND FUNCTION OF THE REGULATORY COMPONENTS OF SKELETAL MUSCLE USING SITE-DIRECTED MUTANTS OF MYOSIM LIGHT CHAIN, TROPONIN C AND TROPONIN I. Fernando C. Reinach, Dept. de Bioquimica, Instituto de Quimica da Universidade de Sao Paulo, C.P. 20.780 SP Brazil.

Using site directed mutants of cloned troponin C, we have located residues involved in the calcium induced conformational change. Mutations in these residues, which are outside the metal binding sites, alter calcium binding to the isolated troponin C. When these mutants are incorporated into skinned fibers, replacing the endogenous troponin C, alterations in the pCa versus tension curves were observed. To understand the nature of these alterations, calcium dependent binding of troponin C mutants to troponin I were studied using recombinant troponin I produced in E. coli. Based on the study of these mutants a preliminary model for the route of information transfer within the troponin complex will be proposed.

In order to analyse the structural features which confer specificity and affinity to the metal binding sites present in troponin C and myosin light chain 2, we have constructed and analysed hybrid proteins containing different combinations of domains from troponin C and myosin light chain. Calcium binding loops or whole helix-loop-helix (EF hand) were transplanted from the troponin C gene into equivalent positions in the myosin light chain gene. The study of the proteins expressed by these hybrid genes allow the determination of the extent of functional domains within these calcium binding proteins.

Thick Filament Assembly

C A015 DISTRIBUTION OF HEAVY CHAIN ISOFORMS IN NATIVE MYOSIN MOLECULES AND THICK FILAMENTS OF DEVELOPING CHICKEN SKELETAL MUSCLE, Everett Bandman, B. Kerwin, and L.D. Taylor, Department of Food Science and Technology, University of California, Davis, CA 95616.

Myosin, the major contractile protein of the thick filament, is a hexameric protein comprised of two heavy chains and 4 light chains. The heavy chain subunit is represented by a highly homologous family of isoforms which are differentially expressed during muscle development. We have produced a library of monoclonal antibodies which distinguish between the different isoforms. Using these antibodies, we investigated the distribution of myosin heavy chain isoforms in native myosin molecules, in native thick filaments, and in myofibrils *in situ* during chicken muscle development. In order to determine whether myosin could be composed of two different heavy chain isoforms we developed a double antibody sandwich ELISA. Purified monoclonal antibodies were bound to a microtiter plate and incubated with myosins purified from muscle at different stages of development. Bound myosin was then probed with biotinylated antibodies specific to epitopes present in the same isoform, or with antibodies specific to epitopes of different isoforms. Bound biotinylated antibodies were detected with streptavidin peroxidase. Our results demonstrate that very little myosin heavy chain heterodimer exists within cells expressing multiple isoforms. Studies of isolated thick filaments from muscle at these stages decorated with gold-conjugated monoclonal antibodies demonstrated that filaments could be heterogeneous in myosin content. Similar results were obtained with ultracryosections of developing muscle using gold-conjugated antibodies. However, the distribution of the different isoforms was not random in either isolated filaments or in myofibrils *in vivo*. The absence of heavy chain heterodimers in myosin synthesized *in vivo* suggests that either the mRNAs for different isoforms are spatially segregated within the cell, or that different heavy chain isoforms cannot form dimers. The latter possibility is being investigated using an *in vitro* assay for myosin assembly. Our observations on the non-random distribution of different isoforms within isolated thick filaments and myofibrils *in situ* is not compatible with a simple exchange process occurring *in vivo*. Our studies suggest that the assembly of myosin containing structures during muscle cell growth may be an ordered process occurring at specific sites within the muscle cell.

CA 016 SEQUENCE ANALYSIS OF cDNAs ENCODING AVIAN SKELETAL C-PROTEIN AND 86 KD PROTEIN: INTRACELLULAR MEMBERS OF THE IMMUNOGLOBULIN FAMILY. Steven Einheber, Kevin T. Vaughan, and Donald A. Fischman. Dept. of Cell Biology and Anatomy, Cornell University Medical College, New York, New York, 10021.

Thick filaments in vertebrate skeletal muscle are associated with a number of accessory proteins, many of which have no known function. In chicken muscle, these include: C-protein, myomesin, M-protein, M-CK, and 86kd protein. With the exception of M-CK, the primary sequence of these proteins is unknown. It has been suggested that C-protein may play a role in thick filament assembly, alignment, or muscle contraction. 86 kd protein has been shown to colocalize with C-protein in a series of periodic stripes in the crossbridge zone of the thick filament. We have begun a molecular genetic analysis of these proteins to learn more about their structure and function. cDNAs for C-protein and 86 kd protein have been isolated from a neonatal chicken pectoralis cDNA library. Sequence analysis of the C-protein clone reveals that it contains nine regions of internal homology. Six of these resemble immunoglobulin domains and three resemble fibronectin domains. Until recently, these sequence motifs have only been described in extracellular and cell-surface proteins. However, similar domains have now been reported in other proteins associated with the thick filament including smooth muscle myosin light chain kinase, twitchin and titin (Einheber and Fischman; Benian et al.; Trinick, unpublished results). In addition, preliminary sequence comparison of the 86 kd and C-protein clones indicate close homology in their carboxyl segments. Thus, the 86 kd protein may be another intracellular member of the C-2 set of the immunoglobulin family. (Supported by NIH AM32147 and MDA)

CA 017 DIFFERENTIAL DISTRIBUTION OF SUB-SETS OF MYOFIBRILLAR PROTEINS IN NON-STRIATED AND STRIATED MYOFIBRILS, Howard Holtzer, Thomas Schultheiss, Zhongxiang Lin, and Donald A. Fischman, Dept. of Anatomy, University of Pennsylvania, Philadelphia, PA 19104; and Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021.

Cultured cardiac myocytes were stained with antibodies to sarcomeric α-actinin, troponin-I, α-actin, myosin heavy chain (MHC), myomesin, C-protein, MAbs to two different titin epitopes, and vinculin. Attention was focused on the distribution of these proteins with respect to non-striated myofibrils (NSMFs) and striated myofibrils (SMFs). In NSMFs, α-actinin is found as longitudinally-aligned, irregular ~0.3μm aggregates. Such aggregates are associated with α-actin, troponin-I, and titin. These I-Z-I-like complexes are also found as ectopic patches outside the domain of myofibrils in close apposition to the ventral surface of the cell. MHC is found outside of SMFs in the form of ~1.6μm long fibrils. The temporal-spatial distribution and accumulation of the MHC-fibrils with respect to the I-Z-I-like complexes varies greatly along the length of the NSMFs. Unlike the constant ratio of MHC to I-Z-I proteins that obtains in SMFs, there is a vast excess of I-Z-I proteins in NSMFs and ectopic patches. The transition between the terminal ~1.7μm sarcomere of any given SMF and its distal NSMF-tip is abrupt and is marked by a characteristic narrow α-actinin Z-band and vinculin-positive adhesion plaque.

