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Specific attachment of desmin filaments to desmosomal plaques in cardiac myocytes

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Intercellular junctions which are similar in ultrastructure and protein composition to typical desmosomes have so far only been found in epithelial cells and in heart tissue, specifically in the intercalated disks of cardiac myocytes and at cell boundaries between Purkinje fiber cells. In epithelial cells the cytoplasmic side of desmosomes, the 'desmosomal plaque', represents a specific attachment structure for the anchorage of intermediate filaments (IF) of the cytokeratin type. Cardiac myocytes do not contain cytokeratin filaments. In primary cultures of rat cardiac myocytes, we have examined by immunofluorescence and electron microscopy, using single and double label techniques, whether other types of IF are attached to the desmosomal plaques of the heart. Antibodies to desmoplakin, the major protein of the desmosomal plaque, have been used to label specifically the desmosomal plaques. It is shown that the desmoplakin-containing structures are often associated with IF stained by antibodies to desmin, i.e., the characteristic type of IF present in these cells. Like cytokeratin filaments in epithelial cells, desmin filaments attach laterally to the desmosomal plaque. They also remain attached to these plaques after endocytotic internalization of desmosomal domains by treatment of the cells with EGTA. These desmin filaments do not appear to attach to junctions of the *fascia adhaerens* type and to nexuses (gap junctions). These observations show that anchorage at desmosomal plaques is not restricted to IF of the cytokeratin type and that IF composed of either cytokeratin or desmin, specifically attach, in a lateral fashion, to desmoplakin-containing regions of the plasma membrane. We conclude that special domains exist in these two IF proteins that are involved in binding to the desmosomal plaque.

Key words: heart muscle/desmin/desmosomes/intermediate filaments/cell junctions

Introduction

Intermediate-sized filaments (IF), i.e., unbranched cytoplasmic filaments of 7-11 nm diameter, represent a major component of the 'insoluble cytoskeleton' thought to be involved in establishing and maintaining specific cellular shape and architecture (for reviews, see Lazarides, 1982; Franke *et al.*, 1982a; Holtzer *et al.*, 1982; Osborn *et al.*, 1982). These IF are frequently seen in conspicuous morphological relationships to membranes and other cell organelles such as mitochondria (Zerban and Franke, 1977; Chen *et al.*, 1982; Lee *et al.*, 1979; David-Ferreira and David-Ferreira, 1980; Mose-Larsen *et al.*, 1982), nuclei (Franke *et al.*, 1982a) and the plasma membrane (Farquhar and Palade, 1963; Staehelin, 1974; Lazarides and Hubbard, 1976). Such observations have led to the hypothesis that IF may be integral components of an architectural framework governing the positioning and relationships of cellular components (Chen *et al.*, 1982; Lazarides, 1982; Singer *et al.*, 1982). An especially clear case of membrane-attachment of IF is the anchorage of bundles of cytokeratin filaments (tonofilaments) at the cytoplasmic side of desmosomes. These attachment sites are characterized by a dense plaque structure, the desmosomal plaque (Farquhar and Palade, 1963; Kelly, 1966; Staehelin, 1974; Skerrow and Matoltsy, 1974; Drochmans *et al.*, 1978). This plaque is specific for the desmosomal domain, be it located in desmosomal junctions, in hemidesmosomes or in endocytosed desmosome-derived vesicles, and contains some characteristic constitutive proteins. The most prominent of these proteins are the desmoplakins, polypeptides of high mol. wt. (215 000 and 250 000 in some epithelial tissues; Franke *et al.*, 1981a; 1982b, 1983; Müller and Franke, 1983). Antibodies to such desmosomal plaque proteins define and identify desmosomal components not only in epithelial cells but also in the heart, specifically in the intercalated disks and between Purkinje fiber cells (Franke *et al.*, 1981a, 1982b, 1983) where structures similar to the desmosomes of epithelial cells have been repeatedly described by electron microscopists (Fawcett and Selby, 1958; Sjöstrand *et al.*, 1958; Fawcett and McNutt, 1969; Ericksson and Thornell, 1979; for reviews, see Campbell and Campbell, 1971; Manasek, 1976).

Present evidence indicates that, in epithelial cells, cytokeratin IF specifically attach to desmosomal plaques but not to other plasma membrane domains (Staehelin, 1974; Franke *et al.*, 1978a, 1981a, 1982b, 1983; Geiger *et al.*, 1983) whereas actin-containing microfilaments are associated with the vinculin-rich plaques lining junctions and focal regions of the *adhaerens* type (Bretscher and Weber, 1978; Geiger *et al.*, 1981, 1983). Cytokeratins are also specifically attached to desmosomal plaques in various cultured epithelial cell lines (e.g., HeLa, MDCK, MDBK, BMGE-H) which express both types of IF proteins, cytokeratins and vimentin. In such cells, cytokeratin filament bundles terminate at the desmosomes whereas vimentin filaments are clearly excluded from these regions (Franke *et al.*, 1979b, 1979c, 1981b, 1982a; Osborn *et al.*, 1980; Schmid *et al.*, 1983), indicating that cytokeratin IF but not vimentin IF have the information for specific attachment to these membrane domains. Using immunolabelling techniques, desmin has been localized in cardiac muscle to Z-lines and close to the plasma membrane of the intercalated disk (Lazarides and Hubbard, 1976; Franke *et al.*, 1980; Carlsson *et al.*, 1982). However, conclusions as to a specific association with one of the various types of junction present in the intercalated disk cannot be made, because of the complexity of this junction-rich zone (cf., Fawcett and McNutt, 1969; Tokuyasu *et al.*, 1981; Colaco and Evans, 1981). Therefore, we have used primary cultures of cardiac myocytes to examine the details of the relationship between desmin filaments and the desmosomal plaques.

