Caveolae-associated proteins in cardiomyocytes: caveolin-2 expression and interactions with caveolin-3

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Caveolae are 50- to 100-nm flask-shaped invaginations of the plasma membrane that dynamically regulate specialized functions at the cell surface. Caveolae are particularly abundant in terminally differentiated cells (i.e., adipocytes, squamous epithelial cells, and muscle cells), where they participate in vesicular transport and serve as platforms to organize and regulate signal transduction by G protein-coupled receptors and receptor tyrosine kinases (5, 26). Recent studies implicate caveolae as nucleation centers for signaling by G protein-coupled receptors and receptor tyrosine kinases in cardiomyocytes (14, 20, 31). Progress in this area has benefited from the development of biochemical methods to isolate caveolae, taking advantage of their distinctive lipid and protein compositions. Caveolae are highly enriched in cholesterol and lipids with saturated acyl side chains ( sphingolipids and glycosphingolipids); these lipids pack tightly to form a liquid-ordered phase that resists solubilization by nonionic detergents such as Triton X-100 (at low temperatures). Caveolae microdomains are further defined by their principal structural protein, caveolin, a multigene family of immunologically distinct 21- to 24-kDa isoforms that can be used to track caveolae vesicles during biochemical purification.

Caveolin-1 is the ubiquitous isoform detected in tissues as full-length caveolin-1α and an ~3 kDa smaller caveolin-1β isoform [generated through an alternative transcription initiation site at an internal methionine at position 32 (5)]. Caveolin-1 self-assembles to form high-molecular-mass homoooligomers that directly bind glycosphingolipids and cholesterol and (through protein-protein and protein-lipid interactions) drive caveolae biogenesis. Caveolin-3 is the structurally related muscle-specific caveolin gene family member whose role in striated muscle cells is largely analogous to that of caveolin-1 in nonmuscle cells. Caveolin-1 or -3 expression in cells that lack caveolin/caveolae is sufficient to sculpt vesicles with morphological features of caveolae; both caveolin-1 and caveolin-3 act as molecular scaffolds, to sequester and regulate signaling by certain lipid-modified signaling proteins (Go subunits, Ras and Src (26)).

Caveolin-2 is a structurally related caveolin family member with a tissue distribution that largely overlaps with caveolin-1 (25). However, the functional properties of caveolin-2 are quite distinct from those of caveolin-1 or caveolin-3. Caveolin-2 is detected as a mixture of monomers and dimers that are retained in the Golgi complex or lipid droplets in cells that lack caveolin-1 or -3; caveolin-2 alone does not assemble into high-molecular-mass oligomers or drive caveolae formation (13). However, in most native cells that coexpress caveolin-1 and -2, caveolin-2 is recovered in stable Triton-insoluble heterooligomeric complexes along with caveolin-1 (24). Caveolin-2 might be expected to interact in a fashion analogous to caveolin-3 and influence caveolae biogenesis in striated muscle cells, including cardiomyocytes. However, there is as yet no consensus in the literature as to whether cardi-
omyocytes express caveolin-2. Although several laboratories detected caveolin-2 mRNA or protein in cardiac preparations (7, 12, 17), some investigators argue that endothelial cells and fibroblasts (rather than cardiomyocytes) are the source of caveolin-2 in intact heart preparations (7, 17). Indeed, Yarbrough et al. (30) recently identified caveolin-3, but not caveolin-1 or caveolin-2, by immunoblot analysis with isofrom-selective antibodies and low-density vesicles purified from isolated adult rat cardiomyocytes.

Flotillin-1 and flotillin-2/epidermal surface antigen (ESA) comprise another family of ~45-kDa caveolae-associated proteins. Although not structural homologs of caveolin, flotillins act as functional homologs to drive caveolae-like vesicle formation when expressed alone in insect cells; in cells that coexpress flotillins and caveolins, flotillin-1 and flotillin-2 form stable complexes that are communoprecipitated (along with caveolin-2) by the caveolin-1 antibody (29). Although flotillin-1 (which is abundant in striated muscle tissues) and flotillin-2 (which is relatively ubiquitous) are both detected in extracts from mouse myocardium and neonatal rat cardiomyocyte cultures (19, 29), a role for flotillins in the formation and/or structural organization of caveolae-like vesicles in muscle cells has never been considered.

