## MOLECULAR BIOLOGY OF MUSCLE DEVELOPMENT

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## *Cellular Aspects of Myogenesis*

**0490** REQUIREMENTS FOR MUSCLE GENE ACTIVATION BY PUTATIVE TRANS-ACTING REGULATORS, Helen M. Blau, Choy-Pik Chiu, Grace K. Pavlath, and Edna Hardeman, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

We initially reported that muscle gene expression could be induced in fibroblasts. This was achieved by producing heterokaryons, stable multinucleated somatic cell hybrids formed by the fusion of mouse muscle cells (C2) with human amniotic fibroblasts. This heterokaryon system is particularly advantageous for analyzing requirements for cell specialization, since the chromosomes and cytoplasmic regulatory molecules of both cell types remain present.

We examined the requirements for muscle gene activation in heterokaryons using four different strains of human fibroblasts (primary cells from amniotic fluid, fetal and adult skin and MRC-5 fibroblasts from fetal lung), chondrocytes and keratinocytes. Eight distinct human muscle gene products were detected including two sarcomeric actins ($\alpha$-cardiac and $\alpha$-skeletal), four myosin light chains ($LCI_s$, $LC2_s$, $LC2_f$, and $LC_{emb}$), the M-subunit of the contractile enzyme creatine kinase, and a cell surface antigen, 5.1H11. A single cell analysis revealed that the efficiency of activation was high; as many as 95 ± 1% of heterokaryons containing at least one human fibroblast nucleus expressed human muscle 5.1H11. This activation did not require significant DNA synthesis. Muscle gene activation occurred regardless of gene dosage, i.e., the ratio of the nuclei of the two cell types contained inside a heterokaryon. The induction of expression of two human muscle genes was detectable at the transcriptional level. Finally, muscle gene expression could be induced in a previously non-responsive cell type, by pre-treatment with 5-azacytidine.

We conclude that the expression of differentiated functions characteristic of muscle can be induced in several different specialized nonmuscle cells and that this activation occurs in the absence of a round of DNA replication. This suggests that alterations in chromatin structure requiring significant DNA synthesis are not necessary for muscle genes in these cells to be accessible to and respond to putative trans-acting regulatory factor(s) present in differentiated muscle cells. In contrast, in at least one cell type, muscle genes or the genes that regulate their expression, are not receptive to muscle regulatory factors, unless the cells are pretreated with 5-azacytidine which presumably results in a reduction in the level of DNA methylation. Finally, contrary to expectation, an excess of mouse muscle nuclei was not required for activation. We conclude that the nuclei of a number of cell types exhibit considerable plasticity in response to putative trans-acting regulators and are capable of being reprogrammed to express muscle functions.

Supported by NIH grants HD18179 and GM07149 and the Muscular Dystrophy Association.

**0491** MITOGENIC REGULATION OF SKELETAL MUSCLE DIFFERENTIATION, S. Hauschka, C. Clegg, R. Lim, J. Chamberlain, J. Jaynes, C. Bulinski, T. Linkhart, and G. Merrill, Dept. of Biochemistry, University of Washington, Seattle, WA 98195.

The terminal differentiation of MM14 mouse myoblasts is regulated by a repression mechanism mediated by fibroblast growth factor (FGF). FGF, but not other mitogens such as EGF and PDGF, prevents differentiation and stimulates proliferation. FGF represses muscle differentiation directly, rather than indirectly via proliferation, because the continuous exposure of non-cycling myoblasts to FGF prevents their differentiation. When deprived of FGF log phase MM14 cells continue to traverse the cell cycle at their normal rate; but after a 2-3 h lag they cease entering S phase and accumulate in the $G_1$ compartment. $G_1$ phase cells deprived of FGF for longer than 3 h commit to terminal differentiation and will not re-enter the cell cycle when FGF is restored. Proof that cells commit to terminal differentiation in $G_1$ and that the commitment process does not require DNA synthesis following exposure to a differentiation-inducing environment is based on the observation that mitotic cells plated into FGF-free medium differentiate without further replication. 5-6 h after FGF removal (2-3 h after the initiation of commitment) myocytes accumulate detectable M-creatine kinase mRNA, and within another 1-3 h they stain positively for AChR, MHC, CK and $\alpha$-actin, concurrently EGF receptor and TK disappear. Loss of specific mitogen receptors would provide an attractive mechanism for maintaining myocytes in a permanent post-mitotic state.

To determine whether the post-mitotic myocyte phenotype is mediated via a diffusible regulator, myocytes were fused with $G_1$ myoblasts or with various non-myogenic cells, exposed to FGF-rich medium, and assayed for DNA synthesis. (Myocyte x $G_1$ myoblast) heterokaryons failed to replicate DNA even at myocyte:myoblast nuclear ratios of 1:3. The post-mitotic phenotype is thus strongly dominant to the proliferation phenotype of $G_1$ myoblasts. In contrast, (myocyte x non-myogenic cell) heterokaryons replicated DNA and could be shown to reexpress the normally inactive myocyte TK gene. The post-mitotic myocyte phenotype is thus not dominant to the proliferation phenotype of non-myogenic cells. However, if (myocyte x non-myogenic) heterokaryons were maintained in FGF-free medium for 6-18 h prior to the addition of FGF, increasing percentages of the heterokaryons exhibited dominance of the post-mitotic phenotype. The component responsible for causing the post-mitotic state of myocytes is thus also capable of establishing this phenotype in non-myogenic cells if permitted sufficient time prior to the activation of proliferation signals. These studies suggest a model for the normal regulation of myogenic differentiation in which a component from the FGF-mediated replication pathway represses myoblast differentiation; whereas in the absence of FGF, differentiation is activated and a diffusible factor is produced which counteracts the mitogenic response system; thus conferring the post-mitotic myocyte phenotype to the committed cell.

0492 SOMATIC CELL GENETIC ANALYSIS OF MYOGENESIS, Woodring E. Wright, Department of Cell Biology, University of Texas Health Science Center, Dallas, Texas 75235

A variety of studies from our and other laboratories (1-4) indicate that differentiated skeletal muscle cells contain trans acting factors capable of inducing muscle structural gene expression in non-muscle cells following the fusion of the two cells to form a heterokaryon. Cells thus continue to express at least some of the regulatory molecules controlling myogenesis even after differentiation has occurred, and these molecules are capable of activating previously silent muscle genes in non-muscle cell types in the absence of DNA synthesis. Cell hybrid studies between differentiation competent L6 myoblasts (65% of the cells differentiate under standard conditions) and differentiation defective L6 myoblast variants (0.05% differentiate under standard conditions) demonstrate that the hybrids express an intermediate 2% probability of differentiation. A molecular model designed to explain these results required that differentiation defective myoblasts produce reduced levels of the regulatory factors controlling the decision to differentiate (5). Since differentiation defective cells are generated with high frequency, unless there is an intrinsic directionality to this process this also implied that variants overexpressing the factors should be spontaneously generated. Gene dosage heterokaryon experiments involving myoblasts grown in 5-bromodeoxyuridine (BUdR) (6) suggested that cells overexpressing these factors should be able to differentiate in higher than normal concentrations of BUdR. Multiple cycles of selection in the presence of increasing concentrations of BUdR resulted in the isolation of L6 variants able to differentiate in 30 times the normal clonal inhibitory concentration of BUdR (7). The density shift of DNA on cesium chloride gradients showed that these cells were incorporating BUdR relatively normally. As predicted from our theoretical model, cell hybrids formed between these cells and differentiation defective cells exhibited very high (>80%) probabilities of differentiation. Cells overexpressing these factors would be expected to differentiate rather than divide once the inhibitory influence of BUdR was removed. BUdR-resistant cells cloned in the absence of BUdR formed very small colonies, often containing 20 nuclei of which half were already fused to form a myotube. The availability of variants having an amplified expression for the factors regulating the decision to differentiate should greatly facilitate the molecular cloning of those factors.

1. J. Cell Biol. 98:427 (1984) 2. Exp. Cell Res. 151:55 (1984) 3. Cell 32:1171 (1983)
4. Cell 37:879 (1984) 5. J. Cell Biol. 98:436 (1984) 6. J. Cell Biol. 96:1571 (1983)
7. J. Cell Biol., in press (1985)

## *Cell Biology of Myogenesis*

0493 MULTIPLICATION STIMULATING ACTIVITY, OVINE SOMATOMEDIN AND INSULIN PROMOTE SATELLITE CELL PROLIFERATION IN VITRO, Ronald E. Allen, M. V. Dodson and K. L. Hossner, University of Arizona, Tucson, AZ 85721.

The ability of rat skeletal muscle satellite cells to proliferate in response to insulin, multiplication stimulating activity (MSA) and ovine somatomedin (oSm) was evaluated in vitro. In the presence of medium containing 3% horse serum and dexamethasone, all three polypeptides stimulated proliferation in a dose-dependent manner; in the absence of dexamethasone, however, only insulin was able to stimulate proliferation. In addition, satellite cell proliferation was examined in serum-free medium, and half-maximal stimulation was achieved at concentrations of 6 ng/ml, 15 ng/ml and 1295 ng/ml of oSm, MSA and insulin, respectively. Furthermore, at half-maximal concentrations of the respective polypeptides, insulin and oSm stimulated greater cell proliferation than MSA. In the presence of saturating concentrations of insulin in serum-free medium, oSm did not stimulate further proliferation; however, a dose-dependent stimulation of proliferation, over and above maximal insulin stimulation, was observed with MSA. These experiments demonstrate a direct action of insulin-like growth factors on satellite cells and provide indirect evidence for action through both IGF receptor types.

0494 CHARACTERIZATION OF MUSCLE CELL CLONES DEFECTIVE IN EXPRESSING THE ACETYLCHOLINE RECEPTOR, Roy A. Black and Zach W. Hall, U. of Calif.-San Francisco, San Francisco, CA 94143

We have isolated variants of the C2 muscle cell line which are defective in expressing the acetylcholine receptor (PNAS, in press). Clone 23 shows only about 15% of the wild-type surface level of receptor but accumulates an intracellular pool about five-fold greater than that of the wild type. In pursuing the nature of the defect in this clone, we have found that its acetylcholinesterase is externalized normally. Moreover, co-culturing the cells with either of two receptor-deficient clones fails to correct the defect. It therefore appears that the defect is in the acetylcholine receptor itself.

The two deficient clones we have isolated fail to complement each other. Surprisingly, in a co-culture with 80% of the cells from a deficient clone and 20% from the wild type, the level of receptor is the same as that expressed by a 100% wild-type culture. The level of receptor thus is not determined simply by the number of wild-type nuclei in the culture.

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0495 NERVE DEPENDANCE OF MYOBLAST DIFFERENTIATION IN CULTURES OF SOMITIC CELLS. Giulio Cossu and Elisabetta Vivarelli, Institute of Histology, University of Rome, Italy.

During mouse embryogenesis, differentiated, myosin positive cells appear in the somite at 9.5 days p.c. (Jockusch et al. Exp Biol Med 9, 121, '84). Cultures from somites of 9.5 days or older mouse embryos contain many myosin (or desmin) positive cells. However no myosin positive cells ever appear in cultures from somites of 8.5 days embryos. Thus pre-myogenic cells from this stage do not survive or fail to differentiate in culture. We investigated possible mechanisms which control the transition from a pre-myogenic to a myogenic cell. Cells from somites of 8.5 days embryos were cultured in the presence of 1mM cAMP, or in conditioned medium from a myotube-fibroblast culture, or in the presence of explants of spinal cord or brain extract from 13 days old mouse embryos. The addition of brain extract drastically decreased the survival of somitic cells while all the other conditions stimulated several fold their proliferation. However only the presence of spinal cord caused the appearence of myosin positive cells. In the conditions used, no outgrowth of axons had yet been observed and therefore no contact could occur between nerve and muscle. We conclude that the transition from a pre-myogenic to a myogenic cell from early somites is dependent upon the presence of factors released from the nerve, namely the spinal cord in vitro and likely the contiguous neural tube in vivo.

0496 EFFECT OF MONENSIN ON MYOBLAST FUSION. H. Den, Columbia University College of Physicians and Surgeons, New York NY 10032

Monensin, at a concentration of 0.5-10 uM, completely (100%) and reversibly inhibits fusion of embryonic chick myoblasts invitro. At the same time, monensin administration leads to a marked accumulation of glycopeptides inside the cells and a decrease of those secreted into the medium. Chromatography of the intrazcellularly retained glycopeptides on Con A-Sepharose shows that the increase is most pronounced in the high-mannose fraction. I \
Mild proteolysis of cells labeled with [2-$^{3}$H] mannose releases less radioactivity from the surface of monensin-treated than from control cells, although the amount of total radioactivity is almost four times higher than in the control cell. Since it has now been established that monensin interferes with the intracellular transport of newly synthesized biycoproteins it is assumed that its inhibitory effect is the result of the inability of glycoprotein(s) essential for myoblast fusion to reach the cell surface.

0497 STIMULATION AND INHIBITION OF MYOBLAST DIFFERENTIATION BY SOMATOMEDINS. by J. R. Florini, D. Z. Ewton, and S. L. Falen. Biology Dept., Syracuse Univ., Syracuse, NY.
It is widely believed that mitogens inhibit the differentiation of myoblasts by forcing them to reenter the cell cycle rather than remain in the $G_1$ phase at which fusion occurs. In apparent disagreement with this view, we have reported that the Insulin-like Growth Factors (IGFs) are highly mitogenic for myoblasts and are also potent stimulators of their differentiation; we have shown that this is not a simple result of the greater cell density in IGF-treated cultures. Recently completed concentration dependency studies now offer a possible explanation for this apparent discrepancy. We find that the somatomedins IGF-I and IGF-II, as well as insulin, all stimulate the differentiation of L6 myoblasts at relatively low concentrations, and exhibit a concentration-dependent decrease in this stimulation at higher levels. Treatment of myoblast cultures with IGF-II in the presence of saturating insulin (200 nM) gave half-maximal concentration for inhibition of differentiation (quantitated as units of creatine kinase per mg cell DNA) at 500 nM IGF-II. The half-maximal concentrations for stimulation of differentiation are: IGF-I, 1 nM; IGF-II, 25 nM, and insulin, 13 nM. On the down side of the biphasic curve, 50% of maximal differentiation occurred at: IGF-I, 13 nM; IGF-II, 500 nM, and insulin, 2000 nM. Our current view of these results is that stimulation of differentiation may result from the general increases in anabolic processes stimulated by these hormones, while the decreased differentiation at higher concentrations may be attributed to shortening of the $G_1$ phase of the cell cycle as part of their mitogenic actions. (Supported by grants from the USPHS and the Muscular Dystrophy Assoc.)

0498 SPINAL MOTOR NEURON CELL DEATH AND DEVELOPMENTAL CHANGES IN MYOSIN EXPRESSION, J.A. Hall, R.W. Oppenheim and F.H. Schachat, Departments of Anatomy, Duke University Medical Center, Durham, N.C. 27710 and Bowman Gray Medical School, Winston-Salem, N.C. 27103
Between days 10 and 19 in chick embryonic development there is a transition in myosin expression in pectoralis muscle. The pattern of myosin light chain expression switches from expressing both fast and slow light chains to expressing only the fast light chains characteristic of the muscle in the adult. There is also a change in myosin heavy chain expression. This change occurs during a major event in the maturation of the motor system, the death of half the spinal motor neurons which participate in the polyneuronal innervation of skeletal muscle. In order to determine whether there is a causal relationship between these two events, transitions in myosin expression have been studied in chick embryos treated with curare which prevents spinal motor neuron cell death by reducing neuromuscular synaptic activity. Using monoclonal antibodies and SDS-PAGE to analyze changes in heavy and light chain expression, respectively, we have found that curare treatment does not interfere with the changes in myosin expression. Because removal of spinal motor neurons at day 2 results in complete atrophy of most embryonic muscles by day 8, it is too simple to conclude that the myosin transition is independent of neuromuscular interaction. Rather it appears that either a trophic interaction or a low residual level of synaptic activity is sufficient to induce the change in myosin expression.

0499 HEPARIN INHIBITION OF MYOGENESIS IN CULTURE AND REVERSAL OF THE INHIBITION BY SELECTED MUSCLE OR NERVE EXTRACTS, Elissavet Kardami and Richard C. Strohman, U.C. Berkeley, Department of Zoology, Berkeley, Ca 94720.

In the process of investigating the factors which may modulate muscle gene expression and growth during development and regeneration, we examined the effect of various proteoglycans on chicken skeletal muscle primary cell cultures, in the presence of complete growth medium, on collagen coated plates. Our results have shown that chondroitin sulfate and hyaluronic acid at 50-500 ug/ml, do not significantly affect growth, while heparin, at 90 ug/ml, inhibits muscle cell growth by 50-80%, if added to the cells within 24 h from the time of plating. Heparin has no effect on 6-8 day old myotubes and does not inhibit the attachment of myoblasts or fibroblasts to the collagen substrate. Chicken ALD muscle, dystrophic breast muscle and sciatic nerve extracts reverse heparin inhibition if added to the cells within 3 days from the time of plating, while normal breast muscle extracts have significantly lower heparin reversal potential. The heparin reversal activity (HRA) is distinct from other muscle affecting activities (which are also present in the extracts) and can be selectively removed by heparin--Sepharose affinity chromatography. Heparin-like substances can occur in vivo, and in conbination with HRA may have a physiological role in controlling muscle growth during muscle development or regeneration following injury.-

0500 NERVE AND GROWTH FACTOR REGULATION OF MUSCLE DEVELOPMENT. Reid S. Compton, Peter Merrifield and Irwin R. Konigsberg, Dept. of Biology, Univ. of Virginia.
The development of muscle from a determined myoblast to a physiologically adult muscle fiber involves two distinct processes - differentiation and maturation. Although pure populations of myoblasts differentiate normally in vitro they do not accumulate the adult-specific pattern of contractile protein isoforms indicating that these two processes are differentially regulated. In the past, we have demonstrated that primary cultures of quail myocytes down-regulate contractile protein synthesis as they reenter the cell cycle in response to a mitogenic stimulus. Using an established line of mouse myoblasts which is temperature sensitive for proliferation (ts-36), we have now shown that the down-regulation of contractile protein synthesis in ts-36 can occur without reentry into the cell cycle and that this down-regulation can be induced by FGF but not EGF. Since muscle maturation cannot be mimiced in vitro, we have used a monoclonal antibody to myosin fast alkali light chain to analyse the developmentally regulated expression of $MLC_{3f}$ in quail limb buds grafted onto the chorioallantoic membrane of chick embryo hosts. Since aneural limb bud grafts accumulate only $MLC_{1f}$ while innervated grafts accumulate both the $MLC_{1f}$ and $MLC_{3f}$ isoforms characteristic of adult muscle, we conclude that the accumulation of $MLC_{3f}$ is nerve-dependent. Thus, while muscle differentiation is directly regulated by growth factors like FGF, muscle maturation is regulated by some influence of the nervous system. (This work was supported by grants to IRK from NIH (HD 07083) and MDA.

0501 GENETIC DETERMINANTS OF THE MUSCLE CELL LINEAGE, Andrew B. Lassar and Harold Weintraub, Hutchinson Cancer Center, Seattle, WA 98104

I have been attempting to develop an assay to detect genes responsible for the establishment and maintenance of the muscle cell lineage. In particular I have been working with a line of mouse cells (C3H 10t1/2) which convert into myoblasts, adipocytes, or chondrocytes following treatment with 5-azacytidine. Presumably 5-azacytidine treatment effects the stable hypomethylation of genes which determine these three mesenchymal cell lineages. In an attempt to identify genes which determine the muscle cell lineage I am investigating whether muscle DNA (isolated from an azacytidine derived myoblast) when transfected into the parental C3H 10t1/2 cells will establish a muscle phenotype in the absence of azacytidine treatment. In a complementary study, I am cloning sequences which are expressed in the C3H 10t1/2 myoblasts yet are absent in the parental C3H 10t1/2 cells; the hope being that such sequences are important in establishing the muscle cell lineage.

0502 CONTROL OF CREATINE PHOSPHOKINASE SYNTHESIS BY FIBROBLAST GROWTH FACTORS. Brian Lathrop, K. Thomas*, L. Glaser, Dept. of Biol. Chem., Washington Univ. Sch. of Med., St. Louis, MO 63110, & *Research Labs, Merck Sharp & Dohme, Rahway, NJ 07065.
The regulation of creatine phosphokinase (CPK) expression by polypeptide growth factors was examined in the clonal mouse muscle $BC_3H1$ cell line. After cell growth is arrested by low serum concentrations, these cultures express a variety of muscle-specific proteins. When induced cells are exposed to high levels (20%) of fetal calf serum, synthesis of muscle-specific proteins is repressed with a concomitant reinitiation of cell growth. Pituitary-derived fibroblast growth factor (FGF) was found to cause substantial cell growth and to repress the rate of CPK syntheiss to the same extent as serum when added to quiescent, differentiated cultures. Although pituitary-derived FGF was mitogenic for $BC_3H1$ cells, the rate of cell growth did not correlate with the extent of repression. Highly purified brain FGF (Thomas et al. (1984) PNAS 81:357-361) was also found to repress the rate of CPK synthesis. At a concentration of 4 ng/ml, brain FGF repressed the rate of CPK synthesis to a greater extent than 20% serum when added to quiescent, differentiated cultures. Notably, brain-derived FGF showed no mitogenic activity for $BC_3H1$ cells under these conditions. These results demonstrate that the expression of CPK can be regulated by defined polypeptide growth factors and that mitogenic activiation is not necessary for repression of CPK synthesis. Supported by GM18405.

0503 **DEVELOPMENTAL EXPRESSION OF THREE ACTIN EPITOPES IN THE RAT,** **James L. Lessard and Thomas Sadler*,** Children's Hospital Research Foundation, Cincinnati, OH 45229, *University of North Carolina, Chapel Hill, NC 27514

The developmental and cellular expression of the epitopes for three monoclonal antibodies (C4, B4, HUC 1-3) to actin have been investigated in the rat. In immunoblots, C4 reacts with all six known vertebrate isoactins while B4 and HUC 1-3 react selectively with only the muscle isoforms of this protein. B4 binds preferentially to smooth muscle gamma actin but also reacts with an alpha actin present in vascular smooth muscle, cardiac muscle and skeletal muscle. HUC 1-3 reacts weakly, but similarly, with all four muscle isoactins. Indirect immunofluorescence was carried out using Carnoy's fixed embryos. Using B4 or HUC 1-3, no specific staining of either the primitive heart or gut tube was evident until about day 11 when the myoepicardium shows a greater fluorescence than the surrounding tissues. By day 15, fluorescence in the heart musculature is intense and well defined to the myocardium surrounding the ventricles and the walls of the atria. Staining is also observed in the the walls of the blood vessels and the gut as well as in the region of the lung buds. Interestingly, HUC 1-3 stains presumptive muscle cells in the limb bud at this time but B4 does not. The disparity in the expression of the epitope for B4 and HUC 1-3 in skeletal muscle suggests that the binding specificity for these two antibodies is distinct and that there is an actin form (presumably an alpha actin) that is detectable with HUC 1-3 but not B4. C4 shows a general reactivity with all rat tissues at these stages of development with enhanced fluorescence in presumptive muscle cells. Supported by NIH grants HD 17000 and 17381.

0504 DIVERSITY IN MYOSIN HEAVY CHAIN EXPRESSION IN CULTURED MYOTUBES. Jeffrey B. Miller and Frank E. Stockdale. Stanford Medical School, Stanford, CA 94305.

Both fast and slow isoforms of myosin are found in embryonic day (ED) 8 to 14 chick muscles, but only the fast isoforms are found in myotubes formed in cultures of ED 8 to 14 myoblasts. In a study of early muscle development, however, we found a population of myoblasts that form myotubes which contain myosin with slow heavy chain (SHC) epitopes. Presumptive pectoral muscle of different ages of embryos was dissociated into myoblasts which were grown on collagen-coated plates. After 2 to 13 days in culture, myosin was assayed by immunostaining of myotubes, and by immunoblotting of extracted myosin using monoclonal antibodies specific to fast myosin light chains 1 and 3 (FLC), fast heavy chains (FHC), slow light chain 1 (SLC), or SHC.

About 10% of myotubes formed in myoblast cultures from ED 5 or ED 6 breast muscle synthesize a myosin heavy chain with SHC epitopes. No SHC was found in myotubes in cultures from ED 8 or later breast muscle. SLC was found in most myotubes from myoblast cultures of ED 5 or 6, and some myotubes of ED 8 myoblast cultures, but not in myotubes from myoblasts of cultures ED 12 or later. FLC and FHC were found in most (>90%) myotubes regardless of the embryonic age of the myoblasts donor. The proportion of myotubes that expressed SHC appeared to remain constant during two weeks in culture, but SLC positive myotubes appeared to decrease in number after one week in culture. Conditioned medium did not significantly affect isoforms of myosin expressed in these mass cultures. These results show that myosin with SHC epitopes can be synthesized by cultured myotubes, and suggest that two populations of myoblasts inhabit early pectoralis major with one population disappearing by mid-embryonic development. (Supported by NIH grants AG 02822 and HD 16740 and MDA).

0505 MYODIFFERENTIATION IS SUPPORTED BY INORGANIC ION AS BY IRON-BOUND TRANSFERRIN(Fe-Tf). *Eijiro Ozawa, *Koji Saito, *Yasuko Hagiwara and *°Saoko Atsumi. *Division of Cell Biology, National Center for Nervous, Mental and Muscular Disorders, Tokyo, 187, Japan, and °Department of Anatomy, Yamanashi Medical College, Yamanashi, 409-38, Yamanashi, Japan.

We have shown that Fe-Tf is required not only to myoblasts but also myotubes for their growth and maintenance. Inorganic Fe ion can replace Fe-Tf in these regards, suggesting that protein moiety of Fe-Tf serves as a vehicle of Fe entrance into the cells. Although reports from other laboratories support this idea, there are reports suggesting that Tf protein molecule may play other roles. Therefore, we studied whether Fe can support myodifferentiation similarly to Fe-Tf and whether Tf protein serve to modify the process. Since apoTf did not support cell growth, the effects of Fe-Tf and Fe were compared.

Myogenic cells isolated from 11th day chick embryo breast muscles were cultured in a medium composed of 85% Eagle's MEM and 15% horse serum containing either Fe-Tf from chick egg white (0.2μM) or $FeCl_3$ (100μM). (We have reported that horse Fe-Tf was inert to chick myogenic cells.) The cells grew and differentiated in each medium to almost the same extent in morphological, physiological and biochemical respects. We conclude what is essential in myodifferentiation is Fe and not Tf protein.

0506 HEPARANSULFATE PROTEOGLYCAN AND SKELETAL MYOGENESIS, Douglas Noonan and Ronald J. Przybylski, Department of Developmental Genetics and Anatomy and The Developmental Biology Center, Case Western Reserve University, Cleveland, Ohio 44106.

We are studying the glycosaminoglycans (GAG) and proteoglycans (PG) in primary cultures and cell lines of skeletal muscle as they progress through a proliferative phase followed by cell-cell recognition, alignment and fusion to form multinucleated myotubes. The GAGS of the entire cells and a sub-fraction of their cell surfaces, namely, the substratum attachment sites consist of heparan sulfate (HS), chondroitin-4 and -6 sulfate, hyaluronate and dermatan sulfate. C-6-S is the major component much of which is secreted into the medium. The HS of the cell fraction change remarkably and appear developmentally regulated. SAM HS changes little with myotube formation while the cellular HS increases approximately 2 fold. Cellular HS exists in two forms (based on sensitivity to alkaline borohydride reduction and nitrous acid deamination); as a PG and as a smaller GAG, both of which increase with time in culture. Though little HS GAG is found in a 6 hr pulse with radio sulfate, no clear precursor-product relationship between the PG and the GAG was noted. The cellular HSPG has a Kav of .12 on Sepharose CL-6B whereas the GAG chains liberated from this PG have a Kav of .33, indicating the HSPG consists of 2-4 GAG chains and a small protein core.

