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N-Cadherin/Catenin–Based Costameres in Cultured Chicken Cardiomyocytes

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Abstract N-cadherin is a member of the Ca^{2+}-dependent cell adhesion molecules and plays an important role in the assembly of the adherens junction in chicken cardiomyocytes. In addition to being present at the cell-cell junction, N-cadherin is associated with costameres in extrajunctional regions. The significance of the N-cadherin-associated costameres and whether catenins are components of costameres in chicken cardiomyocytes are not known. In this study, double-labeling immunofluorescence microscopy was used to determine the extrajunctional distribution of both N-cadherin and its cytoplasmic associated proteins, α- and β-catenins, and their relationship to myofibrillar Z-disc α-actinin. N-cadherin, α-, and β-catenins were all found to be present at the extrajunctional region and, in some cases, were codistributed with myofibrillar α-actinin exhibiting a periodic staining pattern. Confocal microscopy confirmed that both N-cadherin and β-catenin colocalized with peripheral myofibrillar α-actinin on the dorsal surface of cardiomyocytes as components of the costameres. Intracellular application of antibodies specific for the cytoplasmic portions of N-cadherin, α-, and β-catenin, either by electroporation or microinjection, resulted in myofibril disorganization and disassembly. These results suggest the existence of N-cadherin/catenin-based costameres in the dorsal surface of cultured chicken cardiomyocytes in addition to the integrin/vinculin-based costameres found in the ventral surface and indicate that the former set of costameres is essential for cardiac myofibrillogenesis. J. Cell. Biochem. 75:93–104, 1999. © 1999 Wiley-Liss, Inc.

Key words: N-cadherin; catenins; costamere; cardiomyocyte; confocal; electroporation

Cadherins are transmembrane glycoproteins which mediate cell-cell adhesion through a Ca^{2+}-dependent mechanism and play a pivot role in morphogenesis [Takeichi, 1988]. The extracellular domain of the cadherins is responsible for homophilic binding to its counter-part on neighboring cells, while the cytoplasmic portion is linked to the actin-based cytoskeleton via a group of proteins called catenins [Ozawa et al., 1989]. At least three catenins, α-, β-, and γ-catenin, with molecular masses ranging from 80–102 kDa, have been identified. α-Catenin has been shown to be a vinculin homologue [Nagafuchi et al., 1991; Herrenknecht et al., 1991]. β-Catenin shares 70 or 63% sequence identity with the *armadillo* gene product of *Drosophilia* and human plakoglobin, respectively [McCrea and Gumbiner, 1991], while γ-catenin and plakoglobin have been suggested to be one and the same protein, as they have a similar molecular mass and pI [Knudsen and Wheelock, 1992; Peifer et al., 1992; Piepenhagen and Nelson, 1993]. The formation of a cadherin-catenin complex is thought to be crucial for the adhesive nature of cadherins [Nagafuchi et al., 1989; Ozawa et al., 1990].

N-cadherin, a member of the cadherin family, is concentrated at the adherens junction of intercalated discs and plays an important role in the assembly of the adherens junction in cardiomyocytes [Volk and Geiger, 1984, 1986]. In addition to its presence at the cell-cell junction, N-cadherin is found at lateral borders in chicken cardiac muscle as periodic patches associated with the peripheral Z-discs [Volk and Geiger, 1984]. In cultured chicken cardiomyocytes, N-cadherin is associated with costameres along peripheral Z-discs at the dorsal and lateral cell

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surfaces [Goncharova et al., 1992]. Costameres were first identified as vinculin-containing rib-like structures which are involved in attaching the Z-discs of peripheral myofibrils to the plasma membrane on the lateral surfaces of cardiac and skeletal muscles [Pardo et al., 1983a,b; Koteliansky and Gnushev, 1983]. In addition, talin, spectrin, α-actinin, and integrin are also found in costameres [Craig and Pardo, 1983; Belkin et al., 1986; Danowski, 1992; McDonald et al., 1995]. An immunoelectron microscopic study further demonstrated the presence of α- and β-catenins in the costameric structure at the lateral membrane of Xenopus cardiac muscle [Kurth et al., 1996].

Several lines of evidence suggest the involvement of N-cadherin in cardiac myofibrillogenesis. Treatment of cultured chicken cardiomyocytes with anti-N-cadherin antibodies inhibits myofibril assembly by reducing the size and number of Z-discs and results in the appearance of α-actinin-containing beaded filaments, both effects being concentration-dependent and reversible [Goncharova et al., 1992]. In a quantitative electron microscopic study, inclusion of anti-N-cadherin antibody in the culture medium was shown to inhibit myofibrillogenesis in chicken cardiomyocytes by reducing the cytoplasmic myofibril content [Soler and Knudsen, 1994]. A recent study of precardiac mesoderm explants from chicken embryos demonstrated that addition of N-cadherin antibodies to the culture medium perturbs precardiac differentiation and myofibrillogenesis by preventing the expression of sarcomeric α-actinin and myosin [Imanaka-Yoshida, 1997; Imanaka-Yoshida et al., 1998]. It is assumed that N-cadherin, by acting through its cytoplasmic associated catenins, functions as a subsarcolemma organization center for myofibrils [Soler and Knudsen, 1994].

Although α- and β-catenins are present in areas other than the intercellular junction in Xenopus cardiac muscles [Kurth et al., 1996] and N-cadherin has been implicated in cardiac myofibrillogenesis [Goncharova et al., 1992; Soler and Knudsen, 1994; Imanaka-Yoshida et al., 1998], it is not known whether catenins are components of costameres in chicken cardiomyocytes and the significance of N-cadherin-associated costameres in myofibrillogenesis remains obscure. In this study, immunofluorescence microscopy was used to explore the distribution of N-cadherin, α-catenin, and β-catenin in cultured chicken cardiomyocytes and their relationship to costameric α-actinin. The results demonstrate that catenins are associated with costameres in chicken cardiomyocytes. The incorporation of specific antibodies by electroporation has been used as an effective method for the large-scale transfer of antibodies into cells for the study of cellular metabolism [Chakrabarti et al., 1989]. When antibodies against N-cadherin, α-catenin, and β-catenin were introduced into cardiomyocytes by either electroporation or microinjection, a marked change in myofibrillar organization and strucutre was seen, suggesting a role for N-cadherin/catenin-based costameres in cardiac myofibrillogenesis.