A titin antibody, T20, which localizes to an epitope at the Z-band in SMFs, precisely co-stains the 0.3μm α-actinin aggregates in ectopic patches and NSMFs. Another titin antibody, T1, which in SMFs localizes to an epitope at the A-I junction, typically does not stain ectopic patches and NSMFs. Where detectable, the T1-positive material is adjacent to rather than part of the 0.3μm α-actinin aggregates. Myomesin and C-protein are found only in their characteristic sarcomeric locations -- even in just perceptible SMFs. These A-band-associated proteins appear to be absent in ectopic patches and NSMFs.

The major features of these myofibrillar protein distributions are also seen in differentiating skeletal muscle cells from standard myogenic cultures, as well as in pigment cells converted into postmitotic myoblasts by the gene MyoD1. In HH Stage 16-17 chick myotomes, standard skeletal myogenic cultures, and MyoD1 converted cells, desmin is expressed prior to the myofibrillar proteins. The myofibrillar proteins are then expressed roughly concurrently. We can detect no significant lag between the appearances of titin, α-actinin, myosin, or any of the other myofibrillar proteins within a single myocyte.

CA 018 **ARCHITECTURE AND ASSEMBLY OF THE SARCOMERE MATRIX**

Kuan Wang. Clayton Foundation Biochemical Institute, Department of Chemistry, and Cell Research Institute, University of Texas at Austin.

The sarcomere of striated muscle has recently been shown to contain a cytoskeletal matrix that coexists with thick and thin filaments and is consisting of two giant proteins, titin and nebulin.

Titin, which appears in solution as extremely long (over 1.0μm), flexible and slender (4nm) strands with axial periodicity, behaves as a thick filament scaffold or template and connects thick filaments to the Z line. Immunoelectron microscopic studies of the disposition of titin epitopes in sarcomeres of various lengths suggest (1) a single titin molecule spans the entire distance from the M line region to the Z line and is parallel but external to thick filaments; (2) the continuous titin filament is intrinsically elastic along the entire length. However, in intact sarcomeres, only the I-band segment exhibits elastic-stretch dependence. The stretching of the titin segment within the A band is prevented, perhaps by its interaction with thick filaments. The lack of elastic-stretch dependence of its epitopes suggests that nebulin constitutes a set of inextensible longitudinal filaments anchored at the Z line.

The structural model of the sarcomere matrix adds a new dimension of complexity to the architecture of muscle sarcomere and is being explored as a structural basis to explain muscle elasticity and myofibrillogenesis as well as other phenomena that cannot be satisfactorily accounted for by the widely accepted two-filament sarcomere model.

Structure and Assembly; Genetic and Developmental Aspects; Protein Interactions

CA 100 COFILIN AND AN ADF-LIKE ACTIN BINDING PROTEIN IN EMBRYONIC CHICKEN SKELETAL MUSCLE: CLONING AND CHARACTERIZATION OF THEIR cDNAS, Hiroshi Abe, Takeshi Endo, and Takashi Obinata, Department of Biology, Chiba University, Yayoi-cho, Chiba 260, Japan.
We previously isolated two actin regulatory proteins with apparent mol wt of 19,000 and 20,000 (called 19K and 20K proteins) from embryonic chicken skeletal muscle and characterized their effects on actin polymerization; 19K protein is very similar to ADF, an actin depolymerizing facter of chicken brain, whereas 20K protein is akin to cofilin which was discovered in mammalian brain. In order to clarify the molecular structures of these proteins, we constructed a λgt11 cDNA library from mRNA of embryonic chicken skeletal muscle. The cDNA clones for 19K and 20K proteins were isolated by screening the library with the antibodies specific for each protein. With these cDNAs as probes, full-length cDNA clones were obtained and their sequences were determined. The deduced amino acid sequence of 20K protein was very similar to that of cofilin of porcine brain, and in addition, it also exhibited high homology with 19K protein, although 19K and 20K proteins shows distinct functional properties. As judged by the nucleotide sequences, it is obvious that the two proteins are derived from different genes. It was observed that the expression of two proteins are regulated differently during the development of skeletal muscle by Northern blotting with cDNA probes and immunoblotting with the antibodies to respective proteins; that is, the expression of 20K protein persists through developmental stages of skeletal muscle, while that of 19K protein is down-regulated during development.

CA 101 TRUNCATED CARDIAC TROPONIN T cDNA EXPRESSION IN SKELETAL MUSCLE, Page A.W. Anderson, Brian K. Kay, and Nadia N. Malouf, Department of Pediatrics, Duke University, Durham, NC 27710, Departments of Biology and Pathology, University of North Carolina, Chapel Hill, NC 27514.

Troponin T (TnT) expression in cardiac muscle has been found to be developmentally and regionally regulated. In chicken myocardium, TnT expression has been shown to be the result of alternative splicing of an N-terminal exon. The importance of this N-terminal heterogeneity in myofibril formation and function in cardiac muscle is unknown. We have recently demonstrated, using one- and two- dimensional PAGE and immunoblots, the presence of five TnT isoforms in rabbit myocardium that appear to differ immunologically in their N-terminal regions from the fast skeletal muscle isoforms. To explore the basis of these differences and their functional significance, we have probed an adult rabbit cardiac gt10 library (kindly provided by Dr. D.H. MacLennan), using a chicken cardiac TnT clone (kindly provided by Drs. T.A. Cooper and C.P. Ordahl). A 1.13 kb cDNA was isolated that encoded a truncated (aa 1-200) rabbit cardiac TnT with > 98% homology to the published adult TnT isoform. We will present our use of this truncated cardiac TnT cDNA in the study of myotube formation in culture, following electroporation of the cDNA into skeletal myoblasts.

CA 102 MUTATIONS IN THE DROSOPHILA TROPONIN I (*heldup*) GENE CAUSE A VARIETY OF DEFECTS IN SARCOMERIC STRUCTURE, Clifford J. Beall and Eric A. Fyrberg, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218
The troponin complex regulates muscle contraction in response to calcium. The complex is composed of three subunits: the calcium binding troponin C, the tropomyosin binding troponin T, and the inhibitory troponin I (TnI). We have analyzed a muscle specific clone derived from the 16F-17A polytene region (1) and show that it encodes at least two TnI isoforms generated by alternate splicing. The sequence of the Drosophila proteins are 25-30% identical to vertebrate isoforms, and about 65% identical to the sequence from another arthropod, the crayfish (2). Drosophila TnIs contain a 30 amino acid N terminal extension relative to vertebrate skeletal muscle isoforms, as do vertebrate cardiac TnIs. Flightless mutations in the *heldup* (*hdp*) gene (3), map to the same genomic region (4). These mutations have been analyzed by PCR amplification of TnI mRNA and electron microscopy of indirect flight muscles. The hdp^3 and hdp^5 alleles lack an alternately spliced mRNA encoding one isoform of the protein. These mutants contain only disorganized filaments with no recognizable sarcomeric structure. The hdp^2 allele is altered by the substitution of valine for alanine at residue 55 (corresponding to residue 25 in rabbit fast skeletal TnI). hdp^2 causes a variety of effects including the production of sarcomeres of incorrect length and width. This phenotype suggests that troponin either has a direct structural role in myofibril assembly, or that the regulation of actin-myosin interactions is important in assembly.