Recent studies in cell culture and genetically engineered mouse models suggest that tight regulation of caveolin-3 expression (and caveolae biogenesis) is critical for normal muscle physiology (4). Accordingly, this study examines caveolin/flotillin isoform expression in cardiomyocytes. The goals were to determine whether caveolin-3 expression is developmentally regulated, whether cardiomyocytes coexpress caveolin-2 and caveolin-3 and whether caveolin-3-caveolin-2 interactions might contribute to caveola biogenesis in cardiomyocytes.

METHODS

The protocols used in this study were approved by the Institutional Animal Care and Use Committee at Columbia University.

Cardiomyocytes were isolated from the hearts of 2-day-old Wistar rats by a trypsin dispersion procedure according to a protocol that incorporates a differential attachment procedure to enrich for cardiomyocytes followed by irradiation (19). Cells were plated at a density of 0.5 × 10^6 cells/ml (high density; to generate a confluent monolayer) or 0.25 × 10^6 cells/ml (low density; to yield cultures with reduced cell-cell contacts) on proamine sulfate-coated culture dishes and cultured in MEM (GIBCO-BRL) with 10% fetal calf serum, 5 × 10^{-5} M hypoxanthine, and 12 mM NaHCO_3. For some experiments, cultures highly enriched in cardiac fibroblasts were obtained from cells adherent to culture dishes during the preplating step (19). Adult rat ventricular myocytes were disaggregated according to methods described previously and used within 1–6 h of isolation (10).

Caveolin-rich membranes were prepared according to a detergent-free purification scheme described previously (19). Briefly, cells from five 100-mm-diameter dishes were scraped into 0.5 M sodium carbonate (pH 11.0; 0.5 ml/dish) and combined for each preparation. The extract was sequentially disrupted by homogenization with a Dounce homogenizer, a Polytron tissue grinder, and a tip sonicator. The homogenate was adjusted to 40% sucrose by adding an equal volume of 80% sucrose prepared in MES-buffered saline [25 mM MES (pH 6.5) and 0.15 M NaCl], placed on the bottom of an ultracentrifuge tube, overlaid with a 5–35% discontinuous sucrose gradient, and centrifuged at 38,000 rpm for 16–18 h in a SW40 rotor (Beckman). After centrifugation, aliquots of fractions were dissolved in sample buffer containing SDS and 2-mercaptoethanol and heated before electrophoresis in SDS-PAGE gels optimized for resolution of low-molecular-weight proteins to detect the distinct molecular forms of caveolin-2 (with a 3-cm-long 10% spacer gel above a 6-cm 16.5% polyacrylamide separating gel). Samples were then transferred to nitrocellulose and immunoblotted with anti-caveolin-1 (MAB 2234), anti-caveolin-2 (MAB 65), anti-caveolin-3 (MAB 26), anti-flotillin, or anti-ESA purchased from BD Transduction Laboratories. Evidence that anti-caveolin-2 (MAB 65) recognizes caveolin-2, but does not cross-react with either caveolin-1 or caveolin-3, has been previously published (24). The anti-β_1-adrenergic receptor antibodies were from Santa Cruz Biotechnology. In Figs. 1 and 3, immunoblot analysis of caveolin proteins was with proteins washed three times each with PBS, and membranes; membranes were probed for caveolin-2, stripped, and reprobed for caveolin-3 in Figs. 2 and 4. Immunodetection was with chemiluminescence.