0507 CHICK SKELETAL MUSCLE CELLS ARE GENERATED IN COHORTS OF SIXTEEN FROM STEM CELLS, LeBris Smith Quinn and Mark Nameroff, University of Washington, Seattle, WA 98195

Direct evidence supporting a cell cycle-dependent lineage model for myogenic differentiation has been obtained from an analysis of large myogenic clones. Such clones typically contain both cycling precursors and post-mitotic, terminally differentiated muscle cells. We counted the total number of cells, and the number of terminally differentiated cells, in individual myogenic clones by means of immunocytochemical staining for the M isozyme of creatine kinase. We found that the number of terminally differentiated cells in each clone was usually equal to, or just short of, an exact integer multiple of sixteen, falling away in an exponential fashion. A chi-squared test showed this distribution to be significantly different from uniform random at the 0.001 level. These findings are not consistent with the model of myogenesis in which a homogeneous population of differentiation-competent precursor cells is postulated. Rather, the results indicate that a self-renewing stem cell exists in the vertebrate skeletal muscle cell lineage; this cell can generate committed precursor cells by means of symmetric and asymmetric cell divisions. Committed cells then give rise to cohorts of exactly 16 terminally differentiated muscle cells by means of four determined, symmetric divisions. These data strongly support the idea that normal myogenic precursor cells are "counting" the number of cell divisions prior to terminal differentiation. These observations contrast sharply with those made on myogenic cell lines, and are of obvious importance in understanding the molecular and genetic bases of muscle differentiation.

0508 RELATIONSHIP BETWEEN RNA METHYLATION AND DIFFERENTIATION IN L5 MYOBLAST LINE, Sigfrido Scarpa[1,2], Sergio Agostini[1], Cristina Morganti[1,2], Arturo Sala[1,2], Roberto Strom[1] and Giulio L. Cantoni[3].
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Differentiation of L5 myoblasts is stimulated by administration of DZA (P.N.A.S. 81, 3064, 1984) and inhibited by Con A. The effect of DZA is probably related to inhibition of one or more methyl-transfer reactions, whereas the mechanism of action of Con A is not known. In T-lymphocytes Con A has been shown to increase RNA methylation (Exp. Cell Res. 140, 137, 1982). It was of interest to compare the effect of these two compounds on RNA methylation in myoblasts in fusing and non-fusing conditions. Cells were labelled with $^{3}HC_{3}$-Methionine and total RNA was extracted. Under both conditions incorporation of the methyl group of methionine in RNA was increased by Con A as compared to controls, whereas treatment with DZA had the opposite effect, namely it decreased the incorporation of the methyl of methionine in RNA by approximately 50%. We tentatively conclude that RNA methylation appears to be inversely related to the induction of differentiation.

0509 COOPERATIVE EFFECTS OF AZACYTIDINE AND HORSE SERUM IN RESTORING THE CAPACITY FOR DIFFERENTIATION IN NON-FUSING L6 MYOBLASTS, Robert J. Smith, Suzanne Mrozak, and Francesco Beguinot, Harvard Medical School, Boston, MA 02215

Cells of the L6 myoblast line progressively lose their capacity for differentiation with increased passage in culture. By subculturing L6 myoblasts continuously for approximately one year in Eagle's MEM with 10% bovine serum (BS), we obtained a sub-line that had lost all capacity for differentiation as evidenced by fusion index = 0, creatine kinase < 1 nmol/min/mg protein, and myosin heavy chain undetectable. The capacity for differentiation in these cells can be restored by culturing them in MEM with 10% horse serum (HS) and $10^{-6}$M 5-azacytidine (AZA): fusion index = 83%, creatine kinase = 69 nmol/min/mg, myosin heavy chain positive. The effect is evident after ≥ 25 population doublings and requires both HS and AZA. If AZA is subsequently eliminated from the medium, cells grown with HS maintain the capacity for differentiation for > 40 population doublings, whereas HS-AZA cells transferred to MEM with BS cease differentiating (50% decrease in fusion index after 15 population doublings). Growth of L6 myoblasts from the fusion-competent parent line in HS-AZA medium results in indefinite persistence of the capacity for differentiation. We conclude that loss of differentiation capacity in L6 cells with increased passage in culture is an epigenetic phenomenon that can be prevented or reversed by the combined effects of HS and the DNA methylation inhibitor AZA. The ability to reversibly alter differentiation capacity with these two agents provides a powerful tool for studies on the control of differentiation and on the importance of coordinate expression of differentiated functions in muscle cells.

0510 INNERVATION OF FIBER TYPES DURING POSTNATAL DEVELOPMENT OF RAT SOLEUS, Wesley J. Thompson, Department of Zoology, University of Texas, Austin, TX 78712

During early postnatal development when muscle fibers are undergoing transitions in the contractile proteins they are expressing, the innervation of these fibers is also changing. By the process of "synapse elimination", fibers change from being polyneuronally innervated to the single innervation seen in the adult. Since a large body of evidence indicates that innervation has a large role in determining the differentiation of muscle fibers both in the adult and during development, it is important to know how the differentiating fiber types are innervated. As a first step toward this goal, I have utilized the glycogen depletion technique to investigate the type identity of the fibers in single motor units in neonatal rat soleus muscle. Employing conventional ATPase histochemistry, two distinct types of fibers are present in the muscle at 8 days after birth. Motor units at this time contain predominantly fibers of one of these two types. This result shows that, even at a time when motor neurons innervate 2-3 times the number of fibers they do in the adult and all fibers are polyneuronally innervated, one can identify two types of motor neurons which segregate their innervation to two, largely different populations of these fibers. Such a segregation could provide a means by which individual muscle fibers receive unambiguous instructions from the nervous system as to the type differentiation they should assume. Alternative explanations include a selective synaptogenesis between predetermined types of motor neurons and muscle fibers. In any case, this finding implies that some type of recognition occurs during synaptogenesis by the different types of motor neurons: either motor neurons recognize each other or they recognize somehow disparate muscle fibers.

0511 MUSCLE SATELLITE CELLS ARE ANTIGENICALLY DISTINCT FROM MYOBLASTS. Frank S. Walsh, Stephen E. Moore and Ramesh Nayak, Molecular Neurobiology Laboratory, Institute of Neurology, Queen Square, London WC1N 3BG.

We have produced a panel of antibodies that define muscle differentiation antigens in an attempt to produce antigenic markers of each of the main muscle cell lineages. Now we wish to determine whether the myoblast lineage can be subdivided further. Four antibodies that react with myoblasts were studied in detail. These were two monoclonal antibodies (McAbs) named 24.1D5 and 5.1H11 and rabbit anti Thy-1 and rabbit anti N-CAM. The test systems chosen for analysis were cell cultures of human muscle that contain myoblasts, fibroblasts and myotubes and cryostat sections of control and myopathic human muscle. All four antibodies were found to react with myoblasts in cell culture. Anti Thy-1 was the only reagent of the four to react with fibroblasts. McAb 5.1H11 and anti N-CAM were similar as they reacted with both myoblasts and myotubes but not fibroblasts. The most specific reagent found was McAb 24.1D5 which reacts with myoblasts only. Cryostat sections of normal and diseased muscle were used to characterise these reactivities further. The only antibody to react with adult skeletal muscle was McAb 24.1D5. Here reactivity was restricted to the small satellite cells that reside beneath the basal lamina of myofibres. Myopathic muscle that contained histochemically identifiable regenerating fibres were found to be reactive with McAb 5.1H11 and anti N-CAM only. Muscle satellite cells are believed to be the source of myoblasts for regeneration in muscle injury. The above data reports the first demonstration that satellite cells are antigenically distinct from myoblasts.

0512 DESMIN IS EXPRESSED IN DIVIDING CELLS IN SKELETAL MUSCLE MASS CULTURES AND CLONES, Zipora Yablonka-Reuveni and Mark Nameroff, University of Washington, Seattle, WA

Desmin is found in both smooth muscle and skeletal muscle cells. In the skeletal muscle lineage, it is commonly believed that desmin appears only in post-mitotic myoblasts and myotubes. Using a combination of [$^3$H]thymidine autoradiography and immunofluorescent staining with an antibody against chick gizzard desmin, we examined individual cells in both primary mass cultures and primary clones established from the pectoral muscles of 10- and 18-day chick embryos. In mass cultures from 10-day embryos, dividing cells containing desmin were only observed occasionally. However, in mass cultures from 18-day embryos, about 5% of the radio-labeled cells were desmin positive. To determine whether these doubly-labeled cells were in the skeletal myogenic or another lineage, similar studies were carried out on myogenic clones established from both 10- and 18-day embryos. Again, very few desmin positive, thymidine labeled cells were observed in clones from 18-day muscle. Skeletal myosin was not detected in such cells when they were immunofluorecently stained with an antibody against skeletal myosin heavy chain. Among the number of intriguing explanations for these findings are: 1. Skeletal myogenic stem cells may be a heterogeneous cell population. 2. Skeletal myogenic stem cells may more frequently give rise to other cell types (i.e., smooth muscle cells and/or fibroblasts) as a function of embryonic age. [This work was supported by grants from the American Heart Association Washington Affiliate and from the National Institutes of Health (#AM28154).]

## *Membranes and Matrix*

0513 THE ROLE OF PROTEOGLYCANS IN MUSCLE DEVELOPMENT. Arnold I. Caplan, David G. Pechak, Mary J. Kujawa, David A. Carrino. Biol. Dept., Case Western Reserve Univ., Cleveland, OH 44106.

In the developing chick embryo, muscle development proceeds via a series of discrete temporal steps in both in vitro and in vivo sites. Single cells committed to the myogenic lineage, myoblasts, proliferate and then fuse one into the other to form multinucleated myotubes. The myotubes increase in girth as more and more molecules are added to the organized macromolecular contractile assemblies. Both myoblasts and myotubes synthesize a unique and unusually large chondroitin sulfate proteoglycan which is deposited outside the cells. Ultimately, the synthesis of this muscle proteoglycan is not observed in adult muscle. Fibroblasts can inhibit the synthesis of muslce proteoglycan as demonstrated when additional fibroblasts are added to myogenic cultures at various stages of development. We have characterized this proteoglycan in molecular detail and believe that it functions to structure water outside the developing myogenic cells in an effort to reserve space for the physical expansion of muscle. This molecule is 3-5 million daltons, has 40-60 chondroitin sulfate chains of 70,000 daltons which are sulfated in the 6-position to the 90 percentile, can form a link-stabilized aggregate with hyaluronate, has both N- and O-linked oligosaccharides, but does not contain keratan sulfate. A monoclonal antibody to cartilage proteoglycan core protein cross reacts with the muscle proteoglycan and shows diffuse immunofluorescent staining over both myoblasts and myotubes in muscle cultures. Current studies attempt to identify the epitope for this antibody on the muscle proteoglycan. A detailed model will be presented to explain the role of this proteoglycan during muscle development.

Supported by grants from MDA and NIH.

0514 MYOBLAST FUSION: THE REQUIREMENT OF A METAL DEPENDENT ENDOPROTEASE, Warren J. Strittmatter and Christine B. Couch, Department of Neurology, Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030

The calcium dependent fusion of cultured rat myoblasts to multinucleate myotubes appears to require the activity of a neutral metalloendoprotease at the time of fusion. Metalloendoprotease inhibitors and synthetic dipeptide substrates prevent myoblast fusion when added to fusion competent myoblasts at the time of fusion. Metalloendoprotease activity has been identified and partially characterized with a fluorogenic protease substrate (Cell 32:257-265). Myoblasts contain both soluble and membrane-associated metalloendoproteases, and these proteases have different inhibitor specifities. Several inhibitors which block myoblast fusion inhibit only soluble and not membrane-associated metalloendoprotease activity in myoblasts. One metalloendoprotease inhibitor has no effect on myoblast fusion and inhibits only the membrane-associated protease. These observations therefore implicate a soluble metalloendoprotease in myoblast fusion. Two soluble metalloendoproteases can be demonstrated by column chromatofocusing, with pI values of 5.9 and 4.8. The soluble metalloendoprotease eluted at pH 5.9 is not inhibited by an inhibitor which blocks fusion, while the soluble metalloendoprotease eluted at pH 4.8 is inhibited. Of the three metalloendoproteases identified in myoblasts, the metalloendoprotease required in myoblast fusion appears to be the soluble metalloendoprotease with a pI of 4.8 (J.B.C. 259:5396-5399). This protease is now being purified from myoblasts to further study its role in fusion.

## *Protein Polymorphism*

0515 DIFFERENTIAL ROLES OF MYOSIN ISOFORMS IN FILAMENT ASSEMBLY, Henry F. Epstein, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

Wild-type body wall muscle cells of Caenorhabditis elegans produce two myosin heavy chain isoforms, A and B, at a constant ratio of 1:4, that form homodimeric myosins. Electron microscopy of negatively stained complexes of isoform-specific antibodies with thick filaments shows that the surface of the 9.7 μm long filament is divided into 5 zones with respect to myosin content: a medial 0.9 μm zone containing myosin A only, two flanking 0.45 μm zones containing both myosins A and B and two polar 4.4 μm zones containing myosin B only.

Biochemical and electron microscopic studies show that at 0.45 M KCl, pH 6.35, the medial all-myosin A region remains with novel core structures extending in a polar fashion, whereas myosin B and paramyosin are solubilized. This differentiation of thick filament structure suggests a sequential model for its assembly. Myosin A and B would be involved in the initial and terminal phases of assembly.

Analysis of mutant thick filaments clarifies the roles of the myosin isoforms. E190 (unc-54 I) thick filaments contain myosin A only and have normal length. E1214 (unc-15 I) mutants produce no paramyosin, and their thick filaments are composed of a medial myosin A region and protruding polar core structures. Dr. Laurel Traeger has studied the unc-52 II mutant SU200 and the sup-3 V E1407 mutant where the molar ratio of A:B is 1:2. The medial myosin A regions increase to 2.2 μm in SU200 and 3.2 μm in E1407. Both kinds of mutant thick filaments have normal length, and in E1407, the myofibrillar structure is normal. The molecular sorting of the myosin isoforms is not necessary for either filament assembly or arrangement. The concentrations and mole ratios of A and B and the underlying substructure are determinants of the myosin distribution. Dr. Sandra Honda has shown by gene-specific hybridization experiments that mRNA levels for A and B chains differ, suggesting potential mechanisms for the regulation of isoform biosynthesis.

The biochemical and structural results suggest that the regulated biosynthesis of the two heavy chains isoforms controls the initiation and termination of thick filament assembly by the differential interactions of the myosin isozymes. The research was supported by grants from the National Institute of Aging, National Institute of General Medical Sciences, and the Muscular Dystrophy Association.

0516 MYOSIN ISOZYME TRANSITIONS IN EMBRYONIC CHICKEN PECTORALIS MUSCLE, Susan Lowey*, Saverio Sartore*, Geraldine F. Gauthier†, Guillermina Waller* and Ann W. Hobbs†, *Rosenstiel Center, Brandeis University, Waltham, MA 02254 and †Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605

Three classes of myosin isozymes appear sequentially during skeletal muscle development: embryonic, neonatal and adult myosin (1). Although differences in primary structure have been demonstrated by biochemical and immunological methods, these myosins have the same overall structure, show extensive sequence homology and display similar enzymatic activities (2). It has generally been assumed that one major "fast type" myosin heavy chain is expressed during embryonic development in ovo, and that this same embryonic myosin is expressed in cultured cells. The reactivity of 10-day embryonic pectoralis muscle with polyclonal antibodies against slow [ALD] myosin has been ascribed, in part, to a minor "slow type" component in embryonic myosin (3). By day 18 of incubation, this "slow" myosin has diminished considerably (4). Here we have used monoclonal antibodies prepared against adult chicken pectoralis myosin to analyze in greater detail the composition of embryonic pectoralis muscle at various stages of development.

An antibody [12C5] specific for the N-terminal 25 kD peptide of the heavy chain reacts strongly with adult and 18-day embryonic myosin, but not with 11 day posthatch myosin (5). Another antibody [5C3], specific for the C-terminal end of the rod, reacts only with adult myosin and not with any of the developmental isozymes, whereas an antibody specific for the $LC2_f$ light chain [7C10] reacts with all variants throughout myogenesis (5). These findings support the hypothesis that three classes of myosin isozymes exist during development. Unexpectedly, radioimmunoassay showed that anti-S1 [12C5] reacted weakly with myosin purified from 10-day chick embryos. Cryosections of 10-day pectoralis muscle also reacted poorly with fluorescein-labeled anti-S1 [12C5] compared to 18-day embryos, which showed strong immunofluorescence. Two other monoclonal antibodies, specific for the 25 kD [10H10] and the 50 kD [13E1] peptides of the heavy chain, reacted poorly or not at all with 10-day embryonic myosin compared to strong reactivity with 18-day embryonic myosin. Cells grown in culture also showed differential binding of antibodies: whereas all myotubes reacted with antibodies to $LC2_f$ and ALD, only some cells were stained with antibody against S1 (12C5). These results suggest that at least two "fast type" myosins are expressed during the embryonic stages of avian myogenesis, and that these isozymes are developmentally regulated both in vivo and in vitro.

1) Whalen, et al., Nature 292, 805, 1981. 2) Lowey et al., J. Musc. Res. Cell Motility 4, 695, 1983. 3) Benfield et al., J. Musc. Res. Cell Motility 4, 717, 1983. 4) Gauthier et al., J. Cell Biol. 92, 471, 1982. 5) Winkelmann et al., Cell 34, 295, 1983.

0517 EXPRESSION OF C-PROTEIN ISOFORMS DURING CHICKEN STRIATED MUSCLE DEVELOPMENT AND ITS DEPENDENCE ON INNERVATION, Takashi Obinata, Maho Kawashima and Osamu Saitoh, Pepartment of Biology, Chiba University, Yayoi-cho Chiba 260, Japan.

Distinct three C-protein isoforms, called fast muscle type, slow muscle type and cardiac muscle type, have been distinguished in adult chicken striated muscles by immunochemical analysis using monoclonal antibodies (Reinach et al., 1982; Kawashima et al., 1984). In this study, we describe the transition of C-protein isoform expression occurring in developing chicken striated muscles by immunocytochemistry.

During chicken embryonic development, cardiac type C-protein isoform was first detected in all striated muscles including cardiac, slow skeletal (ALD) and fast skeletal (PLD and pectoralis) muscles. In cardiac muscle, only cardiac type C-protein was continuously expressed through developmental stages. However, during striated muscle development, C-protein isoform transition characteristic to each muscle type has been observed: during ALD muscle development, only one transition from cardiac isoform to slow muscle isoform, and during pectoralis muscle development, two step transitions namely, from cardiac isoform to slow muscle isoform + fast muscle isoform, occurring during late embryonic development, and then finally to fast muscle isoform alone, occurring postnatal development. The pattern of C-protein isoform expression in cultured striated muscle cells was similar to those in the embryonic muscle tissues and the remarkable C-protein isoform transition as observed during in vivo development was not detected in muscle cultures.

The transition of C-protein isoform expression during postnatal development of pectoralis muscle was markedly affected by muscle denervation. When neonatal muscle was denervated just after hatching, slow C-protein isoform persists, whereas denervation of adult muscle causes the reappearance of slow C-protein within two weeks after surgery. Cardiac type C-protein was scarcely detected in the denervated muscles. In contrast, regenerating muscle cells induced in adult pectoralis muscle by focal cold injury expressed not only two skeletal muscle C-protein isoforms but also cardiac C-protein. These observations indicate that the C-protein expression in pectoralis muscle without innervation goes back to neonatal state but not to embryonic state.

0518 CONTROL OF MYOSIN EXPRESSION DURING RODENT MUSCLE DEVELOPMENT R. Whalen, J. Ajioka, G. Butler-Browne, C. Laurent, D. McCormick, C. Pinset, M. Toutant, G. Riley and S. Watkins
Département de Biologie Moléculaire, Institut Pasteur, 25 Rue du Dr. Roux, 75724 Paris, France

Two types of changes in myosin expression occur during skeletal muscle development. The first involves the induction of embryonic myosin as myoblasts differentiate into myotubes. The second type involves transitions between myosin isoforms as muscle tissue matures.

We have developed a tissue culture system in which rat L6 myoblasts can be obtained as quiescent, undifferentiated cells which can subsequently be induced to fuse in serum-free medium. We can demonstrate that the induction of myosin expression (measured as protein synthesis or accumulation of mRNAs) does not require DNA synthesis. Studies of DNAse I sensitivity have been carried out to determine if changes in the chromatin conformation of muscle-specific genes also occur in the absence of DNA synthesis.

The second type of change in myosin expression is illustrated by the sequential appearance of different myosin isoforms throughout the fetal and post-natal development of skeletal muscle. The appearance of adult fast myosin is regulated, at least in part, by levels of thyroid hormone since chemically-induced hypothyroidism in young rats blocks the transition from neonatal to adult fast myosin. To investigate further the endocrinological factors controlling this transition, we have examined mutant mice manifesting a syndrome of hereditary pituitary dwarfism. In these animals the myosin isozyme transitions of both skeletal and cardiac muscles are affected. These two tissues differ however, in that the transition in skeletal muscle takes place more slowly than in normal mice while the cardiac transition is completely inhibited up to at least 75 days after birth. Injections of thyroxine induce accumulation of the adult isozymes and lead to disappearance of the developmental forms in both tissues within 11-12 days. The characteristics of the dwarf mutation suggest the conclusion that lack of thyroid hormone rather than growth hormone is responsible for the alteration of myosin isozyme transitions. This mouse mutant may be a useful model for further study of the control of myosin isozyme transitions.

## *Protein Polymorphism and Gene Organization*

0519 A THIRD NONMUSCLE ACTIN GENE TRANSCRIBED IN CHICKEN, Derk J. Bergsma, Kun Sang Chang and Robert J. Schwartz, Baylor College of Medicine, Houston, TX 77030

We have identified a novel chicken actin gene by sequence analysis of an actin gene isolated from a chicken genomic library. The actin protein deduced from the nucleotide sequence of this gene very closely resembles the vertebrate cytoplasmic actins; accordingly, we classified this gene as a nonmuscle type. We have adopted the convention of Vandekerckhove et al (1981) for indicating the nonmuscle actins of amphibia, and denoted this gene as type 5. RNA blot analysis demonstrated that the type 5 mRNA transcripts accumulate in adult tissues in a pattern indicative of a nonmuscle actin gene. Southern blot analysis indicated that the type 5 actin gene is a distinct member of the chicken actin multigene family. Inspection of the nucleotide sequence revealed many unexpected features that distinguished the type 5 gene from all of the other vertebrate actin genes examined to date. These unique characteristics included: 1) an alanine codon following the initiation methionine codon, 2) a single intron was found within the 5' untranslated region, but no intron interruptions occured in the coding portion of the gene, and 3) a atypical Goldberg-Hogness box (ATAGAA) proceeded the mRNA initiation terminus. These unusual features have interesting implications for actin gene diversification during evolution.

0520 EXPRESSION OF MOUSE MYOGLOBIN GENE IN G8 CELL LINE. Blanchetot, A., Price, M., Jeffreys, A.J. Genetics Department, University of Leicester, England.

During muscle cell differentiation myoblasts fuse to form myotubes in which activation of muscle specific genes occurs. Up until now the studies on gene expression in muscle cell culture have been mostly devoted to genes coding for contractile proteins.

Using a G8 cell line (mouse embryonic myoblast cell line) we have recently found an induction of transcription of the myoglobin gene (Mb) after differentiation of myoblast to myotubes. In order to study the expression of a gene coding for a muscle specific soluble protein, we have isolated the functional mouse Mb gene from a G8 cell line library using the 3 different seal Mb exons as probes.

Although the length of the gene is shorter, the exon/intron organisation of the G8 Mb gene is similar to that found in human and seal. Using G8 cells as an in vitro transient expression system with pRSV-Cat vector we hope to introduce the mouse Mb promoter - Cat fusion into these cells in order to study the expression and regulation of transcription of the endogenous gene during embryonic muscle differentiation.

0521 TROPONIN T HETEROGENEITY: FOUR FAST TROPONIN T SPECIES IN RABBIT SKELETAL MUSCLE THAT DIFFER IN THE N-TERMINAL REGION. Margaret M. Briggs and Frederick H. Schachat. Department of Anatomy, Duke University Medical Center, Durham, NC 27710.

Immunoblot analysis of myofibrils from the intrinsic musculature of rabbit tongue revealed a family of four species of fast troponin T (TnT). Two of them have been characterized previously. One, $TnT_{2f}$ ($M_r$ 37,000), is the predominant form in fast muscles of the rabbit back and has been sequenced (Pearlstone et al., J. Biol. Chem. (1977) 252, 983-989). The other, $TnT_{1f}$ ($M_r$ 37,500), has been purified and shown to differ from $TnT_{2f}$ in the N-terminal CNBr peptide (Briggs et al., J. Biol. Chem. (1984) 259, 10369-10375). The two newly-identified species are designated $TnT_{cf}$ ($M_r$ 39,000) and $TnT_{3f}$ ($M_r$ 35,000). $TnT_{cf}$ has so far been detected only in tongue, and may be restricted to craniofacial muscles, but $TnT_{3f}$ is also present in the trunk, hindlimb and diaphragm muscles. Time course degradation studies indicated that $TnT_{cf}$ and $TnT_{3f}$ do not arise from proteolysis, and the molecular bases of the heterogeneity were further investigated.

Tongue troponin was [$^{32}$P]-labeled with TnT kinase to help identify the N-terminal region of each TnT. By comparison with CNBr peptides purified from $TnT_{1f}$ and $TnT_{2f}$, stained and autoradiographed SDS-PAGE peptide maps of the four labeled TnT species showed that each one has a different N-terminal CNBr peptide (CB3). There was no apparent difference in the CB2 peptides on SDS-PAGE. The four fast TnT species may thus be members of a family of homologous proteins that differ in the N-terminal region.

0522 CHARACTERIZATION OF EMBRYONIC MYOSIN HEAVY CHAIN GENES FROM CHICKEN. Gabriele E. Bugaisky, Smilja Jakovcic, and Patrick K. Umeda. University of Chicago, Chicago, IL. 60637.

Highly divergent regions of myosin heavy chain (HC) cDNA clones have been used to directly isolate the corresponding genes by homologous recombination. A fragment containing about 300 bp from the 3' region of p251, which encodes the COOH terminus of an embryonic HC isoform, and another 300 bp segment from the 5'-most region of p60, which encodes the S-2 portion of a different embryonic HC isoform were used to screen a chicken genomic library. Two genomic clones, $\pi\lambda$251 and $\pi\lambda$60, were isolated and characterized. The 16 kb insert of $\pi\lambda$251 has been examined by restriction endonuclease mapping and Southern blot analysis. Furthermore, electron microscopic evaluation of p251-$\pi\lambda$251 heteroduplexes reveals that 1.2 kb of the mRNA coding sequences are located within a central 4 kb region of the phage. When this region of the chicken gene is compared to the rabbit cardiac $\alpha$-myosin HC gene, the size and number of introns appear smaller in the chicken; conversely, 2-3 exons of the rabbit gene could correspond to a single chicken exon. Since about 6 kb of genomic insert extends 5' of those sequences that hybridize to p251, it is likely that additional coding regions lie within this genomic clone. DNA sequence analysis of the highly divergent 3' portion of the gene is being used to confirm the identity of clone $\pi\lambda$251.

0523 ISOLATION AND PRELIMINARY CHARACTERIZATION OF AN APPARENT HUMAN CREATINE PHOSPOKINASE B-ISOZYME GENE, Ghaleb H. Daouk, Scott Putney, and Lulu Pickering, Massachusetts Institute of Technology, Cambridge, MA 02139.

Creatine Kinase (CK) is a major enzyme involved in the energy metabolism of the cell. It exists in two major isozymes: M(Muscle) isozyme, found in striated muscle predominantly, and B(Brain) isozyme, found in brain and other non-striated muscle tissue. The heterodimer CKMB exists predominantly in the myocardium.

