CELLULAR AND MOLECULAR BIOLOGY OF MUSCLE DEVELOPMENT

Organizers: Frank Stockdale and Larry Kedes

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Cellular and Molecular Biology of Muscle Development

Cellular and Molecular Biology of Commitment and Cell Lineages

M 001 HETEROKARYONS: A MODEL SYSTEM FOR STUDYING CELL COMMITMENT AND DIFFERENTIATION, Helen M. Blau, Bruce Blakely, Mildred Cho, Simon Hughes, Lydia Pan, Charlotte Peterson, Beat Schaefer, Marilyn Travis, and Steven Webster, Stanford University School of Medicine, Stanford, CA 94305.

Experiments with heterokaryons have demonstrated that the differentiated state can be altered. Such short-term somatic cell hybrids stably retain all of the genes in their normal position within chromosomes. Using this system, we have shown that the expression of nine different muscle genes can be induced in a range of human nonmuscle cells following fusion with muscle cells. Differences in the frequency and time course of gene activation in endodermal, mesodermal and ectodermal cell types suggest differences in the lineage decisions and steps required to generate and maintain them. Diffusible molecules that are transported to nuclei through the cytoplasm presumably mediate the activation of genes observed in heterokaryons, since the nuclei of the two cell types remain separate and distinct. Changes in chromatin structure associated with DNA replication do not appear necessary for trans-activation of muscle genes in all cell types tested, except HeLa cells. However, there are marked differences in gene dosage effects and in the kinetics of novel muscle gene expression which may be due to differences in the requisite number or amount of trans-acting factor(s). In addition to changes in gene expression, major changes in cytoarchitecture are induced, leading to a redistribution of organelles with a central role in cell polarity and intracellular transport. From the pattern of actin and myosin expression, it appears that human fibroblasts incorporated into heterokaryons produce their own muscle regulatory molecules. Using cells isolated with the fluorescence-activated cell sorter, we have shown that developmentally regulated transitions in muscle gene expression are amenable to analysis in pure populations of human primary muscle cells that can be isolated in quantity from normal and diseased muscle tissues. Muscle cells at different stages of development contain distinct regulatory molecules capable of activating different muscle genes. Since mouse cells activate human muscle genes, the regulatory molecules must be conserved in evolution. To isolate stage-specific trans-acting factors, biochemical approaches and assays based on function are being used. Our strategy for isolating these molecules derives directly from heterokaryon experiments that define the requirements for activating well-characterized transfected genes representing different stages of myogenic development: determination, differentiation, and maturation.

M 002 THREE HIERACHIES IN SKELETAL MUSCLE FIBRE CLASSIFICATION: ALLOTYPE, ISOTYPE AND PHENOTYPE, Joseph F.Y. Hoh, Suzanne Hughes, Gregory Hugh and Irene Pozgaj, Department of Physiology, University of Sydney, NSW, 2006, Australia.

Fibres of limb and trunk muscles of mammals may be classified into slow, fast-red and fast-white types, and their phenotypic diversity has hitherto been attributed to different patterns of neural activity. Immunocytochemical analyses of fibres using specific anti-myosin antibodies during development, regeneration and after denervation have revealed a more complex picture. Primary and secondary myotubes were observed to belong to different categories characterized by different patterns of myosin gene expression. There were fast and slow primary myotubes, both of which expressed slow myosin during late foetal life; the former expressed slow myosin transiently, and initiated fast myosin expression at birth, while the latter continued to express slow myosin at maturity. There were also fast and slow secondary myotubes, which were already immunocytochemically distinct at birth. These differences emerge at a time when there is extensive polyneuronal innervation, and when distinct impulse patterns for fast and slow muscles have not yet appeared. These categories of fibres or isotypes behaved differently upon denervation, e.g., slow primaries hypertrophied while other fibres atrophied. Furthermore, with ageing, some slow primaries became phenotypically fast, but upon denervation, reverted to express slow myosin and underwent hypertrophy. The results suggest that the emergence of fibre isotypes is nerve independent, and probably has its roots in diverse lineages of myoblasts. Nerves may subsequently change the phenotype of muscle fibres, but their isotypic characteristics remain unaltered. The jaw-closing muscles of the cat contain superfast fibres which express a unique myosin not found in limb muscles. When superfast muscle was transplanted into a limb muscle bed, regenerating myotubes synthesized superfast myosin independent of innervation. Reinnervation by the nerve to fast muscle led to the expression of superfast and not fast myosin, while reinnervation by the slow nerve led to the expression of a slow myosin immunocytochemically different from limb slow myosin. Thus jaw and limb muscles belong to distinct allotypes, each with a unique range of phenotypic options the expression of which may be modulated by the nerve.

M 003 EXPRESSION OF SLOW AND FAST ISOFORMS OF MHC IN CHICKEN SKELETAL MUSCLE FIBERS OF DIFFERENT MYOBLAST ORIGINS, Frank E. Stockdale, Stanford University School of Medicine Stanford, CA 94305.

Diversity in fiber types of skeletal muscles is rooted in the expression of an array of isoforms of myosin heavy chain and other skeletal muscle proteins. Expression of different fast and slow members of the myosin heavy chain family is responsible for the histochemical recognition of fiber diversity. The temporal and positional or pattern of expression of different myosin isoforms in developing muscle fibers appears to have its developmental origin in diversity among myogenic precursor cells in the early embryo. Skeletal muscles of embryonic and fetal chickens express both fast and slow MHC isoforms. In the chicken there are two slow MHC isoforms, SM1 and SM2, and many fast MHC isoforms. All fibers in the limb of the embryo (primary fibers) express SM1 plus a fast isoform(s) of MHC. Distinctions among primary fibers are based on the expression of SM2 within some fibers which are distributed in specific locations and muscles. On the other hand, fibers formed in the limb of the fetus (secondary fibers) express SM1 and a fast isoform(s) of MHC, but not SM2. Myoblasts isolated from the limbs of the embryo or the fetus and cloned in cell culture form fibers that also have restrictions on slow MHC isoform expression - some fetal myoblasts form colonies that express both a fast MHC isoform(s) and SM1, but not SM2; some embryonic myoblasts form colonies that express a fast isoform(s), and both SM1 and SM2. Patterning of fiber type distribution in anatomic muscles of the developing chicken embryo is due to differential expression of SM2 and may be linked to specific myoblast types.

M 004 EXPRESSION OF A SINGLE TRANSFECTED cDNA CONVERTS FIBROBLASTS TO MYOBLASTS, Robert L. Davis, Stephen J. Tapscott, Harold Weintraub and Andrew B. Lassar, Department of Genetics, Fred Hutchinson Cancer Center, 1124 Columbia St., Seattle , WA, 98104.

5-azacytidine treatment of mouse C3H10T1/2 embryonic fibroblasts, or transfection of these cells with genomic muscle DNA, converts them to myoblasts at a frequency suggesting alteration of one or only a few closely linked regulatory loci. Assuming such loci to be differentially expressed as poly (A)+ RNA in proliferating myoblasts, we prepared subtracted cDNA probes to screen a myocyte cDNA library. Based on a number of criteria, three cDNAs were selected and characterized. We found that expression of one of these cDNAs (which we term MyoD1) transfected into C3H10T1/2 fibroblasts, where it is not normally expressed, is sufficient to convert them to stable myoblasts. Myogenesis also occurs, but to a lesser extent, when this cDNA is expressed in a number of other cell lines. The major open reading frame encoded by this cDNA contains a short protein segment similar to a sequence present in the *myc* protein family. Studies to understand how MyoD1 expression activates the myogenic program as well as how this gene may interact with agents which inhibit myogenesis (ie. oncogenes and BudR) will be discussed.

Extracellular Matrix and Cytoskeleton in Myogenesis

M 005 DISASSEMBLY AND ASSEMBLY OF STRIATED MYOFIBRILS, Zhongxiang Lin, James Eshleman, John Choi, Werner Franke, Donald Fischman, and Howard Holtzer, Dept. of Anatomy, Univ. of Penn., German Cancer Center, and Dept. of Cell Biology, Cornell Med School.

The disassembly and assembly of myofibrils in primary myotubes was analyzed by immunofluorescence and EM after exposure to the phorbol ester, TPA. First signs of sarcomere disruption observed after 2h in TPA as loss of Z-band density. By 5-10h, all I-Z-I complexes were selectively deleted from the myofibrils, leaving behind intact A-bands. The rapid deletion of I-Z-I complexes was inversely related to the assembly of numerous cortical, alpha-actin bodies (CABs), transitory structures 3.0um in diameter. Each CAB consisted of a filamentous core that stained with antibodies to alpha-actin and sarcomeric alpha-actinin, and was surrounded by a discontinuous rim of vinculin. Cell organelles were excluded from these CABs. After 10-20h in TPA the CABs fragmented into granules, and by 30h all alpha-actin and sarcomeric alpha-actinin had been cleared from the myosac. TPA did not affect beta- or gamma-actin-containing the stress fibers or microfilaments in replicating presumptive myoblasts, fibroblasts or myosacs.

Disruption of A-bands first evident after 15h in TPA. Thick filaments of normal length and structure progressively released, and by 40h all A-bands had been broken down into enormous numbers of randomly dispersed, intact, single thick filaments. The breakdown of A-bands correlated with the formation of a few large cytoplasmic patches that invariably showed co-localization with MHC, myomesin, C-protein, and titin. Elimination of all thick filament associated proteins required 60-72h in TPA. Neither Colcemid nor cycloheximide blocked the formation of CABs, while inhibition of oxidative phosphorylation with dinitrophenol and glycerol permeabilization of the myotubes did block CAB formation.

TPA down-regulates the steady state levels of alpha-actin and sarcomeric MHC and their mRNAs, while modestly up-regulating the levels of gamma-actin and its mRNA. Recovery from TPA, well underway after only 24h in normal medium, indicated by the de novo assembly of 1.5 long thick filaments. These nascent single thick filaments were first observed juxtaposed to the myosacs long stress fibers. The transition from staggered, thick filaments into laterally aligned sarcomeric aggregates was tightly coupled to the emerging sarcomeric periodicity of titin, C-protein, alpha-actin and sarcomeric alpha-actinin. A sarcomeric periodicity of less than 1.5 - 1.7um was never observed with any skeletal isoform.

M 006 AVIAN INTEGRIN: ITS FAMILY AND NEIGHBORS: Alan F. Horwitz and Donna Bozyczko, Department of Cell Biology, University of Illinois, 506 Morrill Hall, Urbana, Il 61801.

The avian integrin is a cell surface receptor for extracellular matrix molecules like fibronectin, laminin, vitronectin, and perhaps some collagens. Integrin also serves as a receptor for talin, a cytoskeletal associated molecule thought to participate in the linkage of actin filaments to the cell surface. On transformation with viruses encoding tyrosine kinases, integirn is phosphorylated on tyrosine and shows altered binding properties. On skeletal muscle integrin localizes in regions of myotendonous and neuromuscular junctions where it co-localizes with characteristic subsets of cytoskeletal and extracellular matrix molecules. This distribution is representative of fast twitch muscle. On slow muscle fibers integrin is distributed nearly continuously along the fiber surface implying a large region of sarcolemmal cytoskeletal and/or extracellular matrix interactions. This work was supported by grants from the NIH and MDA.

M 007 EXTRACELLULAR MATRIX OF SKELETAL MUSCLE, Richard Mayne, Somporn Swasdison, Ralph D. Sanderson and Michael H. Irwin, Dept. of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham. AL 35294.

Recent experiments have investigated the requirements for the formation of a continuous basal lamina on the surface of myoblasts grown in culture[1]. Clones of quail myoblasts were grown in a native gel of rat tail tendon collagen, and the formation of a continuous basal lamina was achieved after the addition of fibroblastic cells. These results suggest that a close relationship must exist between myoblasts and fibroblastic cells during muscle development. Such an interaction is not surprising in view of experiments suggesting that mesenchymal tissue of the developing limb bud plays a role in the patterning of developing muscle[2]. In addition, during myogenesis in vivo a basal lamina always surrounds "clusters" of myogenic cells and separates them from the surrounding mesenchymal tissue. Basal lamina is not present on the surface of myoblasts and myotubes that are in contact with each other and form part of a cluster from which both primary and secondary myotubes will eventually develop. Interactions must also exist during myogenesis between developing myofibers and fibroblastic cells of the developing tendon. These are of different embryological origin[3] and yet must interact to form the specialized myotendinous junction. Previous experiments have shown that a continuous basal lamina is present at the myotendinous junction and that the collagen fibers of the tendon do not pass through the basal lamina. More recently, we have shown by immunofluorescent staining that laminin, type IV collagen and heparan sulfate are all present at the myotendinous junction. However, "myotendinous antigen" (also called tenasin or brachionectin) was present throughout the tendon but was not present in the endomysium or perimysium. This contrasts with fibronectin which was present throughout the connective tissue of skeletal muscle. Type I collagen and type VI collagen were present in the tendon and also continued into the endomysium and perimysium. Myogenesis was studied with cells embedded within the center of a collagen gel. Examination of the ends of myotubes by electron microscopy showed marked similarity to myotendinous junctions with extensive infolding of the myotube surface, the presence of a basal lamina and the apparent formation of connections between the myotube and the extracellular matrix of the gel.

Supported by a Grant from the Muscular Dystrophy Association.

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M 008 CELL SURFACE MOLECULES AND SYNAPSE FORMATION AT THE NEUROMUSCULAR JUNCTION, Joshua R. Sanes, Washington University Medical Center, St. Louis, MO 63110

Axons readily form synapses on denervated, but not on already innervated muscle fibers, indicating that muscles regulate their susceptibility to innervation in accordance with their current state of innervation. Axons preferentially reinnervate original synaptic sites, indicating that these sites are attractive to axons. We are studying components of the muscle cell surface that might mediate these interactions.

a) The neural cell adhesion molecule (NCAM) is restricted to synaptic areas in normal adult muscle, but appears extrasynaptically following denervation. In contrast, several other adhesive molecules are present at similar levels on innervated and denervated muscle surfaces. This pattern suggests that muscle might use NCAM to signal their susceptibility to innervation. Consistent with this notion, motor axons bear NCAM and could thus interact homophilically with muscle NCAM. Also, anti-NCAM perturbs nerve-muscle interactions in vitro.

b) Reinnervation of original synaptic sites is guided by components of the muscle fiber's basal lamina, rather than by the muscle fiber itself. Thus, axons preferentially reinnervate original synaptic sites on basal lamina ghosts from which muscle fibers have been removed. A number of antibodies have been generated which stain synaptic but not extrasynaptic portions of muscle basal lamina. Several synaptic antigens thereby defined have been characterized to some extent, but none has yet been critically tested as a "recognition" molecule.

c) How might neurites find original synaptic sites? One potential source of guidance is provided by fibroblasts. Fibroblasts near synaptic sites proliferate following denervation, and accumulate at least 3 adhesive macromolecules: NCAM, J1, and fibronectin. Studies in vitro show that muscle fibroblasts synthesize all 3 molecules, and that the synthetic capacity of these cells is greater when they are prepared from denervated than from innervated muscle. The interstitial deposits of adhesive molecules these perisynaptic fibroblasts produce could influence axons growing through these spaces to reinnervate original synaptic sites.

Together, these results suggest that axons are guided by a hierarchy of muscle-derived cell-surface molecules during reinnervation: NCAM (and soluble growth factors) throughout the muscle, fibroblast-produced molecules in perisynaptic interstitial areas, and basal lamina antigens at synaptic sites. Studies of neurons cultured on cryostat sections of muscle support this notion: neurites are longer on sections of denervated muscle than on sections of innervated muscle, and longer on sections of denervated muscle cut perisynaptically than on sections from synapse-free areas. Furthermore, neurites cease growth upon contact with synaptic sites in the sections. These results in vitro also provide a bioassay for testing effects of antibodies to cell surface molecules on neurite behavior.

M 009 GENERATION OF N-CAM DIVERSITY IN SKELETAL MUSCLE, Frank S. Walsh, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

During skeletal muscle development and tissue remodelling expression of the N-CAM gene is precisely regulated at the levels of gene activity, RNA processing and post-translational modification. Absolute changes in N-CAM polypeptide and mRNA levels accompany the various stages of myogenesis (1,2), and following denervation and lesion of adult myofibres (3). In addition, changes in N-CAM isoform patterns involving transmembrane, phosphatidylinositol-linked and soluble polypeptide species, and altered glycosylation events can be demonstrated (1,4).

Characterization of N-CAM cDNAs spanning full length coding sequences in conjunction with S1-nuclease protection and genomic cloning studies show that polypeptide isoforms are generated by alternative RNA splicing and poly A site selection. In addition, a muscle-specific exon encoding a novel extracellular N-CAM domain (4), and an unusual alternative splicing mechanism generating a secreted N-CAM isoform can be identified.

Combined with altered RNA expression levels and splicing patterns, differential tissue-specific glycosylation further expands the potential for generating diversity of N-CAM expression in skeletal muscle. Eukaryotic gene transfer and site-directed mutagenesis studies may now allow structure-function relationships to be analysed and the role of N-CAM in skeletal myogenesis more clearly defined.

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Myofibrillar Assembly

M 010 REGULATION OF CYTOSKELETAL ASSEMBLY BY THE cAMP-DEPENDENT PROTEIN KINASE, Ned J.C. Lamb, Anne Fernandez, William J. Welch and James R. Feramisco, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724.

Elevating the intracellular levels of cAMP-dependent protein kinase (A-kinase) in Rat Embryo Fibroblasts by microinjection of the purified catalytic subunit, resulted in marked but reversible changes in both cell shape and the organization of the cytoskeleton. Indirect immunofluorescence staining of injected cells revealed the complete loss of filamentous actin staining by 60 minutes after A-kinase injection, at which time the intermediate filaments were heavily bundled and collapsed around the nucleus. Accompanying the rearrangements of these two cytoskeletal networks, there occured a number of changes in protein phosphorylation as assayed by $[^{32}P]H_3PO_4$ metabolic pulse labelling in injected living cells and subsequent analysis of the phosphoproteins by two dimensional gel electrophoresis. Specifically, we have correlated an increase in myosin light chain kinase phosphorylation and a concomitant loss of myosin light chain phosphorylation with the disassembly of the microfilaments. Similarly, a major increase in vimentin phosphorylation accompany the reorganization of the intermediate filaments, which we have correlated to a site specific phosphorylation of two vimentin peptides by A-kinase. Moreover, we have observed an increased phosphorylation of two similar vimentin phosphopeptides in cells at mitosis, following heat shock or after treatment with drugs which depolymerize the microtubules, all of which induce the reorganization of the intermediate filaments. These changes in protein phosphorylation and the organization of the cytoskeleton are discussed in relation to the possible regulation by A-kinase of the microfilament and intermediate filament cytoskeletal systems.

M 011 INCORPORATION OF NEWLY SYNTHESIZED PROTEIN BY MYOFIBRILS IN A CELL-FREE SYSTEM.
Donald A. Fischman, Marina Bouché, Isaac Peng and Steven M. Goldfine. Department of Cell Biology and Anatomy, Cornell University Medical College, New York NY 10021.
A cell-free system has been established for examining the post-translational steps in protein incorporation by myofibrils (Bouché and Fischman, Biophys. J. 49:233a, 1986). Poly(A)RNA from muscle or nonmuscle tissues was translated in a reticulocyte lysate containing ^{35}S-met. Upon terminating protein synthesis, Triton X-100 (0.1%), a protease-inhibitor cocktail, BSA (0.1%), and adult myofibrils or myofilaments were added to the lysate, incubated for 1 hr at selected temperatures and then centrifuged. After washing the pellet, the labeled proteins in both supernatant and pellet fractions were displayed by SDS-PAGE and fluorography. We observed that of the many proteins synthesized in the lysate, only contractile proteins co-pelletted with the myofibrils. The incorporation was saturable, proportional to the concentration of myofibrils added and relatively temperature insensitive. The amount incorporated was a unique property of each protein: it was unrelated to size, amount synthesized, concentration in the myofibril, or location in the sarcomere. Evidence has been obtained for the independent incorporation of myosin subunits into synthetic thick filaments or myofibrils: myosin heavy chains (MHCs), synthesized in the presence or absence of light chains (MLCs), exhibited equivalent incorporation. The same was true for the MLCs. We have begun a comparative analysis of actin isoform incorporation using mRNA populations selected from chicken brain or muscle to synthesize β and γ or α isoforms of actin, respectively. These results indicate that newly synthesized proteins or protein subunits can be incorporated post-translationally into myofibrils in a cell-free system and are compatible with the hypothesis that proteins of the sarcomere are in an exchange equilibrium with a small but kinetically active soluble pool in the cytoplasm.

M 012 DYNAMICS OF MYOSIN EXPRESSION DURING THE INDUCED TRANSFORMATION OF ADULT FAST-TWITCH MUSCLE, Bernhard J. Kirschbaum, Jean-Aimé Simoneau and Dirk Pette, Faculty of Biology, University of Konstanz, D-7750 Konstanz, Federal Republic of Germany.
We investigated at the mRNA and protein level the exchange of fast with slow isoforms of myosin light (LC) and heavy chains (HC) in chronically stimulated fast-twitch muscle of the rat. Using specific cDNA probes for HCIIb, HCIIa, HCβ, HC_{neo} and HC_{emb} [1] we observed by S1-nuclease analysis that the HCIIb transcript was no longer detectable after less than 1 wk of stimulation. By this time, a transient signal was seen with the HC_{neo} probe. An increase in mRNA for HCIIa was evident only after the signal for HCIIb had disappeared, and persisted in longer stimulated muscles. An elevation of the level of the slow HCβ mRNA was detectable only after prolonged (>5 wk) stimulation. Using specific cDNA probes for LC1f/LC3f, LC1sb [2,3], and LC2f [4], as well as by in vitro translation we observed that changes in the levels of mRNAs for fast LCs occurred only after 2 wk of stimulation. By this time, a significant increase in mRNA for LC1f was accompanied by a decrease in LC3f mRNA. The message for LC2f was stable until 5-6 wk, but decreased thereafter. Increases in LC1sb mRNA were detectable only after 4 wk of stimulation. The changes detected at the protein level mostly resembled those at the mRNA level. Exceptions were LC3f and LC2s. The decrease in the amount of LC3f exceeded that of its mRNA and the increase in the amount of LC2s, which occurred in long-term stimulated (>5 wk) muscles, could not be assigned to an increase in the amount of its in vitro translatable mRNA. Electrophoretic analyses of the amounts of HCIIb, HCIIa and HCβ indicated sequential changes similar to those at the mRNA level. The altered expression of these heavy chains together with that of the fast and slow LC isoforms fit well with the isomyosin patterns of the respective muscles, as well as the histochemically assessed changes in fiber population. However, it remains to be clarified to which extent histochemical profiles of normal and transformed fibers are comparable with regard to the underlying myosin HC composition [5]. In summary, this study shows that, at the transcriptional level, the changes in the expression of myosin heavy chains precede those of the light chains. These alterations mostly correspond to changes at the mRNA level and point to the significance of transcriptional regulation. However, posttranscriptional regulatory steps can not be excluded in some cases.

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M 013 USE OF FLUORESCENT PROBES TO STUDY MYOFIBRILLOGENESIS, Joseph W. Sanger, Jean M. Sanger, Balraj Mittal, Jeffrey S. Dome, Michelle Ng and Sung-ki Lee, Laboratory for Cell Motility Studies, Department of Anatomy, and Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6058.

The microinjection of fluorescently labeled contractile proteins into muscle cells makes it possible to follow sarcomeric components as they are assembled, maintained and modified in living cells. Our approach has been to isolate proteins of the thick filaments, thin filaments and Z-bands and attach to them fluorescent dyes that enable them to be visualized inside living cells. After microinjection, the fluorescent proteins exchange with endogenous proteins and label the structures in the cell containing those proteins. Components of the sarcomere in a particular area of a single cell can then be monitored for extended periods of time using low light-level video cameras and image processing techniques to observe changes that occur during processes like growth, ruffling of cell margins, drug treatment, etc. Questions can be addressed such as whether stress fibers serve as scaffolding during myofibrillogenesis or whether nascent myofibrils grow from short sarcomeric patterns (similar to those of stress fibers) to longer sarcomeric patterns characteristic of contractile myofibrils. By examining such processes in living cells as well as in fixed cells, we can extend our understanding of cellular events beyond interpretations that are based solely on reconstructions from static images of fixed cells.

M 014 INCORPORATION AND DYNAMICS OF FLUORESCENTLY LABELED MYOSIN AND ITS LIGHT CHAIN IN LIVING CELLS, Y.-L. Wang, N.M. McKenna, C.S. Johnson and M.E. Konkel, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Myosins purified from skeletal and smooth muscles were fluorescently labeled and microinjected into living cells. Even though skeletal muscle myosin is relatively insoluble *in vitro* under physiological salt conditions, it dispersed readily from injection sites and incorporated into the A-bands of sarcomeres in myotubes. Following proteolytic digestion, the ability to incorporate was maintained in the rod portion, diminished in light meromyosin, and abolished in heavy meromyosin, indicating that light meromyosin in conjunction with the S2 portion was responsible for the incorporation. Incorporation was also observed with fluorescently labeled gizzard myosin following microinjection into 3T3 or IMR-33 cells. The myosin was detected both in bands along stress fibers and in fine beads forming a submembranous sheath. Skeletal muscle myosin microinjected into these cells showed similar localization. Time lapse studies indicated that myosin at the periphery of fibroblasts can be very labile, reorganizing in some cases in less than 60 secs. Myosin beads along stress fibers seemed to be more stable, showing no change in shape or location for more than 1 hr. However, analysis of fluorescence recovery after photobleaching revealed that the half-time of recovery was shorter than 20 sec both along stress fibers and in the submembranous sheath. This result indicates that individual myosin molecules may undergo constant exchange among different structures. In contrast, myosin associated with myofibrils in muscle cells showed little recovery over 4 hrs, suggesting that once the molecule was incorporated into myofibrils, the binding was very stable.

Similar experiments were performed with purified myosin regulatory light chains (LC20) from smooth muscle. In cardiac muscle cells, fluorescent LC20 was localized in sarcomeric structures. In 3T3, PtK cells and chick embryonic fibroblasts, the localization of LC20 was also similar to that of myosin. In addition, when injected PtK cells entered cell division, LC20 fluorescence became diffuse initially and then became concentrated in the cleavage furrow at telophase. These observations suggest that myosin light chains may undergo constant exchange among the heavy chains, and provide a high degree of flexibility for the regulation of actin-myosin interactions. (Supported by a MDA grant and NSF grant DCB-8510673).

Neuromuscular Interactions in Muscle Development

M 015 REGULATION OF ACETYLCHOLINE RECEPTORS IN DEVELOPING MYOTUBES, Gerald D. Fischbach, David Harris and Douglas Falls, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis MO 63110.

Cholinergic neurites induce the accumulation of ACh receptors at developing neuromuscular junctions in vivo and in vitro. In chick ciliary ganglion-myotube cocultures, this interaction is evident within a few hours after contact is first established. In this system, the majority of receptors at developing synapses are "new" molecules that were not exposed on the cell surface prior to contact. Migration and trapping of "old" receptors occurs but it plays a relatively minor role.

We have purified a polypeptide of approximately 42,000 Mr from chick brain that increases the rate of receptor insertion into the surface membrane of chick myotubes. The maximal increase amounts to 4-6 fold and a clear effect is evident at 10pM. The acetylcholine-receptor-inducing-activity (ARIA) increases α subunit mRNA levels (detected with anti-sense RNA probes in a nuclease protection assay) but does alter the amounts of δ or γ mRNA. In addition to the major protected α mRNA fragment, a larger species was detected and this also increased dramatically following treatment with ARIA. If this fragment represents a nuclear precursor, then it is likely that ARIA exerts a direct effect on gene transcription. In situ hybridization experiments indicate significant variation between myotubes and perhaps along the length of individual myotubes in response to ARIA.

M 016 AGRIN Uel J. McMahan. Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305.

Studies conducted in this laboratory have demonstrated that the portion of a muscle fiber's basal lamina sheath that occupies the synaptic cleft at the neuromuscular junction has stably bound to it molecules that direct the formation of postsynaptic apparatus on regenerating muscle fibers. Accordingly, if muscles are damaged in ways that spare the basal lamina sheaths of the muscle fibers, the new muscle fibers that develop within the sheaths form aggregates of acetylcholine receptors (AChR) and acetylcholinesterase (AChE) where they contact the synaptic sites on the sheaths despite the absence of axon terminals. The extracellular synaptic organizing molecules in the synaptic basal lamina may be identical to those molecules that mediate the nerve-induced formation of AChR and AChE aggregates in developing myofibers during synaptogenesis in the embryo and they may also be involved in the maintenance of the postsynaptic apparatus in the adult.

Our studies have also led to the identification of agrin, a protein that is extracted from the synapse-rich electric organ of Torpedo californica and that may be similar to the AChR- and AChE-aggregating molecules in the basal lamina at the neuromuscular junction. For example, agrin is found in basal lamina containing fractions of the electric organ, it induces the formation of patches in cultured myotubes that contain a high concentration of AChRs, AChE and other components of the postsynaptic apparatus, low levels of a similar factor are found in extracts of muscle, and monoclonal antibodies directed against agrin recognize molecules highly concentrated in the synaptic basal lamina at the neuromuscular junction in vivo. We have now purified agrin to homogeneity, determined its n-terminal amino acid sequence, and prepared oligonucleotide probes with the aim of using molecular genetic techniques to characterize agrin and study how the expression of agrin is regulated during development and regeneration.

I will document the above findings, discuss our progress in characterizing agrin and present results showing that the cell bodies of motor neurons contain agrin-like molecules, consistent with the hypothesis that the agrin-like molecules in the synaptic basal lamina are produced by motor neurons and released by their axon terminals.

M 017 REGULATION OF MYOSIN ISOFORM EXPRESSION BY INNERVATION AND THYROID HORMONE, Robert G. Whalen, Natalie Cambon, LynSue Kahng, Edouard Prost, Steven D. Russell, Sanjay Sesodia and Shin'ichi Takeda, Department of Molecular Biology, Pasteur Institute, 75724 Paris, France

The expression of the different mammalian myosin heavy chain isoforms, and the control of the transitions among them, is influenced by innervation and thyroid hormone levels. The neonatal to adult fast MHC transition in newborn rats is independent of continued innervation. However, daily injections of thyroid hormone T3 can accelerate the appearance of the mRNA for the adult fast IIB MHC by up to 3 days in the gastrocnemious muscle. This precocious induction is also independent of innervation; furthermore innervated and denervated muscles respond to different levels of T3 in similar ways, indicating no substantial influence of innervation on this induction. In young adult rats, the soleus muscle contains essentially only the slow and fast IIA mRNAs. However, denervation will lead to the appearance of low levels of fast IIB and neonatal mRNA. Although a one-week treatment of T3 has little effect on fast IIB or slow mRNAs in innervated muscles, denervation appears to confer T3 sensitivity to these mRNAs. This dominant effect of innervation is also observed in experiments on regenerating rat soleus muscle. Following muscle degeneration after treatment with notexin, regeneration and reinnervation will occur, and slow myosin is reaccumulated as the exclusive form. However, if the regenerating soleus muscle is denervated, fast myosin accumulates but no slow myosin is found. Thus, this regenerating muscle has the capacity to make either slow or fast myosin. If hyperthyroidism is induced by T3 injection while regeneration is occurring, the reinnervated muscle will still accumulate only slow myosin protein, demonstrating that the slow nerve influence can override the potential effect of T3. To further characterize the response of muscle tissue to thyroid hormone, we have obtained cDNA clones coding for the T3 receptor from libraries of mouse muscle tissue and the C2 cell line. One clone shows a striking sequence similarity to the previously reported receptor cDNA isolated from rat brain. Northern blotting and in situ hybridization are being used to determine the tissue distribution and developmental regulation of the muscle receptor mRNA.

Receptors and Channels in Muscle Growth and Function

M 018 REGULATION OF THE NA^+/K^+-ATPase and Ca^{2+}-ATPase IN DEVELOPING SKELETAL MUSCLE, Doug Fambrough, Barry Wolitzky, Joe Taormino, Kunio Takeyasu, Karen Renaud, Andy Barnstein, Zaven Kaprielian, and Norm Karin, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

The sodium pump and the SR calcium pump are evolutionarily related cation transport ATPases, both of which are essential for muscle function. The sodium pump is largely responsible for setting the transmembrane ion gradients of Na^+ and K^+ necessary for excitability; while the calcium pump is involved in accumulation of Ca^+ in the sarcomplasmic reticulum (the calcium released in excitation-contraction coupling and reaccumulated in relaxation.) The expression of these cation transport ATPases is augmented in concert with muscle differentiation, and there are changes in the expression of pump isoforms during the maturation of muscle fiber types. In these respects, the regulation of these membrane proteins shows parallels with the regulation of contractile protein isoforms. The sodium pump is under fine regulatory control in skeletal muscle. Up- and down-regulation are mediated by a complex set of mechanisms that include alterations in transcriptional rates, regulation of subunit assembly, regulation of incorporation into sarcolemma, and modulation of degradation rate. Analysis of sodium pump regulation has been complicated by the occurrence of isoforms of each of the subunits. Do these isoforms have any physiological relevance, and are their structural differences important in any regulatory mechanism? We are approaching these questions in part by expressing DNAs encoding each subunit and isoform in mouse cells where the expression is detected with avian-specific monoclonal antibodies and function is measured as ouabain-sensitive sodium pump activity against the mouse's background of ouabain-resistant sodium transport. The calcium pump appears to be more resistant to regulatory change. We have used the calcium pump as a marker with which to study domains of nuclear influence upon membrane characteristics in muscle. For these studies, again, an interspecies hybrid system has been devised, involving expression of avian DNA encoding the calcium pump in mouse myogenic cells. These studies suggest a surprisingly rapid interchange of membrane components that argue against the importance of nuclear domains in muscle biology.

M 019 SITE-SPECIFIC MUTATION OF THE Ca^{2+} ATPase of SARCOPLASMIC RETICULUM, [#]Kei Maruyama, [#*]Junichi Fujii, [*]Michihiko Tada and [#]David H. MacLennan. [#]Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G1L6 and [*]First Department of Medicine, Osaka University School of Medicine, Osaka 553, Japan.

We have used expression and site-specific mutagenesis of amino acids in fast-twitch and slow-twitch/cardiac isoforms of the Ca^{2+} ATPase of rabbit sarcoplasmic reticulum to gain insight into the structure and function of these enzymes. Since the activity of the slow-twitch/cardiac isoform is regulated by phospholamban, we have begun to use coexpression and mutagenesis of the two proteins in order to gain insight into the mechanism by which they interact. Full length cDNAs encoding the fast-twitch (1) and slow-twitch (2) isoforms or phospholamban (3) were ligated into the expression vectors pcDX (4) or p91023(B) (5) and expressed transiently in COS-1 cells. Short segments of the cDNA were excised, mutated and sequenced before being returned to their proper position in the expression vector. Microsomal fractions were isolated after 48 or 72 hours and shown to contain immunoreactive Ca^{2+} ATPases or phospholamban. Microsomes from cells transfected with normal cDNA were able to carry out ATP-dependent, oxalate-stimulated Ca^{2+} uptake at rates of about 75 nmoles/min/mg protein, at least 15 fold above the rate in nontransfected microsomes. Ca^{2+} uptake was abolished by the Ca^{2+} ionophore A23187.

We have found that the slow-twitch/cardiac and fast-twitch isoforms have similar intrinsic Ca^{2+} pump activities. The two alternatively spliced forms of the fast-twitch Ca^{2+} ATPase (2) were found to catalyse identical rates of Ca^{2+} transport in spite of their very different carboxyl-termini. Coexpression of phospholamban and the slow-twitch Ca^{2+} ATPase, achieved by either cotransfection or by transfection with one vector containing both cDNAs, led to an increase in the Ca^{2+} concentration required to stimulate Ca^{2+} transport.

Asp^{351}, the site of formation of the catalytic acyl phosphate in the ATPase was mutated to Asn, Glu, Ser, Thr, His or Ala. In every case, Ca^{2+} transport activity and Ca^{2+}-dependent phosphorylation were eliminated. Ca^{2+} transport was also eliminated by mutation of Lys^{352} to Arg, Gln or Glu or by mutation of Asp^{351}-Lys^{352} to Lys-Asp. Mutation of Lys^{515}, the site of FITC modification in the enzyme, resulted in diminished Ca^{2+} transport as follows: Arg, 60%; Gln, 25%; Glu, 5%. Several mutations of acidic residues in the amino terminal portion of the ATPase have been carried out in order to map the Ca^{2+} binding site in the enzyme.

(1) Brandl, C.J. et al. (1986) Cell 44, 597. (2) MacLennan, D.H. et al. (1985) Nature 316, 696. (3) Fujii, J. et al. (1987) J.Clin. Invest. 79, 301. (4) Okayama, H., and Berg, P. (1983) Mol. Cell Biol. 3, 280. (5) Wong, G.G. et al. (1985) Science 228, 810.

M 020 CELLULAR AND MOLECULAR CHANGES INITIATED BY ACETYLCHOLINE RECEPTOR CLUSTERING, Lee L. Rubin, Donna T. Anthony and Giovanna Marazzi, Laboratory of Neurobiology, The Rockefeller University, New York NY 10021.

An early event in nerve-muscle synapse formation is the accumulation of high concentrations of synaptic acetylcholine receptors (AChRs). This accumulation is initiated by an external factor, probably of neuronal origin, acting in a manner which remains to be elucidated completely. After AChRs have clustered at newly formed synapses, the level of non-clustered AChRs declines markedly, producing an even larger AChR concentration gradient between synaptic and extrasynaptic regions. Our recent work has been directed at trying to understand the way in which muscle cells initially cluster AChRs and the way in which non-synaptic AChRs are then selectively eliminated. For these experiments, we have made use of these observations: a) AChRs on normal cultured embryonic muscle cells can be induced to cluster following treatment with factors derived from a variety of sources; b) once AChRs have clustered, a subset of nuclei and Golgi localize beneath the cluster and cease their normal movement along the muscle cells. These sub-cluster organelles provide a potential source of newly synthesized AChRs for direct insertion into the cluster, suggesting a mechanism for replacing degraded AChRs; c) when cultured chick muscle cells are transformed with Rous sarcoma virus they become completely unable to cluster AChRs, apparently because of the absence in these cells of a novel non-myofibrillar tropomyosin we have identified and characterized; d) the total number of AChRs is determined by the level of muscle contractile activity, with increased contraction causing a decrease in AChR synthesis; e) the effects of contraction can be reproduced by simply increasing calcium levels in muscle cells so that if non-contracting muscle cells are treating with a calcium ionophore, their level of AChR synthesis also decreases. We will describe our recent studies on the cytoskeletal reorganization which takes place subsequent to AChR clustering. We will also discuss how our observations on AChR clustering and synthesis lead to a hypothesis explaining how muscle cells can differentially regulate AChR synthesis in synaptic and extrasynaptic regions.

M 021 DEGRADATION AND NEURAL CONTROL OF "NEW" ACH RECEPTORS INSERTED INTO DENERVATED NEUROMUSCULAR JUNCTIONS. M. M. Salpeter and S.-L. Shyng. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

A progressive increase in degradation rate of acetycholine receptors is seen at vertebrate neuromuscular junctions after denervation. This could reflect the combined rates of "original" junctional receptors present before denervation, and "new" receptors inserted into the denervated junction (Levitt & Salpeter, Nature 291: 239, 1981). Studies on degradation of original receptors in sternomastoid muscle of the mouse, using a gamma-counting technique, have shown that for about 10 days after denervation the original receptors retain an 8 to 10 day degradation half life ($t_{\frac{1}{2}}$) equivalent to that of innervated junctional receptors. Thereafter degradation accelerates to a $t_{\frac{1}{2}}$ of 2.5 days. Reinnervation reverses the $t_{\frac{1}{2}}$ of these prelabeled original receptors back to 8 days (Salpeter et al., J. Cell Biol. 103:1399, 1986).

The degradation rate of new receptors cannot be studied exclusively by gamma counting which assumes uniform distribution of extrajunctional label, since postdenervation extrajunctional receptors develop preferentially near the endplate. With EM autoradiography however the degradation rate of receptors localized on the postjunctional folds can be studied without being affected by the extrajunctional receptors. Using the mouse sternomastoid muscle we first inactivated all original receptors by topically applying non-radioactive α-bungarotoxin (BGT). The nerve was cut and ligated to prevent reinnervation. New receptors were similarly labeled with 125-I-BGT 6 and 14 days later, and their degradation $t_{\frac{1}{2}}$ determined by EM autoradiography. At both times, when the "original" receptors were degrading with a $t_{\frac{1}{2}}$ of 8 and 2.5 days respectively, the new receptors were found to have a $t_{\frac{1}{2}}$ of ~ 1 day. Thus "new" junctional receptors inserted into denervated neuromuscular junctions degrade as do embryonic or postdenervation extrajunctional receptors. EM autoradiography also shows that the total site density of the postdenervation junctional receptors remains essentially unchanged and that new and original receptors are interspersed on the postjunctional membrane.

To study the effect of reinnervation, the muscle was denervated by repeated nerve crush. Reinnervation occured 3 to 6 days after the last crush. The $t_{\frac{1}{2}}$ of labeled new receptors, which initially was 1 day, increased after innervation, first to 2.9 days (by day 5), and then to 9.4 days (by day 12). We conclude that, as with original receptors, reinnervation can slow the degradation of new receptors already inserted into the postjunctional membrane. Furthermore there seems to be an intermediate degradation $t_{\frac{1}{2}}$ of ~ 3 days, both in original receptors after denervation and in new receptors after reinnervation. (NIH grant NS 09315)

Cardiac Myogenesis

M 022 MULTIPLE CIS-ACTING REGULATORY REGIONS CONTROL THE TISSUE-SPECIFIC AND HORMONALLY REGULATED EXPRESSION OF THE SARCOMERIC MHC GENES.

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The pattern of expression of seven distinct myosin heavy chain (MHC) genes was examined in the cardiac and skeletal muscles of rats subjected to different stimuli. It was observed that the specific response to developmental, hormonal (T3) and physiological stimuli varies not only among the different MHC genes, but also in a given gene when it is expressed in different muscle types. To identify putative cis-acting sequences involved in the regulated expression of these genes, we have constructed α-, β-, and embryonic skeletal MHC CAT reporter gene plasmids and assayed their transient expression in different muscle and non-muscle cells, in the presence of absence of T3 and of the co-transfected placental T3 receptor driven by the MSV promoter. By a systematic deletion, insertion and recombination analysis of intragenic and 5' upstream sequences, we have identified several regulatory domains with distinct functional properties distributed over 1.5 kb of the 5' end flanking region of these genes. Expression of the emb-MHC gene constructs is developmentally regulated with the myogenic program along with its endogenous counterpart which is only expressed in differentiated myotubes. Expression of the α-MHC gene constructs is restricted to cardiocytes and is dependent on the presence of high levels (5-30nM) of T3. Expression of the β-MHC gene constructs was observed only in the skeletal myogenic cells where the endogenous gene is expressed at very low levels.

Expression of these gene constructs is controlled by three main regulatory elements: i) the promoter element which directs transcription initiation but does not support by itself efficient expression; ii) the muscle-specific restrictive elements which act as negative regulators because, when present, allow expression of the constructs only in the muscle cells; however, they do not play a role in determining high levels of expression; iii) the upstream regulatory region which determine levels of expression and is composed of several positive and negative regulatory elements. In addition, co-transfection of any of the embryonic and β-MHC gene constructs with the T3 receptor results in 80% inhibition of their expression. In contrast, superinduction of the α-MHC gene constructs is observed when they are co-transfected with the T3 receptor. These observations demonstrate that tissue-, developmental-, and hormonal-regulated expression of the MHC genes is regulated by distinct control elements, which are likely to be targets for DNA-binding regulatory proteins which, in turn, may exert their function in a tightly interactive manner.

M 023 REGULATION OF MYOSIN HEAVY CHAIN GENE EXPRESSION. Eugene Morkin, Joseph J. Bahl, and Bruce E. Markham. University Heart Center, University of Arizona, Tucson AZ 85724
Triiodothyronine (T_3) regulates the expression of ventricular myosin isoenzymes by causing an accumulation of α-myosin heavy chain (MHC) mRNA and inhibiting expression of β-MHC. These alterations are associated with a marked increase in myocardial contractile performance. Because of the diverse actions of T_3 in vivo, hormonal control of MHC expression was studied in primary cultures of fetal rat heart cells. T_3 was able to regulate MHC expression without the need for other exogenous factors. Transfection of these cells with pSVOMCAT, an α-MHC/CAT fusion gene, indicated that the sequences necessary for expression are contained in the 5' flanking region. Moreover, co-transfection with a clone encoding the anti-sense message for c-erb A, the putative T_3 nuclear receptor, inhibited expression of pSVOMCAT.

To further evaluate control of α-MHC gene expression, a gel mobility-shift assay was used to identify a nuclear protein binding factor (BF1) in cardiac myocytes which binds specifically to a region of α-MHC known to be important for efficient induction by T_3. Only one binding site for BF1 was found in 3.3 kbp of DNA from position +421 to -2942 in the α-MHC gene. The site was located by methylation interference experiments to a sequence from -599 to -576. BF1 also could be extracted from several other cell types, including rat and human fibroblasts, GH1 cells and L_6E_9 myoblasts and myotubes. Multiple copies of a synthetic double-stranded sequence containing this site were cloned into $pA_{10}CAT$. About 4-5 copies of BF1 were able to act on a heterologous promoter, causing expression of CAT activity which could be induced about 7-fold by T_3. Interestingly, the sequences in this binding site are similar to those for the chicken ovalbumin gene upstream promoter (COUP) factor. Thus, BF1 may be a transcription factor which plays a role in in regulation of α-MHC expression by T_3.

M 024 CONTRACTILE MECHANISMS OF THE NORMAL AND ABNORMAL MAMMALIAN HEART : STUDY OF MOLECULAR CHANGES, Ketty Schwartz, Anne-Marie Lompré, Jean-Jacques Mercadier, Jane-Lyse Samuel, Stefano Schiaffino, D. de la Bastie, and L. Rappaport. I.N.S.E.R.M. Unité 127, Paris 75010, France.
Increased pressure or volume loading of the heart induces hypertrophy and improved contraction efficiency, respectively due to quantitative and qualitative changes in the genetic expression of the cardiac myocyte. The qualitative changes most frequently studied are the isomyosin shifts, which lead to a slower and more efficient myocardial contraction, the isoCPK shifts which might account for the increased efficiency of energy transfer and the isoactin shifts whose functional significance is not yet known. In tissues for which there is a potential for down-regulation of α myosin heavy chain (MHC) expression, i.e. rat ventricles (1) and human atria (2), there is a transition from αMHC to βMHC, and these transitions, which are mainly regulated by pretranslational mechanisms (3),persist as long as the overload is maintained. In experimental hypertrophy, the isoactin shifts involve a transitory accumulation of transcripts from the α skeletal muscle (α Sk) actin gene, which occurs mainly at the onset of hypertrophy (5). In situ molecular hybridization has shown that the accumulation of βMHC and αSk actin mRNAs does not occur in the same regions nor at the same time : following aortic coarctation, the accumulation of αSk actin mRNA precedes that of βMHC mRNA by at least 12 hours and is distributed evenly thoughout the left ventricle, whereas βMHC mRNA accumulation is detected in the endocardium only 24 hours after surgery. In addition, the atrial natriuretic factor (ANF) gene, which is normally expressed only in fetal ventricles, is reactived in adult pressure overloaded ventricles, and the accumulation of ANF mRNA follows a bimodal time course, with a peak at day 4. The overall picture that emerges from these studies is that the same initial signal, hemodynamic overload, induces in adult ventricles a pattern of myogenesis, which is characterized by an uncoordinated and multifactorial reactivation of the fetal phenotype of several proteins, and which leads to an improvment of the working conditions of the cardiac pump.
1-Lompré AM et al. Nature, 1979, 282, 105 ; 2-Mercadier et al. J. Amer. Coll. Cardiol., 1987, 9, 1024 ; 3-Izumo S., Lompre AM et al. J. Clin. Invest., 1987, 79, 970 ; 5-Schwartz K. et al. Circ. Res., 1986, 59, 551.

M 025 THE EFFECT OF FUNCTIONAL LOAD ON THE EXPRESSION OF MYOSIN HEAVY CHAINS (MHC), Radovan Zak, Department of Medicine and Pharmacol. & Physiol. Sciences, The University of Chicago, IL 60637.
The increase in functional load placed on both cardiac and skeletal muscles is accompanied by rapid and substantial activation of overall gene expression. This can be demonstrated by measuring the rate of organ growth, accumulation and synthesis of total proteins, total RNA and by assays of DNA-dependent RNA polymerase. The poly(U)-poly(A) hybridization assay indicates that the concentration of mRNA per unit of tissue increases but it does not change when expressed per unit of RNA. Thus, the accelerated muscle growth is accompanied by an increase in the number of translation units rather than by preferential transcription of mRNA. The increased functional load placed on a muscle is also accompanied by selective changes in expression of individual genes. In the case of MHC, the adaptive growth results, depending on the kind of stimulus, in replacement of some MHC isoforms by others. Following treatment with an excess of thyroid hormone (which favors MHC-alpha), when the change in growth rate is small (5% per days), the reciprocal switch in myosin isoforms is accomplished by repression of MHC-beta and activation of MHC-alpha genes. In pressure overload (which favors the MHC-beta), when the change in growth rate is large (30% in 4 days), the synthesis of MHC-alpha remains unaltered while that of MHC-beta is increased. In the case of skeletal muscle when a very high rate of growth can be achieved by functional overload (80% in 4 days), the rate of synthesis of both MHCs (SM-1 and SM-2) is increased during the phase of sustained muscle growth. The isomyosin switch-over takes place because the synthesis of the dominant species is activated four times more than that of the species which is being eliminated. These data indicate multiplicity of gene regulating mechanisms which vary according to the growth stimulus. In order to analyze the role of growth regulating factors accurate measurements of gene expression are required. In the second part of this presentation, the assessment of currently available methods will be presented.