Results

Primary cultures of cells dissociated from cardiac tissue

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contain myocytes as well as other cell types such as fibroblasts and endothelial cells. Rat cardiac myocytes present in such cultures continue to express desmin, as found in primary cultures of embryonic cardiac myocytes of chicken (Lazarides, 1978).

We have recently prepared guinea pig antibodies to desmoplakin(s) from bovine muzzle epidermis which, unlike previous antibody preparations against desmosomal plaque proteins from this tissue (Franke *et al.*, 1981a), cross-react with desmosomal plaque proteins of taxonomically distant species (Franke *et al.*, 1982b; Müller and Franke, 1983). The specific reaction of these antibodies with rat desmoplakin is shown on cryostat sections through frozen rat heart tissue (Figure 1a). The exclusive binding to regions of intercalated disks is obvious, often revealing punctate substructures which by immunoelectron microscopy can be identified as desmosomal plaques (cf., Franke *et al.*, 1982b). Other antibodies showing strong reaction on intercalated disks such as anti-actin, anti-vinculin and anti-α-actinin do not co-localize with desmoplakin antibodies but stain the fuzzy plaques associated with adjacent junctions of the *fascia adhaerens* type (data not shown; see also Tokuyasu *et al.*, 1981).

Colonies of rat cardiac myocytes growing *in vitro* also specifically react with desmoplakin antibodies along cell-to-cell boundaries (Figures 1b,c,e and 2a). In addition, these cells sometimes exhibit punctate fluorescence, occurring in variable frequencies and variable patterns, deep in the cell interior (Figures 1e and 2a; for explanation see below). Free cell margins are usually free of desmoplakin-positive reaction sites (Figure 1b), similar to cultured epithelial cells (Franke *et al.*, 1982b). Three different antibodies (guinea pig and rabbit) against desmin showed similar fibrillar arrays in the cytoplasm of these cells. We have examined by double antibody labelling the spatial relationship of the muscle-type IF, i.e, desmin IF, and these desmosomes, using guinea pig antibodies to desmoplakins and rabbit antibodies to desmin. Both preparations of rabbit antibodies to desmin gave identical results in this study. Desmin-positive reaction occurs in irregular fibrillar arrays throughout the cytoplasm (e.g., Figures 1d,f and 2b) and is often observed in close proximity to desmoplakin-positive 'dots', i.e., desmosomes (Figures 1c – f, 2a and b). Short desmin-positive fibrillar streaks, often traceable for only a few micrometers, can be seen to originate from such desmoplakin-positive dots (e.g., arrows in Figures 1c – f and 2a,b).

These cultured cardiac myocytes were negative with various antibody preparations to cytokeratins and vimentin (not shown), in agreement with the negative results obtained from antibody staining of sections through heart tissue. However, the presence of some vimentin in these cells cannot be excluded on the basis of negative immunolocalization data only, as vimentin has been reported to occur in Z-lines of other striated muscles by some authors (for discussion, see Holtzer *et al.*, 1982; Lazarides *et al.*, 1982; Osborn *et al.*, 1982).

Removal of calcium from the culture medium with, for example, 4 mM EGTA resulted in splitting of desmosomes and the internalization of desmosomal domains by endocytosis in a manner similar to that described in epithelial cells (cf., Kartenbeck *et al.*, 1982) and in heart tissue (Muir, 1967). After such treatment, the cytoplasm of the cultured myocytes is characterized by the accumulation of desmoplakin-positive 'dots' (obviously representing desmosome-derived vesicles), which are associated with desmin-positive fibrillar strands (inserts in Figure 2a and b).

Electron microscopy of cultured cardiac myocytes (Figure 3) reveals cell-to-cell boundaries with dense arrays of the various types of junction, including desmosomes, also characteristic of the intercalated disk of the myocardium. Close inspection shows that the myofibrillar bundles, specifically the actin microfilaments, terminate at the dense plaque regions of the *fasciae adhaerentes* (A in Figure 3a). In contrast, desmosomal junctions (D in Figure 3) are intimately associated with bundles of IF which approach these junctions either laterally or at, seemingly, right angles (Figure 3b). Evaluation of serial sections has shown that many of these desmosome-attached IF bundles are rather short (0.5 – 3 μm), clearly shorter than typical tonofibrils of epidermal and other epithelial cells.