Complementary DNA (cDNA) for several CK genes have been isolated and sequenced. In particular the rabbit CKM and CKB isozymes cDNAs have been cloned and sequenced in our laboratory. We have used a 180 bp rabbit CKM cDNA subclone encoding the highly concerved reactive cysteine ("Active site") region as a probe to screen a human genomic library. Several genomic clones hybridized to the probe, and further DNA sequence analysis of a segment of one of our clones has almost complete homology of amino-acid codons to the corresponding rabbit CKB cDNA sequence. Furthermore, most of the predicted amino-acid substitutions between the rabbit M and B isozymes, in the residues that flank the reactive cysteine, were also observed in our human genomic clone. The preliminary restriction map and architecture of this clone are presented.

0524 DEVELOPMENTAL ALTERATIONS OF 6-PHOSPHOFRUCTO-1-KINASE ISOZYMES IN RAT HEART AND SKELETAL MUSCLE, George A. Dunaway and Thomas P. Kasten, Southern Illinois University School of Medicine, Springfield, IL 62708

The alterations in the homotetrameric and heterotetrameric isozymes of 6-phosphofructo-1-kinase (PFK) have been studied in fetal, neonatal, and adult heart and skeletal muscle. The nature of these isozymes was investigated by chromatographic and immunological characterization of the native isozymes and by partial purification of the PFK isozymes in each tissue at different stages of maturation. From the partially purified preparations, the levels of each of the three PFK subunits were determined by resolution by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 7.5% slab gels, visualization by silver staining, and quantification by scanning gel densitometry with a laser densitometer interfaced with a computer. From the amounts of each of the 3 possible subunits in the purified PFK isozymes, the theoretical distributions of each of the 15 possible isozymes was predicted by statistical analysis assuming the appropriate polynomial probability distribution. PFK activities in adult heart (15.3 units/g) and adult skeletal muscle (80.2 units/g) were 2.3-fold and 27-fold greater than found in fetal heart and muscle, respectively. Chromatographic and immunological studies suggested that fetal heart and muscle contain all of the three possible PFK subunits. During maturation of skeletal muscle, two of the subunits were progressively lost and the muscle-type subunit was increased dramatically. Maturation of skeletal muscle was characterized by a shift from the complex mixture of homotetrameric and hybrid PFK isozymes which were present in fetal muscle to a single homotetrameric form in adult muscle which is composed of the muscle-type subunit. Heart maturation was accompanied by an increase in the muscle subunit and a decrease in the other type of subunits, liver-type and C-type. The result in adult heart is a complex mixture of PFK isozymes which appears to be different from that found in fetal heart.

0525 THE EXPRESSION OF MYOSIN HEAVY CHAIN ISOFORMS IN A NERVE-MUSCLE CULTURE SYSTEM
Marion S. Ecob & Robert G. Whalen, Muscular Dystrophy Research Laboratories, Newcastle & Institut Pasteur, Paris.

When adult mouse muscle fibers are cocultured with embryonic mouse spinal cord, the muscle regenerates to form myotubes which develop cross-striations and contractions. The adult isoform of fast myosin is present in cultures which have been contracting for at least 10 days (Ecob et al., Differentiation 25, 84-87, 1983). We have now shown that the adult fast isoform was not present in young myotubes, but that its appearance was coincident with the development of cross-striations and contractions, both of which are cord-dependent phenomena. Moreover, the adult isoform was never found in myotubes of a similar age in culture, which had regenerated in the absence of spinal cord. The expression of the adult isoform therefore appeared to require the spinal cord.

When cultures were established and maintained in the continuous presence of d-tubocurarine, there was a five-fold decrease in the percentage of fibers which contained the adult fast myosin. One interpretation of these results is that nerve trophic effect creates an environment in which the muscle can express the adult fast isoform of myosin, but that full expression may require activity in the muscle fibers. We cannot exclude that a neurotrophic effect is the sole requirement for the expression of fast myosin, the accumulation of which may have been impaired by the potential secondary effects of the d-tubocurarine. It is unlikely that the low levels of fast myosin were the result of spontaneous contractions, but further drug studies and the electrical stimulation of myotubes cultured without nerve may clarify these issues.

0526 MYOSIN LIGHT CHAIN ISOZYMES IN DROSOPHILA DEVELOPMENT, Scott Falkenthal[1] and Norman Davidson[2], Dept. of Genetics, Ohio State University, Columbus, Ohio, 43210[1], Dept. of Chem. Cal Tech, Pasadena, CA 91125[2].

The total sequence of the Drosophila melanogaster myosin alkali light chain (MLC-ALK) gene has been determined. By sequence comparisons with an MLC-ALK cDNA clone and by S1 nuclease analyses the pattern of introns and exons within the gene has been deduced. There are multiple polyadenylation signals which can account for most of the observed heterogeneity in the lengths of the mRNAs. In the 3' half of the gene, there is developmental regulation of two alternative splicing patterns which result in mRNAs that translate to give proteins with two alternative 14 amino acid carboxy-terminal sequences. One splicing pattern produces an mRNA which translates into a protein used for both larval and adult musculature, whereas the other splicing pattern is used for the latter stage only. Comparison of the gene structure of the Drosophila MLC-ALK to that of chicken reveals striking similarities in terms of intron position and number.

0527 TORPEDO ACETYLCHOLINESTERASE: ISOLATION OF cDNA CLONES WITH ANTIBODIES RAISED AGAINST AN AChE ACTIVE SITE SYNTHETIC DECAPEPTIDE, Sandra M. Gaston, John Tamkun, Steve Burden, and Phillips W. Robbins, The Massachusetts Institute of Technology, Cambridge, MA 02139, and Richard Lappin, The Rockefeller University, New York, NY 10021

A lambda gtll library generated from Torpedo electric organ poly A+ RNA has been screened with a polyclonal antisera directed against the active site of Electrophorus AChE. The antisera was prepared by immunization with a synthetic decapeptide whose sequence corresponds to the active site serine region of Electrophorus AChE (Schaffer et al., Biochemistry 12: 2946, 1973). The synthetic polypeptide preparation was crosslinked to thyroglobulin carrier prior to injection. The resulting anti-decapeptide antisera reacts with a 68K MW polypeptide in Western blots of Torpedo electric organ total protein. Three independant recombinant lambda gtll clones have been isolated with this antisera, with cDNA inserts of approximately 170, 290 and 350 base pairs. These cDNA inserts are being further characterized prior to their use as molecular probes.

0528 Genomic Myoglobin Genes. David A. Konkel, Gregory R. Alsip, and Roy Pugh. Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

Myoglobin (mb) is the monomeric heme-binding globin superramily protein involved in muscular oxygen storage and transport. The synthesis of mb in red skeletal, smooth, and cardiac (but not fast white) muscle appears under control of nerve-muscle interaction. To allow study of these regulatory processes, we previously reported isolation of five unique, partially overlapping clones from a chicken genomic library by probing with the seal mb exon two. Southern blotting and restriction map analysis suggests that one clone, λCM13.1, spans most of two discrete mb-like genes, while the other clones may represent pseudogenes. DNA sequence analysis of λCM13.1 is now well underway, using directed overlapping Bal 31 deletion fragments for dideoxy sequencing. Analysis of about 1/3 of the clone has not completely identified the mb-coding regions, so it remains unclear which gene is expressed. The sequencing reveals that in addition to the two mb-like genes, λCM13.1 contains an as yet unidentified pseudogene marked by a long polyA tract closely preceded by two 5/6 matches to the canonical AATAAA polyA addition signal.

We have also isolated putative mb-containing clones from a mouse genomic library, and are preparing to extend our studies into that system to provide a basis of comparison with results from our chicken system. (Supported by NIH grant NS17026)

0529 REPETITIVE SEQUENCES IN THE MYOSIN HEAVY CHAIN GENES OF WHITE LEGHORN CHICKENS Keith E. Kropp and Jeffrey Robbins, University of Missouri-Columbia, Columbia, MO 65212

Recently, two fast-white myosin heavy chain promoter regions have been sequenced from White Leghorn Chicken (1). When the sequences were compared, the second intron downstream from the start of translation was found to be highly conserved between the two clones. We have attempted to determine the significance and possible function of a 24 bp repetitive sequence found within the conserved intron. The oligonucleotide (24 bp) was used to probe for the presence of the sequence in the other MHC genes and to determine its approximate location with respect to the start of translation. A genomic southern was performed to determine the reiteration frequency. Finally, a northern was done to see if the 24 bp sequence was transcribed into mRNA. Preliminary results showed that the 24 bp sequence is highly repeated in the genome, and is located within the structural sequences of other myosin heavy chain genes. The correlation of the presence of this sequence with respect to the tissues and developmental stages at which the genes are transcribed is being explored.

1. Gulick, J.D., Kropp, K.E. and Robbins, J. (submitted).

0530 CARDIAC MYOSIN GENES: STATISTICAL ANALYSIS OF MOLECULAR EVOLUTION, James E. Levin and Patrick K. Umeda, University of Chicago, Chicago, IL 60637

The evolution of myosin heavy chain (HC) genes has been studied by examining the distribution of DNA sequence differences between closely related isoforms. In both the rabbit and the rat, statistical analysis of ventricular alpha and beta cDNA sequences reveals that differences tend to accumulate in clusters rather than being scattered at random. Even translationally silent differences are clustered when two genes from a single species are compared, but they are randomly distributed in cross-species comparisons. This result, as well as the analysis of clustering in over 100 comparisons of coding sequences from other gene families, suggests that gene conversion or similar events have acted on parts of the myosin HC genes. Heteroduplex analysis, S1 nuclease protection studies, and DNA sequencing have been used to localize several exon boundaries within the rabbit genes. Individual exons exhibit widely variable levels of sequence divergence, but within each exon, translationally silent changes appear to be scattered at random. Thus, the overall sequence divergence probably does not accurately reflect the length of time since these genes have diverged from their common ancestor. Instead, individual exons may have their own evolutionary history. Because of this, it is difficult to determine whether highly homologous regions are conserved due to functional constraints on the proteins, or are the result of chance homogenization of the genes.

0531 ACCUMULATION OF MYOSIN LIGHT CHAIN 3 IN DEVELOPING LIMB MUSCULATURE IS NERVE-DEPENDENT. Peter A. Merrifield and Irwin R. Konigsberg, University of Virginia.
In the chick, myosin alkali light chains ($MLC_{1f}$ and $MLC_{3f}$) are both transcribed from the same gene, however this transcription is differentially regulated during development (Nature 308, 333. 1984). $MLC_{1f}$ accumulates exclusively in the limb muscle of the early chick embryo; $MLC_{3f}$ is first detected at day 16 at approximately the time at which other contractile protein isoform switches reportedly occur (J. Cell Biol.95, 763. 1982; J. Biol. Chem. 257, 545. 1982.). We have used a monoclonal antibody to an epitope common to both alkali light chains to map the accumulation of $MLC_{1f}$ and $MLC_{3f}$ in the limb muscle of developing quail embryos and in aneural and innervated quail limb buds grafted onto the chorioallantoic (CAM) of chick embryos. Using immunoblot analysis, we demonstrate that $MLC_{1f}$ is present in the limb throughout early development and that $MLC_{3f}$ is first detected at day 11. Limb buds from three day old quail donors (without neural tube) grafted onto the CAM of seven day old chick hosts to a chronological age of 15 days develop only embryonic muscle - in that $MLC_{1f}$ accumulates exclusively. When limb buds are dissected to contain neural tube and grafted under identical conditions, they develop mature muscle as indicated by the accumulation of both $MLC_{1f}$ and $MLC_{3f}$. This is the first positive evidence that innervation is essential to normal muscle maturation in vivo. ( Supported by grants to IRK from NIH ( HD 07083 ) and the Muscular Dystrophy Association of America.)

0532 SUP-3: A MUTATION WHICH ELEVATES MYOSIN LEVELS IN NEMATODE MUSCLE IS CLOSELY LINKED TO A MYOSIN HEAVY CHAIN GENE, David M. Miller, MRC Laboratory of Molecular Biology, Cambridge, CB2 2QH, England.
Four different types of myosin heavy chain (MHC) are produced in C. elegans: MHC A and MHC B in the body wall muscle; MHC C and MHC D in the pharyngeal muscle. The complete nucleotide sequence of the MHC B (unc-54) gene and partial sequences of three homologous MHC genes (myo-1, myo-2, myo-3) have been determined (Karn et al. PNAS 80: 4253, 1983). The MHC isoforms encoded by these additional genes have now been identified. Hind III fragments from each MHC gene were ligated into the plasmid pUR 288 and expressed as fusion peptides with $\beta$-galactosidase. The reactivity of the hybrid proteins with monoclonal antibodies was tested on western blots. The reactivity of MHC B specific antibodies verified the assignment of the unc-54 gene. Antibody 5-6, which is specific to MHC A, reacts with the fusion peptide encoded by a 460 bp fragment from the S-2 region of myo-3 but not with fusion peptides from homologous regions of the other three genes. Similar experiments have suggested that myo-1 and myo-2 encode MHC D and MHC C. In situ hybridization data have localized myo-3 to a region of chromosome V encompassing the sup-3 gene (D. Albertson, unpub.). Mutations in the sup-3 locus result in a 2-3 fold elevation of MHC A (Waterston et al. in Diseases of the Motor Unit p747, 1982). To test the possibility of linkage between these two loci, restriction digests of sup-3 deficiency DNA (eDf1/+) were probed on Southern blots with cosmids containing the myo-3 gene. The sup-3 DNA exhibits ~15 kb Pst I and ~8 kb Bcl I fragments that are not present in wild type DNA. These results favor the hypothesis that sup-3 is a cis-acting DNA sequence that modulates the expression of the myo-3 gene.

0533 THE EFFECT OF THE SUP-3 SUPPRESSOR ON MUSCLE LATTICE STRUCTURE AND MYOSIN HEAVY CHAIN ACCUMULATION, Anthony Otsuka, Department of Genetics, University of California at Berkeley, Berkeley, Ca. 94720
The sup-3 suppressor improves the locomotion of several paramyosin (PM), null myosin heavy chain B (MHCB) and unc-87 mutants in Caenorhabditis elegans. Our analysis of muscle sections from suppressed PM mutants demonstrated regions of improved muscle lattice structure. Shortened myosin-containing filaments present in PM mutants appeared to elongate to normal length in the presence of sup-3. Studies by R. Waterston and his colleagues have shown that accumulation of a second body-wall myosin heavy chain, MHCA, is increased in suppressed MHCB mutants. We have found that the MHCA to MHCB ratio is increased in PM mutants (2.0 fold), as might be expected from the localization of MHCA to the midlength region of wild type thick filaments. In suppressed PM mutants, the ratio is increased even further (3.1 fold), suggesting that MHCA is preferentially incorporated into thick filaments. In strains containing only the sup-3 suppressor, the MHCA to MHCB ratio is increased relative to wild type (2.3 fold), demonstrating that sup-3 has a direct effect on the accumulation of MHCA. In suppressed MHCB null mutants, suppression may result from an increase in the number of thick filaments containing only MHCA. In suppressed PM mutants, suppression may be due to elongation of shortened thick filaments, although it is not clear how MHCA accumulation is involved in this process.

0534 MYOSIN HEAVY CHAIN GENE PROMOTERS, Jeffrey Robbins, James Gulick, Keith Kropp and Nancy Valente, University of Missouri-Columbia, Columbia, MO 65212

A comparative study of the chicken myosin heavy chain gene promoters and the 5'-flanking squences is underway. A total of 18 myosin promoters have been isolated and isoform specific probes prepared from the 5'-nontranslated regions. Although there are limited homologies present in the 5'-flanking sequences of some of the genes, the use of probes derived from the non-conserved regions allow detection of specific isoform expression at the RNA level and unambigious identification of the corresponding gene. These 5' flanking sequences are being analyzed in terms of their homologies and differences, and subsequently correlated with the genes' patterns of transcription during development and differentiation in the different muscle types.

0535 CLONING OF A SEA URCHIN MYOSIN HEAVY CHAIN GENE, Sam J. Rose, Marvin J. Rosenberg, Diane Chen, Cathy Shapiro, Eric H. Davidson and Roy J. Britten, California Instit. of Technology, Pasadena, Calif. 91125

Using a Drosophila probe, a coding region for sea urchin (Strongylocentrotus purpuratus) myosin heavy chain has been isolated from a genomic Charon 4 library. The area of homology is very near the carboxy terminus of the coding region and represents 75% nucleotide sequence and 70% amino acid sequence conservation between Drosophila melanogaster and S. purpuratus. Hybridization of these sequences can be observed at high criterion and occurs in an area not yet implicated as containing a specific functional role in the protein product.

0536 CHARACTERIZATION OF DEVELOPMENTAL AND DISEASE-RELATED MYOSIN HEAVY CHAIN ISOZYMES BY HPLC. Rushbrook, J.I., Weiss, C., Wadewitz, A.G. and Stracher, A., Department of Biochemistry, SUNY-Downstate Medical Center, 450 Clarkson Ave., Brooklyn, N.Y. 11203.

We have identified an atypical heavy chain in the myosin of the affected fast white muscle fibers of genetically dystrophic chickens which is clearly unrelated to the normal embryonic isoform (1,2). Bandman has recently presented evidence that it is, in fact, the later-appearing neonatal isoform.

In order to further characterize both developmental and disease-related variants we have developed HPLC procedures for the purification and study of myosin and various of its proteolytic fragments. Using a reverse-phase system separating the major tryptic fragments of S-1, we have identified 50kDa species characteristic of normal and neonatal myosins. HPLC analysis of thermolysin digests of these fragments indicates minimal sequence differences between adult and neonatal heavy chains.

By these procedures, the atypical dystrophic heavy chain is found to resemble strongly the neonatal isoform. Surprisingly, it constitutes as much as 40% of the heavy chain quotient in adult chickens. (Supported by 1 R01 NS 843801 to A.S.)

(1) Rushbrook, J.I, and Stracher, A. (1979) Proc. Natl Acad . Sci (USA) 76, 4331-4334. (2) Rushbrook, J.I., Yuan A. I and Stracher, A. (1981) Cell. Motil.1, 399-416. (3) Bandman (1984) Muscle and Nerve 7, 312-326.

0537 ANALYSIS OF HUMAN SKELETAL MUSCLE MYOSIN HEAVY CHAIN GENES, Lino J. Saez and Leslie A. Leinwand, Albert Einstein College of Medicine, New York, N.Y. 10461.

cDNA and genomic clones encoding human skeletal muscle myosin heavy chains (MHC) have been isolated and characterized. Using the human MHC cDNAs as probes, we have isolated a total of 9 non-overlapping human genomic MHC clones. This includes the 4 clones we previously described (Leinwand et al, 1983). 12 MHC cDNA clones were isolated from a human skeletal muscle cDNA library (provided by L. Kedes). At least 8 of these appear different by restriction endonuclease analysis. Two were selected for further analysis. DNA sequence and hybridization analyses demonstrate that pHMHC A3 and pHMHC Z2 represent two different, but closely related, MHC mRNAs that are expressed in adult human skeletal muscle. These two cDNA clones contain 2000 and 2600 nucleotides of the 3' ends of these mRNAs, respectively. The amino acids encoded in the DNA sequence of pHMHC A3 show a high degree of homology with the protein sequences of the carboxy terminal portions of skeletal and cardiac muscle MHCs from chicken, rat and nematode. This homology extends into the 3' untranslated region of the mRNAs from human, chicken and rat. The human skeletal muscle MHC sequence is more homologous to the chicken skeletal muscle sequence than it is to the rat cardiac muscle sequence indicating that these tissue-specific forms have evolved independently.

0538 HETEROGENEITY OF CONTRACTILE PROTEINS AND THE NATURE OF SKELETAL MUSCLE FIBER DIVERSITY. F. H. Schachat, M. M. Briggs, G. E. Moore, M. S. Diamond and P. W. Brandt, Depts. of Anatomy, Duke University, Durham, NC 27710 and Columbia University, New York, New York 10032.

Electrophoretic analysis of myofibrillar proteins from several rabbit fast skeletal muscles led to the identification of multiple fast troponin Ts. In Briggs et al. [J. Biol. Chem., 259, 103975 (1984)] two species of rabbit fast skeletal muscle troponin T (TnT), $TnT_{1f}$ and $TnT_{2f}$, were characterized. Here, the distribution of these fast TnT species and the $\alpha_2$ and $\alpha\beta$ tropomyosin (Tm) species found in fast muscle [Bronson and Schachat, J. Biol. Chem., 257, 3937 (1982)] is characterized in fast muscles and in single muscle fibers.

SDS-PAGE analysis shows that the presence of each fast TnT species is associated with the presence of a different Tm dimer: $TnT_{1f}$ with $\alpha\beta$ Tm and $TnT_{2f}$ with $\alpha_2$ Tm. Comparison of fiber type distributions with the patterns of expression of the fast TnT-Tm combinations shows that they cannot be explained by differences in the distribution of type IIA and type IIB fibers. The absence of a correlation between histochemical typing and the composition of the thin filament $Ca^{+2}$-regulatory complex is more apparent in individual fast muscle fibers where both fast TnT-Tm combinations are expressed in a continuum. This continuum of expression is inconsistent with the concept of distinct fiber types. But, it appears to be related to differences in the $Ca^{+2}$-cooperativity of fast fibers as revealed by physiological studies of skinned fibers.

0539 A NEW CALCIUM DEPENDENT SWITCH DURING MURINE MUSCLE DEVELOPMENT S.P.Scordilis, M.T.Anderson, E.B.Goodwin, E.A.Rowland, M.H.Cole and A.Guile, Dept. Biol. Sci., Smith College, Northampton, Mass. 01063

A major metabolic change of some calcium dependent proteins involved in various aspects of actomyosin regulation occurs between one and two weeks after birth in murine skeletal muscle. Myosin, myosin light chain kinase (MLCK), a $Ca^{2+}$dependent neutral protease (CDNP) and calmodulin (CaM) were characterized in mice from 18 days gestation to 8 weeks after birth. Myosin exhibited two isoforms, embryonic and adult: the light chain patterns (pI,mw) were identical, whereas the heavy chain CNBr peptides were different. Concomitant with this structural change, the actin-activated ATPase activity of the embryonic isomyosin depended on P-light chain phosphorylation, whereas that of the adult did not. Over the same time course the MLCK goes from a $Ca^{2+}$ independent form to a $Ca^{2+}$/CaM dependent one. Using myosin heavy chain as a substrate, CDNP was first detected 2 weeks after birth. This CDNP has a pCa for half maximal activation of 6.5 and is inhibited by trifluoperazine. Similarly, the amount of CaM increases from the embryo to a peak at birth and then decreases to adulthood. These results demonstrate that a concerted change (switch) occurs in several calcium ion dependent proteins which function in actomyosin regulation.

This work was supported by grants to SPS from the Muscular Dystrophy Association and the Blakeslee Fund of Smith College.

0540 STRUCTURE AND EXPRESSION OF HUMAN AND RAT HEART MYOSIN LIGHT CHAIN GENES, Leanne Cribbs[1], Pamela Delaney[2], Gloria Leon[3], Charmaine Mendola[1], Manuel Krauskopf[3], Ivan Balazs[4] and M.A.Q. Siddiqui[1], Roche Institute of Molecular Biology, Nutley, NJ[1], Montclair State College, Montclair, NJ[2], Universidad Austral de Chile, Valdivia, Chile[3], Actagene, Inc., Elmsford, NY[4].

Using the coding sequence of chicken heart myosin light chain ($MLC_2$) cDNA as probe, we have isolated cDNA clones from the human and rat heart cDNA libraries constructed in our laboratory. The entire base sequence of the clones, pHLC419 and pRLC249, specific for human and rat heart respectively was determined. Northern hybridization analysis of RNA probed with pRLC249 indicated no significant differences between normal and spontaneously hypertense rat heart $MLC_2$ mRNA during the rat embryonic development. A human chromosomal gene for $MLC_2$ was also isolated by screening the human DNA recombinant library. The human $MLC_2$ gene is represented in one copy per genome and appears to be located on chromosome 8. The gene structure and organization of human $MLC_2$ gene is currently under investigation.

0541 TWO HUMAN FETAL MYOSIN ISOZYMES RECOGNIZED BY MONOCLONAL ANTIBODIES, Laura Silberstein and Helen M. Blau, Department of Pharmacology, Stanford University School of Medicine, Stanford, Ca 94305.
The existence of multiple myosin isozymes in the development of mammalian striated muscle has been previously documented biochemically, immunologically, and most recently at the molecular level. Yet, the precise number of isoforms based on gene number may well be an underestimate of the total and little is known regarding the expression and distribution of these proteins during muscle development. We describe here two monoclonal antibodies which recognize distinct human skeletal myosin heavy chain isozymes detectable only during early development in human skeletal muscle. The specificity of these antibodies was verified by ELISA assay, Western blots, and in cryostat sections of human quadriceps muscle. Monoclonal antibody F.16 recognizes fetal, but not neonatal or adult, myosin. Monoclonal antibody F.33 reacts strongly with fetal, weakly with neonatal, and not at all with adult myosin. The distribution of the two myosins recognized by these antibodies is quite distinct in immunohistochemical studies of tissue sections. We conclude that there are at least two distinct myosin heavy chains with different fiber distributions present early in human muscle development.
This work was supported by awards from the Muscular Dystrophy Association and the National Institutes of Health.

0542 ACTIN GENES IN DIFFERENT DROSOPHILA SPECIES, Ann Sodja, Ana M. Mildner, Laura Elsenboss, Dept. of Biological Sciences, Wayne State University, Detroit, MI 48202
In order to gain a deeper understanding about the evolution of actin gene structure and function, we have initiated a comparative study of actin genes in different Drosophila species. Southern blotting/hybridization experiments suggest that the number and/or organization of these genes differs in other species from that in Drosophila melanogaster (Dm). Several actin containing recombinant phages from the respective genomic libraries have been isolated and are being analyzed, among others, with respect to their coding, intervening and adjacent sequences.
The similarities and differences observed in their structure and expression will be related to our existing data on the actin genes of Drosophila melanogaster. This data should enable us to identify structurally and functionally important features in actin gene expression and evolution.

0543 **MODULATION OF MYOFIBRILLAR ATPASE ACTIVITY ASSOCIATED WITH TRANSITIONS IN ISOFORMS OF TROPONIN I IN DEVELOPING DOG HEART WITH NO CHANGE IN ISOMYOSIN COMPOSITION.** **Solaro, R.J., Kumar, P., Blanchard, E.M. and Martin, A.F. The University of Cincinnati, College of Medicine, Cincinnati, OH 45267**

Developmental changes in the expression of isoforms of thick filament proteins in striated muscle have been identified in a number of animal species. We have examined the myosin $Ca^{2+}$-ATPase activity, the actomyosin $Ca^{2+}$-dependent Mg-ATPase activity and the protein composition in myofibrils isolated from hearts of adult and perinatal dogs. Although there were no differences in the $V_{max}$ of the ATPase activities between myofibrils from adult and perinatal hearts, the preparations showed significant differences in their response to acidic pH. Reductions in pH from 7.0 to 6.5 significantly reduced the sensitivity to $Ca^{2+}$ of the adult heart myofibrils, whereas the same change in pH had essentially no effect on the perinatal heart preparations. We found, using pyrophosphate gels, no difference in ventricular myosin isomyosin composition between adult and young animals. Examination of the myofibrillar proteins by SDS-PAGE indicated the presence of two forms of TnI in dog ventricle. The relative proportions of these isoforms of TnI changed substantially during development, suggesting that alterations in thin filament regulation may be the factor responsible for the difference in response to acidic pH. This was confirmed by filament displacement studies in which a ten fold excess of skeletal myosin was mixed with adult perinatal heart actomyosin preparations. These hybrid preparations showed the same differential response to acidic pH as the native preparations, indicating a lack of thick filament involvement in this effect. Thus, changes in TnI isoforms may play a significant role in modulating myofibrillar properties during cardiac development.