1. Falkenthal et al., M.C.B. 4, 956.
2. Kobayashi et al., J.B.C. 264, 1551.
3. Deak et al., J.E.E.M. 69, 61.
4. Homyk and Emerson, Genetics 119, 105.

CA 103 REVERSIBLE ORGANIZATION OF DESMIN FILAMENTS IN CULTURED RAT ATRIAL AND VENTRICULAR MYOCYTES. A. Borisov[1] and T. Saetersdal[2]. [1]Institute of Cytology, Leningrad, USSR; [2]University of Bergen, Norway.
Desmin (D) distribution was studied by immunofluorescence microscopy in freshly isolated and cultured atrial (A) and ventricular (V) myocytes from rats of different ages. Desmin-containing intermediate filaments (IF) were found at Z-band levels and in areas of intercalated discs (ID) in both cryostat sections and in freshly isolated adult cells. After 2-5 d in vitro, the adult cells began to lose this cross-striated pattern of D distribution. At this period a diffuse staining reaction was observed and significantly less reactivity for D was found at the ID, especially in A myocytes. Although embryonic and early neonatal myocytes exhibited prominent D-containing IF, very few of these IF were confined to the Z-band levels. After 2-3 wk in culture, some of the V cells (both newborn and adult) exhibited D cross-striations, predominantly in the central regions of the myocytes where some mature myofibrils were present. Extensive myofibrillogenesis was observed near ruffles and cytoplasmic processes of the plasma membrane and these sites contained radial arrays of D IF. Where such processes of adjacent cells came into contact, intercellular junctions formed which contained D and appeared to be forming ID. In contrast to V myocytes, A cells rarely reformed D cross-striations of ID in culture. During mitosis of A, embryonic and neonatal V cells the myofibrils partially disassembled and D-containing IF were localized into small aggregates widely dispersed around the spindle but there was considerable variability between different cells of the culture.

CA 104 RELATIONSHIP OF MYOSIN mRNA AND MYOFIBRIL ASSEMBLY IN RAPID GROWTH AND REPAIR. Brenda R. Eisenberg, David J. Dix and Paul H. Goldspink. Department of Physiology, University of Illinois at Chicago, IL 60680.
The relationship between myofibril assembly and rapid growth may be regulated by anatomical distribution of mRNA. Slow myosin message (sMHC) was localized using in situ hybridization (ISH) with a biotinylated riboprobe detected in the light microscope with streptavidin-alkaline phosphatase and in the electron microscope by 5 nm IgG gold conjugates. Rabbits were immobilized for 4 days in full plantarflexion to stretch tibialis anterior muscle. In all fibers there is a perinuclear gradient of sMHC mRNA and the normal subsarcolemmal annulus of sMHC mRNA increased dramatically. In damaged fibers sarcomeres are misaligned and polysomes are seen clustered between the myofibrils in EM. In LM of stretched fibers ISH shows mRNA in patchy concentrations within the core of some fibers. Ultrastructural distribution of MHC mRNA was examined by ISH in papillary muscles growing rapidly under the influence of thyroid hormone. Specific cytoplasmic densities of myosin mRNA were calculated by counting clusters of 5 or more gold particles over respective tissue components. The density in the inter-myofibrillar spaces is 3 times the myofibrils but no A band accumulation was seen. It appears that myosin is synthesized all along the entire fibril periphery and is not coupled directly to myofibril assembly. Supported by NIH HL40880, American Heart and Muscular Dystrophy Associations.

CA 105 STRUCTURAL STATES OF THE Z BAND IN CARDIAC AND SKELETAL MUSCLE. Margaret A. Goldstein, John P. Schroeter, J.P. Bretaudiere*, Lloyd H. Michael, and Ronald L. Sass**, Department of Medicine, Baylor College of Medicine, Houston, TX 77030, *Department of Pathology, Univ. of Texas Medical School, Houston, TX 77030, and **Department of Biology, Rice Univ., Houston, TX 77251.
We have shown that the two structural states of the Z band lattice seen in cross section (ss and bw) in cardiac and skeletal muscle are related to the contractile state of the muscle. The lattice responds to active tension but resists passive deformation. The change in Z band form and dimension is correlated with cross-bridge binding. We have used two-dimensional image processing techniques on digitized electron micrographs to compare each lattice form in cardiac and skeletal muscle with both real space averaging and fourier filtering methods. The purpose of these studies is to enhance structural features that are concomitant with the observed changes in dimension. All images from all lattices showed an approximate four-fold symmetry. Every image showed cross-connecting filaments of greater or lesser curvature which appeared to connect each axial filament to its four nearest neighbors. In the ss form, the cross-connecting filaments overlap in projection, with the area of overlap lying half-way between axial filaments. All ss images exhibited a reduced Z band spacing compared to the bw images. This decrease in spacing is sufficient to cause the cross-connecting filament overlap observed in the ss images. The images revealed differences in the appearance of axial filaments in adjacent rows which would maintain thin filament polarity within the Z lattice. We conclude that the Z band is an essential and dynamic part of the myofibril.

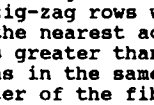
CA 106 NON-UNIFORM BINDING OF PHALLOIDIN TO MYOFIBRIL THIN FILAMENTS, Marion L. Greaser and Benita E. Schnasse, Muscle Biology Laboratory, University of Wisconsin, Madison, WI 53706

Previous studies (Wilson et al. Biochem. Cell Biol. 65:376, 1987) reported that fluorescent phalloidin stained myofibrils in patterns inconsistent with the known locations of thin filament actin. In the present work, rabbit psoas, bovine psoas, and bovine cardiac myofibrils were incubated with rhodamine or fluorescein phalloidin. Non-uniform fluorescence patterns were observed in most myofibrils from both skeletal and cardiac muscle. The intensities were usually greatest at the Z lines and at the free ends of the thin filaments. The non-uniformity of staining occurred over a wide range of phalloidin to actin ratios (0.03 to 1.0). Extraction of myofibrils with phosphate-pyrophosphate solutions removed the phase dense A bands, altered the position of titin after staining with monoclonal 9D10, but left the phalloidin patterns intact. The patterns were unchanged in the presence or absence of Ca^{++}. Tryptic or chymotryptic digestion of myofibrils removed the phase dense Z lines and also reduced the phalloidin staining at the Z lines. Although the cause of the differences in phalloidin binding in different regions of the thin filament remain to be identified, these studies demonstrate that actin monomers (presumably due to associated proteins) are not all equivalent in the sarcomere.

