MOLECULAR MECHANISMS OF CARDIAC GROWTH AND HYPERTROPHY

Organizers: Robert Roberts and Michael Schneider

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Poster Sessions

Molecular Mechanisms of Cardiac Growth and Hypertrophy

Keynote Address (joint)

H 001 REGULATION OF REGULATORS, David Baltimore, The Rockefeller University, New York, New York 10021 and the Whitehead Institute for Biomedical Research, Cambridge, MA. 02142. The most conceptually challenging issue of differentiation is not how gene expression is regulated. -- Jacob and Monod largely solved that problem -- but how the regulators are regulated. To achieve a differentiated, stable phenotype requires that a cell program numerous genes in quite precise ways, using robust regulatory strategies to assure long-term stability. In no cellular system is there a satisfactory description of regulation, but as more regulators are isolated, and the genes encoding them are cloned, elements of regulatory strategies are emerging. One is that regulators occur in structurally-related families and that often members of a family interact among themselves. The leucine zipper, helix-loop-helix and rel-related proteins (especially NF-κB) are examples. Also regulators may be sequestered by inhibitors (as IκB for NF-κB or Id for helix-loop-helix proteins) and therefore, the regulation of the inhibitor becomes a crucial component of the system. Certain regulators positively activate themselves, contributing to stability. Also, when their products are not required, many, maybe all, genes are maintained in the non-expressed state by active silencing methods and counteracting silencing may be a crucial aspect of allowing gene expression. Individual genes are affected by a medley of regulators, thus the ensemble of gene products that define any one differentiated state is produced by a multitude of regulators, each maintained by its own particular set of interactions.

Cell Fate Decisions (joint)

H 002 MYOGENESIS IN THE MOUSE, Margaret Buckingham, Gary Lyons, Marie-Odile Ott and Françoise Catala, Department of Molecular Biology, Pasteur Institute, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

We have examined the temporal and spatial expression of muscle genes during myogenesis in the mouse embryo, using *in situ* hybridization on tissue sections. The first striated muscle to form is the heart. In the cardiac tube from 7.5 days the myosin and actin isoforms of the adult heart are already co-expressed. Once the heart has acquired atrial/ventricular compartments different myosin gene transcripts begin to show spatial restriction. This is an asynchronous process (1). At no time are transcripts of the MyoD1 family detectable in the heart. The first skeletal muscle to form is the myotome, in the central region of the somites. Myotomal cells, as well as skeletal muscle cells in muscle masses elsewhere in the body originate from the dermomyotome of the somite. In contrast to the heart, different muscle structural gene transcripts appear asynchronously over a period of several days in the myotome (2). Transcripts of myogenic regulatory genes also accumulate with distinct kinetics (3). Myf-5 (in collaboration with E. Bober, H. Arnold) is the only muscle gene sequence detected in the dermomyotome, prior to formation of the myotome (4). Transcripts are present in some cells throughout the dermomyotome, but are concentrated in the dorsal lip region from which myotomal cells originate. Myf-5 transcripts are also present precociously in premuscle masses in the limb buds and elsewhere. At later stages of muscle development myf-5 is no longer detectable; myogenin, MyoD1 and myf-6 are the major myogenic regulatory sequences in foetal skeletal muscle. These results will be discussed in the context of muscle cell lineage determination and of the transcriptional regulation of muscle genes. The myosin alkali light chain (MLC1A) gene (5) expressed in the heart and also in embryonic skeletal muscle, provides an example of a regulatory element containing tandemly arranged consensus sequences binding myogenic factors of the MyoD1 family located in the proximal promoter. However examination of the central CArG-like sequence of this element indicates that myogenin (in collaboration with W. Wright) may be implicated in an additional regulatory phenomenon during skeletal myogenesis.

1) Lyons et al., 1990, J. Cell Biol., 111:1465-1476; 2) Lyons et al., 1990, J. Cell Biol. In press; 3) Sassoon et al. (1989) Nature, 341:303-307; 4) Ott et al. (1990) submitted; 5) Barton et al., 1988, J. Biol. Chem., 263:12669-12676.

H 003 THE ROLE OF THE ACTIVIN FAMILY IN MESODERM FORMATION IN XENOPUS. J.C. Smith, J.B.A. Green, G.-D. Guex, G. Howes, H.V. New, M.G. Sargent. National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

The mesoderm of amphibian embryos, including muscle, notochord, kidney and blood, is formed in response to an inductive interaction in which cells of the vegetal hemisphere of the blastula act on overlying equatorial cells. The activins, members of the TGFβ superfamily, may be involved in this process. In the adult, the activins are known to modulate the release of follicle stimulating hormone from the pituitary and to promote erythroid differentiation. In the *Xenopus* embryo, we and others (Asashima *et al.*, *Roux's Arch. Devl Biol.* **198**, 330-335, 1990; Thomsen *et al.*, *Cell* in press) have shown that activins A and B can induce isolated presumptive ectoderm to form mesodermal cell types.

In this report we discuss how the activin family might be involved in specifying different regions of the *Xenopus* mesoderm. As markers we use probes for various mesodermal cell types as well as homeobox and zinc finger genes. Different concentrations of activin activate these genes to different extents. We also consider whether activin protein is present in the early embryo, and whether it can influence the expression of other members of the TGFβ superfamily in a cascade mechanism.

H 004 POSITIONAL INFORMATION IN LIMB MORPHOGENESIS, Lewis Wolpert, Department of Anatomy and Developmental Biology, University College and Middlesex School of Medicine, Windeyer Building, London W1P 6DB, UK.

Pattern formation in chick wing development can be understood in terms of the cells acquiring positional values along the antero-posterior and proximo-distal axes. For the antero-posterior axis there is a signal from the polarizing region which may involve retinoic acid as a morphogen; for the proximo-distal axis the proposed specification of position may depend on time in the progress zone. The relationship of positional values to homeobox gene expression will be discussed.

In addition to positional information there may be a primitive prepattern for both cartilage and muscle.

Muscle cells have an origin different from other cells in the limb, migrating in from the somites. Muscle patterning appears to involve migration of muscle cells and varying adhesive interactions with connective tissue cells. Unlike connective tissue cells, which are non-equivalent, muscle cells seem to be equivalent.

Most of the interactions in limb development can be classified in terms of STOP, GO, STAY and POSITION. The complexity of the limb lies in cellular responses rather than cellular interactions.

Molecular Mechanisms of Cardiac Growth and Hypertrophy

Cellular and Molecular Biology of Commitment (joint)

H 005 HIERARCHICAL CONTROL OF MUSCLE GENE EXPRESSION, Eric N. Olson, Thomas J. Brennan, Tushar Chakraborty, T.-C. Cheng, Peter Cserjesi, Diane Edmondson, Li Li, Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Establishment of a skeletal muscle phenotype appears to involve a regulatory cascade that culminates with the induction of a battery of genetically unlinked muscle-specific genes. We have used the muscle creatine kinase (MCK) enhancer as a target to study the mechanism whereby the muscle regulatory factor myogenin activates muscle-specific transcription. Myogenin, like other members of the MyoD family, binds to the concensus sequence CANNTG, which is present in the core of the MCK enhancer and in the control regions of numerous other muscle-specific genes. The affinity of myogenin for DNA increases dramatically in the presence of the widely expressed helix-loop-helix (HLH) protein E12, with which it forms heterodimers. Full activity of the MCK enhancer appears to require cooperative interactions between myogenin (or other members of the MyoD family) and other muscle-specific, as well as ubiquitous enhancer-binding factors. One such factor may be the myocyte-specific factor MEF-2, which binds an A+T-rich element in the MCK enhancer that is conserved in other muscle-specific enhancers. MEF-2 expression is activated by myogenin, suggesting that it may function at an intermediate level in the myogenic regulatory cascade. To define the domains of myogenin responsible for muscle-specific transcription, DNA-binding, and cooperative interactions with other enhancer-binding factors, we have generated an extensive series of myogenin mutants by site-directed mutagenesis and domain-swapping. These mutants have revealed regions in the amino and carboxy termini of myogenin that contribute to transcriptional activation and have defined two amino acids (alanine^{86}-threonine^{87}) in the basic region that impart muscle specificity. Mutagenesis of either of these residues converts myogenin from an activator to an inhibitor of myogenesis. The role of E12 in muscle-specific gene expression has also been investigated by creation of E12 mutants that dimerize with myogenin and abolish its ability to bind DNA. These mutants, which inhibit the ability of myogenin to activate myogenesis in transfected fibroblasts, are being examined for their effects on differentiation of cell types from other lineages. In addition to the positive roles played by myogenin and E12, myogenesis is also subject to negative control by growth factors, which can inhibit myogenin expression. Analysis of the 5' flanking region of the myogenin gene has revealed regions that confer muscle-specificity and growth factor responsiveness. Growth factors also can suppress myogenin's actions in cells that express it constitutively. Mechanisms through which growth factor signals may silence myogenin as an activator of muscle-specific genes include, induction of inhibitory HLH proteins such as Id, induction of immediate early genes like *fos* and *jun* that can trans-repress muscle-specific genes, repression of other cellular factors with which myogenin cooperates to activate target genes, changes in protein phosphorylation, and suppression of myogenin's transcription activating properties independent of DNA-binding. The involvement of each of these mechanisms in growth factor-dependent repression of myogenesis will be discussed.

Growth Factors and Signal Transduction (joint)

H 006 CHRONIC INFLAMMATION, PDGF, TGFß, AND SMOOTH MUSCLE PROLIFERATION, Russell Ross, Edouard J. Battegay, Elaine W. Raines, Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195

Atherosclerosis is thought to be a specialized form of chronic inflammation. Smooth muscle proliferation is a hallmark of atherosclerosis and is responsible for restenosis following bypass surgery and PTCA. A number of cells present in developing lesions of atherosclerosis, including activated endothelium, smooth muscle, monocyte/macrophages, and platelets, are capable of elaborating a number of growth-regulatory molecules, including the different dimeric forms of PDGF and at least one form of TGFß. Both PDGF-B-chain transcript and protein and TGFß transcripts are increased in lesions of atherosclerosis. In cultured quiescent smooth muscle cells, TGFß modulates smooth muscle proliferation by altering expression of both PDGF A-chain and both PDGF cell-surface receptors. The amount of TGFß per cell determines whether the smooth muscle response to TGFß is proliferation or inhibition of proliferation. Released PDGF is cleared very rapidly from the circulation. However, smooth muscle cells and endothelial cells can selectively store PDGF on extracellular binding sites. This extracellular compartmentalization appears to be mediated through the alternatively spliced exon 6 of the PDGF-A chain and a homologous region in exon 6 of the PDGF-B chain. Thus directional migration and proliferation of smooth muscle in response to PDGF can be modulated by other growth-regulatory molecules and by the form of PDGF secreted. Such regulation may determine the outcome of chronic inflammation that precedes and accompanies fibroproliferative diseases such as atherosclerosis. Supported in part by NIH grants HL-18645 and HL-03174.

H 007 TGF-β IN THE CARDIOVASCULAR SYSTEM. Michael B. Sporn, Anita B. Roberts, Thomas S. Winokur, Kathleen C. Flanders, James K. Burmester, Ursula I. Heine, Lalage M. Wakefield, David Danielpour, Su Wen Qian

The three isoforms of TGF-β (TGF-β1, -β2, and -β3) are omnipresent, multifunctional peptide signalling molecules. Recent studies indicate that they play an important role both in the development and the activities of the cardiovascular system. Their normal and pathologic physiology in both the heart and the peripheral vascular system will be discussed. Special emphasis will be given to the role of TGF-β in response to ischemic or anoxic injury of the heart.

H 008 MOLECULAR MECHANISMS OF TRANSCRIPTIONAL REGULATION IN YEAST, Kevin Struhl, Ph.D., Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115

The yeast GCN4 protein binds to upstream promoter sequences of 30-100 genes involved in amino acid biosynthesis and coordinately induces their transcription in response to amino acid starvation. GCN4 binds as a dimer to a 9-bp region, with optimal binding to the palindrome ATGACTCAT. The 60 C-terminal amino acids of GCN4 are sufficient for specific DNA binding and also for dimerization. The GCN4 DNA-binding domain is similar in sequence to the jun oncoprotein, the oncogenic version of the vertebrate AP-1 transcription factor. Moreover, GCN4 and jun bind the same DNA sequences, and jun efficiently activates transcription in yeast cells indicating a basic similarity in the molecular mechanism of eukaryotic transcriptional activation. The GCN4 DNA-binding domain contains a "leucine zipper" region that is sufficient for dimerization; however, the leucine residues are not critical.

In addition to the DNA-binding domain, transcriptional activation by GCN4 requires a short acidic region in the center of the protein. Acidic regions of 35-40 amino acids are sufficient for full activation when fused directly to the DNA-binding domain. The activation region is a repeated structure composed of small units that act additively which presumably interacts with other proteins of the transcriptional machinery.

The *his3* promoter contains two functionally distinct TATA elements, T_R and T_C, but only T_R can activate transcription in combination with GCN4 or GAL4. The sequence TATAAA is sufficient for T_R to activate transcription, but almost all single bp substitutions abolish function. Interestingly, the TATTTA double mutant is functional and 3 mutations activate transcription in combination with GCN4 but not GAL4. Finally, a wide variety of sequences completely unrelated to TATAAA can serve as the T_R element. These observations suggest that multiple proteins can perform the TATA function for transcriptional activation.

H 009 FOS-JUN STORY, Lynn J. Ransone, Jane Visvader, V.J. Dwarki, Kim Morley, Penny Wamsley and Inder M. Verma, Molecular Biology & Virology Laboratory, The Salk Institute, La Jolla, CA 92037.
Gene expression is modulated by the specific interaction of nuclear proteins with unique regulatory sequences *in the genome. Nuclear oncoproteins* fos *and* jun (AP-1) cooperate in forming a very stable heterodimeric complex that binds to the AP-1 site with increased affinity. The "leucine zipper" domain of both fos and jun is necessary for the formation of this heterodimer. We have undertaken a mutational analysis of (1) individual leucine residues, (2) neighboring amino acids within the "leucine zipper" domain, and (3) residues in the highly basic domain of both the fos and jun proteins, to examine the contribution of these amino acids to the formation of a stable fos/jun heterodimer, the formation of jun homodimers and the binding potential of the heterodimeric complex to the AP-1 site. Mutations of single residues within the "leucine zipper" domain had no effect on protein complex formation. However, mutagenesis of the first leucine of the heptad repeat in either fos or jun drastically reduced binding of the complex to DNA. Mutations within the fos and jun basic regions and alteration of the spacing between the basic and "leucine zipper" domains indicate that the basic region of fos has a crucial role in determining the DNA binding affinity of the transcriptional complex. This is further substantiated by making site specific mutations in the basic amino acids in fos protein. We have also generated fos-jun chimeras to access the role of "leucine zipper" in the formation of homodimer. Mutation in the leucine zipper domain affects not only the protein-protein association, but also DNA binding. Conversely mutation in the DNA binding domain influences Fos-Jun heterodimer formation. We have generated DNA binding mutants of Jun (JunΔRk) which are functional transdominant negative mutants. F9 cells producing JunΔRk cannot be differentiated by retinoic acid. The role of fos/jun complex in transcriptional transactivation will be discussed.

Ion Channels

H 010 TRUNCATING K^+ CHANNELS AND *ras*/GAP CHANNEL MODULATORS, Arthur M. Brown, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

What parts of a channel protein can be removed without affecting voltage sensitivity or ionic selectivity? What parts of the GTP-ase activating protein GAP can be removed without affecting its ability to modulate channel proteins? We studied these problems using *drk1* a voltage-sensitive, delayed rectifying K^+ channel of 853 amino acids cloned from adult rat brain (1,2) and human GAP (3) which modulates an inwardly rectifying muscarinic atrial K^+ channel (K^+[ACh]) in adult guinea pig (4). Removal of 101 to 139 amino acids from the N-terminus slowed activation of *drk1* cRNA expressed in Xenopus oocytes 10- to 100-fold without changing voltage sensitivity or apparent ion selectivity. Removal of 338 amino acids from the C-terminus speeded activation slightly. A doubly truncated mutant in which these deletions were combined expressed wild type currents. Extension of either N- or C-terminal deletions into putative transmembrane segments 1 or 6 respectively abolished expression. A core region of less than half the protein is therefore responsible for the fundamental properties of *drk1*.

GAP blocked K^+[ACh] currents by uncoupling the muscarinic receptor from G_k the heterotrimeric G protein activator of this channel. GAP did not bind to purified G_k leading us to investigate the effects of human *ras* p21 a known target for GAP. *ras* also blocked K^+[ACh] channels. The effects of GAP were blocked by an anti-*ras* antibody, the effects of GAP were blocked by an anti-*ras* antibody and the effects of both antibodies were overcome by the combination of *ras*/GAP. GAPette a truncated form of GAP consisting of the C-terminal 334 amino acids has one-fifth the GTP-ase activity of GAP but was virtually ineffective as a channel blocker. Therefore the catalytic domain is not responsible for the channel blocking effects of GAP. One possibility is that *ras*/GAP together form a cellular signaling molecule and we are presently defining the region of GAP essential for blockage of the K^+ channel. Supported by NIH grants - HL 37044, NS 23877, HL 39262 and HL 36930.

References:

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H 011 MOLECULAR PROPERTIES OF VOLTAGE SENSITIVE Na^+ AND Ca^{++} CHANNELS. Catterall, William A., Department of Pharmacology, University of Washington, Seattle, WA 98195.

Electrical signals in the form of transmembrane ionic currents are important regulators of cellular function. The voltage-gated ion channels include the Na^+ channels, Ca^{++} channels, and K^+ channels. Voltage-sensitive Na^+ and Ca^{++} channels mediate inward movements of Na^+ and Ca^{++} that are responsible for the depolarizing phase of the action potential in excitable cells, and Ca^{++} moving into the cell through Ca^{++} channels acts as a signal for multiple intracellular events. Activation of K^+ channels terminates the action potential and repolarizes the cell. Na^+ channels isolated in functional form from mammalian brain consist of a complex of an α subunit (260 kDa) in association with a $\beta 1$ subunit (36 kDa) and disulfide-bonded to a $\beta 2$ subunit (33 kDa). The α subunit is common to Na^+ channels from all tissues that have been studied, and mRNA encoding it is sufficient to direct the synthesis of functional sodium channels in *Xenopus* oocytes and mammalian cells. Ca^{++} channels isolated from skeletal muscle transverse tubules consist of an α_1 subunit (175 kDa), which is similar is primary structure to the α subunit of the Na^+ channel, can form a functional voltage-gated channel, and contains the receptor sites for calcium channel modulators. It is associated with phosphorylated β (54 kDa) and glycosylated γ (30 kDa).subunits which are encoded by separate genes and with a disulfide-linked glycoprotein complex of α (143 kDa) and δ (27 kDa), which are encoded by the same gene and formed by post translational proteolytic processing.

The homologous primary structures of the α subunit of the Na^+ channel and the α_1 subunit of the Ca^{++} channel contain 4 repeated homologous domains containing multiple transmembrane segments. Site-directed, antipeptide antibodies have been used to probe the transmembrane organization of Na^+ channels and to identify separate sites at which cAMP-dependent phosphorylation, binding of α-scorpion toxins, and binding of specific antibodies modulate rapid Na^+ channel inactivation. Similar experimental approaches have identified two size forms (212 kDa and 175 kDa) the Ca^{++} channel $\alpha 1$ subunits and have identified cAMP-dependent phosphorylation sites and a receptor site for phenylalkylamine Ca^{++} channel blockers in those subunits. Highly conserved S4 α-helical segments in each domain of all members of the voltage-gated ion channel family are both hydrophobic and positively charged and are postulated to traverse the membrane and form the voltage-sensing elements of the channels according to a "Sliding Helix" model of voltage-dependent gating. These studies begin the development of a functional and topological map for the principal subunits of the voltage-sensitive ion channels.

H 012 Molecular Studies of Voltage-gated Potassium Channels.
Ehud Isacoff, William Kimmerly, George Lopez, Diane Papazian*, Yuh Nung Jan and Lily Yeh Jan.
Howard Hughes Medical Institute and the Department of Physiology and Biochemistry, UCSF. *Department of Physiology, UCLA.

Potassium channels represent a diverse group of ion channels found in most eukaryotic cells studied. They are often regulated by second messengers and are involved in a wide range of cellular functions including secretion, heart beat, neuronal excitability and synaptic plasticity. Because of the scarcity and heterogeneity of these potassium channels, we approached the cloning of a potassium channel gene by taking advantage of Drosophila genetics. Cloning of this gene, Shaker, and its homologs in different vertebrates and invertebrates by a number of researchers allowed molecular studies of the mechanism underlying potassium channel diversity as well as identification of structural elements involved in channel functions such as voltage-dependent activation, inactivation, and ion permeation. A progress report of our studies will be presented.

H 013 PUMPS AND CHANNELS IN THE SARCOPLASMIC RETICULUM

David H. MacLennan

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The sarcoplasmic reticulum regulates the concentration of Ca^{2+} ions within muscle cells through a Ca^{2+} pump, (Ca^{2+} ATPase), a Ca^{2+} release channel (ryanodine receptor) and a series of luminal Ca^{2+} sequestering proteins (calsequestrin, calreticulin, sarcalumenin, HCP). Regulation of Ca^{2+} ion concentration, in turn, regulates muscle contraction. We have cloned cDNAs encoding many of the sarcoplasmic reticulum proteins from both skeletal and cardiac muscles and have made deductions concerning their secondary structures and active sites from their amino acid sequences deduced from their cDNAs. These deductions have led to models for Ca^{2+} transport and release which are being tested through expression of the proteins in functional form, mutagenesis and measurement of altered functions. This work is most advanced with the Ca^{2+} pump from fast-twitch skeletal muscle. Mutation of about 250 of the 1000 amino acids making up the protein has indicated that the sites of high affinity Ca^{2+} binding are located in the center of the transmembrane domain and are made up from residues contributed by transmembrane sequences M4, M5, M6 and M8. The ATP binding site appears to be located in the headpiece and is composed of a series of loop sequences connecting alternating alpha helices and beta strands. In our model, Ca^{2+} transport occurs through binding to high affinity sites accessible to the cytoplasm in the E1 conformation followed by release to the lumen from low affinity sites which form during the ATP-induced transition of the protein from the E1 to the E2 conformation.

Cloning of cDNA encoding the ryanodine receptor has led to the prediction that up to 5% of the 5000 amino acids might exist in transmembrane sequences near the COOH-terminal end of the protein, while the remainder are cytoplasmic, making up the foot structures connecting the sarcoplasmic reticulum and the transverse tubule. We have localized the gene for this protein to human chromosome 19q13.1 and we have shown linkage between the RYR1 gene and the predisposing gene for malignant hyperthermia (MH). Detection of mutations in the RYR1 gene, leading to MH, should provide insight into the regulatory region of the Ca^{2+} release channel.

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Growth Factors and Oncogenes in Myocardium

H 014 MYOCARDIAL HYPERPLASIA IN TRANSGENIC MICE, M.E. Steinhelper, E. Katz, N. Lanson*, W. Claycomb*, and L.J. Field; Krannert Institute of Cardiology, 1001 West 10th street, Indianapolis, IN, 46202-2859, and *Department of Biochemistry, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112-1393. To assess the proliferative capacity of adult cardiomyocytes, transgenic animals were generated in which expression of the SV40 Large T antigen oncogene (T-ag) was targeted to atrial myocytes with the Atrial Natriuretic Factor promoter (ANF-TAG mice), or to both atrial and ventricular myocytes with the alpha-cardiac Myosin Heavy Chain promoter (MHC-TAG mice). In both models, atrial T-ag expression results in unilateral right atrial tumorigenesis. The tumors are comprised of differentiated, proliferating myocytes as evidanced by tritiated thymidine uptake, immunohistological, ultrastructural and molecular analyses. Moreover, the atrial tumor cells can be propagated as subcutaneous transplants in syngeneic animals, and cells derived from these transplantable tumor lineages proliferate in culture. The cultured cells retain numerous phenotypes characteristic of highly differentiated myocardiocytes, and exhibit spontaneous electrical and contractile activities. The tumor cell lines can be passaged repeatedly and still give rise to differentiated, proliferating cultures. The cells cannot be passaged in culture indefinately. However, they can be re-introduced into syngeneic animals, and the resulting tumors once again can be passaged in culture. We have maintained several atrial tumor cell lineages for a period 4 years in this mannor. In the MHC-TAG mice, T-ag expression is observed in both atrial and ventricular cardiomyocytes. Atrial expression results in pathology similar to that observed in the ANF-TAG mice. Ventricular hyperplasia is also observed in this model, indicating that ventricular myocytes are responsive to T-ag. In addition, the MHC-TAG ventricular myocytes produce tumors in syngenic animals. Tumorigenesis in both transgenic models is highly dependent upon genetic background, thereby providing a potential tool with which to identify genes that regulate myocardiocyte proliferation. These models demonstrate that adult myocardiocytes can re-enter the cell cycle and retain a differentiated phenotype.

Molecular Mechanisms of Cardiac Growth and Hypertrophy

H 015 **GROWTH FACTOR CONTROL OF CARDIAC GENE TRANSCRIPTION.** Michael D. Schneider. Departments of Medicine, Cell Biology, and Mol. Physiol. & Biophysics, Baylor College of Medicine, Houston, TX 77030

The selectivity and specificity of gene regulation in cardiac muscle by heparin-binding and transforming growth factors resembles the characteristic program of fetal gene induction during myocardial hypertrophy produced by load. In cultured neonatal rat cardiac myocytes, basic FGF and TGFβ1 provoked up-regulation of genes encoding four embryonic ventricular proteins such as β–myosin heavy chain, skeletal α-actin (SkA), smooth muscle α-actin, and atrial natriuretic factor, in concert with down-regulation of 2 adult ventricular protein genes, α-myosin heavy chain and the sarcoplasmic reticulum Ca^{2+}-ATPase. Despite induction of β–myosin heavy chain, smooth muscle α-actin (SkA), and atrial natriuretic factor similar to that provoked by basic FGF, acidic FGF down-regulated expression of the cardiac and skeletal α-actin genes. This continuum of responses and the disparities elicited by basic versus acidic FGF diverge from both the uniform suppression of striated muscle genes and the corresponding effects of basic and acidic FGF, observed in skeletal muscle cells. Transfection of neonatal rat cardiac myocytes with characterized portions of the chicken α-actin promoters established that both positive and negative control of striated α-actins by growth factors may be predominantly transcriptional, and that less than 200 bp of 5'-flanking DNA sufficed for reciprocal control of SkA transcription by recombinant acidic versus basic FGF. To delineate more precisely DNA elements involved in FGF control of SkA in cardiac muscle, neonatal rat cardiac myocyte cultures were transfected with deletion-gap mutations in SkA promoter elements that contribute to expression in skeletal muscle. Three positive cis-acting elements—an upstream TA-rich region, upstream CCAAT box-associated repeat (CBAR), and, especially, the downstream CBAR (DCBAR)—were crucial to basal expression in both cardiac and skeletal muscle (45, 24, and 9% of wild-type promoter activity, respectively). To ascertain whether the DCBAR is sufficient for up-regulation by one or both FGFs, a 28 bp fragment of the chick SkA promoter (-100/-73), centered on the DCBAR, was positioned upstream from a neutral promoter and transfected into neonatal ventricular cells. Activity in vehicle-treated control cells was ~5% of wild-type SkA promoter activity and >50-fold above background. Recombinant basic FGF augmented activity 4.5-fold (p=0.003). Thus, the DCBAR, which resembles the serum response element of the c-*fos* gene, is sufficient for basal expression in neonatal cardiac cell cultures in the absence of agonist, and for induction by recombinant basic FGF. Although induction of c-*fos* has been postulated to mediate cardiac hypertrophy in vitro and in vivo, the function of fos protein as a transcription factor necessitates formation of a dimer with one of several related *jun* proteins. Basal expression of *jun*B » c-*jun* was detected in adult control rats. Following suprarenal aortic constriction, transient induction of c-*jun* and *jun*B mRNA was observed (maximal at 1 and 2 hr, respectively), returning to basal expression by 4 to 24 hr. In the cultured neonatal ventricular cells, induction of c-*fos* and *jun*B was ascertained by RNA hybridization after stimulation with either acidic FGF or basic FGF, at levels at least equal to those in parallel cultures of cardiac fibroblasts. It remains to be proven whether potential disparities exist in the induction of c-*jun* and, consequently, in the proportion of complexes containing *jun* versus *jun*B. Thus: [1] the c-*jun* and *jun*B proto-oncogenes are induced in myocardium by a hemodynamic load in vivo or FGFs in vitro, providing potential dimerization partners for c-*fos*; [2] the DCBAR suffices for basal expression of SkA in cardiac myocytes and for up-regulation by basic FGF, suggesting a role for the serum response factor in control of this α-actin gene; [3] together, these investigations implicate candidate *cis*-acting sequences and transcription factors for more mechanistic tests of trophic signal transduction in cardiac muscle cells.

H 016 MECHANICAL LOAD INDUCES SPECIFIC GENE EXPRESSION AND CELLULAR HYPERTROPHY IN CARDIAC MYOCYTES, Yoshio Yazaki, Issei Komuro, Masahiko Kurabayashi, Cardiovascular Division, Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

During the process of cardiac hypertrophy, the expression of specific genes, such as protooncogenes and fetal-type genes of contractile proteins was induced as well as an increase in protein synthesis. To examine whether mechanical stimuli directly induce specific gene expression in the heart, we cultured rat neonatal cardiomyocytes in elastic silicone dishes and stretched these adherent cells. Myocyte stretching stimulated expression of the protooncogene, c-fos, in a stretch length-dependent manner, followed by an increase in amino acid incorporation into proteins. The stimulation of c-fos gene expression was recognized by 5% length-stretch of culture dishes and the maximum stimulation was obtained by 20% stretch. This protooncogene expression by stretching was observed abundantly in cardiac myocyte-rich fraction rather than in non-muscle cell-rich fraction. The fetal-type gene, skeletal alpha-actin, was also re-expressed by myocyte stretching. Therefore, these results suggested that stretching cardiac myocytes in vitro could substitute for hemodynamic overload in vivo. c-fos mRNA levels were enhanced within 15 min by stretching, peaked at 30 min, and declined to undetectable levels by 240 min. The level of skeletal alpha-actin mRNA was accumulated 4 hrs after stretching, and gradually increased up to two days during stimulation. The transfected chloramphenicol acetyltransferase gene linked to up-stream sequences of the c-fos gene including its promoter indicated that sequences containing serum response element were required for efficient transcription by stretching. The run-on assay using myocyte nuclei confirmed the enhanced transcriptional activity of the gene. The accumulation of c-fos mRNA by stretching was inhibited by protein kinase C inhibitors, such as H-7 and staurosporin, but not by protein kinase A inhibitors. The pretreatment with phorbol esters also inhibited the stretch-induced expression of the c-fos gene. The pretreatment with epidermal growth factor or forskolin did not reveal any effect on the expression of the gene. Furthermore, an increase in inositol phosphate levels in myocytes was observed within one min after stretching. These results suggest that mechanical stress might directly stimulate protein kinase C activity via phospholipase C activation and thus, might regulate gene expression in cardiac hypertrophy.

Cardiac Myogenesis and Development

H 017 RETROVIRAL ANALYSIS OF CARDIAC LINEAGES AND GENE EXPRESSION, Donald A. Fischman, Takashi Mikawa, Andrei Borisov and Anthony M.C. Brown, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021. A replication-defective retroviral vector (CXL), based on the avian spleen necrosis virus, and expressing β-galactosidase, was used for clonal analysis of the embryonic chicken heart. This virus can be grown to high titers (>10^5cfu), efficiently infects avian cells and contains no helper virus. We report here on the use of this vector to measure doubling times of myocyte clones in the early heart (E2-6 days) and for the 3-D reconstruction of myocyte colonies during heart wall morphogenesis. For the analysis of cell doubling times, embryos were infected with CXL on days 1,2 and 3 of development and the hearts fixed on day 6. After X-gal and Hoechst 33258 staining, the number of cells/lacZ+ colony was tabulated from histological serial sections. Colony size increased exponentially in the first week with a calculated cell doubling time of 15-16 hr. No nonmuscle colonies were observed in the heart after CXL infection of cardiac mesoderm between stages 4-6; only myocytes were lacZ+. By analyzing the ratio of lacZ+/lacZ- cells, we estimate that stage 4 embryos must contain in excess of 10^3 myocyte precursors. Embryos infected with CXL developed normally and hatching efficiency was unimpaired. The mean wet weight of CXL-infected hearts was not significantly less than controls. Myosin expression and cross-striated appearance of the lacZ+ cells in vivo and in vitro was no different from lacZ- cells. It appears that the proliferative and differentiative properties of the CXL-infected cells are not significantly different from uninfected cells. Three types of myocyte colonies were observed in 3-D reconstructions: a) colonies without interrupted intercellular connections, usually along the periphery of ventricular and atrial walls; b) colonies that extend from the ventricular periphery through to the trabeculae, often interspersed with lacZ- cells; c) dispersed colonies in the center of the ventricular wall. Some of the elongated colonies exhibit two orthogonal orientations at different layers of the ventricular wall indicating that a single clone can participate in formation of two or more layers of the wall. Supported by NIH HL37675, The March of Dimes and the Aaron Diamond Foundation.