Molecular and Cellular Basis of Muscle Regeneration and Isoform Switching

M 026 MYOSIN HEAVY CHAIN (MHC) EXPRESSION IN REGENERATING AVIAN MUSCLE: INFLUENCE OF MYOGENIC AND NON-MYOGENIC FACTORS. Everett Bandman, Lisbeth C. Cerny AND Tracy Bennett. University of California, Davis, CA 95616.
Numerous isoforms of skeletal MHC have been found in all vertebrate species examined. In the chicken at least 3 fast isoforms that are sequentially expressed in the pectoralis major (PM) and 2 slow isoforms that are expressed in the developing anterior latissimus dorsi (ALD) have been well characterized. This may be a minimum number since there have been some reports that other embryonic and adult isoforms exist in chicken skeletal muscle. Although the identification of the myosins involved in PM and ALD transitions is clear, the factors that determine both the expression and repression of a particular isoform are unclear. We have produced a monoclonal antibody library that enables us to study MHC transitions at the cellular, ultrastructural and molecular levels employing a variety of immunochemical approaches. In order to address the questions involving regulation of MHC transitions, we have examined the myosin composition of different fast and slow muscles during development in normal and dystrophic chickens as well as in regenerating fibers in innervated and denervated fast and slow muscle beds. We have found that the fast MHC transitions that characterize the PM are not universal among other fast muscles and that adult chicken fast fibers may contain any one or any combination of the isoforms found in the developing pectoralis. These isoforms may be expressed in a number of different patterns during development although the major isoform of the embryonic PM is universally expressed in embryonic fast and slow muscle fibers. Regenerating innervated PM fibers recapitulate myosin expression during development. However, denervated regenerating PM fibers fail to repress isoforms characteristic of immature muscle. These results suggest that innervation of fast fibers regulates myosin isoform composition by repressing the accumulation of inappropriate isoforms. Regulation of myosin isoforms in slow muscle fibers is distinct from that of fast fibers. The developing ALD expresses the embryonic fast isoform in addition to slow isoforms, however, no other fast isoforms are expressed. Similar results were obtained in regenerating and denervated muscle. Apparently, PM and ALD fibers may only express different subsets of the myosin heavy chain family. These results suggest that: (1) myoblasts are predetermined to give rise to fibers that can only express a subset of MHCs, or that (2) there are non-myogenic factors other than innervation that restrict MHC expression. Experiments that explore these 2 hypotheses will be discussed. (Supported by grants from NIH (AR31731) and MDA to EB.

M 027 ISOMYOSIN SWITCHING WITH AND WITHOUT THE INVOLVEMENT OF SATELLITE CELLS. Brenda R. Eisenberg, John M. Kennedy, Mary P. Wenderoth, David J. Dix and Zhaoying Lin. Departments of Physiology, Rush Medical College and University of Illinois, Chicago IL 60612.
Signals for myosin isoform switching are numerous and include developmental changes, hormone levels and alterations in the rate or amount of work demanded. We have produced switches in isoform content of striated muscle cells to determine mechanisms and structures involved in the isoform exchange. The questions addresed are 'How does the cell maintain work output during the exchange' and 'Are satellite cells responsible for expression of the new isoform or is the myocyte nucleus reprogrammed '? The models are 1) rabbit ventricular myocytes under the influence of thyroid hormone, 2) chicken anterior latissimus dorsi muscle under the influence of over-loading (stretching), and 3) rabbit tibialis anterior muscle under the influence of long-term nerve stimulation. Methods of study include quantitative ultrastructural analysis, immuno-fluorescent and immuno-electron microscopy, and in situ hybridization of mRNA. The ability to work during isoform switching is explained by the finding that myofibrils are not dismantled but rather a rapid exchange of the new isoform for the old in every thick filament occurs throughout the cell. In cardiac myocytes the new myosin isoform is uniformly distributed even though myosin mRNA is found to be non-uniformly distributed. Results from in situ hybridization in cardiac myocytes shows that myosin mRNA radiates in spokes from the nucleus whereas in normal skeletal muscle the highest concentration of myosin message is found in subsarcolemmal zones. Satellite cells may play a role in isoform switching in skeletal muscle under conditions of activation (damage or rapid hypertrophy). However, satellite cells cannot be essential for this switching process since cardiac myocytes which have no such cells readily undergo changes with thyroid treatment. Regardless of the signal to the myonucleus which triggers transcription of the new isomyosin, it appears that the new myosin mRNA must be distributed to specific cell locations for translation then diffusion and rapid exchange of isomyosin into fibrils follows. Therefore, regulation of the isoform exchange could be at the transcriptional, translational and assembly stages. Supported by grants from NIH and the American Heart Association.

M 028 MOLECULAR GENETIC APPROACHES TO MYOSIN FUNCTION, Leslie A. Leinwand, Rebecca Feghali, Ilene Karsch, Regina Sohn and Elizabeth McNally, Albert Einstein College of Medicine, Department of Microbiology and Immunology, 1300 Morris Park Avenue, Bronx, New York, 10461.
Myosin heavy chains (MHC) in vertebrates comprise a diverse group of proteins showing both tissue-specific and developmentally regulated expression. These proteins are encoded by multigene families consisting of 10-15 members. In order to define the program of MHC expression in human cardiac and skeletal muscle, we have isolated MHC cDNA clones from various tissues and developmental stages. The lengths of these clones range from 2.0kb up to 4.0kb. Through cDNA analysis we have found three forms of MHC expressed in human cardiac muscle and a minimum of five MHCs expressed in human skeletal muscle. The genes encoding the α and β cardiac myosin heavy chains have been isolated and found to map to chromosome 14. The genes encoding one adult and one embryonic skeletal myosin heavy chain have also been isolated and found to map to chromosome 17. RNA analysis with gene-specific probes shows that each gene has a unique pattern of expression. Amino acid sequence analysis of the carboxy termini of these clones indicates that the myosins encoded by them range in homology from 56% up to 98%. A major focus of our research is to understand the functional significance of these sequence differences. Our approach has been to express eukaryotic contractile proteins in *E. coli* and to analyze them for function. Varying portions of the human fast fiber myosin rod have been cloned into plasmid expression vectors and assayed for assembly. We have localized the assembly competence region of the myosin rod to a 20,000 dalton segment in the carboxy terminus of the myosin molecule. To study force generation at a molecular genetic level, we have expressed a segment of heavy meromyosin (115,000 daltons) from the α cardiac MHC cDNA. It is expressed in *E. coli* as an intact soluble protein. Functional assays will include myosin light chain binding and actin-activated ATPase activities.

M 029 ADENOVIRUS-5 E1A GENE PRODUCTS SUPPRESS MYOGENIC DIFFERENTIATION AND SPECIFICALLY INHIBIT TRANSCRIPTION FROM MUSCLE SPECIFIC PROMOTERS, Keith A. Webster, George E.O. Muscat, and Larry Kedes, The MEDIGEN project, Department of Medicine, Stanford University School of Medicine and VA. Medical Center, Palo Alto, CA 94304. Segments of the Ad-5 early region genes including the complete E1 region, the E1A region, and individual fragments coding for the two major E1A proteins, were transfected into rodent L8 and C2 myoblasts. Transformed cell colonies which expressed E1A mRNAs proliferated at the same rate as the wild type cells but were unable to fuse to form myotubes. Northern analyses demonstrated that biochemical differentiation was also inhibited in the E1A expressing lines insofar as muscle specific transcripts were suppressed by greater than 99%. Housekeeping like genes such as the cytoplasmic actins, glycolytic enzymes, and α-tubulin were minimally effected.

Effects of the E1A products upon gene expression were analysed in transient expression assays by cotransfecting C2 cells with the E1A expressing plasmids and constructs containing sarcomeric or cytoplasmic actin promoters linked to the chloramphenicol acetyltransferase (CAT), gene. Previous reports from this laboratory have documented that proliferating C2 myoblasts, unlike the L8 line, are fully competent in the transient expression of heterologous genes fused to the muscle specific actin promoters (Minty, Blau & Kedes, Mol.Cell Biol. 6:2137, 1986). The E1A plasmids strongly inhibited the transient expression directed by skeletal and cardiac α-actin promoters but had minimal effects upon the expression of corresponding cytoplasmic actin constructs or of the MMTV promoter. Transient expression analyses of a series of sarcomeric actin promoter deletions demonstrated that the putative E1A target in these promoters contains the consensus sequence, "$CC(AT)_6GG$" which may constitute a core recognition element involved in the developmentally regulated expression of sarcomeric actins in myogenic cell lines. We will discuss evidence that the E1A proteins operate by inhibiting the formation of functional complexes between specialized transcriptional control elements and their DNA binding proteins.

Transcriptional Processing in Muscle Gene Regulation

M 030 POST TRANSCRIPTIONAL REGULATION OF EXPRESSION OF TROPOMYOSIN IN MUSCLE AND NON-MUSCLE TISSUE, A.R. MacLeod, Ludwig Institute for Cancer Research, MRC Centre, Hills Road, Cambridge, CB2 2QH, U.K.

We have identified 5 polypeptides in human fibroblasts which are structurally related to tropomyosins from muscle sources. These 5 polypeptides are the products of 4 distinct human tropomyosin genes. Of the 5 polypeptides, 2 are derived from a single gene by alternative splicing while the other 3 are each produced by a single gene. However all tropomyosin genes are expressed by an alternative splicing mechanism which appears to function primarily on a tissue-specific basis. Thus it has been suggested that the appearance of the muscle-specific alternative splice of these tropomyosin genes is due to the induction in muscle of trans-acting factors which overide the "default" splicing pathway seen in non-muscle tissue. However there is evidence to suggest that non-muscle cells can carry out accurate alternative splicing of muscle gene transcripts. For example we have found low level expression of a skeletal muscle α-tropomyosin in human fibroblasts. This suggests that factors which regulate mRNA stability may play a significant role in regulating the tissue-specific accummulation of alternative transcripts of tropomyosin genes.

M 031 REGULATORY ELEMENT(S) INVOLVED IN THE REGULATION OF MYOSIN ALKALI LIGHT CHAIN GENE FAMILY, Yo-ichi Nabeshima
Division of Gene Technology, National Institute of Neuroscience, Kodaira, Tokyo 187, JAPAN

In the process of skeletal muscle development, many contractile protein genes are activated to form functional myotubes. To study the molecular machanisms of these gene regulation, we joined the sequence of the 5'-flanking and upstream region of the chicken myosin alkali light chain gene to the structural gene for chloramphenicol acetyltransferase (CAT). The fusion gene was introduced into primary cultured chick myocytes and quail myoblasts which transformed by a temperature sensitive mutant of Rous Sarcoma Virus (RSV ts NY 68) and the transientry expressed CAT activity was assayed. The introduced fusion gene was highly expressed only in the differentiated skeletal muscle cells, but not in the myoblasts and non-muscle cells. The cis-acting regulatory region responsible for the enhanced expression in response to the myocyte differentiation was determined by the experiments with the external and internal deletion mutants of the fused gene. Furthermore, the protein(s) which bound to the cis-acting DNA sequence was analysed by gel shift assay method.

M 032 CHARACTERIZATION OF CIS-ACTING SEQUENCES THAT REGULATE THE TISSUE SPECIFIC EXPRESSION AND REPRESSION OF GENES DURING MYOGENESIS. Bruce M. Paterson, Liliana DePonti, Wolfgang Quitschke, Barbara Winter, Lin Ze-Yu, Ruedi Billeter and Juanita Eldridge. Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

During myogenesis muscle-specific genes are transcriptionally activated and more than 80% of the genes expressed in the myoblast are inactive in differentiated cells. We have characterized important cis-elements involved in the transcriptional activation of the α-cardiac and α-skeletal actin genes, the myosin light chain 1f/3f gene, and the transcriptional suppression of the β-actin gene during myogenesis. Relatively short sequence elements ranging from 40 to 150 base pairs provide the major control regions, although other distal elements may be involved in modulating gene expression. It is important to characterize the trans-acting factors that interact with these control regions in order to understand the regulatory mechanisms. Relevant results will be presented on this issue.

Transcriptional Regulation of Muscle Gene Expression

M 033 ACTIN AND MYOSIN GENE EXPRESSION DURING STRIATED MUSCLE DEVELOPMENT IN THE MOUSE

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During the development of striated muscle the actin and myosin multigene families undergo transitions in isoform expression (1,2). We have used in situ hybridization to investigate this situation at early stages of myogenesis in the mouse. Cardiac actin transcripts are already accumulated in the primitive heart tube (7.5 days) and in somites (from 8.5 days). Skeletal actin transcripts become abundant in the myotome region of the somites from 9.5 days. The cardiac actin gene is therefore one of the first adult muscle genes to be activated during mammalian embryogenesis. We have described a mutant cardiac actin locus in the BALB/c mouse which results in reduced levels of cardiac actin and correspondingly higher levels of skeletal actin in the adult heart (3,4). This mutation also has repercussions, although less striking, during cardiac and skeletal muscle development.

The myosin alkali light chains (MLC), like the actins, show a cardiac/skeletal flexibility of expression during myogenesis. We have isolated and sequenced the atrial/foetal MLC (5) gene, thus obtaining the first protein sequence for this isoform. The ventricular/slow skeletal MLC gene (6) has also been isolated. Comparison of the 5' upstream sequences of these genes with those of the fast skeletal muscle MLC gene, and the cardiac and skeletal actin genes, indicates the presence of common sequences which may be involved in their regulation.

A functional analysis of mouse MLC and actin gene sequences has been carried out in primary cultures from mouse skeletal muscle, using 5' flanking sequences linked to a CAT indicator gene. We conclude that the fast skeletal MLC gene has two functional promoters. For $MLC1_F$ 1200 bp, but not 470 bp of sequence 5' to the Cap site are sufficient to confer tissue and developmental specificity while for $MLC3_F$ 438 bp suffice. All muscle gene promoters tested showed developmental regulation in the primary culture system. In contrast, variable results were obtained in muscle cell lines; in all cell lines tested the $MLC1_F$ proximal region was inactive, the α-cardiac actin promoter is not regulated in C2 cells, whereas other muscle promoters are correctly regulated. We conclude that muscle cell lines do not provide a reliable test system for functional activity. These experiments also indicate that the 5' promoter region of different muscle genes have different regulatory requirements.

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M 034 REGULATION OF GENE EXPRESSION DURING MYOGENESIS,

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We have been investigating the mechanisms of gene expression responsible for the emerging phenotype of the differentiating muscle cell. Evidence will be presented that a complex battery of muscle specific control elements regulates the expression of the α-actins and other muscle specific genes. These interact with trans-acting, modulating complexes made up of both general transcription factors as well as muscle-specific, accessory factors. We will describe the specific sequences involved, the locations and binding contacts of the modulating complexes and the characteristics of the functional transcriptional proteins.

Cellular and Molecular Biology of Muscle Development

M 035 CIS- AND TRANSACTING ELEMENTS INVOLVED IN THE REGULATION OF EXPRESSION OF MUSCLE-SPECIFIC GENES, Uri Nudel, Danielle Melloul, Valerian Nakar, Rebecca Aft-Konigsberg, Doron Lederfine and David Yaffe, Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Using the DNA-mediated gene transfer technique and chimeric gene constructs containing the promoter region of muscle-specific genes, we have shown that this region contains sufficient information to confer tissue- and stage-specific expression of the genesin transfected cells. To further analyze the cis-acting control elements, we introduced deletions in the promoter region (between -730 and -40) of a skeletal muscle actin/chloramphenicol acetyltransferase (CAT) chimeric gene, and introduced them into myogenic cells. Analysis of the expression of these constructs reveals the existence of several elements which are involved in the activation of the genes in differentiated cells. It seems also that some sequences suppress the expression of the genes in undifferentiated myogenic cells and in nonmuscle cells. Some of these sequences have the properties of a muscle gene-specific enhancer. Experiments were initiated to search for transacting factors which bind specifically to muscle gene control regions. Using the electrophoresis mobility shift assay technique, we found that nuclear extracts from differentiated myogenic cells contain protein(s) which bind specifically to DNA fragments containing the promoter region of the skeletal muscle actin gene and the MLC-2 gene. The actin gene fragment and the MLC-2 fragment compete for the binding of the same protein(s). By footprinting analysis we identified several regions in these fragments which bind nuclear proteins. Extensive sequence homology was detected between the protected regions of the actin gene and the MLC-2 gene.

M 036 CIS-ACTING ELEMENTS AND TRANS-ACTING FACTORS INVOLVED IN TRANSCRIPTIONAL CONTROL OF ALPHA STRIATED ACTIN GENES, Robert J. Schwartz, Department of Cell Biology, Baylor College of Medicine, Baylor College of Medicine, Houston, TX 77030.

In this study we investigated tissue restricted regulation of the chicken α-striated actin genes. A series of unidirectional 5' deletion and linker scanner mutations of the α-skeletal and α-smooth muscle actin upstream regions were inserted into the expression vector pSVoCAT, and were used to map DNA sequences that are required in cis for cell type specific expression. Chick embryonic myoblasts (CEM) and passaged fibroblasts (CEF) were suitable recipients for transfection with these CAT recombinants, since endogenous skeletal α-actin mRNA accumulated to more than 20-fold higher in CEM than in CEF cultures. Conversely, α-smooth muscle messenger arose to the highest levels in CEF cultures. The results of the transfection studies defined a cis-acting control region that contributed to the cell-specific expression of the chicken skeletal α-actin gene. Its 5' border was positioned 202 bp preceding the α-actin RNA cap site, which was congruent with the boundary of what was designated the core promoter element. The magnitude of CAT activity that accumulated in CEF cultures within any of the α-skeletal constructions was diminished about 90% relative to the level produced in transfected CEM cultures. With this approach we identified a critical subcomponent of the α-actin promoter which included a portion of a 16 base pair DNA motif termed the "CCAAT"-box associated repeat (CBAR). Remarkably, this CBAR element was found to be evolutionarily conserved as an indirect repeat within the 5'-flanking region of all known muscle-specific vertebrate actin genes. This characteristic as well as the loss of transcriptional activity by the insertion of a linker within this region of the α-skeletal actin gene strongly suggests that CBAR's have general regulatory importance for potentiating transcription. In the case of the α-smooth muscle actin gene the first 122 nucleotides of 5' flanking DNA were found to contain a "core" promoter capable of accurately directing high levels of transcription in both fibroblasts and myotubes. The activity of this core promoter is modulated in fibroblasts by a "governor" element(s) located at least in part between nucleotides -257 and -123. This region of the avian α-smooth muscle actin genes is responsible for providing at least a 95% degree of tissue restricted expression between fibroblasts and myoblasts. A smaller DNA (~ 75% homology) upstream CBAR is sufficient to suppress expression of the core promoter in CEM cultures. Thus, the upstream CBAR may play a negative role in the transcriptional regulation α-smooth muscle actin gene. We are now trying to determine how these highly conserved CBAR elements may play both a positive and negative role in the tissue restricted regulation of the α-striated actin genes.

M 037 TRANS-ACTING FACTORS THAT CONTROL MUSCLE ACTIN GENE EXPRESSION.

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To characterize the diffusible *trans*-acting factors involved in muscle gene regulation we have investigated the proteins that bind to the actin promoters. Using an electophoretic mobility shift assay, eight complexes were identified between nuclear proteins and a 300 bp segment of the chick skeletal actin promoter. We have focused on two of these complexes because they form with sequences that are located within a minimal functional promoter fragment and their presence seems to be regulated in a tissue specific manner. Using gel filtration chromatography and methylation interference footprinting we found that two factors interact with the same site in the promoter. The 100 Kd muscle actin promoter factor 1 (MAPF1) is the predominant binding activity in non-muscle nuclear extracts. Nuclear extracts from muscle cells form an additional complex that results from the binding of a 35 Kd protein (MAPF2). The binding of either protein is prevented by the methylation of any one of five guanine residues that are situated within a hexanucleotide segment from 78 to 83 bp's upstream from the start of transcription. MAPF1 and MAPF2 will compete for complex formation with the common binding site.

Th MAPF binding site is located within a 144 nucleotide promoter segment which is sufficient to confer muscle-specific gene expression to a reporter gene in transfected cells. Mutations in the footprint sequence which eliminate detectable binding decrease expression by a factor of 25 to 50 in skeletal muscle, but do not elevate the low level of expression found in non-muscle cells. These results show that the factor binding site contributes to the activation of expression in muscle cells. The MAPF binding site overlaps with the 3'-portion of a dyad symmetry element (DSE) that is highly conserved in muscle actin genes and is similar to DSE's in non-muscle promoters. Investigations with the muscle and non-muscle DSE's suggest that related factors may interact with these elements.

Gene Regulation in Animal Genetic Systems

M 038 MODULATION OF MYOSIN ASSEMBLY AND SYNTHESIS, Henry F. Epstein, Joseph P. Ardizzi, Sandra Honda, Ronald V. Abruzzese, Gary C. Berliner, Douglas L. Casey and Irving Ortiz. Department of Neurology and Division of Neuroscience, Baylor College, of Medicine, Houston, Texas 77030

The nematode Caenorhabditis elegans contains body-wall and pharyngeal muscles which exhibit organized sarcomeres and may be termed "striated". The sex, anal-intestinal and gonadal sheath muscles do not exhibit sarcomeres and may be termed "smooth". Monoclonal antibodies specific to myosin heavy chain isoforms A,B,C and D and to paramyosin localized these specific gene products to the different muscles. Paramyosin is present in all the muscle cells. MHC C and D are limited to pharyngeal muscle. All other muscle cells contain MHC A and B. The specific molecular properties of MHC A and B and paramyosin are compatible with multiple structural arrangements at the filament and subcellular levels.

One explanation for such modulation of assembly is the interaction of additional structural proteins with the major components. In body wall muscle, MHC A and B are differentially localized to specific filament regions. Paramyosin forms a domain underlying MHC. Genetic and physical dissociation of the thick filaments reveal a distinct core structure by electron microscopy. At least six additional protein bands are detected by SDS-PAGE in the mutant filaments lacking paramyosin or MHC B and in salt-dissociated wild-type filaments in which 90% of MHC and 75% of the paramyosin have been solubilized.

Expression of the unc-54 (MHC B), myo-3 (MHC A) and unc-15 (paramyosin) genes has been studied at the levels of runon transcription in isolated nuclei, accumulated mRNAs by hybridization and relative protein levels by immunoblots in embryos, L1 and L4 stages. unc-54, myo-3 and unc-15 RNAs accumulate at ratios of about 6:1:1 in embryos, 23:4:1 in L1 and 36:13:1 in L4. The nuclear transcription assays at stages L1 and L4 both show unc-54, myo-3 and unc-15 at relative activities of about 4:2:1. The accumulated proteins at these stages show a ratio of MHCB: MHCA: paramyosin of about 4:1:3. In embryos, the proteins are in equal abundance. Thus, the relative expression of the three thick filament genes changes from transcriptional to mRNA to protein levels suggesting that in addition to tissue-specific transcriptional activation, modulation occurs at post-transcriptional and translational levels. Supported by grants from the National Institutes of Health, the Muscular Dystrophy Association and the Welch Foundation.

M 039 MOLECULAR GENETICS OF DROSOPHILA THIN FILAMENT FORM AND FUNCTION, Eric Fyrberg, Department of Biology, The Johns Hopkins University, Charles and 34th Streets, Baltimore, MD 21218.

We are employing molecular genetic techniques to study structure-function relationships for actin, alpha-actinin, and the troponin-tropomyosin complex. Evidence that particular actin isoforms are specialized for contraction and/or assembly within particular cellular and developmental contexts will be presented. Characterization of normal and mutant alleles of troponin-T and tropomyosin will be described. We will also describe molecular genetic investigations of the cytoskeleton. In particular, characterization of normal and mutant alleles of alpha-actinin will be presented, as will the use of anti-morphic alleles to study cytoskeletal function during embryogenesis.

Molecular Biology of Human Muscle Disease

M 040 EXPRESSION OF THE MURINE DUCHENNE MUSCULAR DYSTROPHY GENE IN THE MUSCLE AND BRAIN OF NORMAL AND MUTANT MDX MICE. Jeffrey S. Chamberlain, Joel A. Pearlman, Richard A. Gibbs, Joel E. Ranier, Nancy J. Farwell, and C. Thomas Caskey, Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX. 77030.

We have isolated cDNA clones corresponding to the 5' 2.5kb of the murine Duchenne muscular dystrophy (mDMD) mRNA. These clones cross-hybridize well with the human DMD gene and are versatile in detecting deletions, duplications and RFLPs in DMD patients and carriers. Numerous genomic clones have been isolated from various portions of the human DMD gene, and are being used to develop novel diagnostic procedures which will be discussed. cDNA clones have been utilized to map the mDMD gene to a region of the mouse X-chromosome that also contains the muscular dystrophy mutation mdx. We have also examined the tissue specific expression of the mDMD gene in normal and three different strains of mutant mdx mice. mDMD mRNA is detectable in skeletal and cardiac muscle, and at very low levels in the brain of normal mice. All three strains of mdx mice display vastly reduced levels of apparently normal sized mDMD mRNA in both muscle and brain, when compared to the relative levels of muscle -phosphorylase kinase and hypoxanthine phosphoribosyltransferase mRNAs. These results strongly suggest that the mdx mutations are within the mDMD gene, and that mdx mice are an animal model for human Duchenne/Becker muscular dystrophy. The presence of DMD mRNA in brain tissue implies that DMD associated mental retardation may be a direct result of the failure of a mutant DMD gene product to provide a crucial function in the central nervous system.

M 041 BIOCHEMISTRY AND PHYSIOLOGY OF DUCHENNE MUSCULAR DYSTROPHY, Eric P. Hoffman[§], C. Michael Knudson[&], Kevin P. Campbell[&] and Louis M. Kunkel[§], [§]Children's Hospital, Harvard Medical School, Boston, MA 02158, and [&]University of Iowa, Iowa City, Iowa 52242.
Using cloned cDNAs corresponding to the mouse homologue of the Duchenne muscular dystrophy locus in humans, polyclonal antibodies have been raised in sheep and rabbits against 60 kd and 30 kd fragments of the DMD protein product. These antibodies recognize a common 400 kd protein in cardiac, skeletal, and smooth muscle which represents approximately 0.002% of total muscle protein. The protein, dystrophin, is completely absent in mdx mice and in most DMD-affected individuals, and exhibits a reduced size and/or abundance in most patients afflicted with a milder form of DMD, Becker muscular dystrophy. Dystrophin is highly conserved across evolution, exhibiting the same large size in many mammalian species, birds and amphibians. Dystrophin is a structural component of skeletal muscle 'triads', and therefor implicates Ca++ imbalances as the primary pathophysiological process in the dystrophic process. This protein is smaller, much less abundant and immunologically unrelated to another large triad-localized protein, the ryanodine receptor.

M 042 MITOCHONDRIAL MYOPATHIES:DISEASES OF TWO GENOMES. D.C. Wallace, X. Zheng, J. Schoffner, M. Lott, K. Li and S.N. Neckelmann. Depts. of Biochemistry and Neurology, Emory University School of Medicine, Atlanta,GA 30322.

Mitochondrial myopathy (MM) is associated with degeneration of the oxidative, striated, muscle fibers and the proliferation of highly abnormal mitochondria. Adjacent glycolytic, muscle fibers are unaffected. MM occurs in conjuction with a variety of neurological symptoms and correlates with deficiency in oxidative phosporylation (OXPHOS). OXPHOS consists of 5 complexes plus the adenine nucleotide translocator (ANT). Each complex is assembled from multiple polypeptides, most encoded by the nuclear DNA, but 13 encoded by the mitochondrial DNA (mtDNA). Nuclear DNA OXPHOS genes, such as the ANT, can include tissue-specific isoforms. Mitochondrial DNA genes fix mutations 10 to 17 times faster, are maternally inherited, and segregate from mixed intracellular populations leading to variable cellular phenotypes.

MM associated with myoclonic epilepsy (ME) is the best candidate for a mtDNA mutation. The patients show muscle weakness, lactic acidosis, bilateral deafness, ataxia, and progressive dementia. All individuals along the maternal lineage have MM, but their neurological symptoms vary greatly. 31P-NMR and excercise stress tests show that these patients rapidly exhaust the oxidative capacity of their muscle. Enzyme assays on skeletal muscle mitochondria indicate a variable deficiency in Complex I + IV with the extent of the metabolic defect correlating with the severity of the neurological symptoms. This pedigree could be explained by mutant mtDNAs mixed with normal mtDNAs and segregating along the maternal lineage.

MM associated with lethal, neonatal, lactic acidosis is the best candidate for a nuclear DNA OXPHOS mutation. One infant was normal at birth and born to normal parents with a normal daughter. He died at three months of progressive lactic acidosis, hepatomegally, and cardiomyopathy. His muscle showed a total deficiency in Complexes I and IV. This case could be explained by a neonatal switch from a normal fetal OXPHOS isoform to a defective adult isoform.

These two predigrees suggest a dichotomy of MM. The mtDNA mutations show maternal inheritance, variable expression, and progression. The nuclear DNA mutations show mendelian inheritance, quantized expression, and tissue and developmental stage specific expression.

M 043 THE DUCHENNE MUSCULAR DYSTROPHY LOCUS: A 2000 kb GENE WITH A 400 kDa PROTEIN, Ronald G. Worton, Arthur H.M. Burghes, Elizabeth E. Zubrzycka-Gaarn, Dennis E. Bulman, Henry J. Klamut, Sharon E. Bodrug and Peter N. Ray, Department of Genetics and Research Institute, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada, and George Karpati, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Duchenne muscular dystrophy (DMD) and its milder form, Becker muscular dystrophy (BMD) are allelic X-linked muscle wasting disorders. The responsible gene has been recently cloned from knowledge of its map location at band Xp21 in the short arm of the X chromosome. The cDNA recognizes a 14 kb transcript from fetal and adult skeletal muscle, as well as from fused myotubes in myogenic cell cultures. In total the cDNA probe recognizes over 60 Hind III bands on genomic digests of human DNA indicating a minimum of 60 exons in the gene. One or more exons are deleted in over 50% of boys with DMD or BMD and several translocation exchange points in females with the disease map within the gene. One of the translocation exchange points maps between exons 7 and 8 in a 105 kb intron. Two others map between exons 1 and 2 in an intron of unknown size. The size of the transcript predicts a 400-450 kDa protein product. Antibodies specific for the N-terminal region of the DMD gene product have been generated by immunization of rabbits with synthetic peptides (prepared by J. Talbot and R. Hodges, University of Alberta) based upon the cDNA sequence data at the 5' end of the gene. A second set of antibodies specific for the same region have been generated using as immunogen, fusion protein produced from cDNA subcloned into two different fusion vectors. By Western blot analysis, these antibodies recognize the fusion product as well as a 400 kDa protein from adult skeletal muscle. In studies of cultured muscle, the antibodies label the cell surface of fused myotubes. In studies by one of us (GK) an avidin-biotin based immunoperoxidase technique on cryostat sections of human muscle biopsies has revealed specific labelling of the plasma membrane (sarcolemma). Similar sections from DMD patients revealed reduced or negative staining. The DMD gene thus seems to encode a large protein component of the muscle cell membrane. The function remains to be elucidated.

Cellular and Molecular Biology of Muscle Development

Cell Lineages, Extracellular Matrix, Cytoskeleton, and Myosin Structure - I

M 100 MUSCLE CELL COMPONENTS DICTATE HEPATOCYTE GENE EXPRESSION AND CYTOARCHITECTURE IN HETEROKARYONS, Bruce T. Blakely, Steven C. Miller, Grace K. Pavlath, and Helen M. Blau, Stanford University School of Medicine, Stanford, CA 94305.

Major changes in cytoarchitecture and gene expression were induced in short-term heterokaryons. When human hepatocytes were fused with mouse muscle cells, the hepatocyte Golgi apparatus changed from its usual polar location to a uniformly perinuclear location typical of striated muscle. Human liver albumin was extinguished and the expression of the human muscle cell surface antigen 5.1H11 was induced without DNA replication or cell division. Coexpression of liver and muscle proteins was rarely observed. These novel findings provide insight into the regulation of gene expression and the targeting and localization of organelles with a central role in cell polarity, intracellular transport and secretion.

M 101 CELL-SUBSTRATE ATTACHMENT VIA THE FIBRONECTIN RECEPTOR PROVIDES THE SIGNAL FOR TERMINAL MYOGENIC DIFFERENTIATION. David Boettiger, Mindy George-Weinstein, and A. Sue Menko, University of Pennsylvania, Philadelphia, PA 19104.

Replicating myoblasts have two options, they may continue to replicate and initiate another cell cycle or they may withdraw from the cell cycle and initiate the terminal phases of differentiation. The terminal phases of differentiation involve the initiation of synthesis of most of the major muscle specific proteins and fusion to form mature myotubes. The signal to differentiate involves the binding of the fibronectin receptor, integrin, to the substrate providing an anchor for the assembly of the cytoskeletal elements and initiating synthesis of the major myogenic products. Normally this is accomplished by the binding of integrin to fibronectin. This interaction can be manipulated by the CSAT monoclonal antibody which binds to integrin. Soluble CSAT prevents terminal differentiation by blocking the interaction of integrin and fibronectin; substrate bound CSAT promotes terminal differentiation by substituting for substrate bound fibronectin. The results demonstrate that extracellular matrix receptors participate in the transmission of developmental triggers using a novel signalling system.

M 102 MYOBLASTS OF A CELL LINE GENERATED FROM AN ADULT MOUSE PARTICIPATE IN EMBRYONIC DEVELOPMENT, R. Buttner[1], F. Brem[2], K. Muller[1], and K.H. Westphal[1], 1: Laboratorium fur molekurlare Biologie - Genzentrum, Am Klopferspitz, D-8033 Martinsried, FRG. 2: Institut fur Tierzucht und Tierhygiene, Veterinarstrasse, D-8000 Munich, FRG.
We have studied the ability of myoblasts from a thymus reticulum derived muscle cell line (Abstract from Muller K. et al.) to respond on differention signals in mouse blastocysts and embryos and subsequent participation in regular organogenesis. Our experimental system comprises a myoblast cell line showing spontaneous in-vitro differentiation to muscle cells, which we have transfected with the plasmid p HVI SV 3V. After injection of one to two cells in blastocysts and 100 to 200 cells in 7 to 9 days old embryos we were able to detect in a variety of tissues of 8 to 16 weeks old mice plasmid marker DNA by different techniques. Probing genomic DNA on Southern blots with p HVI SV 3V we detect identical signals in all organs except from brain in two out of six mice having received the myoblasts in the blastocyst state, whereas in mice injected with the myoblasts as embryos the signals are much weaker and restricted to single organs. By transfection of genomic DNA from mice injected as blastocysts into E. coli DH 1 we are able to rescue plasmids with identical resistances and cross-hybridisation properties to p HVI SV 3V. Both the restriction pattern of rescued plasmids and the signals obtained from genomic DNA suggest that the marker DNA must have undergone substantial rearrangement. Our results show for the first time that non-malignant cells from an adult mammal introduced into blastocysts can participate in embryonic development and organogenesis. Presently we are characterizing the differentiation on a cellular level in tissue sections in order to investigate the cell lineage of the injected cells. The experimental rationale we used might generally be able to detect cells in an adult organism which can participate in embryogenesis and may lead to important new insights into the differentiation potential of those cells.

M 103 MYOFIBRILLAR REPAIR IN VIVO WITHOUT CELLULAR REGENERATION, Stirling Carpenter, George Karpati, Bassem Yamut, Montreal Neurological Institute, Montreal, Quebec, Canada, H3A-2B4.

We have devised an experimental model for studying selective breakdown of myosin (Muscle & Nerve 10:428-438, 1987) and the reconstitution of myofibrils without necrosis or formation of new cells in adult rat muscle in vivo. The sciatic nerve is crushed at the sciatic notch, causing denervation of leg muscles for approximately 2 weeks, after which reinnervation occurs. During the period of denervation 2-3mg/Kg/day of dexamethasone is administered. This combined treatment causes marked atrophy of muscle fibers in solei and plantares associated with profound depletion of myosin filaments as assessed by electron microscopy and electrophoresis, with relative preservation of all other myofibrillar elements; elastic filaments appeared to be retracted to the N_2 line. With reinnervation of the muscle and cessation of dexamethasone, reconstitution of myofibrils will take place. The stages in this repair will be described.

M 104 REPRESSION OF MUSCLE GENES IN PRIMARY AND IN TRANSFORMED NON-MUSCLE CELLS, Mildred Cho, Lydia Pan, and Helen M. Blau, Stanford University School of Medicine, Stanford, CA 94305.

Heterokaryons, the short-term fusion products of muscle and nonmuscle cells, have been used to study the activation and repression of muscle-specific gene expression in non-muscle cells. Although all primary non-muscle cell types tested in our laboratory show activation of previously dormant muscle genes upon fusion with myotubes, the malignant HeLa cell line only expresses these genes when treated with 5-azacytidine before heterokaryon formation. This finding suggests that, in this transformed cell type, there exists an additional mechanism for repressing and maintaining muscle genes in a silent state. In general, the unresponsiveness of endogenous muscle genes to muscle-specific trans-acting factors could be due to the state of the genome or to negative factors contributed by the transformed non-muscle cell. We are testing whether the repression of muscle genes observed in HeLa cells and the relief of this repression by 5-azacytidine is a general property of transformed cells and whether different mechanisms underlie the repression of genes in neoplastic and in normal diploid primary cells.

M 105 DESMIN EXPRESSION IN THE ELECTRIC ORGANS OF Electrophorus electricus, L., Manuel Luís Costa, Cláudia dos Santos Mermelstein, Vivaldo Moura Neto, Carlos Chagas Filho, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ, Brazil, 21941

Desmin is the intermediate filament protein typical of muscle cells. We extracted it from the electric organs of the electric eel Electrophorus electricus, L. This desmin was shown, by immunoblotting and peptide digestion, to be very similar to the chicken gizzard desmin. In two dimensional poliacrylamide gel electrophoresis it displayed five isoforms instead of two or three expressed in mammalian or avian muscles. Moreover, we found that, although the five isodesmins were present in the three kinds of the eel's electric organs, the expression pattern of isoforms was significantly different, according to isoelectricfocalization and densidometric analysis. We propose that the changes in expression of isodesmins are closely related to the physiological demandings of these organs.

M 106 EXPRESSION OF BASEMENT MEMBRANE AND CYTOSKELETON COMPONENTS IN NORMAL AND DYSTROPHIC HUMAN MUSCLE CELL, Christine Delaporte, Brigitte Dautréaux, Andrée Rouche and M. Fardeau, Inserm U. 153, Paris - France.
In Duchenne Muscular Dystrophy (DMD), cultured muscle cells have an abnormal growth. The main abnormality occurs during myoblast fusion which is delayed and results in small unbranched myotubes containing few nuclei. Scanning electron microscopy showed marked differences in the cell surfaces of myoblasts (Mb) and myotubes (Mt) : the normal Mb surface was rough with thick villi, whereas dystrophic Mb were smooth with long thin spicules. Dystrophic Mt were extremely flat and did not exhibit anchorage spindles. Fibronectin, heparan sulfate proteoglycan (HSPG), laminin, collagen IV, tubulin and desmin were tested by immunofluorescent staining with specific monoclonal antibodies. In dystrophic cells, fibronectin and HSPG were significantly reduced. In controls, these molecules covered the cultures in a dense meshwork, but in dystrophic cultures there were only sparse filaments along some Mt. Collagen IV and laminin showed a punctate distribution in DMD Mt when they were present along the entire Mt surface in controls. Tubulin, desmin and vimentin were much less abundant in dystrophic than in control Mt. Moreover these cytoskeleton molecules were poorly organized with rounded cytoskeleton free zones. Although these are not the primary defects they could explain the lower adhesiveness of dystrophic cells and their limitations in ability to fuse.

M 107 ISOLATION AND INITIAL CHARACTERIZATION OF A cDNA CLONE ENCODING AVIAN SKELETAL MUSCLE C-PROTEIN. Steven Einheber and Donald A. Fischman. Cornell University Medical College, New York, NY 10021.

C-protein is a myosin/thick filament associated protein exhibiting a periodic distribution along the middle third of each half A band. Understanding of its function has been limited by a lack of detailed information concerning its primary structure. To establish its structure and define tissue- and stage-specific isoforms, we have isolated and begun to characterize a cDNA clone encoding the fast muscle isoform of this protein. A λgtll cDNA library, constructed from one week post-hatch chicken pectoralis muscle, was screened with a polyclonal antiserum directed against gel-purified C-protein. A clone (λC-86), with a 3kb insert, was isolated and its encoded protein proven to be immunoreactive with several polyclonal antisera against C-protein and a monoclonal antibody (MF-1) specific for the fast muscle isoform. Identity of the clone was established further by hybridization to methylmercury hydroxide gel fractions enriched in C-protein mRNA and by hybrid selection. In preliminary northern blot analyses λC-86 hybridized to mRNA of approximately 4kb from 19-day embryonic, one week post-hatch, and adult chicken pectoralis muscle. It also hybridized to mRNA of similar size in rat quadriceps muscle. Restriction maps and sequence analysis will be presented. (supported by NIH grant RO1 AM32147 and a grant from MDA)

M 108 CHARACTERIZATION OF A BIPOTENT DERIVATIVE OF C2C12 MYOBLASTS. Karen J. Farmer and Woodring E. Wright, University of Texas Southwestern Medical Center, Dallas, TX 75235.
We describe here a C2C12 myoblast variant that behaves as an at least bipotent stem cell in that up to 50% of individual subclones contain mixed populations of myotubes and adipocytes. Mixed clones are still present after three cycles of subcloning. Colonies differentiate within 14 days to adipocytes, myocytes, or both when shifted to low serum medium. Addition of insulin to low serum medium increases the number of adipocytes relative to myocytes, but does not suppress the development of mixed colonies. Growth in dexamethasone for 10 days prior to transfer to low serum produced dense colonies composed of small "cobblestone" cells that are neither adipocytes nor myocytes. Addition of dexamethasone to low serum medium repressed differentiation of all colonies. Determined monopotent adipoblast and myoblast sublines are easily obtained by subcloning a mixed colony. Studies of the changes in regulatory patterns that occur when the bipotent stem cell versus its determined progeny are fused to differentiated chick myocytes are in progress.

M 109 COMMITMENT TO FORMATION OF DISTINCT MYOTUBE TYPES IN CHICKEN SATELLITE CELLS, Jeffrey L. Feldman and Frank E. Stockdale, Stanford University School of Medicine, Stanford, CA 94305.

Myoblast diversity has been demonstrated among embryonic and fetal myoblasts, but little has been discussed with respect to diversity among satellite cells. We have used an in vitro system to analyze muscle fiber formation from myogenic progenitors isolated from adult muscles (satellite cells). Myoblasts can be classified on the basis of the fast and/or slow myosin heavy chain (MHC) isoform or isoforms expressed in the myotubes they form in vitro. Satellite cells were isolated from the adult pectoral muscle (PM, primarily fast fibers) and anterior latissimus dorsi (ALD, primarily slow fibers) muscles of the chicken. The types of muscle fibers formed by the satellite cells were determined by immunocytochemistry with mAbs specific for fast and slow isoforms of MHC. Chicken satellite cells isolated from both the PM and the ALD initially formed myotubes that expressed only fast MHC. After the cells were either serially passaged or subcloned and passaged over an extended culture period of three weeks, however, 10-25% of the myotubes formed from ALD-derived satellite cells expressed both fast and slow MHC isoforms (fast/slow myotubes) and 1-2% expressed only slow isoforms (slow myotubes), whereas all the myotubes formed from PM-derived satellite cells continued to express only fast MHC. Thus, it appears that, much as there are diverse types of embryonic and fetal myoblasts, there are diverse types of chicken satellite cells that can form muscle fibers of different types and that the satellite cells found in adult fast and slow muscles may be different.

M 110 HEMIN ENHANCES DIFFERENTIATION AND MATURATION OF CULTURED REGENERATED SKELETAL MYOTUBES, Vicky L. Funanage, Nancy A. Schroedl, Susan M. Smith, James J. Kirwin, Priscilla A. Moses, and Charles R. Hartzell, Alfred I. duPont Institute, Wilmington,DE 19899

Regeneration of damaged or diseased skeletal muscle occurs by activation and differentiation of satellite cells, specialized cells located on the surface of muscle fibers. Satellite cells from regenerating skeletal muscle differentiate in culture to form contracting, cross-striated myotubes. Myotube maturation normally takes about two weeks in culture, but is accelerated to only five to seven days by the addition of hemin to the culture medium. Hemin causes a number of morphological changes in primary satellite cell cultures. These include an increase in the number, size, and alignment of myotubes and an increase in the number of myotubes that exhibit contractions and cross-striations. These morphological changes are accompanied by several biochemical changes, which include increases in the protein and mRNA for the muscle-specific isoenzyme of creatine kinase, an increase in palmitate oxidation, and increases in the specific activities of enzymes involved in aerobic metabolism. These biochemical changes cannot be duplicated by the addition of transferrin or ferrous ion to the culture medium. Thus, hemin exerts unique and extensive effects on skeletal myotube differentiation and metabolism. However, the effects of hemin are not restricted to regenerated skeletal myotubes as hemin elicits similar morphological and biochemical changes in cultured chick embryonic myoblasts and, to a lesser extent, rat L6 myoblasts.

M 111 IN VIVO STUDIES PROVIDE EVIDENCE AGAINST THE QUANTAL MITOSIS MODEL OF MYOGENESIS, Miranda D. Grounds, John K. McGeachie, University of Western Australia, Perth, Western Australia, 6009.

Autoradiographic studies were carried out on regenerating muscles of adult chickens. Three different muscles of 10 hens were injured with cut lesions, and tritiated thymidine (1uCi/g) injected at various times after injury. Detailed comparison of grain counts of premitotic nuclei, and of postmitotic myotube nuclei in samples removed 10 days after injury (when labelled precursors had fused to form myotubes), revealed how many times labelled precursors had divided before fusing into myotubes. DNA synthesis in muscle precursors was initiated 30 hours after injury. The grain counts of myotube nuclei showed that many precursors labelled at the onset of precursor proliferation had divided only once, or twice before fusing into myotubes. These observations contrast with studies of myogenesis in tissue cultured embryonic(1) and adult(2) chick muscle which propose that muscle stem cells divide at least 4 times, and undergo a final "quantal" mitosis before fusion. Our data from mice(3) and chickens show that the "quantal" mitosis model described by Quinn et al (1985) does not apply to myogenesis of mature muscle in vivo, implying that this model may only be a function of conditions in tissue culture.

1. Quinn, L.S., Holtzer, H. and Nameroff, M. (1985). Nature 313: 692-694.
2. Yablonka-Reuveni, Z., Quinn, L.S., Nameroff, M. (1987). Dev. Biol. 119: 252-259.
3. Grounds, M.D. and McGeachie, J.K. (1987). Cell Tiss. Res. In press.

M 112 COMMITMENT IN AVIAN MIGRATORY MYOBLAST POPULATIONS, E. Janet Hager and Frank E. Stockdale, Stanford University Medical School, Stanford, CA 94305.
Avian embryonic myoblasts constitute a heterogeneous population of cells that give rise to myotubes in cell culture expressing different myosin heavy chains (MHC). Embryonic myoblasts appear to be committed to distinct fast, mixed fast/slow and slow myogenic cell lineages because the progeny of an individual myoblast form only a single type of myotube in clonal culture. It is unknown if each of the three embryonic myoblast types identified in vitro form different primary fiber types in the developing embryo. As development proceeds, embryonic myoblasts are superceded by a distinct population of fetal myoblasts which become the predominant myoblast type during fetal muscle (secondary fiber) formation. There is substantial evidence that both the primary and secondary skeletal muscle fibers of avian limbs form from cells (myoblasts) that migrate from adjacent somites. It is unknown if a single precursor cell or multiple types of myoblasts migrate from the somites to the forming limb buds. To determine the path of cell migration, the myoblast origin of primary and secondary muscle fibers of different types, and the lineage relationships between embryonic and fetal myoblasts, we are establishing methods to stably mark different clonal populations of myoblasts. Two approaches for cell marking are in progress: firstly, stable transfection of quail myoblasts of known MHC expression with a β-galactosidase marker gene and secondly, use of the fluorescent vital dye CFSE. Marked embryonic and fetal myoblasts, committed to fast or fast/slow lineages, will be injected into somites and limb buds. The developmental fate and myogenic properties of the injected cells and their progeny will be determined both in vivo and in vitro using immunochemistry with MHC-specific monoclonal antibodies.

M 113 ANALYSIS OF PROTEIN SECRETION DURING MYOGENESIS, Anne E. Hughes, Colin A. Graham, W. H. Irwin McLean and Norman C. Nevin, Queen's University, Belfast BT9 7AB, U.K.
The changes which occur in expression of cellular proteins during myogenesis are well documented. We are interested in the role of the extracellular matrix proteins during differentiation. Two-dimensional polyacrylamide gel electrophoresis identifies over fifty proteins secreted by myoblasts in culture. Preliminary characteristics of these proteins are presented, as are the changes which occur during myogenesis.

