Intracellular Ca\(^{2+}\) release in muscle is governed by functional communication between the voltage-dependent L-type Ca\(^{2+}\) channel and the intracellular Ca\(^{2+}\) release channel by processes that are incompletely understood. We previously showed that sorcin binds to cardiac Ca\(^{2+}\) release channel/ryanodine receptors and decreases channel open probability in planar lipid bilayers. In addition, we showed that sorcin antibody immunoprecipitates ryanodine receptors from metabolically labeled cardiac myocytes along with a second protein having a molecular weight similar to that of the \(\alpha_1\) subunit of cardiac L-type Ca\(^{2+}\) channels. We now demonstrate that sorcin biochemically associates with cardiac and skeletal muscle L-type Ca\(^{2+}\) channels specifically within the cytoplasmically oriented C-terminal region of the \(\alpha_1\) subunits, providing evidence that the second protein recovered by sorcin antibody from cardiac myocytes was the 240-kDa L-type Ca\(^{2+}\) channel \(\alpha_1\) subunit. Anti-sorcin antibody immunoprecipitated full-length \(\alpha_1\) subunits from cardiac myocytes, C2C12 myotubes, and transfected non-muscle cells expressing \(\alpha_1\) subunits. In contrast, the anti-sorcin antibody did not immunoprecipitate C-terminal truncated forms of \(\alpha_1\) subunits that were detected in myotubes. Recombinant sorcin bound to cardiac and skeletal HIS\(_6\)-tagged \(\alpha_1\) C termini immobilized on Ni\(^{2+}\) resin. Additionally, anti-sorcin antibody immunoprecipitated C-terminal fragments of the cardiac \(\alpha_1\) subunit exogenously expressed in mammalian cells. The results identified a putative sorcin binding domain within the C terminus of the \(\alpha_1\) subunit. These observations, along with the demonstration that sorcin accumulated substantially during physiological maturation of the excitation-contraction coupling apparatus in developing postnatal rat heart and differentiating C2C12 muscle cells, suggest that sorcin may mediate interchannel communication during excitation-contraction coupling in heart and skeletal muscle.

The release of Ca\(^{2+}\) from muscle sarcoplasmic reticulum (SR)\(^3\) is the principal link between electrical excitation of the sarcolemma and mechanical activation of the myofilaments, a process known as excitation-contraction (E-C) coupling. Ca\(^{2+}\) release from stores in the cardiac SR occurs via Ca\(^{2+}\) release channels that are referred to as ryanodine receptors (RYRs). In the heart, Ca\(^{2+}\) release from the SR is largely triggered by Ca\(^{2+}\) influx at the plasma membrane via voltage-dependent L-type Ca\(^{2+}\) channels that are also dihydropyridine receptors (DHPRs) (1–7). In skeletal muscle, DHPRs serve as voltage sensors to detect depolarization of the sarcolemmal transverse tubules (T-tubules) and provide the physical impetus for opening of the SR Ca\(^{2+}\) release channels (1, 8–11). In both tissues, the geometry and close spatial relationship between sarcosomal L-type channels and SR RyRs is critical in determining the time course of Ca\(^{2+}\) release and E-C coupling; however, the molecular mechanisms that mediate interchannel communication are incompletely understood (1, 12–14). The possibility that additional proteins or factors might be interposed to facilitate cross-talk has often been suggested (1, 8, 12).

Sorcin, a 22-kDa Ca\(^{2+}\)-binding protein first identified in multidrug-resistant cells, is widely distributed among mammalian tissues, including heart and skeletal muscle (15–19). At the subcellular level, sorcin localizes to T-tubule junctions of cardiac SR (19) and co-localizes with brain RyR in rat brain caudate-putamen nucleus (20, 21). We previously demonstrated that introduction of sorcin into nonmuscle cells confers the property of caffeine-activated intracellular Ca\(^{2+}\) release, suggesting a role for sorcin in modulating RyR function (19). This hypothesis was further strengthened by the demonstration that sorcin completely inhibits ryanodine binding to cardiac RyRs and substantially decreases the open probability of the Ca\(^{2+}\) release channels reconstituted in lipid bilayers (22). Sorcin is, therefore, one of a group of modulators of RyR gating that includes calmodulin and FK506-binding protein, ligands that bind to a RyR domain that connects directly to a cytoplasmic extension of the transmembrane assembly of the receptor (23–27).