Electron microscopic examination of cultured cardiac myocytes treated with EGTA (see above) shows the same phenomenon as described for cultured epithelial cells, namely the appearance of numerous cytoplasmic vesicles associated with a 'cap' of dense plaque material and tufts of IF (Figure 4a – e; for epithelial cells, see also Kartenbeck *et al.*, 1982; cf., Overton, 1968; Seman and Dmochowski, 1975). Frequently, one IF bundle can be associated with two or more of these vesicles (Figure 4a). While most of these vesicles present an inflated, electron-translucent cavity, some vesicles still have an identifiable internal dotted line reminiscent of the midline structure of desmosomal junctions (Figure 4c,d). Both grazing sections (e.g., Figure 4e) and cross-sections (Figure 4b) give the impression that these IF bundles laterally abut on the vesicle-associated plaques, similar to the situation described for intact desmosomal junctions.

Electron microscopy of myocardium as well as of cultured cardiac myocytes has shown that desmoplakin is localized in the plaques associated with desmosomal junctions (small gold particles in Figures 4f – h; see also Franke *et al.*, 1982b) as well as with intracellular desmosome-derived vesicles induced by EGTA treatment (Figure 4i – n). Staining with antibodies to desmin, using two different antibody preparations and three different immunoelectron microscopic procedures, consistently showed reaction with bundles of IF associated with desmosomal plaques (larger gold particles in Figure 4f – h; dense label in Figure 4i – n). When antibodies to cytokeratin and vimentin were examined with the same techniques no significant staining of the cardiac IF bundles and their attachment plaques was observed (not shown).

Discussion

Our results demonstrate that the IF specifically associated with the desmosomal plaques of cardiac myocytes are desmin filaments. The use of primary cultures of cardiac myocytes has been helpful in elucidating the relationship between desmosomes and IF since in these cells the complicated interdigitation of the junction-rich plasma membrane of the intercalated disk is replaced by more smoothly-contoured cell-to-cell boundaries. From our findings, we conclude that in cardiac myocytes the same principle of specificity and mutual exclusiveness of junction-attachment is prevalent as in epithelial cells: actin-containing microfilaments terminate at the vinculin-rich plaques of *adhaerens*-type junctions, whereas IF terminate at desmoplakin plaques of desmosomes (see also Staehelin, 1974; Franke *et al.*, 1979a, 1981a, 1982b; Tokuyasu *et al.*, 1981; Geiger *et al.*, 1983). This principle also