M 114 SPATIAL IMMUNOLOCALIZATION OF CYTOSKELETAL PROTEINS DURING CARDIAC MYOGENESIS IN VITRO, Yuji Isobe, Dino A. Messina and Larry F. Lemanski, SUNY Health Science Center, Syracuse, NY 13210.
The localizations of several cytoskeletal proteins such as α-actinin, tubulin and spectrin were studied in newborn cultured and adult dissociated hamster heart muscle cells by light and three-dimensional electron microscopy. Developing newborn cells were grown on coverslips and gold EM grids while adult cells were kept in suspensions. These cells were permeabilized chemically or physically, incubated with primary antibodies against cytoskeletal proteins, and then indirectly immunolabeled with colloidal gold probes. In some specimens, silver enhancement was performed for light microscopic evaluation. Cells cultured on grids were critical point dried while those on coverslips were freeze-dried and then replicated with platinum and carbon. Adult dissociated cells were first freeze-fractured and deep-etched and then replicated. They were viewed with transmission electron microcope using tilting specimen stage. α-actinin and spectrin were found on and around myofibrillar Z bands where intermediate filaments converge, whereas tubulin staining was seen along microtubules. In addition, α-actinin staining was found at the intersection points of actin and intermediate filaments. These results suggest that α-actinin and spectrin interact with cytoskeletal elements to form highly organized three dimensional structures. (This work was supported by NIH grants HL32184 and HL37702 and a grant from the AHA.)

M 115 TWO DIFFERENT POPULATIONS OF MULTIPLY-INNERVATED FIBERS IN ADULT EXTRAOCULAR MUSCLE ARE LABELLED WITH A MONOCLONAL ANTIBODY SPECIFIC FOR α-CARDIAC HEAVY CHAIN. J. Jacoby, J. Davidowitz, R. Zak*, S. Shafiq+ and G. Philips. NYU Med Ctr, New York, NY 10016, *U Chicago, Chicago, IL 60637 and +SUNY-Downstate Med Ctr, Brooklyn, NY 11203.

Monoclonal antibodies (McAB) against myosin heavy chains (HC) distinguish two populations of multiply-innervated fibers (MIFs) in extraocular muscle (EOM). EOMs of mammals are composed of two layers, global and orbital. Each layer contains a population of MIFs which respond to depolarization with slow, sustained contractures. In rabbit, a McAB raised against the α-cardiac myosin HC by R. Zak labels ventricle and atrium, and strongly labels all global and the majority of orbital MIFs, but does not label either psoas "fast-twitch" or soleus "slow-twitch" muscle. These fibers exhibit an acid-stable ATPase activity. A second antibody, isolated by S. Shafiq against chicken anterior latissimus dorsi muscle (ALD), strongly labels all orbital but not global MIFs, and also does not label either psoas or soleus muscle. McABs specific for fast-twitch muscle fibers label the remaining populations of fibers in EOM. These fibers, as expected, exhibit an alkali-stable ATPase activity. Some, but not all of the ALD-McAB labelling fibers that do not label with the α-cardiac McAB also label with fast-twitch McABs. This double labelling occurs primarily in the region of the endplate band, where these fibers exhibit an alkali-stable ATPase. This work is supported by grants to J. Jacoby from NIH and The American Heart Association, NYC Affiliate.

M 116 DEVELOPMENTAL EXPRESSION OF FAST AND SLOW Ca^{2+}-ATPase ISOFORMS IN OVO AND IN VITRO IN CHICKEN SKELETAL MUSCLE. Z. Kaprielian and D. M. Fambrough, Dept. of Biology, The Johns Hopkins University, Baltimore, MD.

The expression of fast and slow isoforms of the sarcoplasmic reticulum Ca^{2+}-ATPase was studied in the developing chick embryo and in tissue-cultured myotubes. Monoclonal antibodies specific for each isoform were used as probes of protein expression. Analysis of expression of Ca^{2+}-ATPase isoforms in chick thigh muscles by immunofluorescence microscopy revealed that all muscle fibers expressed both isoforms during their development. Primary generation muscle fibers expressed predominantly the slow isoform. Secondary generation fibers expressed both isoforms at comparable levels. It was not until late in embryonic development that Ca^{2+}-ATPase isoform specific mAb's defined different populations of fibers. Immunoblot analysis of embryonic thigh muscle proteins indicated that the expression of the slow isoform varied little from embryonic day (ED) 6 to 19, while expression of the fast isoform increased dramatically just prior to ED19. Tissue cultured myotubes derived from ED12 chick thigh muscle myoblasts, plated at high density, expressed both isoforms of the Ca^{2+}-ATPase at very similar levels. Clonal analysis of myoblasts taken from early (ED6) and late (ED12) chick thigh muscles showed that all muscle colonies expressed both forms, consistent with in ovo results. These results suggest that no correlation exists between expression of Ca^{2+}-ATPase isoforms and myogenic lineages in developing avian muscle.

M 117 **REPLICATING MYOBLASTS EXPRESS A MUSCLE-SPECIFIC PHENOTYPE,** Stephen J. Kaufman and Rachel F. Foster, University of Illinois, Urbana, IL 61801.

Monoclonal antibodies and immunofluorescence microscopy were used to determine that replicating newborn rat myoblasts express a muscle-specific phenotype. To identify replicating cells, incorporation of 5-bromo-2-deoxyuridine (BrdUrd) into DNA was assayed using anti-BrdUrd antibody. H36, a developmentally regulated, muscle-specific, integral membrane protein (J.C.B. 100:1977), and desmin were scored as markers of the myogenic phenotype. Cells grown in BrdUrd for 90 min that stained with anti-BrdUrd antibody (ie., B^+ cells) also stained with anti-H36 and anti-desmin (R11) antibodies (ie., $H36^+$ and $R11^+$ cells). The proliferative state of $H36^+$ and $R11^+$ myoblasts was equal to the percent B^+ cells in the entire population, indicating that the expression of H36 and desmin are uniformly characteristic of replicating myoblasts. Inhibition of protein synthesis before and during growth in BrdUrd did not alter the frequency of R11 and H36 immunofluorescence in B^+ cells. Thus desmin and H36 were present in the replicating myoblasts prior to the onset of growth in BrdUrd. These results were confirmed using $H36^+$ cells selected by flow cytometry: these purified $H36^+$ myoblasts replicate, express desmin and differentiate. Similar results were obtained with mouse myoblasts. Desmin expression in these mammalian cells differs from that in chick embryo myoblasts: only a small proportion of replicating chick myoblasts express desmin. That replicating mammalian myoblasts have a muscle-specific phenotype serves to define a distinct stage in myogenic development and a specific cell in the myogenic lineage. It further implies that there is a regulatory event activated during myogenesis that is required for expression of those genes whose products distinguish the replicating myoblast.

M 118 ISOLATION AND CHARACTERIZATION OF A HUMAN NONMUSCLE MYOSIN HEAVY CHAIN cDNA CLONE, Leslie A. Leinwand[†], Claudia Saez[†] and Jeanne Myers[*], University of Pennsylvania, Philadelphia, Pennsylvania 19104, [†]Albert Einstein College of Medicine, Bronx, New York 10461.

Myosin heavy chains are ubiquitous in eukaryotes where, in conjunction with other contractile proteins, they are responsible for force generation. This force manifests itself in diverse cellular processes ranging from cytokinesis to muscle contraction. While numerous molecular probes have been isolated that encode striated muscle myosin heavy chains, no clones have been isolated which encode vertebrate nonmuscle myosin heavy chains. This is due in part to the lack of extensive sequence homology between muscle and nonmuscle myosin. We have isolated a 1.4 kb cDNA clone froma human fibrosarcoma cDNA library which encodes a portion of the nonmuscle myosin heavy chain rod. DNA sequence analysis indicates that periodic features ofthe nonmuscle myosin rod are similar to striated muscle myosin despite the absence of direct sequence homology between them. RNA analysis indicates that this gene is expressed in all cell types tested as a 6 kb mRNA. In human cells, there appear to be two mRNA species present which differ from each other by only 100 nucleotides. Analysis of genomic DNA indicates that nonmuscle myosin heavy chains are encoded by one or a very small number of genes; or the members of a multigene family are quite divergent. This is in contrast to the large number of vertebrate sarcomeric myosin heavy chain genes and the high degree of sequence conservation among the members.

M 119 **pBU65, AN EARLIER FACTOR TRANSIENTLY EXPRESSED DURING MYOGENESIS IN CULTURE AND IN FETAL DEVELOPMENT,** **V.K. Lin, L.M. Miranda, D. Sassoon* and W.E. Wright, U.T. Southwestern Med. Ctr., Dallas, TX and *Pasteur Inst. Paris, France.** **We have used subtraction-hybridization to isolate factors expressed in L6 rat myoblasts following a stimulus to differentiate but before overt biochemical differentiation begins. We have obtained a clone, pBU65, which is transiently expressed in differentiation-competent cells but not in their differentiation-defective derivatives. During muscle differentiation in culture, its transcript appears 24 hr before myosin heavy chain transcripts can be detected, then it peaks and declines as myotube formation begins. pBU65 is expressed twenty-fold higher in fetal skeletal muscle than in adult skeletal muscle, and, except for low levels in fetal brain, its expression is not detectable in any other tissues examined. In situ hybridization to mouse embryo sections show that pBU65 is expressed in presumptive muscle tissue as early as 8.5 days p.c. This pattern of expression suggests that pBU65 codes for a structural or regulatory factor important during an early event in myogenesis, and represents the earliest known marker for the process of terminal myogenesis. DNA sequence analysis of pBU65, which is 21 n.t. shorter than its native mRNA, indicated that there is no significant homologies to known sequences. It codes for a protein with a predicted size of 32.5 kDa, and in vitro transcription/translation yielded a protein with an M_r of 30 kDa. We are presently preparing an antibody to the protein encoded by pBU65 to be used in cytochemical analysis. In addition, the cloning and characterization of the gene corresponding to pBU65 is in progress.**

M 120 MODULATION OF MUSCLE DIFFERENTIATION BY CELL-MEMBRANE-SUBSTRATUM INTERACTIONS, A. Macieira-Coelho and J. P. Wahrmann, Department of Cell Biology, Faculty of Health Sciences, Linköping, Sweden and Institute of Molecular Biology, INSERM, Paris, France.

The differentiation of cells from the L6 mouse cell line could be modulated plating the cells on a protein polymer covered either with positively or negatively charged substances. Differentiation proceeded on the negatively charged surfaces but stopped on the positive ones. Differentiation could be stopped at different times during the early stages, seeding positively charged beads on the cells. The results showed that cell shape is not the limiting factor for the division and differentiation of L6 myoblasts. Several experiments suggested that this substratum does not have a direct effect on cell metabolism but rather controls cell division and differentiation through physical forces triggered at the membrane level. The results favor the hypothesis previously proposed (Int. Rev. Cyt. 83, 1983, p. 183) that conformational changes originated at the cell membrane are transmitted through the cytoskeleton to the nuclear cage, where they help to create the chromatin conformation favorable for gene expression.

M 121 MOLECULAR CLONING OF mRNAs FOR HUMAN CARDIAC MYOSIN HEAVY CHAINS AND LIGHT CHAINS: REGULATION OF EXPRESSION DURING DEVELOPMENT AND HYPERTROPHY. Masahiko Kurabayashi, Hidetsugu Tsuchimochi, Yoshio Yazaki. University of Tokyo, Tokyo, Japan. The present study was undertaken to understand the molecular basis of myosin heterogeniety in human myocardium and the molecular mechanisms responsible for their isoform switches during development and pressure overloaded hypertrophy. We have isolated and characterized α- and β-myosin heavy chains (MHCs), atrial and ventricular alkali myosin light chains (MLC1) from a cDNA library of human fetal heart and characterized. Comparison of the nucleotide and deduced amino acid sequences between α- and β-MHC shows 95.1%, 96.2% homology, respectively. The overall nucleotide and amino acid sequence homology between the coding regions of atrial and ventricular MLC1 mRNA is 77.2%, 76.7%, respectively. By Northern blot analysis using the isoform-specific noncoding region probes, expressions of individual isoform in normal, developing and diseased heart are examined. α- and β-MHC mRNA is exclusively expressed at all ages in atrium and ventricle, respectively. β-MHC mRNA is also expressed in slow skeletal muscle and adult atrium but scarecely expressed in fetal atrium. Furthermore, we demonstrated that the expression of β-MHC mRNA is increased in pressure overloaded atrium and that of α-MHC mRNA is correspondingly decreased. From the comparison of the degree of transition from α- to β-MHC analyzed by Northern blot with that visualized by antimyosin immunofluorescence in the tissue section obtained from the same patient, we show that changes in α- and β-MHC protein are, at least in part, regulated at pretranslational level. RNA blot analysis shows that transition from atrial to ventricular MLC1 also occurs in hypertrophied atrium. Finally, we demonstrate that fetal light chain 1 and the atrial MLC1 are encoded by the same gene.

M 122 ELECTROPHORETIC AND IMMUNOLOGICAL ANALYSIS OF SECRETED PROTEINS WHICH DISTINGUISH FIBROBLASTS FROM MYOBLASTS, W. H. Irwin McLean, Colin A. Graham, Anne E. Hughes and Norman C. Nevin, Queen's University, Belfast, U.K.
It is difficult to identify myoblasts and fibroblasts morphologically. We have examined the proteins which are secreted by these cell types. Two-dimensional polyacrylamide gel electrophoresis reveals over fifty groups of proteins. Many are common to both cell type however several proteins show cell type-specific secretion. Antibodies against these proteins can distinguish myoblasts and fibroblasts by immunofluorescence. These antibodies will facilitate the assessment of cells in primary and clonal muscle cultures from normal and dystrophic biopsies.

M 123 CO-EXPRESSION OF MULTIPLE MYOSIN HEAVY CHAIN ISOFORMS IN CULTURED QUAIL MYOTUBES AND MYOGENIC CLONES, Peter Merrifield, William Sutherland and Irwin Konigsberg, Department of Biology, University of Virginia, Charlottesville, VA 22901.
Myosin heavy chain (MHC) is encoded by a multigene family and exhibits polymorphisms which are tissue and develpmental stage specific. We have raised monoclonal antibodies (Mabs) to the MHCs expressed in cultured quail myotubes and used them to demonstrate that at least five distinct MHC isoforms are expressed in these cells. Epitope mapping demonstrates that these MHCs are very similar to those expressed in adult heart (α and β cardiac), adult ALD (slow), adult extraocular (superfast) and adult pectoralis (fast type IIB) muscles. Differentiated myotubes co-express adult α cardiac, β cardiac and slow MHCs immediately after myogenic fusion. Shortly thereafter, adult superfast MHC can be detected. While adult fast type IIB MHC can be detected in cultures maintained for longer than 9 days, it never accumulates to the levels observed in post-hatch quail. Although myoblasts differentiate at different rates in different media, co-expression of multiple isoforms can be detected in myotubes grown in defined (F12 PVP/Insulin), differentiation-promoting (1-10 MEM) and growth-promoting (10-15 MEM) medium. Clonal analyses reveal that myoblasts obtained from 9 day quail embryos form clones which co-express all isoforms, suggesting that myoblasts have the capacity to express all isoforms under standard culture conditions. This pattern of MHC expression in cultured quail myotubes is consistent with a mechanism of differentiation which involves coordinate gene activation followed by selective repression of certain isoforms during subsequent muscle maturation. (Supported by NIH and MRC).

M 124 SYNERGISM BETWEEN MYOGENIC AND NON-MYOGENIC CELLS DURING MUSCLE FORMATION IN VIVO, Jennifer E. Morgan and Terence A. Partridge, Department of Histopathology, Charing Cross and Westminster Medical School, London W6 8RF.

Enzymatic disaggregation of neonatal mouse muscle yields a mixture of myogenic and non-myogenic cells which can be separated on discontinuous Percoll gradients to give one fraction enriched in myogenic cells and one non-mygenic fraction. These two fractions and the unseparated cell preparation were injected into freeze-killed extensor digitorum longus muscles of athymic (nu/nu) mouse hosts and examined 70 days later. The amount of new muscle formed at the graft site was estimated (wet wt. x % muscle in tissue sections) and its origin determined by the use of allotypic isoenzymes of Glucose-6-Phosphate Isomerase (GPI) as markers. Freeze-killed muscles not injected with cells contained small amounts of new-formed muscle, derived entirely from host tissue. The amount of muscle formed was doubled, surprisingly, by injection of either the enriched myogenic or the non-myogenic fraction: in the former case the muscle was mainly of donor origin, in the latter it appeared to be entirely host, the donor cells having made no detectable direct contribution. Injection of the unfractionated cell suspension gave the highest yield of new muscle at the graft site: this muscle was derived from both the injected cells and from host tissues. We conclude that the non-myogenic cells present in muscle can enhance myogenesis in regenerating muscles.

M 125 SUPPRESSION OF ONCOGENE EXPRESSION IN MUSCLE CELLS DURING DIFFERENTIATION INDUCED CESSATION OF PROLIFERATION, K. Muller, J. Espen and K.-H. Westphal, Laboratorium fur molekulare Biologie-Genzentrum, Am Klopferspitz D-8033 Martinsried FRG.
The increased level of expression of the nuclear oncogenes p53, c-myc, c-fos and of the B2 repeat containing RNA has been correlated with the transformed status and the degree of tumorgenicity of several cell types. In untransformed cells the expression of these oncogenes and of the B2 repeat RNAs correlates with cellular proliferation. We introduce a new experimental system, the AM1 C1 2 thymus reticulum derived myoblasts (Wekerle H. et al., Nature 256, 493-494, 1975), which is unique in its ability to generate differentiated non proliferative cells without any changes in the exogenous mitogen concentration. We demonstrate that the transformation like high level expression of p53, c-myc and of the transformation/proliferation associated B2 repeat RNAs (but not c-fos) is severely suppressed by the muscle cell differentiation process. Expression of the SV40 large Tantigen in the myoblasts blocks myogenic differentiation, but does not alter oncogene expression at RNA level and p53 protein level is kept similar to that of transformed cells. The data suggest that transformation like high level expression of p53, c-myc and of the B2 repeat containing RNAs is neither sufficient to keep muscle cells proliferating nor to transform them, and the expression of the oncogenes is under the control of genes induced in the muscle cell differentiation process without any direct influence of the exogenous growth factor concentration. The observations show the superior role of a physiological differentiation process in the control of proliferation and transformation and have important implications on the functions of nuclear oncoproteins and the B2 repeat containing RNAs in proliferation and transformation. We are further exploring the intrinsic ability of this cell system in suppression of oncogene expression and the differentiation properties by introducing cells of this type into blastocytes and embryos (abstract by R. Buttner et al).

M 126 ENHANCED THYMIDINE INCORPORATION INTO SKELETAL MUSCLE SATELLITE CELLS OF PIGS TREATED WITH TESTOSTERONE AND RATS SELECTED FOR RAPID GROWTH, D.R. Mulvaney and M.T. Gore, Alabama Agricultural Experiment Station, Auburn University, AL 36849.
Postnatal replication of satellite cell (SC) nuclei and their intrusion into myofibers results in increased myonuclei numbers and DNA, which endows muscle with greater protein synthetic potential. Our objectives have been to characterize the differences in SC metabolic activity in pigs treated with androgen and rats that differ in rates of growth. Because of sexual dimorphism in muscle growth, analysis was made on the effects of castration and testosterone propionate (TP) administration on SC proliferative activity (PA). Thirty-six neonatal pigs were randomly assigned to the following treatment groups: sham implanted gonadally intact males (B), sham-implanted castrated males (C) or castrated males implanted with testosterone propionate (C+TP). Four pigs from each group were sacrificed at 7, 14 or 21 d of age after a 6-h continuous infusion of ^{3}H-thymidine. Myofibers isolated from the triceps brachii were prepared for satellite cell enumeration by light microscope autoradiography. A developmental decline in SC metabolic activity occurred in all groups, however, the greatest decline occurred in C ($P<.01$). A treatment-by-age interaction was observed for percentage of labeled nuclei. Castration reduced total and labeled nuclei per mm myofiber ($P<.05$), and C + TP had a higher percentage of labeled nuclei than C (2.8 vs 2.2%; $P<.05$). Triceps brachii muscles from 21 d B and C + TP were 120% ($P<.05$) of C. In other studies, male rat pups selected for rapid or slow rates of growth for 12 generations were injected with ^{3}H-dThd at 1, 2 and 3 wk age and muscle fibers prepared as above. Percentage tagged nuclei was higher ($P<.01$) in muscle of fast compared to slow growing rats and declined with age in both strains ($P<.01$). Results indicate that growth of skeletal muscle is dependent on SC mitotic activity and that genetic selection for rapid growth as well as administration of TP, enhances this activity in young animals.

M 127 FUNCTIONAL ASSAYS FOR IDENTIFYING TRANS-ACTING FACTORS THAT MEDIATE MUSCLE DETERMINATION AND DIFFERENTIATION, Lydia Pan, Charlotte Peterson, Beat Schaefer, Joyce West and Helen M. Blau, Stanford University School of Medicine, Stanford, CA 94305

Development entails a series of determination and differentiation steps. Little is known about the factors which regulate these steps. Heterokaryon experiments suggest that trans-acting factors from a muscle cell can diffuse through the cytoplasm and activate the expression of muscle genes in a fibroblast nucleus (Blau et al., 1983, Cell 32:1171-1180; Blau et al., 1985, Science 230:758-766; Pavlath and Blau, 1986, J. Cell Biol. 102:124-130; Hardeman et al., 1986, Cell 47:123-130). We are attempting to identify and characterize these factors by assaying for their function. Experiments involving cybrids, enucleated muscle cells fused to fibroblasts, will determine if the activation of muscle genes in the fibroblast nucleus is a stable, heritable event. This would imply that the changes in gene expression are at the level of cell determination. The activation of endogenous muscle genes observed in heterokaryons and cybrids is being mimicked by DNA transfection and RNA microinjection into fibroblasts leading to the identification of the underlying factors and the genes encoding them. To identify factors acting at the level of differentiation, we are stably introducing into nonmuscle cells several specific muscle promoters linked to reporter genes (Hardeman et al., J. Cell Biol., in press). Activation of these genes via transfection and microinjection is being monitored at the single-cell level by microscopy and with the fluorescence-activated cell sorter. The recent findings by others that many factors act indirectly and do not bind DNA underscore the need for assays for tissue-specific trans-acting factors based on function.

M 128 MYOGENESIS *IN VIVO* FROM THE MOUSE C2 MUSCLE CELL–LINE.

Terence A. Partridge*, Jennifer E. Morgan*, Stephen E. Moore[§] & Frank,S.,Walsh[§],
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The capacity of the murine C2 myogenic cell-line to form muscle *in vivo* has been investigated. Various numbers of C2 cells were inserted into regenerating muscle autografts in athymic (nu/nu) host mice. Hosts were selected such that they differed in Glucose–6–phosphate (GPI) isoenzyme allotype from C2 cells. This enabled us to identify host and donor contributions to the regenerated muscle. The presence of a 'hybrid' GPI indicated that donor C2 cells had fused with the regenerating host muscle fibres. When C2 cells were injected into freeze killed muscle or into ethicon–coated vicryl surtures, they gave rise to large masses of densely packed muscle fibres. In graft sites examined several months after injection, tumours of C2 origin were found: we are attempting to suppress this neoplastic behaviour of C2 cells while retaining the muscle fibres formed from these cells. Currently, we are investigating whether C2–derived muscle fibres become innervated and phenotypically mature. If they do, this should prove a valuable model system for the study of gene expression and for the experimental manipulation of such gene expression in innervated, mature skeletal muscle.

M 129 **BASIC DIFFERENCES BETWEEN CHICKEN AND MAMMALIAN MUSCLES**

Rudolf Billeter, Marius Messerli, Adrian Puntschart, Eva Wey, Hans M. Eppenberger and Jean-Claude Perriard, Institute for Cell Biology, Swiss Federal Institute of Technology, CH-8093 Zuerich, Switzerland.

We have determined the fiber type composition of seven major chicken thigh muscles with the histochemical methods myofibrillar ATPase, α -Glycerolphosphatedehydrogenase (glycolytic capacity) and NADH-Tetrazoliumreductase (oxidative capacity). Chicken muscles are composed principally of a mixture of type II (fast twich) and type III (slow tonic) fibres. This is in contrast to mammalian muscles, which are composed of type II (fast twich) and type I (slow twich) fibres, while type III fibres are only found in extraocular muscles. In chicken, however, type I fibres are rare, they were found only in m. sartorius and m. adductor medialis.

Chicken type III fibres have a high oxidative capacity, but display varying glycolytic capacities: In muscles with many type III fibres, their glycolytic capacities are low, while in muscles with few type III fibres, they are rich in glycolytic enzymes. These results suggest that in chicken, type III fibres take the place of type I fibres in mammalian muscle.

M 130 **Myogenic Determination of the Multipotential 10T1/2 Cell Line By a Cloned Human DNA Locus, *myd.***
Deborah F. Pinney, Stephen F. Konieczny, Keith E. Latham, Sonia Pearson-White and Charles P. Emerson, Jr.
University of Virginia, Charlottesville, VA 22901

Embryonic cells become committed to specific lineages through a process termed determination. In this study we demonstrate a genetic basis of mesodermal determination and identify the genes involved in myogenic determination. Stable myogenic cell lines were derived at a high frequency by transfection of a cloned multipotential mouse embryo cell line, C3H 10T1/2, clone 8 (10T1/2) with cloned human DNA linked to a selectable neomycin-resistance gene. These cell lines proliferate in culture, yet maintain the capacity to differentiate and express characteristic muscle-specific proteins. Thus, we conclude that myogenic determination has a simple genetic basis: a single myogenic determination gene *myd*, is activated, thereby establishing a muscle-specific regulatory program that mediates the activation of muscle genes when these determined myoblasts differentiate. We have taken two distinct approaches to further characterize the myogenic determination event: a cDNA cloning approach and isolation of the human genomic *myd* clone. We constructed a cDNA library from myogenic cells derived from 10T1/2 cells by 5-azacytidine treatment. Myoblast-specific cDNAs, including a *myd* encoding cDNA, were identified using a differential screening protocol. The cDNAs isolated allow us to investigate the function of the gene products involved in myogenic determination and to determine the sequence of events when 10T1/2 cells are converted to stable myogenic cell lines. Isolation of the original human *myd* genomic clone allows us to investigate *myd* gene organization and the regulatory elements required for tissue specificity and appropriate developmental expression.

M 131 CHARACTERIZATION OF CHICK MYOGENIC CELL COMPARTMENTS. LeBris Smith Quinn and Mark Nameroff, University of Washington, Seattle, WA 98195.

Myogenic cells from the pectoral muscles of 10-11 day and 17-18 day chick embryos can be distinguished on the basis of at least five criteria: (1) Clonal analysis reveals that cells from 10-11 day embryos form mostly small myogenic clones which contain 1-16 muscle cells, while cells from 17-18 day embryos form large myogenic clones which contain hundreds of precursors and terminally differentiated muscle cells. (2) In mass cultures, cells from 10-11 day embryos undergo terminal differentiation in an asynchronous fashion with a peak of differentiation on day 3, whereas mass cultures of cells from 17-18 day embryos differentiate relatively synchronously after four days of culture. (3) Myogenic cells from the two sources exhibit different polypeptide synthetic patterns, as assayed by SDS-polyacrylamide gel electrophoresis. (4) Exposure of cells from 10-11 day embryos to 60 μg/ml Merocyanine 540 plus white light results in nearly complete inhibition of myogenesis (by myogenic cell death), while cells from 17-18 day embryos are resistant to this treatment. (5) Exposure of 10-11 day cell cultures to 100 pM TGF-B for 30 hours results in a delay in terminal differentiation, but the same treatment has no effect on the kinetics of terminal differentiation in cultures from 17-18 day embryos. We infer that cells which give rise to the large clones are stem cells, and that they are precursors to the cells which form small clones ("committed" cells). The above-identified differences are evidence of physiological and biochemical changes which occur as cells traverse sequential stages in the myogenic lineage.

M 132 CLONING OF A VERTEBRATE NONMUSCLE MYOSIN HEAVY CHAIN,
Ralph V. Shohet, David A. Brill, Yvette A. Preston and Robert S. Adelstein, NHLBI, NIH, Bethesda, MD 20892.

We screened a chicken brush border lamda gt11 cDNA library (from P. Matsudaira, MIT) with affinity purified antibodies raised against human platelet myosin. Two clones of 4.1 and 4.3 kb hybridized to each other and to a 23 bp oligonucleotide probe derived from the amino acid sequence of a tryptic fragment of human platelet light meromyosin. Northern analysis using poly A+ from chicken brush border and kidney showed hybridization with a 6.5 kb message. Partial sequencing (500 bp) of several restriction fragments reveals a 70-85% nucleotide and predicted amino acid similarity to a recently characterized avian smooth muscle myosin heavy chain (M. Yanagisawa et al.,J. Mol. Biol.,in press). A portion of our clone hybridizes to a single, 5.5 kb, EcoRI fragment on a chicken genomic southern.

M 133 **ROLE OF EXTRACELLULAR MATRIX IN THE DEVELOPMENT OF NEUROMUSCULAR JUNCTIONS,** T.Somasekhar, Y.Ramamohan, B.K.C. Sagar and Sarala Das Dept. of Neuropathology, National Institute of Mental Health and Neurosciences, Bangalore, India.

An electronmicroscopic investigation was carried out on the myotomal muscles of tadpole of frog (Rana cyanophlictis)to study the development of neuromuscular junctions NMJ's with emphasis on the role of extracellular matrix (ECM). In unhatched tadpoles (58 hours) the NMJ's showed tiny axon terminals with synaptic vesicles and a few dense core vesicles without any post synaptic specializations. The morphological differentiation of pre - and post synaptic components of NMJ's appeared incomplete even in 34 day old tadpoles. The basal lamina was initially seen only at the sites of NMJ's. Fibroblasts and collagen fibrils of the ECM were found often in the vicinity of newly formed NMJ's. A sequential fine structural study on the development of NMJ's will be presented highlighting the role of ECM.

M 134 MYOSIN GENE EXPRESSION IN MAMMALIAN CARDIOGENESIS, Lauren J. Sweeney., Dept. of Anatomy, Loyola Univ. School of Medicine, Maywood, IL 60153.

Results presented here demonstrate that myosin expression can be utilized to investigate questions of cell lineage and plasticity of those cell lineages in cardiogenesis in both mammalian and avian models. We have previously shown that atrial myocytes express a different myosin phenotype than ventricular myocytes from the earliest stages of differentiation in the embryonic heart of the chicken, and further that this phenotype is distinct from that of the adult heart (Sweeney et al., Circ. Res. 61:287-295,1987). The results presented here indicate that such a phenotypic difference also pertains to the embryonic mouse and rat hearts. Immunofluorescence methodologies were employed to localize myosin HC expression to specific myocytes, and gel electrophoresis and immunoblot transfers to confirm the expression of HCa and HCb. The monoclonal antibodies (McAb) employed to make these discriminations included McAb 37, which has previously been determined to be HCa specific, and McAbs CCM 52 and HPM9, which react with differing affinities with both HCa and HCb. Results showed that both the rat and mouse atrial myocardia expressed the fast HCa isoform (with high ATPase activity) throughout embryonic and fetal life, while the embryonic and fetal ventricular myocardia expressed only the slower HCb (with 2-3 fold lower ATPase activity). In addition, the atrial myocardium expressed HCb from the onset of differentiation. This phenotype is in contrast to that of the neonatal and adult mammalian heart, in which the HCa and HCb isoforms are expressed in both atria and ventricles in varying percentages. Furthermore, the fact of such differential expression of myosin isoforms in embryonic atrial and ventricular myocytes provides a developmental model for examining the commitment of mammalian myocytes to their contractile protein phenotypes. Such studies will allow us to determine how early in development the plasticity of myosin expression which pertains in neonatal and adult mammalian myocytes is established.

M 135 EXPRESSION OF SLOW TONIC MYOSIN IN MUSCLE SPINDLE FIBRES EARLY IN MAMMALIAN DEVELOPMENT, Lars-Eric Thornell, Barbara Kay Grove, Fatima Pedrosa, University of Umeå, S-901 87 Umeå, Sweden; Gill Butler-Browne, Paris, France; Gertej Dhoot, Birmingham, England and Donald A. Fischman, New York, USA.

Muscle spindles contain highly specialized intrafusal muscle fibers which show a unique isomyosin and M-band composition and have special interactions with both sensory and motor neurons compared to extrafusal fibers. We have examined the developmental expression of isomyosins and M-bands protein in muscle spindles in human and rat fetuses. Fetal muscles at different stages of development were serially cryosectioned and immunostained with polyclonal or monoclonal antibodies against different isomyosins and M-band proteins. Primary myotubes destined to become intrafusal fibers could be distinguished on the basis of a unique expression of slow tonic myosin in human fetuses as early as 10-11 weeks of gestation and in rats at 17 days of gestation. In the human fetuses, this occurred prior to the differentiation of extrafusal fibers into fiber types which could be detected at 14-16 weeks of gestation by the appearance of slow twitch myosin concomitant with the reduction of neonatal myosin in primary generation fibers. Diversification with respect to M-band occurred still later. Rats denervated at birth and examined at 4 weeks of age did not show expression of slow tonic myosin in the denervated muscles. Thus we propose that a special lineage of myotubes is predetermined or influenced early by sensory neurons to form the primary generation of intrafusal fibers in muscle spindles whereas the secondary generation fibers and the M-band characteristics are modulated by motor neurons.

M 136 **Architecture of Sarcomere Matrix in Skeletal Muscle--Evidence that Nebulin Constitutes a Distinct Set of Nonextensible Filaments in Parallel with Titin Filaments,** K. Wang and J. Wright. Clayton Foundation Biochemical Institute & Department of Chemistry, The University of Texas at Austin, Austin, TX 78712

Nebulin, a giant myofibrillar protein (0.6 x 10^6 Mr subunit) abundant in skeletal muscles, has been proposed as a component of a set of cytoskeletal filaments that coexist with thick and thin filaments within the sarcomere. A monospecific antiserum was used to localize nebulin in mechanically skinned single fibers from rabbit psoas muscle at electron microscopic resolution. These labeled fibers exhibited six to seven pairs of transverse bands of various intensities within each sarcomere. Studies of their axial distribution at different sarcomere lengths indicated that nebulin epitopes maintained a fixed distance to the closest Z line, suggesting a nonextensible elongated structure rigidly attached to the Z line. Since nebulin does not exhibit the types of elastic stretch found in the I band domain of titin filaments, it must constitute a distinct set of filaments in parallel, but not in series, with titin filaments. Thus the sarcomere may have two sets of non-actomyosin filaments: a set of A-segment- (or thick filament-) linked titin filaments, and a set of I-segment- (or titin filament-) linked nebulin filaments. This four-filament sarcomere model has many interesting structural and functional implications. For example, it suggests that titin, but not nebulin, is the primary source of sarcomere elasticity. It raises the possibility that titin and nebulin might act as organizing templates (or scaffolds) as well as length-determining factors for the assembly of A-segments and I-segments, respectively, during muscle development.

M 137 IDENTIFICATION OF SATELLITE CELL-SPECIFIC ANTIGENS, Zipora Yablonka-Reuveni, Department of Biological Structure, University of Washington, Seattle, WA 98195

Satellite cells are the myogenic precursor cells in mature muscle and are positioned between the basement membrane and the plasma membrane of the muscle fiber. The similarity between these cells and the embryonic myoblasts is not clear. We have recently reported on the isolation of satellite cells from chicken pectoral muscle employing Percoll density centrifugation (Yablonka-Reuveni et al., Dev. Biol. 119, 252-259, 1987). This approach yields satellite cells free of myofibril debris and fibroblasts. We now report on the isolation of four monoclonal antibodies which react specifically with satellite cells from chicken pectoral muscle but not with myoblasts or myotubes from 10-day-old chicken embryos. Percoll-isolated satellite cells were used to immunize Balb/c mice. Screening for satellite cell-specific antibodies was performed using 1-2-day-old myogenic cultures from the pectoral muscles of 10-day-old embryos and adults. When assayed on frozen sections from adult muscle, three antibodies reacted positively with satellite cells but not with other cells in the tissue. A fourth antibody reacted with satellite cells and also with a specific region in the blood vessels observed in the muscle sections. We conclude that isolated myogenic cells from adult muscle indeed represent the "classical" satellite cells. Using these antibodies, we are now studying the time of appearance of satellite cells during muscle development. (Supported by NIH grant #AR-28154 and by University of Washington Graduate School Research Fund)

Cell Lineages, Extracellualr Matrix, Cytoskeleton, and Myosin Structure - II

M 200 **CARDIAC TROPONIN T ISO-PROTEINS STRUCTURE/FUNCTION**
Parker B. Antin and Charles P. Ordahl. UCSF, SF, CA 94143

Sarcomerogenesis and maturation involves the sequential expression and assembly of an ordered series of contractile iso-proteins. A major unresolved question in muscle developmental biology is the functional significance of these myofibrillar iso-protein switches. Toyota and Shimada showed that chicken cardiac troponin T (cTNT) dissappears during early skeletal muscle development (1) in response to neurogenic cues (2). Molecular analysis of cTNT mRNA indicated that the cTNT protein which appears in embryonic skeletal muscle is an embryo-specific variant of cTNT (cTNTemb) that contains an internal 10-amino acid segment absent from cTNTadult (3). We have made anti-peptide antibodies which recognize the embryo-specific peptide of cTNTemb. Using this antisera, in conjunction with an antibody recognizing all cTNT isoproteins (1), we now demonstrate by immunoblot analysis that cTNTemb has an apparent molecular weight of approximately 37 kD and is the predominant cTNT isoform expressed in both embryonic heart and skeletal muscle. cTNTemb subsequently disappears from skeletal and cardiac muscle late in fetal development. In heart it is replaced by the 36 kD cTNTadult isoform which persists throughout adult life. These findings using anti-peptide antibodies specific for cTNTemb corroborate previous predictions of cTNT iso-protein switching based upon mRNA analysis (3). Techniques are now being developed to adapt this antibody for immunofluorescence studies to determine the sites of incorporation of both isoforms into the myogenic cytoskeleton. To develop a system to manipulate cTNT protein expression in vivo, we have cloned full length cDNAs into an avian retroviral expression vector to allow expression of cTNT iso-proteins, and mutants, to be forced in a variety of cell types. Infected fibroblasts show expression of cTNT mRNA under viral control. Experiments are underway to use anti-cTNT antibodies to analyze the expression of virally encoded cTNT iso-proteins. Supported by grants from NIH(CPO) and NIH Fellowship(PBA); Refs: 1.JCB 91[1981]497; 2.Cell 33[1983]297; 3. Cooper & Ordahl, JBC 260[1985]11140.

M 201 DEVELOPMENT OF THE EXTENSOR TIBIAE NEUROMUSCULAR SYSTEM IN THE EMBRYONIC GRASSHOPPER, Eldon E. Ball*, Camilla M. Myers*, and Paul M. Whitington+: *Developmental Neurobiology, R.S.B.S., Australian National Univ., Canberra City, A.C.T. 2601, Australia; +Dept. of Zoology, Univ. of New England, Armidale, N.S.W. 2351, Australia

The large extensor tibiae muscle (ETi) of the metathoracic legs powers the jump of the grasshopper. It is innervated by four identified neurons whose pattern of innervation, transmitters, and approximate time of arrival at the muscle are all known. The muscle forms as a giant syncytium which begins to split up at approximately 50% of embryonic development (Ball & Goodman, Dev. Biol. 111,399-416 (1985)). We are studying the development of innervation of the ETi by two of the identified neurons by using dye fills of these cells at intervals of a few percent of development from the time they first send out axons. Their axons make several interesting and consistent "mistakes", which are then rapidly corrected. Ablation experiments, followed by embryo culture, are currently in progress to determine causal relationships during the establishment of the adult pattern of innervation.

M 202 MYOSIN EXPRESSION AND PHYSIOLOGICAL PROPERTIES OF REINNERVATING AND REGENERATING RAT SOLEUS MUSCLE Christine E. Davis and John B. Harris, Muscular Dystrophy Research Laboratories, Newcastle General Hospital, Newcastle upon Tyne, England

Degeneration, followed by regeneration of the soleus muscle of female rats (6-8 weeks) is induced by injection of Notechis scutatus venom. Degeneration is complete by 24 hrs and regeneration and differentiation by 21 days. 95-100% of soleus muscle fibres normally react with antibodies to slow myosin and up to 30% may also cross react with antibodies to fast myosin. By contrast, the fibres of regenerated muscles react only with slow antimyosin and not with fast antimyosin. Denervated regenerating soleus muscles react only with fast antimyosin. We wish to know whether we can alter myosin expression in regenerating muscles by manipulating the duration of denervation and whether changes in myosin expression are reflected in changes in contractile properties.

Single crushes were made to the sciatic nerve of one hind limb of rats to produce a transient denervation of the muscle. Multiple crushes were used to vary the period of denervation. At various times after denervation the mechanical properties and the numbers of fibres reacting with slow and fast myosin antibodies were determined. Preliminary results suggest delaying reinnervation for approximately 10 days resulted in a reinnervated soleus muscle in which the number of muscle fibres reacting with slow antimyosin was unchanged but the number of fibres cross reacting with fast antimyosin was increased to 85%. There was no obvious change in the speeds of contraction or relaxation of the muscles. This apparent dislocation between the biochemical and the physiological definition of the muscle is being explored.

M 203 VISUALIZATION OF CARDIAC VENTRICULAR MYOSIN HEAVY CHAIN HOMODIMERS AND HETERODIMERS BY MONOCLONAL ANTIBODY EPITOPE MAPPING. Claude A. Dechesne, Patrice Bouvagnet, J.O.C. Leger, Doris Walzthöny and Jean J. Leger. INSERM U. 300, Faculté de Pharmacie, 34100 Montpellier, France.

The dimeric organization of cardiac ventricular myosin heavy chain (MHC) was investigated using monoclonal antibodies (Mabs), highly specific of either α or β MHC. Ten epitopes spanning the myosin rod were mapped by electron microscopy of rotary shadowed replicas of Mab complexed with human and/or rat ventricular myosins. Rat ventricular myosin was simultaneously labeled by one of the anti-α and one of the anti-β MHC Mab. These two Mabs were directed against epitopes that were clearly separated on the MHC rod. $\alpha\alpha$ MHC and $\beta\beta$ MHC homodimers were visualized in complexes consisting of two molecules of the same Mab bound to one myosin molecule. $\alpha\beta$ MHC heterodimers were visualized in complexes formed by one molecule of each of the two Mab bound to one myosin molecule. This visualization of cardiac myosin molecules clearly demonstrates the homodimeric and heterodimeric arrangement of α and β MHC, as initially proposed by Hoh et al. (1979).

M 204 THE REPLACEMENT OF C-PROTEIN ISOFORMS IN THE SARCOMERES OF DEVELOPING CHICK PECTORALIS MUSCLE. J.E. Dennis and D.A. Fischman, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021.

The distribution of contractile protein isoforms in developing myofibrils is of interest to models of sarcomere assembly, remodeling and turnover. Since the "slow" and "fast" isoforms of C-protein, recognized by McAbs ALD-66 and MF-1 respectively, are found in the same myofibers of early posthatch chick pectoralis muscle (PM) (Obinata et al., 1984, Dev. Biol. 101:116-124) but the "slow" isoform disappears from the PM by 2-weeks post-hatching, we have been able to compare the loss of this isoform from individual sarcomeres in the same or adjacent myofibers by immunoelectron microscopy. The sarcomere pattern of the "slow" isoform in the 3-d posthatch PM is identical to that observed in adult slow ALD muscle (Dennis et al., 1984, J. Cell Biol. 98:1514-1522) suggesting that the sarcomere pattern is a property of the C-protein isoform and not its interactive myosin heavy chain. At 3-d virtually all PM myofibers exhibit strong and identical staining with ALD-66. By 8-d, however, the intensity and pattern of staining varies between adjacent fibers. Of importance, is the fact that within single myofibers all of the sarcomeres exhibit identical reactivity with the McAb. These results suggest that replacement of the C-protein isoforms within a single cell occurs coordinately along all of the myofibrils and not by excision of whole sarcomeres. (Supported by NIH AM32147 and MDA).

M 205 THE EXPRESSION OF PARVALBUMIN IN NERVE-MUSCLE COCULTURES. Marion Ecob-Prince, Aileen Brown and Ekkehard Leberer*. Muscular Dystrophy Group Research Laboratories, Newcastle-upon-Tyne, England, NE4 6BE and *University of Toronto, Canada.

Parvalbumin is a high affinity Ca^{2+}-binding protein present only in vertebrates and is highly enriched in fast muscle. This localization has prompted the suggestion that parvalbumin may function to facilitate the rate of muscle relaxation. In rabbits, parvalbumin is associated only with fast contracting muscle and its expression appears to be neurally-regulated (Biochem. J. 235:67-73, 1986).

We have now confirmed that parvalbumin is also associated with fast fibres in mouse muscle in vivo and have investigated its expression in mouse muscle that has regenerated in cocultures with mouse spinal cord cells. These regenerated fibres are histochemically mature and contain adult fast myosin without the developmental isoforms (J. Cell Biol. 103:995-1005, 1986).

Using fixed frozen sections of these cultures, we have been unable to demonstrate parvalbumin in the regenerated mature fibres. This preliminary result will now have to be substantiated using freeze-substituted cultures from which we are sure the highly-soluble parvalbumin has not been lost.

Supported by Muscular Dystrophy Group of Great Britain.

M 206 IFM(2)2: AN ASSEMBLY DEFECTIVE MUTATION THAT MAPS TO THE MYOSIN HEAVY CHAIN GENE. Scott Falkenthal and Miyoung Chun, The Ohio State University, Columbus, OH 43210.

Ifm(2)2 is a homozygous viable mutation that affects myofibrillar assembly in the indirect flight muscle (IFM) and no other muscles of Drosophila melanogaster. No sarcomeric structures assemble due to the complete absence of thick filaments; however, thin filaments and Z discs do accumulate. Remarkably, the thin filaments maintain their normal position along the longitudinal axis of the fiber. The major thick filament proteins (myosin heavy chain, MHC, and myosin light chains, MLC) fail to accumulate in the IFM. Using a molecular complementation assay we have determined that this mutation maps to the muscle specific myosin heavy chain gene at chromosome band 36B. Pulse labeling studies have demonstrated that the MHC protein is not synthesized in the mutant IFM, whereas the MLC proteins are synthesized at normal rates. The rate at which the myosin light chains are degraded must be very high, because we fail to detect their accumulation. Therefore, the stability of the myosin light chains is absolutely dependent upon their assembly with the myosin heavy chain. The transcriptional activity of the MHC gene in this mutant will be discussed.

M 207 A NONMOTILE ZEBRAFISH MUTANT AFFECTING EARLY STAGES OF MYOFIBRILLOGENESIS, Adam Felsenfeld and Mark Curry, Institute of Neuroscience, University of Oregon, Eugene, 97403

What steps are necessary for the correct orientation and assembly of the mature myofibril? One of the first cytoskeletal structures observed in myofibrillogenesis consists of bundles of long thin filaments called 'stress fiber like structures'(SFLS)(1,2).It has been suggested that SFLS have a role in orienting the myofibril. Are SFLS required for myofibril formation? To address this, we have examined early events in myofibrillar development in both wild type zebrafish and a nonmotile mutant in which skeletal muscle cells develop to contain myofilaments which are not organized into longitudinally arrayed myofibrils. Assembly of myofibrils in wild type embryos occurs first precisely between 14 and 14.5 hours after fertilization in an identifiable population of axial muscle cells lying just next to the notochord. The first steps of this assembly are ultrastructurally similar to those described in other organisms. Initial assembly occurs along SFLS's, as identified by both electron microscopy and staining with rhodamine-phalloidin. The immature myofibril initially contains thick filaments interspersed longitudinally among the SFLS but not in proper register with each other. In contrast, the same cells in the mutant at this stage stain poorly with rhodamine-phalloidin, suggesting an absence of SFLS. Electron microscopic observation of mutant muscle at this time reveals a lack of both SFLS and oriented thick filaments. These observations are consistent with the hypothesis that SFLS are necessary for correct assembly of mature myofibrils. (Supported by NSF grant BNS-8512370 and NIH grant NS17963).

1. H.B.Peng, et al (1981) Developmental Biology **88**, 121-126
2. P.Antin, et al (1986) J. Cell Biology **102**, 1464-1479

M 208 ASSEMBLY OF CYTOSKELETAL PROTEINS IN MUSCLE CELLS. William B. Isaacs and Alice B. Fulton, University of Iowa, Iowa City, IA 52242.

We have extended earlier studies of cotranslational assembly of cytoskeletal proteins by studying assembly in vivo. In cultured embryonic chicken muscle cells, some myosin heavy chains form RNase and puromycin resistant associations with the cytoskeleton during translation. For brevity, this fraction of stably associated nascent chains will be said to assemble cotranslationally (details in PNAS, 1987, 84:6174-6178).

We have studied assembly in vivo of four other proteins, vimentin, tubulin, alpha actinin, and titin, which differ extensively in assembly. Fraction of given protein that assembles cotranslationally: vimentin, 35-42%; tubulin, 0%; alpha-actinin, 0%; titin, 70-80%. A smaller fraction of vimentin is cotranslationally assembled in the transformed muscle cell line, rhabdomyosarcoma, than in cultured muscle. Post-translational assembly kinetics of vimentin also vary with cell type; $t_{1/2}$ of assembly varies from 6 to >22 min.

These results reveal several properties of cotranslational assembly: 1. Not all cytoskeletal proteins assemble cotranslationally. 2. Not all cotranslationally assembled proteins are muscle specific. 3. Neither size nor charge determines cotranslational assembly. 4. A given protein can follow different assembly paths in different cellular environments. These results reduce the chance that cotranslational assembly is an artefact of extraction and imply that cotranslational assembly is under cellular control. Taken with studies of mRNA localization, these results imply a different mode of cytoskeletal assembly and control than the model of self-assembly from solution most used to date.

M 209 IN VITRO STUDIES OF HYBRIDS OF CARDIAC TROPONIN C AND CALMODULIN, Samuel George, Tomio Ono, John Putkey, Richard Cook and Anthony Means, Division of Cell Biology and Cardiology, Baylor College of Medicine, Houston, TX 77030.

