Contractile Elements

CE01

COMPARISON OF VENTRICULAR AND ATRIAL ADULT RAT CARDIOMYOCYTES IN LONG-TERM CULTURE AND IN COCULTURE: CELL-CELL CONTACT PROTEINS AND ANF STORAGE AND SECRETION.

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Adult rat cardiomyocytes (ARC) in long-term culture undergo a sequence of morphological changes starting from a rod-shaped form and resulting in a nearly confluent layer of contracting cells. During redifferentiation the cells also resume hormonal activity; the activation of a fetal gene program a.o. the reexpression of atrial natriuretic factor (ANF) is reminiscent of that seen in overload hypertrophy in vivo. Cell-cell contact sites form intercalated-disc like structures with typical expression of adhesion and gap-junctional proteins. Cell contacts between cells in atrial or in ventricular ARC cultures as well as in cocultures show no difference in composition regarding N-Cadherin or Connexin-43 as shown by immunocytochemistry. In cocultures metabolic and electrical coupling through gap junctions seems to be functional as it is seen by the transfer of lucifer yellow. The two cell types in their redifferentiated form can be discriminated by the distribution of ANF and by the expression of the atrial-specific myosin light chain II. The disparate influence of longterm administration of bFGF or IGF-I on ANF expression in ventricular and atrial ARC is demonstrated.

CE02

ANALYSIS OF BIOGENESIS OF HEART CYTOARCHITECTURE "IN VIVO" BY CONFOCAL MICROSCOPY

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During myofibril assembly in cultured neonatal rat cardiomyocytes (NRC) and adult rat cardiomyocytes (ARC) numerous stress fiber-like structures (SFLS) are present suggested to serve as a scaffold for the new myofibrils. The SFLS are found inserting at vinculin positive sites, also called subsarcolemmal adhesion plaques (SAPs), which play an important role as potential nucleation sites in the generation of new myofibrils. Since some of these structures might be adaptation to culture conditions and not always reflect the structural requirements of cardiac myofibrillogenesis, the emergence of myofibrils was also investigated in the developing heart.

Whole mount preparations of early stages of chicken heart rudiments, when beating starts, stained with antibodies against myofibrillar and cytoskeletal proteins were analysed by confocal microscopy. We have found that in the chicken cardiac rudiments the nascent myofibrils are in close association with membranes and are not specifically associated with the perinuclear region, in contrast to the myofibrils of cultured cells. There is not an extensive network of SFLS and also the vinculin positive sites are less prominent if compared to the situation in cultured cardiomyocytes.

CE03

EFFECTS OF IGF, FGF AND T3 ON CYTOSKELETAL AND MYOFIBRILLAR STRUCTURES IN ADULT RAT CARDIOMYOCYTES IN CULTURE

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Adult rat ventricular cardiomyocytes in long-term culture undergo extensive remodelling concomitant with re-expression of fetal cytoskeletal and myofibrillar proteins. In controls and with insulin-like growth factor (IGF-I), the myofibrils follow the actin stress fibre-like (sfl) structures in growing out into the cell periphery. In contrast, basic fibroblast growth factor (bFGF) and triiodothyronine (T3) restrict myofibrillar growth with a sharp boundary to the perinuclear region. Nevertheless these cells contract more rapidly than controls. Cells with a sharp myofibrillar boundary amount to 1-12% in controls or with IGF-I, but with bFGF they reach 50% and with T3 even 74%. Staining of T3 or bFGF treated cells with an antibody against pan-myosin shows cross-striation of myosin in myofibrils in the cell center as well as a non-striated pattern along the sfl-structures into the periphery. The fetally occuring α-smooth muscle actin (α-sm-actin) becomes transiently reexpressed in the sfl-structures in control cells. It is down-regulated by IGF-I, whereas bFGF and T3 have the opposite effect. With bFGF or T3 the most dense packing of α-sm-actin is found just outside the myofibrillar area. In these cases α-actinin shows a similar intense staining outside the boundary .

CE04

INTERACTION OF THE REGULATORY LIGHT CHAIN WITH THE MYOSIN HEAVY CHAIN

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The regulatory light chain (RLC) can be crosslinked to the heavy chain (HC) with thiol-specific bifunctional reagents with a length of more than 6Å. We showed that the RLC gets crosslinked via its Cys-128 to the Cys-815 which is located directly upstream from the IQ motif in the HC that it characteristic for calmodulin binding sites (Schaub et al., Biophys. J. 68: 329s, 1995). The binding site on the HC comprises 35 amino acid residues from 808-842 and is highly hydrophobic (Rayment et al., Science, 216: 50-58, 1993). To test the binding site on the RLC we produced the following fragments: 1–127 (containing the EF-hand domains I, II and II), 128–169 (domain IV), 12–169 (containing all 4 domains but missing the N–terminus) and 60–169 (Domains II, III and IV). Cleavage points were verified by micro-sequencing. Binding of the fragments was tested on myosin whose RLC was removed previously. Neither fragment 1–127 nor 128–169 did recombine. In contrast, fragments 12–169 and 60–169 bound in stoichiometric amounts. Fragment 84–169 was found to associate with the HC by Michell et al. (Eur. J. Biochem. 161: 25-35, 1986). RLC domains I–III are then not sufficient for binding. Domain IV alone does not bind either, but is essential for association with myosin. The RLC binding site must be distributed over a stretch containing domains III and IV with their connector regions.

CE05

A MYOSIN-II HEAVY CHAIN GENE FROM *HYDRA VULGARIS* (HYDROZOA, COELENTERATA)

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A cDNA library of *Hydra vulgaris* was screened with a monoclonal antibody against myosin from the marine Cnidarian *Stomotoca atra* (V. Schmid, Basel). Positive clones were sequenced and analyzed using GCG. The nucleotide and the deduced amino acid sequences showed high homologies to myosins from other organisms. The clones represent overlapping fragments of a single 'myosin II heavy chain'-gene with one single open reading frame over the 1617 sequenced base pairs. The nucleotide codon usage shows the *Cnidaria*-characteristic strong bias for A or T in the third positions (70%) of the putative *Hydra*-myosin gene. The deduced amino acid sequence includes the typical heptad repeat of hydrophobic amino acids found in the α-helical coiled coil tail domain of myosin-II molecules. In situ hybridizations on whole polyps and on dissociated cells showed no region or cell type specific expression of the gene.

CE06

Contractile Cells in the Mammalian Lung

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Smooth muscle cells have been shown to display important differences in phenotype and in their protein composition. In particular within the vascular wall, smooth muscle cell heterogeneity is a well investigated phenomenon. So far, little is known about the functional reasons for the observed differences.

We have investigated the expression of genes encoding for contractile and cytoskeletal proteins in the mammalian lung. This organ contains different populations of smooth muscle cells in major blood vessels and in the bronchial tree, while distal regions hold cells with partial smooth muscle characteristics (myofibroblasts). In an attempt to correlate the presence of so called 'smooth muscle markers' with contractility of these cells, we have compared the protein profiles of pulmonary smooth muscle cells and myofibroblasts in vivo with the expression of such markers in a human foetal lung cell line (IMR-90). We have found that not only in the expression of smooth muscle markers but also in contractile efficiency these cells hold a position intermediate between that manifested by fully differentiated smooth muscle cells and fibroblasts.