H 018 REGULATED EXPRESSION OF MUSCLE GENES IN EMBRYONAL CARCINOMA CELLS DEVELOPING INTO CARDIAC MYOCYTES IN CULTURE. Michael W. McBurney and Giovanna Pari, Departments of Medicine and Biology, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.
Cells of the P19 line of embryonal carcinoma can be induced to differentiate in culture. Retinoic acid induces the development of neuroectodermal cell types (neurons and astroglia) while dimethyl sulfoxide treated cells develop primarily into mesodermal and endodermal cell types including cardiac muscle. The cardiac myocytes co-express atrial and ventricular myosin light and heavy chains along with both cardiac and skeletal actin indicating that these cells resemble embryonic cardiocytes. Chimeric genes comprised of the human cardiac actin promoter driving a reporter gene were transfected and stably integrated into undifferential P19 cells and these transformants subsequently induced to differentiate. Expression of the reporter genes was markedly enhanced in cardiac myocytes. Deletion analysis of the cardiac actin promoter indicated that efficient expression in cardiocytes required a number of promoter elements in addition to those previously identified by transient expression assays in cultured skeletal myoblasts. In particular, it appears that all 4 of the CarG elements [CC(ATrich)$_6$GG] in the cardiac actin promoter play important roles in ensuring efficient expression in cardiocytes.

H 019 THE ANF GENE AS A MARKER FOR SIGNAL TRANSDUCTION IN THE HEART, Mona Nemer, Jacques Drouin, Stefania Argentin, Ali Ardati and Stéphane Tremblay, Laboratory of Molecular Genetics, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7

Tissue-specific gene expression plays a key role in growth and differentiation of cardiac muscle cells, as reflected by the expression of specific genes during the different stages of heart development. In addition, within the heart itself, atria and ventricles express specific subset of these genes suggesting that despite their common embryological origin, atrial and ventricular myocytes follow distinct paths of differentiation.

In the past few years, we have used the atrial natriuretic factor (ANF) gene as a model to study signal transduction in the endocrine heart. ANF, a peptide hormone with potent natriuretic, diuretic and vasodilatory - hence hypotensive - properties, is the major secretory product of the heart. The ANF gene is expressed primarily in atrial and ventricular myocytes where it is under differential hormonal and developmental control. In particular, we have found that ventricular expression of the ANF gene correlates positively with growth stimulation and inversely with cardiac differentiation: ANF mRNA and peptides decrease precipitously during the first week of postnatal development in parallel with the decrease in mitotic activity of ventricular cells. However, in the adult ventricle, ANF gene expression is dramatically induced in all conditions of cardiac growth stimulation or cardiac hypertrophy. Thus, the ANF gene appears to be a good marker to study tissue-specific expression during various stage of heart development. In order to define the regulatory elements of the ANF gene, we have fused ANF promoter fragments up to -3.7 kb to the human growth hormone gene and transfected these constructs into primary cardiocyte cultures derived from newborn atria and ventricles. The ANF promoter was functional only in cardiac cells and sequences up to -1.6 kb were required for full activity. Using a combination of mutagenesis and *in vitro* binding of nuclear extracts to ANF upstream fragments, we have identified several promoter elements which contribute to cardiac-specific expression and to modulation during cardiac growth and differentiation. One of these elements, which is present in several copies in functionally relevant promoter regions, binds cardiac-specific nuclear protein(s). The distribution of this protein correlates perfectly with the relative expression of the ANF gene in various cardiac tissues and is higher in newborn versus adult ventricles. These findings suggest that this cardiac protein is itself target for developmental regulation and thus, it may be involved both in tissue-specific and developmental control of ANF and possibly other cardiac genes.

H 020 TROPONIN SWITCHING IN THE DEVELOPING HEART. Stefano Schiaffino, Simonetta Ausoni, Costanza De Nardi, Paolo Moretti, Marina Campione and Luisa Gorza.

Institute of General Pathology and CNR Center for Muscle Biology and Physiopathology, University of Padova, 35100 Padova, Italy.

Developmental changes in the troponin complement of the rat heart were investigated at the protein and mRNA level. Both troponin T (TnT) and troponin I (TnI) undergo isoform switching during cardiac development. One major fetal and two major adult cardiac TnT isoforms were detected by specific monoclonal antibodies. A TnT cDNA clone, isolated from an adult rat heart cDNA library, was found to be identical in the 3'-untranslated and 3'-coding region but divergent in the 5'-coding region from the cardiac TnT cDNA clones described by Jin and Lin (JBC 264, 14471, 1989). The findings suggest that at least three distinct TnT isoforms differing in the COOH-terminal region can be generated by alternative splicing of 5' exons of the cardiac TnT gene. A TnI cDNA clone was also isolated from the same library and its expression was determined by RNA blot analysis. Cardiac TnI mRNA was barely detectable in the 18d fetal heart and accumulated in the neonatal and adult stages. The fetal and neonatal but not the adult heart contained large amounts of slow skeletal TnI transcripts, detected by oligonucleotide probes specific for the 5'- and 3'-untranslated regions of slow skeletal TnI mRNA. The findings are consistent with a developmental switching from the slow skeletal to the cardiac TnI gene in the rat heart. Developmental changes in TnT and TnI composition can be expected to affect Ca^{2+}-sensitivity and response to inotropic stimulation of the developing heart.

H 021 THE EXPRESSION OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS AND THEIR COGNATE RECEPTOR ARE DOWNREGULATED DURING MYOGENIC DIFFERENTIATION. James W. Moore, Craig Dionne, Michael Jaye, Judith L. Swain, Depatments of Medicine and Microbiology, Duke University, Durham, NC 27710, and Dept. of Molecular Biology, Rorer Inc., King of Prussia, PA 19406

Acidic and basic fibroblast growth factors (FGFs) are members of a family of proteins that exert pleiotropic effects in a range of cell types including skeletal myocytes. Previous studies demonstrate that exogenously supplied FGFs stimulate proliferation of myoblasts and inhibit their differentiation in culture, but little information is available concerning endogenous expression of FGFs by skeletal myocytes. We first examined FGF expression in tissues. Acidic and basic FGF mRNAs were found to be expressed in murine and rat skeletal muscle, and expression was demonstrated to vary with the tissue and species examined. Myogenic cell lines were then analysed to determine if FGFs are expressed in myoblasts, and if so, whether expression is regulated during myogenic differentiation. Murine Sol 8 and rat L6 myoblasts were found to express acidic and basic FGF mRNAs, and the expression of both growth factors was downregulated at the transcript level during myogenic differentiation. A decrease in expression of the mouse homologue of the human FGF receptor paralleled the decrease in acidic and basic FGF mRNAs in Sol 8 cells, indicating that the decrease in FGF receptor abundance previously observed during myogenic differentiation is regulated at the mRNA level. The results suggest that a coordinate decrease in endogenously produced acidic and basic FGFs and their cognate receptor may participate in the regulation of myogenic differentiation. Furthermore,the observation that expression of a myogenic determination gene, myogenin, increases as FGF transcripts decline, together with previous data demonstrating suppression of myogenin expression by FGF, suggest a mechanism whereby endogenously produced FGFs may exert their effect on differentiation.

Adaptation to Mechanical Load and Trophic Hormones

H 022 TRANSCRIPTIONAL REGULATION OF THE ANF AND MLC-2 GENES DURING CARDIAC GROWTH AND DEVELOPMENT, K. R. Chien, Department of Medicine and Center for Molecular Genetics, UCSD, La Jolla, CA. 92093

To study transcriptional mechanisms during myocardial growth and development, we have extensively characterized two cardiac genes during α-adrenergic mediated hypertrophy of neonatal rat myocardial cells. In ventricular myocytes, α_1 adrenergic stimulation induces a 10-fold increase in ANF release, and a 15-25-fold increase in ANF mRNA, as assessed by RNase protection studies. A rat cardiac sodium channel gene is not up-regulated, indicating that inducibility is not a property of all cardiac muscle genes. Transient expression studies with constructs containing nested deletions of the ANF and MLC-2 promoter regions fused to a luciferase reporter gene, has identified sequences within their respective 5' flanking regions, sufficient to confer α adrenergic inducible expression to the luciferase reporter. While transient assays in non-muscle cells indicate inducible regulatory sequences in the ANF gene can be segregated from sequences which mediate cardiac specific expression, a similar set of cis regulatory elements mediate inducible and cardiac specific expression of the MLC-2 gene. Mutational analyses and studies with neutral promoter constructs, indicate that a conserved 28bp element (HF-1) in the MLC-2 gene is both necessary and sufficient for cardiac specific expression. Mobility gel shift and competition studies reveal that cardiac nuclear extracts contain HF-1 binding activity which is qualitatively distinct from that in skeletal muscle and CV-1 cells, suggesting that the cardiac factors which recognize HF-1 may be expressed in a tissue specific fashion. Point mutations in HF-1 abolish the ability to confer tissue specificity, as well as binding by cardiac nuclear extracts, further documenting the functional importance of HF-1. Future studies examining the relationship between tissue specific and inducible expression in the ANF and MLC-2 genes should be valuable in delineating the mechanisms which regulate cardiac gene expression.

H 023 MUSCLE-SPECIFIC AND THYROID RESPONSIVE CONTROL ELEMENTS IN THE CARDIAC MYOSIN HEAVY CHAIN GENES. Vijak Mahdavi, W.Reid Thompson and Bernardo Nadal-Ginard, Department of Cardiology Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston MA 02115.

The focus of our research is the elucidation of the molecular mechanisms that regulate the expression of the cardiac α- and β-myosin heavy chain (MHC) genes. Mutations in this gene locus, in human, are responsible for certain forms of familial hypertrophic cardiomyopathy. The cardiac MHC genes are regulated in an antithetic fashion by developmental, hormonal as well as pathological stimuli. In addition, they display tissue-specific interspecies differences in their pattern of expression.

Using a combination of transient and stable transfection assays, we have found that cardiac and skeletal muscle-specific expression of the β-MHC gene, in human and rat, is controlled by two distinct regulatory domains which can function independently. The basal promoter exerts a negative function that resctricts expression of promiscuous enhancers to muscle cells. A 170bp muscle-specific enhancer, within the 5'upstream sequences, constitutes a positive element. Using a series of deletion and mutation constructs to test the functional significance of protein binding sites detected by DNaseI footprinting, we found that the enhancer consists of two interactive subdomains. A 22 bp sequence element in the first positive subdomain is identical to the AP5/GT-II site of the SV40 enhancer is sufficient to confer cardiac and skeletal muscle specificity to heterologous promoters. This element, which is strongly footprinted in muscle and non-muscle nuclear extracts, looses its function upon mutation in the AP5/GT-II site. The second positive subdomain contains SP1 and NFe binding sites and also confers cardiac and skeletal-specific expression to heterologous promoters. Maximal level of expression, however, requires the cooperative interaction of both subdomains. In addition, in contrast to other muscle-specific enhancers, the β-MHC enhancer is not responsive to the myogenic determinant factors MyoD1 and myogenin. These data suggest novel regulatory pathways for genes expressed in cardiac and skeletal muscles.

Thyroid hormone-mediated induction of the α-MHC gene involves the direct interaction of its T_3 responsive element (TRE) with T_3 receptors. We have identified in the 5' upstream sequences of the β-MHC gene an element which binds T_3 receptors with high avidity. Surprinsingly, this element consists of one half of the positive TRE found in the α-MHC gene and, when duplicated, functions as a positive TRE. The role of this element in the T_3-mediated repression of the β-MHC gene is currently investigated.

H 024 REGULATION OF MYOSIN HEAVY CHAIN AND ACTIN ISOGENES EXPRESSION DURING CARDIAC GROWTH AND HYPERTROPHY, Ketty Schwartz, Lucie Carrier, Catherine Chassagne, Claudine Wisnewsky, Jean-Jacques Mercadier and Kenneth R. Boheler, INSERM U 127, Hôpital Lariboisière, 75010 Paris, FRANCE

Changing the working conditions of the cardiac pump results in a mechanogenic transduction that involves not only increased growth, but also a complex pattern of gene reprogramming. The expression of the contractile element genes is modified, and until now, most attention has been focused on myosin heavy chain isogenes. Less is known concerning the isoactins. Our studies were designed to characterize the pattern of expression of sarcomeric isoactins and to determine whether there is a common regulatory pathway between myosin heavy chain and actin genes. For this, we have analyzed by primer extension assays the respective mRNA levels of α-skeletal and α-cardiac actins in human and rat ventricles submitted to several physiological and pathological conditions. Cardiac development was studied in patients with no apparent cardiovascular problems (6 fetal and young children and 11 adults) and in rats at birth to 24 months of age. We found that both actin isogenes are always co-expressed and that the pattern is species specific and changes during development. In man, at 28 weeks *in utero*, α-skeletal actin represent <20% of the total sarcomeric actin, it increases to 30-40% after birth and becomes the predominant isoform of young and adult hearts (61 ± 8%). In rat, α-skeletal actin averages 50% at birth and remains stable during the first two to three weeks. It decreases thereafter to <5% after two months of age and is expressed at approximately the same level in aged animals. Since we recently found that thyroxine hormone up-regulates the α-skeletal actin gene in adult rat heart (Winegrad et al. Proc. Natl. Acad. Sci. USA 1990, 87, 2456) we analyzed hypothyroid development and observed that it does not significantly modify this pattern, at least during the first two weeks of age. The effect of hemodynamic load was studied in hypertrophied hearts from 15 patients with end-stage heart failure: they exhibited the same isoactin pattern as the control ones, and there was no difference between patients with dilated cardiomyopathies (n = 5), ischemic heart disease (n = 5) or with diverse etiologies (n = 5). In adult rats, α-skeletal actin accumulates at the onset of pressure overload hypertrophy (Schwartz et al. Circ. Res. 1986, 59, 551) and we are unable to say if human hearts synthesize an even higher percentage of α-skeletal actin in earlier stages of heart diseases. Comparison of all the above results with those previously reported for α- and β-myosin heavy chains indicate that myosin heavy chain and actin multigene families are both expressed in a species specific fashion but are independently regulated within a species. The level of regulation, transcriptional and/or post-transcriptional will be discussed in view of recent results from run-on assays from isolated rat nuclei.

H 025 REGULATION OF MITOCHONDRIAL GENE EXPRESSION IN SKELETAL AND CARDIAC MUSCLE, R. Sanders Williams[1], Kang Li[1],William E. Kraus[2], and Brian Annex[2]. [1]Molecular Cardiology Research Laboratories, The University of Texas Southwestern Medical Center, Dallas, TX 75235, and [2]Duke University Medical Center, Durham, NC 27710. Hypertrophic growth of the myocardium requires accelerated mitochondrial biogenesis in conjunction with addition of sarcomeres if a balance between energy consumption and energy production by oxidative metabolism is to be maintained. In non-failing myocardium, we have observed that expression of mitochondrial genes is closely coupled to the extent of hypertrophy produced by a variety of stimuli, including thyroid hormome, acute pressure overload, the compensatory response of residual viable myocardium following myocardial infarction, and forced over-expression of the c-myc proto-oncogene in transgenic animals. Failure to augment expression of mitochondrial genes could restrict the extent of hypertrophic growth that can be sustained without decompensation and heart failure, and abnormalities of the mitochondrial genome have been observed in inherited and acquired cardiomyopathies. In contrast, mitochondrial gene expression in skeletal muscles is dissociated from hypertrophic growth during the adaptive responses to different types of work overload. We are seeking to define the molecular signaling pathways that link changes in work load to changes in expression of mitochondrial genes in mammalian striated muscles. Previous studies have revealed that the abundance of mitochondrial DNA (and hence mitochondrial gene dosage), as well as the structure of mitochondrial DNA (formation of a DNA triplex in the D-loop region), are subject to both developmental and physiological regulation. Current studies are focused on the regulation of expression of nuclear genes that encode proteins important for replication and transcription of mitochondrial DNA, and on efforts to establish methods for strategic mutations of mitochondrial DNA within mammalian cells.

H 026 ACTIVATION OF THE α-MYOSIN HEAVY CHAIN GENE BY cAMP IS DEPENDENT UPON PREEXPOSURE OF CARDIOMYOCYTES TO SERUM, Mahesh P. Gupta, Madhu Gupta, Smilja Jakovcic and Radovan Zak*, Department of Medicine, University of Chicago, Chicago, IL 60637
The adrenergic nervous system is known to play an important role in the redistribution of cardiac myosin isoforms. We have used cultured fetal rat heart myocytes to investigate the role of cAMP on the α-myosin heavy chain (MHC) gene expression. The effect of forskolin or 8-Br-cAMP on the expression of MHC mRNA was determined by Northern blot analysis using 20mer oligonucleotide probes, complementary to the unique 3' untranslated sequences of the rat α- or β-MHC mRNA. Treatment of cultured myocardial cells grown in a medium containing 5% calf serum either with 10uM forskolin or 1mM 8-Br-cAMP increased, the abundance of α-MHC mRNA 3 to 4 fold. This effect was dose and time dependent and was further potentiated when the 8-Br-cAMP was given together with 0.5mM 3-isobutyl-1-methyl xanthine, a phosphodiesterase inhibitor. Interestingly, no appreciable change was observed in the level of β-MHC mRNA. Pretreatment of cells with 25mM K^+ which blocks contractile activity of cells did not prevent the increase in α-MHC mRNA caused by cAMP. In nuclear run-on assays, a 70% increase in the transcription rate of the cardiac MHC gene was observed following treatment of cells with 10uM forskolin for 12h. The activation of α-MHC mRNA expression was seen only when cells were plated in medium containing serum. In contrast, when cardiomycytes were plated and grown in defined medium no effect of cAMP on α-MHC mRNA levels was observed. The results demonstrate the preferential induction of cardiac α-MHC gene expression by cAMP and suggest an important role of serum components in transcriptional activation of α-MHC gene. (* Invited Speaker, R. Zak)

Molecular Control of Muscle Gene Expression - I (joint)

H 027 **REGULATION OF ALTERNATIVE SPLICING OF CONTRACTILE PROTEIN GENES.** Bernardo Nadal-Ginard, Christopher W.J. Smith, James G. Patton and Mary Mullen. Howard Hughes Medical Institute. Department of Cardiology, Children's Hospital. Departments of Pediatrics, Cell and Molecular Physiology, Harvard Medical School. Boston, MA. U.S.A.

Alternative pre-mRNA splicing has emerged in recent years as a widespread device for regulating gene expression and generating protein diversity. In addition, it has provided new insights into some fundamental aspects of splicing. This mode of regulation is particularly prevalent in muscle cells, where genes such as Troponin T are able to generate up to 64 different isoforms from a single transcriptional unit. The α-tropomyosin gene has proven particularly useful for the analysis of the mechanisms involved in this type of regulation. Exon 2 and 3 of this gene are a mutually exclusive pair that are sterically inhibited from splicing together by the presence of an abnormally positioned upstream branch point. Exon 3 is incorporated constitutively as the default choice in all tissues except in smooth muscle cells where it is replaced by exon 2.

Analysis of a large variety of constructs containing this region of the α-TM gene in different cell types and in vitro cell-free system demonstrated that the default exon selection is determined through competition between the two exons rather than down-regulation of exon 2. Exon 3 out-competes exon 2. This behavior is determined by the nature of the polypyrimidine tract in front of exon 3 as well as by the sequence of the branch point. Thus, the relative quality of the pyrimidine tracts is the primary determinant of default exon 3 selection, with the relative branch point sequences also playing a role. This role of the pyrimidine tracts is mediated by trans-acting factors. Using U.V. cross-linking a 55 kd protein has been identified, partially sequence and cloned, that binds to the branch point/polypyrimidine tract. The binding of this protein to a large number of different intron sequences demonstrates a perfect correlation between affinity for this factor and splice site strength. These results suggest that the binding of this factor plays an important role in determining splice site selection and, at least in part, determines the default splicing pattern of α-TM.

How is the regulated pattern determined? In vivo expression studies demonstrate that the switch to the regulated selection of exon 2 involves inhibition of the splice site elements of exon 3 and the sequences involved in this down-regulation have been identified.

H 028 **TRANSCRIPTIONAL REGULATION IN EMBRYONIC CARDIAC AND SKELETAL MUSCLE**

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Cardiac and skeletal myocytes transcriptionally activate the gene encoding cardiac troponin T (cTNT) early in embryonic development. During mid-fetal development, the cTNT gene is up-regulated in cardiac cells and repressed in skeletal muscle cells. We have used transfection and DNA-protein binding experiments to elucidate the cis elements and trans factors which govern expression of this gene in these two related cell types. In embryonic skeletal muscle cells cTNT promoter activity is dependent upon both copies of a conserved M-CAT motif (5'-CATTCCT-3') residing between nucleotides -101 and -60 of the "minimal" cTNT promoter (-101 to +38; all numbers relative to transcription initiation site at +1). This minimal cTNT promoter is inactive in embryonic myocardial cells unless an additional 47 nucleotide "cardiac element" is included. The cardiac element resides 200 nucleotides upstream and is capable of conferring activity to the cTNT minimal promoter in cardiac cells in an orientation- and position-independent manner. An AT rich motif within the cardiac element may be important for its activity. Expression in cardiac cells is also dependent upon both M-CAT motifs indicating that activity in cardiac cells requires collaboration between these motifs and the upstream cardiac element. Sequence motifs which appear to be similar to other muscle regulatory motifs such as CArG (serum response factor binding site) or MEF-1 (Myfkin binding site) can be deleted without significant loss of cTNT promoter in either cardiac or skeletal muscle cells. Thus, cell-specific expression of the cTNT promoter in cardiac and skeletal muscle appears not to require direct interaction with any of the known myogenic determination factors. Two novel factors, the M-CAT binding factor (MCBF) and the cardiac element binding factor (CEBF), bind to their respective cis elements in a sequence specific fashion. These results indicate that both common and different cis elements and trans factors are required for expression of the cTNT promoter in embryonic cardiac and skeletal muscle.

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H 029 DIFFERENTIAL REGULATION OF MYOSIN LIGHT CHAIN EXPRESSION BY SPECIFIC MYOGENIC FACTORS. Nadia Rosenthal, Bruce M. Wentworth, James C. Engert and Uta Grieshammer, Department of Biochemistry, Boston University School of Medicine, Boston MA 02118.

Expression of the myosin light chain 1/3 (MLC1/3) gene locus is regulated both developmentally and fiber-specifically during mammalian skeletal myogenesis. The two transcripts encoding MLC1 and MLC3 are activated sequentially from two widely spaced promoters during embryogenesis, and persist in adult fast fibers, whereas transcription of MLC1 is repressed in adult slow fibers. Activation of MLC transcription appears to be dependent on a strong muscle-specific enhancer element, located 3' to the locus in both rats and humans, over 24 kb away from the upstream MLC1 promoter. Analysis of transgenic mice carrying multiple copies of an MLC1 promoter-CAT transcription unit driven by the MLC enhancer has demonstrated the ability of the MLC enhancer to activate gene expression at the appropriate developmental stage, exclusively in the skeletal muscles of these animals. The MLC enhancer includes three CANNTG consensus sites (A, B and C) which bind MyoD, as defined by bacterial fusion protein-DNA interactions in vitro. Specific pairs of the three sites (A and C, or B and C) are required for enhancer function, both in muscle cells and in non-muscle cells cotransfected with a MyoD or myogenin expression vector. The MLC enhancer is less responsive to trans-activation by a myf5 expression vector, and is not trans-activated by a MRF4/myf6 expression vector. Analysis of the interaction of individual sites with muscle and non-muscle nuclear proteins reveals multiple, distinct protein complexes formed with each site. Only two of the sites (A and C) interact with native MyoD or myogenin present in differentiated muscle extracts. This suggests a hierarchy of function among the three sites within the MLC enhancer that involves known myogenic factors, as well as other nuclear proteins yet to be characterized. We are currently investigating whether different protein interactions with the MLC enhancer may influence promoter choice, resulting in the differential transcription of MLC1 and MLC3 during muscle development and in various adult fiber types.

Molecular Genetics of Myocardial Disease

H 030 GENE THERAPY OF CARDIOVASCULAR DISEASE. David A. Dichek. Molecular Hematology Branch, National Heart Lung and Blood Institute, Bethesda, MD 20892.

We are using retroviral vector-mediated gene transfer to develop new therapeutic strategies for the treatment of intravascular thrombosis and hypercholesterolemia. A retroviral vector containing a human t-PA cDNA was constructed and used to transduce adult sheep endothelial cells. Analysis of the conditioned medium of t-PA transduced cells using t-PA antigen and activity assays as well as zymography demonstrated a large increase in fibrinolytic activity (1-2 orders of magnitude) which is clearly attributed to the overexpression of human t-PA protein. In order to begin to test the ability of these fibrinolytically enhanced cells to prevent thrombosis in vivo we developed protocols for seeding the cells onto intravascular stents and vascular grafts and for quantitating cell retention after exposure to physiological flow conditions. In separate experiments a retroviral vector containing a human LDL receptor cDNA was constructed and used to transduce primary skin fibroblasts from Watanabe Heritable Hyperlipidemic rabbits (an LDL receptor deficient strain). Correction of the rabbits' genetic defect was confirmed by in vitro assays including immunoprecipitation and LDL binding. Autogenous gene corrected cells reimplanted into donor rabbits survived for at least 4 weeks in vivo, as determined by both immunohistochemistry and recovery of LDL-binding cells from the implants. The potential of these two approaches for the treatment of thrombosis and hypercholesterolemia will be discussed.

H 031 MOLECULAR GENETICS OF MYOTONIC DYSTROPHY. M. Benjamin Perryman[1], Linda Bachinski[4], M. Dolores Cortez[1], Jacqueline Dubel[2], Patrick W. Dunne[2], Pu Liu[4], Shang-Wu Wung[2], Tetsuo Ashizawa[2], A. Craig Chinault[3], J. Fielding Hejtmancik[5], Michael J. Siciliano[4], Henry F. Epstein[2], Departments of Medicine[1] and Neurology[2], and the Institute of Molecular Genetics[3], Baylor College of Medicine, LMOD/NEI, National Institutes of Health[5], Bethesda, MD, and Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center[4], Houston, Texas, One Baylor Plaza, Rm. 506C, Houston, TX 77030.

Myotonic muscular dystrophy (DM) is the most common form of dystrophy producing cardiac as well as skeletal muscle dysfunction. Data from the several laboratories of the MDA Working Group have established a consensus order of identified genes and anonymous markers in the 19q 13.2-13.3 region surrounding the DM locus to be: 19qcen--ApoC2-MCK-ERCC1-DM-D19S51-D19S50-D19S22-19qter. We have devised several experimental approaches to isolate expressed sequences from this region in order to produce polymorphic DNA probes, isolate yeast artificial chromosomes (YACs) containing them, and identify potential candidate genes for DM. A cDNA library was prepared from the human-hamster cell line 20XP3542-1-4 containing the DM region. cDNA synthesis was primed with consensus 5' intron splice sequences and the resulting hncDNA was screened with human-specific repetitive DNA sequences. Human cDNA clones shown to be distal to MCK by hybridization to a broken human chromosome 19-rodent hybrid cell lines were chosen for further analysis. One such clone, 1A5 was sequenced, and oligonucleotide PCR primers region were synthesized. These primers with a 40 bp GC rich clamp at the 5' end identify a two-allele polymorphism in human genomic DNA by denaturing gradient gel electrophoresis. The same oligonucleotide primers without the GC clamp were used to screen a human genomic YAC library by polymerase chain reaction (PCR). A clone containing a 370 kb YAC hybridizing to both 1A5 and MCK was isolated. Human Alu repeat sequence-vector arm PCR has produced a 200 bp and a 150 bp product from the right and left end of the clone, respectively. Once orientation of the YAC clone has been established a walk will be initiated in the direction of the telomere using PCR primers derived from the Alu-vector arm PCR products. Other PCR primers for D19S51 which flanks the DM locus distally are being used to isolate additional YAC clones. To identify human cosmid clones which contain candidate genes, a human placental library was screened with a chromosome 19q-specific minisatellite sequence. Eighty-eight cosmids and 120 cosmids containing human DNA inserts derived from the 20XP3542-1-4 line were blotted onto nylon membranes and probed with repetitive deficient cDNA prepared from human tissues affected in DM, adult and fetal skeletal muscle, cardiac Purkinje fibers, and lens epithelium. Five cosmids gave a strong hybridization signal with three or more of the cDNA probes while two hybrid cosmids are strongly positive with two of the cDNAs. In addition, at least seven cosmids show hybridization at the level of lower abundance cDNAs (dystrophin and spectrin) with all four cDNA probes. Supported by MDA (H.F.E., J.F.H., M.J.S.) and VA (T.A.) grants and Bugher Foundation (D.M.C.) and MDA (P.W.D.) postdoctoral fellowships.

H 032 MUTATIONS IN CARDIAC MYOSIN HEAVY CHAIN GENES CAUSE FAMILIAL HYPERTROPHIC CARDIOMYOPATHY, Gary Tanigawa, Anja Geisterfer-Lowrance, Susan Kass, Scott D. Solomon, John A. Jarcho, Hans-Peter Vosberg*, William McKenna**, J. G. Seidman and Christine E. Seidman, Departments of Medicine and Genetics, Harvard Medical School, Boston, MA 02115; *Max-Planck Institut for Medical Research, Heidelberg, Germany; **St George's Medical School, London, U.K.

Familial hypertrophic cardiomyopathy is an autosomal dominant disorder that is characterized by unexplained myocardial hypertrophy, a wide spectrum of clinical symptoms, and early mortality. We have demonstrated that a gene responsible for this disorder is on chromosome 14 and is closely linked to the cardiac myosin heavy chain (MHC) genes in two unrelated families. Studies have recently identified two independent mutations in the α and β cardiac (MHC) genes in these kindreds. In one family an unequal cross-over event has produced a novel α/β MHC fusion gene in all affected individuals (1). In the second family, a missense mutation in the β cardiac MHC gene converts a highly conserved arginine residue at amino acid 403 to a glutamine residue (2). Collectively these data demonstrate that myosin gene mutations cause FHC in some families. Analyses of additional families indicate that FHC is a genetically heterogeneous disorder (3). In these kindreds the FHC locus is not linked to chromosome 14 band q1. Further studies should provide new clues to important structure-function relationships of the myosin molecule and provide insights into potential candidate genes that may also cause FHC.

1. Tanigawa et al, Cell 62:991-998, 1990.
2. Geisterfer-Lowrance et al, Cell 62:999-1006, 1990.
3. Solomon et al, J. Clin. Invest. 86:993-999, 1990.

Thrombosis and Thrombolysis

H 033 MODULATORS OF THROMBOSIS IN ACUTE CORONARY SYNDROMES AND CORONARY DISEASE PROGRESSION,Valentin Fuster, M.D.,Ph.D. Mount Sinai Medical Center,NY,NY 10029. Angiography in patients with unstable angina or myocardial infarction with subtotal coronary occlusion often reveals eccentric stenoses with irregular borders suggesting ruptured atherosclerotic plaques and thrombosis, as documented by angioscopy and at autopsy. In addition, rapid changes in the angiographic patterns preceeding and following the acute coronary syndromes suggest rapid dynamic processes. We have studied these processes in an 'ex vivo' perfusion chamber, in an 'in vivo' swine model, and in humans. Our results, combined with those of others, suggest that local and systemic risk factors at the time of plaque disruption favor the degree of thrombogenicity,and so the different clinical syndromes. That is, a limited amount of risk factors may only lead to a small thrombus with subsequent fibrotic organization, and so the growing of an atherosclerotic plaque; a moderate amount of risk factors may lead to an occlusive but labile thrombus, and so to unstable angina and non Q-wave infarction; large amount of risk factors may lead to an occlusive and fixed thrombus, and so to Q-wave myocardial infarction.

Such local and systemic risk factors that contribute thrombogenecity following plaque rupture are: 1)Rheological Factors - a) The more severe the stenotic lesion after plaque rupture the higher the local shear rate, which enhances platelet deposition and thrombus formation. b) Platelet deposition and thrombosis are particularly favored if the site of rupture includes the apex of the stenotic plaque with its high shear rate, while the stasis in the post-stenotic region favors propagation of thrombus with a major fibrin component. 2)Degree of Plaque Damage - Plaque rupture produces a rough surface and exposes collagen type I and tissue factor; these stimulate occlusive thrombus which is either fixed or labile depending on the degree of damage or amount of collagen type I and tissue factor exposed. 3)Residual Thrombus - After spontaneous or pharmacological reperfusion, surface of the residual thrombus is very thrombogenic and may contribute to reocclusion; this is because thrombin generated during the original clotting and which remained bound to fibrinogen-fibrin, after reperfusion is again exposed to the circulatory blood. 4)Systemic Factors Enhancing Thrombogenecity - There is clinical and experimental evidence that three systemic factors at the time of plaque disruption may enhance thrombogenecity:a)the level of serum epinephrine (i.e., in stress, smoking, early AM),b)the level of serum cholesterol and c)impaired fibrinolysis resulting from high serum lipoprotein(a) and high levels of plasminogen activator inhibitor.

Thus,in coronary disease local and systemic risk factors are being identified as modulators of thrombosis at the time of coronary plaque disruption.The amount of risk factors explain the various degrees of thrombogenicity and so the various coronary syndromes.

H 034 LOCALIZATION OF THE GENE FOR FAMILIAL HYPERTROPHIC CARDIOMYOPATHY TO CHROMOSOME 14q1 IN A DIVERSE AMERICAN POPULATION. J. Fielding Hejtmancik, Paul A. Brink, Jeffrey Towbin, Rita Hill, Lucy Brink, Grazyna Z. Czernuszewicz, Terry Tapscott, Anatole Trakhtenbroit, M. Benjamin Perryman, Robert Roberts, Section of Cardiology, Baylor College of Medicine, Houston, Texas, 77030.