Calmodulin (CaM) and cardiac troponin C (cTnC) are two members of the EF hand superfamily of Ca^{++} binding proteins. CaM and cTnC are structurally similar with 55% direct amino acid identity. Unlike CaM, cTnC does not bind Ca^{++} in its first domain. and also has an 8 amino acid extension at its N-terminus . CaM is ubiquitous in eukaryotic cells, and regulates a broad range of intracellular processes. cTnC is limited primarily to the troponin-tropomyosin complex of slow skeletal and cardiac muscle, and does not activate CaM-dependent enzymes. In order to evaluate the molecular basis for the inability of cTnC to activate CaM dependent enzymes, we have constructed several bacterial expression vectors for chicken CaM and cTnC, and hybrids of these two proteins. The hybrids include: (1) first domain of cTnC, last three domains of CaM (TaM), (2)first domain of CaM, last three domains of cTnC (CaT), and (3) a truncated CaM in which the first domain is removed (aM). CaM and TaM activate CaM dependent - cyclic GMP phosphodiesterase (PDE) to the same maximal extent, with almost identical apparent Kms. cTnC and CaT required 10^3 higher concentrations than CaM to activate PDE, whereas aM did not activate PDE. We conclude that: (1) Ca^{++} binding to the first domain of CaM is not necessary to activate PDE, (2) neither the inactive Ca^{++} binding site nor the N-terminal extension of cTnC constitute the basis for its inability to activate PDE, (3) the conformation imparted by the first domain of CaM is necessary to activate PDE but can be substituted by the dissimilar region of cTnC.

M 210 **TRANSCRIPTION OF GLYCOGEN PHOSPHORYLASE GENES IN FREELY GRAFTED RAT SKELETAL MUSCLE.** Frederic Gorin, Christopher Matthews, Rebecca Mullinax, Richard Carlsen. University of California, School of Medicine, Davis, Ca.

Regenerating skeletal muscle grafts differ from developing muscle in having lower oxidative and glycolytic enzymatic activities. Wagner,et.al.(J.Neurol.Sci.34:373) demonstrated that the activities of several key glycolytic enzymes, including glycogen phosphorylase, fail to return to control levels in stabilized free grafts of rat EDL. The grafts also show physiological deficits which suggest incomplete reinnervation of the regenerated muscle fibers. We have isolated total RNA from freely grafted rat EDL at different times following the procedure. We determined, using rat cDNA probes specific for the different isozymes of phosphorylase, that muscle (M) phosphorylase mRNA is nearly undetectable up to 28 days after grafting. Histological and physiological evaluation of the grafts indicated that the tissue was revascularized, but was in the early stages of reinnervation. Stimulation of the peroneal nerve evoked a twitch response that was approximately 12% of the force produced by the contralateral control EDL. These observations indicate that phosphorylase enzymatic activity is not restored to normal levels in free muscle grafts because the normal myogenic induction of M- phosphorylase gene expression may not occur. The data also suggest that transcription of this gene may depend on neurotrophic influences provided by the motor nerve.

M 211 ASSEMBLY OF TITIN, MYOSIN, ACTIN, AND TROPOMYOSIN INTO MYOFIBRILS IN CULTURED CHICK CARDIOMYOCYTES, Marion L. Greaser[1], Susan E. Handel[1], Seu-Mei Wang[1], Edward Schultz[1], Jeannette C. Bulinski[2], Jim J.-C. Lin[3], and James L. Lessard[4], [1]Univ. of Wisconsin, Madison, WI 53706; [2]UCLA, Los Angeles, CA 90024; [3]Univ. of Iowa, Iowa City, IA 52242; [4]Childrens Hospital, Cincinnati, OH 45229.

Myofibril assembly was followed in cardiomyocytes by double immunofluorescence using different antibody combinations. Muscle (M) tropomyosin (TM) and alpha actin were initially found in stress fiber like structures (SFLS) without M-myosin or titin. The beginning of sarcomere formation was marked by the appearance of periodic breaks in the TM and actin staining at the level of the Z lines. At the same time a pair of titin staining bands was associated with each developing A band (myosin staining) even at the 2 or 3 sarcomere stage. As the myofibrils matured, the actin and M-TM staining was progressively excluded from the A band region. Similarly the myosin staining pattern transformed from one in which the A bands had uniform intensity to the mature state in which stronger staining was concentrated in the M line region. The titin staining achieved its mature state prior to that obtained with the other major myofibrillar proteins, suggesting titin may play an important role in initiating sarcomere assembly. In some cases SFLS were positively stained for M-TM without alpha actin staining, indicating that synthesis of these two proteins was not tightly coupled. Gamma actin and nonmuscle-TM were sometimes found in sarcomeric patterns. Their assembly into developing myofibrils brings into question the current designations of "sarcomeric" proteins.

M 212 SUPERFAST MYOSIN HEAVY CHAIN EXPRESSION IN A NERVE-MUSCLE CULTURE SYSTEM, Mark A. Hill, J.F.Y. Hoh and Marion S. Ecob-Prince, Muscular Dystrophy Group Research Laboratories, Newcastle-upon-Tyne, England, NE4 6BE.

The myosin heavy-chain (MHC) has many isoforms which are expressed in a fibre type and developmental specific manner. It is known that innervation influences MHC isoform expression both during muscle development as well as in the adult. This neural control of MHC expression may be mediated in either a trophic- or activity-dependent manner.

Using antibodies which are specific to superfast, fast and slow MHC isoforms, we have found that the majority of fibres of adult cat posterior temporalis muscle contain superfast myosin and a minority (<5%) contain adult slow. We have now cocultured the posterior temporalis muscle with embryonic mouse spinal cord cells. The regenerated posterior temporalis fibres develop into cross-striated, contracting fibres containing both adult fast and slow but not superfast MHC isoforms. These preliminary results are now being extended to question the control of superfast MHC expression and the role of the nerve in this process.

Supported by Muscular Dystrophy Group of Great Britain.

M 213 LOCALIZATION OF TALIN IN PERMEABILIZED AND LIVING NON-MUSCLE CELLS, Rick S. Hock, Jean M. Sanger and Joseph W. Sanger, Department of Anatomy and Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6058.

To investigate the role of talin in the attachment of stress fibers to the cell membrane of non-muscle cells, purified chicken gizzard talin was covalently labeled with the fluorescent dye lissamine rhodamine sulfonyl chloride. The fluorescently-labeled protein (talin-LR) was then repurified on Sephadex G-25 and DEAE-cellulose to remove free dye and denatured protein. In acetone permeabilized epithelial cells (PtK_2), talin-LR localized to attachment plaques and junctional regions between cells. In permeabilized fibroblasts (gerbil fibroma and embryonic chick), talin-LR strongly bound to attachment plaques, membrane ruffles, and foci. Talin-LR was rapidly (less than 3 hr) incorporated into attachment plaques when microinjected into living PtK_2 cells. During mitosis and cytokinesis, the attachment plaques disappeared and then subsequently reappeared upon spreading of the two daughter cells. Preliminary experiments indicate that this probe will allow for a differentiation between a class of stable attachment plaques and a second more dynamic class that is found around the nucleus and at the actively moving cell periphery. Utilizing this probe, experiments are in progress to assess the involvement of talin in the establishment and maintenance of the cell membrane attachment sites for myofibrils in living skeletal and cardiac muscle.

M 214 TITIN IN EMBRYONIC MUSCLE: ANTIBODY LOCALIZATION AND PRELIMINARY ISOLATION OF A cDNA PROBE, **Ted W. Huiatt, Michelle A. Kurpakus and Greg R. Sater, Muscle Biology Group, Iowa State University, Ames, IA 50011.**

Titin is a megadalton, myofibrillar protein that is thought to comprise a third set of elastic filaments in the sarcomere. To study the role of titin in myofibril assembly, a series of double-label immunofluorescence experiments were done to localize simultaneously titin and actin, titin and muscle myosin, and titin and nebulin in embryonic chick myogenic cell cultures. Results demonstrated that titin and myosin were organized at approximately the same time into myofibril-like patterns and that organization of both proteins occurred before the organization of actin into discreet I-bands. Assembly of both titin and muscle myosin occurred along stress-fiber-like arrays of actin. In contrast, nebulin organization occurred either at the same time or later than the organization of actin and nebulin organization appeared to be correlated with the appearance of phase dense Z-lines in nascent myofibrils. To further characterize titin, we have isolated a cDNA clone for titin mRNA. A cDNA library was constructed from embryonic chick cardiac muscle RNA using random priming instead of the more commonly used oligo-(dT) priming, a procedure that allows construction of very large cDNA's. The cDNA was then randomly digested with a combination of restriction endonucleases to obtain fragments of appropriate size, and the fragments were inserted into the expression vector gt11. Screening of the resulting library with polyclonal anti-titin antibodies resulted in identification of several positive clones, one of which has been isolated. Further characterization of this clone by Northern blot analysis is in progress. **(Supported in part by grants from AHA and MDA.)**

M 215 ASSEMBLY OF CYTOSKELETAL PROTEINS IN MUSCLE CELLS. William B. Isaacs and Alice B. Fulton, University of Iowa, Iowa City, IA 52242.

We have extended earlier studies of cotranslational assembly of cytoskeletal proteins by studying assembly <u>in vivo</u>. In cultured embryonic chicken muscle cells, some myosin heavy chains form RNase and puromycin resistant associations with the cytoskeleton during translation. For brevity, this fraction of stably associated nascent chains will be said to assemble cotranslationally (details in PNAS, 1987, 84:6174-6178).

We have studied assembly <u>in vivo</u> of four other proteins, vimentin, tubulin, alpha actinin, and titin, which differ extensively in assembly. Fraction of given protein that assembles cotranslationally: vimentin, 35-42%; tubulin, 0%; alpha-actinin, 0%; titin, 70-80%. A smaller fraction of vimentin is cotranslationally assembled in the transformed muscle cell line, rhabdomyosarcoma, than in cultured muscle. Post-translational assembly kinetics of vimentin also vary with cell type; $t_{1/2}$ of assembly varies from 6 to >22 min.

These results reveal several properties of cotranslational assembly: 1. Not all cytoskeletal proteins assemble cotranslationally. 2. Not all cotranslationally assembled proteins are muscle specific. 3. Neither size nor charge determines cotranslational assembly. 4. A given protein can follow different assembly paths in different cellular environments. These results reduce the chance that cotranslational assembly is an artefact of extraction and imply that cotranslational assembly is under cellular control. Taken with studies of mRNA localization, these results imply a different mode of cytoskeletal assembly and control than the model of self-assembly from solution most used to date.

M 216 CLONING, CHARACTERIZATION OF HUMAN VENTRICULAR ALKALI MYOSIN LIGHT CHAIN (HVLC1) cDNA AND THE PREDICTED SECONDARY PROTEIN STRUCTURE OF HVLC1, George Jackowski, Eva Hoffmann, Qin-Wei Shi and Peter M. Olley, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada, M5G1X8 and Department of Pediatrics, University of Alberta, Edmonton, Canada, T6G2B7.

This report deals with the isolation and characterization of a cDNA clone for the ventricular alkali myosin light chain (HVLC1) and compares the predicted secondary protein structure of HVLC1 to human skeletal (HSLC1) and chicken cardiac (CMLC1) muscle myosin light chain one proteins. To achieve this goal a human ventricular cDNA library was constructed, yielding 2.4 x 10^{-5} recombinants. Using a 17 mer oligodeoxynucleotide probe corresponding to a conserve region of VLC1 portion of the known partial sequence of HVLC1 protein, fourteen cDNA clones encoding for HVLC1 were isolated, one of which, pCD HVLC1, represents a full-length transcript of HVLC1 mRNA and the 3' uncoding region. Southern blot hybridization of restricted human DNA demonstrates that the VLC1 exists as one gene copy in man. The derived amino acid sequence shows 63% homology with HSLC1 and 81% homology with CMLC1.

Although a fair amount of information on the function of the regulatory myosin light chain (MLC2) has been obtained, the function of the alkali myosin light chain (MLC1) is currently unknown. We decided to determine the predicted secondary structure of HVLC1, HSLC1 and CMLC1 which would assist us in determining the role these proteins play in the regulation of the contractile process. A comparison of the predicted secondary structures demonstrated that the carboxylic end of HVLC1 and HSLC1 contain three identical coiled hinge regions while the amino ends of the proteins were different, the HVLC1 amino end was globular and the HSLC1 amino end was planar. The two cardiac myosin light chains, human (HVLC1) and chicken (CMLC1), were similar in the amino end. They both had a predicted globular head region while in the carboxylic end, the chicken (CMLC1) had a two coiled hinge region. These studies suggest that the myosin light chain one (MLC1) molecule is a flexible hinged molecule and that tissue specificity resides in the amino end of the molecule.

Supported by MRC grant #MA-8559, G. Jackowski is an MRC Scholar.

M 217 CHARACTERIZATION OF TWO HUMAN HEAVY CHAIN cDNAs THAT ARE EXPRESSED SPECIFICALLY IN FETAL SKELETAL MUSCLE, I. Karsch-Mizrachi*, L. Silberstein#, M. Travis#, H. Blau#, and L. Leinwand*, Albert Einstein College of Medicine, Bronx, New York; #Stanford University School of Medicine, Stanford, CA.

In vertebrates, sarcomeric myosin heavy chains (MHCs) are developmentally regulated and can be distinguished by immunochemical and molecular genetic reagents. Monoclonal antibodies (mAbs) have been generated that recognize epitopes present specifically in fetal, neonatal or adult human muslce fibers. Two of the fetal specific mAbs, F1 and F2, recognize MHCs that are expressed only prenatally. In order to identify DNA sequences encoding developmentally regulated MHCs, F1 and F2 were used to screen a gt11 human skeletal muscle cultured cell expression cDNA library. Two distinct cDNA clones, FS-1 and FS-2, were isolated with F1 and F2 mAbs, respectively. FS-1 encodes the 3' 3.5 Kb of the 5.8 Kb full length MHC mRNA. The insert of FS-2 is 1.9 Kb and corresponds to the portion of the myosin rod represented by the 5' half of FS-1. The FS-1 cDNA clone has been characterized for expression exclusively in fetal skeletal muscle. The DNA sequence of this clone is at least 70% homologous to other vertebrate MHCs. When compared to the rat embryonic skeletal MHC gene sequence (Strehler et al (1986) J. Mol. Biol. 190, 291-317), FS-1 shows 88.2% nucleic acid sequence homology and 95% amino acid homology. This indicates that the human FS-1 gene is likely to be the analog of this rat gene. The gene encoding FS-1 has been isolated.

M 218 MODULATION OF THE CA^{2+} BINDING PROTEIN PARVALBUMIN IN EMBRYONIC AND ADULT MUSCLES OF *Xenopus*, Brian Kay, Lawrence Schwartz, Jeanne Alexander and Anne-Marie Pret. University of North Carolin, Chapel Hill, NC 27514.

Parvalbumin (PV) is an intracellular 12 kD protein with two high affinity Ca^{2+}-binding domains. PV is first detectable at stages 24-25 of development, a time when myotomal muscles are differentiating and contractile activity occurs spontaneously in embryos. While the majority of muscle fibers express PV, there is a subset which does not. The PV(+) and PV(-) fibers are organized in a very stereotyped pattern depending on the specific muscle group. Histochemical staining of tadpole muscles indicate that PV containing fibers correspond to fast-twitch skeletal muscles, whereas those without PV are slow-twitch type. The basis of the differential expression is most likely transcriptional, as evidenced by the lack of parvalbumin mRNA in PV(-) muscle cells by *in situ* hybridization experiments. From the analysis of 2D-PAGE western blots and cDNA clone sequence analysis, it appears that one molecular form of PV is expressed in tadpole tail muscles, and two additional forms are expressed in the developing limb muscles of metamorphosing tadpoles. We are attempting to modulate the cellular levels of this protein in embryos and adults in several manners. When tadpoles are raised in the presence of turbocurare, PV expression in myotomal muscles is uneffected. In contrase, when the gastrocnemius muscle was denervated in adults, PV levels dropped approximately 70% within 4 days. We are presently injecting embryos with plasmids engineered for constitutive expression of parvalbumin.

M 219 EVOLUTIONARY CONSERVATION AND MOLECULAR MAPPING OF EPITOPES THAT CHARACTERIZE FAST AND SLOW MYOSIN HEAVY CHAINS, Jeffrey B. Miller, Stephanie B. Teal and Frank E. Stockdale, Stanford University, Stanford, CA 94305.

We used mAbs F59, S46, and S58; immunoblotting; and molecular genetics to identify structural regions of myosin heavy chains (MHCs) that (1) have been conserved throughout vertebrate evolution and (2) are characteristic of either fast or slow isoforms. Fast and slow MHC isoforms from chickens and rats were electrophoretically separable and individual MHC isoforms always reacted either only with mAb F59 (all fast isoforms) or only with mAbs S46 and/or S58 (all slow isoforms). When tested against individual MHCs with known ATPase activites, mAb F59 reacted with the high ATPase, fast MHC isoforms (e.g. rat α-cardiac, chicken adult fast) and mAbs S46 and S58 reacted with the low ATPase, slow isoforms (e.g. rat β-cardiac/slow skeletal, chicken slow MHC2). Fast and slow MHCs were expressed in tissue-specific patterns in skeletal and cardiac muscles of chickens and rats of all ages. In both the skeletal and cardiac muscles of all classes of vertebrates - from hagfish to humans - but not in invertebrates or chordates, MHCs were expressed that carried either the fast F59 epitope or one or both of the slow S46 and S58 epitopes. The F59 epitope was on the S1 fragment and the S46 and S58 epitopes were on the rod. The F59 epitope was localized to a.a.s 205-277 (near the ATP binding site) by tryptic analysis and by expressing fragments of cDNA encoding the S1 of chicken embryonic fast MHC. Newly isolated cDNA clones encoding the S46 and S58 epitopes are being characterized. These regions, conserved through ∿400 million yr of vertebrate evolution, may be determinants of the distinct enzymatic and structural functions of fast and slow MHC isoforms (supported by NIH and MDA).

M 220 STRUCTURE AND EXPRESSION OF MYOSIN HEAVY CHAIN GENE (S) IN SMOOTH MUSCLE TISSUES, Ryozo Nagai, Philip Babij, Rainu Kaushal and Muthu Periasamy, Department of Physiology and Biophysics, University of Vermont, Burlington, VT 05405.

We have isolated and sequenced cDNA clones encoding light meromyosin (LMM) of a rabbit smooth muscle myosin heavy chain (MHC). Comparisons of nucleotide sequences in this region with rabbit skeletal and nematode MHC showed 47.8% and 43.5% homology, suggesting that smooth and sarcomeric MHC genes diverged from a common ancestor. Amino acid sequences of rabbit smooth muscle MHC share approximately 30% homology with LMM of rabbit or rat skeletal muscle. Interestingly, the LMM of smooth muscle MHC shows very similar periodic distributions of hydrophobic and charged residues as sarcomeric MHC together with a high potential for α-helical formation, indicating an α-helical coiled-coil structure throughout the region. We have found that this smooth muscle MHC gene is expressed both in vascular and nonvascular smooth muscle tissues by S1 nuclease mapping analysis. Furthermore, S1 nuclease mapping indicated that another species of MHC mRNA is coexpressed in all smooth muscle tissues. We have recently identified a cDNA clone for the second species of smooth muscle MHC mRNA. We are also characterizing a number of genomic clones to find whether or not there is a multigene family for smooth muscle MHC. (Supported by PHS R29-38355)

M 221 ULTRASTRUCTURAL ANALYSIS OF DROSOPHILA MUSCLE MUTANTS SUGGESTS THERE IS A COMPLEX PATTERN OF MYOSIN HEAVY CHAIN ISOFORM UTILIZATION, Patrick T. O'Donnell, Kaname Mogami* and Sanford I. Bernstein, Biology Department and Molecular Biology Institute, San Diego State University, San Diego, CA 92182 and *Department of Physics, University of Tokyo, Hongo, Bunkyo, Tokyo, 113, Japan.

The analysis of muscle mutants in *Drosophila* offers a powerful approach to understanding the function of contractile proteins. Functional trochanter (jump) and indirect flight muscle (IFM) are not required for viability or fertility and dominant mutations which affect their function can be readily isolated. We have examined the ultrastructure of the IFM and jump muscle in four dominant-flightless, homozygous-viable muscle mutants. All four mutations are genetically inseparable from known mutations within the single muscle myosin heavy chain (MHC) gene on chromosome two. We have found that IFMs of these mutants accumulate little or no MHC, lack thick filaments and show no organized myofibrils. These data strongly suggest that the genetic lesions are within the MHC gene. Although all four mutations have similar effects on IFM structure, they each affect the jump muscle in strikingly different ways. Wild type jump muscle is composed of 18 cells, four of which are smaller than the others. Ultrastructural analysis of transverse sections indicates the myofilament lattice and myofibril organization appear nearly identical for all 18 cells. The mutation *Ifm(2)2* affects only the structure of the four smaller cells resulting in a reduction in thick filament number and myofibril size. The mutation *b85* affects the structure of all 18 cells resulting in the absence of thick filaments and the lack of any myofibril organization. This mutation does not disrupt all muscle types since the ultrastructure of the direct flight muscle of*b85* is normal. The *b82* mutation causes an absence of thick filaments and myofibrils in the four smaller cells and disorganized myofibrils in the remaining cells. Finally, the mutation *29IF* has no apparent effect on the structure of the jump muscle and interestingly is the only one of the four mutants capable of jumping. We have previously shown that the MHC gene encodes an RNA that is alternatively processed in a tissue and stage-specific manner. These mutations we have described may lie in the alternatively spliced exons of the MHC gene or in tissue-specific promoter elements. Use of some alternative exons or promoter elements may be shared between the IFM and jump muscle while use of others may be unique to the IFM. Lastly, our results strongly suggest that although similar in structure, the two groups of cells within the trochanter muscle probably perform different functions. One possibility is that the four smaller cells represent the antagonist to the remaining cells which generate the force required for jumping. Supported by grants from NIH and MDA.

M 222 REGULATION OF ACTIN ASSEMBLY IN DEVELOPING SKELETAL MUSCLE: ISOLATION OF TWO ACTIN REGULATORY PROTEINS FROM EMBRYONIC CHICK SKELETAL MUSCLE. Takashi Obinata and Hiroshi Abe, Department of Biology, Chiba University, Yayoi-cho, Chiba 260, Japan.
Purified monomeric G-actin polymerizes spontaneously into F-actin in vitro under physiological salt and pH conditions, and in adult skeletal muscle, the vast majority of actin is in a F-form and constitutes thin filaments of myofibrils. Our previous study, however, showed that the concentration of G-actin in embryonic muscle is much higher than the critical concentration for polymerization of purified actin and the large amount of G-actin is partly due to inhibitor(s) for actin polymerization (Shimizu and Obinata, J. Biochem. 99, 751-759, 1986). In this study, we succeeded in purifying two actin regulatory proteins, the mol. wt. of which are 19 KDa and 20 KDa, respectively, from embryonic chick skeletal muscle by a combination of DNase I affinity, gel filtration and hydroxyapatite column chromatographies. The amount of both proteins in muscle tissue decreased with age. Their effects on actin-polymerization were examined by viscometry, light scattering, and electron microscopy etc. The 19 KDa protein made a complex with G-actin, and thereby inhibited actin-polymerization and caused disassembly of actin filaments. The 20 KDa protein also inhibited the actin filamentogenesis at slightly alkaline pH, while at neutral pH, it bound to F-actin to cause modulation of its filamentous structure and inhibition of tropomyosin-binding to F-actin. Suppression of actin polymerization by 19 KDa protein was removed by myosin (HMM). From these results, we propose that in developing muscle, actin assembly is dually controlled by inhibitor(s) and an accerelator (myosin); this mechanism enables ordered assembly of actin and myosin at early phase of myofibrillogenesis.

M 223 REGULATION OF MYOSIN ASSEMBLY BY ACTIN FILAMENT NETWORKS, Joel D. Pardee, Kevin T. Vaughan, Rohit K. Mahajan, and Julie A. Johns, Cornell University Medical College, New York, NY. 10021.
Since myosin thick filaments are formed in the actin-rich cortex of non-muscle cells and post-fusion myocytes, we have examined the effects of actin filament networks on Dictyostelium and muscle myosin assembly. Fluorescence energy transfer (FET) and light scattering assays indicate that myosin assembly is accelerated approximately 5 fold by actin filaments when 1 mM ATP or AMPPNP is present. Assembly rates are linearly dependent on F-actin concentration between 0-1 uM, with a stoichiometric ratio of approximately 10 moles actin per mole myosin giving maximum assembly acceleration. In contrast, actin filaments in the presence of ADP abolish myosin assembly. Addition of severin to mixtures of actin filaments and assembling myosin causes rapid fragmentation of actin filaments and an immediate cessation of accelerated myosin assembly. FET assays employing donor-labeled myosin and acceptor-labeled F-actin demonstrate that individual myosin molecules associate with actin filaments at a rate equivalent to the accelerated myosin assembly rate, suggesting that myosin to actin binding precedes thick filament formation. Electron microscopic studies support a model of actin filament mediated myosin assembly. In ADP, myosin monomers rapidly decorate F-actin, preventing self-association of myosin. ATP accelerated assembly results in contracted acto-myosin filament complexes. Of great interest is the observation that AMPPNP accelerated assembly produces thick filaments that are enclosed within cylindrical arrays of actin filaments resembling primitive stress fiber structures. (Supported by NIH grants GM 32458 and AM 32147.)

M 224 EFFECT OF SCIATIC CRUSH IN YOUNG RATS AND MICE ON FIBRE TYPE DISTRIBUTION, David J. Parry, Dept. of Physiology, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.
Lowrie et al. J. Physiol. 331, 51 (1982) and 349, 397 (1984) reported Tibialis Anterior (TA) and extensor digitorum longus (EDL) muscles of rats which had reinnervated following a sciatic crush at 5-6 days were predominantly oxidative. They inferred that immature fibres were irreversibly damaged by the high firing rate of the reinnervating "fast" axons. However, their findings were based almost entirely upon SDH staining. We have repeated these experiments using immunohistochemistry with monoclonal antibodies to myosin heavy chains (MHC), and confirmed that there is marked atrophy of II_B fibres in TA and EDL, but not in gastrocnemius and plantaris. Since II_B fibres first appear at about 5-6 days there may be a critical age at which sciatic crush may induce atrophy of a given fibre type. II_A fibres which were unaffected by the sciatic crush at 5-6 days do not appear in rat muscles until many days later. We, therefore, crushed sciatic nerves of rats ranging from 4-14 days and examined fibre type distributions in TA, EDL and triceps surae. When sciatic crush was performed before 7 days of age virtually all II_B fibres in TA and EDL were atrophic. In older (10-14 d) rats the TA and EDL were increasingly spared. Since MHC expression is more precocious in the mouse similar experiments were carried out in this species. Even with sciatic crush at 3 days , II_B fibres were found in near normal numbers and sizes following reinnervation. It is suggested that the timing of the sciatic crush relative to initiation of II_B MHC expression may be important in determining whether II_B fibres survive reinnervation. (Supported by MDAC).

M 225 HIGH RESOLUTION CHROMATOGRAPHIC ANALYSIS OF MYOSIN ISOFORMS IN AVIAN FAST-TWITCH MUSCLES. J. I. Rushbrook, C. Weiss and T.T. Yao., SUNY Health Science Center at Brooklyn, New York 11203.

The myosin isoform content of the pectoralis major (PM), posterior latissimus dorsi (PLD) and adductor superficialis (AS) muscles have been analyzed by anion-exchange chromatography of S-1 and reverse-phase analysis of S-1 tryptic fragments. Histochemically, these muscles contain the fiber types: PM-99% IIB; PLD-87% IIB, 13% IIA; AS-10% IIB, 87% IIA, 3% I. PM myosin contained single major LC1 and LC3 S-1 isoforms indicative of a single type IIB heavy chain. The PLD chromatogram contained seven peaks and at least four heavy chain species. Two peaks corresponded to the major PM peaks and therefore are type IIB isoforms. The remainder did not correspond to either slow tonic or PM post-hatch isoforms.

The AS S-1 profile was variable from preparation to preparation. There was little of the type IIB isoform and no peaks corresponding to slow tonic or PM post-hatch isoforms. In the different preparations there were at least six different components, a number of which co-eluted with peaks in the PLD preparations and some of which were probably derived from type IIA isoforms. A preliminary analysis of 16-day PM embryonic myosin reveals a minimum of four components, several of which may correspond to unidentified species in the PLD and PM profiles. N-terminal sequencing of the S-1 tryptic fragment 20 kDa from the PLD muscle has identified to date two species differing in primary sequence.

M 226 THE CUTICLE LOCOMOTOR STRUCTURES AND ENERGY TRANSDUCTION DURING FLEXING OF THE NEMATODE BODY, Matthew Ryuntyu, Department of Agronomy and Soil Science, University of New England, Armidale. N.S.W. 2351.

A model of the spiral distribution of cuticular ribbons in the manner of pistons has been proposed (Ryuntyu, 1984. 8 Eur. Cong. EM, Budapest, 3:2061-2062). Fibrous spirals do not neutralize muscular forces but direct them along the hydrstatic skeleton. The functions of the cuticular spiral are equivalent to the functions of circular muscles. The extension and contraction of the spirals along the body is automatic in character and takes places without the additional expenditure of energy apart from the initial muscular force. The phenomenon of flexing is an individual instance of this process (Ryuntyu, 1984. 8 Eur. Cong. EM, Budapest, 3:2063-2064). This has enabled a model of the liquid-crystal cuticular medium to be adapted to include spirals. These cuticular spirals can be seen to be "suspended" in this medium between the epicuticle and the infracuticle strata. Locomotion in nematodes is related to the process of wave-formation on the body surface and takes place through mechanical contact between the muscle segments and the matrix elements by the mesocuticle. The process is programmed at the micromorphological level through the specific structural organisation of the matrix elements. Contraction of muscle segments induces stretching of the ribbons, which compress the gaps of the canals between themselves. This causes the contents of the canals to be pressed (sucked) out into the external environment.

M 227 EFFECT OF C-PROTEIN ON MYOSIN THICK FILAMENT ASSEMBLY AND STABILITY, Anu D. Saad, Elizabeth Zlotchenko and Ignatius Tan, Cornell University Medical College, New York, NY 10021.

The effect of C-protein on thick filament assembly and stability was examined by comparing the assembly and exchange properties of synthetic myosin thick filaments in the absence and presence of added C-protein. Fluorescence energy transfer (FET) studies using assembly competent myosin molecules labeled with donor (5-(2-((iodoacetyl)aminoethyl) aminonapthalene-1-sulfonic acid) or acceptor (5-iodoacetamidofluorescein) fluorochromes indicate that the presence of C-protein does not affect the polymerization of myosin into thick filaments. To examine exchange, myosin and C-protein were combined in a 1:1 molar ratio and formed into filaments containing either donor-labeled or acceptor-labeled myosin. Donor labeled filaments were then combined with acceptor labeled filaments and the exchange of myosin monitored by the decrease in donor fluorescence. Myosin exchange in filaments containing C-protein was significantly lower than that in myosin only filaments. FET exchange experiments using copolymers containing fluorescently labeled C-protein and unlabeled myosin demonstrate that C-protein exchanges extensively and rapidly between synthetic thick filaments. Further studies using immunoelectron microscopy and immuno-precipitation with the anti-C-protein monoclonal antibody MF1 suggest that C-protein is localized at or close to the surface of myosin thick filaments. These results suggest that C-protein may bind to the exterior of thick filaments and stabilize the myosin molecules within these filaments.

M 228 THE EPITOPE PATTERN OF TWO UNIQUE ACTINS IN INTESTINAL EPITHELIAL CELLS IS HIGHLY CONSERVED, N.M. Sawtell, J.L. Lessard, B. Richardson, S. Dingle and A.L. Hartman, Children's Hospital Research Foundation and University of Cincinnati, Cincinnati, OH 45229.

Actin has evolved from a single protein into a family of at least six distinct vertebrate isoforms. Based on amino acid sequence data and gene structure, the vertebrate isoactins segregate into two major classes, the "cytoplasmic" or nonmuscle actins and the "α-like" or muscle actins. We have identified two unique actin isoforms in rat intestinal brush border. These actins, when examined by N-terminal peptide mapping and isoelectric focusing appear to be the vertebrate cytoplasmic isoforms. However, these actins have a pattern of epitopes that distiquishes them from the known vertebrate actins. They contain an epitope defined by Mab 1-1 is which common to the vertebrate muscle actin forms but not present in the known vertebrate cytoplasmic isoforms whhile two additional vertebrate muscle actin epitopes, defined by Mabs 1-2 and B4, are not present on these brush border actins. Intestinal epithelial cells in all species examined thus far, both vertebrate (primates, rodents, avians, reptiles, amphibians, boney fish) and invertebrate (arthropods, annelids, mollusks, echinoderms), contain actin in their intestinal brush borders having this immunoreactive phenotype. Moreover, preliminary data demonstrate that like the brush border, invertebrate muscle actin contains the 1-1 epitope but not the B4 epitope. These data suggest that the 1-1 epitope appeared early on a subset of cytoplasmic-type actins and was retained throughout the evolutionary divergence of these cytoplasmic actins to the vertebrate "α-" muscle actins. Supported by NIH grants HD17000 and AR36486.

M 229 MYOFIBRIL ASSEMBLY IS LINKED WITH VINCULIN, α-ACTININ AND CELL-SUBSTRATE CONTACTS IN CARDIAC MYOCYTES IN VITRO, Masaru Terai and Yutaka Shimada, Departments of Anatomy and Pediatrics, School of Medicine, Chiba University, Chiba 280, Japan.

In order to study the pattern of distribution of myofibrils in cardiac myocytes in vitro, we stained the cultures with various combinations of the specific antiactin drug phalloidin and antibodies against myosin heavy chain, troponin C, α-actinin and vinculin, and examined in fluorescence and interference reflection microscopy.

Filament bundles with and without cross-striations in cardiac myocytes were reactive with the antibodies against the sarcomeric proteins and, thus, were regarded as nascent myofibrils. The pattern of distribution of these fibrillar structures was generally correlated with the shape of the cells. They were aligned parallel to the long axis of the cell within the straight part of the cells. In polygonal areas, they were prominent spanning the cellular diagonals but some were also observed along the perimeters. Both ends of nascent myofibrils terminated at the sarcolemmal areas where the cell formed focal contacts with the substrate. At these sites, vinculin and α-actinin, the proteins thought to be involved in the attachment of actin to the membrane, were always present.

These results indicate that the formation of focal contacts and the accumulation of the adhesion plaque proteins at these sites are prerequisite to or, at least, correlated with polymerization and regular alignment of sarcomeric proteins. It is proposed that myofibrillogenesis occurs along the inferred stress lines that are generated between the sites of focal cell contacts.

M 230 MYOSIN EXPRESSION IN SLOW AVIAN MUSCLES FOLLOWING LOSS OF CONTRACTILE ACTIVITY. Noriko Shimizu, Angela Connold, Suzanne Kamel, Gerta Vrbova and Radovan Zak, University of Chicago, Chicago, IL. 60637 and University College, London. The chicken slow tonic muscle (ALD) consists of multiply innervated muscle fibers and expresses two myosin isoforms, SM1 and SM2. The SM1 isoform predominantly expressed in the embryo, and is gradually replaced by SM2 isoform after hatching. In order to examine the effect of contractile activity on the transition of myosin isoform, ALD was denervated by sectioning the brachial plexus or inactivated by blocking of acetylcholine receptors with α-bungarotoxin (α-Btx) in the presence of intact innervation. A loss of innervation accelerates the developmental shift towards the predominance of SM2 isoform. Similar changes were seen in the muscles paralyzed with α-Btx. We also examined a regional effect of α-Btx on myosin isoform expression. A localized application of α-Btx caused the greatest decline of the SM1 isoform in the center region of the toxin treatment. These results suggest that contractile activity generates factors controlling myosin isoform expression and that also the nuclei of multinucleated cells are not synchronized but are regulated by local factors.

M 231 DIFFERENCES IN THE DEVELOPMENTAL EXPRESSION OF THE MUSCLE ISOFORMS OF CREATINE KINASE AND ACTIN IN CULTURED MYOBLASTS, John C. Szucsik, G. Ayra and James L. Lessard, Children's Hosp. Res. Fnd., Cincinnati, OH 45229.

We have identified a monoclonal antibody (Mab) designated 2B6 which is specific for the M isoform of creatine kinase in the chicken. This antibody was used to follow the appearance of the M isoform of creatine kinase in primary cultures of chick myoblasts. Myoblasts were obtained from day 11 chick embryo thigh muscle. On days 3, 6, and 9 of culture samples were fixed for indirect immunofluoresence using Mab's 2B6 or B4 which is specific for the muscle isoforms of actin (Lessard, in press). Our results indicate that the developmental expression of the M isoform of creatine kinase is not as tightly coupled to myoblast fusion as the muscle isoforms of actin. The M isoform of creatine kinase can be seen in unfused myoblasts while this was rarely seen for the muscle isoforms of actin. Also, subtle differences in cellular localization were observed for these proteins. While both Mab's exhibited staining of filamentous structures in myotubes, the staining for the M isoform of creatine kinase displayed a strong perinuclear localization that was not seen with the muscle isoforms of actin.

M 232 REINNERVATION OF MUSCLE FIBER TYPES IN THE NEWBORN RAT SOLEUS, Wesley J. Thompson§, Lena Carmen Soileau§, Laura Silberstein¶, and Helen M. Blau¶, §Dept. of Zoology, Univ. of Texas, Austin TX 78712 and ¶Department of Pharmacology, Stanford Univ. School of Medicine, Stanford, CA 94305.

We have examined the selectivity of reinnervation of fiber types in rat soleus muscle denervated by crush of the soleus nerve 2 days after birth. The fibers innervated by single, regenerated motor axons were identified by use of glycogen depletion approximately 2 weeks following denervation. The types of fibers were determined by use of immunohistochemistry employing anti-myosin antibodies and, in some cases, by use of myofibrillar ATPase staining. Two distinct types of fibers are present in soleus at 2 days and through the next 16 days of normal postnatal development. These fiber types are retained in a denervated muscle for the period of time required for reinnervation. Although 40% of the fibers are lost from the muscle during reinnervation, we find no evidence for interconversion of muscle fiber types. Nonetheless, 10 of the 12 single motor units examined had fiber type compositions which were markedly biased toward one or the other of these two types; the bias in these units could not be explained by chance reinnervation. On the basis of topographical distribution of the muscle fibers in each of these units, the motor axons reinnervated a novel set of fibers. We interpret these findings to mean that neonatal soleus motor neurons reinnervate fiber types in a selective manner. This selective innervation may explain the bias in the fiber type composition of normal motor units during early postnatal development.

M 233 SPATIAL AND TEMPORAL REGULATION OF MYOSIN HEAVY CHAIN ISOFORM EXPRESSION DURING HUMAN SKELETAL MUSCLE DEVELOPMENT, Steven Webster, Marilyn Travis, Cecelia Webster, Kevin Rich, Laura Silberstein, Helen M. Blau, Stanford University School of Medicine, Stanford, CA 94305.

To increase our understanding of the developmental regulation of human muscle proteins, we produced monoclonal antibodies to human myosin heavy chain (MHC). Six groups of monoclonal antibodies recognizing MHC with distinct developmental and fiber-type specific patterns of expression have been identified. The cDNAs encoding fusion proteins recognized by these monoclonal antibodies have been isolated and the regulation of the corresponding genes is being characterized (see Karsch-Mizrachi et al., this volume). We have determined the progression of human MHC expressed in vivo and induced in vitro (Silberstein et al., 1986, Cell 46:1075-1081). In addition, we are defining the developmental regulation of myosin gene expression by putative stage-specific trans-acting factors in heterokaryons. The expression of myosins in human muscle cells is being determined with cells from fetal, neonatal and adult stages isolated in quantity using the fluorescence-activated cell sorter. To examine potential myoblast heterogeneity we are carrying out clonal analyses of the heritable patterns of MHC expression by myoblasts isolated from muscle tissues at different stages of human development. This approach should ultimately lead to the dissection of the regulation, structure and function of members of the human myosin heavy chain gene family at a molecular level in the course of normal and dystrophic human muscle development.

M 234 FIBROBLAST EXTRACELLULAR MATRIX MODULATES MYOBLAST DIFFERENTIATION IN VITRO, S.L. Welles and B.P. Toole, Dept. of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA 02111.

Stage 38 chick pectoral fibroblast (FBL) extracellular matrices modulate myoblast (MYBL) fusion and creatine phosphate kinase (CPK) activity. Control MYBL plated on gelatin commence fusion at 40 hrs. and fusion is completed at 64 hrs. post-plating. MYBL fusion is inhibited if cells are plated on top of post-confluent FBL, but this inhibition can be reversed by treating the FBL with 0.01U Streptomyces hyaluronidase/ml. Conversely, MYBL plated on EGTA- or DOC-derived basal matrices commence fusion at 15 hrs. and fusion is completed by 40 hrs. CPK activity also varies as a function of FBL matrix in a manner which supports our morphological observations. CPK activity is either inhibited by 50% or increased to 130% of the control value if MYBL are plated on top of either the hyaluronate (HA)-rich FBL layer or the FBL basal matrix, respectively. The inhibition of the CPK activity by the FBL layer can be partially reversed by hyaluronidase treatment. FBL membrane solubilization in 0.5% DOC yields two populations of HA binding proteins with K_d=5.5 X 10^{-8}M and 1.1 X 10^{-10}M. Wheat germ agglutinin affinity chromatography yields a 10-20 fold partial purification of the HA binding activity. PAGE and Western blotting using a polyclonal antibody which blocks binding of HA to SV3T3 membranes shows a 66 kd protein as one potential HA-binding protein. We hypothesize that prior to myogenesis, FBL-bound HA prevents myoblast fusion. Decreases in HA at the time of differentiation would allow MYBL to interact with other matrix molecules, which could promote fusion and production of differentiation-specific proteins.

Muscle Membranes Receptors, Growth Factors and Cardiac Myogenesis

M 300 DIFFERENTIAL REGULATION OF MUSCLE GENE EXPRESSION BY v-myc AND v-src ONCOGENES Germana Falcone, Franco Tatò and Stefano Alemà, Università "La Sapienza" and Istituto di Biologia Cellulare, CNR, Rome, Italy.

In order to examine the relationship between in vitro transformation and skeletal muscle differentiation, we have recently developed clonal strains of quail myogenic cells harbouring functionally different viral oncogenes. The most striking consequence of transformation, irrespective of the oncogene involved, was a dramatic reduction in the proportion of cells attaining terminal differentiation. Specific differences, however, were observed in the cellular mechanisms of this differentiative block between v-src, encoding a membrane-bound tyrosine kinase, and v-myc, a DNA-binding nuclear protein.Moreover, by the use of the appropriate conditional and nonconditional transformation mutants, we have also established culture conditions which allow fast differentiation of replicating myoblasts. The regulation by different oncogenic products of muscle-specific gene expression at the transcriptional and posttranscriptional levels was examined by cloned DNA hybridizing to mRNA species that accumulate in differentiating cells. The results of these experiments will be presented.

This work was supported by grants from the CNR (PF-MPR and Oncologia; PS Neurobiologia) and the AIRC.

M 301 STAGE SPECIFIC BLOCKAGE OF AVIAN CARDIAC MYOGENESIS IN VITRO. Arlene Gonzalez-Sanchez and David Bader. Cornell University Medical College, New York, NY

In an effort to identify possible stage specific events in the commitment of cardiogenic splanchnic mesoderm to the cardiac myocyte lineage, cardiogenic mesoderm with subjacent endoderm from stage 4 to stage 8 chick embryos was isolated and grown in vitro. The tissue fragments were grown in DMEM with 10% fetal bovine serum in the presence or absence of the tumor promotor TPA, an agent known to inhibit differentiation in a number of developmental systems. At various times after plating, tissues were examined for the presence of beating heart cells and were stained with monoclonal antibodies directed against sarcomeric myosin heavy chain. Greater than 95% of the tissue fragments grown in the absence of TPA contained beating cardiac myocytes regardless of the age of the donor embryo. In contrast, cultured cardiogenic cells from stage 4 embryos grown in the presence of TPA did not exhibit spontaneous contractility and did not contain sarcomeric myosin. In addition, these differentiation blocked cardiogenic cells became highly proliferative and maintained a "fibroblast-like" morphology. With the increasing age of the donor embryo, cardiogenic cells exhibited increasing insensitivity to TPA treatment. When cardiogenic cells from stage 8 embryos were grown in the continuous presence of TPA, greater than 85% of tissue fragments differentiated into cardiac myocytes. The differential sensitivity of cardiogenic cells to the tumor promotor TPA suggests that maturing cardiac myoblasts are not homogeneous in their differentiative potential but exhibit stage specific transitions in their commitment to the cardiac lineage. This work was supported by grants from the NIH (HL35776 and HL36775) and the New York Heart Association.

M 302 THYROID HORMONE INDUCTION OF THE α-MYOSIN HEAVY CHAIN GENE REQUIRES BINDING FACTOR 1. Joseph J. Bahl, Bruce E. Markham, Kevin J. Garvey and Eugene Morkin. University Heart Center, University of Arizona, Tucson, Arizona 85724.

α-Myosin Heavy Chain Binding Factor 1 (α-MHCBF1) binds specifically to a site located form -599 to -576 in a thyroid hormone sensitive region of the rat α-MHC promoter. We have constructed a 24-bp synthetic binding site (SBS) which exhibits in vitro binding characteristics similar to the natural site. One to 5 copies have been inserted into $pA_{10}CAT.2N$ adjacent to the SV40 early promoter. The cloned binding site sequences were transfected into primary fetal rat cardiomyocytes and their effect on expression monitored by determination of choramphenicol acetyltransferase activity (CAT). The results indicate that a single SBS in either orientation had no effect on CAT activity. A slight increase in CAT activity was observed with multiple copies suggesting that SBS has only weak enhancer activity for the SV40 promoter. SBS also was cloned into a plasmid vector and cotransfected into cardiocytes with pSVOMCAT, which contains 3.3-kbp of the 5′ flanking sequences of the α-MHC gene fused to CAT, in the presence and absence of 10 nM T_3. SBS in 4:1 molar excess caused a 60% decrease in T_3-inducible CAT activity and at an 8:1 ratio almost abolished activity. Thus, α-MHCBF1 is a positive trans-acting regulatory factor required for thyroid hormone induction of α-MHC gene expression. The lack of significant enhancer activity suggests that other factors are likely to be involved.

M 303 α_1-ADRENERGIC REGULATION OF GENE TRANSCRIPTION IN HEART CELLS Nanette H. Bishopric, Carlin S. Long , Paul C. Simpson and Charles P. Ordahl.

Many embryonic/perinatal muscle isogenes are re-expressed during experimental cardiac hypertrophy in the adult rat. The molecular signals governing isogene switching during cardiac hypertrophy are unknown. In order to identify these signals, we studied expression of the alpha-actin genes in a cell culture model of heart muscle cell hypertrophy. In cultured neonatal rat heart cells, as in the adult rat heart in vivo, expression of the cardiac alpha-actin (cACT) isogene was predominant over that of the skeletal alpha-actin (sACT) isogene (1). α_1-adrenergic stimulation induced hypertrophy in these cells, accompanied by a 10.6-fold increase in the level of sACT messenger RNA (mRNA). In contrast, cACT mRNA increased by only 2.6-fold and cytoskeletal actin mRNA by 1.8-fold (1). To determine whether this effect was transcriptional or post-transcriptional, nuclear run-on transcription assays were performed. After 12 hours of stimulation with 2 uM norepinephrine (NE), the cultured heart muscle cells demonstrated a 5.6-fold ($p<0.005$) increase in sACT gene transcription. This increase was prevented by concurrent α_1-adrenergic blockade. Isoproterenol, a selective beta-adrenergic agonist, did not stimulate sACT gene transcription. In the same experiments, there was no measurable effect of NE on cACT gene transcription. These results establish that the α_1-adrenoceptor mediates embryonic/perinatal isogene switching at the transcriptional level.

Supported by grants from NIH (CPO & PCS), NIH and Merck/American College of Cardiology Fellowships (NB), and an American Heart Assn Fellowship (Calif. Affil.) (CSL).

Reference: (1) Bishopric NB, Simpson PC and Ordahl CP (1987). J. Clin Invest. 80:1194-1199.

M 304 PRESSURE OVERLOAD STIMULATED CARDIAC HYPERTROPHY (CH) LEADS TO A RAPID INCREASE IN THE mRNA FOR FIBRONECTIN, Kenneth R. Boheler, Wolfgang H. Dillmann, and Alice Barrieux, Univ of California, San Diego , San Diego, CA 92103.

Aortic constriction rapidly leads to myocyte hypertrophy and non-myocyte hypertrophy and hyperplasia. To determine if fibronectin (FN), an extracellular matrix protein, could be involved in this growth process, we performed the following experiments. Cardiac RNA was isolated from rats following 1, 3, 8, and 14 days of aortic stenosis (AS) or sham surgery (controls). Through Northern blot analysis, total cardiac RNA was probed with a rat specific ^{32}P-labelled FN cDNA clone. While no signal was detectable in autoradiograms of control RNA after a six day exposure, a strong signal was present in RNA isolated from hypertrophied myocardium. This increase in hybridizable mRNA is correlated with the increase in heart mass but is transient in duration. Preliminary studies based on 2-dimensional gel separation and Western blot analysis of FN using anti-rabbit FN antibodies also indicate a shift in pI of sham vs. AS rats (6.5 vs. 6.38, respectively). Conclusions: 1) The mRNA for FN is CH responsive; 2) the protein for cardiac FN may be modified with the onset of CH; and 3) these changes in FN predominance and expression may play a critical role in the development of cardiac hypertrophy.

M 305 ALTERNATIVE 3'-UNTRANSLATED REGIONS IN TWO RAT CYTOPLASMIC γ-ACTIN cDNA CLONES, Chester W. Brown, Kirk M. McHugh and James L. Lessard, Children's Hospital Research Foundation, Cincinnati, OH 45219.