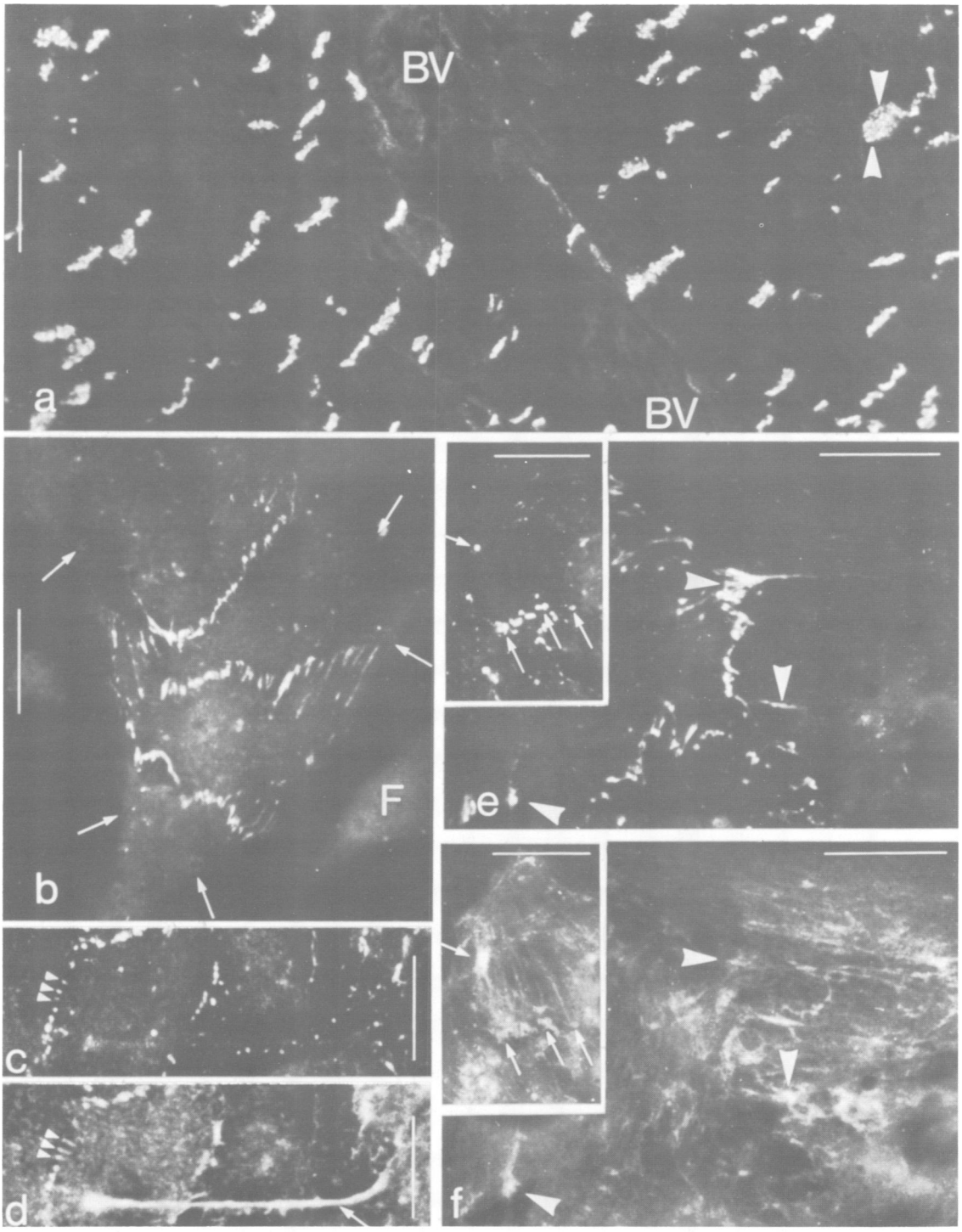

Fig. 1. Immunofluorescence microscopy of a cryostat section through frozen rat heart tissue **(a)** and of whole mount preparation of cultured rat cardiac myocytes grown on cover slips **(b – f)** after incubation with guinea pig antibodies raised against bovine desmoplakin **(a,b,c,e)** alone **(a,b)** or in combination with rabbit antibodies to porcine **(d)** or human **(f)** desmin on cultured rat cardiac myocytes **(c – f)**. Guinea pig antibodies were visualized by rhodamin-**(c)** or FITC-**(e)** coupled second antibody, and staining obtained with rabbit antibodies to desmin was visualized by FITC-**(d)** or rhodamin-**(f)** coupled second antibody. Heart tissue **(a)** shows strong desmoplakin reaction at the intercalated disks of myocardiac cells and absence of staining on fibroblasts capillaries, arterioles and other blood vessels (BV in **a**). Small punctate arrangements of single desmosomes are sometimes revealed in flat-sectioned intercalated disks **(a,** arrowheads in the upper right). Colonies of cultured rat cardiac myocytes show desmoplakin staining at cell-to-cell boundaries but not on free cell margins (arrows in **b,e**). Sometimes, intracellular 'dots' are seen **(c,e)**, representing hemidesmosomal equivalents or endocytosed desmosome-derived vesicles. Non-myocyte cells such as fibroblasts are devoid of desmoplakin antibody reaction (F in **b**). Comparison of the staining obtained after double immunofluorescence reaction shows that the small regions which are decorated by the antibodies to desmoplakin **(c,e)** are also stained by the antibodies to porcine desmin **(d;** (vi) of Materials and methods; used at ~100 μg/ml, at which no cross-reaction with vimentin has been observed) and human desmin **(f;** (v) of Materials and methods). Desmin staining in addition shows fibrillar structures extending from the desmoplakin-positive sites into the cytoplasm (e.g., arrows and arrowheads in **c** and **d** or in **e** and **f**; this relationship is specifically resolved in the inserts of **e** and **f**). Note the specificity of the antibodies used: the antibodies to desmoplakin **(c)** do not stain the cell extension heavily stained by desmin antibodies (arrow in **d**). Bars denote 50 μm **(a)** and 20 μm **(b – f)**.