0544 VARIATION IN ACETYLCHOLINESTERASE ACTIVITY AND ITS LOCALIZATION IN A SUBSYNAPTIC GRADIENT ACCORDING TO TWITCH CHARACTER. Heather R. Stephens and Victor Gisiger, Dept d'Anatomie, Universite de Montreal, Montreal H3C 3J7 Quebec

In comparison to its fast-twitch counterparts, the soleus muscle of the normal mouse and rat exhibits a distinct content in acetylcholinesterase (AChE) characterized by a low activity, a low proportion in the molecular form $G_4$ and a relative increase in $A_8$. In addition, the soleus nerve showed a significant reduction in both total AChE activity and $G_4$ content as compared to nerves of fast-twitch muscles (Gisiger & Stephens, J. Physiologie., 78: 720, 1982-3). In the dystrophic 129/ReJ mouse, the fast muscles exhibit an AChE content typical of the soleus muscle and the AChE content of the nerves to fast muscles is similar to that to the normal soleus. Thus, there is a precise correlation between AChE present in a motor nerve and its muscle, as well as between the level of enzyme activity in these structures and their twitch properties. Moreover, this nerve-muscle correlation is maintained in the dystrophic condition. To search for sub-cellular correlates of the AChE molecular forms, we used two approaches. The first was a correlated biochemical-cytochemical analysis of corresponding serial muscle sections, which revealed that most of the $G_4$ was concentrated in sections containing motor endplates and that the AChE endplate reaction was accompanied by perijunctional activity within the fiber at the light microscopic level. The second approach, an ultrastructural cytochemical study, showed reaction product within the sarcoplasmic reticulum of endplate zones; it was maximal subsynaptically and decreased rapidly in a symmetrical manner distal to the endplate. The intensity and spread of this gradient were greater in fast-twitch muscles, suggesting that $G_4$ may be found in this region.

0545 DIFFERENTIAL CONTROL OF THE EXPRESSION OF SLOW AND FAST MYOSIN ISOENZYMES IN ADULT RODENT SKELETAL MUSCLE, D.B. Thomason, R.W. Tsika, and K.M. Baldwin, University of California, Irvine, CA 92717

Four myosin isoenzymes can be identified in rodent skeletal muscle based primarily on their light chain composition: one associated with slow fibers and three with fast fibers. Both the absolute and relative amounts of the isoenzymes change in ankle extensor synergists depending on the pattern of stress applied to the muscle. Chronic weight bearing or its elimination predominantly affects slow myosin expression, while phasic use patterns (e.g., running) have greatest impact on fast myosin expression. In normal muscles containing a spectrum of slow and fast isoenzymes, steady-state levels of mRNA coding for the myosin light chains (as determined by in vitro translation) correlate with the myosin isoenzyme profile. These results indicate transcriptional or post-transcriptional, pre-translational control of myosin isoenzyme expression. In addition, the differential response of the isoenzymes to combinations of stress patterns (e.g., chronic disuse coupled with phasic use) is indicative of different mechanisms for the control of slow and fast myosin expression.

0546 STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF MYOSIN ISOZYME SPECIFIC EPITOPES, Donald A. Winkelmann and Susan Lowey, Rosenstiel Research Center, Brandeis University, Waltham, MA 02254

The analysis of myosin with monoclonal antibodies has helped to establish the diversity of myosin isozymes in developing muscle tissues and to detect transitions in myosin expression. We have applied this approach to the study of avian fast skeletal muscle myosin by preparing monoclonal antibodies which react with epitopes in the head and rod regions of the myosin heavy chain (HC) and with myosin $LC2_f$ (Winkelmann et al., Cell 34 295, 1983). A group of antibodies which react with the 25Kd (12C5,10H10,4H7,6E2) and the 50Kd (13E1,13F6) tryptic fragments of the myosin head have been mapped on myosin by electron microscopy of rotary shadowed antibody-myosin complexes. The sites for three epitopes in the 25Kd fragment, including one within 5Kd of the N-terminus of the myosin HC, are clustered on the head 145±20Å from the head-rod junction. An epitope in the 50Kd fragment (13F6) maps even further out on the head. The accessibility of these epitopes on S-1 bound to actin was probed with $F_{ab}$ fragments; none of these sites are on the contact surface between the head and actin. Two antibodies (12C5 & 4H7) have distinctive effects on the $K^+$EDTA- and $Ca^{2+}$-ATPase activities of myosin which mimic the effects on activity of modification of the reactive sulfhydryl, SH-1. 12C5 also inhibits the actin-activated ATPase. None of the other antibodies to the head affect myosin ATPase activity. Anti-LMM (5C3) identifies an adult specific epitope on the C-terminus of the rod. This antibody markedly affects myosin filament formation. These results identify epitopes located in functionally important regions on myosin which change on isozymes expressed during development.

0547 The Structure of cDNA Clones from Rabbit Skeletal Muscle

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cDNA clones corresponding to the C-terminal end of myosin heavy chain mRNA from rabbit adult skeletal muscle have been obtained using cDNA synthesis primed with oligo(dT). cDNA clones corresponding to the internal sequence of MHC were obtained with specific primers. The primary structure of these cDNA clones was determined and compared to the DNA sequence of MHC cDNA clones from other sources and to the protein sequence data available.

## *Gene Organization*

0548 ACTIN AND MYOSIN GENES : THEIR EXPRESSION DURING MYOGENESIS IN THE MOUSE

Margaret Buckingham, Serge Alonso, Paul Barton, Arlette Cohen, Philippe Daubas, Ian Garner, Benoît Robert and André Weydert, Pasteur Institute, Paris (France)

During the maturation of skeletal muscle fibres in vivo genes encoding different striated muscle isoforms are expressed. In the mouse, transcripts of a foetal skeletal myosin heavy chain (MHC) gene are replaced at birth by those coding for an adult skeletal MHC (1). Actin and myosin alkali light chain (MLC) mRNAs and proteins, characteristic of adult heart muscle accumulate with skeletal isoforms in late foetal skeletal muscle. The cardiac ($\alpha_C$) actin gene continues to be expressed in new born skeletal muscle while only the adult fast skeletal MLC mRNAs are detectable after birth. Prior to this the cardiac atrial $MLC1_A$ and the fast skeletal $MLC1_F$ are the major foetal isoforms (2). Each of these multigene families therefore follows a distinct and non-synchronous developmental strategy. In mammalian muscle cell lines (C2, T984, L6), the types of striated muscle MLC and actin genes expressed vary with the line. In vivo the expression of genes encoding cardiac isoforms is not confined to developing skeletal muscle ; $MLC1_S$ of adult soleus is encoded by the same gene as the cardiac $MLC1_V$ isoform.

Using a genetic approach we have looked at the linkage relationships and chromosomal localization of actin and myosin genes in the mouse. Genes expressed in the same adult cardiac or skeletal, or foetal skeletal, muscle phenotypes are not linked. The actins and MLCs are encoded by dispersed multigene families. Cardiac and skeletal MHC genes are also not linked.

For studies on the structure and regulation of muscle genes we have concentrated on the skeletal/cardiac interplay of gene expression in the actin and MLC families. $MLC1_F$ and $MLC3_F$ are encoded by a single gene (3); two mRNAs are generated by initiation at different 5' promoters followed by differential splicing of exons coding for the distinct $-NH_2$ termini of the two proteins. Sequencing of the $MLC1_A$ ($MLC1_{emb}$) gene (2) is in progress, and should provide the first amino acid sequence of this protein. Comparison of the coding sequences of mouse $\alpha_C$, $\alpha_{SK}$ and β actin mRNAs indicates differences in codon usage, with conservation of part of the 5' coding region. The 5' promoter regions of the $\alpha_C$ and $\alpha_{SK}$ actin genes have been sequenced. In the former a $(TG)_{24}$ stretch of potential "Z" forming DNA is found upstream from the CAAT box. In some mus 1 mouse lines, the whole 5' end of this gene including upstream promoter sequences is duplicated raising questions on regulation since the two promoters are potentially functional. Different actin and MLC gene promoter regions have been introduced into $SV_0CAT$ vectors and are currently being used in transfection experiments in muscle cell lines in order to examine sequences necessary for the regulation of individual genes during myoblast differentiation, and to compare regulatory phenomena between these cardiac/skeletal gene pairs.

(1) Weydert et al. (1983) J.B.C. 258, 13867-13874 ; (2) Barton et al. (1984) J. Muscle Res. & Cell Motility, in press ; (3) Robert et al. (1984) Cell 29, 129-140.

0549 MOLECULAR BIOLOGY OF HUMAN MYOGENESIS, Larry Kedes, Peter Gunning, Adrian Minty, Robert Wade, William Bains, Harry Erba, Department of Medicine, Veterans Administration Medical Center and Stanford Medical School, Palo Alto, CA 94304, and Edna Hardeman, Department of Pharmacology, Stanford Medical School, Stanford, CA 94305. Different isoforms of contractile proteins accumulate during the formation of skeletal muscle fibers and their differentiation. We and others have examined the kinetics of transcription and accumulation of mRNAs for a number of muscle specific contractile proteins. This talk will consider the mechanisms that regulate these tissue specific events and present data that extends the observations to 20 abundant human adult skeletal muscle mRNAs. Using cDNAs isolated from a human muscle cDNA library we have observed that most of these abundant transcripts are markedly different, either in quantity or size, from those expressed in non-muscle cells or in myoblasts. These clones do not code for contractile proteins for which hybridization probes are available. The general nature and implications of these findings will be discussed. We have investigated the tissue specific regulation of contractile protein genes by reintroducing them into both muscle and non-muscle cells. A description of these experiments will present evidence for muscle specific factors that interact with regions upstream from the human cardiac actin gene.

0550 CLONING MUSCLE RELATED GENES OF CAENORHABDITIS ELEGANS, Robert H. Waterston, Donald G. Moerman, Guy Benian, G. Ross Francis and Robert Barstead, Department of Genetics, Washington University, St. Louis, MO 63110

Mutant alleles of more than 25 genes affecting muscle structure have been identified in Caenorhabditis elegans. The protein produced for four of these genes has been discovered and the DNA sequence of the genes for the myosin heavy chains and for actins have been determined. Sequence analysis of mutant genes combined with other studies is providing new insight into the function of these proteins and their regulation.

The protein products of the other genetically identified genes is as yet unknown. Monoclonal antibodies produced to a variety of muscle components have revealed significant new features of C. elegans muscle structure. We are now trying to recover the genes that encode these polypeptides through the use of cDNA and genomic libraries in expression vectors. These clones will then be used to determine the chromosomal location of the genes through linked polymorphorisms or in situ hybridizations. This position will be used to investigate the relationship of these genes to the genetically defined elements.

Our recent identification of a transposon based mutator system in C. elegans provides an alternative approach to establishing gene:product relationships for additional genes. We have shown that the repetitive element Tc1 transposes at useful frequencies in the Bergerac strain of C. elegans. A favored target is the muscle related gene unc-22, which mutates spontaneously in this background at a rate of $10^{-4}$. Using Tc1 as a probe against filter transfers of mutant DNA we have been able to identify and to clone the unc-22 fragment containing the newly inserted Tc1. The flanking unique sequence DNA has been used to recover about 50 kb of DNA from the region. Fourteen independent spontaneous unc-22 mutations have been analysed and each appears to be due to the insertion of a Tc1 element but the site of insertion is different for each allele examined. The sites are scattered over a region of about 15-20 kb, indicating that unc-22 may encode a large polypeptide. Results from Northern analysis are consistent with this notion, and indicate that unc-22 produces an mRNA of >10,000 nts.

Recovery of spontaneous mutations in other genes has been possible and we are currently determining if Tc1 transposition is the basis for some of these mutations. If so it should be feasible to recover DNA from these genes as well by using Tc1 as a tag and thereby to determine the protein product of additional genetic elements involved in specifying C. elegans muscle structure.

## *Gene Organization*

0551 **MUSCLE GENE EXPRESSION IN DROSOPHILA AND IN VERTEBRATES.** **N. Davidson, B.J. Bond, S. Falkenthal,[2] V.P. Parker,[2], C. Rozek,[3] and S.B. Sharp, Department of Chemistry, California Institute of Technology, Pasadena, CA 91125**

**Several different mechanisms have been adapted by Drosophila melanogaster for the generation of diversity in the isoforms of muscle proteins that are expressed in different developmental stages and tissues. There are six actin genes and they are dispersed in the genome. Each gene codes for a slightly different protein and each exhibits a unique tissue and temporal pattern of expression during development. In contrast, there is only one myosin heavy chain gene (MHC), one myosin alkali light chain gene (MLC-ALK), and one myosin DTNB light chain gene (MLC-2). There are developmental differences in splicing patterns at the 3' ends of the MHC and MLC-ALK genes; in both cases, differing splicing choices result in different amino acid sequences at the carboxy termini of the isoforms. There appears to be only one MLC-2 peptide used in muscles in all developmental stages. Some developmental differences in polyadenylation sites, of unknown significance, are also observed. Results will also be reported on the application of gene transfer methods for the study of regulated expression of vertebrate actin genes in myogenic cell lines.**

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0552 SARCOMERIC MYOSIN HEAVY CHAIN GENE FAMILY ORGANIZATION AND PATTERN OF EXPRESSION. V.Mahdavi, E.E. Strehler, M. Periasamy, D. Wieczorek, S.Izumo, S.Grund, A.P.Chambers, M.A.Strehler, and B. Nadal-Ginard. Dept. of Cardiology, Children's Hospital and Dept. of Pediatrics, Harvard Medical School,Boston,MA 02115.

The sarcomeric myosin heavy chains (MHCs), which exhibit different levels of ATPase activity are encoded by a closely related multigene family from which 7 numbers have been identified and characterized by the isolation of cDNA and genomic recombinant clones, DNA sequencing, blot analysis and $S_1$-nuclease mapping. In the rat, the main tissue and/or developmental specificity of these seven genes is as follows: embryonic (fetal), perinatal, fast oxydative (type IIA), fast glycolytic (type IIB) and super-slow (extraocular muscle) skeletal; fetal (β) and adult (α) cardiac. Interestingly, the fetal cardiac (β) and slow skeletal muscle (type I) MHCs are encoded by the same gene. A similar identity is found between the adult ventricular (α) and atrial MHC mRNAs which are also encoded by the same gene.

The MHC genes appear to map to a single chromosome and at least two of them α- and β- cardiac, are closely linked in the genome (1). Each of these genes is ∿25kb long and their coding sequences are interrupted by many introns (40 in the case of embryonic gene, (2) whose location is highly conserved among MHC genes. Interestingly, each gene contains a unique and specific sequence that comprise the COO- terminal peptide and 3' untranslated region of the mRNA in a separate exon (3). These and other gene-specific sequences have been used to study the expression of the seven MHC genes by the $S_1$-nuclease mapping technique. Each MHC gene displays a pattern of expression that is tissue-specific and developmentally regulated, with more than one MHC gene expressed in each muscle and developmental stage. Moreover, with the exception of the super-slow gene that has a very specific pattern of expression, the other genes are all expressed in more than one tissue.

The expression of cardiac and skeletal MHC genes can be modulated by thyroid hormone, presumably at the transcriptional level (4). Surprisingly, however, the same gene expressed in different muscles responds differently to the hormone. In hypothyroid animals the expression of the α-cardiac MHC gene is repressed in the ventricles but not in the atria. Hyperthyroid states repress the expression of the fetal cardiac gene in the ventricle but not in slow skeletal muscle. This behavior of the MHC genes provides an interesting model in which to study the DNA sequences which are responsible for the developmental, hormonal and tissue-specific regulation of these genes.

(1) Mahdavi, V. et al. (1984) Proc.Natl.Acad.Sci. U.S.A. 81, 2626-2630. (2) Strehler, E.E. et al. (1984) J.Biol.Chem., in press. (3) Nadal-Ginard, B. et al. (1982) in Muscle Development: Molecular and Cellular Control (Pearson, M.L., and Epstein, H.F.,eds.), pp. 143-168. (4) Lompre, A.M. et al. (1984) J.Biol.Chem. 259, 6437-6446.

0553 MOLECULAR ANATOMY OF MYOSIN HEAVY CHAIN GENES, Patrick K. Umeda, James E. Levin, Achyut M. Sinha, Leanne L. Cribbs, Douglas Darling, David J. Ende, Huey-Juang Hsu, Evelyn Dizon, and Simlja Jakovcic, Departments of Medicine, and Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637.

Cardiac and skeletal muscles contain multiple molecular forms of myosin heavy chains (HCs) that are encoded by a multigene family. The expression of these genes follows a characteristic pattern during development, and may be altered by physiologic and environmental stimuli. To determine the molecular mechanisms that regulate the expression of individual myosin HC genes, we have isolated genomic clones specifying the high ATPase HCα and the low ATPase HCβ of rabbit ventricles. A gene library was screened with probes from cDNA clones specifying α and β myosin HCs, and 7 strongly hybridizing clones were studied in detail. Two overlapping clones contained over 25 kbp of the α HC gene. Within this region, electron-microscopic heteroduplex analysis and DNA sequencing identified at least 32 exons encoding approximately 90% of the mRNA. A β HC genomic clone containing 20 exons from the 5' region of the gene has also been characterized. Corresponding exons of the α and β HC genes are similar in size, but the introns showed marked differences in their length and sequence divergence. A similar organization of exons in human as well as chicken HC genes suggests that this complex gene structure is a general characteristic of HC genes from higher eukaryotes. By DNA sequence analysis, several exons from the 5' portions of both rabbit genes have been precisely determined. Between the two cardiac HC genes, this region shows greater overall as well as silent site sequence divergence than the 3' portion coding for the light meromyosin part of the HC. In both genes, the 5' exon identified by heteroduplex analysis encodes 67 amino acids of the N-terminus of the protein. S1 nuclease mapping using an end-labeled probe from the N-terminal exons indicates that the coding sequences extend 9 and 12 nucleotides (nt) upstream of the N-termini of the beta and alpha HC genes, respectively. At the 5' boundary of this exon, both genes contain consensus splice acceptor sequences. Furthermore, primer extension experiments using a synthetic oligonucleotide complementary to the first seven codons of the β HC gene, reveals a major cDNA of approximately 120 nts with the β HC mRNA. A similar size product was not observed with either α HC mRNA, or rat liver poly(A) RNA. The results indicate that the 5' untranslated region of the β HC gene is interrupted by intervening sequences, and also suggests the presence of a corresponding intron in the α HC gene. Experiments are now underway to delineate the transcriptional initiation sites of both genes and putative 5' flanking regulatory regions. In addition, the coding and intervening sequences identified from our sequence analysis are being used to examine the transcription of the α and β HC genes in isolated nuclei.

## *Gene Expression*

0554 MYOSIN POLYMORPHISM DURING MUSCLE DEVELOPMENT, MATURATION, AND DISEASE, Everett Bandman, University of California, Davis, CA 95616

My laboratory has studied the myosin heavy chain isoform composition of normal and diseased skeletal muscle during differentiation and maturation in order to determine the factors which regulate myosin expression. To complement our biochemical techniques we have produced numerous monoclonal antibodies, many of which are monospecific for fast and slow developmental isoforms. Reacting western blots of myosin heavy chain peptide maps with these monoclonal antibodies yields "immunofingerprints" characteristic of each myosin isoform. Our results reveal considerable heterogeneity in the sequence of myosin expression during development of fast muscle fibers. The embryonic to neonatal to adult myosin transition characteristic of the pectoralis major muscle is not observed in other fast twitch muscles such as the posterior lattissimus dorsi and the lateral head of the gastrocnemius. Muscle pathology clearly alters myosin expression, most often resulting in the expression of myosins characteristic of immature muscle. While often this is the result of regeneration resulting in the induction of embryonic myosin, avian dystrophy appears to actually inhibit the neonatal to adult transition in the pectoralis. Thus determining the primary lesion of avian dystrophy may yield important clues to the regulation of myosin expression during normal development. Other experiments studying myosin expression in vitro and a unique autoimmune disease involving antibodies to masticatory muscle myosins will be described.

0555 **MYOSIN LIGHT CHAIN GENE EXPRESSION IN CARDIAC AND SKELETAL MUSCLE.** Paul J R Barton, Mark Fiszman, Benoit Robert & Margaret E Buckingham. Institut Pasteur Paris FRANCE.
We have isolated clones from a cDNA library of mouse cardiac mRNA, that encode part of the adult atrial (LC1A) and ventricular (LC1V) myosin light chain mRNAs. One of these clones (pC6) containing part of the atrial LC1A mRNA sequence shows hybridization to a mRNA in 18 day fetal skeletal muscle that is of the same size and which shows the same thermal stability of hybridization as the atrial LC1A mRNA. Plasmid pC6 also hybdridizes to RNA from cultured myotubes and the in vitro translation product of this RNA comigrates with the fetal isoform LC1emb expressed in these cells. Two dimensional gel analysis of adult atrial and fetal skeletal proteins show LC1A and LC1emb to be indistinguishable in the mouse. Hybridization of pC6 to mouse genomic DNA indicates the presence of a single genomic locus and we conclude that the atrial light chain LC1A and the fetal isoform LC1emb are encoded by the same gene and are probably identical.
The ventricular type cDNA clone pA29 has been identified as containing part of the LC1V mRNA sequence by hybrid selected in vitro translation. Hybridization of this clone to other adult muscle RNAs shows LC1V mRNA to be present in soleus muscle which contains a mixture of fast and slow fibre types but is absent from RNA of fast skeletal muscle. Analysis at the protein, RNA and gene level indicates that the ventricular form LC1V and the slow skeletal muscle form LC1S in soleus muscle are encoded by the same gene and are probably identical. Genetic analysis in the mouse shows that the genes coding for LC1A (LC1emb), LC1V (LC1S) and the fast skeletal muscle LC1F/LC3F gene are located on separate mouse chromosomes. We are currently sequencing the genes encoding these cardiac isoforms.

0556 MUTATIONS IN THE MYOSIN HEAVY CHAIN GENE OF DROSOPHILA INDICATE THIS GENE IS REQUIRED FOR BOTH LARVAL AND ADULT DEVELOPMENT, S.I.Bernstein*, P.T.O'Donnell*, K.Mogami+, T.R.F.Wright+, and C.P.Emerson,Jr.+, *Biology Dept., San Diego State University, San Diego, CA 92182 and +Biology Dept., University of Virginia, Charlottesville, VA 22901
Drosophila has a single myosin heavy chain (MHC) gene which maps to a chromosomal region known to contain several dominant flight muscle mutations (Bernstein et al., Nature 302: 393, 1983). We have analyzed the DNA of several of these mutants and found four which possess abnormalities in their MHC gene. Each of these mutations is lethal when homozygous. Death occurs at the late embryonic or larval stage of development. The most severe mutation (Mhc-1)has a 100 bp deletion near the 5' end of the gene. No abnormal MHC RNA is detected in Mhc-1/+ pupae, but the level of thoracic MHC protein is reduced to half that of wild-type. The three other mutations result from DNA insertions in introns near the 5' end of the gene. The DNA insertions possess transcription termination sites that result in the production of truncated MHC transcripts. Since mutant/+ pupae accumulate levels of MHC protein intermediate between that of Mhc-1 and wild-type, we hypothesize that transcription sometimes proceeds to the normal termination site and that introns (including the transcribed DNA insertion) are spliced out. This would result in the production of some normal MHC mRNA. Our results prove that the single MHC gene of Drosophila is required for both larval and adult development.

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0557 GENE EXPRESSION IN STRIATED MUSCLE OF IMMOBILIZED LIMBS OF RATS, Frank W. Booth, University of Texas Medical School, Houston, Texas 77225

Fixation of the hindlimbs of rats in plaster of paris results in the atrophy of the gastrocnemius muscle, which has been immobilized at, or less than, resting length. During the first 6 hours of limb immobilization, synthesis rates of mixed proteins, actin and cytochrome c in the muscle are significantly decreased. The contents of α actin mRNA and cytochrome c mRNA in the gastrocnemius are not significantly changed at the 6th hour of hindlimb immobilization. These results suggest that a decrease in muscle size because of disuse is not initiated by a decrease in mRNA content.

0558 A SINGLE TROPONIN T GENE GENERATES MULTIPLE PROTEIN ISOFORMS BY A NOVEL PATTERN OF ALTERNATIVE SPLICING. R.E.Breitbart,H.T.Nguyen,R.M.Medford,V.Mahdavi, and B.Nadal-Ginard, Dept. of Cardiology, Children's Hospital and Dept. of Pediatrics, Harvard Medical School, Boston, MA 02115.

Alternative splicing of primary RNA transcripts has been shown to be an important mechanism for the generation of protein diversity from single genes for myosin light chain, α-tropomyosin, and troponin T.

The troponin T gene, in particular, expresses at least four mRNA sequences, differing in their inclusion or exclusion of five separate mini exons located in the 5' portion of the gene. The detailed splice junction pattern of these exons, in fact, demonstrates the potential for multiple additional combinatorial arrangements, and several of them are evident on S1 mapping analysis. Moreover, there are another two duplicated mini exons further downstream, coding for aa 229-242, which are incorporated in a mutually exclusive fashion, effectively doubling the number of protein isoforms which could be produced from this gene. The pattern of alternative splicing evidenced here rules out a processive scanning model of splice site recognition.

A minimum of two of the troponin T mRNAs share 5' untranslated sequence, suggesting that a common promoter may initiate transcription of more than one mature message. If this is the case, then trans-acting factors must be involved in the control of alternative splicing of the primary transcript.

0559 DEVELOPMENTAL REGULATION OF TISSUE SPECIFIC ISOFORMS OF TROPONIN T MEDIATED BY DIFFERENTIAL SPLICING PATHWAYS, Elizabeth Bucher, Kenneth Hastings, and Charles P. Emerson Jr.

Two cDNAs and a genomic clone of the muscle regulatory protein, troponin T, are being characterized by DNA sequence analysis and S1 mapping of RNA transcripts during quail muscle development. Comparison of two cDNA clones isolated from a quail embryonic myofiber library reveals that their nucleotide sequences are identical except for 38 nucleotides encoding amino acids 228 to 242, near the troponin T carboxy terminus. The cDNA data is consistent with a mechanism involving alternative splicing of a single gene transcript.

S1 mapping demonstrates developmental transitions of two Tn-T mRNAs that these two cDNAs represent. Cultured myofibers, embryonic pectoralis, and embryonic leg coexpress the transcripts. In contrast, adult leg and adductor preferentially express one mRNA transcript over the other, while adult pectoralis exculsively expresses one form. This transition of isoforms implies a mechanism involving precise developmental control of internal splice choices during muscle maturation.

Characterization of the Quail Tn-T genomic clone is consistent with a single gene encoding these troponin T isoforms. Continued analysis of the gene should be useful in determining the molecular basis for troponin T isoform generation.

0560 CLONING AND CHARACTERIZATION OF A FULL-LENGTH MOUSE MUSCLE CREATINE KINASE cDNA, J.N. Buskin, J.B. Jaynes, J.S. Chamberlain, and S.D. Hauschka, Department of Biochemistry, University of Washington, Seattle, Washington 98195

We have previously reported cloning partial cDNAs for the mouse muscle isozyme of creatine kinase (M-CK). These clones were used to isolate cDNAs which, in composite, extend from the 5' untranslated region of the mRNA through the 3' poly A tail. The exact 5' end of the mRNA has been determined by primer extension. The entire cDNA, ~1400 bp excluding poly A, has been sequenced. Portions of the gene have also been sequenced; exon sequences correspond exactly to the cDNA sequence. Comparison of genomic and cDNA sequences has been used to determine the exact location of splice sites. The mouse M-CK DNA sequence and the deduced amino acid sequence of the protein have been compared to sequences reported for other species. Consistent with immunological and DNA hybridization experiments, these comparisons have demonstrated the high degree of conservation between the M-CKs of various vertebrate species.

0561 INDUCTION OF MUSCLE CREATINE KINASE IS TRANSCRIPTIONALLY REGULATED FOLLOWING MITOGEN WITHDRAWAL FROM MOUSE MYOBLASTS, J.S. Chamberlain, J.B. Jaynes, J.N. Buskin, and S.D. Hauschka, Dept. of Biochemistry, University of Washington, Seattle, WA 98195.