We have screened a size-fractionated pBR322 cDNA library from rat stomach using the universal actin probe-pHMαA-1. Two cDNA clones encoding cytoplasmic γ-actin were isolated, as determined from the sequence analysis. One (clone 4) is 1210 base-pairs long and extends from amino acid 155 to the end of a 556 base-pair 3'-untranslated (3'UT) region. The other (clone 15) is 1500 base-pairs long and spans from amino acid 4 to the end of a 386 base-pair 3'UT region. Interestingly, these two clones contain strikingly different 3'UT ends. A 223 base-pair region beginning with the stop codon of each cDNA is identical in both cDNAs and shows strong homology with the same 3'UT portion of the human cytoplasmic γ-actin mRNA. In clone 15 this is followed by a 130 base-pair sequence that is not found in clone 4. Both clones then contain an identical 33 base-pair sequence. Clone 4 contains an additional 300 base-pair sequence that is not found in clone 15. An understanding of the molecular basis for two distinct 3' UT sequences of these cytoplasmic γ-actin cDNAs may lead to important inferences concerning the expression of this protein.

M 306 CONTRACTILE PROTEIN SYNTHESIS OF ADULT CARDIAC MYOCYTES IN CELL CULTURE, Lawrence B. Bugaisky and Ronald S. Hall. University of Alabama at Birmingham, Depts. of Pathology, Cell Biology and Anatomy. Birmingham Alabama.

Adult rat cardiac myocytes undergo a morphological transformation when placed into cell culture which results in loss of their typical cylindrical shape. This alteration in shape does not represent cellular "dedifferentiation" as has been previously suggested, but more likely is an adaptation to the cell culture environment. The fact that these myocytes are highly differentiated is supported by the following observations. The relative pattern of total protein synthesis as examined by one-dimensional gel electrophoresis following incorporation of ^{35}S-methionine is qualitatively similar throughout the first 10 days in culture (the period of time when the greatest morphological changes occur). Only the V1 cardiac myosin isoform is observed for the first 8-10 days in culture and the expression of only this isoform can be extended at least 2 weeks by growth of the cells in serum-free medium. Analysis of the cells by 2-dimensional gel electrophoresis following ^{35}S-methionine incorporation demonstrates synthesis of α-actin, α and β tropomyosin and cardiac light chains 1 and 2. Unlike embryonic heart in vivo, no embryonic skeletal light chain has been observed. Additionally, using isoform specific probes for α-actin we have observed the presence of mRNA for cardiac actin while skeletal actin message, if present, appears to exist in low amounts. Thus, adult cardiac myocytes in culture express the full range of contractile proteins expected of mature cells in vivo and do not express obvious quantities of those contractile protein isoforms common to less differentiated muscle. (Supported by N.I.H. grant HL37320 and A.H.A.)

M 307 TWO MITOGENIC SIGNALLING PATHWAYS REGULATE PROLIFERATION AND DIFFERENTIATION IN MUSCLE CELLS. Joe A. Connolly, Gilles Simard, Andrew Sue-A-Quan, Helen Tai, K.A. Zito and D.J.Kelvin, University of Toronto, Toronto, Canada.

BC3H1 is a non-fusing muscle cell line which proliferates in 20% FBS but differentiates when the serum is removed. Differentiation is blocked when cells are treated with the mitogen fibroblast growth factor (FGF). Interestingly, Pertussis Toxin (PT) blocks proliferation and induces differentiation in 20% FBS, and this is apparently due to the ADP-ribosylation of a 40Kd membrane protein. Using PT, we distinguish two mitogenic signalling pathways: 1. FGF stimulates proliferation and blocks differentiation in a completely PT-insensitive fashion; 2. preliminary experiments suggest thrombin stimulates proliferation and this is sensitive to PT. We have previously found that transfection of BC3H1 cells with the Ha-ras oncogene promotes proliferation and blocks differentiation. Where does ras and/or other G proteins function in these pathways regulating proliferation and differentiation? In ras transfected cells, PT does not induce differentiation or block proliferation, although a 40 Kd membrane protein is ADP-ribosylated. We conclude that two signal-transducing pathways, one sensitive to PT, regulate proliferation and differentiation in BC3H1 muscle cells. It would appear that ras functions as a G protein in the PT-insensitive pathway or downstream from the PT substrate.

M 308 ACTH IS A SPECIFIC MITOGEN FOR MYOGENIC CELLS, G. Cossu, G. Cusella, M. Bouchè, M.I. Senni and C. Boitani. Institute of Histology, University of Rome, Italy.

The mechanisms regulating the proliferation of adult myogenic cells are still poorly understood. Among known mitogens, only Fibroblast Growth Factor and somatomedins are known to be mitogenic for myogenic cells, while several potential mitogens are being characterized. In a search for possible effects on myogenic cells of other known molecules such as growth factors, hormones or neuropeptides, we have observed that peptides derived from proopiomelanocortin (ACTH, α, β and γ MSH, but not β endorphin) are potent mitogens for both satellite cells and embryonic myoblasts. The effect has been measured by counting the number of cells in myogenic clones or measuring the amount of skeletal myosin in high density cultures grown in 5% horse serum $\pm$ the growth factor to be tested. The half dose of ACTH is in the nanomolar range and its effect is potentiated by suboptimal doses of FGF. ACTH is not mitogenic for vertebral chondroblasts, muscle or dermal fibroblasts. While a mitogenic effect of other neuropeptides (bombesin, substances P and K) on mesodermal cells have already been reported, this is the first evidence that a peptide hormone can act as a mitogen for a non-target cell. The possible significance of the mitogenic effect of ACTH on satellite cells relies on the fact that any severe injury to skeletal muscle puts the animal under a stress condition and the released ACTH, by acting selectively on satellite cells, would be advantageous for muscle regeneration.

M 309 ESTABLISHMENT AND CHARACTERIZATION OF A POTENTIAL CARDIAC MUSCLE CELL LINE Joseph B. Delcarpio and William C. Claycomb, Departments of Anatomy and Biochemistry and Molecular Biology, L.S.U. Medical Center, New Orleans, LA 70112. Acquisition of a permanent cell line which retains the basic characteristics of the cardiac muscle cell and which can be subcultured would be invaluable for studies of the heart. To this end we have established a number of preliminary cell clones which have been derived from rat and human cardiac muscle cells. Ventricular cardiac muscle cells were isolated from fetal and neonatal rat hearts and from 16-18 week old human fetal hearts. Cells were then fused to L-M (TK^-) mouse sarcoma cells via a modification of the polyethylene glycol technique used to create hybridomas. Hybrids were grown in selective media containing hypoxanthine, thymidine and aminopterin (HAT). Only those cells which acquired the TK gene survived in HAT media. Clones were selected and subcultured based on their morphological resemblance to cardiac muscle cells. In combination with and in addition to fusion, we introduced a temperature sensitive mutant gene of the Rous Sarcoma Virus (v-src) and the selectable marker gene (neo) into our cells via electropermeablization. This gene has been used to transform other cell types at the permissive temperature of 35°C. When grown at the nonpermissive temperature of 41°C the cells cease replication and undergo differentiation. We are now testing these clones for cardiac specific muscle gene expression.

M 310 EXPRESSION OF CARDIAC THIN FILAMENT PROTEINS DURING DEVELOPMENT OF EUTHYROID AND HYPOTHYROID RATS. Dieckman, L.J. and Solaro, J. Univ. Cincinnati, College of Medicine, Cincinnati, OH 45267-0576.

Many factors have been found that alter the distribution of myosin isoforms in cardiac thick filaments, yet the regulation of the isoform population of cardiac thin filament proteins has not been described. There is recent evidence for the existence of developmentally related transitions in isotype population of cardiac thin filament proteins, especially TnI (Solaro et. al. Circ. Res., 58:721, 1986). In view of the importance of thyroid state as a determinant of myofilament structure and function, we have compared the developmental changes which occur in cardiac myofilaments prepared from euthyroid rats and rats made hypothyroid by treatment with 6-n-propyl-thiouracil. SDS-PAGE of myofibrils purified from hearts of 7, 14, 21 and 28 day old animals indicate that both euthyroid and hypothyroid rats display a developmentally related shift towards the "adult" isoform of TnI. However, hypothyroid rats display a lower percentage of the adult form of TnI at 14, 21, and 28 days of age. A functional correlate of the isotype population of TnI, is a relative insensitivity of Ca-activation to acidic conditions. We therefore measured myofibrillar ATPase activity at pH 7.0 and 6.5, and compared Ca-activation curves of euthyroid and hypothyroid myofibrils. Half maximal activating Ca concentrations for myofibrils prepared from 14 and 28 day old control hearts were reduced 2.3 fold and 3.0 fold respectively, when pH was reduced from 7.0 to 6.5. However, Ca-activation of myofibrils prepared from 14 and 28 day hypothyroid rats was reduced only 1.7 fold and 1.8 fold. Our results indicate that functionally significant transitions in thin filament isotypes of rat heart myofibrils occur in the neonatal period. In addition, our results provide the first evidence that expression of these thin filament isoforms is related to thyroid state.

M 311 SV40 T ANTIGEN INDUCES DNA SYNTHESIS AND MITOSIS IN TERMINALLY DIFFERENTIATED MYOTUBES, Takeshi Endo* and Bernardo Nadal-Ginard, Howard Hughes Medical Institute, Children's Hospital, Harvard Medical School, Boston, MA 02115; *Present address: Chiba University, Chiba, Chiba 260, Japan.

During terminal differentiation, cultured skeletal muscle myoblasts cease DNA synthesis, irreversibly withdraw from the cell cycle (commitment), and fuse to form multinucleated myotubes. Certain viral and cellular oncogene products have been shown to regulate cellular growth and differentiation. Some of them not only transform cultured cells but also induce host cell DNA synthesis and mitosis in quiescent (GO-arrested) cells. To address whether DNA synthesis and mitosis can be induced in terminally differentiated cells by simian virus 40 (SV40) T antigen, we transfected mouse C2 myogenic cells with recombinant DNA molecules encoding temperature-sensitive (ts) SV40 T antigen under the control of Zn^{2+}-inducible metallothionein promoter (pMtSVTtsNeo) and obtained stably transformed cell clones (C2SVTts). Induction of T antigen with Zn^{2+} at permissive temperature (33°C) suppressed both biochemical and terminal differentiation of C2SVTts cells. When preformed myotubes were shifted to the medium containing Zn^{2+} at permissive temperature, high levels of DNA synthesis in the nuclei and mitotic figures of metaphase were observed within the first 24 hr. In addition, some nuclei containing a portion of the cytoplasm popped out of the myotubes. By 48 hr after the induction of T antigen, many nuclei remaining in the myotubes turned to small fragments or showed large smear appearances and the myotubes degenerated. These results imply that terminally differentiated myotubes are still able to reenter the cell cycle and proceed to M phase via GO/G1 and S phases, although these situations are lethal to the committed cells.

M 312 GENE EXPRESSION AND MEMBRANE RECEPTORS FOR INSULIN-LIKE GROWTH FACTORS IN NEONATAL RAT HEART, Gary Engelmann[a], Keith Boehm[b], Joyce Haskell[c], Philip Khairallah[a], and Judith Ilan[b], [a]Cleveland Clinic Foundation & [b] Case Western Reserve University, Cleveland, OH, 44106 and [c] University of S. Carolina, Columbia, SC, 29208

Growth and maturation of neonatal cardiomyocytes is a complex process that includes the final replicative "window" and initial events associated with maturation. Insulin-like growth factors (IGFs) are a major circulating and endogenous polypeptide growth family found in fetal and neonatal development. Final cardiomyocyte cell number is established during the first 5-7 days, a period of abnormal growth in the spontaneously hypertensive rat (SHR). Ventricular transcripts for IGF-I and IGF-II (day 3) and Type-1 membrane receptor levels (crosslinking and binding assays) (days 1-7) were elevated in the SHR, yet biochemical indices of replication are deficient. Ventricular tissue and cardiomyocyte IGF gene expression were verified by in-situ hybridization. Serum-free cultures of cardiomyocytes respond to exogenous IGF stimulation of DNA and protein synthesis, yet this response is blunted in the SHR. Because neonatal SHR cardiomyocyte hyperplasia is reduced, post-IGF receptor events may be responsible for this deficiency or accelerated maturation into a permanently post-mitotic cell is mediated by elevated IGF synthesis and receptors in an autocrine/paracrine manner of growth regulation. Both aspect of cardiomyocyte development are currently being investigated. Supported by AHA Grant-in-Aid, Northeast Ohio Affiliate (GE)

M 313 NEONATAL RAT VENTRICULAR TRANSFORMING GROWTH FACTOR-BETA GENE EXPRESSION AND IN-VITRO EFFECTS ON CULTURED CARDIOMYOCYTES, Gary Engelmann[a], Keith Boehm[b], Maria Sparks[c], Frank Ruscetti[c], and Judith Ilan[b], [a]Cleveland Clinic Foundation & [b]Case Western Reserve University, Cleveland, OH 44106 and [c]NCI, Frederick, MD

Growth of the cardiomyocyte during fetal/neonatal time periods represents a composite collection of interactive events eventually yielding a finite population of muscle cells. The final replicative component and initiation of cellular maturation occur during the first 5-7 neonatal days. There are no known signals for these events, yet transforming growth factor-beta (TGF-ß) is known to be a "multifunctional" regulator of cellular proliferation. Based on the negative regulatory aspects of TGF-ß on mesenchymal cell proliferation, we found that TGF-ß mRNA transcripts are abundantly expressed in neonatal rat heart at the time when cardiomyocyte replication is nearly complete and the transition to hypertrophy begins. Concomitant with the elevated TGF-ß gene expression, insulin-like growth factor (IGF) gene expression levels also decline. Exogenous IGFs stimulate cardiomyocyte DNA and protein synthesis in-vitro. We determined that co-incubation with TGF-ß resulted in an inhibition of the IGF-mediated stimulation of DNA and protein synthesis. Maximal TGF-ß inhibitory effects were seen at, or near, 1 ng/ml with 10 ng/ml stimulation with either IGF-I or MSA. TGF-ß modulation of growth factor mediated replicative or maturational effects can be hypothesized to occur in an autocrine or paracrine manner within the neonatal rat heart. Supported by AHA Grant-in-Aid, Northeast Ohio Affiliate (GE)

M 314 AGRIN-RELATED MOLECULES IN NORMAL AND ANEURAL DEVELOPING MUSCLE. J. R. Fallon. and C. E. Gelfman, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.
Agrin is a molecule derived from the extracellular matrix of Torpedo electric organ that organizes AchR and AchE on cultured muscle cells. Immunocytochemical studies with monoclonal anti-agrin antibodies have established that in normal and damaged adult muscle molecules closely related if not identical to agrin are localized in the synaptic basal lamina. These results provide strong evidence that agrin or related molecules play a role in directing the regeneration of the neuromuscular junction. The present studies are aimed at determining the role of agrin in the development of the synapse. The localization and time of appearance of agrin-related molecules has been compared to that of AchR clusters in the developing chick hindlimb. Examination of serial sections of the entire thigh from embryos of stage 24-30 (embryonic day 4.5-7) revealed that monoclonal antibodies directed against Torpedo agrin stain throughout the dorsal muscle mass from as early as stage 24, before the appearance of AchR clusters. At all stages examined greater than 95% of the AchR clusters co-localized with agrin-related molecules. In order to assess the role of innervation in the expression of agrin related molecules embryos with aneural hindlimbs were generated by spinal cord extirpation at stage 17. Examination of these embryos at stage 35 revealed that agrin related molecules were present in the aneural muscles and were localized at AchR clusters. Thus the presence of nerve in early development is not necessary for the expression of agrin-related molecules in muscle. These experiments demonstrate that in developing muscle agrin or a closely related molecule is 1) expressed before AchR clusters are detected and 2) co-localized with the earliest AchR clusters formed and 3) are synthesized by muscle cells. These results suggest that agrin may play a role in directing the initial events of synapse formation during development.

M 315 EFFECTS OF ORTHOVANADATE (OV) ON ACETYLCHOLINE RECEPTOR (AChR) CLUSTERS, Giovanna Marazzi, Chiara Bisiani, Carlo Sala and Guido Fumagalli,Department of Pharmacology, School of Medicine, 20129 Milano, Italy.
Adhesion plaques are dynamic structures that mediate cell contact with the substrate; their morphologic pattern can be modified by conditions that increase tyrosine phosphorilation of associated proteins such as cell transformation or treatment with orthovanadate (OV),an inhibitor of tyrosine phosphatases. Since in rat myotubes most of the AChR clusters are associated to such structures,we asked whether increased levels of phosphotyrosine could affect AChR cluster stability.Rat myotubes in culture were incubated in 25-100 μM OV; this had no effect on AChR total amount and turnover but the number of cluster per dish was reduced:the effect was seen with all the concentrations tested and was maximal after 6 h.The proportion of AChR associated to the culture substrate(determined by the method of Bloch JCB 102,1986) was also significantly decreased: the effect was dose and time dependent and was maximal after 18 h in 100 μM OV. No significant modification of the size of the clusters remaining after OV was seen suggesting that disassembly of individual clusters was rapid and sudden. Western blot analysis of affinity purified receptor using monospecific antiphosphotyrosine antibodies showed a significant increase of phosphotyrosine on the β subunit of AChR which was maximal after 18 h incubation in 100 μM OV. These data suggest that tyrosine phosphorilation of the receptor occurs on the β subunit and that this event may have functional implications on AChR cluster stability.

M 316 A MUSCLE CELL VARIANT DEFECTIVE IN GAG BIOSYNTHESIS IS ALSO DEFECTIVE IN SPONTANEOUS CLUSTERING OF ACETYLCHOLINE RECEPTORS, Herman Gordon, Mark T. Lupa & Zach W. Hall, Dept Physiology, Univ. Calif., San Francisco, CA 94143.

We have isolated a genetic variant from the C2 muscle cell line that is defective in its incorporation of free sulfate, a marker of glycosaminoglycan (GAG) biosynthesis. Further characterization shows the variant to be defective in incorporation of glucosamine, suggesting a failure to synthesize the carbohydrate backbone of GAGs. Nonetheless, the variant differentiates, fuses into polynucleate myotubes, and expresses acetylcholine receptor (AChR) on its cell surface. When the AChRs were stained with rhodamine-conjugated α-bungarotoxin, the fluorescence was found to be distributed evenly over the surfaces of the variant myotubes with only occasional micro-aggregates or clusters. This is in marked contrast with wildtype C2 myotubes which spontaneously and profusely form clusters of AChR. When co-cultured with embryonic mouse spinal cord explants or with dissociated e.d. 8 chick ciliary ganglion neurons, the variant myotubes form extensive clusters, many of which are associated with nerve contacts. The existence and properties of this genetic variant suggest a role for GAGs in synaptogenesis. We are presently pursuing the biochemical and cell biological characterization of these GAGs.

Supported by grants from the NIH and MDA.

M 317 DEXAMETHASONE-DEPENDENT INHIBITION OF DIFFERENTIATION OF SKELETAL MYOBLASTS BEARING STEROID-INDUCIBLE N-ras ONCOGENES, L. A. Gossett and E. N. Olson, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Differentiation of skeletal myoblasts is prevented by constitutive expression of mutationally activated ras proteins, which have been postulated to lead to persistant activation of specific intracellular growth factor cascades. To investigate the mechanisms involved in ras-dependent repression of myogenesis, the C2 muscle cell line was transfected with a plasmid containing a mutationally activated human N-ras oncogene under transcriptional control of the steroid-sensitive promoter of the mouse mammary tumor virus long terminal repeat. Clonal cell lines that differentiated normally in the absence of dexamethasone, but which failed to differentiate in the presence of dexamethasone were isolated and analyzed. Addition of dexamethasone to myoblasts bearing steroid-inducible ras oncogenes prevented myotube formation and induction of muscle-specific gene products and induced N-ras in a dose-dependent manner (ED_{50}=7 nM). Myoblasts released from ras-dependent repression, by removal of dexamethasone, underwent fusion and induced muscle-specific gene products in a manner indistinguishable from control C2 cells and did not require a round of DNA synthesis to be reprogrammed prior to differentiation. Together, these results indicate that oncogenic ras proteins directly activate an intracellular cascade that impinges on the differentiation program and that the signals generated by ras are highly transient. Current studies involve modulation of the level of normal ras proteins in C2 cells and analysis of the influence of different growth factors on the differentiation program.

M 318 REGULATION OF DIFFERENTIATION OF THE BC_3H1 MUSCLE CELL LINE THROUGH CYCLIC AMP-DEPENDENT AND -INDEPENDENT PATHWAYS, J. S. Hu and E. N. Olson, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

The muscle cell line, BC_3H1, accumulates a variety of muscle-specific gene products, but does not undergo fusion, following withdrawal from the cell cycle in medium containing low levels of mitogens. Previous studies demonstrated that fetal calf serum, fibroblast growth actor (FGF), and type β transforming growth factor (TGF-β) inhibit expression of muscle-specific genes and stimulate expression of the proto-oncogenes, c-myc and c-fos, in BC_3H1 cells. In the present study, the potential involvement of cyclic AMP (cAMP) in growth factor-mediated regulation of proliferation and differentiation was examined in BC_3H1 cells. Addition of dibutyl cAMP (db-cAMP), 8-bromo-cAMP, or compounds that increase intracellular cAMP levels, i.e., forskolin, prostaglandin E1 (PGE1), and cholera toxin to BC_3H1 cells prevented expression of the muscle-specific gene products, muscle creatine kinase (MCK) and nicotinic acetylcholine receptor. Cell proliferation was neither stimulated nor inhibited by cAMP at concentrations that inhibited myogenesis. The inhibitory effect of cAMP was dose-dependent and reversible and was potentiated by the phosphodiesterase inhibitor, 3-isobutryl-1-methylxanthine (IBMX). Cyclic AMP and PGE1 also stimulated transient expression of the c-myc and c-fos proto-oncogenes. Intracellular cAMP levels were unaffected in the presence of FGF and TGF-β suggesting that these growth factors inhibit differentiation of BC_3H1 cells through cAMP-independent pathways. The dose-dependence for inhibition of differentiation by TGF-β also was unaffected in the presence of IBMX. Together, these data indicate that muscle-specific gene expression can be regulated in BC_3H1 cells via distinct pathways; a cAMP-dependent pathway and a cAMP-independent pathway, the latter of which is utilized by TGF-β and FGF.

M 319 HEPARIN-BINDING GROWTH FACTORS IN CARDIAC COMPARTMENTS. Elissavet Kardami, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3.

Many highly vascularized tissues have been found to contain high concentrations of heparin-binding growth factors (HGFs); best studied members of this family of growth factors are acidic and basic fibroblast growth factors (a-,b-,FGF). The precise local function of HGFs is not clear, but it seems probable that they serve a paracrine or autocrine function, particularly during angiogenesis and skeletal muscle regeneration. I have found that extracts from adult chicken and rat hearts contain HGFs; rat cardiac HGFs are recognized by antibodies raised against the (1-24) N- terminal peptide from bovine bFGF, in immunoblotting (Western) assays. Biological activity assays, using bFGF sensitive cell types in culture (rabbit fetal chondrocytes, avian skeletal myoblasts) indicate that growth factor concentration is highest in the atrial extracts, and slightly higher in the right ventricle compared to the left ventricle. These findings are intriguing because they suggest a correlation between the less differentiated state and better DNA synthetic ability of the atrial myocyte and HGF concentration; as a first step towards examining this possibility, I have tested the effects of bFGF on embryonic and neonatal cardiomyocyte proliferation, in culture: bFGF stimulates atrial and ventricular myocyte DNA synthesis and proliferation. Study of the regulation of bFGF action on cardiac muscle by other factors and the extracellular matrix may explain the inability of ventricular muscle to regenerate after injury.

M 320 CLONING OF AVIAN Ca-ATPase cDNA AND EXPRESSION IN MOUSE Ltk-CELLS AND THE MURINE MUSCLE CELL LINE C2C12, Norman J. Karin, Zaven Kaprielian and Douglas M. Fambrough, Johns Hopkins University, Baltimore, MD 21218.

A cDNA library in λgt10 was constructed from poly(A)$^+$RNA isolated from day 18 chick embryo skeletal muscle. The library was screened with oligonucleotides synthesized based on the amino acid sequence of rabbit Ca-ATPase. A clone of 2.6kb which encoded ~75% of the fast fiber isoform was isolated and used to re-screen the library. The full-length Ca-ATPase cDNA, constructed from three overlapping clones, contains 50bp of 5' untranslated sequence, 3kb of coding sequence and 350bp of 3' untranslated sequence, including the poly(A) tail. The cDNA was inserted into a eukaryotic expression vector downstream from the SV40 early promoter and transfected into mouse Ltk- cells and mouse C2C12 myoblasts. The pattern of expression, detected by species-specific monoclonal antibodies, was similar in the two cell types and closely resembled nuclear envelope/endoplasmic reticulum paradigm of endogenous Ca-ATPase expression. C2C12 cells carrying the avian cDNA co-localized the avian and endogenous Ca-ATPases throughout differentiation in vitro.

M 321 METALLOENDOPROTEASE INHIBITORS THAT BLOCK THE DIFFERENTIATION OF L_6 MYOBLASTS INHIBIT INSULIN DEGRADATION BY THE ENDOGENOUS INSULIN-DEGRADING ENZYME, Celik Kayalar and William T. Wong, University of California, Los Angeles, CA 90024

The rat skeletal muscle cell line, L_6 myoblasts, proliferate, reach confluency, fuse to form myotubes and express a number of muscle-specific proteins. We have shown that this differentiation process is blocked by specific metalloendoprotease inhibitors (1). We now demonstrate that metabolizing L_6 myoblasts and their cell extracts degrade insulin to acid-soluble fragments mainly by a non-lysosomal pathway. About 90% of the insulin-degrading activity resides in the cytoplasm and is due to a 110 kd enzyme known as the insulin degrading enzyme (IDE)(2).

The same metalloendoprotease inhibitors that block the differentiation of L_6 myoblasts also inhibit their insulin-degrading activity in a) metabolizing cells; b) cell extracts; c) immunoprecipitates of cytoplasmic extracts obtained by an anti-IDE monoclonal antibody.

BC_3H1 is a mouse smooth muscle-like cell line that differentiates in culture but that does not fuse. Essentially the same results are obtained with these cells with respect to the inhibition of insulin degradation and of differentiation by specific metalloendoprotease inhibitors.

These results suggest that the insulin-degrading enzyme in L_6 and BC_3H1 cells is the metalloendoprotease whose activity is required for the initiation of the morphological and biochemical differentiation in these systems.

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2. Duckworth, W.C., Heinemann, M.A., and Kitabchi, A.E. (1972). Proc. Natl. Acad. Sci. USA. 69, 3698-3702.

M 322 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF ATRIAL NATRIURETIC FACTOR EXPRESSION IN CULTURED HUMAN HEART CELLS, D. Stave Kohtz, M. Renate Dische and Bruce Goldman, Mount Sinai School of Medicine, New York, NY 10029

Human cardiac myocytes were enzymatically dissociated from fetal autopsy material and cultured in mitogen-rich medium. A strain of cells (HAM-1) was derived that proliferated in mitogen-rich medium and differentiated in mitogen-poor medium. When co-cultured with a human neuroblastoma cell line (SH-SY5Y) in mitogen-poor medium, HAM-1 cells secreted atrial natriuretic factor (ANF). Nuclear run-off transcription analyses indicated that transcriptional activation of the ANF gene occurred when HAM-1 cells were switched from mitogen-rich to mitogen-poor medium, regardless of the presence or absence of co-cultured neuroblastoma cells. Northern blot analyses, however, indicated that ANF mRNA accumulated only in HAM-1 cells that were co-cultured with neuroblastoma cells in mitogen-poor medium. In contrast, AMF mRNA did not accumulate in HAM-1 cells that were cultured alone. The results indicate that neuronal cells can influence the expression of ANF by differentiating human cardiac myocytes through a regulatory pathway affecting ANF mRNA stability.

M 323 EFFECTS OF RECOMBINANT IGF-I AND IGF-II ON PROLIFERATION, DIFFERENTIATION, AND PROTEIN TURNOVER *IN VITRO* Claire E. Kotts, William H. Phillips, Ramnath Seetharam, Robert A. Heeren, Alan M. Easton, Edith Y. Wong, Robert J. Collier, Gwen G. Krivi, and Clifton A. Baile, Animal Sciences Division, Monsanto Company, St. Louis, MO 63198

The insulin-like growth factors (IGFs) are thought to be involved in the regulation of muscle growth. We compared the effects of recombinant IGF-I and IGF-II on proliferation, differentiation, and protein turnover in L6 myoblasts and myotubes. While both growth factors stimulated proliferation, kinetic analysis of the responses resulted in half-maximal activities at concentrations of 1.7 and 8.6 nM for IGF-I and IGF-II, respectively. Maximal stimulation with IGF-I or IGF-II resulted in cell numbers 223% and 193% of controls, respectively. Protein accumulation in myotubes was 145% of controls in cultures treated for 48 hours with IGF-I at 5 nM or greater; half-maximal activity occurred with 0.5 nM IGF-I. Protein accumulation was 161% of controls at the highest level of IGF-II tested (50 nM). IGF-I increased the incorporation of ^{3}H-Tyr into myotube protein and decreased the release of ^{3}H-Tyr from pre-labeled proteins indicating that changes in net protein accumulation resulted from effects on both synthesis and degradation rates. The onset and extent of differentiation was enhanced in a dose dependent manner in cultures treated with 0.2-10.0 nM IGF-I. Maximal creatine phosphokinase activity was reached on day 10 in cultures treated with 10 nM IGF-I compared to day 14 for controls. Effects of IGF-II on differentiation are currently being assessed. Differential effects of these two growth factors may be a function of changes in receptor population or number during myogenesis.

M 324 MOLECULAR CLONING OF THE 53,000 DALTON GLYCOPROTEIN OF THE SARCOPLASMIC RETICULUM, Ekkehard Leberer, *N. Michael Green, Elizabeth Zubrzycka-Gaarn, #Kevin P. Campbell and David H. MacLennan, Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, M5G 1L6, Canada; *National Institute for Medical Research, Mill Hill, London NW7 1AA, UK; and #Department of Physiology and Biophysics, University of Iowa, Iowa City IA 52242.

We used polyclonal and monoclonal antibodies against the sarcoplasmic reticulum-associated 53,000 dalton glycoprotein (GP-53) to isolate a cDNA clone encoding the protein from an expression library prepared from rabbit fast-twitch skeletal muscle mRNA. An exact match was found between its deduced amino acid sequence and four amino acid sequences, including the amino terminal sequence, that we obtained by analysis of peptides isolated from the purified protein. The cDNA clone hybridized to two species of mRNA of approximately 3.6 and 5.0 kb in length. Both mRNA species were present in fast-twitch and slow-twitch skeletal muscle and cardiac muscle. The cDNA clone encodes a processed protein of 453 residues with a molecular weight of 52,421 daltons and an amino-terminal signal sequence. The protein contains two potential glycosylation sites, supporting the earlier observation that the mature protein contains two oligosaccharide chains of the compositon: Man 9, GlcNAc2. The molecule is strongly polar throughout but is nearly neutrally charged. Structural analysis of the predicted amino acid sequence suggests that GP-53 is an extrinsic membrane protein bound peripherally to the luminal side of the sarcoplasmic reticulum membrane. Extraction studies with EGTA suggest that the interaction between GP-53 and the luminal surface of the membrane is Ca^{2+}-dependent.

M 325 INVOLVEMENT OF A 37 KD TROPOMYOSIN-LIKE PROTEIN IN ACETYLCHOLINE RECEPTOR CLUSTERING, Giovanna Marazzi, Frédérique Bard and Lee L. Rubin, The Rockefeller University, New York, N.Y. 10021.

Our laboratory has shown that chick muscle cells transformed with Rous sarcoma virus (RSV) are unable to form clusters of acetylcholine receptors (AChRs) and are missing a 37 KD tropomyosin-like protein. In attempts to clarify the role of this protein in the formation and maintenance of AChR clusters, we have microinjected a monoclonal antibody specific for this protein into cultured chick muscle cells. Injection was followed by treatment with a factor derived from Torpedo electric tissue that causes AChRs to cluster on control cells. Cells were then labeled with a FITC-conjugated second antibody to identify injected cells and with rhodamine α-bungarotoxin to localize AChRs. Preliminary results indicate that injection blocks the formation of new clusters, but does not affect pre-existing clusters. Cells microinjected with nonimmune immunoglobulin or with monoclonal anti-tubulin antibodies are still able to cluster AChRs, indicating that the injections per se do not interfere with clustering. These data suggest that the 37 KD tropomyosin-like protein may play an important role in promoting the formation of AChR clusters. To further test the role of this protein, we are now microinjecting the purified protein itself into RSV-transformed cells to see whether this will enable them to acquire the ability to cluster AChRs.

M 326 HUMAN PLACENTAL c-erbA cDNA SEQUENCES MODULATE α-MYOSIN HEAVY CHAIN GENE EXPRESSION IN CULTURED RAT HEART CELLS. Bruce E. Markham, Kevin J. Garvey, Joseph J. Bahl and Eugene Morkin. University Heart Center, University of Arizona, Tucson, Arizona 85724.

Triiodothyronine (T_3) regulates the expression of the α-myosin heavy chain (MHC) gene at the transcriptional level. The role of the nuclear T_3 receptor has been examined using human placental c-erbA cDNA cloned in the antisense orientation into pRSVCAT. When transfected into primary cultures of rat fetal cardiomyocytes, the antisense construct (pRSVhEACAT$^-$) gave a slight but reproducible background of chloramphenicol acetyltransferase (CAT) activity. The addition of T_3 (10nM) did not significantly affect the level of background activity. The effect of this clone on α-MHC expression was assessed by cotransfection with pSVOMCAT, which contains 3.3 kbp of α-MHC 5' flanking DNA fused to CAT coding sequences. Transfected fetal rat myocytes were incubated for 24 hours prior to the addition of T_3 to the medium to allow for anti-erbA expression. Cotransfection of pSVOMCAT with increasing amounts of pRSVhEACAT$^-$ (2.5 to 20ug) produced a progressive decrease in T_3-inducible CAT activity to background levels. CAT activity was not altered when M13MP19 RF DNA was used in place of pRSVhEACAT$^-$. These results are consistant with a decrease in endogenous receptor levels and indicate that the c-erbA product is involved in T_3 induction of α-MHC gene expression.

M 327 **ION-MEDIATED AND MINERALOCORTICOID HORMONE REGULATION OF Na^+-K^+- ATPase α-1 GENE EXPRESSION IN VASCULAR SMOOTH MUSCLE**, Russell M. Medford, Bradford C. Berk, Mark B. Taubman and Ronald A. Hyman, Brigham and Women's Hospital, Harvard Medical School, Boston, Mass. 02115.

The Na^+-K^+-ATPase (NAKA) plays a central role in the regulation of intracelluar Na^+, ($[Na^+]_i$). Reciprocal regulation of NAKA by $[Na^+]_i$ represents an important example of ion-mediated regulation of a eukaryotic gene. To increase $[Na^+]_i$, NAKA was inhibited by exposure of cells to ouabain (1mM). This significantly increased $[Na^+]_i$ from 24 to 38 mM in cultured rat aortic vascular smooth muscle cells (VSMC) after 30'. α-1 mRNA was directly assayed by Northern filter hybridization using an α-1 specific cDNA probe. Treatment of rat VSMC for 30' with ouabain was followed by a rapid, transient 3-4 fold induction of α-1 mRNA levels that returned to basal levels within 4 hrs. In contrast, treatment of rat VSMC with the mineralocorticoid aldosterone (2 μM) resulted in a slow, sustained, 3-4 fold induction of α-1 mRNA, first detected 4 hrs following treatment. These results suggest that small alterations in $[Na^+]_i$ in VSMC are associated with increased expression of α-1 mRNA. The dramatic kinetic differences suggest that ionic and mineralocorticoid regulation of α-1 mRNA accumulation occurs by different mechanisms. The molecular mechanisms which account for increased NAKA activity following stimulation of cell growth are unknown. Growth factors stimulate Na^+ influx via Na^+-H^+ exchange causing a transient increase in $[Na^+]_i$ at 30' of a similar magnitude as 1mM ouabain. This data suggests the increase in $[Na^+]_i$ due to growth factor stimulation of Na^+-H^+ exchange may contribute to induction of NAKA mRNA in VSMC.

M 328 THE ACh RECEPTOR-ASSOCIATED 43K PROTEIN IS MYRISTOYLATED IN RODENT MUSCLE CELLS. Linda S. Musil and John P. Merlie. Washington Univ. School of Medicine, St. Louis, MO 63110.

Torpedo electric tissue contains 43K, a peripheral protein of MW= 43,000 associated with the cytoplasmic face of post-synaptic membranes at areas of high nicotinic acetylcholine receptor (AchR) density. 43K has been implicated in the establishment and/or maintainance of these AchR clusters. A protein of similar size and subcellular location has been identified in vertebrate skeletal muscle cells using antibodies raised against isolated **Torpedo** 43K. Cloning of **Torpedo** and mouse 43K cDNAs revealed that the amino terminus of both proteins contains a sequence predicted to be an excellent substrate for **N**-myristoyltransferase, the enzyme believed to mediate the covalent attachment of the C14 fatty acid myristate to amino terminal glycine residues of certain intracellular proteins. This observation, along with the finding that isolated **Torpedo** 43K is blocked to Edman degradation, suggested that 43K may be myristoylated. To test this hypothesis, mouse muscle BC3H1 cells were incubated for 4hr with ^{3}H-myristate, lysed, and immunoprecipitated with polyclonal or monoclonal antibodies raised vs isolated **Torpedo** 43K. A single ^{3}H labeled species was recovered that comigrated on SDS-PAGE with 43K immunoprecipated from BC3H1 cells metabolically labeled with ^{35}S-cys. Precipitation of this band was specifically competed by pH 11 extracts of **Torpedo** postsynaptic membranes, a highly enriched source of 43K. Approximately 95% of the ^{3}H labeled material released from isolated BC3H1 43K by acid methanolysis was extractable in organic solvents and eluted from a C18 reverse-phase HPLC column exclusively at the position of the methyl myristate standard. Thus, 43K contains authentic myristic acid rather than an amino- or fatty- acid metabolite of ^{3}H-myr. The ^{3}H-myr cannot be removed from 43K by prolonged incubation in SDS, BME, methanolic KOH, or hydroxylamine (pH 7.0 or 10.0), indicating an amide linkage between myristate and 43K which is typical of proteins myristoylated on amino terminal glycine residues. Fatty acid acylation of 43K seems to be independent of cell surface AchR expression since 43K is labeled with ^{3}H-myr to a similar extent in C2, BC3H1, and L6 muscle cell lines, which differ dramatically in cell surface AchR number and/or clustering capacity. Covalently linked myristate may be responsible for the high affinity of purified 43K for membranes despite the absence of a notably hydrophobic amino acid sequence. LSM supported by 5T32NSO7129.

M 329 THE COMMITMENT OF SKELETAL MUSCLE CELLS TO A PERMANENT POST-MITOTIC PHENOTYPE IS CORRELATED WITH THE LOSS OF CELL SURFACE FIBROBLAST GROWTH FACTOR RECEPTORS. Bradley B. Olwin*, Patricia Noel, and Stephen D. Hauschka. Department of Biochemisitry, University of Washington, Seattle, WA 98195, and *Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, 53706.

Acidic and basic fibroblast growth factors (aFGF and bFGF) are potent repressors of skeletal muscle differentiation. Although aFGF and bFGF repress terminal differentation of the mouse MM14 skeletal muscle cell line and some primary human and chick skeletal muscle cells, they are only partially effective at repressing differentiation of the mouse C2C12 skeletal muscle cell line and have no effect on the differentation of the rat L6 skeletal muscle cell line. To examine whether a loss of growth factor receptors could account for the loss of responsiveness to mitogenic stimuli, we analyzed proliferating and differentiated muscle cells for the presence of FGF receptor (FGFR). Crosslinking and equlibrium binding of ^{125}I-aFGF to intact myoblasts identified a 165 kDa FGFR on MM14 and C2C12 cells, but failed to identify a receptor on L6 myoblasts. Terminally differentiated MM14 and C2C12 cells did not exhibit detectable cell surface or particulate membrane FGFR indicating that a total loss of cell surface FGFR accompanies skeletal muscle differentiation. In contrast, differentiation of BC3H1 cells (a mouse cell line which undergoes biochemical differentiation upon FGF removal but does not aquire a permanent post-mitotic phenotype) is not accompanied by a loss of cell surface FGFR. Since quiescent, undifferentiated MM14 cells also retain their FGFR, the loss of cell surface FGFR is not associated with either reversible cessation of proliferation or biochemical differentiation. These data thus support a correlation between loss of FGFR and acqusition of a permanent post-mitotic phenotype. A preliminary analysis of potential FGF-dependent signal transduction pathways indicates that neither phorbol esters (PMA) nor pertussis toxin affect MM14 cell proliferation or differentiation. Thus, FGF-mediated repression of myogenesis is unlikely to be mediated via activation of protein kinase C or a pertussis toxin-sensitive GTP coupling protein.

M 330 EXPRESSION OF THE PREPROENKEPHALIN A GENE IN HAMSTER CARDIAC MUSCLE. Michel Ouellette and Léa Brakier-Gingras, Département de Biochimie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Enkephalins are opioid peptides which participate in the regulation of myocardial function. They are synthesized under the form of a precursor, preproenkephalin A, and their major site of synthesis is in the adrenal glands and the brain. Poly(A)$^+$RNA was isolated from the heart of hamsters and analyzed by Northern blot, using as a probe a 918 base-pair HincII fragment of human preproenkephalin cDNA. It was shown that the preproenkephalin A gene is also transcribed in the heart (atria and ventricles) of hamsters. Two forms were detected among the transcripts: a minor one, which was about 1400 nucleotides in size, which is common to the adrenal glands and the brain; a major one, which was about 900 nucleotides in size. Using cardiomyopathic hamsters, we have observed that the expression of the preproenkephalin A gene is altered in the heart during the development of congestive heart failure. Our results support the hypothesis that locally synthesized enkephalins could be involved in the regulation of the heart function. We are now studying the molecular mechanisms which account for the difference in size between heart preproenkephalin transcripts and their counterparts in the brain or the adrenal glands.

M 331 *CONTRACTION OF INTACT AND SKINNED CHICK MYOTUBE IN CULTURE*, Eijiro Ozawa and Koji Saito, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187, Japan.

Little is known about the contractility of myotubes grown in vivo or in vitro. We developed methods to record contraction of cultured myotubes, and studied their contractility. (1) A glass grain was laid on a myotube. Displacement of the grain, caused by contraction, was detected and recorded through a phototransistor. Membrane potential changes were recorded simultaneously. Many myotubes showed a prolonged depolarization, a spike discharge or both in response to stimulation with a depolarizing pulse. Contractions were elicited by the active membrane potential changes and also by passive depolarizations induced by weak stimuli. Caffeine evoked contractions in a medium containing 1.8 mM Ca. Caffeine contracture, however, decreased or disappeared in a Ca-free medium in 80 per cent of the myotubes. The results suggest that Ca ions for the contraction were delivered from an intracellular store, probably the sarcoplasmic reticulum, and that the ability of the store is low in accumulating and releasing Ca ion. (2) By recording tension generated in a saponin-treated myotube, sensitivity of the contractile system to divalent cations was examined. Tension increased with Ca ion concentration from 1 to 100 μM. The order of potency in generating tension was Ca>Sr>Ba. Dose-response curves were similar to those of a fast twitch fiber rather than a slow tonic fiber.

M 332 EXPRESSION OF IGF-1 REGULATED PROTEINS AND MESSENGER RNAS IN MUSCLE William H. Phillips, Claire E. Kotts, and Gwen G. Krivi, Biological Sciences, Monsanto Company, St. Louis, MO 63198

As a primary mediator of somatotropin action, the 70 amino acid polypeptide known as IGF-1 (insulin-like growth factor-1) stimulates muscle cell proliferation and enhances the process of myogenesis *in vitro*. In addition, the binding of IGF-1 to cell surface receptors on cultured myoblasts or myotubes augments overall protein accumulation due to increased rates of protein synthesis and inhibition of intracellular proteolysis. Although the metabolic responses caused by this growth factor are well documented, the actual molecular mechanisms underlying the IGF-1 control of proliferation and muscling by IGF-1are not understood. In order to identify both early and late changes in gene expression induced by IGF-1, we will report results of a time course study to analyze the regulation of individual transcription and translation products. Proliferating L6 myoblasts and differentiated L6 myotubes were treated for 0, 1, 4, and 36 hours with recombinant IGF-1 (10nM and 50nM respectively) in media supplemented with 2% fetal calf serum. ^{35}S-methionine incorporation experiments were performed on cultures exposed to exogenous IGF-1 for 36 hours, confirming previously published results that protein synthesis rates double; further analysis by two dimensional polyacrylamide gel electrophoresis revealed a 2-3 fold increase in at least one myotube protein (≈18,000 kDa) compared to untreated controls. Experiments are underway to examine rapid changes in both myoblast and myotube protein patterns following very brief exposures (≤ 4 hr) to IGF-1. Since changes noted in the protein patterns of IGF-1 treated cultures may reflect differences in messenger RNA populations, total RNA was also extracted from the IGF-1 treated cultures at similar time points. Northern analysis is being performing to examine the temporal expression of a number of oncogenes and other muscle-specific genes under the control of IGF-1.

M 333 A RAPID AND SENSITIVE ASSAY FOR THE QUANTITATION OF MYOBLAST RECOGNITION AND ADHESION, J.A. Pizzey and F.S. Walsh, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

Myoblast fusion occurs in discrete stages, beginning with cellular recognition and adhesion. Most quantitative analyses of these processes in vitro involve long periods of incubation and thus are not always suitable for analyzing early events in cell recognition. An assay using Couette viscometry is described in which the kinetics of C2 myoblast recognition can be accurately determined. The use of this technique to measure intercellular adhesion has generally been limited to developmental studies and several conditions had to be modified to suit the present application. Firstly, the effects of cell dissociation in low Ca^{2+} (0.7mM) and Ca^{2+}-free media on subsequent adhesion are described; the initial rate of subsequent aggregate formation is nearly 3-fold greater in the latter case. Secondly, the effects of serum on early (5-30 minutes) myoblast adhesion are presented, and finally a recovery period is required to allow for the translocation of surface components removed by trypsinization prior to the adhesion assay. As a result of establishing the base-line conditions for this assay, we have also been able to support earlier findings which suggest that initial intercellular adhesions are mediated by surface-components which are trypsin-resistant, although these may be augmented at a later stage by stronger, trypsin-sensitive mechanisms.

M 334 RETROVIRAL-MEDIATED EXPRESSION OF FOREIGN MEMBRANE PROTEINS IN C2 MUSCLE CELLS. Evelyn Ralston and Zach W. Hall, Dept. of Physiology, Univ. Calif. San Francisco, CA. 94143

The dramatic remodeling of cellular architecture that takes place upon muscle cell fusion provides a unique system in which to address questions of membrane organization and control As a tool to study such questions, we have developed several muscle cell lines expressing non muscle membrane proteins. Cells of the mouse muscle line C2 were infected with the Moloney murine sarcoma virus vectors CD8-MV7 and CD4-MV7 (gifts of D. Littman) encoding the T-lymphocyte antigens CD8 and CD4, respectively, and containing a gene for neomycin resistance. Colonies were selected for resistance to the antibiotic G418 and screened for surface expression of CD8 or CD4 by a red bloood cell rosetting assay. A high fraction of the resistant colonies also expressed the antigen (> 90% for CD8; > 75% for CD4). The CD8-expressing C2 lines have been characterized in detail by immunofluorescence and by immunoprecipitation of surface or metabolically labeled cells. CD8 is expressed in both myoblasts and myotubes and assembles into homodimers. The intracellular distribution of CD8 shows a Golgi-like pattern. Surface expression of CD8 appears generally uniform on both myoblasts and myotubes. In myotubes, however, CD8 is also found in clusters that coincide with acetylcholine receptor clusters. Supported by NIH and MDA grants.

M 335 EXPRESSION OF MULTIPLE FORMS OF THE $(Na^+ + K^+)$-ATPase IN CHICK SKELETAL MUSCLE. Karen J. Renaud, Kunio Takeyasu, Michael M. Tamkun, Andrew M. Barnstein, and Douglas M. Fambrough. The Johns Hopkins University, Baltimore, MD 21218.

Monoclonal antibodies were raised against both the α- and β-subunits of adult chicken kidney $(Na^+ + K^+)$-ATPase. We have also isolated a set of genes from a chicken genomic library, and cDNAs from chicken brain, muscle, fibroblast, and kidney libraries. These encode three forms of the α-subunit and one form of the β-subunit. Expression of some of these cDNAs in E.coli as β-galactosidase fusion proteins has enabled us to make some correlations between antibody binding sites on the protein subunits and cDNA sequences encoding those sites. Results from this "epitope mapping", along with immunofluorescent staining of tissue sections and immunoblot data show that at least three molecular forms of the α-subunit, and two of the β-subunit, are recognized by the antibodies, and that these forms are expressed in a cell-type specific manner. In particular, slow twitch muscle fibers express a distinct form of the α-subunit which is also found in peripheral neuron, kidney, and heart, but not in fast twitch fiber or in brain. Fast twitch muscle fiber contains another form of the α-subunit that also exists in brain. Currently, peptide maps of monoclonal antibody affinity purified subunits are being produced in order to further distinguish between the various forms of the subunits.

M 336 CALCIUM CHANNEL DIVERSITY IN DEVELOPING SKELETAL MYOBLASTS: SEQUENTIAL EXPRESSION OF THREE CHANNEL SUB-TYPES AND COORDINATE SUPPRESSION BY TRANSFORMING GROWTH FACTOR β. M.D. Schneider*†, J.M. Caffrey†, and A.M. Brown†, Departments of *Medicine, *Cell Biology, and †Physiology & Molecular Biophysics, Baylor College of Medicine, Houston, Texas.