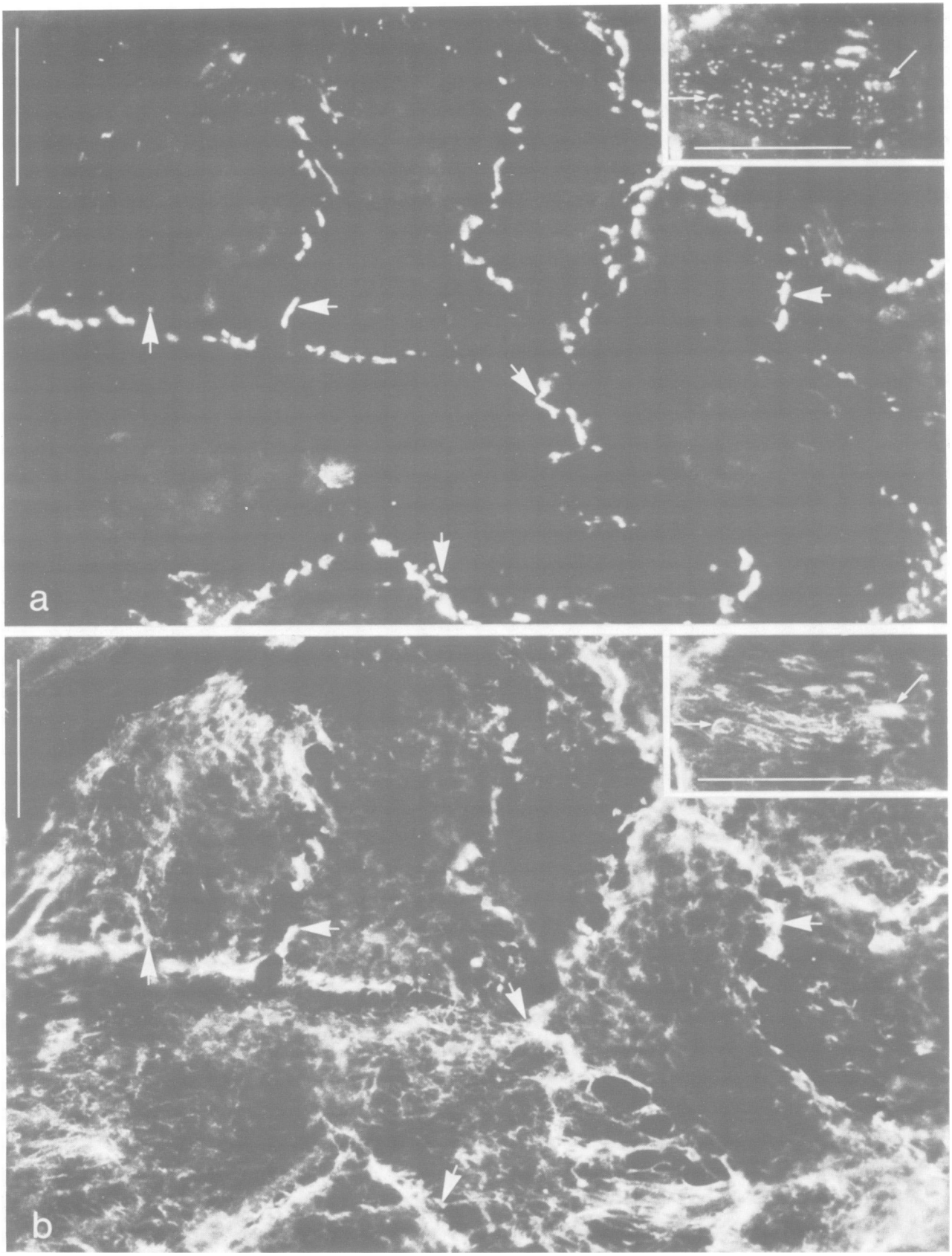

Fig. 2. Double label immunofluorescence microscopy on cultured rat cardiac myocytes. **a** Fluorescence obtained with guinea pig antibodies to desmoplakin visualized by FITC-coupled second antibodies; **b** same cells visualized by reaction with rabbit antibodies to desmin visualized by rhodamin-coupled second antibodies. Desmosomal reaction sites **(a)** are also decorated by desmin antibodies **(b)** but the latter also display staining of a fibrillar meshwork which extends throughout most of the cytoplasm. Some examples of the co-localization of desmoplakin and desmin antibodies as well as of desmin fibril association with desmoplakin-positive sites are denoted by arrows. Structural correspondence of desmoplakin-positive 'dots' and desmin fibrils is also seen after treatment of the cells with 4 mM EGTA for 120 min (demonstrated in the inserts in the upper right corners). Fluorescent dots revealing desmosomal protein (insert in **a**) appear to be associated with filamentous structures stained with the desmin antibodies (insert in **b**). Bars, 20 μm.