CA 107 ELASTIC BEHAVIOR OF TITIN FILAMENTS DURING THICK FILAMENT MOVEMENT IN ACTIVATED SKELETAL MUSCLE Robert Horowits, Koscak Maruyama* and Richard J. Podolsky NIAMS, NIH, Bethesda, MD 20892 and *Chiba University, Chiba, Japan

Titin (also called connectin) is a huge striated muscle protein that binds to thick filaments and links them to the Z-disc. We used a monoclonal antibody to study the behavior of titin in both relaxed and activated skinned rabbit psoas fibers by immunoelectron microscopy. In relaxed fibers, antibody binding is visualized in the I-band as two extra striations per sarcomere arranged symmetrically about the M-line. These striations move away from both the nearest Z-disc and the thick filaments when the sarcomere is stretched, confirming the elastic behavior of titin within the I-band of relaxed sarcomeres as previously observed by several investigators. When the fiber is activated, thick filaments in sarcomeres shorter than 2.8 μm tend to move from the center to the side of the sarcomere. This translocation of thick filaments within the sarcomere is accompanied by movement of the antibody label in the same direction. In that half sarcomere in which the thick filaments move away from the Z-disc, the spacings between the Z-disc and the antibody and between the antibody and the thick filaments both increase. Conversely, on the side of the sarcomere in which the thick filaments move nearer to the Z-line, these spacings decrease. Regardless of whether I-band spacing is varied by stretch of a relaxed sarcomere or by active sliding of thick filaments within a sarcomere of constant length, the spacings between the Z-line and the antibody and between the antibody and the thick filaments increase with I-band length in identical manner. These results indicate that the titin filaments remain bound to the thick filaments in active fibers, and that the elastic properties of titin are unaltered by calcium ions and cross-bridge activity.

CA 108 IN SITU DISSOCIATION OF THICK FILAMENTS AND LATTICE EXPANSION IN RABBIT SKELETAL MUSCLE, Richard J. Podolsky and Maria L.F. Barbosa, Laboratory of Physical Biology, NIAMS, NIH, Bethesda, Md. 20892. Rigor muscle fibers expand laterally when ionic strength is lowered and/or pH is raised. In fibers transferred from a 50 mM solution at pH 7.0 to one at pH 8.5, the thick filaments also expand and become hollow cylinders where they overlap with thin filaments. This contrasts with isolated myosin filaments, which dissociate into 3 subfilaments at low ionic strength (Maw and Rowe, 1980. Nature 24: 412). The difference in dissociation pattern is presumably due to the presence of rigor crossbridges which connect each myosin filament to six surrounding actins and cause the myosin filament to break into more than three subfilaments. In a 10 mM solution at pH 8.5 the thin filament lattice is no longer made of regular hexagons. Rather, actins arrange themselves in zig-zag rows which are mirror images of each other. The distance between the nearest actins in different zig-zag rows, d_1, is greater than the distance between nearest actins in the same row, d_2. This property makes the diameter of the fibril increase more than the X-ray lattice spacing, d. Lattice and thick filament expansion can both be reversed by adding 3mM Mg^{++}. The results confirm the importance of electrostatic interactions in maintaining the integrity of the thick filament backbone and show that this structure is stabilized by Mg^{++}.



CA 109 EXCHANGE OF FLUORESCENTLY-LABELED MYOSIN BETWEEN SYNTHETIC FILAMENTS AND INTACT MYOFIBRILS, Anuradha D. Saad and Ignatius P. Tan, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York 10021.
The exchange of myosin between synthetic thick filaments and intact myofibrils was examined by fluorescence microscopy. Myosin purified from adult chicken pectoralis muscle was labeled with rhodamine-isothiocyanate and prepared into synthetic thick filaments. These were combined with myofibrils in a microscope slide chamber and the exchange of myosin out of synthetic filaments and into myofibrils was followed by observing the increase in A-band fluorescence over time. Extensive exchange of rhodamine-labeled myosin into myofibrils was detected when filaments were mixed with myofibrils in buffer containing MgATP (0.125M KCl, 10mM PO_4, pH 6.8, 0.1mM PMSF, 2.5mM EGTA, 5mM $MgCl_2$, 5mM ATP). The exchange was specific for the A-bands and no attachment of labeled myosin to the I-bands was observed. ATP was required for exchange of myosin into myofibrils. Experiments carried out in the presence of GTP or ADP resulted in no significant A-band fluorescence. Exchange of myosin between synthetic filaments (in the absence of myofibrils) did not require MgATP. When the rhodamine-labeled filaments were crosslinked using EDC, exchange was inhibited and incorporation of the fluorescently-labeled myosin into the A-bands of the myofibrils could not be detected. These results indicate that myosin molecules can readily exchange between synthetic thick filaments and intact myofibrils. The exchange reaction requires the presence of dissociated crossbridges and is presumably mediated by a kinetically active pool of soluble myosin. (supported by the MDA and NIH AM37653)

CA 110 THE ASSEMBLY OF SKELETAL MUSCLE TROPOMYOSIN IS PATHWAY DEPENDENT. IDENTITY OF SEQUENCES THAT DETERMINE HOMO- AND HETERODIMER FORMATION, Fred Schachat, Edward K. Williamson, and H. Richard Brown, Department of Cell Biology, Duke University Medical School, Durham, North Carolina 27710
In vivo the skeletal muscle tropomyosin dimer assembles from and subunits forming both homo- and heterodimers. The amino acid sequence homology of the subunits, and the presumption that assembly of coiled-coil proteins would be equilibrium-controlled led a priori to the assumption that the subunits would sort randomly. However, the composition of Tm dimers in rabbit skeletal muscles shows that the heterodimer is the preferred in vivo species, while most slow "equilibrium-type" renaturation procedures reveal a clear preference for homodimer formation. These experimental results show that there are multiple pathways for Tm dimer formation and suggest that formation of the Tm dimer may be kinetically-directed. Studies on Tm renaturation indicate that different regions of the subunits specify hetero and homodimer formation. A sequence of 30 to 40 amino acids in the N-terminal region of the Tm subunits appears to specify heterodimer assembly, while the sequences around the cysteine at position 190 appear to be responsible for homodimer assembly. The identification of the sequences that specify heterodimers is supported by studies on non-muscle tropomyosins, while a model based on a conformational free energy scale for core positions in coiled-coil proteins appears to explain the formation of homodimers.

CA 111 SEQUENCE REQUIREMENTS FOR MYOSIN FILAMENT ASSEMBLY, R.Sohn and L.Leinwand. Dept. of Microbiology and Immunology. Albert Einstein College of Medicine, Bronx, New York 10461.
To determine the sequence requirements for myosin filament assembly, we have expressed portions of a human skeletal myosin heavy chain (MHC) rod in *E. coli*. Four fragments of a cDNA clone encoding an adult human fast skeletal myosin isoform have been cloned into the pIN expression vector (Nakamura and Inouye (1982) EMBO J. 1: 771). Two of these constructs also express proteins resulting from an internal initiation site, bringing the total number of segments of the rod examined to five. Of the 1937 amino acids of the MHC, the constructs include DNA fragments encoding: I. residues 1082-1471; II. residues 1589-1902 (internal start); III. residues 1274-1729; IV. residues 1589-1729 (internal start); and V. residues 1709-1902. Bacteria harboring these constructs overexpress their respective proteins at 10-50 mg/liter of culture. These proteins have been purified and analyzed for assembly as assessed by their solubility properties and paracrystalline organization. Within the same bacteria, fragments can behave independently with regard to salt dependent assembly. Proteins II and IV are insoluble in 50 mM KCl while proteins I, III, and V are soluble. Protein II demonstrates a salt dependence identical to that of rabbit LMM, suggesting that the sequences necessary for myosin assembly are restricted to these 313 amino acids. The paracrystals formed by protein II have the characteristic 430 A periodicity, but appear qualitatively different from rabbit LMM paracrystals. To define further the size and sequence requirements for assembly, construct II was deleted from the carboxyl end to generate 6 additional constructs. These constructs express proteins from ~8kD to ~30kD spaced ~5kD apart. These constructs will be analyzed for their assembly properties.