Familiar hypertrophic cardiomyopathy, is an inherited autosomal dominant disease which is markedly varied in its clinical manifestations. It is estimated that about 70% to 80% of the patients do not have significant clinical symptoms. Furthermore, the ventricular hypertrophy as determined on echocardiography may not be detectable until they are of adult age. Familiar hypertrophic cardiomyopathy is the leading cause of death in the young. Attempts to isolate the gene responsible for this disorder has been difficult in part due to the varied phenotypic expression of this disease. Previous studies utilizing serum markers indicated that in a Japanese family the gene for hypertrophic cardiomyopathy was linked to the HLA region. Studies performed in North America and in Europe have failed to show any linkage of the disease to the HLA region. In our initial studies with serum markers we confirmed conclusively that the gene responsible for HCM in our families was not linked to the HLA region. Recent application of restriction fragment length polymorphism (RFLP) markers has provided provocative results with localization to chromosome 18 (Japan), 16 (Italy), 14 (French/Canadian) and most recently 2 (NIH) suggesting genetic heterogeneity and possible racial influence. Interpretation remains speculative until one or more of these loci is confirmed in unrelated pedigrees by independent investigators. Thus, we studied 9 unrelated families composed of varied ethnic origin across the USA. There were a total of 174 individuals with 58 affected. The diagnosis of HCM was based on the presence of ventricular hypertrophy without obvious cause as detected by echocardiography. DNA, from each individual, was digested with restriction enzymes Taq1 or BamH1 and analyzed by Southern blots followed by hybridization with probes TCRA, MYHβ, D14S25, and D14S26. Multipoint linkage analysis showed a maximum lod score of 5.6 occurring 8 cM from D14S25 between D14S25 and TCRA. This corresponds to an odds ratio of 398,000 to 1. The 95% confidence limits extend from 1 cM beyond D14S25 to 22 cM beyond D14S25 (2 cM from TCRA). While recombinants occurred with each marker in at least one family, there is no statistically significant evidence favoring genetic heterogeneity among the families but it cannot be definitively excluded. Thus, the probability of linkage to 14q1 is less than 99%. This suggests that the gene causing FHCM in much of the American population is localized within this region. It is known that myosin heavy chain β, a candidate gene for FHCM, is also within the region of chromosome 14q1. In 2 families, obligate recombinants were noted for the MYHβ marker. These are being investigated for linkage to loci on other chromosomes as suggested for FHCM by others.

H 035 *Engineering of the contact sites of tissue plasminogen activator and its serpin PAI-1*

Joseph F. Sambrook, Rhonda Bassel-Duby, Robert Gerard, Mary-Jane Gething, Betsy Goldsmith and Edward Madison. Department of Biochemistry, University of Texas Southwestern Medical School, Dallas USA 75235-9038.

The surfaces of the catalytic domains of serine proteases that activate zymogens are decorated with several loops of amino acids, each four to nine residues in length. These loops are absent from more generic enzymes such as trypsin and chymotrypsin, which are not especially selective in their choice of protein substrates. We have used a combination of molecular modelling and site-directed substrates. We have used a combination of molecular modelling and site-directed mutagenesis to investigate whether these loops are involved in the formation of specific contacts between serine proteases and their protein substrates and inhibitors. The serine protease domain of tissue-type plasminogen activator (t-PA) interacts with two other proteins -plasminogen (its substrate) and plasminogen activator inhibitor 1, a member of the serpin superfamily. One of the five loops of amino acids projecting from the protease domain of t-PA ($_{296}$KHRRSPGER$_{304}$) maps near the mouth of the active site of the enzyme. The homologous region of trypsin is known to interact with the Kazal-type inhibitor BPTI *(Huber et al, J. Mol. Biol. 89, 73-101, [1974]).* To investigate role of this loop in substrate recognition and formation of inhibitory complexes, we have used site-directed mutagenesis to generate variants of t-PA and either lack this loop entirely or carry a variety of amino acid substitutions. None of these changes has any significant effect on the rate of catalysis of t-PA or on the affinity of the enzyme for its natural substrate, plasminogen. However, alteration or removal of the loop drastically reduces the rate of interaction between t-PA and a number of serpins, including PAI-1. The most extreme phenotype is shown by a mutant in which three positively-charged amino acids (Lys_{296},Arg_{298} and Arg_{299}) are replaced with glutamic acid residues. The rate of association of this triple mutant with PAI-1 is slowed by a factor of at least 10^4 and its catalytic activity is completely resistant to inhibition by the serpin. Based on these and other results, we propose that 296-304 loop is not involved in catalysis but plays a crucial role in the formation of inhibitory complexes between t-PA and PAI-1. The region of PAI-1 that is proposed to interact with the 296-304 loop of t-PA contains several acidic residues (E_{350}, E_{351} and D_{355}). We have therefore explored the possibility that a mutant PAI-1 containing a compensating change could efficiently inhibit the "serpin-resistant" mutants of t-PA. Interestingly, substitution of E_{350} or E_{351} with Arg generates serpins that can efficiently interact with and inhibit the enzymatic activity of both wild-type and "serpin-resistant" t-PAs.

Late Abstracts

Commitment and Differentiation of Avian Cardiac Progenitor Cells. David Bader, Michael Montgomery, Judith Litvin, Joseph Bisaha, David Goldhammer and Charles Emerson Jr. Cornell University Medical College, NYC, N.Y.

Identification of genes regulating cardiac differentiation and the activation of cardiac-specific gene expression is the key to understanding cardiac development. Early in vertebrate development, mesodermal cells become committed to the cardiogenic lineage. Soon thereafter, these committed precursors begin to differentiate into cardiac myocytes. Our data demonstrate, in molecular terms, that cardiac progenitor cells become committed just prior to gastrulation (stages 3-4) and terminally differentiate between stages 7-15. Immunofluorescence with MF-20 (an antibody specific to sarcomeric myosin heavy chain, MHC) and reverse transcription-polymerase chain reaction (RT-PCR) using oligos to a cardiac-specific MHC demonstrate the earliest **potential** for cardiac myosin expression occurs at stage 3 and that the earliest expression in vivo of a cardiac-specific myosin is at stage 7.

The Helix-Loop-Helix (HLH) gene family has been shown to be critical for the differentiation of several cell-lineages and for regulation of cell-specific gene expression. We present data suggesting the presence of two genes related to this family in the developing heart:
1) The twist gene, first identified in Drosophila and later in Xenopus, is required for mesodermal differentiation. RT-PCR with gene-specific primers using RNA from stage 4 lateral-plate mesoderm or one week post-hatch chick hearts amplified a product that hybridized at low stringency with Drosophila twist cDNA by Southern blot. This protein conserved across species and present during gastrulation (and apparently much later) may be as critical for commitment and differentiation processes in the vertebrates as it is in Drosophila; 2) Using an antiserum against the conserved second helix of the myogenic HLH, we provide immunochemical evidence that an HLH protein(s) (called H2) is specifically expressed during the period when committed cardiac progenitors are converted to differentiated myocytes (stages 7-15). Western blot and immunoprecipitation analyses demonstrate the presence of a 50kD protein which is specifically expressed at this time and is reactive with this antibody. Electrophoretic mobility shift assays (EMSA) show the stage-specific expression of a DNA binding protein(s) interactive with muscle-specific enhancer sequences and that this DNA/protein complex specifically reacts with the anti-second helix antibody. Our goal is to clone these genes and analyze their functions at the molecular level during cardiogenesis. Taken together, these studies should provide an understanding of the cellular and molecular basis of cardiac myogenesis.

SIGNAL TRANSDUCTION BY PDGF RECEPTORS INVOLVES DIMERIZATION AND ASSOCIATION WITH SIGNALING MOLECULES, L.T. Williams, J.A. Escobedo, V.A. Fried, H. Ueno, and W.M. Kavanaugh Howard Hughes Medical Institute and Cardiovascular Research Institute, University of California San Francisco, CA 94143 and Department of Cell Biology and Anatomy, New York University, Valhalla, New York 10595

To study the role of receptor dimerization in signal transduction we have used mutants of the PDGF β receptor (PDGFR) that bind PDGF but lack intrinsic kinase activity. When co-expressed with wild type receptors, these mutants formed heterodimers with wild type PDGFR in a PDGF-dependent manner. A heterodimer consisting of wild type PDGFR and a PDGFR with a truncated tyrosine kinase domain was defective in autophosphorylation, whereas the ligand-induced homodimer of wild type receptor was autophosphorylated. When co-expressed with wild type receptors in Xenopus oocytes, the mutant PDGFR abolished signal transduction by wild type PDGFR but did not affect signal transduction by wild type FGF receptors (FGFR). A similar mutant of the FGFR blocked signal transduction by wild type FGFR but not by PDGFR. These findings show that receptor dimerization is required for PDGF receptor signal transduction, suggest that autophosphorylation is intermolecular reaction between the components of a receptor dimer and establish a unique approach to inhibit receptor function in vivo.

Autophosphorylated PDGFR associates with several cellular molecules and phosphorylates some of them on tyrosines. We have recently focused on an 85 kDa protein (P85) that associates with ligand-activated PDGFR and is one of the major tyrosine-phosphorylated proteins in PDGF-stimulated cells. We purified P85 using tyrosine-phosphorylated PDGFR as an affinity reagent and cloned the cDNA that encodes P85. P85 contains two SH2 domains but no identifiable kinase motifs. Thus P85, like GTPase activating protein, may associate with PDGF receptor through its SH2 domains. We showed that P85 binds to a 16 amino acid segment of PDGFR the that contains phosphotyrosine at position 719. This segment of PDGFR also binds phosphatidylinositol 3 kinase (PI3 kinase), an enzyme that has been implicated in the regulation of growth of both normal and transformed cells. P85 expressed in cos cells competes with PI3 kinase and 110 kDa protein for binding to the receptor. Thus P85 is either a subunit of PI3 kinase or an antagonist that competes with PI3 kinase for binding to the receptor. Thus signaling seems to involve ligand-induced receptor dimer formation, intermolecular autophosphorylation of receptors, and the binding of SH2 domain-containing proteins to autophosphorylation sites on the receptor. The SH2 domains appear to recognize phosphotyrosine in a specific sequence context.

Molecular Mechanisms of Cardiac Growth and Hypertrophy

Cardiac Development: Differentiation

H 100 THE DEVELOPMENTAL CHANGES IN THE EXPRESSION OF RABBIT CARDIAC TROPONIN T ISOFORMS AND THE FORCE-pCa RELATIONSHIP ARE RELATED, Page A.W. Anderson, Rashid Nassar, Nadia N. Malouf*, Michael B. Kelly and Annette E. Oakeley, Departments of Pediatrics and Cell Biology, Duke University, Durham, NC 27710, Department of Pathology*, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514

The cardiac troponin T (TnT) isoforms are expressed in a developmentally and regionally regulated manner. This heterogeneity has not been shown previously to affect the biophysical properties of the myocardium. In this study we take advantage of the finding that in rabbit myocardium the neonatal period is associated with changes in the expression of four TnT isoforms, named TnT 1 through 4 based on electrophoretic mobilities. Twelve ventricular strands from rabbits 2-5 days old were skinned with Triton-X. The force-pCa relationship was measured at sarcomere lengths 2.0-2.4 μm. The pCa_{50} and the Hill constant were determined by a least squares fit of the Hill equation. The proteins of each strand were subjected to SDS-PAGE, and the Western blot probed with a cardiac specific monoclonal antibody, MAb13-11. The amounts of the TnT isoforms relative to total TnT were determined by densitometric scanning of the Western blots (TnT 1, 8.7$\pm$2.8%;TnT 2, 40.7$\pm$7.1%;TnT 3, 3.9$\pm$1.5%;TnT 4, 46.7$\pm$8.8%). The pCa_{50} was 5.85$\pm$.17 (mean $\pm$ SD), the Hill constant 2.6$\pm$.4 at a sarcomere length of 2.28$\pm$.15 μm. Linear regression of pCa_{50} against %TnT 2 gave a slope of 0.17 ($p<0.013$, $r=.7$). These data demonstrate a relationship between the cardiac TnT isoforms and the sensitivity of the myofilaments to calcium. We are isolating rabbit cardiac TnT cDNA with the intent of determining the structural differences among TnT isoforms, in order to test for their functional effects.

H 101 REGULATION OF SARCOPLASMIC RETICULUM GENE EXPRESSION DURING CARDIAC DEVELOPMENT AND THYROID STRESS. Masashi Arai, Kinya Otsu*, David H MacLennan*, Norman R Alpert, and Muthu Periasamy. Dept of Physiology & Biophysics, Univ of Vermont, VT 05405, *C H Best Institute, 112 College St, Univ of Toronto, Ontario, Canada M5G 1L6.

To understand mechanisms regulating the expression of sarcoplasmic reticulum proteins, we quantitated the expression level of mRNAs for ryanodine receptor (calcium release channel), sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA), phospholamban, and calsequestrin during cardiac development and in hyper- and hypothyroidic heart and skeletal muscle of rabbit. During development, cardiac muscle expressed exclusively the cardiac isoform of ryanodine receptor, cardiac/slow-twitch Ca^{2+}-ATPase (SERCA2), and cardiac calsequestrin. Their relative expression level continuously increased during progression from the late fetal to adult heart. In contrast, skeletal muscle expressed both fast-twitch and cardiac/slow-twitch isoforms of Ca^{2+}-ATPase and calsequestrin in fetal and neonatal stages. The cardiac/slow-twitch isoforms were repressed in the adult fast-twitch skeletal muscle. However, skeletal muscle expressed exclusively the skeletal isoform of ryanodine receptor at all stages of development.

Changes in thyroid hormone level significantly affected the expression of Ca^{2+} regulatory proteins of sarcoplasmic reticulum. Both the cardiac ryanodine receptor and Ca^{2+}-ATPase mRNA were up-regulated in hyperthyroidic hearts and down-regulated in hypothyroidism. Phospholamban mRNA decreased both in hyper- and hypothyroidism. Calsequestrin mRNA level did not change much in hyper- and hypothyroidic hearts. The expression of these genes in skeletal muscle was similarly affected by thyroid hormone. These data suggest that the expression of sarcoplasmic reticulum proteins are regulated at the pretranslational level.

In conclusion, our studies indicate in general that the expression of sarcoplasmic reticulum proteins are regulated both qualitatively and quantitatively in development and under physiological stress.

H 102 **EXPRESSION OF THE HOMEOBOX GENE HOX-7 IN THE EMBRYONIC HEART**

Paul J. R. Barton, [1] Benoit Robert, [2] Robert Thompson, and Magdi Yacoub, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY U.K., [1] Institut Pasteur, Paris France, and [2] Medical University of South Carolina, Charleston, SC USA.

The homeobox gene Hox-7 has been shown to be expressed in a number of tissues during early embryogenesis in vertebrates. In particular, expression is prominent in the developing limb bud, in the branchial arches and in the endocardial cushion tissue of the heart (Robert *et al.,* EMBO J **8**:91 1989). We have further investigated expression in the heart by *in situ* hybridization of Hox-7 specific probes on sections of chick embryo. During early development the atrioventricular (AV) cushions are formed by cells which delaminate from the endothelium in the region of the AV canal. These cells invade the extracellular matrix (cardiac jelly) which separates the endothelial and myocardial cell layers, where they multiply and form the mesenchyme cell population of the developing AV cushions. It is generally agreed that this marks the onset of events leading to septation and to the formation of the AV valves. Hybridization of sections of embryonic chick heart show that Hox-7 is expressed both in the proliferating mesenchyme cell population, and in the overlying endothelial cells. The timing and distribution of Hox-7 expression is consistent with it playing a specific role in cushion formation, and thereby with the onset of cardiac septation and valve formation.

H 103 DIFFERENT GENE ACTIVATION MODE FOR ATRIAL NATRIURETIC FACTOR *versus* STRUCTURAL HEART MUSCLE GENES. P.H. Boer, M.L. de Bold and A.J. de Bold.
University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Ontario, K1Y 4E9, CANADA.

P19 embryonal carcinoma cells were used as a tissue culture model for cardiac myogenesis. Striated muscle structure, spontaneous beating activity and positive immunofluorescence with MF20 myosin heavy chain antibody appeared after 6 days in culture. Stable RNA was isolated throughout the differentiation program and Northern blots were hybridized with actin and ANF gene specific probes. Rapid cardiac α-actin gene induction occurred from day 6, reaching maximum mRNA levels at day 8-9. In contrast, ANF gene transcripts - while undetectable in undifferentiated cells - accumulated to very low levels detectable with PCR techniques. Because ANF transcripts accumulated in P19 cells transfected with a cDNA expression plasmid, lack of mRNA may be due to lack of ANF promoter induction. This was verified by nuclear run-on assays during the time course of differentiation. Thus, in differentiated P19 cells with transcriptionally activated sarcomeric protein genes we have not found a high rate of ANF gene transcription as may be found at certain stages in heart development. Therefore, it appears that high level expression of the ANF gene in the P19 cell requires the intervention of yet undefined factors. Supported by the Medical Research Council of Canada and the Ontario Heart and Stroke Foundation.

H 104 **CLONING OF cDNAs ENCODING CARDIAC TRANSCRIPTION FACTORS BY POLYMERASE CHAIN REACTION.** Nigel J. Brand, Nina Dabhade, Magdi Yacoub and Paul Barton, National Heart and Lung Institute, Dovehouse St, London SW3 6LY, U.K.

Many genes encoding proteins of the contractile apparatus of striated muscle have been cloned and characterised, yet relatively little is known about the transcription factors regulating their expression during development or in response to external stimulii such as workload. This is especially true of cardiac muscle. We aim to isolate cDNA clones encoding transcription factors that may be implicated in cardiac gene regulation by using the polymerase chain reaction (PCR) in conjunction with sets of degenerate oligonucleotides specific for conserved domains found in known transcription factors, including helix-loop-helix (H-L-H), zinc-finger, POU-domain and homeobox-containing families. Initial experiments have concentrated on the search for cardiac equivalents of the myogenic determination factor family (typified by MyoD1) recently identified in developing skeletal muscle, using oligonucleotide sets specific for the H-L-H domain together with a variety of RNA sources, including adult and foetal heart. To date, no such sequences have been detected in cardiac RNA. This finding is intriguing as some genes, such as atrial myosin light chain and creatine kinase, are expressed in both skeletal and cardiac muscle, and it has been demonstrated that their promoters contain DNA sequences to which MyoD1 binds. Parallel studies are aimed at identifying potential POU-like sequences associated with cardiac development and induced hypertrophy in the adult.

H 105 CARDIAC SPECIFIC GENE EXPRESSION IN MURINE EMBRYONIC HEART, Daniel J. Dumont, Alexandra L. Joyner and Martin L. Breitman,
Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, CANADA.
We set out to understand early cardiac development by using PCR technology in an attempt to clone candidate genes which are expressed specifically in the developing murine heart. RNA samples extracted from heart isolated at 9.5 and 12.5 days of embryonic development were used as template for RT-PCR (reverse transcription linked to the polymerase chain reaction). The degenerate oligonucleotides used in these studies were specific for two gene families. (1) Tyrosine kinases- Tyrosine kinases are known to play a major role in controlling cellular proliferation and differentiation. We have cloned several previously characterized tyrosine kinases and several novel tyrosine kinases that are expressed specifically in early heart. (2) Activins- The second family of genes cloned belong to the TGF-beta super-family. Activins are the most potent inducers of embryonic mesoderm described to date and thus may play an important role in controlling heart development. We have been successful in cloning activin B from 10 day old embryonic heart. In situ RNA hybridization analysis will be carried-out to determine the expression patterns of some of the identified genes.

H 106 CARDIAC MYOCYTE CELL LINE FORMATION. Gary L. Engelmann[a], Maria C. Birchenall-Roberts[b], Francis W. Ruscetti[b], and Allen M. Samarel[a]. [a]Loyola University, Department of Medicine, Maywood, IL, 60153 and [b]NCI, Laboratory of Molecular Immunoregulation, Frederick, MD, 21701-1013

Ventricular myocyte development in the mammalian fetus is characterized by rapid, near-terminal proliferative events and initiation of cellular maturation. The results are such that the neonatal heart contains a finite number of post-mitotic cardiomyocytes adaptive to hypertrophic growth stimuli. Analysis of the growth and maturation of the embryonic and fetal cardiomyocyte has been limited due to the lack of a suitable cell line which reflects the cardiac muscle lineage. To that end, primary cultures of 16-day gestation fetal cardiomyocytes were infected with v-*myc* or v-H-*ras* containing retroviruses, which resulted in the formation of three separate cardiomyocyte cell lines (CLEM-1102A, BWEM-1102C, and ELEM-658HH). The CLEM and BWEM cell lines were from separate v-*myc* infected cultures; whereas the ELEM cell line was from a v-H-*ras* infection. The initial morphological, biochemical and molecular characterization of the cell lines is to be presented. The cell lines express the following cardiac-related genes to varying degrees and with levels at or near those found in fetal or neonatal cardiomyocyte primary cultures: Troponin C, α-Cardiac Actin, Egr-1, ß-MHC, and ANF. Each cell line is MyoD negative. Their morphology reflects an immature state of myocyte development. All three lines proliferate (CLEM & BWEM with minimal serum) with >20 passages attained, and metabolic/immunofluorescent labeling supports their cardiac lineage. For the first time, probable cell lines of cardiomyocytes have been generated and will facilitate the analysis of this unique cell type of the cardiovascular system.

H 107 DEVELOPMENTAL CHANGES IN FORCE AND CALCIUM SENSITIVITY OF SKINNED TRABECULAE FROM CHICKEN HEART, R.T.H. Fogaça, T.M. Nosek and R.E. Godt, Department of Physiology & Endocrinology, Medical College of Georgia, Augusta GA 30912.

Small trabeculae were dissected from left ventricles of chickens from embryonic day 7 to adult (51 days post-hatching). Trabeculae were tied to a force transducer and chemically skinned with Triton X-100 detergent. At pH 7.0 and 22° C, maximal calcium-activated force (Fmax), normalized to cross-sectional area, was 3-5 kPa from embryonic days 7-17, and increased four-fold up to the time of hatching (day 21). Fmax subsequently decreased to 6.5 kPa in the adult. As in rabbit heart (McAuliffe et al., *Circ. Res. 66*: 1206, 1990), calcium sensitivity decreased with developmental age, consistent with the switching of troponin T isoforms from embryonic to adult (Sabry & Dhoot, *J. Mol. Cell. Cardiol. 21*: 85, 1989). Reduction of solution pH to 6.5 decreased both Fmax and calcium sensitivity to a greater exent in the adult than at embryonic day 7. A similar effect of pH on calcium sensitivity with development has been observed in rat heart and has been attributed to switching of troponin I isoforms (Solaro et al., *Circ. Res. 63*: 779, 1988). Our data are consistent with this possibility since, in the chick ventricle, the embryonic isoform of troponin I predominates at embryonic day 7, while only the adult form remains in the adult ventricle (Sabry & Dhoot, *J. Musc. Res. Cell Motil. 10*: 85, 1989). Thus developmental changes in calcium activation are similar in mammalian and avian myocardium. (Support: NIH grants AR 31636, HL/AR 37022, and HL 36059).

H 108 CARDIAC BUT NOT SKELETAL ACTIN IS EXPRESSED IN HUMAN FETAL HEART MUSCLE CELLS IN VIVO AND IN VITRO, Bruce Goldman and John Wurzel, Department of Pathology, Temple University Medical School, Philadelphia, PA, 19140

Re-expression of fetal genes is typical of many models of cardiac hypertrophy. One such gene is the skeletal isoform of sarcomeric actin (α-skeletal actin, SA), which is normally expressed in growing (fetal and neonatal) rat hearts. To study the developmental regulation of actin isoform expression in humans, we performed Northern analyses on RNA extracted from first and second trimester human fetal hearts, from cultured human fetal ventricular muscle cells, and from adult cardiomyopathic ventricles. Cultured cells were studied in both high-mitogen and serum-free media. Hybridization with a ^{32}P-labeled isoform-specific cDNA probe demonstrated SA mRNA only in the adult samples; α-cardiac actin and cytoskeletal actins were clearly expressed in all extracts. Culture in medium containing 20% fetal bovine serum did not induce SA expression in ventricular cells, but a switch to serum-free medium at the same passage level increased the relative expression of the cardiac isoform. SA is undetectable in the prenatal human heart, and the developmental regulation of actin expression in the human heart may be fundamentally different from that in other mammals.

H 109 SHARED CONTRACTILE PROTEIN ISOFORMS DURING RAT HEART AND HINDLIMB DEVELOPMENT : ARE THESE "ASSEMBLY ISOFORMS"? Edna C. Hardeman, Colin J. Sutherland, and Monica L. Gordon, Muscle Development Unit, Children's Medical Research Foundation, P.O. Box 61, Camperdown N.S.W. 2050, Australia.

We have examined the level of mRNAs of a number of contractile protein isoform genes during rat heart and hindlimb develoment. A pattern is emerging from our data in which the same isoform for each contractile protein gene family is expressed during an early developmental window in both striated muscles. For example, $MLC2_{fast}$, the predominant isoform in fast twitch skeletal muscle, and TnI_{slow}, the predominant isoform in slow twitch skeletal muscle, are both expressed in developing rat heart from embryonic day 10 (E10). At this time each is the major isoform from their respective gene families. We show that these are also the major isoforms from their respective gene families in early rat skeletal muscle development and in cultured skeletal muscle cells. It thus appears that developing skeletal and cardiac muscle have a common developmental programme of isoform gene expression. We propose that the particular isoforms which comprise this shared early programme have properties which favour *de novo* sarcomere assembly in both types of striated muscle and as such we have termed them "assembly isoforms". The appearance of the mature contractile protein phenotype which emerges later in development in both striated muscles may do so in response to specific functional requirements.

H 110 MC29-immortalized clonal avian heart cell lines.

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Our group recently demonstrated that the myelocytomatosis virus, MC29, specifically induces cardiac rhabdomyosarcomas in avian embryos provided that the virus is inoculated before embryonic day 4 (1). Cells in the tumor display muscle-specific antigenic markers but no myofibrillar organization. We established quail clonal heart muscle cell lines from these cardiac rhabdomyosarcomas (2). The clones were characterized by means of antibodies 13F4 and MF20, which are markers of striated muscle cells. These muscle antigens progressively disappeared with time in culture. The cardiac clones were maintained *in vitro* for more than 60 generations (6 months) without morphological changes. Cardiomyocytic differentiation could be reinduced in culture, by associating the avian cardiac cells with 3T3 cells in a defined synthetic medium. Muscle markers were then reexpressed in all cardiac cells as soon as day one after coculture although multiplication of cardiac cells continued unabated. This is distinctive of cardiac clones since MC 29-infected quail skeletal myoblasts or quail fibroblasts exhibited a split response to 3T3 association, i.e. decreased growth and enhanced differentiation. The cardiac clones were maintained *in vitro* for more than 60 generations (6 months) without morphological changes. To our knowledge, this is the first description of clonal embryonic avian heart cell lines.

(1) Saule *et al.* (1987) *Proc. Natl. Acad. Sci. USA.* **84**, 7982-7986.
(2) Jaffredo *et al.*. MC29-immortalized clonal avian heart cell lines can partially differentiate *in vitro*. Exp. Cell Res. in press.

H 111 NEGATIVE AND POSITIVE ELEMENTS INVOLVED IN NONMUSCLE SPECIFIC GENE EXPRESSION.

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Vimentin is a member of the intermediate filament family normally expressed in cells of mesenchymal origin and is down regulated during myogenesis. Known cis-acting factors which contribute to vimentin's gene expression are: 1) GC box proximal promoter elements (+1bp to -161bp) which bind Sp1, 2) the proximal enhancer element (-161bp to -321bp), and 3) the vimentin silencer element (-568bp to -608bp) which binds the silencer protein (95kDa). Previously, we have shown that the DNA binding activity of the silencer protein increases during myogenesis [Farrell et al., MCB 10:2349-2358, 1990]. In this report, we have identified a 75bp enhancer region upstream of the silencer element which enhances transcriptional activity. This distal enhancer element (DEE) overrides the silencing effect of the negative element in functional CAT assays in nonmuscle cells. As determined by gel mobility shift assays, DEE binds a specific protein (DEEP), which has an apparent size of 140 kDa as determined by Southwestern blots. DNase I protection experiments localize DEEP's binding to a 29bp region. The protein distribution and activity of DEEP is opposite to that of the silencer protein. DEEP decreases as vimentin expression decreases while the silencer protein increases during myogenesis. Nuclear extracts from 3 day old chicken cardiac tissues display low DEEP binding activity and increased silencer protein binding activity. Therefore, the DEE binds a 140 kDa protein which overrides the silencer protein in nonmuscle cells and contributes to the down regulation of the vimentin gene during myogenesis.

H 112 RESCUE OF ABERRANT MYOFIBRILLOGENESIS IN CULTURED HEARTS OF CARDIAC MUTANT SALAMANDERS BY EXOGENOUS RNA, Larry F. Lemanski, Margaret E. Fransen and Sherrie M. LaFrance, Department of Anatomy and Cell Biology, SUNY Health Science Center at Syracuse, NY 13210.

In the mexican axolotl (salamander), Ambystoma mexicanum, embryos homozygous for the cardiac lethal gene c form hearts that lack normal sarcomeres in the ventricle and atrium. Although mutant hearts contain normal amounts of actin, myosin, troponin and alpha-actinin, they are deficient in sarcomeric tropomyosin. Previous studies revealed that mutant hearts can be rescued by in vitro culture in the presence of normal anterior endoderm (a presumed inducer of heart differentiation) or medium conditioned by normal anterior endoderm; rescue was characterized by the formation of normal sarcomeres and the onset of myocardial contraction. Further analysis indicated that the rescuing activity in conditioned medium was caused by RNA and that RNA extracted from normal anterior endoderm (but not embryonic liver or neural tube) was also active. Subsequent experiments have demonstrated that in addition to normal anterior endoderm RNA, RNA from juvenile axolotl heart and adult sheep heart also correct the mutant defect, resulting in larger accumulations of tropomyosin. Present studies analyze the dose response of mutant hearts to the exogenously applied RNAs: positive results have been obtained using concentrations between 1-400 ng/microliter. In addition to characterizing the size class of the active RNA, oligo d(T) fractionations of the total RNAs are in progress to determine whether the active fractions contain poly (A)+ sequences. These studies will enable us to determine whether the molecular mechanism of rescue of mutant hearts is the same for endoderm-derived and heart-derived RNAs. (Supported by NIH Grants HL-32184, HL-37702 and an AHA Grant-in-Aid to LFL.)

H 113 EXPRESSION OF SARCOPLASMIC RETICULUM Ca^{2+}ATPase AND CALSEQUESTRIN GENES IN RAT HEART DURING ONTOGENIC DEVELOPMENT AND AGING. Anne-Marie Lompré*, Francine Lambert* and Ketty Schwartz+,*INSERM U275, ENSTA-Ecole Polytechnique, 91120- Palaiseau- FRANCE. +INSERM U127, Hopital Lariboisière, 75010 Paris- FRANCE.

Expression of sarcoplasmic reticulum Ca^{2+}ATPase and calsequestrin genes was studied in hearts of rats of different ages. We show that, unlike what has been described for fast skeletal muscle, alternative splicing of the Ca^{2+}ATPase gene transcript expressed in heart is not developmentally regulated. A single calsequestrin mRNA isoform is also detected in heart. The relative amount of Ca^{2+}ATPase and calsequestrin mRNA was quantitated by dot blot analysis and normalized to 18S ribosomal RNA and to a myocyte specific mRNA: myosin heavy chain (MHC). The concentration of Ca^{2+}ATPase mRNA increases 10 times at the end of fetal life, is stable during adulthood and decreases by 56% in 24 months old rats. The concentration of calsequestrin mRNA vary only slightly during development indicating that the genes coding for two SR proteins evolve independently. In the fetal-neonatal period, the increase in Ca^{2+}ATPase mRNA concentration is parallel to the increase in total MHC mRNA suggesting a common regulatory mechanism while the decrease observed in senescent rat is independent of any quantitative change in MHC mRNA.

H 114 STIMULATION OF AXOLOTL CARDIAC DEVELOPMENT BY DEFINED GROWTH FACTORS IN VITRO, Anthony J. Muslin and Lewis T. Williams, HHMI, CVRI, University of California, San Francisco, 94143.

Cardiac tissue is formed by the differentiation of mesoderm into cardiac myocytes which eventually lose their capacity to proliferate. Previous studies have demonstrated that the large embryos of various amphibian species could be used to examine heart formation from mesoderm in vitro and that cardiac development requires an inductive interaction between endoderm and mesoderm. We have used the axolotl (salamander) to study cardiac induction in vitro and the role of growth factors in this process. Explants of presumptive cardiac mesoderm from neurula-stage embryos were isolated and placed in hanging drop cultures. Early neurula (stage 14) mesodermal explants rarely formed beating heart tissue in culture. However, when human platelet-derived growth factor or transforming growth factor beta1 was added to mesodermal explants, beating tissue formation occured much more frequently. Addition of retinoic acid, insulin, or basic fibroblast growth factor to explants did not enhance beating tissue formation. This system provides an approach to the identification of genes that regulate growth and differentiation in cardiac tissues.

H 115 EVIDENCE REGARDING THE PRESENCE OF A MYO D-LIKE TRANSCRIPTIONAL ACTIVATOR IN PRIMARY RAT CARDIOCYTES.

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The transfected human cardiac actin (HCA) gene achieves tissue-specific expression in the skeletal myogenic C2 cell line upon interaction of its minimal promoter with at least three transcriptional regulators. Two of them, Sp-1 and SRF or SRF-like factors are ubiquitously represented while MyoD confines its expression only to skeletal muscle tissue and myogenic cell lines. Point mutations in the HCA DNA binding sites for Sp-1, SRF and MyoD result in silencing the promoter transfected in C2 cell line. The HCA promoter-CAT chimeric constructs are transcriptionally active when introduced in primary cardiocytes where neither MyoD transcripts nor a cognate protein have been detected. To ascertain whether the promoter is differently *regulated in skeletal myogenic and myocardial environments* the HCA mutants for Sp-1,SRF and MyoD binding sites were introduced in primary rat cardiocytes and failed to be activated. These results suggest that the same regulatory elements utilized for the skeletal myogenic expression are involved in the cardiac activation of the HCA promoter and that a regulatory factor present in cardiocytes activates transcription via a MyoD binding site located in the HCA promoter.