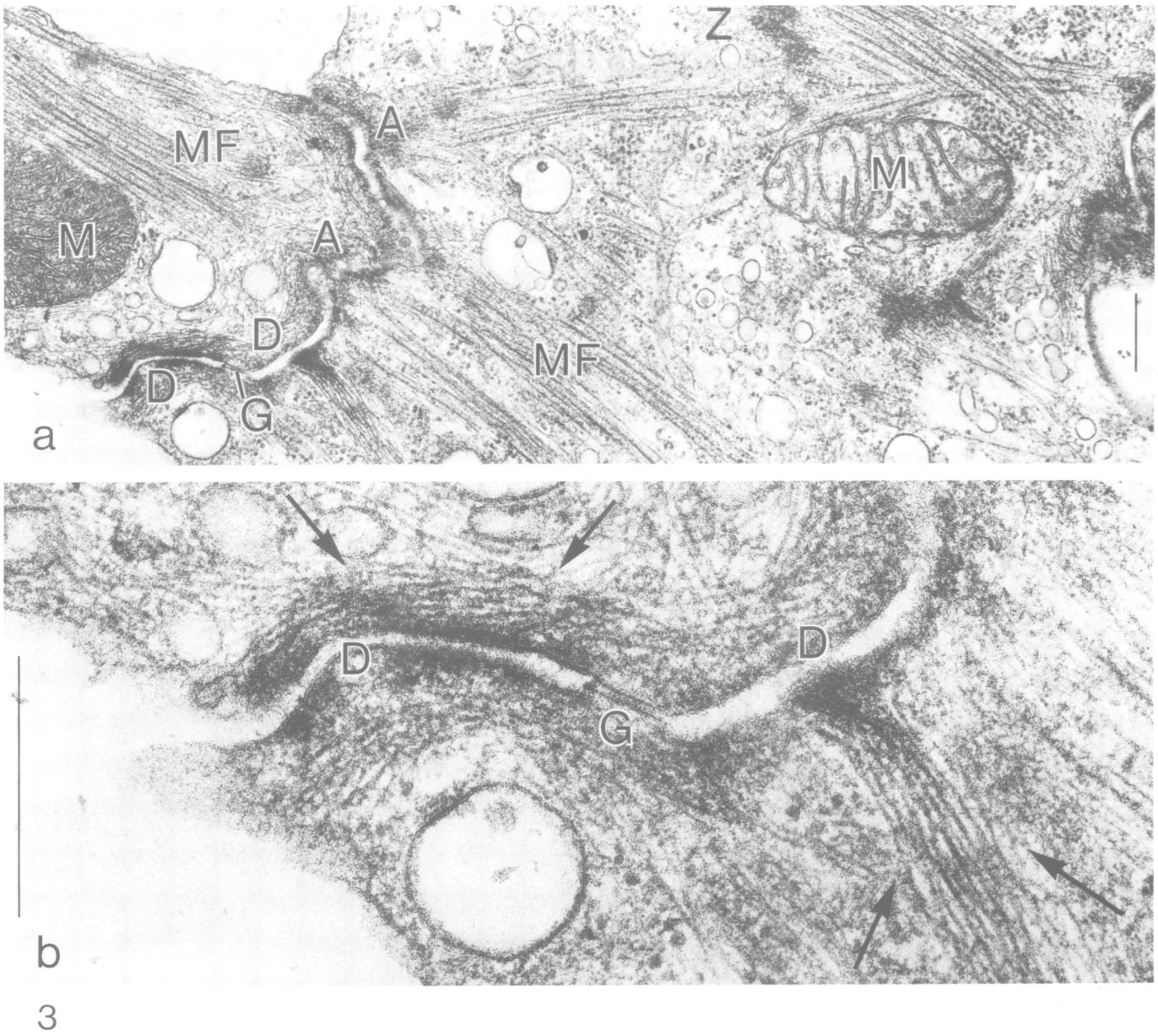

Fig. 3. Electron micrographs of cultured cardiac myocytes. **a** Survey picture showing the organization of the junctional complexes containing desmosomes (D), gap junctions (G), and *fasciae adhaerentes* (A). Bundles of myofibrils (MF) run toward the *adhaerens* junctions (A) but not toward desmosomes. Bundles of IF are associated with the desmosomal plaques (D) seen, at higher magnification, in lateral attachment (pairs of arrows in upper left of **b**) or in seemingly terminal attachment (arrows in lower right of **b**) Z, disorganized Z-line structures (**a**); M, mitochondrion (**a**). Bars denote 0.4 μm.

explains the sporadic observations in the literature that myocardiac IF run in directions different from those of the myofilament bundles, in particular in the vicinity of the intercalated disks (e.g., Fawcett and McNutt, 1969; Forbes and Sperelakis, 1975; Behrendt, 1977; Moses and Claycomb, 1982). The functional importance of the anchorage of desmin IF to desmosomal junctions in the myocardium, but not in other myogenic differentiations such as cross-striated skeletal muscle and smooth muscle is not understood. It may be related to the special tension and stress to which this tissue is continuously exposed. It is also interesting to note in this context that the precardiac cells of the embryo are also different from other myogenic cell populations in that they are not formed from freely migratory mesenchymal cells but from a special group of mesodermal cells integrated into a coherent cell sheet classified as 'secondary epithelium' (for reviews see DeHaan and Sachs, 1972; Manasek, 1976).

Our observations also indicate an homology between desmin IF and cytokeratin IF as concerns their specific association with desmoplakin-plaques. This is in line with recent reports of common immunological determinants (Pruss *et al.*, 1981) and of homologies in the amino acid sequences of desmin and a certain human epidermal keratin polypeptide (Hanukoglu and Fuchs, 1982) and sheep wool keratin (Geisler and Weber, 1982). It will be interesting to see which molecular region in these two classes of IF protein contains the determinant(s) for this membrane-attachment specificity. This is all the more interesting since vimentin, which seems to be much more closely related to desmin in terms of primary sequence homology (Geisler and Weber, 1981), does not selectively associate with the desmosomal plaques present in cultured epithelial cells expressing both cytokeratin and vimentin IF (for references see Introduction). The molecular basis for the specific attachment of a group of cytoskeletal