We have examined the regulation of creatine kinase (CK) induction in differentiating MM14 mouse myoblasts. Trace amounts of the BB isozyme of CK are present in proliferating myoblasts, but mitogen withdrawal from the cultures triggers a rapid induction of both MM- and MB-CK. Antibodies raised against mouse MM-CK cross-react with MM- and BB-CK from human, rabbit, chicken, and mouse, suggesting the presence of highly conserved sequences in these enzymes. However, a cDNA clone containing only the 3' non-translated region was identified with CK antibodies and is specific to mouse M-CK as judged by solution hybridization and dot blot analysis. This clone was therefore used to quantitate M-CK mRNA levels during myoblast differentiation and to isolate the M-CK gene. Proliferating myoblasts contain less than 10 molecules per cell of M-CK mRNA. Induction of the message precedes the increase in M-CK protein and is first detectable 5 h after mitogen withdrawal (2-3 h after the first cells commit to terminal differentiation and 7 h before fusion begins). Message levels peak 24 h after mitogen withdrawal at 1100 molecules per nucleus, and then decline. Hybridization of nuclear run-off transcripts from isolated myoblast and myocyte nuclei to M-CK clones demonstrates that the increase in mRNA levels is accompanied by an increase in the rate of transcription of the M-CK gene.

0562 RNA PROBES TO DEVELOPMENTALLY REGULATED MUSCLE GENES, John R. Coleman and Charles P. Ordahl, Division of Biology and Medicine, Brown University, Providence, RI 02912 (JRC) and Department of Anatomy, UCSF, San Francisco, CA 94143 (CPO), USA

Sequences from cDNA clones coding for chicken muscle proteins have been inserted into vectors containing the bacteriophage promoter SP6 and transcribed to generate $^{32}P$- and $^{3}H$-labelled RNA probes of high specific activity for use in RNA blot and in situ hybridization analyses. Sequences inserted to date include the 3'-untranslated end of skeletal α-actin (260 bp), the 3'-untranslated end of cardiac troponin T (140 bp), and a coding sequence from the 5' end of M-creatine kinase (240 bp). Sequences were inserted into vectors in both orientations and transcripts of both DNA strands were made using SP6 RNA polymerase. Blot hybridization analyses using poly(A)+ RNA demonstrate that all three genes are activated during skeletal muscle cell differentiation in ovo. Cardiac troponin T expression ceases in skeletal muscle as development progresses, but continues in cardiac muscle, confirming the findings of Cooper and Ordahl (Science, In Press). In situ hybridization analyses of the cellular patterns of gene expression during skeletal muscle differentiation in vitro are in progress. (Supported by NIH Grant #GM-32018 to CPO and NSF Grant #PCM-82-15972 to JRC)

0563 A SINGLE CARDIAC TROPONIN T GENE IS GOVERNED BY TWO DIFFERENT REGULATORY PROGRAMS AND GENERATES TWO mRNAs VIA ALTERNATE SPLICING

Thomas A. Cooper and Charles P. Ordahl
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A cDNA clone of a low-abundance mRNA expressed transiently during early skeletal muscle development (Class B mRNA-106A4; Ordahl et. al. PNAS 77:4516, 1980) has been found to encode a cardiac isoform of troponin T by amino acid sequence analysis. In contrast to its regulation in skeletal muscle development 106A4 is strongly up-regulated during cardiac development indicating that it is a cardiac isoform of troponin T. The mRNA in both tissues is the product of a single gene (Cooper and Ordahl, Science, in press). We have isolated the cloned gene and identified all exons by nucleotide sequencing. Work in progress to determine whether a single promoter initiates transcription in both tissues will be presented. In addition, nucleotide sequence of cDNA clones indicates that this gene generates two mRNAs in cardiac muscle via developmentally regulated alternate splicing near the 5' end of the mRNA. In contrast to rat skeletal muscle troponin T (Medford et. al., Cell 38: 409,1984), we have found no alternative splicing within the 3' end of the mRNA. We are currently characterizing the splicing patterns in both cardiac and skeletal muscle. Toyota and Shimada (Cell 33:297,1983) have shown that down-regulation of cardiac troponin T expression in cultured embryonic skeletal muscle is under neurogenic control. We are designing experiments to identify the genic components responsive to innervation.

0564 GENE SPECIFIC EFFECT OF COLLAGEN INHIBITION ON MYOGENESIS, J. James Donady and Sandy Timmerman, Biology Dept., Wesleyan University, Middletown, CT 06457

Primary cultures of Drosophila myoblasts are inhibited from completing myogenesis, including myotube fusion, by cell-substrate contact with vertebrate collagen (type I). The treatment, which causes the cells to float, is reversible. The analysis of two muscle-specific genes, actin (57A) and myosin heavy chain (38B), shows different responses to the absence of substrate attachment. The actin gene activity is not affected by collagen treatment. Hybridization of a 3' gene-specific probe to dot blots of poly(A)+ RNA from treated cells shows no detectable decrease in transcripts compared to control cells of the same age (myotube fusion stage). However, the myosin heavy chain gene activity shows a dramatic decrease in poly(A)+ RNA in treated cells. Therefore, specific gene activity is affected by alteration of cell-substrate adhesion and/or cell shape. The effects of this alteration on transcription or RNA processing, as well as on other coordinately regulated genes, are under investigation.

0565 THE NUCLEOTIDE SEQUENCE OF THE CHICK ALPHA-CARDIAC ACTIN GENE: THE MAJOR ISOFORM EXPRESSED IN EMBRYONIC AVIAN SKELETAL MUSCLE, Juanita Eldridge, Zendra Zehner, and Bruce M. Paterson, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

The entire nucleotide sequence of the chicken α-cardiac actin gene has been determined. This is the first complete sequence of a cardiac actin gene that includes the promotor region, all the introns, and the polyadenylation site. The α-cardiac actin gene is the major isoform expressed in developing embryonic avian skeletal muscle. The gene contains 6 introns, 5 of which interrupt the coding region at amino acids 41, 150, 204, 267, and 327. The first intron is in the 5' noncoding region and is 438 base pairs in length.

The α-cardiac actin gene encodes an mRNA of approximately 1400 base pairs with a 5' and a 3' untranslated region of 59 and 184 nucleotides, respectively. Like the chicken α-skeletal actin gene, the α-cardiac actin gene has the Cys codon between the initiator ATG and the codon for the N-terminal amino acid (Asp) of the mature protein. There are no major sequence homologies from the CAAT to the TATA regions between the chicken skeletal and cardiac actin genes; however, the 3' region contains a short sequence homology, TCAGGATGAC, reported for other actin genes. This short sequence does not appear to be muscle specific when compared to sequences in the National Sequence Data Base.

0566 HUMAN ACTIN GENE ORGANIZATION AND DIFFERENTIAL EXPRESSION, H.P. Erba, P. Gunning, L. Kedes, Dept. of Medicine, Stanford University, Stanford, CA 94305, J. Leavitt, Linus Pauling Institute, Palo Alto, CA 94306, R. Eddy and T. Shows, Roswell Park Memorial Institute, Buffalo, N.Y. 14263.

The genes for human γ-cytoplasmic and α-skeletal actins have been characterized in order to study their expression in vitro. We have detected variations in steady-state levels of γ and β actin mRNAs in various tissues. In one case, a 5-fold elevation of γ actin mRNA levels accompanied spontaneous transformation of rat-2 fibroblasts. The complete sequences of two γ actin cDNAs, isolated from a human fibroblast library, have been determined. These two cDNAs differ by only two silent substitutions in the codons for amino acids 305 and 309. An isotype-specific probe from the 3'UTR of the γ-cytoplasmic actin cDNA hybridizes to 14 EcoRI fragments in the human genome at low stringency, but to a single 25 Kbp EcoRI fragment at 50C below the Tm. The EcoRI fragments of lower homology have been cloned in λ vectors and appear by restriction enzyme mapping to be processed pseudogenes. Hybridization of the γ-specific probe to mouse-human synkaryons indicates dispersion of the pseudogenes in the human genome. A single pseudogene maps between q21 and p11 of the human X chromosome. The α-skeletal gene has been isolated from a human genomic library by in vivo recombination with a πVX plasmid containing the 3'UTR of the α-skeletal actin cDNA. The sequence of the human α-skeletal gene and its 5' flanking region has been determined.

0567 STRUCTURE AND EXPRESSION OF ACTIN GENES DURING TRAUMATIC REGENERATION.
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Laboratoire de Biologie de la Differenciation Cellulaire, LA CNRS 179, Faculté des Sciences de Luminy - 13288 MARSEILLE CEDEX 9 - France.

In order to understand the variations of the terminal differentiation programme of muscle cells during regeneration of two species (a Polychaete Annelid : Owenia fusiformis, and an Amphibian : Pleurodeles waltlii) we clone their actin genes. From Owenia we obtained 10 genomic clones and two cDNA clones from Pleurodeles muscle RNAs. By determining the sequence of Pleurodeles $\alpha$ actin cDNA clones we noticed an important concervation of the skeletal $\alpha$ actin 3'non coding sequence, with the corresponding genes of binds and mammals (Pleurodeles is an ancestral form of lower vertebrate). This conservation occurs in a special structure (block of 100 nucleotides GC rich region followed by a 130 nucleotides AT rich region ; an average conservation of 80 % occurs mainly in AT rich blocks of $\simeq$ 20 nucleotides). We assume from this result that 3' non coding region may play a role in terminal differentiation processes. Different possible models will be discussed. Using these probes we have determined the variations of expression of these genes during traumatic regeneration. Parallel studies are done in the lab. on the structure and evolution of invertabrates myosin heavy chain genes.

0568 ENHANCER ACTIVITY ASSOCIATED WITH THE CYTOPLASMIC β-ACTIN GENE. Nevis L. Fregien and Norman Davidson, California Institute of Technology, Pasadena, CA 91125.

When Myoblasts in culture are induced to differentiate into myotubes, a number of muscle specific genes are activated. At the same time several genes, including the cytoplasmic β-actin gene, are turned off. We have examined the β-actin gene for enhancer sequences which may be utilized for the control of expression of the β-actin gene in myoblasts and myotubes. Fragments of the chick β-actin gene were inserted ajacent to the bacterial neomycin resistance gene which uses a weakened herpes simplex virus thymidine kinase gene promoter. The resultant plasmids are assayed for their ability to transform myoblasts (BC3H-1 and C2/C12 cells) to G-418 resistance. Increased transformation frequencies were observed when the entire β-actin gene or certain fragments of it are placed next to the weakened promoter. The enhancing activity does not appear to be myoblast specific since it is also observed in fibroblasts (Ltk$^-$ cells). Measurements of the amount of RNA in myoblasts and myotubes showed that the thymidine kinase promoter is not shut off in myotubes. The best results were obtained when the 5' end of the β-actin gene, including the promoter and the first intron, was placed in parallel orientation to the thymidine kinase promoter. This construction gave the highest transformation efficiency and showed marked reduction of expression during differentiation.

0569 GENE EXPRESSION SURROUNDING THE SINGLE MYOSIN HEAVY CHAIN GENE OF DROSOPHILA: A REGION OF SEVERAL FLIGHT MUSCLE MUTATIONS, Elizabeth L. George and Charles P. Emerson, Jr., University of Virginia, Charlottesville, VA 22901

Several dominant mutations that disrupt myofibrils of the indirect flight muscles of Drosophila have been isolated by Mogami and Hotta (1981). These mutations cluster at two loci in the genome, one of which is the site of the single copy myosin heavy chain gene (Bernstein, et al., 1983). More precise genetic mapping suggests that these mutations map outside of the MHC gene. We have cloned 75 kb of genomic DNA surrounding the MHC gene, and several distinct RNA's in this region are specific to embryonic muscle. Some of these genes express multiple transcripts according to developmental stage, including two genes with an additional myofiber-specific transcript. We are currently investigating these genes further to determine if they are the site of any of the flight-muscle mutations.

0570 ISOLATION AND CHARACTERIZATION OF THE RAT SARCOMERIC MYOSIN HEAVY CHAIN GENE FAMILY. S.H. Grund, M. Periasamy, E. Strehler, D. Wieczorek, D. Bois, V. Mahdavi, and B. Nadal-Ginard. Dept. of Cardiology, Children's Hospital, Dept. of Pediatrics, Harvard Medical School, Boston, MA 02115.

In order to understand the molecular mechanisms underlying developmental- and tissue-specific expression of the sarcomeric myosin heavy chain (MHC) multigene family, we have isolated and characterized MHC genomic clones from a rat genomic library. Comparison of genomic clone sequences with those from previously characterized MHC cDNAs indicates that we have isolated MHC genomic clones specific for embryonic, adult types IIA and IIB fast, an MHC genomic clone uniquely expressed in the rat extraocular eye muscle, and the cardiac ventricular α- and β- myosin heavy chains. $S_1$ mapping analysis demonstrates that these individual MHC genomic clones are expressed in a developmental and/or tissue specific manner consistent with their assigned specificity. However, the β-cardiac MHC gene is also expressed in adult soleus muscle. The embryonic MHC gene, which has been completely sequenced, spans approximately 25kb and is composed of 39 exons and 40 introns. To try to define sequences necessary for regulated expression of MHC, we have compared 5' flanking sequences of the embryonic, adult fast red and two cardiac MHC genes. We have reported previously that the sarcomeric MHC gene family is located on a single chromosome in the mouse and humans and that the two cardiac ventricular MHC genes are located 4kb apart. Preliminary evidence suggests that at least two of the skeletal MHC genes are also closely linked ( ∿4kb).

0571 MYOSIN GENE EXPRESSION IN CHICK MUSCLES. James Gulick and Jeffrey Robbins, University of Missouri, Columbia, MO 65212

Synthetic oligonucleotides complementary to the mRNA coding for two chicken fast-white myosin heavy chain genes are being used to study the process of differential transcription. The oligonucleotides (15-mers) correspond to the 5' non-coding region of the two genes. When used in "dot-blot" analyses with genomic clones containing different MHC promoter sequences they demonstrated isoform specificity in the different muscle types. Southern transfers of chicken genomic DNA were used to verify the absolute specificity of each probe. Each oligonucleotide was annealed to adult and embryonic breast, leg and cardiac mRNA, extended with reverse transcriptase and the products analyzed on a sequencing gel. The resulting extension products allow us to determine when and at what level these genes are expressed in the different muscle types.

0572 ISOLATION AND ANALYSIS OF EXPRESSION OF cDNAs ENCODING ABUNDANT HUMAN SKELETAL MUSCLE mRNAs. P. Gunning[1], E. Hardeman[2], J. Garrison[3], R. Wade[1], H. Blau[2] and L. Kedes[1], Departments of Medicine[1] and Pharmacology[2], Stanford University, Stanford, CA 94305 and Department of Pharmacology[3], University of Virginia, Charlottesville, VA 22908

We have utilized novel features of the Okayama-Berg cDNA cloning vehicle to isolate cDNA clones encoding abundant human skeletal muscle mRNAs. The method allowed us to isolate these clones without using radiolabeled RNA or DNA probes and simultaneously enriched for full-length cDNAs. Twelve cDNAs encode mRNAs which are sarcomeric muscle specific (heart plus skeletal muscle) whereas six encode mRNAs which are skeletal muscle specific. Twelve cDNAs are expressed in non-muscle and muscle cells. Analysis of the expression of these sequences during human myogenesis revealed a variety of regulatory programs. Only four clones recognize RNAs which are expressed at similar levels in fibroblasts, myoblasts, myotubes and adult skeletal muscle. The remainder recognize mRNAs which are either up or down regulated in steady state levels by over 10-fold during myogenesis. A surprising number (13) cDNAs recognize multiple transcripts of different sizes and at least six of these detect mRNAs whose transcript size changes during myogenesis. Genomic blot analysis indicates that some of these changes reflect different transcripts generated by a single gene.

0573 ACTIVATION OF HEART GENES DURING SKELETAL MUSCLE MYOBLAST DIFFERENTIATION, Pat L. Hallauer and Charles P. Emerson, Jr., Biology Department, University of Virginia, Charlottesville, VA 22901

A key feature in the developmental regulation of skeletal muscle gene expression is the coordinate activation of a diverse set of skeletal muscle contractile protein genes during myoblast fusion. We have used a cDNA clone analysis to ask whether the homologous cardiac muscle gene set may be included in this process. cDNA clones corresponding to 15 different mRNAs whose abundance increase during quail skeletal myoblast differentiation were probed with hot cDNA made from adult quail heart or liver RNA. Three of the cDNA clones hybridized extensively and exclusively with the cardiac RNA sequence probe. Analysis by Northern blotting, S1 nuclease and DNA sequencing techniques identified these three clones as encoding cardiac isoforms of myosin heavy chain, actin and troponin C. Thus, at least three "heart" genes are activated during skeletal muscle myoblast fusion. We propose that the coactivation of homologous skeletal and cardiac muscle genes is a simple consequence of their original creation by gene duplication.

0574 TRANSCRIPTIONAL ACTIVATION OF SARCOMERIC ACTINS IN STABLE HETEROKARYONS. E.C. Hardeman, C.-P. Chiu and H.M. Blau. Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.

We previously reported that the synthesis of human muscle proteins can be induced in heterokaryons formed by the PEG-mediated fusion of mouse muscle and human nonmuscle cells. To date, we have detected the synthesis of six human muscle-specific proteins in these stable somatic cell hybrids. In order to determine whether activation occurs at the transcriptional or at the post-transcriptional level, we examined the accumulation of messenger RNAs from two human sarcomeric genes,those encoding the a-cardiac and a-skeletal muscle actins. These two actins coexist in heart and skeletal muscle tissues in vivo, but a-cardiac actin is the major sarcomeric actin in embryonic skeletal muscle and a-skeletal actin is the major sarcomeric actin in adult skeletal muscle. Using actin isotype and species-specific cDNA probes together with Northern blot analysis we monitored the timecourse and relative amounts of the two muscle-specific transcripts in heterokaryons. Our results showed that the synthesis of both human muscle actin gene messenger RNAs was induced in heterokaryons and both transcripts accumulated with time after heterokaryon formation. No human-specific sarcomeric actin transcripts were detected in either parental cell type prior to fusion. In addition, a-cardiac actin was the predominant of the two actin transcripts which is in agreement with expression during *in vitro* myogenesis. We conclude that activation of both human sarcomeric actin genes in mouse muscle x human nonmuscle heterokaryons occurs at the transcriptional level and that the expression of these muscle-specific genes parallels that of pure myogenic cultures.

0575 DNA FROM NORMAL AND DYSTROPHIC CHICKEN IS NOT IDENTICAL 3' DISTAL TO THE FAST-MHC GENE. S. Heywood and D. Zezza, The University of Connecticut, Storrs, CT 06268

A subclone containing the 3' terminus of the fast isoform of myosin heavy chain (MHC) gene obtained from a dystrophic chicken library was used to select a 20 kb downstream genomic fragment from normal chickens. It was found that an overlapping restriction fragment from the dystrophic DNA failed to hybridize to $pCMtcRNA_{102}$ (a cDNA clone of $tcRNA_{102}$, one of several small RNAs found to be associated with MHC-mRNA in an mRNP particle) while the downstream overlapping restriction fragment of the DNA from normal chicken contained sequences hybridizing to $pCMtcRNA_{102}$. A 4.1 kb subclone was subsequently isolated from the normal chicken DNA and compared to its homologous DNA from the dystrophic chicken. Restriction map analysis suggests that both the dystrophic DNA and the normal chicken DNA are from the same area of the genome; however, significant differences are observed in the size of some of the restriction fragments. These data suggest that a coding sequence for $tcRNA_{102}$ is located 3' distal to the fast-MHC gene in normal chickens. On the contrary, this area of the genome is perturbed and lacks this sequence in the DNA of dystrophic chickens. Although these genomic alterations are observed in cloned DNA, major differences have not been detected in genomic blots of normal and dystrophic DNA. This may result from multiple copies of $tcRNA_{102}$ genes present in the genome. An analysis of the RNAs associated with MHC-mRNA in the MHC-mRNP from normal and dystrophic chick muscle suggests that these differences observed in the DNA can also be detected in the transcription products. This work supported by NIH grant #HD03316-16.

0576 REGULATION OF MYOSIN HEAVY CHAIN GENE EXPRESSION IN CAENORHABDITIS ELEGANS, Sandra Honda and Henry F. Epstein, Baylor College of Medicine, Houston, TX 77030
The mode of action of two putative regulatory loci affecting myosin heavy chain (MHC) synthesis is being investigated in order to understand the relationship between the two body wall myosins during thick filament assembly. Unc-52 II mutants display a phenotype of progressive paralysis due to retarded sarcomere construction. Biochemically, these mutants synthesize lower levels of MHC B (unc-54 gene product) during late larval stages relative to wild type. Using unc-54 specific probes from the coil-coil region of a myosin genomic clone, it appears that these decreased levels of MHC B reflect lower amounts of steady state unc-54 mRNA by Northern blots. To assess whether such differences are transcriptional, a nuclear runoff transcription system has been established. Approximately 70-80% of the $\alpha^{32}$P-UTP-incorporating activity of isolated nuclei can be attributed to RNA polymerase II in $\alpha$-amanitin inhibitor studies. Work is in progress to determine unc-54 gene activity in unc-52 mutants. Similar studies are now under way to examine a second regulatory mutant, sup-3 V, which suppresses unc-54 I mutations be elevating levels of MHC A.

0577 CLONING AND CHARACTERIZATION OF THE MOUSE MUSCLE CREATINE KINASE GENE, J.B. Jaynes, J.S. Chamberlain, J.N. Buskin, J.E. Johnson, and S.D. Hauschka, Dept. of Biochemistry, University of Washington, Seattle, Washington 98195.

The gene coding for the muscle-specific isoform of creatine kinase (M-CK) in mouse has been cloned and its transcriptional unit analyzed. A minimum of six introns are excised from the primary transcript (which is at least 12 kb in length), one from the 5' non-translated portion of the mRNA and the others from its protein coding region. The gene contains sequences within introns which are reiterated to varying degrees in the genome, and some of these are highly transcribed in nuclei isolated from 3T3 cells, from myoblasts, and from myotubes.

Portions of the protein coding sequence of the mRNA are homologous to human M-CK as judged by cross-hybridization of cDNA subclones with human genomic DNA. Portions of M-CK also cross-hybridize with mRNA of the mouse brain-specific isozyme (B-CK) as judged by dot blot analysis, while the 3' non-translated region of M-CK does not cross-hybridize. Northern blot analysis of mouse muscle and brain RNAs shows that 1) the M-CK and B-CK messages are similar in size (1600-1700 bases), and 2) no cross-hybridization with different sized RNAs is detected with probes from either the protein coding region or the 3' non-translated region of M-CK cDNA.

Experiments are underway to determine which portions of the gene are essential for its regulated expression during differentiation of MM14 mouse myoblasts in culture.

0578 PROPER DEVELOPMENTAL ACTIVATION OF A QUAIL TROPONIN I GENE INTRODUCED INTO MULTIPOTENTIAL AND MYOGENIC MOUSE CELL LINEAGES, Stephen F. Konieczny and Charles P. Emerson, Jr., University of Virginia, Charlottesville, VA 22901
DNA-mediated transfection experiments were undertaken to examine the molecular mechanisms which coordinate contractile protein gene expression during skeletal muscle determination and differentiation. The questions of interest are whether the determinitive events which lead to the formation of myogenic lineages are essential for the activation of the contractile protein genes or whether these genes can be properly activated solely as a response to the differentiative event itself. A quail troponin I (TnI) contractile protein gene, containing only 530 bp of 5' and 1.5 kb of 3' flanking sequences, was stably transfected into the mouse multipotential cell line, 10T1/2, and into a myogenic cell line derived by 5-azacytidine conversion of 10T1/2 cells (Konieczny and Emerson, Cell 38:791-800, 1984). Differentiated myofiber cultures of transfected clones exhibit up to a 100 fold increase in quail TnI transcripts compared to the low TnI levels found in myoblast and 10T1/2 cultures. Transfected multipotential 10T1/2 cells converted with 5-azacytidine into a clonal line of determined myoblasts also exhibit a 100 fold accumulation of TnI transcripts in myofiber cultures only. Nuclei run-on assays show that the myofiber-specific TnI message accumulation is due to differential activation of transcription during differentiation. These results demonstrate that the myogenic determinative events are not essential to the differentiation-specific activation of contractile protein genes. In addition, they suggest that myofiber-specific factors, which interact with cis-acting information residing within the quail TnI gene itself, solely are responsible for this developmental activation.

0579 ACTIN GENE TRANSCRIPTION IN REVERTANTS OF C. ELEGANS ACTIN MUTANTS, Michael Krause and David Hirsh, University of Colorado, Boulder Colorado 80309

The extensive molecular biology available for Caenorhabditis elegans muscle in conjunction with feasible biochemistry has made the worm an attractive system for the study of muscle development. We have chosen to look at one aspect of muscle development; the regulation of actin gene expression. C. elegans has four actin genes and each has been cloned and sequenced. In addition, actin mutants and revertants have recently been identified. By comparing actin gene transcription in wild-type, mutant and revertant animals, we hope to gain insight into the molecular events regulating these genes. We have focused our analysis on four revertants of actin mutants in which reversion was accompanied by gross rearrangements in one or more of the actin genes.

All four revertants have an abnormal pattern of actin gene transcription during post-embryonic development. These differences predominantly result from fluctuations in the relative abundance of actin transcripts. In at least two revertants there is also a temporal change in actin message abundance in contrast to the temporally invariant wild-type pattern. Furthermore, we have been able to demonstrate that either of two actin genes compensates transcriptionally in response to alterations in the level of transcription of the other gene.

0580 V-MOS TRANSFORMATION OF NIH 3T3 CELLS INDUCES AN ALTERED CHROMATIN STRUCUTRE IN THE $\alpha_2$(I) COLLAGEN PROMOTER, G. Liau and B. de Crombrugghe, NIH Bethesda MD 20205

The collagens are structural proteins involved in extracellular matrix formation in animal tissues. We have isolated the $\alpha_2$(I) and $\alpha_1$(III) collagen genes and have used as a model system to examine the developmental regulation of these genes, the inhibition of their synthesis after oncogenic transformation of cultured fibroblasts. There is good evidence to suggest this inhibition is transcriptionally mediated. We have examined the chromatin structure around the transcriptional start site of the $\alpha_2$(I) collagen gene in control and V-mos transformed mouse fibroblasts. NIH 3T3 cells contain a DNase I hypersensitive site mapping between -250 to +100. Restriction enzyme sites within this region are readily accessible to digestion in isolated nuclei. Dde 1, Mnl 1, and Hinf 1 sites, which are dispersed throughout the 3 Kb region probed, are only accessible in the -350 to +45 region. An Xba 1 site at -505 and a Bgl II site at -350 are also accessible. Nuclei isolated from V-mos transformed fibroblasts contain open sites from -101 to -114 when probed with Apa I, Hinf 1, and Dde 1 respectively and is, in addition, accessible to Hinf 1 at -250. However, the Bgl II site at -350 and the Xba site at -505 are now almost completely protected from digestion. An Sph 1 site at +58 is also substantially less accessible than in NIH 3T3 nuclei. Our in vivo restriction enzyme map suggests that V-mos transformation induces an alteration in chromatin structure around the promoter of the $\alpha_2$(I) collagen gene. The protection of the upstream region could be due to binding of a protein which may in turn alter the transcriptional efficacy of the $\alpha_2$(I) collagen gene.

0581 STRUCTURE OF THE HUMAN MUSCLE TROPOMYOSIN GENE FAMILY. Girish Modi and Frank S. Walsh, Molecular Neurobiology Lab, Inst Neurology, London and Kathleen Talbot and Alexander R. MacLeod, Ludwig Inst, Cambridge.

