IMMUNOCYTOCHEMISTRY OF CYTOSKELETAL PROTEINS OF ADULT RAT CARDIOMYOCYTES IN VITRO AFTER CELL ISOLATION AND TREATMENT WITH DRUGS

THESIS

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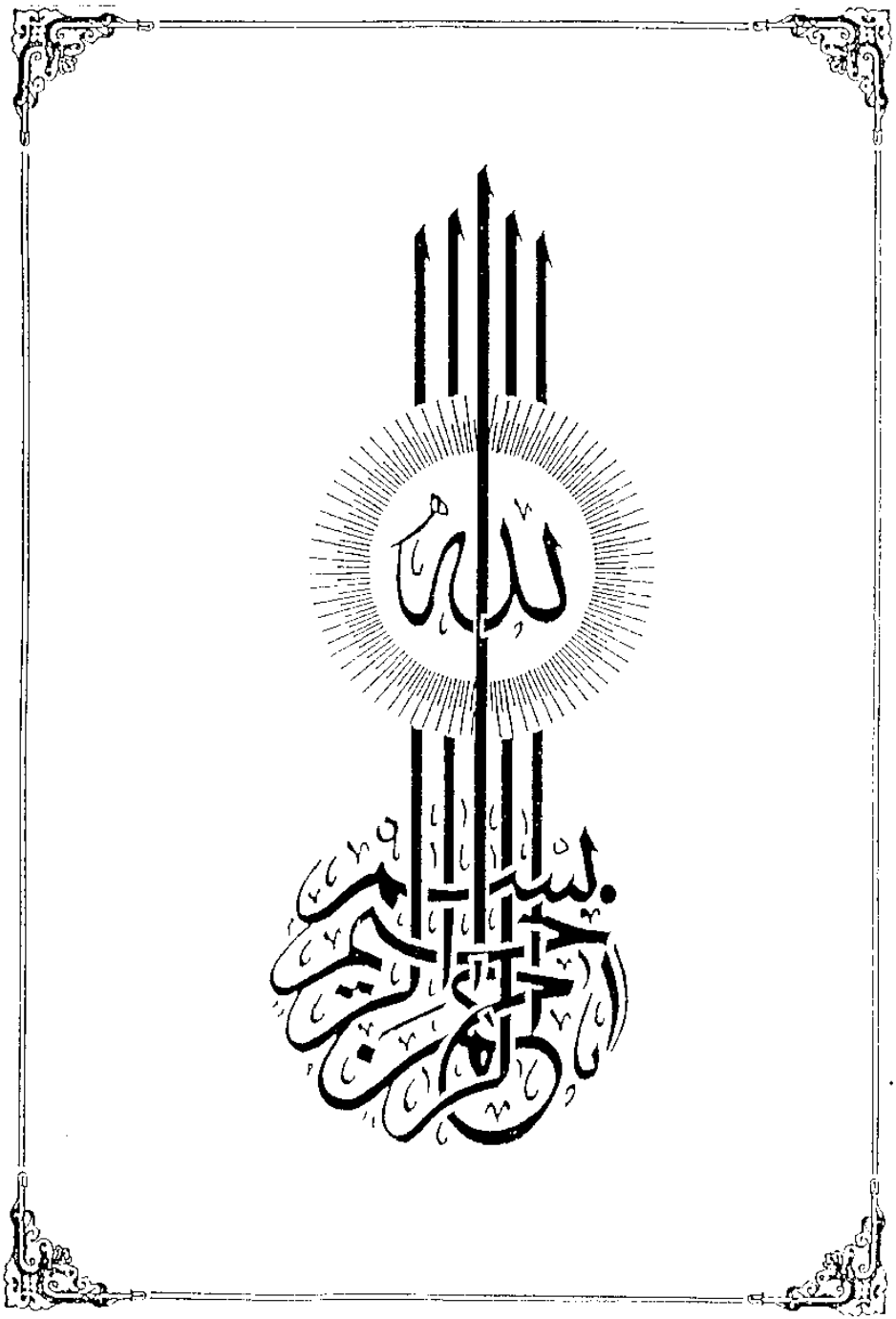

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Introduction and Aim of Work

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The isolation and cultivation of adult rat myocardial cells has been the subject of many improvements as a culture model of differentiated mammalian cells (Moses and Kasten 1979).

The enzymatic dissociation of these cells was found to disassemble the myofibrillar cytoskeleton, followed later by its reassembly during the culture period (Nag, Cheng, Fischman and Zak, 1983).

The effect of the tumor promoting factor 12- 0 - tetrodecanoyl - phobol- 13 - acetate (TPA) on the myofibrillar apparatus has been studied on many cell culture models. Croop, Toyama, Dlugosz and Holtzer (1980); Croop, Dubyak, Toyama, Dlugosz, Scarpa and Holtzer (1982); and Lin, Eshleman, Forry- Schaudies, Duran, Lessard and Holtzer (1987) had worked on chick embryo breast muscles, while Toutant and Sobel (1987), had worked on limb muscles of neonate mouses .