MATERIALS AND METHODS

Cell Culture

Chick cardiomyocyte cultures was prepared from 7- to 9-day-old chick embryos as previously described [Lin et al., 1989]. Heart tissue fragments were isolated and washed with Ca^{2+}/Mg^{2+}-free Hank's balanced saline solution (HBSS), then minced and incubated with 0.05% trypsin. After enzymatic digestion, cells were mechanically separated by repeated passage through a Pasteur pipette and maintained in a nutrient medium (10% fetal bovine serum, 1% penicillin and streptomycin, 2 mM glutamine in MEM containing Earle's salts). The cells were then collected by centrifugation at 1,000 rpm for 10 min, resuspended in 10 ml of nutrient medium, preplated on a 10 cm Petri dish and maintained in a 5% CO_2 incubator at 37°C for 1 h. After attachment of fibroblasts, the suspended cells were collected, diluted with nutrient medium, and grown on collagen-coated coverslips at a density of $2.0–3.5 \times 10^5$ cells/35 mm Petri dish in a 5% CO_2 incubator at 37°C. On the day after plating, the nutrient medium was replaced with glutamine-free medium (10% fetal bovine serum, 1% penicillin, and streptomycin in MEM containing Earle's salts), then the medium was replaced every second day.

Immunofluorescence Microscopy

Cardiomyocyte cultures were fixed for 10 min at room temperature with 10% formalin in phosphate-buffered saline (PBS). After washing in PBS, the cultures were simultaneously blocked and permeabilized for 15 min at room temperature with 5% nonfat dry milk in PBS containing 0.1% (v/v) Triton X-100, as previously described [Wu et al., 1996]. Incubation with the primary antibody was performed at 37°C for 1 h. Rabbit

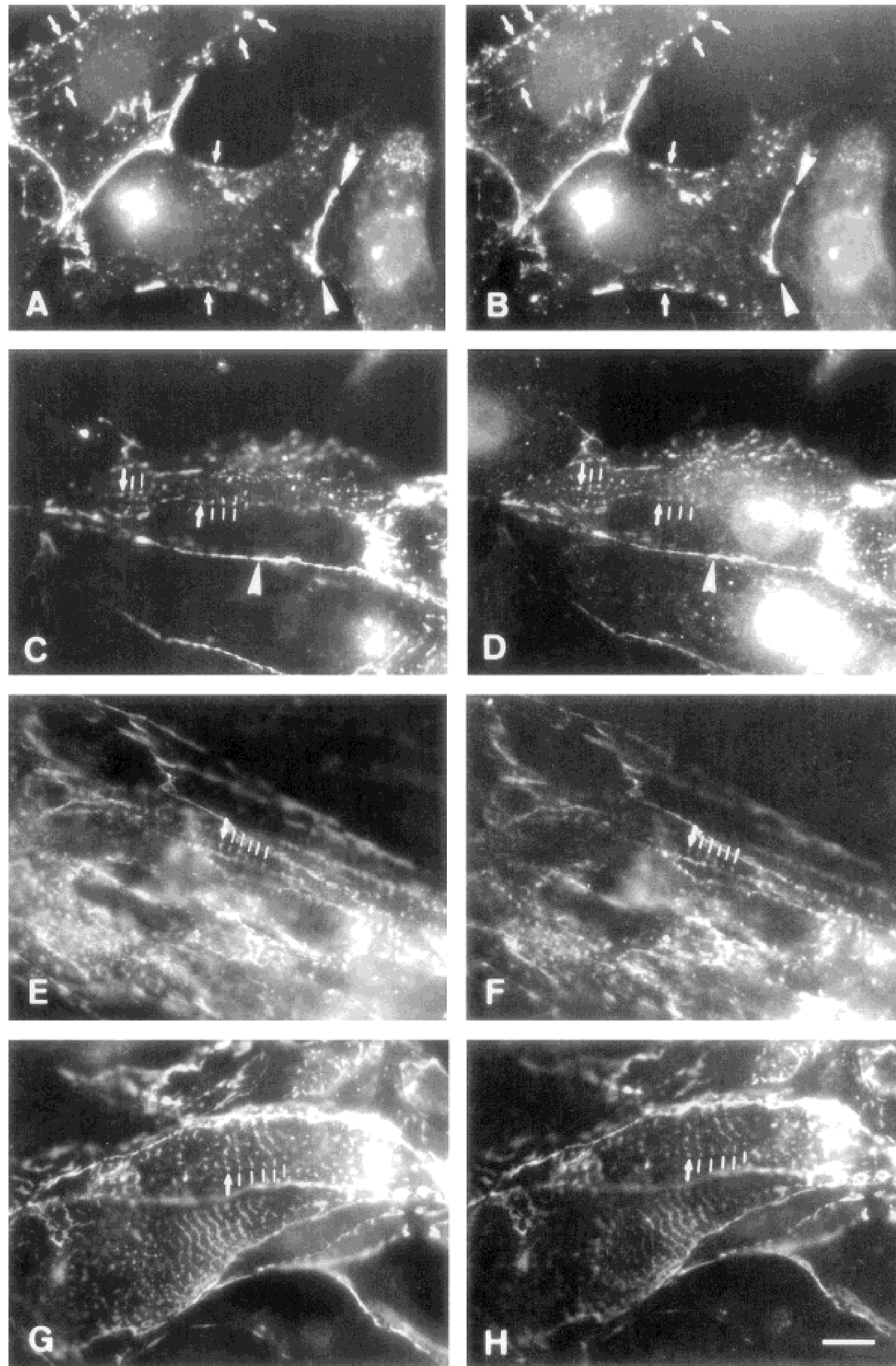

Fig. 1. Double-labeling immunofluorescence microscopy of N-cadherin, α-catenin, and β-catenin in cardiomyocytes. Day 2 **(A–D)** and day 5 **(E–H)** cardiomyocytes double-stained for N-cadherin (A,C,E,G), and α-catenin (B,F) or β-catenin (D,H), respectively. The arrowheads indicate the presence of N-cadherin, α-catenin, and β-catenin at cell-cell junctions. The arrows indicate the extrajunctional distribution of N-cadherin, α-catenin, and β-catenin. The parallel bars indicate periodic staining for N-cadherin (C,E,G), α-catenin (F), or β-catenin (D,H). Scale bar = 10 μm.