Human α and β-TM clones were isolated from a human skeletal muscle cDNA library. Positive clones were identified by hybridisation with rabbit α and β-TM clones. Three specific profiles of hybridisation were found. These were fragments that reacted with the rabbit α-TM probe, fragments that were α, β cross-reactive and fragments that were β-specific. A 1.2Kb PVU II-Hind III fragment from a β-TM specific clone was isolated and subcloned into M13mp8 and sequenced by the Sanger procedure. Extensive amino acid sequence homology was found with the known sequence of rabbit β-TM and the human cDNA contains sequence information from amino acid 150 to the polyA tail. A 0.6Kb PVU II-Hind III fragment of the α-TM clone is being sequenced. Southern analysis of Hind III digested human DNA revealed 6 bands of 16, 14, 8.5, 6.5, 3 and 0.6Kb for α-TM and 4 bands of 16, 4.2, 3 and 0.6Kb for β-TM. To attempt to analyse the structure of these genomic sequences further a Hind III genomic library of human DNA cloned in $\lambda_{2558}$ was screened with the α and β-TM cDNA fragments. A number of clones have been isolated and are being analysed. Southern analysis of Hind III digested human and mouse DNA showed that different bands are detected across species suggesting that these genes may be assigned to a specific human chromosome and to determine whether the α and β genes are linked. These studies are now in progress.

0582 TROPOMYOSIN GENE EXPRESSION IN QUAIL MYOFIBERS AND FIBROBLASTS, Sonia H. Pearson-White, Patricia Hallauer, and Charles P. Emerson, Jr., University of Virginia, Charlottesville, VA 22901.

We are investigating the molecular basis for the regulated expression of the muscle-specific contractile protein genes in the quail. Two cDNA clones were isolated from a quail myofiber library by differential hybridization with myofiber and myoblast cDNA. Comparison with published nucleotide sequences suggests that cC101 is a muscle-specific alpha-tropomyosin, while cC102 is a fibroblast tropomyosin. Is the fibroblast isoform generally expressed in myofibers together with the muscle-specific form? These clones share a region of sequence identity of more than 350 nucleotides. Do these two forms arise by differential splicing of a single gene, or do the several tropomyosin genes suggested by a genomic Southern blot produce single transcripts with long identical regions maintained by gene conversion or natural selection? New myofiber and fibroblast full-length cDNA libraries were constructed and the nucleotide sequence of clones homologous to cC101 and cC102 is being determined. Regions specific to each isoform will be used as specific probes to identify which isoforms are expressed in which cell types.

0583 MECHANISM(S) OF ALTERNATIVE SPLICING OF MLC $1/3_f$ GENE PRIMARY TRANSCRIPTS. M. Periasamy, E.E. Strehler, and B. Nadal-Ginard. Dept. of Cardiology, Children's Hospital, Dept. of Pediatrics, Harvard Medical School, Boston, MA 02115.

In fast skeletal muscle a single gene locus encodes two different developmentally regulated alkali myosin light chain isoforms (MLC 1 and 3). The structural organization of MLC 1/3 gene implies alternative splicing of the primary transcript in order to produce either $MLC1_f$ or $MLC3_f$ mRNAs. By in vitro transcription analysis, we have shown that $MLC1/3_f$ gene has two transcriptionally active promotors specific for MLC1 and 3. Structurally these promotors are ∿10kb apart and can give rise to two mRNA precursors of significantly different primary structure both in size (∿10kb and ∿20kb) and sequence. So it may well be, the selective use of a specific promotor could determine a particular splicing pathway via secondary or tertiary structure of the primary transcript. In order to elucidate the role of RNA structure and conformation on RNA processing, we have constructed $MLC1_f$ and $MLC3_f$ minigenes with several structural modifications. These minigene constructs are currently being studied for their ability to produce correctly spliced MLC1 or 3 mRNAs when introduced into different mammalian cells. These experiments will allow to distinguish between cis - and trans-acting mechanisms involved in alternative splicing.

0584 STRUCTURE AND ORGANIZATION OF THE α-TROPOMYOSIN GENE: DIFFERENTIAL SPLICING OF "ISOTYPE-SWITCH EXONS" INVOLVING ALTERNATIVE SPLICING PATHWAYS ACCOUNTS FOR EXPRESSION OF THREE DEVELOPMENTAL- AND TISSUE-SPECIFIC α-TM ISOFORMS. N. Ruiz-Opazo and B. Nadal-Ginard, Dept. of Cardiology,Children's Hospital, Dept. of Pediatrics, Harvard Medical School, Boston, MA 02115.

α-tropomyosin (α-TM)is composed of two subunits, with approximate MW of 33,000 daltons each. This protein in conjunction with the troponin complex plays a central role in the $Ca^{2+}$-dependent regulation of muscle contraction. Two previously characterized α-TM cDNA clones isolated from smooth and skeletal muscle were completely sequenced and used as probes in $S_1$ nuclease mapping studies to assess the developmental and tissue-specific expression of the α-TM gene. These studies have defined the molecular structure of three distinct α-TM mRNA isoforms each being shown to have common as well as amino-proximal- and carboxy-terminal isotype specific regions. Common and isotype-specific cDNA sequences were then utilized as probes to isolate genomic recombinant clones spanning approximately 60 kb of the rat genome. Southern blots of genomic DNA and α-TM λ-genomic recombinant clones have revealed that the common and isotype-specific sequences are present in a single copy per haploid genome. Further analysis has shown that the α-TM gene is approximately 50kb long. The common region comprises only a small portion of the genomic sequences (∿5kb). In marked contrast, the isotype-specific regions are encoded in multiple exons(upstream and downstream from the common region)spanning the remaining 45kb of the α-TM gene. These results demonstrate that three α-TM mRNA isoforms identified are produced from the same gene by differential splicing of a set of exchangeable "isotype-switch exons" involving complex alternative splicing pathways.

0585 METHYLATION: A POTENTIAL REGULATOR OF GENE EXPRESSION DURING MYOCYTE DIFFERENTIATION, Richard L. Sabina, Nalini Desai, and Edward W. Holmes, Duke University Medical Center, Durham, NC 27710

L-6 is an established cell line which can differentiate into myotubes under the appropriate culture conditions. The studies described here have tested several classes of compounds as potential modulators of differentiation in the L-6 model. Myogenesis was assessed by morphology and expression of the muscle specific proteins, creatine kinase (CK) and AMP deaminase (AMP-D).

Results: 1) 5-azacytidine (azaCR) and its deoxyribose analog are potent inducers of myogenesis. The development of intact myofibrils and onset of spontaneous contractions appeared earlier in azaCR-treated cells compared to untreated cells. CK and AMP-D activities increased earlier and reached higher levels - 55 and 500-fold increases, respectively, in azaCR-treated vs 10-fold lower increases in untreated cells. 2) The concentration of azaCR which maximally stimulates myogenesis is $10^{-6}$M. Higher concentrations are toxic and lower concentrations are ineffective. 3) DNA is hypomethylated in azaCR-treated relative to control cultures. 4) Cytosine arabinoside suppresses the increase in CK and AMP-D activities. Insulin, epinephrine, and acetylcholine did not affect differentiation.

Conclusions: 1) Agents which lead to hypomethylation (azaCR) of DNA enhance differentiation, while agents which lead to hypermethylation (cytosine arabinoside) suppress differentiation. 2) In cells already committed to myogenesis, methylation of DNA may regulate expression of genes encoding for muscle specific proteins.

0586 A NOVEL CYTOPLASMIC TRANSLATION INHIBITORY RNA & RNP OF CHICK EMBRYONIC MUSCLE: PROPERTIES AND POSSIBLE ROLE IN MYOGENESIS, S. Sarkar, C. Jayabaskaran & S. Dasgupta, Boston Biomedical Research Institute, Boston, MA 02114.

The mRNA and protein levels for the developmentally regulated fast myosin light chain $LC_3$ show a noncoordinate relationship during chick embryonic breast muscle development (FEBS Lett., 149, 22, 1982). In our attempts to probe for possible "regulatory" macromolecules involved in $LC_3$ synthesis, we have isolated and characterized a novel cytoplasmic translation inhibitory 10 S RNP (iRNP) containing a 4 S RNA (iRNA) species from chick embryonic muscle (JBC, 256, 5077, 1981). 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 mRNA binding to the 43 S preinitiation complex (Met-$tRNA_f$ .40 S ribosome .eIF2) (JBC, 258, 15141, 1983). The biochemical properties of iRNA and iRNP indicate that they represent a novel class of cytoplasmic macromolecules. The nucleotide sequence of three iRNA subspecies (136, 110 and 76 nucleotides respectively) show that they are distinct from other eukaryotic small RNAs. Several iRNA subspecies contain 5'-ppp termini indicating that they are bonafide gene transcripts. The iRNA subspecies hybridize with muscle mRNAs in "dot blots". The iRNA and iRNP exert a negative control on the translation process by inhibiting a rate limiting step in protein synthesis i.e., selection of specific mRNA for ribosome loading. The relevance of these results to the regulation of myogenesis *in vivo*, and in particular $LC_3$ mRNA translation will be discussed.

0587 REGULATION OF INTACT AND HYBRID α AND β ACTIN GENES INSERTED INTO MYOGENIC CELLS. S. B. Sharp, Caltech, Pasadena, CA 91125; T. A. Kost, Norden Labs, Lincoln, NB 68501; S. H. Hughes, Frederick Cancer Research Facility, Frederick, MD 21701; C. P. Ordahl, UCSF, San Francisco, CA 94143; N. Davidson, Caltech, Pasadena, CA 91125.

In order to determine which regions of cytoplasmic and skeletal muscle chicken actin genes contain cis-acting sequences important in regulating their developmentally timed expression, we have transferred intact and hybrid genes into a myogenic cell line and monitored their expression. Each of four genes, intact β-actin, intact skeletal α-actin, a 5'α-3'β hybrid, and a 5'β-3'α hybrid, was inserted into a plasmid containing the gene for G418 resistance, and transferred into the mouse myoblast-like cell line, BC3H-1. Transformant colonies from each of the transfers were pooled and expanded. Gene expression in the undifferentiated and differentiated states was monitored by RNA gel blots using gene specific probes from the 3' untranslated regions of the chick α and β genes. The intact α and β genes were differentially regulated. β mRNA was present in uninduced cells, and just as for the endogenous mouse β mRNA,was substantially reduced in differentiated cells. Chicken α message was not appropriately upregulated upon induction. It, like β, was present in uninduced cells, but unlike β, showed no dramatic decrease in abundance upon differentiation. Preliminary results with the hybrid genes indicate that the β promoter is stronger than the α promoter in undifferentiated cells, and that most of the information for down-regulation upon differentiation resides in the 3' half of the β gene.

0588 TISSUE-SPECIFIC EXPRESSION OF A PROCESSED CALMODULIN GENE, Joseph P. Stein, Division of Endocrinology, University of Texas Medical School, Houston, Texas

Two calmodulin genes exist in the chicken genome. One, CL1, codes for an authentic calmodulin protein that is expressed in all tissues of the chicken. The other, CM1, appears to be a processed copy of CL1, since it contains no introns and is flanked by 9 bp direct repeats. CM1 codes for a calmodulin-like protein (CaML) that has 19 amino acid differences from other vertebrate calmodulins, some of which are located in residues critical for calcium binding. The possible function(s) of this protein is currently under investigation. Interestingly, the CM1 gene is expressed only in cardiac and skeletal muscle tissue, albeit at a significantly lower level than the authentic (CL1) calmodulin gene. 500 bp upstream from the AUG initiator codon is a 200 bp internally repetitive sequence, made up primarily of multiple repeats of the tetranucleotide sequence TGGA. This repeat sequence appears to be unique in the chicken genome. We are currently testing it for possible enhancer activity. The reasons for the muscle-specific expression of this processed calmodulin gene are being investigated.

0589 IDENTIFICATION OF A cDNA REPRESENTING A HUMAN X-LINKED MUSCLE SPECIFIC SEQUENCE, R. Wade[1], P. Gunning[1], J. Garrison[2], T. Shows[3] and L.H. Kedes[1], Stanford University School of Medicine[1], Stanford, CA 94305, University of Virginia[2], Charlottesville, VA 22908, Roswell Park Memorial Institute[3], Buffalo, N.Y. 14263.

We have sought to identify and localize muscle-specific genes along the human X chromosome. Clones isolated from a human adult cDNA library (see abstract by Gunning et al) were used as probes against Southern blots of DNA from human cell lines containing either a single X chromosome (1X) or four X chromosomes (4X) (provided by L. Kunkel and S. Latt, Harvard). The cDNA clones were scored for differential hybridization to restriction fragments of 4X over 1 X containing DNA. In this manner, a number of potentially X-linked cDNA clones were selected for further analysis utilizing Southern blots of DNA from human/rodent hybrid cell lines containing defined portions of the human X chromosome. A muscle cDNA clone has been found to share homology with a sequence located along the short arm of the X chromosome. Northern blots of total RNA from human fibroblasts, myoblasts, and adult muscle detect at least six distinct transcripts that hybridize with the cDNA probe, some of which are muscle-specific. Southern blot analysis of human DNA indicates that the sequence, while present in at least two loci, is not representative of a large multigene family, suggesting that the variety of transcripts are due to complex transcriptional or post-transcriptional processes. The nature of these processes is under investigation. Additionally, the potential value of this clone as a probe for the detection of restriction fragment length polymorphisms (RFLPs) useful as cosegregating markers of X-linked disorders is being pursued.

0590 CHARACTERIZATION OF THE MYOSIN HEAVY CHAIN GENE FAMILY IN RAT EXTRAOCULAR MUSCULATURE. D.F. Wieczorek, M. Periasamy, and B. Nadal-Ginard, Dept. of Cardiology, Children's Hospital, Dept. of Pediatrics, Harvard Medical School, Boston, MA 02115.

Myosin heavy chain (MHC), the main component of the thick filament of the sarcomere, is encoded by a highly conserved multigene family of 7-10 members which in mouse and human appears to be clustered onto a single chromosome. Each of the different sarcomeric MHC genes displays a pattern of expression that is tissue specific and developmentally regulated.

We have isolated and characterized an MHC genomic clone, designated λ10B3 which was found to be expressed solely in the extraocular musculature of the rat. DNA sequencing analysis indicates this clone contains sequence which codes for amino acid 1697 to the carboxyl-terminal amino acids, which corresponds to the rod portion of the light meromyosin molecule. $S_1$-nuclease mapping experiments have shown that this gene is induced in the first month of post-natal life and is expressed through adult life. Additionally, the extraocular muscle fibers in the adult were found to simultaneously accumulate a minimum of 6 different MHC mRNAs transcribed from different genes, including embryonic, neonatal, adult skeletal and cardiac muscle isoforms. This coexpression of multiple transcripts corresponding to embryonic, neonatal, and adult MHC isoforms in the extraocular musculature offers new insights into the phenotypic expression of the MHC multigene family.

0591 NUCLEOTIDE HOMOLOGY BETWEEN CHICK EMBRYONIC SKELETAL AND CARDIAC MHC cDNAs, Barry J. Zadeh, Arlene Gonzalez-Sanchez, Tomoh Masaki and David Bader, Cornell University Medical College, New York, N.Y. 10021

A library of DNA clones was prepared from mRNA isolated from embryonic chick heart. Filter hybridization with pMHC-25, a MHC cDNA developed by Medford et al. (PNAS 77:5749, 1980), was used to identify MHC recombinants. One of these, designated pCMHC-1, had been identified and characterized by sequence analysis, restriction mapping and Southern hybridization. Southern blots of pCMHC-1 with pMHC-25 confirm the insert contains MHC coding sequences. Endonuclease digestion with various nucleases produces restriction fragments identical with those previously reported for an embryonic chick skeletal muscle MHC cDNA (Kavinsky et al., J.B.C. 258:5196, 1983). Sequence analysis shows exact nucleotide homology between these cDNAs in the coding region near the COOH terminus. Dot blots and northern hybridization studies indicate that the mRNAs of embryonic ventricle and skeletal muscle display similar hybridization with pCMHC-1. These experiments demonstrate that exact nucleotide homology exists in the COOH terminus coding region of the ventricular and skeletal muscle MHC mRNA in the chick embryo. Further studies are necessary, however, to determine the degree of homology in other coding regions and in the non-coding regions of these mRNAs.

0592 INTERMEDIATE FILAMENT GENE EXPRESSION DURING MYOGENESIS, Zendra Zehner, Tom Pavelitz, John Ning, Ruth Real and Rita Colella, Medical College of Virginia, Richmond, VA 23233

Previously, we have reported on the characterization of the single copy vimentin gene. In chicken this gene produces multiple functional mRNA's from the differential utilization of several polyadenylylation sites. We have shown that three of the four possible sites are utilized in in vivo RNA isolated from several tissues whereas site 1 is not functional. This may be due to the fact that site 1 lacks a downstream sequence capable of forming a hairpin structure with the poly A consensus sequence as postulated by Nevins et al. Currently, we are completing the sequence of the chicken vimentin gene (approximately 1850 nts of coding) comprised of 9 exons covering 8 Kb of DNA. A comparison of the chicken and hamster gene reveals a marked conservation of nucleotide (80%) and amino acid (90%) sequence. This remarkable homology is further exemplified both in the conservation of exon size and preference for codon untilization with valine being the only exception.

By comparing the predicted amino acid sequence of chicken vimentin and desmin, we have synthesized two oligonucleotides to areas unique to desmin. These probes have been used to screen a gizzard cDNA library. At least two clones hybridize specifically to both probes and we are currently examining their sequence by the dideoxy chain termination method.

By Northern analysis we have compared the expression of these intermediate filament genes in various mouse and rat cell lines during myogenesis. In addition we have analyzed proliferative myoblasts and differentiated myotubes for the expression of several oncogenes during differentiation.

## *Gene Expression*

**0593** EXPRESSION OF HUMAN CYTOSKELETAL TROPOMYOSIN GENES, Alexander R. MacLeod, Ludwig Institute for Cancer Research, Cambridge, England, CB2 2QH.

Cytoskeletal extracts of human fibroblasts contain five polypeptides with the physical and chemical characteristics of tropomyosin. These have been called the heat stable cytoskeletal proteins (hscp) and numbered according to their apparent molecular weight of 30,000 (hscp30pl and hscp30nm), 32,000 (hscp32), 34,000 (hscp34), and 36,000 (hscp36) [1]. cDNA clones corresponding to hscp30pl, hscp30nm and hscp36 have been isolated by cross-hybridization to a variety of cloned heterologous tropomyosin sequences. Analysis of these cDNA's indicates that hscp30pl and hscp30nm are isoforms of the 247 amino-acid non-muscle type tropomyosin. They are coded for by large mRNA's of 3.0 kb and 2.5 kb, which show very little cross-hybridization. The large size of these mRNA's is accounted for by very long 3' non-coding regions. By comparison hscp36 is a 284 amino-acid muscle-type tropomyosin similar if not identical to a tropomyosin expressed in smooth muscle [2]. The mRNA encoding hscp36 is strikingly different from the non-muscle type tropomyosin mRNA's being 1.1 kb in length and having a 3' non-coding region of only 80 bp. cDNA sequences corresponding to hscp32 and hscp34 have not yet been isolated and it remains to be determined whether these represent genuine non-muscle tropomyosins. Each of the three mRNA's for which cloned cDNA probes are available are encoded by separate functional genes. Each of these functional genes is associated with a family of pseudogenes mostly of the RNA-copy type. The function of tropomyosin in the cytoskeleton is unknown but is now of considerable interest in view of the altered expression of tropomyosin in transformed cells. The occurrence of both muscle and non-muscle-type tropomyosins suggests at least two separate functions for tropomyosin in the cytoskeleton. An analysis of these functions using in vitro mutagenesis is now being undertaken.

1. Talbot, K. and MacLeod, A.R. (1983) J. Mol. Biol., 164: 159-174.
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**0594** A 5'-FLANKING SEQUENCE FROM THE HUMAN CARDIAC ACTIN GENE DIRECTS HIGH-LEVEL TRANSCRIPTION SPECIFICALLY IN CELLS OF MYOGENIC ORIGIN, Adrian J. Minty and Larry Kedes, Dept. of Medicine, Stanford University, Stanford, CA 94305

We have characterized sequences surrounding the transcription start site of the human cardiac actin gene. The 5'-untranslated region of this gene is interrupted by an intron of approx. 600 base-pairs and the first exon of the gene shows a very high CpG content. The promoter activity of the region of 450 base-pairs upstream from the cap site has been analyzed by using this sequence to replace the SV40 early promoter in the plasmid pSV2CAT. The levels of transient CAT expression from this vector, relative to those from pSV2CAT, are significantly higher in the mouse C2 myogenic cell line than in the mouse L fibroblastic cell line or the rat pCG2 neuronal cell line. The increased transcriptional activity in C2 cells can be seen even at the proliferating myoblast stage and can be eliminated by co-transfection with increasing amounts of the cardiac actin gene. We thus conclude that a positively acting regulatory factor in muscle cells interacts with a region near the start of the gene to promote high-level transcription, and that this factor can be present before the time of maximal cardiac actin expression. Experiments are in progress to develop assays for the gene encoding the putative regulatory factor. When the human cardiac actin gene is stably introduced into mouse C2 cells by transfection of a plasmid containing the gene inserted in the vector pSV2neo, this gene shows a high-level constitutive expression. No increase in expression is seen on myoblast differentiation, although the endogenous mouse cardiac actin gene is up-regulated. This suggests that the endogenous gene is subject to a second level of regulation which represses its activity in myoblasts.

0595 EXPRESSION AND REGULATION OF THE CHICKEN ACTIN GENES IN HETEROLOGOUS MYOGENIC AND NONMYOGENIC CELL LINES, B. M. Paterson, A. Seiler-Tuyns, R. Billeter, and J.D. Eldridge, Laboratory of Biochemistry, National Cancer Institute, Bethesda, MD 20205

During myogenesis, synthesis of the β-cytoplasmic actin isoform is greatly reduced whereas expression of the α-sarcomeric actins begins with the onset of differentiation. We have isolated and characterized three of the six actin genes in the chicken: the β-cytoplasmic gene, the α-cardiac gene, and the α-skeletal gene. The genes have been unambiguously defined by nucleotide sequence. Utilizing a specific restriction enzyme fragment from the 5' coding exon of each gene as a primer, we have developed a primer extension assay that is diagnostic for the steady state transcript level from each of the actin genes. α-cardiac actin is the predominant sarcomeric isoform expressed in embryonic chick breast muscle both in vivo and in vitro (greater than 90%). Five weeks post hatch there are no detectable cardiac actin transcripts in breast muscle: the only measurable sarcomeric actin transcripts represent α-skeletal actin. Low levels of beta actin mRNA are still detectable. Thus, there is a sarcomeric actin isoform switch during the embryonic to adult muscle transition. In order to define those regions in the sequence of each gene responsible for the regulation during differentiation and the sarcomeric isoform switch, the three actin genes were introduced into the C2 murine myogenic cell line, into L-cells, and into the rat L-6 myogenic cell line using the SV-40 derived vector, PSV2-gpt. Primer extension and S1 assays were used to determine levels of actin gene expression in the various lines. Data will be presented on preliminary regulation studies with emphasis on the cell background and its effect on the levels of regulation and expression of the various actin genes.

0596 EXPRESSION OF THE RAT SKELETAL MUSCLE MYOSIN LIGHT CHAIN 2 GENE IS DEVELOPMENTALLY RETULATED IN TRANSGENIC MICE. Moshe Shani, Department of Cell Biology, The Weizmann Institute of Science, P.O. Box 26, 76100, ISRAEL.

To study the control elements involved in tissue and stage specific expresion of genes during myogenesis the rat myosin light chain 2 gene was introduced into mice via microinjection into fertilized eggs. Four transgenic mice, carrying various copy numbers of the injected DNA were obtained. Three of these mice transmitted the inserted gene to about 50% of the progeny, while the fourth so far did not and is probably a mosaic.

To determine whether the introduced gene was developmentally regulated, RNA isolated from eight tissues of three of these mice was analyzed for the presence of the rat transcripts. In the offspring of two out of the three transgenic mice, the introduced gene was expressed specifically only in skeletal muscle. These results indicate that the inserted cloned gene includes cis-acting sequences that are sufficient to specify the current expression of this gene.

*Gene Expression*

0597 **THE GENE(S) CODING FOR SMOOTH AND SKELETAL $\alpha$ TROPOMYOSIN: EXPRESSION DURING EMBRYONIC DEVELOPMENT AND STRUCTURAL ORGANIZATION.** **Marc Y. Fiszman[1], Elissavet Kardami[2] and Marguerite Lemonnier[1], [1]Mol. Biol. Dept., Pasteur Institute ,Paris, France, [2]Zool. Dept.,Univ. California, Berkeley, CA 94720**

Affinity purified antibodies against $\alpha$ tropomyosin from chicken Pectoralis major muscle ($\alpha_F$-TM) recognize specifically the 43 000 daltons tropomyosin from gizzard, hereafter denominated $\alpha$ gizzard tropomyosin ($\alpha_{Gz}$-TM) according to a recently proposed nomenclature (1). Limited tryptic cleavage provide evidence that these antibodies recognize sites located on the -COOH half of the $\alpha_F$ and the $\alpha_{Gz}$ tropomyosins. Using a cDNA which represents the 3' untranslated region and 90% of the coding sequence of the mRNA coding for $\alpha_F$ tropomyosin (2), we have characterized the mRNA coding for $\alpha_{Gz}$ tropomyosin which is a 2.0kb molecule. The 1.2kb mRNA ($\alpha_F$-TM) and the 2.0kb mRNA show greater than 90% homology over 500 nucleotides in their 3' coding sequence. However, the two mRNA have totally distinct 3' untranslated regions. We will show that during skeletal muscle development the two mRNA are being synthesized at the same time. However the rate at which they are expressed is modulated so that in young embryos (5-8 days in ovo) the two messengers are present in equal amount while in older embryos the mRNA corresponding to the skkeletal muscle tropomyosin is the major form. It is interesting to note that this phenomenon does not exist during gizzard development since only the 2.0kb mRNA can be detected. On the other hand, the 1.2kb mRNA is the only mRNA to be accumulated when myoblasts differentiate to form myotubes in vitro. To try to gain more information on the mechanisms which govern these patterns of expression we have started to isolate and analyze the gene(s) which code(s) for these mRNAs. By screening a chicken genomic library in $\lambda$Charon 4A, we have isolated a recombinant phage which carries a genomic sequence which corresponds to the common region of the two mRNAs and which also corresponds to the two different 3' untranslated regions of the two messengers. Such a structure is compatible with either two genes which are intermingled or a single gene which codes for the two messengers by differential splicing. This alternative will be solved when the 5' end of the gene(s) will be analyzed.

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0598 GENETIC AND MOLECULAR ANALYSES OF DROSOPHILA ACTIN GENE FUNCTION, Eric A. Fyrberg, The Johns Hopkins University, Baltimore, Maryland 21218.

Drosophila melanogaster, in addition to lending itself well to genetic investigations, provides an excellent system of muscle fibers in which to systematically analyze mutations affecting sarcomere formation. Much of the thoracic volume of adult flies is occupied by a series of 26 muscle fibers which vibrate the wings rapidly during flight. These fibers, referred to as the indirect flight muscles (Deak, 1982) are required only for flight, not for viability or fertility. For this reason mutations which specifically disrupt flight muscles can frequently be propagated as homozygotes, thereby facilitating their investigation.

Our recent efforts have focussed on more precisely defining the regulation and function of a flight muscle-specific actin gene, termed act88F, which is expressed only within developing flight muscle fibers. To learn more about the molecular mechanisms governing transcription of this gene we are conducting three related lines of inquiry: (1) We are defining the 5' flanking sequences requisite for proper regulation. This involves deleting appropriate regions of the cloned gene and assessing function after reintroduction into germline DNA. (2) We are characterizing the molecular lesion in a cis-acting, down-regulated act88F allele recovered from the flightless mutant raised. (3) We are beginning genetic screens for trans-acting loci which disrupt the developmental regulation of the act88F gene, using gene fusions which confer easily recognized nullo-activity phenotypes (Bonner et al., 1984).