Anti-sorcin antibody immunoprecipitates two proteins from metabolically labeled cardiac myocytes (19), one of which we previously identified as the 565-kDa cardiac RyR (19), and the other (~220 kDa) displayed an electrophoretic mobility similar to that of the major, pore-forming \(\alpha_1\) subunit of the cardiac L-type Ca\(^{2+}\) channel (\(\alpha_{1C}\)) (28, 29). Here we directly test the possibility that the unidentified protein is \(\alpha_{1C}\) by analyzing the

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¶ The abbreviations used are: SR, sarcoplasmic reticulum; DHPR, dihydropyridine receptor; E-C coupling, excitation-contraction coupling; HEK, human embryonic kidney; MHC, myosin heavy chain; RyR, ryanodine receptor; TBST, Tris-buffered saline with Tween 20; T-tubule, transverse tubule; PBS, phosphate-buffered saline.
interaction of sorcin and the α1 subunits of cardiac and skeletal muscle Ca\(^{2+}\) channels. The ability of sorcin to interact with sarcolemmal L-type channels, in concert with its modulatory effect on RyR gating, positions sorcin as a candidate regulator of interchannel cross-talk.

**MATERIALS AND METHODS**

**Cell Lines and Cultured Cardiac Myocytes**—COS-1 cells were obtained from ATCC (Rockville, MD) and grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Culture conditions for human embryonic kidney (HEK) 293 or Sf9 insect cells have been described (29–31). Mouse C2C12 myoblasts (32–34) were maintained in Dulbecco’s modified Eagle’s medium containing 15% fetal bovine serum and were transferred to Dulbecco’s modified Eagle’s medium containing 2% horse serum to initiate differentiation, a process that serves as a model of normal myogenesis. Formation of multinucleated myotubes was observed within 48 h after medium transfer. Preparation of rat cardiac myocytes was carried out according to published procedures (19, 35).

**Membrane Preparations**—Sf9 and HEK 293 cell membranes were prepared as described previously (29, 30). Fractionation of rat heart tissue for soluble and crude membrane components was carried out by differential centrifugation according to our published procedures (29).

**Antibodies and Expression Vectors**—Preparation and characterization of sorcin antibodies (19); of SKN, SKC, Card I, and Card C antibodies; and of α1C and α1S expression vectors has been described previously (30, 36). Briefly, the SKN and SKC antibodies recognize N-terminal and C-terminal domains on the α1S subunit (36), while the Card I and Card C antibodies recognize internal and C-terminal domains of the α1C subunit (37). The RyR antibody was a generous gift of Dr. Gerhard Meissner (University of North Carolina) (37). The anti-myosin heavy chain (MHC) antibody FS9 has been described (34). Anti-polyhistidine was purchased from Sigma, and horseradish peroxidase-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and used according to the manufacturer’s directions.

**Western Blot Analysis and Immunoprecipitation**—Soluble fractions of cultured cells and tissues, as well as solubilized membrane fractions (described above) were prepared for analysis in buffer A. Samples containing 60 μg of protein (38) were separated by gel electrophoresis on 6% (for analysis of α1 subunits, RyR, and MHC) or 11% (for sorcin and HIS\(_{S}\)-tagged proteins) polyacrylamide gels under denaturing conditions (39). Proteins were transferred to nitrocellulose, and the membranes were processed for Western blotting with detection by chemiluminescence (Amersham Pharmacia Biotech) as described previously (30). Additional details are given in the figure legends. For immunoprecipitation assays, aliquots of protein samples in buffer A containing 100 or 200 μg of protein in a total of 0.25 ml of buffer A were incubated with 1 μg of antibody raised against peptides from either the N or C terminus of sorcin (19) for 1 h at 4 °C. Antibody-antigen complexes were precipitated with protein G (Sigma), washed with buffer A, and solubilized in Laemmli (39) buffer at room temperature for 15 min. Immunoprecipitated proteins were analyzed by Western blot as described above and in the figure legends. Molecular weight markers were purchased from Amersham Pharmacia Biotech or Bio-Rad.