Recently the effect of TPA on the morphology of cultured terminally differentiated adult rat cardiac myocytes had been investigated (Claycomb and Moses, 1988). Their work was mainly concerned with the effect of the drug on DNA synthesis and their

few ultrastructural observations showed that the drug reversibly dismantles the myofibrillar apparatus of these cells.

The aim of the present work was to study the dedifferentiation - redifferentiation phenomenon of the cardiomyocytes after TPA treatment employing recent techniques as immunofluorescence. This will likely add to our understanding of the process of differentiation of this cell model.

Review of Literature

I- THE CONTRACTILE APPARATUS OF THE SARCOMERE

Organization of the Sarcomere (Rodriguez and Ferrans, 1985)

Some of the components of the individual myofibrils were identified during the past century by light microscopic observation of longitudinal sections of striated muscle. The pattern of striations gave rise to two distinctly different bands when observed with polarized light. Accordingly, these bands were named anisotropic or A-band and isotropic or I-band. Ultrastructurally, the A-band corresponded to that area of the myofibril where the thick filaments were present which were made of protein myosin . The I-band was the area of thin filaments which were made of three proteins;G-actin,tropomyosin,and troponin. A third component was identified by the name Z-disc or band (from the German Zwischenscheibe = middle or dividing disc) which bisected the I-band. Parallel to this structure, at either side of the Z-disc, there was another landmark, the N-line (Nebenscheibe = neighbouring disc), which bisected each of the halves of the I-band.The A-band was bisected by an electron-dense M-line (Mittellinie = middle line) which is a focal thickening in the middle of each thick filament. At each side of the M-line, another line could be distinguished which consisted of a lighter zone where no cross-bridges occurred between individual thick filaments. This zone was designated L-line or pseudo-H (Helle = light or clear) zone.

Fig:A is the authers representative diagram for all of the above .

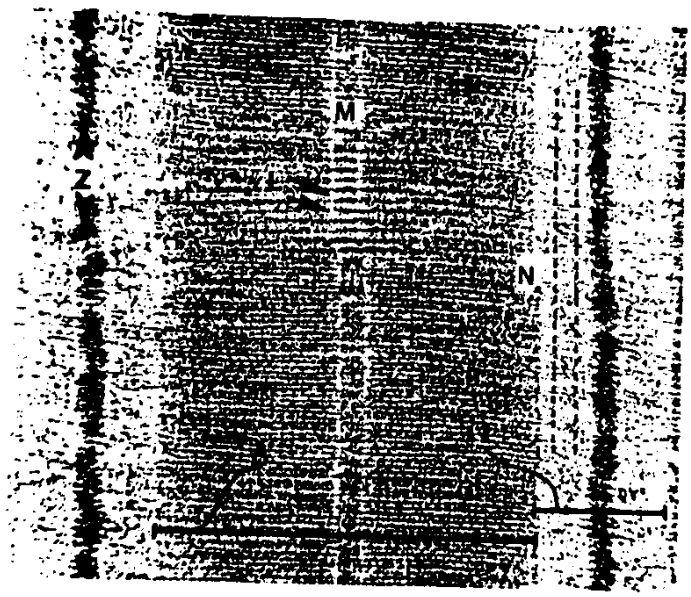

Fig:A (Rodriguez and Ferrans,1985)

A representative diagram of a cardiac sarcomere . The A-band(A) is the wide central band and it is bisected by the M-band(arrow M). The M-line shows 5 fine divisions(small arrow-heads). The limits of the sarcomere are given by the two Z-bands(large arrow-heads),and these Z-bands, in turn bisect the I-bands. Each half of the I-band formed by the dividing Z-band shows a darker zone which corresponds to the N-line and is seen outlined by the vertical dotted lines.

II. THE CYTOSKELETAL SYSTEM OF CARDIAC MUSCLE

The cytoskeleton of eucaryotic cells classically includes three elements; microtubules, intermediate filaments, and actin-containing microfilaments (Rappaport and Samuel, 1988).

Microtubules

Until recently, microtubules and their ultrastructure in cardiac muscle have been referred to in a relatively few publications (Page, 1967; Ferrans and Roberts, 1973; Rybicka, 1978).

Microtubules are made up of the protein tubulin and other regulatory proteins called microtubule-associated proteins (MAPs). Tubulin is a heterodimer of alpha and beta subunits (Luduena, 1979).