CA 112 MOLECULAR INTERACTIONS IN ASSEMBLIES OF ALPHA-HELICAL COILED-COILS IN MYOSIN AND INTERMEDIATE FILAMENTS,
Murray Stewart, Simon Atkinson, Simon Clarke, Rob Moir, Roy Quinlan, Reinhard Rachel and Sue Whytock, Medical Research Council Laboratory of Molecular Biology, Hills Rd, Cambridge CB2 2QH, ENGLAND.
The interactions between the alpha-helical coiled-coil rod domains of myosin and intermediate filament proteins dominate assembly of these molecules into functional higher aggregates. We have employed a combination of structural and molecular biology methods to define these interactions and to explore the molecular basis of coiled-coil aggregation. Electron microscopy and computer image processing of crystals and paracrystals has defined key elements of molecular structure, such as the coiled-coil pitch, and molecular interaction geometries. Whole proteins and defined fragments of myosin rod, glial fibrillary acidic protein (GFAP) and nuclear lamins have been expressed in high yield in *E. coli*. Directed mutagenesis has been used to produce material for crystallisation and to explore the role of particular repeating features of the coiled-coil sequences in macromolecular assembly *in vitro* and *in vivo*.

CA 113 cDNA CLONING OF 86 KD PROTEIN, A THICK FILAMENT-ASSOCIATED PROTEIN IN CHICKEN SKELETAL MUSCLE,
Kevin T. Vaughan, Steven Einheber and Donald A. Fischman. Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York, 10021.
In addition to myosin, thick myofilaments in chicken striated muscle contain at least 6 other proteins: C-protein, myomesin, M-protein, M-CK, titin and 86 kd protein. Except for M-CK, none of these proteins has a clearly defined function. 86 kd protein is a myosin-binding protein, restricted to fast twitch muscles, which is located in the C-region of the sarcomere along nine of the 43 nm stripes, seven of which are shared with C-protein (Bahler et al., J.Mol.Biol.186:381,1985). A partial clone for 86kd protein was isolated from a neonatal chicken muscle cDNA library. This clone of 1 kb hybridizes with a muscle-specific mRNA of 2.3 kb, a message size sufficient to encode the 86 kd protein. A full length cDNA was isolated from the same library using the partial clone as a probe. Sequence information from this full length clone reveals significant homology to a C-protein cDNA (Einheber and Fischman, unpublished results): with over 50% identity at the 3' end. Some regions within this sequence show complete identity. The deduced amino acid sequence is very homologous to the deduced amino acid sequence of the C-protein cDNA; 49% identity over 350 amino acids, with an additional 15% conservative changes. Notable features of this amino acid sequence include homology to repeats found in the immunoglobulin and fibronectin family of proteins. (Supported by NIH AR32147 and MDA).

CA 114 MYOSIN FILAMENT ASSEMBLY PROBED WITH MONOCLONAL ANTIBODIES.
Donald A. Winkelmann and Stephanie A. Silos. Department of Pathology, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854.
Anti-rod monoclonal antibodies were used to probe the interactions involved in myosin filament assembly, stability and structure. The epitopes defined by three antibodies have been mapped to the S2 subfragment, and the epitope for a fourth antibody maps very near the C-terminus of the rod. Myosin filaments (1 μm long) were formed by stepwise dilution of adult chicken pectoralis myosin to physiological salt conditions. Myosin was either incubated with IgG antibody prior to filament formation, or preformed filaments were incubated with antibody for periods of up to 24 hours. Electron microscopy revealed that the anti-LMM antibody blocks normal filament assembly by forming stable 0.27 μm long filaments. This antibody does not appear to periodically label filaments, but does cause a slow shift of the filament population from long filaments to short filaments. Two of the anti-S2 antibodies also block filament assembly, forming short filaments with a labeling pattern which distinguishes them from the short anti-LMM filaments. The diameter of these intermediate sized filaments is similar to that observed for myosin subfilaments. These anti-S2 antibodies label myosin filaments with an apparent repeat of 14 nm, and they do not affect filament stability. In contrast, the third anti-S2 does not interfere with normal filament assembly or stability but does periodically label filaments. These results demonstrate the separability of the nucleation and elongation interactions involved in myosin filament formation, stress the importance of the C-terminus of the LMM subfragment in filament assembly, and demonstrate that sites within the S2 subfragment can affect post-nucleation assembly interactions. (Supported by grants from the NIH and the MDA)

CA 200 ANALYSIS OF THE PARAMYOSIN AND MYOSIN HEAVY CHAIN GENES OF *DROSOPHILA MELANOGASTER*. K.D. Becker, P.T. O'Donnell and S.I. Bernstein, Biology Dept. & Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

Using an antibody made against *Lethocerous* paramyosin (provided by B. Bullard), we have isolated a 2 kilobase (kb) cDNA from a lambda gt11 bacteriophage expression library, which we believe to encode *Drosophila* paramyosin. Limited DNA sequencing of this clone has identified a single open reading frame with 20-30% identity, at the amino acid level, to other alpha-helical proteins, such as the rod region of vertebrate and invertebrate myosin heavy chains. Our clone also has 29% amino acid identity with paramyosin from the fluke, *Schistosoma mansoni*. The *Drosophila* clone hybidizes to mRNAs of ~3.2 and 3.5 kb. Both are large enough to encode the ~100 kilodalton paramyosin protein. Using the original clone as a probe, 6 additional cDNAs, with inserts of 2.5 to 3.8 kb, have been isolated. Further analysis should elucidate the structure of the two size classes of paramyosin mRNA. The position of the gene(s) is currently being identified by *in situ* hybridization to polytene chromosomes.

Using P element-mediated transformation, we have produced transgenic lines carrying the wild-type myosin heavy chain (MHC) gene in either of two vectors. Lines with the G418 resistance gene as a marker do not fully rescue the dominant flightless phenotype of MHC mutants, even when the gene has been mobilized to various chromosomal locations. However, gel electrophoresis of proteins isolated from mutant indirect flight muscle (that normally fails to produce MHC) indicates that the transgene is producing protein at ~20-30% of wild-type levels. The second MHC-P element clone used contains the 22 kb MHC gene with more 5' and 3' flanking sequences. Flies transformed with this construct are currently being characterized to determine whether the presence of additional flanking regions result in higher levels of MHC production. (Supported by NIH grant GM32443, an MDA Research Grant, and an Established Investigatorship to S.I.B. from AHA.)