**In Vitro Binding Assays**—Fusion proteins of the L-type Ca\(^{2+}\) channel α1C and α1S C-terminal domains were expressed in bacteria with vectors constructed by ligation of a genomic BglII–BamHI fragment of α1C (GeneBankTM accession number X05921) into the BamHI site of pQE (QIAGEN, Valencia, CA). The proteins were expressed with an N-terminal HIS\(_{S}\) tag fused to amino acids 1622–2171 (40) of the α1C or α1S C terminus or to amino acids 1497–1873 (40) of the α1C C terminus. For immobilization of fusion peptides on Profab Ni\(^{2+}\) resin (Invitrogen Corp., San Diego, CA), bacterial lysates containing the fusion proteins were prepared according to the manufacturer’s directions by sonication and freeze-thaw in phosphate-buffered saline (PBS) at pH 7.8 (binding buffer). Cleared supernatants were applied to washed resin beds by batch absorption, and treated resin samples were washed three times with binding buffer, twice with PBS at pH 6.0, and three times with PBS at pH 7.4. Aliquots of 10 μg of recombinant sorcin (41) in PBS (pH 7.4) were then combined with the protein-containing resins for 30 min at 4 °C. Resins were washed three times with PBS (pH 7.0), and bound proteins were eluted in 0.5 M imidazole. HIS\(_{S}\)-tagged α1C, a cyclin-dependent kinase inhibitor (42), was used as a negative control. Eluted proteins were analyzed by Western blot after electrophoresis on 11% acrylamide. Nitrocellulose membranes containing transferred proteins were sequentially probed with antisera against α1C and β1 subunits of the anti-polyhistidine antibody and then with rabbit polyclonal antibody to a peptide from the C terminus of sorcin (19).

**PREPARATION AND TRANSFERENCE OF HIS\(_{S}\)-TAGGED C-TERMINAL FRAGMENTS OF α1C**—A fragment (designated fragment A) of the C-terminal cytoplasmic domain of α1C (amino acids 1622–2171) (41) was removed from the α1C expression vector (30) and cloned in frame into the pHISA expression vector (Invitrogen), which added 30 amino acids, including the HIS\(_{S}\) epitope, at the C terminus. Fragments B (amino acids 1622–1772), C (amino acids 1749–1978), D (amino acids 1622–1772), and E (amino acids 1622–1748) from the α1C C terminus were prepared by polymerase chain reaction and cloned into pHISA. Polymerase chain reaction primers included restriction sites to facilitate subsequent cloning. The following primer pairs were used: fragment B, sense GGAACACCTGGAGAAGCAGCAGAC (also used in reverse direction) and antisense GGCATCTAGATCCTCAACGCGATTGTCG; fragment C, sense GCGAGACCTCTCCCGACAGCCCTCACTAGC and antisense GCTTCTGACAGGAGGTGGGGGAATGCG; fragment D, antisense GCTCTAGATCAGTAGGAGGTCCACACCATCCGTCTCTG; fragment E, antisense GCTCTAGAATCTCCACCTGCGATTGTCG. Plasmids encoding the fragments were transiently co-transfected with the full-length sorcin vector pRCD-MYSOR (19) in COS-1 cells or alone in HEK cells with the use of lipofectamine (Life Technologies, Inc.). Association of sorcin and fragments was studied by immunoprecipitation with sorcin antibody and Western blot analysis.

**RESULTS**

**Sorcin Antibody Immunoprecipitates α1C**—We previously showed that sorcin antibody recovered two proteins from rat cardiac myocytes metabolically labeled with \(^{85}\)S)methionine, a 565-kDa protein shown to be the RyR and an unidentified ~220-kDa protein (19). Here we demonstrate that a single protein recognized by Card I, an α1C-specific antibody shown to recognize the 240-kDa α1C (29, 30), was immunoprecipitated from rat heart tissue by sorcin antibody (Fig. 1A, lane 1). Card C, an antibody directed against the C terminus of α1C (30), also recognized this protein (see Fig. 4). Results of immunoprecipitations carried out in the absence of Ca\(^{2+}\) (not shown) were indistinguishable from those shown in Fig. 1A. Rat heart contained the 22-kDa sorcin, which co-migrated with recombinant sorcin (41) (Fig. 1B, lane 1), and an 18-kDa species (Fig. 1B, lane 1). Both sorcin forms were detected in most rat (and mouse) heart samples; tissues from some animals contained only the 22-kDa form (19).