According to recent ultrastructural studies, microtubules were claimed to be more numerous than previously reported (Goldstein and Entman, 1979; Goldstein,Schroeter and Sass,1979; Goldstein and Cartwright, 1982; Forbes and Sperelakis, 1983; Samuel ,Bertier ,Bugaisky, Marotte, Swynghedauw, Schwartz and Rappaport, 1984; Cartwright and Goldstein, 1985). They had diameters ranging from 24 to 30 nm in transverse sections, they appeared rounded and could be resolved with the aid of markham rotation to reveal 13 subunits. Microtubules were found to be particularly abundant around the nucleus. They formed helical enwrapments around the myofibrils and were closely associated with mitochondria, sarcoplasmic reticulum, and T-tubules in the vicinity of the Z lines. A few longitudinally-oriented parallel microtubules were found between the myofibrils.

Immunofluorescent localization of tubulin in frozen sections of intact myocardium revealed the cardiomyocytes to have fewer microtubules than the non-myocytes. In the cardiomyocyte cytoplasm, tubulin was localized as a ring around the nucleus and as random punctate elements or short strands. A few axial microtubules having a helical configuration were also described (Rappaport and Samuel, 1988).

Intermediate Filaments

Filaments intermediate in diameter between microtubules and microfilaments, although recognized earlier (Heuson-Stiennon, 1965), were described in detail in developing skeletal muscle by Ishikawa (1968).

Intermediate filaments have been reported in a number of ultrastructural studies of adult cardiac muscle (Viragh and Challice, 1969; Ferrans and Roberts, 1973).

Five classes of intermediate filaments are known to exist: 1) Desmin, the major muscle type first isolated from smooth muscle (Cooke, 1976; Lazarides and Hubbard, 1976), which is also called skeletin (Small and Sobieszek, 1977). 2) Vimentin filaments found in fibroblasts, developing muscle, and many cells of mesenchymal origin (Bennett, Fellini, Croop, Otto, Bryant, and Holtzer, 1978; Franke, Weber, Osborn, Schmid, and Freudenstein, 1978). 3) Keratin tonofilaments in cells of epithelial origin (Franke et al., 1978). 4) Glial filaments of astrocytes (Shelanski and Liem, 1979). 5) Neurofilaments present in neurones (Eriksson, Kjorell, Thornell, and Stigbrand, 1980).

According to immunofluorescent and immunoelectron microscopic studies on cardiac muscle, desmin was localized in the vicinity of the Z-lines, where it was suggested to align the

Z-lines of adjacent myofibrils together causing their lateral registration (Granger and Lazarides, 1978; Lazarides, 1980). Desmin was also shown to connect the Z-lines to the intercalated discs, sarcolemma, nucleus, and mito-chondria (Lazarides, Granger, Gard, O'Connor, Breckler, Price, and Danto, 1982; Tokuyasu, 1983; Tokuyasu, Dutton, and Singer, 1983; Danto and Fischman, 1984; Thornell, Johanssen, Eriksson, Lehto, and Virtanen, 1984). Using immunogold-labelling combined with SEM, desmin-containing filaments were affiliated with myofibrils at the Z-lines in embryonic chick heart (Sugi, 1989).

Non-Myofibrillar Actin Filaments

The non-muscle beta and gamma actins were found to exist as well in cardiac and skeletal muscle cells (Woodroofe and Lemanski, 1981; Weins and Spooner, 1983), where they were thought to play a role in sarcomerogenesis (Lessard, Scheffter, Engel, and Tepperman, 1983).

CULTURE OF ADULT CARDIAC MYOCYTES

The complex organization and interactivity of cells in cardiac tissue have limited the extent to which individual myocardial cells could be characterized. Progress in cellular cardiology required experimental preparations that circumvent problems such as cellular heterogenecity, intracellular coupling (i.e., electrical and mechanical intracellular coupling made it impossible to distinguish effects due to intrinsic cellular properties from those due to cell interactions), and multicompartmentalization lead to impossible control of the interstitial milieu. A variety of experimental preparations were used by cellular cardiologists. Tissue cultures from immature hearts, dating back 75 years (Burrows, 1910; Goss, 1931; Szepsenwol, 1946) were among the earliest *in vitro* preparations. The use of enzymes by Dulbecco,(1952); and Moscona,(1952) to isolate single cells for cultures were more recent notable events. Their method applied to chick embryo heart in 1955 by Cavanaugh and to neonatal rat heart by Harary and Farley in 1960, produced the first cultures from isolated cardiac cells. Subsequently, many other workers used cultured chick embryo and neonatal rat cells in culture e.g., Dehaan and Hirakow,(1972);and Kasten,(1972) as well as cells from embryonic and neonatal mouse (Boder and Ellis, 1972), human embryo (Halbert, Bruder, and Thomson, 1973) and hamster (Bester and Gevers, 1975) . Immature cells, however, differ from adult myocardial cells (Claycomb, 1983).

A more accurate model of adult myocardium was sought, at first, in isolated cells from adult animals. The isolation of