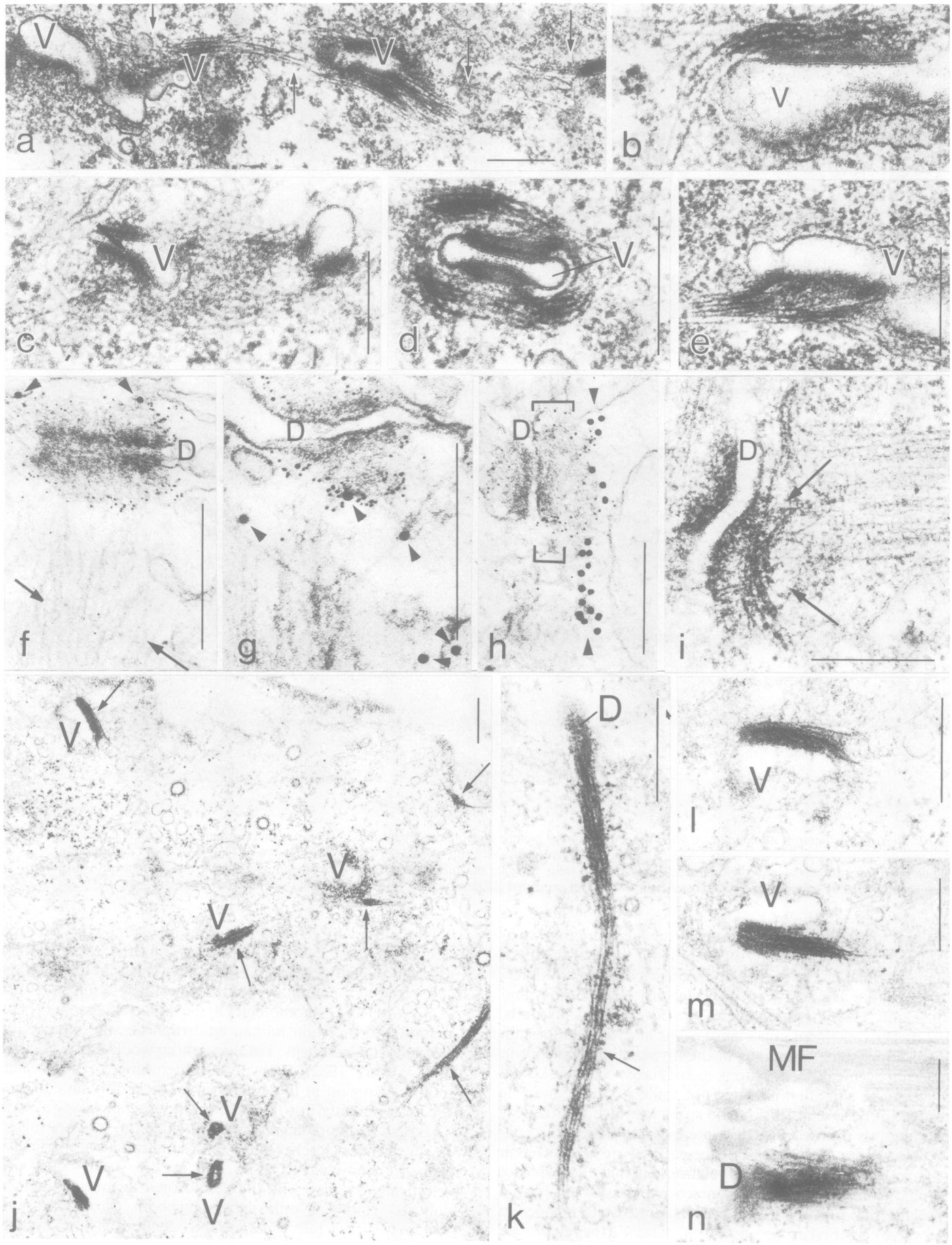
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Fig. 4. Electron micrographs showing desmin filament-desmosome relationship (**a–e**). Cultured cardiac myocytes after treatment with 4 mM EGTA for 120 min. After this treatment numerous vesicles (V) with densely stained 'caps' of desmosomal plaque material in association with IF bundles appear in the cytoplasm, representing internalized vesiculated desmosome-derived membrane domains (**a–e**). Often IF bundles connecting different plaques on the same or on different vesicles are observed (**a,c**). In some cases the typical desmosomal midline structure is still revealed (left vesicle in **c;d**). The lateral insertion of IF bundles abutting on the desmosomal plaque is recognized in grazing (**e**) as well as in cross- (**b**) sections. (**f–h**) Desmosomes of intercalated disks of frozen cardiac tissue after double labelling with antibodies to desmoplakin visualized by 5 nm gold particles and antibodies to desmin visualized by ~20 nm large gold particles. Note dense labelling of desmoplakin in plaques of desmosomes (D; denoted by bracket in **h**) and more dispersed filament labelling with desmin antibodies (arrowheads in **f** and **g**) but heavy desmin labelling in **h** (gold particles denoted by arrowheads). The desmin label is more distant from the membrane than the desmoplakin label. Note also absence of label on other structures including myofibrils (denoted by arrows in **f**). (**i–n**) Electron microscopic localization of antibodies to desmin, using decoration with second antibody (IgG), followed by lead citrate staining (**i–m**) or peroxidase-coupled IgG (**n**), in cultured myocytes, showing reaction on tufts of IF (arrows) associated with the plaques of desmosomal junctions (**i**, arrows) or with internalized vesicles of EGTA-treated cells (**j–n**). MF, myofibril. Bars represent 0.3 μm.

elements, desmin and cytokeratin IF at the desmosomal plaque is presently not understood.

Materials and methods

Cell cultures

The preparation of rat heart muscle cells and the fractionation of myocytes will be published in detail elsewhere (Moser *et al.*, in preparation). Hearts from 2–3 days old Wistar rats were cut into pieces, washed twice with phosphate buffered saline (PBS) and incubated overnight at 0–4°C in trypsin (No. 210/234; Boehringer Mannheim, FRG) dissolved in PBS (pH 7.8). The tissue pieces were then cut into smaller pieces and rinsed twice with PBS (pH 7.2) in order to remove erythrocytes. DNase I (No. 18550 Serva, Heidelberg, FRG) was then added (1 mg/ml), and the suspension stirred for 30–45 min. After a further 10 min incubation with trypsin solution the suspension was drawn through a large diameter injection needle (Braunule® 2G14, Braun, Melsungen, FRG). The small tissue pieces disaggregated and a dense cell suspension was formed which was filtered immediately through a glass filter funnel (G1, siliconized) in order to remove remaining crude tissue pieces. The filtrate was diluted with culture medium (HAM's F12; No. 210/161, Boehringer) and washed twice with this medium, each time collecting the cells by centrifugation at 400 *g* for 5 min.