We are also characterizing the molecular nature of several act88F alleles isolated previously as dominant flightless mutants (Mogami et al., 1981). By correlating alterations in the encoded aberrant actin isoform with the observed defects in flight muscle myofibrils we hope to learn more about actin assembly and function (see Karlik et al., 1984).

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0599 ACTIN GENE REGULATION DURING AMPHIBIAN EMBRYOGENESIS, Tim Mohun, S. Brennan, J.B. Gurdon, MRC Molecular Embryology Group, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

The actin gene family is regulated in three distinct ways during frog embryogenesis. Firstly, those genes encoding cytoskeletal actin proteins are transcribed during oogenesis to provide a maternal store of mRNA. Secondly, muscle-specific actin genes are activated in the early embryo in those cells destined to form embryonic muscle. Thirdly, by the time adult muscle tissues are formed, muscle actin gene expression is restricted according to muscle type.

To study the molecular mechanisms of such controls we have isolated five fragments of genomic frog DNA each containing a different actin gene. These have been characterised by DNA sequencing and examined for putative regulatory sites that determine the differing patterns of actin gene expression. Microinjection of cloned genes into frog eggs has been used to study the regulatory activity of these sites. We have also studied the regulation of the actin gene family using embryological techniques. Cells in the early embryo destined to contribute to the mesoderm tissue (and hence embryonic muscle) are able to activate the muscle-specific actin genes at the appropriate time after egg fertilisation irrespective of whether the normal embryonic cell interactions are maintained. By removing various portions from early embryos and examining the activity of the actin genes in the remaining "partial" embryos we have found that the ability to activate muscle actin genes is localised to a particular region of cells from the earliest times. Indeed, by ligating fertilised eggs along various planes with respect to the egg axis and examining the transcriptional activity of the nucleated egg fragments during their subsequent development, we have found that all the requirements for activation of muscle actin genes in later development appear to be localised in a sub-equatorial region of the fertilised frog egg.

Gurdon, J.B., Brennan, S., Fairman, S., and Mohun, T. (1984). Early commitment to α-actin gene transcription in Xenopus development. Cell, in press.
Mohun, T.J., Brennan, S., Dathan, N., Fairman, S., and Gurdon, J.B. (1984). Cell type-specific activation of actin genes in the early amphibian embryo. Nature, in press.
Gurdon, J.B., Mohun, T.J., Fairman, S., and Brennan, S. (1984). All components required for the eventual activation of muscle-specific actin genes are localized in the subequatorial region of an uncleaved Amphibian egg. Proc. Nat. Acad. Sci. USA, in press.

0600 ALTERNATIVE SPLICING: A COMMON MECHANISM FOR THE GENERATION OF CONTRACTILE PROTEIN DIVERSITY FROM SINGLE GENES. B.Nadal-Ginard, R.E.Breitbart, E.E. Strehler, M. Periasamy, N.Ruiz-Opazo. Dept. of Cardiology,Children's Hospital and Dept. of Pediatrics, Harvard Medical School, Boston, MA 02115.

Three different contractile protein genes that are members of multigene families-myosin light chain 1 and 3,MLC1/3 troponin T, and α-tropomyosin, (α-TM)-have been shown to generate multiple mRNAs by a process of alternative splicing. Each of these mRNAs encodes different tissue-specific and developmentally regulated protein isoforms. In the case of MLC1/3 gene, two different promoters, spaced ∿10kb apart generate primary transcripts widely different at their 5' end whose secondary structure could be involved in determining the splicings pattern for MLC1 and MLC3 mRNAs. The troponin T gene generates a minimum of 4 proteins with different aa sequences upstream from aa 40. In addition, there are two duplicated mini exons, coding for aa 229 to 242, that could double the number of protein isoforms produced by this gene. Some of the mRNAs share 5' untranslated sequences strongly suggesting that they are generated from the same primary transcript. If this is the case, trans-acting factors must be involved in the tissue and developmental regulation of the splicing pathways.

A single α-TM gene encodes for three different α-TM molecules:one smooth and two striated skeletal and cardiac. All three proteins are identical between aa 81 and 257,while the remainder of the sequence is produced by alternative splicing of "isotype switch exons" in different combinations. These exons code for "isotype switch peptides" that,with the exception of the carboxyl terminal sequences,precisely correspond to single actin-binding domains of TM.

The nature of the splicing events involved in the production of multiple mRNAs from these three genes conclusively rules out a processive scanning model of splice site recognition. The use of different promoters could,in principle,predetermine a particular splicing pathway via secondary or tertiary structure of the primary transcript. This mechanism,however,does not explain alternative pathways of splicing of transcripts from the same promoter site.

The ability to generate subtly different proteins from a single gene that,in turn, belongs to a multigene family, highly increases the potential for myofibrillar diversity that most likely plays a significant, but as yet poorly understood, role in muscle physiology.

0601 CONTROL OF EXPRESSION OF MUSCLE-SPECIFIC GENES: ANALYSIS BY THE DNA-MEDIATED GENE TRANSFER TECHNIQUE, Uri Nudel, Danielle Melloul, David Grinberg and David Yaffe, Deaprtment of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

We have previously demonstrated that the expression of fused genes containing about 2/3 of the skeletal muscle actin gene plus 730 bp 5' flanking DNA is developmentally regulated in transformed myogenic cells. A DNA fragment extending from 730 bp upstream from the cap site to the beginning of the first intron of the skeletal muscle actin gene spliced to the structural gene coding for chloramphenicol acetyl transferas (CAT) is sufficient to confer the tissue- and stage-specific expression of the CAT gene (1). Likewise, a chimeric gene containing 1 kb DNA, including the cap site and upstream sequences of the fast muscle myosin light chain 2 gene fused to the bacterial CAT gene, is developmentally regulated in transfected myogenic cells. At least 10-fold increase in CAT activity during differentiation of the transfected myogenic cultures was observed. On the other hand, the expression of a fused mouse/human β-globin gene (which is developmentally regulated in murine erythroleukemic cells (2)) decreased 2—4-fold upon differentiation of most of the transformed myogenic clones.

To test the evolutionary conservation of the DNA sequences specifying the developmentally regulated expression of the skeletal muscle actin gene, a recombinant plasmid containing the chick skeletal muscle actin gene (obtained from Dr. C. Ordahl) was introduced into rat myogenic cells. In a significant number of isolated clones, the accumulation of chick actin mRNA increased greatly during differentiation. This demonstrates that the control mechanism of expression of muscle-specific genes has been conserved for at least 300 million years.

A detailed map of the regulatory region of the skeletal muscle actin gene and of the myosin light chain 2 gene, constructed by insertion of various deletions in the 5' upstream region, will be presented, and the molecular mechanism for regulation of muscle-specific genes will be discussed.

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## *Myofibrillar Assembly and Morphogenesis*

0602 REGULATION OF ACTIN AND MYOSIN SYNTHESIS DURING REGENERATIVE GROWTH OF MUSCLE GRAFTS, David A. Essig, George H. Jones, and Timothy P. White. The University of Michigan, Ann Arbor, MI 48109.

The regulation of the synthesis of whole muscle and contractile proteins was studied during regenerative growth induced by autografting of the rat soleus muscle. An inverse relationship between polysome concentration and muscle protein mass was observed throughout growth without a change in polysomal translational activity. Exercise conditioning increased muscle protein mass at 42 and 56 days postgrafting and was associated with an elevated polysome concentration without a change in polysome activity. These data were consistent with pretranslational control of overall synthesis rates. From 14 to 56 days postgrafting, polysomal actin synthesis of grafts was similar to control value and was correlated with mRNA content measured by dot blotting. Myosin heavy chain (MHC) synthesis in grafts was depressed relative to control value. In 14 and 28 day grafts and in control muscles, synthesis was in proportion to mRNA content and suggested that depressed rates of MHC synthesis in grafts was mediated by mRNA availability. At 56 days, synthesis of MHC was not in proportion to mRNA levels and was consistent with translational control. MHC and actin synthesis during regenerative growth of grafts was not coordinated and contrasted with patterns previously observed during normal myogenesis.

0603 THE ASSEMBLY OF TITIN DURING MYOFIBRILLOGENESIS IN CULTURED SKELETAL MUSCLE CELLS, Marion L. Greaser, Seu-Mei Wang, and Edward Schultz, University of Wisconsin, Madison, WI 53706

Mononucleated myogenic cells were obtained from the leg muscles of 14 day old rats and maintained in cell culture for four to six days. The location of titin in these cells and in developing myotubes was determined using a monoclonal antibody and indirect immunofluorescence. This particular antibody stained the A-I junction region of adult rat myofibrils. The antibody stained the majority of post-mitotic myoblasts and all myotubes, but did not stain fibroblasts. Titin was observed to occur in mixed patterns of filamentous, fibrillar, and distinct "doublet" banding (two perpendicular stained bands per sarcomere). The fibrillar pattern showed staining of bundles parallel to the long axis of the cell. Double-label-immunofluorescence indicated that these bundles also contained actin, myosin, troponin, and α-actinin. A transition between an obscure banding and a clean "doublet" pattern was often found at the tapered ends of nascent myofibrils. The "doublet" arrangement predominated in mature myotubes and was identical to that found in mature muscle myofibrils. The present studies suggest that titin assembly occurs with the following sequence: (1) newly synthesized titin is distributed in diffuse filaments throughout the cell, (2) the filaments coalesce into oriented bundles with fairly uniform staining, (3) an irregular, patchy staining on the bundles appears, suggesting some longitudinal rearrangement, (4) the titin molecules are aligned in the mature "doublet" pattern. The fact that titin periodicity in some bundles preceeded the appearance of actin banding suggests that titin may play an important role in sarcomere assembly.

0604 IMMUNOCHEMICAL ANALYSIS OF MYOSIN GENE EXPRESSION IN CAT TEMPORALIS MUSCLE TRANSPLANTED INTO FAST AND SLOW LIMB MUSCLE BEDS J.F.Y. Hoh, S. Hughes, C. Chow, P.T. Hale, R.B. Fitzsimons and S. Schiaffino, University of Sydney, N.S.W. 2006, Australia

Cat jaw muscles contain a superfast myosin which is structurally distinct from myosins found in limb muscles. Since nerves to limb muscles control the expression of fast and slow myosin genes, we examine the question of whether these nerves are capable of inducing the synthesis of fast or slow myosins when the temporalis muscle is transplanted into limb muscle beds. Muscles were examined immunohistochemically using antibodies reacting specifically against superfast, slow, fast or foetal myosin heavy chains. Early regenerates of Marcaine treated temporalis in either bed reacted with antibodies against foetal, slow, superfast but not fast myosins. In the fast muscle bed, the reactivity of the transplant against foetal and slow myosins progressively decrease subsequently, so that by 11 weeks most of the muscle fibres contain only superfast myosin. In contrast, temporalis muscle regenerating in the slow muscle bed showed a predominance of slow myosin reactivity at 10 weeks post-operatively. Limb muscles transplanted into limb muscle beds do not synthesize superfast myosin. These experiments suggest that satellite cells from temporalis muscle are preprogrammed to express superfast myosin, and that this pattern of myosin gene expression can be altered by a slow limb nerve but not by a fast nerve.

0605 Light Chain Distribution of the Developmental Myosin Isozymes in the Rat. Alan M. Kelly and Neal A. Rubinstein, Pennsylvania Muscle Institute, University of Pennsylvania, Philadelphia, PA 19104.

We have examined the LC complement of each of the developmental isozymes f1-f4 and s1 and s2 using 2D SDS-PAGE. In the diaphragm at birth, f3 and f4 have the same LCs: predominantly $LC_{1f}$, $LC_{1emb}$, $LC_{2f}$, and traces of $LC_{3f}$. f2 contains $LC_{1f}$ + $LC_{1emb}$ in equal proportions to $LC_{3f}$. f1 is a homodimer of $LC_{3f}$. The proportions of f1 and of $LC_{3f}$ increase with development. In the soleus at birth, f1-f4 have the same mobility as in the diaphragm but have different LCs. All four isozymes have $LC_{1s}$ + $LC_{1f}$ in equal proportions to one another. f3 and f4 additionally contain $LC_{1emb}$ and $LC_{2f}$ plus traces of $LC_{3f}$. In f2 the proportion of $LC_{3f}$ is increased, and this is further increased in f1. f1 and $LC_{3f}$ are eliminated by 15 days. Fast isozymes in adult soleus also contain combinations of fast and slow LCs. In the soleus at birth, slow isozymes s1 and s2 contain a predominance of $LC_{1s}$ with smaller proportions of $LC_{1f}$, $LC_{1emb}$, $LC_{2s}$, and $LC_{2f}$. Fast LCs are rapidly eliminated and are not found in the adult slow isozymes. A single slow band, SM1, is found in the diaphragm at all stages. At birth, SM1 has approximately equal proportions of $LC_{1s}/LC_{2s}$ and $LC_{1f}/LC_{2f}$. $LC_{1f}$ and $LC_{2f}$ decline slowly with development; and in the adult only traces of $LC_{1f}$ remain. In the adult sternomastoid, significant proportions of $LC_{1f}$ and $LC_{2f}$ remain in SM1, as if the long-term process of myosin remodelling and specialization is less complete than in other muscles.

0606 IDENTIFICATION OF A NEW HIGH MOLECULAR WEIGHT Z LINE PROTEIN INVOLVED IN MYOFIBRILLOGENESIS. Pamela A. Maher, Gerald Cox and S.J. Singer, University of California at San Diego, La Jolla, CA.

We have generated a number of monoclonal antibodies (Mcab) to a purified preparation of the fascia adherens domains of the intercalated disk membranes of chicken cardiac muscle. Among these Mcab were several which by immunofluorescence and immunoelectron microscopy labeled the intercalated disk membranes and the Z lines in cardiac muscle, the Z lines of skeletal muscle, and the dense bodies and dense plaques of gizzard smooth muscle. Two of these antibodies (Mc 20 and Mc 284) were directed against the same muscle-specific antigen that appeared as a pair of polypeptide bands of 340 kd and 325 kd on SDS-polyacrylamide gels. With cultures of developing skeletal muscle myotubes from 12-day chick embryos, immunofluorescent labeling with Mc 20 or Mc 284 showed long narrow stretches of Z line-like periodicity just under the surface of the myotube within 24 hr after plating, corresponding to an early stage of myofibril formation before cross-striations were clearly visible by phase contrast or Nomarski optics. At this same early stage, immunofluorescent labeling for α-actinin showed no such Z line-like periodicity. Only after 4-6 days in culture did both α-actinin and Mc 20 (and 284) immunofluorescent labeling co-localize to the Z lines of well-developed myofibrils. We propose that this new protein is a component of the Z lines and may function more critically than α-actinin during the embryonic organization of the myofibril. We have suggested the name zeugmatin(ζευγμα ≡ yoking)for this protein because of its association with the end-on attachment of microfilament bundles at the Z line.

0607 HEAT SHOCK INDUCES RAPID CHANGES IN CELL MORPHOLOGY IN MYOGENIC CELLS IN CULTURE. Dianne M. Rausch, Richard I. Morimoto, and Sidney B. Simpson, Jr., Department of Biochemistry, Molecular, and Cell Biology, Northwestern University, Evanston, IL. 60201.

Cultures of muscle progenitor cells isolated from lizard (Anolis carolinensis) tail regenerates were examined for their response to elevated temperatures during myogenesis. These cells proliferate indefinitely in culture at 31° C if maintained at a sub-confluent density. If the cells grow to high density, they withdraw from the cell cycle and synthesize myosin, a marker of muscle cell differentiation. Exposure of proliferating and differentiating cells to 41° C results in a rapid and dramatic change in cell morphology. The cells become highly adhesive to the plastic culture dishes, and assume a flattened, fibroblast-like morphology. This change in cell shape is most apparent with cells in advanced stages of myogenesis, where aggregates of rounded, pre-differentiative cells as well as the bipolar, elongated myotubes also flatten and assume a shape characteristic of early, proliferating myoblasts. This change in morphology is initiated within ten minutes after the temperature shift, and is reversible with similar kinetics. Analysis of protein synthesis by labeling with $^{35}S$-methionine and SDS-PAGE reveals the induction of heat shock proteins with no apparent change in the pattern of pre-existing protein synthesis.

## *Myofibrillar Assembly*

**0608** STRUCTURE-FUNCTION RELATIONSHIPS OF 'MINOR PROTEINS' OF THE MYOFIBRIL.
Hans M.Eppenberger,Martin Bähler,and Theo Wallimann, Dept. of Cell Biology, Swiss Fed. Inst. of Technology, CH-8093 Zurich, Switzerland.
The A-band of a myofibril consists of thick myosin filaments and a number of additional elements obviously made up by minor proteins like C-protein, H-protein (1) and M-band proteins (2-4) as well as a 86 kD protein recently identified by us (5). Some of these 'minor proteins' are believed to be involved in the assembly and maintenance of the thick filament arrays and/or may have enzymatic and regulatory activities. In a first survey A-segments, native thick filaments, frayed filaments, bare zone assemblages, repolimerized bare zone assemblages as well as completely disassembled and again reassembled thick filaments were investigated for the presence of the 3 known M-band proteins (6) by the colloidal gold labelling technique. Incubation with anti-M-protein and anti-myomesin antibodies resulted in heavy labelling of all thick filament types mentioned with the exception of the completely disassembled and again reassembled thick filaments. MM-creatine kinase, an integral component of the intact M-band structure, was detectable on isolated native thick filaments with lower frequency and to a variable extent. M-protein and myomesin are considered important for the assembly and structural maintenance of thick filaments as well as for anchoring additional M-band proteins like MM-creatine kinase. Such a role of the 2 high MW M-band proteins receives additional support on the one hand from experiments performed with myogenic cell cultures in the presence of phorbol ester, where myofibrils break up with time (7), on the other hand from microscopic studies on the myofibril status in hereditary myopathic hamsters (8). In both cases, the A-bands seem to resist disintegration as long as the M-band proteins can be shown by antibody reaction to still be present in the M-band.
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**0609** IMMUNOCYTOCHEMICAL ANALYSIS OF THICK FILAMENT ISOFORM TRANSITIONS IN REGENERATING AVIAN MUSCLE,
Donald A. Fischman and Anuradha D. Saad, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021.
The posterior and anterior latissimus (PLD and ALD) muscles of adult chickens were focally cold injured with a steel rod cooled in liquid nitrogen. Contralateral muscles received sham operations. At 2,5, and 8 days after freeze injury, control and injured muscles were dissected, frozen in liquid $N_2$-cooled isopentane and transversely, serial-sectioned for immunocytochemistry using the avidin-biotin-horseradish peroxidase procedure. Myosin heavy chain isoforms were examined with monoclonal antibodies (McAbs) MF20, MF30 and ALD58: C-protein isoforms with ALD66 and MF1. The results of our previous work on the developing pectoralis muscle are shown in Table I (Bader et al., J. Cell Biol. 95:763, 1982). Antibody MF20 stained all muscle fibers (fast and slow) at all stages of regeneration and development. MF30, which did not react with 12dE pectoralis muscle, did stain fibers of the PLD and ALD at all stages of regeneration we examined. MF14, which is adult fast-twitch-specific, did not stain 5-day-old PLD regenerates, but did transiently stain the ALD muscle. Thus, regenerating PLD muscle undergoes a myosin heavy chain antigenic transition analogous to developing pectoralis muscle but this transition occurs precociously. The slow-specific McAb, ALD58, did not stain control or regenerating PLD muscle. Early in ALD regeneration, at stages when the MF14 epitope was clearly evident, staining with ALD58 was quite weak. At this same period in ALD regeneration, our results suggest the synthesis of fast-type C-protein (MF1 positive staining) but underproduction of slow-type C-protein (weak ALD66 staining). These results show that during the regeneration of slow (ALD) muscle, there occurs a period (5th-8th day) in which fast-type MHC is expressed. The present study indicates that, additionally, a fast-type C-protein is also expressed at this period. Only late in the second week of regeneration are the mature, slow MHC and C-protein fiber-type characteristics observed. Our investigation complements the parallel experiments of Toyota and Shimada (Cell and Tissue Res. 236:549, 1984) with troponin isoforms and clearly demonstrate that regenerating adult muscle undergoes sequential changes in gene expression which resemble those transitions during normal development.

TABLE I:

| Antibody | Antigen | 12dE | 16dE | 1d | 7d | 14d | Adult |
|---|---|---|---|---|---|---|---|
| MF20 | MHC | + | + | + | + | + | + |
| MF30 | MHC | - | +/- | + | + | + | + |
| MF14 | MHC | - | - | - | + | + | + |
| ALD58 | C-Pr | ND | | | | | |
| ALD66 | C-Pr | - | + | + | - | - | - |
| MF1 | C-Pr | - | +/- | + | + | + | + |

**0610** USING A COCARCINOGEN (TPA) AND A CARCINOGEN (EMS) TO PROBE MYOFIBRILLOGENESIS, H. Holtzer, P. Antin, A. Dlugosz, S. Forry-Schaudies, J. Eshlemen, V. Nachmias, Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

The carcinogen EMS and the co-carcinogen TPA reversibly and selectively alter the morphology and lineage specific synthetic program of definitive myogenic cells in culture. Myogenic cells maintained in the presence of these agents fuse to form vast, high ly flattened, isodiametric multinucleated myosheets. Immunofluorescence, EM and biochemical analysis reveal that myosheets fail to synthesize, or to accumulate mRNAs coding for, myosin heavy chain, LC1, LC2, alpha and beta tropomyosin or troponin C. Synthesis and accumulation of desmin, vimentin and tubulin are unaffected: microtubules and intermediate filaments are present in a meshwork throughout the myosheets. Prominent, well separated stress fiber-like structures (SFLS) subtend the upper plasma membrane of myosheets and can be greater than 0.5 mm in length. These SFLS bind phalloidin, anti-alpha-actinin, and anti-brain myosin in patterns similar to SFLS in normal fibroblasts.

When TPA or EMS are removed from the culture medium, myosheets resynthesize muscle-specific contractile proteins and assemble them into functional myofibrils. Immunofluorescence and EM studies reveal that the earliest assembly of myofibrils occurs in association with the submembranous SFLS: anti-brain myosin and anti-LIM overlap throughout long stretches of these SFLS-myofibril structures: at times one end of a phalloidin positive structure may exhibit staining properties of SFLS, at the other end of a striated myofibril. The early forming SFLS-myofibril complexes rapidly lose their intimate association with the upper plasma membrane and are displaced into sarcoplasm where newly assembled thick and thin filaments are added peripherally to the emerging nascent myofibrils. With further maturation, the previously prominent SFLS disappear over the belly of the myotube and remain only in the growth tips and pseudopodial extensions of myosheets. A similar temporal and topographical relationship between SFLS and emerging myofibrils has been observed in skeletal and cardiac myoblasts. Based on this data, a complex, multistage molecular model is presented for myofibrillar assembly and maturation.

**0611** TRANSITION OF HEAVY CHAIN OF GIZZARD SMOOTH MUSCLE MYOSIN ISOFORMS DURING DEVELOPMENT AND THEIR THICK FILAMENT GENESIS AS COMPARED WITH SKELETAL MUSCLE, Tomoh Masaki, Reiko Suzuki, Hiromi Takano-Ohmuro, Takeshi Tanaka, Institute of Basic Medical Sciences, The University of Tsukuba, Niihari-Gun, Ibaraki-Ken 305, Japan

Myosin heavy chain molecules undergo isoforms transitions during striated muscle development (1).

To study further on this change of myosin molecule, adult chicken cardiac myosin epitopes in embryonic skeletal muscle, which were not detected in adult skeletal myosin, were examined during myogenesis. These adult cardiac myosin epitopes in embryonic skeletal myosin molecule distributed in both head and tail region of molecule, and disappeared during development.

Heavy chain of embryonic and adult gizzard smooth muscle myosin was also differ from each other (2,3). The pattern of peptide fragments of embryonic myosin heavy chain produced by α-chymotryptic digestion in the presence of SDS was similar but distinguishable from that of adult myosin heavy chain. It was different from that of brain or fibroblast myosin. One of the monoclonal antibodies against the myosin heavy chain of adult chicken gizzard muscle, G17-6, barely binds embryonic myosin. However, its epitope rose gradually during development of gizzard smooth muscle. In addition, size of the embryonic peptide containing this epitope is different from that of adult peptide. These results suggest that transition of gizzard myosin isoform occurs at least three steps during development.

The in vitro assembly of myosin purified from embryonic and adult gizzard smooth muscle was studied as compared with that of the skeletal myosin.

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## *Cytoskeleton and Morphogenesis*

0612 EXPRESSION OF M-BAND PROTEINS DURING MYOGENESIS, J.C.Perriard, Institute for Cell Biology, Swiss Fed. Inst. Technology,CH-8093 Zurich,Switzerland.

Three components of the m-band of the sarcomere have been identified: the 185 kd myomesin (1), the 165 kd M-protein and the MM-isoenzyme of creatine kinase (MM-CK). Myomesin and M-protein were shown to be expressed in cross-striated muscle of chicken, but not in smooth muscle and non-muscle tissue. The kinetics of accumulation of myomesin and M-protein in developing breast and heart muscle as well as in myogenic cell cultures was investigated by immunoblotting and ELISA techniques. Myomesin was detectable in early stages of muscle development, the expression of M-protein,however,appears to have a delayed occurence. These newly expressed proteins were found in the m-bands of the nascent myofibrils of differentiated myogenic cells.

Creatine kinase exists in multiple molecular forms, the BB-CK is found in embryonic tissue and in a number of adult tissues including chicken heart. The MM-CK,however,is found exclusively in tissues containing skeletal muscle cells. During muscle development and "in vitro" myogenesis, CK isoenzymes switch from BB-CK to M-CK containing isoenzymes until in adult muscle all of the non-mitochondrial CK activity is MM-CK. The CK subunits are products of at least 2 different genes and are regulated by changing transcriptional activity. The isoenzyme switch can be first characterized by an increase in the rate of B-CK synthesis. As cells reach terminal differentiation, B-CK synthesis is repressed and M-CK expression is induced (2). The regulation of this isoprotein switch was further investigated using recombinant DNA techniques. The cDNA for M-CK was cloned using conventional techniques(3) and analysis of the nucleotide sequence allowed the elucidation of the amino acid sequence(4). B-CK cDNA clones were identified in a λ gt11 cDNA expression library using specific anti-BB-CK antibodies. The nucleotide sequence derived amino acid sequence for B-CK was compared to M-CK and extended homologies were found. The cloned cDNA sequences were used as probes to follow the expression of the CK genes in differentiating myogenic cells. In order to understand the regulation of these genes, detailed knowledge of the CK gene structures will be necessary and studies to this end are in progress.

MM-CK is found exclusively in chicken skeletal muscle and part of it is bound to the m-band of the sarcomere,capable of regenerating "in vitro" the ADP that is produced during muscle contraction to ATP(5). The B-CK isoprotein cannot replace M-CK in this structure. The primary structure of M-CK appears to define the properties that allow organelle specific association. Using microinjection methods to transfer isolated M-CK mRNA as well as "in vitro" generated transcripts of M-CK cDNA into chicken heart cells, devoid of endogenous M-CK, or into hetero-specific muscle cells, this isoprotein specific association is being studied.

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0613 CYTOSKELETAL ORGANIZATION IN EMBRYONIC MUSCLE CELLS IN VITRO REVEALED BY THE FREEZE-DRY REPLICA ELECTRON MICROSCOPY, Yutaka Shimada and Yuji Isobe, Department of Anatomy, School of Medicine, Chiba University, Chiba 280, Japan.

The characteristic cross-striated appearance of myofibrils in skeletal and cardiac muscle cells arises as a result of the regular assembly of contractile and regulatory proteins. Major parts of these muscle proteins are now recognized as the most commonly encountered elements in nonmuscle cytoskeletons. Thus, myofibrils can be regarded as a specialized form of cytoskeletons differentiated especially for cellular contraction. In this study, we have attempted to examine the spatial cytoskeletal organization relating to myofibril formation in embryonic chick skeletal muscle cells; such a study appears to be essential for a better understanding of the phylogeny of the cellular motile systems.