For immunoprecipitation, cardiomyocytes from one 100-mm-diameter dish were rinsed with ice-cold PBS and harvested by the addition of 0.7 ml of extraction buffer (10 mM Tris-Cl (pH 8), 150 mM NaCl, 50 μg/ml aprotinin, 0.1 mM leupeptin, 50 μg/ml benzamidine, 2 mM phenylmethylsulfonfnyl fluoride, 50 μM pepstatin A, 0.1 mM sodium vanadate, 50 mM NaF, 1% Triton X-100, and 60 mM octyl glucoside). Scraped cells were sonicated and centrifuged at 4°C for 15 min at maximal speed in a microcentrifuge. The supernatant was washed three times with washing buffer [10 mM Tris-Cl (pH 8), 150 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100], and bound proteins were eluted with SDS-PAGE sample buffer and subjected to immunoblot analysis as described above.

For immunofluorescence studies, cardiomyocytes isolated from neonatal ventricles were plated on fibronectin-coated glass coverslips; the adherent cardiac fibroblasts obtained during the preplating step were cultured for 3 days, resuspended, and plated on fibronectin-coated glass coverslips in parallel. Cells were fixed with 3% paraformaldehyde in PBS at room temperature for 30 min and permeabilized with 0.1% Triton X-100 in PBS containing 0.2% BSA for 10 min. Non-specific binding sites were blocked by incubating coverslips for 30 min in PBS containing 0.2% BSA, 0.1% Triton X-100, and 10% normal goat serum. Cells were then incubated overnight at 4°C with mouse monoclonal anti-caveolin-1 (MAB 2234, 1:50), anti-caveolin-2 (MAB 65, 1:20), or anti-caveolin-3 (MAB 26, 1:1,000) alone or with polyclonal anti-α-actinin (1:50; Santa Cruz) raised in the rabbit (to minimize cross-reactivity with distinctly tagged secondary antibodies for double-labeling experiments). Primary antibodies were diluted in PBS with 0.1% Triton X-100 and 0.2% BSA. Cells were incubated three times (10 min each) with PBS, and primary antibodies were visualized by incubation with appropriate secondary antibodies conjugated to Alexa dyes (Molecular Probes, Eugene, OR); Alexa Fluor 594 goat anti-rabbit IgG (1:1,000) was used to visualize α-actinin, and Alexa 488 Fluor goat anti-mouse IgG (1:1,000) was used to visualize actinin.
caveolin isoforms. Slides were mounted with 20 mg/ml propyl gallate in 90% glycerol and images were captured with a cooled charge-coupled device camera (Princeton Instruments, Trenton, NJ) mounted on a Nikon TE 200 microscope (40× neofluor/1.3 NA objective) and processed with Metamorph imaging software.

RESULTS

Previous studies established that caveolin-3 is recovered from neonatal rat cardiomyocyte cultures in light vesicular membranes, along with flotillin-1 and flotillin-2/ESA (19). Because changes in caveolin-3 or flotillin expression in cardiomyocytes are predicted to impact on caveolae biogenesis, initial studies examined the ontogeny of caveolin-related protein expression in ventricular myocardium. Caveolin-2, an accessory protein for caveolin-1 that has been considered a non-muscle cell contaminant in cardiac samples, was also considered in these studies. Figure 1A shows that caveolin-2, caveolin-3, flotillin-1, and flotillin-2 were readily detected in extracts from neonatal and adult ventricular myocardium. Caveolin-3 and caveolin-2 were detected at low levels in immature ventricles (from day 2 to day 15), relative to the much higher levels of expression in 2-mo-old adult ventricles. In contrast, flotillin-1 and flotillin-2/ESA expression was similar in all samples. The absence of a substantial age-dependent difference in the expression of flotillins suggests that age-dependent changes in caveolin-2 and caveolin-3 abundance cannot merely be dismissed as an artifact resulting from nonspecific changes in caveolae-associated protein recovery (due to changes in myofibrillar protein expression and/or cell structure).