H 116 THE ROLE OF TROPONIN C IN THE RESPONSE OF CARDIAC MYOFILAMENTS TO DEACTIVATION BY INCREASED PROTON AND PHOSPHATE CONCENTRATIONS

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We tested the hypothesis that the myofilament deactivating effects of acidic pH and elevated inorganic phosphate, conditions associated with depressed cardiac function, are due to depressions in the amount of Ca-bound to troponin C (TNC). Our approach involved nearly complete exchange of skeletal (s) for cardiac (c) and cardiac for skeletal TNC in myofibrils from heart and fast skeletal muscle. Acidic pH induced a bigger shift in pCa50 (half maximal Ca-concentration) in cardiac than in skeletal myofibrils. Yet these shifts were nearly the same in both c-myofibrils and s-myofibrils when the native TNC was exchanged with either cTNC or sTNC. Moreover, soleus myofibrils that contain cTNC in the native state had much less of a shift in pCa50 than cardiac myofibrils. To test whether these differences are due to TNI, the inhibitory TN subunit, we measured Ca-bound to cTNC and sTNC using fluorescent probes as a reporters of bound Ca. Acidic pH had a bigger effect on Ca-binding to cTNC than sTNC, and in both cases, the effect was even bigger when measured in the TNI-TNC complex. These results indicate that the effect of acidic pH on myofilament activation, depends in part on thin filament proteins other than TNC, most likely TNI. Effects of elevated inorganic phosphate from 1 to 20 mM, a change known to alter the pCa-force relation, had no influence on Ca-binding by cardiac myofibrils. This result indicates that altered cross-bridge kinetics and binding may influence the pCa-activation relation independent of TNC.

H 117 INHIBITOR OF DNA-BINDING (Id) mRNA IN CARDIAC MUSCLE: EVIDENCE FOR A HELIX-LOOP-HELIX NUCLEAR PROTEIN IN DIFFERENTIATED CARDIAC CELLS.

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Id (Inhibitor of DNA-binding) is a recently described "helix-loop-helix" (HLH) protein that regulates heterodimer formation with other HLH proteins, blocks their ability to bind to DNA, and subsequently acts as a transcriptional regulator. Other functions of Id have yet to be described. Id expression has been previously shown to be highest in proliferating cells and diminished in differentiated cells. We have analyzed Northern blots prepared from a variety of adult rat tissues and have determined that the murine Id cDNA probe identifies a doublet band of 1.1 kb and 1.25 kb in heart. A single transcript of 1.25 kb is present in testis, brain, and kidney. Negligible levels of Id mRNA are detected in liver, intestine, and skeletal muscle. Heart had the highest level of hybridization intensity of all samples tested. When heart tissue is dissociated into nonmuscle and muscle cells, the majority of the 1.1 kb hybridization signal partitions to the nonmuscle sample while the cardiac muscle sample retains the 1.25 kb message as well as some of the 1.1 kb message. Adult ventricular cardiac muscle cell cultures (14 days) were also examined for the presence of Id mRNA, and found to contain stronger hybridization levels than the adult freshly dissociated muscle sample. The discovery of Id mRNA in differentiated ventricular cardiac muscle suggests a new avenue of investigation in the search for transcriptional regulators in cardiac tissue.

H 118 ISOLATION AND CHARACTERIZATION OF CHICKEN VENTRICULAR MYOSIN HEAVY CHAIN cDNA: A NEW MEMBER OF THE MYOSIN MULTIGENE FAMILY.

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The myosin heavy chain (MHC) is a major structural component of the contractile apparatus and is present in tissue specific isoforms encoded by a multigene family. One of the earliest forms of MHC found in developing and regenerating skeletal muscle is immunologically indistinguishable from the MHC expressed in the ventricle of adult chickens. To evaluate whether they are the same, we first characterized the adult ventricular MHC. By screening a chicken heart cDNA expression library with monoclonal antibodies specific to MHC we have isolated overlapping clones encompassing 3.2 kb that encode the S2 and LMM region of the MHC. Northern blot analysis of RNA probed with a 1.6 kb cDNA insert displayed a specific 6.5 kb band in the ventricle, weak signal in the atrium and no detectable signal in either fast-twitch or slow-tonic muscles. However, RNA from fetal skeletal muscle and from muscle regenerating after cold injury revealed a band of identical mobility. Amino acid sequence comparison to known cardiac isoforms of MHC indicates at most 77% similarity. Comparison to known sarcomeric MHC sequences using the Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1990) reveals the chicken ventricular MHC to be a newly identified, distinct member of the myosin gene family. Recently, we have isolated cDNA clones from a regenerating skeletal muscle cDNA library and preliminary sequence analysis shows 96% homology to that of the ventricular cDNA. If indeed the ventricular and ventricular-like MHC are the product of the same or closely related genes, characterization of a gene expressed in both cardiac and skeletal muscle during development offers the ability to define the factors responsible for tissue specificity.

Supported by NIH grant HL-20592.

H 119 X-LINKED CARDIOMYOPATHY: MOLECULAR GENETIC EVIDENCE OF LINKAGE TO THE DUCHENNE MUSCULAR DYSTROPHY LOCUS. Jeffrey A. Towbin, Paul Brink, Bruce Gelb, Xue Min Zhu, Jeffrey S. Chamberlain, Edward R.B. McCabe, Michael Swift. Baylor College of Medicine, Dept. of Pediatrics and Institute for Molecular Genetics, Houston, Texas; University of North Carolina, Dept. of Genetics, Chapel Hill.

X-linked dilated cardiomyopathy (XLCM) is a primary myocardial disease which presents with early-onset and rapid progression in males, occasional late onset in females, and absence of skeletal myopathy. The purpose of this study was to evaluate a family with XLCM using molecular genetic techniques to localize the XLCM gene locus. A linkage study using X chromosome DNA markers was initiated in a previously reported (Berko & Swift, 1987) 5-generation XLCM pedigree (63 members, 11 affected, 5 manifesting carriers). The pedigree was analyzed for potential strength using computer-generated maximal lod scores and showed appropriate linkage power. Fifty X-linked polymorphic probes spanning the X-chromosome were analyzed and demonstrated linkage of XLCM to the 5′ region of the Duchenne muscular dystrophy (DMD) locus at Xp21.2. Using the probe XJ1.1 (DXS206) a lod score of +2.77 at θ=0 was obtained. Supportive data for linkage was also provided by the proximal DMD cDNAs and remaining XJ series probes which also had lod scores greater than +2.0 at θ=0. No deletions have been found. Western blotting using antibody to the proximal dystrophin protein against human cardiac tissue demonstrates no protein expression in XLCM. We conclude that XLCM is due to abnormality of the 5′ dystrophin gene, either a promoter defect, point mutation, or alternative splicing. This is the first demonstration of a dystrophin abnormality in patients with cardiomyopathy and no skeletal muscle disease.

H 120 **CLONING AND DEVELOPMENTAL EXPRESSION OF HUMAN CARDIAC TROPONIN I.** William J. Vallins, Nigel J. Brand, Nina Dabhade, [1]Nigel Spurr, Magdi Yacoub and Paul J. R. Barton. National Heart and Lung Institute, Dovehouse Street, London SW3 6LY U.K. and [1]I.C.R.F. South Mimms, Herts. EN6 3LD U.K.

We have used the polymerase chain reaction (PCR) with degenerate oligonucleotide sets complementary to regions conserved between troponin protein isoforms, in order to clone a cDNA for human cardiac Troponin I (TnI). PCR amplification from human ventricular muscle RNA resulted in a cDNA fragment of predicted size and which was shown to encode cardiac TnI by DNA sequencing. Full length cDNAs were subsequently isolated from a human ventricular muscle cDNA library using the PCR-derived fragment as probe. From these the complete amino acid sequence of human cardiac TnI has been deduced. This shows a high degree of homology with other TnI isoforms, and has the expected N-terminal extension with characteristic sites for phosphorylation. Genomic analysis shows that cardiac TnI is encoded by a single copy gene located on human chromomsome 19. Analysis of its developmental expression in the human heart shows that cardiac TnI forms a major transcript as early as 20 weeks gestation. This contasts with the situation in rat and chick where there is a developmental switch from expression of slow skeletal type TnI to cardiac TnI late in cardiac development, and implies that if there is a similar switch in man, it occurs before 20 weeks gestation.

H 121 TISSUE-SPECIFIC DIFFERENTIAL EXPRESSION OF MULTIPLE SPECTRIN ISOFORMS IN FETAL AND ADULT HUMAN CARDIAC AND SKELETAL MUSCLE, Tomas Vybiral, Douglas L. Casey, P. Deitiker, P.W. Dunne, Robert Roberts, Henry F. Epstein, Departments of Neurology and Medicine, Section of Cardiology, Baylor College of Medicine, Houston, TX, 77030

Spectrins are membrane-associated actin-binding proteins, which have been associated with receptors and ion channels in erythrocytes and brain. We report the characterization of human fetal and adult cardiac and skeletal muscle by Western blots of 4.5% SDS-PAGE of biopsy samples with erythroid- and brain-specific anti-spectrin antibodies. Fetal and adult septal and ventricular cardiac muscles exhibit five erythroid spectrin bands. Their apparent molecular masses, are 284, 274, 270, 255 and 240 kD. Also, two brain-reactive spectrin bands, 284 and 274 kD, are detected in heart. In contrast, fetal and adult deltoid and vastus lateralis skeletal muscle exhibits an alternate isoform pattern: Of four erythroid bands (284, 274, 270, 240 kD) detected in fetal tissue, only the 284, 270 and 240 kD isoforms persist into adulthood. One prominent brain-reactive (284 kD) fetal isoform dramatically decreases in reactivity in adult tissue. In order to further understand the mechanism and potential implications of this developmental and tissue-specific differential expression, three cardiac cDNA libraries were screened with spectrin cDNA probes and 13 cardiac spectrin cDNA-clones have been isolated and characterized. In conclusion, human fetal and adult cardiac and skeletal muscles express multiple distinct spectrin isoforms. This differential expression may explain the specific functional and morphological differences between myocardial and skeletal muscle membrane cytoskeletons. This work was supported by NHLBI/SCOR Heart Failure Grant to H.F.E. and R.R. and an AHA/Bugher Foundation Fellowship to T.V.

Molecular Mechanisms of Cardiac Growth and Hypertrophy

Growth Factors: Ion Channels

H 200 ANGIOTENSIN II-MEDIATED CARDIAC HYPERTROPHY IN ADULT RATS, Kenneth M. Baker, David E. Dostal, Mitchell I. Chernin, Andrea L. Wealand, Kathleen M. Conrad, Weis Center for Research, Geisinger Clinic, Danville, PA 17822, Bucknell University, Lewisburg, PA 17837

Angiotensin II (AII) has been reported to have direct hypertrophic actions in mammalian and avian myocardium. In this study we determined if AII had receptor-mediated effects on stimulating cardiac hypertrophy independent of mechanical hypertrophic stimuli (increased cardiac afterload). Angiotensin II (1.8 μg/h) was infused into Sprague-Dawley rats for 7 and 14 days. Following 7 and 14 days of AII infusion, there were significant increases of 19 and 21%, respectively, in left ventricular mass as indexed by left ventricular/body weight ratios. The AII-induced increases in left ventricular mass were blocked by the nonpeptidic AII receptor antagonist Dup 753, and unaffected by the angiotensin converting enzyme inhibitor enalapril maleate or the vasodilator, hydralazine. The induction of left ventricular cardiac hypertrophy by AII was independent of AII-induced increases in vascular resistance, in that, animals receiving the vasodilator, hydralazine, had normotensive mean arterial pressures but still developed significant left ventricular hypertrophy in response to AII infusion. Angiotensinogen mRNA activity was decreased in the left ventricle following 7 days of AII infusion. These data suggest that AII has a direct, receptor (AT_1)-mediated hypertrophic effect on rat heart that is independent of cardiac afterload. Angiotensin II appears to have a negative feedback on inhibiting production of angiotensinogen mRNA in the left ventricle.

H 201 **PRESENCE OF STRETCH-ACTIVATED CHANNELS IN FRESHLY ISOLATED ADULT RAT HEART CELLS.** **J. O. Bustamante, M. L. de Bold, P.H. Boer and A. J. de Bold.** University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Ontario, K1Y 4E9, CANADA

Acute changes in the rate of release of atrial natriuretic factor (ANF) follow mechanical stretch of atrial muscle. The events recognized by atrial cardiocytes during stretch, and leading to the increase of ANF release, are not known. To determine whether ion fluxes could be related to this process, we looked for the presence of stretch-activated channels (SACs) in freshly isolated adult rat cardiocytes. Experimental results obtained from cell-attached and excised (inside-out) patches, demonstrated the existence of cation-selective SACs in atrial and ventricular cells. Cell-attached patches in medium M199, showed SACs with reversal potential ~-40 mV indicating that: 1) K^+ is about 5 times more permeable than Na^+, and 2) at normal resting potentials (~-75 mV) Na^+ would move inwardly upon membrane stretch, despite the lower permeability to Na^+ as compared to K^+. When Na^+ in the pipette solution is isotonically replaced with Ca^{2+}, no SAC activity was observed. The SACs were completely blocked by 100 μM extracellular Gd^{3+} and 50 μM intracellular diltiazem but not by 100 μM extracellular TTX. Under conditions that "dissect" ionic currents, two major classes of SACs were found: one that is selective for K^+ and another that is selective for Na^+. The single channel conductance of the SACs did not depend on the membrane voltage but there was a threshold of -60 mV for the Na^+-selective SACs which suggests a relationship of these channels with the normally voltage-dependent Na^+ channels (VDNaCs). Additional experiments designed to monitor the effects of stretch on VDNaCs showed that stretch causes the disappearance of VDNaC activity and appearance of Na^+-selective SACs, raising the question of whether the latter type of channels are VDNaCs modified by stretch. Supported by the New Brunswick and Ontario Heart and Stroke Foundations and the Medical Research Council of Canada.

H 202 BASIC FIBROBLAST GROWTH FACTOR IN THE DEVELOPING AND OVERLOADED HEART. Peter Cummins, Anne Logan*, Metin Avkiran** and Bernadette Cummins, Departments of Physiology and *Clinical Chemistry, University of Birmingham, Birmingham, B15 2TT, UK, and **Heart Research Unit, St Thomas's Hospital, London, UK.

Basic fibroblast growth factor (bFGF) has been implicated in cardiac growth and myocyte proliferation. Atrial and ventricular heart chambers may differ in their ability to undergo hyperplasia both during normal development and as a consequence of pressure overload in the neonate. The levels of bFGF in developing heart chambers are still a matter of debate. A radioimmunoassay and enzyme linked immunoassay have been used to determine bFGF in bovine atria and ventricles of hearts from 11 weeks gestation to term (40 weeks) for comparison with adult values. Fetal bFGF levels in both chambers (range 300-700 ng/g) were consistently higher than in the adult (range 150-350 ng/g). Levels in fetal atria were also higher than in fetal ventricles but this appeared to be reversed in the adult. Results using both assays which employed different bFGF antibodies raised against recombinant whole bFGF or a synthetic N-terminal peptide gave consistently higher values in the adult than previously published. Cardiac hypertrophy induced by pressure overload in neonatal rats (age range 7-45 days post partum) is being investigated to determine the role of bFGF, acidic FGF and transforming growth factor β in cardiac enlargement and whether they influence the transition from hyperplasia to hypertrophy of existing cells. Both immunoassay and immunocytochemistry are being used to measure levels and to localise areas of growth factor production.

H 203 TRANSFORMING GROWTH FACTOR BETA INHIBITS EXPRESSION OF CARDIAC TNI IN CULTURED CARDIOMYOCYTES. [1]Lynda J. Dieckman, [2]Anne M. Murphy and [3]Gary L. Engelmann. [1,3]Dept. of Medicine, Loyola Univ. Medical Ctr., Maywood, IL 60153. [2]Dept. of Pediatrics, Washington Univ. Medical Ctr, St. Louis, MO 61330.

Recent evidence suggests that transforming growth factor beta (TGF-B) can regulate growth and maturation of many cell types. While TGF-B peptides and gene transcripts have been reported from neonatal to adult hearts, a specific role for TGF-B in heart growth and maturation remains to be determined. Therefore, the purpose of the following studies has been to test the direct effect of TGF-B on cardiac myocyte maturation. One index of cardiomyocyte maturation is an increase in expression of the "adult" form of cardiac TNI (cTNI) and a decrease in expression of the "perinatal" form slow skeletal TNI (ssTNI). We have used primary cultures of neonatal rat cardiomyocytes untreated or treated with 1 ng/ml TGF-B. Using ^{32}P labelled cDNA probes which react specifically against either ssTNI or cTNI, we conducted Northern/slot blot analysis of total RNA of cultured cardiomyocytes. While there was no inhibition of expression of cTNI transcripts at 6 hours, expression of cTNI in myocytes treated with TGF-B for 24, 48 or 72 hours, was decreased 32.9%, 48.7% and 52.2%, respectively (n=2). Unlike cTNI, transcripts for ssTNI were not reduced by TGF-B treatment. To estimate the amount of myofibrillar protein per total cellular protein, samples were taken prior to and after myofibrillar purification, and TGF-B treated cells (72 hr) displayed slightly lower myofibrillar protein/total protein ratio (0.21) than controls (0.27) (n=1). Finally electron microscopy indicated that there are no major ultrastructural differences between TGF-B treated and control cells. Our results indicate that TGF-B influences developmental changes in contractile protein isoforms of cardiac myocytes.

H 204 DIFFERENTIAL EFFECTS OF TRANSFORMING GROWTH FACTOR-BETA AND PHORBOL MYRISTATE ACETATE ON CARDIAC FIBROBLASTS: REGULATION OF FIBRILLAR COLLAGEN mRNAs AND EXPRESSION OF IMMEDIATE EARLY GROWTH RESPONSE GENES, Mahboubeh Eghbali, Ronald Tomek, Cheryl Woods and Brijesh Bhambi, Michael Reese Hospital, Cardiovascular Institute, University of Chicago Pritzker School of Medicine, Chicago, IL 60616

In this study, we have investigated the effect of transforming growth factor-beta (TGF-β_1) on collagen gene expression in cultured cardiac fibroblasts and have compared this effect to that of mitogenic agent, phorbol myristate acetate (PMA). The regulation of collagen types I and III gene expression was examined by using cDNA probes to rat α_2 (I) and mouse α_1 (III) procollagens. Quiescent cultured cardiac fibroblasts from rabbit heart were treated with TGF-β_1 (10-15 ng/ml) and PMA (200 ng/ml). After 24 hours of treatment with TGF-β_1, the abundance of mRNA for pro α_2 (I) and pro α_1 (III) collagens were increased by 112% ($p<0.001$) and 97% ($p=0.05$) respectively, in treated fibroblasts compared to those in untreated cells. PMA-treated cells showed an opposite response in that, a 42% ($p=0.01$) decrease in mRNA levels for pro α_2 (I) collagen was observed. The abundance of mRNA for pro α_1 (III) collagen was not affected by PMA. Cycloheximide reversed increased collagen gene expression by TGF-β_1, whereas, inhibitory effect of PMA on collagen type I mRNA did not change with cycloheximide. Treatment with PMA led to the induction of mRNAs for early growth response gene (Egr-1) and protooncogenes c-fos and c-jun, in cardiac fibroblasts. Using Egr-1 antibody and immunofluorescent light microscopy, this protein was localized to the fibroblasts nuclei after 5 hours of treatment with PMA. Expression of these immediate early response genes was not induced in cardiac fibroblasts following TGF-β_1 treatment. The results of this study suggest that TGF-β_1 in the myocardium may play an important role in regulation of collagen biosynthesis and hence myocardial fibrosis. These findings may further imply that increase in collagen biosynthesis in the heart, may be independent of fibroblasts response to immediate growth enhancing events such as induction of transcriptional regulator Egr-1 and proto-oncogens c-fos and c-jun that accompany cardiac myocyte hypertrophy.

H 205 ADULT RAT CARDIOMYOCYTES IN CULTURE DISPLAY MITOCHONDRIA WITH PARACRISTALLINE INCLUSIONS, HIGHLY ENRICHED FOR CREATINE KINASE.Monika Eppenberger, Inka Riesinger, Marius Messerli, Patrick Schwarb, Jean Claude Perriard, Theo Wallimann and Hans M. Eppenberger, Dept. Cell Biology, ETH, 8093 Zürich, Switzerland.

In adult regenerating cardiomyocytes in culture, in contrast to fetal cells, mitochondrial creatine kinase (Mi-CK) was expressed. In the same cell two populations of mitochondria, differing in shape, in distribution within the cell and in content of Mi-CK could be distinguished. Immunofluorescence studies using antibodies against Mi-CK revealed a characteristic staining pattern for the two types of mitochondria: Giant, mostly cylindrically shaped, and, as shown by confocal laser light microscopy, randomly distributed mitochondria exhibited a strong signal for Mi-CK, whereas small, "normal" mitochondria, localized in rows between myofibrils, gave a much weaker signal. Transmission electron microscopy of the giant mitochondria demonstrated paracrystalline inclusions located between cristae membranes (intracristal inclusions). Immunogold labeling with anti-Mi-CK antibodies revealed a specific decoration of these inclusions for Mi-CK. It was assumed, that the appearance of the paracrystalline inclusions, accompanied by the accumulation of Mi-CK as a major component, represented a pathological, stress-induced situation, similar to the one observed in certain myopathies. However, addition of 20 mM creatine, the substrate of Mi-CK, to the essentially creatine-free culture medium caused the disappearance of the giant cylindrically shaped mitochondria as well as of the paracrystalline inclusions, accompanied by an increase of the intracellular level of creatine. Replacement of creatine in the medium by the creatine analogue and competitor ß-guanidinopropionic acid caused the reappearance of the enlarged mitochondria. It is believed and will be discussed here, that the concentration of Mi-CK at the inclusions of giant mitochondria represents a compensatory effect of the cardiomyocytes to cope with a metabolic stress situation caused by low intracellular creatine levels.

H 206 HUMAN CONNEXIN43: STRUCTURE-FUNCTION ANALYSIS USING SITE-DIRECTED MUTAGENESIS AND EUKARYOTIC TRANSFECTION. Glenn I. Fishman[1], David C. Spray[2] and Leslie A. Leinwand[3], Departments of Medicine[1], Neurosciences[2] and Microbiology and Immunology[3], Albert Einstein College of Medicine, Bronx, NY 10461. Members of the connexin (Cx) multigene family encode proteins which assemble into gap junction (GJ) channels. To identify the molecular basis for GJ channel behavior in the human (H) heart, we used site-directed mutagenesis to generate mutant cDNAs of H-Cx43 and expressed the encoded proteins by stable transfection in the communication deficient H-hepatoma cell line, SKHep1. Previously, we reported that cell lines expressing wild-type H-Cx43 display two distinct unitary conductance values of about 60 pS and 95 pS. Truncation mutants with either 80 or 138 amino acids removed from the carboxy-terminus alter the unitary conductance value to about 170 pS or 50 pS, respectively, but do not change the modest voltage dependence. (Fishman et al, 1990 *J Cell Bio* 111: 589-598; Moreno et al, 1990, *Soc Neurosci Abs*; Fishman et al, *submitted*). These results suggested that the cytoplasmic tail was an important determinant of the unitary conductance value, but did not comprise the putative voltage sensor. To test the hypothesis that residues in the amphipathic third transmembrane domain participate in forming the aqueous channel pore, cell lines were generated harboring expression plasmids encoding proteins with single amino acid substitutions in this region, specifically ^{161}Phe to Gly and ^{158}Ser to Asp, Lys or Phe. For each of the four constructs, cell lines were identified which expressed immunoreactive protein at levels similar to those expressing wild type H-Cx43. Furthermore, like the wild-type protein, all four mutants appeared to be post-translationally modified by phosphorylation. However, unlike transfectants expressing wild-type H-Cx43 or either of the truncation mutants, none of these cell lines were capable of Lucifer Yellow dye transfer. These results suggest that these mutations in the third transmembrane domain disrupt either membrane insertion, channel assembly, or more likely, ion permeation through the aqueous channel. Current studies are directed toward distinguishing among these possibilities through electrophysiological analysis of these cell lines.

H 207 **ACTIVATION OF THE α-MYOSIN HEAVY CHAIN GENE BY cAMP IS DEPENDENT UPON PREEXPOSURE OF CARDIOMYOCYTES TO SERUM,** **Mahesh P. Gupta, Madhu Gupta, Smilja Jakovcic and Radovan Zak*, Department of Medicine, University of Chicago, Chicago, IL 60637**

The adrenergic nervous system is known to play an important role in the redistribution of cardiac myosin isoforms. We have used cultured fetal rat heart myocytes to investigate the role of cAMP on the α-myosin heavy chain (MHC) gene expression. The effect of forskolin or 8-Br-cAMP on the expression of MHC mRNA was determined by Northern blot analysis using 20mer oligonucleotide probes, complementary to the unique 3' untranslated sequences of the rat α- or β-MHC mRNA. Treatment of cultured myocardial cells grown in a medium containing 5% calf serum either with 10uM forskolin or 1mM 8-Br-cAMP increased, the abundance of α-MHC mRNA 3 to 4 fold. This effect was dose and time dependent and was further potentiated when the 8-Br-cAMP was given together with 0.5mM 3-isobutyl-1-methyl xanthine, a phosphodiesterase inhibitor. Interestingly, no appreciable change was observed in the level of β-MHC mRNA. Pretreatment of cells with 25mM K^+ which blocks contractile activity of cells did not prevent the increase in α-MHC mRNA caused by cAMP. In nuclear run-on assays, a 70% increase in the transcription rate of the cardiac MHC gene was observed following treatment of cells with 10uM forskolin for 12h. The activation of α-MHC mRNA expression was seen only when cells were plated in medium containing serum. In contrast, when cardiomycytes were plated and grown in defined medium no effect of cAMP on α-MHC mRNA levels was observed. The results demonstrate the preferential induction of cardiac α-MHC gene expression by cAMP and suggest an important role of serum components in transcriptional activation of α-MHC gene. (* Invited Speaker, R. Zak)

H 208 ENDOTHELIN PROMOTES PHOSPHOINOSITIDE HYDROLYSIS AND C-FOS EXPRESSION IN ISOLATED ADULT CARDIAC VENTRICULAR MYOCYTES OF TWO MAMMALIAN SPECIES, Linda G. Jones, John D. Rozich and George Cooper IV, Department of Medicine, Medical University of South Carolina, Charleston, SC 29425

Although the mechanisms underlying cardiac hypertrophy are not well understood, recent studies suggest that specific cellular growth factors play a critical role in this process. We wish to report that the endothelial product, endothelin (ET), may have a role in growth regulation of the heart since in isolated adult feline and rat cardiac ventricular myocytes, endothelin stimulates phosphoinositide-specific phospholipase C activity and c-fos expression. Both of these intracellular signals have been shown to be important in cell growth responses. In cells labeled overnight with [^{3}H]inositol, ET promotes a 2-4 fold increase in the accumulation of the inositol phosphates in a dose-dependent manner with an EC_{50} of ~5 nM and a maximal effect by 100 nM. The time course of accumulation of the inositol phosphates is similar to that observed for other receptor-mediated PI responses in the heart i.e. an increase in the inositol polyphosphates (IP_2, IP_3 and IP_4) is observed at early time points (by 30 seconds) whereas an increase in inositol monophosphate is not observed until after 1 minute of exposure to endothelin. In addition, results obtained by Northern blot analysis demonstrate that exposure of isolated cardiac myocytes to 100 nM ET for 30 minutes increases the expression of the nuclear proto-oncogene c-fos in both species. These data provide evidence that endothelin promotes both the early, cell surface signaling events and the later nuclear signal transduction events believed important in growth regulation and suggest that ET may have a role in cardiac hypertrophy.

H 209 ROLE OF Na+ INFLUX IN STIMULATING CONTRACTILE PROTEIN SYNTHESIS BY ISOLATED ADULT CARDIOCYTES, R.L. Kent, P.L. McCollam, D.L. Mann, and G. Cooper, IV; Div. of Cardiology, VA Medical Center and Gazes Cardiac Research Inst., Medical Univ. of South Carolina, Charleston, SC

The mechanism(s) whereby hemodynamic load enhances protein synthesis in cardiac hypertrophy is unknown. We have shown that stretching papillary muscles will lead to increased Na^+ influx and increased synthesis of general and contractile muscle proteins. We have linked both protein synthesis and the Na^+ influx through stretch-activated cation channels by inhibiting both of them with either 500 μM streptomycin or 10 μM gadolinium, two inhibitors of stretch-activated cation channels. In the present study we sought to extend our findings by determining whether Na^+ influx would directly stimulate general and contractile protein synthesis in isolated adult cardiac myocytes, or cardiocytes. Cultured feline adult cardiocytes were treated with 1 μM veratradine, a fast Na^+ channel opener, which doubled $^{22}Na^+$ influx. Protein synthesis was determined by measuring the incorporation of [^{3}H]phenylalanine into both general proteins and contractile proteins. Four hours of treatment with 1 μM veratradine resulted in a 23% increase in general protein synthesis ($P < 0.05$) and a 40% increase in myosin heavy chain synthesis ($P < 0.05$). These effects of veratridine on protein synthesis were abolished by pretreating the cells with 20 μM tetrodotoxin, a fast Na^+ channel blocker. Thus, these studies show that Na^+ influx can enhance general and contractile protein synthesis in cultured adult cardiocytes, and thus support our hypothesis that Na^+ influx may be an early signal coupling mechanical load to hypertrophic cardiocyte growth.

H 210 SUSTAINED CONTRACTILE ACTIVITY NEGATIVELY REGULATES THE EXPRESSION OF THE CALCIUM RELEASE CHANNEL GENE IN SKELETAL MUSCLE. William E. Kraus, James W. Moore and Andrew R. Marks. Dept. of Medicine, Duke University, Durham, NC 27710, Depts. of Molecular Biology and Medicine, Mt. Sinai School of Medicine, NY, NY 10029.

The recent cloning of the skeletal muscle calcium release channel (CRC) has facilitated rapid progress in understanding mechanisms of excitation-contraction coupling and regulation of CRC gene expression during myocyte differentiation. Indirect motor nerve stimulation (INS) of rabbit hind limb muscle is an excellent model for examining the CRC gene response to the physiologic stimuli generated by increases in contractile work. We measured CRC transcript levels in total RNA derived from muscles subjected to INS at 10 Hz for 3 days. CRC transcript abundance was reduced approximately 10-fold (n=5) when compared to the contralateral, unstimulated limbs, which served as internal controls. The dramatic negative regulation of CRC gene expression contrasts with α-actin, myoglobin and aldolase A, none of which are significantly altered at 3 days; nor are there any significant changes in muscle weight, total protein or RNA content. In light of the recent observation that fibroblast growth factor (FGF) is elevated by INS, it is of interest that FGF deinduces CRC in an *in vitro* model (Marks, *et al.* , in press). These results indicate that the CRC is one of the earliest genes regulated in skeletal muscle during increased contractile activity, and suggest that CRC gene expression, possibly regulated by FGF, may play a role in the adaptive response of skeletal muscle to increases in contractile work.

H 211 REGULATION OF IGF-1 EXPRESSION DURING JUVENILE CARDIAC GROWTH BY THYROID AND GROWTH HORMONE IN THE HYPOPHYSECTOMIZED RAT,
Joel M. Kupfer and Stanley A. Rubin Department of Medicine, Division of Cardiology, Cedars-Sinai Medical Center Los Angeles, CA 90049.

Insulin like growth factor 1 (IGF-1) plays an important role in somatic growth. The regulation of cardiac IGF-1 expression was studied in hypophysectomized (hypox) juvenile rats, supplemented for 14 days with physiological doses of growth hormone (GH), thyroid hormone (T3), or the combination GH/T3. Compared to placebo treated hypox rats cardiac weight was significantly increased by 21%, 25%, and 80% in the T3, GH, and T3/GH groups, respectively ($P<0.05$). RNA was extracted from hearts at 30 hours and at 14 days and IGF-1 expression quantitated and compared to hypox-placebo treated rats by a solution hybridization RNase protection assay using a 322 bp fragment from the alternatively spliced 5'untranslated region. Compared to hypox placebo animals at 30 hours GH significantly induced a 3.2 fold increase in IGF-1 expression compared to only a modest 1.5 fold increase by T3. In contrast at 14 days IGF-1 expression was greater by 4.3 fold in the T3 group compared to only 1.8 fold in the GH group. IGF-1 expression in the T3/GH group was similar to GH alone at 30 hours or to T3 alone at 14 days. In contrast to its effects on IGF-1 expression T3 induced changes in myosin heavy gene expression were rapid and complete by 30 hours. These results suggest that both GH and T3 are potent inducers of cardiac IGF-1 although with dramatically different time courses. Furthermore the delayed onset of T3 action with respect to IGF-1 expression suggest that the signalling transduction pathways for GH and T3 are different and that T3 effects are probably not directly mediated through transcriptional properties of its nuclear receptor. Finally our data suggest that autocrine/paracrine induction of IGF-1 may represent a common pathway through which GH and T3 regulate cardiac growth.

H 212 HYPOTHYROIDISM FAVOURS EXPRESSION OF HIGH MOLECULAR WEIGHT BASIC FGF IN THE HEART. Lei Liu and Elissavet Kardami. Department of Physiology, U. of Manitoba & St. Boniface Gen. Hosp. Res.Centre, 351 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6.