polyclonal anti-α-catenin antiserum (C-2801), rabbit polyclonal anti-β-catenin antiserum (C-2206), and mouse monoclonal anti-N-cadherin antibody (C-2542, clone GC-4) were all purchased from Sigma Chemical Co. (St. Louis, MO). The production and characterization of a rat anti-α-actinin antiserum has been previously described [Wang et al., 1998]. Double-immunostaining was performed using an appropriate combination of the rabbit and rat antisera and the mouse monoclonal antibody. After washing in PBS, the cultures were incubated for 1 h at 37°C with a 1:100 dilution of FITC-conjugated goat anti-rabbit IgG antiserum (Sigma) or FITC-conjugated, mouse IgG pre-absorbed, goat anti-rat IgG antiserum (Vector, Burlingame, CA), as appropriate. After a brief wash, the cultures were then incubated for 1 h at 37°C with a 1:100 dilution of biotinylated, rat IgG pre-absorbed, goat anti-mouse IgG antiserum, followed by a 1:100 dilution of avidin-conjugated Texas red (Vector) for another 30 min at 37°C. After washing in PBS, the cultures were mounted using a mixture of 2% n-propyl gallate in 60% glycerol and 0.1 M PBS (pH 8.0). The immunofluorescence-labeled cultures were analyzed and photographed using either a Leica microscope equipped with epifluorescence or a

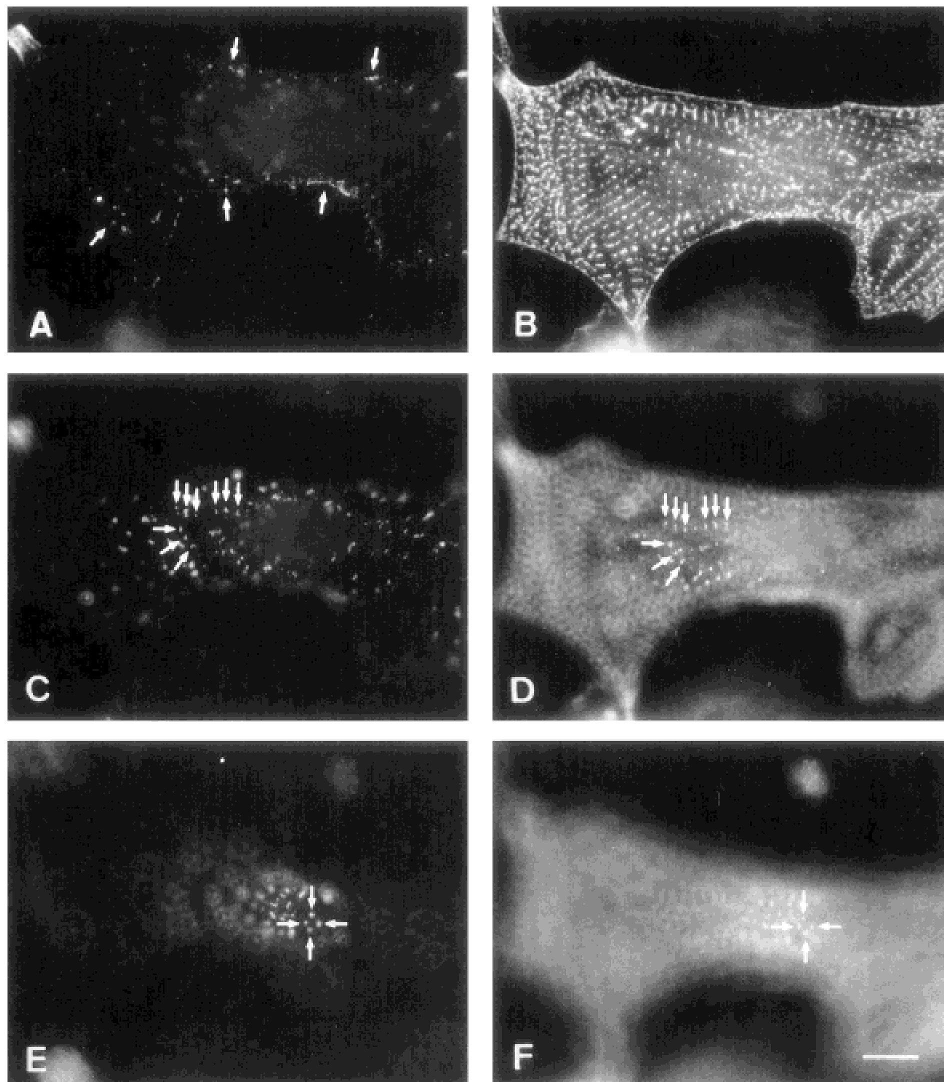

Fig. 2. Colocalization of N-cadherin and α-actinin in cardiomyocytes. Day 2 cardiomyocytes double-labeled for N-cadherin (A,C,E) and α-actinin (B,D,F). The focal planes were placed on the ventral surface **(A,B)**, middle level **(C,D)**, or dorsal surface **(E,F)** of the same cell. The arrows in A indicate the peripheral distribution of N-cadherin. The arrows in C and E indicate the same positions on the Z-discs as the arrows in D and F, respectively. Scale bar = 10 μm.

Zeiss laser scan microscope. In control cultures, primary antibodies were omitted and all control groups exhibited negligible immunoreactivity. The FITC signal was not detected in the Texas red channel and vice versa.

Laser Scan Confocal Microscopy

Immunofluorescence-labeled cells were analyzed using an inverted laser scanning microscope (Zeiss LSM 410 invert, Carl Zeiss, Oberkochen, Germany), equipped with both argon ion (488 nm) and HeNe (543 nm) lasers. For double-labeling, the confocal overlay mode was used, and images from two different channels, one green and the other red, collected simultaneously on the same focal plane. Colocalization of two labeled antigens was detected as a single yellow image when the images from both channels were overlaid. For each image, the cells were optically sectioned from the ventral to the dorsal surface at intervals of 1 μm.

Electroporation and Microinjection of Antibodies Into Cardiomyocytes

Electroporation was performed according to a modification of a standard procedure [Celis, 1994] as previously described [Wang et al., 1997]. The antibodies used were a rabbit poly-