Whether the expression of voltage-gated Ca^{2+} channels during myogenesis is regulated by mechanisms akin to those that control the formation of other muscle-specific gene products is unknown (Science 236:570). We have identified four phases of Ca^{2+} channel expression in mononucleate C2 muscle cells, following mitogen withdrawal at sub-confluent density. [1] No Ca^{2+} channels were expressed in proliferating myoblasts. [2] "Transient" Ca^{2+} channels were induced within 24 hours of mitogen withdrawal, 1 to 2 days before appearance of "fast" and "slow" channels. [3] All three components of Ca^{2+} current coexisted in biochemically differentiated, unfused C2 cells after 3-5 days. [4] "Transient" channels subsequently were deinduced: only "fast" and "slow" channels were found following mitogen withdrawal for 14 days. Formation of all three components of Ca^{2+} current was suppressed reversibly by transforming growth factor β. Thus, expression of distinct Ca^{2+} channel sub-types occurs sequentially during myogenesis, requires neither fusion nor interaction with neurons, and can be regulated by transforming growth factor β, a peptide which, independently of cell proliferation, prevents the activation of muscle-specific genes.

M 337 **TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF THE EXPRESSION OF MYOFIBRILLAR PROTEIN GENES DURING CARDIAC HYPERTROPHY IN RATS.**

***+Rajamanickam, C., +Arun Subramaniam, *Rajendra Wadgaonkar, *M.A.Q. Siddiqui. +School of Biological Sciences, Madurai Kamaraj, University of Madurai, India. *Department of Anatomy and Cell Biology, S.U.N.Y.-Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, N.Y. 11203.** **Studies on RNA transport in vitro from nuclei from normal and aorta constricted hypertrophic hearts have shown that an enhanced transport of RNA occurs from hypertrophic heart nuclei. Hybridization analysis of the RNA transported in vitro using specific cDNA probes showed that both α and β MHC mRNAs and MLC_1 and MLC_2 mRNAs are transported to a greater extent from hypertrophic heart nuclei than from normal nuclei. Also, the transport in vitro of the above sequences from the normal nuclei is stimulated upon incubating the normal nuclei with a partially fractionated 100,000 x g supernatant obtained from the hypertrophic heart. Further, hybridization of the above mRNA sequences in the RNA from nuclear, polysomal and post-polysomal fractions from normal and hypertrophic heart and the run-on transcription in vitro in isolated nuclei, have shown that both transcriptional and post-transcriptional regulation operate in the expression of the respective genes.**

M 338 EFFECT OF 3,3',5-TRIIODO-L-THYRONINE AND cAMP ON THE EXPRESSION OF ALPHA AND BETA MYOSIN HEAVY CHAIN GENES IN THE RAT VENTRICULAR CARDIAC MUSCLE CELL Wendell R. Smith and William C. Claycomb, Department of Biochemistry and Molecular Biology, L.S.U. Medical Center, New Orleans, LA 70112. 3,3',5-Triiodo-L-thyronine (T_3) and cAMP are known to influence differentiation of the rat ventricular cardiac muscle cell. During early development of the rat there is a switch in the expression of the alpha and beta myosin heavy chain (MHC) genes. Beta MHC is dominant during late fetal and early neonatal development, while alpha MHC is dominant in the adult. T_3 is able to influence the expression of the MHC isoforms both in vivo and in vitro. Tissue levels of cAMP increase during development of the rat heart and it has previously been proposed that cAMP is a primary effector for the terminal differentiation of the cardiac muscle cell. We have investigated the effect of T_3 and cAMP on the expression of alpha and beta MHC mRNA in primary cultures of neonatal and adult ventricular cardiac muscle cells. When T_3 was added to the cultures of adult and neonatal cardiac muscle cells, the levels of alpha MHC message increased while the levels of beta MHC message decreased. cAMP increased the amount of alpha MHC message but had no effect on the expression of beta MHC message. These results indicate that there are at least two different mechanisms by which the expression of MHC genes are controlled in these cells, a coordinated control influenced by T_3 and an uncoordinated control under the influence of cAMP.

M 339 PREPROENKEPHALIN mRNA EXPRESSION IN DEVELOPING AND CULTURED RAT VENTRICULAR CARDIAC MYOCYTES Jeremy P. Springhorn and William C. Claycomb, Dept. of Biochemistry and Molecular Biology, L.S.U. Medical Center, New Orleans, LA 70112 Heart muscle tissue has previously been reported to contain the highest level of preproenkephalin (ppEnk) mRNA of any tissue in the adult rat. We have determined that it is present in the actual ventricular cardiac muscle cells of the heart and is developmentally regulated. The developmental expression of ppEnk mRNA was observed to be low throughout the first 17 days of early neonatal development and, at 23 days postpartum, decreased to almost zero. A low level of expression was again observed by 30 days and, by adulthood, reached a level in excess of 50 fold over the 23 day time point. ppEnk mRNA was actively expressed in primary cardiac muscle cell cultures prepared from both neonatal and adult rats. Its steady state level in cell cultures was observed to be increased by cyclic AMP and isobutyl methyl xanthine, while enkephalinamide decreased its expression. The phorbol ester, TPA, elicited a transient effect (i.e. an increase was observed at 4 hours and returned to control levels by 24 hours). We speculate that enkephalin may play a multi-functional role in the differentiation of neonatal cardiac muscle cells and in the terminally differentiated adult heart cell. We demonstrate that the primary heart culture systems employed in this study will be useful models with which to explore both transcriptional and translational regulation of ppEnk mRNA.

M 340 ISOLATION AND CHARACTERIZATION OF cDNA CLONES CODING FOR TROPONIN C AND I FROM CHICKEN CARDIAC MUSCLE, Naoji Toyota[1,2], Yutaka Shimada[2] and David Bader[1], [1]Dept of Cell Biology & Anatomy, Cornell Univ Medical College, NY, NY. [2]Dept of Anatomy, School of Medicine, Chiba Univ, Chiba, Japan.

We have isolated cDNA clones coding for chicken cardiac troponin C and I (CTNC and CTNI, respectively) from a gt11 cDNA library prepared from cardiac muscle of 19 day embryos. Nucleotide sequence analysis was carried out to determine the sequence of the proteins.

CTNC contained a 5' non-translated region of 18 nucleotides, the entire coding region, and a 3' non-translated region of 143 nucleotides. The amino acid sequence deduced from the nucleotide sequence was similar to that reported for troponin C from bovine cardiac muscle except one amino acid. The amino acid and nucleotide sequences of CTNC were nearly identical to those reported for troponin C from chicken slow skeletal muscle except several nucleotides in non-translated regions. The strong conservation of the amino acid and nucleotide sequences of chicken cardiac and slow muscles suggests that troponin C of these muscles are transcribed from the same gene or similar but distinct genes which diverged recently.

CTNC contained the coding region of 600 nucleotides and a 3' non-translated region of 117 nucleotides. Comparison of the amino acid sequences of CTNI and four troponin I isoforms revealed that CTNI contained two kind of regions: strongly conserved and considerably diverged regions. The variant regions in the sequence were in the middle and the COOH-terminal region of the protein. The differences in the sequence of CTNI isoforms may define distinct functional domains among embryonic and adult troponin I.

M 341 LONGITUDINAL GROWTH OF MUSCLE FIBERS IN VITRO INDUCED BY MECHANICAL ACTIVITY
H.H. Vandenburgh and P. Karlisch, Brown Univ. and Miriam Hospital, Providence, RI.

Embryonic skeletal muscle cells from avian pectoralis muscle are grown in tissue culture in a device which mechanically stretches the substratum on which the cells grow. When the elastic substratum is continuously stretched in one direction at a rate which simulates in vivo elongation of growing bone(0.35 mm/hr for 70-78 hr) the developing myotubes are stimulated to grow longitudinally from a mean myotube length of 0.71 + 0.31 to 1.72 + 0.68 mm ($p < .001$). Mean myotube diameters in control and stretched cultures are not significantly different. The stretch-stimulated myotubes have significantly decreased fiber length per nucleus ratios and reduced mean inter-nuclear distances compared to unstimulated controls. Myotube nuclei per culture well is significantly increased by growth of the cells on the stretching substratum (104%, $p < .001$). Together, these data indicate that muscle cell fusion has been stimulated in the stretched cultures. The number of total cell nuclei (myoblast, fibroblast, and myotube) per culture well is also increased in the stretched wells (61%, $p < .001$), indicating a stimulation of cell proliferation. We propose that during limb development the close coupling between bone length and muscle fiber length results from the growing bone mechanically stimulating two processes in the attached muscle tissue: the rate of cell proliferation, and the rate of muscle cell fusion. Stretch appears to stimulate cell proliferation by reducing contact inhibition of cell division and possibly by stimulating the production of endogenous muscle growth factors.
(Supported by NIH Grant AR36266 and NASA Research Grant NAG2-414)

M 342 MYOSIN GENE SWITCHING IN CARDIAC MYOCYTE HYPERTROPHY IN VITRO
Lawrence E. Waspe, Neal White, Charles P. Ordahl and Paul Simpson, UCSF, SF, CA 94143.

We have shown that α1-adrenergic receptor stimulation causes cellular enlargement without hyperplasia in primary cultures of neonatal rat ventricular myocytes (1). Models of cardiac hypertrophy produced in vivo by hemodynamic overload demonstrate increased synthesis of contractile proteins and their mRNAs, as well as a switch towards the myosin isozyme with lowest ATPase activity (V3) (2). To determine if comparable myosin switching occurs during hypertrophy induced in vitro we quantitated cardiac α- and β-myosin heavy chain (MHC) mRNAs and their respective proteins in cultured myocardial cells treated with the α1-adrenergic receptor agonist norepinephrine (NE). Myocytes exposed to 2 uM NE for 24-72 hr showed a 2.0- 2.4 fold increase in total cellular protein and RNA content vs control ($p<0.01$). MHC protein was increased 2.1-fold (33.0 vs 16.1 pg/cell), and total MHC mRNA increased 2.5- fold. NE-treated myocytes had a V1/V3 ratio of 0.93 as compared with a ratio of 2.17 for control cells, indicating that myosin isozyme switching occurs during induced cardiac myocyte hypertrophy in vitro. To determine if this switch is mediated at the pre-translational level, the relative amounts of α- and β-MHC mRNA were quantitated with an S1 nuclease protection assay using a single synthetic oligonucleotide complementary to both iso-mRNAs. The ratio of α/β-MHC iso-mRNA in control myocytes was 1.54, but after 24 hr of NE this ratio fell to 0.37. Thus, the observed myosin isozyme switching in vitro is attributable to alteration in the steady state levels of the cognate MHC iso-mRNAs. NE induction of V3 and β-MHC mRNA was blocked by an α1-adrenergic antagonist but not by the β-antagonist, propranolol, demonstrating that the observed MHC switching is mediated via the α1-adrenergic receptor. These results indicate that the α1-adrenergic receptor may function as a cellular link to gene switches involved in myocardial hypertrophy.

References : (1) Simpson [1983] J Clin Invest 72:732. (2) Lompre et al [1979] Nature 282:105.

M 343 SUPPRESSION OF ONCOGENE EXPRESSION IN MUSCLE CELL DIFFERENTIATION
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The increased level of expression of the nuclear oncogenes p53, c-myc, c-fos and of the B2 repeat containing RNA has been correlated with the transformed status and the degree of tumorgenicity of several cell types. In untransformed cells the expression of these oncogenes and of the B2 repeat RNAs correlates with cellular proliferation. We introduce a new experimental system, the AM1 Cl 2 thymus reticulum derived myoblasts (Wekerle H. et al., Nature 256, 493-494, 1975), which is unique in its ability to generate differentiated non proliferative cells without any changes in the exogenous mitogen concentration. We demonstrate that the transformation like high level expression of p53, c-myc and of the transformation/proliferation associated B2 repeat RNAs (but not c-fos) is severely suppressed by the muscle cell differentiation process. Expression of the SV40 large Tantigen in the myoblasts blocks myogenic differentiation, but does not alter oncogene expression at RNA level and p53 protein level is kept similar to that of transformed cells. The data suggest that transformation like high level expression of p53, c-myc and of the B2 repeat containing RNAs is neither sufficiant to keep muscle cells proliferating nor to transform them, and the expression of the oncogenes is under the control of genes induced in the muscle cell differentiation process without of any direct influence of the exogenous growth factor concentration. The observations show the superior role of a physiological differentiation process in the control of proliferation and transformation and have important implications on the functions of nuclear oncoproteins and the B2 repeat containing RNAs in proliferation and transformation.
We further exploring the intrinsic ability of this cell system in suppression of oncogene expression and the differentiation properties by introducing cells of this type into blastocytes and embryos (abstract by R. Büttner).

M 344 SINGLE MYOBLASTS OF A ADULT MOUSE MUSCLE CELL LINE PARTICIPATE IN EMBRYONIC DEVELOPMENT, R. Büttner[1] , F. Brem[2] , K. Müller[1] , K.H. Westphal[1]
1 : Laboratorium für molekulare Biologie - Genzentrum, Am Klopferspitz, D - 8033 Martinsried, FRG. 2 : Institut für Tierzucht undTierhygiene, Veterinärstrasse, D - 8000 Munich, FRG.

We have studied the ability of myoblasts from a thymus reticulum derived muscle cell line (Abstract from Müller K. et al.) to respond on differention signals in mouse blastocysts and embryos and subsequent participation in regular organogenesis.Our experimental system comprises a myoblast cell line showing spontaneous in-vitro differentiation to muscle cells, which we have transfected with the plasmid p HVI SV 3V. After injection of one to two cells in blastocysts and 100 to 200 cells in 7 to 9 days old embryos we were able to detect in a variety of tissues of 8 to 16 weeks old mice plasmid marker DNA by different techniques. Probing genomic DNA on Southern blots with p HVI SV 3V we detect identical signals in all organs except from brain in two out of six mice having received the myoblasts in the blastocyst state, whereas in mice injected with the myoblasts as embryos the signals are much weaker and restricted to single organs. By transfection of genomic DNA from mice injected as blastocysts into E. coli DH 1 we are able to rescue plasmids with identical resistances and cross-hybridisation properties to p HVI SV 3V. Both the restriction pattern of rescued plasmids and the signals obtained from genomic DNA suggests that the marker DNA must have undergone substantial rearrangement. Our results show for the first time that non-malignant cells from an adult mammal introduced into blastocysts can participate in embryogenic development and organogenesis. Presently we are characterizing the differentiation on a cellular level in tissue sections in order to investigate the cell lineage of the injected cells. The experimental rationale we used might generally be able to detect cells in an adult organism which can participate in embryogenesis and may lead to important new insights into the differentiation potential of those cells.

M 345 STRUCTURE AND EXPRESSION OF THE MYOCARDIAL SR Ca^{2+} ATPase GENE. Angel Zarain-Herzberg*, Ryozo Nagai*, Norman Alpert*, Muthu Periasamy* and David H. MacLennan**.
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**Banting and Best Dept. of Medical Research, C.H. Best Institute, Toronto, Ontario, Canada M5G 1L6.

In order to understand the physiological and pathological regulation of the cardiac sarcoplasmic reticulum (SR) Ca^{2+} ATPase it is necessary to know the different isoforms that are expressed and the genes encoding them. Two different genes encode SR Ca^{2+}ATPase of fast-twitch and slow-twitch skeletal muscle. The fast-twitch Ca^{2+}ATPase gene in rabbit has been extensively characterized.

It has been suggested that slow-twitch and a cardiac muscle Ca^{2+}ATPase may be products of a single gene, based on nucleotide sequence homology at the 3' untranslated region with cardiac mRNA. In this study we determined the phenotypes of Ca^{2+}ATPase mRNA during development and pathological conditions, such as cardiac hypertrophy and atrophy using the above probes. We found that only the slow-twitch isoform is expressed in the myocardium. We demonstrate that the level of slow-twitch/cardiac Ca^{2+} ATPase mRNA synthesis increases under thyroxine-induced hypertrophy, but decreases in pressure overload hypertrophy and in cardiac atrophy induced by hypothyroidism. Our data suggest that SR undergoes reorganization in myocardial adaptation to hypertrophy.

To gain insight into the mechanisms regulating the cardiac SR Ca^{2+}ATPase expression, we have isolated genomic clones and performed structural analysis of the gene. In addition, we are characterizing the upstream regulatory regions of the gene by introducing defined portions of the gene into muscle cell cultures.

Regulation of Muscle Genes and Protein Expression - I

M 400 DEFINITION OF CIS-REGULATORY SEQUENCE ELEMENTS IN THE CARDIAC MYOSIN LIGHT CHAIN 2 GENE: REQUIREMENT FOR TISSUE-SPECIFIC EXPRESSION AND BINDING OF TRANS-ACTING FACTORS, Hans H. Arnold, Thomas Braun, Egbert Tannich and Gregor Buschhausen-Denker, Department of Toxicology, Hamburg University Medical School, Grindelallee 117, 2000 Hamburg 13.

The function of the cardiac MLC 2 gene promoter from chicken was analyzed in transfected or microinjected myogenic tissue culture cells by the transient expression of CAT enzyme activity. Progressive deletion analysis of 2.1 Kb of 5'upstream sequences revealed that the sequence between nucleotide -64 and the start site for transcription is required and sufficient for muscle specific promoter activity. A detailed functional dissection of this region by in vivo competition experiments shows that 2 essential elements can be discerned. Gel retardation assays and DNAseI footprints with nuclear extracts from various tissues and culture cells suggested the binding of at least 2 protein factors or complexes one at the atypical TATA motif of the gene promoter and one in the region -65 to -35. The protein binding at both regions can be prevented by specific competitor DNA but not with neutral plasmid DNA or poly dI/poly dC. Our results suggest that in addition to the TATA motif at least one additional closely spaced sequence element interacts with nuclear factors and is needed for the muscle specific activation of the chicken regulatory MLC 2-A gene.

M 401 ALKALI MYOSIN LIGHT CHAINS IN HUMAN ARE ENCODED BY A MULTIGENE FAMILY THAT INCLUDES THE SMOOTH- AND NONMUSCLE, AND THE EMBRYONIC OR ATRIAL LIGHT CHAINS,
Hans H. Arnold, Ulla Seidel, Eva Bober, Susan Lenz and Peter Lohse, Department of Toxicology, Hamburg University, Medical School, Grindelallee 117, 2000 Hamburg 13.
A set of cDNA clones coding for alkali myosin light chains was isolated from fetal and adult human skeletal muscle libraries. DNA sequence analysis and the expression pattern of individual clones revealed at least 4 different types of clones corresponding to fast fiber type MLC_1 and MLC_3; the embryonic MLC also expressed in fetal ventricle and adult atrium; a group of nonmuscle like MLCs ubiquitously expressed in all cells; a smooth-muscle like MLC, closely related to the nonmuscle forms. The gene coding for $MLC_{1/3}$ is unique and located on human chromosomel 2. The $MLC_{emb/atr.}$ gene is also present only once per haploid genome. In contrast, the smooth- and nonmuscle LC isoforms are encoded by a subfamily of multiple genes. The isolation and analysis of at present four different loci has shown 1 functional gene and 3 processed pseudogenes. The functional locus gives rise to the smooth muscle as well as the nonmuscle isoform by differential splicing of a small exon encoding the C-terminus of the nonmuscle LC. Several cDNA clones were isolated that were also different at their 5'ends resulting from alternative splicing pathways, e.g. exon-exclusion or use of an internal 3'acceptor site. Functional implications of these results will be discussed.

M 402 CHARACTERISATION AND PROMOTER ANALYSIS OF STRIATED MUSCLE MYOSIN ALKALI LIGHT CHAIN GENES. Paul J R Barton, Philippe Daubas, Arlette Cohen, Benoît Robert and Margaret Buckingham. Pasteur Institute 28 Rue du Dr Roux, 75724 Paris France.
We have isolated and analysed the three principal myosin alkali light chain genes expressed in mouse striated muscle: (i) the MLC1F/MLC3F gene expressed in adult fast skeletal muscle, (ii) the MLC1A/MLC1emb gene expresed in cardiac atrial muscle and in fetal striated muscle, (iii) the MLC1V gene expressed in cardiac ventricular muscle and in slow skeletal muscle (where the isoform is known as MLC1S). The structure and sequence of the MLC1A/MLC1emb gene has been determined and provides the first amino acid sequence data for this isoform for any species. Comparison with the published amino acid sequences of MLC1F and MLC1V reveals a close structural and evolutionary relationship between MLC1A/MLC1emb and MLC1V. The occurrence of a specific atrial isoform is limited to mammals and analysis of the MLC1A/MLC1emb gene indicates the adoption of a new splice site that may represent a recent (evolutionary) adaptation to this role. Comparison of the MLC1F, MLC3F MLC1V and MLC1A gene promoters reveals four types of shared sequence element (i) Sequences showing homology with the viral E1A enhancer core sequence, (ii) A shared sequence common to all MLC1 type genes, including genes from other species, but which is absent from other muscle gene promoters, (iii) sequences shared between MLC1A and MLC1V only, (iv) sequences shared between MLC1A, MLC1V and the α-cardiac actin gene. Transient assay type transfection experiments using the MLC1F and MLC3F promoters show that regulatory elements sufficient to direct tissue specific and developmentally regulated expression are contained within the first 1200bp and 423bp upstream of the respective cap sites of the MLC1F/MLC3F gene.

M 403 FACTORS THAT RECOGNIZE THE MUSCLE CREATINE KINASE PROMOTER.
Pamela Benfield, Cathy Earhart, Robert Horlick, Mark Mitchell and Mark Pearson. E. I. duPont de Nemours and Company, Central Research and Development, Wilmington, Delaware 19898.
Nuclear extracts from both myogenic (L6 and C2) and non-myogenic cells (L cells and HeLa) contain sequence specific binding activities that recognize a series of sequences immediately upstream of the muscle creatine kinase promoter. The characteristics of this binding are different in myogenic and non-myogenic cells and also appear to be different between differentiated myotubes and undifferentiated myoblasts. The exact sites of binding have been determined by DNAaseI footprinting. In order to assay the potential function of these binding proteins we have constructed mutant promoters in which the binding sites are deleted or mutated. These mutant promoters have been linked to the CAT reporter gene and introduced into differentiating myogenic cells (C2 and L6), and also into non-myogenic cells (L cells). Our results indicate that these sequences probably do not function in the up-regulation of the muscle creatine kinase gene that accompanies fusion. However, they do appear to exert an effect on the level at which the gene is expressed in all cell types assayed. We have also examined the effect of these mutations on *in vitro* transcription driven by the muscle creatine kinase promoter in HeLa cell extracts. HeLa extracts transcribe the muscle creatine kinase promoter very inefficiently. Mutations that result in an increase in CAT expression as judged by the gene transfer experiments described above also lead to improved transcription efficiency in the HeLa system. The possible role of sequences in the immediate upstream region of the muscle creatine kinase gene upon its expression in myogenic and non-myogenic cells will be discussed.

M 404 EVOLUTION OF THE GENE, ENCODING THE Ca^{2+}-BINDING PARVALBUMIN, M.W. Berchtold, Universität Zürich-Irchel, 8057 Zürich, Switzerland

Parvalbumin is a small acidic protein expressed in high levels in fast skeletal muscles and in certain cells of the central nervous system and of some endocrine glands.This protein belongs to a family of high affinity Ca^{2+}-binding proteins which are structurally related. Recently, we described the intron/exon organization of the rat parvalbumin gene and established its relationship to other genes for Ca^{2+}-binding proteins. In addition, we localized the human gene on chromosome 22 by the somatic cell hybrid technique (Berchtold et al. J.B.C. 262, 8696, 1987). To analyze the evolution of the parvalbumin gene a human parvalbumin clone was isolated from a chromosome 22 specific library. Four of five expected exons, including 400 bp upstream of the putative transcription start site and 90% of the coding region were found within a single 7.5 Kb Eco R1 fragment. Splice site positions with respect to the amino acid sequence were identical in the human and rat parvalbumin gene. The coding region has 92% sequence homology. Only 9 conservative aminoacid replacements could be predicted from exon sequencing. One stretch of the human promoter (-79 to -110) is identical to the rat counterpart. This region has a high homology to the promoter of the gene for myosin light chain 3F (Nabeshima et al. Nature 308 , 333, 1984). This gene is expressed in fast contracting/relaxing muscle fibres (anaerobic, type IIb), the cell type which exhibits also highest parvalbumin expression. We conclude that this promoter region might be important for the tissue and cell type specific expression of parvalbumin.

M 405 CHARACTERIZATION OF THE PROTEIN FACTORS THAT INTERACT WITH THE CARDIAC ACTIN PROMOTER, Linda M. Boxer and Laurence H. Kedes, The Medigen Project, Department of Medicine, Stanford Medical School and Veterans Administration Medical Center, Palo Alto, CA 94304.

Gel mobility shift experiments have been used to identify protein factors that interact with the regions of the cardiac α-actin promoter that are involved in the developmental regulation of this gene. These factors bind to a 20 base pair region located at -93 from the start site of transcription. This region contains a CArG box (for $CC(A/T)_6GG$) which has been shown to be required for muscle specific transcription of this gene by in vivo transfection experiments. The protein factor that binds to this sequence has a molecular weight of approximately 65,000 as determined by SDS gel analysis. Its native molecular weight has also been determined. A CArG box binding factor is also present in nuclear extracts from nonmuscle cell lines, and studies are underway to determine whether this is the same factor that has been identified in nuclear extracts from muscle cells. Gel mobility shift competition experiments have shown that this factor also interacts with the human skeletal α-actin promoter as well as with several nonmuscle-specific promoters which also contain CArG box sequences (β-actin, γ-actin, and c-fos). These studies suggest that the developmental regulation of the cardiac α-actin gene is a complex process.

M 406 IDENTIFICATION OF TWO ALTERNATIVE SPLICING EVENTS THAT GENERATE FUNCTIONALLY DIFFERENT FAST TNT ISOFORMS. Margaret M. Briggs, Philip W. Brandt, and Frederick H. Schachat, Duke University Medical Center, Durham, NC 27710.

The response of permeabilized rabbit fast skeletal muscle fibers to calcium is in part determined by the troponin T (TnT) and tropomyosin (Tm) isoforms they express. There are three major fast TnT isoforms in rabbit muscle, designated TnT_{1f}, TnT_{2f}, and TnT_{3f} by their mobility on 2D gels, and two major tropomyosin forms, α_2 and $\alpha\beta$. Several of the TnT and Tm isoforms are expressed in each muscle fiber; and their proportions vary dramatically from fiber to fiber. The three major TnT isoforms are N-terminal variants generated by alternative splicing. We have combined amino acid sequencing with peptide mapping and selective antibodies to establish that each TnT has a unique N-terminal amino acid sequence, and that TnT_{2f} and TnT_{3f} are each related to TnT_{1f} by single alternative splicing events. We have found that regardless of the muscle from which they were dissected, fibers expressing primarily TnT_{2f} and α_2 Tm exhibit very steep pCa/tension relations, with a high value of the Hill coeficient, n_H. In contrast, fibers with higher proportions of either TnT_{1f} or TnT_{3f} and $\alpha\beta$ Tm have correspondingly lower n_H values. We believe that the expression of different TnT isoforms is a key element in the continuum of physiological properties exhibited in skeletal muscle fibers.

M 407 **The quail fast troponin T and α-tropomyosin genes generate developmentally regulated mRNAs by restricting multiple splice choices.** Elizabeth A. Bucher, Sonia Pearson-White, Fabienne Charles de la Brousse and Charles P. Emerson Jr., University of Virginia, Charlottesville, Va 22901.

In the troponin complex the N-terminal region of troponin T (TnT) binds troponin I and the C-terminal region binds tropomyosin and troponin C (1). These functional domains of fast skeletal TnT have variable amino acid sequences. This variability is generated from a single mRNA transcript by combinatorial splicing of multiple exons encoding amino acids internal to the N- terminus and alternative splicing of exons encoding amino acids internal to the C-terminus of the protein (2,3,4). Sequence and S1 protection analysis of the quail fast TnT demonstrate precise developmental control of alternatively spliced exons and of splice choices near the 5' end during quail muscle development. *Cis* -acting sequences in introns may locally direct regulated splicing of individual exons by interacting with cellular factors. Alternatively, global events such as regulation of troponin pre-mRNA structures may direct and restrict 5' and 3' splice events. The relationship between 5' and 3' splice events was tested by primer extension experiments. These studies demonstrate that splice choices near the 5' end are clearly related to 3' alternatives and, further, that not all possible combinations are detected. The 5' and 3' restrictions of splice choices show developmental stage and muscle-type specificity.

Similar to TnT, cDNA analysis of the quail α-tropomyosin (α-Tm) demonstrates that this gene also exhibits multiple regulated splice events, including an exon encoding a protein domain that interacts with TnT (1). Three regions of α-Tm are alternatively spliced, however, only 4 of 8 possible combinations are detected in a tightly controlled tissue-specific manner(5). Thus, both TnT and α-Tm exhibit tissue-specific restriction of multiple splice choices from a single mRNA transcript. α-Tm is differentially spliced in skeletal muscle, smooth muscle, and non-muscle tissues with only one major spliced form expressed in all skeletal muscle types. In contrast, TnT mRNA differential splicing occurs in distinct skeletal muscle types suggesting that restrictive splicing of these two genes is not directed by a common mechanism. (1) Perry, In: Myology (1986). (2) Breitbart et al., Cell 41:67-82 (1985). (3) Wilkinson et al., Eur. J. Biochem. 143: 47-56 (1984). (4) Hastings et al., J. Biol. Chem.260: 13699-13703 (1985). (5) Pearson-White and Emerson, J. Biol. Chem. in press (1987).

M 408 ALTERNATIVE PROMOTOR USAGE BY ALDOLASE A DURING DIFFERENTIATION IN C2C12 CELLS, Melissa C. Colbert and Elena Ciejek, University of Rochester, Rochester, NY 14642

Aldolase is a ubiquitous glycolytic enzyme whose isoforms, A, B and C, show both tissue specific distribution and developmental regulation. The A isozyme has the widest tissue distribution and is abundant in skeletal muscle. In the human and the rat there is compelling evidence that this gene is controlled by at least two separate promotors, resulting in mRNAs of two different sizes. Promotor usage appears to depend upon tissue type and developmental state. The longer form of mRNA is expressed in embryonic muscle and non-muscle tissues; the shorter form mRNA is expressed only in muscle. We have been studing the regulation of aldolase A promotor usage in cultures of mouse C2C12 cells. Analysis of mRNA isolated from sub-confluent cultures of C2C12 myoblasts by Northern blot shows that aldolase A is transcribed as the longer message, consistant with usage of the embryonic promotor. RNA isolated from C2C12 myotubes(7 days in differentiation medium) shows an induction of total aldolase message. Primer extension off mRNAs isolated from both myoblasts and myotubes indicates that muscle specific aldolase A mRNA is absent from myoblasts and that myotubes contain both embryonic and muscle specific messages. Thus it appears that during myogenesis *in vitro* an alternative promotor is activated as is seen *in vivo*. Furthermore, the embryonic, non-muscle promotor, normally turned off *in vivo* remains active in myotubes. We have studied the appearance of these specific mRNAs in cells isolated at 24 hour intervals over 7 days of differentiation and compared the activation of muscle specific aldolase A with that of skeletal actin. Our results show that muscle specific aldolase A mRNA levels increase within 24 h of the appearance of skeletal actin.

M 409 **CIS REQUIREMENTS FOR pre-mRNA ALTERNATIVE SPLICING**
Thomas A. Cooper, Michael H. Cardone, and Charles P. Ordahl, UCSF, SF,CA 94143.

Alternative splicing is characteristic of many muscle-specific pre-mRNAs. How a specific exon is "marked" for alternative splicing is unknown, however, at least a portion of the information for this process must be contained in cis within the pre-mRNA. The cardiac troponin T (cTNT) gene transcribes a single pre-mRNA that contains one alternative exon (exon 5). Alternative splicing generates two cTNT mRNAs which differ only in the inclusion or exclusion of exon 5. To define cis-acting elements required for the alternative splicing of cTNT exon 5, we have established a transfection system for the cTNT pre-mRNA. A modified cTNT gene has been constructed and transfected into primary cultures of chick embryo fibroblasts, chick skin fibroblasts, as well as chick embryonic breast muscle. Transcription is driven by a Rous sarcoma virus promoter/enhancer cassette. Surprisingly, in fibroblast as well as in muscle cultures, both cTNT alternative splice products are expressed at equivalent levels. Exon 5 is recognized as an alternative exon even in non-muscle cells which normally never process the cTNT pre-mRNA. These results establish that the cis elements which distinguish alternative and constitutive exons within the muscle-specific cTNT pre-mRNA do not require muscle-specific factors in trans to function. To define the cis-acting elements involved, systematic deletion analysis of the transfected gene encoding the cTNT pre-mRNA has been performed. These analyses have determined that the alternative splice is independent of cTNT sequences upstream of exon 4 and downstream of exon 6 as these regions can be replaced by comparable regions from other constitutively spliced genes. In addition, three mutations between exons 4 and 6 have disrupted the exclusion of exon 5 leading to its constitutive inclusion. These mutations appear to have disrupted an "alternator" function within the cTNT pre-mRNA. The possible mechanisms by which these regions govern alternative splicing will be discussed. Supported by grants from NSF and NIH (CPO) and the Bank of America Giannini Foundation (TAC).

M 410 STUDIES ON THE STRUCTURE, EXPRESSION AND EVOLUTION OF MUSCLE-SPECIFIC GLYCOGEN PHOSPHORYLASE GENES, Michael M. Crerar, Emina S. David, John W. Hudson, Kathleen Hefferon, Kathryn Matthews, G. Brian Golding, Department of Biology, York University, North York, Ont. Canada, M3J 1P3 and Tom Glaser, David E. Housman, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, 02139. Mammalian glycogen phosphorylases comprise a gene family whose members exhibit differential expression across a wide variety of tissues. At least three isozymes exist; muscle, liver and brain [foetal], named after the tissues where they predominate. As a start in examining the regulation and evolution of this family, we have isolated antibodies and cDNAs for all three isozymes from the rat. Using these as probes, we have observed that the muscle isoform, the sole isozyme produced in adult skeletal muscle, varies dramatically in amount in this tissue depending on the developmental stage, fibre type and with chronic nerve stimulation. Cardiac muscle expresses both muscle and brain isozymes which also exist in varying amounts in a wide variety of other tissues. Tissue-specific variation in expression appears governed by mRNA accumulation. Mapping studies in the mouse show that muscle, brain and liver phosphorylase genes are situated on separate chromosomes indicating that separate cis-acting elements must govern their selective expression. A reconstruction of the phylogenetic history of the family suggests that muscle and brain isozymes are more closely related than the liver isozyme and that duplications that gave rise to this gene family predate the mammalian radiation. Supported by the following grants: NSERC and Muscular Dystrophy Association of Canada to M.M.C., NSERC to G.B.G and NIH to D.E.H.

M 411 IDENTIFICATION OF CIS-ACTING ELEMENTS MEDIATING MUSCLE- AND STAGE-SPECIFIC EXPRESSION OF THE CARDIAC β/SLOW SKELETAL MYOSIN HEAVY CHAIN GENE, Leanne L. Cribbs, Noriko Shimizu, Courtland E. Yockey, Smilja Jakovcic and Patrick K. Umeda, University of Chicago, Chicago, IL 60637.
The cardiac β-myosin heavy chain (HC) is expressed in developing skeletal muscles and is the major isoform found in mammalian slow-twitch fibers. To understand the mechanisms controlling myosin HC expression during muscle differentiation and those involved in specifying the adult patterns of fiber-specific expression, we have begun to define the cis-regulatory regions of the rabbit cardiac β gene by DNA transfection experiments. A chimeric gene was constructed by fusing 684 bps of 5' coding and flanking sequences to the gene for chloramphenicol acetyltransferase (CAT). In primary chick skeletal muscle cultures, the transfected gene is expressed only following terminal differentiation and is suppressed when myogenesis is inhibited by BUdR. RNase protection experiments show that transcription of the exogenous β promoter parallels the induction of the endogenous skeletal α-actin gene. Analyses of 5' deletion mutants indicate that muscle-specific activation is mediated by cis-acting regulatory elements within 300 bps of the cap site. Within this region, linker-scanner mutants identify at least two regions necessary for transcription. The first lies between -275 and -261, and the second is near the CAAT box of the gene. The results indicate that at least two cis-acting elements are necessary for both muscle- and stage-specific expression. Currently, we are analyzing the specific role of each element and their interactions with trans-acting factors.

M 412 MOLECULAR CLONING OF THE CHICKEN SLOW SKELETAL MUSCLE MYOSIN LIGHT CHAIN (LC_{1s}) AND THE CHARACTERIZATION OF ITS EXPRESSION DURING DEVELOPMENT, Nicholas Papadopoulos, James Myres, and Michael T. Crow, The University of Texas Medical School, P.O. Box 20708, Houston, Texas 77225.

Using a monoclonal antibody specific for the alkali myosin light chain of avian slow skeletal muscles, we have screened lambda cDNA expression libraries constructed from embryonic muscle tissue to isolate several full-length cDNA clones for the light chain. Northern blot analyses of these clones showed that they react specifically with mRNA(s) from adult slow skeletal muscle, embryonic and adult cardiac(ventricular) muscle, as well as embryonic skeletal muscles. The amino acid sequence of the mRNA as deduced from the nucleic acid sequence of the cDNAs displayed a strong homology with the sequences of various other alkali light chains. S1 nuclease analyses with probes to both the 3' and 5' regions of the cDNAs failed to detect any differences in the length of the fragments protected by mRNA from slow skeletal or cardiac muscle and Southern blot analyses using these same 3' and 5' specific probes showed only a single band of hybridization. Taken together, these data suggest that the slow skeletal and cardiac alkali myosin light chains are encoded by the same gene. Unexpectedly, major differences between the deduced amino acid sequence of the cDNAs and the actual amino acid sequence data published for the adult ventricular alkali light chain were noted. The accumulation of mRNA for the light chain increased during the development of both skeletal and cardiac muscles, and, in the case of skeletal muscle, was blocked by the addition to the embryo of reagents that blocked functional neuromuscular contacts. Funded by grant HD # 20710 to M.T.C.

M 413 REGULATION OF MYOGLOBIN GENE EXPRESSION, B.H. DEVLIN, M.B. BOLSTER, W.E. Kraus, F.C. Wefald, and R.S. Williams, Duke University, Durham, NC 27710.

Myoglobin is a muscle specific, heme containing protein that facilitates the diffusion of oxygen into muscle. The level of myoglobin is modulated by oxygen tension, heme availability, and contractile activity. Using mouse C2 myogenic cells, we have demonstrated that Mb mRNA is first detected during differentiation. Furthermore, we have shown that steady state levels of Mb mRNA in the cells is increased 2-4 fold in response to hypoxic conditions. Increasing heme availability reduces the level of Mb mRNA even though the level of Mb protein increases with heme availability in L6 cells (1). Our results indicate the major regulatory mechanisms of Mb synthesis are pretranslational. In addition, we have used CAT constructs to demonstrate the cis-acting regulatory regions of the human myoglobin gene responsive to differentiation signals are contained in a 2kb 5' flanking fragment. Trans-acting factors have been identified in regulatory regions by gel retardation experiments and sites of interaction of binding proteins have been identified by methylation protection and interference experiments.

(1) Graber, S.G. and R.C. Woodruff (1986) JBC 261: 9150-9154.

M 414 MYOSIN HEAVY CHAIN mRNA DISTRIBUTION IN SLOW AND FAST FIBER TYPES OF RABBIT SKELETAL MUSCLE DETECTED BY IN SITU HYBRIDIZATION. D.J. Dix and B.R. Eisenberg, Dept. of Physiology, Rush Medical College, Chicago, IL. Information concerning amount and distribution of myosin heavy chain (MHC) mRNA is obtained in frozen sections by hybridization with a probe transcribed from a 1.1 kb cDNA from rabbit cardiac ã myosin gene. Filter-bound RNA extracts from various rabbit muscles revealed this probe to hybridize well to slow ã and ß myosin (cardiac and soleus), and a fast 2A and 2B mixture (tibialis anterior), but poorly to fast 2B (psoas). The RNA probe is labelled by the incorporation of biotin-11-UTP into the transcript and is detected with streptavidin-alkaline phosphatase. Both a complementary probe (cRNA) which hybridizes at high stringency and an mRNA probe which does not hybridize are transcribed from the same template. The MHC cRNA probe yields a mosaic staining pattern on mixed type muscles but a uniform staining pattern on homogenous muscles such as soleus. Serial sections used for fiber typing by immuno- and histochemistry show a correlation of MHC cRNA stain with fiber type: 2B fibers are pale, 2A fibers are intermediate or dark, and slow fibers are dark. MHC cRNA hybrids are localized around the periphery of all stained fibers. A poly(U) probe binds all fibers of mixed muscle and results in a less distinct mosaic. Therefore, the mosaic hybridization pattern appears to be related to both probe isoform specificity and total MHC mRNA content of a fiber type. Supported by AHA and HL 35728.

M 415 ISOLATION AND CHARACTERIZATION OF A CHICKEN "PERINATAL" -SPECIFIC MYOSIN HEAVY CHAIN cDNA, Steven Einheber, G. Raman, Richard Nickowitz and Donald A. Fischman, Cornell University Medical College, New York, NY 10021.

Myosin heavy chain (MHC), a major component of the muscle contractile apparatus, is known to undergo isoform switching during development. Using a rat skeletal muscle MHC probe, (pMHC 25),(Medford et al.,1980,PNAS 77:5749) we have isolated a 2.2 kb cDNA clone (λHC-1) from a one week post-hatch (PH) chicken pectoral λgt10 cDNA library (Reinach and Fischman, 1985, J.Mol.Biol.181:411). Northern blot analysis of total RNA from different developmental stages (embryo to adult) revealed that λHC-1 hybridized preferentially to MHC message from one week PH pectoral RNA. Partial nucleotide sequence analysis of the 3' end, including the region encoding the carboxy terminus and the downstream untranslated region, revealed that this clone differed from published embryonic MHC isoform sequences in the untranslated region. In RNAase protection assays a 300 nucleotide 3' end fragment was fully protected by one week PH pectoral RNA whereas 13-d embryonic and adult pectoral RNA's afforded only partial protection. Prolonged exposures revealed very low level expression at the 13-d embryonic stage. We conclude that λHC-1 encodes an MHC isoform that is preferentially expressed in the pectoralis major muscle of chickens in the "perinatal" period. Supported by NIH grant AM32147 and by a Norman and Rosita Winston Foundation fellowship to GR.

M 416 TRANSCRIPTIONAL REGULATION OF A MUSCLE ACETYLCHOLINE RECEPTOR SUBUNIT GENE,Sylvia M. Evans, Paul D. Gardner, Stephen Fox Heinemann, and James W. Patrick, The Salk Institute, San Diego, CA 92138.

The muscle nicotinic acetylcholine receptor (AChR) is a major component of the neuromuscular junction. The major embryonic AChR has the subunit composition $\alpha_2\beta\gamma\delta$. There is some evidence that the AchR at the mature neuromuscular junction substitutes the ϵ-subunit for the γ-subunit. In a fusing mouse muscle cell line, C2Cl2, the amount of each embryonic subunit mRNA increases four-to five-fold upon myoblast fusion into myotubes. This increase could be a result of transcriptional activation by transacting factors. To investigate the existence of positive and/or negative transacting factors, we have focussed on the regulatory region of the γ-subunit gene. The γ-subunit is of interest because we suspect it is turned on with development and turned off by innervation. In addition, the amounts of γ-subunit mRNA in embryonic muscle are high relative to the other subunit mRNAs, and appear to be tightly controlled. We have spliced the γ-subunit gene control region (γ^P) in front of a selectable marker, the neomycin resistance gene (neo) on a plasmid bearing a marker for hygromycin resistance (hyg). This plasmid, pHygγ^PNeo, and a control plasmid, pHyg Neo, were independently transfected into mouse muscle C2Cl2 cells and mouse fibroblast 3T3 cells. Stable hygromycin resistant cell lines selected after transfection with pHygγ^PNeo were scored for neomycin resistance: 13/20 C2Cl2 cell lines were neomycin resistant, before and after myoblast fusion; 2/17 3T3 cell lines were neomycin resistant. Neomycin resistance in log phase C2Cl2 transfectants could be due to a high copy number of the γ^PNeo gene. Transfectants with the control plasmid were neomycin resistant in 2/10 cases for both C2Cl2 and 3T3 cell lines. The existence of positive transacting factors in C2Cl2 cells can now be tested by polyethylene glycol mediated fusion of C2Cl2 cells to a 3T3/pHygγ^PNeo cell line. This 3T3 -derived stable transfectant is hygromycin resistant, neomycin sensitive, yet carries the γ^PNeo hybrid gene intact, as determined by genomic Southern blot analysis. Activation of the neomycin gene in the 3T3 cell line by fusion to C2Cl2 cells would indicate the presence of trans-activators of the γ-subunit gene in C2Cl2 cells. The ability to monitor the transactivation of the γ-subunit gene promoter element in the 3T3 transfectant by selection for neomycin resistance may permit the selection of cDNA clones encoding the transactivating factors.

M 417 REGULATION OF VIMENTIN GENE EXPRESSION DURING MYOGENESIS. Francis X. Farrell Christina M. Sax, Richard J. Garzon, Zendra E. Zehner, Virginia Commonwealth University, Richmond, VA, 23298.

During myogenesis, the intermediate filament proteins (IFP) vimentin and desmin are differentially expressed. The muscle specific IFP desmin is dramatically increased, while vimentin protein levels decrease. Northern analysis has shown that this expression pattern correlates with mRNA levels during musle development. We have analyzed the elements that control vimentin gene expression during myogenesis by the use of complete genes (which differ in the length of their flanking region), a truncated gene (mini-gene), and 5'-flanking sequences fused to CAT. These constructions have been tested by their transfection into the muscle cell line L6E9. Previously, we have shown that these constructions are appropriately expressed in L-cells (fibroblasts), but not in a rat hepatoma cell line (MH1C1) where vimentin is not expressed. We find that the truncated gene which contains 0.8 kb and 0.7 kb of 5'- and 3'-flanking sequences, respectively, and 20% of internal sequences (80% deleted) is down-regulated to a similar extent as the complete gene. Moreover, the region -292 to -132 fused to CAT confers transcriptional down-regulation during L6E9 differentiation. Vimentin mRNA levels decrease an average of 83% as the cells proceed from myoblasts to the myotube stage. This is in contrast to the chicken tk and beta-actin genes where internal and 3'-sequences are required for decreased expression during myogenesis. We conclude that the decrease in vimentin mRNA levels during myogenesis is due in part to a reduction in transcriptional initiation which is controlled by sequences located in the 5'-end of the chicken vimentin gene.

M 418 TROPOMYOSIN GENE EXPRESSION IN CHICKEN EMBRYOS AND DURING MYOGENIC IN VITRO, M.Y. Fiszman, M. Lemonnier, D. Libri, T. Meinnel & R. Savino, Department of Molecular Biology, Pasteur Institute, Paris (France).

We have studied the expression of α and β skeletal tropomyosin genes in various chicken embryonic tissues and during differentiation of skeletal muscle cells in culture. In the embryo, these genes are transcribed in skeletal and smooth muscles but not in non muscle tissues. However, in culture, they are transcribed in all cell types. In non muscle cells and in undifferentiated muscle cells their transcription products correspond to the mRNA detected in smooth muscles (2.0 Kb for α and 1.3 Kb for β) while in differentiated myotubes the transcripts are those detected in skeletal muscle (1.4 for α and 1.6 for β). We have also isolated a cDNA which correspond to the mRNA coding for the α-tropomyosin of slow skeletal muscle. This mTNA (1.4 Kb) is expressed in Anterior Latissimus Dorsi (ALD) and in cultures of RVS transformed quail myoblasts but not in primary cultures of either quail or chick muscle tropomyosin. Evidence will be presented that these two mRNA originated from the same transcript by an alternative splicing mechanism.

M 419 GENE EXPRESSION OF HUMAN FAST SKELETAL TROPONIN C Reinhold Gahlmann and Larry Kedes, MEDIGEN Project, Department of Medicine, Stanford Medical School and Veterans Administration Medical Center, Palo Alto, CA 94305.

We have cloned the human genes encoding slow and fast skeletal troponin C. Fast skeletal troponin C is expressed exclusively in skeletal muscle while the slow skeletal troponin C gene is expressed both in skeletal and cardiac muscle. A construct containing 6 kbp 5'-flanking sequences of the fast skeletal troponin C gene and a reporter gene (chloramphenicol acetyltransferase) was transfected into cells of two myogenic cell lines, C2C12 and H9c(2-1). The cell lines C2C12 and H9c(2-1) were originally isolated from mouse adult skeletal muscle and rat embryo heart, respectively. Transient expression of the troponin C-CAT-chimera and of a human α-cardiac actin promoter-CAT chimera was compared. While both hybrid-genes are transcribed in C2C12 myoblasts and myotubes at a similar level, only the human α-cardiac actin promoter but not the fast skeletal troponin C promoter is active in H9c(2-1) cells. This result suggests that different regulatory mechanisms control the expression of cardiac actin and fast troponin C in striated muscle cells. The mapping of the regulatory sequences important for muscle type specific activity of the fast skeletal troponin C promoter will be presented.

M 420 GENERATION OF MULTIPLE ISOFORMS FROM THE SINGLE MUSCLE MYOSIN HEAVY CHAIN GENE IN *DROSOPHILA*. Elizabeth L. George, Margaret B. Ober and Charles P. Emerson, Jr., University of Virginia, Charlottesville, VA 22901.