Basic fibroblast growth factor (FGF) is a family of multifunctional polypeptides, stimulating cell division and affecting differentiation of many cell types including cardiomyocytes. Lacking a conventional sequence for secretion, bFGF is believed to act intracellularly. Originally characterized as an 16-18 kda form, bFGF is now known to exist also in amino-terminal extended, higher molecular weight, versions (20-26 kda); this has raised the possibility of different roles. We recently found that high or low MW bFGFs predominate in extracts from neonatal or adult rat hearts, respectively (Doble and Kardami, in preparation). Because thyroid hormone regulates muscle gene expression and differentiation and its blood levels rise dramatically during the post-natal development of the rat, we addressed the question of whether it may regulate expression of cardiac bFGFs. Here we report that extracts from hypothyroid rat hearts contain greatly increased levels of high MW bFGF compared to euthyroid controls. Thyroid hormone has a direct effect on myocyte bFGF expression: 1 nM triiodothyronine promotes expression of low MW bFGF by cultured cardiomyocytes. In addition, thyroid hormone stifles the bFGF-induced prolongation of the proliferative potential of the cardiomyocyte. These data imply that one of the ways by which thyroid hormone promotes cardiac muscle differentiation is by regulating expression of endogenous bFGFs in the heart.

H 213 A β-ADRENERGIC RECEPTOR STIMULATES PARACRINE GROWTH FACTOR ACTIVITY FOR CARDIAC MYOCYTES. Carlin S. Long, Marietta Paningbatan, and Paul C. Simpson. Dept of Medicine, Univ of CA and the San Francisco VA Medical Center, San Francisco, CA 94121

Catecholamines have long been implicated in the process of myocardial hypertrophy. We have previously reported that cultured neonatal rat cardiac myocytes hypertrophy in response to α_1-adrenergic receptor stimulation. Moreover, this α_1-stimulation leads to the re-expression of fetal/neonatal contractile protein isogenes and certain cellular oncogenes, similar to that seen with pressure-load hypertrophy in vivo. We have also recently identified a heparin-binding growth factor produced by neonatal rat heart non-myocytes (NMCs) which leads to myocyte hypertrophy in culture. Since cardiac NMCs also contain adrenergic receptors (both α and β) on their cell surface, we were interested in determining whether adrenergic stimulation of these NMCs could in some way modify the growth response of the myocytes. Neither Phenylephrine (PHY) nor Isoproterenol (ISO) caused an increase in the per cell contents of total protein in pure cultures of NMCs (1.01-fold and 1.03-fold over control, respectively). Serum-free medium was conditioned by the NMCs in the absence (NMC-CM) or presence of 0.2 μM ISO (ISO-CM). Growth-promoting activity was increased by 70% in ISO-CM in comparison with NMC-CM (1.74-fold over control for NMC-CM vs 2.27-fold over control for ISO-CM). The ISO-CM effect was not abrogated by the inclusion of the β-adrenergic antagonist propranolol (2.0 μM) in the assay medium (2.23-fold over control). Medium conditioned in the presence of PHY (PHY-CM) also showed an increased activity over NMC-CM; however, this increase was solely due to the presence of PHY in the medium, being blocked by the presence of an α_1-adrenergic antagonist (WB4101) in the assay medium. Using an RIA assay which detects β-stimulated cAMP generation, we have determined that NMCs contain both β_1 and β_2 adrenergic receptors, in contrast to previously published data. We believe that this is the first report suggesting that β-adrenergic stimulation of cardiac NMCs produces growth factor activity for cardiac myocytes. This may reflect a paracrine mechanism relevant in vivo.

H 214 ISOFORM EXPRESSION OF THE α_1 SUBUNIT OF THE VOLTAGE-DEPENDENT DIHYDROPYRIDINE SENSITIVE CALCIUM CHANNEL IN SKELETAL MUSCLE, Nadia N. Malouf, Ceal N. Hainsworth, Debbie K. McMahon and Brian K. Kay, Departments of Pathology and Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514

The α_1 subunit is the principle transmembrane polypeptide of the voltage-dependent dihydropyridine sensitive calcium channel (VDCC) in skeletal muscle. Its cDNA and deduced amino acid sequences are homologous to other voltage-sensitive ion channel subunits and together are believed to be members of one gene family. The prototype structure of the α_1 subunit of the VDCC contains four homologous domains with six transmembrane spanning segments within each domain. Here, we present evidence that at least two isoforms of the α_1 subunit of the VDCC are present in skeletal muscle and that the expression of these isoforms is developmentally regulated. cDNAs corresponding to the α_1 subunit have been isolated from a newborn rabbit skeletal muscle library and sequenced. The nucleotide sequence was found to be identical to the sequence reported from adult muscle (Tanabe *et al.* Nature 328:313, 1987) except that the newborn transcript was 2 kb shorter than the adult. cDNA sequences and RNA-polymerase chain reaction experiments indicate that the deleted segment corresponds to the inner two repeats of the adult four-repeat prototype and extends from II_{S2} to IV_{S2}. This abbreviated transcript encodes a polypeptide of 150,000 molecular weight, compared with the 170,000 characterized in the adult. Using RNA-PCR experiments, Northern and Western blots, we demonstrate that this abbreviated isoform is present in both adult and newborn muscle. The abbreviated two domain isoform is predominant in newborn muscle while the larger four domain isoform is the major species in the adult.

H 215 EXPRESSION OF THE RYANODINE RECEPTOR/JUNCTIONAL CHANNEL COMPLEX IS REGULATED BY FIBROBLAST GROWTH FACTOR IN BC3H1 CELLS Andrew R. Marks, Aki Saito, Bernardo Nadal-Ginard, Sidney Fleischer and Mark B. Taubman Brookdale Center for Molecular Biology and Dept. of Medicine Mt. Sinai Medical Center, N.Y., N.Y. 10029, Dept. of Molecular Biology, Vanderbilt University, Nashville TN and Howard Hughes Medical Institute, Children's Hospital, Boston, MA 02115.

The ryanodine receptor/junctional channel (JCC) complex in skeletal muscle is encoded by a 16 kb mRNA. The JCC is comprised of four identical protomors each with a MW of 565,000 Da giving the structure a mass of ~2.3 million. We have previously demonstrated that the JCC mRNA is present in BC3H1 cells, a mouse tumor cell line which expresses striated, smooth and non-muscle isoforms of many of the contractile protein genes. The JCC is the equivalent to the foot structure of the sarcoplasmic reticulum involved in excitation-contraction coupling, as visualized by electron microscopy (EM). Many of the contractile protein genes and the dihydropyridine-sensitive calcium channel are reversibly regulated by serum withdrawal (induction) and readdition (inhibition) in BC3H1 cells. In the present study we show that JCC mRNA accumulation is induced within 48 hrs. by withdrawal of serum in BC3H1 cells, and is inhibited within 48 hrs. by addition of fibroblast growth factor (FGF, 50 ng/ml), but not by EGF or PDGF. Expression of the JCC protein in response to serum withdrawal was determined by EM of fixed, stained BC3H1 cells. The JCC protein was also induced by serum withdrawal within 5 days and deinduced by readdition of serum within 5 days. These results indicate that the JCC gene is negatively regulated by FGF, and that both the mRNA and protein for this large structure have relatively short half-lives. CRC mRNA was induced ~24 hr. before that of contractile genes (eg. skeletal a-actin) suggesting that the calcium channel may be required for contractile protein gene expression.

H 216 DIFFERENTIAL REGULATION OF Na^+,K^+-ATPASE α-1 GENE PROMOTER ACTIVITY BY STEROID HORMONE RECEPTORS: EVIDENCE FOR ONCOGENE/STEROID HORMONE TRANSCRIPTIONAL FACTOR INTERACTIONS R.M. Medford, L. Olliff, N. Weisberg and R. Hyman, Department of Medicine, Division of Cardiology, Emory University, Atlanta, GA. 30322

Regulation of active ion transport mediated by the Na^+,K^+-ATPase (NAKA) gene family is an important feature of multiple adaptive and pathophysiological processes such as cardiac hypertrophy, hypertension and oncogenic transformation. Analysis of the proximal promoter elements of the rat NAKA α-1 subunit gene suggests this regulation may be mediated by several classes of interacting transcriptional regulatory factors. Elements of the rat NAKA α-1 promoter and upstream regions (coords $^-481$-$^+197$) were attached to the bacterial reporter gene CAT (pA1P). Consistent with an increase in α-1 mRNA levels, a several fold induction in pA1P CAT activity was observed in v-mos oncogene transformed (MOS) compared with untransformed, Balb/c-3T3 fibroblasts (A31). The ability of MOS cells to mediate the regulation of pA1P activity by inducible, transcriptional regulatory factors was also dramatically altered. Either the human mineralocorticoid receptor (hMR) or glucocorticoid receptor (hGR) was introduced into cells by transfection using expression vectors. Transient co-transfection of pA1P with hMR into A31 cells, followed by 48 hours in DME containing 10% bovine calf serum, induced pA1P derived CAT activity compared with transfection by pA1P alone. In contrast, co-transfection with hGR repressed pA1P CAT activity. MOS cells exhibited a different pattern of transcriptional regulation. Co-transfection of both pA1P and hMR into MOS cells decreased, rather than increased, pA1P derived CAT activity. Similar to A31 cells, co-transfection with hGR repressed pA1P CAT activity. These results suggest that hMR, but not the hGR, can either positively or negatively regulate α-1 promoter activity through interactions with trans-acting transcriptional regulatory factors that differ between normal and transformed cells. Deletion mapping studies suggest that this occurs within an evolutionarily conserved promoter element containing overlapping and adjacent steroid response elements, AP-1 and ATF-like DNA-binding sequences.

H 217 INHIBIN α- and βA-SUBUNITS ARE SELECTIVELY EXPRESSED IN THE RAT HEART Helene Meunier and Bingruo Wu, Department of Endocrinology, Research Institute, The Hospital for Sick Children, Toronto, CANADA M5G 1X8.

Inhibin and activin are glycoprotein hormones produced mainly in the gonads. Inhibin is active as a dimer which comprises one α-subunit and one (βA or βB) of two possible β-subunits. Activin is a homo (βA/βA, βB/βB) or heterodimer (βA/βB) of the β-subunits. These two related glycoprotein hormones act in opposite ways to modulate follicle stimulating hormone (FSH) release from the pituitary gland. They are also members of the TGF-β gene family. Consequently, inhibin and activin exert diverse actions in a number of tissues as growth factors, namely in the hemaopoietic system and in ovarian follicles. Recently, activin was shown to promote survival of teratoma cells (P19) and some types of nerve cells, as well as to induce differentiation of the mesoderm in early amphibian embryos. Using Northern blot analysis, we investigated the presence of inhibin subunits in the adult rat heart, a tissue derived from the differentiated mesoderm. Inhibin α-subunit mRNA was detected in the atria and ventricles, but was found to be more abundant in the right compartments of the heart. Very low levels of inhibin βA-subunit mRNA could be detected in the atria, but remained undetectable in the ventricles. These observations demonstrate, once again, independent patterns of expression for the α- and βA-subunits, and suggest a local action of inhibin subunits in the rat heart.

H 218 MOLECULAR CLONING OF cDNA ENCODING THE Ca^{2+} RELEASE CHANNEL (RYANODINE RECEPTOR) OF RABBIT CARDIAC MUSCLE SARCOPLASMIC RETICULUM, Kinya Otsu and David H. MacLennan, The Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Canada M5G1L6

We have cloned and sequenced cDNA encoding the rabbit cardiac ryanodine receptor. The cDNA, 16,532 bp in length, encodes a protein of 4,969 amino acids with a Mr of 564,711. Although the molecular weight is almost identical to that of the skeletal isoform, the protein is 63 amino acids shorter and the deduced amino acid sequences are only 66% identical. The two isoforms are co-linear over much of their length and the predicted secondary structures and hydropathy pofiles do not differ in any significant respect. A potential ATP binding domain was identified at residues 2619-2652, a potential phosphorylation site was identified at residue 2809 and potential calmodulin binding sites were identified at residues 2775-2807, 2877-2898 and 2998-3016. We suggest that a modulator binding domain in the protein lies between residues 2619 and 3016. Northern blot analysis of mRNA from a variety of tissues demonstrated that the cardiac isoform is expressed in heart and brain, but at very low levels, if at all, in fast- and slow-twitch skeletal muscle, smooth muscle, and non-muscle tissues.

H 219 A 28 bp ELEMENT OF THE SKELETAL α-ACTIN PROMOTER ENCOMPASSING A CCAAT BOX-ASSOCIATED REPEAT SUFFICES FOR INDUCTION BY BASIC FGF IN CARDIAC CELL CULTURE. Thomas G. Parker, King-Lau Chow, Robert J. Schwartz, and Michael D. Schneider, Departments of Medicine, Cell Biology, and Physiology & Molecular Biophysics, Baylor College of Medicine, Houston, TX, 77030

Unlike their suppression of muscle-specific genes in skeletal myoblasts, fibroblast growth factors (FGFs) selectively provoke fetal gene expression in cardiac myocytes, including skeletal α-actin (SkA), as seen with pressure-overload hypertrophy *in vivo*. We have recently demonstrated that basic and acidic FGF exert reciprocal control over SkA transcription in cardiac myocytes, via the proximal 202 nucleotides of 5'-flanking sequence. To further define DNA elements involved in lineage-specific control of SkA by FGFs, neonatal rat cardiac myocyte cultures were transfected with deletion-gap mutations of elements in a -421/+24 nt chick SkA promoter which contribute to expression in skeletal muscle. Excision of an upstream TA-rich region, upstream CCAAT box-associated repeat (CBAR), GC-rich region (a negative regulatory element), and downstream CBAR produced residual CAT activity, compared to the wild-type promoter, of 0.45 ± 0.025 (p=0.04), 0.24 ± 0.053 (p=0.05), 1.04 ± 0.21 (p=NS), and 0.087 ± 0.025 (p=0.03), respectively. Thus, all 3 of the positive elements, particularly the downstream CBAR, which is similar to the c-*fos* serum response element, are crucial to basal expression in both cardiac and skeletal muscle. To ascertain whether the downstream CBAR is sufficient for up-regulation by one or both FGFs, 28 bp of the chick SkA promoter (-100/-73), containing the downstream CBAR, was positioned upstream from a neutral promoter and transfected into ventricular cell cultures. Basal chloramphenicol conversion was 1.24 ± 0.295% (~5% of wild-type SkA promoter activity). Recombinant basic FGF (25ng/ml) augmented activity 4.5-fold (5.56 ± 1.74 % conversion; p=0.003), whereas acidic FGF produced no significant change. Therefore, an isolated 28 bp element of the SkA promoter encompassing the downstream CBAR is sufficient for basal expresssion in cardiac cell cultures and for inducibility by recombinant basic FGF.

H 220 ACTIVATION OF Na^+/H^+ EXCHANGE BY PROTEIN KINASE C IN CULTURED NEONATAL RAT HEART CELLS DURING DEVELOPMENT, Shey-Shing Sheu and Virendra K. Sharma, Department of Pharmacology, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642

Activation of Na^+/H^+ exchange by protein kinase C (PKC) has been proposed as a signal for cell growth and development. Using fluorescence digital imaging microscopy and a pH indicator BCECF, the effect of phorbol ester on intracellular pH (pH_i) in ventricular myocytes cultured from 1 day-old rat was studied. 100 nM phorbol 12,13-dibutyrate (PDBu) increased pH_i from 7.10 ± 0.04 to 7.92 ± 0.07 (n = 5) in 1-2 day cultured myocytes and increased pH_i from 7.15 ± 0.05 to 7.55 ± 0.06 (n = 5) in 14-16 day cultured myocytes. This effect of PDBu on pH_i was completely abolished by the pretreatment of heart cell with 20 μM dimethylamiloride, a Na^+/H^+ exchange inhibitor. PDBu also failed to increase pH_i in myocytes pretreated with 0.1 μM staurosporine, a PKC inhibitor. However, pH_i recovery from NH_4Cl-induced acidification has similar time course in both age groups of myocytes. The data suggest that activation of PKC by PDBu leads to an increase in pH_i via Na^+/H^+ exchange. The greater PDBu-induced change in pH_i in younger myocytes is not due to the higher Na^+/H^+ exchange activity in these cells. It is probably due to a higher PKC activity and/or a higher sensitivity to PDBu.

H 221 REGULATION OF CARDIAC RYANODINE RECEPTOR BY PROTEIN KINASE-DEPENDENT PHOSPHORYLATION, Munekazu Shigekawa, Toshiyuki Takasago, Toshiaki Imagawa, Ken-ichi Furukawa,and Tarou Ogurusu, Department of Molecular Physiology, National Cardiovascular Center Research Institute, Suita, Osaka 565, Japan

Protein kinase-dependent phosphorylation of the ryanodine receptor /Ca^{2+} release channel in the canine cardiac microsomes was examined. The observed level of the phosphorylation with 1 mM [γ-^{32}P]ATP by cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG) or protein kinase C (PKC) was comparable to the maximum level of [^{3}H]ryanodine binding in cardiac microsomes, whereas the level of the phosphorylation by endogenous calmodulin (CaM)-kinase was about 4 times larger. Phosphorylation by PKA, PKG, and PKC increased [^{3}H]ryanodine binding in cardiac microsomes by 22 ± 5, 17 ± 4 and 15 ± 9% (average ± S.D., n = 4-5), respectively. In contrast, incubation of microsomes with 5 μM CaM alone and 5 μM CaM plus 1 mM ATP decreased [^{3}H]ryanodine binding by 38 ± 14 and 53 ± 15% (average ± S.D., n = 6), respectively. Phosphopeptide mapping and phosphoamino acid analysis provided evidence suggesting that PKA, PKG and PKC predominantly phosphorylated serine residue(s) in the same phosphopeptide (peptide 1), whereas the endogenous CaM-kinase phosphorylated serine residue(s) in a different phosphopeptide (peptide 4). Photoaffinity labeling of microsomes with photoreactive [^{125}I]-labeled CaM revealed that CaM bound to a high molecular weight protein, which was immunoprecipitated by a monoclonal antibody against the cardiac ryanodine receptor. These results suggest that protein kinase-dependent phosphorylation and CAM play an important regulatory role in the function of cardiac sarcoplasmic reticulum Ca^{2+} release channel.

H 222 CHANGES IN GENE EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS WITH MYOCARDIAL HYPERTROPHY, Francisco J. Villarreal, and Wolfgang H. Dillmann, Department of Medicine, University of California San Diego, Medical Center, San Diego, CA 92103

Cardiac hypertrophy results in an increased expression of mRNA's for the extracellular matrix (ECM) proteins fibronectin (FN) and collagens type I and III. This leads to an increased layout of ECM proteins and an important remodeling of the cardiac interstitium. FN is thought to form the matrix on which collagen is laid down and transforming growth factor beta 1 (TGFβ1) might be involved in the stimulation of ECM protein production in the myocardium. This study tests the hypothesis that increases in mRNA for TGFβ1 and FN should precede increases in collagen mRNA. We studied the expression of ECM protein genes for collagen type III (which binds avidly to FN) and FN genes in a pressure overload model of myocardial hypertrophy. Northern blot analysis of total RNA from sham operated and thoracic banded hypertrophied animals was used to assess changes in mRNA for these genes. TGFβ_1 mRNA increased sharply (approximately threefold) from day 1 after aortic banding, decreasing to control levels after 5 days. FN gene expression increased twofold from day 1 and reached a peak at day three, with mRNA levels decreasing after seven days. Collagen III increased at day 3 (threefold) persisting at high levels 7 days after banding. This study suggests that increased layout of ECM proteins with myocardial hypertrophy might begin as early as 1 day after banding, with increased production of FN followed by the deposition of collagen. Increased expression of ECM genes might be secondary to higher levels of TGFβ proteins present in the myocardium.

H 223 GROWTH FACTOR ACTIVATION AND STRUCTURE-FUNCTION STUDIES OF THE Na^+/H^+ ANTIPORTER ANALYSED BY cDNA MUTAGENESIS.

Shigeo WAKABAYASHI, Claude SARDET, Pierre FAFOURNOUX and Jacques POUYSSEGUR, *Centre de Biochimie-CNRS, Université de Nice, Parc Valrose, 06034 Nice, France.*

The Na^+/H^+ antiporter is a ubiquitous plasma membrane transporter that regulates intracellular pH and is activated by diverse mitogenic and oncogenic signals through an increased affinity for H+ at the intracellular 'modifier' site. Our recent cloning and immunological studies established that the human Na+/H+ antiporter is a phosphoglycoprotein of 815 amino acids with an N-terminal domain that contains ten putative transmembrane spanning segments followed by a large carboxy-terminal cytoplasmic domain (315 amino acids). Mitogenic stimulation by either EGF or thrombin stimulates the phosphorylation of the antiporter exclusively on serine residues and on the same phosphopeptides, suggesting early integration of distincts transmembrane signals. To study the structure-function of the Na+/H+ antiporter and the molecular mechanism for growth factor activation, a set of cDNA deletion mutants within the cytoplasmic domain have been generated and expressed in antiporter-deficient fibroblasts revealing the following interesting features. Deletion of the complete cytoplasmic domain: 1) preserves Na+/H+ exchange activity, 2) shifts the pK of the 'modifier' site to the acidic range and 3) abolishes growth factor activation. Results from more refined deletion and point mutants aimed at mapping the key cytoplasmic region modified by mitogenic stimulation will be presented. In conclusion we have defined two functional domains: the transmembrane domain that has all the features required to catalyze amiloride-sensitive Na+/H+ exchange with a build-in 'H+-sensor' and the cytoplasmic regulatory domain that determines the set point value of the exchanger. We propose that activation of the antiporter in response to external stimuli and oncogenic transformation occurs via a phosphorylation-induced conformational change of the regulatory domain.

H 224 **THE CARDIAC CONDUCTION SYSTEM IN THE RAT EXPRESSES THE α3 ISOFORM OF THE Na,K-ATPase;** Raphael Zahler, Michael Brines, Michael Kashgarian, Edward J. Benz, Jr., and Maureen Gilmore-Hebert, Depts of Medicine, Human Genetics, and Pathology, Yale University School of Medicine, New Haven, CT 06510

Rat heart contains all three known isoforms of the α subunit of the Na,K-ATPase. The physiologic roles of these isoforms, and their localization to specific cell types in the heart, are not well understood; however, the relatively increased sensitivity of the rat α2 and α3 isoforms to ouabain, and the predominance of α3 in neural tissue, suggest that this isoform may be expressed in the cardiac conduction system. Thus, neonatal rat hearts were frozen and plastic embedded; 10μ sections were cut onto glass slides. Tissue was fixed in paraformaldehyde, deproteinated, and hybridized with 3'-end labeled antisense 30-mer oligonucleotide probes specific for each of the three rat Na,K-ATPase isoforms. Washing was performed in solutions containing mercaptoethanol and EDTA at 60^{o}. Emulsion-dipped slides were evaluated by light microscopy after 4-8 days' exposure and staining with hematoxylin/eosin.

Results: Signal was observed diffusely in perinuclear areas of cardiac ventricular myocytes probed for the α1 isoform of Na,K-ATPase. When sections were probed for α3, the AV node, bundle of His, and proximal main bundle branches had significantly greater signal than adjacent working ventricular muscle fibers. In contrast, the α2 isoform appeared to localize to AV valves and papillary muscles. Competition experiments with excess unlabeled oligonucleotide confirmed that signal was specific for each isoform. Thus, mRNA for each of the α isoforms of the Na,K-ATPase can be localized to individual cells of cardiac tissue by in-situ hybridization; this may yield clues as to their function in the heart. The α3 isoform is expressed preferentially in the conduction system.

Molecular Mechanisms of Cardiac Growth and Hypertrophy

Cardiac Hypertrophy: Gene Expression

H 300 INTERACTING POSITIVE AND NEGATIVE TRANSCRIPTIONAL REGULATORY ELEMENTS AND DNA-BINDING PROTEINS OF THE NA^+,K^+-ATPASE α-1 GENE PROMOTER M. Ahmad, L. Olliff, N. Weisberg and R. M. Medford, Department of Medicine, Division of Cardiology, Emory University, Atlanta, GA. 30322

A characteristic, but poorly understood, feature of Na^+,K^+-ATPase (NAKA) gene regulation is its wide range of expression between different tissue types, normal and transformed cells, and physiological or developmental states. We have tested the hypothesis that multiple, interacting elements of the rat α-1 promoter mediate this complex regulatory response. By transfecting a deletion series of the rat NAKA α-1 promoter (coords $^-$481-$^+$197)-CAT reporter gene construct into multiple cell types, we have identified at least two positive and one negative transcriptional regulatory domains regulating α-1 gene transcription. Each regulatory domain correlates with an evolutionary conserved, putative DNA binding element ,termed a proximal upstream cluster (PUC). Consistent with our hypothesis, each of these three PUCs exhibits differential tissue- and oncogenic transformation-specific regulation of α-1 gene transcription. To determine whether these functional differences were due to differences in specific trans-acting factors, DNA binding-gel mobility shift assays were performed using the PUC-1 positive regulatory domain (coords $^-$56 - $^-$102). PUC-1 contains multiple, adjacent and overlapping elements homologous to AP-1, ATF and steroid and thyroid hormone response elements. Nine specific bands (A1-A9) were identified, with both quantitative and qualitative differences, from the nuclear extracts of multiple cell types. Suggestive of transcriptional factor interactions, band A5 demonstrates binding co-operativity in untransformed, Balb/c-3T3 fibrolbasts (A31) but not in v-mos (MOS) transformed fibroblasts. Intriguingly, a new DNA binding complex, termed the transformation specific response element (TSRE), was found in both MOS and H-ras transformed fibroblasts, but not in untransformed A31 cells. Competition assays suggest that the TSRE may be an ATF-like element. The diversity of types, amounts and interactions of PUC-1 *nuclear binding* proteins may contribute to the observed complex regulation of NAKA α-1 gene expression.

H 301 THE C-*JUN* AND *JUN*B PROTO-ONCOGENES ARE INDUCED IN MYOCARDIUM BY A HEMODYNAMIC LOAD IN VIVO AND FIBROBLAST GROWTH FACTORS IN VITRO. Fiona M. Black, Thomas G. Parker, Lloyd H. Michael, Robert Roberts, and Michael D. Schneider. Departments of Medicine, Cell Biology, and Molecular Physiology and Biophysics, Baylor College Of Medicine, Houston, TX 77030

Up-regulation of the nuclear oncogenes, c-*myc* and c-*fos*, in cardiac muscle has been previously established in models of myocardial hypertrophy induced in adult heart by pressure overload and in cultured neonatal cells by various agonists. However, transcriptional control by c-*fos* requires the formation of heterodimers with related "leucine zipper" proto-oncogenes such as c-*jun* or *jun*B. We therefore determined myocardial expression of c-*jun* and *jun*B following hemodynamic overload, by Northern hybridization with full-length murine cDNAs. Suprarenal aortic constriction of male Sprague-Dawley rats (250-300 g) for up to 30 d produced a gradual increase in left ventricular mass (~23% increase in LV mass/body mass at 9 d), associated with appropriate up-regulation of the fetal contractile protein genes, α-skeletal actin and β-myosin heavy chain. Basal expression of *jun*B » c-*jun* was detected at constant levels in sham-operated animals. Transient induction of c-*jun* and *jun*B mRNA was observed (maximal at 1 and 2 hr, respectively), with a return to basal expression by 4 to 24 hr. Densitometry estimated a peak 20-fold increase in c-*jun* and a 2.5-fold increase in *jun*B. Thus, pressure overload induces not only the proto-oncogene, c-*fos*, but also its obligatory dimerization partners, c-*jun* and *jun*B, whose functional properties differ. To investigate the relationship between these findings and growth factor signalling in neonatal ventricular myocytes in culture, cardiac cells were incubated in serum-free medium for 48 hr then were exposed to recombinant acidic or basic FGF (25 ng/ml). Acidic FGF up-regulated c-*fos* >15-fold, c-*jun* >5-fold, and *jun*B >5-fold (maximal at 30 min); peak levels of expression were no greater in purified cardiac fibroblasts that were similarly treated. Basic FGF likewise up-regulated c-*fos* and *jun*B, with less conclusive effects on c-*jun* in the cardiac myocyte cultures. Thus, the transcription factors, c-*jun* and *jun*B, are induced in ventricular myocardium by a hemodynamic load in vivo or recombinant FGFs that evoke a corresponding "fetal" program of cardiac-specific genes in vitro. Taken together, these results establish additional congruities between growth factor signalling mechanisms and events triggered acutely by load, promote the hypothesis that functional *fos*/*jun* dimers are induced in ventricular myocardium as an immediate-early response in one or both models, and identify candidate genes for mechanistic tests to disrupt growth factor signalling in myocardial cells.

H 302 TRANSCRIPTIONAL REGULATION OF THE SARCOMERIC ACTIN AND THE MYOSIN HEAVY CHAIN GENES IN ISOLATED RAT CARDIAC NUCLEI, Kenneth R. Boheler, Catherine Chassagne, Lucie Carrier and Ketty Schwartz, INSERM U127, Hôpital Lariboisière, 75010 Paris FRANCE

In the heart, mRNA accumulations for sarcomeric actins and myosin heavy chains (MHC) are subject to diverse regulatorial processes. To study cardiac transcriptional regulation, we have isolated nuclei (Liew et al., Am J Physiol. 1983; 244: C3.) from intact hearts or livers of 23 day old rats from which nuclear run-on assays (Konieczny and Emerson, Mol Cell Biol. 1985; 5: 2423) have been performed. The isolated ^{32}P labelled transcripts were hybridized against single stranded clones containing sequences specific for cardiac (α_C) and skeletal (α_S) actin, α and β MHC, and fibronectin (FN) in both the sense and antisense orientations. The nuclei were found to be highly active and sensitive to α-amanitin inhibition. Using 15-20 x 10^6 nuclei, ^{32}P incorporation into cardiac transcripts plateaus at 35 minutes and is about one fifth of that seen from equal numbers of hepatocyte nuclei. Cardiac transcripts range in size form 200 to >6000 nucleotides and no detectable RNase or DNase activities were present in the run-on assays. In hepatocyte nuclei, only FN transcripts were detected. Preliminary results from myocyte nuclei show the presence of α but not β MHC transcripts and corresponds to what has been previously reported accumulated in 3 week old rat hearts. The nuclear transcripts for both α_C and α_S actin are present in proportions roughly corresponding to the accumulations of mature message which were determined in parallel by primer extension analysis according to Ordahl (Dev Biol. 1986; 117: 488). FN transcripts were not detected in the myocyte nuclei. We conclude that regulation of the α_C and α_S actins, and the α and β MHCs is largely transcriptional and independently regulated in the rat heart.

H 303 **CARDIAC EXPRESSION OF NUCLEAR PROTO-ONCOGENES AFTER CHRONIC ISOPROTERENOL INFUSION**

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Cardiac hypertrophy is triggered by numerous factors including increased wall stress and adrenergic hormones. However, cellular signal transduction leading to growth of myocytes is poorly understood. In this study, we examined cardiac expression of several nuclear proto-oncogenes in response to infusion of isoproterenol (ISO). ISO (5 mg/kg) was infused into rats with the help of an implanted osmotic pump. Northern blot analysis of left ventricular RNA hybridized with c-myc and c-fos probes revealed a rapid but transient induction, expression lasted 8 hours after starting ISO infusion. A similar time course of expression was observed in case of fos-B and hsp-70. However members of the jun family showed a differential expression pattern, c-jun and jun-D were detected in normal heart, but were not induced by ISO, whereas jun-B was stimulated by ISO, but was not expressed in normal heart. We conclude, that ß-adrenergic stimulation leads to a specific expression pattern of nuclear proto-oncogenes, which may be different in other hypertrophy models.

H 304 COLLAGEN EXPRESSION AND ACCUMULATION IN THE NEONATAL RAT HEART, Wayne Carver, Thomas K. Borg and Louis Terracio, Department of Anatomy, Cell Biology and Neurosciences and Department of Pathology, Univ. of South Carolina School of Medicine, Columbia, SC.

The neonatal heart undergoes a number of morphological and biochemical alterations in response to increased pressure and volume following birth. Prominent among these is the elaborate development of the myocardial extracellular matrix (ECM). The ECM, composed predominantly of interstitial collagen, forms a three-dimensional, stress tolerant network in the first weeks of neonatal development. The present study utilizes biochemical (hydroxyproline and gel electrophoresis) and molecular (Northern and in situ hybridization) techniques to examine the synthesis and accumulation of the interstitial collagens during this period of 'physiological hypertrophy' of the neonatal rat heart. These experiments indicate that 1) the heart weight/body weight ratio (used as a measure of cardiac hypertrophy) increases in the first weeks after birth. 2) The hydroxyproline content of the heart increases rapidly during this period. 3) The ratio of collagen type III/type I decreases from relatively high in the neonatal heart to adult levels by 2 months of age. 4) Type I and type III mRNA levels increase immediately after birth and decline by 3 weeks of age.

H 305 EXPRESSION OF THE α1, α2,α3 AND β-SUBUNIT mRNAs OF THE Na,K-ATPase IN HYPERTROPHIED RAT HEARTS.