To confirm the identity of the cardiac protein recognized by Card I and Card C, we examined whether α1 subunits could be immunoprecipitated with sorcin antibody from Sf9 cells infected with a recombinant baculovirus directing expression of α1C or from HEK 293 cells transiently expressing full-length α1C. This ability would require the presence of endogenous sorcin species in those nonmuscle cells. We found that sorcin antibody recognized an 18-kDa protein in HEK 293 cells (Fig. 1B, lane 2), similar in size to the 18-kDa protein in heart, and an ~30-kDa protein in Sf9 cells (Fig. 1B, lane 3). The 22-, 24-, and ~30-kDa bands in lane 1 were not detected on Western blots with anti-polyhistidine antibody (data not shown). Card I recognized a protein immunoprecipitated by anti-sorcin antibody from α1C-expressing Sf9 (Fig. 1A, lane 4) or HEK 293 cells (Fig. 1A, lane 6). The immunoprecipitated bands co-migrated with bands detected by Card I by direct Western blot of proteins from Sf9 and HEK 293 cells heterologously expressing the α1C subunit (data not shown). No
Sorcin Associates with L-type Ca\textsuperscript{2+} Channels

**Fig. 1.** Sorcin antibody immunoprecipitated endogenously expressed α1C and α1S from heart tissue and C2C12 cell myotubes, respectively, and heterologously expressed α1 subunits from Sf9 and HEK 293 cells. A, Western blots with Card I antibody. Immunoprecipitation of 200-μg samples of membrane proteins from rat heart (lane 1), α1C-expressing Sf9 (lane 2), uninfected Sf9 (lane 3), and HEK 293 cells (lane 10), and HEK 293 cells (lane 2), and α1S (lanes 2) lysed in buffer A (see "Materials and Methods"). Myoblasts (lanes 1 and 5) and fully differentiated myotubes (lanes 2 and 6) were analyzed. lanes 1, 2, 5, and 6 show soluble proteins (60 μg/lane) from C2C12 cells lysed in buffer A (see "Materials and Methods"). Myoblasts (lanes 1 and 5) and fully differentiated myotubes (lanes 2 and 6) were analyzed. Lanes 3 (blasts) and 4 (myotubes) show proteins immunoprecipitated with sorcin antibody from 200 μg of C2C12 cell protein. Immunoprecipitation of 200-μg samples of membrane proteins from uninfected Sf9 (lane 7), α1C-expressing Sf9 (lane 8), untransfected HEK 293 (lane 10), or α1C-expressing HEK cells (lane 11) with sorcin antibody is depicted. See the Fig. 1A legend for Western blot conditions.

Specific proteins identified by Card I were recovered by immunoprecipitation of α1C-expressing Sf9 cells with preimmune serum (Fig. 1A, lane 2) or by immunoprecipitation of uninfected Sf9 (Fig. 1A, lane 3) or untransfected HEK cells (Fig. 1A, lane 5) with anti-sorcin antibody. These results strongly suggested that a heretofore unidentified protein immunoprecipitated with sorcin antibody from metabolically labeled cardiac myocytes (19) was α1C.

The cardiac α1C subunit has been previously reported to exist in two forms in isolated cardiac membranes (29, 43). A minor fraction of the cardiac α1C is the full-length protein of ~240 kDa that can be recognized by both the Card I and Card C antibodies (29), while the major fraction of α1C in cardiac membranes is a C-terminal truncated protein of ~190 kDa that reacts with Card I but not Card C (29). Interestingly, in the experiments described here, only the full-length form of the α1C was immunoprecipitated by the anti-sorcin antibody (Fig. 1A), suggesting a potential role of the C terminus for the α1C/sorcin interaction.