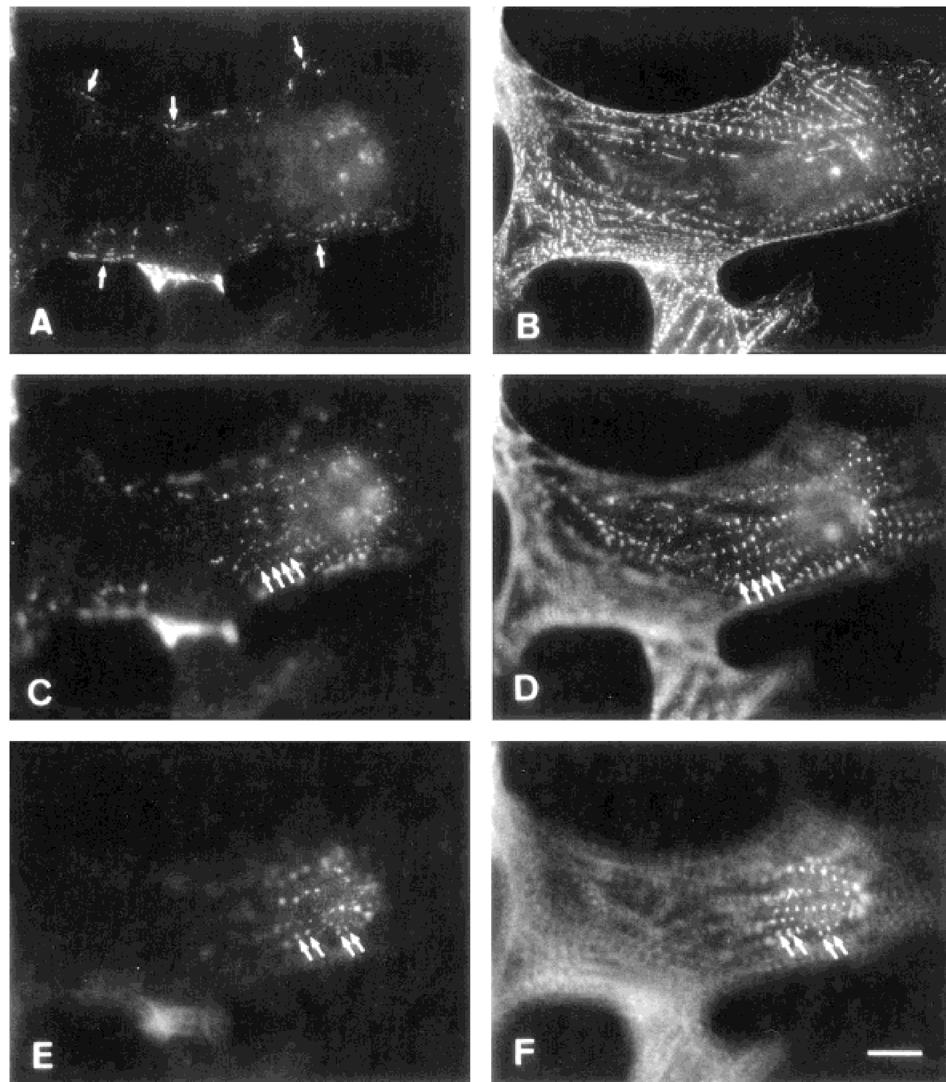

Fig. 3. Colocalization of β-catenin and α-actinin in cardiomyocytes. Day 2 cardiomyocytes double-labeled for β-catenin (A,C,E) and α-actinin (B,D,F). the focal planes were placed on the ventral surface **(A,B)**, middle level **(C,D)**, or dorsal surface **(E,F)** of the same cells. The arrows in A indicate the peripheral distribution of β-catenin. The arrows in C and E indicate the same positions on the Z-discs as the arrows in D and F, respectively. Scale bar = 10 μm.

clonal anti-pan-cadherin antiserum (C-3678, Sigma), a rabbit polyclonal anti-α-catenin antiserum (C-2801), and a rabbit polyclonal anti-β-catenin antiserum (C-2206). The anti-pan-cadherin antiserum was raised against a synthetic peptide of 24 amino acids corresponding to the C-terminal of chicken N-cadherin [Geiger et al., 1990]. Cardiomyocytes cultured on coverslips were washed twice with Hepes buffer saline (HBS; 21 mM Hepes, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM glucose, pH 7.5), then the coverslips and the attached cardiomyocytes were transferred to an electroporation cuvette containing 1 ml of antibody (1:10 dilution in HBS). After incubation for 5 min on ice, the cardiomyocytes were electroporated at room temperature in a cell porator (Gibco BRL, Gaithersburg, MD) with a brief pulse of 750 V/cm and a duration of 0.7~0.9 msec using the settings of an initial voltage of 300 V, a low resistance, and a capacitance of 60-μF. In electroporation controls, the antibody was replaced by normal rabbit IgG (Sigma), or HBS. Microinjection was performed using a micromanipulator with a glass micropipette, as previously described [Nakagawa and Takeichi, 1997], the volume of antibody-containing solution injected per injection being approximately 100 nl. In microinjection controls, the antisera were replaced by normal rabbit serum (Sigma) or HBS. Incorporated IgG was labeled using an FITC-conjugated goat anti-rabbit IgG. After electroporation, the cardiomyocytes were left in the electroporation cuvette on ice for 15 min. After a brief wash with HBS to remove excess antibody, the cultures were transferred to a 35