CA 201 FUNCTIONAL ANALYSIS OF ALTERNATIVELY EXPRESSED EXONS OF THE MYOSIN HEAVY CHAIN LOCUS IN *DROSOPHILA* Mary Beth Davis, Gregg A.Hastings, Margaret B. Ober, and Charles P. Emerson, Jr. Department of Biology, University of Virginia, Charlottesville, VA 22901

The single copy *Drosophila* muscle myosin heavy chain (MHC) gene has a complex exon structure that produces a diversity of larval and adult muscle MHC isoforms through regulated alternate RNA splicing.[1] *In situ* hybridization studies using exon-specific probes have identified a restricted and mutually-exclusive pattern of expression of the alternative exons in adult thoracic muscles, indicating that at least three MHC isoforms are generated (G. Hastings and C. Emerson, manuscript in preparation). The indirect flight muscles contain a uniquely expressed combination of alternate exons. A different isoform is shared by the tergal depressor of the trochanter and direct flight muscle #51. In contrast, a third isoform is shared by two functionally distinct muscle types, direct flight muscle #52 and the esophagus. Germline transformation is being used to investigate the potential functional significance of this regulated expression. Based on the *in situ* hybridization results, a series of cDNA fragments corresponding to the putative muscle specific MHC isoforms are being fused *in vitro* to the MHC promoter, and the ability of the minigenes to rescue both flight and viability in various MHC mutants will be tested.

[1]George, et al. (1989), Mol. Cell Biol. **9**, 2957.

CA 202 STAGE, TISSUE, AND CELL TYPE SPECIFICITY OF MYOSIN HEAVY CHAIN PRE-mRNA SPLICING IN *DROSOPHILA*. K.A. Edwards, W.A. Kronert, E.S. Roche, P.T. O'Donnell, L. Wells & S.I. Bernstein, Biology Dept. & Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

We are investigating patterns of alternative splicing of muscle myosin heavy chain (MHC) transcripts in *Drosophila*. The MHC gene contains five families of homologous, tandemly repeated exons of identical size interspersed among 13 single copy exons. Analysis of 12-24 hour embryonic cDNAs shows that only one member of each family is spliced into any given mRNA, and that at least four different combinations of exons are used, which would encode variant amino acid sequences in three regions of the globular head. Exons 9a, 9b, and 9c encode protein regions that are 86-91% identical and lie near the actin binding sites. In larvae, 9b and 9c are used, with 9b predominant. In adults, *in situ* hybridization and northern blots show: 9a is the exclusive form found in the indirect flight muscles (IFM); 9a is co-expressed with 9b in the jump muscle and certain other thoracic muscles; 9b is expressed in all muscles but the IFM; 9c is expressed at a low level in non-thoracic MHCs. Interestingly, the jump muscle has two cell types that express 9a and 9b at different levels. We have identified three dominant flightless mutants with point mutations in 9a. Mhc^7 has a stop codon that appears to cause complete turnover of 9a-containing mRNAs. Mhc^9 changes an invariant glutamic acid to a lysine, and results in loss of thick filaments from muscles in which 9a predominates, indicating this residue is critical for the assembly or integrity of thick filaments. Mhc^{11} has a splice donor mutation that results in the accumulation of an aberrantly sized IFM transcript that includes all three copies of 9 and their associated introns up to a cryptic donor site following 9c. Thus the use of 9b and 9c is blocked in the IFM. In Mhc^{11} jump muscle, all transcripts appear to bypass 9a in favor of 9b. Thus there are distinct alternative splicing mechanisms in different muscles. The tissue-specific use of these highly homologous exons implicates several specific amino acid residues in modulating function in this area of the myosin head. (Supported by NIH grant GM32443, an MDA Research Grant, and an Established Investigatorship to S.I.B. from AHA.)

CA 203 DEEP-ETCHING REPLICA IMMUNOGOLD ELECTRON MICROSCOPIC STUDY OF DESMIN DURING CARDIAC MYOFIBRILLOGENESIS IN CULTURED NORMAL AND CARDIOMYOPATHIC HAMSTER HEART CELLS, Guan R. Hou and Larry F. Lemanski, Department of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210

Genetically cardiomyopathic (CM) hamsters, which carry an autosomal recessive gene, suffer from chronic congestive heart failure which results in premature death. Previous studies on cultured cardiac myocytes demonstrate that cardiomyopathic heart cells have significant myofibril disarray as well as fewer numbers of cellular projections when compared to normal. Since myofibrillogenesis occurs in the complex filamentous network of the cytoskeleton, desmin intermediate filaments, a major component of the cytoskeleton in cardiac myocytes, could play a role in the initiation, assembly or alignment of myofibrils. In the present study, we identified intermediate filaments using immunogold staining for desmin in combination with deep-etching replica electron microscopy. This method enabled us to examine the spatial distribution of desmin during myofibrillogenesis at very high resolution. Normal cardiac myocytes at 5 days in culture show well-organized myofibrils, with most intermediate filaments situated in the spaces between the myofibrils; however, CM myocytes show poorly organized myofibrils and the intermediate filaments appear to be arranged in random patterns. Normal cardiac myocytes after 9 days in culture show an increased organization of myofibrils with more myofilaments being packed together in parallel arrays. Intermediate filaments are detectable between the myofibrils in both normal and CM hearts after 9 days in culture; however, the filaments appear to be more randomly arranged in the CM cells. Moreover, the 9 day CM cells contain myofibrils that appear similar to those in 5 day normal cultures. Results of the present study indicate possible abnormalities in the arrangements of desmin filaments in cardiomyopathic hamster heart cells in culture, which may be related to the myofibril disarray exhibited by these cells. (Supported by NIH grants HL 32184, HL 37702 and an American Heart Association Grant to LFL).

CA 204 CLATHRIN LOCALIZATION IN THE SARCOMERE DURING MYOFIBRIL ASSEMBLY, Stephen Kaufman and Rachel F. Foster. Departments of Microbiology and Cell Biology, University of Illinois, Urbana, IL 61801. Immunofluorescence microscopy has been used to demonstrate the localization of clathrin in repetitive bands that appear soon after the fusion of skeletal myoblasts into multinucleate fibers. This organization of clathrin has been found in cultures containing myotubes that develop from explants of newborn rat hindlimb cells and in myotubes derived from the L8E63 myogenic line. Clathrin bands were also prominent in skinned fibers prepared from adult rat soleus muscle. Clathrin banding was localized in the sarcomere as a doublet, one element on either side of the Z line. An additional band within the A band, was evident in some fibers. Conditions that disrupt clathrin baskets or prevent its assembly likewise disrupt the assembly of clathrin in bands. The assembly of clathrin into sarcomeric bands occurs early in the development of the myofibrillar apparatus. Quantitation of the appearance of clathrin banding in primary cultures of myotubes indicates that it precedes that of other myofibrillar proteins and that assembly takes place in the following order: clathrin, titin, myosin and actin. The assembly of clathrin as well as myosin, titin and actin into sarcomeric bands is inhibited by chloroquine. Based on these results and considering the role of clathrin in intracellular transport and its capacity to interact with actin and a-actinin, we suggest that clathrin has diverse roles in the assembly, integrity and functioning of the sarcomere and its integration with the sarcolemma. The early assembly of clathrin into bands further suggest that clathrin may also function in the early organization of the contractile system. Supported by NIH grant GM28842.