**Sorcin Antibody Immunoprecipitates Full-length α1S—**We next addressed the question of whether the α1C/sorcin association was α1C isoform-specific. SKN, an α1C-specific antibody generated against the N terminus of α1S and shown to recognize full-length (214-kDa) and C-terminal truncated (170–190-kDa) forms of α1S (31, 36, 44), recognized three proteins in C2C12 myotubes (Fig. 1C, lane 2). None of these proteins were detected in undifferentiated C2C12 myoblasts (Fig. 1C, lane 1); up-regulation of the α1S subunit during skeletal muscle development has been established (45, 46). The anti-sorcin antibody immunoprecipitated only the most slowly migrating form that was detected in myotubes, while lower M\textsubscript{s} forms that were reactive with SKN were not recovered (Fig. 1C, lane 4). The SKC antibody, directed against the α1S C terminus, recognizes the full-length 214-kDa α1S subunit but does not detect the C-terminal truncated forms of α1S that are commonly observed in skeletal muscle (44, 47). In the present experiments, SKC recognized only the full-length subunit in C2C12 myotubes (Fig. 1C, lane 6), a band of similar size recovered by sorcin antibody (Fig. 1C, lane 4). The M\textsubscript{s} of full-length α1S has been established by Ferguson plot analysis to be 214 kDa, although under most conditions it migrates anomalously as an ~190-kDa protein (44). From these data, we concluded that the lower M\textsubscript{s} bands in C2C12 myotubes that were immunoreactive with SKN, but not with SKC, were C-terminal truncations of α1S, and that the sorcin antibody only immunoprecipitated full-length α1S. These results suggested that an intact C terminus in α1S was necessary for interaction with sorcin. As in the α1C studies, we examined whether sorcin antibody would recover α1S heterologously expressed in Sf9 and HEK 293 cells. SKN (and SKC, not shown) recognized a single protein immunoprecipitated by sorcin antibody from α1S-expressing Sf9 (Fig. 1C, lane 9) and HEK 293 (Fig. 1C, lane 11) cells. The bands comigrated with proteins recognized by SKN and SKC by direct Western blot (data not shown). No specific proteins identified by SKN were recovered by sorcin antibody immunoprecipitation of uninfected Sf9 (Fig. 1C, lane 7), Sf9 infected with α1C (Fig. 1C, lane 8), or untransfected HEK cells (Fig. 1C, lane 10). The results are consistent with the interpretation that sorcin associates with the α1S and α1C subunits and that an intact C terminus in each protein is necessary for this interaction.

**Association of Sorcin with α1C/C Termi- nus Domains—**In order to test the hypothesis that sorcin interacts with C-terminal domains of α1C and α1S, HIS\textsubscript{6}-tagged C terminus fragments of α1C and α1S were immobilized on Ni\textsuperscript{2+} resin and incubated with recombinant sorcin protein. As shown in Fig. 2, recombinant sorcin (22 kDa) specifically bound to immobilized α1S (Fig. 2,
The irrelevant HIS6-tagged p27 protein was observed (Fig. 2, respectively). In contrast, no specific association of sorcin with was confirmed and extended in a mammalian expression sys-

skeletal

antibody consistently immunoprecipitated fragment A, extend-

frgments depicted in the

indicates an IgG artifact. Sorcin’s putative (26 kDa) were recovered by sorcin antibody. The

D (amino acids 1622–1772) (31 kDa), and E (amino acids 1622–1748) (amino acids 1622–2171) (70 kDa), B (amino acids 1622–1872) (37 kDa),

cipitated proteins are in the second set of

sorcin, Fig. 1

HEK transfected cells.

antibody immunoprecipitated the fragments shown from both COS and COS-1 cells transiently co-transfected with full-length sor-

C terminus, it is noteworthy that the cardiac and skeletal α1 isofoms are highly homologous (40) in the domain

lanes 1C C terminus

α1C binding domain is within

A–E lanes

Membrane proteins were fractionated on 11% gels. The

arrows indicate 22-kDa sorcin. The 18-kDa form.

B. Western blot with sorcin antibody. Aliquots containing 60 μg of protein from soluble fractions of isolated rat cardiac myocytes lysed in buffer A 1 day (lanes 1 and 2), 2 days (lanes 3 and 4), and three days after plating (lanes 5 and 6) were analyzed. Lanes 2, 4, and 6 contain proteins from cells maintained in the presence of 10 mM verapamil (VRPL). The arrow indicates 22-kDa sorcin.

implicated in the α1/sorcin interaction.