To isolate myocytes the cell pellet was resuspended in 'saline isotonic Percoll' (SIP; Pharmacia, Uppsala, Sweden) prepared according to the data given by the supplier. This cell suspension (2 ml aliquots containing 10^7 cells) was layered in the bottom portion of a step gradient consisting of 3 ml of 80% SIP/20% PBS and 50 ml of 50% SIP/50% PBS in 12 ml siliconized glass centrifuge tubes. After centrifugation at 400 *g* for 30 min, a top layer containing non-muscle cells, a middle layer (buoyant density $d_w^{20} = 1.079$) containing myocytes myoblasts (purity: 92–98%) and an erythrocyte-rich pellet could be distinguished. After washing twice with culture medium, the cells were explanted at densities of 1–5 x 10^5 cells/ml in multiwell plates containing collagen-coated glass coverslips. Cultures were grown for 5–17 days in HAM's 712 supplemented with 10% horse serum and the medium was changed every 2 days.

Tissue

Rat hearts were obtained from freshly killed neonatal and adult animals, cut into small pieces and directly frozen in isopentane cooled with liquid nitrogen (about −150°C; Franke *et al.*, 1979a).

Antibodies

The following antibodies were used: (i) guinea pig antibodies to desmoplakins from bovine muzzle (Franke *et al.*, 1982b, 1983; Müller and Franke, 1983); (ii) guinea pig antibodies to vimentin of murine or human origin (Franke *et al.*, 1979c); (iii) guinea pig antibodies raised against bovine epidermal prekeratin (Franke *et al.*, 1980) and murine hepatic cytokeratin D (Franke *et al.*, 1981c); (iv) guinea pig antibodies raised against desmin from chicken gizzard which cross-reacted with mammalian desmin (Franke *et al.*, 1980); (v) rabbit antibodies to desmin from human uterus which specifically react with desmin but not with vimentin as demonstrated previously (Gabbiani *et al.*, 1982; Quinlan and Franke, 1982); (vi) affinity-purified rabbit antibodies to desmin from smooth muscle of porcine stomach (kindly provided by J.V.Small and J.DeMey, Institute of Molecular Biology, Austrian Academy of Science, Salzburg, Austria).

Microscopy

Indirect immunofluorescence microscopy of acetone-fixed (10 min, −20°C) cryostat sections of rat myocardium was performed as described (Franke *et al.*, 1981a).

Cultured cardiac myocytes were grown on coverslips and processed for indirect immunofluorescence microscopy after fixation in methanol (10 min, −20°C) and acetone (15 s, 20°C) following the procedures described for cryostat sections (see above). For double immunofluorescence microscopy, guinea pig antibodies to desmoplakin and rabbit antibodies to desmin were applied successively. Fluorescein isothiocyanate (FITC)-coupled rabbit antibodies (Miles, Frankfurt, FRG) to guinea pig Ig were added first, followed by rhodamin-coupled goat antibodies (Cappel, Cochranville, PA) to rabbit Ig. Alternatively, rhodamin-coupled goat antibodies to guinea pig Ig (Cappel) were used first, followed by FITC-coupled goat antibodies (Miles) to rabbit Ig.

For electron microscopic immunolocalization, cells grown on coverslips were fixed with 2% formaldehyde and 0.05% glutaraldehyde in PBS (pH 7.4) for 60 min and washed several times in PBS containing 50 mM NH_4Cl, 0.05% saponin, and 0.2% gelatine (Louvard *et al.*, 1982). Rabbit antibodies to desmin from porcine stomach were allowed to react for 30 min at room temperature. Cells were washed again in PBS (with the same additives as specified above) and incubated for 30 min with a 1:20 dilution of fluorescein-coupled goat antibodies to rabbit IgG. Prior to a final fixation in 2.5% glutaraldehyde (50 mM sodium cacodylate, pH 7.2) for 30 min, the cells were rinsed in PBS. Post-fixation was performed in 2% OsO_4 (50 mM cacodylate buffer) for 2 h. Alternatively, cells were processed for electron microscopic immunolocalization using rabbit antibodies to desmin by the peroxidase procedure as described (Kartenbeck *et al.*, 1981, 1982).

For double label electron microscopic immunolocalization, cryostat sections from heart tissue of newborn rats were fixed for 10 min in acetone (−20°C) and then incubated simultaneously with guinea pig antibodies to desmoplakins and with rabbit antibodies to desmin from human uterus for 30 min. Sections were washed three times with PBS for 10 min and incubated with goat IgG (1 mg/ml; Miles) for 25 min, prior to the simultaneously performed incubation with both goat IgG against guinea pig IgG which were coupled to small gold colloidal particles (5 nm diameter) and against rabbit IgG coupled with larger gold particles (20 nm, diameter; each 20 μg/ml; Janssen Pharmaceutica, Beerse, Belgium) for 12 h at room temperature. After extensive washing with PBS, sections were fixed with glutaraldehyde and processed for thin sectioning.

For electron microscopy of ultrathin sections, cells grown on cover slips were fixed, dehydrated, embedded and sectioned as described (Franke *et al.*, 1978b).

Dissociation of cells and desmosomal junctions

EGTA (4 mM) was used for dissociating cells (for details see Kartenbeck *et al.*, 1982). After various times of incubation (0, 10, 30 or 120 min) at 37°C cells were fixed for light and electron microscopy.

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