CA 205 DEVELOPMENT OF CONNECTIN (TITIN) IN RELATION TO THE A AND Z BAND FORMATION IN CARDIAC MYOCYTES IN VITRO. M. Komiyama[1,2], K. Maruyama[3] and Y. Shimada[1], [1]Department of Anatomy, School of Medicine, Chiba University, Chiba 280; [2]Department of Anatomy, Faculty of Physical Education, The International Budo University, Katsuura 299-52; [3]Department of Biology, Faculty of Science, Chiba University, Chiba 260, Japan.

The relationship of connectin with A and Z band formation of myofibrils was studied in embryonic chick cardiomyocytes in vitro by double-fluorescence staining with various combinations of phalloidin and antibodies to connectin, myosin, α-actinin and troponin C (TnC). During premyofibril stages, α-actinin dots and diffuse actin (phalloidin and anti-TnC) staining was detected first, and then myosin and connectin dots appeared at nearly identical distribution. Later, α-actinin dots were aligned on phalloidin- or anti-TnC-positive non-striated fibrils, which were unreactive or reactive weakly with antimyosin or anticonnectin. As α-actinin dots aggregated to form Z lines, myosin and connectin became to exhibit typical striations. No difference in staining patterns was observed with two kinds of monoclonal antibodies against different domains of connectin filaments (4C9 and SM1) at early phases. As myosin staining showed clear A bands, connectin epitopes became arranged in polarized positions. The results indicate that primitive I-Z-I complexes appear prior to assembly of connectin and myosin filaments, and then connectin filaments developing intimately with myosin become associated with Z lines. Thus, the putative elastic protein connectin may play some roles in integrating myosin filaments with preexisting I-Z-I brushes. Occasional absence of A and connectin bands between two Z lines indicates that connectin is not a preformed scafforld of myofibrils, upon which sarcomeric proteins are added.

CA 206 ACTIN AND MYOSIN LIGHT CHAIN BINDING SITES ON HUMAN CARDIAC MYOSIN HEAVY CHAIN FRAGMENTS SYNTHESIZED BY E. COLI, Bernard Cornillon, Patrick Eldin, Martine Le Cunff, Monique Anoal, Jocelyne Léger, Dominique Mornet, Hans-Peter Vosberg* and Jean J. Léger, Institut National de la Santé et de la Recherche Médicale, INSERM U.300, Faculté de Pharmacie, Montpellier, France, * Max Planck Institute, Heidelberg, Federal Republic of Germany.
To investigate the structural basis of the functional differences between cardiac isomyosin, fragments of human cardiac cDNAs coding for myosin β heavy chain and ventricular light chain 1 (a gift from P. Gunning) were amplified by PCR, inserted in the coding portion of the β-galactosidase gene and expressed in E. Coli. The different synthesized protein fragments were detected by monoclonal or polyclonal antibodies specific for the particular myosin light and heavy chain domains being studied. Certain of these antibodies, called "functional" antibodies, positively or nagatively influence myosin functions, such as ATPase activity, actin binding, and myosin light-heavy chain interactions. Several synthetic myosin heavy chain fragments either in solution or blotted on a solid support, interacted with myosin light chains (or fragments) and with actin. Using the differential reactivities of some monoclonal antibodies with free light chains and native myosins, several light chain domains interacting with the myosin heavy chains were also identified. Dissection, isolation and reconstitution of some of the multiple functions within the cardiac myosin molecule are therefore possible using this approach combining molecular genetics and hybridoma technology. The next steps will involve a comparison with cardiac myosin subunits, as soon as the corresponding cDNA becomes available, mutagenesis studies involving putative "functional" amino acids, and injection of chimeric constructs into cardiac cells.

CA 207 **CELLULAR DISTRIBUTION OF SMOOTH MUSCLE ACTINS DURING MAMMALIAN EMBRYOGENESIS: EXPRESSION OF THE α-VASCULAR BUT NOT THE γ-ENTERIC ISOFORM IN DIFFERENTIATING STRIATED MYOCYTES,** J.L. Lessard and N.M. Sawtell, Children's Hospital Research Foundation, Basic Science Research, Cincinnati, Ohio 45229. The cellular distribution of the α-vascular and γ-enteric smooth muscle actin isoforms was analyzed in rat embryos from gestational day (g.d.) 8 through the first neonatal week by *in situ* antigen localization using isoactin specific monoclonal antibodies. The α-vascular actin isoform was first detected on g.d. 10 in discrete cells lining the embryonic vasculature. By g.d. 14 this isoform was also present in the inner layers of mesenchymal cells condensing around the developing airways and gut. The γ-enteric actin, however, was not detected until g.d. 15 when cells surrounding the developing aorta, airways, and gut labelled with the γ-enteric specific probe. There was continued expression of these two actin isoforms in regions of developing smooth muscle through the remainder of gestation and first neonatal week at which time their distribution coincided with that found in the adult. In addition to developing smooth muscle, the α-vascular actin isoform was expressed in differentiating striated muscle cells. On g.d. 10, there was intense labelling with the α-vascular specific probe in developing myocardiocytes and, within 24 hours, in somitic myotomal cells. Although significant levels of this smooth muscle actin were present in striated myocytes through g.d. 17, by the end of the first postnatal week, α-vascular actin was no longer detectable in either cardiac or skeletal muscle. Thus, the normal developmental sequence of striated muscle cells includes the transient expression of the α-vascular smooth muscle actin isoform. In contrast, the γ-enteric smooth muscle actin was not detected at any time in embryonic striated muscle. The differential timing of appearance and distribution of these two smooth muscle isoforms indicates that their expression is independently regulated during development. In addition, this is the first demonstration that developing mammalian striated muscle cells utilize a smooth muscle actin in early myofibrillogenesis.