To resolve previous uncertainties regarding the source of caveolins identified in cardiac tissues, caveolin/flotillin expression also was examined in cardiomyocytes isolated from intact ventricles. Figure 1B shows that caveolin-2, caveolin-3, flotillin-1, and flotillin-2/ESA immunoreactivity were abundant in extracts from cultured neonatal and isolated adult ventricular myocytes. Caveolin-2 was detected predominantly as the full-length isoform in ventricular tissue but as multiple distinct bands, with mobilities corresponding to full-length caveolin-2α, caveolin-2β (believed to be generated by alternative translation initiation from a single RNA species, analogous to the mechanism described for caveolin-1), and caveolin-2γ [an isoform with an as yet unknown structure (11)] in neonatal rat cardiomyocyte cultures. Caveolin-2 also was detected as full-length caveolin-2α and the smaller caveolin-2β isoforms in adult rat cardiomyocytes. Remarkably, the abundance of caveolin-2 and caveolin-3 was relatively similar in neonatal cardiac cultures and in isolated adult cardiomyocytes. The substantially higher levels of caveolin-2 and caveolin-3 in neonatal cardiomyocyte cultures than in postnatal day 2 ventricular tissue suggests that caveolin-2 and caveolin-3 are induced in parallel during cardiomyocyte culture. Indeed, Fig. 2 shows that caveolin-2 and caveolin-3 expression increased markedly between day 1 and day 4 of culture; flotillin-1 expression is relatively more constant in comparison. Previous studies established that caveolin-1 expression is markedly influenced by cell density in NIH 3T3 cells [in which caveolins play an important role to regulate growth and tumor formation (6)]. However, caveolin-2 and caveolin-3 levels differ little in cardiomyocyte cultures grown at low or high density for 4 days; although Fig. 2 shows a modest reduction in caveolin-2 and caveolin-3 expression at day 6 in the high-density cultures relative to the low-density cultures, this difference was not consistently observed in other experiments. Of note, a caveolin-2 isoform whose mobility corresponds to that of caveolin-2γ was particularly prominent in cardiomyocytes that failed to adhere to substrate during the first 24 h of culture; this form of caveolin-2 is detected only at much lower levels in adherent neonatal cardiomyocyte cultures.

Changes in the level of expression of certain cardiomyocyte gene products have been attributed to a postnatal surge in thyroid hormone secretion (8). Because the β-adrenergic receptor-Gα-adenylyl cyclase complex targets to caveolae in cardiomyocytes [and caveolin-3 regulates the catalytic activity of cardiac adenylyl cyclase isoforms (3, 14, 28)], we examined whether thyroid hormone regulates caveolin/flotillin expression.
Figure 3 shows that thyroid hormone does not alter caveolin or flotillin isoform expression in cardiomyocyte cultures (under conditions in which the predicted thyroid hormone-dependent change in β1-adrenergic receptor expression is prominent). These results indicate that the developmental regulation of caveolin isoform expression is not mediated by thyroid hormone. These results also argue that structural changes in the abundance of caveolae on the cell surface are unlikely to contribute to thyroid hormone-dependent changes in sympathetic tone in cardiomyocytes.

Cardiomyocytes (which express caveolin-3 but not caveolin-1) provide a convenient assay system to determine whether caveolin-2 forms complexes with caveolin-3 and contributes to caveolae biogenesis. In non-muscle cells, caveolin-2 assembles into high-molecular-mass heterooligomers that are recovered in light vesicular fractions only when coexpressed with caveolin-1; caveolin-2 is excluded from light vesicles in cells that lack caveolin-1 (15). In light of previous reports that caveolin-2 does not coassemble into high-molecular-mass heterooligomers with caveolin-3 [under conditions in which caveolin-1-caveolin-2 interactions are prominent (24)], we expected to recover caveolin-2 in the heavy fractions of bottom-loaded sucrose gradients (separate from caveolin-3). However, with an established biochemical fractionation scheme that uses homogenization in sodium carbonate followed by equilibrium centrifugation to separate caveolin-3-enriched membranes from other cell membranes and cytosolic proteins, we recovered caveolin-2 (including the more rapidly migrating isoforms) in low-density vesicles along with caveolin-3 (Fig. 4A). However, it should be noted that both caveolin-2 and caveolin-3 proteins were readily detectable in fivefold greater amounts of protein pooled from the heavy sucrose gradient fractions (fractions 8–13, Fig. 4A, right). Of note, caveolin-1 was not detected in the low-density vesicle fraction prepared from cardiomyocyte cultures, although it is abundant in a similar vesicle fraction prepared from fibroblasts used as positive controls (Fig. 4B). These results argue that the cardiomyocytes themselves (rather than a low level of caveolin-2-enriched contaminating fibroblasts) must be the source of caveolin-2 detected in these experiments.