Increase in Sorcin Expression During Muscle Development—To further probe potential relationships between sorcin and L-type Ca2⁺ channel α1 subunits, we examined the development- al expression of sorcin during physiological maturation of E-C coupling in developing muscle. It is well established that the expression of sarcolemmal and SR channel proteins is regulated in concordance with a program of muscle maturation (1, 45, 46, 48–52). Sorcin abundance in postnatal rat heart substantially increased from day 1 to day 12 after birth (Fig. 4A). Both the 22- and 18-kDa species, depicted in Fig. 1B, increased in parallel. The α1C subunit was readily detectable as early as postnatal day 1 and also increased in abundance during this time period (Fig. 4A), in agreement with previous reports (45).

Sorcin was not present in neonatal rat cardiac myocytes 24 h after establishment in culture (Fig. 4B, lanes 1 and 2) but was detected on subsequent days as the cells commenced beating (Fig. 4B, lanes 3 and 5). In contrast to the intact heart (Fig. 1B, lane 1, and Fig. 4A) and freshly isolated adult cardiac myocytes (data not shown), only the 22-kDa form was observed in neo- natal myocytes. We next examined whether contractile arrest, produced by treating cells with verapamil (51), would affect sorcin expression. As shown in Fig. 4B, lanes 4 and 6, the level of sorcin was substantially reduced in verapamil-arrested cells.

The 22-kDa sorcin (the 18-kDa form was not detected) also accumulated in differentiating mouse skeletal muscle C2C12 cells (Fig. 5). RyR expression increased in parallel with sorcin, whereas myosin heavy chain was only detected after a 48-h period in mitogen-depleted medium (Fig. 5), consistent with previous reports (33, 52). Full-length and C-terminal truncated forms of the α1S subunit were expressed in early stages of differentiation and were up-regulated in parallel during pro- gression to fully differentiated myotubes (Fig. 5, lanes 3 and 4).

DISCUSSION

The voltage-sensing L-type Ca2⁺ channels in T-tubules play a major role in E-C coupling in both heart and skeletal muscle (1, 28). The close apposition of the L-type channels and RyRs in specific spatial relationships is required to accurately bridge
Sorcin Associates with L-type Ca\(^{2+}\) Channels

FIG. 5. Western blots of C2C12 cells with antibodies raised against RyR (565 kDa), MHC (200 kDa), \(\alpha_{1C}\) (214-kDa and truncated forms), and sorcin (22 kDa). Cells were lysed in buffer A, and 60 \(\mu\)g of protein were applied to each lane for each antibody. SKN antibody was used to detect \(\alpha_{1C}\), and other antibodies were described under “Materials and Methods.” Myoblasts (lane 1), cells in mitogen-depleted medium for 6 or 24 h (lanes 2 and 3, respectively), and fully differentiated myotubes 48 h after mitogen depletion (lane 4) were analyzed. RyR, MHC, and \(\alpha_{1C}\), were analyzed on 8% gels, and sorcin was analyzed on 11% gels. The legend to Fig. 1A and Ref. 19 describe additional Western blot conditions.

The results of the present study indicate that the Ca\(^{2+}\) channel function is not known. We have no evidence that sorcin antibody recovered functional channels. The cytoplasmically oriented C terminus of \(\alpha_{1C}\) has been shown to be involved in Ca\(^{2+}\)-sensitive channel inactivation, and a 142-amino acid segment from amino acids 1572–1717 in the \(\alpha_{1C}\) C terminus has been suggested to be required for inactivation (62). The putative sorcin-binding domain delineated in this report is within the margins of the potential inactivation domain, and suggests that sorcin’s involvement in this aspect of L-type Ca\(^{2+}\) channel regulation should be investigated.