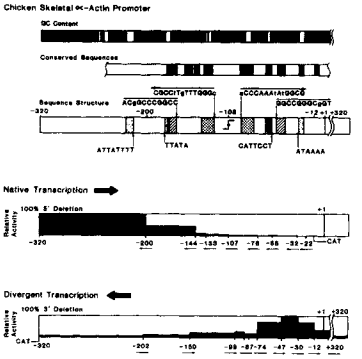
DNA sequence analysis of the single muscle myosin heavy chain (MHC) gene(1) of *Drosophila* reveals at least three sets of alternatively spliced exons in the N-terminal globular head. This is the first demonstration that alternative splicing generates diversity in the multiple functional domains of the MHC head, and thus suggests novel amino acid sequence requirements for specific muscles during development. cDNA sequence analysis and RNA hybridization studies with exon-specific probes establish that the related exons are spliced into mRNA by an alternative and mutually exclusive, rather than combinatorial, mechanism. Of the three sets of alternatively spliced exons, exon 7 is unique because there are four versions in the MHC gene, thus providing potential to generate at least 16 distinct protein isoforms within the MHC globular head. Patterns of stage and tissue specificity, as well as restriction of the possible isoforms generated, suggest that alternative RNA splicing plays an important role in the generation of MHC isoforms in the diverse muscle types of the fly.

To study the transcriptional control of the single muscle MHC gene, we have constructed a MHC promoter/alcohol dehydrogenase fusion gene for P-mediated germline transformation. In transformed flies this MHC fusion gene directs expression of the *Drosophila* alcohol dehydrogenase (adh) gene in the muscles of young larvae, late pupae and adults. This temporal expression of the fusion gene parallels endogenous MHC gene expression(2,3,4), and adh activity is restricted to muscle tissue. Thus, the cis-acting transcriptional control signals for stage and muscle specificity of the MHC gene are located very near the 5' end of the gene and may also be within the first intron. Further analysis of this fusion gene is in progress to determine if multiple regulatory elements direct the expression of the single MHC gene in the functionally diverse larval and adult muscle types. (1. Bernstein et al. Nature **302**:393,1983 2. Rozek and Davidson Cell **32**:23, 1983 3. Bernstein et al. Mol. Cell. Biol. **6**:2511, 1986 4. Wassenberg et al. J. Biol. Chem. **262**:10741, 1987)

M 421 MOLECULAR BASIS OF MYOSIN HEAVY CHAIN TRANSITIONS IN SKELETAL MUSCLE HYPERTROPHY. Patricia Gregory, Jacques Gagnon, David Essig, Suzanne Kamel, Gwen Prior, Patrick Umeda and Radovan Zak, University of Chicago, Chicago, IL 60637. To understand the molecular mechanisms which regulate the transition in myosin heavy chain (MHC) isozymes during skeletal muscle hypertrophy, we measured fractional synthesis rates (k_s) of MHC isozymes, actin, and total protein in the chicken ALD muscle after attachment of a lead weight to the wing. In addition, we studied the expression of SM1 and SM2 MHC mRNAs using a specific cDNA probe, and translated polyribosomes *in vitro* from normal and overloaded muscles. Although the k_s of total protein was almost doubled at 24 hours, both MHC isozymes had a depressed k_s. The k_s of actin was doubled at 24 hours, implying a discoordinate response of the contractile proteins to the overload stimulus. By 72 hours the SM1 protein was decreased by half; the k_s of both MHC isozymes were doubled, indicating a delayed stimulation of myosin synthesis. Although the k_s of SM2 was doubled by 48 hours, the SM1 k_s was increased only in those muscles which had lost a substantial portion of SM1 protein, suggesting that rapid turnover of individual proteins results in a faster transition in muscle phenotype. Results of nuclease protection analyses are consistent with differential gene expression, as indicated by a concurrent decline in the relative amount of SM1 mRNA. Furthermore, the results of *in vitro* translation of polyribosomes parallel the results obtained *in vivo*.

M 422 CHARACTERIZATION OF THE CHICKEN SKELETAL ALPHA-ACTIN PROMOTER REGION, James M. Grichnik and Robert J. Schwartz, Baylor College of Medicine, Houston, TX 77030

Serial deletions of the chicken skeletal actin gene has revealed a promoter region (-202 to -12) provides tissue restricted transcriptional activity. Sequences within this region exhibit partial dyad symmetry about an axis at nucleotide -108. Divergent transcriptional activity allowed us to define the 3' regulatory boundary without introducing surrogate promoter sequences. Serial 3' deletions which bisected the element (-91 5' CCAAATATGG 3' -82) markedly reduced divergent activity. Disruption of the upstream counterpart (-127 3' CCAAAgAAGG 5' -136) resulted in a marked decrease in native orientation activity. These element pairs, which we describe as CCAAT Box Associated Repeats (CBARs), are conserved in all vertebrate sarcomeric α-actin genes and may act in a cooperative manner to facilitate transcription. This analysis also revealed that divergent transcriptional activity was dependent upon the deletion of sequences 3' to -47. We suggest that sequences proximal to the skeletal alpha-actin gene's transcription start site serve to position/orient RNA polymerase.



M 423 Interaction of Protein Factors with the Human Cardiac α-Actin Promoter. Thomas A.Gustafson and Laurence H. Kedes. Stanford University Department of Medicine and Veterans Administration Medical Center, Palo Alto, CA 94304.

The human cardiac α-actin promoter is involved in the developmental regulation of the gene in muscle cells. In this study we have utilized gel mobility-shift, methylation interference, and DNAseI protection assays to examine protein factor interaction with the promoter. All assays demonstrated specific interaction of nuclear factors with a region of the promoter encompassed by nucleotides -93 to -113 bp from the transcriptional start site. This region contains a $CC(A/T)_6GG$ element, termed a CArG box, which has previously been implicated in the muscle-specific transcriptional regulation of the gene using functional assays. Surprisingly, the CArG binding factor was found to be present in nuclear extracts of all cell types examined, including those of muscle (C2, L8 and L6) and nonmuscle origin (Hela, Ltk- and HuT12). Competition studies showed that the CArG binding factor also interacts with the human skeletal α-actin promoter and the c-fos serum responsive element but not with a histone CAAT box or with the SV40 enhancer. Thus, a region of the human cardiac α-actin promoter known to be functionally involved in muscle-specific and developmental regulation of the gene appears to interact with factor(s) which are neither muscle- nor gene-specific, suggesting a more complex mode of regulation than previously envisioned.

M 424 SERUM-FREE MEDIA FOR GROWTH AND DIFFERENTIATION OF HUMAN MUSCLE SATELLITE CELLS, Richard G. Ham and Judy A. St. Clair, University of Colorado, Boulder, CO 80309, and Helen M. Blau, Stanford Univ., Stanford, CA 94305.

We have studied extracellular requirements for growth and differentiation of normal human skeletal muscle satellite cells (HMSC) in detail. An optimized nutrient medium, MCDB 120, has been developed that is superior for growth of HMSC both with serum and with serum-replacing supplements. A serum replacement (SR-1), consisting of insulin, dexamethasone, EGF, fetuin, and serum albumin, supports clonal growth in MCDB 120 equivalent to that obtained with optimal amounts of serum and embryo extract. FGF (or pituitary or embryo extract) is required for growth with serum, but has only a small effect in MCDB 120 + SR-1. A doubly supplemented medium containing both SR-1 and 5% dialyzed fetal bovine serum in MCDB 120 supports far better clonal growth than either SR-1 or serum + embryo extract. Differentiation is inhibited in MCDB 120 + SR-1, with or without added serum. However, such cultures exhibit good differentiation, determined both visually (formation of multinucleate myotubes) and biochemically (increased creatine kinase specific activity), within 3-6 days after the medium is changed to Dulbecco's modified Eagle's medium plus 10 μg/ml insulin (DMEI). Differentiation in MCDB 120 + insulin is only slightly less than in DMEI. We are exploring the inhibition of differentiation by SR-1, which appears to involve more than one component. FGF plays a central role in control of growth and differentiation of widely studied mouse model systems. Our data show that FGF is far less important for growth of HMSC, and suggest that it is also less important in the control of their differentiation.
Supported by a grant from the Muscular Dystrophy Association.

M 425 EXPRESSION OF A QUAIL TROPONIN I GENE IN TRANSGENIC MICE, P.L.Hallauer, K.E.M.Hastings*, and A.C.Peterson, Ludwig Institute for Cancer Research, Montreal, and *Montreal Neurological Institute, McGill University.
A cloned quail genomic DNA fragment containing the fast skeletal muscle troponin I gene as well as 530 bp of 5'-flanking and 1500 bp of 3'-flanking DNA was microinjected into mouse zygote pronuclei. Eight transgenic mouse lines were produced in which the quail gene was incorporated at unique loci in 1-30 copies. Quail troponin I mRNA is present in skeletal muscle in all 8 transgenic lines and is manyfold more abundant in the gastrocnemius, a fast muscle, than in the soleus, a slow muscle. In a line carrying a single copy of the transgene expression in fast skeletal muscle is the only significant expression in the animal (including heart).(Multicopy lines express an aberrant transcript in several additional tissues). We conclude that the tissue-specific expression of the troponin I gene in terms of striated muscle vs non-striated-muscle tissues, cardiac vs skeletal muscle, and fast vs slow skeletal muscle, is based on mechanisms involving cis-acting DNA elements. Moreover these elements are located within the gene itself, or within 530 bp upstream, or 1500 bp downstream of the gene. In addition these elements, and the regulatory mechanisms in which they function, were apparently fully established in the common ancestor of birds and mammals and have been functionally conserved in both lineages.

M 426 CHARACTERIZATION OF A cDNA CLONE REPRESENTING SKELETAL MUSCLE MYOSIN LIGHT CHAIN KINASE, B.P. Herring, J.T. Stull and M.H. Nunnally, Dept. of Physiol., Univ. of Texas Southwestern Med. Ctr. at Dallas, Dallas, TX 75235.
The 18-20 kDa light chain subunits of mammalian myosin can be phosphorylated by the Ca^{2+}/CaM-dependent enzyme myosin light chain kinase (MLCK). Various isoforms of this enzyme have been proposed to exist in different animal species and also in different tissues within a given species. The structural relationship between the different isoforms has, however, not been clearly defined. In order to investigate this problem, a 1.8 kB cDNA clone has been isolated from a rat skeletal muscle cDNA library. This clone corresponds to the C-terminal half of MLCK. Northern analysis of skeletal muscle RNA revealed that this clone hybridized to a 3.3 kB RNA species. The hybridization was specific for skeletal muscle RNA, as no hybridization was detected in equivalent quantities (20 μg) of RNA isolated from rat uterus, liver or heart. Although 0.5 μg of RNA isolated from fast skeletal muscle was sufficient to detect hybridization, no hybridization could be detected in 50 μg of RNA isolated from heart. Fast skeletal muscle produced a 2-3 fold greater hybridization signal than slow skeletal muscle. This ratio is in agreement with the reported enzyme activities and amounts of MLCK determined by Western analysis. These data suggest that a single form of myosin light chain kinase is present in both fast and slow twitch skeletal muscle and that this may not only be distinct from the smooth and non-muscle isoforms, but also from the cardiac enzyme. Supported in part by HL06296.

M 427 IN VIVO ANALYSIS OF MYOSIN HEAVY CHAIN GENE TRANSCRIPTIONAL REGULATION IN *D. MELANOGASTER*. Norbert Hess, David Becker & Sanford I. Bernstein. Biology Department & Molecular Biology Institute, San Diego State University, San Diego, CA 92182.
We are using the P element transformation system to study the temporal and tissue specific expression of the muscle myosin heavy chain (MHC) gene in *Drosophila melanogaster*. This gene is single copy in the haploid genome and produces protein isoform diversity via alternative splicing of a single primary transcript. We are mapping the promotor region of the MHC gene in order to use it as a tissue specific promotor for various MHC gene constructs. We have made several MHC-lac Z gene fusions and these are being inserted into the *Drosophila* genome to define a MHC 5' region fragment that retains promotor activity and tissue specific expression. In order to utilize the entire MHC gene for mutant rescue experiments, we have performed extensive restriction enzyme analysis of several overlapping MHC-λ clones. Using this information we have assembled the 22kb MHC gene with its 3' and 5' flanking regions in a plasmid vector which will be utilized for germline transformation. These experiments are designed to demonstrate MHC gene expression at positions other than its normal chromosomal location. We are also using the P element system to analyze alternative splicing of the MHC RNA 3' end. Our analysis focuses on determining which sequences are necessary for correct splicing in specific tissues and developmental stages. We have modified the unusual splice acceptor site of the alternatively spliced 3' penultimate exon. P element vectors containing the wild type and modified 3' ends of the gene driven by a heat shock promotor have been transformed into wild type *D. melanogaster* for analysis in vivo. These constructs are expressed and spliced at different developmental stages. We are currently mapping the splice junctions of these transcripts to define whether the mutations affect alternative splicing. [Supported by NIH grant GM32443]

M 428 REGULATION OF THE RAT MUSCLE CREATINE KINASE PROMOTER DURING MYOGENESIS. R. A. Horlick and P. A. Benfield, E. I. du Pont de Nemours & Company, Central Research and Development, Wilmington, Delaware 19898.
The ability of the rat muscle creatine kinase (CKM) promoter to drive the CAT gene in transient transfections of mouse C2 cells has been studied. A minimum of -1.1 kb of DNA 5' to the (CKM) transcription start site is required in order to see up-regulation of expression of the reporter CAT gene in differentiating C2 cells. Nuclear extracts from C2 myotubes, but not HeLa not L- cells are able to shift the mobility on acrylamide gels of a small 150 bp fragment derived from this 1.1 kb region.
In limited DNase digestion assays, C2 myotube nuclear extracts protected a small dCdA-rich region of 23 nucleotides. Deletion analysis shows an area immediately surrounding and including the footprinted region to be required for up-regulation of the CAT gene in differentiating C2 cells. Data suggest this regulatory sequence is not an enhancer but rather functions in an orientation and distance-dependent manner.
A 400 bp promoter-derived fragment (which includes the footprinted region) placed upstream to a brain creatine kinase promoter-CAT construct is not sufficient to confer up-regulation during differentiation of C2 cells.

M 429 ACETYLCHOLINE RECEPTOR REGULATION IN RAT PRIMARY MUSCLE IN RESPONSE TO RAT BRAIN EXTRACT AND ASCORBATE: SPECIFIC REGULATION OR A DIFFERANTIATION RELATED EFFECT? Ora Horovitz and Miriam Salpeter, Cornell University, Ithaca, NY 14853.

Differentiation of muscle cells from mononucleated myoblasts to multinucleated myotubes is accompanied by the expression of muscle specific gene products. Among these are the α-actin, myosin light chain$_2$ (MLC_2), muscle specific creatine phosphokinase (M-CPK), and the acetylcholine receptor (AChR). It is still not clear what factors are involved in the induction and the regulation of these muscle specific genes and whether they are regulated individually or as a group. In this study we show that rat embryonic brain extract (RBE) and one of its active components, ascorbate, are involved in the regulation of a number of such muscle specific genes. In rat muscle cells treated with either of these factors, a 2-3 fold increase in m-RNA levels of the AChR α-subunit, the α-actin the MLC_2 and the CPK was observed. An increase in α-actin m-RNA levels was also detected in treated chick primary cultures. Since innervation is essential for growth and maintainance of muscles, the effect of RBE and ascorbate suggests that the regulation of these genes by innervation may work via chemical signals rather than by electrical stimulation. We also confirm previous studies which have shown that in L_5 cells the brain factors stimulate only the AChR m-RNA but not that of the other proteins. This difference may lead to studies for understanding the differential regulation of muscle specific genes. **Supported by N.I.H. GM 10422.**

M 430 EXPRESSION OF SKELETAL MUSCLE mRNAs ANALYZED BY IN SITU HYBRIDIZATION, J. B. Lawrence and R. H. Singer, Department of Anatomy, University of Massachusetts Medical School, Worcester, Massachusetts 01605
We have used in situ hybridization to analyze the expression of muscle-specific mRNAs directly within individual cells during skeletal muscle differentiation in vitro. Cells isolated from the pectoral muscle of 11-12 days chicken embryos were fixed at various times after plating and hybridized with H^3, S^{35} or biotin-labelled probes. The probes detected mRNAs for myosin heavy chain (MHC), the skeletal isoform of alpha actin (SKact), and the cardiac isoform of actin (Cact). It has been shown (Paterson and Eldridge, Science, 224:1436; Hayward and Schwartz, J. Cell Biol. 102:1485) that Cact is the predominant isoform in early embryonic chicken skeletal muscle, wheaeas SKact is the major isoform in adult muscle. Our analysis of these mRNAs at the single cell level shows that Cact mRNA is expressed prior to myofibre formation, with greater than 20% of mononucleated cells positive for this mRNA just prior to the period of active fusion. Furthermore, Cact mRNA is detected prior to detection of MHC mRNAs. MHC mRNA was detected in a maximum of 5% of mononucleated cells but was expressed in myofibres beginning on day 2 of culture. Many single cells expressing cardiac actin mRNA are morphologically distinct from the highly elongated, bipolar single cells containing myosin heavy chain message. Immunofluorescence performed on the same cultures shows that cardiac actin mRNA is detected in single cells in which the corresponding protein is not detected. Analysis of these mRNAs by in situ hybridization has allowed us to define more precisely the sequence of events involved in muscle differentiation.

M 431 MOLECULAR CLONING AND CHARACTERIZATION OF A Ca^{2+} ATPase FROM THE RAT HEART SARCOPLASMIC RETICULUM, Diane de la Bastie, Ketty Schwartz, Anne-Marie Lompre, INSERM Unite 127, 75010 Paris, France.

cDNA clones covering the entire length of the Ca^{2+}ATPase mRNA were isolated from an adult rat heart cDNA library using a cDNA probe from the 3' end of the rabbit slow/cardiac ATPase mRNA (a gift from Dr. D. Mac Lennan). Some of these clones were characterized by sequence analysis which revealed strong homologies with the rabbit slow/cardiac ATPase. Few divergences were however observed in the phosphorylation and nucleotide binding sites. The tissue specificity of expression of these clones was studied by Northern blot and S1 mapping analysis. Two messages of different size were detected by blot analysis of total RNA from various tissues : one of about 4Kb is expressed in heart (atria and ventricles) and in slow skeletal muscle (soleus), another of about 3.7Kb is detected in fast skeletal (TFL), and both isoforms are present in a mixed muscle (diaphragm). It should be noted that the fast mRNA is barely detected by our cDNA clones, which indicates that the fast and slow isoATPases are very divergent over all their sequence. We also observed that a 4Kb mRNA is present in smooth muscle, but this mRNA, by S1 mapping analysis, is different from the one present in heart.

M 432 TRANSCRIPTIONAL REPRESSION OF AN EMBRYO SPECIFIC ISOGENE.
Carlin S. Long and Charles P. Ordahl, Univ. of Calif. San Francisco. San Francisco, Ca 94143.
Gene switching during myogenesis involves both activation and repression of specific contractile protein genes. In those cases studied, activation of contractile protein genes is governed at the level of transcription. In order to study the mechanisms of repression, we have analyzed the gene encoding the thin filament protein cardiac Troponin-T (cTNT) which disappears during embryonic chick skeletal muscle development (1). Previous work has shown that the disappearance of cTNT protein is attributable to an abrupt, 20-fold decrease in the steady state level of cTNT mRNA between day 14 and day 18 in ovo (2). We have used transcriptional run-on assays to measure the rate of transcription of the cTNT gene in nuclei isolated from embryonic skeletal muscle and heart muscle at days 12, 14, 16 and 18 in ovo. In embryonic heart development, cTNT gene transcription increases 13-fold between embryonic days 12 and 18 (18.8 ppm day 12 vs 251 ppm day 18), in good agreement with the observed 20-fold increase in cTNT mRNA (2). In embryonic skeletal muscle, however, over this time course, the rate of transcription of the cTNT gene decreases at least 12-fold (46.1 ± 2.4 ppm hybridized at day 12 vs 3.8 ± 0.5 ppm at day 18), while the skeletal alpha-actin gene, assayed in the same nuclei, undergoes a 10-fold increase in transcription (43.9 ± 8.8 ppm hybridized at day 14 vs 430 ± 6.1 ppm at day 18). These results demonstrate that the developmentally programmed disappearance of the cTNT protein and mRNA during embryonic muscle development is due to transcriptional repression of the cTNT gene. Therefore, we conclude that in addition to previously suggested post-transcriptional control mechanism for down-regulation of the skeletal muscle thymidine kinase gene (3), repression at the transcriptional level is also an important mechanism in myogenic gene switching. Supported by grants from the NIH (CPO) and AHA/Ca Affil.#86-N25 (CSL).
References: (1) Toyota,N.& Shimada, T. 1981. J.Cell Biol. 91:497-504. (2) Cooper,T.A. & Ordahl, C.P.1984 Science 226:979-982..(3) Groudine,M. & Casimir,C. 1987. Nucleic Acids.Res. 12:1427-1446.

M 433 ISOLATION OF cDNAS ENCODING MUSCLE TRANS-ACTING FACTORS, Gary E. Lyons, Michael Ittmann*, Claudio Basilico* and Laurence H. Kedes, Stanford University, Palo Alto, CA; *New York University, New York, NY.

A DNA construct consisting of the human cardiac alpha-actin gene promoter linked to a gene encoding a cell-cycle regulatory protein was stably transfected into a temperature-sensitive mutant of the BHK cell line which has the capacity to divide at 33°C but arrests in G_1 of the cell cycle at 39.5°C. The cell-cycle gene product rescues the temperature-sensitive cells from arrest at the non-permissive temperature. Subclones with an integrated chimeric cardiac actin-cell cycle gene are being tested for their ability to express the gene in the presence of muscle-specific *trans*-acting factors when the clones are fused with C2 myotubes to form stable heterokaryons. An Okayama-Berg expression vector cDNA library was made from the C2 mouse muscle cell line which constitutively expresses muscle-specific positive regulatory factors which interact with the cardiac alpha-actin gene promoter. The muscle cDNAs will be transfected into the mutant BHK cell line containing the integrated cell-cycle gene, and cells will be selected at 39.5°C for their ability to undergo mitosis. The specific cDNAs that engender the expression of the cell-cycle gene will be isolated and characterized.

M 434 UPSTREAM REGIONS REQUIRED FOR CARDIAC TROPONIN T GENE EXPRESSION
Janet H. Mar, Parker B. Antin , William J. Odegard and Charles P. Ordahl. UCSF, SF, CA 94143.
The chicken gene encoding cardiac troponin T (cTNT) is expressed in both cardiac and skeletal muscle during early embryonic development, but is specifically repressed in skeletal muscle during later stages of development. To determine if the cis-acting sequences governing transcription of this gene in these two related cell types are the same, we have transfected promoter/upstream segments of the cTNT gene linked to the bacterial chloramphenicol acetyltransferase (CAT) gene into primary cultures of early embryonic cardiac and skeletal muscle cells. Using this transient expression system, CAT activity directed by the cTNT promoter/upstream region is between two and three orders of magnitude higher in cardiac or skeletal muscle cells than in fibroblast cells, indicating that cis elements responsible for cell-specific expression reside in this region of the cTNT gene. The high degree of cell specificity of CAT expression governed by the cTNT promoter was confirmed immunocytochemically using a monoclonal CAT antibody. Deletion experiments demonstrate that different regions in proximity to the cTNT promoter are required for expression in skeletal and cardiac muscle cells. An 82 nucleotide upstream segment has been shown to be both necessary and sufficient for skeletal muscle specific transcription. A separate 61 nucleotide upstream segment is required for expression in cardiac muscle cells but is not required for expression in skeletal muscle cells. A third region, residing farther upstream, contains an enhancer element which is active in both cardiac and skeletal muscle cells, but is inactive in fibroblast cells. Therefore, transcription of the cTNT promoter in early embryonic cardiac and skeletal muscle cells is governed by both shared and distinct cis-acting regulatory sequences, suggesting that similar and different trans factors are involved in the expression of the cTNT gene in the two muscle cell types.
Supported by NIH grants to CPO and NIH postdoctoral fellowships to JHM and PBA.

M 435 ISOLATION AND CHARACTERIZATION OF SMOOTH MUSCLE α-AND γ-ACTIN cDNAs IN RAT,
K.M. McHugh, K. Crawford, and J.L. Lessard, Children's Hosp. Res. Fdn., Cincinnati, OH 45229

The isoactins provide a useful model system to examine the mechanisms involved in gene regulation because there are at least six distinct actins which are differentially expressed during embryonic development. We have recently isolated and sequenced two rat stomach cDNAs that encode the two smooth muscle actins (vascular α-actin and enteric γ-actin). The amino acid sequence of the vascular α-actin is encoded by 1131 nucleotides and the protein sequence is identical to that reported for the chicken vascular α-actin. The 188 nucleotide 3' UT region shows little sequence similarity with the 3' UT region of the chicken vascular α-actin, but strong sequence similarity (91%) with the mouse vascular α-actin 3' UT region. The 5' UT region is 42 bp in length and shows some modest similarities with both the human and chicken vascular α-actin 5' UT sequences. By comparison, the amino acid sequence of the enteric γ-actin is encoded by 1128 nucleotides and the protein sequence shows a single amino acid difference (Gln->Pro at residue 358) from that reported for the chicken enteric γ-actin. The 3' UT region is 83 bp in length and shows little sequence similarity to the other isoactins except for an inverted region of 49 bp which is 76% similar to the β-cytoplasmic isoform of actin. The 5' UT region is only 15 bp in length and displays no apparent sequence similarities to any other isoactins. Northern blot analysis with a vascular α-actin specific probe shows high levels of vascular α-actin mRNA in the rat stomach, uterus, and aorta with low to undetectable levels in the skeletal muscle, kidney, heart, brain, and liver. Similar blots with an enteric γ-actin specific probe show high levels of enteric γ-actin mRNA in the stomach and uterus with low to undetectable levels in the aorta, skeletal muscle, kidney, heart, brain, and liver.

M 436 THE HUMAN SKELETAL AND CARDIAC α-ACTIN PROMOTERS INTERACT WITH COMMON TRANS-ACTING FACTORS,
George Muscat and Larry Kedes, The Medigen Project, Department of Medicine, Stanford Medical School and Veterans Administration Medical Center, Palo Alto, CA 94304.

Trans-acting factors that modulate and mediate the tissue-specific expression of the human skeletal α-actin gene have been identified by an *in vivo* competition assay and an electrophoretic mobility shift assay in a myogenic cell line. These rate-limiting factors interact with defined cis-acting regions that correspond to sequences required for the transcriptional modulation of this sarcomeric α-actin gene in muscle cells. Several fragments that interacted with positive factors contained the sequence CC(A+T rich)$_6$GG (designated a CCArGG box), which is found in the promoter region of several muscle associated genes and shown to be a binding site for positive trans-acting factors in the human cardiac α-actin gene. The factor(s) bound by skeletal α-actin sequences between -153 and -36 were found to be also essential for the maximal activity of the cardiac α-actin promoter and the SV40 enhancer *in vivo* in muscle cells.Gel shift experiments indicated that the factor bound by this sequence, was also specifically competed by a 30 bp fragment containing the first cardiac α-actin CArG motif.In contrast,*in vivo* competition assays indicated that HuT-12 fibroblastic cells contain functionally distinct factor(s) that are used by SV40 but not by the skeletal α-actin cis-acting sequences. The existence of functionally different factors in these cells may explain the muscle cell specificity of this sarcomeric gene.

M 437 **DISTINCT TISSUE-SPECIFIC MITOCHONDRIAL ISOFORMS OF CREATINE KINASE ARISE BY A GENETIC MECHANISM.** Johann P. Hossle, Gabriela Wegmann, Theo Wallimann, Hans M. Eppenberger and Jean-Claude Perriard, Institute for Cell Biology, Swiss Federal Institute of Technology, CH-8093 Zuerich, Switzerland.

Creatine kinases play a key role in energy metabolism of contractile and neural tissues. In striated chicken muscle a mitochondrial form (Mi-CK) is coexpressed with one of the cytosolic forms (M-CK or B-CK) and the different isoenzymes are linked by a metabolic "phosphocreatine circuit". The cDNA sequence encoding the complete mature Mi-CK of chicken striated muscle was isolated by immunoscreening from a λgt11-cDNA library representing skeletal muscle poly(A)$^+$ RNA. The Mi-CK aminoacid sequence deduced from the cloned striated muscle Mi-CK cDNA is the first complete Mi-CK sequence established to date.

Immunoblot analysis of 2D-gels of mitochondrial preparations from chicken heart, skeletal muscle and brain demonstrated different Mi-CK isoproteins in muscle and brain. S1 protection experiments using the cloned Mi-CK cDNA sequence as hybridization probe strongly suggested, that the dinstinct Mi-CK isoproteins are encoded by multiple tissue-specific Mi-CK transcripts. The differences at the nucleotide level between the corresponding Mi-CK messengers of muscle and neural tissues were found to be extensive, since no crosshybridization on RNA blots was observed. The results obtained indicate, that distinct Mi-CK isoproteins arise by differential splicing events or that multiple genes are subjected to tissue-specific control of expression.

M 438 **CHARACTERIZATION OF A MUSCLE-SPECIFIC ENHANCER IN THE MYOSIN LIGHT CHAIN 1/3 LOCUS.** N. Rosenthal, M. Donoghue, H. Ernst and B. Wentworth. Howard Hughes Medical Institute/Children's Hospital, Boston, MA 02115

Two skeletal myosin light chains, MLC1 and MLC3, are generated from a single 21 kb gene by transcription from two different promoters and alternate splicing of the pre-mRNAs. To define DNA sequences involved in MLC transcriptional control, we constructed a series of plasmid vectors in which segments of the rat MLC locus were linked to a CAT gene and assayed for expression in muscle and non-muscle cells. Whereas sequences proximal to the two MLC promoters do not appear to contain tissue-specific regulatory elements, a 500 bp DNA segment downstream of the MLC locus dramatically increases CAT gene expression in differentiated myotubes but not in undifferentiated myoblasts or non-muscle cells. The ability of this segment to activate gene expression to high levels in a distance-, promoter-, position-, and orientation-dependent way defines it as a strong muscle-specific enhancer element. A detailed analysis of the 500 bp enhancer region is currently underway to locate precisely the sequences responsible for the tissue specificity of the element, and to identify potential binding sites for muscle-specific transcription factors.

M 439 LOCALIZATION OF ACTIN GENE EXPRESSION IN EARLY AVIAN EMBRYOS, D.L.Ruzicka[1,2] and R.J.Schwartz[2], Harvard Med. Sch., Boston, MA[1] and Baylor Coll. Med., Houston, TX[2].

Developmental transitions in actin gene expression in tissues of early avian embryos have not been well-characterized because experiments analyzing RNA from whole embryos do not allow conclusions regarding the cellular localization of transcripts. To address questions of tissue specificity and early cell commitment to actin gene expression, paraffin sections of chick embryos were hybridized in situ to cloned 3'-specific, single-stranded M13 probes for β-cytoplasmic and α-actin (cardiac, skeletal and smooth muscle) gene transcripts. β-cytoplasmic mRNA is abundant and ubiquitous in early embryonic chick tissues, whereas α-actin genes exhibit stage- and tissue-specific differences in expression. Cardiac α-actin gene expression has a relatively discrete onset at (Hamilton-Hamburger) stage 9 and transcripts are preferentially enriched in the presumptive epimyocardium of the fusing heart primordia. Accumulation of skeletal muscle α-actin mRNA in the heart lags behind α-cardiac, with a modest, localized rise in signal occurring after stage 12. Expression of both actin species later increases in presumptive myoblasts when myogenic cells are migrating out of the somites. Unexpectedly, the epimyocardial tissue of the developing cardiovascular system is also enriched in smooth muscle α-actin transcripts before stage 12, as are the migrating myotomal cells of later stages. However, α-smooth mRNA in the heart becomes strongly localized in the conus by stage 12, indicating that its expression is selectively down-regulated elsewhere in regions of the heart not destined to form vascular structures. Thus, α-actin gene expression is detected during histogenesis and precedes physiological functioning in muscle tissues, although regulation of expression varies for each gene.

M 440 RESOLUTION OF CYTOPLASMIC TRANSLATION INHIBITORY RNA OF CHICK EMBRYONIC MUSCLE INTO BIOLOGICALLY ACTIVE SUBSPECIES. Q.L. Wu, M. Raychowdhury, M.S. Eller and S. Sarkar, Department of Anatomy and Cellular Biology, Tufts University Medical School, Boston, MA 02111.

We have isolated a novel cytoplasmic translation inhibitory 10 S RNP (iRNP) containing a 4 S RNA (iRNA) species (size range 60-140 nucleotides) from chick embryonic muscle. Both iRNA and iRNP are potent inhibitors of in vitro translation of a variety of mRNAs. The inhibition is due to a specific effect on a single step in the initiation phase, i.e. binding of mRNA to the 43 S preinitiation complex. The iRNA has no effect on the phosphorylation of the alpha subunit of eIF2 phosphorylation pathway. The proteins of iRNP do not show any translation inhibitory activity and do not immunoreact with six monospecific autoimmune antibodies (RNP, Sm, Pm-1, SSA, SSB and SCL-70). Thus, iRNP is distinct from SnRNP particles. Several iRNA subspecies have been resolved by HPLC and PAGE as biologically active subspecies. The 140-nucleotide iRNA subspecies inhibits translation of muscle mRNAs more strikingly than globin or poliovirus mRNA. The other subspecies show differential inhibition of globin mRNA translation. Several iRNA subspecies contain 5'-ppp termini and show sequence microheterogeneity at the 3' and 5' regions. The resolved iRNA subspecies hybridize with muscle poly (A) mRNA and globin mRNA in dot-blots but not with 28 S, 18 S, 4 S, and 5 S RNAs of chick muscle. It is suggested that iRNA may act as eukaryotic "anti-mRNA" and its role is to regulate the cytoplasmic mRNA levels, thus modulating the translation pattern and augmenting the transcriptional control of contractile protein gene expression during myogenesis.

M 441 IN SITU ANALYSIS OF MUSCLE GENE EXPRESSION IN MOUSE EMBRYOS, David Sassoon, Ian Garner, Stefano Schiaffino°, Serge Alonso, Paul Barton, and Margaret Buckingham. Insitut Pasteur, 25 rue du Dr. Roux. Paris, France Cedex 15, °Institute of General Pathology, University of Padova, Italy

We have been investigating early gene transcription of the contractile muscle protein families using the method of in situ hybridization on mouse embryo and fetal tissue sections. Striated muscle is among the first distinct tissue types formed in the mouse, in particular, the heart (7.5 days post coitum) and the somites (8-13 days p.c.). Using cRNA ^{35}S-labelled probes specific to cardiac and skeletal actin (5'NC), we observe that the 7.5 day embryo expresses high levels of cardiac actin in the primitive heart tube. Skeletal actin is expressed in the heart in a heterogeneous manner. By 8.5 days, we can begin to follow somite formation. We observe that this actin gene pair follows different programs of expression in cardiac versus skeletal muscle tissue. Skeletal actin is weakly detected in the heart at all stages of development thus far examined, whereas both actins are strongly present in somites, myotomes, and fetal skeletal muscle. In somites corresponding to segments already in the process of forming myotomes, cardiac actin expression is high in both somites and myotomes whereas skeletal actin is high only in myotomes and either absent or very weak in the somites. Using probes to β-MHC (3' NC) and MLC1A (1 emb, 3'NC), we observe that by 13.5 days, β-MHC is only detected in the ventricle whereas MLC1A is strongly expressed in both venticle and atria. This would imply that the mechanisms governing the compartmentalization of atrial and ventricular gene expression are different for these two multigene families. We have previously demonstrated that a duplication in the promotor of the α-cardiac gene in BALB/c mice results in the reduced expression of cardiac actin and a compensatory increase in skeletal actin in adult heart tissue. In situ analyses of cardiac tissue in both wild-type and BALB/c mice reveal that skeletal actin transcripts are not equally present in all heart cells but are highly expressed in various regions of the heart. In contrast, cardiac actin transcripts are equally distributed in all cells of the heart suggesting that the compensatory response is under localized control presumably due to local physiological factors. We are currently extending these studies as well as examining other myosin heavy and light chain gene transcription.

M 442 STRUCTURAL AND FUNCTIONAL ANALYSIS OF MYOSIN LIGHT CHAIN 2: IDENTIFICATION OF TRANSCRIPTION ACTIVATION/REPRESSION ELEMENTS.

Gadot, M., Danishefsky, K., Zarraga, A.M., Datta, K., Lin, E., Qasba, P. and Siddiqui, M.A.Q. S.U.N.Y.-Health Science Center at Brooklyn, Department of Anatomy and Cell Biology, 450 Clarkson Avenue, Brooklyn, N.Y. 11203. **Recently, we have isolated and characterized the chicken cardiac MLC_2 gene and demonstrated its promoter activity following transfection of cell lines. We have examined here the functional properties of two dA-dT rich sequences and a CCAAT-like sequence, CCAAAAGTGG in MLC_2 gene, by constructing several mutant MLC_2 upstream sequences with deletions/substitutions of specific DNA elements using oligonucleotide-directed site specific mutagenesis. S1 nuclease protection assays of the RNA transcribed by the mutant promoters showed that the initiation of transcription of MLC_2 gene is totally dependent upon the presence of the distal dA-dT rich sequence (TATTTTTA), but not on the proximal TATTTATT sequence. The latter sequence, which is located close to their mRNA start site (-4bp), is most likely involved in selectivity of the transcription initiation site. Deletion of CCAAAAGTGG sequence results in initiation of transcription at an additional site (-40) without effecting the initiation at +1, suggesting that this element is required for repression of transcription at (-40). Chloromphenicol acetyl transferase (CAT) competition experiments based on co-transfection of a muscle cell line with plasmids containing the MLC_2 promoter sequence fused to CAT reporter gene and competitor DNAs indicate that the regulatory cis-elements in MLC_2 participate effectively in binding with trans-acting regulatory factors. The presence of trans-acting nuclear proteins was also demonstrated by direct DNA-protein mobility retardation assays.**

M 443 Structure, Sequence, and Expression of the Human Skeletal alpha-Actin Gene.
Alan Taylor, Harry Erba, and Larry Kedes. The MEDIGEN Project, Department of Medicine, Stanford University School of Medicine and Veterans Administration Medical Center, Palo Alto, California 94304.
Regulation of the actin multigene family involves the recognition of regulatory sequences which specify the tissue-type and developmental program of expression for each actin isotype. In order to investigate the underlying regulatory mechanisms, the human skeletal alpha-actin gene has been cloned and sequenced, including 700 nucleotides of the 5' regulatory region. Structurally, this actin gene is composed of seven exons with one large intron in the 5' untranslated region which is characteristic for the sarcomeric actins. The intact gene was shown to be developmentally regulated when transfected into the heterologous rat L8 myogenic cells indicating a highly conserved regulatory system. The DNA sequence was compared to other actin genes and several regions of sequence similarity were identified, particularly within regions shown to be important for gene expression. Most notable among the conserved sequences are the CC(A/T rich)6GG motifs which have demonstrated interactions with trans-acting transcriptional factors. This motif has also been identified in several other genes, some of which have demonstrated factor binding to this same motif. The contribution of transcriptional verses post-transcriptional regulatory control during muscle gene expression is also being addressed.

M 444 HUMAN VENTRICULAR\SLOW TWITCH MYOSIN ALKALI LIGHT CHAIN GENE, William L. Fodor, Seharaseyon Jegatheesan, Basil T. Darras, Uta Francke and Elio F. Vanin, Department of Biochemistry, Ohio State University, Columbus, OH and Department of Human Genetics, Yale University School of Medicine, New Haven, CT.

The human ventricular\slow twitch isoform myosin alkali light chain (MLC) gene was isolated from a partial Eco RI library using the rat fast twitch skeletal isoform cDNA as a probe. The gene spans a total of approximately 6kbp of DNA and consists of 7 exons (ranging in size between 27 to 183bp) and 6 introns (ranging in size between 66bp to 2.2kbp). This is similar to the exon-intron arrangement that has been seen in the fast twitch skeletal isoforms from a number of different species. Exons 1 through 6 code for 43, 9, 50, 24 and 8 amino acids while the last exons is entirely non-coding. The fact that exon 1 codes for the first 43 amino acids is consistent with the fact that the ventricular\slow twitch isoform is a MLC1 type isoform. A Xba I-Eco RI fragment from the 3' end of the Eco RI fragment containing the gene was used as a probe against Hind III and Bgl II digests of DNA from 12 different rodent-human somatic cell lines. From these results obtained we were able to localize the human ventricular\slow twitch MLC isoform gene to chromosome 3. This is consistent with the localization of the mouse ventricular\slow twitch gene to chromosome 9.

M 445 CLONING AND EXPRESSION ANALYSIS OF HUMAN SLOW AND FAST-FIBER SKELETAL MUSCLE TROPONIN I GENES, Robert Wade and Larry Kedes, The Medigen Project, Department of Medicine, Stanford Medical School and Veterans Administration Medical Center, Palo Alto, CA 94304.

The fast-fiber and slow-fiber skeletal muscle isoforms of Troponin I are products of separate genes. We have isolated full-length cDNAs and genomic clones representing each of the human skeletal muscle TnI isoforms. Initial studies have focused on the slow-fiber TnI gene. The human slow-fiber TnI gene spans ca. 14 kb and encodes a mature mRNA of ca. 950 bases. A transfected chimeric gene comprised of 4 kb of TnI-slow 5' promoter sequence linked to the bacterial CAT gene is expressed at high levels in differentiated cultures of the mouse C2 myogenic cell line. Deletion analysis is being conducted to identify 5' regulatory sequences important to the high level and tissue-specific expression of the human TnI-slow gene. In addition, mini-genes are being constructed to investigate the possible role of intragenic sequences involved in Tn I gene expression. Comparative structural analysis of the human fast-fiber TnI gene is being performed to delineate evolutionarily conserved features of TnI genes that may play an important role in their regulation.

Regulation of Muscle Genes and Protein Expression - II; Molecualr Aspects of Muscle Disease

M 500 THE ABUNDANCE OF CALMODULIN mRNAs IS REDUCED IN PHOSPHORYLASE KINASE DEFICIENT SKELETAL MUSCLE. Patrick K. Bender and Charles P. Emerson, Jr., Department of Biology, University of Virginia.

In the I/Lyn mouse strain an x-linked mutation results in an absence of phosphorylase kinase (PhK) activity in skeletal muscle. Immunoblots of extracts from the deficient tissue demonstrate that the catalytic subunit (gamma) of PhK is absent. Moreover, radioimmunoassay shows that there is a 40% reduction in calmodulin protein. In normal skeletal muscle, an estimated 40% of the calmodulin is associated with PhK. This stoichiometric reduction in calmodulin indicates that the expression of calmodulin is coordinated with the expression of the PhK enzyme. We have investigated the coordinate expression of calmodulin by comparing the heterogeneity and abundance of calmodulin mRNAs in normal and deficient skeletal muscle. Hybridization of a calmodulin cDNA to northern blots, demonstrated that there are four distinct molecular weight species of calmodulin mRNA in normal tissue. All four of these species are present in the deficient tissue and none of them are preferentially reduced. However, quantitation of the relative abundance of the calmodulin mRNA to actin mRNA demonstrates that there is a 45% reduction in all four calmodulin mRNA species. This reduction is proportional to the reduction in calmodulin protein. These results indicate that the expression level of the calmodulin mRNAs is coordinated with the expression of its major enzyme target in skeletal muscle--PhK. This coordination may involve a feedback mechanism which regulates either the transcription or message processing of the calmodulin mRNAs with the levels of the PhK protein.

M 501 MAPPING OF TRANSLOCATION BREAKPOINTS IN FEMALES WITH DUCHENNE MUSCULAR DYSTROPHY USING A 5' cDNA CLONE. S.E. Bodrug, A.H.M. Burghes, R.G. Worton, University of Toronto and the Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

Duchenne and Becker muscular dystrophies (DMD and BMD) are allelic muscle wasting disorders, the gene for which is located at Xp21. Twenty-one females with DMD or BMD have X-autosome translocations with the X chromosome breakpoint at Xp21. Somatic cell hybrid mapping indicates that the translocation breakpoints are scattered throughout the large (~2000 kb) DMD/BMD gene. A cDNA clone corresponding to the 5' end of the DMD/BMD gene was used to probe Southern blots of DNA from hybrids carrying translocation chromosomes. A t(X;21)(p21;p12) translocation in a patient with BMD was found to break within a 110 kb intron between exons 7 and 8. No other translocations have yet been mapped within this intron. Two other DMD translocations, t(X;5)(p21;q35) and t(X;11)(p21;q13) were found to break between the first and second exon of the gene, indicating perhaps that a similar mechanism was involved in the origin of these two translocations. Alternatively, the first intron may be quite large, accounting for two translocation breakpoints in the same intron. The t(X;21) translocation has been cloned and sequenced, and a small amount of DNA (< 100 bp) is missing from the translocated chromosomes. The tetranucleotide CGGC was found in the immediate vicinity of the t(X;21) breakpoint on both translocation chromosomes, making it a candidate recognition sequence for an enzyme involved in the translocation process. The question of whether the t(X;5), t(X;11) and t(X;21) translocations arose by the same mechanism is being addressed by identifying, cloning and sequencing the t(X;5) and t(X;11) translocation breakpoints.

M 502 DETECTION OF MUTATIONS IN THE DMD LOCUS USING cDNA PROBES. A. Burghes, C. Duff, X. Hu, HJ. Klamut, E. Zubrzycka-Gaarn, DE. Bulman, PN. Ray and R.G. Worton. The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

Several cDNA clones have been isolated from the DMD locus by probing an adult muscle cDNA library with unique fragments from the phage clone XJ10 in the DXS206 locus. Subsequent cDNA clones were obtained by walking from the original cDNA clone on the same cDNA library. Sequence comparison with fetal cDNA isolated in Kunkel's laboratory indicates that the adult and fetal clones are identical. These cDNA clones have been used to screen 130 patients with DMD or BMD. Deletion, duplication and insertion mutants have been found, the duplication frequency thus far is approximately 1/3 of the deletion frequency. The frequency for deletion and duplication for various regions of the gene is being determined. The exons that were deleted or duplicated for various patients with BMD or DMD were determined. Two patients with DMD have been found to be deleted for the first exon and promoter region. The cDNA clone has provided material for generating oligopeptides and fusion proteins that have been used to produce antibodies against the DMD protein. These antibodies are currently being used to screen DMD and BMD patients with known gene alterations as well as patients with no known alteration to determine changes at the protein level. In addition, selected patients are being studied at the RNA level.

M 503 EXPRESSION OF THE MURINE DUCHENNE MUSCULAR DYSTROPHY GENE IN THE MUSCLE AND BRAIN OF NORMAL AND MUTANT MDX MICE. Jeffrey S. Chamberlain, Joel A. Pearlman, Richard A. Gibbs, Joel E. Ranier, Nancy J. Farwell, and C. Thomas Caskey, Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX. 77030.
We have isolated cDNA clones corresponding to the 5' 2.5kb of the murine Duchenne muscular dystrophy (mDMD) mRNA. These clones cross-hybridize well with the human DMD gene and are versatile in detecting deletions, duplications and RFLPs in DMD patients and carriers. Numerous genomic clones have been isolated from various portions of the human DMD gene, and are being used to develop novel diagnostic procedures which will be discussed. cDNA clones have been utilized to map the mDMD gene to a region of the mouse X-chromosome that also contains the muscular dystrophy mutation mdx. We have also examined the tissue specific expression of the mDMD gene in normal and three different strains of mutant mdx mice. mDMD mRNA is detectable in skeletal and cardiac muscle, and at very low levels in the brain of normal mice. All three strains of mdx mice display vastly reduced levels of apparently normal sized mDMD mRNA in both muscle and brain, when compared to the relative levels of muscle γ-phosphorylase kinase and hypoxanthine phosphoribosyltransferase and HPRT mRNAs. These results suggest that the mdx mutations are within the mDMD gene, and implicate expression of the DMD gene in DMD associated mental retardation.

M 504 MUSCULAR DYSGENESIS IN MICE IS A DEVELOPMENTAL MUTATION, Nirupa Chaudhari and Kurt G. Beam, Colorado State University, Ft. Collins, CO 80523.
In skeletal muscle, sarcolemmal depolarization leads to contraction via a series of events termed excitation-contraction (E-C) coupling. Muscular dysgenesis (*mdg*) in mice is a single gene mutation which specifically elimitates E-C coupling. *Mdg* muscle has previously been shown to exhibit a depressed number of dihydropyridine (DHP)binding sites and a lack of the DHP-sensitive slow calcium current. It was our premise that this mutation would allow us to identify genes for the DHP receptor and other E-C coupling proteins.
A normal skeletal muscle cDNA library was screened by differential colony hybridization to identify mRNA sequences accumulating to different concentrations in normal and dysgenic muscle. Sequences of two classes (representing only 5% of all clones) were isolated - those whose mRNAs are under-expressed and those which are over-expressed in mutant relative to normal skeletal muscle. The concentration of respective mRNAs was examined during normal muscle development. In all cases, we found that sequences which are under-expressed in mutant muscle are also those which are activated late during muscle differentiation. Conversely, mRNAs which are over-expressed in mutant muscle accumulate early during muscle development and disappear as differentiation proceeds. Thus, dysgenic skeletal muscle appears to be developmentally arrested in a portion of its genetic program, which probably corresponds to the genes encoding E-C coupling and related proteins. The role, during development, of these genes is being examined through a combination of cell and molecular biological approaches. Supported through grants from Muscular Dystrophy Association and National Institutes of Health (Ns 24444) to KGB.