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In the pressure overload model of rat heart hypertrophy, alterations of the Na,K-ATPase properties lead to a higher ouabain sensitivity of the enzyme and to a lower dissociation rate constant of the high affinity ouabain binding site than those observed in the adult rat heart. These properties are similar to those seen in the neonatal rat heart (Charlemagne D, Maixent M, Preteseille M and Lelievre L. J. Biol. Chem. 1986; 261:185-189).To determine if these functional modifications are related to an altered expression of the Na,K-ATPase α isoform genes and particularly of the α3 isoform specifically expressed in the neonatal rat heart, Northern- and slot-blot analyses of RNA isolated from sham-operated and hypertrophied rat left ventricles were performed using subunit- and isoform-specific cDNA probes. To better correlate changes in gene expression, the data are expressed relatively to the amount in β myosin heavy chain mRNA of each heart. The level of the predominant α1 isoform and that of the β-subunit are unchanged by mild or severe hypertrophy. The α2 mRNA coding for a high affinity ouabain isoform is decreased by 40 % (hypertrophy < 50%, β MHC mRNA increase < 5 fold) and 60 % (hypertrophy > 50 %, β MHC mRNA increase > 5 fold) in compensatory phase of hypertrophy. The neonatal α3 isoform mRNA, which is extremely low in adult heart is increased up to two fold during severe hypertrophy but it is unlikely that this increase might totally account for the altered properties of the enzyme.

H 306 STRUCTURE AND EXPRESSION OF THE RAT ISO-ATRIAL NATRIURETIC PEPTIDE (iso-ANP) GENE. Lina Dagnino, Jacques Drouin and Mona Nemer. Laboratoire de génétique moleculaire. Institut de recherches cliniques de Montréal, Montréal (Quebec) CANADA, H2W 1R7

Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and rat iso-ANP belong to a family of potent vasodilatory, natriuretic and diuretic peptide hormones secreted by cardiomyocytes. These peptides are structurally and biologically similar, and their respective physiological roles remain unclear. However, differential regulation of ANP and iso-ANP gene expression may reflect distinct physiological or pathophysiological roles. We isolated the rat iso-ANP gene to investigate its pattern and mechanisms of expression. Although the structural organization of the iso-ANP gene resembles that of the ANP and BNP genes, there is little homology between the 5'-flanking sequences in the rat ANP gene and those in the iso-ANP gene. In adult atria and ventricles, iso-ANP mRNA constitutes about 1% and 30%, respectively, of ANP transcripts. Cardiac iso-ANP mRNA levels do not change substantially during post-natal development, whereas ventricular ANP transcripts decrease about 100-fold shortly after birth. In spontaneously-hypertensive rats (SHR), iso-ANP mRNA increases with age and development of the hypertensive state, reaching levels 2- and 3-fold higher, respectively, in the atria and ventricles of 16 week-old animals than in age-matched normotensive rats. We are currently investigating the effect of cardiac hypertrophy on iso-ANP gene expression. Since the iso-ANP gene constitutes an interesting model to study the expression of cardiac genes, we are also conducting experiments to identify 5'-flanking sequences responsible for its tissue-specific expression. Supported by the Canadian Heart Foundation and the Medical Research Council of Canada

H 307 MECHANISMS FOR *IN VIVO* INDUCTION OF MYOCYTE HYPERTROPHY FOLLOWING EPINEPHRINE INFUSION, Theresa A. Deisher and Michael B. Fowler, Department of Hematology, University of Washington, Seattle, WA 98195 and Division of Cardiology, Stanford University, Stanford, CA 94305

Subcutaneous two week infusion of epinephrine (6.0 µg/kg/min) in rats causes cardiac hypertrophy, determined morphometrically by measuring myocyte cell width or indirectly indicated by an increased heart to body weight ratio. Epinephrine-induced increases in myocyte cell width were not prevented by simultaneous infusion of ß-adrenergic antagonists, calcium channel antagonists, membrane stabilizers, superoxide anion scavengers or endothelial-derived relaxing factor precursors. However, the heart-to-body weight ratio was reduced with several of these simultaneous infusions compared to animals infused with epinephrine alone, indicating that, although widely utilized, this index is not an accurate indication of cardiac hypertrophy when treatment groups have not gained equivalent amounts of body weight. While not preventing epinephrine-induced myocyte hypertrophy, ß1- adrenoceptor and calcium channel blockade, D-propranolol, rhSuperoxide dismutase and L-arginine did reduce mortality and cardiomyopathy associated with epinephrine treatment, indicating that the observed myocyte hypertophy may be mediated through a system independent of increased afterload or direct adrenergic stimulation, perhaps the local cardiac renin-angiotensin system. Furthermore, following the rats for an additional two weeks after cessation of the epinephrine infusion reveals that histological evidence of cardiomyopathy has begun to spontaneously regress, cardiac adrenergic contractile responses have been partially restored, but the myocyte hypertrophy has not regressed. The hypertrophy may be a protective adaptation, rather than a pathologic cellular response to epinephrine.

H 308 HIGH CORONARY PRESSURE INDUCES A COORDINATE INCREASE IN TOTAL AND CONTRACTILE PROTEINS SYNTHESIS AND OF PROTO-ONCOGENE AND HSP68 GENE EXPRESSION IN THE ISOLATED RAT HEART. Claude Delcayre, Nguyen Van Thiem, Christian Mouas, Josiane Bercovici and Bernard Swynghedauw, INSERM U 127, Hopital Lariboisiere, 41 Bvd de la Chapelle, 75010 Paris, FRANCE.

In vivo pressure overload induces an increase in cardiac proto-oncogenes and stress proteins gene expression which may initiate the long-term genetic changes observed in hypertrophy. As these early messages can also be evoked by humoral factors or anaesthetic stress, their study requires in vitro models. To know whether mechanical stimulus is directly linked to specific gene expression, the expression of proto-oncogenes and stress protein HSP68, together with synthesis of total proteins, myosin heavy chain (MHC) and actin were studied in beating or KCl-reversibly arrested isolated rat hearts perfused under various coronary pressures.

We used a model of isolated retroperfused heart, oxygenated by a hollow fiber cartridge to eliminate the occurence of hypoxic stress. The system was adapted to measure the synthesis of total and major contractile proteins and the accumulation of RNA in the same heart. After a 2 hours perfusion, incorporation of ^{14}C-phenylalanine was measured into total proteins, and in MHC and actin after separation by preparative electrophoresis; the expression of specific mRNAs was followed by Northern blot analysis.

Hearts were perfused under a coronary pressure of 60 mm Hg for 2 hrs. When arrested hearts were compared to beating ones, synthesis of total proteins and actin were unchanged, whereas that of MHC was decreased by 24%. HSP68 expression was very low in both beating and arrested hearts. c-fos was expressed at a low level in beating hearts and undetectable in arrested hearts.

When hearts were perfused under120 mm Hg for 2 hrs, the synthesis of total proteins was stimulated by 46%, and the expression of both c-fos and HSP68 were strongly increased to reach similar levels in both beating and arrested hearts. In contrast, the synthesis of MHC and actin were stimulated by 59 and 68%, respectively, in beating hearts only.

Our results show that the synthesis of total proteins, c-fos and HSP 68 are mainly under the dependance of passive stretch, whereas the synthesis of myosin heavy chain and actin are mainly under the dependance of contraction. They strongly suggest a relationship between the enhanced c-fos and HSP68 expression and the change in protein synthesis.

H 309 TRANSCRIPTIONAL REGULATION OF THE HUMAN β-MYOSIN HEAVY CHAIN GENE
J.G. Edwards, I.L. Flink, S.-y. Cheng*, K.-h. Lin* J.J. Bahl and E. Morkin, University Heart Center, University of Arizona College of Medicine, Tucson, AZ 85724, and the *Laboratory of Molecular Biology, National Cancer Institute, National Institute of Health, Bethesda, MD 20892.
In humans, the myosin heavy chain (MHC) isoforms of the heart are encoded by the α-MHC and β-MHC genes, with the latter being predominantly expressed in ventricular myocardium. In other species, the relative levels of the isoforms are sensitive to several stimuli including T_3, growth factors, and work overload. We have studied regulation of plasmids containing 5' flanking sequences from the β-MHC human gene fused to the CAT gene by transfection into fetal rat heart cells. By functional analysis, gel mobility shift and DNAse "footprinting" using c-erbA products expressed in E. coli, we have identified several cis-acting elements, including: 1) a strong positive element located between -281/-292 that is responsible for constitutive expression; 2) a negative regulatory region located -300/-335; 3) multiple TREs located in the regions -34/-77 and -343/-468. Cotransfection of fetal rat heart cells with plasmids coding for c-erbAα1 and c-erbAβ1 T_3 receptors enhanced T_3-mediated repression. Interaction of various trans-acting factors with these cis-elements in the β-MHC promoter may account for the pattern of expression in vivo.

H 310 MYOCYTE EXPRESSION OF ICAM-1: CRITICAL FACTOR IN INFLAMMATORY INJURY,
Mark L. Entman, Keith Youker, Carol J. Toman, Arthur L. Beaudet, Caryl L. Lane, William J. Dreyer, Scott Shappell, Addison A. Taylor, Roger D. Rossen, C. Wayne Smith and Donald C. Anderson, Baylor College of Medicine, Houston, Texas 77030.
Neutrophil (PMN) adherence to cardiac myocyte is critical to inflammatory cardiac injury and requires cytokine induction of the myocyte (Entman et al., J.C.I., 85:1497-1506, 1990). A partial ICAM-1 cDNA was prepared from endotoxin (LPS) elicited dog spleen by PCR amplification using degenerate oligomers of conserve sequences in human and murine ICAM-1. The product has 74% and 60.9% sequence identity with human and murine ICAM-1 for domains 2-4, respectively. Northern blots showed very low basal myocyte ICAM-1 mRNA (3.1 kb) with induction elicited by IL-β (10 U/ml), IL-6 (2 U/ml), TNFα (400 U/ml) and LPS (40 ng/ml). The expression of ICAM-1 was related to reperfusion injury by the observation that post-ischemic cardiac lymph induced ICAM-1 mRNA and that that effect is inhibited by an IL-6 neutralizing antibody. In all cases, ICAM-1 induction closely paralleled induction of PMN-myocyte adherence and was blocked by monoclonal antibodies to ICAM-1 (CL18/6) and CD18 (R 15.7). The resultant CD18-ICAM-1 adherence was obligate for neutrophil induced myocyte injury and myocyte lipid peroxidation and was specifically blocked by monoclonal antibodies which inhibited adherence. Our evidence suggests that this peroxidation results from direct transfer of superoxide anion from PMN to myocyte since 1) it is not quenched by extracellular SOD-catalase 2) extracellular superoxide is not detected. Cytokine induction of ICAM-1 on cardiac myocytes appears to be an obligate step in the inflammatory injury associated with ischemia reperfusion injury.

H 311 IDENTIFICATION OF A CIS-ACTING ELEMENT THAT INTERACTS WITH A 97 KD TRANSCRIPTION FACTOR TO CONSTITUTIVELY REGULATE THE HUMAN CARDIAC β-MYOSIN HEAVY CHAIN GENE,
Irwin L. Flink, John G. Edwards, Misa Kimura and Eugene Morkin, University Heart Center, University of Arizona Health Sciences Center, Tucson, AZ 85724.
The human cardiac β-myosin heavy chain (β-MHC) gene is constitutively expressed in ventricular myocardium. A 20-base pair strong positive element (SPE) that may be responsible for this expression has been identified by transfection into fetal rat heart cells of fusion plasmids containing 5'-flanking sequences of the β-MHC gene fused to the CAT gene. The SPE, which is centered at -288, is conserved in the human, rabbit, and rat β-MHC genes, but is not found in the 5'-flanking sequences of the α-MHC gene in any of these species. Mobility shift analysis using a synthetic oligonucleotide containing SPE and nuclear extracts from rabbit and rat ventricle and liver demonstrated a band that is specifically competed by an oligonucleotide containing the unlabeled SPE sequence but not by sequences from the α-MHC promoter. Additionally, blotting of $NaDodSO_4$/PAGE-fractionated nuclear proteins, probed with radiolabeled SPE DNA, revealed sequence-specific binding of a 97 Kd protein from ventricular myocardium and liver. These results suggest that in the human β-MHC gene the SPE is an important factor in constitutive expression; its cognate trans-acting factor is not muscle or species specific.

H 312 **CAN STRUCTURAL REMODELING OF CARDIAC MYOCYTES IN RATS WITH ARTERIOVENOUS FISTULAS BE PREVENTED?** Lino Gonçalves, Freire Santos, António Cabrita, Fátima Pacheco, Mário Freitas, Luís Providência, João Patrício. Department of Cardiology, University Hospital of Coimbra, Coimbra, Portugal.

The aim of this study was to evaluate the efficacy of captopril in preventing cardiac myocyte remodeling in rats with arteriovenous fistulas as far as we know not previous reported. Thirty male Wistar rats weighing approximately 270 g were distributed in three groups: group A (control), group B (fistula) and group C (fistula+captopril). An end to side fistula between the femoral artery and vein was produced in the right thighs of rats from groups B and C .Two rats were excluded at the end of the study, because their fistula were not patent. The other rats were sacrificed arresting the hearts in diastole. Hearts were quickly removed for pathologic study. Body weight increase was similar in all groups. Mean heart weight was significantly increased ($p<0.01$) in the group B when compared with group A, but was similar in groups A and C. Mean heart weight/body weight ratio was also significantly increased ($p=0.014$) in group B when compared with group A, and was similar in groups A and C. Ventricular wall thickness was similar in group A and C, but had increased in group B, particularly in the right ($p=0.004$) and left ventricle ($p=0.03$). Using a point counting technic we calculated the volume fraction of cytoplasm in myocytes from different heart segments. The volume fraction of cytoplasm in group B increased in the right ventricle ($p=0.002$), septum ($p=0.051$), and left ventricle ($p=0.032$), when compared with group A. There were no differences ($p=$ N.S.) between the volume fraction of the cytoplasm in group A and C. In conclusion, captopril prevents the structural remodeling of cardiac myocytes in rats with arteriovenous fistulas.

H 313 IN VITRO TREATMENT OF CARDIAC MYOCYTES WITH CONDITIONED MEDIUM FROM NEURONAL CELL LINES: A MODEL FOR NEURONAL REGULATION OF CARDIAC MYOCYTE GROWTH AND CONTRACTILITY, Steven H. Green[1], Dianne L. Atkins[2], Departments of [1]Biology and [2]Pediatrics, University of Iowa, Iowa City, IA 52242

Sympathetic innervation of cardiac myocytes *in vitro* induces growth and changes in contractility. To establish a model system which will allow purification and identification of the neuronal factor(s) responsible for mediating this regulation, we have initiated studies utilizing neuronal conditioned medium (CM) instead of neuronal explants. We have used CM from PC12 cell cultures, a neuronal cell line with several advantages over primary sympathetic neuronal cultures: PC12 cells are a homogenous neuronal population; can be maintained in a neuronal state in serum-free, NGF-supplemented medium; and are cholinergic so effects mediated by adrenergic mechanisms are absent. PC12 CM induces contractility changes in myocytes qualitatively similar to those induced by innervation: Measurement of contraction frequency (CF) and contraction amplitude (CA) were obtained with video motion analysis of spontaneously contracting cells in standard media or CM. Results were analyzed by nested ANOVA. By 72 h, PC12 CM decreased CF ($P=0.02$) and increased CA ($P= 0.03$), qualitatively similar changes are observed in innervated myocytes. A concomitant change in cellular metabolism is detected as a change in uptake of ^{3}H-2-deoxyglucose. Cardiac myocytes metabolically labeled with ^{35}S-methionine during treatment with PC12 CM exhibit an increase in a single molecular species: a 23 kDa protein. Induction of this species occurs over a period temporally correlated with the contractility changes observed following innervation or treatment with PC12 CM. We find that cardiac myocyte troponin isoforms change *in vitro* with a time course similar to that seen *in vivo*, however, this change is not temporally correlated with changes in contractility nor is it affected by CM. Thus, PC12 CM reproduces the effects of innervation on cardiac myocyte contractility. Contractility changes *in vitro* appear to be correlated with metabolic changes and with a change in expression of a 23 kDa protein.

H 314 MYOCARDIAL REPERFUSION INDUCES THE EXPRESSION OF PROTO-ONCOGENES AND THE GLUCOSE TRANSPORTER GENE IN CANINE HEART, Youichi Katoh, Kazuhide Yamaoki, Issei Komuro, Masahiko Kurabayashi, Eitetsu Hoh, Tomoichiro Asano, Hiroshi Yamaguchi and Yoshio Yazaki, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan.

Recently, involvement of c-*fos* and c-*myc* in the mechanically- and hormonally- induced cardiac hypertrophy has been implicated. Brain-type glucose transporter (GT) has been also shown to be associated with cellular growth. To examine whether specific genetic regulatoly mechanisms are associated with cardiac ischemia and reperfusion, we examined the expression of c-*fos*, c-*myc*, and GT genes in the canine heart after occlusion of the coronary artery and reperfusion. Only 20 to 80 min of occlusion or several times repeats of sequential 5 min occlusion and 15 min reperfusion of the left circumflex coronary artery, total cellular RNA was extracted from ischemic and non-ischemic areas of ventricles. c-*fos* and c-*myc* mRNAs were abundantly expressed only in the reperfusion area, not in the only ischemic area. The expression of GT mRNA was moderately increased in reperfusion area. Our data is the first to reveal that myocardial reperfusion may trigger a growth signal, suggesting that common transduction pathway was involved, at least in part, in the cellular response to ischemia-reperfusion and hypertrophic signals.

H 315 HORMONAL MODULATION OF A GENE INJECTED DIRECTLY INTO RAT HEART
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We have demonstrated that the rat heart is capable of expressing reporter genes following direct injection of plasmid DNA. In the present study, we asked if an injected gene could be expressed from a cellular promoter in a hormonally responsive manner. The hearts of three groups of rats were co-injected with 100 μg each of plasmid DNA containing the chloramphenicol acetyl transferase (CAT) gene coupled to the constitutive Rous sarcoma virus LTR, and the firefly luciferase (luc) gene coupled to the rat α-cardiac myosin heavy chain promoter (-613 to +32) which has been shown in tissue culture to be thyroid hormone responsive. Group 1 animals were treated with propothiouracil (PTU); group 2 animals were treated with PTU until the time of cardiac injection and then given triiodothyronine; group 3 were age matched controls. Animals were sacrificed five days following cardiac injection and heart lysates assayed for CAT and luc activities. Relative to euthyroid controls, the ratio of luc to CAT activity was reduced 40% in group 1 and increased two fold in group 2 animals ($p < 0.05$). These data demonstrate that the α-myosin heavy chain promoter confers thyroid hormone responsiveness and suggest that this direct injection technique can be used to map gene promoters in vivo.

H 316 REGULATION OF ANGIOTENSINOGEN GENE EXPRESSION IN CARDIAC CELLS. Zeng Xiu Liu, Ming-Dong Zhou, M.A.Q. Siddiqui, and Ashok Kumar, Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, NY 11203.

Renin-angiotensin system (RAS) plays an important role in the control of blood pressure and fluid balance. The octapeptide hormone, angiotensin-II, which is one of the most active vasopressor substance known, is synthesized from its precursor molecule angiotensinogen by the combined proteolytic action of renin and angiotensin converting enzyme. Although angiotensinogen is normally synthesized in the liver, recent studies have shown that it is also synthesized in the heart. Angiotensin-II also induces hypertrophy in cardiac and vascular smooth muscle cells and it has been suggested that locally synthesized angiotensinogen in the heart may be converted into angiotensin-II that may induce cardiac hypertrophy. In support of this hypothesis increased levels of angiotensinogen mRNA are observed in the heart of SHR. In order to understand the regulation of angiotensinogen gene expression in cardiac cells we have constructed recombinant DNA molecules where 688 b.p. promoter region of rat angiotensinogen gene was fused to chloramphenicol acetyl transferase (CAT) gene. Transient transfection of these DNA molecules in primary cultures of cardiac cells have shown that this promoter region is active in the expression of CAT gene in Cardiac Cells. We are now analyzing cis-acting DNA elements involved in the expression of angiotensinogen gene in cardiac cells.

H 317 REGULATION OF THE HUMAN ALPHA AND BETA CARDIAC MYOSIN HEAVY CHAIN GENES, John D. Mably, M.J. Sole and C.C. Liew, Laboratory for Molecular Cardiology, Departments of Clinical Biochemistry, Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada M5G 1L5.
The genes for the two human cardiac myosin heavy chain isoforms, alpha and beta, have been isolated and characterized. These genes demonstrate both developmental and tissue-specific expression yet their regulation is not well defined. For this study, serial deletions of the alpha and beta 5'-promoter regions (from approx. -1000bp to +100bp) were generated with Bal31 nuclease. Nuclear extracts were prepared from heart and liver tissue, as well as from HeLa cells. The Bal31 generated fragments were then used with the aforementioned nuclear extracts in DNase I footprinting assays to define putative *cis*-acting regulatory sequences. DNase I footprint analysis of one clone demonstrated a potential binding sequence at approximately -914 to -906 and a similar sequence at -825 to -818 of the alpha myosin heavy chain gene promoter. This sequence (5'-GAAAAATCT-3' or 5'-GAAAATCT-3') is very A/T rich, as is the CArG/CBAR motif found in many other sarcomeric protein genes. This footprint has been demonstrated using not only HeLa cell extract, but also rat heart and liver nuclear extracts. Further work will involve the determination of the specificity of binding of this and other *cis*-element(s) to *trans*-acting factor(s) by means of gel shift analysis with specific and non-specific competing oligomers.

H 318 CHANGES IN THE CONSTITUTIVE LEVEL OF C-MYC EXPRESSION DO NOT ALTER THE HYPERTROPHIC RESPONSE TO EITHER T3 OR cAMP IN NEONATAL CARDIAC MYOCYTES IN CULTURE. Nicholas V. Matiuck, Judith L. Swain, Duke University Medical Center, Durham, NC 27710

The c-myc protooncogene has been implicated in the induction of cardiac myocyte hypertrophic growth by a variety of stimuli. We and others have previously demonstrated that c-myc is transiently induced during the hypertrophic growth response to T3 and dibutryl cAMP (dcAMP). To test the hypothesis that alterations in c-myc expression directly influence hypertrophic growth, we studied cardiac myocytes derived from transgenic animals exhibiting elevated cardiac c-myc expression. Myocytes derived from c-myc transgenic (TG) and nontransgenic (nonTG) neonatal hearts were cultured at similar densities, and the hypertrophic response to 72 hrs of treatment with T3 (10nM) and dibutyryl cAMP (dcAMP) (1 mM) assessed. T3 increased protein content by 30±27% in the TG myocytes (n=5), compared to 32±10% in the nonTG myocytes (n=8). The response to dcAMP was 26±16% for TG (n=8) and 31±14% for nonTG (n=10) (both p>.05). In addition, the protein content per plate was similar for the TG and nonTG myocytes. Thus the hypetrophic growth response to T3 and dcAMP in vitro is not altered by increasing the level of constitutive c-myc expression in cardiac myocytes. These results suggest that the c-myc protooncogene does not modulate the hypertrophic growth response to T3 and dcAMP in cardiac myocytes in vitro.

H 319 CARDIAC RELAXATION AND SARCOPLASMIC RETICULUM FUNCTION IN RATS WITH CHRONIC GROWTYH HORMONE HYPERSECRETION Jean-Jacques Mercadier, Anne-Marie Lompré, José Timsit, Bruno Riou and Yves Lecarpentier. INSERM U 127, Paris and INSERM U 275, LOA-ENSTA, Palaiseau, France.
Chronic growth hormone (GH) hypersecretion in rats results in a unique pattern of cardiac adaptation which allows the myocardium to improve its contractile performance and economy simultaneously, thanks to myosin phenoconversion to V3 and an increase in the number of active enzymatic sites (Timsit et al., J. Clin. Invest. 86:507-515, 1990). To further characterize the alterations of cardiac contractile function during GH hypersecretion, we studied the relaxation of papillary muscles isolated from the left ventricle of rats bearing a GH-secreting tumor. Similar rats were used to determine the function of the sarcoplasmic reticulum (SR) as described previously (de la Bastie et al., Circ. Res. 66:554-564,1990). 18 weeks after injection of GC cells, the body weight of tumor-bearing rats had doubled compared to controls (436±14 vs. 217±6, p<0.05), whereas the heart weight to body weight ratio was unchanged. The isolated papillary muscles displayed a slightly improved relaxation with a 19% increase in the maximal speed of relaxion at low load (3.97±0.47 vs. 3.34±0.49 Lmax/sec, p<0.01), which suggested an improved function of the SR. This was in sharp contrast with a decrease in the phosphorylated intermediate of the SR Ca2+-ATPase (9.0+0.7 vs. 12.2+0.4, p<0.01) which indicates for the number of active calcium pumps. This decrease in the phosphorylated intermediate correlated linearly with a decrease in the calcium transport (r=0.79, p<0.001). At last, we found a 50% decrease in the amount of the SR Ca2+-ATPase mRNA related to that of the 18S ribosomal RNA (p<0.05). These results suggest that during chronic GH hypersecretion in rat, a normal cardiac relaxation at low loading conditions is due to post-translationnal regulation of the SR Ca2+-ATPase or to other mechanisms which are responsible for the cytosolic calcium decay during relaxation.

H 320 HEMODYNAMIC REGULATION OF MYOSIN HEAVY CHAIN GENE EXPRESSION IN THE TRANSPLANTED HEART. Kaie Ojamaa, Irwin Klein and Chull Hong. North Shore University Hospital/Cornell, Manhasset, NY, 11030

Heterotopic rat cardiac iosgrafts (TRANS) are hemodynamically unloaded hearts that undergo rapid cardiac atrophy and a significant reduction in the rate of protein synthesis. The appearance of V3 myosin isoform by 3 days after transplantation suggested that transcription of the beta-myosin heavy chain (MHC) gene had been induced. To explore the regulation of MHC gene expression during atrophy we have extracted and quantitated total LV RNA. Northern analysis was used to measure total MHC mRNA (MyRNA) by hybridization to a 500 bp cDNA probe coding for an homologous region of alpha- and beta-MHC genes. Oligonucleotide probes specific for alpha-MHC and beta-MHC transcripts were used to determine relative levels of expression of the 2 MHC genes. Three days after transplantation when LV size had significantly decreased by 19% and total TRANS LV RNA was 12% lower, the level of total MyRNA was not significantly different. In contrast, LV alpha-MyRNA content was significantly decreased by 60% and beta-MyRNA was induced to yield an 11-fold increase in the TRANS. Thus, during hemodynamic unloading of the heart, transcription of MHC genes appeared to be specifically regulated, simultaneous with changes intotal cardiac protein synthesis rates.

H 321 INCREASE IN THE CARDIAC PREPROENKEPHALIN A mRNA LEVELS DURING INFARCT DEVELOPMENT. Pierre Paradis[1], Pierre Bélichard[2], Jean-Lucien Rouleau[3] and Léa Brakier-Gingras[1], Université de Montréal[1] and Hôpital du Sacré-Coeur[2], Montréal and Université de Sherbrooke[3], Sherbrooke, Canada.

Enkephalins have been implicated in the regulation of cardiovascular function. The preproenkephalin A (ppENK) gene, which encodes for the precursor of enkephalins, is transcribed in rat hearts. To investigate whether changes in ppENK gene expression accompany the development of myocardial infarction, the left anterior descending coronary artery was ligated in 8 week-old Wistar male rats. The activity of the sympathoadrenergic system is known to increase during myocardial infarct development. The release of catecholamines related to this activation could be noxious by contributing to expand the infarcted zone. The levels of ppENK mRNA were measured by Northern blot analysis with a homologous ppENK cDNA probe, in myocardial samples obtained from infarcted and sham-operated control rats, 3 hours after the surgery. In control hearts, the levels of ppENK mRNA were higher in the ventricles than in the atria. Regional differences were apparent in the ventricles, the levels of ppENK being higher in the ventricular septum and the left ventricle free wall than in the right ventricle free wall. In infarcted hearts, the levels of ppENK mRNA were increased about 2-fold in all compartments except in the right ventricle free wall, where the increase was about 4-fold. The changes in ppENK gene expression could be related to the increased sympathoadrenergic activity which accompanies myocardial infarct development, since the transcription of this gene is regulated by second messengers linked to the signalling pathways of the sympathoadrenergic system. Enkephalins are known to prevent the release of catecholamines from sympathoadrenergic nerve terminals and we propose that the increase in ppENK gene expression could be part of a compensatory mechanism which attempts to prevent excess release of catecholamines and therefore to minimize the size of the infarcted zone.

H 322 IDENTIFICTION AND PARTIAL PURIFICTION OF A NUCLEAR FACTOR THAT RECOGNIZES MULTIPLE REGULATORY PROMOTER ELEMENTS. PANKAJ QASBA, ERSHEN LIN, M.A.Q.SIDDIQUI. Department of Anatomy and Cell Biology. SUNY Health science Center at Brooklyn, New York 11203.

The chicken cardiac myosin chain-2(MLC-2) gene promoter contians a sequence motif CCAAAAAGTGG (element A) which shares a striking homology with the evoltionarily conserved sequence CC(A/T)6 GG, (CArG-box). By sequential and site-directed deletion of specific nucleotides,we have established that element A is involved in the positive regulation of the MLC-2 gene transcription. Three specific nuclear protein factors, (ABF1-3) recogize element A with sequence specificity. One of these factors, ABF-1 reconizes the core element A sequence AAAAGT,while other ABFs have a relaxed sequence reqirements.The protein factor ABF-3 recognizes another sequence in MLC-2 promoter, element-P, which is also involved in positive regulation of the MLC-2 gene. This was demonstrated by hetrologous competion of the DNA binding by titration with elements A and P DNAs. It appears that the binding of a single regulatory protein (ABF-3) to multiple cis-DNA elements sharing little sequence homology introduces a functional diversity in MLC 2 promoter.

H 323 REGULATION OF CARDIAC SARCOPLASMIC RETICULUM Ca++ ATPase EXPRESSION BY THYROID HORMONE AND RETINOIC ACID, Daniel K. Rohrer, and Wolfgang H. Dillmann, Dept. of Medicine, University of California at San Diego, San Diego CA 91203.

Both thyroid hormones and retinoids are required for proper mammalian development, as well as maintainence of the differentiated state in the adult. Thyroid hormones in particular are known to control the postnatal development of sarcoplasmic reticulum (SR) Ca++ transport activity, and also regulate cardiac SR Ca++ ATPase mRNA expression in the adult. These findings were expanded by testing for regulation in an in vitro cardiac myocyte preparation, where it was found that thyroid hormone(T3) could regulate SR Ca++ ATPase levels to a degree similar to that seen in vivo. T3 was found to regulate expression at very low doses (EC50 ~30pM), and this effect was mimicked qualitatively by retinoic acid (RA), a mechanistically related hormone(EC50 ~2nM). Cis-acting sequences proximal to the promoter for this gene were isolated and characterized, and a 5' region of this gene capable of conferring T3 dependent transactivation was defined by deletional analysis. The T3 regulated reporter constructs are not RA regulated, suggesting independent mechanisms of action for T3 and RA. Cross-regulation of T3 responsive genes by RA as a general phenomena is suggested by RA's ability to positively regulate cardiac α myosin heavy chain (αMHC) in myocytes. Furthermore, a well defined thyroid response element (TRE) derived from the αMHC gene is not transactivatable by RA. The ability of RA to transactivate expression in this system was verified by the use of a palindromic TRE. These findings clearly demonstrate that while gene regulation can display hormonal redundancy, the mechanisms governing such regulation need not be redundant.

H 324 CLONING AND EXPRESSION OF A GENE CONTAINING THE IDENTIFIER ELEMENT IN THE JUVENILE RAT HEART, Stanley A. Rubin, Division of Cardiology, Cedars-Sinai Medical Center, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90048

The identifier (I.D.) element is a repetitive sequence found in the rodent genome, which was originally believed to be a marker of brain-specific transcripts. I- used a subtractive hybridization strategy to obtain clones from a juvenile rat heart cDNA library, which had been prepared from a hypophysectomized (HYPX) animal treated by both growth hormone (GH) and thyroid hormone (l-T3). A clone was isolated which contained a 0.6 kB insert, and which included a 70 base sequence with high homology to the consensus sequence of the I.D. repetitive element. A computer search of the other portion of the sequence showed no homology with the GenBank/EMBL data base, suggesting that the clone was otherwise unique. A slot blot of total cardiac RNA extracted from HYPX, l-T3 treated, GH treated or l-T3/GH treated rats showed a six-fold induction of expression only in the l-T3/GH treated heart. These data suggest that a transcript containing the I.D. sequence is expressed in the juvenile rat heart stimulated by hypophyseal hormones. The function of this repetitive element, and the sequence of which it is a part, remains to be elucidated.

H 325 STRETCHING OF CARDIAC MYOCYTES: EFFECT ON THE ATRIAL NATRIURETIC PEPTIDE SECRETION FROM THE ATRIAL AND VENTRICULAR CELLS, Heikki Ruskoaho, Pentti Mäntymaa, Päivi Kinnunen, Tarja Taskinen[1] and Olli Vuolteenaho[1], Departments of Pharmacology and Toxicology and Physiology[1], University of Oulu, SF-90220 Oulu, Finland

The major site of atrial natriuretic peptide (ANP) biosynthesis is the atria of the heart, yet stimulation of ventricular synthesis and storage of ANP has been shown in ventricular hypertrophy. To define the effect of stretch on ANP release from ventricular cells, we measured the secretory response to graded passive myocardial stretch in isolated, atrialectomized hearts of one-year-old spontaneously hypertensive (SHR) rats. By this age, SHR showed marked induction of the ANP coding gene in the hypertrophic ventricular tissue, as reflected by the elevated levels of immunoreactive ANP and ANP mRNA. The hearts were studied at three different left ventricular pressures (15, 25 and 75 mm Hg) by increasing the volume of the left ventricular balloon for 4 hours. To examine the effect of stretch on ANP release from atrial myocytes, the modified perfused rat heart preparation that enabled the stepwise distension of the right atrium was used. The increase in atrial pressure by 3.8 mm Hg for 4 hours resulted in a 2.5-fold increase in the perfusate ANP. During constant atrial stretch, the greatest increase in ANP release was observed after 30 min of distension, thereafter ANP secretion decreased reaching control values after 2 hours of stretch. In contrast, stretch of ventricles produced only a rapid, transient (from 2 to 5 min), pressure-dependent increase in ANP secretion. As left ventricular pressure rose from 0 to 25 mm Hg, a 2.2-fold increase in ANP release into the perfusate was observed. Chromatographical analysis revealed that the ventricles as well as atria release both before and during stretch primarily the 28 amino acid ANP-like peptide into the perfusate. This study shows that both atrial and ventricular stretch stimulate ANP secretion and that the kinetics of the secretory response differs between atrial and ventricular cells.