Figure 3. Thyroid hormone regulates β1-adrenergic receptor (β-AR), but not caveolin or flotillin, protein expression in cardiac cultures. Neonatal rat cardiomyocyte cultures were grown in serum-free medium containing 10^-12 or 10^-8 M 3,5,3’-triiodothyronine (T₃) for 5 days to induce hypo- or hyperthyroidism according to methods described in detail previously (18). Immunoblot analysis was on particular fractions prepared as described previously (18).

**Fig. 3.** Thyroid hormone regulates β₁-adrenergic receptor (β-AR), but not caveolin or flotillin, protein expression in cardiac cultures. Neonatal rat cardiomyocyte cultures were grown in serum-free medium containing 10^-12 or 10^-8 M 3,5,3’-triiodothyronine (T₃) for 5 days to induce hypo- or hyperthyroidism according to methods described in detail previously (18). Immunoblot analysis was on particular fractions prepared as described previously (18).
Immunocytochemistry was used as an additional strategy to exclude the possibility that a contaminating nonmuscle cell is the source of caveolin-2 immunoreactivity in cardiomyocyte cultures. Figure 5A shows that the fibroblasts (with α-actinin-decorated stress fibers) stain for caveolin-1 (but not caveolin-3), whereas cardiomyocytes (with organized sarcomeric banding patterns) stain for caveolin-3 (but not caveolin-1). Caveolin-3 is detected in cardiomyocytes as scattered puncta throughout the cytoplasm and at the cell surface (with surface staining for caveolin-3 particularly prominent at sites of cell–cell contact, where caveolae are described to morphologically concentrate). In contrast, the anti-caveolin-2 antibody stained both cell types with similar intensity but with different patterns. Caveolin-2 is detected as a punctate stain throughout the cytosol and along the cell surface in fibroblasts but as a diffuse cytosolic (and an occasional more intense juxtanuclear) stain in cardiomyocytes. Differences between the caveolin-2 staining pattern in cardiac fibroblasts and cardiomyocytes (and between the caveolin-2 and caveolin-3 staining patterns in cardiomyocytes) were unexpected. However, this result is somewhat reminiscent of results recently reported for skeletal muscle, in which caveolin-3 is detected exclusively at the cell surface and caveolin-1 stains the cytoplasm (12).

Although the immunocytochemical studies identify differences in the subcellular distributions of caveolin-2 and caveolin-3, the biochemical studies suggest that at least some fraction of caveolin-2 might associate and coassemble in heterooligomeric complexes with caveolin-3 in cardiomyocytes (because caveolin-2 targets with caveolin-3 to low-density membranes). To determine whether caveolin-3 interacts in vivo with caveolin-2, cardiomyocytes were solubilized in octyl glucoside (a detergent thought to release caveolin proteins from membranes by displacing endogenous lipids such as glycosphingolipids and cholesterol from membranes). Extracts were subjected to immunoprecipitation with an antibody that selectively recognizes caveolin-3 (and does not cross-react with other caveolae-associated proteins); anti-caveolin-3 immunoprecipitates were subject to Western blot analysis with antibodies that recognize caveolin-2. Figure 6 shows that immunoprecipitation with anti-caveolin-3 effectively clears caveolin-3 immunoreactivity from the cell lysate and leads to the coimmunoprecipitation of caveolin-2 protein. However, not all of the caveolin-2 protein is cleared from the lysates under these conditions, suggesting either that the caveolin-3–caveolin-2 interaction is not sufficiently stable to withstand the immunoprecipitation protocol or that caveolin-2 exists as distinct caveolin-3-interacting and free pools in cardiomyocytes. We also performed the converse experiment; although we were not able to identify conditions to effectively immunoprecipitate all caveolin-2 protein with the anti-caveolin-2 IgG (data not shown), we found that immunoprecipitation of caveolin-2 results in the coprecipitation of caveolin-3. Controls demonstrating that caveolin-3 does not coimmunoprecipitate with an irrelevant mouse IgG1 antibody have been published (19).
DISCUSSION