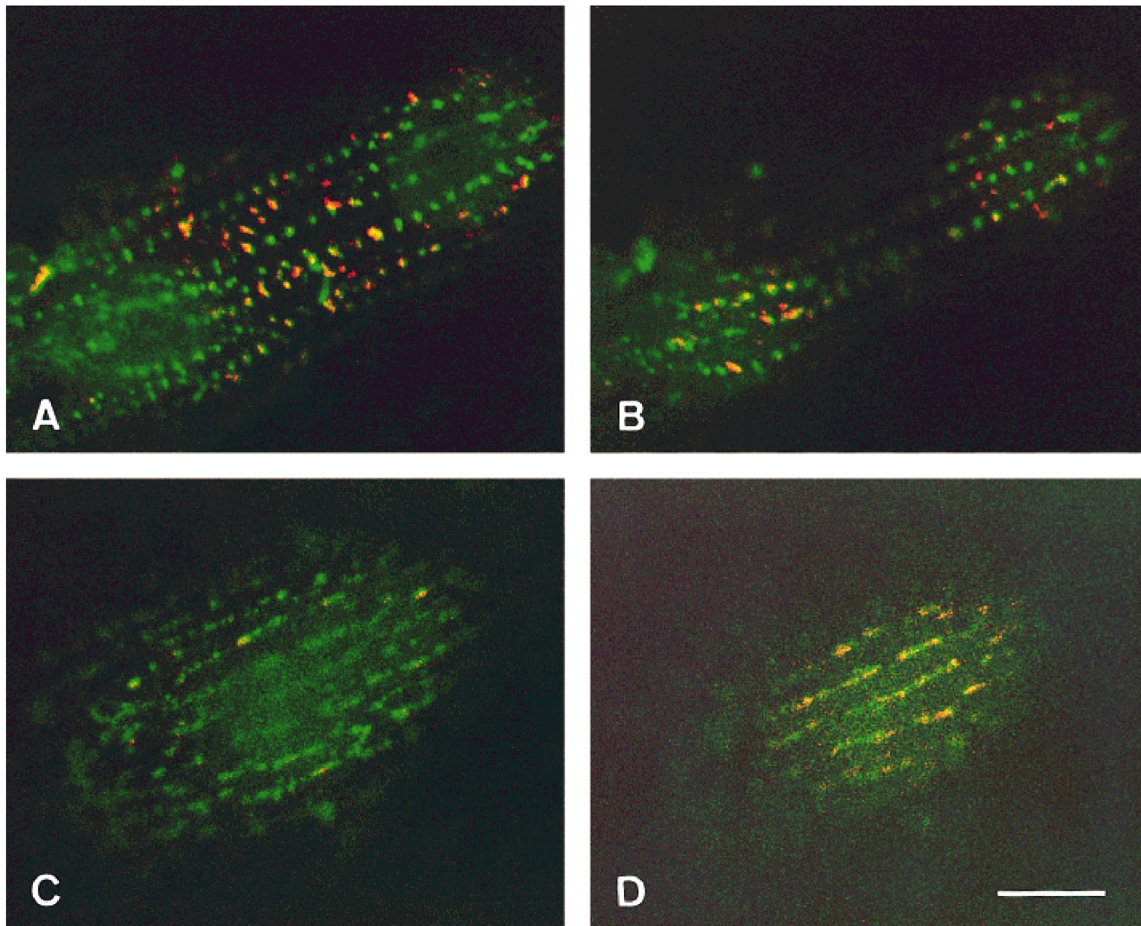

Fig. 4. Confocal micrographs of N-cadherin, β-catenin, and α-actinin localization in cardiomyocytes. **A,B:** Cardiomyocytes double-labeled for N-cadherin (red) and α-actinin (green). **C,D:** Cardiomyocytes double-labeled for β-catenin (red) and α-actinin (green). The focal planes were scanned through the dorsal-most cell surface in B and D. The focal planes in A and C are 1 μm below B and D, respectively. In some areas, N-cadherin or β-catenin reactivity overlaid α-actinin reactivity (yellow images), indicating a precise colocalization of N-cadherin and α-actinin (A,B), or β-catenin and α-actinin (C,D). Scale bar = 10 μm.

mm Petri dish containing nutrient medium and, together with the microinjected cardiomyocytes, incubated in a humidified atmosphere of 5% CO_2 at 37°C for another 1 h before processing for immunofluorescence microscopy.

RESULTS

Costameric Localization of N-Cadherin, α-Catenin, and β-Catenin in Cardiomyocytes

Positive staining for N-cadherin, α-catenin, and β-catenin was seen at cell-cell junctions in cardiomyocytes. Intense staining for N-cadherin (Fig. 1A,C) colocalized with staining for α-catenin (Fig. 1B) and β-catenin (Fig. 1D) along the cell-cell contacts corresponding to the junctional regions. In cardiomyocytes from day 2 cultures, staining of N-cadherin was also seen in the extrajunctional region as small spots or short bands, dispersed in the cytoplasm (Fig. 1A, arrows), and colocalized with the α-catenin staining (Fig. 1B, arrows). Occasionally, a periodic staining pattern of N-cadherin (Fig 1C, arrows and short bars), colocalized with β-catenin staining (Fig. 1D, arrows and short bars), was seen in developing cardiomyocytes from day 2 cultures. In cardiomyocytes at a more advanced stage of development (day 5 cultures), the periodic staining of N-cadherin (Fig. 1E,G), co-localized with both α-catenin (Fig. 1F) and β-catenin (Fig. 1H), was more conspicuous. In addition to its presence at cell-cell junctions and sarcomeric Z-discs, α-actinin is also a marker for the peripheral Z-discs of costameres [Danowski et al., 1992]. In order to further clarify the relationship of the extrajunctional N-cadherin and catenins to costameres, double-labeling studies were performed for N-cadherin or β-catenin and α-actinin, and focal planes selected at various levels from the ventral to the dorsal side of the cardiomyocytes. When the focal plane was close to the ventral surface, staining for extrajunctional N-cadherin (Fig. 2A) and β-catenin (Fig. 3A) was distributed at the cell periphery along the cortical region, whereas the extensive sarcomeric staining of α-actinin was associated with the myofibrillar Z-discs (Figs. 2B and 3B), which showed no N-cadherin or β-catenin staining.

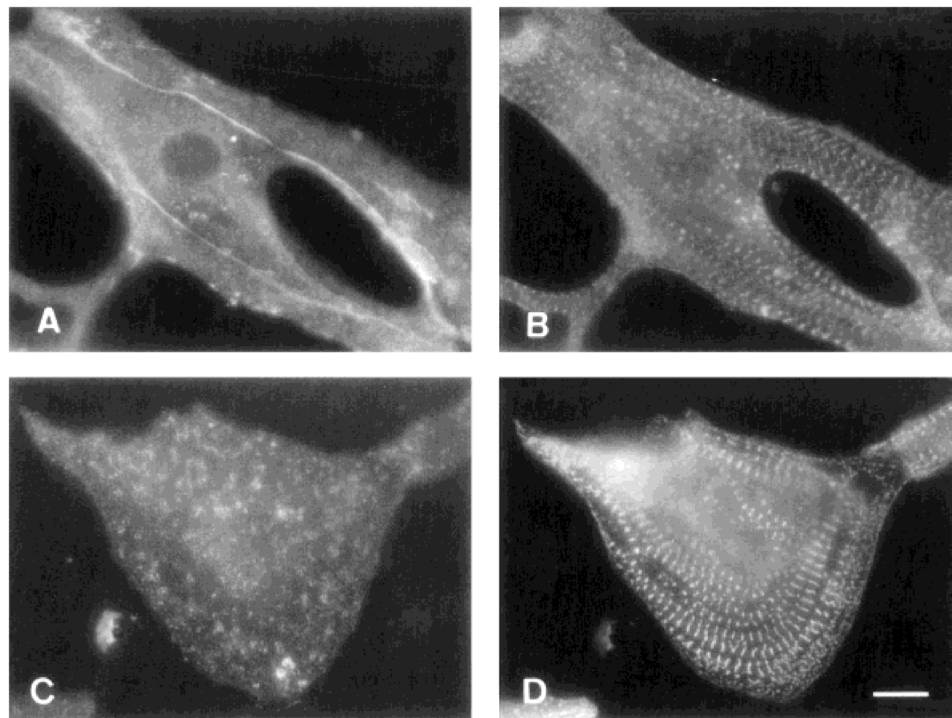

Fig. 5. Electroporation control of IgG incorporation into cardiomyocytes. Cardiomyocytes electroporated with HBS buffer **(A,B)** or HBS containing normal rabbit IgG **(C,D)**. The buffer-treated cells were double-labeled for α-catenin (A) and α-actinin (B). In the normal IgG-treated cells, the incorporated rabbit IgG (C) was visualized using an FITC-conjugated goat anti-rabbit IgG secondary antibody, while the myofibrils were stained for α-actinin (D) using a rat anti-α-actinin primary antibody and a Texas red-conjugated goat anti-rat IgG secondary antibody. The cardiomyocytes in both groups exhibit a uniformly arranged normal staining pattern for myofibrillar α-actinin (B,D). Scale bar = 10 μm.