H 326 EFFECT OF CONTRACTILE ARREST ON MYOFIBRILLAR PROTEIN GENE EXPRESSION IN CULTURED NEONATAL RAT VENTRICULAR MYOCYTES. Allen M. Samarel and Gary L. Engelmann, Department of Medicine, Loyola University Medical Center, Maywood, IL 60153

Contractile protein gene expression in cultured neonatal rat ventricular myocytes was examined in spontaneously beating cells, and cells arrested with verapamil (V, 10 μM) or KCl (50 mM). Neonatal myocytes were isolated, and high-density primary cultures initiated and maintained under serum-free conditions. Spontaneous beating was arrested by addition of V or KCl, and total RNA was isolated after 6, 24, and 48 hr of continuous treatment. In other studies, myocytes were treated with V or KCl for 24 h, the agent was removed, and the cells cultured for an additional 24–48 hr. Total RNA was analyzed by Northern and slot-blot hybridization analysis with 32P-labeled oligonucleotide probes for myosin heavy chain α (MHC-α), MHC-β, α-cardiac actin (α-CA) and 18S-rRNA, and with cDNA's for cardiac troponin-I (cTNI) and cardiac troponin-C (cTNC). V or KCl-mediated contractile arrest was not associated with cell toxicity (as assessed by ultrastructural and biochemical criteria), nor with reduced recovery of total RNA per culture. V or KCl-mediated contractile arrest was associated with an inhibition of myocyte growth, and a dramatic, time-dependent reduction in the relative concentrations of both MHC-β and α-CA mRNA's. In contrast, mRNA levels encoding MHC-α, cTNI and cTNC appeared relatively unaffected. Restitution of contractile activity resulted in myocyte growth and a near complete return of MHC-β and α-CA mRNA levels to those observed in contracting cells. Thus, contractile arrest modified the expression of specific contractile protein genes independent of the method used to inhibit contractile activity. Studies are now underway (using promoter-reporter gene constructs and activators of potential signal transduction pathways) to define the molecular signals involved in work-induced myocyte gene expression and cellular growth.

H 327 **Reexpression of a fetal pattern of fibronectin during the development of rat cardiac hypertrophy induced by pressure overload.**

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Pressure overload induces in cardiac muscle, quantitative and qualitative changes in the genomic expression of myocytes and an accumulation of proteins of the extracellular matrix. One of them, fibronectin (FN), mainly synthetized by non-muscle cells, accumulates soon after imposition of a pressure overload in ischemic areas of the ventricle. Two isoforms of FN that arise from a single gene by alternative splicing, are considered adult (EIIIA) and fetal (EIIIB) forms. The aim of this study was to determine: 1) wether pressure overload stimulates the reexpression of EIIIB in adult heart and 2) the distribution of the 2 FN mRNAs.

Pressure overload was induced in 3 week-old rats by aortic stenosis.Using *in situ* hybridization procedure, we show that FN EIIIA and EIII B mRNAs are relatively abundant in vascular and interstitial cells of fetal ventricle but undetectable in the ventricle of 3wk old rats. A few days after aortic stenosis, both isoforms accumulate in defined areas of the ventricle and in the walls of coronary arteries. Our results demonstrate that non muscle cells, like the myocytes, do respond to pressure overload by reexpressing fetal gene products and suggest that both types of cells utilize similar adaptative mechanisms during the development of cardiac hypertrophy.

H 328 LEFT VENTRICULAR HYPERTROPHY AND STRIATED MUSCLE ISOACTIN GENE EXPRESSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT Ruth I. Stolz, Kelly M. Crawford, James L. Lessard, Division of Cardiology, University of Cincinnati Medical Center and Division of Basic Sciences, Children's Hospital Medical Center, Cincinnati, OH 45267

The objective of the study is to determine striated muscle isoactin gene expression in the left ventricle (LV) and the right ventricular (RV) free wall of spontaneously hypertensive rats (SHR). Eight adult SHR were compared to eight age-matched control Wistar-Kyoto (WKY) rats. In SHR, systolic blood pressure measured by tail cuff is elevated compared to blood pressure in WKY (162 ± 3 vs. 108 ± 3 mm Hg; mean ± SEM; $p < 0.0005$). Left ventricular hypertrophy also characterizes SHR, which have a significantly higher LV/body weight ratio than WKY (4.00 ± 0.54 vs. 2.54 ± 0.08 gm/kg; mean ± SD; $p < 0.0005$). The RV free wall/body weight ratio, however, does not differ between SHR and WKY (0.65 ± 0. 13 vs. 0.59 ± 0.06 gm/kg; mean ± SD).

Total cellular RNA was isolated for dot blot analysis from LV and RV free wall. Specific oligonucleotide probes were designed to identify sequences in the 3' untranslated regions of the α-cardiac actin mRNA and the α-skeletal actin mRNA. Compared to WKY ventricles, SHR ventricles show similar elevations of α-cardiac actin mRNA levels. Levels are 5.0 fold higher in SHR LV than in WKY LV, and 5.2 fold higher in SHR RV free wall than in WKY. Preliminary results indicate that α-skeletal actin mRNA levels are at least 4-5 fold higher in SHR LV and RV free wall compared to WKY.

These results demonstrate alterations in striated muscle isoactin gene expression in this genetic model of hypertension and pressure-overload left ventricular hypertrophy. Both α-cardiac and α-skeletal actin mRNA levels are higher in SHR LV and RV free wall than in WKY ventricles.

H 329 REGULATION OF THE RABBIT CARDIAC MYOSIN HEAVY CHAIN β-GENE PROMOTER, Jody M. Welborn, Rhonda L. Carter, Mitchell T. Gore, R. Scott Hall and Patrick K. Umeda, University of Alabama at Birmingham, Birmingham, AL 35294.

Alterations in cardiac myosin heavy chain (HC) gene expression occur during cardiac muscle development and growth. To understand the molecular mechanisms that control expression of these genes, we are characterizing the cis- and trans-acting regulatory elements of the cardiac myosin HCβ promoter. Previous studies involving DNA transfection of neonatal rat cardiac myocytes identified a suppressor (-311 to -294) and 3 downstream positive regulatory domains. Using an avidin-biotin-DNA binding assay, we have characterized a strong thyroid hormone (T3) receptor binding site in the suppressor. This site also appears to bind T3 receptor in cardiac nuclear extracts. In the transfection assay, the deletion of the binding site not only derepresses β-myosin HC promoter activity, but also abolishes the down-regulation of the β myosin HC promoter by thyroid hormones. The results indicate that the suppressor region mediates thyroid hormone effects on the cardiac β-myosin HC gene transcription. The modulation of promoter function by T3 probably involves two positive domains downstream of the suppressor (-284 to - 274 and -275 to -266). Gel retardation studies and methylation footprinting indicate that both domains contain the binding sites for distinct proteins. Interestingly, extracts from cardiac and embryonic skeletal muscles exhibit differential binding to these regions. The latter results suggests different factors or interactions modulate this gene in the two muscle types. The identification and characterization of the trans-acting factors interacting with regulatory domains of the promoter provides a basis of delineating the molecular mechanism of thyroid hormone modulation of this gene.

H 330 THYROID HORMONE REGULATION OF CARDIAC SARCO(ENDO)PLASMIC RETICULUM Ca^{2+}-ATPase GENE EXPRESSION. Angel Zarain-Herzberg*, Jane Bacchieri and Muthu Periasamy, Department of Physiology, Univ. of Vermont, Burlington, Vermont 05405; and *Division of Cardiovascular Sciences, St. Boniface Research Centre, Univ. of Manitoba, Winnipeg, Manitoba R3C 1L7

We have recently demonstrated using a rabbit animal model that the relative level of cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase mRNA (SERCA2a) is increased approximately two fold in thyrotoxic cardiac hypertrophy, whereas in hypothyroidism its relative level of expression is decreased by 50% compared to euthyroid animals. To further understand the mechanism of thyroid hormone action, we have analyzed the effect of thyroid hormone on the SERCA2 gene expression, using rat primary cardiocyte cultures. When primary cardiocytes cultures were grown in a medium containing 5% fetal bovine serum depleted for thyroid hormone (T_3), the relative level of SERCA2a mRNA was decreased to 81% compared to cells grown in 5% normal fetal bovine serum. In contrast, the SERCA2a mRNA level was increased by 168% when cardiocytes grown in T_3 depleted medium were supplemented with 50 nM exogenous L-T_3. In order to understand if thyroid hormone directly influence the expression of the SERCA2 gene by a thyroid hormone receptor mediated mechanism, we have begun an analysis of the promoter and upstream regulatory region of the SERCA2 gene by co-transfection of a expression plasmid containing the 5' flanking region of the SERCA2 gene and the thyroid hormone receptor cDNA in a expression plasmid into skeletal and cardiac muscle cells. Preliminary results suggest that thyroid hormone receptor increases the activity of our chimeric SERCA2 gene construct. However, we were unable to demonstrate a T_3 dependent activation of the co-transfected thyroid hormone receptor cDNA. Additional studies (e.g. nuclear run-on) are necessary to demonstrate a transcriptional effect of T_3 in the expression of this gene.

Myocardial Disease: Thrombolysis

H 400 **DYSTROPHIN EXPRESSION IN DIAPHRAGM AND CARDIAC PURKINJE FIBERS: EVIDENCE THAT ALTERNATIVELY SPLICED ISOFORMS ARE INVOLVED IN DISEASE.** Roger D. Bies[1,2], Jeffery S. Chamberlain[3], M. Dolores Cortez[2], Robert Roberts[2], C. Thomas Caskey[1], Institute for Molecular Genetics[1], Department of Cardiology[2], Baylor College of Medicine, Houston, TX 77030, Department of Human Genetics[3], University of Michigan Medical School, Ann Arbor, MI 48109.

Patients with Duchenne muscular dystrophy (DMD) die from complications of respiratory failure, arrythmias, heart block, and cardiomyopathy. We found that dystrophin, the defective protein in DMD, is expressed in both diaphragm and cardiac Purkinje fibers. Furthermore, different alternatively spliced isoforms are found in these two tissues. PCR of dystrophin mRNA's from diaphragm and Purkinje fibers was performed and compared to heart, skeletal muscle, and brain. We studied a region of dystrophin 3' mRNA (Bases 10,432 to 10,761) that undergoes alternative splicing in a distinct tissue specific pattern which is found to be highly conserved between murine and human tissues. For comparison, this region is primarily expressed as a full length mRNA in skeletal muscle, while at least three alternatively spliced isoforms are found in brain. The major brain form results from excision of 330 bp. Whole heart tissue shows a splicing pattern with both the muscle and brain predominant isoforms. As predicted, analysis of murine diaphragm showed only expression of the full length isoform prominent in skeletal muscle. In contrast, human cardiac Purkinje fibers express the 330 bp spliced isoform prominent in brain without expressing the 'muscle' isoform.. This unique finding suggests that alternatively spliced isoforms of dystrophin are biologically significant, and impart a tissue specific function which influences both muscle and specialized conduction tissue performance. The lethal manifestations of respiratory and cardiac conduction disease in DMD suggests that dystrophin function is important in diaphragm and Purkinje fibers. The focus of dystrophin cDNA expression experiments should include alternatively spliced isoforms to unravel the function of dystrophin in different tissues.

H 401 A MODEL OF HYPERTROPHIC CARDIOMYOPATHY IN FAMILIES OF GOLDEN RETRIEVER DOGS. K.K. Brown, C. Jones, J.E. Shaffer, H.J. Leighton. Glaxo Research Laboratories, Research Triangle Park, NC 27709.

We have used echocardiographic analysis to quantitate the effects of heritable left ventricular outflow tract (LVOT) obstruction in pure bred golden retrievers (GR). Of eight GR studied, three had marked evidence of LVOT obstruction and impaired ventricular relaxation. The changes in posterior wall dynamics are present in related GR without evidence of LVOT and with only minimal to mild left ventricular hypertrophy. FAC in this subset of animals appears to decrease with age. The mean values for affected GR vs. age-matched normal mongrels were as follows: peak wall thinning rates, normalized to instantaneous thickness, ($-9.28\ sec^{-1}$ vs. $-2.85\ sec^{-1}$); decreased peak wall thickening rate ($7.86\ sec^{-1}$ vs. -3.76^{-1}), decreased chamber filling rate, (12.01 cm/sec vs. 7.94 cm/sec); and decreased time-to-peak filling rate (48 msec vs. 80 msec). Mean fractional chamber area change (FAC) from end-diastole to end-systole (measured from the short axis view, high papillary muscle level) is 52.6% in control animals and 70.3% in affected GR. Electrocardiographic abnormalities, in affected GR include: ST segment changes and ventricular arrhythmias which are exacerbated by sudden increases in heart rate and afterload. Overall, in a clinical population of GR, sudden deaths prior to 4 yrs. of age are not uncommon. We feel the affected dogs will serve as a unique model in which to compare the structural and functional abnormalities to those found in human hypertrophic cardiomyopathy as well as provide insight as to appropriate pharmacologic interventions that may be useful in the treatment of this disorder.

H 402 CAPTOPRIL REDUCES FIBROSIS OF THE NON-INFARCTED MYOCARDIUM AFTER MYOCARDIAL INFARCTION, Jack P. Cleutjens, Mat F. Daemen, Kees van Krimpen, Jos F. Smits, Fred T. Bosman, University of Limburg, Maastricht, The Netherlands

A myocardial infarction in the rat, induced by ligation of the left coronary artery, leads to structural adaptations of the non-infarcted myocardium, including transient proliferation of interstitial cells and myocardial hypertrophy. We studied the role of fibrosis in this process of remodeling by measuring the amount of interstitial collagen in the myocardium, by Sirius red staining of tissue sections and morphometrical analysis, with or without treatment with the ACE inhibitor Captopril. A sustained increase in the collagen positive area after infarction was found in both the left ventricle (septum) and right ventricle from 7 days on to a threefold increase after 14 days compared to the sham operated animals. Continuous treatment with Captopril (500 µg/kg/hr) resulted in a decrease of fibrosis in the non-infarcted myocardium almost back to sham level compared to the non-treated infarcted rats. These findings demonstrate that remodeling of the left ventricle after MI includes fibrosis of the non-infarcted myocardium and that ACE inhibition reduces fibrosis. Increased myocardial fibrosis may have functional implications and will be determined by measurement of hemodynamic parameters.

H 403 SIGNAL-AVERAGED ELECTROCARDIOGRAPHY IMPROVES PATIENT CHARACTERIZATION IN HYPERTROPHIC CARDIOMYOPATHY AND AIDS LOCALIZATION OF A DISEASE GENE TO CHROMOSOME 2p IN A LINKAGE ANALYSIS STUDY, Neal D. Epstein, Mark Leppert*, Henry J. Lin, Ray White*, Jean Marc Lalouel*, Arthur Nienhuis, Lameh Fananapazir, National Institutes of Health, Maryland, and *Howard Hughes Medical Institute, Salt Lake City

We have previously reported that subjects who are obligate carriers of the gene for hypertrophic cardiomyopathy (HC) by virtue of their position in the pedigree, may have an abnormal signal-averaged electrocardiogram (SAE) but normal echocardiogram. To determine whether detection of late potentials (LPs) by SAE improves identification of patients with HC and thus assists localization of HC gene by linkage analysis studies, we performed SAE in 28 members of an HC family in whom multilocus linkage analysis had localized a disease gene to chromosome 2p (lod score 3.09 at Θ=0). SAE was performed using a Corazonix Predictor with a 25 Hz bidirectional filter. LPs were presents if the filtered QRS duration was $\geq$110 ms, Root-Mean-Square voltage was $\leq$25 μV, or Low Amplitude Signals (<40 μV) were $\geq$35 ms. Eleven (39%) individuals were found to have late potentials. Nine of these 11 had normal 2-D echocardiograms. When these 11 individuals were scored as affected, a significant increase occurred in the odds in favor of linkage (from 1000:1 to 10,000:1; lod score 4.0 at Θ=0), thus strengthening the evidence that the disease gene localizes to chromosome 2p in this family.

We conclude that this result lends credence to the ability of SAE to detect affected individuals without LV hypertrophy. Localization of an HC gene to chromosome 2p suggests several candidates for the critical gene, the most likely of which is ß fodrin.

H 404 REGULATION OF ATRIAL NATRIURETIC POLYPEPTIDE (ANP) GENE EXPRESSION IN ATRIUM AND VENTRICLE OF MURINE VIRAL MYOCARDITIS AFTER COMBINATION THERAPY OF IMMUNOMODULATOR Tsugiyasu Kanda, Tomoyuki Yokoyama, Shigeto Naito, Tadashi Suzuki, Kazuhiko Murata. Gunma University School of Medicine, Gunma, 371 JAPAN

Expression of the ANP gene is augmented in both atrium and ventricle of murine viral myocarditis due to encephalomyocarditis (EMC) virus. To elucidate the regulation of ANP synthesis in the heart on immunotherapy, we measured the ANP mRNA level in atrium and ventricle of 8-week-old C3H mice after EMC virus i.p. inoculation with or without treatment of immunomodulator. In saline treated group, expression of ANP gene was induced in ventricle (30-fold vs uninfected mice) rather than in atrium (3-fold) on 7 days after infection. Combination therapy of OK432, an immunomodulator, and interferon (IFN) improved motality and reduced myocardial damage. In OK432+IFN treated group, ANP gene expression was limited both in ventricle (6-fold) and in atrium (1.3-fold) on 7 days.

Conclusion: Immunomodulating therapy by OK432+IFN reduced gene expression of ANP in ventricle rather than in atrium on viral myocarditis, suggesting the restoration of ANP mRNA by immunological treatment.

H 405 CARDIAC MITOCHONDRIAL mRNA AND ULTRASTRUCTURAL CHANGES IN RATS TREATED WITH HIGH-DOSE AZT. William Lewis, Thomas Papoian, Basilio Gonzalez and Wayne W. Grody, Department of Pathology, UCLA School of Medicine, Los Angeles CA 90024-1732.

Rats were given drinking water *ad libitum* with and without zidovudine (azidothymidine, AZT) in concentration of 1 mg/ml. Mean AZT consumption was 111mg/kg/day. After 35 days of AZT therapy, myocardial RNA was rapidly extracted from both tap water controls and AZT-treated rat hearts and was subjected to Northern analysis. Blots were probed with a battery of cDNA probes specific for selected elements and compartments of the cardiac myocyte including (a) the sarcomeric thin filament: α_C-actin cDNA, troponin C (TnC) cDNA, (b) mitochondria: mitochondrial creatine kinase cDNA (mt-CK; courtesy of Dr. A Strauss, Washington U.), mitochondrial malate dehydrogenase cDNA (mMDH; courtesy of Dr. A. Strauss' group), mitochondrial genomic cDNA (mt-GX; courtesy of Dr. Sandy Williams, UTSW School of Medicine) and (c) glycolytic enzymes: glyceraldehyde-3-phosphate dehydrogenase (G3PD) cDNA. In parallel, transmission electron microscopy of samples from glutaraldehyde perfusion- fixed hearts from AZT-treated and control rats was performed. Results showed a relatively selective depression in cytochrome b mRNA hybridization signal from AZT-treated rat hearts probed with mt-GX cDNA. Neither 16S nor 12S mitochondrial rRNA signals from the AZT-treated rat hearts were significantly changed from those of untreated control rat hearts. Similarly, G3PD, α_C-actin, TnC, mt-CK, and mMDH RNA signals were found to be unaltered in Northern blots of extracted myocardial RNA from the AZT-treated group when compared to blots from untreated control rat hearts. Parallel transmission electron microscopy of perfusion-fixed hearts from AZT-treated and control rats revealed disruption of mitochondrial cristae but preserved sarcomeres and nuclei. High-dose AZT may disrupt cardiac mitochondrial structure and function by altering expression of mitochondrial mRNAs.

H 406 IN VIVO GENE TRANSFER INTO CANINE CORONARY AND PERIPHERAL ARTERIES, Chang S. Lim, Gregory D. Chapman, Roger S. Gammon, Joseph B. Muhlstein,. Robert P. Bauman, Richard S. Stack, Judith L. Swain, Department of Medicine, Duke University Medical Center, Durham, NC 27710.

In vivo techniques for genetically modifying endothelial cells may offer treatments for cardiovascular disease. Therapeutic strategies would include suppression of thrombosis and restenosis through the use of gene therapy. This study was conducted to develop techniques for direct *in vivo* gene transfer into coronary arteries. cDNA encoding luciferase was inserted 3' to the CMV promoter in an expression vector. Using liposome-mediated transfection, this plasmid was transferred into surgically exposed canine femoral and coronary arteries. After 3 days, the vessels were explanted from the dog and analyzed for luciferase activity. Assays of 8 femoral arteries and 2 coronary arteries revealed a mean expression of 19±9 pg and 30±1 pg of luciferase, respectively. Luciferase activities in nontransfected control vessels were negligible. In subsequent studies, we have accomplished direct gene transfer into coronary arteies using a percutaneous catheter delivery system. Thus we demonstrate a simple and effective method for the direct transfer of nucleic acids into vascular cells *in situ*.

H 407 **CONSTRUCTION OF A RADIATION MAP OF HUMAN CHROMOSOME 14 IN SOMATIC CELL HYBRIDS USING POLYMERASE CHAIN REACTION.** Adolph Mares, Jr., J. Fielding Hejtmancik, M. Benjamin Perryman, Susan A. Ledbetter, David H. Ledbetter, Robert Roberts.

Hypertrophic cardiomyopathy (HCM), a clinically heterogeneous disease, has been mapped to chromosome 14. To develop a physical map of chromosome 14 including the HCM locus, we used the technique of irradiation and fusion gene transfer, a method for the generation and transfer of chromosome fragments from one cell to another. The parent cell, HHW890, a mutant Chinese hamster ovary cell line containing human chromosomes 5 and 14, was used. Counterselection with 20 μM sodium chromate against cells containing human chromosome 5 yielded a somatic cell hybrid containing only human chromosome 14, MHR14. The presence of human chromosome 14 was confirmed by polymerase chain reaction (PCR) amplification of human short and long interspersed repetitive sequences using either Alu or L1Hs (IRS-PCR), and by G-banded chromosome analysis. MHR14 was lethally irradiated with 6,000 rads to produce fragments of chromosome 14 and subsequently fused to an hypoxanthine phosphoribosyl transferase deficient Chinese hamster cell line using polyethylene glycol. Fifty-one monoclonal radiation hybrids were isolated after selection in HAT medium. Forty of 51 hybrids tested contained Alu elements, and 12 of these 40 hybrids contained L1Hs elements. Polymerase chain reaction identified three of the 40 hybrids which contained CRI436, a marker near the HCM locus, 2 which contained locus D14S34, and 5 which contained myosin heavy chain β. Thus, radiation hybrids provide a means to produce chromosome fragments amenable to characterization by polymerase chain reaction. These techniques provide a specific and sensitive means to physically map genes such as HCM on chromosome 14 and, furthermore, are equally applicable to the mapping of diseases throughout the human genome.

H 408 LINKAGE ANALYSIS OF TWO FAMILIES OF HYPERTROPHIC CARDIOMYOPATHY

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Two families of Hypertrophic Cardiomyopathy were analyzed.Ultrasonography was performed to establish the statute of each member of the families.DNA was extracted digested with restriction enzymes,charged into agarose gels and transferred on GENE SCREEN filters.Two loci were explored:a/ Locus 14q1 using probes CRIL329, CRIL436,CRIC70 and pH2.3 (N. Engl. J. Med. 1989 321 1372-1378). b/ Locus 16q22 using probes Hp2 alpha and D16S4 (J. Med. Genet. 1990 27 363-366).Results were discussed using LINKAGE computer programm.

H 409 **A HIGH MOLECULAR WEIGHT SERUM PROTEIN INDUCED DURING CARDIAC HYPERTROPHY IN PATIENTS' WITH ATRIAL AND VENTRICULAR SEPTAL DEFECTS**
R. Prabhakar, M. Mariappan and C. Rajamanickam, Department of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, Tamilnadu, India.

We have previously reported the appearance and disappearance of a high molecular weight protein of 135 Kd in the serum, during developing caridac hypertrophy in aorta constricted rats (Archiv. Biochem. Biophys. 1990). At present, using antibody raised against the rat protein we have identified the presence of a similar protein in the sera of patients with Atrial Septal Defect (ASD) and Ventricular Septal Defect (VSD). The western blot analysis of the sera showed that this high molecular weight protein is present in considerable amounts in patients' sera when compared to the normal sera. Among the patients' sera this protein is present in induced levels in the sera of VSD patients than in the sera of ASD patients. Partial peptide map analysis of rat and human proteins reveals that these proteins may share extensive homology. Amino acid sequence analysis which is being carried out will further substantiate our results. The possible biochemical and molecular mechanistic roles played by this protein in myocardial cells in the development of cardiac hypertrophy in humans will be discussed.

H 410 LEFT ATRIAL THROMBOSIS IN TRANSGENIC MICE EXPRESSING THE EBV NUCLEAR ANTIGEN LEADER PROTEIN. Peter F. Searle and David Huen, Department of Cancer Studies, University of Birmingham Medical School, Birmingham B15 2TJ, England.
Epstein-Barr virus (EBV) is carried asymptomatically by the majority of the human population, but has been linked with Burkitt's lymphoma, nasopharyngeal carcinoma and several proliferative disorders in immunodeficient individuals. As part of an investigation into the role of specific EBV genes in cellular immortalisation and tumorigenesis we produced several lines of transgenic mice expressing the EBV-nuclear antigen-leader protein under the control of a mouse metallothionein promoter. The transgenic mice produced significant amounts of LP in many tissues including liver, kidney, heart, brain, spleen, thymus and skin, as shown by western blotting and immuno-fluorescence. Surprisingly, transgenic mice in five of these lines die prematurely after 1-2 days of visible symptoms, at ages ranging in different lines from around 4 months to a year. The morbidity and death are apparently caused by the presence of large organised fibrin thrombi within the left atrium of the heart. So far we have not detected any underlying defect in blood coagulation, and the severity of the condition (as reflected in earlier death) correlates with the level of LP expression in the heart, suggesting a local rather than systemic cause of the thrombosis. We have not detected any tumours or other abnormality in the structure of the heart on dissection or in light microscope sections. We suggest that LP may affect the expression of host genes in the atrium leading to fibrillation-induced thrombosis.

H 411 EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN MURINE HEARTS WITH ACUTE MYOCARDITIS CAUSED BY COXSACKIE-VIRUS B3, Yoshinori Seko, Ko Okumura, Fumimaro Takaku and Yoshio Yazaki, Third Department of Internal Medicine, University of Tokyo and Department of Immunology, Juntendo University, Tokyo, Japan.
Cell-mediated autoimmunity has been strongly implicated in the pathogenesis of viral myocarditis. Cell-cell contact and adhesion is required in the immune response, and ICAM-1, which is a ligand for LFA-1, plays an important role in this process. We investigated the expression of ICAM-1 in murine hearts with acute myocarditis caused by Coxsackievirus B3. We also analyzed the induction of ICAM-1 in cultured myocytes by cytokines by immunofluorescence and Northern blotting. ICAM-1 was strongly induced in ventricular myocytes and fibroblasts in acute viral myocarditis. This was also confirmed in vitro. These data suggested that the expression of ICAM-1 in heart cells facilitate adhesion and cytotoxicity of the infiltrating immune cells and may lead to dilated cardiomyopathy.

H 412 CARDIAC GENE EXPRESSION IN HYPOTENSIVE TRANSGENIC MICE OVEREXPRESSING ATRIAL NATRIURETIC FACTOR, Mark E. Steinhelper and Loren J. Field, Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN 46202-2859.

We have induced chronic increases (up to 10 fold) in the plasma concentration of the atrial natriuretic factor (ANF) hormone by targeting ANF expression to the liver of transgenic mice. Mice that inherit and consitutively express chimeric ANF genes are significantly hypotensive, by as much as 30 mm Hg, as compared to their nontransgenic siblings (Hypertension 16:301-307). Previous studies have shown that cardiac function can be altered by acute ANF infusion as well as by hemodynamic load. However, the chronic effects of elevated plasma ANF on cardiac structure, function, and gene expression are not known. Accordingly, the hearts of our transgenic mice have been examined to address these issues. We have determined that the transgenic mice have smaller hearts than their age-, sex-, and body weight matched nontransgenic siblings. This observation was specific for the heart; other major organs, such as the liver, kidney, and brain, did not differ between the transgenic and nontransgenic adults. Additional studies of cardiac growth during development should determine whether the small mass of the transgenic hearts results from decreased hyperplasia, decreased hypertrophy, or a combination of both. Finally, the expression of specific genes that are regulated differentially during cardiogenesis and in response to hemodynamic load are being evaluated.

H 413 TRANSCRIPTION OF NONMUSCLE MYOSIN HEAVY CHAIN DURING RESTENOSIS, Lawrence Weir, Guy Leclerc, and Jeffrey M. Isner, Tufts University School of Medicine, St. Elizabeth's Hospital, Boston MA 02135.

The major factor responsible for restenosis following angioplasty is the proliferation of vascular smooth muscle. Associated with this process is the replacement of smooth muscle myosin with the expression of nonmuscle isoforms. This is consistent with evidence that nonmuscle myosin is required for cytokinesis. Using cDNA clones for human nonmuscle myosin heavy chain isoforms (designated A and B) as hybridization probes we have investigated the regulation of expression of nonmuscle myosin heavy chain at the level of transcription. Samples of human atherosclerotic material obtained percutaneously with a mechanical atherectomy device were analysed for levels of mRNA for nonmuscle myosin heavy chain in restenotic lesions compared to native, nonproliferative lesions. We find that both isoforms are apparently transcriptionally activated during restenosis. More than simply a marker for restenosis in patients with vascular disease, the expression of nonmuscle myosin could be a key element of the abberrant proliferative phenotype of smooth muscle.

Late Abstracts

CARDIAC MUSCLE CROSSBRIDGE MECHANICAL INTERACTIONS DETERMINE ACTIN FILAMENT VELOCITY IN VITRO. N. Alpert, J. Peterson, and D. Warshaw. Univ. of Vermont, Physiol. & Biophys., Burlington, VT 05405

Alterations in hypertrophied cardiac muscle mechanics may be related to the proportion of V1 and V3 myosin isoforms expressed within a muscle fiber. From previous studies, we proposed that V3 myosin cycles slower and spends a greater fraction of its cycle in a high force state (i.e. increased duty cycle) compared to the V1 isoform. To directly test this hypothesis, we used an in vitro motility assay in which fluorescently labeled actin filaments were observed sliding over mixtures of V1 and V3 myosin monomers adhered to a glass coverslip. V1 cardiac myosin was prepared from thyroxin-treated rabbits, whereas V3 myosin was obtained from pressure overloaded rabbit hearts. Using V3 myosin, actin filament velocity (1um/s) was 1/3 that observed with V1 myosin, which correlated with differences observed in both Ca^{+2}- and actin-activated myosin ATPases. When actin filaments interacted with mixtures of V1 and V3 myosins, actin velocity was graded with the V3 myosin predominating. The modulation of actin velocity may reflect a mechanical interaction between the two isoforms. We have developed a quantitative crossbridge mechanical interaction model (Warshaw et al., J. Cell Biol. 111:453, 1990) for the motility assay based on the force:velocity (F:V) relations of the muscles from which the myosin was isolated. To test the model's predictive capabilities, F:V were obtained from thyroxin-treated and pressure overloaded rabbit papillary muscles to constrain the model parameters. The motility data suggest that V3 myosin cycles slower and has a greater duty cycle than the V1 isoform. In addition, when using myosin mixtures, the prime determinant of velocity modulation is associated with the myosin having the greater duty cycle. This may have profound implications for cardiac muscle power, economy, and efficiency. (Support:HL28001(NA);HL07647(JP);HL45161(DW))

FETAL CALF SERUM IMPROVES CONTRACTION AND K+ HOMEOSTASIS IN CULTURED VENTRICULAR MYOCYTES RECOVERING FROM METABOLIC INHIBITION

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To investigate factors contributing to impaired function of myocytes after recovery from prolonged ischemia, we subjected cultured chick ventricular myocytes (CVM) to prolonged metabolic inhibition (MI) (20mM 2-deoxyglucose plus 1mM cyanide for 1 hour) and then allowed cells to recover for 5 days in serum free medium. Cells remained viable as assessed by low LDH release, but failed to exhibit spontaneous contractions. To examine whether recovery from injury produced by MI could be influenced by culture conditions, we also allowed CVM to recover from MI in medium containing 6% fetal calf serum (FCS). We measured magnitude of contraction, K+ content (nmol/mg protein) and $^{42}K+$ uptake (nmol/mg protein/2 min) in both groups and in control cells. Contractions were vigorous and synchronous in CVM recovered in FCS, and FCS also increased myocyte K+ content and $^{42}K+$ uptake.

	Contractions	K+ contents	K+ uptake
No MI-SFM	+++	714±33*	47.6±3.4*
MI-SFM	-	501±47	37.3±1.9
MI-FCS	++++	681±31*	59.8±3.0*

(n=5~9, means ± SEM; *=p<0.05 vs MI-SFM

We conclude that alterations in K+ homeostasis may contribute to functional abnormalties in CVM recovering from MI, and exposure of CVM to components of FCS improves recovery of K+ homeostasis after prologned MI.