CA 208 A RETROVIRAL VECTOR SUITABLE FOR CELL LINEAGE ANALYSIS OF AVIAN CARDIAC DEVELOPMENT, Takashi Mikawa, Anthony Brown and Donald A. Fischman, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021.
For cell lineage analysis of avian cardiac development, a recombinant retrovirus is a suitable tool for delivery of a stable reporter gene. The vector is required to be replication-defective and to have a high level of reporter gene expression. Using the cloning vector pJD214 (J.P.Dougherty and H.M.Temin, 1986) based on the avian spleen necrosis virus (SNV), we have developed a replication-defective recombinant retrovirus (CXL) which carries a bacterial β-galactosidase gene, lacZ. We have obtained CXL producing helper cell lines by transfecting pCXL DNA into SNV packaging cell lines which have almost no sequence homology to the vector. The virus titer in the culture medium of clonal transfected cells was approximately 2 x 10^5 active virions per ml as determined by infection of cultured chicken embryo fibroblasts. CXL virus also efficiently infected dog and rat cells. We have introduced the new vector by direct injection into the cardiogenic areas of quail and chicken blastodiscs in ovo. Four to seven days later, infected embryos were fixed and stained. LacZ positive cells formed colonies in the heart wall. Dilution of virus decreased the number of lacZ positive colonies, while the number of cells in a single colony increased during development. Serial sections of the stained hearts are being analyzed by computer-assisted reconstructions to develop a 3-D map of progeny derived from a single parental cell.
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CA 209 MASSIVE CHANGES IN MYOSIN HEAVY CHAIN COMPOSITION DURING EARLY DEVELOPMENT IN THE CHICK, Julie Ivory Rushbrook, Cipora Weiss and Tsai-Tse Yao, Department of Biochemistry, SUNY Health Science Center at Brooklyn, NY 11203.

Prospective mechanisms for myofibril assembly must consider not only turnover in the mature muscle fiber where protein concentration and isoform composition are stable but situations of dynamic change such as may occur during exercise and development. During development, not only is there rapid growth of tissue but frequently major changes in isoform composition occur. Information on the number and rapidity of such changes is important for the construction of an assembly mechanism which maintains the integrity of a fiber while permitting extensive remodelling. Using high resolution anion-exchange of myosin S-1 we have identified six myosin heavy chain species occurring sequentially during development in the type IIB fibers of the pectoralis major muscle. Four species are present at 15 days in ovo. One (perihatching) becomes the major species from 19-days in ovo to 3-days post-hatch. A fifth species (post-hatch) emerges as significant between 3- and 5-days post-hatch and is the only form apparent at 8-days post-hatch. The post-hatch isoform continues as the major form through 32-days post-hatch when the sixth (adult) becomes significant. The last three forms have been compared by N-terminal sequencing of CNBr peptides and shown to differ in primary sequence. Massive changes in heavy chain composition thus occur at two stages, from 15- to 19 days in ovo, and from 3-days to 8-days post-hatch.

CA 210 NOVEL MUSCLE-TYPE SPECIFIC PROTEINS OF DROSOPHILA. Jim O. Vigoreaux, Judith D.Saide*, Agnes Ayme-Southgate, Katrin Valgeirsdottir, and Mary Lou Pardue. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139 and *Department of Physiology, Boston University School of Medicine, Boston, MA 02118. All insect muscles are striated; however, different muscle types are clearly evident. In Drosophila the highly specialized indirect flight muscle (IFM) is distinguished by its asynchronous mode of contraction and short sarcomere length. The synchronous muscles include the supercontractile muscles, such as the larval body wall, and the tubular muscles, such as the adult leg and direct flight muscle. The muscle types differ in isotypes of a number of contractile proteins. In addition, we have identified several novel proteins that are specific for particular types of muscle. One protein, Z(210), is found in the Z bands of IFM and certain tubular muscle but not in the perforated Z-bands of supercontractile muscle. Another novel protein, the 20,000 D mp20, is found in synchronous, but not asynchronous, muscle. Antibody analyses show that this protein is localized over the A-band in intact muscle but is easily extracted during fractionation of muscle cells. Interestingly, asynchronous muscle also has a small protein, approximately 27,000 D (mp27), which exists as various isoforms that localize over the A-band in intact muscles. Although the localization of mp20 and mp27 suggests that these proteins may play similar roles in the two different types of muscle, we can detect no strong relationship between the proteins either from the gene sequence or from antibody cross-reaction.

CA 300 ELECTROPHORESIS AND ORIENTATION OF F-ACTIN IN AGAROSE GELS Julian Borejdo* and Henry Ortega, Cardiovascular Research Institute, University of California, San Francisco, Ca. 94143 (*present address: Baylor University Medical Center, Baylor Research Foundation, 3812 Elm St., Dallas, Tx. 75266).

F-actin was electrophoresed on agarose gels. In the presence of 2 mM $MgCl_2$ and above pH 8.5 F-actin entered 1% agarose; when the electric field was 2.1 V/cm and the pH was 8.8, F-actin migrated through a gel as a single band at a rate of 2.5 mm/hr. After the electrophoresis actin was able to bind phalloidin and heavy meromyosin (HMM) and it activated Mg^{2+}-dependent ATPase activity of HMM. The mobility of F-actin increased with the rise in pH. The orientation of fluorescein (5-IAF) labeled F-actin was measured during the electrophoresis by the fluorescence detected linear dichroism method. F-actin showed transient orientation which critically depended on the position of F-actin in the electrophoretic band: large F-actin polymers which migrated slowly through agarose gave the best orientation, while small polymers which migrated rapidly gave no orientation at all. The degree of orientation also depended on the strength of the electric field. These results showed that it was possible to electrophorese native F-actin on agarose gels and that the electric field oriented filaments so that the absorption dipole of the 5-IAF dye bound to actin assumed an orientation largely parallel to the direction of the electric field.

CA 301 ALTERED CA^{2+} REGULATION AND THIN-FILAMENT PROTEINS IN MYOFIBRILS FROM ANOXIC RAT HEARTS. M.V. Westfall, E. Davis, R.J. Solaro. Dept. of Physiology and Biophysics, University of Illinois Medical School-Chicago, Chicago, IL 60680.

Altered thin-filament activity may contribute to changes in contractile function during anoxia. Myofibrils were isolated from rats hearts which were made anoxic for 60 min at 37°C and analyzed for myofibrillar Ca^{2+}-dependent Mg^{2+}-ATPase activity. Hearts incubated in saline at 0.5°C and non-incubated hearts served as controls.

	pH 7.0				pH 6.5			
Treatment	n	pCa_{50}	Hill n	Max. ATPase nmol/mg/min	n	pCa_{50}	Hill n	Max. ATPase nmol/mg/min
Control	7	6.59±0.50	0.97±0.09	121.89± 8.69	7	6.27±0.32	0.71±0.10	113.92±6.09
Anoxia	4	6.82±0.82	1.34±0.13*	131.65±32.07	4	6.34±0.53	1.08±0.16*	109.77±4.66

*$p \leq 0,05$ vs control using ANOVA and post-hoc Newman-Keuls test

Values from both control groups were pooled as all indices of ATPase activity were not different. Maximum ATPase activity and pCa_{50} were not changed at either pH but the Hill coefficient (Hill n) increased in myofibrils from anoxic hearts compared to controls at both pH 7.0 and 6.5. Analysis of myofibrillar preparations by SDS-PAGE showed evidence for degradation of troponin-I (TnI), troponin-T (TnT) and actin during anoxia with little change in myosin light chains I and II or tropomyosin. Myofibrils from anoxic hearts, but not controls contained two protein bands which were identified as partially degraded products of TnI and TnT with Western blots. TnI and TnT proteolysis during anoxia may alter thin filament-Ca^{2+} interactions to increase the Hill n. Supported by NIH Grants HL05059, HL22231 and HL22619.