When the focal planes were moved progressively toward the middle level of the cardiomyocytes, staining of N-cadherin (Fig. 2C) and β-catenin (Fig. 3C) appeared in the central regions of the cardiomyocytes and some of the N-cadherin and β-catenin immunoreactive spots colocalized with the periodic α-actinin staining (Figs. 2D and 3D). When the focal plane was close to the dorsal portion of the cell, N-cadherin (Fig. 2E) and β-catenin (Fig. 3E) staining apparently co-localized with α-actinin staining on the costameric Z-discs (Figs. 2F and 3F). In order to clarify the relationship of N-cadherin and β-catenin to the costameres, double-labeled cardiomyocytes were subjected to laser scanning confocal microscopic analysis. As the focal planes were scanned through the dorsal surface of the cardiomyocytes, staining for N-cadherin (Fig. 4A,B) and β-catenin (Fig. 4C,D) was found to be superimposed on that for α-actinin on the costameric Z-discs.

Incorporation of N-Cadherin, α-Catenin, and β-Catenin Antibodies Induce Disassembly of Myofibrils in Cardiomyocytes

Inclusion in the culture medium of antibodies directed against the extracellular domain of N-cadherin has been shown to inhibit myofibril assembly in cardiomyocytes [Goncharova et al., 1992]. To determine the role of the costamere-associated catenins in myofibril organization, antisera against N-cadherin, α-catenin, and β-catenin were incorporated into cardiomyocytes by electroporation. In controls, electroporation of the cardiomyocytes with buffer alone (Fig. 5A) or buffer containing normal rabbit IgG (Fig. 5C) had no apparent effect on myofibril organization, as shown by the normal staining pattern for myofibrillar α-actinin (Fig. 5B,D). In addition, no apparent change in cell morphology was seen by phase-contrast microscopy after antibody incorporation (data not shown). However, when antisera against the cytoplasmic portion of N-cadherin were used for electroporation on days 2 (Fig. 6A,B) and 5 (Fig. 6C,D) cultures, clusters of α-actinin (Fig. 6B,D, arrowheads) and α-actinin–containing premyofibrils (Fig. 6D, arrows) were detected. A marked change in myofibrillar structure was noted when α- (Fig. 7A) or β-catenin (Fig. 7C) antisera were electroporated into day 2 cardiomyocytes, with most of the myofibrils being disassembled, as shown by the disorganized α-actinin staining pattern (Fig. 7B,D). A dramatic effect of anti-

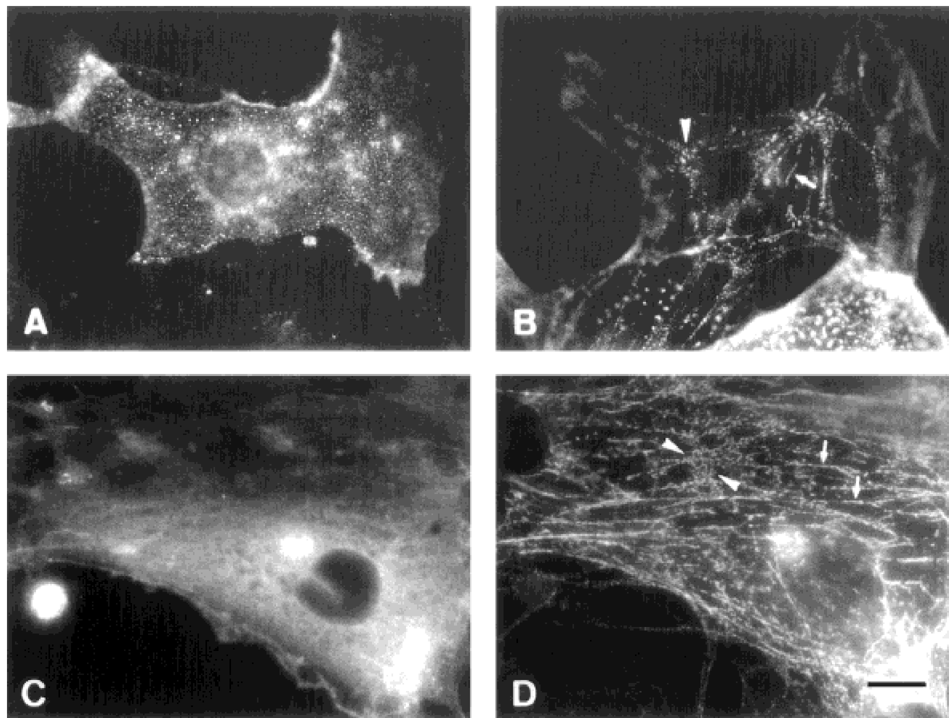

Fig. 6. Incorporation of antisera against N-cadherin into cardiomyocytes by electroporation. Day 2 **(A,B)** and day 5 **(C,D)** cardiomyocytes containing incorporated antisera against the cytoplasmic portion of N-cadherin (A,C) and double-stained for α-actinin (B,D). The arrows indicate α-actinin-containing filamentous structures. The arrowheads indicate clusters of myofibrillar α-actinin. Scale bar = 10 μm.

body incorporation was seen in cardiomyocytes at a more advanced developmental stage (day 5 cultures). When antisera against α- (Fig. 7E) or β-catenin (Fig. 7G) were used, discontinuous patches of α-actinin, representing fragmented myofibrils, were found along the disarrayed myofibrils (arrows in Fig. 7F,H). To further confirm the effect on myofibrils, antibodies were microinjected into cardiomyocytes. Microinjection of a normal rabbit serum (Fig. 8A) into day