We found that sorcin expression increases in abundance in both developing heart and differentiating muscle cells. The contractile machinery in developing rodent heart is activated largely by Ca\(^{2+}\) entering the cell through L-type channels (1, 49). As the T-tubule/SR system gradually develops, contraction becomes more dependent on SR Ca\(^{2+}\) release activated by Ca\(^{2+}\) entry through sarcolemmal channels (49). The increase in sorcin expression in postnatal rat heart coincides with the developing Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism, consistent with a putative role for sorcin in L-type channel/RyR interchannel communication. The increase in sorcin expression during C2C12 myotube formation, commensurate with L-type Ca\(^{2+}\) channel and contractile apparatus development, is in accord with a role for sorcin in interchannel communication in skeletal muscle as well. A program of coordinate expression of myocyte proteins involved in contraction and Ca\(^{2+}\) regulation has been suggested (51). Treatment of heart cells with verapamil initiates divergent expression of proteins that may be involved in this program along with contractile arrest (51). Whether sorcin, whose expression was substantially reduced in verapamil-arrested myocytes, is a component of that Ca\(^{2+}\) regulation program remains to be determined.

Sorcin may undergo Ca\(^{2+}\)-mediated dynamic changes in structure and subcellular localization, allowing it to either simultaneously or sequentially interact with L-type Ca\(^{2+}\) channels and RyR in response to changing Ca\(^{2+}\) levels. [Ca\(^{2+}\)] changes in the sarcolemmal/SR microenvironment may result in conformational changes and translocation of sorcin from cytoplasmic to membrane locations (41, 61) in a manner analogous to the Ca\(^{2+}\)-mediated conformational modification of recoverin (63). Ca\(^{2+}\)-mediated dimerization of sorcin, shown to occur in vitro (60), may also occur in vivo. In addition, the recent demonstration of sorcin binding to annexin VII (64), thought to play a role in E-C coupling in skeletal muscle (65), and our demonstration that calmodulin exerts an additive effect on sorcin’s inhibition of ryanodine binding to cardiac RyR (22) suggests that sorcin may function in conjunction with other channel accessory proteins. Whether these proteins might include L-type Ca\(^{2+}\) channel subunits other than \(\alpha_{1}\) is speculative; direct interaction between sorcin and other channel subunits was not in evidence.

Our studies demonstrating that sorcin interacts with both sarcolemmal L-type Ca\(^{2+}\) channels and SR Ca\(^{2+}\) release channels suggest a role for sorcin in interchannel communication. Sorcin may act as a sensor of the T-tubule junctional environment to ultimately participate in regulation of intracellular Ca\(^{2+}\) mobilization and E-C coupling.

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REFERENCES

1. Bers, D. M. (1993) Excitation-Contraction Coupling and Cardiac Contractile Force. Kluwer Academic Publishers, Boston.

2. Gyorke, S., and Fill, M. (1995) Science 265, 807–809.

3. Lipp, P., and Niggli, M. (1994) Proc. Natl. Acad. Sci. 91, 5792–5799.

4. Okada, K., Kinosita, K., and Hayashi, M. (1993) J. Cell Biol. 121, 1263–1277.

5. Shieh, E. F., and Cohen, M. A. (1994) J. Biol. Chem. 269, 11997–12000.

6. Zamparelli, C., Zamparelli, A., and Piacentini, F. (1994) Cell Calcium 15, 305–316.

7. Nakajima, I., and Inoue, K. (1994) J. Biol. Chem. 269, 24171–24177.

8. Kao, C. S., and Kao, L. (1994) J. Biol. Chem. 269, 18542–18549.

9. Nakajima, I., and Inoue, K. (1994) J. Biol. Chem. 269, 18542–18549.

10. Zamparelli, C., Zamparelli, A., and Piacentini, F. (1994) Cell Calcium 15, 305–316.

11. Nakajima, I., and Inoue, K. (1994) J. Biol. Chem. 269, 18542–18549.

12. Kao, C. S., and Kao, L. (1994) J. Biol. Chem. 269, 18542–18549.

13. Zamparelli, C., Zamparelli, A., and Piacentini, F. (1994) Cell Calcium 15, 305–316.

14. Kao, C. S., and Kao, L. (1994) J. Biol. Chem. 269, 18542–18549.

15. Zamparelli, C., Zamparelli, A., and Piacentini, F. (1994) Cell Calcium 15, 305–316.