DENSITY-DEPENDENT REGULATION OF THE SKELETAL α-ACTIN AND α-MYOSIN HEAVY CHAIN GENES BY ß- AND α_1-ADRENERGIC PATHWAYS DURING MYOCARDIOCYTE HYPERTROPHY.

Nanette H. Bishopric*§ and Keith A Webster§ Dept. of Medicine, *Department of Veterans Affairs 111-C, U.C. San Francisco, 4150 Clement St., San Francisco, CA 94122 and §SRI International, Menlo Park, CA.

The skeletal α-actin (skα-actin) gene is induced in several models of myocardial hypertrophy *in vivo* and *in vitro*. In contrast, α-myosin heavy chain (α-MHC) gene expression is not up-regulated during norepinephrine (NE)-induced *in vitro*. In high-density primary neonatal rat myocardial cells (1.2 x 10^5 cells/cm^2), 48 hour exposure to 4μM NE induced cellular hypertrophy, increased skα-actin gene expression by 8-10 fold, and increased expression of a transfected full-length (-2000 to +187) human skeletal α-actin gene promoter linked to the marker gene CAT. NE and the ß-adrenoceptor-selective agonist isoproterenol were equally potent, and the order of potency of adrenergic antagonists was propranolol >> yohimbine>terazosin, consistent with a ß-adrenergic effect. Intracellular cyclic AMP and c-*fos* and c-*jun* mRNAs were also transiently increased by ß-adrenergic stimulation. Interestingly, sustained levels of cAMP achieved by addition of 2 mM dibutyryl cAMP did not increase skα-actin mRNA despite significant effects on cell growth and a 10-20 fold induction of proenkephalin mRNA. Expression of the α-MHC promoter (-3000 to exon I) in high density cells was strongly (>75%) inhibited by NE via an α_1-adrenoceptor dependent mechanism, demonstrating the integrity of this pathway under high density culture conditions. The receptor specificity of NE effects on growth and actin gene expression was a function of cell density: at low density (0.25-0.30 x 10^5 cells/cm^2), these effects were largely mediated by the α_1-adrenoceptor. We conclude that the ß-adrenoceptor has a major role in regulation of myocardiocyte hypertrophy and gene induction in culture which is dependent on cell density-related factors.

ATRIAL NATRIURETIC PEPTIDE (ANP) LOCALIZATION DURING INDUCED CARDIAC HYPERTROPHY IN RATS, Jennifer Breckler and Frank Torrano, Department of Biology, San Francisco State University, San Francisco, CA 94132

The development of cardiac hypertrophy may indirectly involve the ANP system in atrial cells. Cardiac hypertrophy was induced by a 50% reduction in abdominal aortic diameter between the renal arteries in groups of rats (n=4 each) which were later examined at 3,6, and 9 weeks post-operatively. Ventricles and left/right atria were treated with tetrodotoxin and compared for myocardial cell ultrastructure, and for atrial specific granules (ASG) using anti-ANP conjugated with colloidal gold for immunoelectron microscopy. Control animals (n=3) were sham-operated and sacrificed at the same time periods. Left ventricular weight index was greatest in the experimental group (0.55 ± .13) versus sham group (0.42 ± .01) at 6 weeks post-operatively. Atrial specific granule diameter in the left atria was significantly decreased in all experimental groups when compared to sham-operated animals using one-way ANOVA. In addition, the left atria in the 9-week post-operative group showed a significantly lowered number of ASG per cell profile compared to the right atrium. These findings suggest that during hypertrophy, ANP storage diminishes and ANP release is increased, which is possibly triggered by an increase in left atrial stretch.

INACTIVATION OF GAP JUNCTION CHANNELS IN CHICK VENTRICLE CELLS.
Robert L. DeHaan, Russell L. Penrod, and Yan-hua Chen. Department of Anatomy and Cell Biology, Emory University Center for Health Sciences, Atlanta GA 30322.
During development of the chick embryonic ventricle, AP conduction velocity increases roughly 100-fold between 1.5 and 4 days of incubation and another 10-fold throughout the rest of development. To understand the role of gap junction channels in AP propagation at these stages, gap junction channel conductance (G_j) between 7-day ventricle cell pairs was measured with double whole-cell voltage clamp technique as junctional current (I_j) elicited by rectangular pulses of applied transjunctional voltage (V_j). G_j (= I_j/V_j) was highest near 0 mV V_j, and was progressively reduced by application of V_j between 20 mV and 80 mV or between -20 mV and -80 mV. G_j decayed exponentially during voltage steps to 80 mV or -80 mV to about 28% of its value at the beginning of the step ($G_{j\text{-init}}$), reaching a steady-state value ($G_{j\text{-ss}}$) after several seconds. The relationship between $G_{j\text{-init}}$ and V_j was approximately linear between 0 and 80 mV or -80 mV, whereas the relationship between $G_{j\text{-ss}}$ and V_j was bell-shaped with the maximum at 0 mV. Records of single gap junction channels indicated that voltage-dependent inactivation of G_j results from channel closing or shifts of conductance levels from near 240 pS to 160, 80 and 40 pS sub-conductance states. Recovery from inactivation occurs a few seconds after returning to ±10 mV V_j, where 240 pS channels again predominate (Supported by NIH P01 HL27385 to RLD).

MOLECULAR CLONING OF VOLTAGE-GATED EPITHELIAL K CHANNEL ISOFORMS FROM RABBIT KIDNEY AND LLC-PK$_1$ CELLS. Gary V. Desir, Henry A. Hamlin, Robert F. Reilly, Friedhelm Hildebrandt, and Peter Igarashi.
Epithelial, voltage-gated potassium (K) channels have been well-studied using electrophysiological methods, but little is known about their structures. We tested the hypothesis that some of these channels belong to the *Shaker* gene family, which encodes voltage-gated K channels in excitable tissues. From published sequences of *Shaker* proteins in *Drosophila*, rat and mouse brain, we chose regions which were conserved between species. Based on these protein sequences, degenerate oligonucleotides flanking the putative voltage sensor (S-4) were synthesized and used as primers for the polymerase chain reaction. Five *Shaker*-like cDNAs were amplified from rabbit kidney cortex and 3 from LLC-PK$_1$, an epithelial cell line derived from pig kidney. Each partial-length rabbit kidney cloned cDNA is ~850 base pairs long. The deduced amino acid sequences contain 5 putative transmembrane segments and are 79 -97 % identical to 2 *Shaker*- isoforms expressed in rat brain (RBK1 and RBK2). Sequence similarity is greatest in the putative transmembrane segments, S1-S5. Importantly, the S-4 segment, the putative voltage gate is highly conserved in all 5 cDNAs. Southern hybridization of the different isoforms to genomic DNA indicates that they are encoded by different genes. The partial length LLC-PK$_1$ cDNAs are ~450 bp long and the deduced amino acid sequences are 77-99% identical to the rabbit cDNAs. This is, to our knowledge, the first demonstration that *Shaker*-like genes are expressed in renal epithelial cells. These genes most likely encode voltage-gated K channels involved in renal epithelial K transport.

CHARACTERIZATION OF CDNA AND GENOMIC CLONES OF HUMAN VENTRICULAR MYOSIN LIGHT CHAIN 2, Y. Du, Q-L. Wu, M. Raychowdhury and S. Sarkar
Dept. of Anatomy & Cell Biology, Tufts University Health Sc. Campus, Boston, MA 02111

Myosin light 2 (MLC2), coded by a multigene family, serves as a characteristic marker for fiber-specific myosin isozymes. As a first step towards the characterization of the gene for human ventricular MLC2 (HVLC2) and understanding its expression in normal and pathological human myogenesis, a cDNA clone containing 22 5' untranslated (UT) nucleotids, the entire coding region of 498 nucleotides and a 3'UT segment of 243 nucleotides, has been characterized from a lambda gt10 cDNA library of adult human ventricle mRNA. Comparative nucleotide and derived amino acid sequence analysis with other vertebrate cardiac MLC2 indicates that whereas the sequences in the coding region are highly conserved, the 3' and 5' UT segments show considerable divergence. Several characteristic domains in the coding region which are common among different vertebrate cardiac MLC2 are identified. Interestingly, a conserved domain in HVLC2 and human skeletal fast MLC2 cDNAs (nucleotides 108-285; 80% homology among the coded aa 37-96 with a subdomain of 20 aa, 77-96 having 100% homology) has been identified. The HVLC2 is coded by a single copy gene which is expressed in a tissue-specific manner. Southern blot analysis of human genomic clones in EMBL3 vector with the HVLC2 cDNA probe indicates the presence of an 8 kb strongly positive and another 5 kb weakly positive fragments obtained by Xho I digestion of a 13 kb insert from a single genomic clone. The characterization of the gene is now in progress.

Molecular Mechanisms of Cardiac Growth and Hypertrophy

HELIX-LOOP-HELIX PROTEINS IN CARDIAC CELLS, Sylvia M. Evans, Cathy B. Newton, Jackie S. Thorburn, Paul D. Gardner*, and Marc van Bilsen, Department of Medicine, University of California at San Diego, La Jolla, 92093 and *the Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire.

Both cardiac muscle and skeletal muscle derive from embryonic mesoderm. Several members of the helix-loop-helix (HLH) family, including MyoD, MyoD-like proteins (Myogenin, Myf5, MRF), and Twist, are involved in mesodermal determination. Accordingly, we are investigating the possible role of HLH proteins in cardiac determination. By RNase protection analysis, we have determined that transcripts of the HLH members id and pan1/pan2 are present in cardiac cells. Heterodimeric combinations of these widely-expressed proteins and MyoD result in activation or repression of skeletal muscle genes. Transient cotransfection assays performed in primary ventricular myocyte cultures indicate that the cardiac cell context is permissive for the activation of skeletal promoters by exogenous MyoD, suggesting that the cardiac pan1/pan2 RNAs may be functionally expressed. The promoters of two cardiac genes, myosin light chain 2 and atrial natriuretic factor, are not affected by the expression of MyoD in cardiac cells in high and low serum conditions. Cotransfection of id with myoD and reporter plasmids in cardiac cells decreases the myoD stimulation of skeletal promoters. However, id transfection does not affect expression of the two tested cardiac promoters in cardiac cells in either high or low serum conditions.

NUCLEAR LOCALIZATION OF BB CREATINE KINASE IN EMBRYONIC MUSCLE. David L. Friedman and M. Benjamin Perryman. Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

Creatine kinase (CK) isoenzymes participate in the supply of metabolic energy through the rephosphorylation of ADP at sites of high energy utilization. Within muscle cells, multiple isoforms of CK are distributed to specific cellular locations. The association of CK with specific cellular compartments and ATPases is suggestive of its participation in specific metabolic processes. The mitochondrial CK is localized to the outer surface of the inner mitochondrial membrane where it catalyzes the synthesis of creatine phosphate at the expense of matrix generated ATP. The MM isoform is localized to the M line of the myofibril and the sarcoplasmic reticulum, where it is coupled to contraction and calcium transport, however little is known regarding the functional association or subcellular distribution of BB-CK. We therefore analyzed three types of muscle, cardiac, skeletal and smooth muscle, each of which express multiple forms of creatine kinase including BB-CK. Synthetic peptide immunogens derived from the CK sequence were used to produce antisera for the B, M, and mitochondrial CK subunits. These antisera were evaluated by Western blot analysis using purified CK standards and tissue extracts and recognize only a single isoform subunit on immunoblots and show no cross-reactivity with other isoforms of CK. In the developing rat embryo, M and B-CK immunoreactivity is detected first in the myocardium as early as 12 days. At embryonic day 16, B-CK immunoreactivity is also present in myotomes, where the intracellular distribution is both cytoplasmic and nuclear. This is similar to the intracellular distribution of B-CK immunoreactivity in the brain. In the adult rat, BB-CK is expressed in myocardium and smooth muscle, but not skeletal muscle. In smooth muscle the distribution of B-CK immunoreactivity is entirely associated with the nucleus. The nuclear localization of B-CK immunoreactivity is consistent with the presence of a nuclear targetting sequence within the B-CK primary sequence which is highly homologus with the nuclear targetting sequences of receptors for steroids, thyroid hormone, retinoic acid, and vitamin D metabolites. We suggest that BB-CK may serve to supply energy to support nuclear biosynthesis during development.

CALMODULIN-INDUCED ATRIAL ABNORMALITIES IN TRANSGENIC MICE

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Studies in our laboratory have shown that calmodulin (CaM) mediates many of the growth-regulating effects of Ca^{2+} in non-muscle cells grown in culture. Recent studies indicate that transient increases in cytosolic Ca^{2+} levels may trigger the induction of cardiac hypertrophy. To examine the potential role of CaM in cardiac myocyte growth regulation *in vivo*, we produced transgenic mice carrying a CaM minigene regulated by the human ANF promoter. Northern hybridization and immunohistochemical analyses were used to document three lines of transgenic mice in which levels of CaM mRNA and CaM protein were markedly increased over controls. Preliminary light microscopic examination demonstrated bilateral thickening of the atrial myocardium in transgenic offspring from two lines. An increased mass of atrial tissue was further suggested by the presence of markedly enlarged P waves recorded from two electrocardiographic limb leads. Transgenic mice from the third line were the most severely affected, with six of nine animals dying suddenly at 6-30 days of age. Light microscopy of postmortem tissue revealed atrial myocyte hypertrophy and injury, evidenced by the presence of contraction band necrosis, fibrosis, and inflammatory cell infiltrates. In approximately half of these animals, atrial dilatation and mural thrombi were also apparent in the left atrium where pathologic changes were most notable. Immunostaining suggested that among the three groups of transgenic mice, cardiac CaM levels were highest in the most severely affected line. These results indicate that CaM influences the cellular mechanisms governing myocyte growth *in vivo*, but at very high levels may impair myocyte viability.

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MECHANISMS OF ENDOTHELIN ACTIVATION OF SARCOMERE ASSEMBLY AND CARDIAC GENE EXPRESSION IN VENTRICULAR MYOCYTES: A PARACRINE MECHANISM FOR MYOCARDIAL CELL HYPERTROPHY, A. N. Harris, H. E. Shubeita, K. U. Knowlton, C. Glembotski, J. H. Brown and K. R. Chien, Departments of Medicine, Pharmacology and the Center for Molecular Genetics, UCSD, La Jolla, CA. 92093; and the Biology Department and Molecular Biology Institute, SDSU, San Diego, CA. 92182
Previous studies have suggested that endothelin may influence the growth and differentiation of several distinct cell types. To directly examine the effects of endothelin on myocardial cell hypertrophy, we utilized a well-characterized cultured neonatal rat myocardial cell model, in which a hypertrophic response can be assessed by increases in myocardial cell size, an increase in the assembly of an individual contactile protein (myosin light chain-2) into organized contractile units, accumulation of contractile proteins, and the activation of a program of immediate-early gene (c-*fos*/c-*jun*/*Egr*-1) and contractile protein and embryonic gene expression (a 10-20 fold increase in the steady state level of ANFmRNA). Utilizing these criteria we document that endothelin-1 can induce myocardial cell hypertrophy, and can activate the transcription of ANF luciferase and MLC fusion genes in transient assays. Utilizing a series of deletional ANF-luciferase constructs, a 315bp fragment of the ANF 5' flanking region, which contains consensus CRE, AP-1, and Egr-1 sites, has been implicated in inducible expression during myocardial cell hypertrophy. Thus, endothelin-1 stimulation may represent an important paracrine mechanism for cardiac hypertrophy. Utilizing c-*fos* and *Egr*-1 expression and antisense vectors, and co-transfection approaches, the direct role of c-*fos* and *Egr*-1 in endothelin and α adrenergic activation of MLC-2 and ANF gene expression has been studied. Co-transfection of a c-*fos* expression activates an AP-1 dependent luciferase reporter gene (~30 fold), but has no effect on activating the expression of an ANF-luciferase or MLC-luciferase reporter gene. Results of these and related studies of the inducible expression of these two cardiac genes will be presented.

CHANGES IN N-CADHERIN LOCALIZATION DURING EARLY HEART DEVELOPMENT, Kersti K. Linask, Division of Cardiology, Department of Pediatrics, The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA 19104
N-cadherin (N-CD), a Ca^{++}-dependent cell adhesion molecule, has been localized previously to the mesoderm during chick gastrulation and to the beating avian heart. However, a systematic study of N-cadherin localization in the critical early stages of heart development has been lacking. The presented work defines the spatial and temporal expression of N-cadherin in chick and quail heart forming regions between Hamburger and Hamilton stages 4-12, i.e. 18-48 hrs of development. During gastrulation N-CD is localized in the heart forming region mainly at areas of cell-cell junctions in more centrally located areas of the mesenchyme. As development proceeds to form the pericardial coelom (stages 6, 7, and 8), a noticeable high level of N-CD is seen on precardiac cells lining the coelom. This pattern of localization indicates a possible role in the splitting of somatic and splanchnic mesoderm to form the coelom. The localization on precardiac splanchnic mesoderm eventually spreads around the periphery of the cells. Precardiac cells closer to the endoderm do not express N-CD. In the tubular beating heart N-CD is found throughout the myocardium including intercalated disc junctions. Immunostaining of precardiac mesoderm explants after 24 hrs incubation indicates that differentiating quail endothelial cells, as detected by QH-1 antibody, initially express both N-CD and QH-1 antigen. QH-1 is only maintained by the differentiating endothelial cells and N-CD continues to stain the differentiating cardiomyocytes. The N-CD is often closely associated with actin. Immunostaining patterns of N-cadherin during early cardiogenesis suggests a major role in the modeling of the heart forming area. Its continued expression during cell commitment and differentiation of the cardiomyocyte and the loss of its expression on endothelial cells substantiate the contention of its being an important morphoregulatory molecule. [This work was supported by the American Heart Association, Southeastern Pennsylvania Affiliate].

Candidate regulatory proteins expressed during avian cardiac development. Judith Litvin, Michael Montgomery, David Goldhamer, Charles Emerson Jr. and David Bader. Cornell University Medical College, Department of Cell Biology and Anatomy, NYC, New York.

Recent work has identified a helix-loop-helix family of DNA-binding proteins. Several of these proteins have been shown to be important in cell lineage determination (i.e. MyoD1, twist, E12, etc.). We used a polyclonal antibody generated against the second helix (helix II) of the MyoD1 family of proteins and demonstrated by immunofluorescence specific reactivity with cardiogenic cells at stage 11, the initial differentiative period of heart development. Western blot and immunoprecipitation analyses identified a protein of 50Kd.

Using the electromobility shift assay we identified DNA-binding proteins in cardiogenic cells. Total cellular tissue protein extracts from stage 11 and day 14 hearts were incubated with oligonucleotides corresponding to the troponin I and muscle creatinine kinase enhancers that contain the MyoD1 binding site and separated on native polyacrylamide gels. Protein(s) in the **stage 11 heart** specifically bound to both oligonucleotides giving band shifts different for each oligo. In addition, the helix II antibody altered the mobility of one of the bands resulting in a double-shift. This data suggests that regulatory proteins containing epitopes homologous to the conserved HLH motif are present during the early differentiative stages of cardiac development and are candidates for transcriptional activators.

EVIDENCE FOR ACTIVIN BETA$_A$ IN EARLY EMBRYOS AND ENDODERM-CONDITIONED MEDIUM, John Lough, Nighat P. Kokan-Moore and David L. Bolender, Department of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226

To identify endoderm-secreted proteins that may influence morphogenetic processes during embryogenesis, endoderm from stage 5-8 embryos was cultured in defined medium. Fluorography of secreted proteins in the conditioned medium revealed a polypeptide set that was independent of embryonic stage when the explants were removed or duration in culture. More than 10 bands were detected, the most prominent migrating at 200, 25 and 17 kD. ELISA and Western blot analysis for known matrix proteins and growth factors revealed the presence of fibronectin and, most remarkably, activin beta$_A$, which may represent the 17 kD protein. Immunocytochemical assessment revealed that, at stages 5-15, activin beta$_A$ was confined to extracellular matrix in regions subjacent to ectodermal and endodermal cells, with fingerlike projections penetrating the interior of the embryo. These results suggest that activin beta$_A$ is a major secretory product of endoderm in amniotic embryos. Studies are in progress to evaluate the effects of activin beta$_A$ on the in vitro differentiation of pre-cardiac mesoderm, which we have noted to be influenced by the presence of endoderm (Ann. N. Y. Acad. Sci. 588:421; 1990). (Supported by HL 39829)

MOLECULAR CLONING OF THE MOUSE MITOCHONDRIAL CREATINE KINASE USING POLYMERASE CHAIN REACTION SCREENING. Tony S. Ma and Robert Roberts, Section of Molecular Cardiology, Department of Medicine, Baylor College of Medicine, Houston, TX 77030

Polymerase chain reaction (PCR) amplification can be employed to identify DNA sequence from minute quantities of DNA. PCR can be used in conjunction with DNA filter hybridization technique in the cloning and identification of a cDNA sequence from a cDNA library which facilitate significantly conventional cloning and subsequent subcloning procedures. It is known that amplified cDNA library such as the commonly commercially available cDNA libraries may have distorted sequence representation of a particular sequence of interest due to processes such as differential efficiency of phage amplification. We now present the method of screening a λgt11 cDNA library with a single known oligonucleotide sequence. A 30-mer oligonucleotide (5'ATCCAAGAACGAGGCTGGGAGTTCATGTGGAATGA-3') was synthesized based on known conserved sequence between human ubiquitous and sarcomeric mitochondrial creatine kinase (Mito CK), but differed at the 3' nucleotide positions with the cytosolic M and B subunit sequence. This was used in conjunction with the λgt11 forward or reverse sequencing primer to determine the minimal titre of a commercial cDNA library required to obtain a predicted DNA fragment using PCR amplification. Twenty 90mm bacterial lawns were each infected with 500,000 pfu and amplified. The resultant amplified phage sublibrary was tested for the presence of the desired clone. The results show a frequency of 1 positive clone in 1 million pfu in the original library. Five fold excess of the recombinant number in the sublibrary (i.e. 2.5 million pfu) so identified was then used to infect and create a second set of sublibraries each containing 100,000 pfu. Through several rounds of such sublibrary screening with PCR it is then possible to isolate the Mito CK through one single round of filter hybridization at low plaque density. Antisense primer is used to delineate clones with different 5'-extension. These results indicate that with the PCR screening technique one can identify a desired clone, even if the representation is very low which is not feasible with conventional filter hybridization. At the same time, this procedure is not compromised by the known mutation events that can be generated by PCR cloning.

EXPRESSION OF THE α-MYOSIN HEAVY CHAIN GENE IS REGULATED IN PART BY CARDIAC-SPECIFIC TRANSCRIPTION FACTOR 1. **Bruce E. Markham and Rebecca S. Brogan, Department of Physiology, Medical College of Wisconsin, Milwaukee, WI 53226.**

α-Myosin heavy chain (MHC) promoter reconstitution experiments identified a 35-bp DNA sequence element (proximal regulatory element B; PRE B) which conferred expression, in cardiomyocytes, to an otherwise inactive promoter element containing α-MHC sequences from -214 to +111. PRE B augmented both basal and thyroid hormone-induced expression, was active in either orientation, and its activity was not strictly dependent upon its position. This element was found to interact specifically with a protein found only in rat heart nuclear or cardiomyocyte whole cell extracts. Initial characterization of this protein has shown that it binds to a 10-bp sequence within PRE B and that an alteration of this site which sharply decreases the apparent affinity of the protein for this site also results in a loss of "enhancer-like" activity. This protein has been named cardiac-specific transcription factor 1 (C-STF1). C-STF1 has an apparent molecular weight of 35,000. The direct correlation between factor binding and functional activity and the cardiac-limited distribution of C-STF1 strongly suggest that this protein play a role in cardiac-specific expression. Evidence is also presented which demonstrated that other elements act in concert with the C-STF1/PRE B complex to regulate the α-MHC gene in the heart.

Atwist: An Avian homologue of Drosophila twist and Xenopus Xtwi.
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The twist gene (a member of the helix-loop-helix family) first identified in Drosophila is required for mesodermal differentiation. Drosophila embryos homozygous for the twist mutant fail to gastrulate and lack mesoderm and internal organs. A homologous gene, Xtwi, has been cloned in Xenopus and is present only in mesodermal cells.

RT-PCR with gene-specific primers using RNA from stage 5 whole chick embryos (just post-gastrulation) or one week post-hatch chick hearts amplified a product that hybridized strongly with Drosophila twist cDNA by Southern blot. The band, 125bp, is the same size as the band amplified from Xenopus stage 16 RNA, but shorter than the 265bp band amplified from Drosophila 2-6 hour RNA used as positive controls. Northern blot analysis of RNA isolated from embryonic and adult chicken hearts and probed with the Drosophila twist cDNA identifies transcripts of 1.5 Kb in embryonic day 5, 9, 14, posthatch day 7 and adult hearts. Amplifying out of chicken genomic DNA gives a much larger band ($\leq$2kb), therefore the products amplified by RT-PCR represent mRNA and not contaminating genomic DNA. This protein conserved across species and present during gastrulation (and apparently much later) may be as critical for commitment and differentiation processes in the Vertebrates as it is in Drosophila.

DETECTION OF ALL 6 COXSACKIEVIRAL SEROTYPES BY POLYMERASE CHAIN REACTION,
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Epidemiologic evidence supports a role for Coxsackieviral (CV) infection in the pathogenesis of acute myocarditis; in addition, recent reports have suggested that chronic persistence of the virus may occur in patients with idiopathic dilated cardiomyopathy. However, no simple means of detecting viral infection is available. Dot blot hybridization is nonspecific; in situ hybridization is laborious. Accordingly, using oligonucleotide primers with homology to the 5' noncoding region of CV B4, we synthesized viral cDNA by reverse transcription (RT) and amplified by the polymerase chain reaction (PCR) (30 cycles, annealing temperature=72°C). RNA purified from all 6 Coxsackie B serotypes yielded a 234 base-pair band. Southern analysis showed the band to be homologous to a an internal probe specific for the CVB 5' noncoding region. Controls including the related picornaviruses Coxsackie A21, poliovirus, and influenza virus yielded no signal. The origin of the band was confirmed as CV by subcloning and sequencing. Total RNA from weanling mice infected with CV B3 and suckling mice infected with CVB4 produced a 234 base band following RT and PCR; RNA obtained from uninfected hearts yielded no band. Three mg of infected tissue was needed to yield a detectable band; this is less than the weight of a myocardial biopsy sample. PCR of CV is feasible on small samples of myocardial tissue. This will permit determination of the natural history of human myocardial infection and assessment of the role of immunosuppressive therapy in patients with and without persistent CV infection.

STRETCH ACTIVATED CHANNELS OF CHICK CARDIAC MYOCYTES,
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We observed stretch-activated channels (SACs) evoked by membrane stretch in cells from primary cultures derived from chick embryonic heart. There are four classes of SACs based on their conductances and ionic selectivities. With 150 KCl in the pipette, three of them show linear IV curves having conductances of 25, 100 and 200 pS. The remaining one inwardly rectifies and has a conductance of 25 pS at low voltages. We investigated in detail the γ25 channel as this is more sensitive to stretch and is more frequently observed. This channel is weakly selective between K^+ and Na^+. The open channel noise is lower when K^+ carries the current and it is lower still when most extracellualr ions are replaced with sucrose. In addition to the major monovalent cations, Ca^{2+}, Mn^{2+} and Cs^+ are also permeant; but Mg^{2+} and Ba^{2+} are impermeant. The reversal potential (E_{rev}) for this channel is -50$\pm$12 mV in normal saline. The channel is blocked by TTX at a concentration of 12 μm and Gd^{3+} at a concentration of 20 μM. The other channels, γ100 and γ200, are K^+ selective. The P_K/P_{Na} ratio is 80 for the γ100 and 30 for the γ200 channels according to the GHK equation. The inwardly rectifying channel is weakly selective between K^+ and Na^+. These SACs occur in every possible combination in a single patch. It is known that stretching cardiac tissue leads to changes in automaticity and contractility and SACs may account for these effects. SACs with both inward and outward currents may be required to permit calcium influx without causing excessive depolarization.

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PARASYMPATHETIC INFLUENCES ON EMBRYONIC HEART GROWTH *IN OCULO*, Diane C. Tucker and Timothy Love, Dept of Psychology and Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294

The anterior eye chamber culture system provides a unique opportunity to study the interaction between sympathetic and parasympathetic innervation in controlling cardiac growth and differentiation. When grafted *in oculo*, hearts from 12 day gestation embryos attach to the iris and become innervated and vascularized. By most morphologic and biochemical criteria, embryonic heart grafts differentiate into mature myocardium. When sympathetic innervation of heart grafts is prevented by eye chamber sympathectomy (SCGx), growth is severely compromised. To examine the interaction of parasympathetic and sympathetic innervation in controlling graft growth, eye chambers were parasympathectomized by ciliary ganglionectomy (CGx) (N=22). In eye chambers with sympathetic innervation intact, CGx significantly compromised growth (5.46 $\pm$ .84 vs 7.99 $\pm$ 1.33 mm^2). However, in SCGx eye chambers, CGx did not further compromise growth (4.48 $\pm$ 1.03 vs 4.56 $\pm$ .95 mm^2). To determine whether CGx affected graft growth by reducing muscarinic receptor stimulation, host rats were treated chronically with atropine sulfate (5 mg/kg/day) administered by osmotic minipump (N=11) and compared to controls (N=11). The efficacy of the blockade was confirmed by absence of pupillary and graft beating rate responses to light stimulation and to carbachol. Chronic muscarinic blockade did not alter growth of grafts. Together, these experiments suggest that while parasympathetic innervation does interact with sympathetic innervation to influence embryonic heart growth *in oculo*, these effects are not transduced through muscarinic receptor stimulation.

CYCLIC-AMP REGULATES MULTIPLE ADAPTIVE RESPONSES TO HYPOXIA IN CARDIAC MYOCYTES, Keith A. Webster*§ and Nanette H. Bishopric*≠, *Department of Molecular Biology, SRI International, Menlo Park, CA 94025, §Department of Biochemistry, USC School of Medicine, Los Angeles, CA, and ≠Department. of Medicine, V.A. Medical Center 111-C, U.C.S.F., SanFransisco, CA. The occurence of non-contracting yet viable myocardiocytes within ischemic zones of the myocardium is well established. Reduced or eliminated contraction of cells in these regions of lower oxygen tension may in fact be an adaptive, energy conserving mechanism to prevent or postpone irreversible cell damage. The molecular mechanisms which mediate the contractile responses to acute or chronic ischemia/hypoxia are not fully understood. Spontaneously and synchronously beating neonatal rat myocardiocytes stopped contracting after 2 to 3 days exposure to a hypoxic atmosphere (pO_2=10-20mmHg). The period leading to contractile failure was accompanied by a series of metabolic changes including induction of anaerobic glycolysis, a 30-40% decrease in ATP content and a 3 fold decrease in cyclic-AMP (cAMP). Beating resumed following exposure of the cells to isoproterenol (2-5min) or reintroduction of oxygen (6-12H) both of which restored cAMP close to control levels. These changes were not apparent in control primary non-myocardial cells isolated from the same hearts. The depressed level of cAMP in the chronically hypoxic myocardial cells was found to mediate multiple additional effects on cell functions including changes in the expression of cAMP regulated genes, changes in cAMP dependent protein kinase activities, and reduced responses to exogenous cAMP regulators. We propose that cAMP is part of a complex receptor and signalling system that mediates adaptive survival responses in hypoxic, "hibernating" myocardial cells

A CONSERVED 28 bp ELEMENT (HF-1) IN THE RAT CARDIAC MYOSIN LIGHT CHAIN-2 GENE CONFERS CARDIAC SPECIFIC AND α-ADRENERGIC INDUCIBLE EXPRESSION IN CULTURED NEONATAL RAT MYOCARDIAL CELLS. H. Zhu, S. Navankasattusas, A. V. Garcia, R. S. Ross, S. M. Evans and K. R. Chien, Department of Medicine and Center for Molecular Genetics, UCSD, La Jolla, CA. 92093

The rat cardiac myosin light chain-2 gene has served as a valuable model system to study the *cis* and *trans*-acting factors which mediate cardiac specific and inducible expression during myocardial cell hypertrophy. As assessed by RNase protection studies with mouse and rat cardiac MLC-2 cDNA probes, the cardiac MLC-2 gene is expressed in neonatal rat myocardial cells, but not in SOL8 mouse skeletal muscle cells. A 2.7KbMLC-luciferase fusion gene is expressed at high levels in cardiac cells, but not in CV-1 cells (70 fold; cardiac vs. CV-1) and is not significantly upregulated during the differentiation of SOL8 myoblasts to myocytes. In transient assays employing a series of MLC-2 luciferase constructs, a 250 bp fragment has been identified which is sufficient for both cardiac specific and α adrenergic inducible expression. The fragment contains three regions (HF-1, HF-2, HF-3), which are conserved between the chick and rat cardiac MLC-2 genes suggesting their potential role in the regulated expression of this contractile protein gene. As assessed by substitution mutations within each of the conserved regions, the present study demonstrates that HF-1 and HF-2 are important in both cardiac specific and inducible expression (α adrenergic and endothelin-1 induced hypertrophy), while HF-3 has no detectable role in the regulated expression of the MLC-2 gene in transient assays. HF-1 sequences confer cardiac specific expression to a thymidine-kinase luciferase construct, but have no significant effect in the skeletal muscle or non-muscle cell context. The HF-1 element has a ~ 2 fold effect on adrenergic inducible expression in neonatal cardiac cells. Thus, a new cardiac specific element (HF-1) has been identified which plays a role in both cardiac specific and inducible expression.