M 505 MOLECULAR BASIS FOR MYOADENYLATE DEAMINASE DEFICIENCY, P.R.H. Clarke, E.W. Holmes, and R.L. Sabina, Duke University, Durham, NC 27710.
Deficiency of myoadenylate deaminase (MDD) has been associated with a number of neuromuscular diseases and also as an isolated finding in a subset of patients suffering from exertion-induced muscular pain and weakness suggesting the existence of both secondary (acquired) and primary (inherited) enzyme deficiencies. Like many muscle enzymes, MDD activity varies with muscle fiber type, being highest in fast twitch glycolytic muscle and lowest in slow oxidative fibers. Its activity changes in concert with that of glycolytic enzymes under the influence of chronic 10 Hz stimulation in animal preparations. We have shown in the rat that multiple isoforms of MDD exist and that, like many structural/catalytic muscle proteins, maturation of skeletal muscle is associated with a switch of MDD isoform from embryonic to adult mRNA and protein. In order to study the control of MDD expression in primary and acquired deficiencies at the molecular level, we have isolated MDD cDNA from an adult human muscle library and have established lymphoblast cell lines from patients with primary deficiency for genomic DNA study. We will present results for the following investigations: 1) Nucleic acid sequence of human MDD cDNA and derived amino acid sequence of the encoded protein; 2) Isolation and characterization of human MDD genomic sequences; 3) Northern blot and RNAse protection analyses for quantitation of MDD mRNA present in muscle biopsies from controls and from patients with primary and secondary deficiencies and correlation with tissue enzyme activity. These studies will determine whether MDD deficiency results from a defect in the primary structure of the mRNA versus a regulatory defect affecting transcription or isoform switching.

M 506 X-LINKED MUSCULAR DYSTROPHY IN THE DOG AS A MODEL OF DUCHENNE DYSTROPHY, Barry J. Cooper, Beth A. Valentine and Nena J. Winand, Cornell University, Ithaca, NY 14853, Marion Oronizi-Scott, Hansell Stedman and James E. Sylvester, University of Pennsylvania, Philadelphia, PA 19104.

We have identified an X-linked form of muscular dystrophy in the dog which faithfully mimics the phenotypic expression of Duchenne muscular dystrophy of man. Clinical signs, consisting of weakness and limited ability to open the mouth, are first manifested at about 8 weeks of age. With progression, affected dogs develop a stiff gait, muscle atrophy, overextension of the carpus and overflexion of the tarsus. Affected dogs have elevated creatine kinase (CK) levels from the earliest ages tested (2 days). Carrier females have intermittent elevations of CK. Lesions consist of extensive muscle cell necrosis, with swollen hyalinized fibers, coagulation and phagocytosis by macrophages, and regeneration. Alizarin red S staining of frozen sections of affected muscle reveals calcium positive fibers, including both large dark fibers, and some which appear normal by other histologic criteria. Chronic lesions are characterized by fibrosis and fatty infiltration. Carrier females have similar, but qualitatively less severe, lesions. Cardiac lesions resembling the subepicardial fibrosis found in DMD patients have been found in two older dogs so far examined. Breeding studies have confirmed that the disease is X-linked. The genetic relationship between this canine dystrophy and DMD are currently being investigated using Southern, Northern and dot blot techniques. Preliminary analysis using cDNA probes for the human DMD gene reveal that affected dogs lack mRNA while heterozygotes have intermediate levels. This suggests that the canine disease is a genetic analog of DMD. However, Southern blots have so far failed to reveal additional evidence of a mutation in the DMD locus.

M 507 GENOMIC AND FUNCTIONAL ANALYSIS OF THE ANTISENSE RNA (tcRNA 102) FROM CHICK EMBRYONIC PECTORALIS MUSCLE, Stuart Heywood, Carol Scherczinger, and Rebecca Abrahamson, University of Connecticut, Storrs, CT 06268.

Translational control RNA (tcRNA 102) has been found associated with myosin heavy chain (MHC) mRNA in mRNP particles in embryonic chick pectoralis muscle. It has also been shown to stoichometrically inhibit MHC mRNA translation with a high degree of specificity. The two subspecies of tcRNA 102 (which differ in the six 3' terminal bases) accumulate differently during myogenesis. The 5' non-translated region, the AUG initiation code and seven coding bases of the early embryonic fast MHC-mRNA show sequence complementarity to 28 bases at the 3' end of tcRNA 102. In vitro analysis of protein synthesis initiation complexes, using this embryonic MHC-mRNA and oligonucleotides identical to the 3' end of tcRNA 102, as well as primer extension analysis indicates that this tcRNA-like oligomer is capable of hybridizing to MHC-mRNA in vitro and interfering with mRNA binding to ribosome. These results suggests that tcRNA 102 is a naturally occuring antisense RNA for MHC-mRNA. The differential developmental appearance of the mRNA and antisense RNA, as well as the specificity of interaction of tcRNA 102 with the embryonic MHC-mRNA, suggests tcRNA 102 may be involved in MHC isoform switching. Additional experiments concerned with the genes encoding tcRNA 102 suggest that these genes are arranged in tandem and have a low copy number.

M 508 TRANSCRIPT-SWITCHING IS THE BASIS FOR CHANGE IN AMP DEAMINASE ISOFORMS DURING IN VIVO AND IN VITRO MYOCYTE DIFFERENTIATION, R.L. Sabina, E.W. Holmes, Duke Univ., Durham, NC 27710.

It is well established that many muscle-specific genes undergo switching of non-muscle to muscle isoforms during myogenesis, accompanied by a switch in RNA transcripts. Previously we had reported developmental isoforms of AMP deaminase (AMP-D) in skeletal muscle (Marquetant et al, PNAS 84: 2345, 1987). Subsequent cloning of the rat AMP-D cDNA (Sabina et al, JBC 262: 12397, 1987) has also enabled the analysis of transcript expression during myogenesis. The results of these experiments are as follows: 1) In the early embryo in situ or in proliferating myoblasts in culture, a 3.4 Kb transcript is expressed which encodes the embryonic AMP-D peptide. 2) The amount of this transcript increases during neonatal development in situ and during the transition to myotubes in culture. Concurrently, a 2.5 Kb adult muscle transcript begins to appear. During this time, however, all AMP-D activity is immunoprecipitated by antiserum specific for the embryonic isoform 3) Late in neonatal development and in fully differentiated myotubes in culture, the embryonic transcript and the embryonic peptide disappear. During this interval there is an increase in the abundance of the adult muscle transcript and the adult muscle peptide appears. These results establish transcript switching as the basis for the change in AMP-D isoforms during myogenesis. These results also demonstrate that the AMP-D gene is controlled through transcriptional regulation presumably in response to tissue-specific factors. Additionally it appears that the adult muscle AMP-D transcript is subject to post-transcriptional regulation as well.

M 509 PROTEIN CONTENT AND MECHANICS OF SINGLE SKELETAL MUSCLE FIBERS IN DUCHENNE MUSCULAR DYSTROPHY R. Horowits, M. Dalakas and R.J. Podolsky, NIH, Bethesda, MD 20892
We investigated the relation between tension generation and myofibrillar protein content in skeletal muscle fibers from 3 patients with Duchenne muscular dystrophy (DMD), aged 7 to 11 years. The control group to date consists of 3 patients, aged 41 to 59 years, including 2 patients suffering from neurogenic disorders (post-polio disease and facioscapulohumeral muscular dystrophy) with no evidence of active muscle fiber degeneration, and a third with no physical evidence of neuromuscular pathology. The properties of at least 10 single fibers from each patient were measured after chemical skinning. Fiber dimensions and maximum calcium activated force were measured at a sarcomere length of 2.6 μm; resting tension was measured at 4.0 μm. Each fiber was then dissolved in 4000 volumes of an SDS sample buffer. Proteins in each fiber were separated by SDS PAGE, visualized by silver staining and quantitated by scanning densitometry. Active tensions for control and DMD fibers were 1.10 ± 0.06 and 0.85 ± 0.05 kg/cm^2 (±SEM), respectively, and resting tensions were 0.22 ± 0.01 and 0.23 ± 0.01 kg/cm^2, respectively. The 25% decrease in active tension in DMD was accompanied by a 40% decrease in nebulin concentration, from 0.52 ± 0.05% (wt/vol) to 0.30 ± 0.03%. The concentrations of titin, myosin, actin and α-actinin were similar in DMD and control fibers. Muscle fibers from 1 patient with polymyositis, another degenerative muscle disease, exhibited changes in active tension and nebulin concentration similar to DMD. These results suggest that nebulin may play a role in active, but not passive, tension generation. They also suggest that the decrease in active tension and nebulin concentration in DMD may be a general property of degenerative muscle diseases.

M 510 ALLOGENIC MYOBLAST TRANSPLANTATION INTO SKELETAL MUSCLES OF MDX MICE, George Karpati, Yannick Pouliot, Stirling Carpenter and Paul Holland, Montreal Neurological Institute, Montreal, Quebec, Canada, H3A 2B4
Non-dystrophic myoblasts were transplanted into muscles of young dystrophic (MDX) mice to produce mosaic fibers in which the presumably normal gene of the donor nuclei could correct the deleterious effects of the MDX mutation. Myoblasts of a permanent murine cell line (C_2) were cultured with ^{3}H-thymidine producing a 100% nuclear labeling index. 30 μl suspensions of ¼-½ million myoblasts were injected into multiple sites of quadriceps muscles of 5-15 day old MDX mice. Radioautographs of longitudinal sections of the muscles examined at 4, 10, 20, 35, 45 and 60 days later showed heavily labeled nuclei of the donor myoblasts in many host fibers of muscles removed 4-20 days post-injection, implying abundant fusion. Few or no labeled nuclei were seen in fibers of muscles removed 35-60 days post-injection. Since nuclei of muscle fibers do not divide and their DNA has no turnover, the absence of radio-labeled nuclei suggests that the transplanted nuclei eventually disappear from the mosaic fibers. This presumed nuclear elimination may involve an immunological rejection of fiber segments or selective elimination of the aneuploid C_2 nuclei by the host fibers. These experiments suggest that long-term survival of transplanted myonuclei in host fibers require the use of co-isogenic and/or euploid donor cells.

M 511 TISSUE SPECIFICITY OF MYOSIN HEAVY CHAIN TRANCRIPTS IN *DROSOPHILA MELANOGASTER*, Jeffrey A. Kazzaz, Charles E. Rozek, Case Western Reserve University, Cleveland, OH 44106.
The transcripts of the *Drosophila* myosin heavy chain gene are alternately spliced, utilize two polyadenylation sites, and are under developmental control. By using transcript specific probes for in situ hybridization to tissue sections at different stages of development, we demonstrate the tissue specificity of the transcripts.

M 512 EVIDENCE FOR FACTORS THAT BIND TO THE MUSCLE CREATINE KINASE (MCK) ENHANCER AND PROMOTER, D. J. Kelvin, L. A. Gossett, E. A. Sternberg, and E. N. Olson, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Using the bacterial chloramphenicol acetyltransferase (CAT) gene as a reporter in the C2 muscle cell line, an element centered around -1200 base pairs relative to the transcription start site of the mouse MCK gene was identified that exhibits the properties of a muscle-specific enhancer. This enhancer directs high-level expression of CAT from the MCK promoter or SV40 promoter in myotubes, but little or no expression in myoblasts or non-myogenic cells. To identify trans-acting factors that may influence MCK expression, interaction of the MCK promoter and enhancer with factors from nuclear extracts from a variety of myogenic and non-myogenic cells has been examined. Gel retardation assays of the enhancer reveal at least two retarded bands, one of which appears to be specific for myoblasts and myotubes and is absent in non-myogenic cells. Evidence has also been obtained for interaction of a specific factor with the MCK promoter. Factors contained within nuclear extracts from differentiation-defective myoblasts harboring mutationally-activated ras oncogenes are being examined. Specificity of factor binding has been assessed by experiments using specific and non-specific DNA fragments, in addition to synthetic oligomers comprising regions of the enhancer domain. Analysis of the specific binding sites for these factors is being determined by DNase I protection.

M 513 UPREGULATION OF CYTOCHROME C OXIDASE SUBUNIT III mRNA IN OVERLOADED CHICKEN FAST TWITCH MUSCLE. J. M. Kennedy*, D. A. Essig°, V. Martich+, S. Kamel+, P. K. Umeda+, and R. Zak+. The Univ. of Illinois, Depts. of Physiol*. and Physical Ed°, Chicago, IL 60612, and the Univ. of Chicago, Dept. of Medicine+, Chicago, IL 60637.

Complementary DNA clones were constructed from chicken ventricular RNA. Complementary DNA clones for the mitochondrial-encoded chicken cytochrome c oxidase subunit III (CO III) protein were isolated and identified by restriction endonuclease mapping and partial sequence analysis. These clones were used to determine levels of CO III mRNA expression in various chicken muscles by slot blot analysis. The expression of CO III mRNA was relatively low in the fast twitch posterior latissimus dorsi (PLD) muscle and expressed at much greater levels in atrial, ventricular, and slow-tonic anterior latissimus dorsi (ALD) muscles. Hypertrophy of ALD and PLD muscles was induced by the application of an overload to the wing of chickens. After 42 days of overloading, ALD muscle mass was increased from 364 mg in contralateral control muscles to 1,041 mg in overloaded ALD muscles. The massive hypertrophy of ALD muscles was accompanied by small changes in CO III mRNA expression. In contrast to the ALD muscle, the PLD muscle showed a small increase in muscle mass after 42 days of overloading (637 mg in control PLD muscles vs. 708 mg in overloaded PLD muscles). However, the level of CO III mRNA expression was enhanced by over 2-fold in overloaded PLD muscles. These results indicate that expression of the mitochondrial genome is regulated by events associated with muscle overloading and that the oxidative metabolism of fast twitch muscle is augmented by overloading.

M 514 cDNA CLONING AND PRIMARY STRUCTURE OF THE SUBUNITS ALPHA AND BETA OF PHOSPHORYLASE KINASE,
M.W. Kilimann, N.F. Zander, H.E. Meyer, E. Hoffmann-Posorske, J.W. Crabb & L.M.G. Heilmeyer jr., Institute for Physiological Chemistry, Ruhr-University, D-4630 Bochum 1, West Germany

We have cloned and sequenced cDNA molecules encoding the subunits alpha and beta of phosphorylase kinase from rabbit fast-twitch skeletal muscle. Approximately half of the deduced amino acid sequences is confirmed by peptide sequencing. These cDNA molecules will open up ways to characterize the enzyme's isoforms from other tissues and the molecular basis of their expression, and to elucidate the molecular nature of hereditary phosphorylase kinase deficiencies in humans, mice and rats.

M 515 ISOLATION AND CHARACTERIZATION OF GENOMIC CLONES CORRESPONDING TO THE 5' REGION OF THE DUCHENNE MUSCULAR DYSTROPHY GENE LOCUS. H.J. Klamut, S. Bodrug, A.H.M. Burghes, S. Malhotra, R.G. Worton and P.N. Ray. The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

An adult skeletal muscle cDNA clone isolated in our laboratory contains the first 15 exons of the Duchenne muscular dystrophy (DMD) gene. This clone has been used to screen an XXXX-Sau3A cosmid library for corresponding genomic clones. Three overlapping cosmid clones (designated XJC2, XJC5, and XJC12) have been isolated which contain exons 3 to 7 in a region extending more than 100 kb centromeric to the XJ5.1/JMD-1 locus. A fourth, non-overlapping cosmid clone (XJC8) has been isolated which contains both exon 1 and the pERT84-10 locus. A 1.4 kb Hind III-Pst I fragment containing exon 1 has been subcloned into pBSm13 for sequence analysis of exon boundaries and potential upstream promoter regions. Preliminary sequence analysis indicates that this Hind III-Pst I fragment contains a 209 bp region complementary to exon 1, a downstream region of 268 bp corresponding to a portion of intron 1, and 900 bp of upstream sequence representing the potential promoter region. A G+C content greater than 50% is seen in a 235 bp region spanning the 5' end of exon 1. No obvious TATA, CAAT, or SpI transcription factor concensus sequences are observed in the region immediately upstream of exon 1, although a potential C(Ar)G box (common to muscle-specific promoters) is evident approximately 120 bp upstream. Precise definition of the DMD gene transcription start site and functional assays of promoter activity are currently being undertaken.

M 516 In vivo PROTECTION OF ANTISERUM TO CHICKEN GROWTH HORMONE AGAINST THE PROGRESSION OF THE SYMPTOMS IN CHICKENS WITH INHERITED MUSCULAR DYSTROPHY, Eleonora Kurtenbach, Sarah S. Moraes and Sergio Verjovski-Almeida, Dept. Biochemistry, Inst. Biomedical Sciences, Federal University of Rio de Janeiro, 21910 Rio de Janeiro, BRAZIL.

New Hampshire normal (line 412) and dystrophic (line 413) chickens were treated from the 1st to the 5th day after hatching with intraperitoneal injections of highly specific rabbit antiserum to chicken growth hormone (GH). Controls for the two lines were injected with nonimmunized normal rabbit serum. A total of 96 animals were used. Therapy with antiserum significantly prolonged the functional ability of the dystrophic chickens as quantitated regularly by the standardized test for righting ability. A two-way analysis of variance showed that the righting ability and treatment interaction in the population exhibited F values that had significance levels of 0.001 to 0.054 along the third to sixth weeks after hatching. Our data suggest that it is feasible to retard the evolution of the dystrophic disease using anti-GH which possibly reduces the circulating level of GH. Alternative schemes of treatment of different durations and at different moments along the lifetime of the animals will be tested.
Supported by Brazilian National Research Council (CNPq/FINEP)

M 517 CIS AND TRANS REGULATION OF TROPONIN I EXPRESSION IN MYOGENIC DETERMINATION AND DIFFERENTIATION Brian K. Lathrop, Youngwon Nham, Brian A. Lewis, Stephen F. Konieczny*, Charles P. Emerson Jr., University of Virginia, Charlottesville, VA 22901 and *Purdue University, Lafayette, IN 47907

We have examined cis- and trans-acting control elements which regulate the expression of the quail fast troponin I (TnI) gene during myogenic determination and differentation. These studies have been carried out using the pluripotent C310T1/2 cell line, which upon treatment with 5'-azacytidine will convert with high frequency to stable myogenic cell lines. Stable transfection of TnI into 10T1/2 cells and their myogenic derivatives has shown that TnI is expressed only in differentiating myofibers. Deletion analysis has identified an intragenic control region (ICR) within the first and second introns which is required for expression of the gene (Konieczny and Emerson (1987) Mol. Cell Biol. 7:3065-3075).

We now show that the ICR, when fused to a truncated thymidine kinase (tk) promoter and the neo gene, enhances tk promoter activity with or without the TnI promoter and upstream sequences. Furthermore, the ICR shows this activity in myofibers but not in myoblasts or 10T1/2 cells. The ICR therefore contains sufficient information to allow enhancement of promoter activity in a manner dependent on cell type and cell differentiation.

We have examined nuclear extracts from 10T1/2 cells, and 10T1/2-derived myoblasts and myofibers to ascertain whether DNA-binding factors which interact with the ICR would be restricted by cell lineage or the state of differentiation. Using an electrophoretic mobility shift assay, we have identified a DNA-binding factor found only in myoblasts and myofibers and another factor present only in myofibers. Specific DNA sequences to which these factors bind are being determined by "footprinting" analysis, and the function of these factors in the regulation of TnI expression is being determined. We propose a model in which new trans-acting regulatory factors are produced as a result of determination and that these factors interact with additional factors which are regulated by the extracellular environment during differentiation. The combination of these two sets of factors is presumed to be required for the proper regulation of TnI gene expression.

M 518 STAGE-SPECIFIC EXPRESSION OF THE DUCHENNE'S MUSCULAR DYSTROPHY GENE IN HUMAN MUSCLE IN VITRO, A.A. Lev, E.P. Hoffman, C.C. Feener, L.M. Kunkel, R.H. Brown Jr., Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.

A 14 kb cDNA sequence has been isolated as the transcript of the Duchenne's muscular dystrophy (DMD) gene (Koenig et al, Cell, 50:509-517, 1987). Recently, polyclonal antibodies have been generated which recognize distinct regions of the DMD protein (Hoffman et al., Cell, in press). Using a cDNA probe (FSM5-1) and these polyclonal antibodies we have examined human and mouse muscle in vitro for the expression of this gene. All primary human muscle cultures tested express the DMD transcript and protein at the myotube stage, with and without innervation with fetal mouse spinal cord cells or neuroblastoma-glioma hybrid cells (NG108-15). By contrast, proliferating human myoblasts do not express the transcript or protein. Similarly, transcript was not detected in the following lines: skin and muscle fibroblasts of both human and mouse origin, human lymphoblasts, mouse spinal cord cells, NG108-15 cells, and myotubes from the transformed rat L6 cell line. The DMD transcript could not be detected in any of these non muscle cells. These results suggest that an in vitro model of normal and DMD muscle will be a potent system to investigate the developmental control and tissue specificity of DMD gene expression. Such investigations will elucidate the function of this protein and may assist in developing treatments for this lethal disease.

M 519 TISSUE-SPECIFIC REGULATION OF HUMAN GLYCOGEN PHOSPHORYLASE GENE EXPRESSION, Jean M. Lockyer, Tulane University Medical School, New Orleans, LA 70112.

Glycogen phosphorylase catalyzes the phosphorolysis of glycogen to glucose-1-phosphate. Three isozymes have been described: the brain type is found predominantly in brain and embryonic tissue and the liver and muscle forms predominate in liver and muscle tissues, respectively. The brain (or fetal) type has also been shown to be abundant in adult cardiac tissue. We are characterizing the regulation of expression of human muscle and brain phosphorylase in striated muscle. It is postulated that only the embryonic form is expressed in fetal skeletal and cardiac muscle. A subsequent developmental switch takes place whereby the muscle isozyme completely replaces the brain type in adult skeletal muscle and is expressed concurrently with brain phosphorylase in adult cardiac tissue. We have compared the expression patterns of RNA encoding brain and muscle phosphorylase in human fetal and adult cardiac and skeletal muscle tissue. Total RNA was isolated from each tissue and analyzed by northern blots using isozyme-specific oligonucleotide probes. Both cardiac and skeletal muscle fetal tissues exhibited a similar pattern of phosphorylase RNA expression. The major RNA species was detected using the brain-specific probe at a size of 4.1kb. A minor band was observed at a size of 3.6 kb after probing the RNA with the muscle oligonucleotide. Adult skeletal muscle tissue contained a high abundance of muscle phosphorylase RNA with only a very minor amount of the brain type. Adult cardiac tissue showed the presence of almost equivalent amounts of each of the RNA types. We have obtained human genomic clones for both the brain and muscle phosphorylase genes. We are currently comparing the fine structure of their 5' regions and determining elements which may regulate gene expression.

M 520 **EXON 1 SEQUENCES REQUIRED FOR TROPONIN I GENE EXPRESSION**
William Nikovits, Jr. and Charles P. Ordahl, UCSF, SF, CA 94143.

In order to study promoter sequences which are required for expression of the skeletal troponin I (sTnI) gene, we have transfected chimeric genes into embryonic skeletal muscle cells. Putative regulatory regions of sTnI were fused to the chloramphenicol acetyltransferase (CAT) gene and expression was assayed following transient transfection into primary cultures of embryonic skeletal muscle. A chimeric construction containing 1000 upstream nucleotides and all of sTnI exon 1 is active in skeletal muscle cells but is inactive (activity comparable to promoterless pBR-CAT) in fibroblast cells. 5' deletions of upstream flanking sequence to position -450 are fully active in skeletal muscle cells, however, deletion of exon 1 sequence reduces chimeric gene expression 30-fold in skeletal muscle cells. The reduced activity of the exon 1-deleted chimera remains muscle specific, because it continues to be inactive in fibroblast cells. Conserved sequence motifs have been found within the first exon of sTnI and other muscle-specific genes. In order to test the possible regulatory role of such sequences, a series of chimeric genes were constructed in which sTnI exon 1 sequence was progressively deleted from the 3' end. The activity of these constructions shows a progressive diminution with deletion of exon 1 sequence. Sequences within sTnI intron 1 have been implicated in the control of sTnI transcription in stably transfected mouse muscle cell lines (1). Our results indicate that sequences within exon 1 are also required for efficient expression of the sTnI gene in transiently transfected chick embryonic skeletal muscle cells in primary culture. We are currently testing possible mechanism(s) by which exon 1 sequences affect gene expression using chimeras with heterologous promoters and genes.

Reference (1) Konieczny & Emerson [1987] Mol Cell Biol 7:3065.
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M 521 CHARACTERIZATION AND PURIFICATION OF α-TROPOMYOSIN(TM) GENE ENHANCER NUCLEAR BINDING PROTEINS. Sergio Onate-Betancour, Kevin P. Claffey and Nelson Ruiz-Opazo. Boston University Medical Center, Boston MA 02118.
The αTM gene enhancer exhibits complex regulatory activity. It determines the developmental and tissue-specific expression of the αTM transcription unit as well as the developmental and constitutive expression of a second (N5) unit transcribed in the opposite orientation. The αTM-N5 enhancer has a repeating modular structure, 100bp repeat; and is located 700bp and 500bp upstream of the αTM and N5 "TATAA" boxes, respectively. To dissect the mechanisms of differential regulation of 2 transcription units by a common enhancer, we have identified putative nuclear trans-acting factors which bind to the 100bp repeat by protein-DNA blotting analysis. Nuclear extracts from Hep 3B(liver) cells have 6 different nuclear binding proteins ranging from 45kd to 135kd. In contrast, C_2(muscle) cell nuclear extracts have 5 nuclear binding proteins of lower molecular weights 30-45kd of which the 45kd nuclear binding protein is common to both cell lines, while the 4 smaller ones(≃30,32,35 and 40kd) are muscle-specific. To further characterize the muscle-specific nuclear factors, we performed sequence-specific DNA affinity chromatography using the αTM-N5 enhancer 100bp repeat. After a third-pass affinity purification step, we have been able to purify the 5 nuclear factors present in muscle(C_2) cells. Based on silver staining and protein-DNA blotting analysis, we estimate that each of the 5 factors(≃30,32,35,40 and 45kd) has been purified ≃50,000 fold, 80% homogeneity. The ability to identify and purify αTM-N5 enhancer-specific nuclear binding proteins will allow us to progress toward the understanding of the differential regulation of 2 transcription units by a common enhancer.

M 522 EFFECTS OF CONTRACTILE ACTIVITY ON MYOSIN EXPRESSION IN CULTURED CHICK MYOTUBES, Sabine Düsterhöft and Dirk Pette, Faculty of Biology, University of Konstanz, D-7750 Konstanz, Federal Republic of Germany.
Chick myotubes were directly stimulated at a mean frequency of 2 Hz starting at day 5 in culture. Incorporation of ^{35}S-methionine into LC3f was higher in stimulated than in control cultures. Because the amount of in vitro translatable RNA for LC3f was high both in control and stimulated myotubes, these findings suggest that the higher synthesis of LC3f resulted from increased translation. In contrast to the strong increase in LC3f synthesis, only a slight increase of LC3f was electrophoretically detectable in purified myosin or myofibrillar extracts. Immunohistochemistry using an antibody specific for LC3f [1] revealed a much stronger reaction in stimulated than in control myotubes. This discrepancy might be explained by assuming that LC3f, although being synthesized at a higher rate, is only weakly bound to the myosin heavy chain (HC) expressed in stimulated myotubes. Consequently, it might be degraded at a higher rate and also partially be lost during myosin purification. Electrophoretic HC analyses revealed, in stimulated myotubes, an additional HC cross-reacting with antibodies against slow myosin (HPM 9; CCM 52 [2]). Immunohistochemistry with antibodies against different isoforms of the fast HC (Ab's 12C5.3; 5C3.2 [3]) revealed that the embryonic HC had decreased and was partially replaced by the neonatal HC.

1. Silberstein, L. & Lowey, S. (1981) J. Mol. Biol. 148:153-189
2. Kennedy, J.M. et al. (1986) J. Cell Biol. 103:977-983
3. Winkelmann, D.A., Lowey, S. & Press, J.L. (1983) Cell 34:295-306

M 523 HETEROGENEITY OF CHOLINESTERASE mRNA IN HUMAN MUSCLE TISSUES APPROACHED BY MOLECULAR CLONING. Catherine Prody[1,2], Dina Zevin-Sonkin[1], Edwardo Schejter[1,3], Haim Zakut[3], and Hermona Soreq[1]. 1. The Hebrew University, Jerusalem, 2. The Weizmann Inst. of Science, Rehovot, and 3. Edith Wolfson Med. Cent., Tel-Aviv Univ., Israel

To study the molecular origin for the heterogeneity of cholinesterases in human muscle, poly A+ RNA was prepared from different types of fetal and adult and cardiac muscles, and analyzed by blot hybridization using a full length human butyrylcholinesterase (ChE) cDNA probe. Several different sizes of muscle RNA bands hybridized with the ChEcDNA probe under low stringency conditions. A cDNA library was hence prepared from 21 week fetal human muscle poly A+ RNA and screened with ChEcDNA as well as with several oligodeoxynucleotide probes synthesized according to protein sequences in domaines that are heterologous in other cholinesterases. Twelve phages out of $4x10^5$ were found positive with ChEcDNA while five others were picked with the oligodeoxynucleotide probes. When these five were purified and rescreened, they were also positive with ChEcDNA under low stringency conditions. The possibility that these phages include cDNA inserts representing other types of human cholinesterases is currently being examined.

M 524 STORAGE OF A PHOSPHORYLATED FORM OF DESMIN IN A HUMAN MYOPATHY, L. Rappaport*, F. Contard*, C. Delcayre*, FSM Tome, M. Fardeau, INSERM *U 127 and U 153 Paris, France.

An autosomal dominant muscle disorder characterized by the accumulation of an electron-dense granulo-filamentous material facing the Z-lines within the cytoplasm of skeletal muscle fibres has been described in 2 families (Fardeau et al, Rev. Neurol., 1978). These electron-dense structures have been shown to react strongly with antibodies which were raised against gizzard desmin. Dot-blot immunoquantitation of desmin in protein extracts of muscle biopsies showed a three fold increase in the amount of desmin in this muscle disorder. Immunoblots of the 2D polyacrylamide gels of these protein extracts revealed a phosphorylation of desmin with up to 6 isoforms being detected. A similar storage of phosphorylated desmin exhibiting a characteristic striated pattern has also been reported to occur in normal bovine Purkinje fibres (U. Kjovell et al., Eur. J. Cell. Biol. 1987). Thus, it is possible that the change in distribution of intermediate filaments that we have seen in these biopsies is not the direct consequence of either the phosphorylation or the increased amount of desmin although this remains to be studied in more detail together with possible changes in other myofibrillar or cytoskeletal proteins.

M 525 TROPONIN-T VARIANTS ARE ASSOCIATED WITH FUNCTIONAL HETEROGENEITY AMONG SINGLE FIBERS FROM NEONATAL AND ADULT AVIAN SKELETAL MUSCLES. Peter J. Reiser, Marion L. Greaser and Richard L. Moss, University of Wisconsin, Madison, WI 53706.

The genetic basis for the expression of a highly heterogeneous regulatory protein of striated muscle, TnT, believed to be involved in the regulation of the Ca^{2+}-sensitivity of tension generation, has been studied extensively by others. Our objective was to determine the physiological significance of the different forms of TnT in developing and in fully differentiated muscles. Relative tension/pCa curves were constructed by measuring the tension generated by single, chemically-skinned muscle fibers in a series of solutions with varying pCa values. Curve shifts along the pCa axis and/or differences in curve steepness were interpreted as differences in Ca^{2+}-sensitivity. The protein composition of the same fibers was determined using SDS-PAGE. The results indicate that the Ca^{2+}-sensitivities of single fibers from the adult fast-twitch pectoralis major (PM) and posterior latissimus dorsi (PLD) muscles are different from each other and both differ from the Ca^{2+}-sensitivity of the adult slow anterior latissimus dorsi (ALD) muscle. Furthermore, the Ca^{2+}-sensitivities of the neonatal PM and PLD fibers differ from each other and from the respective adult fibers. Neonatal and adult ALD fibers have virtually identical Ca^{2+}-sensitivities. The differences in the Ca^{2+}-sensitivities of the neonatal and adult fast-twitch fibers are consistently associated with differences in TnT isoform composition while no differences are observed in the other components of the troponin complex, i.e., TnI and TnC. In addition, the TnT, TnI and TnC of ALD fibers have identical electrophoretic mobilities at neonatal and adult ages. The results strongly suggest that the specific TnT isoform composition of single muscle cells plays a role in determining the Ca^{2+}-sensitivity of tension generation in developing and in fully differentiated muscle.

M 526 THE α-TROPOMYOSIN (TM) GENE ENHANCER DIFFERENTIALLY REGULATES THE TISSUE-SPECIFIC AND DEVELOPMENTAL EXPRESSION OF TWO TRANSCRIPTION UNITS. Nelson Ruiz-Opazo and Victoria L. Herrera. Boston University Medical Center, Boston MA 02118.

A single promoter region regulates the developmental and tissue-specific expression of the αTM gene. Nucleotide sequence analysis of the 5' flanking region has identified a 100bp repeat 0.7kb upstream of the αTM "CATAT" box. The 100bp repeat consists of 2 modular halves; an upstream 40bp-long poly d(CA/GT) tract and a downstream 60bp-long unique sequence containing a 12bp sequence 9/12 homologous to the GT-I motif of the SV40 viral enhancer. Enhancer activity was tested by the transfection of mouse muscle cell lines(G_8 and C_2C_{12}) and non-muscle cell lines (Hela, Hep3B and 3T3) with chimeric chloramphenicol acetyltransferase(CAT) genes driven by the putative αTM enhancer and subsequent assay for CAT activity. A panel of chimeric αTM+CAT genes consistently detected position-and orientation-independent enhancer activity only in myotubes. Transfection of myoblasts and non-muscle cell lines showed that the αTM enhancer acts in a developmental and tissue-specific manner. Recently, we found a second transcription unit,N5, located 500bp upstream of the αTM enhancer 100bp repeat. N5 is expressed in all tissues tested so far (liver, uterus, heart, brain, kidney). A panel of chimeric N5+CAT genes revealed that the 100bp repeat induced CAT activity from the N5 "TATAA" box in muscle(only in myotubes) and non-muscle cell lines. These results show complex regulatory activity of the N5-αTM gene enhancer: the developmental and tissue-specific expression of the αTM transcription unit, as well as the developmental and constitutive expression of the N5 transcription unit. Furthermore, it shows the involvement of a poly d(CA/GT) tract in the structure and activity of an enhancer.

M 527 AMP DEAMINASE GENE AMPLIFICATION REVEALS CLOSE LINKAGE OF FOUR COORDINATELY REGULATED GENES DURING MYOGENESIS, R.L. Sabina, Duke Univ., Durham, NC 27710.

Coformycin resistance in Chinese hamster fibroblasts is associated with co-amplification of four unknown genes (i.e. W,X,Y_1,Y_2) presumably closely linked to the target gene, AMP deaminase (Debatisse et al, Mol. Cell Biol. 6: 1776, 1986). The recent cloning of rat AMP deaminase cDNA (Sabina et al, J. Biol. Chem. 262: 12397, 1987) has provided the opportunity to further analyze this presumed amplicon. In order to accomplish this goal a coformycin-resistant rat myocyte subline was established from the parental L6 cell line. One stably resistant clonal isolate (i.e. clone 17) exhibits a 7-fold increase in AMP deaminase gene sequences. Hybridization with the four hamster cDNA's for genes W,X,Y_1, and Y_2 indicates that all are co-amplified in clone 17. These results delineate the AMP deaminase amplicon and document its conservation between hamster and rat. As an approach to investigate the identity of these co-amplified genes, their expression was monitored in various tissues and cell types. Surprisingly it was observed that at least three of the genes (i.e. W,X, and Y_2) are coordinately expressed with the embryonic transcript of AMP deaminase in the following pattern: low-level expression in early embryo, increasing during neonatal skeletal muscle development, then ultimately down-regulated in adult skeletal muscle. This temporal pattern of expression is also observed during differentiation of myocytes in vitro. These results establish the coordinate regulation of four closely linked genes during skeletal myogenesis. Furthermore, it suggests that developmentally associated gene clusters may exist in mammalian cells.

M 528 TRANSCRIPTION OF HUMAN CHOLINESTERASE GENES LOCALIZED BY IN SITU HYBRIDIZATION TO NEUROMUSCULAR JUNCTION NUCLEI. Hermona Soreq[1], Edwardo Schejter[1,2], Patrick Dreyfus[1] and Haim Zakut[2]. 1. Dept. of Biological Chemistry, The Life Sciences Inst., The Hebrew Univ., Jerusalem and 2. Dept. of Obst. & Gynecol., The Edith Wolfson Med. Center, Tel-Aviv Univ., Israel

Transcription of the human cholinesterase (ChE) genes was studied in developing muscle by in situ hybridization with (^{35}S)-ChEcDNA, using frozen fetal and adult muscle sections. Neuromuscular junctions were localized by anti-ChE immunofluorescence in the same sections used for hybridization, and confirmed by ChE activity staining in subsequent sections. Active transcription of the ChE genes, revealed by emulsion autoradiography, was co-localized with ChE staining in neuromuscular junctions. Intense labeling could be observed as early as 17w gestation, co-localized with histochemically immature junctions with simple gutter-like structures. Our findings demonstrate that the accumulation of cholinesterases in neuromuscular junctions is due to the induction of ChE genes transcription in perijunctional muscle nuclei.

M 529 REEVALUATION OF THE TROPOMYOSIN ISOFORM DIVERSITY: GENE CLASSIFICATION AND STRUCTURE-FUNCTON RELATIONSHIP, Johannes Sri Widada, Soudhir Colote, Conception Ferraz, Jean-Pierre Liautard, Francois Bonhomme and Jacques Marti, C.R.B.M. du CNRS, U. 249 de l 'INSERM, BP 5051, 34033 Montpellier Cedex, France.

The tropomyosins are a family of highly related proteins present in all types of tissue, although different isoforms are characteristic of particular cell type.

We have cloned and determined the nucleotide sequence of a cDNA encoded by a newly isolated human tropomyosin gene. Evolutionary analysis has allowed us to clarify the classification and evolution of the tropomyosin genes expressed in vertebrates. Our analysis also showed that different functional pressures can be exerted on different part of a single gene, resulting in different evolutionary rates and convergences on the different parts of the molecule. These pressures may arise from genomic constraints and interactions of the tropomyosin with other cellular components i.e. specific proteins.

Finally, at least one of the tropomyosin cDNA clones contains repeat elements in the 3'UTR. We are performing experiences to elucidate whether this sequence accounts for the differential expression of this isoform.

M 530 A 3'NON-CODING SEQUENCE OF A HUMAN COLLAGEN mRNA IS CONSERVED IN TWO REGULATED RAT MUSCLE RNAS AND RECOGNIZED BY NUCLEAR PROTEINS.
Anna Starzinski-Powitz, Thomas Herget, Marion Reich and Katrin Zimmermann, Institute of Genetics, COLOGNE, FRG.
We have identified 2 cytoplasmic RNAs in rat L6 cells encoding for so far unidentified proteins. These 2 RNAs are turned off about 72 hours after induction of L6 myotube formation in serum-free medium. Both RNAs contain a conserved sequence element (97% homologous to human) which is recognized by nuclear proteins from L6 cells as tested with gel retardation assays and DNase I footprints. DNA-binding of the nuclear proteins is not detectable anymore when the 2 RNAs are turned off. Thus, regulation of the nuclear proteins correlates with the transcription (Northern blot analysis) of the sequences which they are able to recognize. Expression of the two RNAs identified by the conserved sequence as well as the nuclear proteins recognizing this sequence can be re-induced by serum-factors 80 hours after transfer of the cells to serum-free medium. The experiments described suggest that serum-free induction of myotube formation in culture provides a useful tool to discriminate between those genes which are more likely to be regulated intrinsically and others, whose expression may require the permanent presence of exogenous factors.

M 531 STRUCTURAL ANALYSIS OF A GENE ENCODING HUMAN MUSCLE NEBULIN, Hansell Stedman, Marion Cronizi-Scott, Kenneth Fischbeck, James Sylvester, Roy Schmickel, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, Karen Browning, Kuan Wang, University of Texas at Austin, Austin, TX 78712, Noelynn Oliver, Satyapriya Sarkar, Tufts University, School of Medicine, Boston, MA 02111.
Using polyclonal antinebulin antisera to screen a human fetal muscle cDNA expression library, we have isolated and characterized nonidentical cloned cDNA fragments. All such cDNAs thus far characterized have mapped to human chromosome two and have detected an approximately 25 kb transcript on Northern blots. Use of the cloned fragments to rescreen another cDNA library has enabled the reconstruction of most of the full length transcript. Preliminary sequence analysis of the clones detected with one probe has identified the transcript's 3' end. Furthermore, cloned subfragments have been used in RFLP detection and construction of a partial long range restriction map of the genomic locus. These findings are discussed with reference to myosin heavy chain and DMD gene structure.

M 532 AN ENHANCER UPSTREAM OF THE MUSCLE CREATINE KINASE (MCK) GENE FAILS TO FUNCTION IN MYOBLASTS BEARING MUTATIONALLY ACTIVATED ras ONCOGENES. E. Sternberg, G. Spizz, and E. N. Olson. M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.
Differentiation of skeletal myoblasts is inhibited by exposure to TGFβ or by expression of mutationally activated ras proteins, which are postulated to couple specific cell surface growth factor receptors with intracellular effectors. To define the signaling pathways and nuclear targets utilized by growth factors to regulate muscle-specific gene expression, the mouse MCK gene was isolated. Sequences from the MCK upstream region were localized that confer muscle- and differentiation-specific expression on a linked reporter gene, (CAT), in the C2 muscle cell line. Deletion mutation of the region upstream of the MCK gene extending from -4800 base pairs to the transcription initiation site revealed a muscle-specific enhancer centered around -1200 that directed high-level expression of CAT in myotubes, but not in myoblasts or non-myogenic cell lines. The MCK enhancer also conferred muscle-specific regulation on the SV40 promoter, but exhibited a 3- to 5-fold preference for its own promoter. Synthetic oligomers comprising different domains of this enhancer, and harboring specific nucleotide mutations, are currently being employed to investigate the precise sequence requirements for enhancer activation. Expression of mutationally activated ras oncogenes in C2 cells was sufficient to extinguish expression of CAT genes under transcriptional control of the MCK enhancer. Similarly, action of the MCK enhancer was repressed in myoblasts exposed to TGFβ. Together, these results suggest that growth factor signals that suppress muscle-specific gene induction,do so by preventing activation of positive regulatory elements required for transcriptional activation of muscle genes.

M 533 GENE EXPRESSION OF THE (Na+K)ATPase IN CULTURED CHICKEN SKELETAL MUSCLE FIBERS, J.Taormino, K. Takeyasu, and D. Fambrough, Johns Hopkins University, Baltimore, MD 21218.

The expression of the (Na+K)ATPase in cultured chicken muscle cells can be altered by varying the demand for ion transport. Veratridine causes an approximate two-fold increase in β-subunit found at the sarcolemma, while tetrodotoxin can rapidly and completely reverse this effect. Using cDNA probes, we have examined the levels of (Na+K)ATPase mRNA. We found that the β-subunit mRNA increases several-fold. The kinetics of this increase parallel the rate of β-subunit protein synthesis. We are also interested in the relationship between the α and β-subunit mRNAs, and regulation within the alpha gene family. Although "kidney-type" α-subunit mRNA increases after exposure to veratridine, this increase is not as great as for β mRNA, and occurs at a later time. Preliminary results suggest that the level of "brain-type" α mRNA in cultured skeletal muscle is not affected by veratridine. Tetrodotoxin has little or no effect on α or β mRNA levels. Using a nuclear run-off assay, we found increases in α and β mRNAs during up-regulation are reflected in increases in transcriptional rates. Tetrodotoxin does not seem to affect (Na+K)ATPase gene transcription. Thus, up-regulation stimulated by veratridine is mediated by increases in transcription rate, production of new (Na+K)-ATPase, and insertion into the plasma membrane, while down-regulation in response to tetrodotoxin is mediated by a different mechanism.

M 534 TEMPORAL EXPRESSION OF TRANSLATIONAL INHIBITORY RNA BY EMBRYONIC SKELETAL MUSCLE, C. R. Vanderburg, and M. A. Nathanson, New Jersey Med. Sch., Newark, N.J. 07103

Previous investigations in this laboratory have shown that the onset of muscle-specific protein synthesis is controlled in part by RNA which has the ability to inhibit protein synthesis in vitro. This RNA has been isolated from embryonic rat thigh muscle from embryos of 20 days gestation. The inhibitory RNA is non-polyadenylated and occurs in a single fraction of 160-2000nt (fraction 4). Mechanistic studies revealed that the inhibitor acts at the level of initiation, apparently through an interaction with components of the translational apparatus. The inhibitor has been thought to be embryo specific, since it was isolated from embryonic muscle and was absent from correspondingly fractionated adult muscle. In the present study, we have investigated its occurrence in rat skeletal muscle from 18 days of gestation through 5-day neonates. The translational status at each stage was assessed by: 1) inhibitory potential of "fraction 4" RNA, 2) total mRNA content per cell, 3) total RNA content per cell, and 4) muscle morphology. Our data indicates that the amount and translational efficiency of mRNAs from each stage were approximately equal and that the total RNA content varied only slightly. However, with regard to the effect of "fraction 4", we find increasing translational inhibitory capacity from 19-20 days in utero and an absence of inhibition in 5-day neonates. Thus, translational efficiency correlates solely with the potency of "fraction 4" RNA. We propose that the transient expression of such inhibitory RNA in developing skeletal muscle acts to fine-tune the synthesis of muscle-specific proteins from those required during assembly of sarcomeric components in the embryo to those required in functional motility.

M 535 REGULATION OF ALTERNATIVELY-SPLICED α-TROPOMYOSIN ISOFORM PRODUCTION BY ENDOGENOUS AND EXOGENOUS FACTORS, David F. Wieczorek, University of Cincinnati Medical School, Cincinnati, Ohio 45267.

The rat α-tropomyosin (α-TM) gene is encoded in 13 exons, 7 of which are alternatively spliced in a tissue-specific manner. This gene arrangement includes two different 3' ends and a promoter which appears very similar to other "housekeeping" promoters in both its pattern of utilization and in its lack of canonical sequence elements. My studies demonstrate that the α-TM gene generates a minimum of 6 different mRNAs each with the capacity to code for a different protein. These distinct TM isoforms are expressed specifically in nonmuscle, smooth, and striated (cardiac and skeletal) muscle cells through alternative mRNA processing.

My investigations addressing the mechanisms and factors which regulate α-TM expression demonstrate that factors produced in neural tissue act at a post-transcriptional level to control α-TM transcript production. In the presence of nerve extract, muscle cells dramatically decrease their production of the striated specific isoform and concomitantly increase a specific cytoplasmic isoform. Furthermore, this regulation of α-TM expression also occurs in nonmuscle cells by differentially regulating multiple cytoplasmic transcripts. These results demonstrate that the mechanisms and processes which regulate α-TM expression are common to many different cell types and cell lineages.

M 536 UNCOUPLING OF MYOTUBE FORMATION AND PBU65 FROM OTHER MUSCLE PROTEIN EXPRESSION, Woodring E. Wright, Gill Cockerill and Massoud Mammoudi U.Texas Southwestern Medical Center, Dallas, TX 75235

Several myogenic cell lines have been selected for their ability to differentiate after incorporating 5-bromodeoxyuridine (BUdR) into their DNA. These lines are thought to overcome the inhibitory effect of BUdR by overproducing the factors whose action is normally inhibited by BUdR. Since the cells were only selected for the ability to form morphologically identifiable myotubes, only those factors in the sequence of events up to and including myotube formation should be resistant to the effects of BUdR. The expression of creatine kinase and acetylcholine receptor activity and the messages for the skeletal myosin heavy chain, troponin T, creatine kinase, acetylcholine receptor alpha subunit and pBU65 were examined in BUdif$^+$ myoblasts that had been grown in the presence versus the absence of BUdR to explore the possibility of the differential expression of these products under these conditions. All of the above factors followed similar time courses of appearance in the the BUdif$^+$ cells grown in the presence of BUdR and in the parental L6 myoblasts. However, although the appearance of pBU65 and myotubes appeared at an accelerated rate in BUdif$^+$ cells grown in the absence of BUdR, the appearance of the muscle-specific proteins was delayed. One consequence of this delay was that the myotubes formed in the absence of BUdR were very unstable and detached soon after forming. Although the detailed molecular mechanism remains unclear, these results demonstrate that myotube formation and pBU65 expression can be uncoupled from the expression of other muscle-specific functions and thus are controlled by a different constellation of regulatory factors.

M 537 DETECTION OF THE DMD GENE PRODUCT(S) IN SKELETAL MUSCLE USING ANTIBODIES PREPARED AGAINST SYNTHETIC PEPTIDES AND FUSION PROTEINS, E.E. Zubrzycka-Gaarn, D.E. Bulman, G. Karpati, A.H.M. Burghes, H.J. Klamut, J. Talbot, R.S. Hodges, P.N. Ray, and R.G. Worton. The Hospital for Sick Children, Toronto, Ontario M5G 1X8 , Canada, Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada and University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

Hydrophilic synthetic peptides were made corresponding to the amino acid sequences deduced from cDNA sequences isolated from the DMD gene locus. Fusion proteins were prepared by subcloning fragments of this cDNA into expression vectors. Antibodies against these peptides and fusion proteins were raised in rabbits. The affinity purified antibodies were used for detection of the DMD gene product(s) in skeletal muscle and other tissues.

Western blotting and immunostaining technique detected a 400 kDa band in detergent extracts of normal human muscle that was not detected in DMD muscle or in normal fibroblasts.

Localization of the DMD protein was carried out using an indirect avidin-biotin based immunoperoxidase technique on cryostat sections of human muscle biopsies and with an indirect immunofluorescence technique on normal human clonal muscle cells grown in culture. Consistently strong sarcolemmal and a weaker endomysial reactivity was present in muscle fibers of 9 normal controls and 14 miscellaneous, non DMD, disease controls. This reactivity was not present with preimmune sera. In four Duchenne biopsies this sarcolemmal reactivity was substantially less or absent. Immunofluorescent staining was observed on the surface of differentiated myotubes in culture and was not seen with preimmune sera or the second antibody alone.