Embryonic chick skeletal muscle cells cultured for 6-7 days on carbon-coated coverslips were used for the present experiment. These cells were extracted alive with 0.5% Triton, fixed with 2% glutaraldehyde and, then, immersed in 70% ethanol. Some samples were treated with heavy meromyosin (HMM) before the fixation step. On these coverslips over the cells, other coverslips without adhering cells were placed. The entire "sandwiches" were frozen by plunging into Freon 22 cooled by liquid nitrogen and, then, were peeled off under liquid nitrogen. Some samples were similarly frozen in Freon 22 without making "sandwiches". After samples were freeze-dried for 60-180 min, replicas were made in a freeze-fracture device.

Two ultrastructurally distinct cytoskeletal domains could be distinguished within developing myotubes, i.e., dense subsarcolemmal and loose endoplasmic filamentous networks. The most predominantly found components in the subsarcolemmal cytoskeletal domain were filaments measuring 8-10 nm in diameter in replicas. They were identified as actin filaments by their

ability to be decorated with HMM. Nearly all the filaments in the endoplasmic domain measuring 12-14 nm were regarded as intermediate filaments by their unique diameter and inability of binding with HMM. In the latter domain, microtubules with a diameter of 22-24 nm were also found. In addition to these two filament classes and microtubules, another type of cytoskeletal component was seen in both the subsarcolemmal and the endoplasmic domains. It was 2-5 nm in diameter and up to 40 nm in length, and crosslinked these cytoskeletal elements with each other and with developing myofibrils. In the latter area, intermediate filaments ran longitudinally as well as obliquely and formed intermyofibrillar filament lattice. Obliquely running intermediate filaments interlinked adjacent myofibrils at and near Z band levels. Some of these intermediate filaments were anchored to the subsarcolemmal actin filament network. We suggest that intermediate filaments found in embryonic muscle cells are an early stage of an apparatus of tension-transmission and anchoring of myofibrils to each other and to the sarcolemma.

0614 INTERMEDIATE FILAMENTS IN DEVELOPING MYOTUBES IN VIVO, K. T. Tokuyasu, P. A. Maher, A. H. Dutton and S. J. Singer, Department of Biology, University of California at San Diego, La Jolla, CA 92093.

Whether or not desmin is in a non-filamentous form in mature myotubes was a subject of serious dispute for several years. The main reason for raising this question was the difficulty in recognizing intermediate filaments (IF) at the level of the Z band where desmin labeling occurred (e.g., 1). We showed by immunoelectron microscopic and ultrastructural studies of chicken striated muscles that IF are indeed present at the level of the Z band and that desmin is largely if not entirely in the form of IF (2, 3). In the developing myotubes, vimentin is present in addition to desmin. Whether vimentin is absent (4) or present (5) in mature myotubes was another matter of dispute. Using anti-vimentin and anti-desmin antibodies cross-absorbed with the heterologous antigen, we showed that vimentin is gradually reduced in concentration as the embryonic myotube matures in vivo and becomes undetectable at the time of hatching (6). We also showed by immunoelectron microscopy that vimentin and desmin coexist in the same IF in developing myotubes (7). In developing myotubes of the 11 day chick embryo, IF are wavy but as a whole, longitudinally directed (7). It has been proposed that longitudinally aligned IF are depolymerized during development and newly generated IF are formed bridging the Z band to the Z band and causing the Z bands to come into lateral registry (1). However, we showed that in some myotubes of the prenatal embryo, myofibrils are already in lateral register but desmin is still widely distributed around the Z line, and that the distributions of desmin and vimentin are gradually confined toward the Z band level during development (6). The labeling of these proteins occurs exclusively in IF (7). These findings indicate that IF remain in the filamentous form but change direction during the development of myotubes, and that the confinement of IF to the Z band occurs subsequent to, not simultaneously with or preceding, the lateral registration of myofibrils in vivo.

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0615 **ARCHITECTURE OF THE TITIN/NEBULIN CONTAINING CYTOSKELETAL LATTICE OF THE STRIATED MUSCLE SARCOMERE -- EVIDENCE OF ELASTIC AND INELASTIC DOMAINS OF THE BIPOLAR FILAMENTS,** K. Wang, J. Wright, R. Ramirez-Mitchell, Clayton Foundation Biochemical Institute, Department of Chemistry, and Cell Research Institute, The University of Texas, Austin, TX 78712

We have prepared a library of monoclonal antibodies directed to four distinct epitopes of rabbit titin and utilized fluorescent techniques and colloidal gold electron microscopy techniques to study the disposition of these distinct epitopes in the sarcomere. We observed that each of four monoclonal antibodies stained a pair of distinct transverse bands within either the A band or the I band. Furthermore, each pair of bands were centered at the M line. These staining data confirmed our earlier conclusion that titin domains are indeed wider than the A band.[1] It can be inferred that titin-containing filaments are centrally symmetric to the M line, and that these parallel longitudinal filaments are aligned transversely within the sarcomere. Furthermore, we observed that the axial disposition of some, but not all, epitopes varied with the sarcomere length. The epitopes within the I band exhibited an elastic stretch-dependence; whereas those within the A band remained fixed in position. These data suggest that in intact sarcomeres, the elastic cytoskeletal filaments are stretchable only in the I band domain. The filament domains within the A band may be prevented from stretching by certain as yet undefined anchoring mechanisms. Supported in part by NIH AM20270, American Heart Association, Texas Affiliate, and an American Heart Association Established Investigatorship.

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## *Human Muscle Genetics; Cardiac Muscle*

0616 IN VITRO DEVELOPMENT OF VENTRICULAR AND ATRIAL MYOCYTES FROM FETAL RABBIT AND RAT. L. Bugaisky and R. Zak. University of Chicago, Dept. of Medicine, Chicago, Ill.

During in vivo cardiac muscle development both the rat and rabbit pass certain morphogenic landmarks at similar times. We have now compared the growth characteristics of atrial and ventricular myocytes in culture from both these animals to determine the relationship if any, between in vitro beating rates, cell proliferation and myosin isozyme composition. Cellswere grown from 20-21 day fetal rats and 22-23 day fetal rabbits in Ham's F-12 + 5% newborn calf serum. Periodic Acid-Schiff staining indicated a higher percentage of ventricular myocytes in rat than rabbit cultures throughout the first five days. In general, a higher percentage of rat than rabbit ventricular cells were observed to label with $^{3}$H-Thymidine ($^{3}$H-Tdr) following a 1.5 hour pulse during the same five days. Additionally, fewer rat atrial than ventricular myocytes labelled with $^{3}$H-Tdr. For the entire period investigated, atrial cells from both rabbit and rat had higher beating rates than ventricular cells from the same hearts. During at least the first three days ventricular cells from both rabbit and rat had similar beating rates as did the atrial cells. This was intriguing in light of the fact that the maximum in vivo heart rates for the two adult animals may vary as much as 100%. Additionally, we have observed by immunofluorescence a highly organized myofibrillar network in atrial cells from rat and rabbit. Analysis of myosin by native gel electrophoresis has indicated that both the rat and rabbit ventricular cells do not undergo major changes in their isozyme composition during the first week in culture.

0617 **PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF DYSTROPHIC MUSCLE REVEALS ALTERATIONS OF COLLAGEN** by Howard Feit, Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx 75235, Masataka Kawai, Columbia Univ., N.Y., N.Y. 10032

**The resting stiffness of chemically skinned avian dystrophic myofiber bundles (superficial pectoral, Line 413, 4 weeks after onset of the disease) as a function of muscle length was much larger than normal. Contractile properties, as characterized by isometric tension and cross-bridge kinetics (sinusoidal length changes), were unaltered. The excess stiffness of dystrophic muscle was not removed by extraction with 0.6 M KI or with 5 M guanidine HCl mixed with 1% mercaptoethanol. Exposure to bacterial collagenase did not affect the stiffness of dystrophic muscle under digestion conditions adequate to destroy the stiffness of tendon. Biochemical evidence suggests that the increased stiffness of dystrophic muscle depends on an abnormal form of collagen which is collagenase-insensitive by virtue of excessive crosslinking. The collagen in dystrophic muscle has been further characterized by amino acid composition, CNBr digestion and immunocytochemistry. The results suggest that muscular dystrophy may involve the biosynthesis by skeletal muscle of an abnormal form of collagen resulting in a mismatch of compliance between the extracellular matrix and the myoplasm. This abnormal form of collagen, which we have named dystrophin, can be easily demonstrated by histological techniques applied to CNBr-treated muscle. Application of these techniques to a muscle biopsy from a preclinical case (10.5 months) of Duchenne dystrophy has revealed the presence of a similar anomalous form of collagen.**

0618 EVIDENCE FOR PROMISCUOUS EXPRESSION IN HUMAN MUSCLE FIBERS AFFECTED BY MYOTONIC DYSTROPHY, Gudrun E. Moore, Frederick H. Schachat and Allen D. Roses, Duke University Medical Center, Durham, NC 27710.

Changes in myosin expression were analyzed by SDS PAGE in single fibers from human myotonic dystrophy muscle. Histochemical changes observed in cross-section of myotonic dystrophy biopsies include slow fiber atrophy, hypertrophy and negative disproportion. For these reasons attention was focused on the slow fiber population with the development of histochemical techniques to pretype single fibers in teased bundles. Using a stain for succinate dehydrogenase activity the fibers can be divided into two distinct groups; the fast-glycolytic fibers, and the fast-oxidative-glycolytic and slow-oxidative fibers. These stained fibers can then be analyzed by SDS PAGE. The pathogenesis of myotonic dystrophy appears to be characterized by an increase in the number of promiscuous fibers (those fibers that express both fast and slow myosins). This promiscuity is rare in normal muscle. By pretyping the muscle fibers this promiscuity was, in 3 out of 4 cases, found to be linked to fibers that have high oxidative capacity. The levels of promiscuity in 4 control samples was less than 1% whereas promiscuity in 6 myotonic dystrophy samples was 10 times greater. Although the observed promiscuity is not necessarily a primary effect of the disease, it appears to be directly linked to the pathogenesis of the myotonic dystrophy as demonstrated histochemically. With regard to this and of interest is the additional observation that two of three family members at risk for myotonic dystrophy also show high levels of promiscuous expression.

0619 $\alpha$-SKELETAL ACTIN GENE TRANSCRIPTS ACCUMULATE IN HYPERTROPHIED ADULT RAT HEARTS, Ketty Schwartz*, Anne-Marie Lompré*, Pierre Bouveret*, Claudine Wisnewsky*, Patricia Oliviero*, Serge Alonso** and Margaret Buckingham**, *U 127 INSERM & **Inst. Pasteur, Paris, France

Recently it has been shown that, in rat and mouse, at the time of birth, transcripts of the cardiac actin gene are accumulated in skeletal muscle, whereas conversely, transcripts of the skeletal actin gene are accumulated in cardiac tissue (1,2). To determine if the two genes are also co-expressed during the active growth of adult tissues, we studied cardiac hypertrophy of the adult heart. Hypertrophy was produced by constricting the abdominal aorta in male rats at 6-8 weeks of age. The animals were sacrified 2,4,8,15 and 30 days after the operation, and compared to age-matched sham-operated controls. The total amount of actin message was quantified by Northern blot analysis using a coding sequence probe derived from cardiac actin mRNA, and the level of skeletal actin mRNA was determined with the 3' non coding region of the corresponding mRNA. Both cDNA probes have been previously characterized (1). We observed that transcripts of the skeletal actin gene are accumulated at low level, if at all, in normal hearts, but at significant levels (x2 to 16) 2,4 and 8 days after aortic banding. At 15 days, skeletal actin mRNA returned to undetectable levels. This finding show that skeletal and cardiac actin genes are co-expressed in adult hypertrophied rat hearts (cf the report on an adult human heart (3)), which might suggest that during periods of fast growth of muscle tissue, a single gene is not sufficient for the synthesis of enough actin. 1)Minty et al. Cell, 1982, 30,185 ; 2) Mayer et al. Nucl. Acids Res. 1984, 12, 1087 ; 3) Gunning et al. Mol. Cell. Biol. 1983, 3, 1985.

0620 ANALYSIS OF STRUCTURE AND EXPRESSION OF HUMAN SARCOMERIC MYOSIN HEAVY CHAIN GENES, Hans-Peter Vosberg and Peter Lichter, Max-Planck-Institut für medizinische Forschung, Heidelberg (FRG)

Myosin heavy chain (MHC) genes exist as multigene families which according to current estimates comprise 8 to 10 non-allelic genes for sarcomeric muscle in mammalian organisms. We are focussing on the human myosin gene family. We isolated a 14.2 kb long genomic sequence from a lambda genomic DNA library using a rabbit myosin cDNA (Sinha et al. PNAS 79, 5847, 1982) as a probe. This human gene (designated gMHC1) contains about 70% of the coding sequence required for a complete myosin chain. The sequence includes at its 5' end the region of the "active thiols", organized in a separate exon, and at its 3' end a short C-terminal exon coding for the 5 C-terminal amino acids and untranslated sequences. DNA sequence data and comparison with myosins and myosin genes of other mammalian species suggest that gMHC1 codes for the human ß myosin chain. The expression of this gene in different human muscle tissues is currently investigated. So far we have found one genomic locus for sarcomeric myosin on the short arm of the human chromosome 17 (1,2-pter) by in situ hybridisation (Rappold and Vosberg Hum.Gentics 65, 195, 1983).

## *Human Muscle Genetics*

0621 DEVELOPING GENETIC MARKERS BASED ON RESTRICTION FRAGMENT LENGTH POLYMORPHISMS NEAR THE LOCUS FOR X-LINKED MUSCULAR DYSTROPHY, Uta Francke, Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

With knowledge of the primary molecular defect lacking, the gene for X-linked muscular dystrophy (Duchenne type, DMD) has been mapped to band Xp21 based on the occurrence of the disease phenotype in female patients who coincidentally have de novo balanced reciprocal translocations between an X chromosome and an autosome, with the breakpoints consistently in chromosome band Xp21 and associated with genetic inactivation of the structurally normal X. With the goal of developing DNA based genetic markers near the DMD locus, we have identified two patients with minor interstitial deletions of parts of band Xp21, a female (1) and a male (2). We have isolated the abnormal X chromosomes in somatic cell hybrids on a Chinese hamster background. The hybrids were screened, by Southern blot analysis, with 20 single-copy cloned sequences derived from human X chromosome clone banks by other investigators and previously localized to the X short arm (3). Six sequences were mapped to one of 3 different regions within band Xp21; 4 of these (2 on each side of the DMD locus, as defined by the translocation breakpoints) recognize DNA polymorphisms and are suitable for linkage studies (2,3). As revealed by the molecular hybridization studies, the deletion in the male patient, who suffered from DMD and from two other X-linked disorders, was entirely contained within the larger deletion in the female patient. Only 1 of the 20 DNA sequences tested was mapped within his deleted segment (2). DNA from this patient will be useful for subtraction cloning strategies with the goal of isolating more probes from this small region and of eventually identifying the sequences involved in these disorders.

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0622 GENETIC ANALYSIS OF X-LINKED NEUROMUSCULAR DISEASE. Louis M. Kunkel, Anthony P. Monaco, William Middlesworth and Samuel A. Latt, Harvard Medical School, Boston, MA 02115

At least three neuromuscular diseases are known to be X-linked; the most common being Duchenne muscular dystrophy (DMD). For these and many other human genetic diseases, no known defective genetic product has been identified. We have undertaken a genetic approach to the understanding of DMD. The locus for DMD is presumed to lie in the area of Xp21 (Jacobs et al. 1981; Davies et al. 1983). Genetic analysis of various RFLP detecting cloned X chromosome DNA fragments has been utilized in families segregating DMD. An accurate map of these fragments and DMD is starting to emerge from numerous genetic studies in our laboratory and others. To date no randomly isolated cloned probe has been shown to be closer than 5 cM to DMD.

We have recently initiated a more direct approach to obtaining cloned DNA fragments nearby DMD. We have utilized a method which allows specific cloning of DNA fragments absent from patients homozygous or hemizygous for chromosomal deletions. The method exploits phenol-dependent accelerated (Kohne et al. 1977) subtractive DNA/DNA reassociation followed by molecular cloning of appropriately reassociated molecules. Five percent of unique sequence segments (4/81) escaping such a competition have been identified as missing from the DNA of a male patient with DMD, Retinitis Pigmentosa, chronic granulomatous disease and a small deletion in the short arm of the human X chromosome. Each fragment has potential to be a diagnostic reagent for one of the diseases affecting this boy. Each, among others obtainable by this approach, may serve as the necessary start points in the eventual cloning of DMD and these other X-linked disease loci.

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**0623** APPROACHING THE DUCHENNE MUSCULAR DYSTROPHY GENE THROUGH A TRANSLOCATION INVOLVING RIBOSOMAL RNA GENES. R.G. Worton, C. Duff, C. Logan, P.N. Ray, V. Kean, J. Sylvester and R.D. Schmickel, The Research Institute, Hospital for Sick Children, Toronto, and Dept. of Human Genetics, University of Pennsylvania, Philadelphia.

Duchenne muscular dystrophy (DMD), the most severe and most common of the dystrophies is an X-linked genetic disorder normally affecting only males. Several females have been described whose disease is the result of an X-autosome translocation with the exchange point in band p21 at or near the site of the dmd locus. In one of these patients the autosome involved is 21 with an exchange point through band p12, the site of a block of tandemly repeated genes encoding 18S and 28S rRNA. In the translocated chromosomes "rDNA" must be adjacent to the DMD region of the X, allowing subclones of rDNA to be used as probes in screening for a junction fragment. We have isolated the two translocation chromosomes in somatic cell hybrids, free of the other rDNA containing human chromosomes, and by Southern blotting have identified the putative junction near the 5 end of the 28S rRNA gene. Libraries of gel purified fragments as well as partial digest libraries of hybrid cell DNA have been constructed and are being screened for the junction fragment. Preliminary experiments indicate that the junction fragments may contain "poison sequences" as they are not present in the libraries at the expected frequencies. Other stratagies including "jump libraries" and cosmids are now being exploited in an attempt to overcome this difficulty. It is anticipated that cloning of the junction fragment will provide a new approach to the DMD gene itself, and at the very least should provide new probes tightly linked to the DMD locus for use in carrier identification and prenatal diagnosis.

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## *Cardiac Muscle Development*

**0624** Structural and Functional Features of Cloned Chicken Muscle Genes: Transfection in Myogenic and Non-myogenic Cells.

H.H. Arnold, B.Winter, H.Glaser, and K.Wiebauer, Dept.of Toxicology, Medical School, University of Hamburg, Grindelallee 117, 2000 Hamburg 13. FRG.

The gene coding for cardiac myosin light chain (MLC2-A) has been isolated from a chicken genomic cosmid library, which was constructed in our laboratory. The MLC2-A gene spans about 4.5 Kb of DNA and contains 5 introns. The 5' and 3'termini have been determined by nucleotide sequence analysis, which revealed two extra codons for methionine and alanine at the N-terminus of the protein (1). The expression of the gene was analyzed in several tissues and during the embryonic development of chicken. RNA isolated from hearts of chicken, rat, and human as well as from chicken ALD muscle hybridized specifically to a gene probe, whereas fast muscle did not show any hybridization.

The methylation pattern of MLC2-A DNA was examined in expressing (heart) and non-expressing tissue (liver) by digestion with methylation sensitive restriction enzymes. No differences of methylation could be detected in either tissue.

The investigation of chromatin structure by limited digestion of intact nuclei with DNAse I exhibited a merely marginally increased sensitivity of the MLC2-A gene, compared to the presumably silent lysozyme gene. Hypersensitive regions around the 5'end of the gene could be detected in heart and liver tissues. Particularly in the 3'flanking sequences, however, we found two additional tissue specific hypersensitive sites in heart compared to the one found in liver.

Constructs between the dominant selectable marker plasmid (geniticin-resistance) and the complete MLC2-A gene including about 2 Kb of flanking sequences at either site were transfected into C2 mouse myoblasts and RNA from selected clones was analyzed for transcripts. The gene appeared to be expressed to a low steady state level of RNA irrespective of the state of cellular differentiation. The -actin gene, specific for striated muscle, and the ß-actin gene, expressed in all cells were introduced into LTK mouse fibroblast together with the cloned Herpes thymidine kinase gene. Whereas the cytoplasmic actin mRNA could be seen on RNA blots, the -actin mRNA was indetectable in these cells. The implications of cellular and gene specific requirements for the expression of muscle genes will be discussed in the light of our results.

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0625 Expression of Myosin Heavy Chains in Atrial, Ventricular, and Purkinje Fiber Myocytes of the Chicken Heart. D. Bader, A. Sanchez, B. Zadeh, M.A. Matheson, N. Toyota. Cornell University Medical College, N.Y.,N.Y. To examine myosin heavy chain heterogeneity in the adult and developing chicken heart, monoclonal antibodies against myosin from the adult chicken were generated and their specificities characterized. The reactivities of four such antibodies with myosin isolated from adult and developing chicken atrial and ventricular myocardia were analyzed by immunoautoradiography, radioimmunoassay, and immunofluorescence microscopy. The following specificities were observed: Atrial myocytes reacted with antibody B1, ventricular myocytes with A19, cardiac myocytes of the cardiac conductive system with ALD58, and all striated muscle cells of the heart with MF20. Immunoautoradiography and immunoprecipitation demonstrated the specificity of these antibodies for the heavy chain. Using anti-ventricular and anti-atrial antibodies, tissue specific expression of myosin heavy chain was first observed at or near the onset of regional differentiation in the heart(stage 10 and 15 respectively). In contrast, the heavy chain epitope recognized ALD58 in the heart conductive system was not detected until the later stages (stage 32) of embryonic development. Once established in atrial, ventricular and conductive system myocytes, tissue specific expression of myosin heavy chain was maintained throughout development and adult life. To determine whether or not tissue specific expression of myosin heavy chain was retained in isolated myocytes in vitro, monolayer cultures were prepared from atria and ventricles at selected stages of development and assayed for myosin heavy chain content using specific antibodies. The atrial specific heavy chain recognized by B1 was not detected in 7d cultured myocytes derived from 7, 11, and 19d embryonic atria. While stage specific variability was observed, the majority of cultured ventricular myocytes lost specific reactivity with the ventricular specific antibody A19. Still, both atrial and ventricular cultures contained cross-striated myocytes which were reactive with MF20 indicating the presence of sarcomeric myosin. The results demonstrate that the immunochemical heterogeneity of heavy chains derived from atrial, ventricular and conductive system myocytes in the intact heart are not retained in atrial and ventricular myocytes in monolayer culture.

0626 CONTROL AND EXPRESSION OF CARDIAC MUSCLE GENES, M.A.Q. Siddiqui, Ana Maria Zarraga, Chandrika Saidapet, Diarmuid Nicholson, Charmaine Mendola and Manuel Krauskopf, Roche Institute of Molecular Biology, Department of Biochemistry, Nutley, NJ 07110

**Cardiac Muscle Gene Expression:** We have previously constructed several recombinant plasmids containing the cDNA sequences for embryonic chicken cardiac muscle myosin light ($MLC_2$) and heavy chain (MHC) mRNAs. Using defined sequences from these DNAs that exhibit tissue specificity, the appearance of cardiac MLC and MHC mRNA during early chick blastodermal development was monitored. Transcripts coding for cardiac MLC and MHC co-accumulate,in amounts sufficient for detection by Northern blot analysis, at about 60 hr of development significantly earlier than the appearance of mRNAs for skeletal muscle isoforms of these proteins. Southern blot analysis of restriction digests of chicken genomic DNA suggests that $MLC_2$ gene is present as a single copy, whereas the MHC sequences are multicopy genes. For further understanding the structure and organization of these genes, genomic clones for $MLC_2$ and MHC were isolated and characterized. In the case of $MLC_2$, a genomic clone was isolated which contains almost the entire coding sequence and about a 6.0 kb region corresponding to the 5'-terminus region of the gene. The expression and regulation of these genes is currently being investigated using transfection and DNA-injection experiments.

**Isolation of Additional Muscle-Specific Clones:** We have used the DNA libraries from human and rat heart tissues to isolate MLC and MHC cDNA clones and the corresponding genomic clones. In the case of human, the $MLC_2$ gene is represented in one copy per genome and appears to be localized on chromosome 8.

**Coordinate Control of Muscle Genes:** Models on eukaryotic gene control envisage a role for repetitive DNA sequence regulated by tissue specific RNA and/or protein. We have recently described the isolation and characterization of a low molecular weight RNA (7S RNA) from embryonic chick heart tissue. The hybridization of 7S RNA specific cloned DNA (pSS48) to chicken DNA suggests that 7S RNA is the product of repetitive chicken genome. However, pSS48 DNA sequence appears to identify several single copy gene transcripts in chicken heart muscle. Sequence analysis of recombinant plasmids containing cDNA for MLC and MHC indicates that short nucleotide stretches common to pSS48 DNA reside in the 3'-untranslated regions of the respective DNAs. Hybridization experiments utilizing the $MLC_2$ and MHC genomic clones showed that additional sequences hybridizable to pSS48 are located both downstream and upstream to, but not within, the coding region of the respective genes. A developmental study utilizing RNAs from blastodermal cells indicates that 7S RNA is transcribed in embryonic cells preferentially earlier than the onset of transcription of genes for MHC, MLC, and actin. These results, taken together, suggest that 7S RNA might be involved in control of muscle gene transcription. That 7S-like sequences in MLC and MHC genes are indeed crucial to transcription is being tested with artificial gene constructs free of these sequences.

0627 EVOLVING PHENOTYPE OF THE HEART. Ashish Nag, Laureen Sweeney, Brenda Eisenberg, Francis Manasek, Radovan Zak. Dept of Medicine, Univ. of Chicago, Chicago,IL 60637

The change in isomyosin profile of avian and mammalian hearts was examined during embryonic period, in primary culture and in serial sections of the entire ventricular wall of adult heart. Two methods have been used to classify myocytes: immunofluorescent staining with Mc Abs specific for heavy chains of V1 and V3 isomyosins and cytochemical staining for $Ca^{2+}$ activated myosin ATPase.

The chicken ventricle showed strong reactivity with anti V3 Ab throughout all stages of embryonic and adult development. The atrium also reacted with anti V3 Ab from its initial stage of incorporation into the heart (st.12) through the 4th day of incubation (st.24). The atrial reactivity then declined so that from 6th day (st.29) through the remaining embryonic and adult life there was no significant atrial reactivity with anti V3 Ab. These data are consistent with our previous observation that the V3 isomyosin is the first to appear in all cross-striated muscles.

In contrast to birds, mammalian heart contains mixture of V1 and V3 isomyosins within both chambers. Using cultured cells we have examined whether myocytes are synchronized with respect to myosin expression. We have examined the isomyosin profile of cells derived from 16 d. old rat embryonic ventricles grown in a synthetic medium in the absence or presence of physiological concentration of thyroid hormone (T4). Myocytes grown in T4 free media for 7 days contained majority of cells showing bright fluorescence after staining with anti V3 Ab as well as weak ATPase activity. A small population of cells, however, reacted strongly with anti V1 Ab and had high ATPase activity. Myocytes grown in T4 supplemented cultures converted, after 7 days,to a predominance of V1 containing cells. Nevertheless, a small population of cells remained which was strongly reactive to V3 isomyosin. These data are consistent with the existence of two populations of myocytes with different responsiveness to T4.

The heterogeneity in myocyte population was examined in ventricular wall of adult (1 yr) rabbit. The isomyosin population varies through the epi- to endocardium of the right ventricular wall with V1 predominating in the outer epicardial third of the wall and lowest amount in the middle third of the wall. A mixture of stained and unstained myocytes is seen in the endo- and subendocardial regions. Myocytes with similar staining properties appear in large clusters, long strands and major anatomical regions. However, heterogeneous myocytes are also seen connected by an intercalated disc. The heterogeneity between two adjacent cells expressing different isomyosin might depend on a stage of genetic reprogramming reflecting the different population of myocytes.