Caveolin-2 has been viewed as an accessory protein for caveolin-1 with no known role in muscle tissues that express caveolin-3 (1, 24). Although caveolin-2 has been considered a nonmuscle cell contaminant when detected in extracts from intact ventricles, this study provides the first evidence that caveolin-2 is expressed by cardiomyocytes and that caveolin-2 can stably incorporate into heterooligomeric complexes with caveolin-3. In neonatal cardiomyocyte cultures, caveolin-2 is detected as full-length caveolin-2/H9251 as well as smaller isoforms. Although these more rapidly migrating caveolin-2 species were tentatively identified as caveolin-2/H9252 and caveolin-2/H9253 (on the basis of their mobilities), it is reasonable to also consider the possible presence of a novel caveolin-2 splice variant (lacking the COOH-terminal 49 amino acids) recently amplified from the mouse heart and other tissues (9), although not yet identified by Northern or Western blot analysis in native tissues. This caveolin-2 splice variant migrates in SDS-PAGE just slightly faster than caveolin-2β; it localizes to intracellular reticular membranes (rather than surface membranes) when expressed in heterologous expression systems, similar to a pool of caveolin-2 protein in cardiomyocytes. A rapidly migrating form of caveolin-2 that comigrates with caveolin-2γ but could represent a proteolytic product of the full-length caveolin-2) accumulates in cardiomyocytes that fail to adhere to substrate during the first 24 h of culture. This caveolin-2 species deserves further study in view of recent evidence that caveolin-2 is a target for regulatory phosphorylation during integrin ligation as well as maturation of the caveolin complex (23).

The identification of caveolin-2 in cardiomyocytes in this study is at odds with conclusions promulgated by two other laboratories whose studies were performed in genetic models of caveolin isoform overexpression and/or gene deletion in mice or cardiomyocytes isolated from the adult rat ventricle (17, 30). Differences in the results obtained in this and previous studies cannot be ascribed to species-dependent differences in caveolin-2 expression, because we detected relatively similar levels of caveolin-2 expression in neonatal rat and neonatal mouse cardiomyocyte cultures (data not shown).

Fig. 5. Cardiomyocytes express caveolin-3 and caveolin-2; caveolin-1 and caveolin-2 are detected in cardiac fibroblasts. A: mixed cultures of cardiomyocytes and cardiac fibroblasts grown on fibronectin-coated glass coverslips were fixed in methanol and double labeled with a polyclonal IgG directed against α-actinin [detected with Alexa Fluor 594 goat anti-rabbit IgG (red channel)] and MAbs against caveolin-1 (MAb 2234), caveolin-2 (MAb 65), or caveolin-3 (MAb 26) [detected with Alexa Fluor 488 goat anti-mouse IgG (green channel)]. α-Actinin was detected to discriminate cardiomyocytes with a highly organized sarcomeric banding pattern from cardiac fibroblasts, which contain α-actinin-decorated actin-containing stress fibers. Cardiomyocytes are identified by arrows; cardiac fibroblasts are identified by arrowheads. *Prominent caveolin-3 staining at a cell-cell contact. B: images showing that pure cardiac fibroblast cultures stain with caveolin-1 and caveolin-2 but not with caveolin-3.
we could identify an occasional cardiac myocytes (albeit at different levels). Finally, although lin-2 is detected in both neonatal and adult rat cardiomyocytes (which do not contain significant levels of cardiac fibroblast contamination). Rather, this study suggests that the divergent results between this and previous studies might be reconciled by two factors. First, it is possible that a relatively diffuse caveolin-2 staining pattern in cardiomyocytes might be obscured (or dismissed as nonspecific) in the context of the more intense membrane staining for caveolin-2 in endothelial cells and other nonmuscle cell elements in immunohistochemical studies of tissue sections. Alternatively, the failure to detect caveolin-2 in some previous studies might be the result of technical factors such as the amount and/or purity of the samples used for immunoblot analysis.