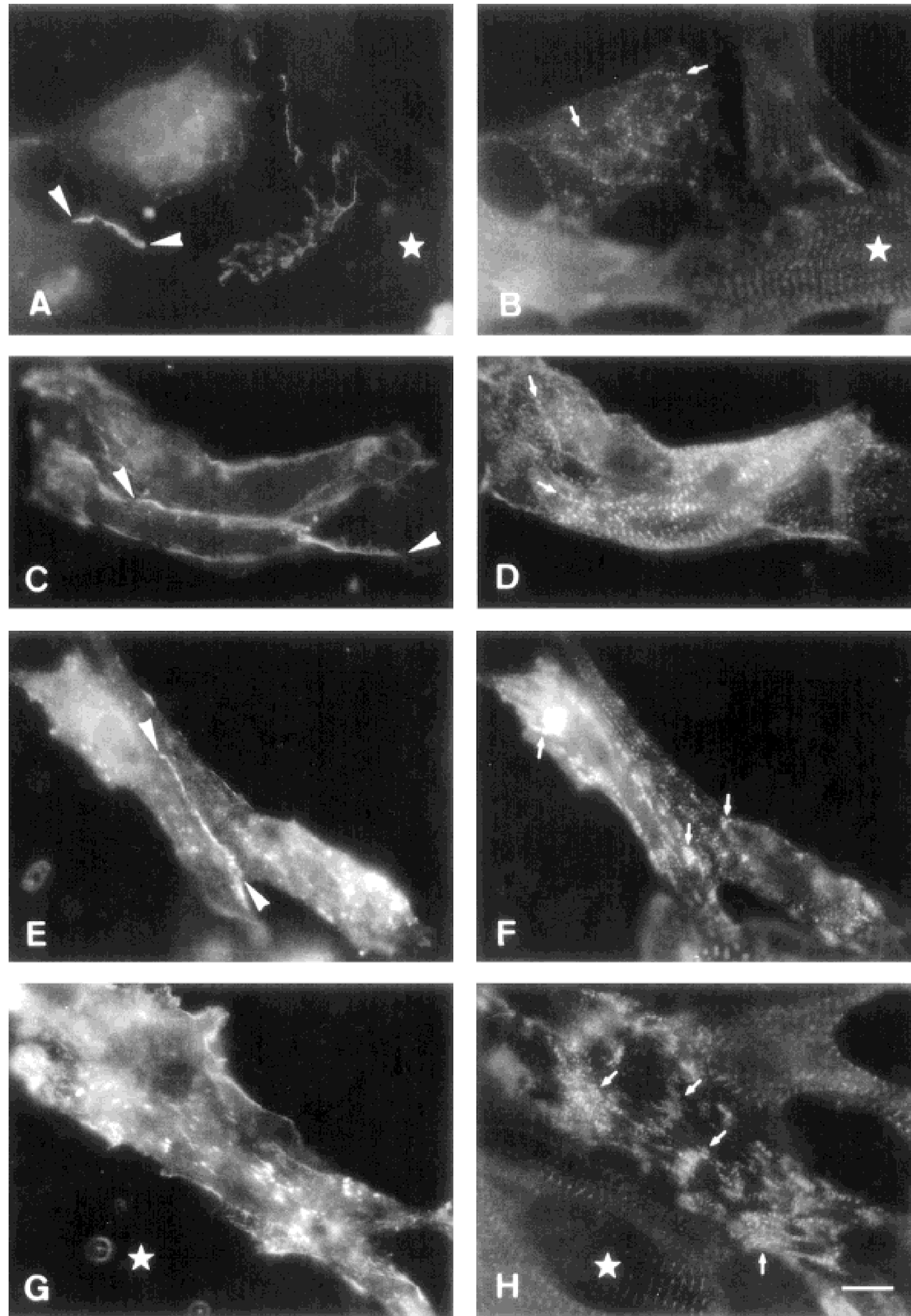

Fig. 7. Incorporation of antisera against α-catenin and β-catenin into cardiomyocytes by electroporation. Day 2 **(A–D)** and day 5 **(E–H)** cardiomyocytes containing incorporated antisera against α-catenin (A,E) and β-catenin (C,G), and double-stained for α-actinin (B,D,F,H), respectively. The arrowheads in (A,C,E) indicate the cell-cell junctions in antibody-incorporated cardiomyocytes. The arrows in (B,D) indicate disorganized α-actinin staining. The arrows in (F,H) indicate clustered staining of the fragmented myofibrillar α-actinin. The asterisks indicate cardiomyocytes without antibody incorporation with a normal periodic α-actinin staining pattern (B,H). Scale bar = 10 μm.

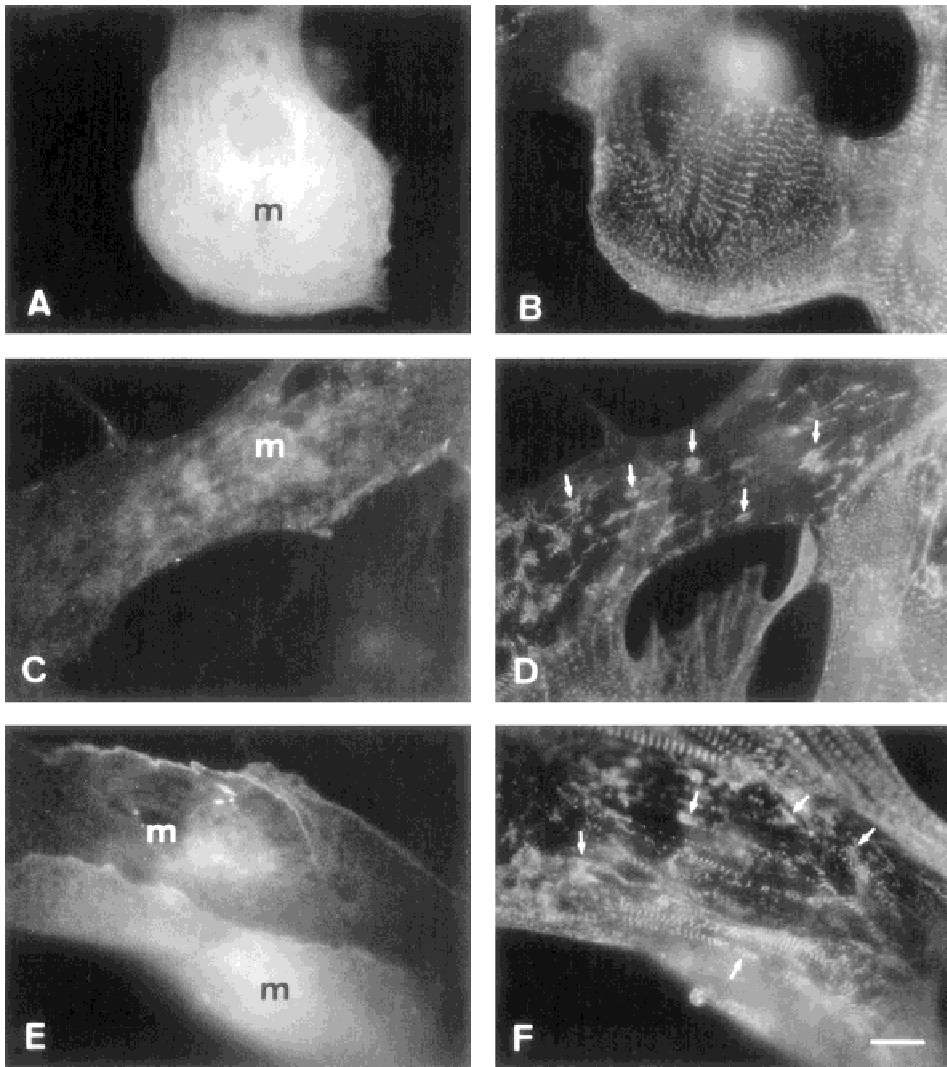

Fig. 8. Microinjection of antisera against α-catenin and β-catenin into cardiomyocytes. Day 3 cardiomyocytes microinjected with normal rabbit serum (A,B) or rabbit antisera against α-catenin (C) or β-catenin (E), and double-stained for the incorporated antibodies **(A,C,E)** and α-actinin **(B,D,F)**. The arrows in (D,F) indicate broken segments of myofibrils. m, microinjected cardiomyocytes. Scale bar = 10 μm.