Changes in caveolin-1 or caveolin-3 expression impact substantially on caveolae biogenesis, but the factors that regulate caveolin isoform expression during development and in disease states remain largely unknown. Engelman et al. (2) reported that caveolin-3 mRNA and protein are first detected in mouse embryos at day 15; although caveolin-1 and caveolin-2 mRNAs are detected at a somewhat earlier stage (day 7), these proteins also are not detected until day 15, identifying caveolins as late markers of differentiation during embryogenesis. Other studies indicate that caveolin-1 expression is downregulated in rapidly growing cells (or in cells transformed by an oncogene), whereas caveolin-1 expression is dramatically upregulated as cells reach confluence or differentiate (6). Caveolin-3 behaves in a similar fashion, in that it is not detected in precursor myoblasts but becomes abundant in differentiated myotubes (24, 27). Results of these studies establish that caveolin-3 and caveolin-2 expression increase with age (through a mechanism distinct from the regulatory effects of thyroid hormone) and during culture of ventricular cardiomyocytes. The results support the notion that caveolin expression is induced during cardiomyocyte differentiation.

Mechanisms that regulate caveolin-2 protein expression have remained even more elusive. The observation that caveolin-2 expression remains constant during oncogenic transformation of NIH 3T3 cells [in which caveolin-1 levels are dramatically downregulated (25)] has been taken as evidence that caveolin-2 mRNA expression is regulated independently of other caveolin isoforms. However, other studies demonstrate that caveolin-2 protein levels are dramatically upregulated (without a change in steady-state mRNA levels) after expression of recombinant caveolin-1 in K562 cells that lack endogenous caveolin-1 (15). Conversely, caveolin-2 protein is reduced in caveolin-1-null and caveolin-1/3 double-knockout mice (16). These studies have been taken as evidence that caveolin-2 protein is stabilized when in heteroligomeric complexes with caveolin-1. The parallel increase in caveolin-3 and -2 expression during cardiac development could suggest a similar regulatory interaction between caveolin-3 and caveolin-2 in cardiomyocytes.

In summary, these studies identify caveolin-2 expression and caveolin-2 interactions with caveolin-3 in cardiomyocytes. The identification of caveolin-2 in a broad range of cardiomyocyte preparations suggests that the previous inclination to dismiss caveolin-2 as irrelevant to the biology of cardiomyocytes may have been premature. Rather, these studies suggest that caveolin-2 might participate with caveolin-3 in the biogenesis of caveolae (and the spatial organization of biochemical and ionic events at specialized surface

Fig. 6. Caveolin-2 and caveolin-3 form stable heterooligomeric complexes. Neonatal cardiomyocyte cultures were lysed and subjected to immunoprecipitation (IP) with anti-caveolin-3 IgG (left). The immunoprecipitate was then probed by immunoblot (IB) analysis with anti-caveolin-2 and anti-caveolin-3. Conversely, lysates were immunoprecipitated with anti-caveolin-2 IgG and then probed for caveolin-2 and caveolin-3 (right). Caveolin-2 and caveolin-3 were coimmunoprecipitated with both antibodies, indicating that caveolin-2 and caveolin-3 form complexes in vivo.
membranes) in cardiomyocytes. However, the distinct immunocytochemical staining patterns for caveolin-2 and caveolin-3 in cardiomyocytes also suggest that caveolin-2 may have additional hitherto unrecognized functions in cardiomyocytes.

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REFERENCES