3 cardiomyocytes had no effect on the myofibrils, as shown by the typical staining pattern of myofibrillar α-actinin (Fig. 8B), whereas the use of antisera against α- (Fig. 8C) or β-catenin (Fig. 8E) caused myofibril disassembly. Large aggregates of α-actinin staining were found along the disarrayed myofibrils in cardiomyocytes (arrows in Fig. 8D,F).

DISCUSSION

In this study, immunofluorescence microscopy was used to explore the extrajunctional distribution of N-cadherin and its cytoplasmic associated proteins, α- and β-catenin, and to determine their role in both the attachment of the myofibril to the sarcolemma and in myofibrillogenesis in cultured chicken cardiomyocytes. Of the members of the cadherin superfamily, N-cadherin was the first to be localized to the intercalated discs of cardiac muscles, where it was primarily associated with the adherens junctions [Volk and Geiger 1984, 1986; Geiger et al., 1990]. In addition to its localization to the intercalated discs, dots of N-cadherin were also noticed along the lateral cell borders in frozen sections of chicken cardiac muscle [Volk and Geiger 1984, 1986; Geiger et al., 1990]. Using computer-aided three-dimensional microscopy, N-cadherin was found to be colocalized with α-actinin of the cortical myofibrils at Z-disc-

membrane contacts on the dorsal cell surface as a component of the costamere in cultured chicken cardiomyocytes [Goncharova et al., 1992]. In the present study, we have demonstrated that not only N-cadherin, but also α-catenin and β-catenin, are components of the costamere in chicken cardiomyocytes.

The α-catenin and β-catenin found in the N-cadherin-associated costameres may play a role in linking the cortical myofibrils to the plasma membrane on the dorsal cell surface. In a study of adult rat cardiomyocytes cultured on a flexible silicon rubber substratum, costameres were shown to be responsible for transmitting force from the contractile apparatus to the extracellular matrix [Danowski et al., 1992]. In the same study, however, some of the cardiomyocytes did not produce substratum wrinkles although the cell surface shifted rhythmically during contraction and relaxation cycles; the authors assumed that these contracting myofibrils were attached to the sarcolemma on the dorsal cell surface [Danowski et al., 1992]. This assumption is further supported by the results of our immunofluorescence study showing that some of the cortical myofibrils were attached to the dorsal sarcolemma by the N-cadherin/catenin-based costameres. In the cell-cell contact areas of fibroblasts, the association of the actin cytoskeleton with the N-cadherin/catenin complex is mediated via a direct linkage between α-actinin and α-catenin [Knudsen et al., 1995]. In cardiomyocytes, the cortical myofibrils may be linked to the dorsal sarcolemma by a direct interaction between the α-actinin of the myofibrils and the α-catenin of the N-cadherin/catenin-based costameres.

Functional assays involving antibody incorporation were then performed to test if the N-cadherin/catenin-based costameres play a role in the attachment of the myofibril to the sarcolemma and in myofibrillogenesis. In contrast to previous studies performed by adding antibodies against the extracellular portion of N-cadherin to the culture medium [Goncharova et al., 1992; Soler and Knudsen, 1994; Imanaka-Yoshida, 1997; Imanaka-Yoshida et al,, 1998], we directly introduced antisera against the cytoplasmic portion of N-cadherin, α-catenin, and β-catenin into cardiomyocytes using electroporation or microinjection. Our results show that incorporation of antibodies by either method caused myofibril disassembly (day 2 cultures; Figs. 6B and 7B,D) or fragmentation (day 5 cultures; Fig. 7F,H). In a model of myofibril assembly, Sanger and colleagues proposed that premyofibrils are initially formed in the subsarcolemmal areas, followed by the appearance of α-actinin–containing Z-bodies along the premyofibrils, and, finally, laterally aligned Z-bodies merge to form the continuous Z-bands of mature myofibrils [Rhee et al., 1993; Dabiri et al., 1997]. Since most of the myofibrils in cardiomyocytes from day 2 cultures are immature, binding of the antibodies to the N-cadherin/catenin-based costameres may prevent the fusion of Z-bodies into Z-bands, as suggested by the presence of the premyofibril-like structures and disorganized α-actinin after antibody incorporation. Since most of the myofibrils are already mature and Z-bands already formed in day 5 cultures, when they bind to the catenins, the incorporated antibodies might hamper the correct association of the N-cadherin/catenin-based costameres, destabilize the linkage between myofibrils and sarcolemma, and result in the discontinuous α-actinin patches seen in the fragmented myofibrils.

The present study demonstrates the existence of an N-cadherin/catenin-based costamere in the dorsal surface of cultured chicken cardiomyocytes in addition to the integrin/vinculin-based costameres found in the ventral surface. Perturbation of the N-cadherin/catenin complex is associated with myofibril disorganization, indicating that this set of costameres is essential for cardiac myofibrillogenesis. The formation and maintenance of costameres and myofibrils in cardiomyocytes have been shown to be regulated by mechanical forces transmitted by the costameres. Mechanically unloading of cardiomyocytes by inhibiting spontaneous contractile activity using a Ca^{2+} channel blocker resulted in the loss of the β_1-integrin/vinculin-based costameres [Sharp et al., 1997]. Contractile arrest also promotes breakdown of cardiac myofibrils and accelerates the turnover of contractile proteins in cultured rat cardiomyocytes [Samarel et al., 1992; Sharp et al., 1993; Simpson et al., 1996]. These results suggest that costameres not only function as mechanical force transducers, but also play a role in biochemical signal transduction for myofibril assembly. Perturbation of the N-cadherin/catenin-associated costameres may affect myofibril assembly through the signal transduction cascade operating via the cadherin/catenin complex. The regulatory role of the N-cadherin/catenin-associated costamere in cardiac myofibrillogenesis requires further investigation.

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